# I. melléklet

**POPPE, L.**, NOVÁK, L., DÉVÉNYI, J., SZÁNTAY, CS.:

Baker's Yeast Mediated Synthesis of (5SR,9S)-5,9-Dimethyl-heptadecane and (5SR,9S)-5,9-Dimethylpentadecane; the Main Sex-Pheromone Components of *Leucoptera scitella* and *Leucoptera coffeella* Enriched in 9S-Isomers,

*Tetrahedron Lett.*, **1991**, *32*, 2643.

Tetrahedron Letters, Vol.32, No.23, pp 2643-2646, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

# BAKER'S YEAST MEDIATED SYNTHESIS OF (5**SR**,9**S**)-5,9-DIMETHYL-HEPTADECANE AND (5**SR**,9**S**)-5,9-DIMETHYL PENTADECANE; THE MAIN SEX-PHEROMONE COMPONENTS OF *Leucoptera scitella* AND *Perileucoptera coffeella* ENRICHED IN 9**S**-ISOMERS

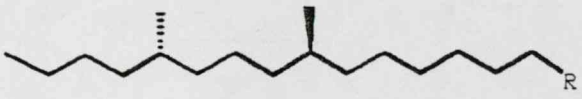
L. POPPE [a], L. NOVÁK [b], J. DÉVÉNYI [b], CS. SZÁNTAY [a,b]

[a] Central Research Institute of Chemistry, 1525 Budapest, P.O. box 17, HUNGARY

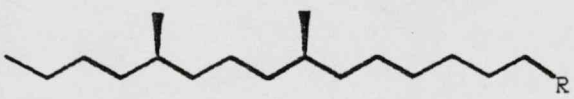
[b] Institute for Organic Chemistry, Technical University of Budapest, 1521 Budapest, Gellert ter 4., HUNGARY

**ABSTRACT**: A mixture of (5**S**,9**S**)-5,9-dimethyl heptadecane (**1a**), the main sex-pheromone component of *Leucoptera scitella*, and its (5**R**,9**S**)-isomer (**2a**) was synthesized conveniently from (**R**)-citronellal (**4**, obtained from racemic citronellal by enantiomer selective baker's yeast reduction) in four steps.
(5**SR**,9**S**)-5,9-Dimethyl-pentadecane (mixture of **1b** and **2b**), a possible sex-attractant of *Perileucoptera coffeella* was prepared analogously.

5,9-Dimethylheptadecane and 5,9-dimethylpentadecane were isolated and identified as the major sex pheromone components of mountain-ash bentwing (*Leucoptera scitella*, Zeller) and *Perileucoptera coffeella* (Guer.-Menev), respectively[1,2]. Although the (5**S**,9**S**) isomer (**1a**) carries the biological




1a: R= Et
1b: R= H



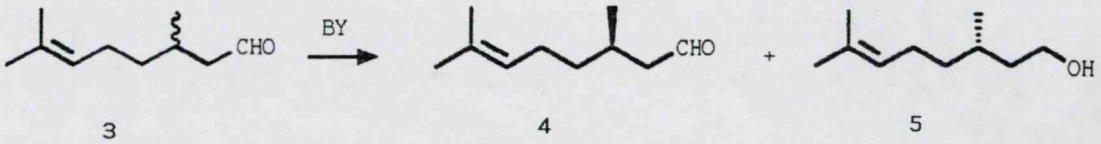

2a: R= Et
2b: R=H

activity, its 1:1 mixtures with all the other stereoisomers also showed essentially the same activity in field trials[3].

Two recent publications reported the synthesis of these compounds as diastereomeric mixtures[2,4]. Leikauf prepared all of the stereoisomers of 5,9-dimethylheptadecane in optically active form[5].

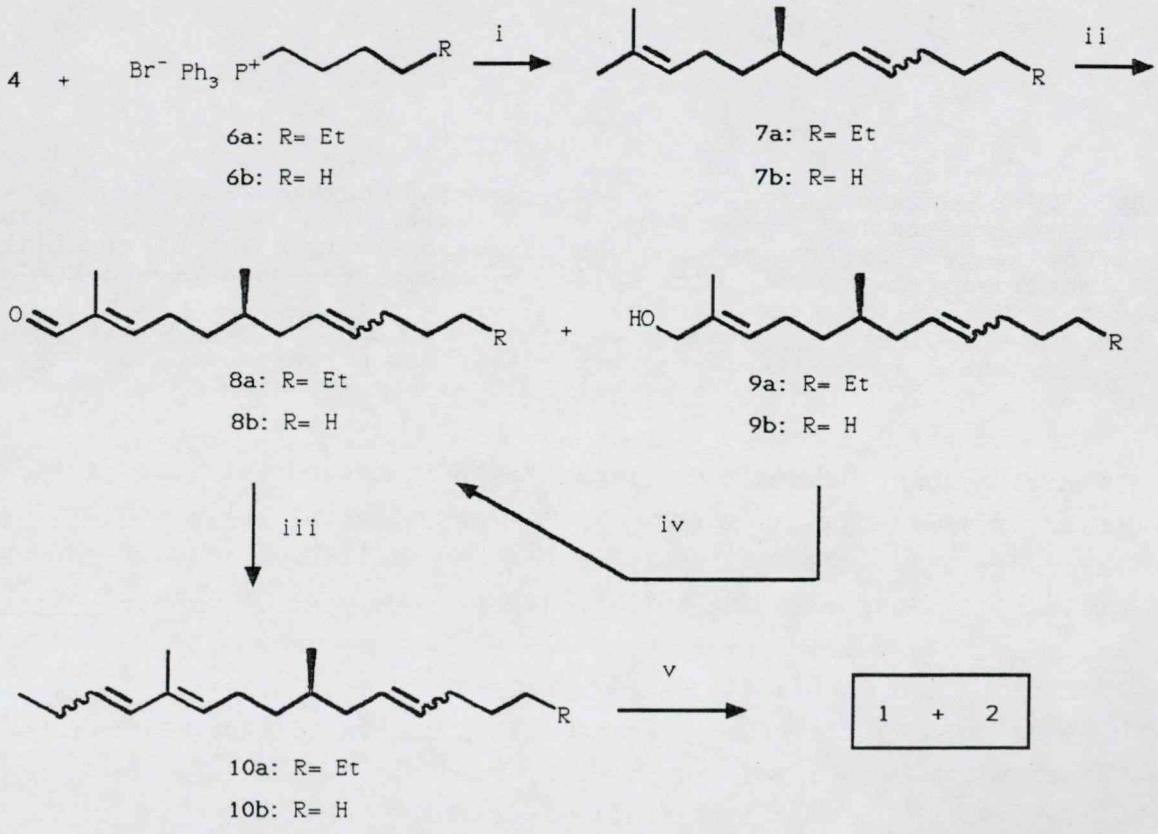
In course of our studies on stereocontrolled synthesis of insect pheromones we elaborated a short and convenient route to (9**S**) isomers of both pheromone components (**1** and **2**).

Racemic citronellal (**3**) was incubated with fermenting Baker's yeast to




3 4 5

afford a mixture of (R)-(+)-citronellal (**4**, 21%, $[\alpha]_D$= +12.7°) and (**S**)-(-)-citronellol (**5**, 33%), which was separated by chromatography.




*Scheme I: i) NaOEt, toluene, azeotropic removal of ethanol, then addition of* **4** *at -50°C, 2 h, r.t.; ii)* $SeO_2$, *EtOH, reflux, 3 h; iii)* $n\text{-}Pr(Ph)_3P^+Br^-$, *NaOEt, toluene, azeotropic removal of ethanol, then addition of* **8a** *or* **8b** *at -50°C, 3 h, r.t.; iv) PDC,* $CH_2Cl_2$, *2 h, r.t.; v) 10% Pd/C,* $H_2$, *MeOH-EtOAc.*

The syntheses of **1a+2a** and **1b+2b** were accomplished as shown in Scheme I. Thus **4** was coupled with the ylide generated[6] from **6a** (77%). The resulting diene **7a**[7] (3:2 mixture of **E**- and **Z**-isomers) was treated with selenium(IV) oxide in refluxing ethanol[8] to give a mixture of aldehyde **8a**[7] (32%) and alcohol **9a**[7] (19%). The latter was easily oxidized to **8a** (piridinium dichromate, $CH_2Cl_2$, 70%). Coupling reaction of **8a** with the ylide generated[6] from propyltriphenylphosphonium bromide yielded triene **10a**[7] (50%, unseparated mixture of geometrical isomers), which on hydrogenation (Pd/C in methanol-ethyl acetate, 83%) gave a mixture[9] of natural pheromone component (**1a**) and its (5**R**,9**S**)-isomer (**2a**)[7].

(**R**)-(+)-citronellal (**4**) also served as a key intermediate in the synthesis of pheromone component **1b**. Here, in the coupling reaction we used the ylide generated from **6a**, and prepared a mixture[9] of (5**S**,9**S**)- and (5**R**,9**S**)-isomers[7] (**1b** and **2b**, respectively) by the same reaction sequence as described above, via the intermediates **7b**, **8b**, **9b**, and **10b** (22% overall yield from **4**).

The mixture of **1a** and **2a** proved as active as (**S**,**S**) isomer (**1a**) alone in field tests. The detailed results will be published elsewhere[10].

**Acknowledgements**: We are grateful to Dr. M. Tóth, Research Institute for Plant-Protection, for carrying out the field tests with the synthetic **1a+2a** sample.

REFERENCES AND NOTES

1) W. Francke, S. Franke, M. Tóth, G. Szőcs, P. Guerin, and H. Arn: *Naturwissenschaften, 74*, 143 (1987).
2) W. Francke, M. Tóth, G. Szőcs, W. Krieg, H. Ernst, and E. Buschmann: *Z. Naturforsch., 43C*, 787 (1988).
3) M. Tóth, G. Helmchen, U. Leikauf, Gy. Sziráki, and G. Szőcs: *J. Chem. Ecol., 15*, 1535 (1989).
4) F. Rama, and L. Capuzzi: *Synth. Commun., 19*, 1051 (1989).
5) U. Leinkauf: Asymmetrische Synthesen mit Estern Sonhaver Bornanole: Stereoselective Synthese des Sexualpheromones von *Leucoptera scitella*. Dissertation, University Heidelberg, 1988.
6) Ylide generation was carried out by adding the corresponding phosphonium bromide to toluene-ethanol containing sodium ethylate prepared in situ followed ethanol removal by azeotropic distillation. For analogous methods see: P. Vinczer, Z. Juvancz, L. Novák, Cs. Szántay: *Acta Chim. Hung. 125*, 797 (1988).
7) All compounds have been full characterized spectrally and by elemental analysis. Selected analytical data are below:

**7a**: IR (film), $\nu_{max}$: 2990, 2945, 2910, 2840, 1640, 1440, 1370 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (d+t,6H), 1.1-1.5 (m,8H), 1,57 (s,3H), 1.65 (s,3H), 1.7-2.3 (m,7H), 5.02 (mc,1H), 5.15-5.40 (m,2H).

**8a**: IR (film), $\nu_{max}$: 2940, 2905, 2840, 2700, 1670, 1630, 1440 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (d+t,6H), 1.0-1.6 (m,8H), 1.69 (s,3H), 1.7-2.5 (m,7H), 5.1-5.4 (m,2H), 6.30 (t,1H), 9.29 (br s,1H).

**9a**: IR (film), $\nu_{max}$: 3330, 2985, 2930, 2900, 2850, 2830, 1660, 1640, 1440, 1365 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (d+t,6H), 1.05-1.6 (m,8H), 1.71 (s,3H), 1.75-2.3 (m,7H), 3.3 (br s,1H,exchangable with $D_2O$), 3.84 (s,2H), 5.1-5.45 (m,3H).

**10a**: IR (film), $\nu_{max}$: 3040, 2980, 2950, 2900, 2890, 1660, 1470, 1380 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (m,9H), 1.0-1.5 (m,8H), 1.70 (s,3H), 1.7-2.4 (m,9H), 4.9-5.9 (m,5H); GLC: $t_R$= 8.04 min (85%) and $t_R$= 9.96 min (15%) [10% SE-52 on CWS 60/80, 2.4 m x 3 mm, $t_K$= 220 C].

**1a+2a**: IR (film), $\nu_{max}$: 2970, 2930, 2870, 1470, 1380 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (m,12H), 1.25 (mc,24H), 1.9 (m,2H); MS (m/z): 268(22)[$M^+$], 211(19), 155(28), 85(59), 71(59), 57(**100**), 43(98), 41(37); GLC: $t_R$= 7.13 min (>98%) [10% SE-52 on CWS 60/80, 2.4 m x 3 mm, $t_K$= 220°C].

**1b+2b**: IR (film), 2970, 2930, 2870, 1470, 1380 $cm^{-1}$; $^1$H-NMR ($CCl_4$, δ): 0.9 (m,12H), 1.25 (mc,20H), 1.89 (m,2H); GLC: $t_R$= 6.67 min (>98%)[10% SE-52 on CWS 60/80, 2.4 m x 3 mm, $t_K$= 180°C].

8) U. T. Bhalerao, H. Rapoport: *J. Am. Chem. Soc. 93*, 4835 (1971); J. Meinwald, K. Opheim: *Tetrahedron Lett.*, 281 (1973).

9) Our attempts for the separation of these mixtures had failed. We assume that saturation of the trienes **10a** and **10b** occured with low diastereoselectivity so ratios of **1a** and **2a** or **1b** and **2b** do not differ significantly from 1:1.

10) So far, we did not get biological results for the mixture of **1b** and **2b**.

(Received in UK 12 March 1991)

# II. melléklet

**POPPE, L.**, NOVÁK, L., KAJTÁR-PEREDY, M., SZÁNTAY, CS.:

Lipase-Catalysed Enantiomer Selective Hydrolysis of 1,2-Diol Diacetates,

*Tetrahedron: Asymmetry*, **1993**, *4*, 2211.

*Tetrahedron: Asymmetry* Vol. 4, No. 10, pp. 2211-2217, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00


# Lipase-Catalyzed Enantiomer Selective Hydrolysis of 1,2-Diol Diacetates

**László Poppe [a], Lajos Novák [b], Mária Kajtár-Peredy [a], Csaba Szántay [a,b]**

[a] Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1521 Budapest, P.O.Box 17, HUNGARY
[b] Institute for Organic Chemistry, Technical University of Budapest, H-1521 Budapest, Gellért tér 4., HUNGARY

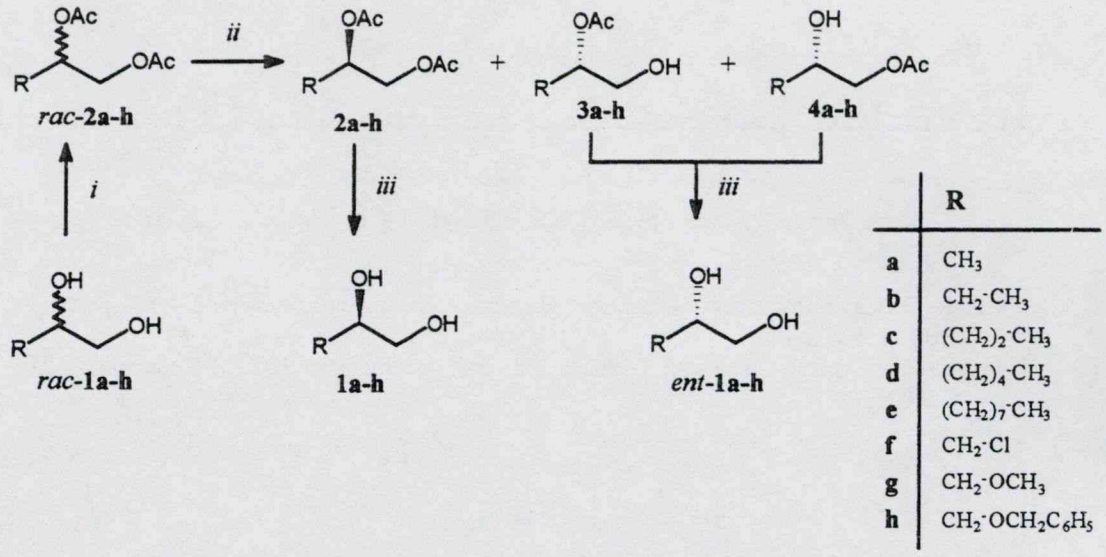
(*Received in UK* 15 *July* 1993)

***Abstract:*** Enantiomer selective hydrolysis of racemic 1,2-diol diacetates (*rac*-**2a-h**) was investigated by using the inexpensive commercial porcine pancreatic lipase. The hydrolysis proceeds with variable regioselectivity but with moderate to good enantioselectivity yielding a mixture of isomeric monoacetates (**3a-h** and **4a-h**) and unchanged diacetate enantiomers (**2a-h**). Evidence was found that both monoacetates (**3a-h** and **4a-h**) are formed with the same sense of enantiomer selectivity.

1,2-Diols are important structural unit or synthetic building block for a large number of biologically active natural or synthetic compounds. The two enantiomers of such compounds possess different biological activity, e.g. while the active enantiomer of pheromone brevicomin contains 1,2-dioxy-butane subunit with *R* configuration the other isomer shows inhibitory properties[1]. Prostacyclin analogs showing platelet-aggregation inhibitory properties were synthesized from (*S*)-1,2-heptanediol[2]. These examples indicate that there is a need for rational method of enantioseparation of racemic 1,2-diols.

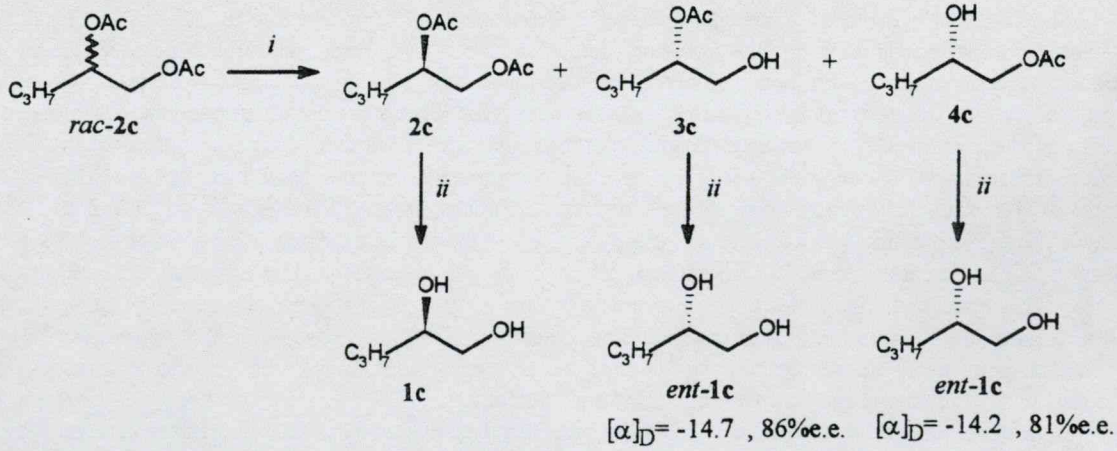
The utility of hydrolases, especially lipases for enantiomer and regioselective transformation of alcohols and related compounds is well known[3]. Recently, lipase catalyzed transformations of 1,2-diol derivatives were studied by several groups. Although hydrolysis[4] or alcoholysis[5] of 1,2-diol diacetates were also investigated, enzymic acylation (transesterification) was chosen as a tool for kinetic resolution of racemic 1,2-diols in the majority of these studies[6-11]. Transesterification methods applying lipase from *Candida cyilindracea* (CcL) in aqueous biphasic system consisting tributyrin as ester component[6], porcine pancreatic lipase (PPL) in ethyl acetate or butyrate[7] or methyl propionate[8] matrix, or lipase from *Pseudomonas* sp. (Amano PS) in tetrahydrofurane containing vinyl acetate and triethylamine[9, 10] have been reported. Acylation of diols by acetic- or butyric anhydride catalyzed by PPL in ether or tetrahydrofurane has also been investigated[11]. Generally, high or exclusive regioselectivity preferring the primary hydroxyl groups has been observed by these enzymic acylations parallel with variable degree of enantiomer selectivity. Contrarily, hydrolysis[4] or alcoholysis[5] of 1,2-diol diacetates by using lipases from *Pseudomonas* sp. (*P. aeruginosa* lipase, and Amano PS, respectively) proceeded with moderate regio- and variable enantiomer selectivity.

In the present study our aim was to investigate the hydrolysis of 1,2-diol diacetates catalyzed by the inexpensive PPL (Scheme 1., Table) with respect mainly to the degree of enantiomer selectivity and applicability. Enantiomer selectivity of hydrolysis could be compared to that observed by enzymic acylation of the parent diols[8] with methyl propionate using the same lipase (PPL) in the case of diols *rac*-**1a,b,c,e.**




**Scheme 1.** *PPL-catalyzed enantiomer selective hydrolysis of 1,2-diol diacetates*
Reagents: *i..)* $Ac_2O$, cat. $H_2SO_4$, reflux, 15 min; *ii.)* PPL, $H_2O$, pH 7, r.t.; *iii.)* cat. NaOMe, MeOH, r.t.

Although enhanced enantiomer selectivity is often observed by acylation of racemic alcohols in organic media in comparison with the hydrolysis of the ester of the same alcohol by the same enzyme[3], in the case of 1,2-diols the situation is opposite. Enantiomer selectivities of hydrolyses of diacetates *rac*-**2a,b,c,e** have proved to be superior to those observed by acylation of the corresponding diols *rac*-**2a,b,c,e** with methyl propionate[8] in each case. Furthermore, our preliminary experiments have shown that the hydrolysis of 1,2-diol diacetate *rac*-**2d** catalyzed by PPL proceeds at least one magnitude faster than the corresponding transesterification of the parent diol *rac*-**1d** in ethyl acetate or methyl propionate with the same enzyme.
The ratio of monoacetate regioisomers (**3** and **4**) obtained by hydrolysis[12] much depends on the constitution of the diacetate *rac*-**2** (Table), contrarily to the exclusive acylation of the primary hydroxyl group in the acylation[8]. The monoacetate regioisomers have proven to be separable by simple vacuum-chromatography[24] from the **3+4c,d,e,h** mixtures. Analysis of each diol products *ent*-**1c** obtained from the separated monoacetates **3c** and **4c** (Scheme 2.) showed that the enantiomer-preferences are the same in the PPL hydrolysis for primary and secondary acetoxy groups.




**Scheme 2.** *Regioselectivity - enantiomer preference correlation in PPL hydrolysis*
Reagents: *i..)* PPL, $H_2O$, pH 7, r.t., 30% conversion; *ii.)* cat. NaOMe, MeOH, r.t.

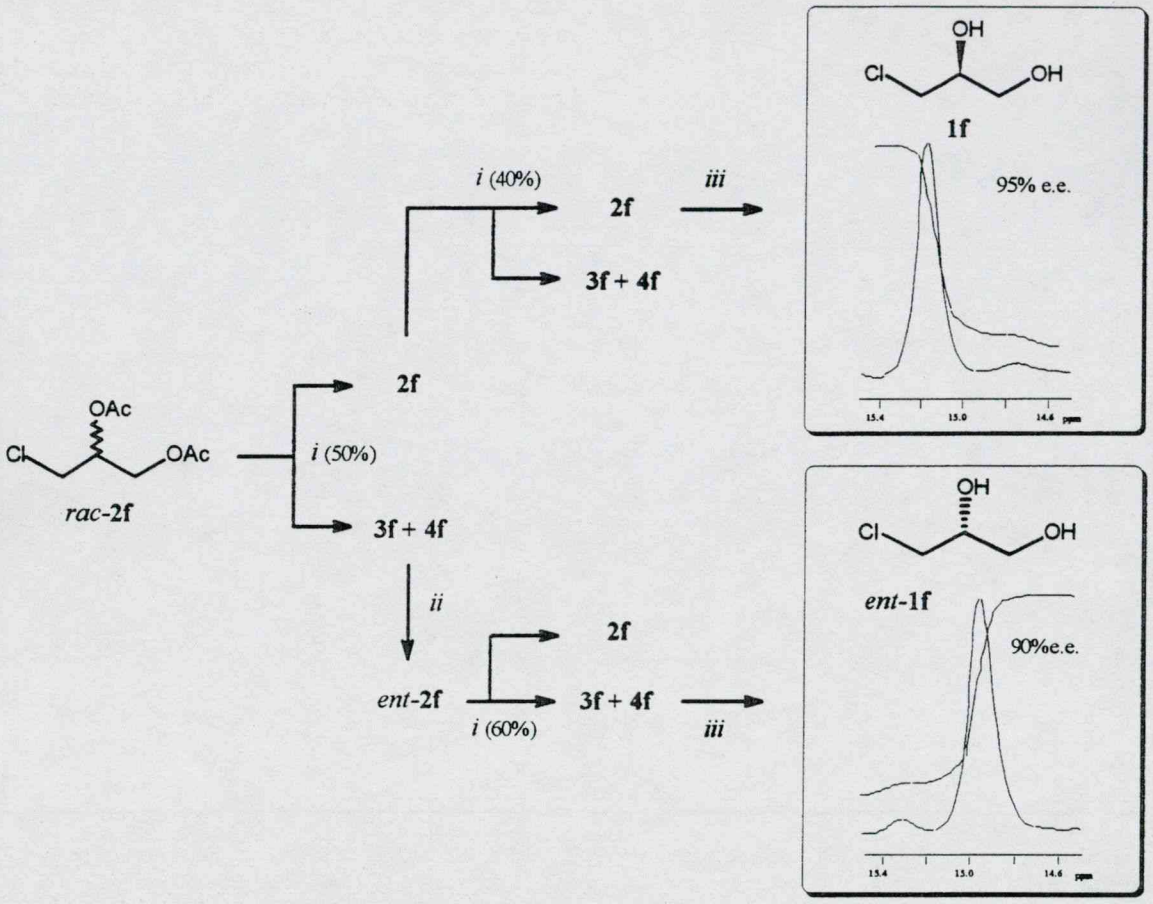
**Table:** PPL-catalyzed enantiomer selective hydrolysis of 1,2-diol diacetates [a]

| Substrate *rac*-2 | Conv. % | 2, Yield[b] % | $[\alpha]_D$ of 1 | e.e. of 1[c], % | Config. of 1 | 3:4 ratio[d] | 3+4, Yield[b], % | $[\alpha]_D$ of *ent*-1 | e.e. of *ent*-1[c], % |
|---|---|---|---|---|---|---|---|---|---|
| a | 50 | 75 | -4.85 [f] | 28 | *R* | 0.62 | 64 | +4.19 [f] | 24 |
| | 30 | | | | | 0.45 | 61 | +5.33 [f] | 30 |
| | 70[e] | 49 | -9.09 [f] | 52 | *R* | | | | |
| b | 50 | 58 | +8.9 [g] | 72 | *R* | 1.1 | 67 | -8.8 [g] | 69 |
| | 30 | | | | | 1.0 | 86 | -10.5 [g] | 82 |
| | 70[e] | 48 | +11.6 [g] | 91 | *R* | | | | |
| c | 50 | 77 | +14.1 [h] | 81 | *R* | 2.2 | 80 | -13.2 [h] | 76 |
| | 30 | | | | | 2.5 | 86 | -14.5 [h] | 85 |
| | 70[e] | 48 | +17.4 [h] | >96 | *R* | | | | |
| d | 50 | 78 | +10.9 [i] | 72 | *R* | 0.57 | 72 | -9.4 [i] | 56 |
| | 30 | | | | | 0.64 | 80 | -11.4 [i] | 68 |
| | 70[e] | 68 | +13.4 [i] | 80 | *R* | | | | |
| e | 50 | 73 | +9.4 [j] | 77 | *R* | 0.75 | 71 | -7.4 [j] | 62 |
| | 30 | | | | | 0.81 | 77 | -9.3 [j] | 78 |
| | 70[e] | 70 | +11.0 [j] | 92 | *R* | | | | |
| f | 50 | 81 | +4.2 [k] | 58 | *S* | 4.4 | 75 | -4.0 [k] | 55 |
| | 30 | | | | | 4.0 | 68 | -4.9 [k] | 68 |
| | 70[e] | 57 | +6.3 [k] | 87 | *S* | | | | |
| g | 50 | 75 | +3.2 [l] | 54 | *S* | 4.3 | 54 | -2.9 [l] | 49 |
| | 30 | | | | | 4.4 | 50 | -4.4 [l] | 75 |
| | 70[e] | 54 | +5.4 [l] | 92 | *S* | | | | |
| h | 50 | 81 | -2.8 [m] | 51 | *S* | 1.7 | 73 | +3.0 [m] | 55 |
| | 30 | | | | | 1.7 | 75 | +3.1 [m] | 57 |
| | 70[e] | 63 | -3.3 [m] | 61 | *S* | | | | |

*a:* reaction conditions: 5-20 mg PPL/mmol substrate, water, pH 7.5, RT, 0.2-3 h. For details see the Experimental section; *b:* isolated yield after separation in respect to the given conversion; *c:* determined by NMR using Eu-shift reagents[13] and/or comparing the measured optical rotatory power with the corresponding literature data given for each diol below; *d:* Isomeric ratio was estimated from the integration of the CO-$CH_3$, -$CH_2$-O, and CH-O signals in the $^1$H-NMR spectra of 3+4 mixtures; *e:* The diacetate fraction separated after hydrolysis to 30% conversion was further hydrolyzed to a degree which corresponds to 70% conversion of the original substrate; *f:* (neat). Maximum value found[14] for (*S*), $[\alpha]_D$ +17.48° (neat); *g:* (c 2.5, ethanol). The highest values found[15] for the pure enantiomers: (*S*), $[\alpha]^{20}_D$ -12.87 (c 2.5, ethanol), (*R*), $[\alpha]^{20}_D$ +12.4 (c 2.5, ethanol); *h:* (c 12, ethanol). Maximum values found for (*R*), $[\alpha]_D$ +16.2 (c 14, ethanol)[16] and for (*S*), $[\alpha]^{25}_D$ -16.1 (c 3, ethanol)[17]. Since our preparation had higher (+17.4°) rotation value as found in the literature optical purity calculations are based on our own value; *i:* (c 12, ethanol). Literature values found for (*R*), $[\alpha]^{20}_D$ +16.8 (c 12, ethanol)[18,19] and for (*S*), $[\alpha]^{22}_D$ -16.6 (c 11.9, ethanol), 100% e.e.[19]; *j:* (c 1, ethanol). Literature value found[20] for (*S*), $[\alpha]^{22}_D$ -11.9° (c 1, ethanol), >94% e.e.; *k:* (c 5, water). Literature values found[21] for (*R*), $[\alpha]^{22}_D$ +7.1 (c 5, water), >94%e.e. and for (*S*), $[\alpha]^{22}_D$ -6.4 (c 5, water), 88% e.e.; *l:* (c 2, ethanol). The highest values found[22] for (*R*), $[\alpha]^{21}_D$ +5.9 (c 1.7, ethanol) and for (*S*), $[\alpha]^{21}_D$ -5.4 (c 2, ethanol); *m:* (c 10, benzene). Maximum value found[23] for (*S*), $[\alpha]^{20}_D$ +5.45 (c 10, benzene).

It is noteworthy, that quite consistent structure-regioselectivity and structure-enantiomer selectivity equations could be obtained for the PPL hydrolysis of diacetates *rac*-**2a-h** by minimizing multilinear equation systems using NMR signals (acetate methyl, O-methyne, O-methylene chemical shifts), calculated (MM2) distances, mass of side substituent R, and TLC Rf value of the diacetates as unconditional parameters.

In case of hydrolyses with moderate enantiomer selectivity a cascade procedure can be applied to enhance the enantiomeric purity. This possibility is illustrated by the tandem hydrolysis of *rac*-**2f** (Scheme 3.).




**Scheme 3.** *Cascade hydrolysis of 1,2-diacetoxy-3-chloropropane (rac-***2f***). [Under formula of diol enantiomers* **1f** *and ent-***1f** *an illustrative part of 400 MHz PMR spectra in the presence of Eu(hfc)*$_3$ *as chiral shift reagent*[13] *are shown]*

Reagents: *i..)* PPL, $H_2O$, pH 7, r.t. (degree of conversion is given in parentheses); *ii.)* $Ac_2O$, cat. $H_2SO_4$, reflux, 15 min.; *iii.)* cat. NaOMe, MeOH, r.t.

Comparing the 90% enantiomeric purity of diol *ent*-**1f** prepared from *rac*-**2f** by the sequence of PPL hydrolysis (to 50% conversion) - reacetylation of the monoacetate fraction **3f+4f** - PPL hydrolysis (to 60% conversion ) to which obtained by the one-step hydrolysis (55%e.e. and 68%e.e. at 50% and 30% conversion, respectively) shows that significant improvement of enantiomeric purity can be achieved using consecutive hydrolyses, naturally, in charge of chemical yield.

From the viewpoint of practical applicability it is worth to mention that in case of *rac*-**2a,b,c,f,g** the diacetate (**2**) and monoacetate (**3+4**) fractions obtained after PPL hydrolysis are conveniently separable by using only extractive methods.

*Conclusions*

Analysis of data on lipase catalyzed hydrolysis of 1,2-diol diacetates compared to the lipase catalyzed acylation of 1,2-diols shows that contrarily to the acylation - hydrolysis of simple racemic alcohols and their esters where a common or very similar transition state for the hydrolysis or acylation is assumable[3] the hydrolytic process is mechanistically quite different from the acylation of the parent diol. The consequences of this difference are the very high regioselectivity parallel with moderate enantiomer selectivity and the poorer acceptance of the 1,2-diols as substrates in case of acylations and moderate and variable regioselectivity parallel with a higher enantiomer selectivity and a higher rate of transformation in case of hydrolyses. It means, that in synthetic procedures requiring high regioselectivity in transformation of 1,2-diols the acylation, while in syntheses needing higher enantiomer selectivity the hydrolysis of the diacetates are the method of choice.

## EXPERIMENTAL

The $^1$H-NMR spectra were measured on JEOL FX-100 (100 MHz) or Brucker AW-80 (80 MHz) spectrometers in $CDCl_3$ solutions containing TMS as internal standard. Enantiomer purity determinations[13] using $Eu(hfc)_3$ as chiral shift reagent were made in dry $d_3$-acetonitrile on a Varian VXR-400 (400 MHz) spectrometer. Optical rotations were determined on a Perkin Elmer 241 polarimeter. Thin-layer chromatography (TLC) was made using Merck Kieselgel 60 $F_{254}$ aluminum sheets. TLC plates were developed using the following solvent systems: hexane-acetone = 5:2, A; diisopropyl ether-acetone = 2:1, B. Spots were visualized by treatment with 3% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative vacuum-chromatography[24] was performed using Merck Kieselgel 60 $F_{254}$. Acetic anhydride and racemic diols (*rac*-**1a,b,c,f**) were purchased from Merck. The other diols (*rac*-**1d,e,g,h**) were synthesized by known procedures. Porcine pancreatic lipase (PPL, Type II) was obtained from Sigma. All solvents used were freshly distilled.

*Acetylation of racemic diols (rac-1a-h): general procedure*

Acetic anhydride (12.4 g, 0.12 mol) was added dropwise to the stirred diol (*rac*-**1a-h**, 0.10 mol) containing one drop of conc. $H_2SO_4$ at a rate providing gentle reflux. After introducing acetic anhydride the mixture was stirred for 15 min and then neutralized by adding sodium acetate. Product was isolated by vacuum distillation in 70-88% yield showing the appropriate IR and $^1$H-NMR spectra.

*rac*-**2a**: yield: 70%, b.p.: 81-82°C (22 mbar/17 torr), *TLC*: Rf(A)= 0.59; *rac*-**2b**: yield: 73%, b.p.: 85°C (15 mbar/11 torr), *TLC*: Rf(A)= 0.58; *rac*-**2c**: yield: 78%, b.p.: 92-94°C (11 mbar/8 torr), *TLC*: Rf(A)= 0.59; *rac*-**2d**: yield: 81%, b.p.: 128-132°C (20 mbar/15 torr), *TLC*: Rf(A)= 0.60; *rac*-**2e**: yield: 81%, b.p.: 132-139°C (4 mbar/3 torr), *TLC*: Rf(A)= 0.62; *rac*-**2f**: yield: 77%, b.p.: 118-122°C (21 mbar/16 torr), *TLC*: Rf(A)= 0.48; *rac*-**2g**: yield: 88%, b.p.: 114-116°C (21 mbar/16 torr), *TLC*: Rf(A)= 0.45; *rac*-**2h**: yield: 72%, b.p.: 138-139°C (4 mbar/3 torr), *TLC*: Rf(A)= 0.59.

*Hydrolysis of racemic diol diacetates (rac-2a-h): general procedure (on 50 mmol scale)*

To a stirred emulsion of 1,2-diol diacetate (*rac*-**2a-h**, 50 mmol) and 80 ml of water PPL enzyme (1 g) was added and the pH value of the mixture was kept constant 7.4 by dropping 1M NaOH solution from an autoburette. After consumpting the desired amount of base (0.4 - 4 h) the mixture was extracted with ethyl acetate (4 x 60 ml). The combined ethyl acetate layers were washed with brine (40 ml) and dried ($MgSO_4$). After evaporating the solvent *in vacuo* the remaining oil was separated either by vacuum-chromatography[23] (*a*) or extraction (*b*) yielding diacetate (**2a-h**) and monoacetate (**3+4a-h**) fractions in 48-85% and 55-85% yield (based on conversion), respectively.

*a*) The remaining oil was applied onto a column filled with silica gel (100 g) and eluted first with hexane-acetone = 10:1 (approximately 1000 ml) then with hexane-acetone = 5:1 eluant mixtures. After analyzing the collected fractions the appropriate parts were combined and evaporated yielding diacetate (**2a-h**) and monoacetates (**3+4a-h**).

*b*) The remaining oil was dissolved in hexane (150 ml) and then extracted with water (3-4 x 150 ml). After reextracting the combined aqueous layers with hexane (100 ml) the unified hexane layers were dried ($MgSO_4$) and evaporated *in vacuo* giving diacetate (**2a,b,c,f,g**). The aqueous layer was then extracted with ethyl acetate (3-4 x 80 ml). Evaporation of the solvent from the combined and dried ($MgSO_4$) ethyl acetate layers *in vacuo* gave monoacetates (**3+4a,b,c,f,g**).

For calculated yields of fractions **2a-h** and **3+4a-h** and isomeric ratio of monoacetates (**3** to **4**) see Table. Physical properties (IR, $^1$H-NMR spectra, TLC) of optically active diacetates (**2a-h**) were similar to the racemic compounds (*rac*-**2a-h**).

*Hydrolysis of 1,2-diacetoxypropane (rac-2a)*

*a*) Hydrolysis of *rac*-**2a**: (10 g) at 50% conversion yielded after extractive separation **2a** (3.75 g) and **3+4a** (2.36 g). **3+4a**: *TLC*: Rf (A) = 0.39, *$^1$H-NMR, δ*: 1.19 (d, J= 6Hz, 1.3H, **4a** -$CH_3$), 1.22 (d, J= 6Hz, 1.7H, **3a** -$CH_3$), 2.07 (s, 1.3H, $CH_3$), 2.09 (s, 1.7H, $CH_3$), 3.61 (d, J= 5Hz, 1.15H, **3a** -$OCH_2$-), 3.8-4.3 (m, 1.3H, **4a** -$OCH_2$- and OCH), 4.7-5.2 (m, 0.31H, **3a** OCH).

*b*) Hydrolysis of *rac*-**2a**: (25 g) at 30% conversion yielded diacetate (11.16 g) and **3+4a** (3.37 g).

*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2a** (3.68 g) and monoacetates (2.41 g).

*Hydrolysis of 1,2-diacetoxybutane (rac-2b)*
*a*) Hydrolysis of *rac*-**2b**: (10 g) at 50% conversion gave after extractive separation **2b** (2.90 g) and **3+4b** (2.54 g). **3+4b**: *TLC*: Rf (A) = 0.40, $^1$*H-NMR*, *δ*: 0.96 (m, 3H, $CH_3$), 1.25-2.0 (m, 2H, $CH_2$), 2.08 (br s, 3H, CO-$CH_3$), 3.55-3.77 (m, 1.05H, **3b** $OCH_2$), 3.78-4.35 (m, 1.4H, **4b** $OCH_2$ and OCH), 4.6-5.05 (m, 0.55H, **3a** OCH).
*b*) Hydrolysis of *rac*-**2b**: (15 g) at 30% conversion yielded diacetate (6.82 g) and **3+4b** (2.93 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2b** (2.14 g) and monoacetates (2.35 g).

*Hydrolysis of 1,2-diacetoxypentane (rac-2c)*
*a*) Hydrolysis of *rac*-**2c**: (10 g) at 50% conversion gave after extractive separation **2c** (3.85 g) and **3+4c** (3.1 g).
*b*) Hydrolysis of *rac*-**2c**: (15 g) at 30% conversion yielded diacetate (6.82 g), **3c** and **4c** (total monoacetates: 3.02 g). Analytical data for the regioisomers separated by vacuum-chromatography on silica gel: **3c**: *TLC*: Rf (A) = 0.39, $^1$*H-NMR*, *δ*: 0.93 (m, 3H, $CH_3$), 1.48 (mc, 4H, 2 $CH_2$), 2.09 (s, 3H, CO-$CH_3$), 3.67 (mc, 2H, $OCH_2$), 4.7-5.2 (m, 1H, OCH); **4c**: *TLC*: Rf (A) = 0.41, $^1$*H-NMR*, *δ*: 0.93 (m, 3H, $CH_3$), 1.45 (mc, 4H, 2 $CH_2$), 2.06 (s, 3H, CO-$CH_3$), 3.7-4.3 (m, 3H, $OCH_2$ and OCH).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2c** (2.14 g) and monoacetates (2.35 g).

*Hydrolysis of 1,2-diacetoxyheptane (rac-2d)*
*a*) Hydrolysis of *rac*-**2d**: (10 g) at 50% conversion gave after separation by vacuum-chromatography **2d** (3.9 g) and **3+4d** (2.9 g). Analytical data for the regioisomers: **3d**: *TLC*: Rf (A) = 0.39, $^1$*H-NMR*, *δ*: 0.89 (m, 3H, $CH_3$), 1.38 (mc, 8H, 4 $CH_2$), 2.08 (s, 3 H, CO-$CH_3$), 3.67 (mc, 2H, $OCH_2$), 4.7-5.2 (m, 1H, OCH); **4d**: *TLC*: Rf (A) = 0.42, $^1$*H-NMR*, *δ*: 0.89 (m, 3H, $CH_3$), 1.41 (mc, 8H, 4 $CH_2$), 2.06 (s, 3H, CO-$CH_3$), 3.7-4.3 (m, 3H, $OCH_2$ and OCH).
*b*) Hydrolysis of *rac*-**2d**: (20 g) at 30% conversion yielded diacetate (11.3 g) and **3+4d** (3.87 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2d** (4.05 g) and monoacetates (2.94 g).

*Hydrolysis of 1,2-diacetoxydecane (rac-2e)*
*a*) Hydrolysis of *rac*-**2e**: (10 g) at 50% conversion yielded after separation by vacuum-chromatography **2e** (3.85 g) and **3+4e** (2.87 g). **3e**: *TLC*: Rf (A) = 0.41, **4e**: *TLC*: Rf (A) = 0.43, **3+4e**: $^1$*H-NMR*, *δ*: 0.89 (m, 3H, $CH_3$), 1.35 (mc, 14H, 7 $CH_2$), 2.06 (s, ca. 1.3H, CO-$CH_3$), 2.08 (s, ca. 1.7H, CO-$CH_3$), 3.64 (mc, 1.2H, **3e** $OCH_2$), 3.75-4.3 (m, 1.45H, **4e** $OCH_2$ and OCH), 4.7-5.2 (m, 0.6H, **3e** OCH).
*b*) Hydrolysis of *rac*-**2e**: (10 g) at 30% conversion gave diacetate (5.2 g) and **3+4e** (1.94 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2e** (2.10 g) and monoacetates (1.87 g).

*Hydrolysis of 3-chloro-1,2-diacetoxypropane (rac-2f)*
*a*) Hydrolysis of *rac*-**2f**: (10 g) at 50% conversion yielded after extractive separation **2f** (4.05 g) and **3+4f** (2.94 g). **3+4f**: *TLC*: Rf (A) = 0.32, $^1$*H-NMR*, *δ*: 2.10 (br s, 3H, CO-$CH_3$), 3.4-3.95 (m, 2.35H, Cl-$CH_2$ and **3f** $OCH_2$), 3.95-4.5 (m, 2.45H, **4f** $OCH_2$ and OCH), 4.8-5.3 (m, 0.2H, **3f** OCH).
*b*) Hydrolysis of *rac*-**2f**: (20 g) at 30% conversion yielded diacetate (10.9 g) and **3+4f** (3.20 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2f** (3.43 g) and monoacetates (3.71 g).

*Hydrolysis of 1,2-diacetoxy-3-methoxypropane (rac-2g)*
*a*) Hydrolysis of *rac*-**2g**: (10 g) at 50% conversion gave after extractive separation **2g** (3.76 g) and **3+4g** (2.10 g). **3+4g**: *TLC*: Rf (A) = 0.30, $^1$*H-NMR*, *δ*: 2.08 (s, 2.45H, **4g** CO-$CH_3$), 2.10 (s, 0.55H, **3g** CO-$CH_3$), 3.37(s, 3H, $OCH_3$), 3.42 (d, J= 5Hz, 1.65H, **4g** $CH_2$-OMe), 3.56 (d, J= 5Hz, 0.35H, **3g** $CH_2$-OMe), 3. 65-4.25 (m, 2.45H, **4g** $OCH_2$ and OCH), 4.8-5.2 (m, 0.2H, **3g** OCH).
*b*) Hydrolysis of *rac*-**2g**: (30 g) at 30% conversion yielded diacetate (14.7 g) and **3+4g** (3.51 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2g** (4.83 g) and monoacetates (4.82 g).

*Hydrolysis of 3-benzyloxy-1,2-diacetoxypropane (rac-2h)*
*a*) Hydrolysis of *rac*-**2h**: (3 g) at 50% conversion yielded after separation by vacuum-chromatography **2h** (1.22 g) and **3+4h** (0.92 g). **3h**: *TLC*: Rf (A) = 0.37, **4h**: *TLC*: Rf (A) = 0.41, **3+4h**: $^1$*H-NMR*, *δ*: 2.04 (s, ca. 1.9H, **4h** CO-$CH_3$), 2.07 (s, ca. 1.1H, **3h** CO-$CH_3$), 3.4-4.3 (m, ca. 4.6H, BnO-$CH_2$, $OCH_2$, and **4h** OCH), 4.51 (s, 2H, $OCH_2Ph$), 4.8-5.3 (m, ca. 0.4H, **3h** OCH), 7.30 (m, 5H, ArH).
*b*) Hydrolysis of *rac*-**2h**: (3.1 g) at 30% conversion gave diacetate (1.87 g) and **3+4h** (0.57 g).
*c*) Hydrolysis of diacetate fraction from *b*) at 57% conversion gave **2h** (0.59 g) and monoacetates (0.62 g).

*Desacetylation of diacetates (2a-h) or monoacetates (3,4a-h) to optically active diols (1a-h) or (ent-1a-h): general procedure*
Acetylated 1,2-diol (**2a-h** or **3,4a-h**; 20 mmol) was dissolved in 0.2% methanolic NaOMe solution (15 ml) and stirred at r.t. for 4 h. After neutralizing the mixture by 1M HCl methanol was evaporated off and the rest was purified by vacuum-chromatography using hexane-acetone= 2:1 as eluant to give diol (**1a-h** or *ent*-**1a-h**) in 70-85% yield.
**1a** or *ent*-**1a**: *TLC*: Rf(A)= 0.15; **1b** or *ent*-**1b**: *TLC*: Rf(A)= 0.20; **1c** or *ent*-**1c**: *TLC*: Rf(A)= 0.22; **1d** or *ent*-**1d**: *TLC*: Rf(A)= 0.27; **1e** or *ent*-**1e**: *TLC*: Rf(A)= 0.29; **1f** or *ent*-**1f**: *TLC*: Rf(A)= 0.20, Rf(B)= 0.68; **1g** or *ent*-**1g**: *TLC*: Rf(A)= 0.11, Rf(B)= 0.37, **1h** or *ent*-**1h**: *TLC*: Rf(A)= 0.29. For optical rotation value, enantiomeric purity and configuration data of the diols (**1a-h** or *ent*-**1a-h**) prepared from the corresponding diacetates (**2a-h**) or monoacetates (**3+4a-h**) obtained by PPL hydrolyses of racemic diacetates (*rac*-**2a-h**) see Table.

**Acknowledgement.** We thank the Hungarian OTKA Foundation for financial support and Dr. Gábor Veress for structure-enantiomer selectivity calculations.

**REFERENCES AND NOTES**

1. Rossi. R., *Synthesis* **1978**, 413.
2. Novák, L., Aszódi, J., Szántay, Cs., *Tetrahedron Lett.* **1982**, 2135; Novák, L., Aszódi, J., Kolonits, P., Szabó, É., Stadler, I., Simonidesz, V., Szántay, Cs.,*Acta Chim. Hung.* **1983**, *113*, 355.
3. Poppe, L., Novák, L., *Selective Biocatalysis: A Synthetic Approach*, Verlag Chemie, Weinheim-New York-Basel-Tokyo, 1992.
4. Iriuchijima, S., Kojima, N., *Agric. Biol. Chem.* **1982**, *46*, 1153.
5. Bianchi, D., Bosetti, A., Cesti, P., Golini, P., *Tetrahedron Lett.* **1992**, *33*, 3231.
6. Cambou, B., Klibanov, A. M., *J. Am. Chem. Soc.* **1984**, *106*, 2687.
7. Cesti, P., Zaks, A., Klibanov, A. M., *Appl. Biochem. Biotechnol.* **1985**, *11*, 401.
8. Janssen, A. J. M., Klunder, A. J. H., Zwanenburg, B., *Tetrahedron* **1991**, *47*, 7409.
9. Theil, F., Ballschuh, S., Kunath, A., Shick, H., *Tetrahedron: Asymmetry* **1991**, *2*, 1301.
10. Theil, F., Weidner, J., Ballschuh, S., Kunath, A., Shick, H., *Tetrahedron Lett.* **1993**, *34*, 305.
11. Ramaswamy, S., Morgan, B., Oehlschlager, A. C., *Tetrahedron Lett.* **1990**, *31*, 3405.
12. Theoretically, acyl migration between the primary and secondary position may occur under the conditions of the enzymic hydrolysis influencing the **3** to **4** ratio. The **3d** to **4d** ratio was found, however, practically independent from the conversion of the hydrolysis of *rac*-**2d** analyzed by GLC with 4 min frequency. This means either no or a very fast equilibration between the monoacetate regioisomers. The latter possibility can be excluded as no isomeric **3h** was detected in the PPL catalyzed hydrolysis of *rac*-**4h** yielding *ent*-**1h** ($[\alpha]^{20}_D$ +2.9 (c 10, benzene), 53%e.e.) after 33% conversion.
13. Sweeting, L. M., Crans, D. C., Whitesides, G. M., *J. Org. Chem.* **1987**, *52*, 2273.
14. Fryzuk, M. O., Boschnich, B., *J. Am. Chem. Soc.* **1978**, *100*, 5491.
15. Mori, K., Sasaki, M., Tamada, S., Suguro, T., Masuda, S., *Tetrahedron* **1979**, *35*, 1601.
16. Levene, P. A., Haller, H. J., *J. Biol. Chem.* **1928**, *79*, 475.
17. Mulzer, J., Angermann, A., *Tetrahedron Lett.* **1983**, *24*, 2843.
18. Levene, P. A., Walti, A., *J. Biol. Chem.* **1932**, *98*, 737.
19. Barry, J., Kagan, H. B., *Synthesis* **1981**, 435.
20. Masaoka, Y., Sakakibara, M., Mori, K., *Agr. Biol. Chem.* **1982**, *46*, 2319.
21. Crans, D. C., Whitesides, G. M., *J. Am. Chem. Soc.* **1985**, *107*, 7019.
22. Goldstein, I. J., Hamilton, J. K., Smith, F., *J. Am. Chem. Soc.* **1957**, *79*, 1190.
23. Hirth, G. Brauer, R., *Helv. Chim. Acta* **1982**, *65*, 1059.
24. Poppe, L., Novák, L., *Magy. Kém. Lapja* **1985**, *40*, 366.

# III. melléklet

POPPE, L., RECSEG, K., NOVÁK, L.:

Convenient Preparation of Monoprotected 1,2-Diols,

*Synth. Commun.* **1995**, *25*, 3993.

SYNTHETIC COMMUNICATIONS, 25(24), 3993-4000 (1995)

# CONVENIENT SYNTHESIS OF MONOPROTECTED 1,2-DIOLS

**László Poppe[a], Katalin Recseg[b] and Lajos Novák[c] ***

[a] Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1526 Budapest, P.O.Box 17, HUNGARY
[b] CEREOL Research & Development Centre, H-1095 Budapest, Kvassay J. út 1., HUNGARY
[c] Institute for Organic Chemistry, Technical University of Budapest, H-1111 Budapest, Gellért tér 4., HUNGARY

***Abstract:*** *Reaction of the protected glycidol derivatives* (**1A-C**) *with a wide variety of Grignard reagents* (**2a-h**) *in the presence of catalytic amount of CuCN provided the corresponding monoprotected diol derivatives* (**3**) *in a highly regioselective manner.*

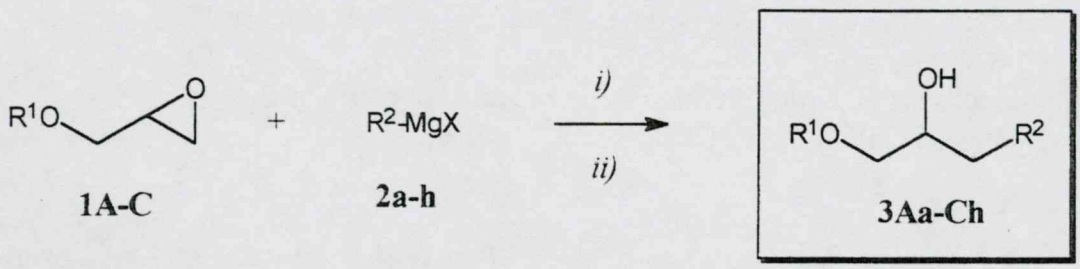
1,2-Diols either in racemic or in enantiomerically pure form are important structural units or synthetic building blocks for numerous biologically active natural or synthetic compounds, and for this reason, they are subject of many recent interests. In the course of our investigations on biocatalytic enantiomer-separation of diverse 1,2-diol derivatives, e.g. 1,2-diol diacetates[1], a need for a general synthetic procedure for the production of such compounds was recognised.

The utility of Grignard reagents for oxirane ring-opening reactions is well known[2]. Ring-opening reaction of oxirane with Grignard reagents in absence[3] or in presence of CuI catalyst[4] was used for two carbon chain elongation. Similar ring-opening reaction of methyloxirane[5] in the presence of CuCN proceeded regioselectively, the oxirane ring was attacked predominantly from the

* To whom correspondence should be addressed.

unsubstituted side. Grignard reagents, mostly in the presence of Cu(I) salt catalysts, opened the oxirane ring of alkyloxiranes[6], vinyloxiranes[7], β-epoxi-sulphones, -sulphoxides or esters[8] or dianhydro sugars[9] also in a regioselective manner. Although, scattered examples for reaction of Grignard reagents with enantiomers of benzyl glycidyl ether (**1A**) exist[10], usefulness and generality of ring opening reaction of protected glycidol derivatives with Grignard reagents for preparation of 1,2-diol derivatives has not been systematically studied.

It was our aim, therefore, to investigate the applicability of the ring opening reaction of various protected glycidol derivatives (**1A-C**) with a selection of Grignard reagents (**2a-h**) yielding the corresponding monoprotected diols (**3**).

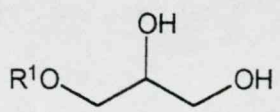



**1A-C** **2a-h** **3Aa-Ch**

| | $R^1$ |
|---|---|
| **A** | $CH_2Ph$ |
| **B** | $SiMe_3$ |
| **C** | $SiMe_2Bu^t$ |

| | $R^2$ |
|---|---|
| **a** | Et |
| **b** | Bu |
| **c** | n-$C_{10}H_{21}$ |
| **d** | i-Pr |
| **e** | t-Bu |
| **f** | $(CH_2)_6OTHP$ |
| **g** | $CH_2Ph$ |
| **h** | Ph |

+




**4A-C**

Conditions: *i..)* cat. CuCN, ether type solvent; *ii.)* saturated $NH_4Cl$
(for details see Table and Experimental)

Benzyl-, trimethylsilyl-, and *tert*-butyldimethylsilyl derivatives of glycidol (**1A-C**, respectively); and Grignard reagents prepared from primary alkyl halides of different lengths, secondary and tertiary alkyl halides, phenyl and benzyl halides, or ω-functionalized alkyl halide (**2a-h**, respectively) were chosen as reaction partners in the present study.

First, the reaction between (*tert*-butyldimethylsilyloxy)methyl oxirane (**1C**) and butylmagnesium bromide (**2b**) (Table, Entries 18-23) was chosen as a typical probe on which effects of solvent, temperature, and amount of CuCN catalyst were examined. It was found that the reaction can be conveniently carried out in ether type solvents in the presence of catalytic amount of CuCN at -15°C within 15 min.

Diethyl ether, tetrahydrofuran and 2-methyltetrahydrofuran were investigated as solvents (Entries 18, 19 and 23, respectively). The desired diol derivative (**3Cb**) was obtained in all three solvents in satisfactory yield. The reaction in diethylether (Entry 18), however, gave a slightly lower yield and more diol byproduct (**4C**). Considering yield, cost, safety, and extractability from water 2-methyl-tetrahydrofuran was chosen as solvent. Next, the effect of amount of CuCN catalyst was studied in tetrahydrofuran at -15°C (Entries 19-21). It was concluded that CuCN should be applied in catalytic (ca. 2 mole%) amount (Entry 19); reactions either in the presence of higher amount of CuCN (Entry 21, 25 mole%) or in the absence of CuCN (Entry 20) gave disappointing results: i.e. much slower reaction and appearance of diverse unidentified byproducts were observed in both cases. Finally, the reaction was carried out at higher temperature (0°C to RT for 2 h, Entry 22) but under this condition a reasonable proportion of ring cleavage product diol (**4C**) was produced parallel with a significant drop in yield of the desired diol derivative (**3Cb**). Consequently, reaction in 2-methyltetrahydrofuran in the presence of 2 mole% CuCN at -15°C for 15 min was chosen as general method for further study with protected glycidol derivatives (**1A-C**) and Grignard reagents (**2a-h**).

Each reaction in the present study performed between glycidol derivatives (**1A-C**) and several Grignard reagents (**2a-h**) (see Table) proved to be highly regioselective, a regioisomeric product arising from attack at the carbon of the oxirane ring bearing substituent was never isolated or detected. A concomitant formation of the corresponding diol byproduct (**4A-C**), however, was observed in the majority of the cases, even if the reaction was conducted under strictly water-free conditions. Our preliminary investigations showed that the relative amount of the diols can be reduced by lowering the temperature from RT or 0°C to -15°C, hence, most of the reactions were investigated at this temperature. Results of ring cleavage reactions of the glycidol derivatives (**1A-C**) with Grignard reagents (**2a-h**) indicate (see Table) that the process is more influenced by the nature of the Grignard reagent and much less sensitive to the kind of protecting group in the glycidol derivative. These reactions seem to be widely applicable, since ring opening with short, medium or long primary alkylmagnesium bromides (**2a, b, c**; Entries 1-3, 9-11, 17-24; respectively) as well as with secondary or tertiary alkylmagnesium halides (**2d, e**; Entries 4,5; 12,13; 25,26; respectively) proceeded with satisfactory to good yields. In the case of the reactions with isopropylmagnesium bromide (**2d**, Entries 4, 12 and 25), however, higher temperature and prolonged time (0°C to RT, 2 h) was needed to obtain

**Table:** Reaction of protected glycidol derivatives (1) with Grignard reagents (2)

| *Entry* | 1 | 2 | (X) | *Conditions*[a] (solvent[c]; CuCN [equiv.]; temp., time.) | | | 3 (4)[b] *Yield* (%) |
|---|---|---|---|---|---|---|---|
| 1 | A | a | Br | Me-THF | 0.02 | -15°C, 15 min | 89 |
| 2 | A | b | Br | Me-THF | 0.02 | -15°C, 15 min | 87(5) |
| 3 | A | c | Br | Me-THF | 0.02 | -15°C, 15 min | 80(9) |
| 4 | A | d | Br | Me-THF | 0.02 | -15°C-RT, 120 min | 44(46) |
| 5 | A | e | Cl | Me-THF | 0.02 | -15°C, 15 min | 61(20) |
| 6 | A | f | Br | Me-THF | 0.02 | -15°C-RT, 120 min | 65(6)[d] |
| 7 | A | g | Cl | Me-THF | 0.02 | -15°C, 15 min | 88(3) |
| 8 | A | h | Br | Me-THF | 0.02 | -15°C, 15 min | 85(4) |
| 9 | B | a | Br | Me-THF | 0.02 | -15°C, 15 min | 83 |
| 10 | B | b | Br | Me-THF | 0.02 | -15°C, 15 min | 82(7) |
| 11 | B | c | Br | Me-THF | 0.02 | -15°C, 15 min | 71(9) |
| 12 | B | d | Br | Me-THF | 0.02 | -15°C-RT, 120 min | 57(24) |
| 13 | B | e | Cl | Me-THF | 0.02 | -15°C, 15 min | 58(19) |
| 14 | B | f | Br | Me-THF | 0.02 | -15°C to RT, 60 min | 59(13)[d] |
| 15 | B | g | Cl | Me-THF | 0.02 | -15°C, 15 min | 87 |
| 16 | B | h | Br | Me-THF | 0.02 | -15°C, 15 min | 88 |
| 17 | C | a | Br | Me-THF | 0.02 | -15°C, 15 min | 93 |
| 18 | C | b | Br | $Et_2O$ | 0.02 | -15°C, 15 min | 75(12) |
| 19 | C | b | Br | THF | 0.02 | -15°C, 15 min | 88 |
| 20 | C | b | Br | THF | 0 | -15°C, 15 min | [e] |
| 21 | C | b | Br | THF | 0.25 | -15°C, 15 min | [e] |
| 22 | C | b | Br | THF | 0.02 | 0°C-RT, 120 min | 54(38) |
| 23 | C | b | Br | Me-THF | 0.02 | -15°C, 15 min | 92 |
| 24 | C | c | Br | Me-THF | 0.02 | -15°C, 15 min | 79(12) |
| 25 | C | d | Br | Me-THF | 0.02 | -15°C-RT, 120 min | 62(29) |
| 26 | C | e | Cl | Me-THF | 0.02 | -15°C, 15 min | 65(18) |
| 27 | C | f | Br | Me-THF | 0.02 | -15°C-RT, 120 min | 63(9)[f] |
| 28 | C | g | Cl | Me-THF | 0.02 | -15°C, 15 min | 89 |
| 29 | C | h | Br | Me-THF | 0.02 | -15°C, 15 min | 91 |

[a] For details on preparation of Grignard reagents and reaction conditions see Experimental. [b] Isolated yields of product(s) separated by chromatography on silica gel. Yield of diol 4 is given between brackets. Single number indicates that no diol (4) was isolated. [c] Me-THF: 2-methyltetrahydrofuran. [d] Beside a minor amount of diol (4) further unidentified byproducts were observed. [e] TLC investigation of the raw product revealed rather low conversion and presence of unidentified byproducts.

satisfactory yields. Similarly good results were achieved in reactions with phenyl- or benzylmagnesium halides (**2g, h**; Entries 7,8; 15,16 and 28,29; respectively). The reactions of glycidol derivatives (**1A-C**) with a Grignard reagent prepared from 1-bromo-6-(2-tetrahydropyranyl)oxy-hexane (**2f**) (Entries 6, 14, 27) affording skeletons functionalized at both ends further illustrate the synthetic usefulness of this process. In reactions with Grignard compound **2f** a prolonged reaction time and higher temperature (-15°C to RT, 2 h) were also required for acceptable yield.

In summary, the highly regioselective ring opening reaction between Grignard reagents (**2a-h**) and protected glycidol derivatives (**1A-C**) proved to be generally applicable yielding 1,2-diol derivatives protected at the primary hydroxyl group (**3Aa-Ch**). These products may conveniently be manipulated further at the free secondary hydroxyl moiety or may provide the corresponding 1,2-diols after deprotection.

## EXPERIMENTAL

NMR spectra were measured on Brucker AW-80 or Varian VXR 400 spectrometers operating at 80 and 400 MHz for $^{1}H$ and 101 MHz for $^{13}C$ in $CDCl_3$ containing TMS as internal standard. IR spectra (ν, film) were recorded on a Spekord IR 20M spectrometer. GLC chromatography was performed on a HP 5890 Series II gas chromatograph equipped with a HP-1 25 m x 0.20 mm, 0.20 μm column and FID ($v_{hydrogen}$= 1.6 ml/min, $t_i$= 140°C, $t_d$= 230°C, 100°C: 1 min, 100-200°C: 5°C/min). Preparative vacuum-chromatography[11] was carried out using Merck Kieselgel 60 (60-200 μm). All isolated products were homogenous by TLC on Merck Kieselgel 60 $F_{254}$ plates and gave satisfactory elemental analysis (C,H) data. Halogen compounds for Grignard reagents **2a-e,g,h** were commercial products from Fluka or Aldrich. Magnesium and 1,2-dibromoethane were supplied by Merck. The protected glycidol derivatives (**1A-C**) and bromide for Grignard reagent **2f** were prepared by known procedures. Dry diethyl ether was obtained from Fluka, tetrahydrofuran and 2-methyltetrahydrofuran were freshly distilled from $LiAlH_4$ and stabilized with 2,6-di-*tert*-butyl-p-cresol.

### *General procedure for ring cleavage of glycidol derivatives by Grignard reagents*

*A) Preparation of Grignard reagents*: A four necked flask containing Mg (0.6 g, 25 mmol) and small pieces of $I_2$ was flamed out by a burner, connected to a dry reflux condenser and cooled down under a slight positive pressure of nitrogen. After cooling, the flask was equipped with a dropping funnel filled with a solution of halogen compound (25 mmol) in 15 ml of solvent indicated in Table and with a second dropping funnel containing 25 ml of pure solvent. A small portion (2-3 ml) of solvent followed by 0.1 ml of
1,2-dibromoethane were introduced into the flask, and after the gas evolution was ceased, pure solvent and solution of the halide were dropped simultaneously. The Grignard reactions were performed at the boiling point of the lowest boiling component or at 50-55°C for 45 min.

*B) Ring cleavage of glycidol derivatives* (**1A-C**) *by Grignard reagents* (**2a-h**): To the resulting solution of the Grignard reagent (**2**), CuCN catalyst (amount indicated in Table) was added at 0°C followed by addition of a solution of the corresponding glycidol derivative (**1**, 20 mmol) in 15 ml of solvent (for temperature and reaction time see Table). The reaction mixture was worked up by pouring into 40 ml of saturated $NH_4Cl$ solution IR: 3300-3750, 3030, 3000, 2935, 1470, 1245, 1090, 845, 805, 710 $cm^{-1}$, $^1$H-NMR: 0.11 (s, 9H, $SiCH_3$), 0.87 (t, 3H, $CH_3$), 1.23-1.50 (br m, 8H, $4CH_2$), 3.34 and 3.58 (mc, 2x1H, $OCH_2$), 3.62 (m, 1H, O-CH).

*1-Trimethylsilyloxytridecan-2-ol* (**3Bc**)
IR: 3300-3750, 3030, 3000, 2935, 1470, 1245, 1090, 845, 805, 705 $cm^{-1}$, $^1$H-NMR: 0.10 (s, 9H, $SiCH_3$), 0.87 (t, 3H, $CH_3$), 1.28 (mc, 18H, 9 $CH_2$), 1.43 (m, 2H, $CH_2$), 3.35 and 3.60 (mc, 2x1H, $OCH_2$), 3.62 (m, 1H, OCH).

*1-Trimethylsilyloxy-4-methylpentan-2-ol* (**3Bd**)
IR: 3350-3750, 3030, 3000, 2925, 1470, 1370, 1250, 1100, 810 $cm^{-1}$, $^1$H-NMR: 0.09 (s, 9H, $SiCH_3$), 0.94 (d, 6H, 2 $CH_3$), 1.14 and 1.41 (mc, 2x1H, $CH_2$-Pr$^i$), 1.73 (mc, 1H, CH), 3.35 and 3.61 (mc, 2x1H, $CH_2$-O), 3.71 (mc, 1H, CH-O).

*1-Trimethylsilyloxy-4,4-dimethylpentan-2-ol* (**3Be**)
IR: 3350-3750, 3030, 3000, 2905, 1465, 1370, 1250, 1100, 805 $cm^{-1}$, $^1$H-NMR: 0.06 (s, 6H, $SiCH_3$), 0.89 (s, 9H, $SiCCH_3$), 0.95 (s, 9H, $CCH_3$), 1.17 and 1.32 (mc, 2x1H, $CH_2$-Bu$^t$), 3.30 and 3.50 (mc, 2x1H, $SiOCH_2$), 3.75 (mc, 1H, CH-OH).

*1-Trimethylsilyloxy-9-(tetrahydro-2H-pyran-2-yloxy)nonan-2-ol* (**3Bf**)
IR: 3750-3350, 3010, 2990, 2920, 1470, 1360, 1100, 1060, 1005, 805, 750 $cm^{-1}$, $^1$H-NMR: 0.13 (s, 9H, $SiCH_3$), 1.30-1.65 (m, 16H, $8CH_2$), 1.70 and 1.83 (mc, 2x1H, $CH_2$), 3.37 and 3.49 (mc, 2x1H, $CH_2O$), 3.35 and 3.58 (mc, 2x1H, $CH_2O$), 3.63 (mc, 1H, CH-O), 3.73 and 3.87 (mc, 2x1H, $CH_2O$), 4.57 (mc, 1H, OCHO).

*1-Trimethylsilyloxy-4-phenylbutan-2-ol* (**3Bg**)
IR: 3300-3750, 3175, 3155, 3105, 3025, 2990, 2920, 1510, 1490, 1455, 1385, 1355, 1240, 1095, 805 $cm^{-1}$, $^1$H-NMR: 0.11 (s, 9H, 2 $SiCH_3$), 1.71 (mc, 2H, $CH_2Ph$), 2.68 and 2.81 (mc, 2x1H $CH_2$-$CH_2Ph$), 3.40 and 3.59 (mc, 2x1H, $CH_2$-O), 3.64 (mc, 1H, CH-O), 7.15-7.35 (m, 5H, ArH).

*1-Trimethylsilyloxy-3-phenylpropan-2-ol* (**3Bh**)
IR: 3300-3750, 3195, 3160, 3115, 3040, 3005, 2930, 1470, 1250, 1100, 810 $cm^{-1}$, $^1$H-NMR: 0.06 (br s, 6H, $2SiCH_3$), 0.89 (s, 9H, $SiC(CH_3)$), 2.77 (dd, 2H, $CH_2Ph$), 3.46 and 3.60 (mc, 2x1H, $OCH_2$), 3.85 (mc, 1H, CH-OH), 7.15-7.35 (m, 5H ArH).

*1-(tert-Butyl-dimethylsilyloxy)pentan-2-ol* (**3Ca**)
GLC: $t_R$= 1.28 min; IR: 3300-3750, 3035, 3000, 2940, 1470, 1370, 1250, 1090, 845, 805 $cm^{-1}$, $^1$H-NMR: 0.05 (s, 6H, $SiCH_3$), 0.88 (s, 9H, $SiCCH_3$), 0.91 (t, 3H, $CH_3$), 1.28-1.53 (m, 4H, $2CH_2$), 3.36 and 3.59 (mc, 2x1H, $OCH_2$), 3.63 (m, 1H, OCH); $^{13}$C-NMR: -5.34 ($SiCH_3$), -5.40 ($SiCH_3$), 14.16 ($CH_3$), 18.30 ($SiC(CH_3)_3$), 18.81 ($CH_2$), 25.89 ($SiC(CH_3)_3$), 34.93 ($CH_2$), 67.32 ($OCH_2$), 71.56 (OCH).

*1-(tert-Butyl-dimethylsilyloxy)heptan-2-ol* (**3Cb**)
GLC: $t_R$= 2.43 min; IR: 3300-3750, 3035, 3000, 2935, 1475, 1250, 1090, 810, 750 $cm^{-1}$, $^1$H-NMR: 0.06 (s, 6H, $SiCH_3$), 0.87 (t, 3H, $CH_3$), 0.88 (s, 9H, $SiCCH_3$), 1.28 (mc 6H, $3CH_2$), 1.40 (m, 2H, $CH_2$), 3.37 and 3.60 (mc, 2x1H, $OCH_2$), 3.62 (m, 1H, OCH); $^{13}$C-NMR: -5.32 ($SiCH_3$), -5.38 ($SiCH_3$), 14.06 ($CH_3$), 18.31 ($SiC(CH_3)_3$), 22.62 ($CH_2$), 25.29 ($CH_2$), 25.90 ($SiC(CH_3)$), 31.97 ($CH_2$), 32.79 ($CH_2$), 67.31 ($OCH_2$), 71.87 (OCH).

*1-(tert-Butyl-dimethylsilyloxy)tridecan-2-ol* (**3Ce**)
GLC: $t_R$= 11.05 min; IR: 3300-3750, 3035, 3000, 2940, 1470, 1250, 1090, 815 $cm^{-1}$, $^1$H-NMR: 0.07 (s, 6H, $SiCH_3$), 0.88 (t, 3H, $CH_3$), 0.90 (s, 9H, $SiCCH_3$), 1.27 (mc, 18H, 9 $CH_2$), 1.43 (m, 2H, $CH_2$), 3.38 and 3.61 (mc, 2x1H, $OCH_2$), 3.62 (m, 1H, OCH); $^{13}$C-NMR: -5.33 ($SiCH_3$), -5.39 ($SiCH_3$), 14.13 ($CH_3$), 18.30 ($SiC(CH_3)_3$), 22.71 and 25.61 ($2CH_2$), 25.89 ($SiC(CH_3)$), 25.90, 29.37, 29.60, 29.62, 29.65, 29.68 and 29.77 (8 $CH_2$), 67.31 ($OCH_2$), 71.86 (OCH).

*1-(tert-Butyl-dimethylsilyloxy)-4-methylpentan-2-ol* (**3Cd**)
GLC: $t_R$= 1.50 min; IR: 3300-3750, 3035, 3000, 2940, 1470, 1375, 1250, 1090, 815 $cm^{-1}$, $^1$H-NMR: 0.08 (s, 6H, $SiCH_3$), 0.89 (s, 9H, $SiCCH_3$), 0.93 (d, 6H, $2CH_3$), 1.14 and 1.38 (mc, 2x1H, $CH_2$), 1.79 (m, 1H, CH), 3.36 and 3.60 (mc, 2x1H, $OCH_2$), 3.72 (m, 1H, OCH); $^{13}$C-NMR: -5.32 ($SiCH_3$), -5.39 ($SiCH_3$), 18.31 ($SiC(CH_3)_3$), 22.21 ($CH_3$), 23.45 ($CH_3$), 24.56 (CH), 25.90 ($SiC(CH_3)_3$), 41.78 ($CH_2$-Pr$^i$), 67.71 ($CH_2O$), 69.97 (CH-O).

*1-(tert-Butyl-dimethylsilyloxy)-4,4-dimethylpentan-2-ol* (**3Ce**)
GLC: $t_R$= 1.70 min; IR: 3350-3750, 3030, 3000, 2925, 1470, 1370, 1250, 1100, 805, 750 $cm^{-1}$, $^1$H-NMR: 0.06 (s, 6H, $SiCH_3$), 0.89 (s, 9H, $SiCCH_3$), 0.95 (s, 9H, $CCH_3$), 1.17 and 1.32 (mc, 2x1H, $CH_2$-Bu$^t$), 3.30 and 3.50 (mc, 2x1H, $SiOCH_2$), 3.75 (mc, 1H, CH-OH); $^{13}$C-NMR: -5.30 ($SiCH_3$), -5.37 ($SiCH_3$), 18.30 ($SiC(CH_3)_3$), 25.91 ($SiC(CH_3)_3$), 30.04 ($CH_2C(CH_3)_3$), 30.07 ($C(CH_3)_3$), 46.20 ($CH_2$-Bu$^t$), 68.38 ($CH_2O$), 69.39 (CH-O).

*1-(tert-Butyl-dimethylsilyloxy)-9-(tetrahydro-2H-pyran-2-yloxy)nonan-2-ol* (**3Cf**)
GLC: $t_R$= 15.84 min; IR: 3750-3350, 3025, 3010, 2940, 1470, 1450, 1375, 1360, 1260, 1110, 1065, 1015, 805, 750 $cm^{-1}$, $^1$H-NMR: 0.08 (s, 6H, $SiCH_3$), 0.90 (s, 9H, $SiCCH_3$), 1.35-1.65 (m, 16H, $8CH_2$), 1.70 and 1.83 (mc, 2x1H, $CH_2$), 3.37 and 3.50 (mc, 2x1H, $CH_2O$), 3.38 and 3.62 (mc, 2x1H, $CH_2O$), 3.63 (mc, 1H, CH-O), 3.73 and 3.87 (mc, 2x1H, $CH_2O$), 4.57 (mc, 1H, OCHO); $^{13}$C-NMR: -5.32 ($SiCH_3$), -5.38 ($SiCH_3$), 18.30 ($SiC(CH_3)_3$), 19.70, 25.51, 25.54 ($3CH_2$), 25.90 ($SiC(CH_3)_3$), 26.19, 29.41, 29.67, 29.74, 30.79, 32.79 ($6CH_2$), 62.32, 67.30, 67.64 ($3CH_2$-O), 71.82 (CH-O), 98.83 (OCHO).

*1-(tert-Butyl-dimethylsilyloxy)-4-phenylbutan-2-ol* (**3Cg**)
GLC: $t_R$= 7.04 min; IR: 3300-3750, 3175, 3155, 3105, 3025, 2990, 2920, 1610, 1490, 1455, 1385, 1355, 1240, 1095, 805, 740 $cm^{-1}$, $^1$H-NMR: 0.06 (br s, 6H, 2 $SiCH_3$), 0.89 (s, 9H, $CCH_3$), 1.71 (mc, 2H, $CH_2Ph$), 2.67 and 2.81 (mc, 2x1H $CH_2$-$CH_2Ph$), 3.42 and 3.61 (mc, 2x1H, $CH_2$-O), 3.64 (mc, 1H, CH-O), 7.15-7.32 (m, 5H, ArH); $^{13}$C-NMR: -5.33 ($SiCH_3$), -5.39 ($SiCH_3$), 18.30 ($SiC(CH_3)_3$), 25.90 ($SiC(CH_3)_3$), 31.87 ($CH_2$), 34.52 ($CH_2$), 67.19 ($CH_2O$), 71.07 (CH-O), 125.80 (ArC), 128.36 (ArC), 128.45 (ArC), 142.07 (ArC-$CH_2$).

*1-(tert-Butyl-dimethylsilyloxy)-3-phenylpropan-2-ol* (**3Ch**)
GLC: $t_R$= 5.28 min; IR: 3300-3750, 3195, 3160, 3115, 3030, 3005, 2930, 1500, 1480, 1465, 1250, 1100, 810, 750 $cm^{-1}$, $^1$H-NMR: 0.06 (br s, 6H, $2SiCH_3$), 0.89 (s, 9H, $SiC(CH_3)$), 2.77 (dd, 2H, $CH_2Ph$), 3.47 and 3.60 (mc, 2x1H, $OCH_2$), 3.88 (mc, 1H, CH-OH), 7.18-7.34 (m, 5H ArH); $^{13}$C-NMR: -5.35 ($SiCH_3$), -5.37 ($SiCH_3$), 18.28 ($SiC(CH_3)_3$), 25.88 ($SiC(CH_3)_3$), 39.57 ($CH_2$), 66.19 ($CH_2O$), 72.78 (CH-O), 126.33 (ArC), 128.42 (ArC), 129.29 (ArC), 138.26 (ArC-$CH_2$).

**Acknowledgement.** We thank the Great Lakes-QO Chemicals Europe for providing 2-methyltetrahydrofuran and the Hungarian OTKA foundation (OTKA 1759/91) for financial support. We are indebted to Dr. Eszter Baiz-Gács for NMR measurements. Zsuzsanna Hencz is also acknowledged for technical assistance.

**REFERENCES AND NOTES**


1. Poppe, L., Novák, L., Kajtár-Peredy, M. and Szántay, Cs., *Tetrahedron: Asymmetry* **1993**, *4*, 2211.
2. *a.* Gaylord, N. G. and Becker, E. I.: *Chem. Rev.* **1951**, *49*, 413; *b.* Gittus G. in "*Houben-Weyl: Methoden der Organische Chemie*", Vol. VII/3, Stuttgart, Thieme, **1965**, pp. 367-487; *c.* Nützel, K. in "*Houben-Weyl: Methoden der Organische Chemie*", Vol. 13/2a, Stuttgart, Thieme, **1973**, p. 343; *d.* Bartók, M. and Láng, K. M. in "*The Chemistry of Heterocyclic Compounds*", Vol 42, Part 3., New York, Wiley, **1985**, pp. 1-196.
3. Bradsher, C. K. and Hunt, D. A: *J. Org. Chem.* **1981**, *46*, 4608.
4. Dawson, I. M., Gregory, J. A., Herbert, R. B. and Sammes, P. B.: *Chem. Commun.* **1986**, 620.
5. Manna, S., Viala, J. Yadagiri, P. and Falck, J. R.: *Tetrahedron Lett.* **1986**, *27*, 2679.
6. *a.* Whitby, R., Yeates, C., Kocienski, P. and Costello, G.: *Chem. Commun.* **1987**, 429; *b.* Sviridov, A. F., Ermolenko, M. S., Yashunsky, D. V., Borodkin, V. S. and Kotchekov, N. K.: *Tetrahedron Lett.* **1987**, *28*, 3835; *c.* Pikul, S., Kozlowska, M. and Jurczak, J.: *Tetrahedron Lett.* **1987**, *28*, 2627.
7. Anderson, R. J.: *J. Am. Chem. Soc.* **1970**, *92*, 4979.
8. Tanikaga, R., Hosoya, K. and Kaji, A.: *Chem. Commun.* **1986**, 429.
9. Wakamatsu, T., Nakamura, H., Nishikimi, Y., Yoshida, K., Noda, T. and Taniguchi, M.: *Tetrahedron Lett.* **1986**, *27*, 6071.
10. *a.* Takano, S., Hirama, M. and Ogasawara, K.: *Heterocycles* **1983**, *20*, 1363; *b.* Takano, S., Moriya, M., Iwabuchi, Y. and Ogasawara, K.: *Tetrahedron Lett.* **1989**, *30*, 3805; *c.* Ichimoto, I., Ohotomo, Y., Iwabuchi, Y., Kirihata, M., Tsuji, H. and Ueda, H.: *Chem. Express* **1989**, *4*, 625; *d.* Abushanab, E. and Sarma, M. S. P.: *J. Med. Chem.* **1989**, *32*, 76; *e.* Chatopadhyay, S., Mamdapur, V. R. and Chadha, M. S.: *Synth. Commun.* **1990**, *20*, 1299; *f.* Takano, S. Moriya, M., Iwabuchi, Y. and Ogasawara, K.: *Chem. Lett.* **1990**, 109; *g.* Staellberg, G. A. M.: *Acta Chim. Scand.* **1990**, *44*, 368.
11. Poppe, L. and Novák, L.: *Magy. Kém. Lapja* **1985**, *40*, 366.


(Received in the UK 02 May 1995)

SYNTHETIC COMMUNICATIONS, 26(11), 2249-2250 (1996)

# ERRATUM

*SYNTHETIC COMMUNICATIONS*, 25(24), PP. 3993–4000 (1995)

"CONVENIENT SYNTHESIS OF MONOPROTECTED 1,2-DIOLS"

László Poppe, Katalin Recseg, and Lajos Novák

On page 3998, between the fifth and sixth lines, an entire page was inadvertently omitted. The missing text appears on the following page.

followed by separation of the layers, extraction with 40 ml of solvent, drying the resulting organic layer over $MgSO_4$. After evaporation off the solvent, the residue was subjected to vacuum-chromatography to yield pure product (for yields see Table). All products were characterized by IR and NMR spectra.

*1-Benzyloxypentan-2-ol* (**3Aa**)
IR: 3300-3800, 3160, 3120, 3035, 3000, 2930, 1500, 1470, 1450, 1370, 1090, 710 $cm^{-1}$, $^1$H-NMR: 0.89 (t, 3H, $CH_3$), 1.28-1.53 (m, 4H, $2CH_2$), 3.33 and 3.54 (mc, 2x1H, $OCH_2$), 3.83 (m, 1H, OCH), 4.55 (s, 2H, O-$CH_2$-Ph), 7.32 (mc, 5H, ArH).

*1-Benzyloxyheptan-2-ol* (**3Ab**)
IR: 3300-3750, 3160, 3120, 3035, 3000, 2930, 1500, 1470, 1450, 1370, 1085, 705 $cm^{-1}$, $^1$H-NMR: 0.88 (t, 3H, $CH_3$), 1.29 (mc, 6H, $3CH_2$), 1.43 (mc, 2H, $1CH_2$), 3.32 and 3.50 (mc, 2x1H, O-$CH_2$), 3.80 (m, 1H, OCH), 4.54 (s, 2H, O-$CH_2$-Ph), 7.33 (mc, 5H, ArH).

*1-Benzyloxytridecan-2-ol* (**3Ac**)
IR: 3300-3750, 3160, 3120, 3030, 3005, 2940, 1500, 1470, 1450, 1375, 1090, 705 $cm^{-1}$, $^1$H-NMR: 0.88 (s, 9H, $SiCCH_3$), 1.28 (mc, 18H, 9 $CH_2$), 1.43 (m, 2H, $CH_2$), 3.31 and 3.51 (mc, 2x1H, $OCH_2$), 3.78 (m, 1H, OCH), 4.52 (s, 2H, O-$CH_2$-Ph), 7.33 (mc, 5H, ArH).

*1-Benzyloxy-4-methylpentan-2-ol* (**3Ad**)
IR: 3300-3800, 3190, 3155, 3110, 3020, 2940, 1500, 1465, 1450, 1385, 1365, 1090, 700 $cm^{-1}$, $^1$H-NMR: 0.88 (d, 6H, $2CH_3$), 1.00-1.28 (m, 2H, $CH_2$), 1.76 (m, 1H, CH), 3.0-3.4 (m, 2H, $OCH_2$), 3.68 (m, 1H, OCH), 4.55 (s, 2H, O-$CH_2$-Ph), 7.32 (mc, 5H, ArH).

*1-Benzyloxy-4,4-dimethylpentan-2-ol* (**3Ae**)
IR: 3300-3750, 3165, 3110, 3020, 3000, 2940, 1500, 1470, 1450, 1380, 1365, 1090, 710 $cm^{-1}$, $^1$H-NMR: 0.95 (s, 9H, $CCH_3$), 1.1-1.35 (m, 2H, $CH_2$-$Bu^t$), 3.0-3.4 (m, 2H, $SiOCH_2$), 3.70 (mc, 1H, CH-OH), 4.54 (s, 2H, O-$CH_2$-Ph), 7.31 (mc, 5H, ArH).

*1-Benzyloxy-9-(tetrahydro-2H-pyran-2-yloxy)nonan-2-ol* (**3Af**)
IR: 3350-3750, 3180, 3160, 3110, 3010, 2990, 2920, 1500, 1470, 1370, 1100, 1060, 1005, 805, 750, 710 $cm^{-1}$, $^1$H-NMR: 1.3-1.5 (m, 14H, $7CH_2$), 1.5-1.9 (m, 4H, 2 $CH_2$), 3.3-3.8 (m, 7H, 3 $CH_2O$ and O-CH), 4.55 (m+s, 3H, OCHO and O-$CH_2$-Ph), 7.3 (mc, 5H, ArH).

*1-Benzyloxy-4-phenylbutan-2-ol* (**3Ag**)
IR: 3300-3750, 3180, 3155, 3115, 3005, 2940, 1500, 1460, 1370, 1095, 710 $cm^{-1}$, $^1$H-NMR: 1.6 (mc, 2H, $CH_2$Ph), 2.5-2.8 (m, 2H $CH_2$-$CH_2$Ph), 3.1-3.5 (m, 2H, $CH_2$-O), 3.63 (mc, 1H, CH-O), 4.43 (s, 2H, O-$CH_2$-Ph), 6.95-7.35 (m, 10H, ArH).

*1-Benzyloxy-3-phenylpropan-2-ol* (**3Ah**)
IR: 3300-3800, 3175, 3150, 3110, 3005, 2930, 1610, 1500, 1460, 1370, 1090, 705 $cm^{-1}$, $^1$H-NMR: 2.72 (d, 1H, one H of $CH_2$Ph), 3.05-3.5 (m, 3H, one H of $CH_2$Ph and $OCH_2$), 3.8 (mc, 1H, CH-OH), 4.40 (br s, 2H, O-$CH_2$-Ph), 7.0-7.35 (m, 10H, ArH).

*1-Trimethylsilyloxypentan-2-ol* (**3Ba**)
IR: 3300-3750, 3035, 3000, 2930, 1470, 1240, 1090, 840, 805, 710 $cm^{-1}$, $^1$H-NMR: 0.10 (s, 9H, $SiCH_3$), 0.90 (t, 3H, $CH_3$), 1.28-1.53 (m, 4H, $2CH_2$), 3.35 and 3.60 (mc, 2x1H, $OCH_2$), 3.63 (m, 1H, OCH).

*1-Trimethylsilyloxyheptan-2-ol* (**3Bb**)

# IV. melléklet

EGRI, G., BAITZ-GÁCS, E., **POPPE, L.**:

Kinetic Resolution of 2-Acylated-1,2-Diols by Lipase-Catalyzed Enantiomer Selective Acylation,

*Tetrahedron:Asymmetry*, **1996**, *7*, 1437.

Pergamon

*Tetrahedron: Asymmetry*, Vol. 7, No. 5, pp. 1437-1448, 1996

0957-4166/96 $15.00 + 0.00

S0957-4166(96)00161-9

# Kinetic Resolution of 2-Acylated-1,2-Diols by Lipase-Catalyzed Enantiomer Selective Acylation

**Gabriella Egri[a], Eszter Baitz-Gács[b], László Poppe[b]***

[a] Department for Organic Chemical Technology, Technical University Budapest, H-1521 Budapest, P.O. Box 91, HUNGARY
[b] Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 17, HUNGARY

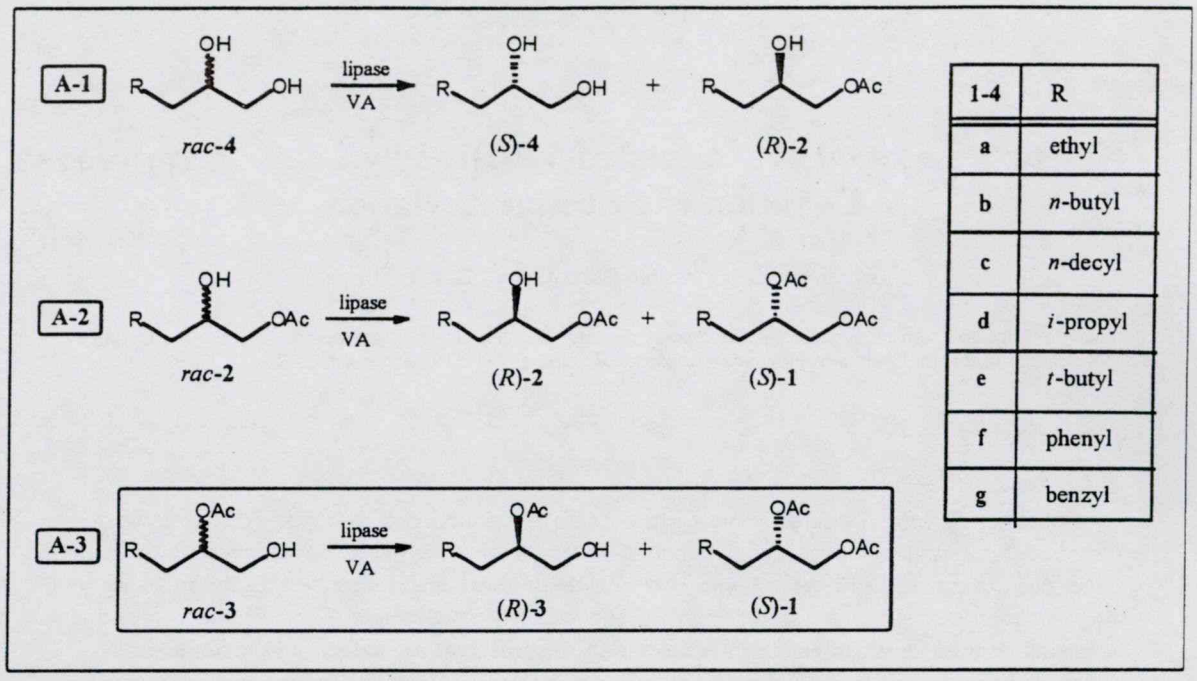
Abstract: Enantiomer selectivity of lipase catalyzed acylation of 2-acylated 1,2-diols was studied. First, acylation of 2-acetoxyheptan-1-ol rac-3b with vinyl acetate was investigated by varying the enzyme and the solvent, showing the highest enantiomer selectivity by using lipase from Pseudomonas fluorescens (PfL) in hexane-vinyl acetate (VA). We have found varying or even reversed enantiomer selectivity for different secondary acyl moieties in 2-acyloxyheptan-1-ols rac-3bA-F. Next, all six possible types of enantiomer selective biotransformations (hydrolysis of diacetate and the two kinds of monoacetetes; acylation of diol and the two kinds of monoacetates) were compared on two model diols rac-4b,d. Among the transformations investigated, acetylation of secondary monoacetates rac-3b,d showed the highest enantiomer selectivity. Finally, PfL catalyzed acetylations of several 2-acetylated 1,2-diols rac-3a-g were investigated under our optimum conditions.


Optically active 1,2-diols are widely used as synthetic building blocks for numerous natural products, pharamaceuticals and fine chemicals. Manufacture of these diols in enantiomerically highly enriched form has primary importance, since the two enantiomers of these compounds may possess markedly different biological activities, e.g. while the active enantiomer of pheromone brevicomin contains 1,2-dioxy-butane subunit with the *R* configuration, the other enantiomer shows inhibitory properties[1]. Consequently, enantiomer selective biotransformations of 1,2-diol derivatives have been the subject of many recent interests.

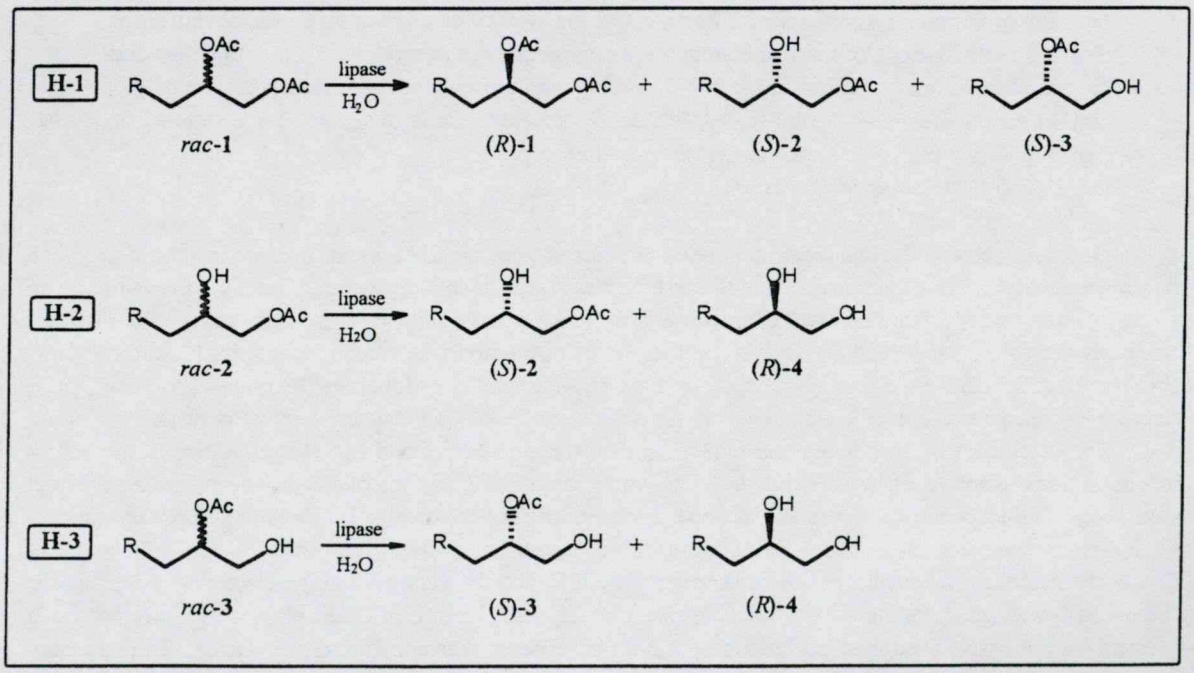
The usefulness of hydrolases, especially lipases, for enantiomer and regioselective transformation of diols and related compounds is well known[2,3]. Enzymatic biotransformations of 1,2-diol derivatives have been extensively studied: lipase-catalyzed acylation (transesterification) of racemic 1,2-diols[4-13] or primary acetates of 1,2-diols[3,13] were chosen as a tool for kinetic resolution in most studies. Hydrolysis[14,15] or alcoholysis[16] of 1,2-diol diacetates, however, have also been investigated. Generally, very high regioselectivity preferring the primary hydroxyl group along with a variable but a usually low degree of enantiomer selectivity has been observed by the lipase-catalyzed acylations of 1,2-diols[3]. The further enzymatic acetylation of the primary acetate products proved to be a slower but a more enantiomer selective process[3,13]. In contrast, hydrolysis of 1,2-diol diacetates proceeded with moderate regioselectivity but the enantiomer selectivity was significantly higher than for acylation of the corresponding 1,2-diol with the same enzyme[15]. This observation, that acylation of the 1,2-diols proved to be less enantiomer selective than the hydrolysis of the corresponding diacetate, suggested us that the size and/or characteristics of the substituent at $C_2$ plays a crucial role in enantiomer selectivity. Based on this hypothesis, we expected a higher enantiomer selectivity in biotransformations of 2-acetates than those of the corresponding enzymatic reactions of derivatives with free 2-hydroxy group.

These remarkable differences in selectivities between different enzymatic reactions of 1,2-diols and their acetate derivatives prompted us to test our above hypothesis and compare the enantiomer selectivities of all the possible lipase-catalyzed kinetic resolutions (acetylations **A1-3** and hydrolyses **H1-3**) of these diol derivatives (Scheme 1).



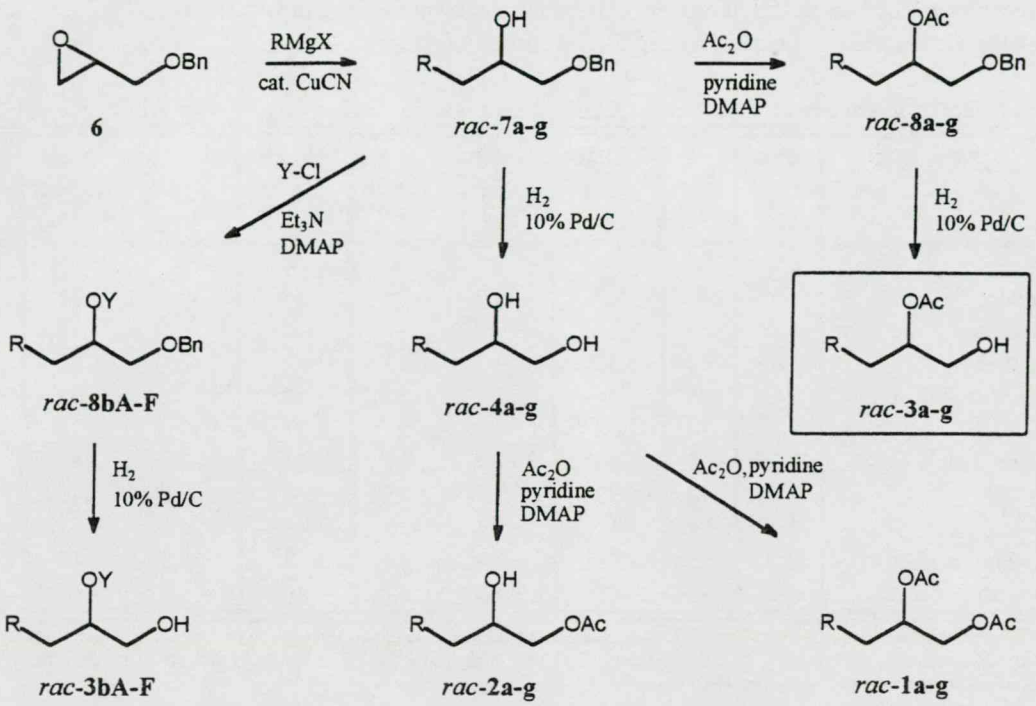



| 1-4 | R |
|---|---|
| a | ethyl |
| b | *n*-butyl |
| c | *n*-decyl |
| d | *i*-propyl |
| e | *t*-butyl |
| f | phenyl |
| g | benzyl |




**Scheme 1.** Lipase-catalyzed enantiomer selective biotransformations of 1,2-diol derivatives (*rac*-**1-4**)

Preparation of the *rac*-1,2-diols *rac*-**4a-g**and their acetate derivatives (Scheme 2) were based on the corresponding 1-benzyloxy-2-alkanols *rac*-**7a-g** which were prepared from racemic benzyl glycidyl ether **6** and the corresponding Grignard reagents[17]. The secondary monoacetates *rac*-**3a-g** were obtained *via* acetylation of the secondary alcohols *rac*-**7a-g** followed by hydrogenolysis. 2-Acylated heptane-1,2-diols *rac*-**3bA-F** were also synthesized in an analogous manner.




**Scheme 2.** Preparation of the *rac*-1,2-diol derivatives utilized in the present study

First, the selectivity of several lipases in acetylation of 2-acetyloxy-heptan-1-ol *rac*-**3b** with vinyl acetate was investigated (Method **A-3** in Scheme 1, Table 1).

**Table 1.** Acetylation of racemic 2-acetoxy-heptan-1-ol *rac*-**3b** with different enzymes

| Enzyme[a] (mg) | Time[b] (h) | c (%) | (*R*)-**3b** Y(%) | Diol from (*R*)-**3b** $[\alpha]_D$ | (*R*)-**3b** ee(%) | (*S*)-**1b** Y(%) | Diol from (*S*)-**1b** $[\alpha]_D$ | (*S*)-**1b** ee(%) | $E_{SP}$[c] |
|---|---|---|---|---|---|---|---|---|---|
| PfL (5) | 1.1 | 46 | 52 | +8.8 | 43 | 40 | -8.8 | 43 | 3.8 |
| PPL (30) | 4.6 | 55 | 33 | +5.7 | 28 | 40 | -5.4 | 26 | 2.2 |
| MjL (30) | 120 | 19 | 78 | +1.4 | 7 | 18 | -6.1 | 30 | 2.0 |
| CcL (30) | 5.3 | 54 | 39 | +3.3 | 16 | 46 | -4.4 | 22 | 1.8 |
| PLE (50) | 20 | 48 | 31 | +2.9 | 14 | 29 | -3.0 | 15 | 1.5 |
| AnL (30) | 20 | 46 | 49 | -0.2 | 1[d] | 41 | +0.3 | 2[d] | 1.0 |
| RaL (30) | [e] | - | - | - | - | - | - | - | - |

[a] PPL: lipase from porcine pancreas, CcL: lipase from *Candida rugosa (cylindracea)*, PfL: lipase from *Pseudomonas fluorescens*, PLE: esterase from pig liver (acetone powder), AnL: lipase from *Aspergilus niger*, MjL: lipase from *Mucor javonicus*, RaL: lipase from *Rhisopus arrhisus*, [b] *rac*-**3b** (200 mg) and the given amount of enzyme were stirred in vinyl acetate (2 ml) at RT. [c] E values ($E_{SP}$) were calculated from an equation[18] containing the enantiomeric excesses of substrate (S) and product (P). Determination of enantiomeric excess values: *i.*) transformation of S and P into the corresponding 1,2-diols, *ii.*) $^1$H-NMR investigation of di-MTPA-esters prepared from the 1,2-diols and (*R*)-MTPA-Cl in pyridine/$CCl_4$. [d] Configuration is opposite to the products obtained with other enzymes. [e] The reaction proved to be extremely slow in comparison with other enzymes

Lipase from *Pseudomonas fluorescens* (PfL) was choosen for the further study, since this enzyme showed both the highest enantiomer selectivity and efficacy among the lipases tested.

Next, the solvent effect on enantiomer selectivity of acetylation of racemic 2-acetoxy-heptan-1-ol *rac*-**3b** with PfL was investigated. Interestingly, no correlation between the solvent polarity (logP) and enantiomer selectivity was found. In this solvent effect study, the inhibitory behaviour of chloroform was also

quite surprising. The best selectivities were obtained in neat vinyl acetate or with hexane as solvent, therefore, this hexane-vinyl acetate 1:1 system was applied in further acylations.

**Table 2.** Effect of solvent on acetylation of racemic 2-acetoxy-heptan-1-ol *rac*-**3b** with PfL

| Solvent | Time[a] (h) | c (%) | (*R*)-**3b** Y(%) | Diol from (*R*)-**3b** $[\alpha]_D$ | ee(%) | (*S*)-**1b** Y(%) | Diol from (*S*)-**1b** $[\alpha]_D$ | ee(%) | $E_{SP}$[b] |
|---|---|---|---|---|---|---|---|---|---|
| chloroform | [c] | - | - | - | - | - | - | - | - |
| *tert*-butanol | 6.75 | 71 | 27 | +3.2 | 16 | 68 | -2.3 | 11 | 1.4 |
| carbon tetrachloride | 4.75 | 66 | 34 | +3.4 | 17 | 67 | -2.6 | 13 | 1.5 |
| diethylether | 4.75 | 64 | 32 | +4.7 | 23 | 58 | -5.0 | 25 | 2.0 |
| 2-methyltetrahydrofuran | 4 | 46 | 44 | +5.2 | 26 | 38 | -6.3 | 31 | 2.4 |
| ethyl acetate | 120 | 34 | 60 | +4.7 | 23 | 38 | -7.7 | 38 | 2.7 |
| tetrahydrofuran | 8.25 | 40 | 46 | +3.0 | 15 | 31 | -8.4 | 41 | 2.8 |
| hexane[d] | 3.55 | 42 | 52 | +5.1 | 25 | 37 | -8.1 | 40 | 2.9 |
| hexane | 6.75 | 54 | 41 | +5.4 | 26 | 49 | -8.3 | 41 | 3.0 |
| hexane-vinyl acetate 1:1 | 1.3 | 55 | 38 | +9.9 | 49 | 48 | -8.1 | 40 | 3.7 |
| vinyl acetate | 1.1 | 46 | 47 | +8.8 | 43 | 40 | -8.8 | 43 | 3.8 |

[a] *rac*-**3b** (200 mg), vinyl acetate (5 mmol) and PfL (5 mg) were stirred at RT in the solvent given in Table. [b] E value calculated from he enantiomeric excesses of substrate (S) and product (P)[18] (see also Table 1). [c] The reaction proved to be extremely slow in omparison with other solvents. [d] Saturated with water

We thought it also worthwhile to investigate the influence of structural features of the 2-acyl moiety on the enantiomer selectivity of the PfL-catalyzed acylation process. Our study with a series of racemic 2-acyloxy-heptan-1-ols *rac*-**3bA-F**; synthesis: (Scheme 2) showed a strong correlation between the size of the acyl moiety in the 2-position and the degree of enantiomer selectivity: a decrease in selectivity ($E_{SP}$) along with the increasing bulkiness was observed. In the case of acylation of the very bulky pivaloyl derivative *rac*-**3bF**, even a reversal in enantiomer preference was found, deduced from the observed (*R*) configuration of the diol **3b** obtained after hydrolysing the product **5*F**.

**Table 3.** Effect of 2-acyl moiety (Y) on acetylation of racemic 2-acyloxy-heptan-1-ols *rac*-**3bA-F** by PfL

| | Y | Time[a] (h) | c (%) | **3b*** **A-F** Y(%) | Diol **3b*** from **A-F** $[\alpha]_D$ | ee(%) | **5*A-F** Y(%) | config. | Diol **5*** from **A-F** $[\alpha]_D$ | ee(%) | $E_{SP}$[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | acetyl | 1.3 | 55 | 39 | +9.9 | 49 | 48 | *S* | -8.1 | 40 | 3.6 |
| **B** | propionyl | 4 | 31 | 55 | +4.0 | 20 | 25 | *S* | -8.7 | 43 | 3.0 |
| **C** | trifluoroacetyl | 120 | 32 | 57 | +3.8 | 19 | 26 | *S* | -8.4 | 41 | 2.9 |
| **D** | phenylacetyl | 2.1 | 34 | 61 | +1.3 | 6 | 31 | *S* | -2.5 | 6 | 1.4 |
| **E** | benzoyl | 3.5 | 49 | 45 | 0 | 0 | 43 | | 0 | 0 | 1.0 |
| **F** | pivaloyl | 4 | 45 | 53 | -1.2 | 6 | 42 | *R* | +0.7 | 4 | 1.1 |

[a] *rac*-**3bA-F** (1 mmol) and PfL (5 mg) were stirred in a solution of vinyl acetate (1 ml) and hexane (1 ml) at RT, [b] E value calculated rom the enantiomeric excesses of substrate (S) and product (P) [18] (see also Table 1).

For testing our starting hypothesis on the crucial role of the 2-acetyl moiety in enantiomer selectivity, derivatives of two different diols - the straight-chain heptan-1,2-diol *rac*-**4b** and the branched-chain 4-methylpentan-1,2-diol *rac*-**4d** - were chosen as models. All six possible types of biotransformations - the three possible kinds of enzymatic transesterifications, such as acetylation of diols *rac*-**4b,d**; method **A-1**, acetylation of primary monoacetates *rac*-**2b,d**; method **A-2**, and acetylation of secondary monoacetates *rac*-**3b,d**; method **A-3** , and the three possible kinds of enzymatic hydrolyses, namely hydrolysis of diacetates *rac*-**1b,d**; method **H-1**, hydrolysis of primary monoacetates *rac*-**2b,d**; method **H-2**, hydrolysis of secondary

monoacetates *rac*-**3b,d**; method **H-3** - were compared in this study (Scheme 1, Table 4). Acetylations were carried out under our standard conditions using vinyl acetate (VA) as an "irreversible" transesterifying reagent and lipase from *Pseudomonas fluorescens* (PfL) and hydrolyses were performed in water at a constant pH of 7.2.

**Table 4.** Lipase catalyzed enantiomer selective hydrolyses (Methods **H-1,2,3**) and acetylations (Methods **A-1,2,3**) of 1,2-diol derivatives (*rac*-**1-4a,b**)

| Substrate | Method [a] | Time (h) | c (%) | S [b] Y(%) | diol from $[\alpha]_D$ | S ee(%) | P [c] Y(%) | diol $[\alpha]_D$ | from P ee(%) | $E_{SP}$ [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| *rac*-**1b** | **H-1** | 0.66 | 51 | 40 | +3.1 | 15 | 36 | -2.7 | 13 | 1.5 |
| *rac*-**2b** | **H-2** | 3.15 | 53 | 45 | -2.7 | 13 | 46 | +2.8 | 14 | 1.5 |
| *rac*-**3b** | **H-3** | 5.3 | 63 | 15 | -4.5 | 22 | 49 | +1.1 | 5 | 1.3 |
| *rac*-**4b** | **A-1** | 0.75 | 24 | 67 | -0.5 | 3 | 21 | +1.9 | 9 | 1.2 |
| *rac*-**2b** | **A-2** | 432 | 44 | 52 | +5.7 | 28 | 41 | -7.0 | 34 | 2.6 |
| *rac*-**3b** | **A-3** | 1.33 | 55 | 39 | +9.9 | 49 | 48 | -8.1 | 40 | 3.6 |
| *rac*-**1d** | **H-1** | 3.3 | 39 | 45 | +9.1 | 30 | 42 | -9.7 | 32 | 2.5 |
| *rac*-**2d** | **H-2** | 13 | 20 | 64 | -0.1 | 0 | 14 | +1.1 | 4 | 1.1 |
| *rac*-**3d** | **H-3** | 25 | 24 | 60 | -0.3 | 1 | 21 | +1.4 | 5 | 1.1 |
| *rac*-**4d** | **A-1** | 3 | 51 | 44 | -9.9 | 32 | 46 | +9.5 | 31 | 2.6 |
| *rac*-**2d** | **A-2** | 192 | 29 | 59 | +6.6 | 22 | 24 | -14.1 | 46 | 3.3 |
| *rac*-**3d** | **A-3** | 1.75 | 41 | 53 | +17.3 | 57 | 37 | -30.2 | 99 | >100 |

[a] **Methods H-1,2,3** (cf. Scheme 1): Racemic substrate (1 mmol) and PfL (5 mg) were stirred in water at RT and the pH was kept at 7.2 by addition of 0.05 M NaOH solution from an autoburette. **Methods A-1,2,3** (cf. Scheme 1): Racemic substrate (1 mmol) and PfL (5 mg) were stirred in a solution of vinyl acetate (1 ml) and hexane (1 ml) at RT. Products of the reactions were isolated by separation on silica gel with a hexane-acetone eluent. [b] S: remaining fraction of racemic substrate, [c] P: product(s) of enzymatic transformation, [d] E values ($E_{SP}$) were calculated from an equation[18] containing the enantiomeric excesses of substrate (S) and product (P) (c.f. Table 1).

Results of the test reactions (Table 4) indicate that, according to our expectations, the highest enantiomer selectivity can be obtained in enzymatic acetylation of the secondary monoacetates *rac*-**3b,d** (Method **A-3**). Interestingly, this process proved to be not only the most selective but one of the fastest as well. In accordance with the previous observations, acetylation of the diols *rac*-**4b,d** (Method **A-1**) proved to be a fast, highly regioselective but less enantiomer selective reaction. Considering this high regioselectivity toward the primary hydroxylic group in lipase-catalyzed acetylation, sluggishness of the acetylation of primary monoacetates *rac*-**2b,d** (Method **A-2**) is not surprising. Among the hydrolytic processes, hydrolysis of the diacetates *rac*-**1b,d** (Method **H-1**) proved to be the fastest and most selective. In accordance with our previous findings in hydrolysis of 1,2-diol diacetates[15], no acyl migration was found either in hydrolyses or acylations[19].

Interestingly, not only the measure but the sense of the enantiomer selectivity of these lipase-catalyzed biotransformations were substrate-dependent. Reversal in enantiomer preference was observed in both series; within hydrolyses the moderate *S*-enantiomer preference of diacetate hydrolysis (Method **H-1**) turned to a slight *R*-preference in hydrolyses of monoacetates (Methods **H-2,3**), while in transesterifications the moderate *R*-enantiomer preference of diol acetylation (Method **A-1**) changed to a higher degree of *S*-enantiomer selectivity in acetylations of monoacetates (Methods **A-2,3**). It is noteworthy, that similar process-dependent change in stereoselectivity, was observed in *Pseudomonas cepacia* lipase-catalyzed hydrolyses and acetylations of 1-O- and 3-O-β-D-glucosyl- and galactosyl-*sn*-glycerols[20].

Finally, dependence of the enantiomer selectivity of PfL-catalyzed acylations of secondary monoacetates *rac*-**3a-g** on the structural features of the 1,2-diol skeleton was investigated. For this study, typical representives of secondary monoacetates having straight-chain alkyl *rac*-**3a-c**, branched-chain alkyl *rac*-**3d,e**, and arylalkyl *rac*-**3f,g** side-chains were prepared. The racemic substrates were then subjected to

PfL-catalyzed reaction under our standard conditions. Results of these reactions (Table 5) indicate that for high enantiomer selectivity a bulky side chain (entries **d-f**) is required. The non-branched substrates (entries **a-c,g**) gave only moderate selectivities indicating that branching in β-position to the acetoxy moiety is essential for good enantiomer differentiation. This finding, that bulkiness of the side chain of 1,2-diols plays a crucial role in enantiomer selectivity, is in agreement with the previous results on acetylation of 1,2-diols or primary monoacetates[3,12,13].

**Table 5.** Effect of side-chain (R) on acetylation of racemic 2-acetylated 1,2-diols (*rac*-**3a-g**) with PfL

| | R | $[\alpha]_{D\ diol}$ (100%ee)[a] | Time[b] (h) | c (%) | (*R*)-**3** Y(%) | diol from $[\alpha]_D$ | (*R*)-**3** ee(%) | (*S*)-**1** Y(%) | diol from $[\alpha]_D$ | (*S*)-**1** ee(%) | $E_{SP}$[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **a** | ethyl | 21.7 | 2.25 | 42 | 32 | +10.5 | 48 | 23 | -13.2 | 60 | 7.0 |
| **b** | *n*-butyl | 20.4 | 1.3 | 55 | 39 | +9.9 | 49 | 48 | -8.1 | 40 | 4.4 |
| **c** | *n*-decyl | 13.4 | 8 | 40 | 41 | +5.6 | 42 | 27 | -7.2 | 54 | 4.5 |
| **d** | *i*-propyl | 30.6 | 1.75 | 41 | 53 | +17.3 | 57 | 37 | -30.2 | >98 | >100 |
| **e** | *t*-butyl | 27.9 | 3.3 | 43 | 52 | +22.7 | 81 | 39 | -27.9 | >98 | >100 |
| **f** | phenyl | 29.6 | 3 | 38 | 57 | +18.9 | 67 | 34 | -29.0 | >98 | >100 |
| **g** | benzyl | 30.8 | 9 | 51 | 43 | +12.9 | 42 | 46 | -13.6 | 44 | 3.8 |

[a] Extrapolated values calculated from specific rotation of diols and from the corresponding enantiomeric excess values obtained from $^1$H-NMR spectra of di-MTPA-esters of the 1,2-diols. Absolute configuration of the 1,2-diols (**4a-d,f,,g**) was determined by comparison with literature rotation values {$[\alpha]_D$ (c, solvent)} of compounds having known absolute configuration: (*S*)-**4a**: -23.2 (1, ethanol)[21], -19.2 (2, ethanol)[22], (*R*)-**4a**: +17.4 (2, ethanol)[2]; (*S*)-**4b**: -20.6 (1, ethanol)[21], (*S*)-**4c**: -10.1 (1.2, methanol)[23], (*R*)-**4c**: +10.1 (1.18, methanol)[23]; (*S*)-**4d**: -31.5 (1, ethanol)[21], (*R*)-**4d**: +13.82 (neat)[24]; (*S*)-**4f**: -36 (1, ethanol)[25], (*R*)-**4f**: +23 (1.03, $CHCl_3$)[26]; (*S*)-**4g**: -34.1 (1, ethanol)[21]. (*S*)-configuration for (-)-**4e** is assumed by analogy with the other members of 1,2-diol series. [b] *rac*-**3** (1 mmol) and PfL (5 mg) were stirred in a solution of vinyl acetate (1 ml) and hexane (1 ml) at RT. [c] E values ($E_{SP}$) were calculated from an equation[18] containing the enantiomeric excesses of substrate (S) and product (P) (c.f. Table 1).

In summary, it can be concluded that acetylation of the easily accessible 2-acetates of 1,2-diols proved to be the best alternative among the six possible types of lipase-catalyzed kinetic resolutions of racemic 1,2-diol derivatives both with respect to enantiomer selectivity and productivity. Lipase-catalyzed acetylation seems to be an ideal choice for obtaining homochiral products from racemic 2-acylated 1,2-diol derivatives having bulkiness in β-position to the acetoxy moiety.

## EXPERIMENTAL.

The $^1$H-NMR spectra were taken on JEOL FX-100 (100 MHz) or Brucker AW-80 (80 MHz) spectrometers in $CDCl_3$ solution containing TMS as internal standard. Enantiomeric purity determinations (cf. Table 1, note c) using di-(*S*)-MTPA esters of the corresponding diols were carried out in $CDCl_3$ solution on a Varian VXR-400 (400 MHz) NMR-equipment. IR spectra were recorded on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin Elmer 241 polarimeter. Thin-layer chromatography (TLC) was made using Merck Kieselgel 60 $F_{254}$ alumina sheets. Spots were visualized by treatment with 3% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative vacuum-chromatography[27] was performed using Merck Kieselgel 60 $F_{254}$. The 1-benzyloxy-alkan-2-ols *rac*-**7a-g** were prepared by a published procedure[17]. Porcine pancreatic lipase (PPL, Type II) was obtained from Sigma. Lipases from *Candida rugosa (cylindracea)* (CcL), *Pseudomonas fluorescens* (PfL), *Aspergilus niger* (AnL), *Mucor javonicus* (MjL), *Rhisopus arrhisus* (RaL), esterase from pig liver (PLE, acetone powder), acetic anhydride, and vinyl acetate were products of FLUKA. All solvents used were freshly distilled.

### PREPARATION OF 2-ACYLATED 1-BENZYLATED 1,2-DIOLS *rac*-**8a-g** AND *rac*-**8bB-F**

*General procedure*: 1-Benzylated-1,2-diol *rac*-**7a-g**, 10 mmol, pyridine (30 mmol, 2.4 ml), and catalytic amount of dimethylaminopyridine were dissolved in hexane and dichloromethane (10 ml, each) followed by a dropwise addition of the corresponding acyl chloride or anhydride (15 mmol) at room temperature. The mixture was kept at 45°C until TLC investigation showed no remaining starting material (20-90 minutes). The

resulting mixture was then diluted with diethyl ether (10 ml) and washed with 5% hydrochloric acid (2x10 ml), saturated $NaHCO_3$ solution (10 ml), and brine (10 ml). The organic phase was dried over $Na_2SO_4$ and solvents were evaporated off *in vacuo*.

*rac-2-Acetoxy-1-benzyloxypentan rac-***8a**

($Ac_2O$) *Yield*: 91%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.90 (m, 3H, $CH_3$), 1.51 (mc, 4H, $2CH_2$), 2.03 (s, 3H, CO-$CH_3$), 3.48 (mc, 2H, $OCH_2$), 4.53 (s, 2H, O-$CH_2$-Ph), 4.9-5.2 (m, 1H, OCH), 7.20-7.34 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3030 (w), 2960, 2872, 1738, 1496 (w), 1454, 1372, 1241, 1106, 1055, 1026, 944, 908, 736; Calcd. for $C_{14}H_{20}O_3$: C 71.16, H 8.53; found: C 70.80, H 8.56.

*rac-2-Acetoxy-1-benzyloxyheptan rac-***8b**

($Ac_2O$) *Yield*: 98%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.93 (m, 3H, $CH_3$), 1.24 (mc, 4H, $2CH_2$), 2.02 (s, 3H, CO-$CH_3$), 3.44 (mc, 2H, $OCH_2$), 4.57 (s, 2H, O-$CH_2$-Ph), 4.9-5.2 (m, 1H, OCH), 7.20-7.37 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3030 (w), 2956, 2931, 2860, 1738, 1496, 1454, 1372, 1242, 1112, 1027, 943, 906, 735; Calcd. for $C_{16}H_{24}O_3$: C 72.69, H 9.15; found: C 72.98, H 9.08.

*rac-2-Acetoxy-1-benzyloxytridecan rac-***8c**

($Ac_2O$) *Yield*: 95%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.87 (m, 3H, $CH_3$), 1.23 (mc, 20H, $10CH_2$), 2.03 (s, 3H, CO-$CH_3$), 3.44 (d, 2H, $OCH_2$), 4.48 (s, 2H, O-$CH_2$-Ph), 4.9-5.2 (m, 1H, OCH), 7.21-7.34 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3030 (w), 2925, 2860, 1740, 1496 (w), 1454, 1371, 1240, 1118, 1096, 1027, 902; Calcd. for $C_{22}H_{36}O_3$: C 75.82, H 10.41; found: C 76.35, H 10.50.

*rac-2-Acetoxy-1-benzyloxy-4-methylpentane rac-***8d**

($Ac_2O$) *Yield*: 94%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.92-0.98 (d, 6H, $2CH_3$), 1.50 (mc, 3H, $CH_2$, CH), 2.04 (s, 3H, CO-$CH_3$), 3.45 (d, 2H, $OCH_2$), 4.52 (s, 2H, O-$CH_2$-Ph), 5.0-5.3 (m, 1H, OCH), 7.19-7.35 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3060 (w), 3030 (w), 2957, 2869, 1737, 1496, 1469, 1454, 1371, 1240, 1115, 1026, 947, 908, 736; Calcd. for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found: C 71.61, H 8.92.

*rac-2-Acetoxy-1-benzyloxy-4,4-dimethylpentane rac-***8e**

($Ac_2O$) *Yield*: 96%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.87 (m, 9H, $3CH_3$), 1.49 (mc, 2H, $CH_2$), 2.00 (s, 3H, CO-$CH_3$), 3.41 (d, 2H, $OCH_2$), 4.44 (s, 2H, O-$CH_2$-Ph), 5.05-5.38 (m, 1H, OCH), 7.21-7.35 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3030 (w), 2955, 2906, 2867, 1737, 1496 (w), 1476, 1453, 1371, 1240, 1204 (w), 1125, 1095, 1052, 1024, 944, 736; Calcd. for $C_{16}H_{24}O_3$: C 72.69, H 9.15; found: C 72.11, H 9.10.

*rac-2-Acetoxy-1-benzyloxy-4-phenylpropane rac-***8f**

($Ac_2O$) *Yield*: 81%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 2.04 (s, 3H, CO-$CH_3$), 2.89 (m, 2H, Ar-$CH_2$) , 3.44 (d, 2H, $OCH_2$) ,4.53 (s, 2H, O-$CH_2$-Ph), 5.02-5.20 (m, 1H, OCH), 7.0-7.5 (m, 10H, $2C_6H_5$); *IR* (film, ν $cm^{-1}$): 3063 (w), 3029, 2933, 2863, 1826, 1737, 1604, 1496, 1454, 1372, 1239, 1124, 1097, 1050, 1029, 958, 896, 747; Calcd. for $C_{18}H_{20}O_3$: C 76.03, H 7.09; found: C 75.88, H 7.14.

*rac-2-Acetoxy-1-benzyloxy-4-phenylbutane rac-***8g**

($Ac_2O$) *Yield*: 96%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 2.01 (s, 3H, CO-$CH_3$), 2.44-2.78 (m, 4H, 2CH2), 3.51 (d, 2H, $OCH_2$), 4.46 (s, 2H, O-$CH_2$-Ph), 4.9-5.2 (m, 1H, OCH), 7.0-7. 2 (m, 10H, $2C_6H_5$); *IR* (film, ν $cm^{-1}$): 3062 (w), 3027, 2933, 2862, 1737, 1603 (w), 1496, 1454, 1372, 1240, 1126, 1099, 1044, 1028, 907 (w), 737; Calcd. for $C_{19}H_{22}O_3$: C 76.48, H 7.43; found: C 76.10, H 7.42.

*rac-1-Benzyloxy-2-propionyloxyheptane rac-***8bB**

($C_2H_5COCl$) *Yield*: 85%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.86 (m, 3H, $CH_3$), 0.96-1.48 (m, 11H, $4CH_2$, $CH_3$), 2.33 (q, 2H, OOC-$CH_2$), 3.42 (d, 2H, O-$CH_2$), 4.55 (s, 2H, O-$CH_2$-Ph), 4.87-5.20 (m, 1H, OCH), 7.18-7.32 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 2956, 2932, 2860, 1737, 1496, 1454, 1367, 1274, 1189, 1112, 1082, 905, 734; Calcd. for $C_{17}H_{26}O_3$: C 73.35, H 9.41; found: C 73.94, H 9.34.

*rac-1-Benzyloxy-2-(trifluoroacetyl)oxyheptane rac-***8bC**

[$(CF_3CO)_2O$] *Yield*: 85%, *$^1H$ NMR* ($CDCl_3$, δ ppm): 0.89 (m, 3H, $CH_3$), 1.06-1.62 (m, 8H, $4H_2$), 3.58 (d, 2H, O-$CH_2$), 4.49 (s, 2H, O-$CH_2$-Ph), 5.0-5.2 (m, 1H, O-CH), 7.18-7.38 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 2959,

2933, 2863, 1785, 1455, 1386, 1342, 1222, 1167, 1110, 1028 (w), 867, 732; Calcd. for $C_{16}H_{21}F_3O_3$: C 60.37, H 6.65, F 17.90; found: C 60.61, H 6.70, F 17.88.

*rac-1-Benzyloxy-2-(phenylacetyl)oxyheptane rac-***8bD**

($C_6H_5CH_2COCl$) *Yield*: 84%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.82 (m, 3H, $CH_3$), 1.01-1.61 (m, 8H, $4CH_2$), 3.6 (m, 4H, O-$CH_2$, O(O)C-$CH_2$-Ph), 4.21 (s, 2H, O-$CH_2$-Ph), 4.85-5.20 (m, 1H, O-CH), 7.1-7.4 (m, 10H, $2C_6H_5$); *IR* (film, ν $cm^{-1}$): 2955, 2930, 2859, 1734, 1496, 1454, 1364, 1257, 1159, 1111, 1029, 908; Calcd. for $C_{22}H_{28}O_3$: C 77.61, H 8.29; found: C 77.92, H 8.35.

*rac-2-Benzoyloxy-1-benzyloxyheptane rac-***8bE**

($C_6H_5COCl$) *Yield*: 95%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.86 (m, 3H, $CH_3$), 1.08-1.67 (m, 8H, $4CH_2$), 3.62 (d, 2H, O-$CH_2$), 4.52 (s, 2H, O-$CH_2$-Ph), 5.17-5.41 (m, 1H, O-CH), 7.12-7.54 (m, 10H, $2C_6H_5$); *IR* (film, ν $cm^{-1}$): 2955, 2930, 2859, 1790, 1716, 1601, 1495, 1452, 1364, 1314, 1274, 1212, 1175, 1106, 1070, 1026, 997, 936; Calcd. for $C_{21}H_{26}O_3$: C 77.27, H 8.03; found: C 77.81, H 7.99.

*rac-1-Benzyloxy-2-pivaloyloxyheptane rac-***8bF**

[$(CH_3C)_3CCOCl$] *Yield*: 82%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.86 (m, 3H, $CH_3$), 1.17 (s, 9H, $3CH_3$), 1.27-1.41 (m, 8H, $4H_2$), 3.48 (d, 2H, O-$CH_2$), 4.52 (s, 2H, O-$CH_2$-Ph), 4.85-5.17 (m, 1H, O-CH), 7.20-7.38 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 2957, 2932, 2861, 1810, 1728, 1496 (w), 1480, 1455, 1366, 1283, 1164, 1111, 1042, 1006, 940, 735; Calcd. for $C_{19}H_{30}O_3$: C 74.47, H 9.87; found: C 74.77, H 9.80.

PREPARATION OF 2-ACYLATED 1,2-DIOLS *rac-***3a-g** AND *rac-***3bB-F**

*General procedure*: To a suspension of 10 % Pd-C catalyst (100 mg) in isopropyl alcohol (20-30 ml), 7-10 mmol of the corresponding 1-benzylated-2-acylated-1,2-diol (*rac-***8a-g** or *rac-***8bB-F**) was added and the suspension was vigorously stirred under hydrogen atmosphere at 40°C for 1-3.5 hours. The catalyst was then filtered off and the solvent was evaporated *in vacuo*. The oily residue was purified by column chromatography with hexane:acetone.

*rac-2-Acetoxypentan-1-ol rac-***3a**

*Yield*: 72%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.95 (m, 3H, $CH_3$), 1.42 (mc, 4H, $2CH_2$), 2.06 (s, 3H, CO-$CH_3$), 3.63 (mc, 2H, $OCH_2$), 4.8-5.1 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3446 (bc), 2960, 2930, 2875, 1738, 1713, 1470, 1435, 1375, 1242, 1126, 1050, 1029, 955; Calcd. for $C_7H_{14}O_3$: C 57.51, H 9.65; found: C 57.22, H 9.59.

*rac-2-Acetoxyheptan-1-ol rac-***3b**

*Yield*: 75%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.92 (m, 3H, $CH_3$), 1.38 (mc, 4H, $2CH_2$), 2.06 (s, 3H, CO-$CH_3$), 3.72 (mc, 2H, $OCH_2$), 4.8-5.1 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3442 (bc), 2955, 2932, 2861, 1739, 1461, 1375, 1242, 1047, 956; Calcd. for $C_9H_{18}O_3$: C 62.04, H 10.41; found: C 62.47, H 10.34.

*rac-2-Acetoxytridecan-1-ol rac-***3c**

*Yield*: 70%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.83 (m, 3H, $CH_3$), 1.20 (mc, 20H, $10CH_2$), 2.04 (s, 3H, CO-$CH_3$), 3.63 (mc, 2H, $OCH_2$), 4.78-5.0 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3452 (bc), 2924, 2854, 1965, 1740, 1719, 1466, 1374, 1241, 1055, 941, 891; Calcd. for $C_{15}H_{28}O_3$: C 69.72, H 11.70; found: C 70.14, H 11.72.

*rac-2-Acetoxy-4-methylpentan-1-ol rac-***3d**

*Yield*: 74%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.92-0.98 (d, 6H, $2CH_3$), 1.50 (mc, 3H, $CH_2$, CH), 2.07 (s, 3H, CO-$CH_3$), 3.62 (mc, 2H, $OCH_2$), 4.8-5.1 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3446 (bc), 2958, 2872, 1739, 1713, 1470, 1432, 1372, 1241, 1171, 1145, 1067, 1025, 952, 879, 820; Calcd. for $C_8H_{16}O_3$: C 59.98, H 10.07; found: C 59.80, H 9.99.

*rac-2-Acetoxy-4,4-dimethylpentan-1-ol rac-***3e**

*Yield*: 81%, $^1H$ *NMR* ($CDCl_3$, δ ppm): 0.87 (m, 9H, $3CH_3$), 1.41 (mc, 2H, $CH_2$), 2.01 (s, 3H, CO-$CH_3$), 3.55 (mc, 2H, $OCH_2$), 4.84-5.16 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3446 (bc), 2955, 2896, 2871, 1736, 1476, 1430 (w), 1368, 1242, 1198, 1083, 1046, 1023, 944, 910; Calcd. for $C_9H_{18}O_3$: C 62.04, H 10.41; found: C 61.92, H 10.43.

*rac-2-Acetoxy-3-phenylpropan-1-ol rac*-**3f**

*Yield*: 65%, *$^1$H NMR* ($CDCl_3$, δ ppm): 2.00 (s, 3H, CO-$CH_3$), 3.61 (mc, 2H, $OCH_2$), 4.04 (m, 2H, Ph-$CH_2$), 4.92-5.20 (m, 1H, OCH), 7.05-7.32 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3445 (bc), 3062, 3028, 2937, 1736, 1604, 1496, 1454, 1431, 1374, 1241, 1086, 1033, 943, 749; Calcd. for $C_{11}H_{14}O_3$: C 68.02, H 7.27; found: C 68.22, H 7.23.

*rac-2-Acetoxy-4-phenylbutan-1-ol rac*-**3g**

*Yield*: 79%, *$^1$H NMR* ($CDCl_3$, δ ppm): 2.00 (s, 3H, CO-$CH_3$), 2.47-2.82 (m, 2H, CH2), 3.71 (mc, 2H, $OCH_2$), 4.02 (m, 2H, Ph-$CH_2$), 4.86-5.02 (m, 1H, OCH), 7.00-7.32 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3443 (bc), 3026, 2948, 1737, 1608 (w), 1496, 1454, 1371, 1244, 1096, 1043, 950, 915; Calcd. for $C_{12}H_{16}O_3$: C 69.21, H 7.74; found: C 68.93, H 7.71.

*rac-2-Propionyloxyheptan-1-ol rac*-**3bB**

*Yield*: 79%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.87 (m, 3H, $CH_3$), 1.06-1.57 (m, 11H, $4CH_2$, $CH_3$), 2.3 (q, 2H, O(O)C-$CH_2$), 3.81 (d, 2H, O-$CH_2$), 4.90-5.06 (m, 1H, OCH); *IR* (film, ν $cm^{-1}$): 3446 (bc), 2956, 2932, 2860, 1737 1463, 1423, 1378, 1342, 1276, 1190, 1125 (w), 1083, 1021, 920, 889, 806; Calcd. for $C_{10}H_{20}O_3$: C 63.80, H 10.71; found: C 64.25, H 10.75.

*rac-2-(Trifluoroacetyl)oxyheptan-1-ol rac*-**3bC**

*Yield*: 81%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.84 (m, 3H, $CH_3$), 1.02-1.51 (m, 8H, $4H_2$), 3.64 (d, 2H, O-$CH_2$), 5.1 (mc, 1H, O-CH); *IR* (film, ν $cm^{-1}$): 3355 (bc), 2960, 2935, 2863, 1788, 1460, 1382, 1345, 1260, 1223, 1170, 1074, 867, 812, 776, 730; Calcd. for $C_9H_{15}F_3O_3$: C 47.37, H 6.62, F 24.97; found: C 46.95, H 6.59, F 25.19.

*rac-2-(Phenylacetyl)oxyheptan-1-ol rac*-**3bD**

*Yield*: 88%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.91 (m, 3H, $CH_3$), 1.04-1.47 (m, 8H, $4CH_2$), 3.58 (s, 2H, Ph-$CH_2$), 3.67 (d, 2H, O-$CH_2$), 4.71-4.98 (m, 1H, O-CH), 7.03-7.40 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3442 (bc), 2955, 2930, 2860, 1733, 1496, 1454, 1259, 1161, 1075, 964, 910; Calcd. for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found: C 71.68, H 8.85.

*rac-2-Benzoyloxyheptan-1-ol rac*-**3bE**

*Yield*: 83%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.92 (m, 3H, $CH_3$), 1.05-1.74 (m, 8H, $4CH_2$), 3.79 (d, 2H, O-$CH_2$), 4.96-5.22 (m, 1H, O-CH), 7.05-7.44 (m, 5H, $C_6H_5$); *IR* (film, ν $cm^{-1}$): 3462 (bc), 2955, 2931, 2860, 1717, 1602, 1451, 1359, 1276, 1177, 1115, 1096, 1070, 1026, 936; Calcd. for $C_{14}H_{20}O_3$: C 71.16, H 8.53; found: C 71.44, H 8.54.

*rac-2-Pivaloyloxyheptan-1-ol rac*-**3bF**

*Yield*: 74%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.88 (m, 3H, $CH_3$), 1.18 (s, 9H, $3CH_3$), 1.22-1.43 (m, 8H, $4H_2$), 3.62 (d, 2H, O-$CH_2$), 4.77-4.98 (m, 1H, O-CH); *IR* (film, ν $cm^{-1}$): 3447 (bc), 2958, 2933, 2872, 2072 (b), 1729, 1708, 1538 (w), 1481, 1461, 1398, 1367, 1285, 1164, 1093, 1061, 1034, 938, 893, 770; Calcd. for $C_{12}H_{24}O_3$: C 66.63, H 11.18; found: C 66.36, H 11.15.

PREPARATION OF 1,2-DIOLS *rac*-**4b,d**

*General procedure*: To a suspension of 10 % Pd-C catalyst (100 mg) in isopropyl alcohol (20-30 ml), 7-10 mmol of the corresponding 1-benzylated -1,2-diol (*rac*-**7b,d**) was added and the suspension was vigorously stirred under hydrogen atmosphere at 40°C for 2-3 hours. The catalyst was then filtered off and the solvent was evaporated *in vacuo*. The oily residue was purified by column chromatography on silica gel with hexane:acetone=1:1.

*rac-Heptan-1,2-diol rac*-**4b**

*Yield*: 89%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.94 (m, 3H, $CH_3$), 1.41 (mc, 4H, $2CH_2$), 3.4-3.8 (m, 3H, O-CH, O-$CH_2$); *IR* (film, ν $cm^{-1}$): 3356 (bc), 2955, 2931, 2860, 1466, 1378, 1133, 1072, 1032, 938, 871; Calcd. for $C_7H_{16}O_2$: C 63.60, H 12.20; found: C 64.11, H 12.22.

*rac-4-Methylpentan-1,2-diol rac*-**4d**

*Yield*: 85%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.87 (d, 6H, $2CH_3$), 1.21 (q, 2H, $CH_2$), 1.77 (mc, 1H, O-CH), 3.4-3.9 (m, 3H, O-CH, O-$CH_2$); *IR* (film, ν $cm^{-1}$): 3355 (bc), 2956, 2930, 2871, 1469, 1386, 1368, 1220, 1171, 1145, 1071, 1028, 948, 920, 882, 842, 734; Calcd. for $C_6H_{14}O_2$: C 60.98, H 11.94; found: C 60.61, H 11.86.

PREPARATION OF 1-ACETYLATED 1,2-DIOLS *rac*-**2b,d**

*General procedure*: The corresponding 1,2-diol *rac*-**4b,d**, (10 mmol), pyridine (25 mmol, 2.4 ml), and catalytic amount of dimethylaminopyridine were dissolved in hexane (10 ml) followed by a dropwise addition of acetic anhydride (10 mmol, 0.95 ml) at room temperature. The mixture was then stirred at room temperature until TLC investigation showed no remaining starting material (20-90 minutes). The resulting mixture was then diluted with diethyl ether (10 ml) and washed with 5% hydrochloric acid (2x10 ml), saturated $NaHCO_3$ solution (10 ml), and brine (10 ml). The organic phase was dried over $Na_2SO_4$ and solvents were evaporated off *in vacuo*. The residue was purified by column chromatography on silica gel with hexane-acetone.

*rac-1-Acetoxyheptan-2-ol rac*-**2b**

*Yield*: 69%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.92 (t, 3H, $CH_3$), 1.2-1.6 (m, 8H, $4H_2$), 2.08 (s, 3H, CO-$CH_3$), 3.8-4.1 (m, 3H, O-$CH_2$, O-CH); *IR* (film, ν $cm^{-1}$): 3447 (bc), 2956, 2933, 2860, 1742, 1458, 1371, 1241, 1137, 1041, 944, 916; Calcd. for $C_9H_{18}O_3$: C 62.04, H 10.41; found: C 62.35, H 10.47.

*rac-1-Acetoxy-4-methylpentan-2-ol rac*-**2d**

*Yield*: 77%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.97 (d, 6H, $2CH_3$), 1.19-1.41 (m, 3H, $CH_2$-CH), 2.06 (s; 3H, CO-$CH_3$), 3.97 (mc, 3H, O-CH, O-$CH_2$); *IR* (film, ν $cm^{-1}$): 3454 (bc), 2957, 2871 (w), 1741, 1469, 1369, 1241, 1171, 1149, 1038, 981, 950, 921; Calcd. for $C_8H_{16}O_3$: C 59.98, H 10.07; found: C 60.22, H 10.08.

PREPARATION OF 1,2-DIOL DIACETATES *rac*-**1b,d**

*General procedure*: The corresponding 1,2-diol *rac*-**4b,d**, (10 mmol), pyridine (50 mmol, 4.0 ml), and catalytic amount of dimethylaminopyridine were dissolved in hexane (10 ml) followed by a dopwise addition of acetic anhydride (25 mmol, 2.4 ml) at room temperature. The mixture was kept at 40°C for 20 minutes and the resulting mixture was then diluted with diethyl ether (10 ml) and washed with 5% hydrochloric acid (2x10 ml), saturated $NaHCO_3$ solution (10 ml), and brine (10 ml). The organic phase was dried over $Na_2SO_4$ and solvents were evaporated off *in vacuo*. The remaining oil was purified by column chromatography on silica gel with hexane-acetone.

*rac-1,2-Diacetoxyheptane rac*-**1b**

*Yield*: 72%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.84 (t, 3H, $CH_3$), 1.16-1.38 (m, 8H, $4H_2$), 2.01 (s, 3H, CO-$CH_3$), 4.1 (mc, 2H, O-$CH_2$), 4.85-5.10 (m, 1H, O-CH); *IR* (film, ν $cm^{-1}$): 2957, 2933, 2862, 1744, 1461, 1371, 1243, 1227, 1126 (w), 1096, 1048, 958, 873; Calcd. for $C_{11}H_{20}O_3$: C 61.09, H 9.32; found: C 60.60, H 9.30.

*rac-1,2-Diacetoxy-4-methylpentane rac*-**1d**

*Yield*: 75%, *$^1$H NMR* ($CDCl_3$, δ ppm): 0.96 (d, 6H, $2CH_3$), 1.2-1.6 (m, 3H, $CH_2$-CH), 2.02 (s; 6H, 2CO-$CH_3$), 3.8-4.3 (m, 2H, O-$CH_2$), 4.95-5.15 (m, 1H, O-CH); *IR* (film, ν $cm^{-1}$): 2959, 2873 (w), 1745, 1558 (w), 1506 (w), 1471, 1431, 1372, 1227, 1097, 1045, 1026, 952, 890; Calcd. for $C_{10}H_{18}O_3$: C 59.39, H 8.97; found: C 58.97, H 9.00.

ACETYLATION OF RACEMIC 2-ACETOXY-HEPTAN-1-OL *rac*-**3b** WITH DIFFERENT ENZYMES

*General procedure*: Racemic 2-acetoxyheptan-1-ol *rac*-**3b** (200 mg) and enzyme were stirred in vinyl acetate (2 ml) at room temperature. After reaching a reasonable conversion (for conversions and reaction times see Table 1) enzyme was filtered off and solvent was evaporated. The residue was subjected to column chromatography on silica gel with hexane-acetone resulting pure diacetate (**1b**) and monoacetate (**3b**) fractions. Spectral data for optically active compounds have not differed significantly from that obtained for the corresponding racemic compounds. Data for type of enzyme (amount of enzyme), **1b**: % yield, **3b**: % yield, are given below. (For determination of absolute configuration and enantiomeric purity *via* the corresponding 1,2-diol, see Table 1 and Experimental, Section on enantiomeric purity determination.)

PfL (5), (*R*)-**3b**: 52, (*S*)-**1b**: 40; PPL (30), (*R*)-**3b**: 55, (*S*)-**1b**: 40; MjL (30), (*R*)-**3b**: 78, (*S*)-**1b**: 18; CcL (30), (*R*)-**3b**: 54, (*S*)-**1b**: 46; PLE (50), (*R*)-**3b**: 48, (*S*)-**1b**: 29; AnL (30), **3b**: 46, **1b**: 41.

ACETYLATION OF RACEMIC 2-ACETOXY-HEPTAN-1-OL *rac*-**3b** IN DIFFERENT SOLVENTS

*General procedure*: Racemic 2-acetoxyheptan-1-ol *rac*-**3b** (200 mg), vinyl acetate (5 mmol) and PfL (5 mg) were stirred in the given solvent (2 ml) at room temperature. After reaching a reasonable conversion (for conversions and reaction times, see Table 2) PfL enzyme was filtered off and solvent was evaporated. The

further work up and analysis of products were carried out as described in the previous section. Data for solvent, (*R*)-**3b**: % yield, (*S*)-**1b**: % yield, are given below.

Chloroform, no reasonable conversion; *tert*-butanol, (*R*)-**3b**: 27, (*S*)-**1b**: 68; carbon tetrachloride, (*R*)-**3b**: 34, (*S*)-**1b**: 67; diethyl ether, (*R*)-**3b**: 32, (*S*)-**1b**: 58; 2-methyltetrahydrofuran, (*R*)-**3b**: 46, (*S*)-**1b**: 38; ethyl acetate, (*R*)-**3b**: 60, (*S*)-**1b**: 38; tetrahydrofuran, (*R*)-**3b**: 46, (*S*)-**1b**: 31; hexane (satd. with water), (*R*)-**3b**: 52, (*S*)-**1b**: 37; hexane, (*R*)-**3b**: 41, (*S*)-**1b**: 49; hexane-vinyl acetate 1:1, (*R*)-**3b**: 38, (*S*)-**1b**: 48.

ACETYLATION OF RACEMIC 2-ACYLOXY-HEPTAN-1-OLS *rac*-**3bA-F** BY PfL

*General procedure*: Racemic 2-acyloxyheptan-1-ol *rac*-**3bA-F** (1 mmol) and PfL (5 mg) were stirred in hexane-vinyl acetate 1:1 (2 ml) at room temperature. After reaching a reasonable conversion (for conversions and reaction times see Table 3) PfL was removed by filtration and solvents were evaporated. The further work up and analysis of products were carried out as described in the previous sections. Data for remaining fraction of substrate **3bA-F**: % yield, and the acetylated products **5*A-F**: % yield, are given below.

(*R*)-**3bA**≡ (*R*)-**3b**, (*S*)-**5*A**≡ (*S*)-**1b**; (*R*)-**3bB**: 55, (*S*)-**5*B**: 25; (*R*)-**3bC**: 57, (*S*)-**5*C**: 26; (*R*)-**3bD**: 61, (*S*)-**5*D**: 31; **3bE**: 45, **5*E**: 43; (*S*)-**3bF**: 53, (*R*)-**5*F**: 42.

HYDROLYSIS OF RACEMIC 1,2-DIOL DIACETATES *rac*-**1b,d** (Method **H-1**), PRIMARY *rac*-**2b,d** (Method **H-2**), OR SECONDARY MONOACETATES (*rac*-**3b,d**; Method **H-3**) BY PfL

*General procedure*: Racemic 1,2-diol diacetate *rac*-**1b,d**, primary *rac*-**2b,d**, or secondary *rac*-**3b,d** monoacetate (3 mmol) and PfL (15 mg) were stirred in water (25 ml) at RT and pH value was kept at 7.2 by addition of 0.05M NaOH solution from an autoburette. When reasonable conversion (preferably around 0.5) was achieved, the mixture was extracted with ethyl acetate (3x30 ml). The combined organic phase was dried over $Na_2SO_4$, and the solvent was evaporated off *in vacuo*. The oily residue was purified by column chromatography on silica gel with hexane:acetone. Yields for remaining fraction of substrates and products are listed below. For configuration and enantiomeric purity analysis of pruducts, see Table 4.

Method **H-1**: (*R*)-**1b**: 40, (*S*)-**2b**+(*S*)-**3b**: 36; (*R*)-**1d**: 45, (*S*)-**2d**+(*S*)-**3d**: 42; Method **H-2**: (*S*)-**2b**: 45, (*R*)-**4b**: 46; (*S*)-**2d**: 64, (*R*)-**4d**: 14; Method **H-3**: (*S*)-**3b**: 15, (*R*)-**4b**: 49; (*S*)-**3d**: 24, (*R*)-**4d**: 21.

ACETYLATIONS OF RACEMIC 1,2-DIOLS *rac*-**1b,d** ( Method **A-1**), PRIMARY *rac*-**2b,d** (Method **A-2**), OR SECONDARY MONOACETATES (*rac*-**3a-g** (Method **A-3**) BY PfL

*General procedure*: Racemic diol *rac*-**4b,d**, (1.5 mmol; Method **A-1**) ), or primary *rac*-**2b,d** (Method **A-2**), or secondary monoacetates *rac*-**3a-g** (Method **A-3**), vinyl acetate (5 mmol) and PfL (5 mg) were stirred in the hexane-vinyl acetate 1:1 (2 ml) at room temperature. After reaching a reasonable conversion (for conversions and reaction times, see Tables 4 and 5) PfL enzyme was filtered off and solvent was evaporated. The further work up and analysis of products were carried out as described at the previous acylations. Yields for remaining fraction of substrates and products are listed below.

Method **A-1**: (*S*)-**4b**: 24, (*R*)-**2b**: 21; (*S*)-**4d**: 51, (*R*)-**2d**: 46; Method **A-2**: (*R*)-**2b**: 44, (*S*)-**1b**: 41; (*R*)-**2d**: 59, (*S*)-**1d**: 24; Method **A-3**: (*R*)-**3a**: 32, (*S*)-**1a**: 23; (*R*)-**3b**: 39, (*S*)-**1b**: 48; (*R*)-**3c**: 40, (*S*)-**1c**: 27; (*R*)-**3d**: 41, (*S*)-**1d**: 37; (*R*)-**3e**: 43, (*S*)-**1e**: 39; (*R*)-**3f**: 38, (*S*)-**1f**: 34; (*R*)-**3g**: 51, (*S*)-**1g**: 46.

DETERMINATION OF THE ENANTIOMERIC COMPOSITION 1,2-DIOL DERIVATIVES

*General procedure*: *Step I: Hydrolysis of the acylated derivative* **1-3a-g**, **3bA-F**, **5*A-F** *to the corresponding 1,2-diols* (*R*)- or (*S*)-**4a-g**: A 10%(v/v) methanolic solution of the appropriate acylated derivative, containing catalytic amount of sodium methylate, was stirred at room temperature overnight. After evaporating off the methanol from the mixture, residue was purifiad by chromatography on a small silica gel column with hexane-acetone yielding (yield was usually over 90 %) pure 1,2-diol [(*R*)- or (*S*)-**4a-g**. For optical rotation values of the optically active 1,2-diols (*R*)- or (*S*)-**4a-g**, see Tables 1-5.

*Step II: Preparation of bis-(S)-MTPA esters of 1,2-diols* **4a-g**: (*R*)-MTPA-Cl (125 μmol), pyridine (150 μmol), and the corresponding 1,2-diol (*R*)- or (*S*)-**4a-g**, (50 μmol) were mixed in $CCl_4$ (1ml) in an ampoule. The ampoule was sealed and kept at 45°C for 1 hours. After cooling to RT, the mixture was diluted with diethyl ether (5 ml) and washed with 5% hydrochloric acid (1 ml), saturated $NaHCO_3$ solution (1 ml), and brine (1 ml). After drying ($Na_2SO_4$) and removing the solvents, the remaining oil was analyzed by $^1$H-NMR. The characteristic signals of the O-$CH_2$- moieties of the bis-MTPA esters (given below) were used for

determination of diastereomeric-composition (reflecting to the enantiomeric composition of the parent diol). For absolute configuration determination of 1,2-diols **4a-g**, see note [a] in Table 5.
Characteristic $^1$H-NMR signals ($CDCl_3$, δ, ppm): (*R*)-**4a**, bis-MTPA ester: 4.54 (dd, 1H), 4.57 (dd, 1H); (*S*)-**4a**, bis-MTPA ester: 4.61 (dd, 1H), 4.64 (dd, 1H); (*R*)-**4b**, bis-MTPA ester: 4.54 (dd, 1H), 4.57 (dd, 1H); (*S*)-**4b**, bis-MTPA ester: 4.61 (dd, 1H), 4.64 (dd, 1H); (*R*)-**4c**, bis-MTPA ester: 4.54 (dd, 1H), 4.57 (dd, 1H); (*S*)-**4c**, bis-MTPA ester: 4.61 (dd, 1H), 4.64 (dd, 1H); (*R*)-**4d**, bis-MTPA ester: 4.54 (dd, 1H), 4.57 (dd, 1H); (*S*)-**4d**, bis-MTPA ester: 4.61 (dd, 1H), 4.64 (dd, 1H); (*R*)-**4e**, bis-MTPA ester: 4.51 (dd, 1H), 4.54 (dd, 1H); (*S*)-**4e**, bis-MTPA ester: 4.55 (dd, 1H), 4.58 (dd, 1H); (*R*)-**4f**, bis-MTPA ester: 4.55 (dd, 1H), 4.58 (dd, 1H); (*S*)-**4f**, bis-MTPA ester: 4.63 (dd, 1H), 4.66 (dd, 1H); (*R*)-**4g**, bis-MTPA ester: 4.56 (dd, 1H), 4.59 (dd, 1H); (*S*)-**4g**, bis-MTPA ester: 4.63 (dd, 1H), 4.66 (dd, 1H).

ACKNOWLEDGEMENT. We thank the Hungarian OTKA Foundation (projects No. 1759 and No. 14894) for financial support. Contribution of Zsuzsanna Hencz to preparation of 1-benzyloxy-2-alkanols is also gratefully acknowledged.

**REFERENCES AND NOTES**

1. Rossi. R., *Synthesis* **1978**, 413.
2. Poppe, L., Novák, L., *Selective Biocatalysis: A Synthetic Approach*, Verlag Chemie, Weinheim, 1992.
3. Theil, F., *Catalysis Today* **1994**, *22*, 517.
4. Cambou, B., Klibanov, A. M., *J. Am. Chem. Soc.* **1984**, *106*, 2687.
5. Cesti, P., Zaks, A., Klibanov, A. M., *Appl. Biochem. Biotechnol.* **1985**, *11*, 401.
6. Ramaswamy, S., Morgan, B., Oehlschlager, A. C., *Tetrahedron Lett.* **1990**, *31*, 3405.
7. Janssen, A. J. M., Klunder, A. J. H., Zwanenburg, B., *Tetrahedron* **1991**, *47*, 7409.
8. Theil, F., Ballschuh, S., Kunath, A., Shick, H., *Tetrahedron: Asymmetry* **1991**, *2*, 1301.
9. Theil, F., Weidner, J., Ballschuh, S., Kunath, A., Shick, H., *Tetrahedron Lett.* **1993**, *34*, 305.
10. Mbappé, M. A., Sicsic, S., *Tetrahedron: Asymmetry* **1993**, *4*, 1035.
11. Hérradón, B., Cueto, S., Morcuende, A., Valverde, S., *Tetrahedron: Asymmetry* **1993**, *4*, 845.
12. Theil, F., Weidner, J., Ballschuh, S., Kunath, A., Shick, H., *J. Org. Chem.* **1994**, *59*, 388.
13. Theil, F., Lemke, K., Ballschuh, S., Kunath, A., Shick, H., *Tetrahedron: Asymmetry* **1995**, *6*, 1323.
14. Iriuchijima, S., Kojima, N., *Agric. Biol. Chem.* **1982**, *46*, 1153.
15. Poppe, L., Novák, L., Kajtár-Peredy, M., Szántay, Cs., *Tetrahedron: Asymmetry* **1993**, *4*, 2211.
16. Bianchi, D., Bosetti, A., Cesti, P., Golini, P., *Tetrahedron Lett.* **1992**, *33*, 3231.
17. Poppe, L., Recseg, K., Novák, L., *Synth. Commun.*, **1995**, *25*, 3993.
18. Rakels, J. L. L., Straathof, A. J. J., Heijnen, J. J., *Enzyme Microb. Technol.* **1993**, *15*, 1051.
19. The primary (**2a-g**) and secondary monoacetates (**3a-g**) were cleanly distinguishable by TLC and separable by LC.
20. Ronchetti, F.: personal communication and poster, BIOTRANS '95, 4-8. Sept. 1995, Warwick, England.
21. Hasegawa, J., Ogura, M., Tsuda, S., Maemoto, S., Kutsuki, H., Ohashi, T., *Agric.Biol.Chem.* **1990**, *54*, 1819.
22. Kometani, T., Morita, Y., Furui, H., Yoshii, H., Matsuno, R., *Chem.Lett.*, **1993**, 2123.
23. Chattopadhyay, S., Mamdapur, V. R., Chadha, M. S., *Bull.Soc.Chim.Fr.*, **1990**, 108.
24. Köppenhoefer, B., Trettin, U., Figura, R., Lin, B., *Tetrahedron Lett.*, **1989**, *30*, 5109.
25. Bergstein, W., Kleemann, A., Martens, J., *Synthesis*, **1981**, 76.
26. Ferraboschi, P., Grisenti, P., Manzocchi, A., Santaniello, E., *Tetrahedron*, **1994**, *50*, 10539.
27. Poppe, L., Novák, L., *Magy. Kém. Lapja* **1985**, *40*, 366.

(*Received in UK* 28 *February* 1996)

# V. melléklet

**Poppe, L.**, Novák, L., Kolonits, P., Bata, Á., Szántay, Cs.:

Convenient Synthetic Route to (+)-Faranal and (+)-13-Norfaranal; The Trail Pheromone of Pharaoh's Ant and Its Congener,

*Tetrahedron*, **1988**, *44*, 1477.

*Tetrahedron* Vol. 44, No. 5, pp. 1477 to 1487, 1988
Printed in Great Britain.

0040–4020/88 $3.00+.00


# CONVENIENT SYNTHETIC ROUTE TO (+)-FARANAL AND (+)-13-NORFARANAL; THE TRAIL PHEROMONE OF PHARAOH'S ANT AND ITS CONGENER [1]

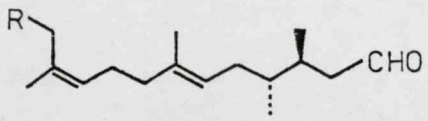
László Poppe [a], Lajos Novák [b], Pál Kolonits [b], Árpád Bata [c] and Csaba Szántay [a,b] *

(*Received in UK* 1 *December* 1987)

[a] Central Research Institute of Chemistry, 1525 Budapest, P. O. box 17., Hungary
[b] Institute for Organic Chemistry, Technical University, 1521 Budapest, Gellért tér 4., Hungary
[c] Department of Biochemistry and Food Technology, Technical University, 1521 Budapest, P. O. box 91., Hungary

Abstract: (+)-Faranal 1a, the trail pheromone of Pharaoh's ant, and its congener, (+)-13-norfaranal 1b were synthetized from chiral building block 4 employing diastereoselective carbon-carbon bond formation. The application of crude pig liver esterase enzyme for the preparation of 4 is also discussed.

(+)-Faranal is the most attractive component of the trail pheromone produced by Pharaoh's ant (Monomorium pharaonis, L.), which is a serious houshold pest in most of the world. This compound has a very high behavioural efficiency and the detection threshold is about 1 pg $cm^{-1}$ of a trail. The structure of (+)-faranal was assigned to be (3S,4R,6E,10Z)-3,4,7,11-tetramethyl-6,10-tridecadienal 1a [2-4].




1a: R= Me
1b: R= H

All four optical isomers of faranal have some biological activity. However, racemic faranal has only one tenth of the trail pheromone activity of the natural product. Surprisingly, the 3-epimer (3R,4R-faranal) was also weakly active and does not interfere with the activity of natural product, since ants follow a trail made of a 1:1 mixture of stereoisomeric compounds. Furthermore, structure modification study showed that among structurally related compounds a 40 : 60 mixture of (3S,4R)- and (3R,4R)-13-norfaranal (1b and its 3-epimer) had also the ability to release trail-following activity [4,5].

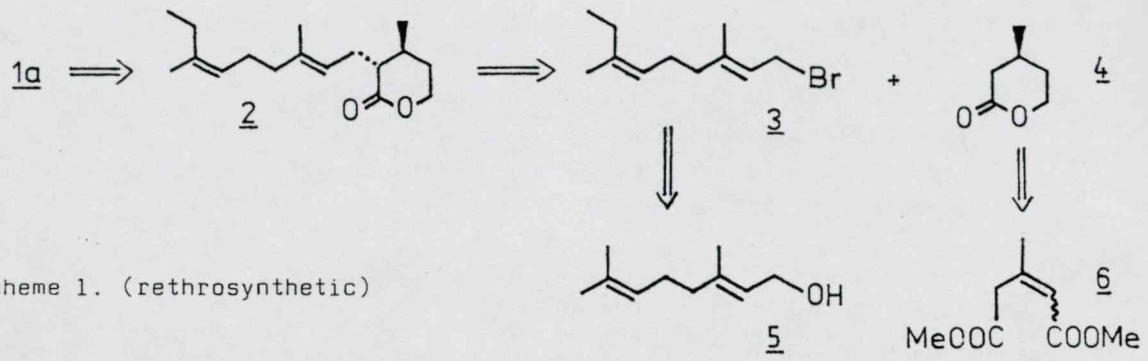
(+)-Faranal has been an attractive synthetic target of considerable current interest because of its challenging structural features and extremely high level of biological activity. Four different synthetic approaches to faranal have been reported. In cooperation with Japanese group, Ritter et al. elaborated the first synthesis of faranal [4]. Actually, this small scale bioorganic synthesis leading to a 40 : 60 mixture of (+)-faranal and its (3R)-epimer, established its absolute stereochemistry.

Mori and Ueda have confirmed the structural assignments of (+)-faranal by synthetising both enantiomers. Their attractive linear approach is rather lenghty and required the chemical resolution of an intermediate [6,7].

Recently, two convergent approaches for the synthesis of racemic faranal

were reported. Knight and Ojhara assembled the sesquiterpenoid skeleton of faranal by Wittig condensation, which yielded an approximately 1 : 1 mixture of (E)- and (Z)-isomers. Racemic faranal and its (6Z)-isomer were then separated by preparative scale g.l.c. [8,9]. In an alternative synthesis of racemic faranal, Baker's group employed the addition of an alkylcopper complex to terminal acetylene for the stereoselective construction of the 6E double bond [10,11].

Recent interest in the use of trail pheromone to increase the rate of toxic bait pick-up has led us to develop new synthetic method for the preparation of enantiomerically pure (+)-faranal and (+)-13-norfaranal (1a and 1b, respectively). Our approach is strategically quite different from the existing ones in the construction of the skeleton of faranal (Scheme 1.). Namely, we formed the

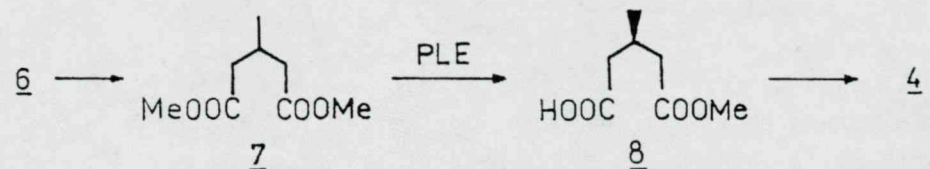



Scheme 1. (rethrosynthetic)

4,5-bond of the molecule stereoselectively by electrophilic enolate alkylation of an appropriately functionalized chiral building block (4) with (Z)-homogeranyl bromide (3).These key intermediates can be easily prepared from geraniol (5) and glutaconic ester (6), respectively.

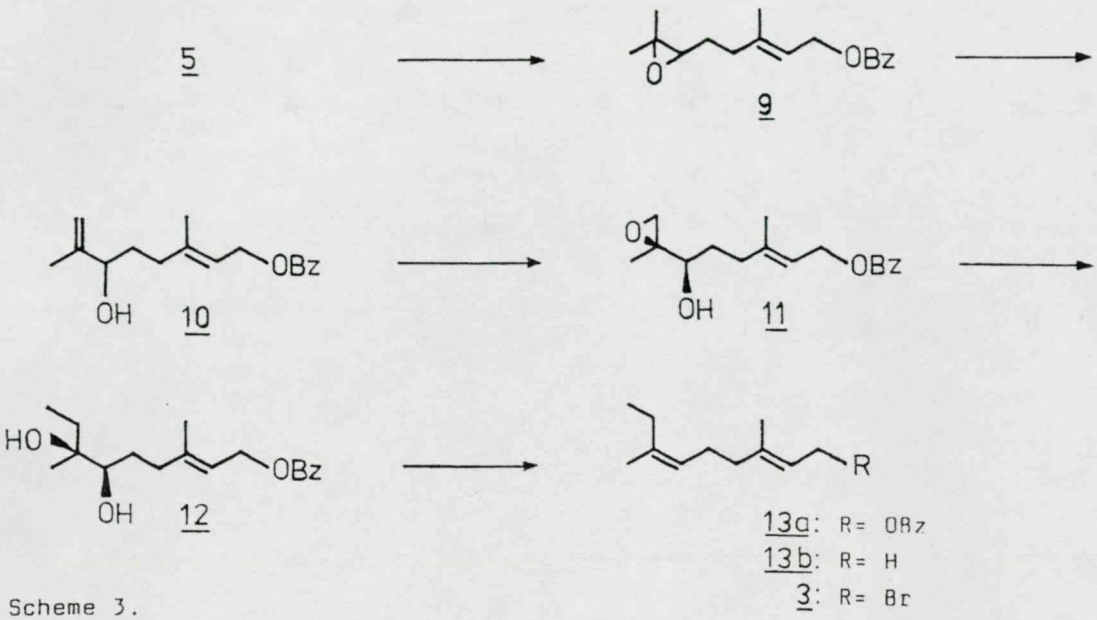
(3S)-Methyl valerolactone (4), our chiral key intermediate, was prepared from dimethyl 3-methylglutarate (7) obtained by catalytic hydrogenation of glutaconic ester (6) [12] (Scheme 2.).

Scheme 2:




Recently, this compound was enantiotopically-selectively hydrolysed with pig liver esterase (PLE) enzyme system [13-16]. The enzyme attacked preferentially at the pro-(S)-methoxycarbonyl group and (R)-monoester (8) was isolated in above 80 % yield and in 80 - 90 % optical purity. However, the commercially available pure pig liver esterase is rather expensive. Therefore, we tried to perform the selective hydrolysis of diester (7) with crude enzyme. Thus, molar amount of diester (7) was suspended in 0.1 M phosphate buffer (pH 7) extract of pig liver acetone powder [17]. The pH value of the resulting mixture was kept within 6.9 - 7.1 range by continuous addition of 10 % sodium hyroxide solution. After consumption of one equivalent of base (approximately 5 h), the mixture was worked up to yield the (R)-enantiomer of monoester (7) in 72 % yield and in 85 % optical purity. Since the optical purity was not satisfactory, the crystalline l-cinchonidine salt of monoester (8) was formed and recrystallized from water-acetone. Acidification of this salt yielded optically pure (R)-monoester (8, e.e.>96 %), which was selectively reduced with sodium in $NH_3$-EtOH [18] or $LiBH_4$ in THF [13] to give (3S)-methyl valerolactone (4).

(Z)-Homogeranyl bromide (3), another key intermediate, was prepared from the readily available geraniol (5) by the combination of known methods with some modification (Scheme 3.).

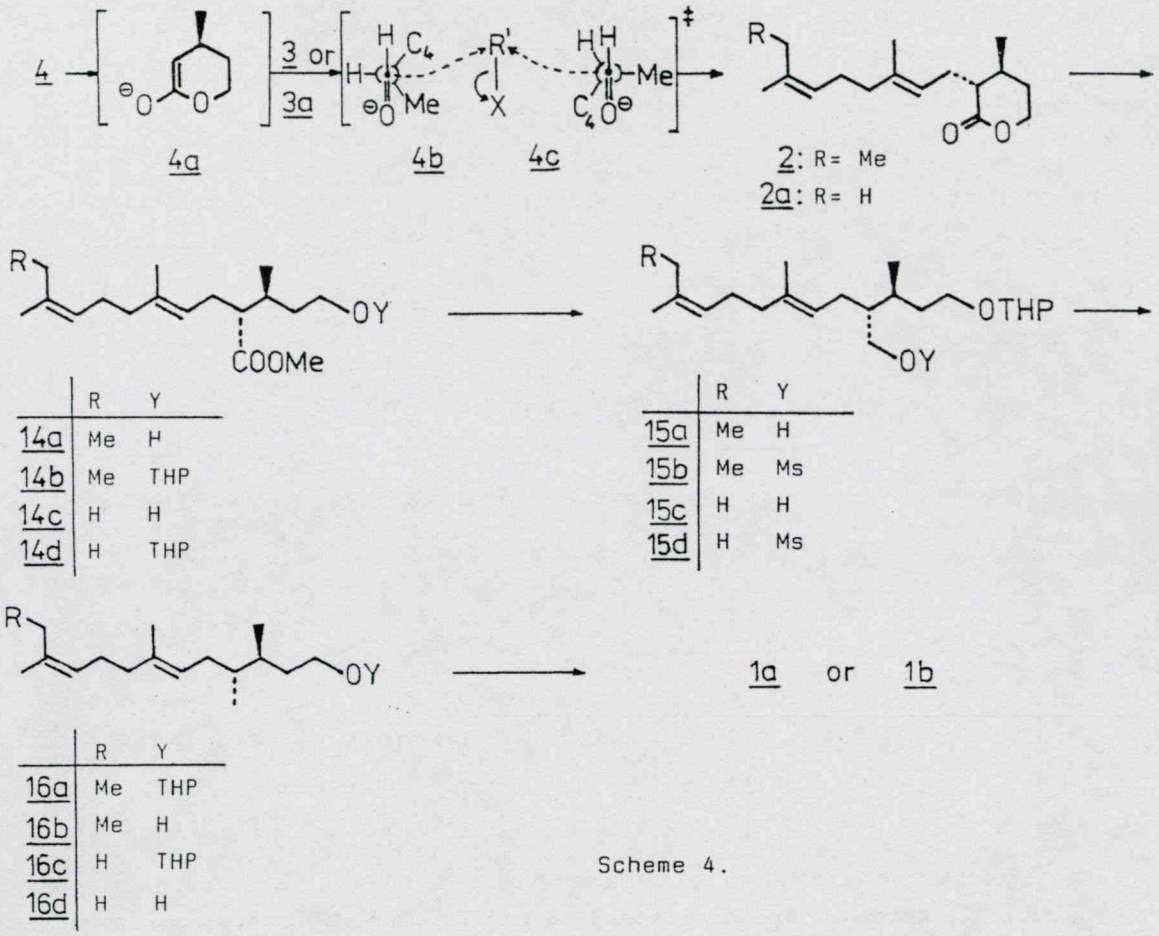



Scheme 3.

Thus, reaction of geraniol with sodium hydride and benzyl bromide gave geranyl benzyl ether [19], which was generally not purified, but directly treated with 3-chloroperoxybenzoic acid to produce epoxy compound (9). A number of reagents (e.g. sodium glass [20], lithium perchlorate [21], lithium diethylamide [22], aluminium isopropoxide [23]) were then examined for the conversion of epoxide (9) to allylic alcohol (10). In our hands, aluminium isopropoxide proved to be superior to the other reagents. The reaction was performed in refluxing toluene and afforded the desired product (10) in good yield. Catalytic epoxidation of alcohol (10) with t-butyl hydroperoxide in the presence of catalytic amount of $VO(acac)_2$ in benzene afforded epoxy alcohol (11), which was then converted into (Z)-homogeranyl benzyl ether (13a) by the sequence involving oxirane reaction with lithium dimethyl cuprate and elimination of the hydroxy groups of the resulting diol (12) by treatment with dimethylformamide dimethyl acetale and acetic anhydride [24]. HPLC analysis showed that the product (13a) was contaminated by less than 8 % of (E)-isomer , which was removed after deprotection by treatment with lithium in liquid ammonia by column chromatography. The resultant (Z)-homogeraniol (13b) was then converted to diastereomerically pure (Z)-homogeranyl bromide (3) by treatment with phosphorous tribromide, in 24 % overall yield (based on geraniol).

Next task was to connect the two building blocks (3 and 4). For this, we plan a diastereoselective carbon-carbon bond formation by an electrophilic enolate alkylation (Scheme 4.).

Enolate (4a) generated from the lactone (4) seemed particularly attractive for this purpose by virtue of its rigidity and conformationally enforced proximity of its reacting center ($C_2$). Furthermore, in the transition state of alkylation only one of the possible conformers (4c, methyl group in perpendicular position ) is stabilized by hyperconjugative interaction. Here, the incoming electrophile preferentially attacks on the opposite side of the plan and renders the (R)-configuration at the newly created chiral center (Scheme 4.).




| | R | Y |
|---|---|---|
| 14a | Me | H |
| 14b | Me | THP |
| 14c | H | H |
| 14d | H | THP |

| | R | Y |
|---|---|---|
| 15a | Me | H |
| 15b | Me | Ms |
| 15c | H | H |
| 15d | H | Ms |

| | R | Y |
|---|---|---|
| 16a | Me | THP |
| 16b | Me | H |
| 16c | H | THP |
| 16d | H | H |

Scheme 4.

Coupling reaction between enolate (4a) generated from the lactone (4) with lithium diethylamide, and (Z)-homogeranyl bromide (3) proceeded as expected to give predominantly the desired anti-isomer (2R,3S)-2, together with a small amount (less than 6 %) of syn-isomer (2S,3S). This high anti stereofacial selectivity was evidenced by the trans relationship found between the substituents of lactone moiety in the product (2), as deduced from the coupling constant value $J_{2,3}$ in NMR spectra, that requires trans-arrangement for this substituent.

Trivial reactions were then used to convert lactone (2) into (+)-faranal (1a). Thus, transesterification of 2 with MeOH and $Et_3N$ led to a hydroxy ester (14a) which was converted to protected ester (14b) by treatment with dihydropyran in the presence of pyridinium p-toluenesulfonate. Reduction of 14b with excess lithium aluminium hidride gave the alcohol (15a) which was converted to the corresponding mesylate (15b) by mesyl chloride in the presence of triethylamine. The mesylate (15b) was reduced with lithium aluminium hidride in refluxing THF and then the protecting group of the resulting ether (16a) was removed by acid catalyzed hydrolysis. Finally, oxidation of the resulting alcohol (16b) with pyridinium dichromate afforded (+)-faranal (1a) in 1.9 % and 3.5 % overall yield (based on 5 and 6, respectively).

Chiral lactone (4) also served as the key intermediate in the first stereocontrolled synthesis of (+)-13-norfaranal (1b)(Scheme 4.). Here, in the electrophilic ester enolate alkylation we used geranyl bromide (3a) and isolated the desired anti-isomer 2b, together with a small amount of syn-isomer (6 %). This was converted to stereoisomerically 94 % pure (+)-13-norfaranal (1b) by the method described above for (+)-faranal, via intermediates 14c, 14d, 15c, 15d, 16c an 16d in 19.4 % overall yield from lactone 4.

## EXPERIMENTAL

IR spectra were obtained with a Specord IR-75 (Carl Zeiss, Jena) spectrophotometer. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL FX-100 FT-NMR instrument at 100 and 25 MHz, respectively, and are reported in ppm downfield from internal TMS. Mass spectra were taken on a JEOL-20K and a JMS-01SG-2 combined GC-MS system at 75 eV ionizing energy. HPLC measurements were carried out on a Du Pont 830 instrument. For the capillary GLC analyses a Packard 428 instrument equipped with FID was used. Thin layer chromatography was carried out using Kieselgel 60 $F_{254}$ on alumina plates (E. Merck Co,FRG) and hexane-acetone=5:0.2(A), hexane-acetone=10:1(B), hexane-acetone=5:2(C) or hexane-ethyl acetate=2:1(D) eluant systems. Spots were visualised by immersing the plates into 5 % ethanolic solution of phosphomolibdenic acid and then heating. All solvents used were freshly distilled and anhydrous, and operations with alkyl lithiums, cuprates, $LiBH_4$ and $LiAlH_4$ were carried out under dry argon atmosphere.

Dimethyl 3-methylglutarate (7)

Dimethyl glutaconate (6,435 g, 2.5 moles) was catalytically hydrogenated by 10 % palladium on active carbon under 4 atm hydrogene pressure at 65°C. After consumption of the calculated 61 l (2.5 moles) hydrogene the catalyst was filtered off and the product was distilled in vacuo to yield 7 (349 g, 91 %) as a colorless oil Bp.: 115°C(0.5 torr); TLC(B):Rf=0.29; IR(film), $\nu_{max}$: 2900, 1725, 1440, 1370, 1260, 1190, 1150, 1080, 1010 $cm^{-1}$; $^{1}$H-NMR($CCl_4$, $\delta$): 1.01 (d,J=5Hz, 3H,-$CH_3$), 2.26 (m,5H, 2-$CH_2$- and 1-CH=), 3.55 (s,6H, 2 $COOCH_3$); MS m/e: 174(2)[$M^+$], 143(100), 142(51), 115(51), 114(92), 101(94), 83(20), 82(28), 73(60), 69(91), 59(75), 55(60), 43(61), 42(38), 41(65), 39(40).

(3R)-Hydrogen-methyl 3-methylglutarate (8)

Pig liver acetone powder[17] (100 g) was homogenized with 0.1 M phosphate buffer (pH=8,1200 mL) and then centrifuged at 3000 g at room temperature for five minutes. To this obtained supernatant (pH value changed to about 7.0 during the extraction process) having 31 U/mL enzyme activity (measured on ethyl butyrate as a substrate at 25°C, pH=8) compound 7 (314 g, 1.8 moles) was added and the pH value of the resulted well stirred emulsion was kept within 6.9-7.1 range by continous addition of 10 % (2.78 M) sodium hydroxide solution. After consumption of 1 equivalent of base (645 mL) the mixture was acidified to pH=2 by the addition of concentrated hydrochloric acid. To the emulsion sodium chloride (150 g) was added and the resulting mixture was centrifuged at 3000 g for five minutes. The precipitated part was washed with ethyl acetate (400 mL) and the supernatant was extracted three times with ethyl acetate (800 mL, each). The combined ethyl acetate solutions were washed with brine (200 mL) and dried over $MgSO_4$. Evaporation of the solvent in vacuo gave crude 8, which was further purified by vacuum-distillation to yield pure 8(213 g, 74 %) as a colorless oil. Bp.: 106-107°C (0.05 torr); $[\alpha]_D^{22}$=0.49°(neat). Lit.[28]: $[\alpha]_D^{22}$=+0.58°(neat, 100 % ee).
Recrystallization of this product (8,12 g, 75 mmol) with l-cinchonidine (22.2 g, 75 mmol) from water (210 mL) containing acetone (60 mL) resulted chrystalline salt of 8 as white needles. Chrystals were then solved in 1M hydrochloric acid (60 mL) and extracted three times with ether (30 mL, each). The combined ethereal solutions were then washed with brine (15 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo optically pure 8 (7.7 g, 64 %) was obtained as a pale yellow oil. $[\alpha]_D^{22}$= 0.57°(neat); TLC (C): Rf= 0.35; IR(film), $\nu_{max}$: 3200, 2900, 1725, 1700, 1440, 1380, 1290, 1200, 1160, 1080, 1010 $cm^{-1}$; $^{1}$H-NMR ($CCl_4$,$\delta$): 1.08 (d,J=5,5Hz, 3H,-$CH_3$), 2.31 (m, 5H,2-$CH_2$- and 1-CH=), 3.62 (s,3H,$COOCH_3$), 11.3 (br s, 1H,COOH); MS m/e: 160(1)[$M^+$], 143(11), 142(25), 129(65), 128(27), 114(66), 101(66), 100(50), 87(18), 83(16), 82(21), 74(67), 72(21), 69(78), 60(13), 59(100), 56(12), 55(42), 45(13), 44(77), 43(33), 42(46), 39(27).

(-)-(3S)-Methylvalerolactone (4)

a) To a stirred and boiling solution of distilled ammonia (700 mL) containing dry ethanol (60 mL) and 8 (32 g, 700 mmol) sodium pieces were added portionwise. After complete sodium addition the mixture was boiled and stirred for 1 h and then solid ammonium chloride was added until the disappearance of the deep blue color of the mixture. Ammonia was evaporated and the residue was dissolved in water (400 mL). The aqueous solution was acidified to pH 2 at 0°C with 50 % $H_2SO_4$ and then extracted three times with ethyl acetate (400 mL, each). Ethyl acetate solutions were combined and washed with brine (80 mL), dried over $MgSO_4$. After evaporation of the solvent in vacuo the residue was vacuum-chromatographed (VLC)[27] (on 200 g of 63-100 μm Kieselgel 60 with hexane-acetone= 10:1 eluant) to yield 4 (12.8 g, 56 %).
Product can only be stored without decomposition at 4°C in diluted aprotic (e.g.ethereal) solution for a longer period.

b) To an ice cooled and stirred solution of 8 (101 g, 0.63 mol) in methanol (500 mL) LiOH $H_2O$

(26.3 g, 0.63 mol) was added portionwise maintaining the inner temperature under 15°C. After complete dissolution of the LiOH $H_2O$ methanol and water was evaporated from the resulted salty product in vacuo to yield dry lithium salt of 8 (100.4 g, 95 %). 80 g (0.48 mol) of this salt was suspended in dry THF (800 mL) under dry argon atmosphere and then $LiBH_4$ (16.5 g, 0.76 mol) was added to the vigorously stirred suspension. The resulted mixture was refluxed for 1 h and after cooling to room temperature poured into ice-water (500 mL), which was then acidified to pH=2 by dropwise addition of concentrated hydrochloric acid. The acidified aqueous mixture was extracted four times with ethyl acetate (400 mL, each) and then the combined organic layers were washed with brine (150 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo and VLC purification (as above) pure 4 (29.9 g, 55 %) was obtained.

$[\alpha]_D^{23}$=-23.5° (c=5.21, $CHCl_3$, from unrecrystallized 8) and $[\alpha]_D^{23}$=-26.9°(c=4.98, $CHCl_3$, from recrytallized 8), Lit.[28]: $[\alpha]_D^{22}$=+27.6° (c=5.72, $CHCl_3$, 3R-enantiomer, 100 % ee); TLC(C):Rf=0.39; IR (film), $\nu_{max}$: 2900, 1720, 1460, 1440, 1400, 1370, 1310, 1280, 1240, 1220, 1160, 1080, 1060, 1000, 920 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 1.09 (d, J=6.5Hz,-$CH_3$), 1.4-2.3 (br m, 5H, 2-$CH_2$- and 1-CH=), 4.0-4.5 (m,2H,-$CH_2$-O); MS m/e: 115(4)[M+1]$^+$, 114(21)[M$^+$],85(1),84(2),71(3),70(23), 55(100),42(83),41(54),39(40).

(2E)-1-Benzyloxy-6,7-epoxy-3,7-dimethyl-2-octene (9)

To an ice-cooled and stirred solution of geranyl benzyl ether[23] (30 g, 0.122 mol) in dry dichloromethane (300 mL) 3-chloro-peroxybenzoic acid (31.5 g, 0.128 mmol, 75 % content ) was added portionwise maintaining the inner temperature under 5°C. After complete addition the resulted mixture was stirred at 0°C for 1 h and then the precipitated white solid was filtered off. Filtrate was washed two times with 10 % sodium hydroxide solution (80 mL, each) and then brine (50 mL). After evaporation of the solvent VLC of the residue (on 300 g of silica gel with hexane-acetone=5:0.1 eluant) resulted 9 (21.9, 69 %) as an oil. TLC (B):Rf=0.36; IR(film), $\nu_{max}$: 2900, 2840, 1660, 1445, 1370, 1240, 1190, 1100, 1080, 1060, 1020, 725, 690 $cm^{-1}$; $^1$H-NMR ($CCl_4$, $\delta$): 1.18 (m, 6H, 2-$CH_3$), 1.4-1.8 (m, 5H,-$CH_2$- and -$CH_3$), 2.12 (mc, 2H,-$CH_2$-), 2.49 (t, J=6Hz, 1H,$C_6$ -CH=), 3.90 (d, J=7Hz, 2H, -$CH_2$-OBz), 4.38 (s, 2H, O-$CH_2$-Ph), 5.33 (t, J=7Hz, 1H,-CH=C), 7.19 (m,5H,Ar-H);MS m/e: 210(1)[M$^+$], 174(6), 154(4), 123(7),107(7), 91(100), 85(13), 71(19), 59(20), 43(7).

(2E)-1-Benzyloxy-6-hydroxy-3,7-dimethyl-2,7-octadiene (10)

A solution of 9 (15.6 g, 60 mmol) and aluminium isopropoxide (12.3 g, 60 mmol) in dry toluene (100 mL) was refluxed and stirred for 8 h. After cooling to room temperature hexane (100 mL) was added to the resulting mixture and then the organic solution was washed with 2M hydrochloric acid (100 mL), saturated $NaHCO_3$ solution (40 mL) and brine (40 mL). The organic layer was then dried over $MgSO_4$ and the solvent was removed in vacuo to give 10 (14.6 g, 93 %) as a pale yellow oil. TLC(C): Rf=0.47; IR (film) $\nu_{max}$: 3400, 2900, 2850, 1670, 1500, 1460, 1380, 1075, 1040, 900, 740, 700 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 1.66 (s, 3H,-$CH_3$), 1.71 (s, 3H,-$CH_3$), 1.95 (mc, 4H, 2-$CH_2$-), 3.57 (m, 1H,=CH-O), 3.99 (d, J=6.5 Hz, 2H,-$CH_2$-OBz), 4.47 (s, 2H, O-$CH_2$-Ph), 4.80 and 4.92 (m,m,2H, C=$CH_2$), 5.38 (t, J=6,5Hz,1H,-CH=C), 7.27 (br s, 5H, Ar-H); MS m/e: 260(1)[M$^+$], 242(1), 174(3), 169(4), 151(13),125(7), 123(10), 109(8), 107(18), 97(6), 95(8), 93(10), 92(17), 91(100), 82(12), 81(19), 71(16), 69(15), 68(11), 67(15), 55(12), 44(22), 42(19).

(2E)-1-Benzyloxy-7,8-epoxy-6-hydroxy-3,7-dimethyl-2-octene (11)

To a solution of 10 (10.4 g, 40 mmol) in dry benzene (100 mL) 20 mg of $VO(acac)_2$ catalyst was added and then t-BuOOH (8.0 g, 90 % content, 80 mmol) was dropped to the resulting mixture over a period of 15 min. After stirring at room temperature for 2 h, additional $VO(acac)_2$ catalyst (20 mg) was added to the reaction mixture and stirring was continued for another 2h. The obtained mixture was then diluted with hexane (100 mL) and washed with 10 % $Na_2CO_3$ solution (30 mL) and brine(30 mL). After drying ($MgSO_4$) and evaporation in vacuo the resulting residue was purified by VLC(on 200 g of 63-200 μm silica gel with hexane-acetone=10:1 eluant) to yield pure 11 (8.6 g, 78 %) as an oil. TLC(C):Rf=0.38; IR (film), $\nu_{max}$: 3350, 2900, 2830, 1660, 1500, 1450, 1380, 1360, 1190, 1060, 1020, 940, 900, 740, 700 $cm^{-1}$; $^1$H-NMR ($CCl_4$, $\delta$): 1.30 (s,3H,-$CH_3$), 1.50 (m,2H,-$CH_2$-), 1.66(s,3H,-$CH_3$), 2.22 (m,2H,-$CH_2$-), 2.61 (mc,2H, $C_8$-$CH_2$-), 3.48 (m,1H,OH), 3.96 (d,J=6.5Hz,2H,-$CH_2$-OBz), 4.36(s,2H,O-$CH_2$-Ph), 5.39 (t,J=6.5Hz, 1H,-CH=C), 7.29 (br s,5H,Ar-H); MS m/e: 276(< 1)[M$^+$], 174(2), 170(2), 169(3), 167(3), 153(3), 141(3), 123(4), 121(4), 111(9), 97(9), 91(100), 87(10), 81(26), 43(31), 41(25).

(2E,6,7-anti)-1-Benzyloxy-6,7-dihydroxy-3,7-dimethyl-2-octene (12)

To a suspension of copper(I) iodide (9.4 g, 48.4 mmol) in dry ether (100 mL) 1.38 M ethereal methyl lithium solution (70 mL, 97 mmol) was added below -20°C under dry argon atmosphere. After stirring at -20°C for 10 minutes the mixture became homogenous and then 11 (6.67 g, 24.2 mmol) in dry ether (30 mL) was added to this solution at -20°C. The resulting mixture was stirred at 0°C for 4 h and then was quenched by addition of 10 % $NH_4Cl$ solution (30 mL). The ethereal layer was washed with two additional portion of 10 % $NH_4Cl$ solution (30 mL,each) and brine (30 mL) and dried over $MgSO_4$. The VLC (100 g of 63-200 μm silica gel , hexane-acetone=10:1 eluant) of the residue of the evaporation in vacuo gave 12 (4.45 g, 63 %). TLC(C):Rf=0.34; IR (film), $\nu_{max}$: 3350, 2900, 2840, 1660, 1450, 1380,

1060, 1000, 730, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 0.91 (t, J=6,5Hz, 3H,-$CH_3$), 1.12 (s, 3H,-$CH_3$), 1.45 (mc, 4H,2-$CH_2$-), 1.65 (s, 3H,-$CH_3$), 2.12 (t, J=6Hz, 2H,-$CH_2$-), 2.36 (br s, 2H,2 OH), 3.35 (m, 1H,=CH -O), 4.01 (d, J=7Hz, 2H,-$CH_2$-OBz), 4.47 (s, 2H, O-$CH_2$-Ph), 5.42 (t, J=7Hz, 1H,-CH=C), 7.28 (br s, 5H, Ar-H); MS m/e: 292(< 1)[$M^+$], 274(3), 219(5), 183(4), 166(3), 163(3), 155(5), 137(5), 112(8), 111(14), 91(100), 81(20), 73(58), 68(30), 57(22), 43(22), 41(15).

(2E,6Z)-1-Benzyloxy-3,7-dimethyl-2,6-nonadiene (13a; Z-homogeranyl benzyl ether)

A solution of 12 (9.8 g, 29.8 mmol) in N,N-dimethylformamide dimethyl acetale (30 mL) was stirred overnight at room temperature and then evaporated in vacuo. To the residue acetic anhydride (30 mL) was added and the solution was boiled and stirred for 8 h. After cooling to room temperature the mixture was diluted with hexane (250 mL) and washed with water (50 mL), two times with 10 % sodium hydroxide solution and brine (50 mL). After drying over $MgSO_4$ the solvent was removed in vacuo and the residue was purified by VLC (100 g silica gel, hexane-acetone=5:0.1 eluant) to yield 13a (5.05 g, 65 %) as an oil. TLC (A):Rf=0.62; IR (film), $\nu_{max}$: 2970, 2930, 2850, 1660, 1450, 1370, 1230, 1100, 1060, 1020, 725, 690 $cm^{-1}$; $^1$H-NMR ($CCl_4$,$\delta$): 0.95 (t, J=6.5Hz, 3H,-$CH_3$), 1.65 (s, 3H,-$CH_3$), 1.72 (s, 3H,-$CH_3$), 2.10 (m, 4H, 2-$CH_2$-), 3.94 (d, J=6.5Hz, 2H,-$CH_2$OBz), 4.46 (s, 2H, O-$CH_2$-Ph),5.05 (m, 1H,-CH=C), 5.33 (t, J=6.5Hz, 1H,-CH=C), 7.29 (br s,5H,Ar-H); MS m/e: 258(11)[$M^+$], 229(2),176(6), 167(7), 150(25), 137(26), 121(17), 91(100), 83(66), 55(66), 41(29); HPLC: $t_R$= 147 s (main component, 95 %, (2E,6E)-isomer:$t_R$= 138 s, 5 %; 250x4.5 mm LiChrosorb RP-8 10 $\mu$m,1 ml/min acetonitrile eluant, $\lambda$ = 254 nm).

(2E,6Z)-3,7-Dimethyl-2,6-nonadien-1-ol (13b; Z-homogeraniol)

To a stirred boiling solution of $LiNH_2$[prepared from lithium (1.4 g, 167 mmol)] in dry ammonia (300 mL) was added a solution of 13a (4.3 g, 16.7 mmol) in dry n-hexane (30 mL). After stirring for 30 min, an excess of ammonium chloride was added, and the mixture was diluted with n-hexane (800 mL). The ammonia was evaporated, and water (150 mL) was added. The separated aqueous layer was extracted with hexane (200 mL) and the hexane layer was washed with saturated $NH_4Cl$ solution (100 mL), and dried over $MgSO_4$. After removing the solvent the residue was purified by low presure liquid chromatography (LPLC) (on 10-40 $\mu$m Kieselgel HR using hexane-acetone=10:1 as eluant) to afford 13b (2.37 g, 84 %). TLC(C):Rf=0.50; IR (film), $\nu_{max}$: 3350, 2980, 2940, 2880, 1660, 1450, 1380, 1000 $cm^{-1}$; $^1$H-NMR ($CCl_4$, $\delta$): 0.96 (t, J=6.5Hz, 3H,-$CH_3$), 1.64 (br s, 6H,2-$CH_3$), 2.01 (m, 4H,2-$CH_2$-), 2.03 (q, J=6.5Hz, 2H,-$CH_2$-), 3.76 (m,1H,OH), 4.03 (d, J=6.5Hz,2H,-$CH_2$-O), 5.07 (m, 1H,-CH=C), 5.37 (t,J=6.5Hz, 1H,-CH=C); MS m/e: 168(1)[$M^+$], 151(1), 150 (1), 137(3), 121(3), 111(2), 107(2), 93(7), 83(32), 67(17), 55(100), 53(18), 43(12), 41(81), 39(38).

(2E,6Z)-1-Bromo-3,7-dimethyl-2,6-nonadiene (3; Z-homogeranyl bromide)

To a stirred solution of 13b (1.68 g, 10 mmol) in dry ether (50 mL) there was added a solution of phosphorous tribromide (1.25 g, 4.5 mmol) in dry ether (5 mL) under dry argon atmosphere at -5°C in darkness. The resulting mixture was stirred at 0°C for 45 min, and then brine (20 mL) was added. After extraction of the aqueous layer with ether (20 mL), the combined ethereal solutions were washed with ice-cooled and saturated $NaHCO_3$ solution (10 mL) and brine (10 mL) and dried over $MgSO_4$. Evaporation of the solvent in vacuo at 0-5°C gave 3 (2.19 g, 95 %)as a pale yellow oil. The product is sensitive for light, heat, wet and was used up immediately for the next step. TLC(hexane): Rf=0.80; IR ($CCl_4$), $\nu_{max}$: 3030, 2980, 2945, 2880, 2870, 1650, 1450, 1380, 1200, 1105, 1060 $cm^{-1}$; $^1$H-NMR ($CCl_4$,$\delta$): 0.90 (t, J=6.5Hz, 3H,-$CH_3$), 1.60 (s, 3H,-$CH_3$), 1.66 (s,3H,-$CH_3$), 1.93 (q, J=6.5Hz, 2H,-$CH_2$-), 2.0 (m, 4H, 2-$CH_2$-), 3.86 (d, J=8.5Hz, 2H,-$CH_2$Br), 4.96 (m, 1H,-CH=C), 5.44 (t, J=8.5Hz, 1H,-CH=C); MS m/e: 232(3), 230(2),[$M^+$], 175(2), 171(2), 151(18), 123(2), 109(2), 95(11), 83(100), 81(17), 68(12), 67(11), 55(72), 41(23).

(2R,3S,2'E,6'Z)-2-(3',7-Dimethyl-2,6-nonadienyl)-3-methyl-5-pentanolide (2)

To a solution of lithium diethyl amide [prepared from diethyl amine (0.60 g, 8.2 mmol) and n-buthyl lithium (8.2 mmol, 1.1 M hexane solution)] in dry THF (20 mL) there was added a solution of 4 (0.94 g, 8.2 mmol, >95 % ee) in dry THF (5 mL) below -70°C, and the resulting mixture was stirred at -78°C for 1 h. Then 3 (1.89 g, 8.2 mmol) dissolved in dry THF (5 mL) and dry HMPA (0.8 mL) was added below -70°C. The resulting mixture was stirred at -78°C for 1 h and then stayed at -30°C overnight. The reaction was diluted with ether (50 mL) and quenched by 10 % hydrochloric acid (20 mL) and the acidic layer was extracted two times with ether (20 mL; each). The combined organic solutions were washed with saturated $NaHCO_3$ solution (10 mL) and brine (20 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the residual oil was chromatographed at low pressure (on 40-60 $\mu$m LiChroprep Si 60 with hexane-acetone=10:0.5 as eluant) to afford 2 (1.07 g, 48 %) as an oil. $[\alpha]^{25}_{546}$= -8.8°, $[\alpha]^{25}_{D}$= -6.8° (c=2.89, $CHCl_3$); TLC(C):Rf=0.58; IR (film), $\nu_{max}$: 2970, 2940, 2890, 1730, 1660, 1450, 1380, 1270, 1200, 1145, 1100, 1060 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 0.95 (t, J=7.5Hz, 3H,-$CH_3$), 1.08 (d, J=6Hz, 3H,-$CH_3$), 1.65 (br s, 6H,2-$CH_3$), 1.7-2.8 (br m, 10H,4-$CH_2$- and 2-CH=), 4.0-4.5 (m, 2H,-$CH_2$-O), 5.09 (m, 2H,2-CH=C); $^{13}$C-NMR ($CDCl_3$): 12.81(-$CH_3$), 16.26(-$CH_3$),20.68(-$CH_3$), 22.85(-$CH_3$), 24.81(-$CH_2$-), 26.15(-$CH_2$-), 27.79(-$CH_2$-), 29.89(-CH<) 30.98(-$CH_2$-), 40.16(-$CH_2$-), 48.32(-CH<), 67.48(O-$CH_2$-), 120.69(-CH=), 123.79(-CH=), 137.16(=C=), 137.72(=C=), 173.69(C=O);

MS m/e: 265(7)[M+1]$^+$, 264(27)[M$^+$], 207(15), 194(7), 183(27), 181(23), 150(18), 127(17), 123(17), 115(16), 114(100), 99(34), 95(21), 93(16), 83(26), 82(19), 81(23), 69(16), 55(75), 43(17), 41(62); GLC: $t_R$= 13,84 min (94 %, (2R,3S)-isomer; $t_R$= 13.67 min, 6 %, (2S, 3S)-isomer; 30 m x 0,25 mm SP-2100 glass capillary column, $t_k$= 160-260°C, 3°C/min, $N_2$).

(2R,3S,2'E)-2-(3,7-Dimethyl-2,6-octadienyl)-3-methyl-5-pentanolide (2a)

Enolate generated from 4(31 g, 0.272 mol, 95 % ee) by $LiNEt_2$ (0.272 mol) and geranyl bromide[25] (59.1 g, 0.272 mol) was coupled as describe for the preparation of 2 to give 2a (34.6 g, 51 %) as a pale yellow oil TLC (C):Rf = 0.57 ; $[\alpha]^{23}_{546}$= -8.6°, $[\alpha]^{23}_{D}$= -6.8° (c=3.35, $CHCl_3$); IR(film), $\nu_{max}$: 2970, 2930, 2880, 1730, 1660, 1450, 1380, 1265, 1140, 1100, 1070 cm$^{-1}$; $^1$H-NMR($COCl_3$, $\delta$); 1.09 (d, J=6Hz, 3H,-$CH_3$), 1.4-1.9 (br m,3H,-$CH_2$- and -CH=), 1.60 (s, 3H,-$CH_3$), 1.65 (s, 3H,-$CH_3$), 2.03 (mc, 6H, 3-$CH_2$-), 2.4 (m, 1H, CH-COO), 4.25 (mc, 2H,-$CH_2$-O), 5.09 (m, 2H,2-CH=C); $^{13}$C-NMR($CDCl_3$): 16.23 ($C_3$,-$CH_3$), 17.67 ($C_7$,-$CH_3$), 20.65 ($C_4$-$CH_3$), 25.71 ($C_8$,), 26.50 ($C_5$,), 27.67 ($C_5$), 29.84 ($C_4$), 30.95 ($C_1$,), 39.89 ($C_4$,), 48.26 ($C_3$), 67.54 ($C_6$), 120.63 ($C_2$,), 124.17 ($C_6$,), 131.31 ($C_7$,), 137.66 ($C_3$,), 173.75 ($C_2$), (main component, ~ 94 %); MS m/e: 250(37) [M$^+$], 207(18), 194(3), 181(30),137(23), 136(23), 127(19), 114(100), 109(26), 99(39), 69(74), 55(25), 41(84); GC: $t_R$= 11.83 min,(2R,3S)-isomer, 94 % ($t_R$= 11,64 min,(2S,3S)-isomer, 6 %, 30 m x 0.25 mm SP-2100 column, $t_k$= 160-260°C, 3°C/min,$N_2$).

Methyl(2R,1'S,4E,8Z)-5,9-dimethyl-2-(1-methyl-3-hydroxy-propyl)-4,8-undecadienoate (14a)

A solution of 2 (1.05 g, 3.98 mmol) in dry methanol (6 mL) and triethylamine (3 mL) was stirred at room temperature overnight, and then methanol and triethylamine was removed by vacuum evaporation. LPLC of the residue on 40-60 µm LiChroprep Si 60 using hexane-acetone=10:1 as eluant gave 14a(0.88 g, 75 %) as an oil. TLC(C):Rf=0.44; IR (film), $\nu_{max}$: 3350, 2950, 2900, 1730, 1660, 1450, 1380, 1185, 1150, 1100, 1050 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 0.95 (m, (d and t), 6H, 2-$CH_3$), 1.4-1.9 (br m, 3H,-$CH_2$- and -CH=), 1.61 (s, 3H,-$CH_3$),1.66 (s, 3H,-$CH_3$), 1.9-2.4 (br m, 9H,4-$CH_2$- and CH-COO), 3.63 (s, 3H, COO-$CH_3$), 3.75 (t, J=6.5Hz, 2H,-$CH_2$-O), 5.07 (m, 2H,2-CH=C); MS m/e: 296(10)[M$^+$], 239(7), 207(10), 195(9), 181(16), 153(8), 135(16), 123(14), 107(14), 97(14), 93(16), 83(44), 82(21), 81(23), 79(16), 69(13), 68(10), 67(22), 55(100), 43(19), 41(65).

Methyl(2R,1'S,4E,8Z)-5,9-dimethyl-2-(1-methyl-3-tetrahydropyranyloxy-propyl)-4,8-undecadienoate (14b)

To a solution of 14a (830 mg, 2.8 mmol) and 2H-dihydropyran (300 mg, 3.6 mmol) in dry dichloromethane (15 mL) was added piridinium tosylate catalyst (30 mg) and the mixture was stirred at room temperature for 6 h. The resulted solution was washed with water (3 mL) and brine (3 mL) and dried over $MgSO_4$. Evaporation of the solvent in vacuo and LPLC of the residue (on LiChroprep Si 60 by hexane-acetone=5:0.1 as eluant) gave 14b (1020 mg, 95 %) as an oil TLC(B):Rf=0.52; IR (film), $\nu_{max}$: 2940, 2880, 1735, 1450, 1440, 1390, 1360, 1330, 1260, 1200, 1170, 1150, 1125, 1080, 1030, 990, 980, 905, 870, 810 cm$^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.93 (d, J=6Hz, 3H,-$CH_3$), 0.96 (t, J=7Hz, 3H,-$CH_3$), 1.4-1.8 (m, 15H, 2-$CH_3$ and 4-$CH_2$- and -CH=), 1.8-2.4 (m, 9H, 4-$CH_2$- and =CH-COO), 3.1-4.0 (m, 4H, 2-$CH_2$O), 3.64 (s, 3H,COO-$CH_3$), 4.57 (m, 1H, O-CH-O), 5.05 (m, 2H, 2-CH=C).

Methyl(2R,1'S,4E)-5,9-dimethyl-2-(1-methyl-3-hyroxy-propyl)-4,8-decadienoate (14c)

2a (32.5 g, 0.13 mol) was converted to 14c (29.2 g, 80 %) as described for the preparation of 14a. TLC(C):Rf=0.46; IR (film), $\nu_{max}$: 3400, 2940, 2900, 1730, 1660, 1440, 1380, 1185, 1150, 1100, 1050 cm$^{-1}$; $^1$H-NMR($CDCl_3$,$\delta$): 0.95 (d, J=6Hz, 3H,-$CH_3$), 1.4-1.95 (br m, 3H,-$CH_2$- and -CH=), 1.60 (br s, 6H, 2-$CH_3$), 1.66 (s, 3H,-$CH_3$), 1.9-2.4 (br m, 7H,3-$CH_2$- and -CH-COO), 3.64 (s, 3H, O-$CH_3$), 3.65 (t, J=6Hz, 2H, O-$CH_2$-), 5.06 (m, 2H, 2-CH=C); MS m/e: 282(10)[M$^+$], 250(6), 239(10), 207(16), 195(11), 181(24), 145(13), 134(20), 113(22), 108(31), 96(19), 93(20), 81(29), 79(16), 69(100), 55(22), 43(12), 41(63), 39(8).

Methyl(2R,1'S,4E)-5,9-dimethyl-2-(1-methyl-3-tetrahydropyranyloxy-propyl) -4,8-decadienoate (14d)

14c (26.0 g, 92 mmol) was converted to 14d (32.8 g,97 %) as described for the preparation of 14b. TLC(B):Rf=0.51; IR(film), $\nu_{max}$: 2940, 2875, 1730, 1665, 1450, 1440, 1385, 1360, 1320, 1260, 1200, 1160, 1140, 1120, 1075, 1030, 995, 980, 905, 875, 810 cm$^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.93 (d,J=6Hz, 3H,-$CH_3$), 1.4-1.8 (br m, 18H, 3-$CH_3$ and 4-$CH_2$- and -CH=), 1.8-2.4 (br m, 7H,3-$CH_2$- and CH-COO), 3.1-4.0 (m, 4H, 2-$CH_2$-O), 3.63 (s, 3H,COO-$CH_3$), 4.55 (m,1H, O-CH-O), 5.05 (m, 2H, 2-CH=C);

(3S,4R,6E,10Z)-4-Hydroxymethyl-1 - tetrahydropyranyloxy-3,7,11-trimethyl-6,10-tridecadiene (15a)

To a stirred suspension of lithium aluminium hydride (0.21 g, 5.4 mmol) in dry ether (8 mL) was added a solution of 14b (1000 mg, 2.7 mmol) in dry ether (3 mL) and the resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by careful dropwise addition of water (3 mL) and then 15 % hydrochloric acid (5 mL) was added to the mixture to dissolve the precipitates. After fast separation the acidic layer was extracted with ether (10 mL ) and the combined ethereal solutions were washed with saturated $NaHCO_3$ solution (5 mL) and brine (5 mL). After drying over $MgSO_4$ the solvent was evaporated in vacuo to afford 15a (803 mg, 87 %) as an oil. TLC(C):Rf=0.64; IR (film), $\nu_{max}$: 3370, 2940, 2910, 2860, 1655, 1440, 1425, 1370, 1340, 1250, 1190, 1170, 1120, 1060, 1020, 970, 895, 860, 800 cm$^{-1}$; $^1$H-NMR($CDCl_3$,$\delta$): 0.89 (d,J=6Hz, 3H,-$CH_3$), 0.95 (t, J=7Hz, 3H,-$CH_3$), 1.3-1.9 (br m, 16H,2-$CH_3$ and 4-$CH_2$- and 2-CH=), 1.9-2.4 (m, 8H, 4-$CH_2$-), 3.1-4.1 (br m, 6H, 3-$CH_2$-O), 4.54 (m, 1H, O-CH-O), 5.07 (m, 2H, 2-CH=C).

(3S,4R,6E,10Z)-4-Mesyloxymethyl-1-tetrahydropyranyloxy-3,7,11-trimethyl-6,10-tridecadiene (15b)

To a stirred solution of 15a (780 mg, 2.21 mmol) and triethylamine (310 mg, 3.09 mmol) in dry ether (6 mL) was added a solution of mesyl chloride (290 mg, 2.54 mmol) in dry ether (3 mL) at 0°C, and the resulting mixture was stirred at room temperature for 1 h. The mixture was then diluted with ether (6 mL) and 10 % hydrochloric acid (6 mL) was added. After separation and extraction of the acidic layer with ether (6 mL) the combined ethereal solutions were washed with saturated $NaHCO_3$ solution (3 mL) and brine (3 mL) and were dried over $MgSO_4$. Evaporation of the solvent in vacuo yielded 15b (836 mg, 88 %). TLC (D):Rf=0.61; IR (film), $\nu_{max}$: 2960, 2940, 2870, 2860, 1670, 1440, 1430, 1350, 1250, 1200, 1180, 1130, 1110, 1070, 1030, 970, 950, 860, 830, 810 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.93 (mc(d and t), 6H, 2-$CH_3$), 1.4-1.9 (br m, 16H, 2-$CH_3$ and 4-$CH_2$ and 2-CH=), 2.0 (mc, 8H, 4-$CH_2$), 2.88 (s, 3H, $SO_2$-$CH_3$), 3.1.-4.0 (br m, 4H, 2-$CH_2$O), 4.06 (d, J=6Hz, 2H,-$CH_2$-OMs), 4.55 (m, 1H, O-CH-O), 5.05 (m, 2H, 2-CH=C).

(3S,4R,6E)-4-Hydroxymethyl-1-tetrahydropyranyloxy-3,7,11-trimethyl-6,10-dodecadiene (15c)

Lithium aluminium hydride reduction of 14d (32.8 g, 89 mmol) according to the method desribed at 15a afforded 15 (27.3 g, 90 %) as an oil. TLC (C):Rf=0.63; IR (film), $\nu_{max}$: 3400, 2940, 2920, 2865, 1645, 1440, 1430, 1370, 1340, 1310, 1250, 1190, 1170, 1150, 1120, 1105, 1060, 1015, 970, 895, 860, 800 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.92 (d, J=6Hz, 3H,-$CH_3$), 1.3-1.95 (br m, 19H, 3-$CH_3$ and 4-$CH_2$- and 2-CH=), 2.03 (mc, 6H, 3-$CH_2$-), 3.2-4.2 (br m, 4H, 2-$CH_2$-O), 3.58 (d, J=6Hz, 2H, O-$CH_2$-), 4.56 (m, 1H, O-CH-O), 5.11 (m, 2H, 2-CH=C).

(3S,4R,6E)-4-Mesyloxymethyl-1-tetrahydropyranyloxy-3,7,11-trimethyl-6,10-dodecadiene (15d)

Mesylation of 15c (26.3 g, 78 mmol) by the process desribed at 15b gave 15d (29.0 g, 89 % as an oil. TLC (D):Rf=0.61; IR (film), $\nu_{max}$: 2960, 2930, 2870, 1670, 1445, 1350, 1250, 1200, 1180, 1130, 1115, 1070, 1060, 1025, 970, 950, 910, 860, 840, 810 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.93 (d, J=6.5Hz, 3H, -$CH_3$), 1.4-1.9 (br m, 19H, 3-$CH_3$ and 4-$CH_2$- and 2-CH=), 2.01 (mc, 6H, 3-$CH_2$-), 2.94 (s, 3H, $SO_2CH_3$), 3.2-4.1 (br m, 4H, 2-$CH_2$-O), 4.11 (d, J=6Hz, 2H, -$CH_2$-OMs), 4.56 (M, 1H, O-CH-O), 5.08 (m,2H,2-CH=C).

(3S,4R,6E,10Z)-1-Tetrahydropyranyloxy-3,4,7,11-tetramethyl-6,10-tridecadiene (16a)

To a stirred suspension of lithium aluminium hydryde (0.30 g, 7.9 mmol) in dry THF (8 mL) was added a solution of 15b (805 mg, 1.98 mmol) in dry THF (8 mL) and the resulting mixture was refluxed for 1h. After cooling the reaction was quenched by careful dropwise addition of water (4 mL). Then 15 % hydrochloric acid (5 mL) was added to solubilize the precipitate and the mixture was extracted three times with ether (12 mL, each). The combined organic solutions were washed with saturated $NaHCO_3$ solutions (4 mL) and brine (4 mL) and dried over $MgSO_4$. After removal of the solvent in vacuo 16a (586 mg, 90 %) was yielded as an oil. TLC (A):Rf=0.61; IR( film), $\nu_{max}$: 2950, 2930, 2870, 1665, 1445, 1430, 1375, 1350, 1315, 1260, 1200, 1160, 1120, 1110, 1080, 1070, 1030, 990, 980, 900, 870, 810 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 0.83, 0.89, 0.95 (d, d, t, 9H, 3-$CH_3$), 1.4-1.9 (br m, 16H, 2-$CH_3$ and 4-$CH_2$- and 2-CH=), 2.0 (mc, 8H, 4-$CH_2$-), 3.2-4.1 (br m, 4H, 2-$CH_2$-O), 4.55 (m, 1H, O-CH-O), 5.10 (m, 2H, 2-CH=C).

(3S,4R,6E,10Z)-3,4,7,11-Tetramethyl-6,10-tridecadien-1-ol (16b)

A solution of 16a (531 mg, 1.58 mmol) and p-toluene sulfonic acid (5 mg) in methanol (10 mL) was stirred at room temperatura overnight. After addition of 10 $\mu$L of triethylamine the solution was concentrated in vacuo and the residue was purified by LPLC (on LiChroprep Si 60 with hexane--acetone=10:1 as eluant) to give 16b (373 mg, 94 %) as an oil. TLC (C):Rf=0.48; $[\alpha]^{22}_{546}$=-5.7°, $[\alpha]^{22}_{D}$=-4.5° (c=4,87, $CHCl_3$); IR (film), $\nu_{max}$: 3350, 2970, 2930, 2880, 1660, 1450, 1380, 1110, 1055, 1015, 1000 $cm^{-1}$; $^1$H-NMR ($CCl_4$,$\delta$): 0.83, 0.88, 0.97 (d, d, t, 9H, 3-$CH_3$), 1.4-1.9 (m, 4H,-$CH_2$- and 2-CH=), 1.59 (s, 3H,-$CH_3$), 1.66 (s, 3H,-$CH_3$) 2.0 (m, 8H, 4-$CH_2$-), 3.46 (s, 1H, OH), 3.55 (t, J=6Hz, 2H,-$CH_2$-O), 5.06 (m, 2H, 2-CH=C); MS m/e: 252(13)[$M^+$] , 223(3), 195(12), 179(5), 177(5), 151(7), 137(35), 123(17), 113(13), 109(19), 99(29), 95(49), 83(100), 69(37), 55(79), 41(32).

(3S,4R,6E)-1-Tetrahydropyranyloxy-3,4,7,11-tetramethyl-6,10-dodecadiene (16c)

Lithium aluminium hydride reduction of the mesylate 15d (29.6 g, 72 mmol) according to the method described at 16a yielded 16c (22.0 g,95 %) as an oil. TLC (A):Rf=0.62; IR (film), $\nu_{max}$:2960, 2940, 2870, 1665, 1450, 1440, 1380, 1350, 1320, 1260, 1200, 1160, 1130, 1110, 1070, 1060, 1030, 990, 900, 870, 810 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.83 (d, J=6Hz, 3H,-$CH_3$), 0.88 (d, J=6Hz, 3H,-$CH_3$), 1.4-1.9 (br m, 19H,3-$CH_3$ and 4-$CH_2$- and 2-CH=), 2.02 (m, 6H, 3-$CH_2$-), 3.2-4.1 (br m, 4H, 2-$CH_2$-O), 4.57 (m, 1H, O-CH-O), 5.12 (m, 2H, 2-CH=C).

(3S,4R,6E)-3,4,7,11-Tetramethyl-6-10-dodecadien-1-ol (16d)

Deprotection of 16c (21.7 g, 67 mmol) by the process described at 16b but purified by VLC the crude product (on 200 g of 63-200 $\mu$m silica gel with hexane-acetone=10:1 as eluant) gave 16d(15.2 g, 96 %) as an oil. TLC (C):Rf=0.49;$[\alpha]^{23}_{546}$=-5.9°,$[\alpha]^{23}_{D}$=-4.6° (c=4.21, $CHCl_3$); IR (film), $\nu_{max}$: 3350, 2980, 2945, 2890, 1660, 1455, 1380, 1205, 1105, 1055, 1010 $cm^{-1}$; $^1$H-NMR ($COCl_3$, $\delta$): 0.82 (d,J=6.5Hz, 3H,-$CH_3$), 0.87 (d, J=6.5Hz, 3H,-$CH_3$), 1.4-1.9 (br m, 4H,-$CH_2$- and 2-CH=), 1.60 (br s, 6H, 2-$CH_3$), 1.68 (s, 3H,-$CH_3$), 2.03 (mc, 6H, 3-$CH_2$-), 3.62 (t, J=6Hz, 2H,-$CH_2$-O), 5.10 (m, 2H,-CH=C); $^{13}$C-NMR ($CDCl_3$): 16.09 ($C_4$-$\underline{C}H_3$ and $C_7$-$\underline{C}H_3$ ), 16.76 ($C_3$-$\underline{C}H_3$), 17.67 ($C_{11}$-$\underline{C}H_3$), 25.71 ($C_{12}$), 26.71 ($C_9$),

31.53 ($C_5$), 33.73 ($C_3$) 35.95 ($C_2$) 38.78 ($C_4$), 39.90 ($C_8$), 61.63 ($C_1$), 123.91 ($C_6$), 124.44 ($C_{10}$), 131.22 ($C_{11}$), 135.32 ($C_7$), (main component, ~ 94 %); MS m/e: 238(11)[$M^+$], 195(12), 177(4), 165(4), 137(7), 123(39), 109(25), 99(24), 95(49), 83(42), 81(37), 69(100), 55(41), 41(48).

(+)-(3S,4R,6E,10Z)-3,4,7,11-Tetramethyl-6,10-tridecadienal (1a; (+)-Faranal)

To a solution of 16b (332 mg, 1.32 mmol) in dry dichloromethane (15 mL) was added piridinium dichromate (505 mg, 1.35 mmol) portionwise at room temperature. After stirring for 3 h the resulting mixture was filtered through a small column containing 15 g of Kieselgel 60 and the column was eluted with ether (50 mL). The resulted solution was then evaporated in vacuo and purified by LPLC (on LiChroprep Si 60 with hexane-acetone=5:0.1 as eluant) to yield 1a (214 mg, 65 %) as an oil that froze keeping at -30°C. Mp.:-25°C, TLC (A):Rf=0.51; $[\alpha]^{24}_{546}$= +19.4°,$[\alpha]^{24}_{D}$= +17.4° (c= 4.12, $CHCl_3$, > 95 % ee), Lit.[7]:$[\alpha]^{23}_{D}$= +16.2° (c= 0,5, hexane, 90 % ee); IR (film), $\nu_{max}$: 2970, 2940, 2880, 2720, 1730, 1655, 1450, 1380, 1120, 1080, 1020 $cm^{-1}$; $^1$H-NMR ($CDCl_3$,$\delta$): 0.84 (d, J=6.5Hz, 3H,-$CH_3$), 0.93 (d, J=6.5Hz, 3H,-$CH_3$), 0.97 (t, J=7Hz, 3H,-$CH_3$), 1.60 (s, 3H,-$CH_3$), 1.67 (s, 3H,-$CH_3$), 1.8-2.6 (br m, 12H,-5-$CH_2$- and 2-CH=), 5.09 (m, 2H, 2-CH=C), 9.74 (t, J=2Hz,-CHO); $^{13}$C-NMR ($CDCl_3$,$\delta$): 12.81 ($C_{13}$), 16.00 ($C_8$-$\underline{C}H_3$), 16.12 ($C_7$-$CH_3$), 17.55 ($C_3$-$\underline{C}H_3$),22.87($C_{11}$-$\underline{C}H_3$), 24.81 ($C_{12}$), 26.27 ($C_9$), 30.97 ($C_5$), 32.02 ($C_3$), 38.49 ($C_4$), 40.11 ($C_8$), 47.43 ($C_2$), 123.12 ($C_6$), 123.91 ($C_{10}$), 135.96 $C_7$), 137.18 ($C_{11}$), 203.21 ($C_1$), (main component, ~ 94 %); MS m/e: 250(6)[$M^+$], 232(2), 221(2), 206(2), 203(3), 193(26), 177(3), 175(8), 137(21), 123(20), 107(11), 95(18), 83(100), 69(22), 55(78),43(17), 41(33); HPLC: $t_R$= 4.12 min (250 x 4.6 mm column, 10 $\mu$m LiChrosorb RP-18, 2.0 ml/min MeOH-water=9:1 eluant, $\lambda$=215 mm/; GLC: $t_R$= 21.11 min, (3S,4R)-isomer, 94 % ($t_R$=20.87 min, (3S,4S)-isomer, 6 %; 40 m x 0.128 mm OV-1 capillary column, $t_k$= 180°C, $N_2$).

(+)-(3S,4R,6E)-3,4,7,11-Tetramethyl-6,10-dodecadienal (1b; (+)-13-Norfaranal)

Oxidation of 16d (10.0 g, 42 mmol) by the method described above afforded 1b (6.63 g 67 %) as an oil. TLC (A):Rf=0.52;$[\alpha]^{22}_{546}$= +19.6°,$[\alpha]^{22}_{D}$= +17.5° (c=4.46, $CHCl_3$); IR (film), $\nu_{max}$: 2980, 2945, 2880, 2720, 1730, 1660, 1450, 1380, 1115, 1080, 1020 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, $\delta$): 0.84 (d, J=6.5Hz, 3H,-$CH_3$), 0.94 (d, J=6.5Hz, 3H,-$CH_3$), 1.59 (s, 6H, 2-$CH_3$), 1.68 (s, 3H, -$CH_3$), 1.8-2.6 (m, 10H, 4-$CH_2$- and 2-CH=), 5.10 (m,2H,2-CH=C), 9.75 (t, J=2Hz, 1H,CHO); $^{13}$C-NMR ($CDCl_3$): 15.97 ($C_4$-$\underline{C}H_3$), 16.09 ($C_7$-$\underline{C}H_3$), 17.55 ($c_3$-$\underline{C}H_3$), 17.67 ($C_{11}$-$CH_3$), 25.71 ($C_{12}$), 26.62 ($C_9$), 31.91 ($C_5$), 32.00 ($C_3$), 38.49 ($C_4$), 39.84 ($C_8$), 47.42 ($C_2$), 123.09 ($C_6$), 124.29 ($C_{10}$), 131.31 ($C_{11}$), 135.90 ($C_7$), 203.21 ($C_1$); (main component, 94 %); MS m/e: 236(4)[$M^+$], 193(22), 175(5), 149(5), 137(5), 123(33), 109(17), 91(23), 69(100), 41(40), GLC: $t_R$= 17.46 min, (3S,4R)-isomer, 94 % ($t_R$= 17.22 min, (3S,4S)-isomer, 6 %; 40 m x 0.128 mm OV-1 capillary column, $t_k$= 180°C, $N_2$).

ACKNOWLEDGEMENTS

*The authors are grateful to Pál Vőfély for his contribution to this work and wish to thank to* Éva Szabó and Jenő Fekete for the MS and HPLC measurements. Financial support from EGIS Pharmaceutical Works, Budapest is gratefully acknowledged.

REFERENCES AND NOTES

1. Part of the present material has appeared in a preliminary communication: L. Poppe, L. Novák, P. Kolonits, A. Batta, and Cs. Szántay, Tetrahedron Lett., 27, 5769 (1986).
2. F.J. Ritter, I.E.M. Brüggemann-Rotgans, P.E.J.Verwiel, C.J. Persoons, and E.Talman,Tetrahedron Lett., 1977, 2617.
3. F.J. Ritter, I.E.M. Brüggemann-Rotgans, P.E.J. Verwiel, E. Talman, F. Stein, J. LaBrijn, and C.J. Persoons, Proc.Int.Congr.Int.Union Study Soc. Insect, 8th, 1977, 41;Chem.Abstr., 89, 1766088 (1978) .
4. M.Kobayashi, T. Koyama, K. Ogura, S. Seto, F.J.Ritter, and I.E.M. Brüggemann-Rotgans, J.Am.Chem. Soc., 102, 6602 (1980).
5. T.Koyama, M. Matsubara, K. Ogura, I.E.M. Brüggemann, and A. Vrielink, Naturwissenschaften, 70, 469 (1983).
6. K. Mori and H. Ueda, Tetrahedron Lett., 22, 461 (1981).
7. K. Mori and H. Ueda, Tetrahedron, 38, 1227 (1982).
8. D.W. Knight and B. Ojhara, Tetrahedron lett., 22, 5101 (1981).
9. D.W. Knight and B. Ojhara, J.Chem.Soc., Perkin Trans.1. 1983, 955.
10. R. Baker, D.C. Billington, and N. Ekanayake, J.Chem.Soc., Chem Commun., 1981, 1234.
11. R. Baker, D.C. Billington, and N. Ekanayake, J.Chem.Soc., Perkin Trans.1., 1983, 1387.
12. C.A. Henrick, W.E. Willy, J.W. Baum, T.A. Baer, B.A. Garcia, Th. A. Mastre, and S. M. Chang, J. Org. Chem., 40, 8 (1975).
13. C.S. Chen, Y. Fujimoto, G. Girdaukas, and C.J. Sih, J.Am.Chem.Soc., 104, 7297 (1982).

14. P. Mohr, M. Tori, P. Grossen, P. Herold, and C. Tamm, Helv.Chim.Acta, 65, 1412 (1982).
15. P. Herold, P. Mohr, and C. Tamm, Helv.Chim.Acta, 66, 744 (1983).
16. C. J. Francis and J.B. Jones, J.C.S. Chem.Comm., 579 (1984).
17. D.J. Horgan, J.K.Stoops, E.C. Webb, and B. Zerner, Biochemistry 8, 2000 (1969).
18. U. Jensen-Korte and H.J. Schäfer, Liebigs Ann.Chem., 1982, 1582.
19. R. M. Coates and M.W. Johnson, J.Org.Chem., 45, 2685 (1980).
20. K. Mori, S. Masuda, and M. Matsui, Agric.Biol.Chem., 42, 1015 (1978).
21. B.C. Hartman and B. Rickborn, J.Org.Chem., 37, 943 (1972).
22. E.E. Van Tamelen and J.P. McCormick, J.Am.Chem.Soc., 92, 737 (1970).
23. S. Terao, M. Shiraish, and K. Kato, Synthesis, 1979, 469.
24. S. Tanaka, H. Yamamoto, H. Nozaki, K.B. Sharpless, R.C. Michaelson, and J.D. Cutting, J.Am.Chem. Soc., 96, 5254 (1974).
25. P. Gosselin, C. Maignan, and F. Roussac, Synthesis, 1984, 877.
26. For recent discussion on stereoselective electrophilic additions to enolates, see: G.J. McGarvey and J.M. Williams: J.Am.Chem.Soc., 107, 1435 (1985), and references cited therein.
27. L. Poppe and L. Novák, Magy.Kém.Lapja, 40, 366 (1985).
28. R.Rossi, A.Carpita and M. Chini, Tetrahedron, 41, 627 (1985).

# VI. melléklet

NOVÁK, L., ROHÁLY, J., **POPPE, L.**, HORNYÁNSZKY, G., KOLONITS P., ZELEI, I., FEHÉR, I., FEKETE, J., SZABÓ, É., ZAHORSZKY, U., JÁVOR, A., SZÁNTAY, CS.:

Naphthalene Analogs of Mevinolin,

*Liebigs Ann. Chem.*, **1992**, 145.

Synthesis of Novel HMG-CoA Reductase Inhibitors, I

# Naphthalene Analogs of Mevinolin

**Lajos Novák[a], János Rohály[a], László Poppe[b], Gábor Hornyánszky[a], Pál Kolonits[a], István Zelei[a], Imre Fehér[a], Jenö Fekete[c], Éva Szabó[c], Uwe Záhorszky[e], András Jávor[d], and Csaba Szántay*[a,b]**

Institute of Organic Chemistry, Technical University[a],
Szt. Gellért tér 4, H-1521 Budapest, XI

Central Research Institute of Chemistry, Hungarian Academy of Sciences[b],
P.O. Box 17, H-1525 Budapest,

Institute for General and Analytical Chemistry, Technical University[c],
Szt. Gellért tér 4, H-1521 Budapest, XI,

Gedeon Richter Chemical Works Ltd.[d],
Gyömröi u. 15–21, H-1103 Budapest,

Institute of Organic Chemistry, University of Karlsruhe[e],
P.O. Box 6380, W-7500 Karlsruhe 1.

Received August 10, 1991

**Key Words:** HMG-CoA reductase inhibitors / Naphthylacetates / Pig liver esterase / Glutarate, 3-hydroxy / Lactones

The title compounds **2** and their corresponding (6*S*) epimers **18** are prepared in several steps by starting with chiral formyl ester **5**, and α-tetralones **10**: (1) coupling reaction with the ylide generated from **11** to yield unsaturated ester **13**, (2) reduction to the corresponding alcohol **14**, (3) addition of the Grignard reagent derived from **14** to formyl ester **5** to afford the hydroxy esters **16** and **17**, and (4) lactonization. This procedure is also used to synthesize the β-naphthyl analogs **29** and **30**. Some results obtained from HMG-CoA reductase inhibitor screening are also reported.

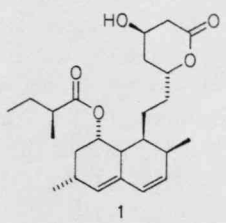
One of the most attractive and efficient way to cure and prevent cardiovascular diseases is the lowering of cholesterol serum levels[1–3]. The recently introduced drugs mevinolin (**1**), synvinolin and eptastatin reduce drastically the cholesterol level by inhibiting the HMG-CoA reductase in the rate-limiting step of cholesterol biosynthesis[4–9]. These fungal metabolites have been an attractive synthetic target of considerable current interest because of their challenging structural features and biological activity. Although, many excellent synthetic approaches to mevinolin and its semisynthetic derivative have been reported[10], these compounds are produced by microbiological procedures.

Various structurally simplified analogs of mevinolin have been synthesized and evaluated for HMG-CoA reductase inhibitory activities. Reports on the structure-activity relationships showed that the (3*R*)-3-hydroxy-δ-valerolactone moiety is essential for biological activity, whereas the highly substituted hexahydronaphthalene ring of mevinolin may be replaced by simplified lipophilic groups. Furthermore, the inhibitory potencies depend on the size and shape of the latter groups[11–13].

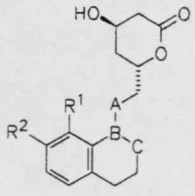
During the course of our program in this area, we have decided to perform the synthesis and biological evaluation of new structural analogs of mevinolin (Scheme 1). In this paper we describe the preparation of naphthalene analogs of the general structure **2** and **3**, which afford some moderately effective inhibitors of HMG-CoA reductase.

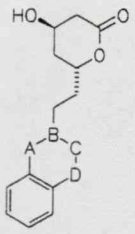
The basic strategy of our synthesis is summarized in Scheme 2. This illustrates the easy generation of the chiral center of the lactone moiety which involves the addition of Grignard reagent **4** to the chiral formyl ester **5** prepared from the prochiral hydroxy ester **6**. The stereochemical features of this addition have not been elucidated previously.

Scheme 1



1





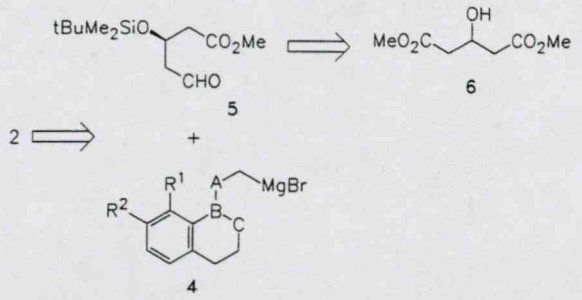
| 2 | A–B–C | $R^1$ | $R^2$ |
|---|---|---|---|
| a | CH=C–$CH_2$(*E*) | H | H |
| b | $CH_2$–CH–$CH_2$ | H | H |
| c | $CH_2$–C=CH | H | H |
| d | $CH_2$–CH–$CH_2$ | OMe | H |
| e | $CH_2$–CH–$CH_2$ | H | OMe |
| f | $CH_2$–CH–$CH_2$ | H | Ph |

| 3 | A–B–C–D |
|---|---|
| a | CH=C–$CH_2$–$CH_2$ |
| b | CH=C–CH=CH |

  0170–2041/92/0202–0145 $ 3.50+.25/0

We have expected that the formation of the (4*R*,6*R*) diastereomer having the same stereochemistry of the lactone moiety as mevinolin would predominate.

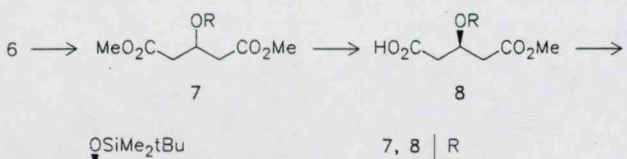
Scheme 2



## Results and Discussion

The key formyl ester intermediate **5** is synthesized in the following manner (Scheme 3). Protection of the hydroxy group of dimethyl-3-hydroxyglutarate (**6**) by silyl ether formation using *tert*-butyldimethylsilyl chloride followed by pig liver esterase-(PLE-)mediated hydrolysis gives monoester **8a** in good yield. The optical purity of the product has been determined by HPLC and $^1$H-NMR analysis of the diastereomeric (*R*)-α-methylamide[14]. Although the enzyme-catalyzed hydrolysis affords the desired (*R*) ester as the major product the enantiomeric purity is rather low (e.e. 52%). Therefore, we have investigated the enzyme-catalyzed hydrolysis of the other protected 3-hydroxyglutarates **7b–d**. In accord with Santaniello's result[15], the highest enantioselectivity has been achieved with the PLE-catalyzed hydrolysis of acetoxy ester **7b**. In this case the (*R*) half ester **8b** has been obtained in 90% e.e. and in 45% chemical yield. Base-catalyzed hydrolysis of **8b** affords the (*R*)-hydroxy ester **8** (R = H), which is treated with *tert*-butyldimethylsilyl chloride to yield the silyl-protected monoester **8a**. Selective reduction of the carboxy function in the latter with diborane affords hydroxy ester **9**, which is converted into formyl ester **5** on treatment with pyridinium chlorochromate.

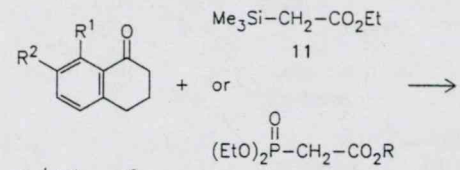
Scheme 3



| 7, 8 | R |
|---|---|
| a | $SiMe_2tBu$ |
| b | COMe |
| c | H |
| d | $CH_2OMe$ |
| e | $COCH_2Ph$ |

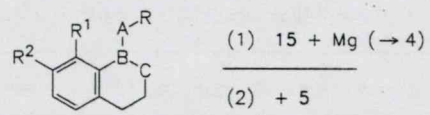
The other building blocks **4** are prepared from known α-tetralone derivatives (Scheme 4). Condensation of α-tetralone (**10a**) with the carbanion generated from ethyl trimethylsilylacetate (**11**) with lithium diisopropylamide provides a 3:2 mixture of (*E*) and (*Z*) isomers of naphthylideneacetates **13a** and **13b** respectively, which are separated by column chromatography. Reduction of the (*E*) isomer **13a** with an excess of lithium aluminum hydride gives the alcohol **14a** which is converted into the corresponding bromide **15a**, the precursor of the desired Grignard reagent, by utilizing phosphorus tribromide.

Scheme 4

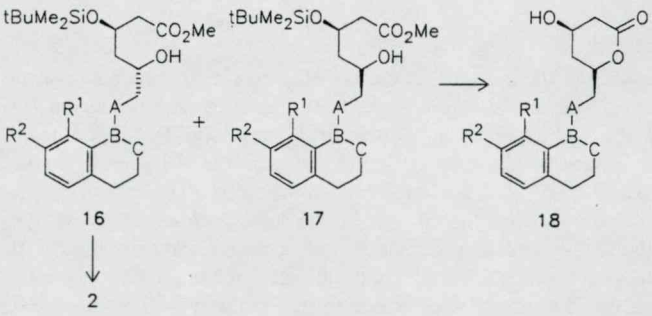


| 10 | $R^1$ | $R^2$ |
|---|---|---|
| a | H | H |
| b | OMe | H |
| c | H | OMe |
| d | H | Ph |

| 12 | R |
|---|---|
| a | Et |
| b | Me |



| | 13 | 14 | 15 |
|---|---|---|---|
| R | $CO_2Et$ | $CH_2OH$ | $CH_2Br$ |



| 13 | 14, 15, 18 | A–B–C | $R^1$ | $R^2$ | 16, 17 |
|---|---|---|---|---|---|
| a | a | CH=C–$CH_2$(*E*) | H | H | a |
| b | – | CH=C–$CH_2$(*Z*) | H | H | – |
| c | b | $CH_2$–CH–$CH_2$ | H | H | b |
| d | c | $CH_2$–C=CH | H | H | c |
| e | – | CH=C–$CH_2$(*E*) | OMe | H | – |
| f | – | CH=C–$CH_2$(*Z*) | OMe | H | – |
| g | d | $CH_2$–CH–$CH_2$ | OMe | H | d |
| h | – | CH=C–$CH_2$(*E*) | H | OMe | – |
| i | – | CH=C–$CH_2$(*Z*) | H | OMe | – |
| j | e | $CH_2$–CH–$CH_2$ | H | OMe | – |
| k | – | CH=C–$CH_2$(*E*) | H | Ph | – |
| l | – | CH=C–$CH_2$(*Z*) | H | Ph | – |
| m | f | $CH_2$–CH–$CH_2$ | H | Ph | – |

The above condensation reaction is also used for the preparation of the naphthylideneacetates **13e,f**, **13h,i**, and **13k,l** from the appropriate α-tetralone derivatives **10b–d**. Cata-

lytic reduction of the (*E*) isomers of these esters **13a, e, h**, and **f** over Pd catalyst yields the corresponding naphthylacetates **13c, g, j**, and **m** which are converted into the desired bromides **15a, b, d, e**, and **f** according to the same sequence of reactions (reduction with lithium aluminum hydride and treatment with phosphorus tribromide).

From the Wittig-Horner reaction of α-tetralone (**10a**) with the less reactive anion of phosphonate ester **12** only traces of (*E*)-naphthylideneacetate **13a** are isolated. Here, the initially formed α,β-unsaturated esters **13a** and **13b** undergo base-catalyzed rearrangement to the thermodynamically more stable β,γ-unsaturated ester **13d** as the major product. The application of the same two-step procedure (reduction and halogenation) to the latter furnishes the corresponding bromide **15c**.

For the coupling of the two building blocks the Grignard reagent **4**, obtained from the bromides **15a–d** with magnesium in tetrahydrofuran, is treated with the chiral formyl ester **5**. The reaction proceeds smoothly and yields 2:1 mixtures of the expected stereoisomers **16a–d** and **17a–d**, which are separated by column chromatography. Desilylation of the separated diastereomers **16a–d** and **17a–d** on treatment with 48% hydrogen fluoride in acetonitrile results in spontaneous lactonization to the target compounds **2a–d** and **18a–d**.

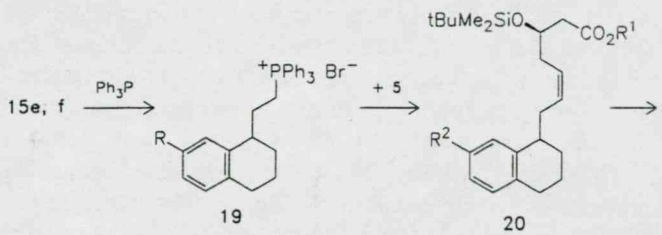
The configurations of these compounds **2a–d** and **18a–d** have been assigned mainly on the basis of the chemical shifts observed for C-4 and C-6 in their $^{13}$C-NMR spectra[16]. For instance, in the chair conformation of the major isomer (4*R*,6*R*)-**2c**, bearing the bulky substituent in equatorial position, the hydroxy group occupies an axial position and exerts a stronger γ-gauche effect on the chemical shift in the 6-position (δ = 75.78) than in the major isomer (4*R*,6*S*)-**18c** (δ = 76.95). Furthermore, the relative $R_f$ values of these diastereomers parallel those of the known similar compounds, which have strengthened our confidence in the assignments of configurations[17,18].

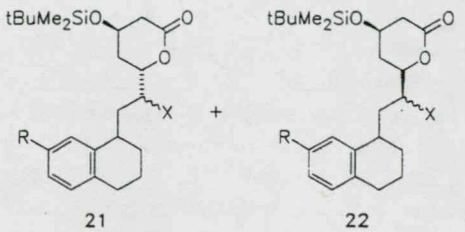
In two cases, the reaction of the bromides **15e, f** with magnesium has failed even by increasing the duration and temperature of the reaction, the sole observed result being the formation of byproducts by self-coupling of the bromide with the Grignard reagent.

Therefore, an alternate route to target compounds **2e, f** has been elaborated (Scheme 5). Reaction of the bromides **15e, f** with triphenylphosphane affords the phosphonium salts **19a, b**, and then the phosphoranes generated from these salts by reaction with butyllithium were treated with formyl ester **5**. Base-catalyzed hydrolysis of the products **20a, b** [(*Z*) isomer >98%] affords the corresponding acids **20c, d** which are subjected to iodolactonization with iodine in wet methylene chloride. The 2:1 mixture of the diastereomeric iodolactones **21a, b** and **22a, b** formed is separated by column chromatography and then separately reduced with tributyltin hydride to give the protected lactones **21c, d** and **22c, d**. Finally, desilylation of the latter with hydrogen fluoride in acetonitrile affords the target compounds **2e, f** and **18e, f**.

The first procedure is also used to prepare lactones with a 2-naphthyl moiety **29a, b** (Scheme 6). Here, β-tetralone (**23**)

Scheme 5




2e, f 18e, f

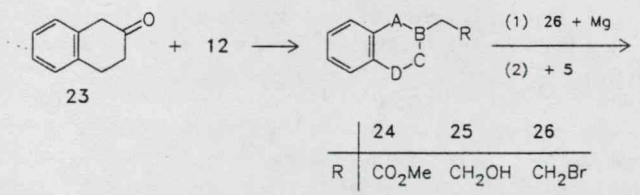
| 19 | R |
|---|---|
| a | OMe |
| b | Ph |

| 20 | $R^1$ | $R^2$ |
|---|---|---|
| a | Me | OMe |
| b | Me | Ph |
| c | H | OMe |
| d | H | Ph |

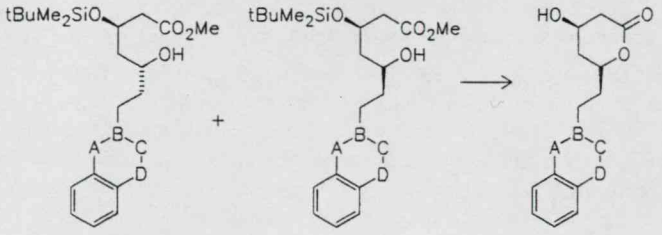
| 21, 22 | R | X |
|---|---|---|
| a | OMe | I |
| b | Ph | I |
| c | OMe | H |
| d | Ph | H |

is treated with the phosphonate ester anion to give the β,γ-unsaturated ester **24a**. Reduction of this ester with lithium aluminum hydride affords the alcohol **25a** which is converted into the corresponding bromide **26a** by reaction with phosphorus tribromide. Treatment of **26a** with magnesium

Scheme 6




| | 24 | 25 | 26 |
|---|---|---|---|
| R | $CO_2Me$ | $CH_2OH$ | $CH_2Br$ |




3

| 24 | A–B–C–D | 25 – 29 |
|---|---|---|
| a | $CH{=}C{-}CH_2{-}CH_2$ | a |
| b | $CH_2{-}CH{-}CH_2{-}CH_2$ | – |
| c | $CH{=}C{-}CH{=}CH$ | b |

furnishes the Grignard compound which is treated with formyl ester **5**. The obtained product is a 2:1 mixture of epimers **27a** and **28a**, which are separated by chromatography. Desilylation of these compounds with hydrogen fluoride and spontaneous lactonization furnish the desired products **3a** and **29a**, respectively.

The unsaturated ester **24a** has also been used as starting material in the preparation of **29b**. In this case catalytic reduction of ester **24a** over Pd catalyst yields a 2:1 mixture of tetrahydronaphthylacetate and naphthylacetate **24b** and **24c**, respectively, by partial disproportionation. As expected, the treatment of **24a** only with Pd catalyst affords a 1:1 mixture of **24b** and **24c**. The separated **24c** is then converted into **3b** according to the method described above for the synthesis of **3a** via the intermediates **25b**, **26b**, and **27b**.

The naphthalene analogs **2**, **3**, **18**, and **29** of mevinolin synthesized here are evaluated as inhibitors of HMG-CoA reductase by using mevinolin as a reference compound[19]. All compounds show moderate inhibitory activity. For example, the $IC_{50}$ value of **2f** is $6 \cdot 10^{-6}$ M. Compounds possessing an unnatural configuration on the lactone moiety (**18** and **29**) reveal no remarkable activity[20].

The authors are grateful to *Györgyi Gavacs* for her contribution to this work. Financial support from *Gedeon Richter Chemical Works. Ltd.*, *Institute for Drugresearch*, and *Hungarian OTKA Foundation* is gratefully acknowledged.

## Experimental

IR spectra were obtained with a Specord IR-75 (Carl Zeiss, Jena) spectrophotometer. — $^1$H- and $^{13}$C-NMR spectra were recorded with a JEOL FX-100 FT-NMR instrument at 100 and 25 MHz; internal standard TMS. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), heptet (h), multiplet (m), and broad (br.). — MS measurements were carried out with a Kratos MS-25 RFA combined GC/MS system (ionizing energy 70 eV, voltage 4 kV). — HPLC chromatographic analyses were performed with a Du Pont 830 instrument equipped with a UV detector or with a Waters 600 instrument equipped with a photodiode array detector 990. Stationary phase: straight-phase (S. P.) Perkin-Elmer Silica 5 μm (25 cm × 4.6 mm) or reverse-phase (R. P.) Finepack C-18 10 μm. — The silica gel used was obtained from Merck and that used for thin-layer chromatography was Kieselgel $PF_{254}$, whilst that employed for column chromatography was Kieselgel 60. — All solvents were dried by means of standard methods, and most reactions were carried out under argon.

*Dimethyl 3-[(tert-Butyldimethylsilyl)oxy]glutarate* (**7a**): To a stirred solution of **6**[21] (17.65 g, 0.1 mol) and imidazole (10.2 g, 0.15 mol) in dry DMF (100 ml) was added *tert*-butyldimethylsilyl chloride (22.6 g, 0.15 mol), and the resulting mixture was stirred at room temp. for 2 h. Crushed ice (300 g) was then added, and the mixture was extracted several times with hexane (500 ml). The organic extracts were combined, dried ($MgSO_4$), the solvent was evaporated under reduced pressure, and the residue was purified by distillation to give **7a** (25.5 g, 58.5%), colorless oil[22]. — B.p. 114–116°C/0.8 Torr. — $n_D^{25} = 1.433$. — IR (film): $\tilde{\nu} = 1720$ cm$^{-1}$ (CO). — $^1$H NMR: $\delta = 0.05$ (s, 6H, $2CH_3$), 0.75 (s, 9H, $3CH_3$), 2.46 (d, $J =$ 6 Hz, 4H, $2CH_2$), 3.58 (s, 6H, $2OCH_3$), 4.45 (t, $J = 6$ Hz, CH).

*1-Methyl Hydrogen (R)-3-[(tert-Butyldimethylsilyl)oxy]glutarate* (**8a**). — *Method A:* Pig liver acetone powder (7.5 g) was homogenized with 0.15 M phosphate buffer (pH = 8, 75 ml) and then centrifugated at 3000 g at room temp. for 1 min. To the obtained supernatant layer (pH value changed to 7.2 g during the extraction process) having 40 U/ml enzyme activity (measured on ethyl butyrate as a substrate at 25°C, pH = 8) **7a** (4.45 g, 15.3 mmol) was added and the pH value of the resulting well-stirred emulsion was kept within the range 6.9–7.1 by continuous addition of a 1 M sodium hydroxide solution. After consumption of 1 equivalent of the base (15 ml) the mixture was acidified to pH = 3 with concd. hydrochloric acid and then centrifugated at 3000 g for 1 min. The supernatant was washed three times with ethyl acetate (50 ml each), and the precipitate was washed once with ethyl acetate (50 ml). The combined ethyl acetate solutions were washed with brine (30 ml) and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded a crude product which was purified by chromatography with hexane/acetone (5:2) as eluent to give pure **8a** (3.6 g, 85%) as light yellow oil[23]. — $[\alpha]_D^{25} = +1.5$ ($c = 4$, $CHCl_3$), $[\alpha]_{546}^{25} = +2.0$ ($c = 4$, $CHCl_3$). — TLC ($CHCl_3$/EtOAc, 3:1): $R_f = 0.47$. — IR (film): $\tilde{\nu} = 3600-2500$ cm$^{-1}$ (COOH), 1740, 1710 (CO). — $^1$H NMR ($CCl_4$): $\delta = 0.08$ (s, 6H, $2CH_3$), 0.86 (s, 9H, $3CH_3$), 2.52 (d, $J = 6$ Hz, 2H, $CH_2$), 2.57 (d, $J = 6$ Hz, 2H, $CH_2$), 3.62 (s, 3H, $OCH_3$), 4.47 (m, 1H, OCH).

*Methyl (3R,1'R)-3-[(tert-Butyldimethylsilyl)oxy]-4-[N-(1'-phenylethyl)carbamoyl]butyrate:* To a stirred mixture of **8a** (0.56 g, 2 mmol), triethylamine (0.44 g, 4.4 mmol), and (*R*)-1-phenylethylamine (0.27 g, 2.2 mmol) in dry dichloromethane (5 ml) was added thionyl chloride (0.27 g, 2.3 mmol), and the resultant solution was stirred at room temp. for 2 h. The reaction mixture was diluted with dichloromethane (50 ml) and then successively washed with 10% hydrochloric acid (5 ml), saturated aqueous sodium hydrogen carbonate solution (5 ml), and brine (5 ml). After drying ($MgSO_4$) the solvent was evaporated in vacuo to yield 0.61 g (81%) of a light yellow oil[14]. — TLC (diisopropyl ether/EtOAc, 10:1): $R_f = 0.32$ and 0.44 [(3*R*) and (3*S*) isomers, respectively]. — HPLC [solvent: hexane/dioxane, 21:4; flow rate: 1 ml min$^{-1}$; detector: UV (254 nm); column: Partisil 5 μ (250 × 4.5 mm)]: $R_t = 21.7$ min (76%) and 23.4 min (24%) [(3*R*) and (3*S*) isomers, respectively]. — IR (film): $\tilde{\nu} = 3300$ cm$^{-1}$ (NH), 1725, 1630 (CO). — $^1$H NMR ($CDCl_3$): $\delta =$ 0.02 (s, 1.5H, 3*S* $SiCH_3$), 0.07 (s, 4.5H, 3*R*, $SiCH_3$), 0.8 [s, 6.75H, 3*R* $C(CH_3)_3$], 0.85 [s, 2.25H, 3*S* $C(CH_3)_3$], 1.44 (d, $J = 6$ Hz, 0.75H, 3*S* $CH_3$), 1.47 (d, $J = 6$ Hz, 2.25H, 3*R* $CH_3$), 2.42 and 2.44 (dd, $J = 6$ Hz, 2H, $CH_2$), 2.57 (d, $J = 6$ Hz, 2H, $CH_2$), 3.63 (s, 0.75H, 3*S* $OCH_3$), 3.67 (s, 2.25H, 3*R* $OCH_3$), 4.49 (m, 1H, OCH), 5.09 (m, 1H, NCH), 6.5 (m, 1H, NH), 7.28 (m, 5H, aromatic H).

*Method B:* To a stirred mixture of **8c** (1.5 g, 9.2 mmol) and imidazole (2.92 g, 43 mmol) in dry DMF was added *tert*-butyldimethylsilyl chloride (3.2 g, 21 mmol), and the resulting mixture was stirred at room temp. for 2 h. The reaction mixture was diluted with ether (40 ml) and then poured into ice/water (40 g). The organic layer was separated, and the aqueous layer was extracted with ether (60 ml). The combined organic layers were washed with a saturated $NH_4Cl$ solution, dried ($MgSO_4$), and the solvent was evaporated in vacuo to yield crude *methyl tert-butyldimethylsilyl 3-[(tert-butyldimethylsilyl)oxy]glutarate* (3.5 g, 97%).

The above described methylsilyl ester was dissolved in a 3:1:1 mixture of methanol, tetrahydrofuran and water (100 ml), and after the addition of potassium carbonate the mixture was stirred at room temp. for 1 h. The organic solvents were evaporated in vacuo, the residue was extracted with ether (30 ml), and then the aqueous layer was acidified with 10% hydrochloric acid (pH = 2.5). The solution was extracted three times with ether (20 ml each), the combined ethereal extracts were dried, and the solvent was evaporated in vacuo to give **8a**. — $[\alpha]_D^{20} = +2.9$ ($c = 2.6$, $CHCl_3$) (90% e.e.).

*Dimethyl 3-Acetoxyglutarate* (**7b**): To a stirred mixture of **6** (4.0 g, 23 mmol) and triethylamine (3.44 g, 34 mmol) in dry ether (40 ml) was added acetyl chloride (3.56 g, 3.25 ml, 45 mmol), and the resulting solution was stirred at room temp. for 18 h. The reaction mixture was diluted with ether (60 ml) and successively washed with 10% sodium hydroxide solution, saturated sodium hydrogen carbonate solution, and brine, and then dried ($MgSO_4$). Evaporation of the solvent gave an oily residue which was purified by column chromatography with hexane/acetone (10:1) as eluent to yield **7b** (4.5 g, 91%) as a colorless oil[24]. – TLC (hexane/acetone. 5:2): $R_f$ = 0.42. – IR (film): $\tilde{\nu}$ = 1735 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 2.0 (s, 3H, $CH_3$), 2.7 (d, $J$ = 6 Hz, 4H, $2CH_2$), 3.67 (s, 6H, $2OCH_3$), 5.48 (m, 1H, OCH).

*Methyl Hydrogen (R)-3-Acetoxyglutarate* (**8b**): Pig liver acetone powder (10 g) was homogenized with 0.15 M phosphate buffer (pH = 8, 100 ml) and then centrifugated at 3000 g at room temp. for 1 min. To the obtained supernatant (pH value changed to 7.1–7.3 during the extraction process) having 40 U/ml enzyme activity (measured on ethyl butyrate as a substrate at 25 °C, pH = 8) **7b** (4.4 g, 20 mmol) was added and the pH value of the resultant vigorously stirred emulsion was kept within the range of 6.9–7.1 by continuous addition of 1 M sodium hydroxide solution. After consumption of 1 equivalent of the base (20 ml), the mixture was acidified to pH = 3 with concd. hydrochloric acid and then centrifugated at 3000 g for 1 min. The supernatant layer was washed three times with ethyl acetate (50 ml each), and the precipitate was washed with ethyl acetate (50 ml). The combined ethyl acetate solutions were washed with brine (40 ml) and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave a crude product which was purified by chromatography with hexane/acetone (10:1) as eluent to yield **8b** (1.85 g, 45%) as a pale yellow oil. – TLC (hexane/acetone, 5:2): $R_f$ = 0.2. – $[\alpha]_D^{25}$ = +5.4 ($c$ = 5.5, $CHCl_3$) (90% e.e.) {ref.[21] $[\alpha]_D^{23}$ = +6.1 ($CHCl_3$) (100% e.e.)}. – IR (film): $\tilde{\nu}$ = 3700–2500 $cm^{-1}$ (COOH), 1730, 1700 (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 2.03 (s, 3H, $CH_3$), 2.71 (d, $J$ = 6 Hz, 2H, $CH_2$), 2.74 (d, $J$ = 6 Hz, 2H, $CH_2$), 3.68 (s, 3H, $OCH_3$), 5.46 (m, 1H, OCH).

*Methyl (3R,1'R)-3-Acetoxy-4-[N-(1'-phenylethyl)carbamoyl]butyrate:* To a stirred mixture of **8b** (0.41 g, 2 mmol), triethylamine (0.44 g, 4.4 mmol) and (*R*)-1-phenylethylamine (0.27 g, 2.2 mmol) in dry dichloromethane (5 ml) was added thionyl chloride (0.27 g, 0.17 ml, 2.3 mmol), and the resulting mixture was stirred at room temp. for 2 h. The reaction mixture was diluted with dichloromethane (50 ml) and then successively washed with 10% hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and brine. After drying ($MgSO_4$), the solvent was removed in vacuo to afford 0.53 g (86%) of a light yellow oil. – TLC (diisopropyl ether/EtOAc, 3:1): $R_f$ = 0.48. – HPLC [Partisil 5 µm (25 cm × 4.5 mm); eluent: diisopropyl ether/$CH_2Cl_2$, 1:4]: $R_t$ = 58.3 min. – IR (film): $\tilde{\nu}$ = 3300 $cm^{-1}$ (NH), 1730, 1635 (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 1.47 (d, $J$ = 7 Hz, 3H, $CH_3$), 1.97 (s, 3H, $CH_3$), 2.56 (d, $J$ = 6 Hz, 2H, $CH_2$), 2.7 and 2.72 (dd, $J$ = 6 Hz, 2H, $CH_2$), 3.67 (s, 3H, $OCH_3$), 5.1 (m, 1H, NCH), 5.45 (m, 1H, OCH), 5.97 (m, 1H, NH), 7.29 (m, 5H, aromatic H).

*Methyl Hydrogen (R)-3-Hydroxyglutarate* (**8c**): To a stirred solution of sodium methoxide (5.4 g, 0.1 mol) in dry methanol (150 ml) was added a solution of **8b** (3.1 g, 15 mmol) in dry methanol (60 ml), and the resultant mixture was stirred at room temp. for 8 h. The reaction mixture was acidified with 10% hydrochloric acid (pH = 2), and the solvent was evaporated in vacuo. The residue was poured into ice/water and then extracted with ether (150 ml). The ethereal solution was dried ($MgSO_4$) and the solvent was evaporated in vacuo to afford **8c** (1.5 g, 62%) as an oil[23]. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.2. – IR (film): = 3480 $cm^{-1}$ (OH), 1720, 1700 (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 2.54 (d, $J$ = 6 Hz, 2H, $CH_2$), 2.6 (d, $J$ = 6 Hz, 2H, $CH_2$), 3.68 (s, 3H, $OCH_3$), 4.45 (quint, $J$ = 6 Hz, 1H, OCH).

*Methyl (R)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxypentanoate* (**9**): To a stirred suspension of sodium tetrahydroborate (0.12 g, 3.6 mmol) in dry bis(2-methoxyethyl) ether (15 ml) was added dropwise a solution of $Et_2O$–$BF_3$ (6 ml) in dry bis(2-methoxyethyl) ether (10 ml) during 3 h, and the diborane generated was swept by a slow stream of argon into a flask containing a cooled solution of **8a** (4.1 g, 15 mmol) in dry tetrahydrofuran (20 ml). After cooling to 0 °C, the reaction was quenched by the addition of ice/water (30 ml), the mixture was basified (pH = 7.8–8) with potassium carbonate solution and then extracted four times with ether (20 ml each). The combined ethereal extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed in vacuo to afford **9** (3.5 g, 90%) as a light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3440 $cm^{-1}$ (OH), 1730 (CO). – $^1H$ NMR ($CCl_4$): $\delta$ = 0.05 (s, 3H, $CH_3$), 0.1 (s, 3H, $CH_3$), 0.85 (s, 9H, $3CH_3$), 1.65 (q, $J$ = 6 Hz, 2H, $CH_2$), 2.4 (d, $J$ = 6 Hz, $CH_2$), 3.0 (br. s, 1H, exchangeable with $D_2O$, OH), 3.55 (s, 3H, $OCH_3$), 3.6 (t, $J$ = 6 Hz, $OCH_2$), 4.22 (quint, $J$ = 6 Hz, OCH). – MS: $m/z$ (%) = 262 (3) [$M^+$], 245 (4) [$M^+$ – OH], 231 (6) [$M^+$ – $OCH_3$], 205 (40) [$M^+$ – $C_4H_9$], 173 (33), 131 (100), 75 (100), 73 (47).

$C_{12}H_{26}O_4Si$ (262.4) Calcd. C 54.92 H 9.99
Found C 54.70 H 9.82

*Methyl (R)-3-[(tert-Butyldimethylsilyl)oxy]-4-formylbutyrate* (**5**): To a stirred solution of **9** (2.5 g, 9.5 mmol) in dry dichloromethane (80 ml) was added pyridinium chlorochromate (15 g) in portions, and the resultant mixture was stirred at room temp. for 1 h. The solution was concentrated in vacuo to a volume of 25 ml and filtered through a short silica gel column. Evaporation of the solvent in vacuo afforded pure **5** (1.54 g, 62%) as a colorless oil. This compound was not stable for a long period of time at room temp. and was stored at 0 °C. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.65. – IR (film): $\tilde{\nu}$ = 1720 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, $3CH_3$), 2.54 (d, $J$ = 6 Hz, 2H, $CH_2$), 2.64 (m, 2H, $CH_2$), 3.66 (s, 3H, $OCH_3$), 4.62 (quint, $J$ = 6 Hz, 1H, OCH), 9.78 (t, $J$ = 2 Hz, 1H, CHO). – $^{13}C$ NMR ($CDCl_3$): $\delta$ = 4.92 ($SiCH_3$), 17.81 [$SiC(CH_3)_3$], 25.56 [$SiC(CH_3)_3$], 42.35 (C-2), 50.83 (C-4), 51.35 ($OCH_3$), 64.96 (C-3), 170.97 (C-1), 200.45 (C-5).

$C_{12}H_{24}O_4Si$ (260.4) Calcd. C 55.35 H 9.29
Found C 55.22 H 9.11

*Dimethyl 3-(Methoxymethoxy)glutarate* (**7d**): By analogy with the procedure used for the preparation of acetoxyglutarate **7b**, compound **6** was protected with chloromethyl methyl ether in 46% yield. – TLC (hexane/acetone, 5:2): $R_f$ = 0.44. – IR (film): $\tilde{\nu}$ = 1740 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): $\delta$ = 2.54 (d, $J$ = 6 Hz, 4H, $2CH_2$), 3.27 (s, 3H, $OCH_3$), 3.63 (s, 6H, $2OCH_3$), 4.27 (m, 1H. OCH), 4.56 (s, 2H, $OCH_2O$). – MS: $m/z$ (%) = 220 (<1) [$M^+$], 189 (13) [$M^+$ – $OCH_3$], 175 (3), 159 (3), 127 (27), 100 (14), 59 (14), 45 (100).

*Methyl Hydrogen 3-(Methoxymethoxy)glutarate* (**8d**): The enzymatic hydrolysis of **7d** was carried out as described for **7b** to yield racemic **8d** in 65% yield. – TLC (hexane/acetone. 5:2): $R_f$ = 0.19. – IR (film): $\tilde{\nu}$ = 3600–2500 $cm^{-1}$ (COOH), 1725. 1690 (CO). – $^1H$ NMR ($CDCl_3$): $\delta$ = 2.66 (d, $J$ = 6 Hz, 4H. $2CH_2$), 3.33 (s, 3H, $OCH_3$), 3.67 (s, 3H, $OCH_3$), 4.37 (m. 1H, OCH), 4.65 (s, 2H, $OCH_2O$). – MS: $m/z$ (%) = 206 (<1) [$M^+$], 175 (11) [$M^+$ – $OCH_3$], 145 (8), 127 (14), 113 (12), 100 (15), 45 (100).

*Methyl (3RS,1'R)-3-(Methoxymethoxy)-4-[N-(1'-phenylethyl)-carbamoyl]butyrate:* This compound was prepared from **8d** and

(*R*)-1-phenylethylamine in 78% yield as described for the synthesis of **8b**. – TLC (diisopropyl ether/acetone, 3:1): $R_f$ = 0.42. – IR (film): $\tilde{\nu}$ = 3300 $cm^{-1}$ (NH), 1730, 1640 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.46 (d, *J* = 7 Hz, 3H, $CH_3$), 2.51 (d, *J* = 6 Hz, 2H, $CH_2$), 2.58 and 2.64 (d, *J* = 6 Hz and d, *J* = 6 Hz, 2H, $CH_2$), 3.26 (s, 1.5H, $OCH_3$), 3.31 (s, 1.5H, $OCH_3$), 3.67 (s, 3H, $OCH_3$), 4.34 (m, 1H, OCH), 4.58 (s, 1H, $OCH_2O$), 4.66 (s, 1H, $OCH_2O$), 5.09 (m, 1H, NCH), 6.27 (m, 1H, NH), 7.28 (m, 5H, aromatic H). – MS: *m/z* (%) = 309 (7) [$M^+$], 278 (11) [$M^+ - OCH_3$], 264 (18), 127 (19), 120 (100), 106 (25), 105 (62), 103 (7), 91 (7), 79 (8), 77 (12), 46 (71).

*Dimethyl 3-(Phenylacetyloxy)glutarate* (**7e**): According to the procedure described for the preparation of **7b**, compound **6** was acetylated with phenylacetyl chloride in 56% yield. – TLC (hexane/acetone, 5:2): $R_f$ = 0.44. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 2.69 (d, *J* = 6 Hz, 4H, 2 $CH_2$), 3.58 (s, 2H, $CH_2$), 3.61 (s, 6H, 2 $OCH_3$), 5.50 (m, 1H, OCH), 7.26 (m, 5H, aromatic H). – MS: *m/z* (%) = 294 (8) [$M^+$], 263 (7) [$M^+ - OCH_3$], 176 (12), 127 (41), 118 (83), 99 (9), 91 (100).

An attempted enzymatic hydrolysis of **7e** resulted only in the formation of **6**.

*Preparation of Ethyl (Naphthylidene)acetates* **13**. – *General Procedure:* To a stirred solution of lithium diisopropylamide, prepared from 2.74 g (3.8 ml, 27.2 mmol) of diisopropylamine in 50 ml of freshly distilled tetrahydrofuran and 17.6 ml of *n*-butyllithium (1.4 M in hexane; 1.73 g, 27 mmol) at −78°C, was added dropwise a solution of 31 mmol of ethyl (trimethylsilyl)acetate (**11**) in 10 ml of tetrahydrofuran over a period of 15 min. After additional 10 min at −78°C, a solution of 12 mmol of the appropriate tetralone **10** in 10 ml tetrahydrofuran was added, and the resulting mixture was stirred at −78°C for 2 h, warmed to −20°C, stirred for 2 h, and then warmed to room temp. in the course of 2 h. The reaction was quenched by the addition of ice/water (100 ml). The organic layer was separated, the aqueous layer was extracted with ether (100 ml), and the combined organic solutions were successively washed with water, 5% sulfuric acid, saturated sodium hydrogen carbonate solution, and water and then dried ($MgSO_4$). Evaporation of the solvent gave a mixture of (*E*) and (*Z*) isomers which was separated or purified by column chromatography with hexane/EtOAc (7:3) as eluent.

*Ethyl (E)-(1,2,3,4-Tetrahydro-1-naphthylidene)acetate* (**13a**): Yield 0.97 g (37%) light yellow oil. – TLC (hexane/EtOAc, 9:1): $R_f$ = 0.63. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:5): $R_t$ = 27.5 min. – IR (film): $\tilde{\nu}$ = 1700 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 1.31 (t, *J* = 7 Hz, 3H, $CH_3$), 1.89 (q, *J* = 6 Hz, 2H, $CH_2$), 2.79 (t, *J* = 6 Hz, 2H, $CH_2$), 3.20 (td, *J* = 6 Hz and 1.5 Hz, 2H, $CH_2$), 4.20 (q, *J* = 7 Hz, 2H, $OCH_2$), 6.33 (t, *J* = 2 Hz, 1H, C=CH), 7.05–7.7 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 14.42 ($CH_3$), 22.76 (C-3'), 28.08 (C-4'), 30.22 (C-2'), 59.64 (O–$CH_2$), 112.53 (C-2), 124.79 (C-5'), 126.34 (C-8'), 129.14 (C-7'), 129.52 (C-6'), 134.23 (C-8a), 141.26 (C-4a), 152.62 (C-1'), 166.94 (C-1). – MS: *m/z* (%) = 216 (74) [$M^+$], 188 (23), 171 (78) [$M^+ - OC_2H_5$], 143 (82), 128 (100), 115 (64), 91 (25), 57 (24).

*Ethyl (Z)-(1,2,3,4-Tetrahydro-1-naphthylidene)acetate* (**13b**): Yield 0.91 g (35%) yellow oil. – TLC (hexane/EtOAc, 9:1): $R_f$ = 0.55. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:5): $R_t$ = 22.5 min. – IR (film): $\tilde{\nu}$ = 1705 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 1.24 (t, *J* = 7 Hz, 3H, $CH_3$), 1.96 (q, *J* = 6 Hz, 2H, $CH_2$), 2.51 (m, 2H, $CH_2$), 2.86 (t, *J* = 6 Hz, 2H, $CH_2$), 4.16 (q, *J* = 7 Hz, 2H, $OCH_2$), 5.79 (t, *J* = 1.5 Hz, 1H, C=CH), 7.0–7.7 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 14.13 ($CH_3$), 23.17 (C-3'), 29.19 (C-4'), 34.98 (C-2'), 59.97 ($OCH_2$), 114.52 (C-2), 124.70 (C-5'), 128.30 (C-8'), 129.32 (C-7'), 129.41 (C-6'), 133.21 (C-8a), 138.97 (C-4a), 155.66 (C-1'), 166.91 (C-1). – MS: *m/z* (%) = 216 (100) [$M^+$], 187 (19), 171 (82), [$M^+ - OC_2H_5$], 143 (68), 128 (91), 115 (60), 103 (14), 91 (18), 73 (17), 55 (26).

*Ethyl (E)-(1,2,3,4-Tetrahydro-8-methoxy-1-naphthylidene)acetate* (**13h**): Yield 1.82 g (40%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.74. – IR (film): $\tilde{\nu}$ = 1695 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.3 (t, *J* = 7 Hz, 3H, $CH_3$), 1.77 (t, *J* = 6 Hz, 2H, $CH_2$), 2.64 (t, *J* = 6 Hz, 2H, $CH_2$), 3.83 (s, 3H, $OCH_3$), 4.15 (q, *J* = 7 Hz, 2H, $OCH_2$), 6.74–7.17 (m, 4H, C=CH and aromatic H). – MS: *m/z* (%) = 246 (25) [$M^+$], 215 (100), [$M^+ - OCH_3$], 201 (30) [$M^+ - OCH_2CH_3$], 187 (92), 158 (44), 128 (54), 115 (60).

$C_{15}H_{18}O_3$ (246.3) Calcd. C 73.15 H 7.37 Found C 73.02 H 7.19

*Ethyl (Z)-(1,2,3,4-Tetrahydro-8-methoxy-1-naphthylidene)acetate* (**13i**): Yield 0.95 g (32%) yellow crystals. – M.p. 60–62°C. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.7. – IR (KBr): $\tilde{\nu}$ = 1700 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.16 (t, *J* = 7 Hz, 3H, $CH_3$), 1.87 (t, *J* = 6 Hz, 2H, $CH_2$), 2.48 (t, *J* = 6 Hz, 2H, $CH_2$), 2.73 (t, *J* = 6 Hz, 2H, $CH_2$), 3.72 (s, 3H, $OCH_3$), 4.2 (q, *J* = 7 Hz, 2H, $OCH_2$), 5.87 (t, *J* = 1.5 Hz, 1H, C=CH), 6.7–7.2 (m, 3H, aromatic H). – MS: *m/z* (%) = 246 (47) [$M^+$], 215 (100) [$M^+ - OCH_3$], 201 (28), 187 (61), 172 (14), 158 (25), 129 (19), 115 (19).

$C_{15}H_{18}O_3$ (246.3) Calcd. C 73.15 H 7.37 Found C 73.10 H 7.21

*Ethyl (1,2,3,4-Tetrahydro-7-methoxy-1-naphthylidene)acetate* (3:1 Mixture of (*E*) and (*Z*) Isomers **13e** and **13f**): Yield 2.25 g (76%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.67 and 0.7. – HPLC (hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 7.22 and 8.74 min. – IR (film): $\tilde{\nu}$ = 1720 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.3 (t, *J* = 7 Hz, 1.8H, $CH_3$), 1.38 (t, *J* = 7 Hz, 1.2H, $CH_3$), 1.9 (m, 2H, $CH_2$), 1.5–3.3 (m, 4H, 2 $CH_2$), 3.75 (s, 3H, $OCH_3$), 4.12 (q, *J* = 7 Hz, 1.2H, $OCH_2$), 4.18 (q, *J* = 7 Hz, 0.8H, $OCH_2$), 5.7 (m, 0.6H, C=CH), 6.2 (m, 0.4H, C=CH), 6.6–7.4 (m, 3H, aromatic H). – MS: *m/z* (%) = 246 (50) [$M^+$], 201 (60) [$M^+ - OCH_2CH_3$], 200 (100) [$M^+ - HOCH_2CH_3$], 186 (22), 172 (48) [$M^+ - HCO_2CH_2CH_3$], 158 (30), 128 (30), 115 (33).

$C_{15}H_{18}O_3$ (246.3) Calcd. C 73.15 H 7.37 Found C 73.32 H 7.28

*Ethyl (1,2,3,4-Tetrahydro-7-phenyl-1-naphthylidene)acetate* (3:1 Mixture of (*E*) and (*Z*) Isomers **13k** and **13l**): Yield 2.81 g (80%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.85. – HPLC (R.P.; MeOH/$H_2O$, 9:1): $R_t$ = 6.0 and 6.15 min. – IR (film): $\tilde{\nu}$ = 1705 $cm^{-1}$ (CO), 1615 (C=C). – $^1H$ NMR ($CCl_4$): δ = 1.18 [t, *J* = 7 Hz, 1.2H, $CH_3$, (*Z*) isomer], 1.25 [t, *J* = 7 Hz, 1.8H, $CH_3$, (*E*) isomer], 1.85 (m, 2H, $CH_2$), 2.4 (m, 2H, $CH_2$), 2.6–3.1 (m, 2H, $CH_2$), 4.05 (q, *J* = 7 Hz, 1.2H, $OCH_2$), 4.08 (q, *J* = 7 Hz, 0.8H, $OCH_2$), 5.81 [m, 0.6H, C=CH, (*Z*) isomer], 6.42 [m, 0.4H, C=CH, (*E*) isomer], 7.0–7.9 (m, 8H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 14.10 [(*Z*) isomer, $CH_3$], 14.36 [(*E*) isomer, $CH_3$], 22.73 [(*Z*), C-3'], 23.34 [(*E*), C-3'], 28.05 and 28.93 (C-4'), 29.84 [(*E*), C-2'], 35.13 [(*Z*), C-2'], 59.70 [(*E*), $OCH_2$], 60.05 [(*Z*), $OCH_2$], 112.65 [(*E*), C-2'], 114.78 [(*Z*), C-2], 123.38, 126.95, 127.27, 127.95, 128.33, 128.68, 128.73, 129.58, 133.30, 134.47, 137.57, 137.98, 139.24, 139.38, 140.70, 140.93, 152.46 [(*Z*), C-1'], 154.65 [(*E*), C-1'], 166.91 (C-1). – MS: *m/z* (%) = 292 (100) [$M^+$], 263 (20) [$M^+ - CH_2CH_3$], 247 (61) [$M^+ - OCH_2CH_3$], 246 (100) [$M^+ - HOCH_2CH_3$], 218 (52), 203 (42), 189 (32), 178 (24), 165 (26), 152 (18), 101 (18), 91 (10), 77 (9).

$C_{20}H_{20}O_2$ (292.4) Calcd. C 82.16 H 6.90 Found C 82.28 H 7.14

*Preparation of Ethyl(dihydronaphthyl)acetates* **13** *and* **24**. – *General Procedure:* To a stirred mixture of ethyl or methyl diethoxyphosphorylacetate (**12a** or **12b**) (0.1 mol) and tetralone **10** or **23**, respectively, (0.1 mol) in dry benzene (80 ml) was added dropwise a solution of sodium methoxide (6.0 g, 0.11 mol) in dry methanol (50 ml), and the resulting mixture was stirred at room temp. for 4 d. The reaction mixture was poured into ice/water (200 ml), the organic layer was separated, and the aqueous layer extracted with ether (300 ml). The combined organic layers were washed with water and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave an oil which was purified by column chromatography with hexane/EtOAc (9:1) as eluent.

*Ethyl (3,4-Dihydro-1-naphthyl)acetate* (**13d**): Yield 12.11 g (56%) yellow oil[25]. – TLC (hexane/EtOAc, 9:1): $R_f$ = 0.7. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 6.0 min. – IR (film): $\tilde{\nu}$ = 1720 $cm^{-1}$ (CO), 1615 (C=C). – $^1H$ NMR ($CCl_4$): δ = 1.18 (t, *J* = 7 Hz, 3H, $CH_3$), 2.3 (m, 2H, $CH_2$), 2.7 (m, 2H, $CH_2$), 3.28 (t, *J* = 1.5 Hz, 2H, $CH_2$), 4.03 (q, *J* = 7 Hz, 2H, $OCH_2$), 5.84 (t, *J* = 3.5 Hz, 1H, C=CH), 7.0 (m, 4H, aromatic H). –

MS: *m/z* (%) = 216 (34) [$M^+$], 171 (20) [$M^+ - C_2H_5OH$], 142 (54), 141 (95), 129 (50), 128 (100), 115 (71), 91 (15).

*Methyl (3,4-Dihydro-2-naphthyl)acetate* (**24a**): Yield 10.52 g (52%) light yellow oil[26]. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.67. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 4.5 min. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO), 1640 (C=C). – $^1H$ NMR ($CCl_4$): δ = 2.3 (m, 2H, $CH_2$), 2.7 (m, 2H, $CH_2$), 3.03 (s, 2H, $CH_2$), 3.6 (s, 3H, $OCH_3$), 7.0 (m, 4H, aromatic H). – MS: *m/z* (%) = 202 (20) [$M^+$], 141 (72) [$M^+ - CO_2CH_3$], 128 (100) [$M^+ - CH_2CO_2CH_3 + H$], 115 (83), 63 (21), 59 (20).

*Preparation of Alkyl (1,2,3,4-Tetrahydronaphthyl)acetates. – General Procedure:* A solution of the appropriate naphthylideneacetate **13** (50 mmol) in dry ethanol (100 ml) was shaken in an atmosphere of hydrogen with palladium/charcoal catalyst (0.4 g) for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The oily residue was purified by column chromatography with hexane/EtOAc (10:1) as eluent.

*Ethyl (1,2,3,4-Tetrahydro-1-naphthyl)acetate* (**13c**): Yield 8.95 g (82%) light yellow oil. – TLC (hexane/acetone, 5:2): $R_f$ = 0.62. – IR (film): $\tilde{\nu}$ = 1725 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 1.2 (t, *J* = 7 Hz, 3H, $CH_3$), 1.8 (m, 4H, $2CH_2$), 2.3–2.9 (m, 4H, $2CH_2$), 3.2 (m, 1H, CH), 4.05 (q, *J* = 7 Hz, 2H, $OCH_2$), 6.95 (m, 4H, aromatic H). – MS: *m/z* (%) = 218 (40) [$M^+$], 144 (97), 131 (100) [$M^+ - C_4H_7O_2$], 130 (89), 129 (65), 128 (33), 115 (17), 91 (16).

$C_{14}H_{18}O_2$ (218.3) Calcd. C 77.03 H 8.31 Found C 76.86 H 8.17

*Ethyl (1,2,3,4-Tetrahydro-8-methoxy-1-naphthyl)acetate* (**13g**): Yield 11.30 g (91%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.75. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.25 (t, *J* = 7 Hz, 3H, $CH_3$), 1.7–2.9 (m, 9H, $4CH_2$, CH), 3.8 (s, 3H, $OCH_3$), 4.15 (q, *J* = 7 Hz, 2H, $OCH_2$), 6.8–7.3 (m, 3H, aromatic H).

$C_{15}H_{20}O_3$ (248.3) Calcd. C 72.55 H 8.12 Found C 72.32 H 7.92

*Ethyl (1,2,3,4-Tetrahydro-7-methoxy-1-naphthyl)acetate* (**13j**): Yield 10.55 g (85%) pale yellow oil[30]. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.73. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 1.25 (t, *J* = 7 Hz, 3H, $CH_3$), 1.75 (m, 4H, $2CH_2$), 2.55 (m, 5H, $2CH_2$, CH), 3.68 (s, 3H, $OCH_3$), 4.05 (q, *J* = 7 Hz, $OCH_2$), 6.4–6.9 (m, 3H, aromatic H). – MS: *m/z* (%) = 248 (95) [$M^+$], 175 (40) [$M^+ - CO_2CH_2CH_3$], 174 (100) [$M^+ - HCO_2CH_2CH_3$], 161 (98) [$M^+ - CH_2CO_2CH_2CH_3$], 160 (92), 146 (30), 134 (30), 115 (30), 103 (18), 91 (28), 77 (18).

$C_{15}H_{20}O_3$ (248.3) Calcd. C 72.55 H 8.12 Found C 72.68 H 8.03

*Ethyl (1,2,3,4-Tetrahydro-7-phenyl-1-naphthyl)acetate* (**13m**): Yield 12.8 g (87%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.85. – IR (film): $\tilde{\nu}$ = 1735 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.22 (t, *J* = 7 Hz, 3H, $CH_3$), 1.85 (m, 4H, $2CH_2$), 2.7 (m, 5H, $2CH_2$, CH), 4.13 (q, *J* = 7 Hz, 2H, $OCH_2$), 7.0–7.6 (m, 8H, aromatic H). – MS: *m/z* (%) = 294 (30) [$M^+$], 220 (43) [$M^+ - CO_2CH_2CH_3 + H$], 207 (30), 193 (18), 140 (100), 91 (16).

$C_{20}H_{22}O_2$ (294.4) Calcd. C 81.60 H 7.53 Found C 81.36 H 7.42

*Methyl (1,2,3,4-Tetrahydro-2-naphthyl)acetate* (**24b**): Yield 7.15 g (70%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.79. – IR (film): $\tilde{\nu}$ = 1735 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 1.5–3.0 (m, 9H, $4CH_2$, CH), 3.55 (s, 3H, $OCH_3$), 6.95 (m, 4H, aromatic H). – MS: *m/z* (%) = 204 (17) [$M^+$], 130 (100) [$M^+ - CH_2CO_2CH_3 + H$], 129 (48), 128 (36), 116 (23), 115 (43), 91 (23).

$C_{13}H_{16}O_2$ (204.3) Calcd. C 76.44 H 7.90 Found C 76.18 H 7.68

*Methyl (2-Naphthyl)acetate* (**24c**): Yield 2.6 g (26%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.67. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CCl_4$): δ = 3.6 (s, m, 5H, $CH_2$, $OCH_3$), 7.0–7.7 (m, 7H, aromatic H). – MS: *m/z* (%) = 201 (9) [$M^+ + 1$], 200 (66) [$M^+$], 142 (12), 141 (100) [$M^+ - CO_2CH_3$], 139 (16), 115 (27), 63 (6), 59 (9).

$C_{13}H_{12}O_2$ (200.2) Calcd. C 77.98 H 6.04 Found C 78.12 H 6.25

*Preparation of 2-(1-Naphthyl)ethanols* **14** *and* **25**. – *General Procedure:* To a stirred suspension of lithium tetrahydridoaluminate (2.9 g, 0.075 mol) in dry ether (50 ml) was added dropwise a solution of the appropriate acetate **13** or **24** (0.04 mol) at 0°C. The resulting suspension was stirred at 0°C for 2 h and then hydrolyzed by sequential dropwise addition of wet ether (40 ml) and 5 ml of 2 N aqueous sodium hydroxide solution. The mixture was filtered, the solids were washed with ether (60 ml), and the combined filtrates and washings were washed with brine (20 ml) and then dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was purified by column chromatography.

*(E)-2-(1,2,3,4-Tetrahydro-1-naphthylidene)ethanol* (**14a**): Yield 4.32 g (62%) yellow oil. – TLC (hexane/acetone, 7:3): $R_f$ = 0.4. – IR (film): $\tilde{\nu}$ = 3350 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 1.9 (m, 2H, $CH_2$), 2.4 (m, 2H, $CH_2$), 2.8 (t, *J* = 6 Hz, 2H, $CH_2$), 4.3 (d, *J* = 6 Hz, 2H, $HOCH_2$), 6.15 (m, 1H, C=CH), 7.15 (m, 4H, aromatic H). – MS: *m/z* (%) = 174 (26) [$M^+$], 157 (21) [$M^+ - OH$], 147 (70), 130 (100) [$M^+ - C_2H_4O$], 128 (58), 115 (54), 77 (18), 63 (17), 51 (16).

$C_{12}H_{14}O$ (174.2) Calcd. C 82.72 H 8.10 Found C 82.47 H 8.02

The corresponding (*Z*) isomer: Yield 4.74 g (68%), oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.54. – IR (film): $\tilde{\nu}$ = 3350 $cm^{-1}$ (OH). – $^1H$ NMR ($CCl_4$): δ = 1.88 (m, 3H, $CH_2$, OH), 2.45 (t, *J* = 6 Hz, 2H, $CH_2$), 2.8 (t, *J* = 6 Hz, 2H, $CH_2$), 4.25 (d, *J* = 6 Hz, 2H, $OCH_2$), 5.52 (t, *J* = 6 Hz, 1H, C=CH), 7.05 (br. s, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 24.13 (C-3'), 29.66 (C-4'), 34.17 (C-2'), 60.37 (C-1), 124.84 (C-5'), 125.20 (C-2), 127.56 (C-8'), 128.38 (C-7'), 128.56 (C-6'), 135.02 (C-1'), 138.77 (C-4a'), 139.06 (C-8a').

*2-(1,2,3,4-Tetrahydro-1-naphthyl)ethanol* (**14b**): Yield 6.49 g (92%) light yellow crystals[28]. – M.p. 36–38°C. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.45. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 17 min. – IR (film): $\tilde{\nu}$ = 3350 $cm^{-1}$ (OH). – $^1H$ NMR ($CCl_4$): δ = 1.4–2.15 (m, 6H, $3CH_2$), 2.5–3.1 (m, 3H, $CH_2$, CH), 3.2 (br. s, 1H, exchangeable with $D_2O$, OH), 3.6 (t, *J* = 6 Hz, 2H, $OCH_2$), 6.85 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.63 (C-3'), 27.64 (C-2'), 29.49 (C-4'), 34.08 (C-1'), 39.61 (C-2), 60.73 (C-1), 125.46 (C-7' and C-6'), 128.47 (C-5'), 129.03 (C-8'), 136.93 (C-4a'), 140.64 (C-8a'). – MS: *m/z* (%) = 176 (19) [$M^+ + 1$], 175 (16) [$M^+$], 157 (23), 131 (100), 130 (43), 129 (36), 115 (42), 91 (34).

$C_{12}H_{16}O$ (176.3) Calcd. C 81.77 H 9.14 Found C 81.58 H 8.91

*2-(3,4-Dihydro-1-naphthyl)ethanol* (**14c**): Yield 4.81 g (69%) light yellow oil. – TLC (hexane/acetone, 5:2): $R_f$ = 0.48. – IR (film): $\tilde{\nu}$ = 3320 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 2.1 (m, 2H, $CH_2$), 2.72 (m, 4H, $2CH_2$), 3.73 (t, *J* = 6 Hz, 2H, $OCH_2$), 5.88 (t, *J* = 4.5 Hz, 1H, C=CH), 7.1 (m, 4H, aromatic H). – MS: *m/z* (%) = 174 (75) [$M^+$], 156 (22) [$M^+ - H_2O$], 141 (63), 129 (100) [$M^+ - C_2H_5O$], 115 (48), 91 (27), 77 (15).

$C_{12}H_{14}O$ (174.2) Calcd. C 82.72 H 8.10 Found C 82.77 H 7.86

*2-(1,2,3,4-Tetrahydro-8-methoxy-1-naphthyl)ethanol* (**14d**): Yield 5.36 g (65%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.47. – IR (film): $\tilde{\nu}$ = 3300 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 1.75 (m, 7H, $3CH_2$, CH), 2.75 (m, 2H, $CH_2$), 3.15 (br. s, 1H, exchangeable with $D_2O$, OH), 3.76 (q, *J* = 6 Hz, 2H, $OCH_2$), 3.78 (s, 3H, $OCH_3$), 6.6–7.1 (m, 3H, aromatic H). – MS: *m/z* (%) = 206 (12) [$M^+$], 188 (5) [$M^+ - OH_2$], 175 (4) [$M^+ - OCH_3$], 161 (100) [$M^+ - OCH_2CH_3$], 115 (27), 91 (27).

$C_{13}H_{18}O_2$ (206.3) Calcd. C 75.96 H 8.79 Found C 75.78 H 8.61

*2-(1,2,3,4-Tetrahydro-7-methoxy-1-naphthyl)ethanol* (**14e**): Yield 7.59 g (92%) light yellow oil[30]. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.43. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 26.7 min. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH). – $^1H$ NMR ($CCl_4$): δ = 1.7 (m, 6H, $3CH_2$), 2.7 (m, 4H, $CH_2$, CH, OH), 3.7 (t, *J* = 6 Hz, 2H, $OCH_2$), 6.3–6.9 (m, 3H, aromatic H). – MS: *m/z* (%) = 206 (40) [$M^+$], 175 (18) [$M^+ - CH_2OH$], 162 (80), 161 (100) [$M^+ - CH_2CH_2OH$], 146 (20), 134 (33), 121 (20), 115 (25), 91 (30), 77 (15).

$C_{13}H_{18}O_2$ (206.3) Calcd. C 75.69 H 8.79 Found C 75.50 H 8.66

*2-(1,2,3,4-Tetrahydro-7-phenyl-1-naphthyl)ethanol* (**14f**): Yield 8.17 g (81%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.4. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 1.6–2.2 (m, 6H, $3CH_2$), 1.9 (br. s, 1H, exchangeable with $D_2O$, OH), 2.76 (m, 2H, $CH_2$), 2.97 (m, 1H, CH), 3.74 (t, *J* = 7 Hz, 2H, $OCH_2$), 7.1–7.6 (m, 8H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.62 (C-3'), 27.67 (C-2'), 29.27 (C-4'), 34.28 (C-1'), 39.75 (C-2), 60.92 (C-1), 124.46 (C-8'), 126.91 (C-5'), 126.97 (C-2'' and C-6''), 127.32 (C-4''), 128.64 (C-3'' and C-5''), 129.61 (C-6'), 136.22 (C-4a'), 138.59 (C-8a'), 141.07 (C-1''), 141.34 (C-7').

$C_{18}H_{20}O$ (252.3) Calcd. C 85.67 H 7.99 Found C 85.40 H 7.75

*2-(3,4-Dihydro-2-naphthyl)ethanol* (**25a**): Yield 6.13 g (88%) light yellow oil[29]. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.4. – HPLC (S.P.: hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 18.5 min. – IR (film): $\tilde{\nu}$ = 3340 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 2.25 (m, 4H, $2CH_2$), 2.6 (m, 3H, $CH_2$, OH), 3.35 (t, $J$ = 6 Hz, 2H, $OCH_2$), 6.08 (s, 1H, C=CH), 6.9 (m, 4H, aromatic H). – MS: *m/z* (%) = 174 (56) [$M^-$], 156 (12) [$M^-$ – $H_2O$], 143 (100) [$M^+$ – $CH_2OH$], 141 (46), 128 (85), 115 (37), 91 (28), 77 (15), 63 (9).

$C_{12}H_{14}O$ (174.2) Calcd. C 82.72 H 8.10 Found C 82.51 H 8.32

*2-(2-Naphthyl)ethanol* (**25b**): Yield 6.75 g (98%) colorless semisolid. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.23. – HPLC (S.P.: hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 18.8 min. – IR (KBr): $\tilde{\nu}$ = 3300 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 1.6 (br. s, 1H, exchangeable with $D_2O$, OH), 3.0 (t, $J$ = 6 Hz, 2H, $CH_2$), 3.9 (t, $J$ = 6 Hz, 2H, $OCH_2$), 7.0–7.85 (m, 7H, aromatic H). – MS: *m/z* (%) = 172 (29) [$M^+$], 141 (100) [$M^-$ – $CH_2OH$], 130 (38), 129 (36), 115 (47).

$C_{12}H_{12}O$ (172.2) Calcd. C 83.68 H 7.03 Found C 83.77 H 7.24

*Preparation of (2-Bromoethyl)naphthalenes* **15** *and* **26**. – *General Procedure:* To a stirred mixture of 2-(naphthyl)ethanol **14** or **25** (0.03 mol) and pyridine (0.3 ml) in dry benzene (5 ml) at 0°C was added dropwise phosphorus tribromide (2.8 g, 1.88 ml, 0.01 mol), and the resulting mixture was stirred at room temp. for 6 h. The reaction mixture was poured into ice/water, the organic layer was separated, and the aqueous layer was extracted with ether (30 ml). The combined organic layers were washed with saturated sodium hydrogen carbonate solution and water and then dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was purified by column chromatography.

*(E)-1-(2-Bromoethylidene)-1,2,3,4-tetrahydronaphthalene* (**15a**): Yield 4.13 g (58%) yellow oil. – TLC (hexane/acetone, 5:1): $R_f$ = 0.78. – $^1H$ NMR ($CDCl_3$): δ = 2.1 (m, 2H, $CH_2$), 2.5 (m, 2H, $CH_2$), 2.8 (td, $J$ = 6 Hz and 1.5 Hz, 2H, $CH_2$), 3.38 (d, $J$ = 6 Hz, 2H, $CH_2$), 5.78 (m, 1H, C=CH), 7.0 (m, 4H, aromatic H).

$C_{12}H_{13}Br$ (237.1) Calcd. Br 33.70 Found Br 33.52

*1-(2-Bromoethyl)-1,2,3,4-tetrahydronaphthalene* (**15b**): Yield 4.81 g (67%) light yellow oil[28]. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.95. – $^1H$ NMR ($CCl_4$): δ = 1.35–2.15 (m, 6H, $3CH_2$), 2.3–3.0 (m, 3H, $CH_2$, CH), 3.17 (t, $J$ = 7 Hz, 2H, $BrCH_2$), 6.75 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.65 (C-3), 27.18 (C-2), 29.48 (C-4), 32.05 (C-1'), 36.06 (C-1), 39.83 (C-2'), 125.72 (C-7), 125.89 (C-6), 128.49 (C-5), 129.26 (C-8), 136.98 (C-4a), 139.58 (C-8a). – MS: *m/z* (%) = 240 (8), 238 (9) [$M^+$], 132 (10), 131 (100) [$M^+$ – $C_2H_4Br$], 129 (9), 128 (8), 115 (9), 91 (8).

$C_{12}H_{15}Br$ (239.2) Calcd. Br 33.42 Found Br 33.08

*1-(2-Bromoethyl)-3,4-dihydronaphthalene* (**15c**): Yield 6.05 g (85%) yellow oil. – TLC (hexane/acetone, 5:2): $R_f$ = 0.95. – $^1H$ NMR ($CCl_4$): δ = 2.2 (m, 2H, $CH_2$), 2.5–3.0 (m, 4H, $2CH_2$), 3.26 (t, $J$ = 6 Hz, 2H, $BrCH_2$), 5.78 (t, $J$ = 3 Hz, 1H, C=CH), 7.0 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 23.05 (C-3), 28.11 (C-4), 31.19 (C-2'), 36.51 (C-1'), 122.04 (C-2), 126.42 (C-7), 126.95 (C-6), 127.35 (C-8), 127.77 (C-5), 133.62 (C-8a), 133.80 (C-4a), 136.60 (C-1).

$C_{12}H_{13}Br$ (237.1) Calcd. Br 33.70 Found Br 33.51

*1-(2-Bromoethyl)-1,2,3,4-tetrahydro-8-methoxynaphthalene* (**15d**): Yield 6.00 g (79%) pale yellow oil. – TLC (hexane/EtOAc, 5:1): $R_f$ = 0.9. – $^1H$ NMR ($CCl_4$): δ = 1.7 (m, 6H, $3CH_2$), 2.1 (m, 1H, CH), 2.7 (m, 2H, $CH_2$), 3.4 (t, $J$ = 6 Hz, 2H, $BrCH_2$), 3.72 (s, 3H, $OCH_3$), 6.5–7.1 (m, 3H, aromatic H). – MS: *m/z* (%) = 270 (9), 268 (10) [$M^+$], 161 (100), 115 (9), 91 (8), 77 (4).

$C_{13}H_{17}BrO$ (269.2) Calcd. Br 29.69 Found Br 29.28

*1-(2-Bromoethyl)-1,2,3,4-tetrahydro-7-methoxynaphthalene* (**15e**): Yield 6.76 g (89%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.85. – HPLC (S.P.: hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 4.2 min. – $^1H$ NMR ($CCl_4$): δ = 1.75 (m, 4H, $2CH_2$), 2.1 (m, 2H, $CH_2$), 2.65 (m, 3H, $CH_2$, CH), 3.4 (t, $J$ = 6 Hz, $BrCH_2$), 3.7 (s, 3H, $OCH_3$), 6.4–7.0 (m, 3H, aromatic H). – MS: *m/z* (%) = 270 (13), 268 (14) [$M^+$], 188 (8) [$M^+$ – HBr], 175 (9), 161 (100) [$M^+$ – $CH_2CH_2Br$], 115 (18), 91 (12), 77 (8).

$C_{13}H_{17}BrO$ (269.2) Calcd. Br 29.69 Found Br 29.62

*1-(2-Bromoethyl)-1,2,3,4-tetrahydro-7-phenylnaphthalene* (**15f**): Yield 7.56 g (80%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.94. – $^1H$ NMR ($CDCl_3$): δ = 1.8 (m, 4H, $2CH_2$), 2.3 (m, 2H, $CH_2$), 2.8 (m, 3H, $CH_2$, CH), 3.55 (t, $J$ = 7 Hz, 2H, $BrCH_2$), 7.0–7.7 (m, 8H, aromatic H).

$C_{18}H_{19}Br$ (315.2) Calcd. Br 25.35 Found Br 25.21

*2-(2-Bromoethyl)-3,4-dihydronaphthalene* (**26a**): Yield 6.55 g (92%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.95. – $^1H$ NMR ($CCl_4$): δ = 2.18 (t, $J$ = 8 Hz, 2H, $CH_2$), 2.69 (t, $J$ = 8 Hz, 2H, $CH_2$), 2.78 (t, $J$ = 7 Hz, 2H, $CH_2$), 3.45 (t, $J$ = 7 Hz, 2H, $BrCH_2$), 6.23 (t, $J$ = 1 Hz, 1H, C=CH), 6.9 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 26.71 (C-3), 27.91 (C-4), 30.54 (C-2'), 40.45 (C-1'), 124.70, 125.66, 126.39, 126.57, 127.13, 134.12, 134.26, 137.86. – MS: *m/z* (%) = 238 (5), 236 (6) [$M^+$], 157 (17) [$M^+$ – Br], 143 (36), 141 (25), 129 (100), 128 (78), 115 (44).

$C_{12}H_{13}Br$ (237.1) Calcd. Br 33.70 Found Br 33.43

*2-(2-Bromoethyl)naphthalene* (**26b**): Yield 5.83 g (83%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.95. – $^1H$ NMR ($CCl_4$): δ = 3.3 (t, $J$ = 6 Hz, 2H, $CH_2$), 3.4 (t, $J$ = 6 Hz, 2H, $BrCH_2$), 7.1–7.7 (m, 7H, aromatic H). – MS: *m/z* (%) = 236 (22), 234 (22) [$M^+$], 155 (43) [$M^+$ – Br], 154 (21), 153 (21), 141 (100) [$M^+$ – $CH_2Br$], 128 (28), 127 (21), 115 (33).

$C_{12}H_{11}Br$ (235.1) Calcd. Br 33.99 Found Br 33.65

*Preparation of Alkyl 3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxynaphthylheptanoates* **16**, **17**, **27** *and* **28**. – *General Procedure:* To a stirred mixture of magnesium turnings (0.24 g, 10 mmol) in dry tetrahydrofuran was added dropwise the appropriate halide **15** or **26** and the resulting mixture was refluxed for 1 h. The solution was cooled to room temp. and added dropwise to a stirred solution of **5** (7.5 mmol) in tetrahydrofuran (10 ml). The resultant mixture was stirred at room temp. for 1 h. The reaction was quenched by the addition of ice/water (30 ml), the organic layer was separated, and the aqueous layer was extracted three times with ether (30 ml each). The combined organic solutions were washed with brine and dried ($MgSO_4$). Evaporation of the solvent gave an oily residue which was shown by HPLC analysis to be a 2:1 mixture of the diastereomers **16** and **17** or **27** and **28**. These isomers were separated by column chromatography.

*Methyl (3R,5R)-(E)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-1-naphthylidene)heptanoate* (**16a**): Yield 0.75 g (24%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.51. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH). – $^1H$ NMR ($CDCl_3$): δ = 0.03 (m, 6H, $2CH_3$), 0.83 (s, 9H, tBu), 2.5 (m, 12H, $6CH_2$), 3.64 (s, 3H, $OCH_3$), 4.26 (m, 1H, OCH), 4.6 (m, 1H, OCH), 5.87 (t, $J$ = 4.5 Hz, 1H, C=CH), 7.15 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 5.09 ($SiCH_3$), 17.84 and 25.68 (tBu), 23.10 (C-3'), 27.96 (C-6), 28.37 (C-4'), 34.51 (C-2'), 36.7 (C-4), 39.37 (C-2), 51.33 ($OCH_3$), 65.08 (C-3), 73.35 (C-5), 122.53 (C-7), 125.42 (C-8'), 126.36 (C-7'), 126.68 (C-6'), 127.59 (C-5'), 134.37 (C-8a'), 135.49 (C-4a'), 136.68 (C-1'), 171.06 (C-1).

*Methyl (3R,5S)-(E)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-1-naphthylidene)heptanoate* (**17a**): Yield 0.47 g (15%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.58. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1730 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.03 (m, 6H, $2CH_3$), 0.8 (m, 9H, tBu), 2.5 (m, 12H, $6CH_2$), 3.63 (s, 3H, $OCH_3$), 4.15 (m, 1H, OCH), 4.51 (m, 1H, OCH), 5.85 (m, 1H, C=CH), 7.1 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 4.86 ($SiCH_3$), 17.15 and 25.56 (tBu), 22.96 (C-3'), 27.87 (C-6), 28.22 (C-4'), 34.36 (C-2'), 38.43 (C-4), 40.04 (C-2), 51.30 ($OCH_3$), 66.19 (C-3), 76.31 (C-5), 122.41 (C-7), 125.45 (C-8'), 126.27 (C-7'), 126.59 (C-6'), 127.50 (C-5'), 134.17 (C-8a'), 135.16 (C-4a'), 136.54 (C-1'), 171.11 (C-1).

*Methyl (3R,5R,1'RS)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-1-naphthyl)heptanoate* (**16b**): Yield 0.63 g (20%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.65. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.09 (s, 6H, $2CH_3$), 0.90 (s, 9H, tBu), 1.79 (m, 10H, $5CH_2$), 2.6 (m, 2H, $CH_2$), 2.76 (m, 2H, $CH_2$), 2.8 (m, 1H, CH), 3.5 (s, 3H, $OCH_3$), 3.95 (m, 1H, OCH), 4.3 (m, 1H, OCH), 6.9 (m, 4H, aromatic H). – MS: *m/z* (%) = 420 (1) [$M^+$], 331 (5) [$M^+$ – $CH_3OH$ – $C_4H_9$], 256 (6), 144 (40), 131 (97), 129 (41), 115 (28), 101 (33), 91 (30), 75 (100).

*Methyl (3R,5R,1'RS)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-1-naphthyl)heptanoate* (**17b**): Yield 0.38 g (12%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.7. – IR (film): $\tilde{\nu}$ = 3360 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CCl_4$): δ = 0.02 (s, 6H, $2CH_3$), 0.8 (s, 9H, tBu), 1.3–2.1 (m, 10H, $5CH_2$), 2.1–3.1 (m, 5H, $2CH_2$, CH), 3.48 (s, 3H, $OCH_3$), 3.9–4.3 (m, 2H, 2OCH), 6.7–7.05 (m, 4H, aromatic H). – MS: *m/z* (%) = 420 (<1) [$M^+$], 331 (6) [$M^+$ – $CH_3OH$ – $C_4H_9$], 256 (6), 144 (44), 131 (93), 129 (38), 115 (27), 101 (35), 91 (28), 75 (100).

*Methyl (3R,5R)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(3,4-dihydro-1-naphthyl)heptanoate* (**16c**): Yield 0.79 g (25%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.6. – IR (film): $\tilde{\nu}$ = 3400 $cm^{-1}$ (OH), 1715 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.07 (s, 6H, $2CH_3$), 0.9 (s, 9H, tBu), 2.5 (m, 10H, $5CH_2$), 3.65 (s, 3H, $OCH_3$), 4.15 (m, 1H, OCH), 4.26 (m, 1H, OCH), 5.9 (t, *J* = 4 Hz, 1H, C=CH), 7.15 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 4.83 ($SiCH_3$), 17.93 and 25.71 (tBu), 23.14 (C-3'), 28.02 (C-6), 28.40 (C-4'), 34.52 (C-7), 36.51 (C-4), 41.98 (C-2), 51.48 ($OCH_3$), 65.05 (C-3), 74.44 (C-5), 122.59 (C-2'), 125.58 (C-8'), 126.39 (C-7'), 126.72 (C-6'), 127.65 (C-5'), 134.44 (C-8a'), 135.49 (C-4a'), 136.78 (C-1'), 171.21 (C-1). – MS: *m/z* (%) = 418 (<1) [$M^+$], 386 (3) [$M^+$ – $CH_3OH$], 254 (11), 169 (15), 143 (23), 142 (100), 141 (22), 128 (16), 101 (20), 75 (29), 73 (17).

*Methyl (3R,5S)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(3,4-dihydro-1-naphthyl)heptanoate* (**17c**): Yield 0.72 g (23%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.66. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.07 (s, 6H, $2CH_3$), 0.88 (s, 9H, tBu), 2.55 (m, 10H, $5CH_2$), 3.66 (s, 3H, $OCH_3$), 4.15 (m, 1H, OCH), 4.3 (m, 1H, OCH), 5.89 (t, *J* = 4 Hz, 1H, C=CH), 7.15 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 4.92 ($SiCH_3$), 17.87 and 25.68 (tBu), 23.10 (C-3'), 28.02 (C-6), 28.37 (C-4'), 34.57 (C-7), 38.63 (C-4), 42.41 (C-2), 51.45 ($OCH_3$), 66.34 (C-3), 76.46 (C-5), 122.56 (C-2'), 125.66 (C-8'), 126.42 (C-7'), 126.74 (C-6'), 127.64 (C-5'), 134.34 (C-8a'), 135.34 (C-4a'), 136.71 (C-1'), 171.29 (C-1). – MS: *m/z* (%) = 418 (<1) [$M^+$], 386 (4), 254 (12), 169 (18), 143 (26), 142 (100), 141 (25), 128 (16), 101 (22), 75 (30), 73 (19).

*Methyl (3R,5S)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-8-methoxy-1-naphthyl)heptanoate* (**16d**): Yield 0.41 g (12%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.8. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1735 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.9 (s, 9H, tBu), 1.2–2.1 (m, 12H, $6CH_2$), 2.7 (m, 3H, $CH_2$, CH), 3.75 (s, 3H, $OCH_3$), 3.9 (s, 3H, $OCH_3$), 4.1 (m, 2H, OCH), 6.6–7.2 (m, 3H, aromatic H).

*Methyl (3R,5S)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(1,2,3,4-tetrahydro-8-methoxy-1-naphthyl)heptanoate* (**17d**): Yield 0.27 g (8%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.84. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1730 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.2–2.1 (m, 12H, $6CH_2$), 2.7 (m, 3H, $CH_2$, CH), 3.7 (s, 3H, $OCH_3$), 3.9 (s, 3H, $OCH_3$), 4.0 (m, 2H, 2OCH), 6.6–7.2 (m, 3H, aromatic H).

*Methyl (3R,5R)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(3,4-dihydro-2-naphthyl)heptanoate* (**27a**): Yield 1.00 g (32%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.53. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CCl_4$): δ = 0.05 (s, 6H, $2CH_3$), 0.8 (s, 9H, tBu), 1.75 (m, 2H, $CH_2$), 2.0–2.8 (m, 10H, $5CH_2$), 3.65 (s, 3H, $OCH_3$), 4.2 (m, 2H, 2OCH), 6.15 (m, 1H, C=CH), 6.9 (m, 4H, aromatic H).

*Methyl (3R,5S)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(3,4-dihydro-2-naphthyl)heptanoate* (**28a**): Yield 0.91 g (29%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.7. – IR (film): $\tilde{\nu}$ = 3400 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CCl_4$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.5–2.8 (m, 12H, $6CH_2$), 3.55 (s, 3H, $OCH_3$), 4.05 (m, 2H, 2OCH), 6.1 (m, 1H, C=CH), 6.85 (m, 4H, aromatic H).

*Methyl (3R,5R)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(2-naphthyl)heptanoate* (**27b**): Yield 1.16 g (37%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3380 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CCl_4$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.8 (m, 4H, $2CH_2$), 2.2–2.8 (m, 4H, $2CH_2$), 3.65 (s, 3H, $OCH_3$), 4.1 (m, 2H, 2OCH), 7.0–7.7 (m, 7H, aromatic H). – MS: *m/z* (%) = 384 (9) [$M^+$], 327 (27) [$M^+$ – $C_4H_9$], 193 (11), 167 (34), 141 (100), 101 (33), 75 (38), 59 (11).

*Methyl (3R,5S)-3-[(tert-Butyldimethylsilyl)oxy]-5-hydroxy-7-(2-naphthyl)heptanoate* (**28b**): Yield 0.97 g (31%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.55. – IR (film): $\tilde{\nu}$ = 3360 $cm^{-1}$ (OH), 1730 (CO). – $^1H$ NMR ($CCl_4$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.8 (m, 4H, $2CH_2$), 2.2–2.8 (m, 4H, $2CH_2$), 3.55 (s, 3H, $OCH_3$), 3.95 (m, 2H, 2OCH), 7.0–7.7 (m, 7H, aromatic H). – MS: *m/z* (%) = 384 (5) [$M^+$], 327 (28) [$M^+$ – $C_4H_9$], 193 (11), 167 (30), 141 (100), 127 (22), 101 (48), 75 (61), 59 (10).

*Preparation of 4-Hydroxy-6-(2-naphthylethyl)-3,4,5,6-tetrahydro-2H-pyran-2-ones* **2a–d**, **18a–d**, **3**, *and* **29**. – *General Procedure:* To a stirred solution of the appropriate ester **16**, **17**, **27**, or **28** (3 mmol) in dry acetonitrile (200 ml) was added 48% hydrofluoric acid (10 ml), and the resultant solution was stirred at room temp. for 2 h. The reaction mixture was diluted with ether (200 ml), cooled to 0°C and mixed with a saturated solution of potassium hydrogen carbonate (70 ml). The organic layer was separated, washed with brine, and then dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography with hexane/EtOAc (3:2) as eluent.

*(4R,6R)-(E)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-1-naphthylidene)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2a**): Yield 0.42 g (52%) yellow oil. – TLC (hexane/EtOAc, 3:2): $R_f$ = 0.18. – IR (film): $\tilde{\nu}$ = 3420 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.9 (m, 4H, $2CH_2$), 2.2 (m, 2H, $CH_2$), 2.6 (m, 6H, $3CH_2$), 4.0 (br. s, 1H, OH), 4.3 (m, 1H, OCH), 4.7 (m, 1H, OCH), 5.88 (t, *J* = 4 Hz, 1H, C=CH), 7.15 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 23.14 (C-3''), 28.05 (C-1'), 28.40 (C-4''), 34.58 (C-2''), 36.01 (C-5), 38.67 (C-3), 62.63 (C-4), 75.79 (C-6), 122.56 (C-2'), 125.61 (C-8''), 126.42 (C-7''), 126.75 (C-6''), 127.68 (C-5''), 134.47 (C-8a''), 135.46 (C-4a''), 136.81 (C-1''), 170.92 (C-2).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 75.12 H 7.21

*(4R,6S)-(E)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-1-naphthylidene)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18a**): Yield 0.58 g (71%) yellow oil. – TLC (hexane/EtOAc, 3:2): $R_f$ = 0.16. – IR (film): $\tilde{\nu}$ = 3440 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.9 (m, 4H, $2CH_2$), 2.2 (m, 2H, $CH_2$), 2.7 (m, 6H, $3CH_2$), 4.25 (m, 2H, 2OCH), 5.89 (t, *J* = 4 Hz, 1H, C=CH), 7.15 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 23.05 (C-3''), 27.99 (C-1'), 28.32 (C-4''), 34.28 (C-2''), 37.68 (C-5), 39.43 (C-3), 63.59 (C-4), 76.40 (C-6), 122.50 (C-2'), 125.75 (C-8''), 126.39 (C-7''), 126.78 (C-6''), 127.68 (C-5''), 134.26 (C-8a''), 135.11 (C-4a''), 136.72 (C-1''), 171.41 (C-2).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 74.82 H 7.14

*(4R,6R,1'RS)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2b**): Yield 0.56 g (68%) light yellow oil. – TLC (hexane/EtOAc, 1:4): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.74 (m, 10H, $5CH_2$), 2.58 (m, 2H, $CH_2$), 2.72 (m, 2H, $CH_2$), 2.8 (m, 1H, CH), 3.77 (br. s, 1H, OH), 4.27 (m, 1H, OCH), 4.68 (m, 1H, OCH), 7.06 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.77 (C-3''), 27.43 (C-2''), 29.51 (C-4''), 31.56 and 31.64 (C-1'), 33.02 (C-2'), 35.50 (C-5), 37.11 and 37.20 (C-1''), 38.43 (C-3), 62.15 (C-4), 76.28 and 76.57 (C-6), 125.45 (C-6'' and C-7''), 128.32 (C-5''), 128.99 (C-8''), 136.95 (C-4a''), 140.40 (C-8a''), 171.46 (C-2). – MS: *m/z* (%) = 274 (3) [$M^+$], 256 (12) [$M^+$ – $H_2O$], 196 (30), 170 (11), 144 (80), 131 (100), 130 (27), 129 (40), 115 (20), 91 (5).

$C_{17}H_{22}O_3$ (274.4) Calcd. C 74.42 H 8.08 Found C 74.71 H 8.25

*(4R,6S,1'RS)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18b**): Yield 0.49 g (60%) light yellow oil. – TLC (hexane/EtOAc, 1:4): $R_f$ = 0.45. – IR (film): $\tilde{\nu}$ = 3400 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.76 (m, 10H, $5CH_2$), 2.5 (m, 2H, $CH_2$), 2.75 (m, 2H, $CH_2$), 2.8 (m, 1H, CH), 3.19 (br. s, 1H, OH), 4.21 (m, 2H, 2OCH), 7.08 (m, 4H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.86 (C-3''), 27.55 (C-2''), 29.60 (C-4''), 31.74 (C-1'), 33.17 (C-2'), 37.24 (C-1''), 37.76 (C-5), 39.49 (C-3), 63.71 (C-4), 77.49 and 77.75 (C-6), 125.61 (C-6'' and C-7''), 128.41 (C-5''), 129.14 (C-8''), 137.07 (C-4a''), 140.38 (C-8a''), 171.12 (C-2). – MS: *m/z* (%) = 274 (6) [$M^+$], 256 (12) [$M^+$ – $H_2O$], 196 (30), 170 (12), 144 (80), 131 (100), 130 (28), 129 (35), 91 (35).

$C_{17}H_{22}O_3$ (274.4) Calcd. C 74.42 H 8.08 Found C 74.54 H 8.17

*(4R,6R)-4-Hydroxy-6-[2-(3,4-dihydro-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2c**): Yield 0.42 g (52%) light yellow oil. – TLC (hexane/EtOAc, 3:2): $R_f$ = 0.25. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.95 (m, 4H, $2CH_2$), 2.2 (m, 2H, $CH_2$), 2.8 (m, 6H,

3 $CH_2$), 4.42 (m, 1H, OCH), 4.79 (m, 1H, OCH), 5.94 (t, $J$ = 4 Hz, C=CH), 7.2 (m, 4H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 23.13 (C-3"), 28.02 (C-1'), 28.37 (C-4"), 34.57 (C-2'), 36.03 (C-5), 38.69 (C-3), 62.53 (C-4), 75.78 (C-6), 122.53 (C-2"), 125.51 (C-8"), 126.39 (C-7"), 126.71 (C-6"), 127.65 (C-5"), 134.46 (C-8a"), 135.46 (C-4a"), 170.97 (C-2). – MS: *m/z* (%) = 272 (6) [$M^+$], 167 (5), 142 (100), 141 (21), 129 (10), 128 (12), 115 (5).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 74.81 H 7.59

*(4R,6S)-4-Hydroxy-6-[2-(3,4-dihydro-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18c**): Yield 0.63 g (77%) light yellow oil. – TLC (hexane/EtOAc, 3:2): $R_f$ = 0.17. – IR (film): $\tilde{\nu}$ = 3440 $cm^{-1}$ (OH), 1725 (CO). – $^1$H NMR ($CDCl_3$): δ = 1.9 (m, 4H, 2 $CH_2$), 2.2 (m, 2H, $CH_2$), 2.7 (m, 6H, 3 $CH_2$), 4.26 (m, 2H, 2OCH), 5.92 (t, $J$ = 4 Hz, 1H, C=CH), 7.2 (m, 4H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 23.04 (C-3"), 37.96 (C-1'), 28.31 (C-4"), 34.41 (C-2'), 37.64 (C-5), 39.42 (C-3), 63.50 (C-4), 76.95 (C-6), 122.47 (C-2"), 125.72 (C-8"), 126.39 (C-7"), 126.74 (C-6"), 127.68 (C-5"), 134.26 (C-8a"), 135.13 (C-4a"), 136.68 (C-1"), 171.52 (C-2). – MS: *m/z* (%) = 272 (6) [$M^+$], 167 (6), 142 (100), 141 (20), 129 (10), 128 (14), 115 (5).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 75.16 H 7.32

*(4R,6R)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-8-methoxy-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2d**): Yield 0.78 g (90%) pale yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.6. – IR (film): $\tilde{\nu}$ = 3440 $cm^{-1}$ (OH), 1730 (CO). – $^1$H NMR ($CCl_4$): δ = 1.1–1.8 (m, 8H, 4 $CH_2$), 2.1–2.4 (m, 4H, 2 $CH_2$), 2.8 (m, 3H, $CH_2$, CH), 3.6 (s, 3H, $OCH_3$), 4.1 (m, 1H, OCH), 4.5 (m, 1H, OCH), 6.5–7.1 (m, 3H, aromatic H).

$C_{18}H_{24}O_4$ (304.4) Calcd. C 71.03 H 7.95 Found C 71.28 H 8.16

*(4R,6S)-4-Hydroxy-6-[2-(1,2,3,4-tetrahydro-8-methoxy-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18d**): Yield 0.43 g (50%) pale yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.44. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1725 (CO). – $^1$H NMR ($CCl_4$): δ = 1.2–1.8 (m, 8H, 4 $CH_2$), 2.3 (m, 4H, 2 $CH_2$), 2.9 (m, 3H, $CH_2$, CH), 3.65 (s, 3H, $OCH_3$), 4.0 (m, 2H, 2OCH), 6.5–7.1 (m, 3H, aromatic H).

$C_{18}H_{24}O_4$ (304.4) Calcd. C 71.03 H 7.95 Found C 71.11 H 8.16

*(4R,6R)-4-Hydroxy-6-[2-(3,4-dihydro-2-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**3a**): Yield 0.48 g (46%) yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3440 $cm^{-1}$ (OH), 1725 (CO). – $^1$H NMR ($CDCl_3$): δ = 1.4–2.0 (m, 4H, 2 $CH_2$), 2.0–2.5 (m, 4H, 2 $CH_2$), 2.6–2.9 (m, 4H, 2 $CH_2$), 2.8 (br. s, 1H, OH), 4.33 (m, 1H, OCH), 4.73 (m, 1H, OCH), 6.24 (br. s, 1H, C=CH), 6.9–7.2 (m, 4H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 27.34 (C-3"), 28.05 (C-4"), 32.53 (C-1'), 33.43 (C-2'), 35.77 (C-5), 38.58 (C-3), 62.45 (C-4), 75.64 (C-6), 122.77, 125.43, 126.28, 126.42, 127.16, 134.32, 134.59, 140.49, 171.06 (C-2). – MS: *m/z* (%) = 272 (37) [$M^+$], 254 (8) [$M^+$ – $H_2O$], 194 (23), 168 (83), 167 (35), 143 (48), 142 (100), 141 (76), 128 (59), 115 (26).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 75.05 H 7.49

*(4R,6S)-4-Hydroxy-6-[2-(3,4-dihydro-2-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**29a**): Yield 0.52 g (64%) light yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.37. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1720 (CO). – $^1$H NMR ($CDCl_3$): δ = 1.4–2.0 (m, 4H, 2 $CH_2$), 2.0–2.6 (m, 4H, 2 $CH_2$), 2.7–3.0 (m, 4H, 2 $CH_2$), 3.31 (br. s, 1H, OH), 4.22 (m, 2H, 2O–CH), 6.23 (s, 1H, C=CH), 6.9–7.2 (m, 4H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 27.26 (C-3"), 28.02 (C-4"), 32.50 (C-1'), 33.29 (C-2'), 37.62 (C-5), 39.43 (C-3), 63.50 (C-4), 76.81 (C-6), 122.97, 125.46, 125.37, 126.45, 127.18, 134.29, 134.47, 140.20, 171.44 (C-2). – MS: *m/z* (%) = 272 (35) [$M^+$], 254 (6) [$M^+$ – $H_2O$], 194 (20), 168 (80), 167 (36), 143 (50), 142 (100), 141 (85), 128 (71), 115 (36).

$C_{17}H_{20}O_3$ (272.3) Calcd. C 74.97 H 7.40 Found C 75.13 H 7.62

*(4R,6R)-4-Hydroxy-6-[2-(2-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**3b**): Yield 0.45 g (55%) yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.42. – IR (film): $\tilde{\nu}$ = 3430 $cm^{-1}$ (OH), 1720 (CO). – $^1$H NMR ($CDCl_3$): δ = 1.4–2.1 (m, 4H, 2 $CH_2$), 2.6 (m, 2H, $CH_2$), 2.7–3.2 (m, 3H, $CH_2$, OH), 4.24 (m, 1H, OCH), 4.69 (m, 1H, OCH), 7.3–7.9 (m, 7H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 31.15 (C-1'), 35.72 (C-5), 37.06 (C-2'), 38.52 (C-3), 62.33 (C-4), 75.29 (C-6), 125.28, 125.99, 126.49, 127.13, 127.39, 127.56, 128.06, 132.01, 133.56, 138.50, 171.08 (C-2). – MS: *m/z* (%) = 270 (44) [$M^+$], 252 (4) [$M^+$ – $H_2O$], 192 (10), 179 (8), 167 (11), 155 (7), 142 (100), 141 (60), 128 (10), 115 (18).

$C_{17}H_{18}O_3$ (270.3) Calcd. C 75.53 H 6.71 Found C 75.34 H 6.92

*(4R,6S)-4-Hydroxy-6-[2-(2-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**29b**): Yield 0.70 g (86%) yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.38. – IR (film): $\tilde{\nu}$ = 3460 $cm^{-1}$ (OH), 1715 (CO). – $^1$H NMR ($CDCl_3$): δ = 1.4–2.6 (m, 4H, 2 $CH_2$), 2.7–3.0 (m, 5H, 2 $CH_2$, OH), 4.15 (m, 2H, 2OCH), 7.2–7.5 (m, 3H, aromatic H), 7.6–7.9 (m, 4H, aromatic H). – $^{13}$C NMR ($CDCl_3$): δ = 31.09 (C-1'), 36.92 (C-2'), 37.70 (C-5), 39.43 (C-3), 63.50 (C-4), 76.23 (C-6'), 125.37, 126.07, 126.60, 127.07, 127.42, 127.59, 128.15, 132.07, 133.56, 138.24, 171.24 (C-2). – MS: *m/z* (%) = 270 (28) [$M^+$], 252 (5) [$M^+$ – $H_2O$], 192 (12), 179 (9), 167 (13), 155 (7), 142 (100), 141 (68), 128 (10), 115 (20).

$C_{17}H_{18}O_3$ (270.3) Calcd. C 75.53 H 6.71 Found C 75.65 H 6.92

*Preparation of Alkyltriphenylphosphonium Bromides* **19**. – *General Procedure:* A mixture of triphenylphosphane (2.62 g, 10 mmol) and the appropriate alkyl halide **15a** or **15b** (9.8 mmol) in dry acetonitrile (10 ml) was stirred at reflux for 40 h. The solvent was evaporated and the residue was treated with ether, and then dried in a vacuum desiccator.

*[2-(1,2,3,4-Tetrahydro-7-methoxy-1-naphthyl)ethyl]-triphenylphosphonium Bromide* (**19a**): Yield 5.1 g (98%) colorless crystals. – M.p. 85°C. – $^1$H NMR ($CDCl_3$): δ = 1.8 (m, 6H, 3 $CH_2$), 2.65 (m, 3H, $CH_2$, CH), 3.45 (m, 2H, $PCH_2$), 3.68 (s, 3H, $OCH_3$), 6.6–7.1 (m, 3H, aromatic H), 7.65 (m, 15H, aromatic H).

*[2-(1,2,3,4-Tetrahydro-7-phenyl-1-naphthyl)ethyl]-triphenylphosphonium Bromide* (**19b**): Yield 5.49 g (97%) colorless semisolid. – $^1$H NMR ($CDCl_3$): δ = 1.95 (m, 6H, 3 $CH_2$), 2.7 (m, 3H, $CH_2$, CH), 3.4 (m, 2H, $CH_2$), 7.1–7.4 (m, 3H, aromatic H), 7.65 (m, 15H, aromatic H).

*Preparation of Alkyl Heptenoates* **20a, b**. – *General Procedure:* To a stirred and cooled (–70°C) suspension of phosphonium bromide **19** (4.2 mmol) in 125 ml of dry tetrahydrofuran was added dropwise 3.4 ml of *n*-butyllithium (1.4 M in hexane, 0.31 g, 4.6 mmol) and the resulting yellow solution was stirred at –20°C for 1 h. After cooling to –70°C, a solution of **5** (1.25 g, 4.82 mmol) in 40 ml of dry tetrahydrofuran was added dropwise, and the mixture was stirred at 0°C for 2 h. The reaction was quenched by the addition of acetic acid (2 ml), and the solvent was evaporated in vacuo. The residue was taken up in ether (200 ml) and filtered, and the filtrate was concentrated. The resulting oil was purified by column chromatography with hexane/EtOAc (7:3) as eluent.

*Methyl (3R,1'RS)-(Z)-3-[(tert-Butyldimethylsilyl)oxy]-7-(1,2,3,4-tetrahydro-7-methoxy-1-naphthyl)-5-heptenoate* (**20a**): Yield 1.42 g (78%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.83. – IR (film): $\tilde{\nu}$ = 1740 $cm^{-1}$ (CO), 1620 (C=C). – $^1$H NMR ($CDCl_3$): δ = 0.05 (s, 6H, 2 $CH_3$), 0.9 (s, 9H, tBu), 1.8 (m, 4H, 2 $CH_2$), 2.45 (m, 6H, 3 $CH_2$), 2.7 (m, 3H, $CH_2$, CH), 3.66 (s, 3H, $OCH_3$), 3.8 (s, 3H, $OCH_3$), 4.2 (m, 1H, OCH), 5.55 (m, 2H, CH=CH), 6.6–7.2 (m, 3H, aromatic H). – MS: *m/z* (%) = 432 (3) [$M^+$], 417 (50) [$M^+$ – $CH_3$], 401 (10) [$M^+$ – $OCH_3$], 375 (30) [$M^+$ – $C_4H_9$], 227 (20), 201 (22), 187 (23), 161 (100), 115 (21), 89 (55), 73 (65).

*Methyl (3R,1'RS)-(Z)-3-[(tert-Butyldimethylsilyl)oxy]-7-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-5-heptenoate* (**20b**): Yield 1.43 g (71%) light yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.67. – HPLC (R.P.; MeOH/$H_2O$, 7:3): $R_t$ = 5.1 min. – IR (film): $\tilde{\nu}$ = 1740 $cm^{-1}$ (CO), 1620 (C=C). – $^1$H NMR ($CDCl_3$): δ = 0.05 (s, 6H, 2 $CH_3$), 0.95 (s, 9H, tBu), 1.1–1.8 (m, 4H, 2 $CH_2$), 2.4 (m, 6H, 3 $CH_2$), 2.85 (m, 3H, $CH_2$, CH), 3.66 (s, 3H, $OCH_3$), 4.2 (m, 1H, OCH), 5.6 (m, 2H, CH=CH), 7.1–7.6 (m, 8H, aromatic H). – MS: *m/z* (%) = 478 (1) [$M^+$], 463 (6) [$M^+$ – $CH_3$], 421 (98) [$M^+$ – $C_4H_9$], 273 (35), 207 (100), 179 (30), 165 (25), 159 (42), 89 (65), 75 (42), 73 (75).

*Preparation of Heptenoic Acids* **20c, d**. – *General Procedure:* To a stirred solution of the appropriate alkyl heptenoate **20a, b** (3.4 mmol) in ethanol (60 ml) was added a 6.7% solution of sodium hydroxide (60 ml), and the resultant mixture was heated under

reflux for 4 h. The ethanol was distilled off in vacuo, the residue acidified with concd. HCl (10 ml) and then extracted with ether (4 × 50 ml). The combined ethereal extracts were washed with brine, dried ($MgSO_4$), the ether was evaporated, and the oily residue was purified by column chromatography with hexane/EtOAc (7:3) as eluent.

*(3R,1'RS)-(Z)-3-[(tert-Butyldimethylsilyl)oxy]-7-(1,2,3,4-tetrahydro-7-methoxy-1-naphthyl)-5-heptenoic acid* (**20c**): Yield 1.21 g (85%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 1715 $cm^{-1}$ (CO), 1620 (C=C). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.7 (m, 4H, $2CH_2$), 2.45 (m, 6H, $3CH_2$), 2.68 (m, 3H, $CH_2$, CH), 3.72 (s, 3H, $OCH_3$), 4.05 (m, 1H, OCH), 5.5 (m, 2H, CH=CH), 6.5–7.1 (m, 3H, aromatic H). – MS: *m/z* (%) = 418 (20) [$M^+$], 361 (40) [$M^+ - C_4H_9$], 287 (10), 269 (35), 227 (35), 201 (25), 187 (45), 161 (100), 75 (60).

*(3R,1'RS)-(Z)-3-[(tert-Butyldimethylsilyl)oxy]-7-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-5-heptenoic acid* (**20d**): Yield 1.12 g (71%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.67. – HPLC (S.P.; hexane/$CH_2Cl_2$/dioxane, 60:40:1): $R_t$ = 7.22 min. [(*Z*) isomer, 95%)] and 8.00 min [(*E*) isomer, 5%)]. – IR (film): $\tilde{\nu}$ = 1715 $cm^{-1}$ (CO), 1615 (C=C). – $^1H$ NMR ($CDCl_3$): δ = 0.08 (s, 6H, $2CH_3$), 1.8 (m, 4H, $2CH_2$), 2.45 (m, 6H, $3CH_2$), 2.8 (m, 3H, $CH_2$, CH), 4.12 (m, 1H, OCH), 5.55 (m, 2H, CH=CH), 7.05–7.7 (m, 8H, aromatic H). – MS: *m/z* (%) = 464 (20) [$M^+$], 407 (40) [$M^+ - C_4H_9$], 333 (30), 315 (25), 273 (50), 207 (100), 179 (30), 165 (28), 145 (22), 75 (80).

*Preparation of Iodo Lactones* **21a,b** *and* **22a,b**. – *General Procedure:* Iodine (9.0 g, 71 mmol) was added to a stirred mixture of potassium iodide (0.61 g, 3.6 mmol), the appropriate heptenoic acid derivative **20c,d** (2.4 mmol), and sodium hydrogen carbonate (1.6 g, 19 mmol) in 40 ml of tetrahydrofuran and 20 ml of water. The resulting mixture was stirred at room temp. for 3 h. A satd. sodium thiosulfate solution (150 ml) was added, and then the mixture was extracted with ether (200 ml). The ethereal solution was washed with brine and dried ($Na_2SO_4$). Removal of the solvent under reduced pressure gave a mixture of the isomers **21a,b** and **22a,b** which were separated or purified by column chromatography with hexane/EtOAc (9:1) as eluent.

*(4R,6S,1'RS,1"RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-methoxy-2-naphthyl)-1-iodoethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**21a**) *and Its (6R) Isomer* **22a**: Yield 1.10 g (84%) pale yellow oil. – TLC (hexane/acetone, 8:2): $R_f$ = 0.28. – IR (film): $\tilde{\nu}$ = 1725 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.15 (s, 6H, $2CH_3$), 1.9 (m, 8H, $4CH_2$), 2.7 (m, 5H, $2CH_2$, CH), 3.15 (m, 1H, ICH), 3.8 (s, 3H, $OCH_3$), 4.2 (m, 1H, OCH), 4.4 (m, 1H, OCH), 6.6–7.2 (m, 3H, aromatic H). – These isomers were not separated.

*(4R,6S,1'RS,1"RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-1-iodoethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**21b**): Yield 0.58 g (41%) light yellow oil. – TLC (hexane/acetone, 9:1): $R_f$ = 0.29. – IR (film): $\tilde{\nu}$ = 3460 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.1 (s, 6H, $2CH_3$), 0.9 (s, 9H, tBu), 1.85 (m, 6H, $3CH_2$), 2.1–2.9 (m, 7H, $3CH_2$, CH), 3.15 (m, 1H, ICH), 4.2 (m, 1H, OCH), 4.35 (m, 1H, OCH), 7.4 (m, 8H, aromatic H). – MS: *m/z* (%) = 590 (<1) [$M^+$], 533 (1) [$M^+ - C_4H_9$], 463 (3) [$M^+ - I$], 331 (20), 207 (100), 179 (15), 165 (13), 101 (60), 75 (60).

*(4R,6R,1'RS,1"RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-1-iodoethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**22b**): Yield 0.24 g (17%) light yellow oil. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1720 (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.1 (s, 6H, $2CH_3$), 0.9 (s, 9H, tBu), 1.9 (m, 6H, $3CH_2$), 2.2–2.9 (m, 7H, $3CH_2$, CH), 3.2 (m, 1H, ICH), 4.15 (m, 1H, OCH), 4.3 (m, 1H, OCH), 7.5 (m, 8H, aromatic H. – MS: *m/z* (%) = 590 (<1) [$M^+$], 533 (1) [$M^+ - C_4H_9$], 463 (5) [$M^+ - I$], 331 (20), 207 (100), 179 (17), 165 (14), 101 (63), 75 (62).

*Deiodination of Iodo Lactones* **21a,b** *and* **22a,b**. – *General Procedure:* To a stirred solution of the iodo lactones **21** or **22** (0.9 mmol) in dry benzene (20 ml) were added consecutively azobis(isobutyronitrile) (0.01 g, 0.06 mmol) and tributyltin hydride (0.75 g, 2.6 mmol) under argon, and the resultant mixture was heated at 60°C for 2 h. Benzene was removed in a rotary evaporator and the oily residue was transferred to a short silica gel column. After standing for 2 h, the product was eluted with hexane/EtOAc (7:3).

*(4R,6R,1'RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-methoxy-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**21c**) *and Its (6S) Isomer* **22c**: Starting with a mixture of **21a** and **21b**, the deiodination afforded a 2:1 mixture of the diastereomers **21c** and **22c**, which were separated by column chromatography.

**21c**: Yield 0.23 g (61%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.7. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.75 (m, 10H, $5CH_2$), 2.6 (m, 5H, $2CH_2$, CH), 3.75 (s, 3H, $OCH_3$), 4.15 (m, 1H, OCH), 4.3 (m, 1H, OCH), 6.5–7.1 (m, 3H, aromatic H).

$C_{24}H_{38}O_4Si$ (418.6) Calcd. C 68.85 H 9.15 Found C 68.56 H 8.92

**22c**: Yield 0.09 g (25%) yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.6. – IR (film): $\tilde{\nu}$ = 1725 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.87 (s, 9H, tBu), 1.75 (m, 10H, $5CH_2$), 2.55 (m, 5H, $2CH_2$, CH), 3.65 (s, 3H, $OCH_3$), 4.1 (m, 1H, OCH), 4.2 (m, 1H, OCH).

$C_{24}H_{38}O_4Si$ (418.6) Calcd. C 68.85 H 9.15 Found C 68.58 H 8.91

*(4R,6R,1'RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**21d**): Yield 0.34 g (81%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.75. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.8 (m, 8H, $4CH_2$), 2.55 (m, 2H, $CH_2$), 2.8 (m, 5H, $2CH_2$, CH), 4.2 (m, 1H, OCH), 4.65 (m, 1H, OCH), 7.35 (m, 8H, aromatic H). – MS: *m/z* (%) = 464 (3) [$M^+$], 407 (10) [$M^+ - C_4H_9$], 389 (12), 332 (8), 315 (10), 297 (18), 273 (22), 247 (80), 233 (20), 220 (40), 207 (100), 192 (20), 179 (25), 165 (30), 101 (65), 75 (60).

$C_{29}H_{40}O_3Si$ (464.7) Calcd. C 74.95 H 8.68 Found C 75.15 H 8.47

*(4R,6S,1'RS)-4-[(tert-Butyldimethylsilyl)oxy]-6-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**22d**): Yield 0.38 g (90%) pale yellow oil. – TLC (hexane/EtOAc, 7:3): $R_f$ = 0.62. – IR (film): $\tilde{\nu}$ = 1730 $cm^{-1}$ (CO). – $^1H$ NMR ($CDCl_3$): δ = 0.05 (s, 6H, $2CH_3$), 0.85 (s, 9H, tBu), 1.8 (m, 8H, $4CH_2$), 2.4 (m, 2H, $CH_2$), 2.75 (m, 5H, $2CH_2$, CH), 4.1 (m, 1H, OCH), 4.2 (m, 1H, OCH), 7.3 (m, 8H, aromatic H). – MS: *m/z* (%) = 464 (3) [$M^+$], 407 (10) [$M^+ - C_4H_9$], 389 (11), 332 (8), 315 (5), 297 (12), 273 (20), 247 (40), 233 (18), 220 (75), 207 (100), 192 (18), 179 (30), 165 (30), 101 (71), 75 (80).

$C_{29}H_{40}O_3Si$ (464.7) Calcd. C 74.95 H 8.68 Found C 75.18 H 8.92

*Removal of the Silyl Protecting Group from the Lactones* **21** *and* **22**. – *General Procedure:* To a stirred solution of the lactone **21d** or **22d** (0.6 mmol) in dry acetonitrile (45 ml) was added dropwise 48% hydrofluoric acid (2 ml), and the resulting mixture was stirred at room temp. for 2 h. After cooling to 0°C, the mixture was neutralized with a satd. sodium carbonate solution, the organic layer was separated, and the aqueous layer was extracted with ether (100 ml). The combined organic layers were washed with brine, dried ($MgSO_4$), concentrated under reduced pressure, and the residue was purified by column chromatography with EtOAc/hexane (4:1) as eluent.

*(4R,6R,1'RS)-4-Hydroxy-[2-(1,2,3,4-tetrahydro-7-methoxy-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2e**): Yield 0.17 g (92%) light yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.6. – IR (film): $\tilde{\nu}$ = 3430 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.73 (m, 10H, $5CH_2$), 2.68 (m, 6H, $2CH_2$, CH, OH), 3.78 (s, 3H, $OCH_3$), 4.36 (m, 1H, OCH), 4.70 (m, 1H, OCH), 6.66 (m, 1H, aromatic H), 6.71 (s, 1H, aromatic H), 6.97 (d, *J* = 8 Hz, 1H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 20.33 (C-3"), 27.70 (C-2"), 28.90 (C-4"), 31.71 and 31.77 (C-1'), 33.17 (C-2'), 36.13 (C-5), 37.70 (C-1"), 38.76 (C-3), 55.40 (O–$CH_3$), 62.77 (C-4), 76.23 (C-6), 111.77 (C-6"), 113.70 (C-8"), 129.44 (C-4a"), 129.93 (C-5"), 141.69 (C-8a"), 157.72 (C-7"), 170.42 (C-2). – MS: *m/z* (%) = 304 (30) [$M^+$], 286 (12) [$M^+ - H_2O$], 200 (8), 174 (68), 161 (100), 159 (75), 128 (15), 115 (18), 91 (20).

$C_{18}H_{24}O_4$ (304.4) Calcd. C 71.03 H 7.95 Found C 71.28 H 8.16

*(4R,6R,1'RS)-4-Hydroxy-[2-(1,2,3,4-tetrahydro-7-methoxy-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18e**): Yield 0.17 g (91%) light yel-

low oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3450 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.7 (m, 10H, $5CH_2$), 2.7 (m, 6H, $2CH_2$, CH, OH), 3.79 (s, 3H, $OCH_3$), 4.30 (m, 2H, 2OCH), 6.65 (m, 1H, aromatic H), 6.72 (s, 1H, aromatic H), 6.98 (m, 1H, aromatic H). – MS: *m/z* (%) = 304 (25) [$M^+$], 286 (8) [$M^+ - H_2O$], 200 (5), 174 (56), 161 (100), 159 (62), 128 (17), 115 (18), 91 (23).

$C_{18}H_{24}O_4$ (304.4) Calcd. C 71.03 H 7.95 Found C 70.85 H 7.68

*(4R,6R,1'RS)-4-Hydroxy-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**2f**): Yield 0.15 g (71%) light yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.5. – IR (film): $\tilde{\nu}$ = 3460 $cm^{-1}$ (OH), 1715 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.75 (m, 10H, $5CH_2$), 2.56 (m, 2H, $CH_2$), 2.77 (m, 2H, $CH_2$), 2.8 (m, 1H, CH), 3.10 (br. s, 1H, OH), 4.25 (m, 1H, OCH), 4.67 (m, 1H, OCH), 7.12 (d, $J$ = 8 Hz, 1H, aromatic H), 7.4 (m, 7H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.74 (C-3''), 27.38 (C-2''), 29.34 (C-4''), 31.80 (C-1'), 33.17 (C-2'), 35.63 (C-5), 37.47 (C-1''), 38.49 (C-3), 62.33 (C-4), 76.40 (C-6), 124.49 (C-8''), 126.95 (C-5''), 127.10 (C-4'''), 128.68 (C-3''' and C-5'''), 129.58 (C-6''), 136.25 (C-4a''), 138.53 (C-8a''), 140.93 (C-1'''), 141.25 (C-7''), 171.15 (C-2). – MS: *m/z* (%) = 350 (50) [$M^+$], 332 (30) [$M^+ - H_2O$], 315 (10), 272 (7), 247 (25), 220 (70), 207 (100), 205 (58), 192 (30), 191 (30), 179 (35), 165 (38).

$C_{23}H_{26}O_3$ (350.4) Calcd. C 78.82 H 7.48 Found C 78.95 H 7.28

*(4R,6R,1'RS)-4-Hydroxy-[2-(1,2,3,4-tetrahydro-7-phenyl-1-naphthyl)-ethyl]-3,4,5,6-tetrahydro-2H-pyran-2-one* (**18f**): Yield 0.20 g (93%) light yellow oil. – TLC (EtOAc/hexane, 4:1): $R_f$ = 0.48. – IR (film): $\tilde{\nu}$ = 3460 $cm^{-1}$ (OH), 1725 (CO). – $^1H$ NMR ($CDCl_3$): δ = 1.79 (m, 10H, $5CH_2$), 2.65 (m, 2H, $CH_2$), 2.77 (m, 2H, $CH_2$), 2.8 (br. s, 1H, OH), 2.87 (m, 1H, CH), 4.15 (m, 2H, 2OCH), 7.12 (d, $J$ = 8 Hz, 1H, aromatic H), 7.2–7.6 (m, 7H, aromatic H). – $^{13}C$ NMR ($CDCl_3$): δ = 19.92 (C-3''), 27.58 (C-2''), 29.34 (C-4''), 31.85 (C-1'), 33.29 (C-2'), 37.56 (C-1''), 37.82 (C-5), 39.52 (C-3), 63.74 (C-4), 77.63 (C-6), 124.58 (C-8''), 127.01 (C-4'''), 128.71 (C-3''' and C-5'''), 129.61 (C-6''), 136.34 (C-4a''), 138.68 (C-8a''), 140.84 (C-1'''), 141.37 (C-7''), 170.86 (C-2). – MS: *m/z* (%) = 350 (25) [$M^+$], 332 (21) [$M^+ - H_2O$], 315 (5), 272 (5), 247 (6), 220 (90), 207 (100), 205 (78), 192 (28), 191 (30), 179 (38), 165 (40).

$C_{23}H_{26}O_3$ (350.4) Calcd. C 78.82 H 7.48 Found C 78.57 H 7.31

*Isolation and Assay of HMG-CoA Reductase:* Microsomes were prepared from livers of rats that had been maintained on rat chow-containing cholestyramine for 7 d. HMG-CoA reductase was solubilized from the microsomes according to the method of Heller and Schrewsberg[31] and purified by means of the method of Kleinsek et al[32]. The enzyme assay was accomplished according to the method of Alberts et al[19], which was slightly modified. Before assay, the compounds were converted into their ring-opened sodium dihydroxycarboxylate salts and then were incubated with 4.7 µg of enzyme (specific activity 18 nmol · $min^{-1}$ · $mg^{-1}$), 50 µM of $^{14}C$-HMG-CoA (specific activity 2.71 mCi/mmol), 2 µM of NADPH in 160 mM of potassium phosphate puffer at 37 °C for 20 min. After the isolation and determination of radioactive mevalonate, the $IC_{50}$ values of the compounds were calculated on the basis of their percent inhibitions. Mevinolin was used as reference compound, its $IC_{50}$ value was $9.1 \cdot 10^{-9}$ M.

## CAS Registry Numbers

**2a**: 137847-53-1 / **2b** (isomer 1): 137847-67-7 / **2b** (isomer 2): 137847-93-9 / **2c**: 137847-95-1 / **2d** (isomer 1): 137847-97-3 / **2d** (isomer 2): 137848-25-0 / **2e** (isomer 1): 137848-08-9 / **2e** (isomer 2): 137848-18-1 / **2f** (isomer 1): 137848-10-3 / **2f** (isomer 2): 137848-20-5 / **3a**: 137847-54-2 / **3b**: 137847-99-2 / **5**: 121980-45-8 / **6**: 7250-55-7 / **7a**: 112904-68-4 **7b**: 90613-44-8 / **7d**: 112904-67-3 / **7e**: 137847-71-3 **8a**: 109744-49-2 / **8b**: 26432-16-6 / **8c**: 87118-53-4 / **8d**: 112904-71-9 / **9**: 119136-74-2 / **10a**: 529-34-0 / **10b**: 13185-18-7 / **10c**: 6836-19-7 / **10d**: 41526-73-2 / **11**: 4071-88-9 / **12a**: 867-13-0 / **12b**: 1067-74-9 / **13a**: 94834-50-1 / **13b**: 94834-49-8 / **13c**: 137847-77-9 / **13d**: 54125-45-0 / **13e**: 137895-06-8 / **13f**: 137847-74-6 / **13g**: 137847-78-0 **13h**: 137847-72-4 / **13i**: 137847-73-5 / **13j**: 137847-79-1 / **13k**: 137847-75-7 / **13l**: 137847-76-8 / **13m**: 137847-80-4 / (*E*)-**14a**: 137847-55-3 / (*Z*)-**14a**: 137847-81-5 / **14b**: 137847-82-6 / **14c**: 4725-34-2 / **14d**: 137847-83-7 / **14e**: 137847-84-8 / **14f**: 137847-85-9 / **15a**: 137847-56-4 / **15b**: 137868-48-5 / **15c**: 137847-86-0 / **15d**: 137868-49-6 / **15e**: 137868-50-9 / **15f**: 137847-87-1 / **16a**: 137847-57-5 / **16b** (isomer 1): 137940-78-4 / **16b** (isomer 2): 137847-88-2 / **16c**: 137847-89-3 / **16d** (isomer 1): 137940-82-0 / **16d** (isomer 2): 137939-86-7 / **17a**: 137847-58-6 / **17b** (isomer 1): 137940-81-9 / **17b** (isomer 2): 137939-72-1 / **17c**: 137847-90-6 / **17d** (isomer 1): 137847-91-7 / **17d** (isomer 2): 137940-84-2 / **18a**: 137847-59-7 / **18b** (isomer 1): 137847-94-0 / **18b** (isomer 2): 137848-11-4 / **18c**: 137847-96-2 / **18d** (isomer 1): 137847-98-4 / **18d** (isomer 2): 137848-26-1 / **18e** (isomer 1): 137848-09-0 / **18e** (isomer 2): 137848-19-2 / **18f** (isomer 1): 137848-21-6 / **18f** (isomer 2): 137848-22-7 / **19a**: 137847-60-0 / **19b**: 137848-01-2 / **20a** (isomer 1): 137847-61-1 / **20a** (isomer 2): 137868-51-0 / **20b** (isomer 1): 137848-02-3 / **20b** (isomer 2): 137848-12-5 / **20c** (isomer 1): 137848-03-4 / **20c** (isomer 2): 137848-13-6 / **20d** (isomer 1): 137848-04-5 / **20d** (isomer 2): 137848-14-7 / **21a** (isomer 1): 137847-62-2 / **21a** (isomer 2): 137939-74-3 / **21a** (isomer 3): 137939-75-4 / **21a** (isomer 4): 137939-76-5 / **21b** (isomer 1): 137940-83-1 / **21b** (isomer 2): 137939-80-1 / **21b** (isomer 3): 137939-81-2 / **21b** (isomer 4): 137939-82-3 / **21c** (isomer 1): 137868-35-0 / **21c** (isomer 2): 137848-15-8 / **21d** (isomer 1): 137848-07-8 / **21d** (isomer 2): 137848-16-9 / **22a** (isomer 1): 137939-76-9 / **22a** (isomer 2): 137939-77-6 / **22a** (isomer 3): 137939-78-7 / **22a** (isomer 4): 137939-79-8 / **22b** (isomer 1): 137848-05-6 / **22b** (isomer 2): 137939-83-4 / **22b** (isomer 3): 137939-84-5 / **22b** (isomer 4): 137939-85-6 / **22c** (isomer 1): 137848-06-7 / **22c** (isomer 2): 137895-05-7 / **22d** (isomer 1): 137939-73-2 / **22d** (isomer 2): 137848-17-0 / **23**: 530-93-8 / **24a**: 41791-31-5 / **24b**: 137939-71-0 / **24c**: 2876-71-3 / **25a**: 63626-01-7 / **25b**: 1485-07-0 / **26a**: 137847-63-3 / **26b**: 2086-62-6 / **27a**: 137847-64-4 / **27b**: 137915-27-6 / **28a**: 137847-65-5 / **28b**: 137847-92-8 / **29a**: 137847-66-6 / **29b**: 137848-00-1 / HGM-CoA reductase: 9028-35-7 / methyl (3*R*,1*R'*)-3-[(*tert*-butyldimethylsilyl)oxy]-4-[*N*-(1'-phenylethyl)carbamoyl]butyrate: 137847-68-8 / (*R*)-1-phenylethylamine: 9131-70-1 / methyl *tert*-butyldimethylsilyl 3-[(*tert*-butyldimethylsilyl)oxy]glutarate: 91424-35-0 / methyl (3*R*,1'*R*)-3-acetoxy-4-[*N*-(1'-phenylethyl)carbamoyl]-butyrate: 137847-69-9 / methyl (3*R*,1'*R*)-3-(methoxymethoxy)-4-[*N*-(1'-phenylethyl)carbamoyl]butyrate: 137847-70-2 / methyl (3*S*,1'*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-4-[*N*-(1'-phenylethyl)carbamoyl]butyrate: 137848-23-8 / methyl (3*S*,1'*R*)-3-(methoxymethyl)-4-[*N*-(1'-phenylethyl)carbamoyl]butyrate: 137848-24-9


[1] B. D. Roth, D. R. Sliskovic, B. K. Trivedi, *Annu. Rep. Med. Chem.* **1989**, *24*, 147–156.
[2] S. M. Grundy, *N. Engl. J. Med.* **1988**, *319*, 24–32.
[3] J. L. Goldstein, M. S. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2804–2814.
[4] A. Endo, M. Kuroda, Y. Tsujita, *J. Antibiot.* **1976**, *29*, 1346–1348.
[5] W. F. Hoffman, A. W. Alberts, P. S. Anderson, J. S. Chen, R. L. Smith, A. K. Willard, *J. Med. Chem.* **1986**, *29*, 849–852.
[6] A. Endo, *J. Med. Chem.* **1985**, *28*, 401–405.
[7] *Drugs Future* **1987**, *12*, 437–442.
[8] *Drugs Future* **1988**, *13*, 531–533.
[9] *Drugs Future* **1988**, *13*, 475–476.
[10] T. Rosen, C. H. Heathcock, *Tetrahedron* **1986**, *42*, 4909–4951.
[11] G. Beck, K. Kesseler, E. Baader, W. Bartmann, A. Bergmann, E. Granzer, H. Jendralla, B. V. Kerekjarto, R. Krause, E. Paulus, W. Schubert, G. Wess, *J. Med. Chem.* **1990**, *33*, 52–60, and references cited therein.
[12] D. R. Sliskovic, J. A. Picard, W. H. Roark, B. D. Roth, E. Ferguson, B. R. Krause, R. S. Newton, C. Sekerle, M. K. Shaw, *J. Med. Chem.* **1991**, *34*, 367–373, and references cited therein.
[13] B. D. Roth, Th. M. A. Bocan, C. J. Blankley, A. W. Chucholowski, P. L. Creger, M. W. Creswell, E. Ferguson, R. S. Newton, P. O. Brien, J. A. Picard, W. H. Roark, C. S. Sekerle, D. R. Sliskovic, M. W. Wilson, *J. Med. Chem.* **1991**, *34*, 463–466, and references cited therein.
[14] T. Rosen, M. Watanabe, C. H. Heathcock, *J. Org. Chem.* **1984**, *49*, 3657–3659.
[15] E. Santaniello, M. Chiari, P. Ferraboschi, S. Trave, *J. Org. Chem.* **1988**, *53*, 1567–1569.
[16] H. O. Kalinowski, S. Berger, S. Braun, *Carbon-13 NMR Spectroscopy*, John Wiley and Sons, New York, **1991**, p. 109 and 116.

[17] W. F. Hoffman, A. W. Alberts, E. J. Cragoe Jr., A. A. Deana, B. E. Evans, J. L. Gilfillan, N. P. Gould, J. W. Huff, F. C. Novello, J. D. Prugh, K. E. Rittle, R. L. Smith, G. E. Stocker, A. K. Willard, *J. Med. Chem.* **1986**, *29*, 159–169.
[18] F. Bonadies, R. Di Fabio, A. Gubbiotti, S. Mecozzi, C. Bonini, *Tetrahedron Lett.* **1987**, *28*, 703–706.
[19] A. W. Alberts, J. Chen, G. Kuron, V. Hunt, S. Huff, C. Hoffmann, J. Rothrock, M. Lopez, J. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Alberts-Schonberg, O. Hensens, J. Hirschfield, K. Hoogsteen, J. Liesch, J. Spinger, *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957–3961.
[20] A more detailed paper about the pharmacological activity of these compounds will be published elsewhere.
[21] S. G. Cohen, E. Khedouri, *J. Am. Chem. Soc.* **1961**, *83*, 4228–4232.
[22] R. Roy, A. W. Rey, *Tetrahedron Lett.* **1987**, *28*, 4935–4938.
[23] P. Mohr, L. Rösslein, Ch. Tamm, *Helv. Chim. Acta* **1987**, *70*, 142–152.
[24] R. P. Evstigneeva, R. S. Livshits, M. S. Bainova, L. I. Zakharkin, N. A. Preobrazhenskii, *Zh. Obsch. Khim.* **1952**, *22*, 1467–1473 [*Chem. Abstr.* **1953**, *47*, 5949c].
[25] M. Marcel, R. Pierre, M. Lee, M. Jacques, P. G. Yvon, *Can. J. Chem.* **1974**, *52*, 2316–2326.
[26] A. S. Sarma, *Indian J. Chem.* **1973**, *11*, 131–133.
[27] As a mixture of isomers: R. Gruber, D. Cagniant, P. Cagniant: *Bull. Soc. Chim. Fr.* **1976**, 1599–1606.
[28] J. Braun, J. Reuter, *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1926–1927.
[29] Th. H. Smith, A. N. Fujiwara, W. W. Lee, H. Y. Wu, D. W. Henry, *J. Org. Chem.* **1977**, *42*, 3653–3660.
[30] P. C. Mukharji, L. N. Saha, K. L. Ghatok: *Indian J. Chem.* **1970**, *8*, 384–388.
[31] R. A. Heller, M. A. Schrewsberg, *J. Biol. Chem.* **1976**, *251*, 3815–3822.
[32] D. A. Kleinsek, S. Ranganathan, J. W. Porter, *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1431–1435.

[193/91]

# VII. melléklet

EGRI, G., FOGASSY, E., NOVÁK, L., **POPPE, L.**:

Synthesis and Lipase-catalyzed Asymmetric Acetylation of 3-Hydroxy-2-hydroxymethylpropanal Acetals,

*Tetrahedron:Asymmetry*, **1997**, *8*, 547.

Pergamon

*Tetrahedron: Asymmetry*, Vol. 8, No. 4, pp. 547–557, 1997

Printed in Great Britain
PII: S0957-4166(97)00018-9
0957-4166/97 $17.00 + 0.00

# Synthesis and lipase-catalyzed asymmetric acetylation of 3-hydroxy-2-hydroxymethylpropanal acetals

Gabriella Egri,[a] Elemér Fogassy,[a] Lajos Novák[b] and László Poppe[c,*]

[a] Department for Organic Chemical Technology, Technical University Budapest, H-1521 Budapest, PO Box 91, Hungary

[b] Department for Organic Chemistry, Technical University Budapest, H-1521 Budapest, PO Box 91, Hungary

[c] Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, PO Box 17, Hungary

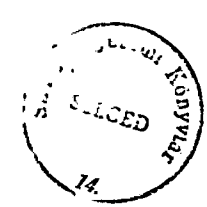
***Abstract:*** Prochiral dialkylacetal derivatives of 3-hydroxy-2-hydroxymethylpropanal **6a–e** were synthesized from the corresponding 2-substituted diethyl malonates **5a–e** and subjected to asymmetric enzymatic acetylation. The diethyl malonates **5a–f** were prepared from diethyl chloromethylenemalonate **3** by using either a one- or a two-step process. Asymmetric acetylation of 3-hydroxy-2-hydroxymethylpropanal diethyl acetal **6b** with several enzymes was studied first, showing the highest enantiotopic selectivity with lipase from *Pseudomonas fluorescens* (PfL). Solvent effect was also investigated: the best selectivity was obtained in a mixture of hexane and diethyl ether. Furthermore, several other acetals **6a–e** were also tested under the optimal acetylation conditions. © 1997 Elsevier Science Ltd. All rights reserved.

Optically active $C_3$ building blocks are of continuously raising interest both in the manufacture of commercial products and in the research of biochemical processes. Products arising from asymmetric functionalization of 3-hydroxy-2-hydroxymethylpropanal dialkylacetals **6a–e** can be favorably used as multifunctional building blocks due to their three sites of different reactivity.

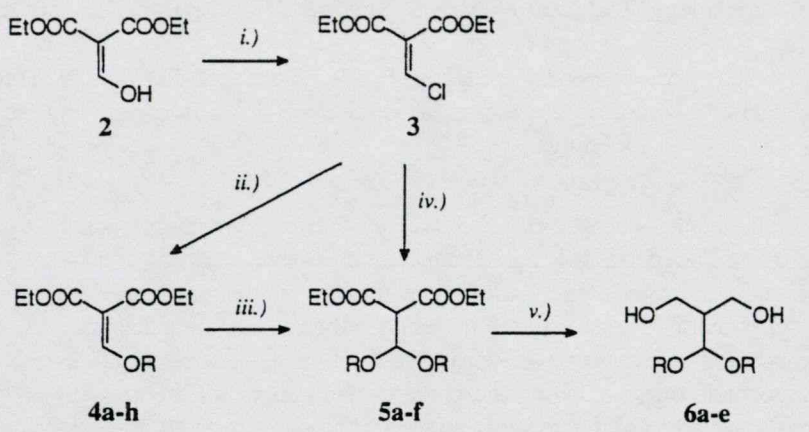
Diethyl ethoxymethylenemalonate **1** is a commercially available compound and can be converted to the desired acetals **6a–e** conveniently in reaction sequences which manifest sometimes unexpected behaviour (Scheme 1). Since charge distribution on the $\alpha$- and $\beta$-carbon centers adjacent to the ether oxygen atom in the vinyl ether type compound diethyl ethoxymethylenemalonate **1** is opposite to the normal vinyl ethers, preparation of diethyl formylmalonate by the generally applied acid-catalyzed methods failed. The desired reaction, however, could be carried out in aqueous NaOH solution[1] smoothly. Diethyl formylmalonate exists, in accordance with previous results, exclusively in its enolic form (i.e. diethyl hydroxymethylenemalonate, **2**. The close similarity of charge distribution on $C_1$ and $C_2$ of this enolic compound **2** to those of its parent vinyl ether **1** may also rationalize why traditional acid-catalyzed acetal formation cannot be applied for further transformations. Diethyl ethoxymethylenemalonate **1**, however, can be directly converted into diethyl (diethoxy)methylmalonate **5b** under basic conditions, e.g. by using sodium ethylate[2,3] or sodium[4] in ethanol. Similarly, diethyl ethoxymethylenemalonate **1** was transformed into the corresponding dimethylacetal dimethylester by base-catalyzed reaction in methanol.[5] Since general methods for the preparation of further acetals were needed, we have chosen the known diethyl chloromethylenemalonate **3**[6], obtained from diethyl hydroxymethylenemalonate **2** by a significantly improved method using thionyl chloride, as a common precursor. A report on transformation of this chloromethylene derivative **3** with an alcohol in the presence of pyridine to the corresponding diethyl alkoxymethylenemalonate[7] prompted us to prepare a series of such alkoxymethylene compounds **4a–h** from which a mild base-catalyzed alkoxide addition yielded the desired acetals **5a–e** smoothly. Bulkiness of the alcohol seems to play a crucial role in these

* Corresponding author. Email: poppe@ch.bme.hu





addition reactions: *iso*-propoxide-, *tert*-butoxide- and phenoxide-addition cannot be accomplished. Attempted preparation of methyl–phenyl or methyl–isopropyl acetals was also unsuccessful either from diethyl methoxymethylenemalonate **4a** or from diethyl (*iso*-propyloxy)methylenemalonate **4g** or diethyl phenoxymethylenemalonate **4h** with the corresponding alcohol. Alternatively, acetals **5a–e** can be obtained directly from diethyl chloromethylenemalonate **3** in a one-pot reaction by using a slight excess (1.1–1.3 equiv.) of sodium hydride in the corresponding alcohol. Cyclic acetal **5c**, however, could only be prepared by the one-pot method. Reduction of diester acetals **5a–e** by lithium aluminum hydride yielded the acetal derivatives of 3-hydroxy-2-hydroxymethylpropanal **6a–e**. Unfortunately, the diallyl acetal **5f** decomposed during this reduction.




| 4-6 | R |
|---|---|
| a | $-CH_3$ |
| b | $-CH_2CH_3$ |
| c | $-CH_2CH_2-$ |
| d | $-CH_2C_6H_5$ |
| e | $-CH_2CH_2CH(CH_3)_2$ |
| f | $-CH_2CH=CH_2$ |
| g | $-CH(CH_3)_2$ |
| h | $-C_6H_5$ |

Reagents: *i.)* $SOCl_2$, DMF; *ii).* ROH, pyridine; *iii.)* ROH, cat. Na; *iv.)* ROH, 1.3 equiv. NaH; *v.)* $LiAlH_4$.

| R | Reaction *iii.)* **4**, Yield % | Reaction *iv.)* **5**, Yield % | Reaction *v.)* **5**, Yield % | Reaction *vi.)* **6**, Yield % |
|---|---|---|---|---|
| **a**, methyl- | 95 | 87 | 92 | 86 |
| **b**, ethyl | 96 | 90 | 93 | 92 |
| **c**, 1,2-ethenyl- | - | - | 94 | 37 |
| **d**, benzyl- | 95 | 80 | 80 | 82 |
| **e**, *i*-amyl- | 95 | 86 | 89 | 74 |
| **f**, allyl- | 94 | 85 | 77 | decomposition |
| **g**, *i*-propyl- | 95 | no reaction | no reaction | - |
| **h**, phenyl- | 91 | no reaction | no reaction | - |

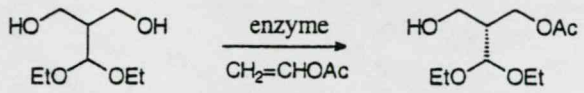
**Scheme 1**. Preparation of 3-hydroxy-2-hydroxymethylpropanal acetals **6a–e**.

After having the desired prochiral diols **6a–e** in our hands, first we tested the asymmetric acetylation of 3-hydroxy-2-hydroxymethylpropanal diethylacetal **6b** with vinyl acetate in hexane using various enzymes (Table 1).

Since the highest selectivity was achieved with PfL, this enzyme was chosen for further studies. Next, the solvent effect on enantiotopic selectivity of this PfL-catalyzed acetylation was investigated (Table 2).

Interestingly, no correlation between the polarity of the solvent and the enantiotopic selectivity of the enzyme was found. Trace water content of the solvent also seems to have no significant influence on the selectivity, and in the protic *tert*-butanol no reaction occurred. Since the best enantiotopic

**Table 1.** Acetylation of 3-hydroxy-2-hydroxymethylpropanal diethylacetal **6b** with various enzymes




**6b** **7b**

| Enzyme[a] (mg) | Time[b] (h) | Yield (%) | ee[c] (%) |
|---|---|---|---|
| PfL (10) | 8 | 71.7 | 66 |
| PPL (30) | 120 | 78.2 | 17 |
| CcL (30) | 120 | 74.9 | 57 |
| PLE (50) | 120 | 75.5 | 52 |
| MjL (10) | 170 | - | - |
| RaL (10) | 170 | - | - |
| Lipase-PS (10) | 8 | 66 | 60 |
| Lipase-AK (10) | 8 | 57 | 57 |

[a] PfL: lipase from *Pseudomonas fluorescens*, PPL: lipase from porcine pancreas, CcL: lipase from *Candida cylindracea*, PLE: pig liver acetone powder, MjL: lipase from *Mucor javonicus*, RaL: lipase from *Rhisopus arrhisus*; [b] 200 mg of acetal **6b** was stirred with the enzyme in vinyl acetate (2 ml) at RT; [c] Enantiomeric excess values were determined by using $^1$H-NMR spectra of (*S*)-MTPA-ester of **7b**.

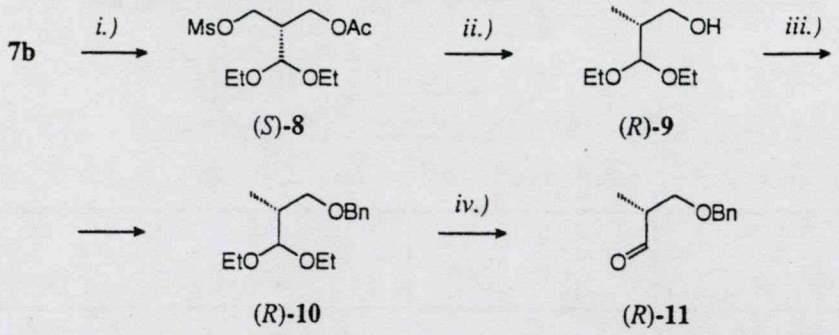
**Table 2.** Acetylation of 3-hydroxy-2-hydroxymethylpropanal diethylacetal **6b** by PfL in various solvents

| Solvent | Time[a] (h) | Yield (%) | ee (%) |
|---|---|---|---|
| hexane | 10 | 95 | 68 |
| hexane : $Et_2O$ (1 : 1) | 10 | 85 | 71 |
| hexane : $(i\text{-}Pr)_2O$ (1 : 1) | 10 | 93 | 69 |
| $CCl_4$ | 10 | 90 | 48 |
| toluene | 20 | 94 | 68 |
| tetrahydrofuran | 20 | 69 | 62 |
| acetonitrile | 20 | 84 | 69 |
| *t*-butanol | 10 | - | - |
| hexane : $(i\text{-}Pr_2O)$ : $H_2O$ (1 : 1 : 0.002) | 20 | 83 | 69 |

[a] **6b** (200 mg) and PfL (10 mg) were stirred in the given solvent (2 ml) and vinyl acetate (0.25 ml)

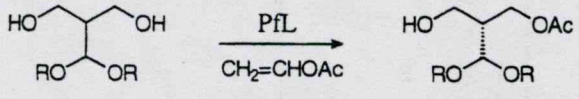
selectivity was achieved in the mixture of hexane and diethyl ether, this solvent mixture was applied in the further studies.

After studying the factors influencing the enantiotopic selectivity of acetylation of the prochiral diol **6b**, absolute configuration of the monoacetate product **7b** was determined by chemical correlation (Scheme 2). The reaction sequence leading to the known (*R*)-(−)-3-benzyloxy-2-methylpropanal (*R*)-**11**[8,9], *via* mesylation, reduction of the mesylate, benzylation and hydrolysis, starting from the monoacetate **7b** proved its (*R*)-configuration.




**Scheme 2.** Determination of the absolute configuration of the optically active monoacetate **7b**. Reagents: *i.)* MsCl, $Et_3N$; *ii.)* $LiAlH_4$; *iii.)* BnCl, NaH; *iv.)* cat. HCl, $AcOH–H_2O$.

**Table 3.** Acetylation of prochiral diols having various acetal-type substituents **6a–d**




**6a-d** **7a-d**

| R | Time [a] (h) | Yield (%) | $[\alpha]_D$ [b] | $[\alpha]_D^{100\%}$ [b,c] | ee (%) |
|---|---|---|---|---|---|
| **a**, methyl- | 12 | 46 | +4.1 | +9.4 | 44 [d] |
| **b**, ethyl- | 10 | 85 | +4.9 | +7.0 | 71 |
| **c**, 1,2-ethenyl- | 7 | 38 | +0.3 | - | ~0 |
| **d**, benzyl- | 21 | 92 | +7.4 | +10.5 | 70 [d] |
| **e**, *i*-amyl- | 24 | 70 | +3.7 | +5.5 | 68 [d] |

[a] 200 mg of **6a-e**, PfL (10 mg), and vinyl acetate (0.25 ml) in hexane:diethyl ether 1:1 (2 ml) was stirred at RT; [b] c=1, acetone; [c] extrapolated values calculated from specific rotation of **6a-e** and from the corresponding enantiomeric excess values obtained from $^1$H-NMR spectra of MTPA-esters of the monoacetates; [d] absolute configuration is assumed to be (*R*) by analogy with that of **7b**.

Finally, several prochiral diols with acetal-type 2-substituents **6a–e** were acetylated using PfL under the optimum conditions (Table 3). Within this series, (*R*)-configuration was assigned to all optically active products **7a,b,d,e**, based on the analogous manner of the enzymatic acetylations and on the same signs of the specific rotations of the products. The lipase-catalyzed reaction yielding monoacetate **7b** with e.e. of 71% from the diethyl acetal **6b** proceeded with the highest enantiotopic selectivity. While di-*i*-amyl and dibenzyl acetals **6d,e** gave similarly good results (68 and 70% e.e., respectively), only a modest enantiotopic selectivity was found in acetylation of the dimethyl acetal **6a**, and almost racemic product **7c** was obtained from the cyclic acetal **6c**. The bulkiness of the acetal-type 2-substituent seems to be decisive for the enantiotopic selectivity: the small substituents gave poor results, the best selectivity was manifested with the medium-size diethyl acetal. Further increase of the bulkiness of the acetal moiety, however, did not increase the selectivity.

In summary, it may be concluded that the enzymatic acetylation of the prochiral 3-hydroxy-2-hydroxymethylpropanal acetals **6a–e** is a convenient method for the preparation of optically active acetals of 3-acetoxy-2-hydroxymethyl-propanal **7a–e**, which may serve as multifunctional chiral building blocks. The highest enantiotopic selectivities were obtained with diethyl and dibenzyl acetals, **6b** and **6e** respectively, using lipase from *Pseudomonas fluorescens* (PfL) and vinyl acetate in a mixture of hexane and diethyl ether. Among the optically active acetals **7a–e**, the dibenzyl acetal **7e**, whose

acetal moiety can be manipulated both by acid-catalysis and catalytic hydrogenation, may have the highest synthetic value.

## Experimental

The $^1$H-NMR spectra were recorded on a Bruker WM-250 spectrometer operating at 250 MHz. Enantiomeric excess values were determined by $^1$H-NMR spectroscopy at 500 MHz on a Bruker DRX-500 spectrometer. All NMR spectra were measured in $CDCl_3$ solution and chemical shift values are expressed in ppm values from TMS as internal standard on the $\delta$ scale. IR spectra of thin film samples were taken on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin Elmer 241 polarimeter. Thin layer chromatography was carried out using Merck Kieselgel 60 $F_{254}$ alumina sheets applying hexane:acetone 10:4 mixture for elution. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative vacuum-chromatography[10] was performed using Merck Kieselgel 60 $F_{254}$. Porcine pancreatic lipase (PPL, Type II) was obtained from Sigma. Lipases from *Candida rugosa (cylindracea)* (CcL), *Pseudomonas fluorescens* (PfL), *Aspergillus niger* (AnL), *Mucor javonicus* (MjL), *Rhisopus arrhisus* (RaL), esterase from pig liver (PLE, acetone powder), diethyl ethoxymethylenemalonate, acetic anhydride, and vinyl acetate were products of Fluka. Lipase PS and Lipase AK were gifts from Amano. All solvents used were freshly distilled.

*Diethyl chloromethylenemalonate 3*

To a solution of diethyl hydroxymethylenemalonate (2, 29.4 g, 157 mmol) and N,N-dimethylformamide (0.5 ml) in toluene (200 ml) thionyl chloride (12.6 ml, 173 mmol) was added dropwise. The reaction mixture was heated under reflux until gas evolution ceased. After removal of the solvent by rotary evaporation, the residue was distilled *in vacuo* yielding 24.7 g (77%) of a colorless oil with characteristic odor. Bp: 64°C (0.2 Torr); *$^1$H-NMR*: 1.29 (t, 3H, $CH_3$), 1.34 (t, 3H, $CH_3$), 4.26 (q, 2H, $OCH_2$), 4.37 (q, 2H, $OCH_2$), 7.47 (s, 1H, =CH–Cl); *IR*: 3080, 2980, 1740, 1610, 1460, 1450, 1370, 1330, 1250, 1210, 1100, 1070, 1020, 910, 870, 840, 750 cm$^{-1}$; Calcd. for $C_8H_{11}O_4Cl$: C 46.50, H 5.37; found C 46.69, H 5.38.

*Preparation of diethyl alkoxymethylenemalonates 4a–h*

*General procedure*

Diethyl chloromethylenemalonate (3, 2.07 g, 10.0 mmol) and pyridine (1 ml) was added to the corresponding alcohol (20 ml) and the resulting solution was stirred at RT for 15 minutes. After removal of the excess alcohol by rotary evaporation, the residue was acidified with 5% HCl (10 ml) and extracted with dichloromethane (3×10 ml). The combined dichloromethane extracts were dried over $Na_2SO_4$ and concentrated in vacuum leaving a colorless oil which was purified by preparative vacuum-chromatography using hexane:acetone 10:1 as eluent.

*Diethyl methoxymethylenemalonate 4a*

Yield: 95%. *$^1$H-NMR*: 1.31 (t, 3H, $CH_3$), 1.36 (t, 3H, $CH_3$), 4.01 (s, 3H, $OCH_3$), 4.15–4.23 (m, 4H, 2 $OCH_2$), 7.55 (s, 1H, =CH–O); *IR*: 2980, 1730, 1640, 1450, 1400, 1380, 1280, 1210, 1140, 1090, 1020, 970, 860, 770 cm$^{-1}$; Calcd. for $C_9H_{14}O_5$: C 53.46, H 6.98; found C 53.26, H 6.96.

*Diethyl ethoxymethylenemalonate 4b*

Yield: 96%. *$^1$H-NMR* and *IR* spectra were in accordance with the literature[11].

*Diethyl benzyloxymethylenemalonate 4d*

Yield: 95%. *$^1$H-NMR*: 1.28 (t, 3H, $CH_3$), 1.33 (t, 3H, $CH_3$), 4.08–4.45 (m, 4H, 2 $OCH_2$), 4.69 (s, 2H, $OCH_2Ph$), 7.25–7.50 (m, 5H, ArH), 7.62 (s, 1H, =CH–O); *IR*: 3500 (br), 2980, 1730, 1630,

1500, 1460, 1370, 1290, 1150, 1090, 1020, 910, 860, 740, 700 $cm^{-1}$; Calcd. for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found C 64.63, H 6.54.

*Diethyl (i-amyloxy)methylenemalonate 4e*

Yield: 95%. *$^1$H-NMR*: 0.94 (d, 6H, 2 $CH_3$), 1.30 (t, 3H, $CH_3$), 1.34 (t, 3H, $CH_3$), 1.30–1.79 (m, 3H, CH and $CH_2$), 4.10–4.43 (m, 6H, 3 $OCH_2$), 7.60 (s, 1H, =CH–O); *IR*: 2960, 1730, 1630, 1470, 1380, 1290, 1180, 1090, 1030, 960, 860, 800 $cm^{-1}$; Calcd. for $C_{13}H_{22}O_5$: C 60.45, H 8.58; found C 60.20, H 8.55.

*Diethyl allyloxymethylenemalonate 4f*

Yield: 94%. *$^1$H-NMR*: 1.30 (t, 3H, $CH_3$), 1.33 (t, 3H, $CH_3$), 4.18–4.40 (m, 4H, 2 $OCH_2$), 4.62 (mc, 2H, $OCH_2$), 5.37 (mc, 2H, =$CH_2$), 5.93 (mc, 1H, =CH–), 7.60 (s, 1H, =CH–O); *IR*: 2980, 1730, 1640, 1590, 1490, 1380, 1250, 1200, 1170, 1080, 1020, 760, 690 $cm^{-1}$; Calcd. for $C_{11}H_{16}O_5$: C 57.89, H 7.07; found C 58.10, H 7.09.

*Diethyl (i-propyloxy)methylenemalonate 4g*

Yield: 95%. *$^1$H-NMR*: 1.20–1.44 (m, 12H, 4 $CH_3$), 4.1–4.3 (m, 5H, 2 $OCH_2$, OCH), 7.66 (s, 1H, =CH–O); *IR*: 2980, 1730, 1630, 1470, 1450, 1380, 1290, 1250, 1190, 1140, 1100, 1030, 920, 850, 790 $cm^{-1}$; Calcd. for $C_{11}H_{18}O_5$: C 57.38, H 7.88; found C 57.62, H 7.90.

*Diethyl phenyloxymethylenemalonate 4h*

Yield: 91%. *$^1$H-NMR*: 1.31 (t, 3H, $CH_3$), 1.37 (t, 3H, $CH_3$), 4.20–4.43 (ms, 4H, 2 $OCH_2$), 7.10–7.48 (m, 5H, ArH), 7.89 (s, 1H, =CH–O); *IR*: 2980, 1730, 1630, 1470, 1450, 1370, 1280, 1240, 1180, 1090, 1030, 970, 940, 860, 770 $cm^{-1}$; Calcd. for $C_{14}H_{16}O_5$: C 63.63, H 6.10; found C 63.37, H 6.12.

*Diethyl dialkoxymethylmalonates 5a–f from diethyl alkoxymethylenemalonates 4a–f*

*General procedure*

To a solution of diethyl alkoxymethylenemalonate (**4a–f**, 10 mmol) in the corresponding alcohol (ca. 5 mmol) catalytic amount (ca. 15 mg) of sodium was added and the resulting solution was stirred at 50°C for 30 minutes. After removal of the excess alcohol by rotary evaporator, 5% hydrochloric acid (5 ml) was added and the resulting mixture was extracted with chloroform (3×5 ml). The combined chloroform extracts were dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo*. The residue was purified by preparative vacuum-chromatography using hexane:acetone 10:1 as eluent resulting a colorless oil.

*Diethyl dimethoxymethylmalonate 5a*

Yield: 87%. *$^1$H-NMR*: 1.29 (t, 6H, 2 $CH_3$), 3.43 (s, 6H, 2 $OCH_3$), 3.74 (d, CH), 4.22 (q, 4H, 2 $OCH_2$), 5.00 (d, 1H, O–CH–O); *IR*: 2980, 2840, 1740, 1610, 1450, 1370, 1310, 1230, 1180, 1090, 1040, 950, 910, 860 $cm^{-1}$; Calcd. for $C_{10}H_{18}O_6$: C 51.27, H 7.75; found C 51.14, H 7.77.

*Diethyl diethoxymethylmalonate 5b*

Yield: 90%. *$^1$H-NMR* and *IR* spectra were in accordance with the literature.

*Diethyl dibenzyloxymethylmalonate 5d*

Yield: 80%. *$^1$H-NMR*: 1.23 (t, 6H, 2 $OCH_3$), 3.91 (d, 1H, CH), 4.18 (q, 4H, 2 $OCH_2$), 4.66 (dd, 4H, 2 $OCH_2Ph$), 5.40 (d, 1H, O–CH–O), 7.2–7.4 (m, 10H, Ar); *IR*: 3030, 2980, 2940, 1750, 1740, 1500, 1450, 1370, 1310, 1100, 1060, 1030, 910, 860, 740 $cm^{-1}$; Calcd. for $C_{22}H_{26}O_6$: C 68.38, H 6.78; found C 68.09, H 6.76.

*Diethyl (di-i-amyloxy)methylmalonate 5e*

Yield: 86%. *$^1$H-NMR*: 0.86 (d, 12H, 4 $CH_3$), 1.15–1.72 (m, 12H, 2 CH, 2 $CH_3$ and 2 $CH_2$), 3.45–3.78 (m, 5H, CH and 2 $OCH_2$), 4.18 (q, 4H, 2 $OCH_2$), 5.09 (d, 1H, O–CH–O); *IR*: 2960, 2870, 1740, 1640, 1470, 1370, 1310, 1180, 1140, 1100, 1070, 1030, 860 $cm^{-1}$; Calcd. for $C_{18}H_{34}O_6$: C 62.40, H 9.89; found C 62.28, H 6.43.

*Diethyl diallyloxymethylmalonate 5f*

Yield: 85%. *$^1$H-NMR*: 1.32 (t, 6H, $2CH_3$), 3.80 (m, 1H, CH), 4.07–4.35 (m, 8H, 4 $OCH_2$), 5.15–5.43 (m, 5H, 2 $=CH_2$ and O–CH–O), 5.90 (m, 2H, 2 =CH–); Calcd. for $C_{14}H_{22}O_6$: C 58.73, H 7.74; found C 58.84, H 7.71.

*Diethyl dialkoxymethylmalonates 5a–f from diethyl chloromethylenemalonate 3*

*General procedure*

To a solution of diethyl chloromethylenemalonate (**3**, 3.1 g, 15.0 mmol) in the corresponding alcohol (15 ml) sodium hydride (0.48 g, 20.0 mmol) was added at 0°C and the resulting mixture was stirred for 30 minutes. After removal of the excess alcohol by rotary evaporator, the residue was neutralized by addition of 5% hydrochloric acid, diluted by water (10 ml) and extracted with chloroform (3×5 ml). The combined chloroform extracts were dried over $Na_2SO_4$ and concentrated. Usually, the product was used in the next reduction step as such. Analytical samples were purified by preparative vacuum-chromatography with hexane:acetone 10:1 as eluent.

*Diethyl dialkoxymethylmalonates 5a,b,d–f*

For yields: see table in Scheme 1; for analytical data: see the preceding section.

*Diethyl (1,3-dioxolan-2-yl)malonate 5c*

Yield: 94%. *$^1$H-NMR*: 1.27 (t, 6H, 2 $CH_3$), 3.68–3.83 (ms, 5H, CH and $OCH_2$–$CH_2O$), 4.21 (q, 4H, 2 $OCH_2$), 5.08 (d, 1H, O–CH–O); *IR*: 3650, 2970, 2840, 1740, 1730, 1620, 1450, 1440, 1370, 1300, 1230, 1180, 1170, 1080, 1040, 940, 920, 860, 750 $cm^{-1}$; Calcd. for $C_{10}H_{16}O_6$: C 51.72, H 6.94; found C 51.93, H 6.93.

*Reduction of the diethyl dialkoxymethylmalonates 5a–e*

*General procedure*

To a suspension of lithium aluminum hydride (0.95 g, 25 mmol) in dry tetrahydrofurane (30 ml) a solution of the diethyl dialkoxymethylmalonate (**5a–e**, 10.0 mmol) in dry tetrahydrofurane (10.0 ml) was added dropwise and the reaction mixture was heated under reflux for 1 hour. After cooling, the reaction mixture was quenched by careful addition of water (5 ml) and the resulting suspension was diluted with ethyl acetate (25 ml). The precipitate was filtered off and the filtrate was dried over $Na_2SO_4$ and concentrated by rotary evaporation. The residue was purified by preparative vacuum-chromatography with hexane:acetone 10:3 as eluent.

*3-Hydroxy-2-hydroxymethylpropanal dimethyl acetal 6a*

Yield: 86%. *$^1$H-NMR*: 2.03 (m, 1H, CH), 3.42 (s, 6H, 2 $OCH_3$), 3.77 (m, 4H, 2 $OCH_2$), 4.48 (d, 1H, O–CH–O); *IR*: 3400 (br), 2940, 2840, 1650, 1460, 1390, 1270, 1190, 1130 $cm^{-1}$; Calcd. for $C_6H_{14}O_4$: C 47.99, H 9.40; found C 48.16, H 9.43.

*3-Hydroxy-2-hydroxymethylpropanal diethyl acetal 6b*

Yield: 92%. *$^1$H-NMR*: 1.15 (t, 6H, 2 $CH_3$), 1.93 (m, 1H, CH), 3.46 and 3.67 (2 m, 4H, 2 $OCH_2$), 3.70 (m, 4H, 2 $OCH_2$), 4.54 (d, 1H, O–CH–O); *IR*: 3390 (br), 2940, 2850, 1650, 1450, 1380, 1270, 1190, 1130, 1060, 970 $cm^{-1}$; Calcd. for $C_8H_{18}O_4$: C 53.91, H 10.18; found C 53.80, H 10.15.

*3-Hydroxy-2-hydroxymethylpropanal 1,2-ethenyl acetal 6c*

Yield: 37%. *$^1$H-NMR*: 2.05 (m, 1H, CH), 3.75–4.03 (m, 8H, 4 $OCH_2$), 4.95 (d, 1H, O–CH–O); *IR*: 3380 (br), 2950, 2890, 1470, 1400, 1240, 1150, 1030, 950, 920 $cm^{-1}$; Calcd. for $C_6H_{12}O_4$: C 48.64, H 8.16; found C 48.75, H 8.18.

*3-Hydroxy-2-hydroxymethylpropanal dibenzyl acetal 6d*

Yield: 82%. *$^1$H-NMR*: 2.10 (m, 1H, CH), 3.77 (m(d), 4H, 2 $OCH_2$), 4.60 (dd, 4H, 2 $OCH_2Ph$), 4.81 (d, 1H, O–CH–O), 7.28 (m, 10H, ArH); *IR*: 3370, 3030, 2930, 2870, 1950, 1500, 1450, 1400, 1290, 1240, 1210, 1140, 1040, 930, 740, 700 $cm^{-1}$; Calcd. for $C_{18}H_{22}O_4$: C 71.50, H 7.33; found C 71.78, H 7.33.

*3-Hydroxy-2-hydroxymethylpropanal di-i-amyl acetal 6e*

Yield: 74%. *$^1$H-NMR*: 0.91 (d, 12H, $4CH_3$), 1.10–1.78 (m, 6H, 2 CH and 2 $CH_2$), 2.09 (m, 1H, CH), 3.39–3.80 (m, 8H, 4 $OCH_2$), 4.61 (d, 1H, O–CH–O); *IR*: 3370 (br), 2960, 2930, 2870, 1740, 1470, 1370, 1240, 1110, 1070, 800 $cm^{-1}$; Calcd. for $C_{14}H_{30}O_4$: C 64.09, H 11.52; found C 64.00, H 11.48.

*Acetylation of 3-hydroxy-2-hydroxymethylpropanal diethyl acetal 6b with various enzymes*

*General procedure*

To a solution of 3-hydroxy-2-hydroxymethylpropanal diethyl acetal (**6b**, 200 mg) in vinyl acetate (2 ml) enzyme (for amount, see Table 1) was added and the resulting suspension was stirred at room temperature (for reaction time, see Table 1). After reaching a reasonable conversion the enzyme was filtered off and the filtrate was concentrated by rotary evaporator. The residue was subjected to column chromatography using hexane:acetone 5:1 as eluant yielding pure 3-acetoxy-2-hydroxymethylpropanal diethyl acetal **7b**. For yields and enantiomeric composition, see Table 1.

*Acetylation of 3-hydroxy-2-hydroxymethylpropanal diethyl acetal 6b in various solvents*

*General procedure:* 3-Hydroxy-2-hydroxymethylpropanal diethyl acetal (**6b**, 200 mg), vinyl acetate (0.25 ml) and lipase from *Pseudomonas fluorescens* (10 mg) were added to the solvent (2 ml; for solvents, see Table 2) and the resulting suspension was stirred at room temperature. Work up of the products was carried out as described in the previous section. For reaction times, yields, and enantiomeric composition, see Table 2.

*Acetylation of 3-hydroxy-2-hydroxymethylpropanal dialkyl acetals 6a–e*

*General procedure:* 3-Hydroxy-2-hydroxymethylpropanal dialkyl acetal (**6a–e**, 200 mg), vinyl acetate (0.25 ml) and lipase from *Pseudomonas fluorescens* (10 mg) were added to a mixture of hexane and diethyl ether (1 ml, each) and the resulting suspension was stirred at room temperature. Work up of the product was carried out as described in the previous sections.

*3-Acetoxy-2-hydroxymethylpropanal dimethyl acetal 7a*

Yield: 46%. $[\alpha]_D$=+4.1, (c=1, acetone), e.e.%=44; *$^1$H-NMR*: 2.10 (s, 3H, $CH_3$), 2.16 (m, 1H, CH), 3.43 (s, 3H, .$OCH_3$), 3.47 (s, 3H, .$OCH_3$), 3.72 (m(d), 2H, $OCH_2$), 4.04–4.27 (m, 2H, $AcOCH_2$), 4.44 (d, 1H, O–CH–O); *IR*: 3465 (br), 2940, 2830, 1740, 1470, 1370, 1240, 1190, 1130, 1040, 980 $cm^{-1}$; Calcd. for $C_8H_{16}O_5$: C 49.99, H 8.39; found C 50.24, H 8.27.

*3-Acetoxy-2-hydroxymethylpropanal diethyl acetal 7b*

Yield: 85%. $[\alpha]_D$=+4.9, (c=1, acetone), e.e.%=71; *$^1$H-NMR*: 1.22 (t, 3H, $CH_3$), 1.23 (t, 3H, $CH_3$), 2.14 (s, 3H, $CH_3$), 2.16 (m, 1H, CH), 3.51–3.57 (m, 2H, $OCH_2$), 3.67–3.80 (m, 4H, 2 $OCH_2$), 4.14 and 4.24 (2×dd, 2H, $AcOCH_2$), 4.58 (d, 1H, O–CH–O); *IR*: 3464 (br), 2980, 2930, 2900, 1740, 1450, 1370, 1240, 1110, 1060, 900 $cm^{-1}$; Calcd. for $C_{10}H_{20}O_5$: C 54.53, H 9.15; found C 54.22, H 9.31.

*3-Acetoxy-2-hydroxymethylpropanal 1,2-ethenyl acetal 7c*

Yield: 38%. $[\alpha]_D$=+0.3, (c=1, acetone); *$^1$H-NMR*: 2.08 (m, 1H, CH), 2.12 (s, 3H, $CH_3$), 3.73–4.15 (m, 6H, 3 $OCH_2$), 4.02–4.28 (m, 2H, $AcOCH_2$), 4.91 (d, 1H, O–CH–O); *IR*: 3450 (br), 2950, 2880, 1730, 1470, 1390, 1240, 1150, 1040 $cm^{-1}$; Calcd. for $C_8H_{14}O_5$: C 50.52, H 7.42; found C 50.71, H 7.69.

*3-Acetoxy-2-hydroxymethylpropanal dibenzyl acetal 7d*

Yield: 92%. $[\alpha]_D$=+7.4, (c=1, acetone), e.e.%=70; *$^1$H-NMR*: 1.92 (s, 3H, $CH_3$), 2.40 (m, 1H, CH), 3.74 (m, 2H, O–$CH_2$), 4.00–4.29 (m, 2H, $AcOCH_2$), 4.57 (dd, 4H, 2 $OCH_2Ph$), 4.71 (d, 1H, O–CH–O), 7.28 (m, 10H, ArH); *IR*: 3470 (br), 2960, 2930, 1740, 1460, 1370, 1230, 1040, 740 $cm^{-1}$; Calcd. for $C_{20}H_{24}O_5$: C 69.75, H 7.02; found C 70.01, H 7.19.

*3-Acetoxy-2-hydroxymethylpropanal di-*i*-amyl acetal 7e*

Yield: 70%. $[\alpha]_D$=+3.7, (c=1, acetone), e.e.%=68; *$^1$H-NMR*: 0.90 (d, 12H, 4 $CH_3$), 1.10–1.78 (m, 6H, 2 CH and 2$CH_2$), 2.04 (s, 3H, $CH_3$), 2.11 (m, 1H, CH), 3.41–3.83 (m, 6H, 3 $OCH_2$), 4.03–4.30 (m, 2H, $AcOCH_2$), 4.61 (d, 1H, O–CH–O); *IR*: 3470 (br), 2960, 2930, 2870, 1740, 1470, 1370, 1240, 1110, 1070, 830 $cm^{-1}$; Calcd. for $C_{16}H_{32}O_5$: C 63.13, H 10.59; found C 63.49, H 10.33.

*Determination of enantiomeric excess of 3-acetoxy-2-hydroxymethylpropanal dialkyl acetals 7a–e*

*General procedure*

To a solution of (*R*)-(−)-MTPA-Cl (38 mg, 0.15 mmol) in carbon tetrachloride (0.35 ml) 3-acetoxy-2-hydroxymethylpropanal dialkyl acetal (**7a–e**, 0.10 mmol; the corresponding racemic samples were obtained from **6a–e** by chemical acetylation using acetic anhydride and pyridine), pyridine (16 mg, 0.2 mmol), and N,N-dimethylaminopyridine (ca. 1 mg) were added and the resulting mixture was heated in a sealed ampoule at 50°C for 3 hours. The reaction mixture was washed with 5% hydrochloric acid (3×0.3 ml), the organic phase was dried over $Na_2SO_4$ and concentrated by rotary evaporator. The residue was analyzed by $^1$H-NMR spectroscopy as such.

*Characteristic $^1$H-NMR signals of 7a–e MTPA-esters (diastereomeric mixtures from racemic monoacetates)*

(*R*)-**7a** MTPA ester: 3.997 (dd, 0.5 H); (*S*)-**7a** MTPA ester: 4.035 (dd, 0.5 H);
(*R*)-**7b** MTPA ester: 4.026 (dd, 0.5 H); (*S*)-**7b** MTPA ester: 4.059 (dd, 0.5 H);
(±)-**7c** MTPA ester: 2.015 (s, 1.5 H), 2.018 (s, 1.5 H);
(*R*)-**7d** MTPA ester: 4.062 (dd, 0.5 H); (*S*)-**7d** MTPA ester: 4.097 (dd, 0.5 H);
(*R*)-**7e** MTPA ester: 4.210 (dd, 0.5 H); (*S*)-**7e** MTPA ester: 4.213 (dd, 0.5 H);

*Determination of the configuration of 3-acetoxy-2-hydroxymethylpropanal diethyl acetal 7b*

*(S)-(+)-3-Acetoxy-2-methanesulfonyloxymethylpropanal diethyl acetal 8*

To a solution of 3-acetoxy-2-hydroxymethylpropanal diethyl acetal (**7b**, 5.12 g, 23.3 mmol; $[\alpha]_D$=+5.0, c=1, acetone), triethylamine (4.0 ml, 29 mmol) and 4-dimethylaminopyridine (50 mg) in dichloromethane (25 ml) a solution of methanesulfonyl chloride (2.2 ml, 28 mmol) in dichloromethane (20 ml) was added dropwise below 25°C and the resulting mixture was stirred at RT for 30 minutes. The reaction mixture was then washed with water (2×10 ml) and the organic layer was dried over $Na_2SO_4$ and concentrated by rotary evaporator yielding 6.7 g (23.4 mmol, 96%) of a colorless oil.

$[\alpha]_D$=+0.9, (c=1, acetone); *$^1$H-NMR*: 1.24 (t, 6H, 2$CH_3$), 2.10 (s, 3H, $CH_3$), 2.40 (m, 1H, CH), 3.02 (s, 3H, $SO_2CH_3$), 3.45–3.78 (m, 4H, 2 $OCH_2$), 4.10–4.45 (m, 4H, 2 $AcOCH_2$), 4.55 (d, 1H, O–CH–O); *IR*: 2980, 2930, 1740, 1460, 1370, 1250, 1180, 1120, 1060, 960, 840, 750 $cm^{-1}$; Calcd. for $C_{11}H_{22}O_7S$: C 44.28, H 7.43, S 10.75; found C 44.15, H 7.41, S 10.78.

*(R)-(+)-3-Hydroxy-2-methylpropanal diethyl acetal* ***9***

To a suspension of lithium aluminum hydride (5.0 g, 131 mmol) in dry tetrahydrofuran (250 ml) a solution of (*S*)-(+)-3-acetoxy-2-methanesulfonyloxymethylpropanal diethyl acetal (**8**, 6.5 g, 21.8 mmol) in tetrahydrofuran (25 ml) was added dropwise under reflux and the resulting mixture was stirred under reflux for 5 min. The reaction was then quenched by careful addition of water (30 ml) and the resulting precipitate was removed by filtration. The filtrate was concentrated by rotary evaporator and the remaining aqueous emulsion was extracted with ethyl acetate (3×30 ml). The combined organic extracts were dried over $Na_2SO_4$ and concentrated. The residue was purified by vacuum-chromatography using hexane:acetone 10:1 as eluent to give 2.6 g (74%) of a colorless oil.

$[\alpha]_D$=+6.2, (c=1, acetone); *$^1$H-NMR*: 0.91 (d, 3H, $CH_3$), 1.24 (t, 4H, $CH_3$), 1.26 (t, 3H, $CH_3$), 2.01 (m, 1H, CH), 3.40–3.88 (m, 6H, 3 $OCH_2$), 4.37 (d, 1H, O–CH–O); *IR*: 3420 (br), 2980, 2930, 2880, 1460, 1370, 1350, 1120, 1060 $cm^{-1}$. Calcd. for $C_8H_{18}O_3$: C 59.23, H 11.18; found C 59.46, H 11.19.

*(R)-(+)-3-Benzyloxy-2-methylpropanal diethyl acetal* ***10***

To a solution of (*R*)-(+)-3-hydroxy-2-methylpropanal diethyl acetal (**9**, 0.81 g, 5.0 mmol) in dry tetrahydrofuran (10 ml) sodium hydride (0.4 g, 10 mmol, 60% in mineral oil) was added and the resulting mixture was heated under reflux for 1 hour. Potassium iodide (1.25 g, 7.5 mmol), tetrabutylammonium chloride (70 mg, 0.25 mmol) and benzyl chloride (0.7 ml, 6.0 mmol) were then added and heating was continued for 1 hour. After cooling, the reaction mixture was concentrated by rotary evaporator and water (3 ml) was added. The resulting emulsion was extracted with dichloromethane (3×5 ml), the combined organic extracts were dried over $Na_2SO_4$ and the solvent was removed to leave 1.15 g (92%) of a colorless oil.

$[\alpha]_D$=+4.2, (c=1, acetone); *$^1$H-NMR*: 1.03 (d, 3H, $CH_3$), 1.20 (t, 4H, $CH_3$), 1.21 (t, 3H, $CH_3$), 2.09 (m, 1H, CH), 3.30–3.82 (m, 6H, 3 $OCH_2$), 4.43 (d, 1H, O–CH–O), 4.50 (s, 2H, $OCH_2Ph$), 7.31 (m, 5H, ArH); *IR*: 2970, 2880, 1450, 1370, 1110, 1060, 1030, 740 $cm^{-1}$; Calcd. for $C_{15}H_{24}O_3$: C 71.39, H 9.59; found C 71.18, H 9.61.

*(R)-(−)-3-Benzyloxy-2-methylpropanal* ***11***

To a solution of water (2 ml), acetic acid (2 ml) and 5% hydrochloric acid (0.1 ml) (*R*)-(+)-3-benzyloxy-2-methylpropanal diethyl acetal (**10**, 0.4 g, 1.6 mmol) was added and the resulting mixture was stirred at RT for 2 hours. The reaction mixture was then extracted with ethyl acetate (2×3 ml) and the combined organic extracts were washed with saturated sodium hydrogen carbonate solution (4 ml) and brine (4 ml). After drying over $Na_2SO_4$ the solvent was removed by rotary evaporator. The oily residue was purified by vacuum-chromatography to yield 0.21 g (74%) of a colorless oil.

$[\alpha]_D$=−17.4, (c=1, chloroform), [(*R*)-(−)-**11**, literature: $[\alpha]_D$=−28.14, (c=1.4, chloroform); $[\alpha]_D$=−28, (c=1, chloroform)]; *$^1$H-NMR*: 1.17 (d, 3H, $CH_3$), 2.68 (m, 1H, CH), 3.67 (m, 2H, $OCH_2$), 4.53 (s, 2H, $OCH_2Ph$), 7.32 (m, 5H, Ar), 9.72 (d, 1H, CHO); Calcd. for $C_{11}H_{14}O_2$: C 74.13, H 7.92; found C 74.21, H 7.90.

**Acknowledgements**

The Hungarian OTKA Foundation (Project Nos: 1759 and 14894) is gratefully acknowledged for financial support. We thank the Amano Enzyme Europe Ltd for the generous donation of Lipase PS and Lipase AK.

**References**

1. Katagiri, N., Akaktsuka, H., Haneda, T., Kaneko, C., Sera, A. *J. Org. Chem.* **1988**, *53*, 5464.
2. Parham, W. E., Reed, L. J. *Org. Synth.* **1955**, *3*, 395:
3. Fuson, R. C., Parham, W. E., Reed, L. J. *J. Org. Chem.* **1946**, *11*, 194.
4. Kremers, J. A., Meijer, E. W. *J. Org. Chem.* **1994**, *59*, 4264.
5. Ozoe, Y., Eto, M. *Agric. Biol. Chem.* **1982**, *46*, 411.

6. Friedrich, K., Thieme, H. K. *Chem. Ber.* **1970**, *103*, 1982.
7. Sheikh, Z., Steel, R., Tasker, A. S., Johnson, A. P. *Chem. Commun.* **1994**, 763.
8. Meyers, A. I., Babiak, K. A., Campbell, A. L., Comins, D. L., Fleming, M. P. *J. Amer. Chem. Soc.* **1983**, *105*, 5015.
9. Kinoshita, M., Arai, M., Ohsawa, N., Nakata, M. *Tetrahedron Lett.* **1986**, *27*, 1815.
10. Poppe, L., Novák, L., *Magy. Kém. Lapja* **1985**, *40*, 366.
11. Juranic, I., Husinec, S., Savic, V., Porter, A. *Collect. Czech. Chem. Commun.* **1991**, *56*, 411.

*(Received in UK 4 December 1996; accepted 7 January 1997)*

# VIII. melléklet

BÓDAI, V., NOVÁK, L., **POPPE, L.**:

Synthesis and lipase-catalyzed enantiotope selective acetylation of 2-benzoyloxy-1,3-propanediol,

*SynLett*, **1999**, 759.

# Synthesis and Lipase-Catalyzed Enantiotope Selective Acetylation of 2-Benzoyloxy-1,3-propanediol

Viktória Bódai[a], Lajos Novák[a], László Poppe*[b]

[a]Institute for Organic Chemistry, Technical University of Budapest, H-1111 Budapest, Gellért tér 4., Hungary

[b]Chemical Research Centre of the Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67, Hungary; E-mail: poppe.szk@chem.bme.hu

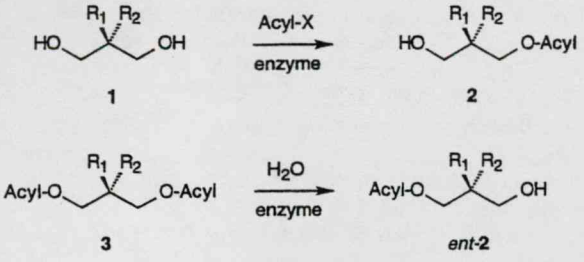
*Received 3 April 1999*

**Abstract:** Preparation and porcine pancreatic lipase (PPL)-catalyzed enantiotope selective acetylation of the prochiral 2-benzoyloxy-1,3-propanediol (**1a**) is described. The reaction with PPL and vinyl acetate gave monoacetate (**2a**) of 96 % e.e.

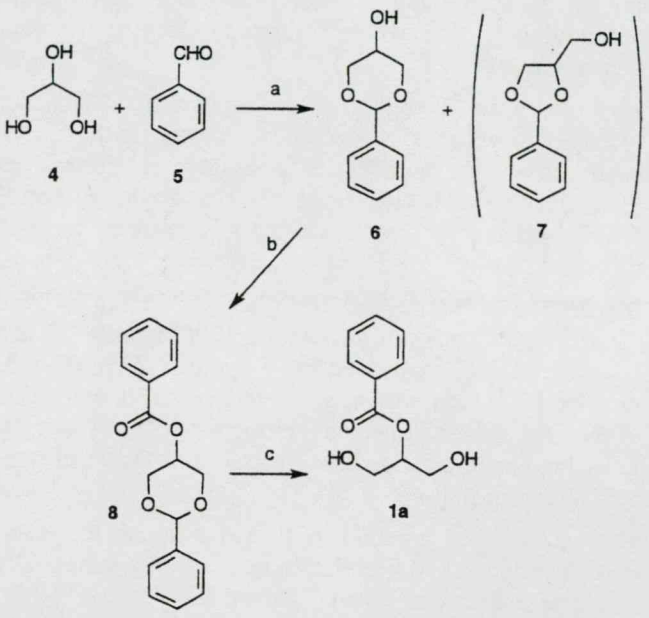
**Key words:** 2-*O*-benzoylglycerol, enantiotope selective, acetylation, lipase

Chiral glycerol derivatives are considered to be useful $C_3$ building blocks for the preparation of homochiral biologically active molecules such as phospholipids[1], phospholipase $A_2$ inhibitors[2], PAF (platelet-activating factor)[3], and many others[4].

Biocatalytical preparation of these chiral $C_3$ units were carried out either by enantiomer selective or enantiotope selective manner. The kinetic resolution of racemic glycerol derivatives such as glycerol acetonide[5,6] , glycerol-2,3-carbonate [7] provided moderate selectivity and 50% theoretical limit of the desired enantiomer. On the other hand, enantiotope selective transformation of prochiral 1,3-propanediols (**1**) or their diacyl derivatives (**3**) provide theoretically 100% of a single enantiomer (**2** or *ent*-**2**).

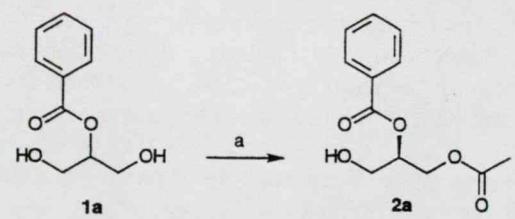



Enzyme-catalyzed acylation of several 2-*O*-alkylglycerol derivatives (**1**, $R_1$,$R_2$= *O*-alkyl, H), such as the 2-*O*-methyl-,[8,9] 2-*O*-ethyl-, [8,9] or 2-*O*-benzylglycerol[8] gave optically active monoacetates (**2**). Hydrolyses of the corresponding diacyl compound (**3**, $R_1$, $R_2$= OBn, H) with different enzymes under various conditions were also performed.[11-15] In the case of the 2-*O*-alkyl substituents, the lipase-catalyzed process proved to be *pro-S* selective. Consequently, acylation of the 2-*O*-benzylglycerol (**1**, $R_1$, $R_2$= OBn, H) provided (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol (**2**, $R_1$= H, $R_2$= OBn) [11] and hydrolyses of the corresponding diacyl derivative (**3**, $R_1$= H, $R_2$= OBn) gave the (R)-enantiomer (*ent*-**2**, $R_1$= OBn, $R_2$= H).[11,12] The slow ra-




(a) cat. cc. $H_2SO_4$, RT, 4 h, 28%; (b) BzCl (1.1 eq.), $Et_3N$ (1.2 eq.), cat. DMAP, $CH_2Cl_2$, RT, 2 h, 96 %; (c) $H_2$, cat. 10 %Pd/C, EtOAc, RT, 8 h, 73 %.

**Scheme 1**




| Entry | Enzyme (mg) | Time | 2a | |
|---|---|---|---|---|
| | | (h) | Y % | e.e. % |
| 1 | Novozym 435 (250) | 0.5 | 5* | 1 |
| 2 | Lipase G (100) | 72 | 5* | 3 |
| 3 | Lipase AK (100) | 1 | 32 | 7 |
| 4 | PsL (100) | 2.5 | 36 | 19 |
| 5 | Lipase N (100) | 72 | 13 | 20 |
| 6 | CcL (50) | 72 | 10 | 33 |
| 7 | PPL (300) | 1 | 63 | 96 |

* According to TLC data, most of the diol **1a** was converted to diacetate.

(a) **1a** (300 mg), enzyme, vinyl acetate (1 ml), THF (3 ml), hexane (3 ml), RT

**Scheme 2**

cemisation (*ca.* 2 %/h) found when optically active (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol (**2**, $R_1$= H, $R_2$= OBn) was incubated in phosphate buffer pH 7 without enzyme is the drawback of the hydrolytic method.[11]

Although the enantiotope selective biotransformations of 2-*O*-alkylglycerol derivatives (**1** or **3**, $R_1$,$R_2$= *O*-alkyl, H) are well documented, no example of enzymic enantiotope selective acylation of 2-O-acylglycerol derivatives (**1**, $R_1$, $R_2$= *O*-acyl, H) was found.

It is worthwile noting that two compounds of this family (**2**, $R_1$, $R_2$= *O*-acyl, H), namely 1-*O*-acetyl-2-*O*-(16-methyl)heptadecanoyl- and 1-*O*-acetyl-2-*O*-(18-methyl)nonadecanoylglycerol, were isolated from *Nicotina benthamiana*.[16]

As a part of our interest in exploring new stereoselective biocatalytic methods, we decided to investigate the lipase-catalyzed acetylation of the 2-*O*-acylglycerol derivatives (**1**, $R_1$,$R_2$= *O*-acyl, H). Hence, 2-*O*-benzoyloxyglycerol (**1a**, $R_1$=OBz, $R_2$=H) was selected as a representative of this class.

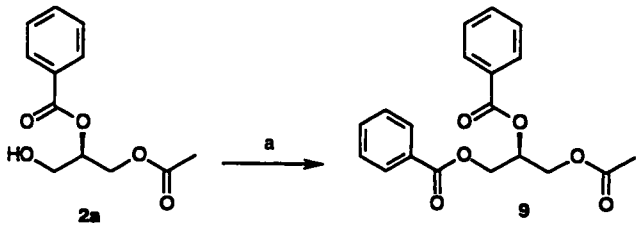
Preparation of the desired diol (**1a**) was straightforward (Scheme 1). Condensation reaction [17] of glycerol (**4**) and benzaldehyde (**5**) provided *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**6**).[18] Consequent benzoylation and deprotection of the benzylidene protected intermediate (**8**)[19] by catalytic hydrogenation yielded the desired diol (**1a**)[20] in pure crystalline form.

With the desired prochiral diol (**1a**) in hand, the enantiotope selectivity of acetylation by several commercially available lipases was tested (Scheme 2).

Among the enzymes investigated, lipase from porcine pancreas (PPL) proved to be the most selective providing almost enantiomerically pure product (**2a**)[21] in good yield (Entry 7). The enantiomeric purity of the product (**2a**) was determined from the $^1$H-NMR signals of its MTPA ester.[22] The composition of the solvent in this reaction catalyzed by PPL played an important role. Since the crystalline diol (**1a**) is poorly soluble in apolar solvents, the reaction was slow in hexane. Enzymatic acetylations using vinyl acetate as acylating agent in more polar solvents like chloroform, ethyl acetate or vinyl acetate gave decreased enantiotope selectivity compared to that obtained in the best solvent system (THF:hexane 1:1).

Prediction of the sense of enantiotopic selectivity seemed to be not obvious for lipase-catalyzed acylation of this new class of prochiral 1,3-propanediols. The lipase-catalyzed acylation of 2-*O*-alkyl-1,3-propanediols (**1**, $R_1$, $R_2$= *O*-alkyl, H) proved to be *pro-S* selective. In the case of 2-alkyl-1,3-propanediols (**1**, $R_1$, $R_2$= alkyl, H) bearing apolar substituent at position 2, enantiotope preference is inverted in a geometrical sense, although as a result of the sequence rules, the affected group is still labelled *pro-S*.[23] Acetylation of the diol bearing 2-*N*-benzyloxycarbonyl group by PPL was found to be *pro-R*.[11]

The absolute configuration of our product (**2a**) was determined by chemical correlation (Scheme 3.).




(a) BzCl (1.1 eq.), $Et_3N$ (1.2 eq.), THF, 0-20°C, 2 h, 88 %.

**Scheme 3**

The optical rotation of our dibenzoyl compound (**9**)[24] ($[\alpha]_D$= -2.78; c = 0.78, methanol) comparing to the literature data for (*S*)-(**9**) ($[\alpha]_D$= -0.8; c = 0.13, methanol)[25]proved its (*S*)-configuration, and therefore (*R*)-configuration of our enzymic product (**2a**).

## Acknowledgement

We thank OTKA (No. T014894 and T025235) for financial support.

## References and Notes

(1) Caer, E.; Kindler, A. *Biochemistry* **1962**, *1*, 518.
(2) Dennis, E. A. *Bio/Technology* **1987**, *5*, 1294.
(3) Hirth, G.; Barner, R. *Helv. Chim. Acta* **1982**, *65*, 1059.
(4) Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447.
(5) Wang, Y. F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 3127.
(6) Bianchi, D.; Bosetti, A.; Golini, P.; Cesti, P.; Pina, C. *Tetrahedron:Asymmetry* **1997**, *8*, 817.
(7) Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *J. Org. Chem.* **1994**, *59*, 1751.
(8) Murata, M.; Terao, Y.; Achiwa, K.; Nishio, T.; Seto, K. *Chem. Pharm. Bull.* **1989**, *10*, 2670.
(9) Terao, Y.; Murata, M.; Achiwa, K.; Nishio, T.; Akamtsu, M.; Kamimura, M. *Tetrahedron Lett.* **1988**, *29*, 5173.
(10) Ghisalba, O.; Lattmann, R.; Gygax, D. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 263.
(11) Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 7200.
(12) Breitgoff, D.; Laumen, K.; Schneider, M. P. *J. Chem. Soc. Chem. Commun.* **1986**, 1523.
(13) Wirz, B.; Schmid, R.; Foricher, J. *Tetrahedron:Asymmetry* **1992**, *3*, 137.
(14) Suemune, H.; Mizuhara, Y.; Akita, H.; Sakai, K. *Chem. Pharm. Bull.* **1986**, *34*, 3440.
(15) Kerschner, V.; Kreiser, W.; *Tetrahedron Lett.* **1987**, *28*, 531.
(16) Tsuzaki, T.; Shinozaki, Y.; Hagimori, M.; Tobita, T.; Shigematsu, H.; Koiwai, A. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1565.
(17) Carlsen, P. H. J.; Soerbye, K.; Ulven, T.; Aasboe, K. *Acta Chem. Scand.* **1996**, *50*, 185.
(18) Data for *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**6**): $\nu_{max}$ (KBr)/cm$^{-1}$ 3285, 3190, 2987, 2920, 2855, 1452, 1391, 1340, 1279, 1239, 1231, 1156, 1089, 1017,996, 977, 948, 930, 831, 808, 741; $\delta_H$ (500 MHz, $CDCl_3$): 3.15 (1H, d, *J*= 10.0 Hz, OH), 3.58 (1 H, br d, *J*= 10.0 Hz), 4.09 (2H, dd, *J*= 12.0 and 1.5 Hz), 4.17 (2H, dd, *J*= 12.0 and 1.5 Hz), 5.54 (1H, s) , 7.36 (3H, m), 7.49 (2H, m). Spectra are in agreement with literature data.[17]
(19) Data for *cis*-5-benzoyloxy-2-phenyl-1,3-dioxane (**7**): m.p. 92-93°C (ethanol); $\nu_{max}$ (KBr)/cm$^{-1}$ 3060, 2990, 2850, 1720, 1595, 1450, 1390, 1360, 1310, 1280, 1265, 1145, 1110, 1010.

790, 750, 710; $\delta_H$ (500 MHz, $CDCl_3$): 4.27 and 4.43 (2H, d, *J*=12 Hz, 2 $CH_2$), 4.96 (1H, s, CH-OBz), 5.72 (1H, s, CH-Ph), 7.39 (3H, m, Ar-H), 7.46 (2H, t, *J*= 7.5 Hz, Ar-H), 7.56 (3H, m, Ar-H), 8.17 (2H, d, *J*= 7.5 Hz, Ar-H).

(20) Data for 2-benzoyloxy-1,3-propanendiol (1a): m.p. 72-73°C (toluene-hexane 2:1); $\nu_{max}$ (KBr)/$cm^{-1}$ 3300, 2950, 2920, 2850, 1720, 1590, 1450, 1350, 1270, 1105, 1020, 955, 705; $\delta_H$ (500 MHz, $CDCl_3$): 2.59 (2H, 2 OH), 3.87 (m, 4H, 2 $CH_2$-O), 5.08 (m, 1H, CH-O), 7.36 (t, 2H, *J*= 7.5 Hz, 2 *m*-Ar-H), 7.50 (t, 1H, *J*= 7.5 Hz, *p*-Ar-H), 7.89 (d, 2H, *J*= 7.5 Hz, 2 *o*-Ar-H).

(21) The prochiral diol (1a, 300 mg, 1.53 mmol) was dissoved in dry THF (3 ml). To this solution vinyl acetate (1 ml), hexane (3 ml) and lipase from porcine pancreas (PPL, 300 mg) were added and the resulting suspension was stirred at RT for 1 h. The lipase (which after washing by acetone and drying proved to be active in a subsequent reaction) was removed by filtration. The oily residue remaining after evaporation of the filtrate was purified by low pressure chromatography on silica gel using hexane - acetone 4 : 1 eluant mixture.
Data for (*R*)-3-acetoxy-2-benzoyloxy-1-propanol (2a, 230 mg, 63 %): $[\alpha]_D$= -27.4 (c 1, ethanol), 96 %e.e.; $\nu_{max}$ (KBr)/ $cm^{-1}$ 3400, 2960, 2910, 2850, 1730, 1720, 1590, 1470, 1450, 1350, 1270, 1110, 1020, 960, 705; $\delta_H$ (500 MHz, $CDCl_3$): 2.04 (s, 3H, O=C-$CH_3$), 3.32 (br s, 1H, OH), 3.85 (m, 2H, $CH_2$-OH), 4.40 (m, 2H, $CH_2$-OAc), 5.33 (m, 1H, CH-O), 7.43 (t, 2H, *J*= 7.5 Hz, 2 *m*-Ar-H), 7.56 (t, 1H, *J*= 7.5 Hz, 1 *p*-Ar-H), 8.04 (d, 2H, *J*= 7.5 Hz, 2 *o*-Ar-H).

(22) Reaction of 2a with (*R*)-MTPA-Cl (1.2 eqv., $CCl_4$, pyridine) gave diastereomeric MTPA esters. Useful signals ($\delta_H$, 500 MHz, $CDCl_3$): 3.517 [s, $OCH_3$, (*R*)-2a MTPA ester], 3.544 [s, $OCH_3$, (*S*)-2a MTPA ester].

(23) Poppe, L.; Novák, L. *Selective Biocatalysis: A Synthetic Approach*; VCH: Weinheim-New York, 1992.

(24) Data for (*S*)-1-acetoxy-2,3-benzoyloxypropane (9): $[\alpha]_D$= -2.78 (c 0.78, methanol); $\nu_{max}$ (KBr)/$cm^{-1}$ 3050, 2950, 1740, 1720, 1600, 1580, 1485, 1450, 1360, 1315, 1250, 1175, 1100, 1070, 1045, 1025, 935, 850, 710, 680; $\delta_H$ (500 MHz, $CDCl_3$): 2.09 (3H,s), 4.47 (2H, mc, $CH_2OAc$), 4.62 (2H, mc, $CH_2OBz$), 5.68 (1H, m, CH-OBz), 7.26 (4H, mc, 4 *m*-ArH), 7.44 (2H, mc, 2 *p*-Ar-H), 8.04 (4H, mc, 4 *o*-Ar-H). Spectra are in agreement with literature data.[25]

(25) Uzawa, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1990**, *55*, 116.

Article Identifier:
1437-2096,E;1999,0,06,0759,0761,ftx,en;L03399ST.pdf

# IX. melléklet

EGRI, G., BÁLINT, J., PEREDI, R., FOGASSY, E., NOVÁK, L. **POPPE, L.:**

Lipase-catalyzed enantiotope selective acetylation of 2-acyloxypropane-1,3-diols. Influence of the acyl moiety on the selectivity,

*J. Mol. Catal. B, Enzymatic*, **1999**, beküldve.

# Lipase-catalyzed enantiotope selective acetylation of 2-acyloxypropane-1,3-diols. Influence of the acyl moiety on the selectivity

Gabriella Egri [1], József Bálint [1], Réka Peredi [1], Elemér Fogassy [1], Lajos Novák [2] and László Poppe [3]*

[1] Department of Organic Chemical Technology, Technical University Budapest, H-1111 Budapest, Műegyetem rkp. 3, Hungary

[2] Institute for Organic Chemistry, Technical University Budapest, H-1111 Budapest, Gellért tér 4, Hungary

[3] Chemical Research Center, Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67, Hungary

* To whom correspondence should be addressed: L. Poppe, *c/o* Institute for Organic Chemistry, Technical University Budapest, H-1111 Budapest, Gellért tér 4, Hungary; Phone: +36-1-4632229, Fax: +36-1-4633297, E-mail: poppe.szk@chem.bme.hu

**ABSTRACT**

Preparation and lipase-catalyzed enantiotope selective acetylation of the prochiral 2-acyloxy-propane-1,3-diols (**1a-h**) is described. A strong influence of the acyl moiety in these diols on the enantiotope selectivity of the porcine pancreatic lipase (PPL)-catalyzed reaction with vinyl acetate was observed. The best result was achieved with 2-(4-methylbenzoyl)oxypropane-1,3-diol resulting monoacetate (**2g**) of ≥98 % e.e.

***Keywords:***

Enzymes and enzyme reactions; acylation; enantioselection; substituent effects.

## INTRODUCTION

In the preparation of homochiral biologically active molecules, such as PAF (platelet-activating factor) [1], phospholipids [2], phospholipase $A_2$ inhibitors [3], and many others [4], chiral glycerol derivatives of high enantiomeric purity might be useful $C_3$ building blocks.

Enantiomer selective biocatalytical methods, e.g. kinetic resolution of racemic glycerol derivatives such as glycerol acetonide [5,6], glycerol-2,3-carbonate [7], provided moderate selectivity and 50 % theoretical limit of the desired enantiomer. On the other hand, enantiotope selective transformation of prochiral 1,3-propanediols (**1**) or their diacyl derivatives (**3**) provide theoretically 100 % of a single enantiomer (**2** or *ent*-**2**) (Fig. 1).

Among the 2-*O*-alkylglycerol derivatives (**1** or **3**, $R_1$,$R_2$= O-alkyl, H), the enantiotope selective biotransformations of 2-benzyloxy substituted compounds are the most studied. Hydrolyses of the corresponding diacyl compound (**3**, $R_1$,$R_2$= OBn, H) with different enzymes under various conditions were performed.[8-11, 13] The slow racemization (*ca.* 2 %/h) found when optically active (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol (**2**, $R_1$= H, $R_2$= OBn) was incubated in phosphate buffer pH 7 without enzyme is the drawback of the hydrolytic method [13]. Enzyme-catalyzed acylation of 2-*O*-benzylglycerol [12-15] (**1**, $R_1$,$R_2$= OBn, H) and other 2-*O*-alkyl- (**1**, $R_1$,$R_2$= *O*-alkyl, H) such as the 2-*O*-methyl- [14,15], 2-*O*-ethylglycerols [14,15] yielding optically active monoacetates (**2**, $R_1$,$R_2$= O-alkyl, H) were also studied. The lipase-catalyzed processes proved to be *pro-S* selective for the 2-*O*-alkylglycerol derivatives. Consequently, acetylation of the 2-*O*-benzylglycerol (**1**, $R_1$,$R_2$= OBn, H) yielded (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol (**2**, $R_1$= H, $R_2$= OBn) [13] and hydrolyses of the corresponding diacyl derivative (**3**, $R_1$= H, $R_2$= OBn) afforded the (*R*)-enantiomer (*ent*-**2**, $R_1$= OBn, $R_2$= H) [8,13].

Although the enantiotope selective biotransformations of 2-*O*-alkylglycerol derivatives (**1** or **3**, $R_1$,$R_2$= *O*-alkyl, H) are well documented, no example of enzymatic enantiotope selective acylation of 2-*O*-acylglycerol derivatives (**1**, $R_1$,$R_2$= *O*-acyl, H) was found. It is worthwhile

noting that two compounds of this family (2, $R_1$,$R_2$= *O*-acyl, H), namely 1-*O*-acetyl-2-*O*-(16-methyl)heptadecanoyl- and 1-*O*-acetyl-2-*O*-(18-methyl)nonadecanoylglycerol, were isolated from *Nicotina benthamiana* [16].

As a part of our interest in exploring new stereoselective biocatalytic methods, we decided to investigate the lipase-catalyzed acetylation of the 2-*O*-acylglycerol derivatives (1a-i, $R_1$,$R_2$= *O*-acyl, H). In our preliminary work, the 2-benzoyloxypropane-1,3-diol (1, $R_1$=OBz, $R_2$=H) was selected as the first representative of this class [17]. This diol was tested with several hydrolases for the enantiotope selective acetylation. The best result was achieved with porcine pancreatic lipase (PPL) and vinyl acetate in hexane-THF yielding monoacetate (2, $R_1$=OBz, $R_2$=H) of 96 % ee [17]. In this study our aim was to investigate the influence of the 2-acyloxy moiety on the enantiotope selectivity of enzymatic acetylation of these prochiral 1,3-diols. Hence, several prochiral carboxylic and sulfonic ester derivatives of glycerol were prepared and tested for enzymatic acetylation.

## EXPERIMENTAL

### *Materials and methods*

The $^1$H-NMR spectra were recorded on a Bruker AW-250 spectrometer operating at 250 MHz. For enantiomeric excess determinations, a Bruker DRX-500 spectrometer operating at 500 MHz was used. All spectra were taken in $CDCl_3$ solution and chemical shift values are expressed in ppm values from TMS as internal standard on δ scale. IR spectra of thin film samples were taken on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin Elmer 241 polarimeter at 20 °C. Thin layer chromatography was carried out using Merck Kieselgel 60 $F_{254}$ alumina sheets (using hexane:acetone 10:4, if otherwise not stated). Spots were visualized by treatment with 5 % ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative chromatographic separations were performed using vacuum-chromatography [18] on Merck Kieselgel 60 (0.063-0.200 mm). Chemicals were

products of Fluka or Aldrich. All solvents used were freshly distilled. CcL (lipase from *Candida rugosa,* formerly *Candida cylindracea*), PPL (lipase from porcine pancreas) and papain were obtained from Sigma. PfL (lipase from *Pseudomonas fluorescens*) was a product of Fluka. Novozym 435 (immobilized lipase of *Candida antarctica*) and Lipozym IM (immobilized lipase of *Mucor miehei*) were gifts from Novo Nordisk. Lipase A (lipase from *Aspergillus niger*), Lipase AK (lipase from *Pseudomonas fluorescens*); Lipase G (lipase from *Penicillinum camambertii*), Lipase M (lipase from *Mucor javonicus*), Lipase N (lipase from *Rhisopus niveus*) and Lipase PS (lipase from *Pseudomonas* sp.) were gifts from Amano.

*cis-5-Hydroxy-2-phenyl-1,3-dioxane* (**6**)

The reaction between glycerol (**4**, 50 g, 0.54 mol) and benzaldehyde (**5**, 50 g, 0.47 mol) according to the known method [19] gave crystalline product (**6**, 30 g, 35 %).
*$^1$H-NMR*: 3.15 (1H, d, *J*= 10.0 Hz, OH), 3.58 (1 H, br d, *J*= 10.0 Hz), 4.09 (2H, dd, *J*= 12.0 and 1.5 Hz), 4.17 (2H, dd, *J*= 12.0 and 1.5 Hz), 5.54 (1H, s) , 7.36 (3H, m), 7.49 (2H, m); *IR* (KBr, $cm^{-1}$): 3285, 3190, 2985, 2920, 2855, 1450, 1390, 1340, 1280, 1240, 1230, 1155, 1090, 1015, 995, 975, 950, 930, 830, 810, 740. (Spectra are in agreement with literature data [19])

*Preparation of cis-5-(aryl- or alkylsulfonyl)oxy-2-phenyl-1,3-dioxanes* (**7a-c**)

*General procedure*: To a solution of *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**6**, 3.73 g, 20 mmol) and triethylbenzylammonium chloride (50 mg) in diethyl ether (25 ml) finely powdered KOH (3.36 g, 60 mmol) was added and the mixture was cooled to –5 °C. At this temperature aryl- or alkylsulfonyl chloride (22 mmol) was added portionwise. The resulting mixture was vigorously stirred at -5 °C for 40 min and at room temperature for 15 min. The white suspension was diluted with ethyl acetate (25 ml) and washed with water (15 ml). The aqueous phase was re-extracted with ethyl acetate (2 x 25 ml). The combined organic layers were dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* afforded the desired acylated products (**7a-c**).

*cis-2-Phenyl-5-(p-toluenesulfonyl)oxy-1,3-dioxane* (**7a**)

According to the general procedure, 6.15 g (92 %) of white crystalline solid was prepared. *M.p.*: 120-123 °C (ethyl acetate); *$^1$H-NMR*: 2.40 (s, 3H, $CH_3$), 4.01 and 4.23 (m($A_2B_2$), 4H, 2 $CH_2$), 4.43 (s, 1H, CH-O), 5.45 (s, 1H, O-CH-O), 7.26-7.65 (m, 7H, ArH), 7.82 (d, 2H, ArH); *IR* (KBr, $cm^{-1}$): 3445, 2860, 1650, 1465, 1395, 1355, 1190, 1175, 1145, 1080, 1015, 985, 930, 745; Calcd. for $C_{17}H_{18}O_5S$: C 61.06, H 5.43, S 9.59; found C 60.89, H 5.42, S 9.61.

*cis-5-Benzenesulfonyloxy-2-phenyl-1,3-dioxane* (**7b**)

According to the general procedure, 4.8 g (75 %) of white crystalline solid was prepared. *M.p.*: 127-130 °C (diethyl ether); *$^1$H-NMR*: 4.09 and 4.27 (m($A_2B_2$), 4H, 2 $CH_2$), 4.52 (s, 1H, CH-O), 5.49 (s, 1H, O-CH-O), 7.26-7.68 (m, 7H, ArH), 7.97 (d, 2H, ArH); *IR* (KBr, $cm^{-1}$): 3445, 2855, 1685, 1640, 1445, 1350, 1185, 1145, 1075, 1015, 910, 870, 850, 755; Calcd. for $C_{16}H_{16}O_5S$: C 59.99, H 5.03, S 10.01; found C 60.07, H 5.02, S 10.03.

*cis-5-Methanesulfonyloxy-2-phenyl-1,3-dioxane* (**7c**)

The general procedure followed preparative vacuum column chromatography (silica gel, hexane:acetone 10:1) yielded 2.22 g (43 %) of white crystals.
*M.p.*: 128-130°C (ethyl acetate); *$^1$H-NMR*: 3.14 (s, 3H, $CH_3$), 4.18 and 4.44 (m($A_2B_2$), 4H, $2CH_2$), 4.68 (s, 1H, CH-O), 5.56 (s, 1H, O-CH-O), 7.37 (mc, 3H, ArH), 7.50 (mc, 2H, ArH); *IR* (KBr, $cm^{-1}$): 3425, 3025, 1450, 1385, 1330, 1170, 1135, 1080, 1015, 980, 955, 940, 910, 870, 740; Calcd. for $C_{11}H_{14}O_5S$: C 51.15, H 5.46, S 12.41; found C 51.24, H 5.47, S 12.43.

*Preparation of cis-5-acyloxy-2-phenyl-1,3-dioxanes* (**7d-i**)

*General procedure*: To a solution of *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**6**, 3.73 g, 20 mmol), pyridine (1.95 ml, 24 mmol) and 4-(dimethylamino)pyridine (50 mg) in dichloromethane (30 ml), acyl chloride (22 mmol) was added at room temperature and the mixture was stirred for 1-12 h. The resulting mixture was washed with 5 % HCl solution (2 x

10 ml), 10 % $Na_2CO_3$ solution (10 ml) and brine (10 ml). After drying over $Na_2SO_4$ and evaporation of the solvent, the solid residue was recrystallized to give white crystals (**7d-i**).

*cis-5-Acetyloxy-2-phenyl-1,3-dioxane* (**7d**)

Yield: 84 %; *M.p.*: 98-101 °C (hexane:ethyl acetate 1:1); *$^1$H-NMR*: 2.16 (s, 3H, $CH_3$), 4.10-4.28 (2 x dd, 4H, $2CH_2$), 4.70 (d, 1H, CH-O), 5.55 (s, 1H, O-CH-O), 7.35 (m, 3H, Ar-H), 7.49 (m, 2H, Ar-H); *IR* (KBr, $cm^{-1}$): 3440, 1730, 1460, 1390, 1375, 1245, 1140, 1085, 1020, 985, 950, 920, 745; Calcd. for $C_{12}H_{14}O_4$: C 64.85, H 6.35; found C 64.79, H 6.36.

*cis-5-Diphenylacetyloxy-2-phenyl-1,3-dioxane* (**7e**)

Yield: 86 %; *M.p.*: 116-119 °C (hexane:ethyl acetate 1:1); *$^1$H-NMR*: 4.12-4.33 (m($A_2B_2$), 4H, $2CH_2$), 4.76 (br s, 1H, CH-O), 5.19 (s, 1H, Ph-CH-Ph), 5.55 (s, 1H, O-CH-O), 7.16-7.56 (m, 15H, Ar-H); *IR* (KBr, $cm^{-1}$): 3435, 1725, 1495, 1450, 1390, 1330, 1310, 1270, 1195, 1160, 1140, 1085, 1020, 745. Calcd. for $C_{24}H_{22}O_4$: C 76.99, H 5.92; found C 76.81, H 5.93.

*cis-2-Phenyl-5-pivaloyloxy-1,3-dioxane* (**7f**)

Yield: 83 %; *M.p.*: 106-111 °C (hexane:ethyl acetate 1:1); *$^1$H-NMR*: 1.29 (s, 9H, $3CH_3$), 4.11-4.27 (m($A_2B_2$), 4H, $2CH_2$), 4.65 (br s, 1H, CH-O), 5.53 (s, 1H, O-CH-O), 7.37 (m, 3H, Ar-H), 7.49 (m, 2H, Ar-H); *IR* (KBr, $cm^{-1}$): 3400, 2985, 1705, 1455, 1395, 1284, 1165, 1140, 1085, 1015, 990, 955, 745; Calcd. for $C_{15}H_{20}O_4$: C 68.16, H 7.63; found C 68.15, H 7.64.

*cis-5-(4-Methylbenzoyl)oxy-2-phenyl-1,3-dioxane* (**7g**)

Yield: 80 %; *M.p.*: 119-122 °C (hexane:ethyl acetate 1:1); *$^1$H-NMR*: 2.41 (s, 3H, $CH_3$), 4.21-4.46 (m($A_2B_2$), 4H, $2CH_2$), 4.94 (br s, 1H, CH-O), 5.61 (s, 1H, O-CH-O), 7.24 (d, 2H, Ar-H), 7.38 (m, 3H, Ar-H), 7.51 (m, 2H, Ar-H), 8.06 (d, 2H, Ar-H),; *IR* (KBr, $cm^{-1}$): 3445, 1775, 1710, 1610, 1455, 1390, 1275, 1210, 1145, 1115, 1085, 1020, 980, 950, 900, 755, 745; Calcd. for $C_{18}H_{18}O_4$: C 72.47, H 6.08; found C 72.34, H 6.07.

*cis-5-Cyclohexanecarbonyloxy-2-phenyl-1,3-dioxane* (**7h**)

Yield: 96 %; *M.p.*: 73-75 °C (hexane); *$^1$H-NMR*: 1.20-1.36 (m, 3H), 1.44-1.56 (m, 2H), 1.65 (mc, 1H), 1.72-1.80 (m, 2H), 1.94-2.0 (m, 2H), 2.45 (mc, 1H, CH-CO), 4.15 and 4.25 (m($A_2B_2$), 4H, 2 $CH_2$-O), 4.69 (br s, 1H, CH-Ph), 5.54 (s, 1H, O-CH-O), 7.32-7.40 (m, 3H, Ar-H), 7.48-7.52 (m, 2H, Ar-H); *IR* (KBr, $cm^{-1}$): 2936, 2880, 1712, 1456, 1392, 1365, 1312, 1248, 1176, 1140, 1084, 1000, 744, 696; Calcd. for $C_{17}$ $H_{22}$ $O_4$: C 70.32, H 7.64; found C 70.18, H 7.72.

*cis-5-Lauryloxy-2-phenyl-1,3-dioxane* (**7i**)

Yield: 60 % (purified by chromatography on silica gel using hexane-acetone 10:1); waxy solid; *$^1$H-NMR*: 0.87 (t, 3H, $CH_3$), 1.20-1.38 (m, 16H, 8 $CH_2$), 1.67 (mc, 2H, $CH_2$), 2.44 (t, 2H, $CH_2$-CO), 4.16 and 4.27 (m($A_2B_2$), 4.72 (br s, 1H, CH-Ph), 5.56 (s, 1H, O-CH-O), 7.33-7.40 (m, 3H, Ar-H), 7.49-7.53 (m, 2H, Ar-H); *IR* (KBr, $cm^{-1}$): 2920, 2880, 1736, 1456, 1432, 1392, 1360, 1276, 1240, 1200, 1176, 1144, 1088, 1016, 744, 696; Calcd. for $C_{22}$ $H_{34}$ $O_4$: C 72.89, H 9.45; found C 73.01, H 9.52.

*Preparation of 2-(aryl- or alkylsulfonyl)oxypropane-1,3-diols* (**1a-c**)

*General procedure*: To a 20 % methanolic solution of the *cis*-5-(aryl- or alkylsulfonyl)oxy-2-phenyl-1,3-dioxane (**7a-c**, 9-18 mmol), equimolar amount of concentrated hydrochloric acid was added and the mixture was refluxed for 1 min. After cooling to room temperature, most of the methanol was removed *in vacuo*, the solution was neutralized with saturated $Na_2CO_3$ solution, and further diluted with water (up to a final volume of 20-30 ml. After complete evaporation of the methanol, the residue was extracted with hexane (2 x 15 ml, removal of benzaldehyde), and with ethyl acetate (3 x 20 ml). The combined ethyl acetate layers were dried over $Na_2SO_4$. Evaporation *in vacuo* resulted the corresponding product (**1a-c**).

*2-(p-Toluenesulfonyl)oxypropane-1,3-diol* (**1a**)

Oil. Yield: 76 %; *$^1$H-NMR*: 2.42 (s, 3H, $CH_3$), 3.74 (m, 4H, $2CH_2$-O), 4.53 (m(t), 1H, CH-O), 7.33 (d, 2H, Ar), 7.81 (d, 2H, Ar); *IR* (film, $cm^{-1}$): 3395 (br), 2950, 1700, 1600, 1455, 1360, 1175, 1095, 1055, 925, 815; Calcd. for $C_{10}H_{14}O_5S$: C 48.77, H 5.73, S 13.02; found C 48.70, H 5.74, S 13.00.

*2-Benzenesulfonyloxypropane-1,3-diol* (**1b**)

Oil. Yield: 74 %; *$^1$H-NMR*: 3.76 (m, 4H, $2CH_2$-O), 4.59 (m(t), 1H, CH-O), 7.56 (t, 2H, Ar), 7.69 (t, 1H, Ar), 7.94 (d, 2H, Ar); *IR* (film, $cm^{-1}$): 3385 (br), 3945, 1450, 1360, 1185, 1095, 1055, 1010, 925, 790, 755; Calcd. for $C_9H_{12}O_5S$: C 46.54, H 5.21, S 13.80; found C 46.52, H 5.20, S 13.79.

*2-Methanesulfonyloxypropane-1,3-diol* (**1c**)

Yield: 69 %; *M.p.*: 65-68 °C (hexane-ethyl acetate 1:1); *$^1$H-NMR* (MeOH-$d_4$): 3.07 (s, 3H, $CH_3$), 3.68 (m, 4H, $2CH_2$-O), 4.53 (m, 1H, CH-O); *IR* (film, $cm^{-1}$): 3385 (br), 1340, 1170, 1085, 1040, 1010, 985, 930, 795; Calcd. for $C_4H_{10}O_5S$: C 28.23, H 5.92, S 18.84; found C 28.28, H 5.93, S 18.83.

*Preparation of 2-acyloxypropane-1,3-diols* (**1d-h**)

*General procedure*: To a 20 % isopropanolic solution of the *cis*-5-acyloxy-2-phenyl-1,3-dioxane (**7d-h**, 16-18 mmol), Pd-C (5 %) was added and the mixture was vigorously stirred under hydrogen at room temperature. After uptaking the calculated amount of hydrogen (2 equivalents), the catalyst was filtered off. Evaporation of the solvent *in vacuo* yielded the corresponding product (**1d-h**).

*2-Acetoxypropane-1,3-diol* (**1d**)

Oil. Yield: 67 %; *$^1$H-NMR*: 2.13 (s, 3H, $CH_3$), 3.81 (m(d), 4H, $2CH_2$-O), 4.89 (mc, 1H, CH-O); *IR* (film, $cm^{-1}$): 3380 (br), 2940, 1735, 1245, 1045, 960, 830; Calcd. for $C_5H_{10}O_4$: C 44.77, H 7.51; found C 44.70, H 7.52.

*2-Diphenylacetoxypropane-1,3-diol* (**1e**)

Oil. Yield: 75 %; *$^1$H-NMR*: 3.74 (mc, 4H, 2$CH_2$-O), 4.87 (mc, 1H, CH-O), 5.06 (s, 1H, Ph-CH-Ph), 7.10-7.28 (m, 10H, Ar-H); *IR* (film, $cm^{-1}$): 3405 (br), 1735, 1495, 1455, 1305, 1190, 1150, 1050, 1010, 745, 700; Calcd. for $C_{17}H_{18}O_4$: C 71.31, H 6.34; found C 71.43, H 6.33.

*2-Pivaloyloxypropane-1,3-diol* (**1f**)

Oil. Yield: 77 %; *$^1$H-NMR*: 1.22 (s, 9H, $CH_3$), 3.77 (m(d), 4H, 2$CH_2$-O), 4.86 (m(t), 1H, CH-O); *IR* (film, $cm^{-1}$): 3415 (br), 2970, 2845, 1710, 1480, 1460, 1400, 1370, 1285, 1170, 1040, 980, 770; Calcd. for $C_8H_{16}O_4$: C 54.53, H 9.15; found C 54.64, H 9.17.

*2-(4-Methylbenzoyl)oxypropane-1,3-diol* (**1g**)

Yield: 76 %; *M.p.*: 84-90 °C (hexane:ethyl acetate 1:1); *$^1$H-NMR*: 2.38 (s, 3H, $CH_3$), 3.90 (m(d), 4H, 2$CH_2$-O), 5.11 (m(t), 1H, CH-O), 7.20 (d, 2H, Ar-H), 7.91 (d, 2H, Ar-H); *IR* (film, $cm^{-1}$): 3410 (br), 1685, 1610, 1460, 1420, 1355, 1305, 1185, 1130, 1085, 1055, 1040, 835, 760, 700; Calcd. for $C_{11}H_{14}O_4$: C 62.85, H 6.71; found C 62.79, H 6.72.

*2-Cyclohexanecarbonyloxypropane-1,3-diol* (**1h**)

Semisolid. Yield: 96 %; *$^1$H-NMR*: 1.19-1.35 (m, 3H), 1.38-1.50 (m, 2H), 1.65 (mc, 1H), 1.71-1.81 (m, 2H), 1.87-1.96 (m, 2H), 2.34 (mc, 1H, CH-CO) 3.75-3.84 (m(dd), 4H, 2$CH_2$-O), 4.89 (m(t), 1H, CH-O); *IR* (film, $cm^{-1}$): 3400 (br), 2936, 2856, 1710, 1452, 1428, 1384, 1312, 1248, 1172, 1136, 1040; Calcd. for $C_{10}H_{18}O_4$: C 59.39, H 8.97; found C 59.29, H 9.02.

*Preparation of racemic 3-acetyloxy-2-acyloxypropan-1-ols* (*rac*-**2a-g**)

*General procedure*: To a solution of the 2-acyloxypropane-1,3-diol (**1a-g**, 5 mmol) in ethyl acetate (10 ml), pyridine (0.45 ml), 4-(dimethylamino)pyridine (25 mg) and acetic acid anhydride (0.47 ml) were added. The mixture was stirred at room temperature for 1 h and then washed with 5 % HCl solution (2 x 5 ml), 10 % $Na_2CO_3$ solution (5 ml) and brine (5 ml). After drying over $Na_2SO_4$ the solvent was removed *in vacuo* and the residue was purified by

preparative vacuum column chromatography (silica gel, hexane-acetone) affording the product (*rac*-**2a-g**) as an oil.

*3-Acetoxy-2-(p-toluenesulfonyl)oxypropan-1-ol* (*rac*-**2a**)

Yield: 51 %; *$^1$H-NMR*: 1.92 (s, 3H, $CH_3$-CO), 2.45 (s, 3H, Ar-$CH_3$), 3.75 (mc, 2H, $CH_2$-O), 4.14-4.26 (m, 2H, $CH_2$-OAc), 4.70 (mc, 1H, CH-O), 7.34 (d, 2H, Ar), 7.82 (d, 2H, Ar); *IR* (film, $cm^{-1}$): 3415 (br), 2955, 1745, 1600, 1360, 1240, 1190, 1175, 1100, 1050, 930, 915, 775; Calcd. for $C_{12}H_{16}O_6S$: C 49.99, H 5.59, S 11.12; found C 49.98, H 5.61, S 11.11.

*3-Acetoxy-2-benzenesulfonyloxypropan-1-ol* (*rac*-**2b**)

Yield: 47 %; *$^1$H-NMR*: 1.92 (s, 3H, $CH_3$-CO), 3.78 (mc, 2H, $CH_2$-O), 4.15-4.28 (m, 2H, $CH_2$-OAc), 4.72 (mc, 1H, CH-O), 7.57 (t, 2H, Ar-H), 7.69 (t, 1H, Ar-H), 7.94 (d, 2H, Ar-H); *IR* (film, $cm^{-1}$): 3415 (br), 2955, 1745, 1450, 1365, 1215, 1190, 1125, 1100, 1050, 930, 790, 755; Calcd. for $C_{11}H_{14}O_6S$: C 48.17, H 5.14, S 11.69; found C 48.24, H 5.14, S 11.72.

*3-Acetoxy-2-methanesulfonyloxypropan-1-ol* (*rac*-**2c**)

Yield: 45 %; *$^1$H-NMR*: 2.12 (s, 3H, $CH_3$-CO), 3.13 (s, 3H, $CH_3$-S), 3.85 (mc, 2H, $CH_2$-O), 4.22-4.41 (m, 2H, $CH_2$-OAc), 4.88 (mc, 1H, CH-O); *IR* (film, $cm^{-1}$): 3520 (br), 3030, 2940, 1745, 1350, 1235, 1175, 1050, 975, 930, 805, 740; Calcd. for $C_6H_{12}O_6S$: C 33.96, H 5.70, S 15.11; found C 33.96, H 5.71, S 15.15.

*2,3-Diacetoxypropan-1-ol* (*rac*-**2d**)

Yield: 54 %; *$^1$H-NMR*: 2.11 (s, 3H, $CH_3$-CO), 2.16 (s, 3H, $CH_3$-CO), 3.71-3.77 (m, 2H, $CH_2$-O), 4.12-4.38 (m, 2H, $CH_2$-OAc), 5.08 (mc, 1H, CH-O); *IR* (film, $cm^{-1}$): 3465 (br), 2960, 1745, 1440, 1375, 1230, 1050, 960,845; Calcd. for $C_7H_{12}O_5$: C 47.73, H 6.87; found C 47.81, H 6.85.

*3-Acetoxy-2-diphenylacetoxypropan-1-ol* (*rac*-**2e**)

Yield: 51 %; *$^1$H-NMR*: 1.96 (s, 3H, $CH_3$-CO), 3.70 (mc, 4H, $2CH_2$-O), 4.16-4.31 (m, 2H, $CH_2$-OAc), 5.05 (mc, 1H, CH-O), 5.07 (s, 1H, Ph-CH-Ph), 7.30 (mc, 10H, Ar-H); *IR* (film,

$cm^{-1}$): 3460 (br), 3030, 2955, 1740, 1585, 1495, 1450, 1370, 1235, 1190, 1150, 1045, 1015, 745, 700; Calcd. for $C_{19}H_{20}O_5$: C 69.50, H 6.14; found C 69.67, H 6.15.

*3-Acetoxy-2-pivaloyloxypropan-1-ol* (*rac*-**2f**)

Yield: 48 %; *$^1$H-NMR*: 1.22 (s, 9H, $3CH_3$), 2.07 (s, 3H, $CH_3$-CO), 3.74 (mc, 2H, $CH_2$-O), 4.19-4.37 (m, 2H, $CH_2$-OAc), 5.07 (mc, 1H, CH-O); *IR* (film, $cm^{-1}$): 3475 (br), 2970, 1730, 1480, 1460, 1400, 1370, 1285, 1235, 1160, 1050; Calcd. for $C_{10}H_{18}O_5$: C 55.03, H 8.31; found C 55.12, H 8.30.

*3-Acetoxy-2-(4-methylbenzoyl)oxypropan-1-ol* (*rac*-**2g**)

Yield: 50 %; *$^1$H-NMR*: 2.08 (s, 3H, $CH_3$-CO), 2.42 (s, 3H, Ar-$CH_3$), 3.87 (m(t), 2H, $CH_2$-O), 4.41 (m(d), 2H, $CH_2$-OAc), 5.31 (m(t), 1H, CH-O), 7.26 (d, 2H, Ar-H), 7.93 (d, 2H, Ar-H); *IR* (KBr, $cm^{-1}$): 3455 (br), 2955, 1715, 1610, 1510, 1445, 1410, 1370, 1275, 1180, 1110, 1045, 1020, 920, 840, 750; Calcd. for $C_{13}H_{16}O_5$: C 61.90, H 6.39; found C 62.04, H 6.41.

*Enzymatic acetylation of 2-acyloxypropane-1,3-diols* (**1a-h**)

*General procedure*: For solvents, enzymes, reaction times and yields, see Tables 2-4. To a solution of the prochiral diol (**1a-h**, 250 mg) in the solvent indicated, vinyl acetate and enzyme were added. After stirring the mixture at room temperature for the given time, the enzyme was filtered off, the solvent was removed from the filtrate in vacuo and the residue was purified by preparative vacuum column chromatography (silica gel, hexane:acetone 10:1.5). For yields, optical rotation and enantiomeric composition of the products (**2a-h**), see Tables 2-4. Spectral ($^1$H-NMR and IR) data for the products (**2a-g**) were indistinguishable from that of the racemic 3-acetoxy-2-acyloxypropan-1-ols (*rac*-**2a-g**).

*3-Acetoxy-2-cyclohexanecarbonyloxypropan-1-ol* (**2h**)

*$^1$H-NMR*: 1.19-1.34 (m, 3H), 1.39-1.52 (m, 2H), 1.65 (mc, 1H), 1.72-1.80 (m, 2H), 1.87-1.96 (m, 2H), 2.07 (s, 3H, $CH_3$-CO), 2.36 (mc, 1H, CH-CO), 3.73 (mc, 2H, $CH_2$-O), 4.21-4.35 (m,

2H, $CH_2$-OAc), 5.08 (mc, 1H, CH-O); *IR* (KBr, $cm^{-1}$): 3464 (br), 2936, 2856, 1736, 1452, 1416, 1372, 1244, 1168, 1048; Calcd. for $C_{12}H_{20}O_5$: C 59.00, H 8.25; found C 59.08, H 8.33.

*Preparation of MTPA esters from the racemic and optically active 3-acetyloxy-2-acyloxypropan-1-ols* (**2a-g**)

The racemic or optically active 3-acetyloxy-2-acyloxypropan-1-ols (**2a-g**, 9-12 mg), pyridine (25 μl) and (4-dimethylamino)pyridine (2 mg) were added to a solution of 5 % (*R*)-MTPA-Cl in carbon tetrachloride (350 μl) and the mixture was heated in a sealed ampoule at 50 °C for 3 h. The resulting mixture was successively washed with 5 % HCl solution (1 ml), saturated $Na_2CO_3$ solution (1 ml) and brine (1 ml). The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated. The diastereomeric ratio of the forming MTPA esters were determined from their $^1$H-NMR spectra (500 MHz, $CDCl_3$, TMS). Several signals used for enantiomeric purity determination are listed in Table 1.

*Determination of the absolute configuration of the monoacetates* (**2a-h**)

*Enzymatic acetylation of 3-benzyloxypropane-1,2-diol* (**9**)[22]

To a solution of 3-benzyloxypropane-1,2-diol (**9**, 9.5 g) in hexane (50 ml), THF (50 ml) and vinyl acetate (25 ml) Lipase-AK (1 g) was added and the mixture was stirred at room temperature for 27 h. The enzyme was filtered off, the solvent was evaporated from the filtrate and the residue was subjected to preparative vacuum column chromatography (silica gel, hexane:acetone 10:0.5 to 10:2) to give (*R*)-1-acetoxy-3-benzyloxypropan-2-ol [**10**, yield: 5.2 g, $[\alpha]_D$= -3.3 (c 1, chloroform); lit.[22]: $[\alpha]_D$= +4.1 (c 1.04, chloroform), enantiomerically pure (*S*)-**10**] and (*S*)-1,2-diacetoxy-3-benzyloxypropane [**11**, yield: 5.2 g, $[\alpha]_D$= +12.4 (c 0.5, chloroform); lit.[22]: $[\alpha]_D$= +14.0 (c 0.5, chloroform), enantiomerically pure (*S*)-**11**].

*Catalytic hydrogenation of (R)-1-acetoxy-3-benzyloxypropan-2-ol* (**10**)

A solution of (*R*)-1-acetoxy-3-benzyloxypropan-2-ol [**10**, 2.0 g, 8.9 mmol, $[\alpha]_D$= -3.3 (c 1, chloroform)] in isopropanol (20 ml) was hydrogenated on 10 % Pd-C (300 mg) at 40 °C for 45 min. The catalyst was filtered off and solvent was evaporated *in vacuo*. Yield: 1.18 g (100 %) of (*R*)-3-acetoxypropane-1,2-diol (**13**)[8] {$[\alpha]_D$= -9.9 (c 2, pyridine)}.

*Bis-sulfonylation of (R)-3-acetoxypropane-1,2-diol* (**13**)

*General procedure:* To a solution of (*R*)-3-acetoxypropane-1,2-diol [**13**, 0.40 g, 3.0 mmol, $[\alpha]_D$= -9.9 (c 2, pyridine)], triethylamine (1.0 ml, 7.2 mmol) and 4-(dimethylamino)pyridine (10 mg) in dichloromethane (3 ml) *p*-toluenesulfonyl chloride (1.26 g, 6.6 mmol, for *ent*-**8a**) or benzenesulfonyl chloride (1.17 g, 6.6 mmol, for *ent*-**8b**) was added and the resulting mixture was stirred at room temperature for 3 h. The mixture was then washed with 5 % HCl solution (2 x 1 ml), 10 % $Na_2CO_3$ solution (1 ml) and saturated $NaHCO_3$ (1 ml). The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo* to leave the product (*ent*-**8a** or *ent*-**8b**) as an oil.

(*S*)-*1-Acetoxy-2,3-di(p-toluenesulfonyl)oxypropane* (*ent*-**8a**)

Yield: 89 %; $[\alpha]_D$= -15.2 (c 1, methanol); $^1$*H-NMR*: 1.91 (s, 3H, $CH_3$-CO), 2.45 (s, 3H, Ar-$CH_3$), 2.46 (s, 3H, Ar-$CH_3$), 4.02-4.23 (m, 4H, $CH_2$-OAc and $CH_2$-OTs), 4.76 (mc, 1H, CH-O), 7.32-7.37 (2 x d, 4H, Ar-H), 7.68-7.78 (2x d, 4H, Ar-H); *IR* (film, $cm^{-1}$): 2960, 1745, 1600, 1455, 1365, 1230, 1190, 1095, 1045, 1000, 935, 815, 765; Calcd. for $C_{19}H_{22}O_8S_2$: C 51.57, H 5.01, S 14.49; found C 51.57, H 5.02, S 14.47.

(*S*)-*1-Acetoxy-2,3-di(benzenesulfonyl)oxypropane* (*ent*-**8b**)

Yield: 90 %; $[\alpha]_D$= -16.0 (c 1, methanol); $^1$*H-NMR*: 1.89 (s, 3H, $CH_3$-CO), 4.07-4.23 (m, 4H, $CH_2$-OAc and $CH_2$-$OSO_2Ph$), 4.79 (mc, 1H, CH-O), 7.48-7.89 (m, 10H, Ar-H); *IR* (film, $cm^{-1}$): 2960, 1745, 1710, 1450, 1370, 1225, 1190, 1035, 1005, 935, 755; Calcd. for $C_{19}H_{18}O_8S_2$: C 49.27, H 4.38, S 15.47; found C 49.34, H 4.37, S 15.46.

*Arylsulfonylation of monoacetates* (**2a,b**) *from enzymatic acetylation of the prochiral diols* (**1a,b**)

*General procedure*: 3-Acetyloxy-2-(*p*-toluenesulfonyl)oxypropan-1-ol [**2a**, 0.33 mmol, $[\alpha]_D$= +9.2 (c 1, methanol)] or 3-acetyloxy-2-benzenesulfonyloxypropan-1-ol [**2b**, 0.33 mmol, $[\alpha]_D$= +6.2 (c 1, methanol)], 4-(dimethylamino)pyridine (1 mg) and triethylamine (0.05 ml) were dissolved in dichloromethane (0.7 ml) and *p*-toluenesulfonyl chloride (0.35 mmol, for **2a**) or benzenesulfonyl chloride (0.35 mmol, for **2b**) was added. The resulting mixture was stirred at room temperature for 3 h, and it was washed with 5 % HCl solution, 10 % $Na_2CO_3$ solution and saturated $NaHCO_3$. The organic phase was dried over $Na_2SO_4$ and solvent was evaporated *in vacuo* to leave the product (**8a** or **8b**) as an oil. Spectral ($^1$H-NMR and IR) data were indistinguishable from those of the above products (*ent*-**8a** or *ent*-**8b**).

(*R*)-*1-Acetoxy-2,3-di(p-toluenesulfonyl)oxypropane* (**8a**): $[\alpha]_D$= +8.8 (c 1, methanol).

(*R*)-*1-Acetoxy-2,3-di(benzenesulfonyl)oxypropane* (**8b**): $[\alpha]_D$= +4.8 (c 1, methanol).

*Preparation of 1-acetoxy-2-acyloxy-3-benzyloxypropanes* (**12d-h**)

*General procedure*: To a solution of (*R*)-1-acetoxy-3-benzyloxypropan-2-ol [**10**, 314 mg, 1.4 mmol, $[\alpha]_D$= -3.3 (c 1, chloroform)], triethylamine (0.23 ml) and 4-(dimethylamino)-pyridine (5 mg) in dichloromethane (1.5 ml) the corresponding acyl chloride (1.54 mmol) was added and the resulting mixture was stirred at room temperature for 1-6 h. The mixture was then washed with 5 % HCl solution (2 x 0.5 ml), 10 % $Na_2CO_3$ solution (0.5 ml) and saturated $NaHCO_3$ (0.5 ml). The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo*. The residue was purified by preparative vacuum column chromatography (silica gel, hexane:acetone 5:1) to give the product (**12d-h**) as an oil.

(*R*)-*1,2-Diacetoxy-3-benzyloxypropane* (**12d**)

Yield: 77 %; $[\alpha]_D$= -15.8 (c 1, methanol); *$^1$H-NMR*: 2.02 (s, 3H, $CH_3$-CO), 2.07 (s, 3H, $CH_3$-CO), 3.58 (m(d), 2H, $CH_2$-OBn), 4.14-4.36 (m, 2H, $CH_2$-OAc), 4.53 (m, 2H, O-$CH_2$-Ph), 5.21 (mc, 1H, CH-O), 7.31 (mc, 5H, Ar-H); *IR* (film, $cm^{-1}$): 2865, 1745, 1455, 1370, 1225, 1100, 1050, 1020, 960, 740, 700; Calcd. for $C_{14}H_{18}O_5$: C 63.15, H 6.81; found C 63.30, H 6.82.

*(R)-1-Acetoxy-3-benzyloxy-2-(diphenylacetoxy)propane* (**12e**)

Yield: 81 %; $[\alpha]_D$= -12.9 (c 1, methanol); *$^1$H-NMR*: 1.89 (s, 3H, $CH_3$-CO), 3.57 (m(d), 2H, $CH_2$-OBn), 4.17-4.32 (m, 2H, $CH_2$-OAc), 4.44 (mc, 2H, O-$CH_2$-Ph), 5.05 (s, 1H, Ph-CH-Ph), 5.33 (mc, 1H, CH-O), 7.21-7.50 (m, 15H, Ar-H); *IR* (film, $cm^{-1}$): 2865, 1745, 1600, 1495, 1455, 1365, 1230, 1190, 1150, 1115, 1045, 975, 740; Calcd. for $C_{26}H_{26}O_5$: C 74.62, H 6.26; found C 74.75, H 6.26.

*(R)-1-Acetoxy-3-benzyloxy-2-pivaloyloxypropane* (**12f**)

Yield: 81 %; $[\alpha]_D$= -14.6 (c 1, methanol); *$^1$H-NMR*: 1.20 (s, 9H, $3CH_3$), 2.03 (s, 3H, $CH_3$-CO), 3.61 (m(d), 2H, $CH_2$-OBn), 4.17-4.33 (m, 2H, $CH_2$-OAc), 4.54 (s, 2H, O-$CH_2$Ph), 5.22 (mc, 1H, CH-O), 7.24-7.38 (m, 5H, Ar-H); *IR* (film, $cm^{-1}$): 2975, 2870, 1810, 1735, 1480, 1455, 1370, 1285, 1235, 1155, 1115, 1045, 740; Calcd. for $C_{17}H_{24}O_5$: C 66.21, H 7.84; found C 66.37, H 7.85.

*(R)-1-Acetoxy-3-benzyloxy-2-(4-methylbenzoyl)oxypropane* (**12g**)

Yield: 78 %; $[\alpha]_D$= -9.2 (c 1, methanol); *$^1$H-NMR*: 2.01 (s, 3H, $CH_3$-CO), 2.35 (s, 3H, Ar-$CH_3$), 3.69 (mc, 2H, $CH_2$-OBn), 4.26-4.49 (m, 2H, $CH_2$-OAc), 4.56 (m(d), 2H, O-$CH_2$-Ph), 5.45 (mc, 1H, CH-O), 7.22 (d, 2H, Ar-H), 7.30 (mc, 5H, Ar-H), 7.92 (d, 2H, Ar-H); *IR* (film, $cm^{-1}$): 2865, 1745, 1720, 1610, 1495, 1455, 1365, 1275, 1230, 1180, 1105, 1045, 910, 840, 750; Calcd. for $C_{20}H_{22}O_5$: C 70.16, H 6.48; found C 70.01, H 6.46.

*(R)-1-Acetoxy-3-benzyloxy-2-cyclohexanecarbonyloxypropane* (**12h**)

Yield: 97 %; $[\alpha]_D$= -12.5 (c 1, methanol); *$^1$H-NMR*: 1.18-1.34 (m, 3H), 1.38-1.51 (m, 2H), 1.64 (mc, 1H), 1.69-1.79 (m, 2H), 1.83-1.93 (m, 2H), 2.02 (s, 3H, $CH_3$-CO), 2.32 (mc, 1H,

CH-CO), 3.58 (mc, 2H, $CH_2$-OBn), 4.18-4.37 (m, 2H, $CH_2$-OAc), 4.53 (mc, 2H, O-$CH_2$-Ph), 5.22 (mc, 1H, CH-O), 7.26-7.37 (m, 5H, Ar-H); *IR* (film, $cm^{-1}$): 2936, 2856, 1740, 1736, 1488, 1452, 1368, 1312, 1292, 1236, 1230, 1168, 1132, 1048, 740, 696; Calcd. for $C_{19}H_{26}O_5$: C 68.24, H 7.84; found C 68.09, H 7.93.

*Catalytic hydrogenation of 1-acetoxy-2-acyloxy-3-benzyloxypropanes* (**12d-h**)

*General procedure*: The 1-acetoxy-2-acyloxy-3-benzyloxypropane (**12d-h**) from the previous reaction was dissolved in isopropanol (3 ml). Catalyst (10 % Pd-C, 10 %w/w of the substrate) was added and hydrogenation was carried out at room temperature for 0.5 to 2 hours. The catalyst was then filtered off and the solvent was evaporated from the filtrate to leave the (*R*)-monoacetates [(*R*)-**2d-h**] in yields between 66 and 82 %. $^1$H-NMR and IR spectra were identical to those of the racemates (*rac*-**2d-g**) or monoacetates from the enzymatic reaction (**2d-h**).

*2,3-Diacetoxypropan-1-ol* [(*R*)-**2d**]: $[\alpha]_D$= -4.6 (c 1, methanol);

*3-Acetoxy-2-(diphenylacetoxy)propan-1-ol* [(*R*)-**2e**]: $[\alpha]_D$= -28.7 (c 1, methanol);

*3-Acetoxy-2-pivaloyloxypropan-1-ol* [(*R*)-**2f**]: $[\alpha]_D$= -8.2 (c 1, methanol);

*3-Acetoxy-2-(4-methylbenzoyl)oxypropan-1-ol* [(*R*)-**2g**]: $[\alpha]_D$= -20.6 (c 1, methanol);

*3-Acetoxy-2-cyclohexanecarbonyloxypropan-1-ol* [(*R*)-**2h**]: $[\alpha]_D$= -6.7 (c 1, methanol).

## RESULTS AND DISCUSSION

Preparation of the desired prochiral 2-acyloxypropane-1,3-diols (**1a-h**) was straightforward (Scheme 1). Condensation reaction [19] of glycerol (**4**) and benzaldehyde (**5**) provided *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**6**). The benzylidene protected secondary alcohol (**6**) was transformed into sulfonic (**7a-c**) or carboxylic esters (**7d-i**) in slightly different ways. The sulfonic esters (**7a-c**) were obtained by reaction of the secondary alcohol

(**6**) with alkyl- or arylsulfonyl chloride and powdered potassium hydroxide in diethyl ether at –5 °C, whereas the carboxylic esters (**7d-i**) were obtained by acylation of the alcohol (**6**) with the corresponding acyl chloride using pyridine and catalytic amounts of 4-(dimethylamino)pyridine (DMAP) in dichloromethane at room temperature. Benzylidene deprotection of the two types of esters (**7a-c** and **7d-i**) was also different. Benzylidene removal from the sulfonic ester intermediates (**7a-c**) was performed by acid hydrolysis with concentrated hydrochloric acid in refluxing methanol, whereas carboxylic esters (**7d-i**) were deprotected by catalytic hydrogenation over 10 % Pd(C) in isopropanol at room temperature, providing the desired prochiral diols (**1a-g**) smoothly. In the case of the lauryl derivative (**7i**), however, deprotection proceeded with immediate acyl migration leading almost exclusively to 1-*O*-laurylglycerol. Although substantial degree of acyl migration was observed by the 2-*O*-diphenylacetyl (**1e**) and 2-*O*-cyclohexanecarbonyl (**1h**) products within days standing in standard glass flasks at room temperature, these prochiral diols (**1e** and **1h**) were suitable for enzymatic transformation immediately after the deprotection.

In our preliminary study the enantiotope selective acetylation of 2-benzoyloxypropane-1,2-diol (**1**, $R_1$=OBz, $R_2$=H), which was considered as the first representant of the prochiral 2-acyloxy-1,3-propanediol family, was investigated [17]. The lipase from porcine pancreas (PPL) proved to be the most selective among the enzymes studied providing the acylated product (**2**, $R_1$=OBz, $R_2$=H) with 96 % enantiomeric purity in good yield. Because of that promising result, the same inexpensive commercial lipase was chosen for the present study of the further 2-acyloxypropanediols (**1a-h**), too.

Composition of the solvent in the PPL-catalyzed acetylation reaction of carboxylic ester type 2-benzoyloxypropane-1,2-diol (**1**, $R_1$=OBz, $R_2$=H) played an important role [17], i.e. enzymatic acetylations in polar solvents like chloroform, ethyl acetate or vinyl acetate gave significantly decreased enantiotope selectivity compared to that obtained in the best solvent system (hexane:THF 1:1). Therefore, we investigated the solvent dependence of the PPL-

catalyzed acetylation process for the prochiral sulfonic ester compounds (**1a-c**) as well. Trends of solvent dependence of the enantiotope selectivity in the acetylation of the *p*-toluenesulfonic ester (**1a**) representing the prochiral 2-sulfonyloxy diols were found similar (Table 2.) to the previous results with the 2-benzoyloxypropane-1,3-diol (**1**, $R_1$=OBz, $R_2$=H) [17]. The degree of selectivity, however, remained significantly lower (31 % ee) even in the best hexane:THF 1:1 solvent system.

After finding common conditions for the lipase-catalyzed enantiotope selective acetylation (PPL, hexane:THF 1:1), the diols (**1a-h**) were subjected to this enzymatic reaction (Table 3., Figure 2.).

Prediction of the sense of enantiotopic selectivity seemed to be not obvious for lipase-catalyzed acylation of this new class of prochiral 1,3-propanediols. The lipase-catalyzed acylation of 2-O-alkyl-1,3-propanediols (**1**, $R_1$,$R_2$= O-alkyl, H) proved to be *pro-S* selective. In the case of 2-alkyl-1,3-propanediols (**1**, $R_1$,$R_2$= alkyl, H) bearing apolar substituent at position 2, enantiotope preference is inverted in a geometrical sense, although as a result of the sequence rules, the affected group is still labeled *pro-S* [20]. Acetylation of the diol bearing 2-*N*-benzyloxycarbonyl [13] or 2-O benzoyloxy [17] group by PPL was found to be *pro-R* selective.

Because of the above discussed uncertainty and since all the products (**2b-h**) except (**2a**) [21] were new compounds, absolute configuration determination of the acetates (**2a-h**) was necessary (Figure 2.). The configurations were determined by chemical correlation starting from (*R*)-1-acetoxy-3-benzyloxypropan-2-ol (**10**). The monoacetate (**10**) with known *R* configuration was obtained from Lipase-AK catalyzed enantiomer selective acetylation of racemic 3-benzyloxypropane-1,2-diol (**9**) [22]. This secondary alcohol (**10**) was acylated into the benzyl protected compounds (**12d-h**) from which debenzylation gave the authentic (*R*)-monoacetates (*R*)-(**2d-h**). Comparing the optical rotation of these (*R*)-(**2d-h**) products with those of obtained from the PPL-catalyzed process proved the *R* configuration of bulkier

carboxylic ester products (**2e-h**), while the smaller 2-acetoxy compound (**2d**) was found to have *S* configuration. The authentic (*S*)-bis-sulfonic esters (*ent*-**8a,b**) were also prepared from (*R*)-(**10**) *via* debenzylation and subsequent bis-sulfonylation of the chiral diol (**13**). The same bis-sulfonic esters having opposite sign of optical rotation (**8a,b**) were obtained by sulfonylation of the enzymatic products (**2a,b**) proving their *S* configuration.

The results listed in Table 3. indicated that the degree and even the sense of enantiotope selectivity of the PPL-catalyzed enantiotope acetylation of the prochiral 2-acyloxypropane-1,3-diols (**1a-h**) was strongly dependent on the moiety at position 2. Data obtained with the carboxylic ester series (**1d-h**) showed that there is a size optima at the size of the 4-benzoyloxy [**1g** → **2g** (≥98 % ee)] moiety for the substituent at position 2. High but somewhat lower *pro-R* selectivity was obtained for the compounds bearing benzoyloxy {(**1**, $R_1$=OBz, $R_2$=H) → (**2**, $R_1$=OBz, $R_2$=H) (96 % ee) [17]} or cyclohexanecarbonyloxy [**1h** → **2h** (>95 % ee)] moieties having similar bulkiness, whereas the more bulky pivaloyloxy [**1f** → **2f** (67 % ee)] or diphenylacetoxy [**1e** → **2e** (16 % ee)] derivatives were transformed with decreased but still *pro-R* selectivity. The small acetoxy [**1c** → **2c** (40 % ee)] moiety resulted in a moderate *pro-S* selectivity. Results for the sulfonic esters (**1a-c**) showed that increasing the bulkiness in the closer vicinity of the prochiral center (i.e. change of the O-CO- to the sterically more demanding O-$SO_2$- structural unit) alters the sense of the enantiotopic preference, too. The prochiral diol with methanesulfonyloxy [**1c** → **2c** (~0 % ee)] moiety was acetylated with no selectivity, while the (*p*-toluenesulfonyl)oxy [**1a** → **2a** (31 % ee)] or benzenesulfonyloxy [**1b** → **2b** (31 % ee)] compounds were acetylated with a moderate *pro-S* selectivity.

The carboxylic ester type 2-(4-methylbenzoyl)oxy product (**2g**) allows the introduction of a leaving group at a primary hydroxylic function of an almost enantiomerically pure glycerol unit. From synthetic point of view, homochiral form of the sulfonic ester products (**2a-c**) bearing a leaving group at position 2 would represent a different valuable class of the chiral

$C_3$ units. Unfortunately, the results for the PPL-catalyzed acetylation of the prochiral sulfonic esters (**1a-c**) providing racemic product for the mesylate (**2c**) or products with low enantiomeric purity for the arylsulfonates (**2a,b**), were disappointing. As a representative of the prochiral sulfonic esters, 2-(*p*-toluenesulfonyl)oxypropane-1,2-diol (**1a**) was therefore investigated with further enzymes (Table 4.).

This study of the acetylation of the prochiral tosylate (**1a**) with further enzymes was only partially successful. Although lipases from *Mucor* sp. [Lipase M (from *Mucor javanicus*) and Lipozyme IM (from *Mucor miehei*)] catalyzed the acetylation with better enantiotope selectivities than that observed with PPL, enantiomeric purities of the product (**2a**, 34 and 42 % ee, respectively) were still too low for synthetic purposes.

## CONCLUSIONS

In conclusion, a strong dependence of the degree and even the sense of selectivity on the 2-acyloxy moiety in the PPL-catalyzed acetylation of the prochiral 2-acyloxypropane-1,3-diols (**1a-h**) was found. The slight increase of size of the 2-acyloxy moiety from benzoyloxy (**1**, $R_1$=OBz, $R_2$=H) [17] to (4-methylbenzoyl)oxy (**1g**) resulted in pronounced enantiotope selectivity (from 96 % ee [17] to ≥98 % ee) providing the $C_3$ compound (**2g**) in almost enantiomerically pure form. Although the diol bearing cyclohexanecarbonyloxy moiety at position 2 (**1h**) was transformed also with high enantiotope selectivity (**2h**, >95 % e.e.), usefulness of this acyl derivative is limited by the slow but significant acyl migration. 1,3-Diols with significantly smaller (**1c,d**) or sterically more demanding (**1a,b,e,f**) moieties at position 2 showed moderate, no or even altered selectivities.

## ACKNOWLEDGMENTS

We thank Amano and Novo Nordisk for generous gifts of enzymes. The Hungarian OTKA Foundation (T-025235 and T-029251) is gratefully acknowledged for financial support. *J. B.* thanks the Zoltán Magyary Foundation and the Hungarian OTKA (D-32705) for postdoctoral fellowships. *G.E.* thanks the Hungarian OTKA foundation for a postdoctoral fellowship (D-29445).

## REFERENCES

[1] G. Hirth, R. Barner, *Helv. Chim. Acta 65* (1982) 1059.

[2] E. Caer, A. Kindler, *Biochemistry I* (1962) 518.

[3] E. A. Dennis, *Bio/Technology 5* (1987) 1294.

[4] J. Jurczak, S. Pikul, T. Bauer, *Tetrahedron 42* (1986) 447.

[5] Y. F. Wang, C.-H. Wong, *J. Org. Chem.53* (1988) 3127.

[6] D. Bianchi, A. Bosetti, P. Golini, P. Cesti, C. Pina, *Tetrahedron:Asymmetry 8* (1997) 817.

[7] M. Pallavicini, E. Valoti, L. Villa, O. Piccolo, *J. Org. Chem. 59* (1994) 1751.

[8] D. Breitgoff, K. Laumen, M. P. Schneider, *J. Chem. Soc. Chem. Commun.* (1986) 1523.

[9] B. Wirz, R. Schmid, J. Foricher, *Tetrahedron:Asymmetry 3* (1992) 137.

[10] H. Suemune, Y. Mizuhara, H. Akita, K. Sakai, *Chem. Pharm. Bull. 34* (1986) 3440.

[11] V. Kerschner, W. Kreiser, *Tetrahedron Lett. 28* (1987) 531.

[12] O. Ghisalba, R. Lattmann, D. Gygax, *Recl. Trav. Chim. Pays-Bas 110* (1991) 263.

[13] Y. F. Wang, J. J. Lalonde, M. Momongan, D. E. Bergbreiter, C.-H. Wong, *J. Am. Chem. Soc. 110* (1988) 7200.

[14] M. Murata, Y. Terao, K. Achiwa, T. Nishio, K. Seto, *Chem. Pharm. Bull. 10* (1989) 2670.

[15] Y. Terao, M. Murata, K. Achiwa, T. Nishio, M. Akamtsu, M. Kamimura, *Tetrahedron Lett. 29* (1988) 5173.

[16] T. Matsuzaki, Y. Shinozaki, M. Hagimori, T. Tobita, H. Shigematsu, A. Koiwai, *Biosci. Biotechnol. Biochem. 56* (1992) 1565.

[17] V. Bódai, L. Novák, L. Poppe, *SynLett* (1999) 759.

[18] L. Poppe, L. Novák, *Magy. Kém. Lapja 40* (1985) 366.

[19] P. H. J. Carlsen, K. Soerbye, T. Ulven, K. Aasboe, *Acta Chem. Scand. 50* (1996) 185.

[20] L. Poppe, L. Novák, *Selective Biocatalysis: A Synthetic Approach*; VCH, Weinheim-New York, 1992.

[21] K. Iguchi, M. Kitade, T. Kashiwagi, Y. Yamada, *J. Org. Chem. 58* (1993) 5690.

[22] B. Herradón, S. Cueto, A. Morcuende, S. Valverde, *Tetrahedron: Asymmetry 5* (1993) 845.

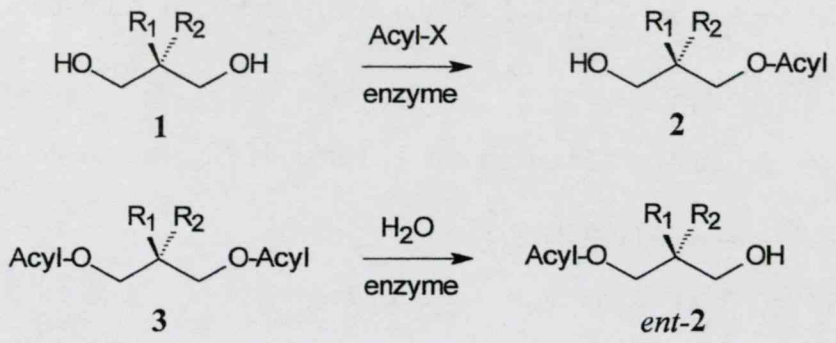
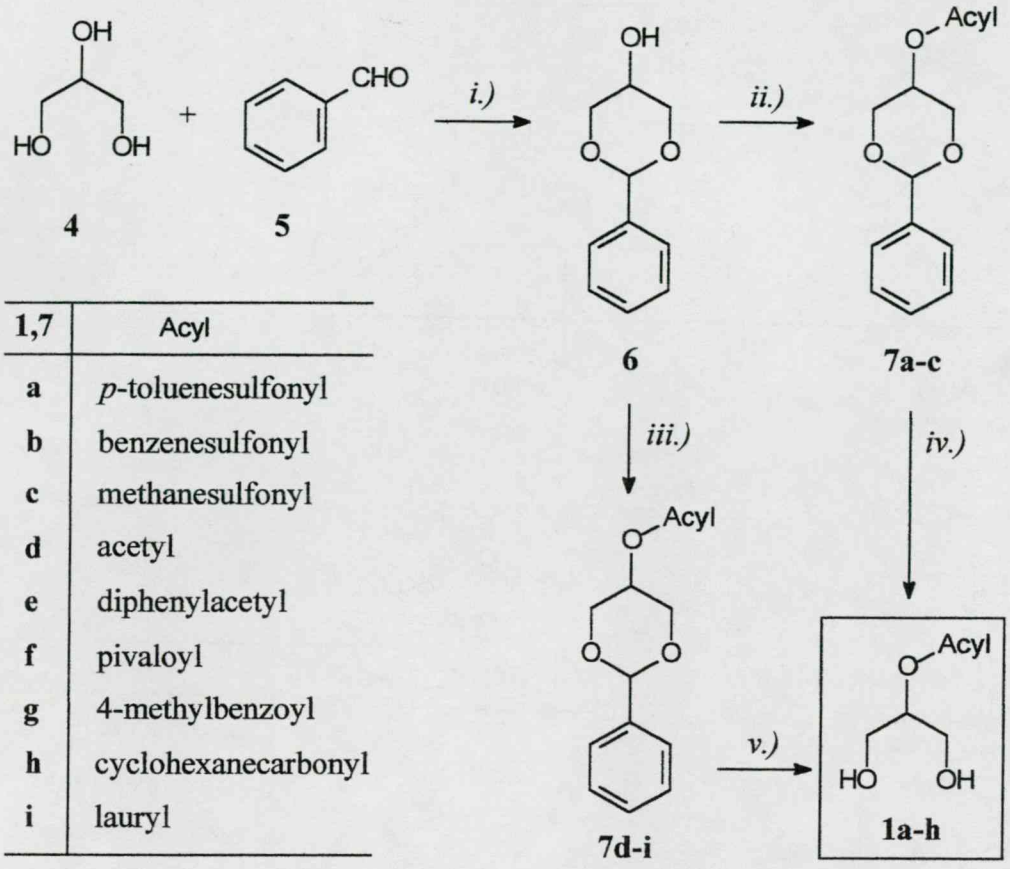
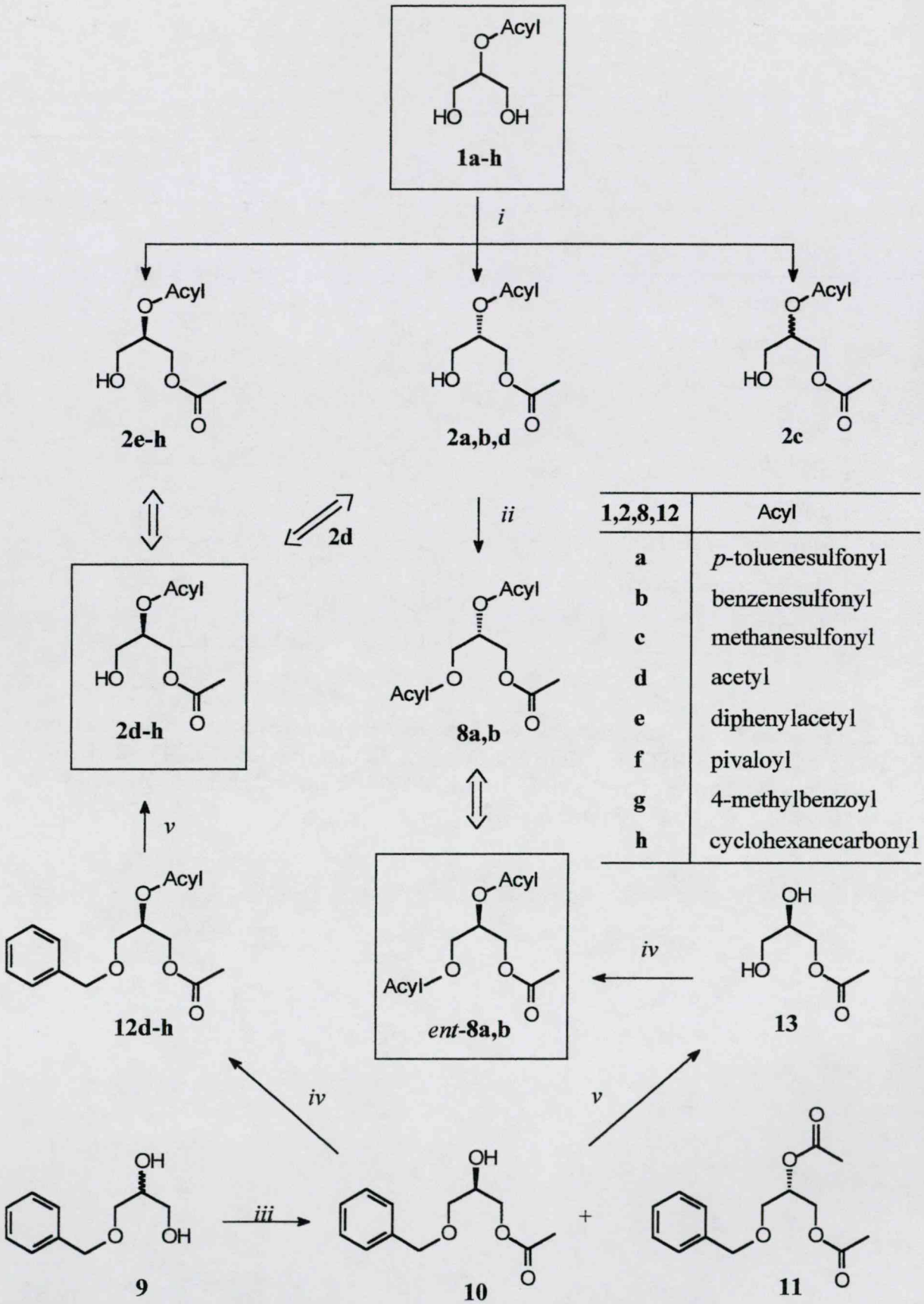



**FIGURE 1.** Enantiotope selective biotransformation of prochiral 1,3-propanediol derivatives (**1** and **2**)




| 1,7 | Acyl |
|---|---|
| **a** | *p*-toluenesulfonyl |
| **b** | benzenesulfonyl |
| **c** | methanesulfonyl |
| **d** | acetyl |
| **e** | diphenylacetyl |
| **f** | pivaloyl |
| **g** | 4-methylbenzoyl |
| **h** | cyclohexanecarbonyl |
| **i** | lauryl |

**FIGURE 2.** Preparation of 2-acyloxypropane-1,3-diols (**1a-h**). *Reaction conditions: i.)* cat. $H_2SO_4$, RT, 5 h, 35 %; *ii.)* $RSO_2$-Cl, KOH, $Et_2O$, -5°C, 40 min, RT, 15 min, 43-92 % ; *iii.)* Acyl-Cl, pyridine, cat. DMAP, $CH_2Cl_2$, RT, 1-12 h, 80-86 %; *iv.)* cc. HCl, MeOH, reflux, 1 min, 69-76 %; *v.)* $H_2$, 10% Pd/C, *i*-PrOH, RT, 67-77 %.




| 1,2,8,12 | Acyl |
|---|---|
| **a** | *p*-toluenesulfonyl |
| **b** | benzenesulfonyl |
| **c** | methanesulfonyl |
| **d** | acetyl |
| **e** | diphenylacetyl |
| **f** | pivaloyl |
| **g** | 4-methylbenzoyl |
| **h** | cyclohexanecarbonyl |

**FIGURE 3.** Enzymatic acetylation of 2-acyloxypropane-1,3-diols (**1a-h**) and configuration of the products (**2a-h**). *Reagents: i.)* PPL, vinyl acetate, hexane:THF 1:1, RT; *ii.)* $RSO_2$-Cl, $Et_3N$, cat. DMAP, $CH_2Cl_2$, RT, 43-92 %; *iii.)* Lipase-AK, vinyl acetate, THF, RT; *iv.)* Acyl-Cl, $Et_3N$, cat. DMAP, $CH_2Cl_2$, RT, 80-86 %; *v.)* $H_2$, 10% Pd/C, *i*-PrOH, RT, 67-77 %.

**TABLE 1.** Determination of the enantiomeric excess of 3-acetyloxy-2-acyloxypropan-1-ols (**2a-g**)

| Compound | $[\alpha]_D$ (c 1, methanol) | E.e. % | $^1$H-NMR signals of **2**-MTPA esters |
|---|---|---|---|
| **2a** | +9.2 | 31 | 1.93 (s); 1.93 (s) |
| **2b** | +6.2 | 31 | 4.59-4.62 (dd); 4.57-4.59 (dd) |
| **2c** | 0 | 0 | |
| **2d** | +1.8 | 40 | 4.56 (dd), 4.61 (dd) |
| **2e** | -2.3 | 16 | 5.01 (s), 4.95 (s) |
| **2f** | -8.0 | 67 | 1.17 (d), 1.18 (d) |
| **2g** | -27.5 | ≥98 | 3.49 (d), 3.52 (d) |

**TABLE 2.** Effect of the solvent on acetylation of 2-(*p*-toluenesulfonyl)oxypropane-1,2-diol (**1a**).

| Solvent | Time | **2a** | | |
|---|---|---|---|---|
| | | Yield % | E.e. % | $[\alpha]_D$ (c 1, methanol) |
| acetonitrile | 7 d | 0 | | |
| chloroform | 7 d | 0 | | |
| diethyl ether | 7 d | 0 | | |
| ethyl acetate | 10 h | 64 | 0 | 0 |
| vinyl acetate | 7.5 h | 67 | 5 | +1.6 |
| THF | 5 h | 77 | 15 | +4.5 |
| *t*-butanol | 5 h | 46 | 16 | +4.7 |
| hexane:THF 1:1 (5 ml) | 7 d | 74 | 31 | +9.2 |

*Reaction conditions*: 0.25g of substrate (**1a**), 200 mg of porcine pancreas lipase (PPL), 0.6 ml of vinyl acetate, 3 ml of solvent, stirring at room temperature.

**TABLE 3.** PPL-catalyzed acetylation of 2-acyloxypropane-1,3-diols (**1a-h**)

| Compound | Time | Yield % | E.e. % * | Configuration | $[\alpha]_D$ (c 1, methanol) |
|---|---|---|---|---|---|
| **2a** | 7 d | 74 | 31 | *S* | +9.2 |
| **2b** | 3 h | 71 | 31 | *S* | +6.2 |
| **2c** | 11 h | 67 | 0 | - | 0.0 |
| **2d** | 6 h | 79 | 40 | *S* | +1.8 |
| **2e** | 2 d | 80 | 16 | *R* | -2.3 |
| **2f** | 6 h | 82 | 67 | *R* | -8.0 |
| **2g** | 11 h | 77 | ≥98 | *R* | -27.5 |
| **2h** | 5 h | 66 | > 95 # | *R* | - 8.7 |

*Reaction conditions*: 0.25 g of substrate (**1a-h**), 200 mg of PPL, 0.8 ml of vinyl acetate, 2.5 ml of THF and 2.5 ml of hexane, stirring at room temperature;

* Determined from the $^1$H-NMR spectra of the **2a-g** MTPA esters. # Determined from optical rotation compared to **2h** prepared from **12h** of known e.e.

**TABLE 4.** Effect of the enzyme on acetylation of 2-(*p*-toluenesulfonyl)oxypropane-1,2-diol (**1a**)

| Enzyme (mg) | Time | **2a** | | |
|---|---|---|---|---|
| | | Yield % | E.e. % | $[\alpha]_D$ (c 1, methanol) |
| Novozym 435 (50) * | 1 d | 0 | | |
| Papain (200) * | 4 d | 0 | | |
| PfL (20) * | 1 d | 0 | | |
| CcL (100) * | 1 d | 64 | 1 | -0.2 |
| Lipase G (100) # | 7 d | 51 | 1 | +0.4 |
| Lipase A (100) # | 7 d | 47 | 2 | +0.6 |
| Lipase AK (50) * | 2 h | 77 | 2 | +0.7 |
| Lipase PS (50) * | 3 h | 72 | 3 | +0.9 |
| Lipase N (100) # | 7 d | 47 | 4 | +1.2 |
| PPL (200) # | 7 d | 74 | 31 | +9.2 |
| Lipase M (50) # | 2 d | 75 | 34 | +10.1 |
| Lipozym IM (50) # | 0.5 h | 82 | 42 | +12.5 |

*Reaction conditions*: 0.25 g of substrate in the solvent, RT; * Solvent: vinyl acetate (2 ml);

# Solvent: THF (2.5 ml), hexane (2.5 ml), vinyl acetate (0.8 ml)

# X. melléklet

EGRI, G., KOLBERT, A., BÁLINT, J., FOGASSY, E., NOVÁK, L., **POPPE, L.**:

Baker's Yeast Mediated Stereoselective Biotransformation of 1-Acetoxy-3-Aryloxypropan-2-ones,

*Tetrahedron:Asymmetry*, **1998**, *9*, 271.

Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 271–283

TETRAHEDRON: *ASYMMETRY*

# Baker's yeast mediated stereoselective biotransformation of 1-acetoxy-3-aryloxypropan-2-ones

Gabriella Egri,[a] Attila Kolbert,[a,b] József Bálint,[a] Elemér Fogassy,[a] Lajos Novák[b] and László Poppe[c,*]

[a]*Department of Organic Chemical Technology, Technical University Budapest, H-1521 Budapest, PO Box 91, Hungary*
[b]*Institute for Organic Chemistry, Technical University Budapest, H-1521 Budapest, PO Box 91, Hungary*
[c]*Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, PO Box 17, Hungary*

Received 12 November 1997; accepted 8 December 1997

**Abstract**

A series of 1-acetoxy-3-aryloxypropan-2-ones **1a–m** were synthesized and subjected to biotransformation by baker's yeast yielding optically active monoacetates **5** or *ent*-**5** and/or diols **4** of moderate to excellent enantiomeric purity. The dependence of the reduction/hydrolysis ratio and stereoselectivity on the size and substitution pattern of the aromatic moiety in the substrate is also discussed. 

3-Aryloxypropane-1,2-diols **4a–m** in enantiomerically pure form are versatile compounds of interest as intermediates in syntheses of pharmaceuticals such as β-receptor blockers, or as chiral fragments in optically active crown ethers. Lipase catalyzed acylation has proved to be a useful tool for enantiomer separation of racemic 3-aryloxypropane-1,2-diol derivatives.[1,2] This kinetic resolution using lipase catalyzed sequential transesterification was found to be highly enantiomer selective for diols having *m*- or *p*-substituted aromatic moieties, whereas the most sterically hindered *o*-substituted derivatives showed decreased enantiomer selectivity or were not accepted as substrates. Without racemization of the remaining enantiomer, however, even highly enantiomer selective processes can provide only 50% of the starting racemate in optically active form.

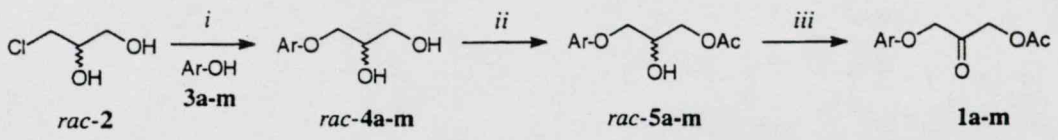
Alternatively, an enantiotope selective method, such as reduction of a prochiral ketone, might yield an optically active product quantitatively. Since acetoxymethyl ketones with phenyl,[3] benzyloxymethyl,[4] or azidomethyl[5] moieties resulted in the corresponding monoacetates of high enantiomeric purity by reduction with baker's yeast, it seemed worthwhile to investigate the analogous biotransformation of 3-aryloxy-1-acetoxypropan-2-ones **1a–m**. Here we report our results on the preparation and baker's yeast mediated stereoselective biotransformation of these ketones.

The 3-aryloxy-1-acetoxypropan-2-ones **1a–m** were prepared by alkylation of the corresponding phenols (**3a–m**) with racemic 3-chloropropane-1,2-diol *rac*-**2**, followed by acetylation of the primary

* Corresponding author. E-mail: poppe.szk@chem.bme.hu

0957-4166/98/$19.00 
*PII:* S0957-4166(97)00626-5

Table 1
Preparation of 3-aryloxy-1-acetoxypropan-2-ones (**1a–m**)




*rac*-**2** *rac*-**4a-m** *rac*-**5a-m** **1a-m**

| | Ar | *rac*-**4a-m** Yield (%) | *rac*-**5a-m** Yield (%) | **1a-m** Yield (%) |
|---|---|---|---|---|
| **a** | phenyl | 81 | 79 | 94 |
| **b** | 1-naphthyl | 82 | 78 | 93 |
| **c** | 2-naphthyl | 79 | 75 | 90 |
| **d** | 2-isopropylphenyl | 77 | 83 | 91 |
| **e** | 2-chlorophenyl | 74 | 73 | 88 |
| **f** | 3-chlorophenyl | 55 | 69 | 94 |
| **g** | 4-chlorophenyl | 81 | 62 | 94 |
| **h** | 2-methylphenyl | 79 | 66 | 90 |
| **i** | 3-methylphenyl | 66 | 75 | 90 |
| **j** | 4-methylphenyl | 71 | 75 | 89 |
| **k** | 3-nitrophenyl | 26 | 68 | 87 |
| **l** | 2,6-dimethylphenyl | 98 | 43 | 79 |
| **m** | 2,4,6-trichlorophenyl | 99 | 42 | 71 |

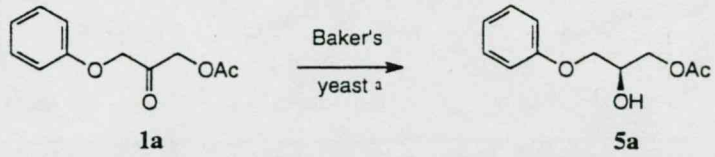
*Reagents*: *i.)* NaOH; *ii.)* $Ac_2O$, DMAP, pyridine; *iii.)* oxalyl chloride, DMSO, $Et_3N$; yields refer to isolated pure products

hydroxyl moiety of the resulting diols (*rac*-**4a–m**) and Swern-oxidation of the monoacetates *rac*-**5a–m** (Table 1).

Having the desired ketones in hand, the effect of the reaction conditions on enantiotope selectivity of the baker's yeast reduction of 1-acetoxy-3-phenoxypropan-2-one **1a** was investigated (Table 2). Under various conditions, the reaction yielded the expected optically active monoacetate **5a** without noticeable hydrolysis, in accordance with the results of the analogous baker's yeast reductions.[3–5]

In baker's yeast mediated enantiotope selective reduction of carbonyl compounds there may be two factors responsible for the incomplete stereoselectivity. Firstly, it may be due to participation of more than one reductase enzyme with different kinetic parameters and eventually opposite selectivity,[6–8] or secondly, the process may be catalyzed by a single enzyme but with incomplete selectivity.[9] In the majority of cases, it turned out that competing enzymes of opposite selectivity was the factor responsible for poor selectivity. Since the kinetic behavior of these competing enzymes is different, the selectivity may be controlled by the reaction conditions. The most frequently used modifications for influencing the selectivity are e.g.: modification of pH;[10,11] application of lyophilized yeast instead of the row cake form;[12,13] use of various additives such as metal salts;[14] allylic alcohol or α,β-unsaturated carbonyl compounds;[12,15] ethyl chloroacetate and similar compounds.[16] In Table 2 the effect of these factors on the enantiotope selectivity is shown. The reaction performed by wet caked baker's yeast at pH 7 (entry 2) provided the best selectivity. This selectivity remained practically unaltered at pH 8, or by adding some salts or allylic alcohol (entries 3 and 5–7). On the other hand, decreased selectivity was observed in the

Table 2
Effect of the reaction conditions on the selectivity of reduction of the 1-acetoxy-3-phenoxypropan-2-one **1a**




**1a** **5a**

| Entry | Yeast | pH | Time | Additive | E.e. % |
|---|---|---|---|---|---|
| 1 | Wet Caked | n.b. [b] | 3 | - | 52 |
| 2 | Wet Caked | 7 | 1 | - | 80 |
| 3 | Wet Caked | 8 | 1 | - | 78 |
| 4 | Lyophilized | 7 | 1.5 | - | 64 |
| 5 | Wet Caked | 7 | 1 | 0.1 M $K_2SO_4$ | 79 |
| 6 | Wet Caked | 7 | 1 | 0.1 M $MgSO_4$ | 80 |
| 7 | Wet Caked | 7 | 2 | 0.3 M allylic alcohol | 79 |
| 8 | Wet Caked | 7 | 2 | 0.15 M ethyl chloroacetate | 37 |

[a] *Reaction conditions:* **1a**, 500 mg; media (0.15M sodium phosphate buffer), 200 ml; baker's yeast, wet (12 g) or lyophilized (2.5 g); sucrose, 5 g. [b] Non buffered tap water.

non-buffered reaction (entry 1), by using lyophilized yeast (entry 4) or by adding ethyl chloroacetate (entry 8).

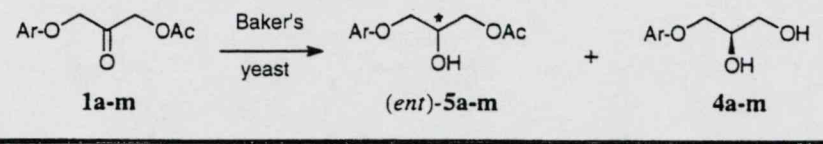
Hence, baker's yeast mediated biotransformations of further 1-acetoxy-3-aryloxypropan-2-ones **1a–k** were performed under the conditions which gave the best selectivity with the unsubstituted phenoxy derivative **1a**. The results of these reactions are summarized in Table 3.

Surprisingly, only the unsubstituted phenoxy-derivative **1a** was reduced without noticeable amounts of hydrolysis. The 2-naphthyloxy-derivative **1b** was not reduced at all, and all the other ketones **1c–m** were transformed not only into the corresponding monoacetates **5** or *ent*-**5** but partially or even fully into the corresponding diols **4** indicating the enzyme action of some hydrolases beside oxidoreductases in these reactions. The differences between the enantiomeric purities or even the configuration of the produced monoacetates **5** or *ent*-**5** and diols **4** imply that in these reactions more than one type of selectivity may be decisive. Analysis of the possible pathways and selectivities is shown in Fig. 1.

The original enantiomeric composition of the monoacetate fraction **5** versus *ent*-**5** is determined by the enantiotope selectivity of the direct reduction ($k_{1,R}$ versus $k_{1,ent\text{-}R}$). The formation of the diol fraction **4** and *ent*-**4** may proceed *via* two alternative routes. The first possible way is the hydrolysis of the monoacetate fraction (**5** and *ent*-**5**) by which the original enantiomeric composition may be altered by the enantiomer selectivity ($k_{2,H}$ versus $k_{2,ent\text{-}H}$) of the hydrolysis. The second alternative way to diols **4** and *ent*-**4** is the non-stereoselective hydrolysis ($k_{1,H}$) of the achiral acetoxy ketone **1** followed by an enantiotope selective reduction ($k_{2,R}$ versus $k_{2,ent\text{-}R}$) of the forming hydroxy ketone **6**.

The reactions of the 1-acetoxy-3-aryloxypropan-2-ones **1a–m** (Table 3) in buffered (pH 7) media with fermenting baker's yeast resulted, with the exception of the 2-isopropyloxyphenoxy compound **1d**, (*S*)-monoacetates **5a,f–j,l,m** and/or of the (*R*)-diols **4b,d–m** indicating the same geometric preference for both products. In the case of the sterically most demanding 1-naphthyl **1b** or phenyl derivatives with at least one substituent in the *o*-position **1d,e,h,l,m**, the formation of the diol **4d,e,h,l,m** of high (>90%

Table 3
Baker's yeast mediated biotransformation of 1-acetoxy-3-aryloxypropan-2-ones (**1a–k**)[a]




**1a-m** (*ent*)-**5a-m** **4a-m**

| | Ar | Time (h) | Monoacetate **5** or *ent*-**5** [b] Yield (%) | %e.e. (config.) | Diol **4** Yield (%) | %e.e. (config.) |
|---|---|---|---|---|---|---|
| **a** | phenyl | 1 | 84 | 83 [c] (*S*) | - | - |
| **b** | 1-naphthyl | 2 | - | - | 78 | >95 [d] (*R*) |
| **c** | 2-naphthyl | 16 | no reaction | | no reaction | |
| **d** | 2-isopropylphenyl | 1 | 36 | 28 (*R*) | 55 | 93 [e] (*R*) [d] |
| **e** | 2-chlorophenyl | 2 | - | - | 82 | >95 [e] (*R*) |
| **f** | 3-chlorophenyl | 2 | 38 | 93 (*S*) | 48 | 81 [e] (*R*) |
| **g** | 4-chlorophenyl | 2 | 65 | 61 (*S*) | 25 | 63 [e] (*R*) |
| **h** | 2-methylphenyl | 2 | 32 | 65 (*S*) | 47 | >95 [e] (*R*) |
| **i** | 3-methylphenyl | 2 | 66 | 77 (*S*) | 26 | 82 [e] (*R*) |
| **j** | 4-methylphenyl | 2 | 36 | 52 (*S*) | 40 | 68 [e] (*R*) [d] |
| **k** | 3-nitrophenyl | 4 | - | - | 80 | >95 [d] (*R*) |
| **l** | 2,6-dimethylphenyl | 1.5 | 60 | >95 (*S*) | 32 | >95 [d] (*R*) |
| **m** | 2,4,6-trichlorophenyl | 6 | 58 | 95 (*S*) | 34 | 92 [d] (*R*) |

[a] *Reaction conditions:* **1a-m**, 500 mg; potassium phosphate buffer (0.15M, pH 7), 200 ml; wet baker's yeast, 12 g; sucrose, 5 g. [b] Configuration and enantiomeric purity of the monoacetates (**5**) were determined from the specific rotation of the corresponding diols (**4**); [c] From $^1$H-NMR spectra of the MTPA ester of **5a**; [d] From optical rotation of the diol [(*R*)-**4**] obtained by alkylating the corresponding phenol (**3**) with (*R*)-3-chloropropane-1,2-diol [(*R*)-**2**, 95 % e.e.][17]; [e] From $^1$H-NMR spectra of the di-MTPA ester of the diol.

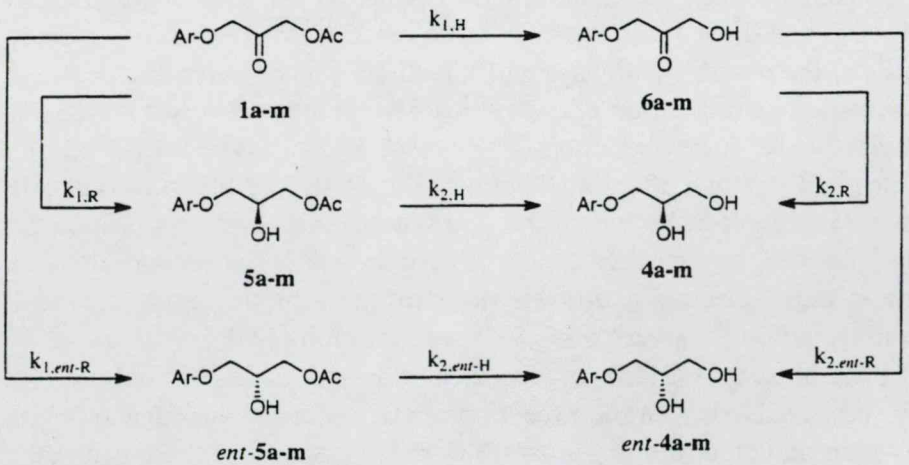



Fig. 1. Baker's yeast mediated reaction of 1-acetoxy-3-aryloxypropan-2-ones **1a–m**

e.e.) enantiomeric purity is common. The reaction of ketone with the more bulky *m*-nitrophenyl moiety **1k** proceeded also with high selectivity, while the compounds with smaller *m*-substitutents of the phenyl moiety **1f,i** were transformed with less remarkable stereoselectivities. The lowest selectivities were found in the reactions of the compounds with *p*-substituted phenyl rings **1g,j**. In three cases, the diol **4b,e,k** was the sole product, while in the other reactions, both the non-hydrolyzed monoacetate *ent*-**5d**, **5f–j,l,m** and the diol **4d,f–j,l,m** were obtained with variable enantiomeric compositions. These results might be best interpreted by assuming first a fast enantiotope selective reduction of the acetoxy ketones **1** with variable degree of (*S*)-enantiomer preference ($k_{1,R}>k_{1,ent\text{-}R}$), followed by an enantiomer selective hydrolysis ($k_{2,H}>k_{2,ent\text{-}H}$) with preference towards the (*R*)-diols **4**.[18] By this assumption, formation of the (*R*)-monoacetate *ent*-**5d** of low enantiomeric excess can also be interpreted without assuming the unlikely configuration-preference change of the reduction within the series of acetoxy ketones **1**. Accordingly, this reaction may be the result of the reduction of the acetoxy ketone **1d** with moderate (*S*)-enantiotope selectivity followed by hydrolysis with high enantiomer selectivity towards the (*R*)-diol **4d**, leaving the non-hydrolyzed (*R*)-monoacetate *ent*-**5d** in excess in the remaining monoacetate fraction.

In conclusion, baker's yeast mediated reaction of the 1-acetoxy-3-aryloxypropan-2-ones **1a–m** proved to be a useful method for the preparation of optically active 3-aryloxypropan-1,2-diol derivatives. This transformation showed high selectivities with the ketones of sterically hindered aryl moieties, while lower selectivities were found for the less hindered ketones with *m*- or *p*-substituted phenyl moieties. Since the opposite tendency (i.e. higher selectivity for the less hindered compounds) was found by the lipase-catalyzed enantiomer selective acylation processes of the similar 3-aryloxypropan-1,2-diol derivatives, the present baker's yeast mediated method seems to be useful for preparing such diols bearing a sterically hindered aryl moiety.

## 1. Experimental

The $^1$H NMR spectra were recorded on a Bruker AW-250 spectrometer operating at 250 MHz. $^1$H NMR spectra for enantiomeric excess determinations were recorded at 500 MHz on a Bruker DRX-500 spectrometer. All spectra were taken in $CDCl_3$ solution and chemical shift values are expressed in ppm values from TMS as internal standard on δ scale. IR spectra of thin film samples were taken on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. Thin layer chromatography was carried out using Merck Kieselgel 60 $F_{254}$ alumina sheets applying hexane:acetone=10:4 (A) or chloroform:methanol=10:0.5 (B) mixtures for elution. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative chromatographic separations were performed using vacuum-chromatography[19] on Merck Kieselgel 60 (0.063–0.200 mm). Phenols, racemic 3-chloropropane-1,2-diol and acetic anhydride were products of FLUKA or Aldrich. All solvents used were freshly distilled. Wet cake or lyophilized form of baker's yeast were from a local store.

### *1.1. Preparation of 3-aryloxypropane-1,2-diols* rac-*4a–m*

To the solution of the phenol derivative **3a–m**, 0.1 mol in ethanol (60 ml) a solution of NaOH (5.0 g, 0.125 mol) in water (20 ml) was added and the resulting mixture was heated under reflux for 10 minutes. Then a solution of racemic 3-chloropropane-1,2-diol *rac*-**2**, 13.3 g, 0.12 mol in ethanol (10 ml) was added within 5 minutes and the mixture was further heated under reflux for between 1 and 2 hours (the progress of the reaction was monitored by TLC; solvent A). After cooling, the volume of the resulting

mixture was reduced to about one third by a rotary evaporator followed by addition of water (60 ml) and extraction with diethyl ether (2×75 ml). The combined organic layers were dried over $Na_2SO_4$ and the solvent was removed. The resulting diols **4a–m** were usually purified by recrystallization from a 1:1 mixture of hexane and diethyl ether. For yields, see Table 1.

*1.1.1.* rac-*3-Phenoxypropane-1,2-diol* rac-***4a***

$^1$H NMR: 3.65–3.85 (m, 2H, $CH_2$–OH), 4.0 (m, 1H, CH), 4.10–4.22 (m, 2H, ArO–$CH_2$), 6.84–7.25 (m, 5H, ArH). IR: 3450 (br), 2910, 1600, 1590, 1500, 1460, 1170, 1120, 1060, 1050, 880, 750, 690 $cm^{-1}$. Calcd for $C_9H_{12}O_3$: C 64.27, H 7.19. Found: C 64.46, H 7.17.

*1.1.2.* rac-*3-(1-Naphthyloxy)propane-1,2-diol* rac-***4b***

$^1$H NMR: 3.86 (m, 2H, $CH_2$), 4.02–4.14 (m, 3H, CH and ArO–$CH_2$), 6.75–8.24 (m, 7H, ArH). IR: 3290 (br), 2940, 1730, 1580, 1510, 1470, 1390, 1270, 1240, 1130, 1110, 1040, 990, 890, 790, 770 $cm^{-1}$. Calcd for $C_{13}H_{14}O_3$: C 71.54, H 6.47. Found: C 71.68, H 6.46.

*1.1.3.* rac-*3-(2-Naphthyloxy)propane-1,2-diol* rac-***4c***

$^1$H NMR: 3.77 (m, 2H, $CH_2$–OH), 3.92 (m, 1H, CH), 4.07–4.26 (m, 2H, ArO–$CH_2$), 7.09–7.73 (m, 7H, ArH). IR: 3400 (br), 2940, 1630, 1600, 1510, 1460, 1260, 1220, 1180, 1060, 1000, 840, 740 $cm^{-1}$. Calcd for $C_{13}H_{14}O_3$: C 71.54, H 6.47. Found: C 71.25, H 6.50.

*1.1.4.* rac-*3-(2-Isopropylphenoxy)propane-1,2-diol* rac-***4d***

$^1$H NMR: 1.24 (d, 6H, $2CH_3$), 3.16, (m, 1H, CH–Ar), 3.74 (m, 2H, $CH_2$–OH), 4.03 (m, 1H, CH), 4.07–4.18 (m, 2H, ArO–$CH_2$), 6.81–7.25 (m, 4H, ArH). IR: 3300 (br), 2960, 2870, 1600, 1500, 1460, 1380, 1240, 1080, 1050, 930, 760 $cm^{-1}$. Calcd for $C_{12}H_{18}O_3$: C 68.55, H 8.63. Found: C 68.28, H 8.66.

*1.1.5.* rac-*3-(2-Chlorophenoxy)propane-1,2-diol* rac-***4e***

$^1$H NMR: 3.78 (m, 2H, $CH_2$–OH), 4.07 (m, 1H, CH), 4.10 (m, 2H, ArO–$CH_2$), 6.88–7.31 (m, 4H, ArH). IR: 3300 (br), 2940, 1590, 1490, 1450, 1300, 1250, 1130, 1070, 1030, 740 $cm^{-1}$. Calcd for $C_9H_{11}O_3Cl$: C 53.35, H 5.47. Found: C 53.40, H 5.46.

*1.1.6.* rac-*3-(3-Chlorophenoxy)propane-1,2-diol* rac-***4f***

$^1$H NMR: 3.75 (m, 2H, $CH_2$–OH), 3.96 (m, 1H, CH), 4.07 (m, 2H, ArO–$CH_2$), 6.69–7.24 (m, 4H, ArH). IR: 3300 (br), 2930, 1600, 1470, 1430, 1280, 1230, 1120, 1060, 890, 860, 770, 680 $cm^{-1}$. Calcd for $C_9H_{11}O_3Cl$: C 53.35, H 5.47. Found: C 53.37, H 5.45.

*1.1.7.* rac-*3-(4-Chlorophenoxy)propane-1,2-diol* rac-***4g***

$^1$H NMR: 3.78 (m, 2H, $CH_2$–OH), 4.01 (m, 1H, CH), 4.10 (m, 2H, ArO–$CH_2$), 6.79–7.26 (m, 4H, ArH). IR: 3300 (br), 2920, 1600, 1490, 1450, 1280, 1240, 1110, 1050, 880, 820 $cm^{-1}$. Calcd for $C_9H_{11}O_3Cl$: C 53.35, H 5.47. Found: C 53.33, H 5.49.

*1.1.8.* rac-*3-(2-Methylphenoxy)propane-1,2-diol* rac-***4h***

$^1$H NMR: 2.23 (s, 3H, $CH_3$), 3.76 (m, 2H, $CH_2$–OH), 4.01 (m, 1H, CH), 4.12 (m, 2H, ArO–$CH_2$), 6.78–7.18 (m, 4H, ArH). IR: 3260 (br), 2930, 1610, 1500, 1460, 1310, 1250, 1120, 1050, 990, 750 $cm^{-1}$. Calcd for $C_{10}H_{14}O_3$: C 65.92, H 7.74. Found: C 65.69, H 7.72.

*1.1.9.* rac-*3-(3-Methylphenoxy)propane-1,2-diol* rac-**4i**

$^1$H NMR: 2.27 (s, 3H, $CH_3$), 3.76 (m, 2H, $CH_2$–OH), 4.00 (m, 1H, CH), 4.08 (m, 2H, ArO–$CH_2$), 6.67–7.18 (m, 4H, ArH). IR: 3290 (br), 2930, 1610, 1590, 1490, 1450, 1290, 1260, 1160, 1060, 910, 860, 770, 690 cm$^{-1}$. Calcd for $C_{10}H_{14}O_3$: C 65.92, H 7.74. Found: C 65.74, H 7.73.

*1.1.10.* rac-*3-(4-Methylphenoxy)propane-1,2-diol* rac-**4j**

$^1$H NMR: 2.25 (s, 3H, $CH_3$), 3.72–4.00 (m, 2H, $CH_2$–OH), 3.96 (m, 1H, CH), 4.13 (m, 2H, ArO–$CH_2$), 7.24–7.87 (m, 4H, ArH). IR: 3390 (br), 1580, 1520, 1440, 1360, 1320, 1250, 1140, 1080, 1010, 870, 820 cm$^{-1}$. Calcd for $C_{10}H_{14}O_3$: C 65.92, H 7.74. Found: C 65.99, H 7.74.

*1.1.11.* rac-*3-(3-Nitrophenoxy)propane-1,2-diol* rac-**4k**

$^1$H NMR: 3.72–4.00 (m, 2H, $CH_2$), 4.13 (m, 3H, $CH_2$ and CH), 7.24–7.87 (m, 4H, ArH). IR: 3390 (br), 1580, 1520, 1440, 1360, 1320, 1250, 1140, 1080, 1010, 870, 820, 740 cm$^{-1}$. Calcd for $C_9H_{11}NO_5$: C 50.71, H 5.20, N 6.57. Found: C 50.56, H 5.22, N 6.59.

*1.1.12.* rac-*3-(2,6-Dimethylphenoxy)propane-1,2-diol* rac-**4l**

$^1$H NMR: 2.21 (s, 6H, $CH_3$), 3.78 (m, 4H, $CH_2$–OH and $CH_2$–OAr), 4.07 (m, 1H, CH), 6.80–7.00 (m, 3H, ArH). IR: 3384 (br), 2925, 1592, 1477, 1264, 1201, 1026, 768 cm$^{-1}$. Calcd for $C_{11}H_{16}O_3$: C 67.32, H 8.22. Found: C 67.38, H 8.19.

*1.1.13.* rac-*3-(2,4,6-Trichlorophenoxy)propane-1,2-diol* rac-**4m**

$^1$H NMR: 3.50–4.55 (m, 5H, $CH_2$–OH, $CH_2$–OAr and CH), 7.25 (s, 2H, ArH). IR: 3312 (br), 2943, 1572, 1553, 1447, 1256, 1053, 858 cm$^{-1}$. Calcd for $C_9H_9Cl_3O_3$: C 39.81, H 3.34. Found: C 39.67, H 3.39.

*1.2. Preparation of 1-acetoxy-3-aryloxypropan-2-ols* rac-**5a–m**

To a solution of 3-aryloxypropane-1,2-diol *rac*-**4a–m**, 50 mmol, pyridine (60 mmol) and (4-*N,N*-dimethylamino)pyridine (100 mg) in methylene chloride (100 ml) acetic anhydride (50 mmol) was added dropwise and the resulting solution was stirred for between 10 and 60 minutes. Then the mixture was washed with 5% hydrochloric acid (2×20 ml), saturated $NaHCO_3$ solution (20 ml) and brine (15 ml). After drying over $Na_2SO_4$ the solvent was removed by rotary evaporation. The residue was purified by preparative vacuum-chromatography by eluting with hexane:acetone 10:1→10:3. Yields of the products *rac*-**5a–m** are given in Table 1.

*1.2.1.* rac-*1-Acetoxy-3-phenoxypropan-2-ol* rac-**5a**

$^1$H NMR: 2.04 (s, 3H, $CH_3COO$), 4.05 (m, 2H, ArO–$CH_2$), 4.09 (m, 1H, CH), 4.08–4.17 (m, 2H, AcO–$CH_2$), 6.76–7.22 (m, 5H, ArH). IR: 3450 (br), 2940, 1740, 1600, 1500, 1370, 1240, 1050, 760 cm$^{-1}$. Calcd for $C_{11}H_{14}O_4$: C 62.85, H 6.71. Found: C 62.66, H 6.70.

*1.2.2.* rac-*1-Acetoxy-3-(1-naphthyloxy)propan-2-ol* rac-**5b**

$^1$H NMR: 2.14 (s, 3H, $CH_3COO$), 4.19 (m, 2H, ArO–$CH_2$), 4.28–4.46 (m, 3H, AcO–$CH_2$ and CH), 6.77–8.25 (m, 7H, ArH). IR: 3450 (br), 1710, 1580, 1460, 1400, 1370, 1270, 1240, 1110, 1050, 950, 790, 270 cm$^{-1}$. Calcd for $C_{15}H_{16}O_4$: C 69.22, H 6.20. Found: C 69.43, H 6.19.

*1.2.3.* rac-*1-Acetoxy-3-(2-naphthyloxy)propan-2-ol* rac-**5c**

$^1$H NMR: 2.12 (s, 3H, $CH_3COO$), 4.14 (m, 2H, ArO–$CH_2$), 4.31 (m, 3H, CH and AcO–$CH_2$), 7.13–7.78 (m, 7H, ArH). IR: 3450 (br), 2940, 1740, 1630, 1600, 1510, 1460, 1390, 1260, 1220, 1180, 1050, 840 cm$^{-1}$. Calcd for $C_{15}H_{16}O_4$: C 69.22, H 6.20. Found: C 69.35, H 6.22.

*1.2.4.* rac-*1-Acetoxy-3-(2-isopropylphenoxy)propan-2-ol* rac-**5d**

$^1$H NMR: 1.10 (d, 6H, $2CH_3$), 2.10 (s, 3H, $CH_3COO$), 3.17 (m, 1H, CH–Ar), 3.84 (m, 2H, ArO–$CH_2$), 4.16 (m, 3H, CH and AcO–$CH_2$), 6.67–7.11 (m, 4H, ArH). IR: 3460 (br), 2960, 2870, 1740, 1490, 1250, 1370, 1240, 1090, 1050, 750 cm$^{-1}$. Calcd for $C_{14}H_{20}O_4$: C 66.65, H 7.99. Found: C 66.45, H 8.02.

*1.2.5.* rac-*1-Acetoxy-3-(2-chlorophenoxy)propan-2-ol* rac-**5e**

$^1$H NMR: 1.94 (s, 3H, $CH_3COO$), 3.91 (m, 2H, ArO–$CH_2$), 4.16 (m, 3H, CH and AcO–$CH_2$), 6.74–7.21 (m, 4H, ArH). IR: 3440 (br), 2950, 1740, 1590, 1490, 1450, 1370, 1250, 1130, 1060 cm$^{-1}$. Calcd for $C_{11}H_{13}O_4Cl$: C 54.00, H 5.36. Found: C 54.16, H 5.34.

*1.2.6.* rac-*1-Acetoxy-3-(3-chlorophenoxy)propan-2-ol* rac-**5f**

$^1$H NMR: 1.97 (s, 3H, $CH_3COO$), 3.81 (m, 2H, ArO–$CH_2$), 4.11 (m, 3H, CH and AcO–$CH_2$), 6.62–7.09 (m, 4H, ArH). IR: 3440 (br), 2950, 1740, 1600, 1480, 1370, 1230, 1070, 1050, 860 cm$^{-1}$. Calcd for $C_{11}H_{13}O_4Cl$: C 54.00, H 5.36. Found: C 53.89, H 5.38.

*1.2.7.* rac-*1-Acetoxy-3-(4-chlorophenoxy)propan-2-ol* rac-**5g**

$^1$H NMR: 2.06 (s, 3H, $CH_3COO$), 3.98 (m, 2H, ArO–$CH_2$), 4.07 (m, 1H, CH), 4.25 (m, 2H, AcO–$CH_2$), 6.77–7.23 (m, 4H, ArH). IR: 3450 (br), 2950, 1740, 1600, 1490, 1370, 1240, 1170, 1090, 1050, 850 cm$^{-1}$. Calcd for $C_{11}H_{13}O_4Cl$: C 54.00, H 5.36. Found: C 54.12, H 5.35.

*1.2.8.* rac-*1-Acetoxy-3-(2-methylphenoxy)propan-2-ol* rac-**5h**

$^1$H NMR: 2.02 (s, 3H, $CH_3COO$), 2.22 (s, 3H, $CH_3$), 3.95 (m, 2H, ArO–$CH_2$), 4.08 (m, 1H, CH), 4.25 (m, 2H, AcO–$CH_2$), 6.75–7.12 (m, 4H, ArH). IR: 3450 (br), 2950, 1740, 1600, 1500, 1460, 1380, 1240, 1190, 1120, 1050, 750 cm$^{-1}$. Calcd for $C_{12}H_{16}O_4$: C 64.27, H 7.19. Found: C 64.40, H 7.20.

*1.2.9.* rac-*1-Acetoxy-3-(3-methylphenoxy)propan-2-ol* rac-**5i**

$^1$H NMR: 1.96 (s, 3H, $CH_3COO$), 2.16 (s, 3H, $CH_3$), 3.79 (m, 2H, ArO–$CH_2$), 3.90–4.10 (m, 3H, CH and AcO–$CH_2$), 6.56–7.00 (m, 4H, ArH). IR: 3450 (br), 2926, 1740, 1600, 1590, 1490, 1460, 1370, 1260, 1160, 1050, 780 cm$^{-1}$. Calcd for $C_{12}H_{16}O_4$: C 64.27, H 7.19. Found: C 64.18, H 7.18.

*1.2.10.* rac-*1-Acetoxy-3-(4-methylphenoxy)propan-2-ol* rac-**5j**

$^1$H NMR: 1.89 (s, 3H, $CH_3COO$), 2.10 (s, 3H, $CH_3$), 3.80 (m, 2H, ArO–$CH_2$), 4.04–4.11 (m, 3H, CH and AcO–$CH_2$), 6.62–6.91 (m, 4H, ArH). IR: 3450 (br), 2930, 1740, 1610, 1510, 1460, 1370, 1240, 1180, 1050, 820 cm$^{-1}$. Calcd for $C_{12}H_{16}O_4$: C 64.27, H 7.19. Found: C 64.21, H 7.18.

*1.2.11.* rac-*1-Acetoxy-3-(3-nitrophenoxy)propan-2-ol* rac-**5k**

$^1$H NMR: 2.12 (s, 3H, $CH_3COO$), 3.72–4.00 (m, 2H, ArO–$CH_2$), 4.14 (m, 1H, CH), 4.31 (m, 2H, AcO–$CH_2$), 7.25–7.83 (m, 4H, ArH). IR: 3330 (br), 2950, 1740, 1620, 1530, 1350, 1240, 1050, 1030, 820, 740 cm$^{-1}$. Calcd for $C_{11}H_{13}O_6N$: C 51.77, H 5.13, N 5.49. Found: C 51.56, H 5.12, N 5.51.

*1.2.12.* rac-*1-Acetoxy-3-(2,6-dimethylphenoxy)propan-2-ol* rac-**5l**

$^1$H NMR: 2.12 (s, 3H, $CH_3COO$), 2.30 (s, 6H, $CH_3$), 3.80 (m, 2H, ArO–$CH_2$), 3.95 (m, 1H, CH), 4.30 (m, 2H, AcO–$CH_2$), 6.90–7.10 (m, 3H, ArH). IR: 3456 (br), 2926, 1740, 1592, 1375, 1243, 1201, 1092, 1046, 771 cm$^{-1}$. Calcd for $C_{13}H_{18}O_4$: C 65.53, H 7.61. Found: C 64.97, H 7.72.

*1.2.13.* rac-*1-Acetoxy-3-(2,4,6-trichlorophenoxy)propan-2-ol* rac-**5m**

$^1$H NMR: 2.05 (s, 3H, $CH_3COO$), 4.10 (m, 3H, ArO–$CH_2$ and CH), 4.30 (m, 2H, AcO–$CH_2$), 7.30 (s, 2H, ArH). IR: 3448 (br), 3076, 2953, 1741, 1553, 1448, 1248, 1047, 1005, 857, 809 cm$^{-1}$. Calcd for $C_{11}H_{11}Cl_3O_4$: C 42.14, H 3.54, Cl 33.92. Found: C 42.25, H 3.50.

*1.3. Preparation of 1-acetoxy-3-aryloxy-propan-2-ones* **1a–m** *by Swern-oxidation*

To a solution of oxalyl chloride (1.35 ml, 15.0 mmol) in methylene chloride (25 ml) dimethyl sulfoxide (2.3 ml, 32.0 mol) in methylene chloride (5.0 ml) was added dropwise at −60°C. After stirring the resulting mixture for 10 minutes, racemic 1-acetoxy-3-aryloxypropan-2-ol *rac*-**5a–m**, 10 mmol in methylene chloride (10 ml) was added at −60°C followed by stirring for 20 minutes at −60°C. Then triethylamine (7.0 ml, 50 mmol) was added and the temperature was increased to room temperature within 30 min. The resulting mixture was washed with water (30 ml) and the aqueous layer was extracted with methylene chloride (20 ml). The combined organic solutions were washed with 5% hydrochloric acid (20 ml), saturated $NaHCO_3$ solution (15 ml) and brine (15 ml). After drying over $Na_2SO_4$ and the solvent was removed in vacuo. The resulting product was usually pure enough for the next step. In several cases the product was purified by preparative vacuum-chromatography using hexane:acetone 10:0.5→10:1 for the elution. For yields of the resulting ketones **1a–m**, see Table 1.

*1.3.1. 1-Acetoxy-3-phenoxypropan-2-one* **1a**

$^1$H NMR: 2.15 (s, 3H, $CH_3COO$), 4.63 (s, 2H, $CH_2$), 4.98 (s, 2H, $CH_2$), 6.87–7.35 (m, 5H, ArH). IR: 1740, 1600, 1500, 1430, 1380, 1240, 1160, 1070, 760, 690 cm$^{-1}$. Calcd for $C_{11}H_{12}O_4$: C 63.45, H 5.81. Found: C 63.58, H 5.79.

*1.3.2. 1-Acetoxy-3-(1-naphthyloxy)propan-2-one* **1b**

$^1$H NMR: 2.11 (s, 3H, $CH_3COO$), 4.62 (s, 2H, ArO–$CH_2$), 4.96 (s, 2H, AcO–$CH_2$), 6.48–8.17 (m, 7H, ArH). IR: 1740, 1630, 1580, 1510, 1420, 1400, 1370, 1310, 1240, 1110, 1040, 1020, 980, 860, 790, 770 cm$^{-1}$. Calcd for $C_{15}H_{14}O_4$: C 69.76, H 5.46. Found: C 69.48, H 5.44.

*1.3.3. 1-Acetoxy-3-(2-naphthyloxy)propan-2-one* **1c**

$^1$H NMR: 2.21 (s, 3H, $CH_3COO$), 4.75 (s, 2H, ArO–$CH_2$), 5.02 (s, 2H, AcO–$CH_2$), 7.04–7.81 (m, 7H, ArH). IR: 1750, 1730, 1630, 1600, 1510, 1430, 1410, 1370, 1300, 1260, 1250, 1220, 1180, 1110, 1080, 1030, 1000, 840, 810, 740 cm$^{-1}$. Calcd for $C_{15}H_{14}O_4$: C 69.76, H 5.46. Found: C 69.57, H 5.45.

*1.3.4. 1-Acetoxy-3-(2-isopropylphenoxy)propan-2-one* **1d**

$^1$H NMR: 1.15 (d, 6H, $2CH_3$), 2.03 (d, 6H, $2CH_3$), 3.25 (m, 1H, CH–Ar), 4.51 (s, 2H, ArO–$CH_2$), 4.92 (s, 2H, AcO–$CH_2$), 6.58–7.19 (m, 4H, ArH). IR: 2960, 1740, 1600, 1590, 1490, 1450, 1370, 1240, 1170, 1090, 1070, 750 cm$^{-1}$. Calcd for $C_{14}H_{18}O_4$: C 67.18, H 7.25. Found: C 67.42, H 7.25.

*1.3.5. 1-Acetoxy-3-(2-chlorophenoxy)propan-2-one 1e*

$^1$H NMR: 2.01 (s, 3H, $CH_3COO$), 4.57 (s, 2H, ArO–$CH_2$), 5.0 (s, 2H, AcO–$CH_2$), 6.72–7.31 (m, 4H, ArH). IR: 1740, 1590, 1480, 1420, 1400, 1370, 1300, 1240, 1160, 1070, 750 cm$^{-1}$. Calcd for $C_{11}H_{11}O_4Cl$: C 54.45, H 4.57. Found: C 54.57, H 4.55.

*1.3.6. 1-Acetoxy-3-(3-chlorophenoxy)propan-2-one 1f*

$^1$H NMR: 2.05 (s, 3H, $CH_3COO$), 4.60 (s, 2H, ArO–$CH_2$), 4.88 (s, 2H, AcO–$CH_2$), 6.62–7.22 (m, 4H, ArH). IR: 2930, 1740, 1590, 1480, 1430, 1380, 1290, 1230, 1170, 1070, 1020, 860, 770, 680 cm$^{-1}$. Calcd for $C_{11}H_{11}O_4Cl$: C 54.45, H 4.57. Found: C 54.61, H 4.58.

*1.3.7. 1-Acetoxy-3-(4-chlorophenoxy)propan-2-one 1g*

$^1$H NMR: 2.05 (s, 3H, $CH_3COO$), 4.56 (s, 2H, ArO–$CH_2$), 4.87 (s, 2H, AcO–$CH_2$), 6.73–7.21 (m, 4H, ArH). IR: 1740, 1600, 1490, 1430, 1370, 1280, 1240, 1160, 1070, 1020, 830 cm$^{-1}$. Calcd for $C_{11}H_{11}O_4Cl$: C 54.45, H 4.57. Found: C 54.39, H 4.57.

*1.3.8. 1-Acetoxy-3-(2-methylphenoxy)propan-2-one 1h*

$^1$H NMR: 2.10 (s, 3H, $CH_3COO$), 2.23 (s, 3H, $CH_3$), 4.60 (s, 2H, ArO–$CH_2$), 4.95 (s, 2H, AcO–$CH_2$), 6.64–7.09 (m, 4H, ArH). IR: 1750, 1600, 1590, 1490, 1430, 1400, 1370, 1240, 1160, 1120, 1060, 850, 810, 750 cm$^{-1}$. Calcd for $C_{12}H_{14}O_4$: C 64.85, H 6.35. Found: C 64.59, H 6.34.

*1.3.9. 1-Acetoxy-3-(3-methylphenoxy)propan-2-one 1i*

$^1$H NMR: 2.02 (s, 3H, $CH_3COO$), 2.17 (s, 3H, $CH_3$), 4.51 (s, 2H, ArO–$CH_2$), 4.87 (s, 2H, AcO–$CH_2$), 6.54–7.07 (m, 4H, ArH). IR: 2920, 1740, 1600, 1590, 1490, 1430, 1370, 1290, 1230, 1150, 1070, 1030, 880, 780, 690 cm$^{-1}$. Calcd for $C_{12}H_{14}O_4$: C 64.85, H 6.35. Found: C 64.90, H 6.35.

*1.3.10. 1-Acetoxy-3-(4-methylphenoxy)propan-2-one 1j*

$^1$H NMR: 2.11 (s, 3H, $CH_3COO$), 2.21 (s, 3H, $CH_3$), 4.57 (s, 2H, ArO–$CH_2$), 4.95 (s, 2H, AcO–$CH_2$), 6.61–7.03 (m, 4H, ArH). IR: 2930, 1740, 1610, 1510, 1430, 1410, 1380, 1290, 1240, 1160, 1070, 1020, 820 cm$^{-1}$. Calcd for $C_{12}H_{14}O_4$: C 64.85, H 6.35. Found: C 64.71, H 6.33.

*1.3.11. 1-Acetoxy-3-(3-nitrophenoxy)propan-2-one 1k*

$^1$H NMR: 2.11 (s, 3H, $CH_3COO$), 4.78 (s, 2H, ArO–$CH_2$), 4.91 (s, 2H, AcO–$CH_2$), 7.19–7.87 (m, 4H, ArH). IR: 1750, 1730, 1530, 1480, 1410, 1350, 1240, 1100, 1030, 870, 810, 740 cm$^{-1}$. Calcd for $C_{11}H_{11}O_6N$: C 52.18, H 4.38, N 5.53. Found: C 52.03, H 4.39, N 5.52.

*1.3.12. 1-Acetoxy-3-(2,6-dimethylphenoxy)propan-2-one 1l*

$^1$H NMR: 2.05 (s, 3H, $CH_3COO$), 2.14 (s, 6H, $CH_3$), 4.36 (s, 2H, ArO–$CH_2$), 4.99 (s, 2H, AcO–$CH_2$), 6.92 (m, 3H, ArH). IR: 2937, 1750, 1734, 1475, 1396, 1231, 1168, 1075, 1058, 788 cm$^{-1}$. Calcd for $C_{13}H_{16}O_4$: C 66.09, H 6.83. Found: C 66.01, H 6.79.

*1.3.13. 1-Acetoxy-3-(2,4,6-trichlorophenoxy)propan-2-one 1m*

$^1$H NMR: 2.18 (s, 3H, $CH_3COO$), 4.60 (s, 2H, ArO–$CH_2$), 5.20 (s, 2H, AcO–$CH_2$), 7.34 (s, 2H, ArH). IR: 3066, 2934, 1739, 1728, 1554, 1456, 1422, 1281, 1259, 1070, 1015, 858, 769 cm$^{-1}$. Calcd for $C_{11}H_9Cl_3O_4$: C 42.41, H 2.91. Found: C 42.36, H 2.95.

### 1.4. Optimization of the reaction conditions for reduction of 1-acetoxy-3-phenoxypropan-2-one 1a

To 200 ml of media (as indicated in Table 2) 1-acetoxy-3-phenoxypropan-2-one **1a**, 500 mg, sucrose (5.0 g), baker's yeast (wet cake 12 g; or lyophilized 2.5 g) and additive (as indicated in Table 2) were added and the resulting mixture was stirred at room temperature for a period indicated in Table 2. Then the reaction mixture was extracted with ethyl acetate (2×150 ml), the combined organic layers were washed with brine (20 ml) and dried over $Na_2SO_4$. After removing the solvent the residue was subjected to preparative vacuum-chromatography (hexane:acetone 10:1→10:5) to yield 75–85% of pure monoacetate **5a**. The enantiomeric composition of the product **5a** (Table 2) was determined by esterification with (*R*)-MTPA-Cl [0.05 mmol scale; triethylamine, cat. DMAP, in $CCl_4$, 50°C, 3 h] and $^1H$ NMR analysis of the **5a**-MTPA-ester [characteristic signals: 2.001 (s, $COCH_3$) for (*S*)-**5a**-MTPA ester; 2.065 (s, $COCH_3$) for (*R*)-**5a**-MTPA ester].

### 1.5. Preparation of optically active 1-acetoxy-3-aryloxypropan-2-ols 5 or ent-5 and/or 3-aryloxypropane-1,2-diols (4 or ent-4) by baker's yeast reaction

General procedure: To 200 ml of sodium phosphate buffer (0.15 M, pH 7) 1-acetoxy-3-aryloxypropan-2-one **1a–k**, 0.5 g, sucrose (5.0 g) and baker's yeast (12.0 g wet) were added and the resulting mixture was stirred at room temperature for a period indicated in Table 3. Work up and chromatographic separation was carried out similarly as described in the preceding section and yielded monoacetate **5** or *ent*-**5** and/or diol **4** or *ent*-**4** fractions. The IR and $^1H$ NMR spectra of the resulting 1-acetoxy-3-aryloxypropan-2-ols **5** or *ent*-**5** and/or 3-aryloxypropan-1,2-diols **4** or *ent*-**4** were similar to those of the corresponding racemic compounds. For yield, enantiomeric composition and configuration of the products, see Table 3.

Enantiomeric composition of several diols **4d–j** were determined from $^1H$ NMR spectra of the corresponding di-MTPA derivatives [esterification with (*R*)-MTPA-Cl: triethylamine, cat. DMAP, in $CCl_4$, 50°C, 3 h; characteristic signals: di-MTPA ester of (*R*)-**4d**: 5.638 (mc, CH–O); di-MTPA ester of (*S*)-**4d**: 5.699 (mc, CH–O); di-MTPA ester of (*R*)-**4e**: 5.669 (mc, CH–O); di-MTPA ester of (*S*)-**4e**: 5.713 (mc, CH–O); di-MTPA ester of (*R*)-**4f**: 5.620 (mc, CH–O); di-MTPA ester of (*S*)-**4f**: 5.665 (mc, CH–O); di-MTPA ester of (*R*)-**4g**: 5.627 (mc, CH–O); di-MTPA ester of (*S*)-**4g**: 5.645 (mc, CH–O); di-MTPA ester of (*R*)-**4h**: 5.641 (mc, CH–O); di-MTPA ester of (*S*)-**4h**: 5.705 (mc, CH–O); di-MTPA ester of (*R*)-**4i**: 5.630 (mc, CH–O); di-MTPA ester of (*S*)-**4i**: 5.675 (mc, CH–O); di-MTPA ester of (*R*)-**4j**: 5.627 (mc, CH–O); di-MTPA ester of (*S*)-**4j**: 5.655 (mc, CH–O)].

For determination of configuration, several (*R*)-diols **4** were prepared from (*R*)-3-chloropropane-1,2-diol [(*R*)-**2**, 95% e.e.] by coupling with the corresponding phenol **3** [(*R*)-**2**: 1.2 mmol, **3**: 1.5 mmol; according to the procedure used for the preparation of the racemic diols].

For direct comparison of the specific rotations, the monoacetate fractions **5** were saponified [1.2 M NaOMe/MeOH, r.t., 10 min, 80–95% yields] to diols **4** (Table 4).

## Acknowledgements

The Hungarian OTKA Foundation (Project Nos: 1759 and 14894) is gratefully acknowledged for financial support. J.B. thanks the Zoltán Magyary Foundation for a postdoctoral fellowship.

Table 4

| 4 | Ar | Solvent (c= 1) | $[\alpha]_D$ (conf.) | | |
|---|---|---|---|---|---|
| | | | [from 5] | [diol fraction] | [from (*R*)-2] |
| **a** | phenyl [a] | ethanol | -9.0 (*R*) | | -10.5 (*R*) |
| **b** | 1-naphthyl [b] | methanol | | -6.8 (*R*) | -6.5 (*R*) |
| **d** | 2-isopropylphenyl [c] | hexane-isopropanol | -4.2 (*S*) | 13.2 (*R*) | 14.1 (*R*) |
| **e** | 2-chlorophenyl [d] | hexane-ethanol 4:1 | | 13.3 (*R*) | |
| **f** | 3-chlorophenyl [e] | ethanol | -9.0 (*R*) | -7.9 (*R*) | |
| **g** | 4-chlorophenyl [f] | methanol | -8.3 (*R*) | -8.7 (*R*) | |
| **h** | 2-methylphenyl [g] | hexane-isopropanol | 12.1 (*R*) | 17.5 (*R*) | |
| **i** | 3-methylphenyl [h] | ethanol | -6.1 (*R*) | -6.5 (*R*) | |
| **j** | 4-methylphenyl [i] | hexane-isopropanol | 6.5 (*R*) | 8.6 (*R*) | 12.1 (*R*) |
| **k** | 3-nitrophenyl [c] | ethanol | | -13.7 (*R*) | -12.3 (*R*) |
| **l** | 2,6-dimethylphenyl [c] | acetone | 2.2 (*R*) | 2.2 (*R*) | 2.1 (*R*) |
| **m** | 2,4,6-trichlorophenyl [c] | acetone | 2.3 (*R*) | 2.2 (*R*) | 2.3 (*R*) |

[a] Lit. [1]: (*R*)-**4a** (98 %e.e.): $[\alpha]_D$= -10.8 (1, EtOH), (*S*)-**4a** (91 %e.e.): $[\alpha]_D$= 10.2 (1, EtOH); [b] Lit.: (*R*)-**4b**: $[\alpha]_D$= -6.76 (1, MeOH)[20], -8.1 (1, MeOH)[21], -8.5 (4.5, MeOH)[22], (*S*)-**4b**: $[\alpha]_D$= 6.7 (1, MeOH)[20], 6.9 (1, MeOH)[21], 7.6 (1, MeOH)[23], 7.7 (1, MeOH)[24], 8.4 (4.5, MeOH)[22]; [c] No published optical rotation data were found; [d] Lit. [1]: (*R*)-**4e** (99 %e.e.): $[\alpha]_D$= 14.0 (1, hexane-EtOH 4:1), (*S*)-**4e** (99 %e.e.): $[\alpha]_D$= -13.4 (1, hexane-EtOH 4:1); [e] Lit. [1]: (*R*)-**4f** (>99 %e.e.): $[\alpha]_D$= -12.7 (1, EtOH), (*S*)-**4f** (98 %e.e.): $[\alpha]_D$= 13.7 (1, EtOH); [f] Lit. [1]: (*R*)-**4g** (95 %e.e.): $[\alpha]_D$= -11.8 (1, EtOH), (*S*)-**4g** (97 %e.e.): $[\alpha]_D$= 12.3 (1, EtOH); [g] Lit. [1]: (*R*)-**4h** (>99 %e.e.): $[\alpha]_D$= 19.8 (0.9, hexane-*i*-PrOH 4:1), (*S*)-**4h** (>99 %e.e.): $[\alpha]_D$= -19.3 (0.9, hexane-*i*-PrOH 4:1); [h] Lit. [1]: (*R*)-**4i** (>99 %e.e.): $[\alpha]_D$= -9.3 (1, EtOH), (*S*)-**4i** (97 %e.e.): $[\alpha]_D$= 9.5 (1, EtOH); [i] Lit. [1]: (*R*)-**4j** (97 %e.e.): $[\alpha]_D$= -9.2 (1, EtOH), (*S*)-**4j** (71 %e.e.): $[\alpha]_D$= 7.5 (1, EtOH).

## References


1. Theil, F., Weidner, J., Ballschuh, S., Kunath, A., Schick, H. *J. Org. Chem.*, **1994**, *59*, 388.
2. Theil, F., Lemke, K., Ballschuh, S., Kunath, A., Schick, H. *Tetrahedron: Asymmetry*, **1995**, *6*, 1323.
3. Manzocchi, A., Fiecchi, A., Santaniello, E. *J. Org. Chem.*, **1988**, *53*, 4405.
4. Aragozzini, F., Maconi, E., Pontenza, C., Scolastico, C. *Synthesis*, **1989**, 225.
5. Sato, T., Mizutani, T., Okumura, Y., Fujisawa, T. *Tetrahedron Lett.*, **1989**, *30*, 3701.
6. Sih, C. J., Chen, C. S. *Angew. Chem., Int. Ed. Engl.*, **1984**, *23*, 570.
7. Shieh, W. R., Gopalan, A. S., Sih, C. J. *J. Am. Chem. Soc.*, **1985**, *107*, 2993.
8. Chen, C. S., Zhou, B. M., Girdaukas, G., Shieh, W. R., Van Middlesworth, F., Gopalan, A. S., Sih, C. J. *Bioorg. Chem.*, **1984**, *12*, 98.
9. McLeod, R., Prosser, H., Fiskentscher, L., Lányi, J., Mosher, H. S. *Biochemistry*, **1964**, *3*, 383.
10. Sakai, T., Nakamura, T., Fukuda, K., Amano, E., Utaka, M., Takeda, A. *Bull. Chem. Soc., Japan*, **1986**, *59*, 3185.
11. Fuganti, C., Grasselli, P., Spreafico, F., Zirotti, C. *J. Org. Chem.*, **1984**, *49*, 543.
12. Nakamura, K., Kawai, Y., Oka, S., Ohno, A. *Bull. Chem. Soc., Japan*, **1989**, *62*, 875.
13. Glänzer, B. I., Faber, K., Griengl, H. *Tetrahedron*, **1987**, *43*, 5791.
14. Nakamura, K., Kawai, Y., Oka, S., Ohno, A. *Tetrahedron Lett.*, **1989**, *30*, 2245.
15. Nakamura, K., Inoke, K., Ushio, K., Oka, S., Ohno, A. *Chem. Lett.*, **1987**, 679.
16. Nakamura, K., Kawai, Y., Ohno, A. *Tetrahedron Lett.*, **1990**, *31*, 267.
17. Poppe, L., Novák, L., Kajtár-Peredy, M., Szántay, Cs. *Tetrahedron: Asymmetry*, **1993**, *4*, 2211.
18. Our preliminary results with baker's yeast reduction of 3-acetoxy-1-benzoyloxypropan-2-one and 1-benzoyloxy-3-hydroxypropan-2-one under similar conditions showed much faster reduction for the acetoxy ketone than for the hydroxy ketone.
19. Poppe, L., Novák, L. *Magy. Kém. Lapja*, **1985**, *40*, 366.
20. Ogg, G. D., Neilson, D. G., Stevenson, I. H., Lyles, G. A. *J. Pharm. Pharmacol.*, **1987**, 378.
21. Nelson, W. L., Bartels, M. J. *J. Org. Chem.*, **1982**, *47*, 1574.

22. Leftheris, K., Goodman, M. *J. Med. Chem.*, **1990**, *33*, 216.
23. Carlsen, P. H. J., Aase, K. *Acta Chem. Scand.*, **1993**, *47*, 737.
24. Iriuchijima, S., Kojima, N. *Agric. Biol. Chem.*, **1982**, *46*, 1153.

# XI. melléklet

BÁLINT, J., EGRI, G. , KOLBERT, A., DIANÓCZKY, CS., FOGASSY, E., NOVÁK, L. **POPPE, L.**:

Baker's Yeast Mediated Reduction of Dihydroxyacetone Derivatives,

*Tetrahedron:Asymmetry*, **1999**, *10*, 4107.

Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 4017–4028

TETRAHEDRON: *ASYMMETRY*

# Baker's yeast mediated reduction of dihydroxyacetone derivatives

József Bálint,[a] Gabriella Egri,[a] Attila Kolbert,[a,b] Csilla Dianóczky,[b] Elemér Fogassy,[a] Lajos Novák[b] and László Poppe[c,*]

[a]*Department of Organic Chemical Technology, Technical University of Budapest, H-1111 Budapest, Müegyetem rkp 3, Hungary*

[b]*Institute for Organic Chemistry, Technical University of Budapest, H-1111 Budapest, Gellért tér 4, Hungary*

[c]*Chemical Research Center, Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, PO Box 17, Hungary*

Received 7 September 1999; accepted 15 September 1999

**Abstract**

Several monoprotected dihydroxyacetone derivatives **4a–d** and their acetates **5a–d** were prepared and subjected to biotransformation with baker's yeast. The simple chemical modification of the substrates (i.e. transforming the relatively small hydrophilic hydroxymethyl group into a larger hydrophobic acetoxymethyl moiety) inverted the sense of enantiotope selectivity of these reductions yielding optically active diols **6a–d**, or their enantiomeric acetates (**7a–d**) and diols (*ent*-**6a–d**), respectively. 

## 1. Introduction

Recently, the synthesis of optically active molecules has attracted interest due to the different effects of enantiomers. In pharmacy, these differences can be observed in therapeutic effects[1] and also in adsorption, metabolism and excretion.[2] Since biological systems are asymmetric catalysts by their nature, biocatalytic methods are widely applied for the preparation of optically active compounds.[3]

In the preparation of homochiral biologically active molecules, such as PAF (platelet-activating factor),[4] phospholipids,[5] phospholipase $A_2$ inhibitors,[6] and many others,[7] chiral glycerol derivatives of high enantiomeric purity might be useful $C_3$ building blocks. Enantiomer selective biocatalytical methods, e.g. hydrolase-catalyzed kinetic resolution of racemic glycerol derivatives such as glycerol acetonide,[8,9] or glycerol-2,3-carbonate,[10] provided moderate selectivity and 50% theoretical limit of the desired enantiomer.

* Corresponding author. E-mail: poppe.szk@chem.bme.hu

0957-4166/99/$ - see front matter 
*PII:* S0957-4166(99)00415-2

Enantiotope selective reduction of prochiral ketones theoretically enables the total conversion of the substrate into a single enantiomer of the product chiral secondary alcohol. Such prochiral ketone precursors of chiral $C_3$ building blocks are protected dihydroxyacetone derivatives. Accordingly, several chiral $C_3$ derivatives have already been prepared by chiral ruthenium complex-catalyzed asymmetric reductions.[11–13] The enantiotope selective baker's yeast reduction might be considered as a convenient biocatalytic alternative to these methods. The 3-methoxy-,[14] 3-benzoyloxy- and 3-(4-nitrobenzoyl)-oxy-[15] 1-hydroxyacetone derivatives and the 3-benzoyloxy-[15] and 3-aryloxy-[16] 1-acetoxyacetone derivatives were reduced by baker's yeast with various results. Although methods from 3-*O*-protected dihydroxyacetone derivatives leading to opposite enantiomeric forms may increase the synthetic value of the process, baker's yeast reduction of the 1-hydroxy- and 1-acetoxy-derivatives of 3-*O*-protected dihydroxyacetones with the same protective group has never been performed.

The known examples for enantiotope selective reduction of hydroxymethyl ketones or their acetates by baker's yeast[14,17–19] showed that ketones with the relatively small and hydrophilic hydroxymethyl group were reduced similarly in a geometrical sense (as a result of the sequence rules, however, the products may have different configuration labels). On the other hand, acetoxymethyl ketones were reduced with the opposite sense of enantiotopic preference. This inversion in the sense of enantiomeric preference was demonstrated by baker's yeast reduction of phenacyl alcohols and their acetates.[19–21] It should be mentioned here that reduction of hydroxymethyl ketones and their acetates by *Geotrichum* sp. 38 was reported to proceed without inversion of the sense of the enantiotopic selectivity.[22]

Since the monoprotected dihydroxyacetone derivatives are precursors of chiral $C_3$ building blocks, and their acetates could presumably be reduced by baker's yeast with opposite enantiopreference, we thought it worthwhile investigating the bioreduction of these compounds. Here we report the baker's yeast reduction of several synthetically useful monoprotected dihydroxyacetone derivatives **4a–d** and their acetates **5a–d**.

## 2. Results and discussion

The preparation of the monoprotected dihydroxyacetone derivatives **4a–d** and their acetates **5a–d** was straightforward starting from dihydroxyacetone **1** as outlined in Fig. 1. Ketones **4a**[15] and **4b** were prepared by monoacylation of the dihydroxyacetone (**1**, existing mostly in dimeric form) by benzoyl chloride and pivaloyl chloride, respectively. The benzyloxymethyl ketone **4c** was obtained[11] from the dimethyl ketal of dihydroxyacetone **2** by subsequent benzylation and acidic deketalization. Monosilylation[23] of dihydroxyacetone **1** provided the ketone **4d** smoothly. The acetoxymethyl ketones **5a–d** were obtained from their hydroxymethyl precursors (**4a–d**, respectively) by simple acetylation.

Since stereoselectivity of baker's yeast-catalyzed reactions may depend considerably on the reaction conditions (pH, solvent, additives, etc.), it was desirable to find the optimum. Hence, reaction conditions of the baker's yeast reduction of 1-benzoyloxy-3-hydroxypropan-2-one **4a** were investigated (Table 1).

The different reaction conditions were chosen by analogies with published modifications of reaction conditions increasing the stereoselectivity of carbonyl-reductions by baker's yeast. Reductions in apolar hydrocarbons with 'non-fermenting' baker's yeast resulted in increased selectivities.[24,25] Such selectivity enhancement was obtained by using $N_2$-atmosphere and cosolvent like DMSO.[15] Some sulfur-containing additives, such as L-cysteine or cysteamine, also produced significant selectivity enhancement in the reductions of 1-acetoxyalkan-2-ones.[26] It should be noted, however, that these additives were also used to suppress the hydrolysis of the ester function in these reactions. Ethanolamine as a possible substitute for the L-cysteine or cysteamine was also tested as an additive.

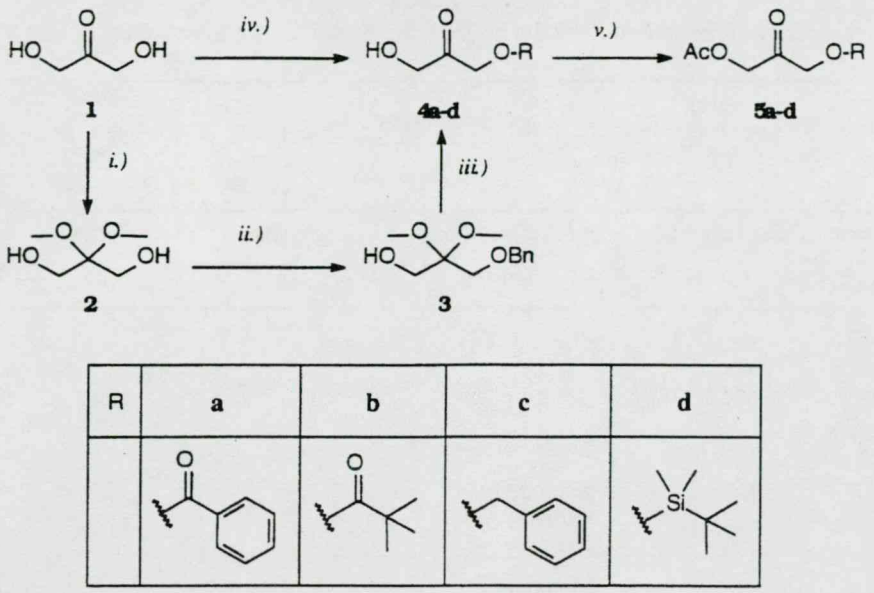



Figure 1. Preparation of hydroxymethyl ketones **4a–d** and their acetates **5a–d**. *Reagents and solvents*: (i) trimethyl orthoformate in MeOH; (ii) BnBr, NaH in THF; (iii) 3 M HCl; (iv) BzCl, cat. DMAP, pyridine (for **4a**), PivCl, cat. DMAP, pyridine (for **4b**) or TBDMSCl, imidazole in THF (for **4d**); (v) $Ac_2O$, cat. DMAP, $Et_3N$ in ethyl acetate (for **5a,c,d**) or AcCl, cat. DMAP, $Et_3N$ in THF (for **5b**)

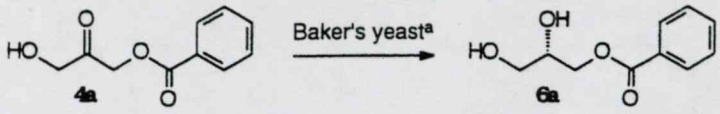
Although alcohol dehydrogenases can operate in ethanol as solvent, no reduction was observed in neat ethanol (Entry 1). Reactions with the traditional 'non-fermenting' yeast (Entry 2) or with 'non-fermenting' yeast in the presence of ethanol as coupled substrate in cofactor regeneration under $N_2$ (Entry 3) proceeded with moderate selectivities. The low isolated yields in hexane (Entries 4, 5 and 8) indicated low productivity/conversion without significant increase in selectivity. The productivities and selectivities of systems containing glucose were better (Entries 6–9). The best selectivity yielding homochiral diol **6a** was achieved by 'fermenting' baker's yeast under anerobic conditions with ethanol as cosolvent and L-cysteine as an additive (Entry 9).

The reaction conditions for the further compounds were optimized in a similar way. The reductions of these ketones **4b–d** and **5a–d** were conducted under the conditions which gave the highest enantiotopic selectivities, as listed in Table 2. The results achieved by the baker's yeast reduction of the hydroxymethyl ketones **4a–d** and their acetates **5a–d** confirmed the previous findings[19–21] and our expectations: the geometrical sense of the enantiotopic preference altered in all the cases when the hydroxymethyl ketones **4a–d** versus their acetates **5a–d** were reduced.

In the case of reduction of acetates **5a–d**, however, substantial amounts of diols *ent*-**6a–d** were also produced. Configuration of these diols *ent*-**6a–d** was opposite to the diols **6a–d** from reduction of the hydroxymethyl ketones **4a–d**. These data confirmed that diols *ent*-**6a–d** were produced mostly by reduction of the acetoxymethyl ketones **5a–d** followed by an enzymatic hydrolysis, and were consistent with our previous results on baker's yeast reduction of 1-acetoxy-3-aryloxypropan-2-ones,[16] where the geometrical sense of enantiotopic preference was the same as for the present ketones **5a–d** and different amounts of hydrolyzed products from enzymatic hydrolysis were also obtained.[27]

Since the hydroxymethyl ketones **4a–d** were reduced substantially slower and with opposite enantiotopic preferences than the acetoxymethyl ketones **5a–d**, our results further support the hypothesis[19–21] assuming that these two classes of ketones are reduced mostly by different enzymes of the baker's yeast system.

Table 1
Dependence of the stereoselectivity on the reaction conditions in reduction of ketone **4a**




| Entry | Yeast (g) | Buffer (Atm.) (ml) | Solvent (ml) | Additive(s) (g) | Time (h) | Y [b] (%) | E.e. [c] (%) |
|---|---|---|---|---|---|---|---|
| 1 | 12 | - | EtOH (100) | - | | no reaction | |
| 2 | 12 | 60 | - | - | 24 | 60 | 56 |
| 3 | 8 | 60 ($N_2$) | EtOH (6) | - | 20 | 56 | 64 |
| 4 | 15 | 15 | hexane (150), EtOH (1.5), DMSO (1.5) | - | 24 | 46 | 77 |
| 5 | 15 | 15 | hexane (150), EtOH (3) | - | 22 | 36 | 85 |
| 6 | 8 | 60 ($N_2$)[d] | EtOH (0.6), DMSO (0.6) | glucose (6) | 4 | 56 | 85 |
| 7 | 8 | 60 ($N_2$)[d] | EtOH (1.2) | glucose (6), ethanolamine (0.12) | 4 | 76 | 86 |
| 8 | 15 | 15 | hexane (150), DMSO (1.5) | glucose (6) | 24 | 42 | 89 |
| **9** | **8** | **60 ($N_2$)[d]** | **EtOH (1.2)** | **glucose (6) L-cysteine (0.3)** | **20** | **80** | **>97** |

[a] Standard conditions: 500 mg of **4a**, 0.15 M pH= 7.0 phosphate buffer, baker's yeast from Budafok factory; [b] Yields refer to products purified by preparative column chromatography; [c] Absolute configuration was taken from Ref. 15; enantiomeric excess was determined from the $^1$H-NMR spectrum of the (*R*)-MTPA ester of **7a**; [d] Substrate was added 30 minutes after starting the fermentation.

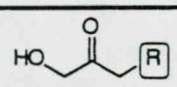
## 3. Conclusions

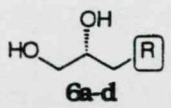
Our results showed that the monoprotected dihydroxyacetone derivatives **4a–d** and their acetates **5a–d** are synthetically useful precursors of different chiral $C_3$ building blocks. In accordance with the previous findings, these hydroxymethyl **4a–d** and acetoxymethyl **5a–d** ketones were reduced by baker's yeast oppositely in a geometrical sense, yielding optically active diols **6a–d**, or monoacetates **7a–d** and the enantiomeric diols *ent*-**6a–d**, respectively.

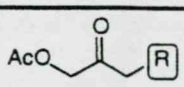
## 4. Experimental

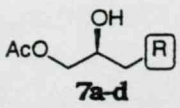
The $^1$H NMR spectra were recorded on a Bruker AW-250 spectrometer operating at 250 MHz. For enantiomeric excess determinations, a Bruker DRX-500 spectrometer operating at 500 MHz was used. All spectra were taken in $CDCl_3$ solution and chemical shifts are expressed in ppm values from TMS as internal standard on δ scale. IR spectra of thin film samples were taken on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. Thin layer chromatography was carried out using Merck Kieselgel 60 $F_{254}$ alumina sheets (using hexane:acetone, 10:4, if not stated

Table 2
Results for baker's yeast reduction of monoprotected dihydroxyacetone derivatives **4a–d** and their acetates **5a–d**

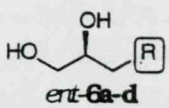


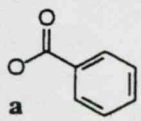
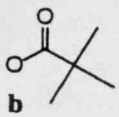
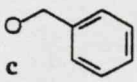
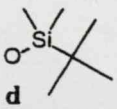




+




| R | Method [a] | Yield % | Conf. | E.e. % | Method [a] | Yield % | Conf. [b] | E.e. % | Yield % | Conf. | E.e. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  **a** | **A** | 80 | *S* | > 97 | **D** | 54 | *R*[b] | 68 | 22 | *R* | 19 |
|  **b** | **B** | 71 | *S* | 72 | **E** | 56 | *R*[b] | >95 | 9 | *R* | 46 |
|  **c** | **A** | 50 | *S* | 55 | **F** | 60 | *S*[b] | 85 | 20 | *R* | 33 |
|  **d** | **C** | 21 | *S* | 59 | **F** | 21 | *R*[b] | > 97 | 25 | *R* | 77 |

[a] *Common conditions*: 500 mg of ketone (**4a-d, 5a-d**), in 0.15 M pH=7 phosphate buffer, baker's yeast from Budafok factory. *Methods*: **A**, 20 h reaction with yeast (8 g) in buffer (60 ml) under $N_2$ containing 2% ethanol, glucose (6 g) and L-cysteine (1 eq.); **B**, 24 h reaction with yeast (12 g) in buffer (100 ml) under $N_2$ containing 2% ethanol and glucose (5 g); **C**, 48 h reaction with yeast (12 g) in buffer (100 ml) under $N_2$ containing 2% ethanol and glucose (5 g); **D**, 3 h reaction with yeast (8 g) in buffer (60 ml) under $N_2$ containing 2% ethanol, 2% DMSO and glucose (6 g); **E**, 1.5 h reaction with yeast (12 g) in buffer (100 ml) containing 5% ethanol and glucose (5 g); **F**, 4 h reaction with yeast (8 g) in buffer (60 ml) under $N_2$ containing 2% ethanol, 2% DMSO and glucose (6 g); [b] Due to a change in group preferences, the absolute configuration *S* in the case of the benzyloxy product **7c** means the same *sense* of stereochemistry as in the case of the other acetates **7a,b,d**. For determination of the configurations, see Experimental section.

otherwise). Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative chromatographic separations were performed using vacuum-chromatography[28] on a Merck Kieselgel 60 (0.063–0.200 mm). Chemicals were products of Fluka or Aldrich. All solvents used were freshly distilled. Baker's yeast manufactured by Budafok factory, Budapest, was obtained from a local store.

### *4.1. 1-Benzoyloxy-3-hydroxypropan-2-one* **4a**[15]

The reaction[15] starting from 1,3-dihydroxyacetone (**1**, 20 g, 222 mmol) yielded the desired ketone (**4a**, 14 g, 32%) as a colorless crystalline solid. Mp: 92–93°C (lit.:[15] 95–97°C); IR (KBr): 3430 (br), 1718, 1602, 1452, 1376, 1278, 1180, 1111, 1072, 924, 811, 709 $cm^{-1}$; $^1H$ NMR: 4.50 (s, 2H, O-$CH_2$), 5.03

(s, 2H, $CH_2$-OBz), 5.53 (br s, 1H, OH), 7.47 (t, 2H, *m*-ArH), 7.58 (t, 1H, *p*-ArH), 8.10 (d, 2H, *o*-ArH). Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.66; H, 5.21.

### 4.2. *1-Hydroxy-3-pivaloyloxypropan-2-one* **4b**

To a stirred solution of 1,3-dihydroxyacetone (**1**, 6.75 g, 75 mmol) and 4-(dimethylamino)pyridine (0.1 g) in pyridine (45 ml) pivaloyl chloride (6.1 ml, 50 mmol) was added dropwise at 24–25°C. After stirring the resulting mixture at room temperature overnight pyridine was evaporated in vacuo. The residue was diluted with ethyl acetate (100 ml) and washed with 5% HCl (2×20 ml). The aqueous phase was re-extracted with ethyl acetate (2×30 ml). The combined organic phases were washed with saturated $NaHCO_3$ solution (30 ml) and brine (30 ml). After drying over $MgSO_4$ the solvent was evaporated and the residual solid was recrystallized from hexane to yield a crystalline product (**4b**, 3.75 g, 43%). IR (KBr): 3408, 2960, 2930, 1735, 1725, 1470, 1360, 1280, 1170, 1070, 880 $cm^{-1}$; $^1H$ NMR: 1.25 (s, 9H, 3 $CH_3$), 4.37 (s, 2H, O-$CH_2$), 4.73 (s, 2H, $CH_2$-OPiv). Calcd for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.40; H, 8.07.

### 4.3. *1-Benzyloxy-3-hydroxypropan-2-one* **4c**[11]

Preparation of the monobenzylated dihydroxyacetone **4a**[11] started from 1,3-dihydroxyacetone (**1**, 2.25 g, 25 mmol) and via the intermediates 2,2-dimethoxypropane-1,3-diol [**2**, 90%, $^1H$ NMR: 3.31 (s, 6H, 2 $OCH_3$), 3.67 (s, 4H, 2 $CH_2O$] and 1-benzyloxy-2,2-dimethoxypropan-3-ol [**3**, 52%, $^1H$ NMR: 3.27 (s, 6H, 2 $OCH_3$), 3.53 (s, 2H, $CH_2$-OBn), 3.70 (s, 2H, O-$CH_2$), 4.58 (s, 2H, $OCH_2Ph$), 7.32 (m, 5H, ArH)] resulted in a homogeneous oily product (**4c**, 1.74 g, 39% overall). IR: 3440 (br), 2869, 1731, 1496, 1434, 1209, 1103, 1028, 741 $cm^{-1}$; $^1H$ NMR: 4.19 (s, 2H, $CH_2$-OBn), 4.47 (s, 2H, O-$CH_2$), 4.60 (s, 2H, $OCH_2Ph$), 7.35 (m, 5H, ArH). Calcd for $C_{10}H_{10}O_4$: C, 66.65; H, 6.71. Found: C, 66.33; H, 6.73.

### 4.4. *1-(tert-Butyldimethylsilyl)oxy-3-hydroxypropan-2-one* **4d**[23]

Silylation[23] from 1,3-dihydroxyacetone (**1**, 2.0 g, 22.2 mmol) provided the desired ketone (**4d**, 1.39 g, 60%) as a colorless oil. IR: 3430 (br), 2970, 2940, 2890, 2870, 1740, 1490, 1270, 1105, 855, 795 $cm^{-1}$; $^1H$ NMR: 0.03 (s, 6H, 2 $CH_3$-Si), 0.91 (s, 9H, 3 $CH_3$), 4.30 (s, 2H, $CH_2$-OTBDMS), 4.49 (s, 2H, O-$CH_2$). Calcd for $C_9H_{20}O_3Si$: C, 52.90; H, 9.87. Found: C; 52.73; H, 9.88.

### 4.5. *Acetylation of hydroxymethyl ketones* **4a–d**

Method A (for **4a**, **4c** and **4d**): To a stirred solution of hydroxymethyl ketone (**4a**, 7 g, 36 mmol), triethylamine (7 ml, 50 mmol) and 4-(dimethylamino)pyridine (0.1 g) in ethyl acetate (70 ml) acetic anhydride (4 ml, 42 mmol) was added dropwise at room temperature and the resulting mixture was stirred for 90 min. The reaction mixture was washed with 10% HCl (2×140 ml), 1 M $Na_2CO_3$ (140 ml) and dried over $Na_2SO_4$. Evaporation of the solvent resulted in an oil. The same procedure was used for ketones **4c** (1.1 g, 6 mmol) and **4d** (1.70 g, 8.32 mmol).

Method B (for **4b**): To a stirred solution of 1-hydroxy-3-pivaloyloxypropan-2-one (**4a**, 2 g, 11.5 mmol), triethylamine (1.75 g, 13.8 mmol) and 4-(dimethylamino)pyridine (50 mg) in THF (25 ml) acetyl chloride (1.08 g, 13.8 mmol) was added dropwise and the mixture was stirred for 90 min. Ethyl acetate (100 ml) and 5% HCl solution (15 ml) were added to the mixture and the forming layers were separated. The aqueous layer was extracted with ethyl acetate (50 ml). The combined organic phases were washed with

saturated $NaHCO_3$ solution (25 ml), saturated $Na_2CO_3$ solution and dried over $Na_2SO_4$. After removal of the solvent, preparative vacuum column chromatography of the residue yielded an oil **5b**.

### 4.6. *1-Acetoxy-3-benzoyloxypropan-2-one* **5a**

Yield: 6.3 g, 75%. IR: 3069, 2991, 2939, 1737, 1729, 1601, 1451, 1417, 1372, 1277, 1228, 1177, 1101, 1052, 1025, 977, 834, 715 $cm^{-1}$; $^1H$ NMR: 2.20 (s, 3H, $CH_3$-CO), 4.86 (s, 2H, $CH_2$-OAc), 5.01 (s, 2H, $CH_2$-OBz), 7.46 (t, 2H, *m*-ArH), 7.58 (t, 1H, *p*-ArH), 8.09 (d, 2H, *o*-ArH). Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12; Found: C, 61.21; H, 5.10.

### 4.7. *1-Acetoxy-3-pivaloyloxypropan-2-one* **5b**

Yield: 1.9 g, 77%. IR: 2970, 1735, 1730, 1725, 1470, 1360, 1470, 1360, 1280, 1215, 1150, 1130, 1055 $cm^{-1}$; $^1H$ NMR: 1.22 (s, 9H, 3 $CH_3$), 2.12 (s, 3H, $CH_3$-CO), 4.69 (s, 2H, O-$CH_2$), 4.70 (s, 2H, O-$CH_2$). Calcd for $C_{10}H_{16}O_5$: C, 55.55; H, 7.46; Found: C, 55.45; H, 7.48.

### 4.8. *1-Acetoxy-3-benzyloxypropan-2-one* **5c**

Yield: 1.23 g, 92%. IR: 3032, 2937, 2860, 1739, 1732, 1455, 1410, 1374, 1235, 1104, 1071, 1027, 742, 700 $cm^{-1}$; $^1H$ NMR: 2.21 (s, 3H, $CH_3$-CO), 4.19 (s, 2H, $CH_2$-OBn), 4.63 (s, 2H, $OCH_2Ph$), 4.94 (s, 2H, $CH_2$-OAc), 7.38 (m, 5H, ArH). Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35; Found: C, 65.00; H, 6.33.

### 4.9. *1-Acetoxy-3-(*tert*-butyldimethylsilyl)oxypropan-2-one* **5d**

Yield: 1.90 g, 93%. IR: 2965, 2940, 2895, 2870, 1755, 1740, 1490, 1420, 1390, 1270, 1250, 1110, 1090, 855, 795 $cm^{-1}$; $^1H$ NMR: 0.03 (s, 6H, 2 $CH_3$-Si), 0.87 (s, 9H, 3 $CH_3$), 2.08 (s, 3H, $CH_3$-CO), 4.19 (s, 2H, $CH_2$-OTBDMS), 4.87 (s, 2H, $CH_2$-OAc). Calcd for $C_{11}H_{22}O_4Si$: C, 53.63; H, 9.00; Found: C, 53.81; H, 9.02.

### 4.10. *Baker's yeast-catalyzed stereoselective reduction of ketones* **4a–d, 5a–d**

#### 4.10.1. *General method*

Yeast was added to the media as indicated in Table 2. After stirring the resulting cell suspension for 30 min, the corresponding ketone (**4a–d**, **5a–d**; 500 mg) was added. When indicated, the substrate was previously dissolved in ethanol (2–4 ml). The reaction mixture was stirred (time given in Table 2). The resulting mixture was extracted with ethyl acetate (2×150 ml), the combined ethyl acetate layers were washed with brine, dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by preparative vacuum column chromatography (hexane:acetone, 10:1) to give oily product(s) in yields indicated in Table 2.

#### 4.10.2. *1-Benzoyloxypropane-2,3-diol* **6a**

IR: 3400 (br), 2952, 1714, 1602, 1452, 1379, 1316, 1278, 1178, 1117, 1071, 1027, 712 $cm^{-1}$; $^1H$ NMR: 3.50–3.80 (m, 2H, O-$CH_2$), 4.12 (m, 1H, O-CH), 4.37 (m, 2H, $CH_2$-OBz), 7.43 (t, 2H, *m*-ArH), 7.54 (t, 1H, *p*-ArH), 8.02 (d, 2H, *o*-ArH).

*4.10.3. 1-Pivaloyloxypropane-2,3-diol* ***6b***

IR: 3450, 2980, 2960, 1730, 1480, 1460, 1370, 1280, 1170, 1045 $cm^{-1}$; $^1H$ NMR: 1.22 (s, 9H, 3 $CH_3$), 3.59 and 3.69 (2 dd, 2H, O-$CH_2$), 3.94 (m, 1H, O-CH), 4.16 (m, 2H, $CH_2$-OPiv).

*4.10.4. 1-Benzyloxypropane-2,3-diol* ***6c***

IR: 3385 (br), 2924, 2867, 1646, 1496, 1453, 1364, 1207, 1074, 925, 865, 738, 698 $cm^{-1}$; $^1H$ NMR: 3.50–3.78 (m, 4H, O-$CH_2$), 3.87 (m, 1H, O-CH), 4.55 (s, 2H, $OCH_2Ph$), 7.35 (m, 5H, ArH).

*4.10.5. 1-(*tert*-Butyldimethylsilyl)oxypropane-2,3-diol* ***6d***

IR: 3400, 2970, 2945, 2895, 2865, 1485, 1275, 1250, 1130, 1090, 850, 795 $cm^{-1}$; $^1H$ NMR: 0.03 (s, 3H, $CH_3$-Si), 0.06 (s, 3H, $CH_3$-Si), 0.88 (s, 9H, 3 $CH_3$), 3.55–3.75 (m, 5H, 2 O-$CH_2$ and O-CH).

*4.10.6. 1-Acetoxy-3-benzoyloxypropan-2-ol* ***7a***

IR: 3462 (br), 3065, 2958, 1727, 1721, 1606, 1452, 1375, 1316, 1276, 1178, 1116, 1071, 1047, 1027, 713 $cm^{-1}$; $^1H$ NMR: 2.11 (s, 3H, $CH_3$-CO), 4.25 (m, 3H, $CH_2$-OAc and O-CH), 4.42 (m, 2H, $CH_2$-OBz), 7.44 (t, 2H, *m*-ArH), 7.58 (t, 1H, *p*-ArH), 8.03 (d, 2H, *o*-ArH).

*4.10.7. 1-Acetoxy-3-pivaloyloxypropan-2-ol* ***7b***

IR: 3400 (br), 2980, 2960, 1730, 1725, 1475, 1450, 1370, 1280, 1170, 1045 $cm^{-1}$; $^1H$ NMR: 1.23 (s, 9H, 3 $CH_3$), 2.09 (s, 3H, $CH_3$-CO), 4.05–4.25 (m, 5H, 2 O-$CH_2$ and O-CH).

*4.10.8. 1-Acetoxy-3-benzyloxypropan-2-ol* ***7c***

IR: 3445, 3063, 3030, 1738, 1496, 1454, 1369, 1244, 1098, 1045, 740, 699 $cm^{-1}$; $^1H$ NMR: 2.08 (s, 3H, $CH_3$-CO), 3.52 (m, 2H, $CH_2OBn$), 4.05 (m, 1H, O-CH), 4.16 (m, 2H, $CH_2OAc$), 4.56 (s, 2H, $OCH_2Ph$), 7.33 (m, 5H, ArH).

*4.10.9. 1-Acetoxy-3-(*tert*-butyldimethylsilyl)oxypropan-2-ol* ***7d***

IR: 3460, 2960, 2935, 2895, 2860, 1755, 1735, 1480, 1405, 1380, 1270, 1250, 1120, 1050, 850, 795 $cm^{-1}$; $^1H$ NMR: 0.02 (s, 3H, $CH_3$-Si), 0.05 (s, 3H, $CH_3$-Si), 0.86 (s, 9H, 3 $CH_3$), 2.04 (s, 3H, $CH_3$-CO), 3.58 (m, 2H, $CH_2$-OTBDMS), 3.76–4.01 (m, 2H, $CH_2$-OAc), 4.09 (m, 1H, O-CH).

*4.11. Determination of enantiomeric excess and absolute configuration of the diols (***6a–d**, ent-**6a–d**) and monoacetates* ***7a–d***

*4.11.1. Enantiomeric excess determination*

*4.11.1.1. (A) MTPA derivatization of the optically active monoacetates* ***7a–d***. A sample of each monoacetate (**7a–d**, 50 μmol, ca.12 mg) with measured optical rotation was converted into its (*R*)-MTPA ester [350 μl of 5% (*R*)-MTPA-Cl solution in carbon tetrachloride, pyridine (25 μl), DMAP (2 mg), 50°C, 3 h]. A similar reaction was carried out with the racemic monoacetates *rac*-**7a–d**. The diastereomer ratio referring to the enantiomeric excess of the monoacetates **7a–d** was determined from the $^1H$ NMR spectra of the MTPA ethers [500 MHz, $CH_3CO$ signals: (*R,R*)-**7a**-MTPA: 2.07 ppm, (*R,S*)-**7a**-MTPA: 2.01 ppm, (*R,R*)-**7b**-MTPA: 2.08 ppm, (*R,S*)-**7b**-MTPA: 2.02 ppm, (*R,S*)-**7c**-MTPA: 2.03 ppm, (*R,R*)-**7c**-MTPA: 1.97 ppm, (*R,R*)-**7d**-MTPA: 2.05 ppm, (*R,S*)-**7d**-MTPA: 1.98 ppm].

*4.11.1.2. (B) Preparation of diacetates* ***8a–d*** *from monoacetates* ***7a–d*** *and diols* ***6a–d*** *or* ent-***6a–d***. Another aliquot of each sample **7a–d** used in the MTPA ee determination was acetylated [**7a–d**: 0.1 g,

$Et_3N$ (1.4 mmol), DMAP (10 mg), $Ac_2O$ (1.1 mmol) in EtOAc (1 ml), at rt, 90 min; purified yields over 90%] to give the corresponding oily diacetate **8a–d** with known enantiomeric excess value.

Diacetates **8a–d** or *ent*-**8a–d** were also prepared from the optically active diols [**6a–d** or *ent*-**6a–d**: 0.1 g, $Et_3N$ (2.8 mmol), DMAP (15 mg), $Ac_2O$ (2.2 mmol) in EtOAc (1 ml), at rt, 90 min; purified yields over 90%] as well. Optical rotations of these diacetates **8a–d** or *ent*-**8a–d** compared to the rotation data of the diacetates **8a–d** with known enantiomeric purities refer to the enantiomeric composition of the corresponding parent diol **6a–d** or *ent*-**6a–d**.

*4.11.2. 1-Benzoyloxy-2,3-diacetoxypropane 8a*

IR: 3065, 2962, 1753, 1747, 1735, 1602, 1452, 1372, 1316, 1260, 1224, 1178, 1115, 1071, 1051, 1026, 713 $cm^{-1}$; $^1H$ NMR: 2.11 (s, 3H, $CH_3$-CO), 2.13 (s, 3H, $CH_3$-CO), 4.20–4.65 (m, 4H, 2 O-$CH_2$), 5.43 (m, 1H, CH-OAc), 7.45 (t, 2H, *m*-ArH), 7.58 (t, 1H, *p*-ArH), 8.04 (d, 2H, *o*-ArH).

*4.11.3. 2,3-Diacetoxy-1-pivaloyloxypropane 8b*

IR: 2970, 1745, 1735, 1725, 1470, 1360, 1280, 1220, 1145, 1040 $cm^{-1}$; $^1H$ NMR: 1.20 (s, 9H, 3 $CH_3$), 2.06 (s, 3H, $CH_3$-CO), 2.08 (s, 3H, $CH_3$-CO), 4.12–4.35 (m, 4H, 2 O-$CH_2$), 5.28 (m, 1H, CH-OAc).

*4.11.4. 1-Benzyloxy-2,3-diacetoxypropane 8c*

IR: 3010, 2925, 2835, 1744, 1727, 1496, 1450, 1365, 1240, 1100, 1030, 950, 735, 695 $cm^{-1}$; $^1H$ NMR: 2.00 (s, 3H, $CH_3$-CO), 2.04 (s, 3H, $CH_3$-CO), 3.56 (m, 2H, $CH_2$-OBn), 4.23 (m, 2H, $CH_2$-OAc), 4.50 (s, 2H, $OCH_2Ph$), 5.18 (m, 1H, CH-OAc), 7.29 (m, 5H, ArH).

*4.12. 1-(*tert*-Butyldimethylsilyl)oxy-2,3-diacetoxypropane 8d*

IR: 2965, 2940, 2895, 2870, 1755, 1485, 1385, 1270, 1245, 1130, 1060, 850, 795 $cm^{-1}$; $^1H$ NMR: 0.03 (s, 3H, $CH_3$-Si), 0.06 (s, 3H, $CH_3$-Si), 0.88 (s, 9H, 3 $CH_3$), 2.05 (s, 3H, $CH_3$-CO), 3.69 (m, 2H, $CH_2$-OTBDMS), 4.09–4.33 (m, 2H, $CH_2$-OAc), 5.05 (m, 1H, CH-OAc).

*4.13. Determination of the absolute configuration of the monoacetates 7a–d and diols 6a–d and* ent-*6a–d*

Since the absolute configuration of a benzoyloxy **6a**,[15] and the benzyloxy compounds **6c**[11,29] and **7c**[30,31] are known, the diacetates **8a,c** prepared either from the monoacetates **7a,c** or the diols **6a,c** and *ent*-**6a,c** were suitable for determination of the absolute configuration of these compounds.

For determination of the absolute configuration of pivaloyloxy compounds **7b**, **6b**, and *ent*-**6b**, an optically active sample of (*R*)-3-acetoxypropane-1,2-diol {$[\alpha]_D$=–9.9 (*c* 2, pyridine) [lit.:[32] $[\alpha]_D$=–9.2, (*c* 1.7, pyridine)] obtained by catalytic hydrogenation from (*R*)-3-acetoxy-1-benzyloxypropan-2-ol manufactured by PfL catalysis[30]} was converted to (*S*)-3-acetoxy-1-pivaloyloxypropane-2-ol *ent*-**7b** proving the absolute configuration of **7b**. Configurations of the diols **6b** and *ent*-**6b** were determined via their diacetates **8b** and *ent*-**8b** compared to the diacetate **8b** from the monoacetate **7b**.

The optical rotation of (*R*)-1-(*tert*-butyldimethylsilyl)oxypropane-2,3-diol **6d** was reported[33] as $[\alpha]_D$=–0.6 (*c* 1.31, $CHCl_3$). Since the small specific rotation values of the polar 1,2-diols are often unreliable,[34] absolute configuration of the diols **6d** and *ent*-**6d** was determined by an independent method. Silylation of the above (*R*)-3-acetoxypropane-1,2-diol with TBDMS-Cl (imidazole/THF) gave (*S*)-1-acetoxy-3-(*tert*-butyldimethylsilyl)oxypropan-2-ol {*ent*-**7d**, $[\alpha]_D$=–14.1 (*c* 1, MeOH)}. The yeast

reduction resulted, however, in (*R*)-monoacetate {**7d**, $[\alpha]_D$=+17.6 (*c* 1, MeOH)}. Acylation of this (*R*)-monoacetate **7d** resulted in (*R*)-diacetate {(*R*)-**8d**, $[\alpha]_D$=+19.8 (*c* 1, MeOH)}. Since a diol fraction {*ent*-**6d**, $[\alpha]_D$=+0.9 (*c* 1, $CHCl_3$); $[\alpha]_D$=+9.2, (*c* 1, methanol)} from another reduction of the acetoxymethyl ketone **5d** also resulted in (*R*)-diacetate {(*R*)-**8d**, $[\alpha]_D$=+19.1 (*c* 1, MeOH)}, this diol *ent*-**6d** should have the (*R*)-configuration. Therefore, the above cited specific rotation data for the (*R*)-1-(*tert*-butyldimethylsilyl)oxypropane-2,3-diol should be revised.

The absolute configurations, enantiomeric excesses and optical rotation values of diols **6a–d** and monoacetates **7a–c** prepared by baker's yeast reduction and their diacetate derivatives **8a–d** are given in Table 3.

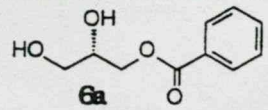
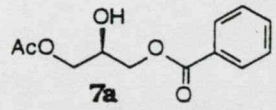
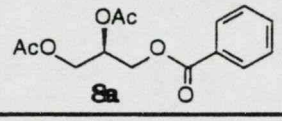
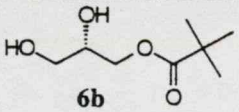
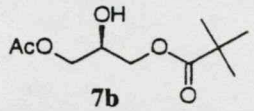
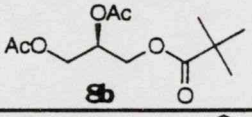
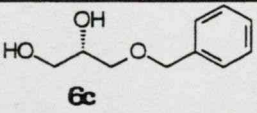
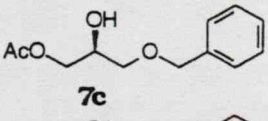
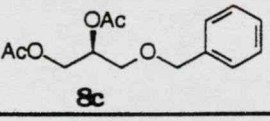
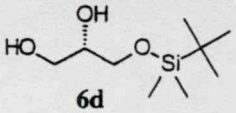
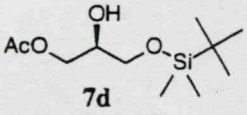
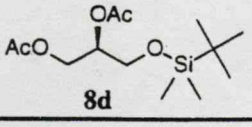
## Acknowledgements

The Hungarian OTKA Foundation (T014894) is gratefully acknowledged for financial support. J.B. thanks the Zoltán Magyary Foundation for a postdoctoral fellowship. G.E. thanks the Hungarian OTKA Foundation for a postdoctoral fellowship (no. D 29445).

## References

1. Coutts, R. T.; Baker, G. B. *Chirality* **1989**, *1*, 99.
2. Burger, A. In *The Basis of Medicinal Chemistry*, Part I; Wolff, M. E., Ed.; Wiley: New York, 1980; p. 1.
3. Poppe, L.; Novák, L. *Selective Biocatalysis: A Synthetic Approach*; VCH, Weinheim: New York, 1992.
4. Hirth, G.; Barner, R. *Helv. Chim. Acta* **1982**, *65*, 1059.
5. Caer, E.; Kindler, A. *Biochemistry* **1962**, *1*, 518.
6. Dennis, E. A. *Bio/Technology* **1987**, *5*, 1294.
7. Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447.
8. Wang, Y. F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 3127.
9. Bianchi, D.; Bosetti, A.; Golini, P.; Cesti, P.; Pina, C. *Tetrahedron: Asymmetry* **1997**, *8*, 817.
10. Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *J. Org. Chem.* **1994**, *59*, 1751.
11. Cesarotti, E.; Antognazza; P.; Pallavicini, M.; Villa, L. *Helv. Chim. Acta* **1993**, *76*, 2344.
12. Karrer, F.; Kayser, H.; Buser, H. P.; Tombo, G. M. R. *Chimia* **1993**, *47*, 302.
13. Buser, H. P.; Spindler, F. *Tetrahedron: Asymmetry* **1993**, *12*, 2451.
14. Waagen, V.; Partali, V.; Hollingsaeter, I.; Huang, M. S. S.; Anthonsen, T. *Acta Chem. Scand.* **1994**, *48*, 506.
15. Aragozzini, F.; Maconi, E.; Potenza, C.; Scolastico, C. *Synthesis* **1989**, 225.
16. Egri, G.; Kolbert, A.; Bálint, J.; Fogassy, E.; Novák, L.; Poppe, L. *Tetrahedron: Asymmetry* **1998**, *9*, 271.
17. Guette, J. P.; Spassky, N. *Bull. Soc. Chim. France* **1972**, 4217.
18. Barry, J.; Kagan, H. B. *Synthesis* **1981**, 453.
19. Manzocchi, A.; Fiecchi, A.; Santaniello, E. *J. Org. Chem.* **1988**, *53*, 4405.
20. Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2469.
21. Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *Tetrahedron* **1994**, *50*, 10539.
22. Wei, Z.-L.; Li, Z.-Y.; Lin, G.-Q. *Tetrahedron* **1998**, *54*, 13059.
23. Schröer, J.; Welzel, P. *Tetrahedron* **1994**, *50*, 6839.
24. North, M. *Tetrahedron Lett.* **1996**, *37*, 1699.
25. Medson, C.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron: Asymmetry* **1997**, *8*, 1049.
26. Hayakawa, R.; Shimizu, M.; Fujisawa, T. *Tetrahedron: Asymmetry* **1997**, *8*, 3201.
27. In connection with the baker's yeast reduction of 1-acetoxy-3-aryloxypropan-2-ones, the enantiomer selectivity of the baker's yeast hydrolysis was studied with several racemic 1-acetoxy-3-aryloxypropan-2-ols. All the investigated racemic aryloxy- [(2-, 3-, and 4-methylphenoxy)-, (2-isopropylphenoxy)- and (1-naphthyloxy)] derivatives were hydrolyzed under the reduction conditions. The hydrolyses proceeded with only a slight (*R*)-enantiomer preference (*E* value=1–3.5) and reached ca. 50% conversion within 2–8 h. Egri, G.; Bálint, J.; Poppe, L., unpublished results.
28. Poppe, L.; Novák, L. *Magy. Kém. Lapja* **1985**, *40*, 366.

Table 3
Enantiomeric excess, specific rotations and absolute configurations for compounds **6a–d**, **7a–d** and **8a–d**

| Compound | Config. | Source | $[\alpha]_D$ (c, solvent) | E.e. % | Method |
|---|---|---|---|---|---|
|  **6a** | *S* | Ref. 15 | +13.7 (2, pyridine) | > 97 | from **8a** |
|  **7a** | *R* | from **8a** | not determined | 68 | from its MTPA ester |
|  **8a** | *R* | from **6a** | +6.8 (1, ethanol) | 68 | from **7a** |
|  **6b** | *S* | from **8b,** | +6.3 (1, ethanol) | 72 | from **8b** |
|  **7b** | *R* | compared to *ent*-**7b**, see text | +5.7 (1, ethanol) | >95 | from its MTPA ester |
|  **8b** | *R* | from **7b** | +0.87 (1, ethanol) | 46 | from **7b** |
|  **6c** | *S* | Ref. 29 | -3.2 (10, benzene) | 55 | from **8c** |
|  **7c** | *S* | Ref. 30 | +3.4 (1, $CHCl_3$) | 85 | from its MTPA ester |
|  **8c** | *S* | from **7c** | +15.2 (1, ethanol) | 85 | from **7c** |
|  **6d** | *S* | from **8d**, see text | -5.8 (1, methanol) | 59 | from **8d** |
|  **7d** | *R* | compared to *ent*-**7d**, see text | +17.6 (1, methanol) | >97 | from its MTPA ester |
|  **8d** | *R* | from **7d** | +15.6 (1, methanol) | 77 | from **7d** |

29. Hirth, G.; Barner, R. *Helv. Chim. Acta* **1982**, *65*, 1059.
30. Herradón, B.; Cueto, S.; Morcuende, A.; Valverde, S. *Tetrahedron: Asymmetry* **1993**, *5*, 845.
31. Laine, D.; Fujita, M.; Ley, S. V. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1639.
32. Breitgoff, D.; Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 1523.
33. Konosu, T.; Oida, S. *Chem. Pharm. Bull.* **1991**, *39*, 2212.
34. Crans, D. C.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 7019.

# XII. melléklet

GLOGE, A., LANGER, B., **POPPE, L.**, RÉTEY, J.:

The Behavior of Substrate Analogues and Secondary Deuterium Isotope Effects in the Phenylalanine Ammonia-Lyase Reaction,

*Arch. Biochem. Biphys.*, **1998**, *359*, 1.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 359, No. 1, November 1, pp. 1–7, 1998
Article No. BB980860

# The Behavior of Substrate Analogues and Secondary Deuterium Isotope Effects in the Phenylalanine Ammonia-Lyase Reaction

Andreas Gloge, Birgid Langer, László Poppe, and János Rétey[1]
*Institut für Organische Chemie, Universität Karlsruhe, Richard-Willstätter-Allee, Postfach 6980, D-76128 Karlsruhe, Germany*

Received February 12, 1998, and in revised form June 24, 1998

**Metacresol and glycine can be thought as a dissection of metatyrosine, which is an excellent substrate of phenylalanine ammonia-lyase (PAL) (B. Schuster and J. Rétey, *PNAS* 92, 8433, 1995). Whereas metacresol was a very weak inhibitor and glycine was inert, simultaneous addition of both compounds led to synergistic inhibition of PAL. [$^2H_5$]Phenylalanine as a substrate showed a kinetic deuterium isotope effect of 9% ($k_H/k_{2_H} = 1.09 \pm 0.01$) while its $K_m$ value was identical to that of the unlabeled substrate. The following substrate analogues were synthesized and assayed with PAL: cyclooctatetraenyl (COT)-D,L)-alanine as well as 2-pyridyl-, 3-pyridyl-, and 4-pyridyl-(L)-alanines. While COT-(D,L)-alanine turned out to be a rather reluctant substrate, all three isomers of pyridyl-(L)-alanines were converted with a comparable or even higher $V_{max}$ than L-phenylalanine into the corresponding pyridyl acrylic acids. Their $K_m$ values were, however, an order of magnitude higher than that of the natural substrate. These results are discussed in terms of the novel mechanism which implies an electrophilic attack of the prosthetic dehydroalanine at the aromatic ring. The heats of formation of the putative sigma complexes of the electrophilic substitution at the pyridine ring have been calculated using semiempirical force-field methods. The results show the feasibility of the proposed mechanism also with the substrate analogues.** © 1998 Academic Press

***Key Words:*** **phenylalanine ammonia-lyase; secondary kinetic deuterium isotope effect.**

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5)[2] catalyzes the reversible elimination of ammonia to form *trans*-cinnamic acid. This important plant enzyme is at the branching point of primary and secondary metabolism, the latter leading to phenylpropanoids like lignin, flavonoids, and coumarins (1, 2).

PAL contains the rare prosthetic group dehydroalanine, which is essential for catalysis. Working with the recombinant enzyme from parsley we showed that dehydroalanine is formed from serine 202 by an autocatalytic posttranslational modification (3). When serine 202 was changed to alanine, threonine, or glycine by site-directed mutagenesis, most of the activity with phenylalanine was abolished. The corresponding mutants were, however, still active with 4-nitrophenylalanine as substrate (4). These results parallel with those obtained with histidine ammonia-lyase (EC 4.3.1.3) catalyzing a similar elimination and having the same prosthetic group (5–7). It has been concluded that a nitro group in the appropriate position activates the abstractable proton in the β-position of the side chain and the role of the dehydroalanine must be similar. Consequently, a mechanism has been proposed (Fig. 1) in which the first chemical step is the Friedel–Crafts type electrophilic attack of the dehydroalanine at the aromatic nucleus (4, 8).

Support for this proposal was provided by the finding that 3-hydroxyphenylalanine (*m*-tyrosine) is an even better substrate for PAL than phenylalanine (4). On the other hand, tyrosine (4-hydroxyphenylalanine), whose OH group is not expected to facilitate an electrophilic attack in position 2 of the phenyl ring, is a rather poor substrate. To submit the new mechanism to further tests we describe experiments using *m*-cresol and glycine as inhibitor and cyclooctatetraenyl-(L)-alanine, 2-pyridyl-, 3-pyridyl-, and 4-pyridyl-(L)-alanines as well as [$^2H_6$]phenylalanine as substrates for PAL.

[1] To whom correspondence should be addressed. Fax: (0)721/608-4823. E-mail: biochem@ochhades.chemie.uni-karlsruhe.de.

[2] Abbreviations used: PAL, phenylalanine ammonia-lyase; COT, cyclooctatetraene; IPTG, isopropyl β-D-thiogalactoside

0003-9861/98 $25.00

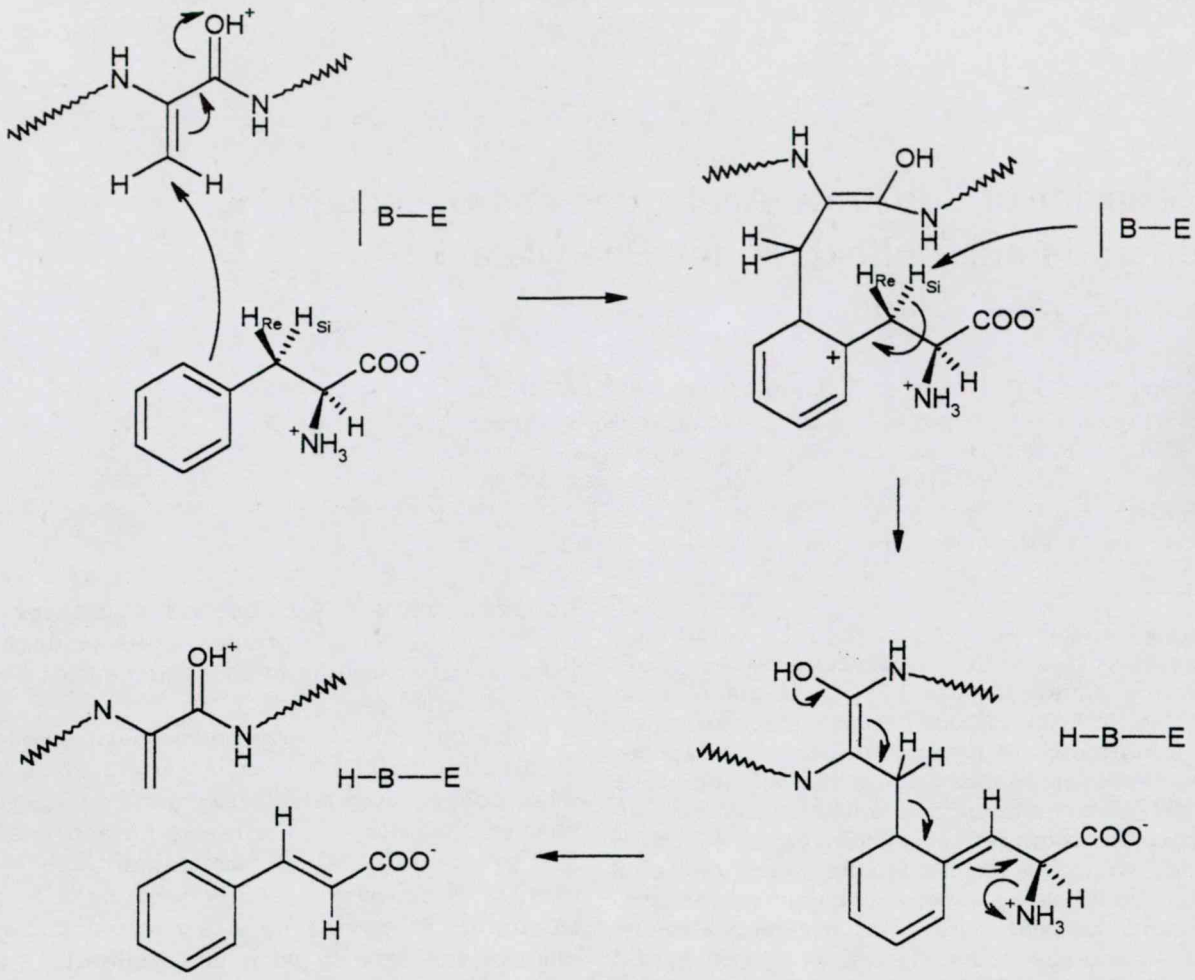



**FIG. 1.** Mechanism of the PAL reaction. Electrophilic attack at the phenyl ring by the dehydroalanine prosthetic group facilitates abstraction of the $\beta$-$H_{Si}$ proton by an enzymic base.

## MATERIALS AND METHODS

*(D,L)-β-Cyclooctatetraenylalanine.* Cyclooctatetraene (COT) was a gift of Professor Gerhard Schroeder (Universität Karlsruhe), (D,L)-β-Cyclooctatetraenylalanine was synthesized as described by methods of Huisgen and co-workers (9), Cope *et al.* (10, 11), and Pirrung and Krishnamurthy (12).

*3-Cyclooctatetraenyl-prop-2-en-1-ol.* This preparation is as reported by Houghton and Waight (13).

*3t-Cyclooctatetraenyl-propenal* **2.** A mixture of 3-cyclooctatetraenyl-prop-2-en-1-ol (337 mg, 2.10 mmol) and active manganese dioxide (14) (3.8 g) in dry ether (100 ml) was stirred for 4 h at room temperature. Filtration and removal of the solvent afforded the aldehyde (316 mg, 95%) as a yellow liquid which was stored at −20°C. $^1$H NMR ($CDCl_3$) δ: 5.7–6.2 (m, 7H), 5.9 (m, 1H), 7.1 (d, 1H), 9.5 (d, 1H).

*3t-Cyclooctatetraenyl-acrylic acid methyl ester* **3.** 3-Cyclooctatetraenyl-propenal (213 mg, 1.35 mmol) was stirred at room temperature with a mixture of sodium cyanide (330 mg, 6.73 mmol), manganese dioxide (2.71 g), and acetic acid (141 mg, 2.35 mmol) in methanol (50 ml) for 90 min. After filtration and removal of the solvent the crude product was dissolved in water (50 ml). The aqueous layer was extracted with ether (3 × 50 ml), and the ether extracts were washed with aqueous $NaHCO_3$ solution and water and dried over anhydrous $Na_2SO_4$. Filtration and removal of ether afforded pure **3** (250 mg, 99%) as a slightly yellow liquid. $^1$H NMR ($CDCl_3$) δ: 3.7 (s, 3H), 5.7 (d, 1H), 5.7–6.1 (m, 7H), 7.3 (d, 1H).

*3t-Cyclooctatetraenyl-acrylic acid* **4.** To a stirred solution of 3*t*-cyclooctatetraenyl-acrylic acid methyl ester (250 mg, 1.33 mmol) in methanol (10 ml) NaOH (254 mg) was added. After stirring for 2 h at room temperature once again NaOH (100 mg) was added. Stirring the mixture at 35°C for 4 h and evaporation of the solvent gave the crude product, which was dissolved in water and acidified with 5% HCl. The resulting yellow precipitate was extracted with ether (3 × 20 ml). The combined organic layers were washed with saturated $NaHCO_3$ solution and water. Drying over $Na_2SO_4$, filtration, and removal of the solvent gave pure **4** as yellow crystalline solid (209 mg, 1.20 mmol, 90%) of high purity. $^1$H NMR ($CDCl_3$) δ: 5.7–6.2 (m, 7H), 5.8 (d, 1H), 7.4 (d, 1H); $^{13}$C NMR ($CDCl_3$) δ: 172.37, 148.39, 140.84, 139.47, 133.89, 133.21, 131.75, 131.33, 130.82, 128.95, 117.10; HR MS, *m/z* 174.0667 (calcd for $C_{11}H_{10}O_2$ 174.0681).

*L-[$^2H_5$]Phenylalanine.* L-[$^2H_5$]Phenylalanine was a generous gift of the Hoechst AG Frankfurt/M.

*Pyridyl-acrylic acids.* 3*t*-(3)Pyridyl-acrylic acid was purchased from Fluka. 3*t*-(2)Pyridyl-acrylic acid and 3*t*-(4)pyridyl-acrylic acid were synthesized by the Knoevenagel reaction (15).

*Pyridyl-(L)-alanines.* Reaction of the three 3*t*-pyridyl-acrylic acids with ammonia catalyzed by PAL afforded the corresponding pyridyl-(L)-alanines (16). Alternatively, the pyridyl-L-alanines were also obtained from Dr. Döbler (Institut für Organische Kalalyseforschung, Rostock, Germany).

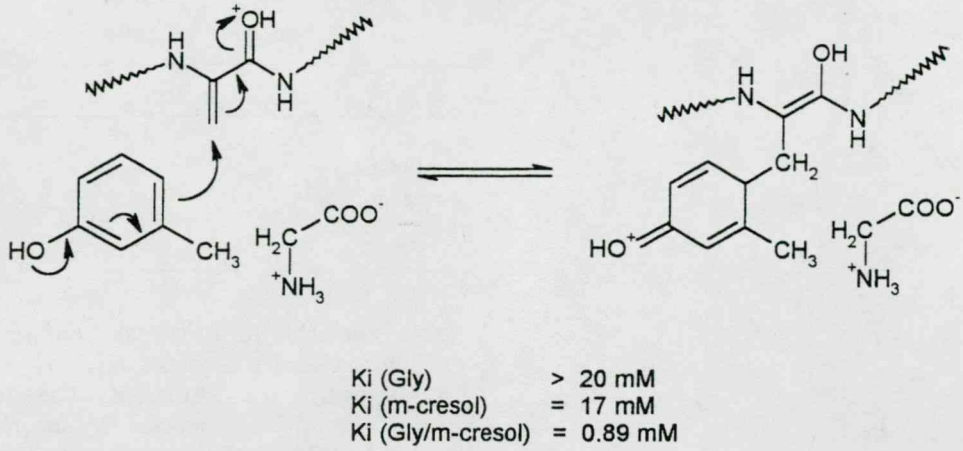



**FIG. 2.** *m*-Cresol and glycine cause a synergistic inhibition of PAL. The formation of a covalent adduct is speculative. A noncovalent tight binding is also possible.

*D,L-β-(2-Pyrimidinyl)alanine.* This preparation is as reported by Haggerty *et al.* (17).

*Bacterial strains, plasmids, and culture conditions. Escherichia coli* BL21(DE3) cells as host cells and pT7.7 as expression vector were used for the expression of wild-type phenylalanine ammonia-lyase (3, 4). For overexpression cells were grown in 1 liter of Luria–Bertani medium (LB) supplemented with ampicillin (85 μg/ml) at 37°C. At an $OD_{600}$ of 1.0, 400 μM isopropyl β-D-thiogalactoside (IPTG) was added. Cells were harvested 4 h after induction. The expression vector pT7.7 was generously provided by Dr. Stanley Tabor (18). The PAL1 gene from a cDNA library from elicitor-treated parsley (*Petroselinum crispum* L.) cells and antibodies against PAL were generous gifts of Prof. Dr. K. Hahlbrock (Max-Planck-Institut für Züchtungsforschung Cologne) and Prof. Dr. N. Amrhein (ETH Zürich), respectively. pT7.7PAL was produced as described by Schuster and Rétey (3).

*Purification.* Transformed *E. coli* BL21 cells were grown in 1 l LB medium containing ampicillin (85 μg/ml) to an $OD_{600}$ of 1.0. Then 0.4 mM IPTG was added. Cells were harvested 4 h after induction by centrifugation at 4500*g*. The cell pellet was resuspended in 10 ml of 10 mM potassium phosphate buffer, pH 6.6, containing: 40 units of Benzonase (Merck, Darmstadt), 5 mM benzamidine, and 0.5 mM phenylmethanesulfonyl fluoride. Sonication (Branson Model 450, 70% power setting, 10 min ice bath) was followed by centrifugation at 30,000*g* for 30 min. Purification was performed as described by Schuster and Rétey (3, 4).

*SDS/PAGE, Western blot, and protein assay.* SDS–PAGE using a 10% polyacrylamide gel was performed according to Laemmli (19) to monitor the purification of PAL. Staining of the gel was carried out with Coomassie brillant blue R 250. Western blotting was performed using the standard laboratory protocol adapted according to Symington *et al.* (20). Protein determinations were performed by measurement of $A_{260}$ and $A_{280}$ according to Warburg and Christian (21).

*Enzyme assay.* PAL activity was determined spectrophotometrically using the absorption of the reaction product cinnamate (22). Standard conditions for the measurement of PAL activity were 0.1 M Tris–HCl buffer, pH 8.8, 0.05–5 mM L-phenylalanine, 30°C. The extinction coefficient ($\epsilon_{290}$) of cinnamic acid is $10^4$ liters $cm^{-1}$ $mol^{-1}$.

*Determination of $V_{max}$ and $K_m$ values.* The kinetic constants were determined by measuring the UV absorption of the produced acrylates (3*t*-(2)pyridyl-acrylic acid, $\epsilon_{(285)}$ = 12,520 liters $cm^{-1}$ $mol^{-1}$; 3*t*-(3)pyridyl-acrylic acid. $\epsilon_{(290)}$ = 8430 liters $cm^{-1}$ $mol^{-1}$; 3*t*-(4)pyridyl-acrylic acid, $\epsilon_{(280)}$ = 8370 liters $cm^{-1}$ $mol^{-1}$; 3*t*-cyclooctatetraenyl-acrylic acid: $\epsilon_{(250)}$ = 20,700 liters $cm^{-1}$ $mol^{-1}$), using 0.05–10 mM of the corresponding amino acid as substrate. Conditions were 0.05–10 mM substrate in 0.1 M Tris–HCl buffer, pH 8.8, at 30°C.

*Determination of the kinetic deuterium isotope effects.* The $K_m$ and $V_{max}$ values were determined spectrophotometrically. The extinction coefficient ($\epsilon_{290}$) of the resulting [$^2H_5$]cinnamate is $10^4$ liters $cm^{-1}$ $mol^{-1}$. To avoid errors, the measurements of the corresponding undeuterated phenylalanine were carried out at the same time. Conditions were 0.05–10 mM substrate in 0.1 M Tris–HCl buffer, pH 8.8, at 30°C.

*Semiempirical force-field calculations.* The sigma complexes and their corresponding methyl-substituted aromatic counterparts were calculated by three different semiempirical force-field methods: MNDO, AM1, and PM3.

*Kinetic investigations of PAL with m-cresol, glycine, and a mixture of m-cresol and glycine. m*-Cresol (5 and 10 mM), glycine (15 mM), or an equimolecular mixture of *m*-cresol and glycine (1 and 7 mM) were added to the enzyme assay. The $K_i$ values were determined by using the standard linearization method of Lineweaver–Burk.

## RESULTS AND DISCUSSION

*Analogous substrates and substrate mimics. m*-Cresol and glycine as mimics of 3-hydroxyphenylalanine (*m*-tyrosine) were probed as inhibitors of PAL (Fig. 2). While glycine hardly inhibited the enzyme and *m*-cresol was a moderate inhibitor ($K_i$ = 17 mM), equimolar amounts of both had a synergistic effect. It can be concluded that the two compounds simultaneously occupy the active site of PAL and compete with the substrate.

Racemic COT alanine was synthesized by the method of Pirrung and Krishnamurthy (12). The expected product of the PAL reaction, cyclooctatetraenyl-acrylate was also synthesized and characterized (Fig. 3). The known allylic alcohol, 3-cyclooctatetraenyl-prop-2-en-1-ol, was prepared starting from cyclooctatetraene according to published procedures (13). Mild oxidation in two steps using the method of Corey *et al.* (23) furnished the methyl ester of cyclooctatetraenyl-acrylic acid which was hydrolyzed to the free acid. Each of the three steps occurred in more than 90% yield.

Determination of the extinction coefficient ($\epsilon$) of the cyclooctatetraenyl acrylate made a kinetic analysis of the PAL reaction with COT-(D,L)-alanine possible. The

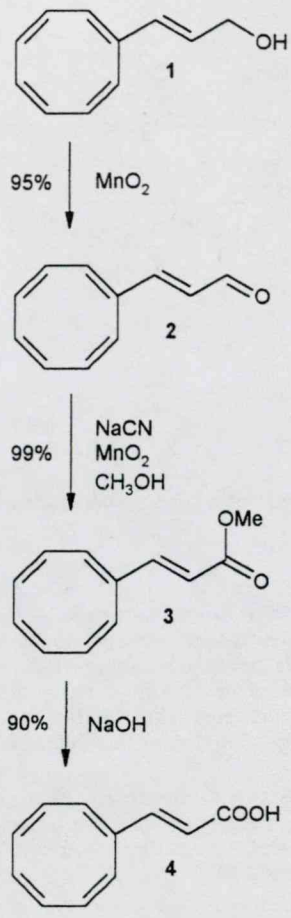



**FIG. 3.** Synthesis of 3*t*-cyclooctatetraenyl-acrylic acid.

very low $V_{max}$ value (0.6% of that for L-phenylalanine) and the 30 times higher $K_m$ value for this substrate show that both binding and turnover are impaired. It

**TABLE I**

Kinetic Constants of Pyridyl-(L)-alanines

| | $K_m/K_{m(L\text{-Phe})}$ | $V_{max}/V_{max(L\text{-Phe})}$ |
|---|---|---|
| L-Phe | 1 | 1 |
| 2-Pyridyl-L-Ala | 22.0 | 0.8 |
| 3-Pyridyl-L-Ala | 41.6 | 2.4 |
| 4-Pyridyl-L-Ala | 12.1 | 1.8 |
| COT-D,L-Ala | 22.6 | 0.0058 |

seems that the binding pocket for the phenyl group is rather flat and the puckered COT ring does not suit into it optimally. Thus both the geometry and the larger size of the cyclooctatetraene ring may place the double bond and the dehydroalanine into an unfavorable steric relationship for the electrophilic attack.

Therefore, the synthesis of the three isomeric pyridyl-(L)-alanines was undertaken (Fig. 4). Following a Japanese patent (16) they can be synthesized from the corresponding acrylates using PAL as catalyst. Accordingly, the three isomeric pyridylacrylates were prepared by known procedures (15). In the preparative conversions by PAL the equilibrium of the reactions was shifted in the desired direction by using high concentrations of ammonia (5 M $NH_3/NH_4^+$).

In such a way the pyridyl-(L)-alanines were obtained in up to 80% yield and were spectroscopically characterized. All three isomers of pyridyl-(L)-alanines turned out to be excellent substrates of PAL (Table I). Although their $K_m$ values were substantially higher than that of L-phenylalanine, the 3- and 4-pyridylalanines had higher $V_{max}$ values. This means that they were less tightly bound by the enzyme, but at saturating concentrations they reacted faster than the natural substrate. At the first glance these results are surpris-

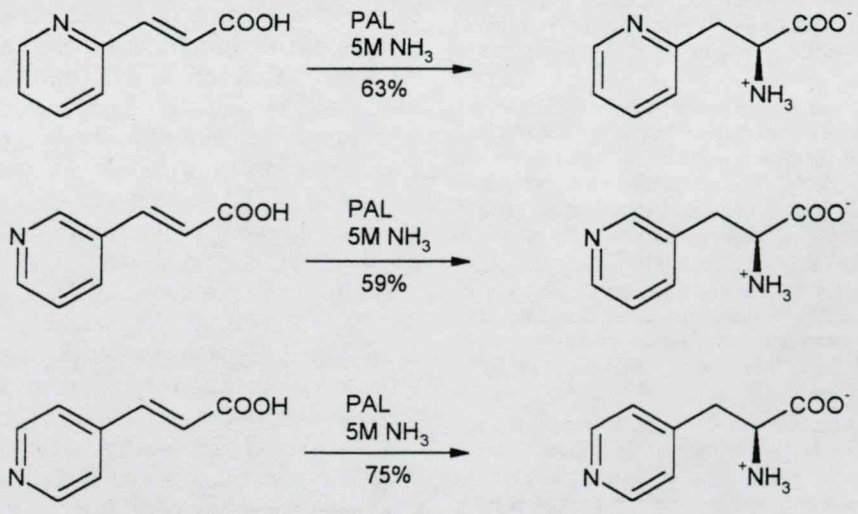



**FIG. 4.** Synthesis of pyridyl-alanines. To 1 mmol acrylic acid in 15 ml of 5 M $NH_4OH$ solution (adjusted by $CO_2$ to pH 10) was added 1 U PAL (*Petroselinum crispum* L.). Eighteen hours of agitation at 30°C, filtration of denatured protein, and removal of the solvent gave the crude product. Purification: recrystallization in $H_2O$/acetone or cation-exchange chromatography (Dowex 50W × 8).

ing, since electrophilic substitution at the pyridine nucleus is more difficult than at benzene. The main reason for it is that the electrophile (most often a proton) adds first to the pyridine nitrogen and the positive charge inactivates the ring for a further electrophilic attack.

Since in solution chemistry it is not feasible to carry out an electrophilic attack at a pyridine ring without first adding an electrophile (e.g., a proton) to the pyridine nitrogen, there is no experimental precedence for the electrophilic attack at a neutral pyridine. Therefore, we carried out three different semiempirical force-field calculations (MNDO, AM1, and PM3) to have an approximate measure for the stability of the $\sigma$ complexes in the corresponding electrophilic substitutions. The differences in the heat of formation ($\Delta H$s) between those of the sigma complexes and those of the aromatic ground states for the methyl-substituted phenyl and the isomeric pyridine rings are shown in Table II (see also Fig. 6). Since the entropy terms for all species should be similar, the $\Delta H$ values can be taken as a measure for the $\Delta G$ values. They show relatively small differences between the phenyl and pyridinyl complexes, but the trend is the expected one; i.e., meta attack in the pyridine ring is more favored than ortho or para attack. It seems, however, that the $V_{max}$ values are normally not determined by the rate of the electrophilic substitution; rather the rate of the dissociation of the enzyme-product complex is at least partially rate-limiting. This is in agreement with a thorough kinetic investigation (24) showing no primary kinetic deuterium isotope effect with [3,3-$^2$H]phenylalanine as substrate.

The $K_m$ values (Table I) suggest also the dissociation of the enzyme product complex being rate-limiting. Among the isomers, 3-pyridyl-(L)-alanine has the highest $K_m$ and $V_{max}$ values. 2-Pyridyl-(L)-alanine is a special case because electrophilic attack at the pyridine nitrogen leads to the inert pyridinium complex. Consequently 2,6-pyrimidinyl-(L)-alanine is not a substrate for PAL, while 3,5-pyrimidinyl-(L)-alanine is a moderately good substrate (A. Gloge and J. Rétey, unpublished).

TABLE II

Semiempirical Force-Field Calculations (MNDO, AM1, and PM3)

| Intermediate | $\Delta H_F$ | $\Delta$ charge of H |
|---|---|---|
| A | 180.6 | 0.048 |
| B | 184.9 | 0.059 |
| C | 185.4 | 0.056 |
| D | 194.3 | 0.072 |
| E | 198.3 | 0.065 |
| F | 212.0 | 0.079 |

*Note.* $\Delta H_F$, difference in the heat of formation between the $\sigma$ complexes and the aromatic ground states. $\Delta$ charge of H, difference in charge of the methyl H atoms between the $\delta$ complexes and the aromatic ground states.

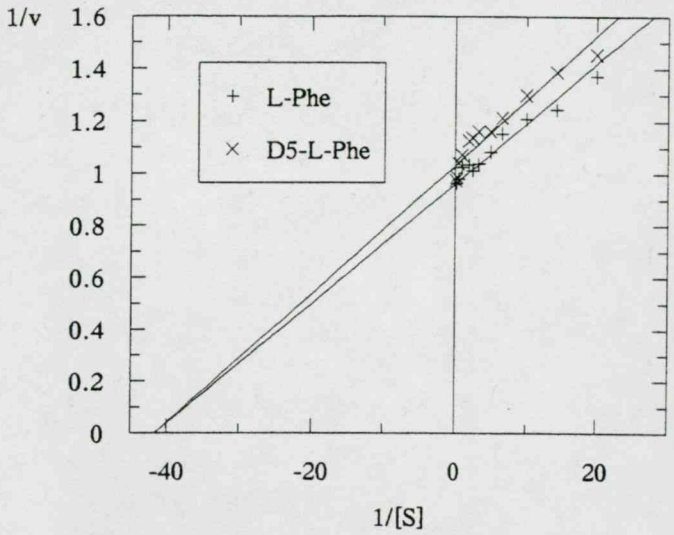



**FIG. 5.** Lineweaver–Burk plot of wild-type PAL with L-phenylalanine and L-[$^2H_5$]phenylalanine as substrates. The reaction rate was determined spectrophotometrically at $\lambda$ = 290 nm.

The $\Delta H$ value for the $\sigma$ complex of a protonated pyridine has been also calculated as 440 kcal/mol, i.e., more than double as high as the neutral pyridine. This is expected since such a complex would carry two positive charges.

For the reasons above we conclude that by placing the pyridine ring into the phenyl-binding hydrophobic pocket, PAL protects it from protonation, thus facilitating the electrophilic attack at the ortho carbon position. On the other hand, the higher polarity of the pyridine ring compared to the phenyl ring decreases the binding affinity, which is reflected by higher $K_m$ values (Table I) and probably higher dissociation rates of the enzyme–product complexes. Accordingly, the $K_i$ values of the pyridylacrylates are by an order of magnitude higher than that of cinnamate (24 ± 3 $\mu$M) (24).

*Secondary kinetic deuterium isotope effect.* To submit the new mechanism involving a Friedel–Crafts type acylation (4, 8) to a further test we measured the kinetic deuterium isotope effect of the PAL reaction with L-[$^2H_5$]phenylalanine as substrate. The Lineweaver–Burk plots were derived from four individual measurements at different substrate concentrations (Fig. 5). The labeled and unlabeled substrates were assayed on the same day and with the same enzyme preparation. Whereas the $K_m$ values were identical, the $V_{max}$ values exhibited a kinetic isotope effect of $k_H/k_D$ = 1.09 ± 0.01. This result strongly indicates that the phenyl ring is involved in the reaction mechanism and that substrate binding is independent of isotopic substitution. The magnitude of the $k_H/k_D$ value is in the range of a secondary deuterium kinetic isotope effect. The origin of such an effect in electrophilic aromatic

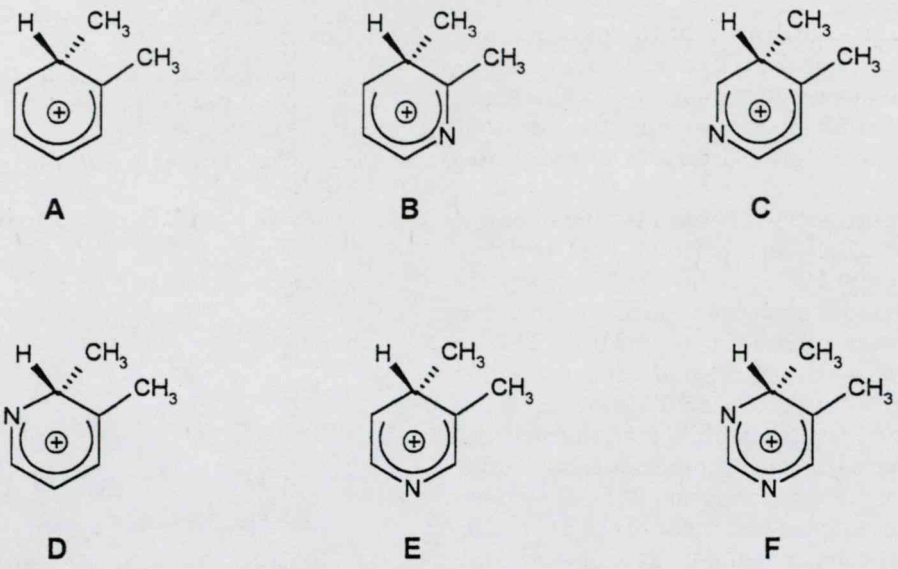



**FIG. 6.** Simplified $\sigma$ complexes as putative intermediates in the electrophilic substitution reactions at phenyl (A), 2-pyridyl (B), 4-pyridyl (C), 3-pyridyl (D and E), as well as 3,5-pyrimidinyl (F) rings.

substitutions has been discussed by several authors (25, 26) and was thoroughly reviewed by Halevi (27). There is agreement that two opposite effects play a role, the rehybridization ($sp^2 \rightarrow sp^3$) and hyperconjugation. While the change of hybridization from trigonal to tetrahedral geometry ordinarily leads to an inverse isotope effect ($k_H/k_D < 1$), this may be overcompensated by hyperconjugation when the hydrogen atom moves out of plane in the transition state. Since the C-D bond contributes less than the C-H bond to the stabilization of the carbenium ion by hyperconjugation, a normal isotope effect ($k_H/k_D > 1$) is to be expected.

Whatever the theoretical explanation for the observed effect, in nonenzymic electrophilic substitution of aromatic compounds similar effects have been observed (28–30) as in the present case. All these results are consistent with the proposed role of the phenyl ring in the PAL reaction and cannot be reconciled with the previously accepted mechanism with no direct involvement of the phenyl ring.

## ACKNOWLEDGMENTS

Financial support from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged. We are grateful to Professor K. Hahlbrock and N. Amrhein for a recombinant plasmid harboring the PAL gene and for antibodies against PAL, respectively; Professor G. Schröder for a gift of cyclooctatetraene; Dr. C. Döbler for samples of chiral pyridyl-(L)-alanines, and the Hoechst AG for [$^2H_5$]pyridyl-(L)-alanine. The paper is dedicated to Professor Meinrad Zenk on the occasion of his 65th birthday.

## REFERENCES

1. Hanson, K. R., and Havir, E. A. (1978) *Rec. Adv. Phytochem.* **12,** 91–137.
2. Hahlbrock, K., and Scheel, D. (1989) *Annu. Rev. Plant Phys. Plant Mol. Biol.* **40,** 347–369.
3. Schuster, B., and Rétey, J. (1994) *FEBS Lett.* **349,** 252–254.
4. Schuster, B., and Rétey, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8433–8437.
5. Langer, M., Pauling, A., and Rétey, J. (1995) *Angew. Chem.* **107,** 1585–1587; *Angew. Chem. Int. Ed. Engl.* **34,** 1464–1465.
6. Langer, M., Reck, G., Reed, J., and Rétey, J. (1994) *Biochemistry* **33,** 6462–6467.
7. Langer, B., Starck, J., Langer, M., and Rétey, J. (1997) *Bioorg. Med. Chem. Lett.* **7,** 1077–1082.
8. Rétey, J. (1996) Naturwissenschaften **83,** 439–447.
9. Gasteiger, J., Gream, G. E., Huisgen, R., Konz, W. E., and Schnegg, U. (1971) *Chem. Ber.* **104,** 2412–2419.
10. Cope, A. C., Burg, M., and Fenton, S. W. (1952) *J. Am. Chem. Soc.* **74,** 173–175.
11. Cope, A. C., Pike, M., and Rugen, D. F. (1954) *J. Am. Chem. Soc.* **76,** 4945–4947.
12. Pirrung, M. C., and Krishnamurthy, N. (1993) *J. Org. Chem.* **58,** 954–956.
13. Houghton, R. P., and Waight, E. S. (1969) *J. Chem. Soc. (C),* 978–981.
14. Attenburrow, J., Cameron, A. F. B., Chapman, J. H., Evans, R. M., Hems, B. A., Jansen, A. B. A., and Walker, T. (1952) *J. Chem. Soc.* 1094.
15. Marvel, C. S., Coleman, L. E., and Scott, G. P. (1955) *J. Org. Chem.* **20,** 1785–1792.
16. Mitsui Toatsu Chemicals, Jpn. Kokai Tokyo Koho J. P. 06, 113, 870 [94, 113, 870] (Cl.C12P13/06), 26 Apr 1994, Appl. 92/271, 476, 09 Oct 1992.
17. Haggerty, W. J., Springer, R. H., and Cheng, C. C. (1965) *J. Heterocycl. Chem.* **2,** 1–6.
18. Tabor, S., and Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
19. Laemmli, U. K. (1970) *Nature* **277,** 680–685.
20. Symingteon, J., Green, U., and Brackman, K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 177–181.

21. Layne, E. (1957) *Methods Enzymol.* **3**, 447–454.
22. Zimmermann, A., and Hahlbrock, K. (1975) *Arch. Biochem. Biophys.* **166**, 54–62.
23. Corey, E. J., Gilman, N. W., and Ganem, B. E. (1968) *J. Am. Chem. Soc.* **90**, 5616–5617.
24. Hermes, J. D., Weiss, P. M., and Cleland, W. W. (1985) *Biochemistry* **24**, 2959–2967.
25. Streitwieser, A., Jagow, R. H., Fahey, R. C., and Suzuki, S. (1958) *J. Am. Chem. Soc.* **80**, 2326–2332.
26. Shiner, V. J. (1959) *Tetrahedron* **5**, 243–252.
27. Halevi, E. A. (1963) *in* Progress in Physical Org. Chem. (Cohen, S. G., Streitwieser, A., and Taft, R. W., Eds.), Vol. 1, pp. 109–216, Wiley Interscience, New York/London.
28. Berliner, E., and Schueller, K. E. (1960) *Chem. Ind.*, 1444.
29. Bonner, T. G., Bowyer, F., and Williams, G. (1953) *J. Chem. Soc.* 2650–2652.
30. Swain, C. G., Knee, T. E. C., and Kresge, J. (1957) *J. Am. Chem. Soc.* **79**, 505.

# XIII. melléklet

GLOGE, A., ZON, J., KŐVÁRI, Á., **POPPE, L.**, RÉTEY, J.:

Phenylalanine Ammonia-Lyase: The Use of Its Broad Substrate Specificity for Mechanistic Investigations and Biocatalysis. Synthesis of L-Arylalanines,

*Chemistry An Europian Journal*, **2000**, közlésre beküldve.

# PHENYLALANINE AMMONIA-LYASE: THE USE OF ITS BROAD SUBSTRATE SPECIFICITY FOR MECHANISTIC INVESTIGATIONS AND BIOCATALYSIS. SYNTHESIS OF L-ARYLALANINES

Andreas Gloge[a], Jerzy Zón[b] Ágnes Kövári [c], László Poppe [c,d] and János Rétey[a]

[a] Prof. Dr. J. Rétey, Dr. A. Gloge
Institut für Organische Chemie der Universität Karlsruhe
Richard Willstätter Allee 2, D-76128 Karlsruhe (Germany)
Tel: (+49) 721-608-2092; Fax: (+49) 721-608-4823; E-mail:
biochem@ochhades.chemie.uni-karlsruhe.de

[b] Dr. J. Zon
Institute of Organic Chemistry, Biochemistry and Biotechnology, Wroclaw University of Technology Wybzeze Wyspianskiego 27, 50-340 Wroclaw Poland

[c] Á. Kövári,
Institute for Organic Chemistry, Budapest University of Technology and Economics
H-1111 Budapest, Gellért tér 4., Hungary

[d] Dr. L. Poppe
Chemical Research Center of the Hungarian Academy of Sciences
H-1025 Budapest, Pusztaszeri út 59-67, Hungary

***Keywords***: chemoenzymatic synthesis; halogenated L-phenylalanines; pyrimidinyl-alanines; enzyme mechanism.

***Abstract***:

Several fluoro- and chloro-phenyl alanines are good substrates of phenylalanine ammonia-lyase (PAL / EC 4.3.1.5) from parsley. The enantiomerically pure L-amino acids were obtained in good yields by reaction of the corresponding cinnamic acids with 5 M ammonia solution (buffered to pH 10) in the presence of PAL. The kinetic constants for nine different fluoro- and chlorophenylalanines do not provide a rigorous proof for but are consistent with the previously proposed mechanism comprising an electrophilic attack of the methylidene-imidazolone cofactor of PAL at the aromatic nucleus as a first chemical step. In the resulting Friedel-Crafts-Type σ-complex the *pro-S* β-proton is activated for abstraction by an enzymic base. Results of semiempirical calculations combined with a proposed partial active site model

showed a correlation between the experimental kinetic constants and the change of polarization of the *pro-S* Cβ-H bond and heat of formation of the σ-complexes, thus making the electrophilic attack at the neutral aromatic ring plausible. Furthermore, while 5-pyrimidinyl-alanine was found to be a moderately good substrate of PAL, 2-pyrimidinyl-alanine was an inhibitor.

## INTRODUCTION

Phenylalanine ammonia-lyase (PAL) catalyses the reversible conversion of L-phenylalanine to *trans*-cinnamic acid. PAL is the key enzyme in the metabolism of phenylpropanoids in plants, since cinnamic acid is the precursor of lignins, flavonoids and coumarins.[1,2] Early biochemical evidence indicated a catalytically essential electrophilic group at the active sites of PAL and the related enzyme histidine ammonia-lyase (HAL). Inactivation by radiolabelled nucleophiles ($K^{14}CN$ and $NaB^{3}H_4$) followed by total hydrolyses of the proteins afforded radioactive products which could be derived from a dehydroalanine residue being the electrophilic prosthetic group.[3,4,5]

Its precursors have been found by site-directed mutagenesis to be Ser 202[6] and Ser 143[7] in PAL and HAL, respectively. Since both enzymes have been overexpressed in *E.coli* cells in active forms, the posttranslational modification takes place autocatalytically.[8,9]

Recently, the 3D structure of HAL has been elucidated by x-ray analysis which revealed that the electrophilic prosthetic group is methylidene-imidazolone (MIO).[10] MIO can be regarded as a modified dehydroalanine. The ring structure and the geometry dictated by the protein conformation prevents the lone pairs of the imidazole nitrogens to delocalize into the α/β unsaturated carbonyl system thus enhancing the electrophilicity of the latter. The driving force for the formation of MIO from the tripeptide Ala142SerGly144 is unknown but not without precedence. The chromophoric imidazolone of the green fluorescent protein[11,12,13] must be formed by a similar autocatalytic process.

The discovery of the electrophilic prosthetic group was followed by the proposal that it reacts with the amino group of the substrate in a type of Michael addition.[14] This reaction would enhance the leaving ability of the amino group, i.e. facilitate the elimination of ammonia, but leaves the question open how the non-acidic β-proton could be abstracted by an enzymic base.

Recently, experimental evidence accumulated in favour of an electrophilic attack at the aromatic nucleus as shown in Scheme 1 for the PAL reaction.[15,16] In the Friedel-Crafts-Type

σ-complex the β-protons of the side-chain are activated for abstraction by an enzymic base. The proton transfer is then followed by ammonia elimination concomitant with restoration of the prosthetic group and the aromaticity of the 6-membered ring.

The question is legitimate, why the β-proton is abstracted leading to an exocyclic double bond while abstraction of the ring proton would straightforwardly lead to re-aromatization. Although the latter would preferentially take place in solution, PAL prevents this reaction by excluding any basic group in the binding site for the phenyl group. Simultaneously, a basic group is correctly positioned to abstract the β-proton of the substrate.

In the present communication we describe the enantiospecific synthesis of various fluoro- and chlorophenylalanines by reversal of the PAL reaction. Kinetic measurements with these new substrates and the results of theoretical calculations and a proposed partial active site model are discussed in terms of the Friedel-Crafts-type mechanism of the PAL reaction.

## Results and Discussion

### Enantioselective Synthesis of L-Phenylalanines Halogenated in the Ring.

Contrary to early claims that the PAL reaction is irreversible[17] it can be reversed by applying high concentrations of ammonia.[18] For preparative conversions the halogenated cinnamic acids were solved in half concentrated ammonia solution whose pH was brought to 10 by bubbling $CO_2$ into it. The reaction was started by addition of recombinant PAL (1-2 iU). After incubation overnight at 37°C the enzyme was removed by boiling and acidification (pH 1.5) followed by filtration. Chromatography on an acidic cation exchange column afforded the enantiomerically pure substituted phenylalanines in moderate to excellent yields. Their e.e. was determined on a chiral column to be more than 99 %. The halogenated cinnamic acids and phenylalanines were characterized by their NMR, and UV spectra, as well as their $R_f$ values. Their structures together with the yields of the isolated phenylalanines are shown in Scheme 2.

### Determination of the Kinetic Constants for the Halogenated Phenylalanines.

The prerequisits for the quantitative measurement of the concentrations of the cinnamic acid products was the determination of their extinction coefficients (ε) at the wavelengths at which a maximal difference existed between those of the arylalanines and the corresponding cinnamic acids (see experimental section). The values were measured in 0.1 M Tris-buffer pH 8.8. These were the conditions also for the kinetic measurements. In columns 4 and 5 of

Table 1 the $V_{max}$ values relative to that of phenylalanine and the $K_m$ values, respectively, are listed.

Inspection of the relative $V_{max}$ values reveals that phenylalanines halogenated in the 3′-position react on PAL significantly faster than the parent compound. This is consistent with an electrophilic attack of MIO at the aromatic nucleus. A halogen substituent in 3′-position facilitates such an attack in ortho or para by stabilisation of the cationoid transition state. Both positions are available for the attack by the sterically fixed MIO due to free rotation of the phenyl ring before substrate binding. This effect is similar to that previously found with a 3′-hydroxyphenylalanine (*m*-tyrosine) as substrate.[15] Halogen substituents in the phenyl ring may also influence the acidity of the β-protons of the side chain. While this effect should activate the β-protons in the neutral ground state, the opposite effect is expected for the cationoid intermediate. In the latter, delocalization of the positive charge to the halogen atoms will decrease electron deficiency in the ring and hence diminish the acidity of the side chain β protons. The acidifying effect of the halogen substituents is however much less than that of a nitrogroup. Anyway, the β-proton abstraction does not seem to be rate determining for good substrates as shown by deuterium labelling.[19] While the attack by MIO is partially rate determining, another rate-limiting step seems to be product release. The kinetic significance of these two steps is substrate-dependent. The $K_m$ values vary only moderately, but roughly increase with increasing numbers of the halogenic substituents.

**Theoretical Calculations**

To demonstrate the feasibility of the electrophilic attack at the halogenated aromatic rings semiempirical calculations for the heat of formation and for the activating effect on the β-protons by the positive charge in the ring were carried out. To facilitate the problem, the calculations (AM1 and PM3) were applied to a simplified model of the putative σ-complexes containing the substrates in their zwitterionic form and a methyl group which corresponds to the carbon of the methylidene moiety of MIO (Scheme 3). Conformational analysis of the zwitterionic form of L-Phe indicated that the most decisive conformation factor for the molecular properties is the dihedral angle (Φ) between the $C_{1'}$-$C_{2'}$ and the *pro-S* $C_\beta$-H bonds (Scheme 3). Results of semiempirical calculations are compiled in columns 2 and 3 of Table 1.

With respect to the σ–complex formation and scissible bond polarization the 2'F, 2'Cl and 4'Cl substituted compounds, particularly the B type sigma complexes are similar to the unsubstituted L-Phe. Accordingly, their kinetic behaviour is similar to the natural substrate.

The putative active site model suggests that the aromatic ring of the substrate is sandwiched between MIO and a phenyl ring of Phe399. The arrangement as shown in Scheme 1 allows maximal overlap between the π system of MIO and the aromatic $C_2$-position. The 2' H of the aromatic moiety might be stabilized in a charge-transfer or π complex type manner without the possibility of rearomatization by its removal. The conserved nature of the analogous phenylalanine in all PAL and HAL sequences (e.g. the GGNFH segment is present in the HAL enzymes of microorganisms such as *P. putida* or *B. subtilis* as well as in mammalian HAL enzymes like mice, rat or human) supports the importance of this amino acid.

In the case of compounds bearing halogen at 3' position, additional hyperconjugation of the 3'-halogen atoms with the aromatic π electrons of Phe399 might also be possible. This effect may lower the energy values listed in Table 1 which were calculated *in vacuo*. In addition, the *pro-S* Cβ-H bond in the ground state of the 3',5'$F_2$ substituted compound is significantly more polarized (ca. 0.040 Mulliken charge units) then in all the other models. In the case of the pentafluoro analogue, the effect of hyperconjugation effect with the aromatic π electrons of Phe399 cannot overbalance the energy demand of the formation of the sigma complex.

For the 5-pyrimidinyl compound relatively high energy is required for formation of the σ complex but the polarization of the scissible *pro-S* Cβ-H is relatively strong. Accordingly, a high $K_m$ value but a normal $V_{max}$ is observed. The inhibition observed with the 2-pyrimidinyl compound is a consequence of the stability of the cationic pyrimidinium complex and the unfavourable proton abstraction from the negatively polarized *pro-S* Cβ-H bond (Scheme 4).

All these correlations are consistent with the intermediacy of the Friedel-Crafts type σ-complexes and the kinetic significance of their formation as partially rate-determining step. Further support for our mechanism was provided by recent publications (20,21).

**Synthesis of and Kinetic Measurements with Pyrimidinylalanines**

The synthesis of β-(2-pyrimidinyl)-D,L-alanine **1** was carried out in six steps following the procedure of Haggerty et al.[22] A chemoenzymatic strategy was applied to the preparation of β-(5-pyrimidinyl)-L-alanine **2**. First of β-(5-pyrimidinyl)-acrylic acid **3** was synthesised starting

from 5-bromo-pyrimidine and *tert*-butylacrylate under Heck conditions. Treatment of the product with trifluoro acetic acid afforded the free β-(5-pyrimidinyl)-acrylic acid **3** which was converted in 57 % yield into the corresponding enantiomerically pure L-alanine-derivative **2** using PAL as a catalyst.

While the 5-pyrimidinyl isomer turned out to be a moderately good substrate of PAL, its 2-pyrimidinyl counterpart **1** was a competitive inhibitor. The corresponding kinetic constants are shown in Table 3. The $K_m$-value of **2** is comparable to the $K_i$-value of **1**.

These results support the postulated ortho-attack of MIO at the aromatic nucleus. When both ortho positions are occupied by a nitrogen atom such an attack leads to a pyrimidinium ion which is so stable that no further reaction occurs (see Scheme 4). The competitive nature of the inhibition requires however that the binding of **2** is reversible.

## EXPERIMENTAL SECTION:

*Recombinant PAL* was overexpressed in *E.coli* and purified as described, first according to Schuster and Rétey[6] and later using the improved method of Baedeker and Schulz.[23]

*Chloro- and fluoro-L-phenylalanines:* Chlorocinnamic acids and fluorocinnamic acids were purchased from Fluka and from Lancaster. Reaction of the chloro- and fluorocinnamic acids with ammonia catalyzed by PAL afforded the corresponding chloro- and fluoro-L-phenylalanines. To a 5 M aqueous $NH_3$-solution, adjusted to pH 10 by $CO_2$, were added 100 mg of the cinnamic acid and 1 U wtPAL (*Petroselinum crispum*). The reaction mixture was agitated overnight at 37°C. The solution was acidified with 5 % HCl to pH 1,5, heated to boiling, filtered and applied to a Dowex 50 cation exchange resin column. The elution occurred with diluted ammonia solution. The crude product was purified with HPLC (Nucleosil 100 C18, 7µm, 250 x 20mm; flow rate: 5 ml/min; load: 20 mg; mobile phase: 0-15 min: 99.9 % $H_2O$ / 0.1 % TFA, 15-90 min: linear increasing gradient to 99.9 % $CH_3CN$ / 0.1 % TFA, retention times: L-phenylalanine: 41.7 min, 2′-fluoro-L-phenylalanine: 42.4 min, 3′-fluoro-L-phenylalanine: 44.1 min, 4′-fluoro-L-phenylalanine: 43.9 min, 2′,6′-difluoro-L-phenylalanine: 42.7 min, 3′,5′-difluoro-L-phenylalanine: 46.5 min, 2′,3′,4′,5′,6′-pentafluoro-L-phenylalanine: 48.4 min, 2′-chloro-L-phenylalanine: 45.3 min, 3′-chloro-L-phenylalanine: 48.0 min, 4′-chloro-L-phenylalanine: 48.7 min). For the determination of the enantiomeric excess we used a chiral column from astec/ict (Chirobiotic T, 250 x 4.6 mm; mobile phase: 70 % $H_2O$ / 30 % EtOH; flow rate: 0,8 ml/min; load: 5 µg; retention times: L-phenylalanine: 7.22 min, 2′-fluoro-L-phenylalanine: 6.87 min, 3′-fluoro-L-phenylalanine: 6.98 min, 4′-fluoro-L-phenylalanine:

7.20 min, 2′,6′-difluoro-L-phenylalanine: 6.42 min, 3′,5′-difluoro-L-phenylalanine: 6.75 min, 2′,3′,4′,5′,6′-pentafluoro-L-phenylalanine: 5.89 min, 2′-chloro-L-phenylalanine: 9.06 min, 3′-chloro-L-phenylalanine: 8.22 min, 4′-chloro-L-phenylalanine: 9.25 min). In all cases ee is over 99 %.

*β-(2-Pyrimidinyl)-D,L-alanine* **1**. Preparation as reported by W. J. Haggerty, R. H. Springer and C. C. Cheng.[20]

*β-(5-Pyrimidinyl)-L-alanine* **2**. β-(5-Pyrimidinyl)-acrylic acid **3** obtained by Heck coupling between 5-bromopyrimidine and *tert*-butyl acrylate, followed by ester-cleavage in TFA.[24]
10 ml of a concentrated, aqueous ammonia-solution were diluted with 10 ml of distilled water. The pH was adjusted to 10.0 by bubbling $CO_2$ into the solution. To the solution were added 64.3 mg (0.428 mmol) β-(5-pyrimidinyl)-acrylic acid **3** and 1 U PAL. After 24 h agitation at 37°C the enzyme was denatured by heat and removed by filtration. The clear solution applied to a Dowex 50 cation exchange resin column. The elution occurred with diluted ammonia solution. The crude product was purified with HPLC (Nucleosil 100 C18, 7μm, 250 x 20mm; flow rate: 5 ml/min; load: 20 mg; mobile phase: 0-15 min: 99.9 % $H_2O$ / 0.1 % TFA, 15-90 min: linear increasing gradient to 99.9 % $CH_3CN$ / 0.1 % TFA, retention times: 23.5 min). The solvent was carefully removed under reduced pressure to give 50 mg (0.246 mmol) of a white solid. The product was obtained as hydrochloride in 57 % yield.

*Determination of $V_{max}$- and $K_m$-values.* The kinetic constants were determined by measuring the UV-absorption of the produced acrylates (*trans*-cinnamic acid: $\varepsilon_{(290)}$ = 10000 liters $cm^{-1}$ $mol^{-1}$; 2′-fluorocinnamic acid: $\varepsilon_{(280)}$ = 12550 liters $cm^{-1}$ $mol^{-1}$; 3′-fluorocinnamic acid: $\varepsilon_{(280)}$ = 13850 liters $cm^{-1}$ $mol^{-1}$; 4′-fluorocinnamic acid: $\varepsilon_{(280)}$ = 15320 liters $cm^{-1}$ $mol^{-1}$; 2′,6′-difluorocinnamic acid: $\varepsilon_{(290)}$ = 3960 liters $cm^{-1}$ $mol^{-1}$; 3′,5′-difluorocinnamic acid: $\varepsilon_{(290)}$ = 5060 liters $cm^{-1}$ $mol^{-1}$; 2′,3′,4′,5′,6′-pentafluorocinnamic acid: $\varepsilon_{(280)}$ = 7910 liters $cm^{-1}$ $mol^{-1}$; 2′-chlorocinnamic acid: $\varepsilon_{(285)}$ = 10770 liters $cm^{-1}$ $mol^{-1}$; 3′-chlorocinnamic acid: $\varepsilon_{(285)}$ = 10680 liters $cm^{-1}$ $mol^{-1}$; 4′-chlorocinnamic acid: $\varepsilon_{(290)}$ = 15790 liters $cm^{-1}$ $mol^{-1}$; β-(5-pyrimidinyl)-acrylic acid: $\varepsilon_{(270)}$ = 11110 liters $cm^{-1}$ $mol^{-1}$) using 0.05-10 mM of the corresponding amino acid as substrate. Conditions: 0.05 mM - 10 mM substrate in 0.1 M Tris-HCl buffer pH 8.8 at 30 °C. $^1$H-NMR (250 MHz; $D_2O$, 25°C): L-phenylalanine: δ = 7.28-7.43 (m, 5H), 3.96 (t, 1H), 3.27 (dd, 1H), 3.08 (dd, 1H), 2′-fluoro-L-phenylalanine: δ = 7.34 (m, 2H), 7.17 (m, 2H), 4.26 (t, 1H), 3.40 (dd, 1H), 3.20 (dd, 1H), 3′-fluoro-L-phenylalanine: δ = 7.38 (m, 1H), 7.08 (m, 3H), 4.19 (t, 1H), 3.32 (dd, 1H), 3.16 (dd, 1H), 4′-fluoro-L-

phenylalanine: δ = 7.26 (m, 2H), 7.10 (m, 2H), 4.28 (t, 1H), 3.29 (dd, 1H), 3.15 (dd, 1H), 2′,6′-difluoro-L-phenylalanine: δ = 7.36 (m, 1H), 6.99 (m, 2H), 4.28 (t, 1H), 3.37 (dd, 1H), 3.26 (dd, 1H), 3′,5′-difluoro-L-phenylalanine: δ = 6.90 (m, 3H), 4.32 (t, 1H), 3.35 (dd, 1H), 3.17 (dd, 1H), 2′,3′,4′,5′,6′-pentafluoro-L-phenylalanine: δ = 4.01 (t, 1H), 3.26 (dd, 1H), 3.19 (dd, 1H), 2′-chloro-L-phenylalanine: δ = 7.48 (m, 1H), 7.34 (m, 3H), 4.05 (t, 1H), 3.45 (dd, 1H), 3.16 (dd, 1H), 3′-chloro-L-phenylalanine: δ = 7.36 (m, 3H), 7.21 (m, 1H), 4.20 (t, 1H), 3.31 (dd, 1H), 3.16 (dd, 1H), 4′-chloro-L-phenylalanine: δ = 7.40 (d, 2H), 7.26 (d, 2H), 3.97 (t, 1H), 3.23 (dd, 1H), 3.10 (dd, 1H).

*Theoretical calculations.* L-Phenylalanine, the halogenated L-phenylalanine derivatives, and their σ-complex models were calculated *in vacuo* using AM1 and PM3 methods. [25,26] Conformational analysis showed two favoured states for zwitterionic structures with antiperiplanar *pro-S* $H_\beta$ -$N^+H_3$ zig-zag arrangement (Scheme 3). All further calculations refer to the arrangement with a fixed Φ = -90 ° torsion angle. Since the parallel results from the two methods were similar, values derived from PM3 calculations are indicated only. In the cases where two distinct σ-complex models may arise by rotation of the substrate prior to the active complex formation, model A represents the structure where the halogen substituent is closer to the $sp^3$ centre in the σ complex.

*The putative active site model* was built from a sequence segment of PAL (SP: P24481) containing the ASGDL active site motif and the F399 by homology-modelling [27] using HAL structure (PDB: 1B8F [10]) as folding template, followed by Gromos [27] and Amber [24] energy minimizations. The ASG structure of the model was finally replaced by MIO taken from the 1B8F HAL structure (less than 0.4 Å deviations in the corresponding atomic positions).

**ACKNOWLEDGEMENTS**

We thank M. Baedeker and G. Schulz (Universität Freiburg) for the improved expression system for PAL[23].

Financial support by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the Hungarian OMFB is gratefully acknowledged.

## References


[1] K.R. Hanson, E.A.Havir, *Recent Adv. Phytochem.* **1978**, *12*, 91-137.

[2] K. Hahlbrock, D. Scheel, *Ann. Rev. Plant Phys. Plant Mol. Biol.* **1989**, *40*, 347-369.

[3] K.R. Hanson, E.A. Havir, *Arch. Biochem. Biophys.* **1970**, *141*, 1-77.

[4] K.R. Hanson, E.A. Havir, *Biochemistry* **1973**, *12*, 1583-1591.

[5] E.A. Havir, P.D. Reid, H.V. March, *Plant Physiol.* **1971**, *48*, 130-136.

[6] B. Schuster, J. Rétey, *FEBS Lett.* **1994**, *349*, 252-254.

[7] M. Langer, G. Reck, J. Reed, J. Rétey, *Biochemistry* **1994**, *33*, 6462-6467

[8] K.R. Hanson, E.A. Havir, *The Biochemistry of Plants* **1981**, *7*, 577-625.

[9] W. Schulz, H.G. Eiben, K. Hahlbrock, *FEBS Lett.* **1989**, *258*, 335-338.

[10] T.F. Schwede, J. Rétey, G.E. Schulz, *Biochemistry* **1999**, *38*, 5355-5361.

[11] M. Ormö, A.B. Cubitt, K. Kallio, L.A. Gross, R.Y. Tsien, S.J. Remington, *Science* **1996**, *273*, 1392-1395.

[12] K.J. Luebke, *Chem. & Biol.* **1998**, *5*, 317-322.

[13] G.J. Palm, A. Zdanov, G.A. Gaitanaris, R. Stauber, G.N. Pavlakis, A. Wlodawer, *Nature Structural Biology* **1997**, *4*, 361-365.

[14] T.A. Smith, F.H. Cordelle, R.H. Abeles, *Arch. Biochem. Biophys.* **1967**, *120*, 724-725.

[15] B. Schuster, J. Rétey, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 8433-8437.

[16] J. Rétey, *Naturwissenschaften* **1996**, *83*, 439-447.

[17] A. Peterkofsky, *J. Biol. Chem.* **1962**, 237, 787-795.

[18] Mitsui Toatsu Chemicals, Jpn. Kokai Tokyo Koho J. P. 06, 113, 870 [94, 113, 870] (Cl.C12P13/06), **26 Apr 1994**, Appl. 92/271, 476, 09 Oct 1992.

[19] J.D. Hermes, P.M. Weiss, and W.W. Cleland, *Biochemistry* **1985**, *24*, 2959-2967.

[20] A. Gloge, B. Langer, L. Poppe, J. Rétey, *Arch. Biochem. Biophys.* **1998**, *359*, 1-7.

[21] A. Lewandowicz, J. Jemielity, M. Kanska, J. Zon, P. Paneth, *Arch.Biochem. Biophys.* **1999**, *370*, 216-221.

[22] W.J. Haggerty, R.H. Springer, C.C. Cheng, *J. Heterocycl. Chem.* **1964**, *2*, 1-6.

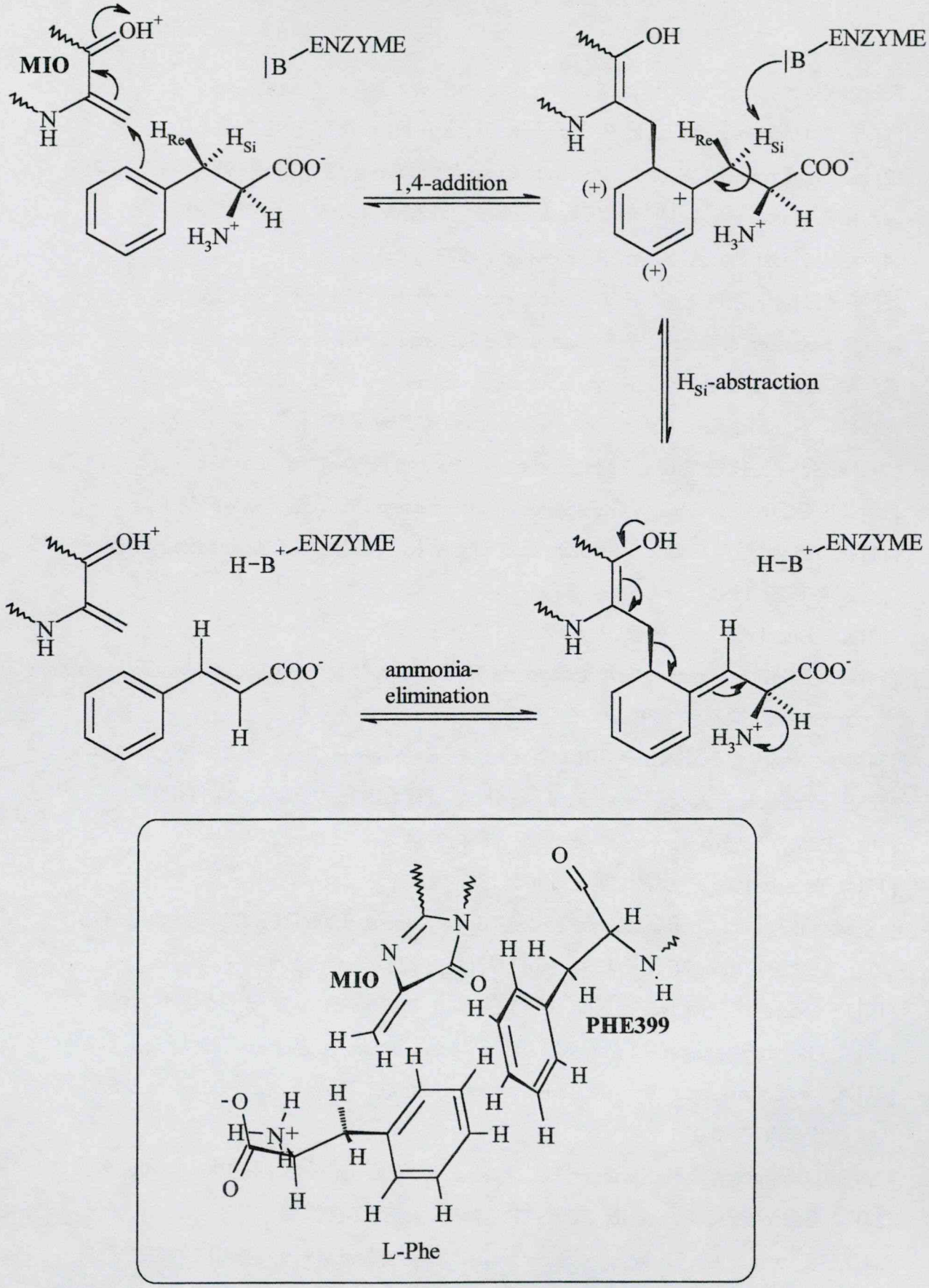
[23] M. Baedeker, G.E. Schulz, *FEBS Lett.* **1999**, *457*, 57-60.

[24] J.E. Plevyak, J.E. Dickerson, R.F. Heck, *J. Org. Chem.* **1979**, *44*, 4078-4080.

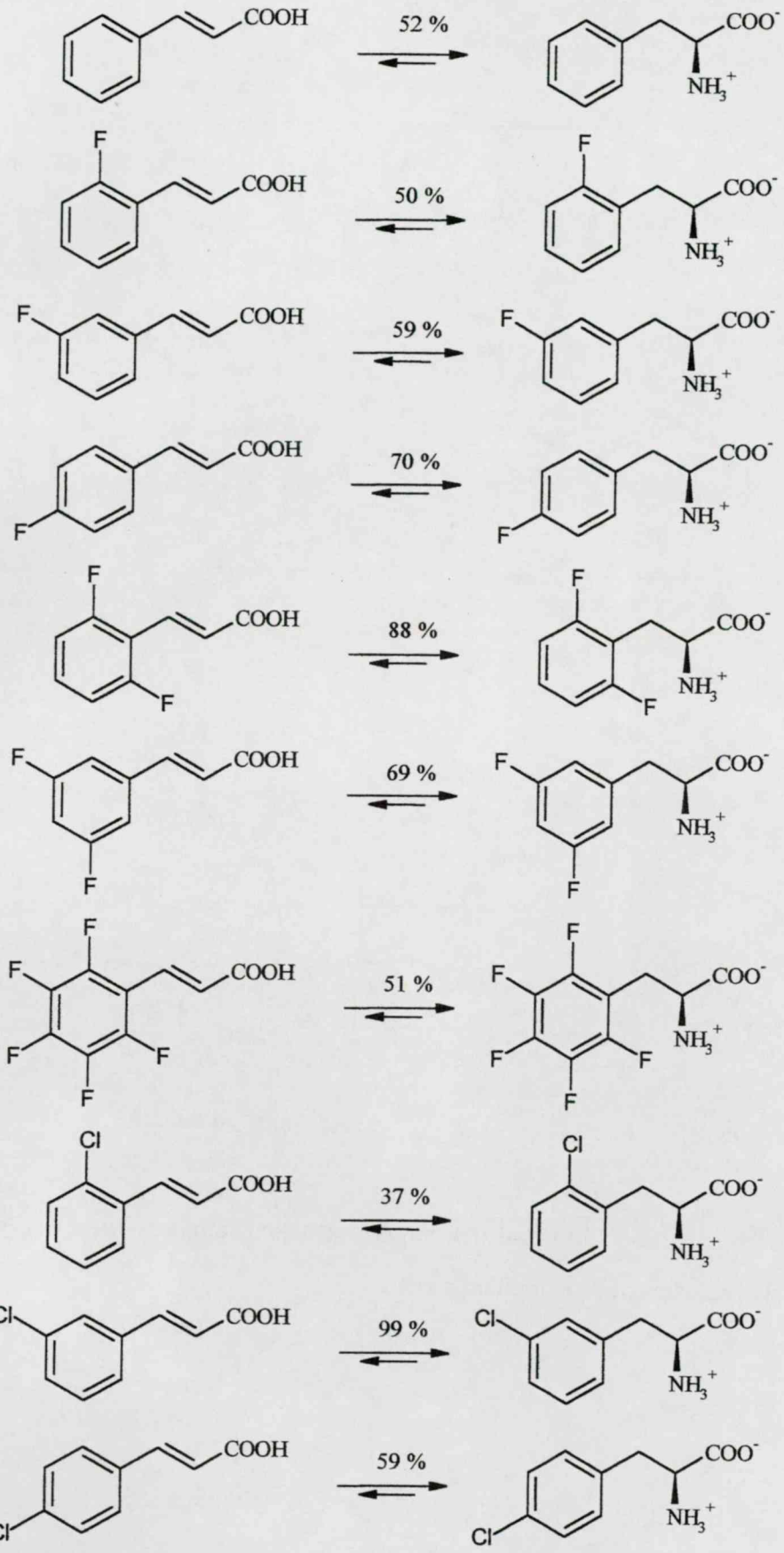
[25] PC Spartan Pro, Wavefunction, Inc., 18401 Von Karman, Suite 370, Irvine, CA 92612, USA.

[26] HyperChem 5.1: Hypercube, Inc., 1115 NW 4th Street, Gainesville, FL 32601 USA.

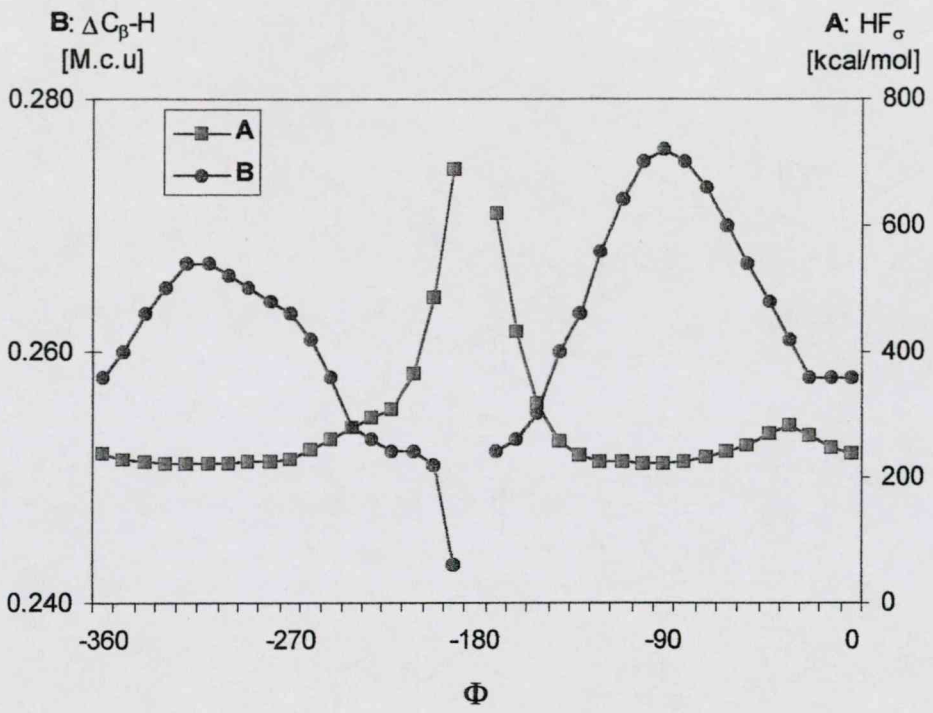
[27] Swiss-PdbViewer, 3.51: N. Guex, M.C. Peitsch, *Electrophoresis* **1997**, *18*, 2714-2723.

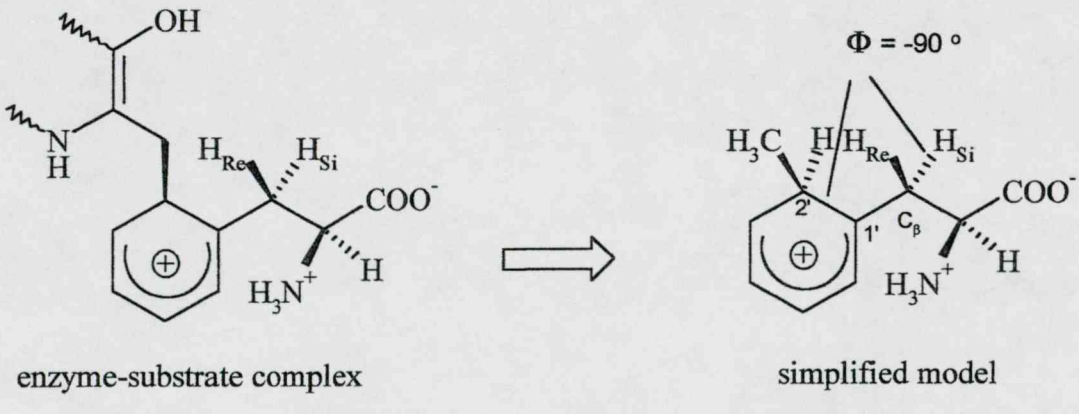



**Scheme 1** Postulated mechanism and partial putative active site model for the reaction catalyzed by phenylalanine ammonia-lyase.




**Scheme 2** Preparative conversion of various halogenated phenylalanines by reversal of the phenylalanine ammonia-lyase reaction.

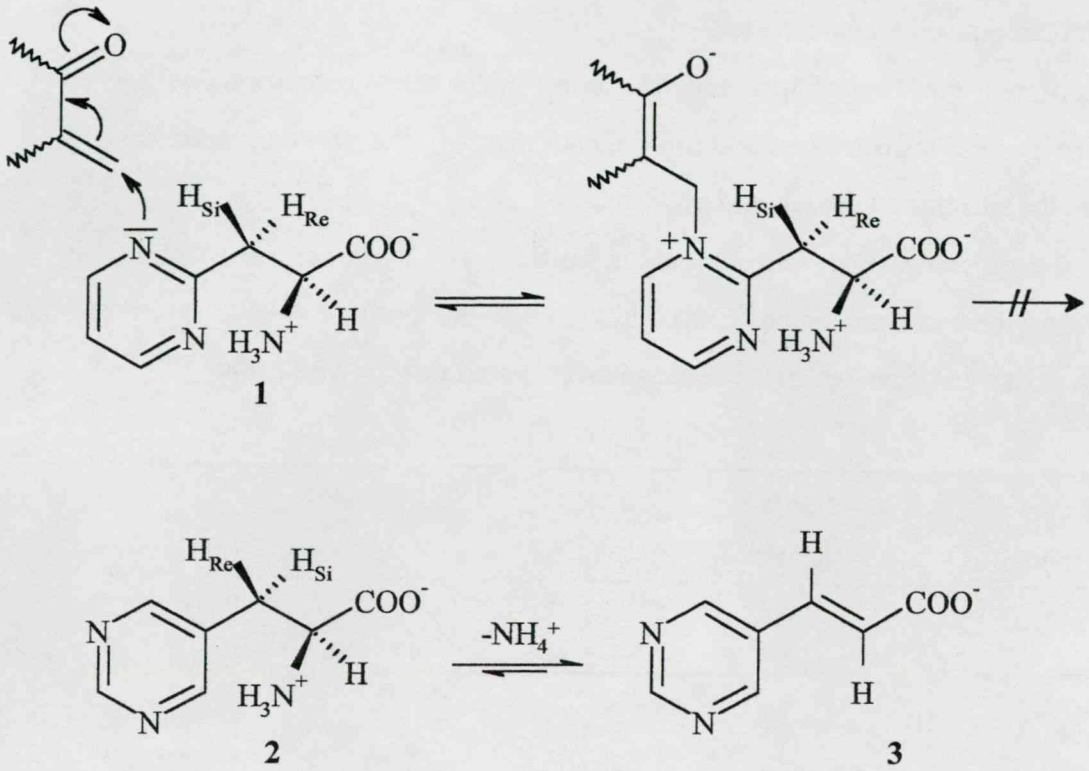



enzyme-substrate complex

simplified model

**Scheme 3** The postulated σ-complex as intermediate of the phenylalanine ammonia-lyase reaction and conformational properties of its simplified model.




**Scheme 4** The postulated unproductive pyrimidinium complex with 2-pyrimidinylalanine **1** and the reaction with 5-pyrimidinylalanine **2**. (Although for the experiments the racemic compounds were used, here only the L-enantiomers are shown which are presumed to be the enzymatically active species).

**Table 1.** Kinetic constants for the various phenylalanine analogues and calculated properties of their active complex models.

$\Delta\Delta H_{\sigma\text{-}g}$: the difference for heat of formation calculated for the active complex model for L-Phe and the ground state L-Phe substrate was substracted from the corresponding difference for the substituted models and substrates;

$\Delta\Delta$ C$\beta$-H$_{\sigma\text{-}g}$: the difference in Mulliken charges on the C$\beta$-H atoms in the substrate substracted from the corresponding difference in the active complex;

$V_{max}/V_{max\text{-}Phe}$: relative $V_{max}$ values of the analogues compared to $V_{max}$ with L-Phe.

| Substrate ($\sigma$ complex model) | | $\Delta\Delta H_{\sigma\text{-}g}$ [kcal/mol] | $\Delta\Delta$ C$\beta$-H$_{\sigma\text{-}g}$ [M.c.u] | $V_{max}/V_{max\text{-}Phe}$ | $K_m$ [mM] |
|---|---|---|---|---|---|
| L-Phe | | 0.0 | 0.184 | 1.00 | 0.033 |
| 2'F-L-Phe | (A) | 15.7 | 0.135 | 1.14 | 0.065 |
| | (B) | 7.8 | 0.129 | | |
| 3'F-L-Phe | (A) | 3.1 | 0.109 | 2.09 | 0.079 |
| | (B) | 1.5 | 0.116 | | |
| 4'F-L-Phe | | 7.6 | 0.128 | 0.56 | 0.010 |
| 2',6'F$_2$-L-Phe | | 21.9 | 0.160 | 0.85 | 0.085 |
| 3',5'F$_2$-L-Phe | | 4.8 | 0.037 | 2.72 | 0.159 |
| 2',3',4',5',6'F$_5$-L-Phe | | 29.9 | 0.141 | 0.16 | 0.076 |
| 2'Cl-L-Phe | (A) | 7.4 | 0.117 | 1.03 | 0.050 |
| | (B) | 5.2 | 0.115 | | |
| 3'Cl-L-Phe | (A) | -1.7 | 0.100 | 2.01 | 0.094 |
| | (B) | -4.2 | 0.102 | | |
| 4'Cl-L-Phe | | 3.2 | 0.126 | 0.82 | 0.045 |
| $\beta$-(5-Pyrimidinyl)-D,L-Ala | | 25.8 | 0.229 | 0.80 | 4.2 |
| $\beta$-(2-Pyrimidinyl)-D,L-Ala | | -24.0 | -0.314 | 0.00 | ($K_i$ = 7 mM) |

# XIV. melléklet

**POPPE, L.**, HULL, W. E., RÉTEY, J.:

Synthesis and Characterization of (5'-Deoxyadenosin-5'-yl)cobalamin (='Adenosylcobalamin') Analogues Mimicking the Transition-State Geometry of Coenzyme-$B_{12}$-Dependent Rearrangements,

*Helv. Chim. Acta*, **1993**, *76*, 2367.

# 168. Synthesis and Characterization of (5′-Deoxyadenosin-5′-yl)cobalamin (= 'Adenosylcobalamin') Analogues Mimicking the Transition-State Geometry of Coenzyme-$B_{12}$-Dependent Rearrangements

by László Poppe[a][c], William E. Hull[b], and János Rétey[a]*

[a]) Lehrstuhl für Biochemie im Institut für Organische Chemie der Universität Karlsruhe, Richard-Willstätter-Allee, D-76128 Karlsruhe

[b]) Zentrale Spektroskopie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg

[c]) On leave from Central Research Institute for Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59–67, H-1525 Budapest

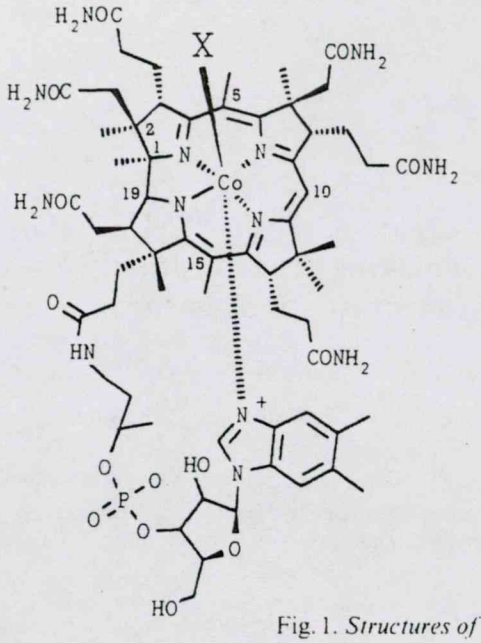
(11.VI.93)

A convergent synthesis of the five novel analogues **1a–e** of (5′-deoxyadenosin-5′-yl)cobalamin (= 'adenosylcobalamin') is described. The analogues **1a–e** carry oligomethylene chains ($C_3$–$C_7$) inserted between the central Co-atom and the 5′-O-atom of the adenosine moiety and are thought to mimick the transition-state geometry in coenzyme-$B_{12}$-catalyzed rearrangement. All five analogues were characterized by NMR, UV, and FAB mass spectrometry.

**Introduction.** – It is generally accepted that the first step in coenzyme-$B_{12}$-dependent enzymic rearrangements is the homolytic cleavage of the Co–C bond of the coenzyme. Recently, a substrate synergysm was shown for methylmalonyl-CoA mutase, *i.e.* homolysis of the Co–C bond in the enzyme-coenzyme complex occurs only upon binding of the substrate [1]. On the basis of EPR measurements, it was postulated that in the activated complex, the paramagnetic centres, *i.e.* $Co^{II}$ and the 5′-$CH_2$ group of adenosine, are at a distance of 6–12 Å [2–4]. Such a drastic change in the coenzyme geometry (and reactivity) must be coupled with a conformational change of the enzyme protein. We devised, therefore, coenzyme-$B_{12}$ analogues mimicking the geometry of the activated complex. In these transition-state or intermediate analogues, the distance between the central Co-atom and the adenosine 5′-O-atom is lengthened by the insertion of a oligomethylene chain. Depending on the length of the chain, the novel analogues are expected to act as more or less strong inhibitors of the coenzyme-$B_{12}$-dependent reactions by binding to the reactive conformation of enzyme proteins.

Here we describe in detail the synthesis and properties of the (5′-deoxyadenosin-5′-yl)cobalamin (= 'adenosylcobalamin') analogues **1a–e** carrying inserts consisting of 3 to 7 $CH_2$ groups between the Co-atom and the 5′-O-atom of adenosine.

**Results and Discussion.** – *Synthesis of the Target [ω-(Adenosin-5′-O-yl)alkyl]cobalamin Derivatives* **1a–e**. On the basis of mechanistic and spectroscopic studies on coenzyme-$B_{12}$-dependent enzymes the transition-state analogues **1a–e** were devised in which the central Co-atom separated from the 5′-*O*-position of adenosine by insertion of shorter or longer $CH_2$ chains ($C_3$ to $C_7$, see *Fig. 1*). Molecular-mechanics calculations showed that the distance between the Co-centre and the 5′-$CH_2$ group of adenosine varies



d$^5$Ado-Cbl: X 5′-deoxyadenosin-5′-yl (Coenzyme $B_{12}$)

HO-Cbl: X OH (Vitamin $B_{12a}$)

**1a–e**: X $-(CH_2)_n-O$

(Transition-state analogues)

Fig. 1. *Structures of substituted cobalamins* (X-Cbl)

from 6.9 to 11.9 Å for the zig-zag chain conformers of **1a–e** consisting of 3–7 $CH_2$ groups, respectively (see *Fig. 2*). Distances in this range were postulated in the activated complex on the basis of EPR measurements [2–4].

To achieve the synthesis of the transition-state analogues a convergent strategy was devised (*Scheme*). Starting from adenosine (**2**) and α,ω-diols **5a–e** ($n$ = 3–7), intermediates **8a–e** ($n$ = 3–7) were prepared which carried an ω-tosyloxy group connected with the 5′-*O*-atom of adenosine through an oligomethylene chain of $C_3$ to $C_7$. Although all reaction steps were conventional, some of them required considerable experimentation for finding optimal conditions. The 2′,3′-*O*-isopropylideneadenosine [5] (**3**) and $N^6$-benzoyl-2′,3′-*O*-isopropylideneadenosine [6–8] (**4**) are known compounds; nevertheless, they were prepared by substantially modified and simplified methods. The protected forms **6a–e** of the α,ω-diols were prepared in moderate yields (39–50%) as colourless oils by an improved method, in analogy with that described for **6d** [9] [10], but involving extractive separation. They showed appropriate $^1$H-NMR characteristics. Subsequent tosylation of **6a–e** with tosyl chloride in pyridine was accompanied with a high degree of elimination. Thus, tosylation was carried out using only a slight excess of pyridine in dry $CH_2Cl_2$ as solvent providing the desired α-(tetrahydro-2*H*-pyranyloxy)-ω-tosyloxy derivatives **7a–e** in 65–81% yield which were characterized by $^1$H- and $^{13}$C-NMR spectroscopy. The key step was the attachment of the tosylates **7a–e** to the doubly protected adenosine **4**. This is essentially a simple $S_N2$ substitution, but the conditions and the quality of the reagents and solvents were crucial. The fully protected chain-lengthened adenosines **8a–e** were obtained in moderate to good yields (60–87%) and characterized by $^1$H- and $^{13}$C-NMR and, in one case (**8d**), by high-resolution mass spectrometry.

The deprotection, and further processing of the intermediates **8a–e**, *via* compounds **9a–e**, **10a–e**, **11a–e**, and **12a–e** is described in detail in the *Exper. Part.* All intermediates were obtained in acceptable yields (56–90%), and their $^1$H- and $^{13}$C-NMR data were consistent with their assumed structures. In the case of compound **10d**, the molecular weight was also confirmed by high-resolution mass spectrometry. The 5′-chain-lengthened adenosine tosylates **12a–e** were then coupled with vitamin $B_{12s}$. The latter was

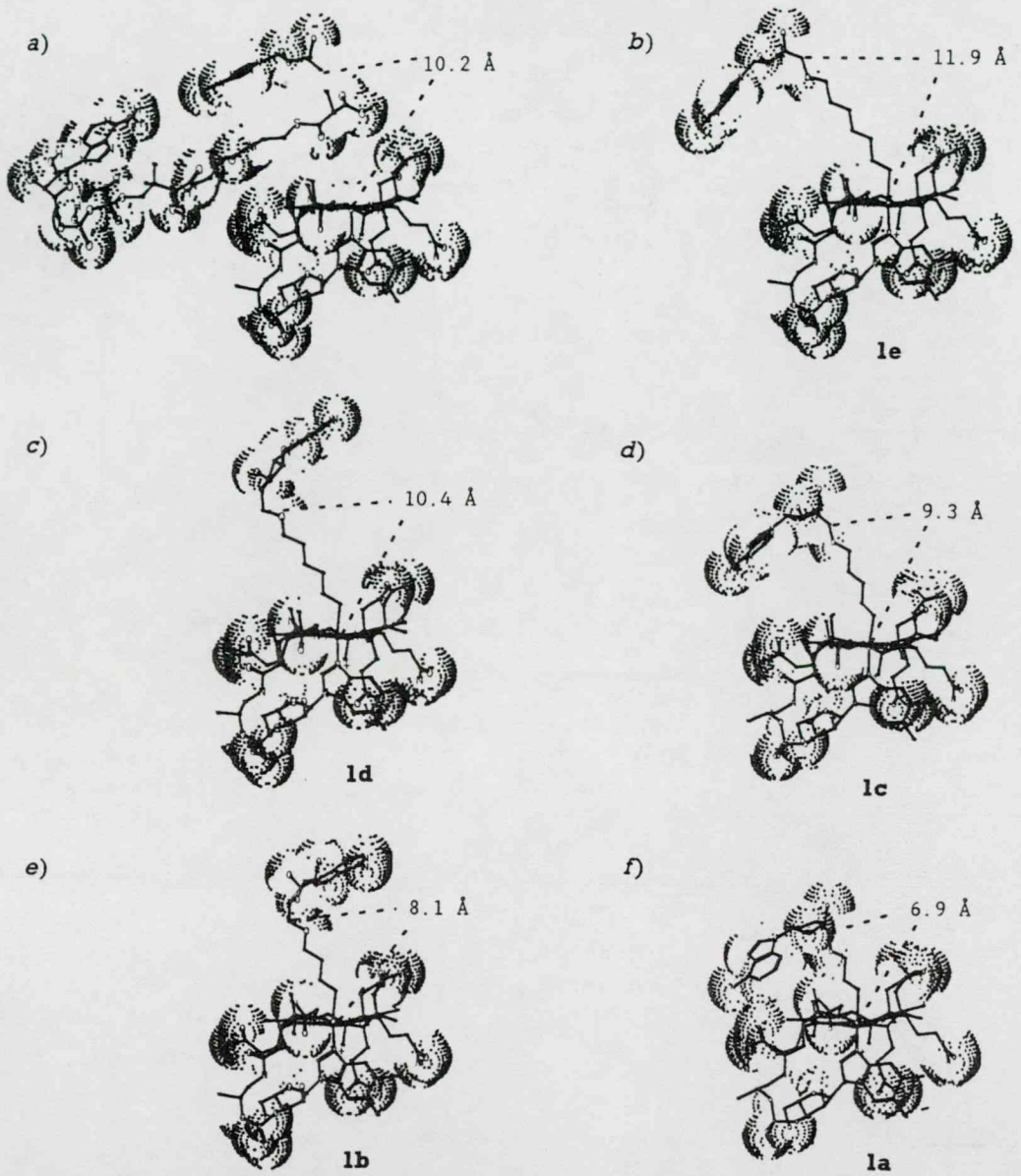
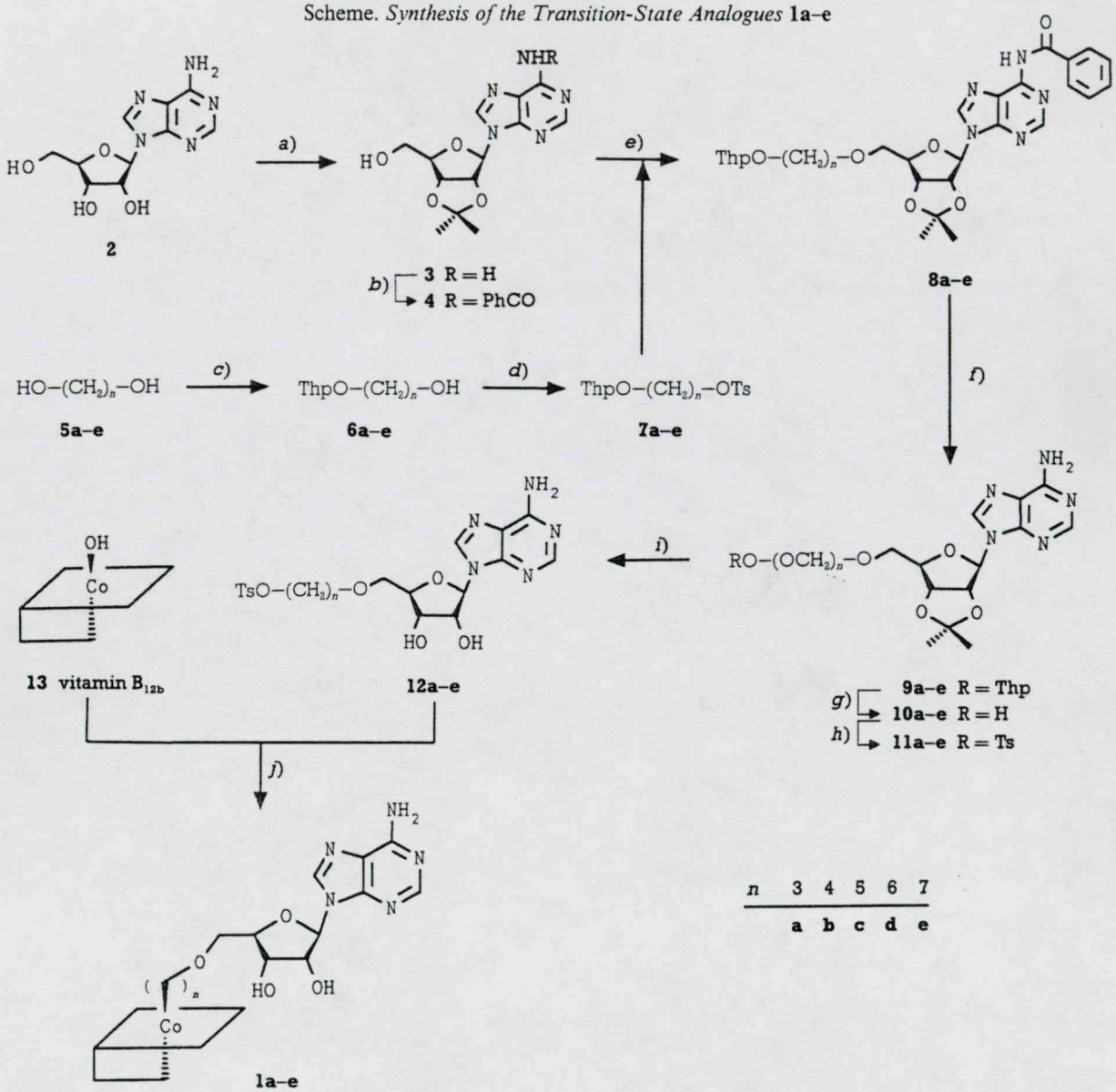



Fig. 2. *Calculated structures and Co–C(5′) distances:* a) *a hypothetical transition state for the methylmalonyl-CoA mutase and* b–f) *transition-state analogues* **1a–e**. Molecular-mechanics calculations were performed on an *IRIS-70-G* computer (*Silicon Graphics*) using PCMODEL 4.0 (*Serena*). X-Ray data of [3-(adenin-9-yl)propyl]cobalamin [17] were applied for building up of the starting structures.

prepared *in situ* from hydroxocobalamin ( = vitamin $B_{12b}$; **13**) by reduction with $NaBH_4$ [11] [12]. In this reaction, pretreatment of the aq. $NaBH_4$ solution with a catalytic amount of a cobalt(II) salt significantly accelerated the rate of the vitamin-$B_{12s}$ formation and increased the yield of the alkylation. After preparative reversed-phase HPLC, the [$\omega$-(adenosin-5′-*O*-yl)alkyl]cobalamins **1a–e** were obtained in 65–80% yield. They were characterized by $^1$H-NMR spectroscopy and, in the case of **1c**, by a COSY spectrum (see below). Fast-atom-bombardment mass spectroscopy (FAB-MS) confirmed not only the expected molecular weights but, owing to the corresponding fragmentation patterns, also the structures of the analogues **1a–e**. Further characterization and purity determination

Scheme. *Synthesis of the Transition-State Analogues* **1a–e**




*a*) Acetone, 70% $HClO_4$ soln., 4-Å molecular sieves. *b*) Pyridine, $Me_3SiCl$; 2. PhCOCl; 3. $MeOH/H_2O$, NaF, $H^+$. *c*) 3,4-Dihydro-2*H*-pyran, THF, cat. TsOH. *d*) TsCl, pyridine, $CH_2Cl_2$. *e*) 1. **4**, NaH, DMF; 2. **7**. *f*) Cat. NaOMe, MeOH. *g*) 2M HCl, MeOH. *h*) TsCl, pyridine, $CH_2Cl_2$. *i*) 10% HCl soln., MeOH, $\Delta$. *j*) 1. **13**, $NaBH_4$, cat. $Co(OAc)_2$, $H_2O$, 2. **12a–e**, $MeOH/H_2O$.

was achieved by UV/VIS spectra and anal. HPLC. The former varied only slightly and were characteristic for alkylated cobalamins [11] [12]. The retention times ($t_R$) in the anal. reversed-phase HPLC were in agreement with the expected differences in polarity. A mixture containing vitamin $B_{12b}$ (**13**), coenzyme $B_{12}$, and all analogues **1a–e** could be cleanly separated into the components with the expected retention times. The analogues **1a–e** are red microcrystalline solids, hygroscopic, and light- and heat-sensitive, but stable when stored in the dark. Aqueous solutions were also stable for days when kept in the dark at 0°.

*$^1$H-NMR Analysis of the [ω-(Adenosin-5′-O-yl)alkyl]cobalamins* **1a–e**. The 1D and 2D COSY $^1$H-NMR spectra of **1a–e** ($n$ = 3–7) were obtained at 500 MHz under conditions close to those used for the published results on coenzyme $B_{12}$ ( = (5′-deoxyadenosin-5′-yl)cobalamin) [13] [14]. The 1D spectra were measured and processed so as to allow precise integration, and for **1a, c, e** ($n$ = 3, 5, 7), the results were accurate enough for the determination of the total number of nonexchangeable protons. During sample preparation, it was noted that the analogues **1a** and **1c** with $n$ = 3 and 5, respectively, were much more soluble than the others, and spectroscopic differences between analogues with even or odd $n$ were also found (see below). Our results and the literature data are summarized in *Tables 1* and *2*. The literature signal assignments provided a starting point for our

Table 1. *500-MHz $^1$H-NMR Data for Coenzyme $B_{12}$ and the Analogues Ado-(CH$_2$)$_n$-Cbl* **1a–e**, Part 1.[a][b])

| | | Chem. shift rel. to TSP[c]) | | | | | |
|---|---|---|---|---|---|---|---|
| | | $CoB_{12}$ | **1a** ($n$ = 3) | **1b** ($n$ = 4) | **1c** ($n$ = 5) | **1d** ($n$ = 6) | **1e** ($n$ = 7) |
| *Corrin Me* | | | | | | | |
| Me(1$^1$) | br. *s* | 0.47 | 0.480 | 0.486 | 0.504 | 0.502 | 0.503 |
| Me(2$^1$) | *s* | 1.36 | 1.364 | 1.356 | 1.375 | 1.376 | 1.377 |
| Me(5$^1$) | *s* | 1.45 | 1.439 | 2.482 | 2.473 | 2.474 | 2.487 |
| Me(7$^1$) | *s* | 1.70 | 1.773 | 1.776 | 1.767 | 1.760 | 1.752 |
| Me(12$^1$) | *s* | 0.87 | 0.841 | 0.861 | 0.767 | 0.813 | 0.801 |
| Me′(12$^1$) | *s* | 1.32 | 1.364 | 1.337 | 1.333 | 1.338 | 1.332 |
| Me(15$^1$) | *s* | 2.43 | 2.367 | 2.305 | 2.381 | 2.344 | 2.388 |
| Me(17$^1$) | *s* | 1.36 | 1.175 | 1.117 | 1.204 | 1.223 | 1.265 |
| *Corrin CH* | | | | | | | |
| CH(3) | *d* | 4.10 | 4.064 | 4.12 | 4.11 | 4.10 | 4.150 |
| CH(8) | *dd* | 3.29 | 3.399 | 3.342 | 3.396 | 3.39 | 3.43 |
| CH(10) | *s* | 5.93 | 5.938 | 5.893 | 5.931 | 5.956 | 5.953 |
| CH(13) | *dd* | 2.89 | 3.033 | 2.954 | 3.012 | 2.960 | 2.99 |
| CH(18) | *dd* | 2.65 | 2.64 | 2.61 | 2.63 | 2.64 | 2.66 |
| CH(19) | *d* | 4.24 | 4.044 | 4.07 | 4.080 | 4.078 | 4.073 |
| *Corrin side-chain CH$_2$* (a = low field, b = high field) | | | | | | | |
| CH$_2$(2$^1$) | *d* 2.41 | | 2.35, 2.31 | 2.36, 2.32 | 2.39, 2.323 | 2.40, 2.34 | 2.41, 2.317 |
| CH$_2$(3$^1$) | *m* 2.06, 1.96 | | 2.08, 1.98 | 2.09, 2.00 | 2.04, 2.00 | 2.02, 1.99 | 2.05, 1.97 |
| CH$_2$(3$^2$) | *ddd* 2.50 | | 2.52, 2.45 | 2.53, 2.45 | 2.57, 2.51 | 2.54, 2.50 | 2.54, 2.48 |
| CH$_2$(7$^1$) | *d* 2.19, 1.72 | | 2.43, 1.910 | 2.47, 1.957 | 2.425, 1.901 | 2.48, 1.924 | 2.46, 1.874 |
| CH$_2$(8$^1$) | *m* 1.75, 0.81 | | 1.84, 0.83 | 1.83, 0.82 | 1.82, 0.82 | 1.80, 0.82 | 1.79, 0.84 |
| CH$_2$(8$^2$) | *ddd* 1.73, 0.88 | | 1.77, 0.936 | 1.77, 0.92 | 1.75, 0.95 | 1.77, 0.95 | 1.70, 0.92 |
| CH$_2$(13$^1$) | *m* 2.22, 2.00 | | 2.06, 2.01 | 2.02, 1.97 | 2.10, 2.00 | 2.10, 2.01 | 2.10, 2.00 |
| CH$_2$(13$^2$) | *ddd* 2.54 | | 2.61, 2.56 | 2.53, 2.48 | 2.53, 2.47 | 2.54, 2.46 | 2.54, 2.46 |
| CH$_2$(17$^1$) | *ddd* 2.45, 2.06[d]) | | 2.44, 2.05 | 2.40, 2.02 | 2.43, 2.05 | 2.42, 2.04 | 2.48, 2.04 |
| CH$_2$(17$^2$) | *ddd* 1.78[d]) | | 2.42, 1.74 | 2.35, 1.68 | 2.40, 1.74 | 2.40, 1.75 | 2.43, 1.76 |
| CH$_2$(18$^1$) | *dd* 2.65 | | 2.66, 2.61 | 2.61, 2.61 | 2.67, 2.62 | 2.65, 2.60 | 2.68, 2.64 |
| *1-Aminopropan-2-ol* (Apr; a = low field, b = high field) | | | | | | | |
| CH$_2$(1)(Apr) | *dd* 3.54, 3.16 | | 3.535, 3.189 | 3.54, 3.191 | 3.538, 3.203 | 3.531, 3.205 | 3.533, 3.188 |
| H–C(2)(Apr) | *m* 4.33 | | 4.353 | 4.358 | 4.359 | 4.36 | 4.353 |
| Me(3)(Apr) | *d* 1.21 | | 1.213 | 1.211 | 1.210 | 1.205 | 1.213 |
| *Total non-exchangeable* H | 81 | | 87[e]) | 89 | 91[e]) | 93 | 95[e]) |

*Table 1* (cont.)

| | | Chem. shift rel to TSP[c]) | | | | | |
|---|---|---|---|---|---|---|---|
| | | $CoB_{12}$ | **1a** ($n = 3$) | **1b** ($n = 4$) | **1c** ($n = 5$) | **1d** ($n = 6$) | **1e** ($n = 7$) |
| *(Dimethylbenzimidazolyl)ribose* (Dbi-Rib) | | | | | | | |
| H–C(2)(Dbi) | *s* | 6.95 | 6.929 | 6.929 | 6.937 | 6.932 | 6.939 |
| H–C(4)(Dbi) | *s* | 6.24 | 6.228 | 6.229 | 6.232 | 6.231 | 6.230 |
| H–C(7)(Dbi) | *s* | 7.16 | 7.169 | 7.157 | 7.162 | 7.159 | 7.159 |
| Me–C(5), | | | | | | | |
| Me–C(6)(Dbi) | *s* | 2.19 | 2.219 | 2.215 | 2.216 | 2.218 | 2.213 |
| H–C(1')(Rib) | *d* | 6.26 | 6.262 | 6.246 | 6.254 | 6.247 | 6.257 |
| H–C(2')(Rib) | *dd* | 4.23 | 4.223 | 4.219 | 4.227 | 4.222 | 4.228 |
| H–C(3')(Rib) | *ddd* | 4.72 | 4.735 | 4.726 | 4.732 | 4.730 | 4.730 |
| H–C(4')(Rib) | *dt* | 4.10 | 4.11 | 4.10 | 4.11 | 4.10 | 4.110 |
| 2 H–C(5')(Rib) | *dd* | 3.88, 3.74 | 3.900, 3.744 | 3.889, 3.736 | 3.898, 3.743 | 3.89, 3.74 | 3.895, 3.745 |
| *Adenosine* (Ade-Rib) | | | | | | | |
| H–C(2)(Ade) | *s* | 8.19 | 8.267 | 8.282 | 8.256 | 8.285 | 8.197 |
| H–C(8)(Ade) | *s* | 8.00 | 8.274 | 8.396 | 8.314 | 8.395 | 8.337 |
| H–C(1')(Rib) | *d* | 5.56 | 6.002 | 6.061 | 6.050 | 6.098 | 6.070 |
| H–C(2')(Rib) | *t(dd)* | 4.54 | 4.685 | 4.726 | 4.696 | 4.659 | 4.703 |
| H–C(3')(Rib) | *t(dd)* | 3.74 | 4.245 | 4.255 | 4.361 | 4.360 | 4.409 |
| H–C(4')(Rib) | *ddd* | 2.54 | 4.11 | 4.167 | 4.204 | 4.258 | 4.275 |
| 2 H–C(5')(Rib) | *dd* | 1.55, 0.57 | 3.535, 3.376 | 3.616, 3.54 | 3.683, 3.559 | 3.765, 3.658 | 3.745, 3.693 |
| *Alkyl-Co* (Abr) | | | | | | | |
| $CH_2$(1")–Co | | | 1.281, 0.49 | 1.37, 0.47 | 1.35, 0.35 | 1.35, 0.50 | 1.33, 0.42 |
| $CH_2$(2") | | | 0.321, −0.181 | 0.35, −0.46 | 0.20, −0.50 | 0.10, −0.49 | 0.10, −0.52 |
| $CH_2$(3") | | | 3.134, 2.936 | 1.22, 1.04 | 0.93, 0.76 | 0.95, 0.79 | 0.87, 0.62 |
| $CH_2$(4") | | | | 3.266, 3.17 | 1.27 | 0.95 | 0.92, 0.86 |
| $CH_2$(5") | | | | | 3.284, 3.22 | 1.31 | 0.96, 0.88 |
| $CH_2$(6") | | | | | | 3.42 | 1.34, 1.32 |
| $CH_2$(7") | | | | | | | 3.42, 3.38 |

[a]) Data for coenzyme $B_{12}$ (6.5 mg in 0.35 ml of 10 mM phosphate/$D_2O$, pD 7.0, 20°) are taken from [13]. This work: *ca.* 1–3 mg of analogue **1a–e** in 0.5 ml of 20 mM phosphate/$D_2O$, pH 7.4, 10°.

[b]) Abbreviations: Apr = 1-aminopropan-2-ol, Dbi = 5,6-dimethylbenzimidazole, Ade = adenine, Rib = ribose, Alk = oligomethylene bridge $(CH_2)_n$ numbered from the Co end. For **1a–e** all assignments were confirmed by observation of the appropriate $^3J$, $^4J$, or $^5J$ cross-peaks in the COSY 2D spectra of at least one analogue.

[c]) TSP = trimethylsilyl propionate; shift values with 3 decimal places were determined from 1D spectra (peak picking); values with 2 decimal places (±0.01 ppm) were estimated from the COSY spectrum.

[d]) The specific assignments for $CH_2(17^1)$ and $CH_2(17^2)$ from ( = $CH_2$(55) and $CH_2$(56), resp. [13]) (based on CH correlations and long-range coupling of C(18) to $CH_2(17^1)$ at *ca.* 2.45 ppm) are probably in error (see text); for the base-off form of coenzyme $B_{12}$ [14], the assignments at pH 2.1 are: $CH_2(17^1)$ at 2.51 and 1.85 and $CH_2(17^2)$ at 2.31 and 1.85 ppm.

[e]) Total proton count confirmed by precise integration.

analysis, and nearly all assignments were independently confirmed through the observation of long-range coupling effects ($^4J$ and $^5J$ in the corrin and benzimidazole rings) in the COSY spectrum (*Footnote 2* in *Table 2*). Using a 60° mixing pulse, multiplet 'tilt' effects could be observed in many cases which allowed vicinal and geminal couplings to be distinguished. The only literature assignments with which we disagree concern the protons $CH_2(17^1)$ and $CH_2(17^2)$. *Bax* and coworkers [13] assigned protons $H_a$–C($17^2$) and $H_b$–C($17^2$) as being nearly equivalent at 1.78 ppm. However, by reason of the integration

Table 2. *J(H,H) and J(P,H) Coupling Constants for Coenzyme $B_{12}$ and the Analogues* **1a–e**[a])

| Coupling | Vicinal and geminal coupling constants in Hz (±0.1) | | | | | |
|---|---|---|---|---|---|---|
| | $CoB_{12}$ | **1a** ($n = 3$) | **1b** ($n = 4$) | **1c** ($n = 5$) | **1d** ($n = 6$) | **1e** ($n = 7$) |
| CH(3)/$H_b$–C(3$^1$) | | 10.4 | | | | |
| CH(8)/$CH_2$(8$^1$)[b]) | | 10.8, 4.9 | 11.5, 5.2 | 11.4, 5.1 | | |
| CH(13)/$CH_2$(13$^1$)[b]) | | 9.2, 2.0 | 7.6, 3.5 | 9.6, 1.7 | | 9 |
| CH(18)/CH(19) | 10.5 | 10.0 | | 10.1 | | 9.9 |
| $H_a$–C(2$^1$)/$H_b$–C(2$^1$) | | –13.3 | | –12.9 | | –12.8 |
| $H_a$–C(7$^1$)/$H_b$–C(7$^1$) | | –13.5 | –13.7 | –13.4 | –13.6 | –13.4 |
| $H_a$–C(1)/$H_b$–C(1)(Apr) | –13.9 | –14.4 | –14.4 | –14.4 | –14.6 | –14.4 |
| $H_a$–C(1)/H–C(2)(Apr) | < 0.9 | 2.7 | | | | 2.7 |
| $H_b$–C(1)/H–C(2)(Apr) | 14.4[c]) | 6.7 | 7.0 | 6.9 | 6.9 | 7.1 |
| H–C(2)/Me(3)(Apr) | 6.3 | 6.4 | 6.4 | | 6.4 | 6.4 |
| H–C(2)(Apr)/P | 7.1 | 7.0 | | | | 7.0 |
| H–C(1′)/H–C(2′)(Dbi-*Rib*) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| H–C(2′)/H–C(3′)(Dbi-*Rib*) | 4.3 | 4.3 | 3.9 | 4.3 | 4.4 | 4 |
| H–C(3′)/H–C(4′)(Dbi-*Rib*) | 8.9 | 8.8 | | 8.7 | | 8.7 |
| H–C(3′)(Dbi-*Rib*)/P | 7.4 | 7.4 | | 7.2 | 7.2 | |
| H–C(4′)/$H_a$–C(5′)(Dbi-*Rib*) | 2.7 | 2.4 | 2.4 | 2.4 | | |
| H–C(4′)/$H_b$–C(5′)(Dbi-*Rib*) | 3.9 | 3.7 | 3.6 | 3.8 | | |
| $H_a$–C(5′)/$H_b$–C(5′)(Dbi-*Rib*) | –13.0 | –13.0 | –12.9 | –13.0 | | –13.0 |
| H–C(1′)/H–C(2′)(Ade-*Rib*) | 3.3 | 4.7 | 5.1 | 4.5 | 4.3 | 4.3 |
| H–C(2′)/H–C(3′)(Ade-*Rib*) | 5.8 | 5.0 | 5.3 | 4.8 | 4.7 | 4.8 |
| H–C(3′)/H–C(4′)(Ade-*Rib*) | 6.7 | 5.2 | 5.1 | 5.3 | 5.2 | 5.2 |
| H–C(4′)/$H_a$–C(5′)(Ade-*Rib*) | < 2.0 | | 2.2 | 2.3 | 2.6 | 2.9 |
| H–C(4′)/$H_b$–C(5′)(Ade-*Rib*) | 9.2 | 6.3 | | 5.3 | 4.9 | 4.9 |
| $H_a$–C(5′)/$H_b$–C(5′)(Ade-*Rib*) | –9.2 | –11.4 | –11.2 | –11.4 | –11.5 | –11.5 |

[a]) See *Footnotes* to *Table 1*; coenzyme $B_{12}$ data is from [14]; in this work, coupling constants were estimated from peak splittings in the 1D spectra wherever possible; the presence of the following long-range couplings was confirmed by COSY cross-peaks for one or more analogues: H–C(4)/H–C(7)(Dbi); H–C(4)/Me–C(5)(Dbi); H–C(4)/Me–C(6)(Dbi); H–C(2)/H–C(4)(Dbi); H–C(2)(Dbi)/H–C(1′)(Rib); H–C(8)(Ade)/H–C(1′)(Rib); Me(2$^1$)/$H_b$–C(2$^1$); Me(7$^1$)/$H_b$–C(7$^1$); CH(13)/Me(15$^1$); CH(13)/Me′(12$^1$); Me(12$^1$)/Me′(12$^1$); CH(10)/Me′(12$^1$); CH(19)/$H_a$–C(2$^1$).

[b]) Assignments of configuration were not made.

[c]) Probably the sum of two coupling constants.

we could clearly see that only 1 H appears near 1.75 ppm, 1 H near 2.05 ppm, and 2 H near 2.45 ppm. In addition, specific correlation peaks for vicinal and geminal couplings involving protons $CH_2$(17$^2$) could be distinguished in the COSY spectrum of **1b**.

Considering the data in *Table 1*, we see that the chemical shifts for the (dimethylbenzimidazolyl)ribose moiety change little with chain length *n* and are very close to the values for the natural coenzyme $B_{12}$. In contrast, the chemical shifts for the adenosine moiety are quite sensitive to the length of the chain due to the expected dependence of anisotropic shielding effects on the distance between the adenosine group and the corrin ring. In coenzyme $B_{12}$ the ribose C(5′) of adenosine is directly bound to Co, whereas in the analogues **1a–e**, it is bound to the ether O-atom of the chain unit, explaining the large difference in shifts of $CH_2$(5′)(Ade-*Rib*). Large shift differences are also observed for H–C(3′)(Ade-*Rib*) and H–C(4′)(Ade-*Rib*). When we consider the changes in chemical

shift for a given adenosine proton as $n$ is increased, an interesting 'alternating' pattern emerges. *E.g.*, for H–C(8)(Ade) starting with $n = 3$, chemical-shift increments for increasing $n$ are +0.12, −0.08, +0.08, −0.06. A similar pattern is found for H–C(2)(Ade) and H–C(1′)(Ade-*Rib*), while H–C(3′)(Ade-*Rib*) shows +0.01, +0.11, 0.00, +0.05 and H–C(4′)(Ade-*Rib*) a monotonic behaviour with increments of 0.057, 0.037, 0.054, 0.017. The 4 corrin protons CH(3), CH(8), CH(13), and CH(19) which point 'up' in the direction of the adenosine group show significant shift differences for the analogues **1a-e** *vs.* coenzyme $B_{12}$. The effect is largest for CH(19) which is close to $H_a$–C(5′)(Ade-*Rib*) (NOE effect) in coenzyme $B_{12}$ [13]. The corrin Me groups Me(12¹)and Me(17¹) also have NOE's with Ade-*Rib* protons in coenzyme $B_{12}$ [13] and show chain-length-dependent shift effects in the analogues. Interesting shift increment patterns are: for Me(12¹), +0.020, −0.094, +0.046, −0.012; for Me(17¹), −0.058, +0.087, +0.019, +0.042; for Me(15¹), −0.062, +0.076, −0.037, +0.044. Again an alternating pattern can be distinguished, and this suggests that the orientation of the adenosine group relative to the corrin ring alternates with increasing methylene chain length, as would be expected, if the chain adopts a relatively stable staggered conformation. It is noteworthy that the solubility of the analogues also shows an alternating pattern with increasing chain length. For the corrin side chains, significant shift perturbations are found only for protons $CH_2(7^1)$ (shown to have NOE's with $CH_2(5^1)$(Ade-*Rib*) coenzyme $B_{12}$ [14]) and $H_a$–C(13¹) which neighbours the perturbed CH(13).

The coupling-constant data of *Table 2* indicate that the most significant differences between coenzyme $B_{12}$ and the analogues **1a–e** occurs in the Ade-*Rib* moiety. The conformation of the ribose ring and the orientation of $CH_2(5')$ group are different in coenzyme $B_{12}$ due to the steric restrictions on attaching the ribose $CH_2(5')$ directly to the Co-atom. These steric constraints are not present when the methylene chain is used for attachment.

Noteworthy are the differences between the chemical shifts of the geminal protons of each chain $CH_2$ group depending on their distance from the Co-atom. Thus, in **1c**, the diastereotopic protons of $CH_2(1'')$–Co exhibit a $\Delta\delta$ of *ca.* 1 ppm, and this is also valid for all other analogues. The $CH_2$ groups in the second ligand sphere of the Co-atom appear at highest field (0.2 and −0.50 ppm, resp.) and show still $\Delta\delta$ values of 0.6–0.7 ppm. The diastereotopy of the $CH_2$ protons in the third, fourth, fifth, sixth, and seventh ligand spheres is still reflected in the $\delta$ values, but is much less pronounced.

**General Discussion and Conclusions.** – The synthesis and use of artificial coenzyme-$B_{12}$ analogues were reported [12] [15]. (Adeninylalkyl)cobalamins, first synthesized by *Hogencamp* [15], show the closest resemblance to the analogues **1a–e** described here and were found to be competitive inhibitors in respect to coenzyme $B_{12}$ in the diol dehydratase reaction [15]. We expect from the novel analogues **1a–e** a stronger binding ability to coenzyme-$B_{12}$-dependent enzymes. In contrast to *Hogenkamp*'s analogues, the present ones contain a ribose moiety that should contribute to binding at the active sites and are closer to the structure of the putative transition state. Preliminary kinetic measurements with methylmalonyl-CoA mutase confirmed the inhibitory capabilities of the novel analogues.

It is noteworthy that analogues of adenosine that are extended at the 5′-site by an alkyl group were found in the naturally occurring hopane series [16]. The role of these compounds in the metabolism of the corresponding bacteria is unknown.

The novel [(adenosin-5′-*O*-yl)alkyl]cobalamins may also serve as ligands promoting the crystallization of coenzyme-$B_{12}$-dependent enzymes. Their detailed biochemical properties and inhibitory behaviour will be published elsewhere.

This work was generously supported by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie*. *L. P.* thanks the *Alexander von Humboldt Foundation* for a postdoctoral fellowship. The authors are grateful to Dr. *U. Zahorszky* for the FAB-MS measurements.

## Experimental Part

1. *General.* Adenosine, $\alpha,\omega$-alkanediols **5a–e**, 3,4-dihydro-2*H*-pyran, benzoyl chloride, toluene-4-sulfonyl chloride, vitamin $B_{12b}$, and $H_2O$-free DMF were products of *Fluka Chemicals*, Switzerland. All sovents were freshly dried and distilled prior to use. HPLC Separations: *Merck-Hitachi-L-6210* pump, *L-4000* UV detector, *D-2500* chromato-integrator, and *Macherey & Nagel* 250 × 4 mm *Nucleosil-10-$C_{18}$* anal. or *Macherey & Nagel* 250 mm × 1″ *Nucleosil-7-$C_{18}$* prep. columns. TLC: *Macherey & Nagel* silica $gel_{254}$ plastic plates; solvent systems: *A*, hexane/acetone 2:1; *B*, hexane/acetone 1:1; *C*, $CH_2Cl_2$/acetone 2:1; *D*, $CH_2Cl_2$/MeOH 10:1; detection by UV light or heating after 3% ethanolic phosphomolybdic acid treatment. Prep. column chromatography (CC) of the intermediates: vacuum CC [18] or flash chromatography (FC) [19]. M.p.: *Büchi* capillary m.p. instrument; uncorrected. UV/VIS Spectra ($\lambda$max($\varepsilon$) in nm): *Perkin-Elmer-Lambda-2* spectrometer; in 0.05M *Tris* buffer (pH 7.5). NMR Spectra: *Bruker-WM-250* or *AM-400* spectrometers for $^1$H and *Bruker-WM-250* spectrometer at 62.90 MHz for $^{13}$C and DEPT experiments; $CDCl_3$ solns. containing $Me_4Si$ as internal standard, unless otherwise stated. Detailed $^1$H-NMR studies of **1a–e**: at 10° and 500 MHz, *Bruker-AM-500* spectrometer; sample preparation in the dark, adding *ca.* 3 mg of each substance to 0.4 ml of 20 mM phosphate/$D_2O$ buffer and adjusting the pH to 7.4 ± 0.05; **1a** and **1c** dissolved completely, **1b** and **1d, e** exhibited much lower solubility (max. 1 mg in 0.5 ml). 1D Spectra: presaturation (3 s) of the residual $H_2O$ resonance, spectral width 7 KHz, 32 K time-domain points, 60° flip angle, 5.3 s repetition time, and 512 transients. Resolution enhancement *via Lorentz-Gauss* lineshape transformation (*Bruker* software) was performed before zero-filling to 64K and *Fourier* transformation. Precise baseline correction, integration, and peak picking were performed using the *Bruker* software routines. COSY 2D Spectra (magnitude-mode) were obtained for each sample using the following conditions: low-power $H_2O$ presaturation during the relaxation delay (2.5 s) and the evolution period, spectral width 4900 Hz, 2K time-domain points in $t_2$, 512 FID's ($t_1$ points) with 24 transients each, mixing pulse flip angle 60°, initial fixed delay of 20 ms in evolution and detection periods to further suppress $H_2O$ and to provide increased intensity for long-range correlations. The data were zero-filled in $t_1$ and, after sine-bell window multiplication, were transformed to give 1*K* × 1*K* magnitude-mode spectra. EI-MS (electron impact) and FAB-MS (fast-atom bombardment): *Finnigan-MAT-90* high-resolution instrument; EI at 70 eV; samples for FAB as 5% glycerol solns.

2. *2′,3′-O-Isopropylideneadenosine* (**3**). A) Adenosine (**2**; 11.0 g, 41.2 mmol), $TsOH \cdot H_2O$ (23.5 g, 124 mmol) and 4 Å molecular sieves (25 g) were mixed in 250 ml of dry acetone and stirred at r.t. After stirring for 2 h (clear soln.→solid precipitate), 12 ml (150 mmol) of pyridine were added (→precipitate nearly dissolved). The mixture was poured on a 11-cm column (∅12.5 cm) filled with neutral $Al_2O_3$ and eluted with 1000 ml of dry MeOH. After evaporation of the product-containing fractions, the solid was recrystallized from acetone: 8.5 g (67%) of pure **3**. TLC: $R_f$ 0.47 (*D*). M.p. 218–220° ([5]: 217.5–218° ($H_2O$)). $^1$H-NMR: (250 MHz, ($D_6$)DMSO): 1.33 (*s*, Me); 1.54 (*s*, Me); 3.54 (*m*, $CH_2$(5′)); 4.20 (*m*, CH(4′)); 4.96 (*dd*, *J*(2′,3′) = 6.1, *J*(3′,4′) = 2.5, CH(3′)); 5.23 (*t*, OH); 5.34 (*dd*, *J*(1′,2′) = 3.3, *J*(2′,3′) = 6.1, CH(2′)); 6.11 (*d*, *J*(1′,2′) = 3.3, CH(1′)); 7.34 (br. *s*, $NH_2$); 8.14 (*s*, CH(2)); 8.32 (*s*, CH(8)). $^1$H-NMR (250 MHz, $CDCl_3$): 1.39 (*s*, Me); 1.64 (*s*, Me); 4.79 (*t*, 1 H, $CH_2$(5′)); 4.97 (*d*, 1 H, $CH_2$(5′)); 4.55 (*s*, CH(4′)); 5.10 (*d*, CH(3′)); 5.21 (*t*, CH(2′)); 5.85 (*d*, CH(1′)); 5.90 (br. *s*, $NH_2$); 6.60 (*d*, OH); 7.84 (*s*, 1 arom. H); 8.33 (*s*, 1 arom. H).

B) Adenosine (**2**; 5.2 g, 19.5 mmol), 70% $HClO_4$ soln. (1.69 ml, 19.5 mmol), and 4 Å molecular sieves (10 g) were mixed in 100 ml of dry acetone and stirred for 2 h at r.t. (→precipitate). A soln. of NaOMe (1.2 g) in MeOH (10 ml) was then added in one portion, the resulting mixture heated to boiling and filtrated, the precipitate washed with 3 × 50 ml of hot acetone, and the combined filtrate slow by cooled to 0° 4.12 g (69%) of white crystalline **3**. Anal. data: as described above.

3. *$N^6$-Benzoyl-2′,3′-O-isopropylideneadenosine* (**4**). A) To a soln. of **3** (3.98 g, 13 mmol) in dry pyridine (15 ml), benzoyl chloride (5.48 g, 4.53 ml, 39 mmol) was added dropwise at r.t. over 15 min. After stirring at r.t. for 4 h, 150 ml of $CH_2Cl_2$ were added. The resulting soln. was washed with 10% HCl soln. (40 ml), sat. $NaHCO_3$ soln. (30 ml),

and brine (30 ml), dried ($MgSO_4$), and evaporated: 7.15 g (95%) of $N^6$,$N^6$,5'-O-*tribenzoyl-2',3'-O-isopropylidene-adenosine*. Yellowish solid. This product was used for the next step without further purification. An anal. sample was obtained by vacuum CC (silica gel (63–200 μm), hexane/acetone 3:1). TLC: $R_f$ 0.68 (*A*). $^1$H-NMR: 1.40 (*s*, Me); 1.63 (*s*, Me); 4.47–4.67 (*m*, $CH_2$(5'), CH(4')); 5.15 (*dd*, *J*(2',3') = 6.7, *J*(3',4') = 4.0, CH(3')); 5.51 (*dd*, *J*(1',2') = 2.9, CH(2')); 6.13 (*d*, CH(1')); 7.24–7.57 (*m*, 3 $H_p$, 6 $H_m$); 7.82 (*m*, 4 $H_o$ of $(Bz)_2N$); 7.93 (*m*, 2 $H_o$ of BzO); 8.13 (*s*, CH(8)); 8.54 (*s*, CH(2)).

To a soln. of $N^6$,$N^6$,5'-*O*-tribenzoyl-2',3'-*O*-isopropylideneadenosine (5.79 g, 10 mmol) in EtOH/$H_2O$ 10:1 (60 ml), finely pulverized NaOH (0.6 g, 15 mmol) was added. The mixture was refluxed for 5 min, and after cooling, the soln. was concentrated to 1/10 volume, diluted with $H_2O$ (30 ml), and extracted with $CH_2Cl_2$ (3 × 40 ml). The extract was washed with $H_2O$ (20 ml) and brine (20 ml), dried ($MgSO_4$), and evaporated and the residue purified by vacuum CC (silica gel, $CH_2Cl_2$/acetone 3:1): 2.1 g (52%) of **4**. White solid. TLC: $R_f$ 0.40 (*C*). M.p. 133–134° ([6]: 132–133° (EtOH); [7]: 151–153° (EtOH)). $^1$H-NMR: 1.38 (*s*, Me); 1.63 (*s*, Me); 3.79 (*dd*, *J* = 2.2, 11, 1 H, $CH_2$(5')); 3.96 (*dd*, *J* = 2.2, 11, 1 H, $CH_2$(5')); 4.52 (br. *s*, CH(4')); 5.07 (*dd*, *J*(3',4') = 3.3, *J*(2',3') = 5.8, CH(3')); 5.22 (*dd*, *J*(1',2') = 5.3, CH(2')); 5.99 (*d*, CH(1')); 7.38–7.62 (*m*, $H_p$, 2 $H_m$); 8.02 (*m*, 2 $H_o$); 8.15 (*s*, CH(8)); 8.74 (*s*, CH(2)). $^{13}$C-NMR: 25.23 (1 Me of $Me_2C$); 27.46 (1 Me of $Me_2C$); 62.70 (C(5')); 81.52 (C(3')); 83.46 (C(2')); 86.48 (C(4')); 93.52 (C(1')); 114.16 ($Me_2C$); 124.07 (C(5)); 128.08 ($C_o$); 128.71 ($C_m$); 132.82 ($C_p$); 133.41 ($C_{ipso}$); 142.64 (C(8)); 150.15 (C(4)); 150.69 (C(6)); 152.23 (C(2)); 165.10 (PhCO).

B) To an ice-cooled soln. of **3** (4.0 g, 13.0 mmol) and 4-(dimethylamino)pyridine (50 mg) in dry pyridine (20 ml), $Me_3SiCl$ (1.76 g, 16.3 mmol, 2.05 ml) was added dropwise over 10 min and the resulting mixture further stirred at r.t. for 1 h. Benzoyl chloride (1.83 g, 13.0 mmol, 1.51 ml) was then added and the mixture cooled to 0° within 10 min. After further stirring at r.t. for 2 h, MeOH/$H_2O$ 6:4 (30 ml), NaF (1.0 g), and $Bu_4NCl$ (100 mg) were added, and stirring at r.t. was continued overnight. The mixture was then diluted with 40 ml of $H_2O$ and the pH adjusted to 2.5 by addition of 5M HCl (*ca.* 40 ml). Extraction with $CH_2Cl_2$ (4 × 50 ml), washing of the combined extracts with 2M HCl (20 ml), sat. $NaHCO_3$ soln. (30 ml), and brine (30 ml), drying ($MgSO_4$), evaporating, and purifying the residue by vacuum CC as described in *Method A* yielded 3.61 g (68%) of **4**.

4. *ω-[(Tetrahydro-2H-pyran-2-yl)oxy]alkan-1-ols* **6a–e**. *General Procedure*. To a soln. of α,ω-alkanediol **5** (0.1 mol) and TsOH (0.1 g) in dry THF (200 ml), 3,4-dihydro-2*H*-pyran (0.1 mol) was added dropwise at 0°. The mixture was stirred at 0° for 1 h and at r.t. for a further h; then 1 ml of $Et_3N$ was added and the solvent evaporated. The residue was taken up in $H_2O$ (15 ml) and MeOH (75 ml) and the bis(tetrahydro-2*H*-pyranyloxy) derivative was removed by extraction with hexane (4–6 × 30 ml). The MeOH was evaporated and the residue diluted with $Et_2O$ (80 ml) washed with $H_2O$ (2–3 × 20 ml) and brine (20 ml), dried ($MgSO_4$), and evaporated: **6** (39–50%), practically homogeneous by TLC.

*3-[(Tetrahydro-2H-pyran-2-yl)oxy]propan-1-ol* (**6a**). According to the *General Procedure*, with propane-1,3-diol (**5a**; 19 g, 0.25 mol, 20 ml) and 3,4-dihydro-2*H*-pyran (8.6 g, 0.10 mol, 9.3 ml): 6.2 g (39%) of **6a**. Colourless oil. TLC: $R_f$ 0.47 (*A*), $R_f$ 0.51 (*B*). $^1$H-NMR: 1.56 (*m*, 2 $CH_2$); 1.6–1.95 (*m*, 2 $CH_2$); 2.94 (*t*, OH); 3.45–3.65 (*m*, $CH_2O$); 3.7–3.96 (*m*, 2 $CH_2O$); 4.61 ('*t*', OCHO).

*4-[(Tetrahydro-2H-pyran-2-yl)oxy]butan-1-ol* (**6b**). According to the *General Procedure*, with butane-1,4-diol (**5b**; 19.7 g, 0.22 mol, 20 ml) and 3,4-dihydro-2*H*-pyran (8.6 g, 0.10 mol, 9.3 ml): 7.9 g (45%) of **6b**. Colourless oil. TLC: $R_f$ 0.49 (*A*), $R_f$ 0.53 (*B*). $^1$H-NMR: 1.34–1.93 (*m*, 5 $CH_2$); 2.53 (br. *s*, OH); 3.36–4.0 (*m*, 3 $CH_2O$); 4.58 ('*t*', OCHO).

*5-[(Tetrahydro-2H-pyran-2-yl)oxy]pentan-1-ol* (**6c**). According to the *General Procedure*, with pentane-1,5-diol (**5c**; 10 g, 96 mmol, 10 ml) and 3,4-dihydro-2*H*-pyran (8.3 g, 99 mmol, 9.0 ml): 8.34 g (46%) of **6c**. Colourless oil. TLC: $R_f$ 0.51 (*A*), $R_f$ 0.55 (*B*). $^1$H-NMR: 1.33–1.91 (*m*, 6 $CH_2$); 1.98 (br. *s*, OH); 3.32–3.94 (*m*, 3 $CH_2O$); 4.56 ('*t*', OCHO).

*6-[(Tetrahydro-2H-pyran-1-yl)oxy]hexan-1-ol* (**6d**). According to the *General Procedure*, with hexane-1,6-diol (**5d**; 15 g, 0.127 mol) and 3,4-dihydro-2*H*-pyran (10 g, 0.12 mol, 10.9 ml): 12.7 g (50%) of **6d**. Colourless oil. TLC: $R_f$ 0.53 (*A*), $R_f$ 0.57 (*B*). $^1$H-NMR: 1.35 (*m*, 2 $CH_2$); 1.4–1.6 (*m*, 4 $CH_2$); 1.91 (br. *s*, OH); 3.3–3.5 (*m*, $CH_2O$); 3.58 (*m*, $CH_2O$); 3.63–3.88 (*m*, 2 $CH_2O$); 4.54 ('*t*', OCHO).

*7-[(Tetrahydro-2H-pyran-2-yl)oxy]heptan-1-ol* (**6e**). According to the *General Procedure*, with heptane-1,7-diol (**5b**; 4.78 g, 36 mmol, 5.0 ml) and 3,4-dihydro-2*H*-pyran (3.0 g, 36.2 mmol, 3.3 ml): 3.6 g (43%) of **6e**. Colourless oil. TLC: $R_f$ 0.54 (*A*), $R_f$ 0.59 (*B*). $^1$H-NMR: 1.2–1.43 (*m*, 3 $CH_2$); 1.4–1.9 (*m*, 5 $CH_2$); 2.49 (br. *s*, OH); 3.26–3.90 (*m*, 3 $CH_2O$); 4.55 (*m*, OCHO).

5. *ω-[(Tetrahydro-2H-pyran-2-yl)oxy] alkyl Toluene-4-sulfonates* **7a–e**. *General Procedure*. To a soln. of **6** (20 mmol) and pyridine (40 mmol) in dry $CH_2Cl_2$ (40 ml), TsCl (4.76 g, 25 mmol) was added portionwise at r.t. and the resulting mixture stirred for 4 h at r.t. Following dilution with $CH_2Cl_2$ (60 ml), the soln. was washed with 5% HCl

soln. (20 ml), sat. $NaHCO_3$ soln. (30 ml), and brine (20 ml), dried ($MgSO_4$), and evaporated and the residue purified by vacuum CC (silica gel, hexane/acetone 3:1): **7** (65–81%).

*3-[(Tetrahydro-2H-pyran-2-yl)oxy]propyl Toluene-4-sulfonate* (**7a**). According to the *General Procedure*, with **6a** (5.5 g, 34 mmol): 8.3 g (78%) of **7a**. Slightly yellow oil. TLC: $R_f$ 0.51 (*A*), $R_f$ 0.75 (*B*). $^1$H-NMR: 1.39–1.85 (*m*, 3 $CH_2$); 1.91 (*m*, $CH_2$); 2.43 (*s*, $MeC_6H_4$); 3.32–3.50 (*m*, $CH_2O$); 3.68–3.80 (*m*, $CH_2O$); 4.14 (*t*, $J = 6.5$, $CH_2OTs$); 4.52 ('*t*', OCHO); 7.33, 7.78 ($A_2B_2$, 4 arom. H). $^{13}$C-NMR: 19.43 (C(4) of Thp); 21.57 ($MeC_6H_4$); 25.37 (C(5) of Thp); 29.24 (C(2)); 30.48 (C(3) of Thp); 62.15 (C(3)); 62.76 (C(1)); 67.75 (C(6) of Thp); 98.82 (C(2) of Thp); 127.87 ($C_o$); 129.85 ($C_m$); 133.04 ($C_p$); 144.76 ($C_{ipso}$).

*4-[(Tetrahydro-2H-pyran-2-yl)oxy]butyl Toluene-4-sulfonate* (**7b**). According to the *General Procedure*, with **6b** (7.8 g, 45 mmol): 9.6 g (65%) of **7b**. Colourless oil. TLC: $R_f$ 0.54 (*A*), 0.77 (*B*). $^1$H-NMR: 1.43–1.92 (*m*, 5 $CH_2$); 2.44 (*s*, $MeC_6H_4$); 3.25–3.52 (*m*, $CH_2O$); 3.61–3.87 (*m*, $CH_2O$); 4.04 (*m*, $CH_2OTs$); 4.53 (*m*, OCHO); 7.32, 7.77 ($A_2B_2$, 4 arom. H). $^{13}$C-NMR: 19.57 (C(4) of Thp); 21.61 ($MeC_6H_4$); 25.42 (C(5) of Thp); 25.66 (C(3)); 26.00 (C(2)); 30.65 (C(3) of Thp); 62.30 (C(4) of Thp); 66.49 (C(6) of Thp); 70.53 (C(1)); 98.81 (C(2) of Thp); 127.86 ($C_o$); 129.83 ($C_m$); 133.14 ($C_p$); 144.70 ($C_{ipso}$).

*5-[(Tetrahydro-2H-pyran-2-yl)oxy]pentyl Toluene-4-sulfonate* (**7c**). According to the *General Procedure*, with **6c** (8.0 g, 43 mmol): 11.9 g (81%) of **7c**. Slightly yellow oil. TLC: $R_f$ 0.57 (*A*), $R_f$ 0.79 (*B*). $^1$H-NMR: 1.34–1.90 (*m*, 6 $CH_2$); 2.43 (*s*, $MeC_6H_4$); 3.26–3.56 (*m*, $CH_2O$); 3.64–3.99 (*m*, $CH_2O$); 4.02 (*t*, $J = 6.4$, $CH_2OTs$); 4.53 ('*t*', OCHO); 7.34, 7.78 ($A_2B_2$, 4 arom. H). $^{13}$C-NMR: 19.66 (C(4) of Thp); 21.61 ($MeC_6H_4$); 22.23 (C(3)); 25.45 (C(5) of Thp); 28.65 (C(4)); 29.03 (C(2)); 30.72 (C(3) of Thp); 62.36 (C(5)); 67.08 (C(6) of Thp); 70.53 (C(1)); 98.87 (C(2) of Thp); 127.84 ($C_o$); 129.83 ($C_m$); 133.15 ($C_p$); 144.69 ($C_{ipso}$).

*6-[(Tetrahydro-2H-pyran-2-yl)oxy]hexyl Toluene-4-sulfonate* (**7d**). According to the *General Procedure*, with **6d** (4.04 g, 20 mmol): 5.62 g (79%) of **7d**. Colourless oil. TLC: $R_f$ 0.60 (*A*), $R_f$ 0.80 (*B*). $^1$H-NMR: 1.31 (*m*, 2 $CH_2$); 1.4–2.0 (*m*, 5 $CH_2$); 2.44 (*s*, $MeC_6H_4$); 3.25–3.51 (*m*, $CH_2O$); 3.62–3.93 (*m*, $CH_2O$); 4.02 (*t*, $J = 6.4$, $CH_2OTs$); 4.54 ('*t*', OCHO); 7.34, 7.78 ($A_2B_2$, 4 arom. H). $^{13}$C-NMR: 19.71 (C(4) of Thp); 21.63 ($MeC_6H_4$); 24.77 (C(4)); 25.09 (C(3)); 25.21 (C(5) of Thp); 28.76 (C(5)); 29.48 (C(2)); 30.76 (C(3) of Thp); 62.43 (C(6)); 67.35 (C(6) of Thp); 70.60 (C(1)); 98.92 (C(2) of Thp); 127.87 ($C_o$); 129.82 ($C_m$); 133.17 ($C_p$); 144.68 ($C_{ipso}$).

*7-[(Tetrahydro-2H-pyran-2-yl)oxy]heptyl Toluene-4-sulfonate* (**7e**). According to the *General Procedure*, with **6e** (3.4 g, 15.7 mmol): 4.18 g (72%) of **7e**. Slightly yellow oil. TLC: $R_f$ 0.62 (*A*), $R_f$ 0.82 (*B*). $^1$H-NMR: 1.27 (*m*, 3 $CH_2$); 1.4–2.0 (*m*, 5 $CH_2$); 2.44 (*s*, $MeC_6H_4$); 3.25–3.51 (*m*, $CH_2O$); 3.62–3.90 (*m*, $CH_2O$); 4.01 (*t*, $J = 6.4$, $CH_2OTs$); 4.55 (*m*, OCHO); 7.34, 7.78 ($A_2B_2$, 4 arom. H).

6. *$N^6$-Benzoyl-2',3'-O-isopropylidene-5'-O-[ω-(tetrahydro-2H-pyran-2-yl)alkyl]adenosines* **8a–e**. *General Procedure*. To a soln. of **4** (1.50 g, 3.65 mmol) in dry DMF (15 ml) under Ar, NaH (120 mg, 5 mmol; 70% content) was added. After stirring at 40° for 5 min, **7a** (4.38 mmol) in dry DMF (1 ml) was added and the resulting mixture further stirred at 50° for 2 h. After evaporation of the main bulk of DMF (4–5 Torr), the residue was purified by FC (silica gel, hexane/acetone 2.1): **8** (60–87%).

*$N^6$-Benzoyl-2',3'-O-isopropylidene-5'-O-[3-(tetrahydro-2H-pyran-2-yl)propyl]adenosine* (**8a**). According to the *General Procedure*, with **4** (0.95 g, 2.3 mmol) and **7a** (0.87 g, 2.76 mmol): 0.76 g (60%) of **8a**. Foamy solid. TLC: $R_f$ 0.46 (*B*), $R_f$ 0.70 (*D*). $^1$H-NMR: 1.38–1.6 (*m*, 3 $CH_2$); 1.41 (*s*, Me); 1.6–1.9 (*m*, 2 $CH_2$); 1.66 (*s*, Me); 3.25–3.9 (*m*, 4 $CH_2O$); 4.49 (*m*, H–C(4')); 4.56 (*m*, OCHO); 4.97 (*m*, H–C(3')); 5.29 (*m*, H–C(2')); 6.27 (*m*, H–C(1')); 7.45–7.6 (*m*, 2 $H_m$, $H_p$); 8.02 (*m*, 2 $H_o$); 8.28 (*s*, H–C(8)); 8.81 (*s*, H–C(2)); 9.38 (br. *s*, NH). $^{13}$C-NMR: 19.61, 19.65 (C(4) of Thp); 25.34 (1 Me of $Me_2C$); 25.38 (C(5) of Thp); 27.20 (1 Me of $Me_2C$); 29.72 (C(2")); 30.65 (C(3) of Thp); 62.37, 62.41 (C(3")); 64.02, 64.18 (C(1")); 68.81, 68.92 (C(6) of Thp); 71.07 (C(5')); 81.85 (C(3')); 85.18, 85.22 (C(2')); 86.22 (C(4')); 91.95, 91.99 (C(1')); 98.89, 98.95 (C(2) of Thp); 114.15 ($Me_2C$); 123.37 (C(5)); 127.93 ($C_m$); 128.77 ($C_o$); 132.70 ($C_p$); 133.70 ($C_{ipso}$); 141.69 (C(8)); 149.51 (C(4)); 151.41 (C(6)); 152.77 (C(2)); 167.76 (C=O).

*$N^6$-Benzoyl-2',3'-O-isopropylidene-5'-O-[4-(tetrahydro-2H-pyran-2-yl)butyl]adenosine* (**8b**). According to the *General Procedure*, with **4** (0.95 g, 2.3 mmol) and **7b** (0.91 g, 2.76 mmol): 0.95 g (73%) of **8b**. Viscous oil. TLC: $R_f$ 0.49 (*B*), $R_f$ 0.73 (*D*). $^1$H-NMR: 1.4–2.0 (*m*, 5 $CH_2$); 1.42 (*s*, Me); 1.65 (*s*, Me); 3.25–3.9 (*m*, 4 $CH_2O$); 4.53 (*m*, H–C(4'), OCHO); 4.96 (*dd*, $J(3',4') = 1.8$, $J(2',3') = 6.4$, H–C(3')); 5.28 (*dd*, $J(1',2') = 1.6$, H–C(2')); 6.28 (*d*, H–C(1')); 7.4–7.6 (*m*, 2 $H_m$, $H_p$); 8.02 ('*d*', 2 $H_o$); 8.27 (*s*, H–C(8)); 8.79 (*s*, H–C(2)); 9.42 (br. *s*, NH). $^{13}$C-NMR: 19.68 (C(4) of Thp); 25.36 (1 Me of $Me_2C$); 25.36 (C(5) of Thp); 26.24 (C(3")); 26.24 (C(2")); 27.21 (1 Me of $Me_2C$); 30.69 (C(3) of Thp); 62.42 (C(4")); 67.10 (C(6) of Thp); 70.96 (C(1")); 71.51 (C(5')); 81.84 (C(3')); 85.21 (C(2')); 86.23 (C(4')); 91.94 (C(1')); 98.92 (C(2) of Thp); 114.14 ($Me_2C$); 123.39 (C(5)); 127.95 ($C_m$); 128.75 ($C_o$); 128.75 ($C_o$); 132.68 ($C_p$); 133.68 ($C_{ipso}$); 141.69 (C(8)); 149.52 (C(4)); 151.41 (C(6)); 152.74 (C(2)); 164.79 (C=O).

*$N^6$-Benzoyl-2',3'-O-isopropylidene-5'-O-[5-(tetrahydro-2H-pyran-2-yl)pentyl]adenosine* (**8c**). According to the *General Procedure*, with **4** (1.23 g, 3.0 mmol) and **7c** (1.23 g, 3.6 mmol): 1.16 g (67%) of **8c**. Foamy solid. TLC:

$R_f$ 0.52 (*B*), $R_f$ 0.75 (*D*). $^1$H-NMR: 1.2–1.4 (*m*, $CH_2$); 1.42 (*s*, Me); 1.4–1.9 (*m*, 5 $CH_2$); 1.66 (*s*, Me); 3.26–3.9 (*m*, 4 $CH_2O$); 4.55 (*m*, H–C(4'), OCHO); 4.96 (*dd*, *J*(3',4') = 1.7, *J*(2',3') = 6.6, H–C(3')); 5.29 (*dd*, *J*(1',2') = 1.8, H–C(2')); 6.28 (*d*, H–C(1')); 7.4–7.6 (*m*, 2 $H_m$, $H_p$); 8.03 ('*d*', 2 $H_o$); 8.29 (*s*, H–C(8)); 8.82 (*s*, H–C(2)); 9.4 (br. *s*, NH).

$N^6$-*Benzoyl-2',3'-O-isopropylidene-5'-O-[6-(tetrahydro-2*H*-pyran-2-yl)hexyl]adenosine* (**8d**). According to the *General Procedure*, with **4** (1.50, 3.65 mmol) and **7d** (1.56 g, 4.38 mmol): 1.89 g (87%) of **8d**. Viscous oil. TLC: $R_f$ 0.56 (*B*), $R_f$ 0.78 (*D*). $^1$H-NMR: 1.15–1.4 (*m*, 2 $CH_2$); 1.42 (*s*, Me); 1.4–1.9 (*m*, 5 $CH_2$); 1.65 (*s*, Me); 3.28–3.9 (*m*, 4 $CH_2O$); 4.54 (*m*, H–C(4'), OCHO); 4.96 (*dd*, *J*(3',4') = 1.6, *J*(2',3') = 6.7, H–C(3')); 5.28 (*dd*, *J*(1',2') = 2.1, H–C(2')); 6.28 (*d*, H–C(1')); 7.46–7.64 (*m*, 2 $H_m$, $H_p$); 8.04 ('*d*', 2 $H_o$); 8.30 (*s*, H–C(8)); 8.82 (*s*, H–C(2)); 9.4 (br. *s*, NH). $^{13}$C-NMR: 19.69 (C(4) of Thp); 25.36 (1 Me of $Me_2C$); 25.44 (C(5) of Thp); 25.82 (C(4")); 25.98 (C(3")); 27.22 (1 Me of $Me_2C$); 29.27 (C(5")); 29.61 (C(2")); 30.73 (C(3) of Thp); 62.37 (C(6")); 67.44 (C(6) of Thp); 70.95 (C(1")); 71.71 (C(5')); 81.86 (C(3')); 85.27 (C(2')); 86.22 (C(4')); 91.95 (C(1')); 98.86 (C(2) of Thp); 114.13 ($Me_2C$); 123.42 (C(5)); 127.95 ($C_m$); 128.76 ($C_o$); 132.67 ($C_p$); 133.69 ($C_{ipso}$); 141.69 (C(8)); 149.51 (C(4)); 151.45 (C(6)); 152.73 (C(2)); 167.78 (C=O). EI-MS: 595 (04, $M^+$), 510 (5), 406 (6), 322 (4), 306 (6), 268 (16), 240 (17), 218 (9), 164 (45), 136 (12), 105 (19), 85 (25), 84 (80), 83 (42), 69 (13), 56 (26), 55 (100), 54 (23), 41 (17), 39 (15). HR-MS: 595.2986 ($M^+$, $C_{31}H_{41}N_5O_7$, calc. 595.3006).

$N^6$-*Benzoyl-2',3'-O-isopropylidene-5'-O-[7-(tetrahydro-2*H*-pyran-2-yl)heptyl]adenosine* (**8e**). According to the *General Procedure*, with **4** (1.23 g, 3.0 mmol) and **7e** (1.33 g, 3.6 mmol): 1.41 g (77%) of **8d**. Viscous oil. TLC: $R_f$ 0.59 (*B*), $R_f$ 0.80 (*D*). $^1$H-NMR: 1.15–1.4 (*m*, 3 $CH_2$); 1.42 (*s*, Me); 1.4–1.92 (*m*, 5 $CH_2$); 1.65 (*s*, Me); 3.3–3.9 (*m*, 4 $CH_2O$); 4.54 (*m*, H–C(4'), OCHO); 4.96 (*dd*, *J*(3',4') = 1.6, *J*(2',3') = 6.6, H–C(3')); 5.28 (*dd*, *J*(1',2') = 2.0, H–C(2')); 6.29 (*d*, H–C(1')); 7.45–7.6 (*m*, 2 $H_m$, $H_p$); 8.03 ('*d*', 2 $H_o$); 8.29 (*s*, H–C(8)); 8.81 (*s*, H–C(2)); 9.3 (br. *s*, NH).

7. *2',3'-O-Isopropylidene-5'-O-[ω-(tetrahydro-2*H*-pyran-2-yl)alkyl]adenosine* **9a–e**. *General Procedure.* A soln. of (3.16 mmol) in MeOH (40 ml) containing NaOMe (0.03 g) was stirred overnight. The product was used in the next step without isolation. An anal. sample (2 ml) was removed and evaporated. The residue was diluted with $CH_2Cl_2$ (10 ml), the soln. washed with $H_2O$ (2 ml) and brine (2 ml), dried, and evaporated, and the residue purified by FC (silica gel, hexane/acetone 1:1): light yellow viscous oil (85–90%; based on the removed proportion of reaction mixture).

*2',3'-O-Isopropylidene-5'-O-[3-(tetrahydro-2*H*-pyran-2-yl)propyl]adenosine* (**9a**). According to the *General Procedure*, with **8a** (740 mg, 1.34 mmol). TLC: $R_f$ 0.18 (*B*), $R_f$ 0.46 (*D*). $^1$H-NMR: 1.41 (*s*, Me); 1.5 (*m*, 2 $CH_2$); 1.64 (*s*, Me); 1.78 (*m*, 2 $CH_2$); 3.3–3.9 (*m*, 4 $CH_2O$); 4.50 (*m*, H–C(4'), OCHO); 4.98 (*m*, H–C(3')); 5.30 (*m*, H–C(2')); 6.18 (*d*, *J* = 1.5, H–C(1')); 6.25 (br. *s*, $NH_2$); 8.06 (*s*, H–C(8)); 8.36 (*s*, H–C(2)). $^{13}$C-NMR: 19.66 (C(4) of Thp); 25.40 (1 Me of $Me_2C$); 25.40 (C(5) of Thp); 27.21 (1 Me of $Me_2C$); 29.78 (C(2")); 30.67 (C(3) of Thp); 62.38 (C(3")); 64.15, 64.21 (C(1")); 68.77, 68.82 (C(6) of Thp); 71.05 (C(5')); 81.79 (C(3')); 85.00 (C(2')); 86.02 (C(4')); 91.39, 91.44 (C(1')); 98.93, 98.96 (C(2) of Thp); 114.11 ($Me_2C$); 119.94 (C(5)); 139.23 (C(8)); 149.47 (C(4)); 153.14 (C(2)); 155.63 (C(6)).

*2',3'-O-Isopropylidene-5'-O-[4-(tetrahydro-2*H*-pyran-2-yl)butyl]adenosine* (**9b**). According to the *General Procedure*, with **8b** (900 mg, 1.59 mmol). TLC: $R_f$ 0.23 (*B*), $R_f$ 0.49 (*D*). $^1$H-NMR: 1.4–1.9 (*m*, 5 $CH_2$); 1.41 (*s*, Me); 1.64 (*s*, Me); 3.3–3.9 (*m*, 4 $CH_2O$); 4.52 (*m*, H–C(4')); 4.55 (*m*, OCHO); 4.97 (*dd*, *J*(3',4') = 1.7, *J*(2',3') = 6.2, H–C(3')); 5.32 (*dd*, *J*(1',2') = 1.4, H–C(2')); 6.29 (*d*, H–C(1')); 6.39 (br. *s*, $NH_2$); 8.07 (*s*, H–C(8)); 8.37 (*s*, H–C(2)). $^{13}$C-NMR: 19.66 (C(4) of Thp); 25.40 (1 Me of $Me_2C$); 25.43 (C(5) of Thp); 26.29 (C(2")); 26.29 (C(3")); 27.21 (1 Me of $Me_2C$); 30.71 (C(3) of Thp); 62.36 (C(4")); 67.14 (C(6) of Thp); 70.95 (C(1")); 71.43 (C(5')); 81.82 (C(3')); 85.00 (C(2')); 86.04 (C(4')); 91.48 (C(1')); 98.85 (C(2) of Thp); 114.09 ($Me_2C$); 119.96 (C(5)); 139.22 (C(8)); 149.46 (C(4)); 153.14 (C(2)); 155.66 (C(6)).

*2',3'-O-Isopropylidene-5'-O-[5-(tetrahydro-2*H*-pyran-2-yl)pentyl]adenosine* (**9c**). According to the *General Procedure*, with **8c** (1.14 g, 1.96 mmol). TLC: $R_f$ 0.29 (*B*), $R_f$ 0.51 (*D*). $^1$H-NMR: 1.15–1.35 (*m*, $CH_2$); 1.41 (*s*, Me); 1.4–1.9 (*m*, 5 $CH_2$); 1.64 (*s*, Me); 3.3–3.95 (*m*, 4 $CH_2O$); 4.52 (*m*, H–C(4')); 4.58 ('*t*', OCHO); 4.99 (*dd*, *J*(3',4') = 1.6, *J*(2',3') = 6.4, H–C(3')); 5.30 (*dd*, *J*(1',2') = 1.5, H–C(2')); 5.77 (br. *s*, $NH_2$); 6.20 (*d*, H–C(1')); 8.05 (*s*, H–C(8)); 8.38 (*s*, H–C(2)). $^{13}$C-NMR: 19.69 (C(4) of Thp); 22.70 (C(3")); 25.39 (1 Me of $Me_2C$); 25.47 (C(5) of Thp); 27.22 (1 Me of $Me_2C$); 29.21 (C(4")); 29.45 (C(2")); 30.75 (C(3) of Thp); 62.40 (C(5")); 67.36 (C(6) of Thp); 71.00 (C(1")); 71.61 (C(5')); 81.84 (C(3')); 85.13 (C(2')); 86.13 (C(4')); 91.67 (C(1')); 98.87 (C(2) of Thp); 114.09 ($Me_2C$); 119.98 (C(5)); 139.40 (C(8)); 149.39 (C(4)); 152.96 (C(2)); 155.24 (C(6)).

*2',3'-O-Isopropylidene-5'-O-[6-(tetrahydro-2*H*-pyran-2-yl)hexyl]adenosine* (**9d**). According to the *General Procedure* with **8a** (1.80 g, 3.03 mmol). TLC: $R_f$ 0.35 (*B*), $R_f$ 0.54 (*D*). $^1$H-NMR: 1.15–1.40 (*m*, 2 $CH_2$); 1.41 (*s*, Me); 1.4–1.9 (*m*, 5 $CH_2$); 1.64 (*s*, Me); 3.3–3.95 (*m*, 4 $CH_2O$); 4.52 (*m*, H–C(4')); 4.57 ('*t*', OCHO); 4.98 (*dd*, *J*(3'.4') = 1.6, *J*(2'.3') = 6.3, H–C(3')); 5.28 (*dd*, *J*(1',2') = 1.3, H–C(2')); 5.8 (br. *s*, $NH_2$); 6.19 (*d*, H–C(1')); 8.06

(*s*, H–C(8)); 8.36 (*s*, H–C(2)). $^{13}$C-NMR: 19.71 (C(4) of Thp); 25.39 (1 Me of $Me_2C$); 25.47 (C(5) of Thp); 25.87 (C(4″)); 26.00 (C(3″)); 27.21 (1 Me of $Me_2C$); 29.33 (C(5″)); 29.61 (C(2″)); 30.76 (C(3) of Thp); 62.38 (C(6″)); 67.50 (C(6) of Thp); 70.96 (C(1″)); 71.68 (C(5′)); 81.84 (C(3′)); 85.11, (C(2′)); 86.10 (C(4′)); 91.58 (C(1′)); 98.85 (C(2) of Thp). 114.05 ($Me_2C$); 119.94 (C(5)); 139.22 (C(8)); 149.46 (C(4)); 153.11 (C(2)); 155.58 (C(6)). EI-MS: 491 (1.6, $M^+$), 476 (3), 462 (12), 306 (9), 218 (23), 164 (100), 136 (25), 85 (23), 55 (10), 43 (6). HR-MS: 491.2726 ($M^+$, $C_{24}H_{37}N_5O_6$, calc. 491.2744).

*2′,3′-O-Isopropylidene-5′-O-[7-(tetrahydro-2H-pyran-2-yl)heptyl]adenosine* (**9e**). According to the *General Procedure*, with **8a** (1.38 g, 2.27 mmol). TLC: $R_f$ 0.39 (*B*), $R_f$ 0.56 (*D*). $^1$H-NMR: 1.15–1.40 (*m*, 3 $CH_2$); 1.42 (*s*, Me); 1.4–1.9 (*m*, 5 $CH_2$); 1.63 (*s*, Me); 3.3–3.95 (*m*, 4 $CH_2O$); 4.51 (*m*, H–C(4′)); 4.56 (‘*t*’, OCHO); 4.96 (*dd*, $J$(3′,4′) = 1.7, $J$(2′,3′) = 6.4, H–C(3′)); 5.29 (*dd*, $J$(1′,2′) = 1.4, H–C(2′)); 5.85 (br. *s*, $NH_2$); 6.19 (*d*, H–C(1′)); 8.06 (*s*, H–C(8)); 8.37 (*s*, H–C(2)). $^{13}$C-NMR: 19.75 (C(4) of Thp); 25.40 (1 Me of $Me_2C$); 25.49 (C(5) of Thp); 25.94 (C(4″)); 26.13 (C(5″)); 27.22 (1 Me of $Me_2C$); 29.23 (C(3″)); 29.34 (C(6″)); 29.66 (C(2″)); 30.79 (C(3) of Thp); 62.44 (C(7″)); 67.62 (C(6) of Thp); 70.98 (C(1″)); 71.75 (C(5′)); 81.86 (C(3′)); 85.13 (C(2′)); 86.12 (C(4′)); 91.68 (C(1′)); 98.88 (C(2) of Thp); 114.06 ($Me_2C$); 120.01 (C(5)); 139.31 (C(8)); 149.32 (C(4)); 153.11 (C(2)); 155.39 (C(6)).

8. *5′-O-(ω-Hydroxyalkyl)-2′,3′-O-isopropylideneadenosines* **10a–e**. *General Procedure*. To a MeOH soln. of crude **9** (from 3.65 mmol of **8**, without isolation), 2M HCl (4 ml) was added and the mixture stirred at r.t. for 2 h. After neutralization (pH 7.5) by sat. $NaHCO_3$ soln., the MeOH, was evaporated. The residue was diluted with $H_2O$ to 20 ml, extracted with $CH_2Cl_2$ (3 × 40 ml), the combined $CH_2CO_2$ soln. washed with brine (20 ml), dried ($MgSO_4$), and evaporated, and the remaining oil purified by FC (silica gel, $CH_2Cl_2$/MeOH 20:1): **10** (56–71%; based on **8**) as yellowish semi-solids.

*5′-O-(3-Hydroxypropyl)-2′,3′-O-isopropylideneadenosine* (**10a**). According to the *General Procedure:* 289 mg (56%; based on **8a**) of **10a**. TLC: $R_f$ 0.28 (*D*). $^1$H-NMR: 1.40 (*s*, Me); 1.64 (*s*, Me); 1.75 (*m*, $CH_2$); 3.4–3.75 (*m*, 3 $CH_2O$); 4.49 (*m*, H–C(4′)); 4.97 (*dd*, $J$(2′,3′) = 6.0, $J$(3′,4′) = 2.1, H–C(3′)); 5.29 (*dd*, $J$(1′,2′) = 1.7, H–C(2′)); 6.18 (*d*, H–C(1′)); 6.5 (br. *s*, $NH_2$); 8.09 (*s*, H–C(8)); 8.33 (*s*, H–C(2)). $^{13}$C-NMR: 25.37 (1 Me of $Me_2C$); 27.17 (1 Me of $Me_2C$); 32.19 (C(2″)); 59.68 (C(3″)); 69.19 (C(1″)); 71.04 (C(5′)); 81.58 (C(3′)); 84.99 (C(2′)); 86.03 (C(4′)); 91.35 (C(1′)); 114.23 ($Me_2C$); 119.74 (C(5)); 139.29 (C(8)); 149.33 (C(4)); 153.24 (C(2)); 155.70 (C(6)).

*5′-O-(4-Hydroxybutyl)-2′,3′-O-isopropylideneadenosine* (**10b**). According to the *General Procedure*: 368 mg (61%; based on **8b**) of **10b**. TLC: $R_f$ 0.31 (*D*). $^1$H-NMR: 1.39 (*s*, Me); 1.5–1.8 (*m*, 2 $CH_2$); 1.66 (*s*, Me); 3.44 (*t*, $J$ = 6.5, $CH_2$(1″)O); 3.5–3.75 (*m*, 2 $CH_2O$); 4.52 (*m*, H–C(4′)); 4.98 (*dd*, $J$(2′,3′) = 6.3, $J$(3′,4′) = 2.3, H–C(3′)); 5.22 (*dd*, $J$(1′,2′) = 1.9, H–C(2′)); 6.26 (*d*, H–C(1′)); 6.8 (br. *s*, $NH_2$); 8.20 (*s*, H–C(8)); 8.35 (*s*, H–C(2)). $^{13}$C-NMR: 25.38 (1 Me of $Me_2C$); 26.23 (C(3″)); 27.19 (1 Me of $Me_2C$); 29.18 (C(2″)); 61.88 (C(4″)); 70.93 (C(1″)); 71.68 (C(5′)); 81.45 (C(3′)); 85.38 (C(2′)); 86.29 (C(4′)); 91.18 (C(1′)); 114.10 ($Me_2C$); 119.51 (C(5)); 139.10 (C(8)); 149.32 (C(4)); 153.27 (C(2)); 155.82 (C(6)).

*5′-O-(5-Hydroxypentyl)-2′,3′-O-isopropylideneadenosine* (**10c**). According to the *General Procedure*: 523 mg (68%; based on **8c**) of **10c**. TLC: $R_f$ 0.36 (*D*). $^1$H-NMR: 1.25–1.65 (*m*, 3 $CH_2$); 1.40 (*s*, Me); 1.65 (*s*, Me); 3.43 (*m*, $CH_2$(1″)O); 3.56 (*dd*, $J$(4′,5′) = 3.5, $J$(5′a,5′b) = 10.0, $H_a$–C(5′)); 3.63 (*t*, $J$ = 6.5, $CH_2$(5″)); 3.73 (*dd*, $J$(4′,5′) = 2.4, $J$(5′a,5′b) = 10.0, $H_b$–C(5′)); 4.53 (*m*, H–C(4′)); 4.97 (*dd*, $J$(2′,3′) = 6.4, $J$(3′,4′) = 2.2, H–C(3′)); 5.22 (*dd*, $J$(1′,2′) = 1.6, H–C(2′)); 5.9 (br. *s*, $NH_2$); 6.27 (*d*, H–C(1′)); 8.19 (*s*, H–C(8)); 8.40 (*s*, H–C(2)). $^{13}$C-NMR: 21.98 (C(3″)); 25.38 (1 Me of $Me_2C$); 27.19 (C(4″)); 29.22 (1 Me of $Me_2C$); 32.47 (C(2″)); 62.17 (C(5″)); 71.01 (C(1″)); 71.88 (C(5′)); 81.42 (C(3′)); 85.57 (C(2′)); 86.65 (C(4′)); 91.55 (C(1′)); 114.02 ($Me_2C$); 119.49 (C(5)); 139.38 (C(8)); 149.40 (C(4)); 153.29 (C(2)); 155.60 (C(6)).

*5′-O-(6-Hydroxyhexyl)-2′,3′-O-isopropylideneadenosine* (**10d**). According to the *General Procedure*: 871 mg (71%; based on **8d**) of **10d**. TLC: $R_f$ 0.39 (*D*). $^1$H-NMR: 1.10–1.85 (*m*, 4 $CH_2$); 1.39 (*s*, Me); 1.66 (*s*, Me); 3.40 (*m*, $CH_2$(1″)O); 3.52 (*dd*, $J$(4′,5′a) = 4.2, $J$(5′a,5′b) = 10.9, $H_a$–C(5′)); 3.62 (*t*, $J$ = 6.5, $CH_2$(6″)); 3.70 (*dd*, $J$(4′,5′) = 2.4, $J$(5′a,5′b) = 10.9, $H_b$–C(5′)); 4.51 (*m*, H–C(4′)); 4.94 (*dd*, $J$(2′,3′) = 6.6, $J$(3′,4′) = 2.3, H–C(3′)); 5.21 (*dd*, $J$(1′,2′) = 1.6, H–C(2′)); 6.23 (*d* + br. *s*, H–C(1′), $NH_2$); 8.15 (*s*, H–C(8)); 8.34 (*s*, H–C(2)). $^{13}$C-NMR: 25.37 (1 Me of $Me_2C$); 25.39 (C(4″)); 25.75 (C(3″)); 27.19 (C(5″)); 29.50 (1 Me of $Me_2C$); 32.48 (C(2″)); 61.84 (C(6″)); 70.95 (C(1″)); 71.61 (C(5′)); 81.65 (C(3′)); 85.48 (C(2′)); 86.41 (C(4′)); 91.59 (C(1′)); 113.99 ($Me_2C$); 119.48 (C(5)); 138.99 (C(8)); 149.39 (C(4)); 153.30 (C(2)); 155.59 (C(6)). EI-MS: 407 (1.6, $M^+$), 306 (9), 218 (23), 164 (100), 136 (25), 85 (23), 55 (10), 43 (6). HR-MS: 407.2151 ($M^+$, $C_{19}H_{29}N_5O_5$, calc. 407.2151).

*5′-O-(7-Hydroxyheptyl)-2′,3′-O-isopropylideneadenosine* (**10e**). According to the *General Procedure*: 611 mg (64%; based on **8e**) of **10e**. TLC: $R_f$ 0.42 (*D*). $^1$H-NMR: 1.25–1.35 (*m*, 3 $CH_2$); 1.35–1.65 (*m*, 2 $CH_2$); 1.41 (*s* Me); 1.66 (*s*, Me); 3.43 (*m*, $CH_2$(1″)O); 3.56 (*dd*, $J$(4′,5′a) = 3.7, $J$(5′a,5′b) = 9.8, $H_a$–C(5′)); 3.69 (*t*, $J$ = 6.5, $CH_2$(7″)); 3.73 (*dd*, $J$(4′,5′b) = 1.8, $J$(5′a,5′b) = 9.8, $H_b$–C(5′)); 4.53 (*m*, H–C(4′)); 4.97 (*dd*, $J$(2′,3′) = 6.3, $J$(3′,4′) = 2.2, H–C(3′)); 5.17 (*dd*, $J$(1′,2′) = 1.6, H–C(2′)); 6.37 (*d*, H–C(1′)); 6.4 (br. *s*, $NH_2$); 8.18 (*s*, H–C(8)); 8.37 (*s*,

H–C(2)). $^{13}$C-NMR: 25.40 (1 Me of $Me_2C$); 25.56 (C(4″)); 26.06 (C(5″)); 29.16 (C(3″)); 29.29 (C(6″)); 29.41 (1 Me of $Me_2C$); 32.51 (C(2″)); 62.05 (C(7″)); 70.85 (C(1″)); 71.56 (C(5′)); 81.46 (C(3′)); 85.68 (C(2′)); 86.28 (C(4′)); 91.47 (C(1′)); 114.02 ($Me_2C$); 119.40 (C(5)); 138.71 (C(8)); 149.39 (C(4)); 153.34 (C(2)); 155.57 (C(6)).

9. *2′,3′-O-Isopropylidene-5′-O-{ω-[(tol-4-yl)sulfonyloxy]alkyl}adenosines* **11a–e**. *General Procedure.* To a soln. of **10** (2.07 mmol) and dry pyridine (3 mmol) in dry $CH_2Cl_2$ (5 ml), TsCl (0.47 g, 2.5 mmol) was added and the resulting mixture stirred at r.t. for 6 h. After diluting with $CH_2Cl_2$ to a volume of 50 ml, the soln. was washed with 5% HCl soln. (10 ml), sat. $NaHCO_3$ soln. (10 ml), and brine (10 ml), dried ($MgSO_4$), and evaporated and the residue purified by CC (silica gel, gradient $CH_2Cl_2$→$CH_2Cl_2$/acetone→acetone (600 ml)); **11** (58–70%) as colourless foamy semi-solid.

*2′,3′-O-Isopropylidene-5′-O-{3-[(tol-4-yl)sulfonyloxy]propyl}adenosine* (**11a**). According to the *General Procedure* with **10a** (250 mg, 0.65 mmol): **11a** (216 mg, 64%). TLC: $R_f$ 0.46 (*D*). $^1$H-NMR: 1.40 (*s*, Me); 1.63 (*s*, Me); 1.77 (*m*, $CH_2$); 2.44 (*s*, $MeC_6H_4$); 3.44 (*t*, $J$ = 6.6, $CH_2$(1″)O); 3.45–3.65 (*m*, $CH_2$(5′)); 3.99 (‘*t*’, $CH_2OTs$); 4.42 (*m*, H–C(4′)); 4.94 (*dd*, $J$(2′,3′) = 6.5, $J$(3′,4′) = 2.7, H–C(3′)); 5.32 (*dd*, $J$(1′,2′) = 2.3, H–C(2′)); 6.10 (br. *s*, $NH_2$); 6.14 (*d*, H–C(1′)); 7.31, 7.73 ($A_2B_2$, 4 arom. H); 7.96 (*s*, H–C(8)); 8.34 (*s*, H–C(2)). $^{13}$C-NMR: 21.67 ($MeC_6H_4$); 25.32 (1 Me of $Me_2C$); 27.16 (1 Me of $Me_2C$); 29.99 (C(2″)); 66.92 (C(3″)); 67.26 (C(1″)); 71.10 (C(5′)); 81.68 (C(3′)); 84.70 (C(2′)); 85.93 (C(4′)); 91.44 (C(1′)); 114.22 ($Me_2C$); 119.93 (C(5)); 127.85 ($C_o$); 129.86 ($C_m$); 132.89 ($C_p$); 139.35 (C(8)); 144.84 ($C_{ipso}$); 149.39 (C(4)); 153.13 (C(2)); 155.53 (C(6)).

*2′,3′-O-Isopropylidene-5′-O-{4-[(tol-4-yl)sulfonyloxy]butyl}adenosine* (**11b**). According to the *General Procedure*, with **10b** (330 mg, 0.87 mmol): **11b** (311 mg, 67%). TLC: $R_f$ 0.49 (*D*). $^1$H-NMR: 1.42 (*s*, Me); 1.4–1.8 (*m*, 2 $CH_2$); 1.65 (*s*, Me); 2.44 (*s*, $MeC_6H_4$); 3.37 (*t*, $J$ = 6.5, $CH_2$(1″)O); 3.4–3.7 (*m*, $CH_2$(5′)); 3.97 (*t*, $J$ = 6.6, $CH_2OTs$); 4.47 (*m*, H–C(4′)); 4.96 (*dd*, $J$(2′,3′) = 6.5, $J$(3′,4′) = 2.5, H–C(3′)); 5.33 (*dd*, $J$(1′,2′) = 2.1, H–C(2′)); 6.15 (br. *s* + *d*, H–C(1′), $NH_2$); 7.33, 7.77 ($A_2B_2$, 4 arom. H); 8.00 (*s*, H–C(8)); 8.34 (*s*, H–C(2)). $^{13}$C-NMR: 21.62 ($MeC_6H_4$); 25.37 (1 Me of $Me_2C$); 25.41 (C(3″)); 25.61 (C(2″)); 27.18 (1 Me of $Me_2C$); 70.19 (C(4″)); 70.59 (C(1″)); 70.97 (C(5′)); 81.70 (C(3′)); 84.80 (C(2′)); 86.01 (C(4′)); 91.43 (C(1′)); 114.19 ($Me_2C$); 119.95 (C(5)); 127.86 ($C_o$); 129.84 ($C_m$); 132.99 ($C_p$); 139.31 (C(8)); 144.75 ($C_{ipso}$); 149.38 (C(4)); 153.09 (C(2)); 155.56 (C(6)).

*2′,3′-O-Isopropylidene-5′-O-{5-[(tol-4-yl)sulfonyloxy]pentyl}adenosine* (**11c**). According to the *General Procedure*, with **10c** (490 mg, 1.25 mmol): **11c** (479 mg, 70%). TLC: $R_f$ 0.53 (*D*). $^1$H-NMR: 1.27 (*m*, $CH_2$(3″)); 1.35–1.47 (*m*, $CH_2$(2″)); 1.41 (*s*, Me); 1.60 (*m*, $CH_2$(4″)); 1.66 (*s*, Me); 2.44 (*s*, $MeC_6H_4$); 3.34 (*t*, $J$ = 6.2, $CH_2$(1″)); 3.56 (*dd*, $J$(4′,5′) = 4.4, $J$(5′a,5′b) = 10.1, $H_a$–C(5′)); 3.66 (*dd*, $J$(4′,5′b) = 2.8, $J$(5′a,5′b) = 10.1, $H_b$–C(5′)); 3.98 (*t*, $J$ = 6.6, $CH_2OTs$); 4.50 (*m*, H–C(4′)); 4.97 (*dd*, $J$(2′,3′) = 6.3, $J$(3′,4′) = 2.4, H–C(3′)); 5.31 (*dd*, $J$(1′,2′) = 2.1, H–C(2′)); 6.19 (*d*, H–C(1′)); 6.22 (br. *s*, $NH_2$); 7.33, 7.78 ($A_2B_2$, 4 arom. H); 8.03 (*s*, H–C(8)); 8.36 (*s*, H–C(2)). $^{13}$C-NMR: 21.62 ($MeC_6H_4$); 21.93 (C(3″)); 25.38 (1 Me of $Me_2C$); 27.19 (1 Me of $Me_2C$); 28.56 (C(4″)); 28.73 (C(2″)); 70.36 (C(5″)); 70.99 (C(1″)); 71.19 (C(5′)); 81.74 (C(3′)); 84.95 (C(2′)); 86.10 (C(4′)); 91.49 (C(1′)); 114.11 ($Me_2C$); 119.98 (C(5)); 127.86 ($C_o$); 129.81 ($C_m$); 133.06 ($C_p$); 139.27 (C(8)); 144.70 ($C_{ipso}$); 149.43 (C(4)); 153.12 (C(2)); 155.62 (C(6)).

*2′,3′-O-Isopropylidene-5′-O-{6-[(tol-4-yl)sulfonyloxy]hexyl}adenosine* (**11d**). According to the *General Procedure* with **10d** (842 mg, 2.07 mmol): **11d** (686 mg, 58%). TLC: $R_f$ 0.56 (*D*). $^1$H-NMR: 1.1–1.3 (*m*, 2 $CH_2$); 1.3–1.47 (*m*, $CH_2$(2″)); 1.41 (*s*, Me); 1.5–1.7 (*m*, $CH_2$(5″)); 1.66 (*s*, Me); 2.44 (*s*, $MeC_6H_4$); 3.37 (*t*, $J$ = 6.4, $CH_2$(1″)); 3.57 (*dd*, $J$(4′,5′) = 4.5, $J$(5′a,5′b) = 10.6, $H_a$–C(5′)); 3.66 (*dd*, $J$(4′,5′) = 2.7, $J$(5′a,5′b) = 10.6, $H_b$–C(5′)); 3.99 (*t*, $J$ = 6.5, $CH_2OTs$); 4.50 (*m*, H–C(4′)); 4.96 (*dd*, $J$(2′,3′) = 6.2, $J$(3′,4′) = 2.4, H–C(3′)); 5.31 (*dd*, $J$(1′,2′) = 2.3, H–C(2′)); 5.95 (br. *s*, $NH_2$); 6.19 (*d*, H–C(1′)); 7.34, 7.78 ($A_2B_2$, 4 arom. H); 8.04 (*s*, H–C(8)); 8.38 (*s*, H–C(2)). $^{13}$C-NMR: 21.63 ($MeC_6H_4$); 25.14 (C(4″)); 25.37 (1 Me of $Me_2C$); 25.39 (C(3″)); 27.20 (1 Me of $Me_2C$); 28.70 (C(5″)); 29.17 (C(2″)); 70.52 (C(6″)); 70.99 (C(1″)); 71.42 (C(5′)); 81.80 (C(3′)); 85.09 (C(2′)); 86.17 (C(4′)); 91.67 (C(1′)); 114.09 ($Me_2C$); 120.00 (C(5)); 127.88 ($C_o$); 129.82 ($C_m$); 133.13 ($C_p$); 139.36 (C(8)); 144.67 ($C_{ipso}$); 149.46 (C(4)); 152.97 (C(2)); 155.35 (C(6)).

*2′,3′-O-Isopropylidene-5′-O-{7-[(tol-4-yl)sulfonyloxy]heptyl}adenosine* (**11e**). According to the *General Procedure*, with **10e** (570 mg, 1.35 mmol): **11e** (497 mg, 61%). TLC: $R_f$ 0.58 (*D*). $^1$H-NMR: 1.1–1.3 (*m*, 3 $CH_2$); 1.35–1.5 (*m* $CH_2$(2″)); 1.42 (*s*, Me); 1.5–1.7 (*m*, $CH_2$(6″)); 1.67 (*s*, Me); 2.44 (*s*, $MeC_6H_4$); 3.37 (*t*, $J$ = 6.4, $CH_2$(1″)); 3.56 (*dd*, $J$(4′,5′) = 4.5, $J$(5′a,5′b) = 10.6, $H_a$–C(5′)); 3.67 (*dd*, $J$(4′,5′b) = 2.7, $J$(5′a,5′b) = 10.6, $H_b$–C(5′)); 3.99 (*t*, $J$ = 6.5, $CH_2OTs$); 4.51 (*m*, H–C(4′)); 4.97 (*dd*, $J$(2′,3′) = 6.2, $J$(3′,4′) = 2.3, H–C(3′)); 5.31 (*dd*, $J$(1′,2′) = 2.2, H–C(2′)); 6.20 (*d*, H–C(1′)); 6.45 (br. *s*, $NH_2$); 7.33, 7.78 ($A_2B_2$, 4 arom. H); 8.07 (*s*, H–C(8)); 8.38 (*s*, H–C(2)). $^{13}$C-NMR: 21.61 ($MeC_6H_4$); 25.18 (C(4″)); 25.38 (1 Me of $Me_2C$); 25.74 (C(5″)); 27.19 (1 Me of $Me_2C$); 28.67 (C(6″)); 28.67 (C(3″)); 29.21 (C(2″)); 70.64 (C(7″)); 70.95 (C(1″)); 71.54 (C(5′)); 81.83 (C(3′)); 85.07 (C(2′)); 86.08 (C(4′)); 91.59 (C(1′)); 114.01 ($Me_2C$); 119.92 (C(5)); 127.86 ($C_o$); 129.78 ($C_m$); 133.03 ($C_p$); 139.18 (C(8)); 144.65 ($C_{ipso}$); 149.40 (C(4)); 153.08 (C(2)); 155.71 (C(6)).

10. *5'-O-{ω-[(Tol-4-yl)sulfonyloxy]adenosines* **12a–e**. *General Procedure.* Derivative **11** (0.24 mmol) was added to a soln. of 10% HCl soln. (0.2 ml) in MeOH (2 ml) and the mixture heated under reflux for 5 min. The mixture was then cooled to r.t. and analyzed by TLC. This 5 min heating/TLC analysis procedure was repeated several times, until the conversion reached a desired degree. The soln. was then cooled and neutralized with sat. $NaHCO_3$ soln. and evaporated. The residue was diluted to 5 ml with $H_2O$ and extracted with $CH_2Cl_2$/acetone 5:2 (3 × 4 ml), the combined extract washed with brine (2 ml), dried ($MgSO_4$), and evaporated, and the residue taken up in $CH_2Cl_2$ (0.5 ml) and purified by FC (silica-gel column (250 × 10 mm), gradient hexane/$CH_2Cl_2$/acetone 1:1:1→$CH_2Cl_2$/MeOH 10:1): 10–30% of recovered **11** and 25–46% of **12**.

*5'-O-{3-[(Tol-4-yl)sulfonyloxy]propyl}adenosine* (**12a**). According to the *General Procedure*, with **11a** (180 mg, 0.35 mmol): 76 mg (46%) of **12a**. Foamy white solid. TLC: $R_f$ 0.32 (*D*). $^1$H-NMR: 1.88 (*m*, $CH_2$(2″)); 2.38 (*s*, *Me*$C_6H_4$); 3.4–3.75 (*m*, $CH_2$(1″), $CH_2$(5′)); 4.08 ('*t*', C*H*$_2$OTs); 4.28 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.64 (*m*, H–C(2′)); 6.08 (br. *s*, H–C(1′)); 6.7 (br. *s*, $NH_2$); 7.24, 7.70 ($A_2B_2$, 4 arom. H); 8.06 (br. *s*, H–C(2) or H–C(8)); 8.08 (*s*, H–C(8) or H–C(2)). $^{13}$C-NMR: 21.58 (*Me*$C_6H_4$); 29.11 (C(2″)); 67.14 (C(3″)); 67.64 (C(1″)); 70.53 (C(5′)); 71.16 (C(3′)); 75.32 (C(2′)); 84.11 (C(4′)); 88.86 (C(1′)); 119.17 (C(5)); 127.83 ($C_o$); 129.91 ($C_m$); 132.71 ($C_p$); 138.95 (C(8)); 144.92 ($C_{ipso}$); 148.92 (C(4)); 152.54 (C(2)); 155.50 (C(6)).

*5'-O-{4-[(Tol-4-yl)sulfonyloxy]butyl}adenosine* (**12b**). According to the *General Procedure*, with **11b** (275 mg, 0.52 mmol): 102 mg (40%) of **12b**. Foamy white solid. TLC: $R_f$ 0.34 (*D*). $^1$H-NMR: 1.65 (*m*, 2 $CH_2$); 2.41 (*s*, *Me*$C_6H_4$); 3.4–3.55 (*m*, $CH_2$(1″)); 3.55–3.8 (*m*, $CH_2$(5′)); 4.02 ('*t*', C*H*$_2$OTs); 4.32 (*m*, H–C(4′)); 4.43 (*m*, H–C(3′)); 4.61 (*m*, H–C(2′)); 6.08 (*d*, *J* = 3.4, H–C(1′)); 6.65 (br. *s*, $NH_2$); 7.28, 7.73 ($A_2B_2$, 4 arom. H); 8.12 (br. *s*, H–C(2) or H–C(8)); 8.17 (*s*, H–C(8) or H–C(2)). $^{13}$C-NMR: 21.60 (*Me*$C_6H_4$); 25.56 (C(3″)); 25.74 (C(2″)); 70.20 (C(4″)); 70.38 (C(1″)); 70.68 (C(5′)); 71.04 (C(3′)); 75.50 (C(2′)); 84.24 (C(4′)); 89.27 (C(1′)); 119.26 (C(5)); 127.83 ($C_o$); 129.88 ($C_m$); 132.86 ($C_p$); 138.95 (C(8)); 144.86 ($C_{ipso}$); 148.79 (C(4)); 152.44 (C(2)); 155.49 (C(6)).

*5'-O-{5-[(Tol-4-yl)sulfonyloxy]pentyl}adenosine* (**12c**). According to the *General Procedure*, with **11c** (440 mg, 0.80 mmol): 145 mg (36%) of **12c**. Foamy white solid. TLC: $R_f$ 0.38 (*D*). $^1$H-NMR: 1.30 (*m*, $CH_2$(3″)); 1.51 (*m*, $CH_2$(2″)); 1.69 (*m*, $CH_2$(4″)); 2.40 (*s*, *Me*$C_6H_4$); 3.4 (*m*, $CH_2$(1″)); 3.45–3.75 (*m*, $CH_2$(5′)); 3.99 (*t*, *J* = 6.4, C*H*$_2$OTs); 4.31 (*m*, H–C(4′)); 4.43 (*m*, H–C(3′)); 4.62 (*m*, H–C(2′)); 6.10 (*d*, *J* = 3.0, H–C(1′)); 6.7 (br. *s*, $NH_2$); 7.27, 7.72 ($A_2B_2$, 4 arom. H); 8.07 (br. *s*, H–C(2) or H–C(8)); 8.18 (*s*, H–C(8) or H–C(2)). $^{13}$C-NMR: 21.60 (*Me*$C_6H_4$); 21.99 (C(3″)); 28.52 (C(2″)); 28.83 (C(4″)); 70.26 (C(1″)); 70.55 (C(5″)); 71.21 (C(3′)); 71.25 (C(5′)); 75.57 (C(2′)); 84.23 (C(4′)); 89.02 (C(1′)); 119.22 (C(5)); 127.82 ($C_o$); 129.85 ($C_m$); 132.91 ($C_p$); 139.05 (C(8)); 144.78 ($C_{ipso}$); 148.34 (C(4)); 152.45 (C(2)); 155.52 (C(6)).

*5'-O-{6-[(Tol-4-yl)sulfonyloxy]hexyl}adenosine* (**12d**). According to the *General Procedure*, with **11a** (643 mg, 1.14 mmol): 148 mg (25%) of **12d**. Foamy white solid. TLC: $R_f$ 0.41 (*D*). $^1$H-NMR: 1.23 (*m*, 2 $CH_2$); 1.47 ('*t*', $CH_2$); 1.55 ('*t*', $CH_2$); 2.41 (*s*, *Me*$C_6H_4$); 3.42 (*t*, *J* = 6.1, $CH_2$(1″)); 3.55–3.75 (*m*, $CH_2$(5′)); 3.96 (*t*, *J* = 6.7, C*H*$_2$OTs); 4.34 (*m*, H–C(4′)); 4.44 ('*t*', H–C(3′)); 4.58 ('*t*', H–C(2′)); 6.10 (*d*, *J*(1′,2′) = 4.0, H–C(1′)); 6.6 (br. *s*, $NH_2$); 7.30, 7.75 ($A_2B_2$, 4 arom. H); 8.08 (br. *s*, H–C(8)); 8.18 (*s*, H–C(2)). $^{13}$C-NMR: 21.61 (*Me*$C_6H_4$); 25.14 (C(4″)); 25.50 (C(3″)); 28.68 (C(5″)); 29.30 (C(2″)); 70.26 (C(6″)); 70.65 (C(1″)); 71.35 (C(3′)); 71.50 (C(5′)); 75.78 (C(2′)); 84.47 (C(4′)); 89.28 (C(1′)); 119.31 (C(5)); 127.84 ($C_o$); 129.83 ($C_m$); 132.99 ($C_p$); 138.40 (C(8)); 144.74 ($C_{ipso}$); 148.85 (C(4)); 152.41 (C(2)); 155.49 (C(6)).

*5'-O-{7-[(Tol-4-yl)sulfonyloxy]heptyl}adenosine* (**12e**). According to the *General Procedure*, with **11e** (461 g, 0.80 mmol): 163 mg (38%) of **12e**. Foamy white solid. TLC: $R_f$ 0.43 (*D*). $^1$H-NMR: 1.2 (*m*, 3 $CH_2$); 1.55 (*m*, 2 $CH_2$); 2.41 (*s*, *Me*$C_6H_4$); 3.43 ('*t*', $CH_2$(1″)); 3.55–3.8 (*m*, $CH_2$(5′)); 3.98 (*t*, *J* = 6.7, C*H*$_2$OTs); 4.33 (*m*, H–C(4′)); 4.46 (*m*, H–C(3′)); 4.64 (*m*, H–C(2′)); 6.13 (*d*, *J*(1′,2′) = 2.7, H–C(1′)); 6.7 (br. *s*, $NH_2$); 7.30, 7.75 ($A_2B_2$, 4 arom. H); 8.08 (br. *s*, H–C(8)); 8.20 (*s*, H–C(2)). $^{13}$C-NMR: 21.60 (*Me*$C_6H_4$); 25.22 (C(4″)); 25.83 (C(3″)); 28.67 (C(5″)); 28.76 (C(2″)); 29.36 (C(6″)); 70.27 (C(7″)); 70.73 (C(1″)); 71.28 (C(3′)); 71.65 (C(5′)); 75.71 (C(2′)); 84.36 (C(4′)); 89.08 (C(1′)); 119.23 (C(5)); 127.84 ($C_o$); 129.83 ($C_m$); 133.02 ($C_p$); 139.02 (C(8)); 144.72 ($C_{ipso}$); 148.85 (C(4)); 152.44 (C(2)); 155.55 (C(6)).

11. *[ω-(Adenosin-5'-O-yl)alkyl]cobalamins* (**1a–e**). *General Procedure* [11][12]. To a soln. of vitamin $B_{12b}$ (**13**; 103 mg, 0.075 mmol) and 2 mg of cobalt(II) acetate in 4 ml of deoxygenated $H_2O$, a soln. of $NaBH_4$ (28 mg, 0.75 mmol) and cobalt(II) acetate (0.5 mg) in 1 ml of deoxygenated $H_2O$ was added under Ar (cherry coloured→brown, then greenish gray) and the resulting soln. stirred at r.t. for 20 min. Then a soln. of **12** (0.10 mmol) in 2 ml of deoxygenated MeOH was added (greenish brown→deep red) and the mixture stirred at r.t. in the dark for 45 min. The soln. was then diluted with 5 ml of 1% AcOH/$H_2O$ and extracted with 50% phenolic $CH_2Cl_2$ (3 × 2 ml). The combined phenolic extracts were diluted with $Et_2O$ (50 ml), and the resulting precipitate was filtered off and washed with dry $CH_2Cl_2$ (2 × 30 ml). Redissolving (with 6 ml of MeOH) and repeating the precipitation with 50 ml of $Et_2O$ yielded crude **1** (81–88%) as light and heat sensitive, hygroscopic, red solids. The crude products were purified by prep. HPLC (λ 280 nm, *v* = 6 ml/min; eluents: 0.01% $CF_3COOH/H_2O$ (*A*) and MeOH (*B*), using a

linear gradient of 30–70% *B* in *A* within 25 min). Typically, a 50-mg portion of crude **1** in 30% MeOH/$H_2O$ (5 ml) was applied onto the column in one run. The product-containing fractions were evaporated in the dark giving the desired pure **1** with 65–80% recovery (direct chromatographic workup of the reaction mixtures without the above described extractive treatment can also be done).

*[3-(Adenosin-5'-O-yl)propyl]cobalamin* (**1a**). According to the *General Procedure*, with **13** (103 mg, 0.075 mmol) and **12a** (48 mg, 0.10 mmol). Purification by HPLC yielded 98.1 mg (80%) of pure **1a**. Anal. HPLC ($v$ = 0.8 ml/min, $\lambda$ 280 nm): $t_R$ 11.4 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.02M $NaH_2PO_4$; *B*, MeOH); $t_R$ 12.6 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.01% ($v/v$) $CF_3COOH/H_2O$; *B* MeOH). UV/VIS: 262.4 (25300), 288.8 (13600), 316.8 (10800), 342.4 (9900), 518.0 (6700). FAB-MS: peak abundance around $[M + H]^+$ (rel. to the most intensive peak of this group): 1637 (6), 1638 (100), 1639 (88), 1640 (83), 1641 (18); fragmentation (the most intensive peaks from the relevant peak groups, normalized to the most intensive peak of the spectrum): 1638 (0.08), 1331 (1), 1070 (0.3), 972 (0.7), 277 (8), 225 (21), 185 (91), 93 (100).

*[4-(Adenosin-5'-O-yl)butyl]cobalamin* (**1b**). According to the *General Procedure*, with **13** (103 mg, 0.075 mmol) and **12b** (49 mg, 0.10 mmol). Purification by HPLC gave 85.4 mg (69%) of pure **1b**. Anal. HPLC ($v$ = 0.8 ml/min, $\lambda$ 280 nm): $t_R$ 12.6 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.02M $NaH_2PO_4$; *B*, MeOH); $t_R$ 13.3 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.01% ($v/v$) $CF_3COOH/H_2O$; *B*, MeOH). UV-VIS: 262.6 (26400), 289.0 (14100), 315.6 (11600), 345.0 (10700), 513.2 (7200). FAB-MS: peak abundance around $[M + H]^+$ (rel. to the most intensive peak of this group): 1651 (3), 1652 (95), 1653 (100), 1654 (47), 1655 (19), 1656 (3); fragmentation (most intensive peaks from the relevant peak groups normalized to the most intensive peak of the spectrum): 1653 (0.3), 1331 (2), 1070 (0.5), 972 (0.2), 369 (3), 277 (10), 185 (98), 93 (100).

*[5-(Adenosin-5'-O-yl)pentyl]cobalamin* (**1c**). According to the *General Procedure*, with **13** (138 mg, 0.10 mmol) and **12c** (66 mg, 0.13 mmol): 148 mg (88%) of crude **1c**. Purification of crude **1c** (120 mg) by HPLC resulted in 92.6 mg of pure **1c**. Anal. HPLC ($v$ = 0.8 ml/min, $\lambda$ 280 nm): $t_R$ 13.3 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.02M $NaH_2PO_4$; *B*, MeOH); $t_R$ 14.2 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.01% ($v/v$) $CF_3COOH/H_2O$; *B*, MeOH). UV/VIS: 262.8 (27400), 289.2 (14500), 314.8 (12400), 344.6 (10700), 511.2 (7500). FAB-MS: peak abundance around $[M + H]^+$ (rel. to the most intensive peak of this group): 1665 (7), 1666 (100), 1667 (87), 1668 (52), 1669 (19), 1670 (1); fragmentation (most intensive peaks from the relevant peak groups, normalized to the most intensive peak of the spectrum): 1666 (0.2), 1331 (2), 1070 (0.8), 972 (1.8), 338 (10), 185 (89), 93 (100).

*[6-(Adenosin-5'-O-yl)hexyl]cobalamin* (**1d**). According to the *General Procedure*, with **13** (103 mg, 0.075 mmol) and **12d** (52 mg, 0.10 mmol): 102.4 mg (81%) of crude **1d**. Purification of a 51-mg portion by HPLC resulting 40.9 mg of pure **1d**. Anal. HPLC ($v$ = 0.8 ml/min, $\lambda$ 280 nm): $t_R$ 14.6 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.02M $NaH_2PO_4$; *B*, MeOH); $t_R$ 15.3 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.01% ($v/v$) $CF_3COOH/H_2O$; *B*, MeOH). UV/VIS: 262.8 (26800), 289.2 (14200), 316.4 (12000), 345.4 (10600), 512.2 (7400). FAB-MS: peak abundance around $[M + H]^+$ (rel. to the most intensive peak of this group): 1680 (85), 1681 (100), 1682 (30), 1683 (2); fragmentation (most intensive peaks from the relevant peak groups, normalized to the most intensive peak of the spectrum): 1681 (12), 1331 (100), 1070 (31), 972 (28), 352 (56), 185 (54), 147 (58), 136 (83), 93 (59).

*[7-(Adenosin-5'-O-yl)heptyl]cobalamin* (**1e**). According to the *General Procedure*, with **13** (138 mg, 0.10 mmol) and **12e** (70 mg, 0.13 mmol): 137.2 mg (81%) of crude **1e**. Purification of a 35-mg portion by HPLC resulted in 22.6 mg of pure **1e**. Anal. HPLC ($v$ = 0.8 ml/min, $\lambda$ 280 nm): $t_R$ 15.8 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.02M $NaH_2PO_4$; MeOH); $t_R$ 16.5 min (0–1 min, 30% *B* in *A*; 1–25 min, 30–70% *B* in *A*; *A*, 0.01% ($v/v$) $CF_3COOH/H_2O$; *B*, MeOH). UV/VIS: 262.6 (28900), 289.2 (15400), 314.8 (13300), 344.8 (11300), 512.0 (8000). FAB-MS: peak abundance around $[M + H]^+$ (rel. to the most intensive peak of this group): 1693 (26), 1694 (84), 1695 (100), 1696 (8), fragmentation (most intensive peaks from the relevant peak groups, normalized to the most intensive peak of the spectrum): 1695 (0.3), 1331 (2.5), 1070 (1), 972 (3.6), 366 (6), 225 (25), 133 (100), 93 (18).

## REFERENCES


[1] Y. Zhao, P. Süch, J. Rétey, *Angew. Chem.* **1992**, *104*, 212; *ibid. Int. Ed.* **1992**, *31*, 215.
[2] G. R. Buettner, R. E. Coffmann, *Biochim. Biophys. Acta* **1977**, *480*, 495.
[3] J. F. Boas, P. R. Hicks, J. R. Pilbrow, *J. Chem. Soc., Faraday Trans. 2* **1978**, *74*, 417.
[4] B. M. Babior, T. H. Moos, W. H. Orme-Johnson, H. Beinert, *J. Biol. Chem.* **1974**, *249*, 4537.
[5] A. Hampton, *J. Chem. Soc.* **1961**, *83*, 3640.
[6] S. Chladek, J. Smrt, *Collect. Czech. Chem. Commun.* **1964**, *29*, 214.
[7] R. S. Ranganathan, G. H. Jones, J. G. Moffat, *J. Org. Chem.* **1964**, *39*, 290.
[8] H. R. Kriecheldorf, M. J. Fehrle, *Makromol. Chem.* **1980**, *181*, 2571.
[9] S. Sakane, K. Maruoka, H. Yamamoto, *Tetrahedron* **1986**, *42*, 2203.
[10] A. Katoh, T. Lu, B. Devadas, S. P. Adams, J. I. Gordon, G. W. Gokel, *J. Org. Chem.* **1991**, *56*, 731.
[11] D. Dolphin, *Methods Enzymol.* **1971**, *18c*, 34.
[12] H. P. C. Hogenkamp, W. H. Pailes, C. Brownson, *Methods Enzymol.* **1971**, *18c*, 57.
[13] M. F. Summers, L. G. Marzilli, A. Bax, *J. Am. Chem. Soc.* **1986**, *108*, 4285.
[14] A. Bax, L. G. Marzilli, M. F. Summers, *J. Am. Chem. Soc.* **1987**, *109*, 566.
[15] T. Toraya, K. Ushio, S. Fukui, H. P. C. Hogenkamp, *J. Biol. Chem.* **1977**, *252*, 963.
[16] G. Flesch, M. Rohmer, *Eur. J. Biochem.* **1988**, *175*, 405.
[17] T. G. Pagnano, L. G. Marzilli, M. M. Flocco, C. Tsai, H. L. Carell, J. P. Glusker, *J. Am. Chem. Soc.* **1991**, *113*, 531.
[18] L. Poppe, L. Novák, *Magy. Kém. Lapja* **1985**, *40*, 366.
[19] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.

# XV. melléklet

**POPPE, L.**, RÉTEY, J.:

[ω-(Adenosin-5'-O-yl)]cobalamins Mimicking the Posthomolysis Intermediate of Coenzyme $B_{12}$-Dependent Rearrangements: Kinetic Investigations on Methylmalonyl-CoA Mutase,

*Arch. Biochem. Biophys.*, **1995**, *316*, 541.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 316, No. 1, January 10, pp. 541–546, 1995

# [ω-(Adenosin-5′-*O*-yl)alkyl]cobalamins Mimicking the Posthomolysis Intermediate of Coenzyme $B_{12}$-Dependent Rearrangements: Kinetic Investigations on Methylmalonyl-CoA Mutase[1]

László Poppe[2] and János Rétey[3]
*Lehrstuhl für Biochemie, Institut für Organische Chemie, Universität Karlsruhe, Karlsruhe, Germany*

Received June 2, 1994, and in revised form September 24, 1994

**Coenzyme-$B_{12}$ analogues carrying oligomethylene chains ($C_3$–$C_7$) inserted between the central Co atom and the 5′-O atom of the adenosine moiety mimicking the putative posthomolysis intermediate in coenzyme $B_{12}$-dependent rearrangements were synthesized and examined for their effects on methylmalonyl-CoA mutase from *Propionibacterium shermanii*. All analogues proved to be inhibitors of methylmalonyl-CoA mutase and in all cases competitive inhibition with respect to coenzyme $B_{12}$ was found. Inhibition constants ($K_i$) were determined by two independent methods and showed in both cases the predicted trend: the $K_i$ values versus chain length had minima at the $C_6$ analogue in which the distance is about 10 Å between the central Co atom and the 5′ carbon of the adenosine, assuming a zig-zag chain conformation. This is the postulated distance between the Co and 5′-methylene paramagnetic centers generated in the methylmalonyl-CoA–coenzyme $B_{12}$ complex after homolytic cleavage of the Co–C bond.** 

***Key Words:*** **[ω-(Adenosin-5′-*O*-yl)alkyl]cobalamins; partial mimics of post-homolysis intermediates; analogues of coenzyme-$B_{12}$; methylmalonyl-CoA mutase (*Propionibacterium shermanii*); kinetic investigations; inhibition constants.**

Coenzyme $B_{12}$ ($CoB_{12}$)[4] is a unique natural product both in respect with its structure and function. By X-ray studies Lehnert and Hodgkin (1) recognized in $CoB_{12}$ a covalent cobalt–carbon bond. It is surprisingly stable in aqueous solution but undergoes homolytic cleavage in $CoB_{12}$-dependent enzymatic reactions (2–4). This homolysis is an essential step of the catalytic cycle. It was suggested that binding to the apoprotein provides the energy for homolysis and also for protection of the highly reactive intermediate from the environment (5). It has also been proposed that the $CoB_{12}$-dependent enzymes promote the cobalt–carbon bond cleavage by application of a stretching force by interaction with the adenosyl moiety and corrin part (6). Babior and his coworkers have suggested on the basis of photolysis and CD studies with (adeninylalkyl)cobalamins and ethanolamine ammonia lyase (6, 7) that the distorting force may originate from the energy released on binding of cobalamins to the active site which induces a conformational change of the enzyme protein and also the adjustment in the corrin ring. In the case of methylmalonyl-CoA mutase (*Propionibacterium shermanii*), however, it was demonstrated that the homolysis of the Co–C bond in the enzyme–$CoB_{12}$ complex occurs only upon binding of the substrate (8). Whatever is the cause of the distorting force responsible for the Co–C bond cleavage, the apoprotein has an energetically favored conformation after homolysis in these complexes. On the basis of ESR studies (9–12), a distance of 6–12 Å has been estimated in the activated complex between the paramagnetic centres, i.e., $Co^{II}$ and the 5′-$CH_2$ group of adenosine.

$CoB_{12}$ analogues mimicking the posthomolysis intermediate may give further information on the structure of the activated complex. We have therefore synthesized

[1] This work was generously supported by the Deutsche Forschungsgemeinschaft, the European Union, and the Fonds der Chemischen Industrie. L.P. thanks the Alexander von Humboldt Foundation for a postdoctoral fellowship (1991–1992) and the Hungarian OTKA Foundation for financial support of a 2-week working visit to Karlsruhe (1993).

[2] Present address: Central Research Institute for Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1525 Budapest, Hungary.

[3] To whom correspondence should be addressed at Lehrstuhl für Biochemie im Institut für Organische Chemie der Universität Karlsruhe, Richard-Willstätter-Allee, D-76128 Karlsruhe, Germany. Fax: 049-721-6084823.

[4] Abbreviations used: $CoB_{12}$, coenzyme $B_{12}$; MDH, L-malate:NAD oxidoreductase.

0003-9861/95 $6.00

$CoB_{12}$ analogues (13) in which the distance between the corrin and the adenosyl moieties, both being essential for binding to the enzyme, were lengthened by insertion of an oligomethylene chain ($C_3$–$C_7$) between the central Co atom and the 5′-*O* atom of adenosine corresponding to the postulated Co–$C_5$ distance of 6–12 Å in the activated complexes. Depending on the chain length, these novel analogues are expected to behave as strong inhibitors of the $CoB_{12}$-dependent reactions by binding to the energetically favored reactive conformation of the enzyme proteins. The strongest inhibition is expected by those members of the homologous series whose structure is closest to the real transition state when bound to the enzyme.

Diverse coenzyme $B_{12}$ analogues have been probed with $CoB_{12}$-dependent enzymes, such as ribonucleotide reductase (14, 15), diol dehydratase (16–26), and ethanolamine ammonia lyase (6, 7). Although the inhibition kinetics of several coenzyme $B_{12}$ analogues was also investigated, it should be mentioned that the majority of previously studied analogues are models of $CoB_{12}$ in its ground state. Exceptions are the adeninyl$(CH_2)_n$cobalamins of longer alkyl chain length (where *n* is between 4 and 9; see Fig. 1) (7, 15, 17, 18) but they may also not be considered as accurate posthomolysis intermediate mimicks since the sugar part of adenosyl moiety, which is likely to contribute substantially to the binding, is lacking. Moreover, according to our molecular mechanics calculations, the length of the alkyl chain in these adeninylalkylcobalamins allows only smaller distances between the corrin and the adeninyl moieties than the corresponding distances postulated in biradical posthomolysis intermediates. Results obtained with these analogues may be extrapolated from one $CoB_{12}$-dependent enzyme to another only with care. This has been demonstrated by the fundamental differences observed between coenzyme $B_{12}$ activities of analogues containing modified $\alpha$-nucleotide base moieties in growth of microorganisms depending on either diol dehydratase or methylmalonyl-CoA mutase (27).

Here we describe in detail the inhibitory behavior of the synthetic posthomolysis intermediate analogues of $CoB_{12}$-carrying oligomethylene chains ($C_3$–$C_7$) inserted between the central Co atom and the 5′-*O* atom of adenosine on methylmalonyl-CoA mutase (*P. shermanii*).

## MATERIALS AND METHODS

*Materials.* Coenzyme $B_{12}$, vitamin $B_{12a}$, adenosine, and succinic anhydride were obtained from Fluka Chemie AG. L-Malate:NAD oxidoreductase (EC 1.1.1.37) (MDH), β-NADH $Li_3$ (NADH), and coenzyme A (CoA) were products of Boehringer Mannheim GmbH. Methylmalonyl-CoA mutase (EC 5.4.99.2), methylmalonyl-CoA epimerase (EC 5.1.99.1), methylmalonyl-CoA carboxyltransferase (EC 2.1.3.1) from *P. shermanii* were isolated and assayed according to previously published methods (28–30). Coenzyme $B_{12}$ analogues ($C_3$–$C_7$) were prepared by alkylation of vitamin $B_{12s}$ with the corresponding tosylates (13). For kinetic measurements coenzyme $B_{12}$ and analogues ($C_3$–$C_7$) were dissolved in bidistilled water at 0–4°C in darkness. The solutions obtained were stable (>95% pure according to HPLC) for weeks when kept under this conditions (13). Structures of cobalamin derivatives used in this study are shown in Fig. 1.

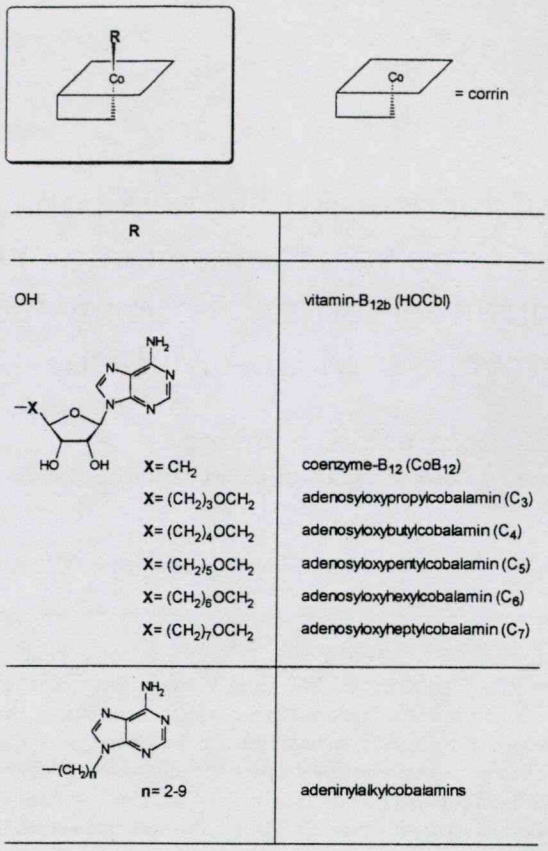



**FIG. 1.** Structures of cobalamin derivatives mentioned in this study.

*Molecular mechanics calculations.* Molecular mechanics calculations were performed by using the MMX modules of PCMODEL 4.0 (Serena, running on a Silicon Graphics Irix 70G workstation) or HyperChem 2.0 (Autodesk, running on an IBM-compatible 486 DX50 PC) program packages.

*Kinetic measurements.* Assay of methylmalonyl-CoA mutase activity in kinetic measurements was based on the method of Zagalak *et al.* (30) with minor modifications. In a microcuvette kept at 30°C Tris–HCl buffer (750 μl, 0.05 M, pH 7.5), sodium pyruvate (100 μl, 0.05 M), NADH (20 μl, 0.01 M), epimerase (20 μl, 2.5 U/ml), transcarboxylase (50 μl, 0.4 U/ml), MDH (10 μl, 30 U/ml), mutase (10 μl, 0.5 U/ml), freshly prepared succinyl-CoA (30 μl, 0.01 M),[5] coenzyme $B_{12}$ (standard, 5 μl, 1 mM; otherwise, 1–5 μl, 0.01–1 mM), and inhibitor (1–5 μl, 0.2–2 mM) solutions were mixed and the decrease of absorbance at 340 nm was recorded for several minutes. All operations with $CoB_{12}$ and the analogues $C_3$–$C_7$ were carried out in the dark. The rate of the succinyl-CoA–methylmalonyl-CoA rearrangement was calculated from

[5] CoA (20 mg) in $NaHCO_3$ solution (1.8 ml, pH 8) was reacted with succinic anhydride (6 mg) at 0°C under argon atmosphere for 30 min, and then the pH value was adjusted to 3.5 with 2 M HCl. The resulting solution was filled up to 2.0 ml with bidistilled water and used as such. HPLC investigation showed that the preparation had about 90% of succinyl-CoA content and starting CoA had been completely consumed.

the change of absorption attributable to the NADH consumption in coupled enzymatic reactions using the equation

$$V = 1000[(\Delta A - \Delta A_0)/\epsilon l],$$

where $V$ is the rate of reaction (nmol·min$^{-1}$·cm$^{-3}$), $\Delta A$ is the change of the absorption (min$^{-1}$), $\Delta A_0$ is the change of the absorption without enzymatic reaction (min$^{-1}$), $\epsilon$ is the absorption coefficient of NADH at 340 nm (6.22 cm$^2$·$\mu$mol$^{-1}$), $l$ is the cell length (1 cm), and 1000 is a conversion factor from micro- to nanomoles. For kinetic calculations a mean rate value measured between 1 and 2 min was used unless otherwise stated.

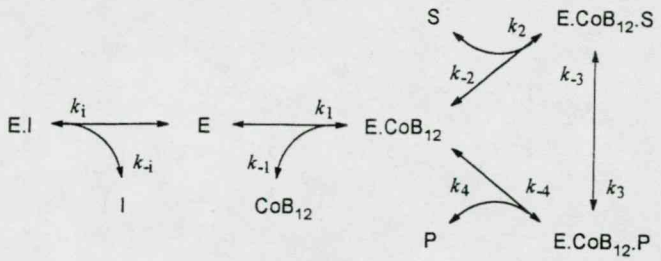
## RESULTS AND DISCUSSION

### *Estimation of the Distance between $Co^{II}$ and 5'-C Radical for $CoB_{12}$-Dependent Enzymes*

The enzymic reactions requiring $CoB_{12}$ have several common features. They catalyze molecular 1,2-rearrangements in which a group and a hydrogen atom are interchanged. In all reactions of this type a key step is the homolysis of the Co–C bond of coenzyme generating $Cbl^{II}$ and the 5'-adenosyl radical. An estimation can be made for the distance between the $Co^{II}$ and the 5'-adenosyl radical in the transition complex after the homolysis if we consider the size of substrate together with the principle of "negative catalysis" (5); i.e., one important role of the apoprotein is to protect the highly reactive intermediates from the environment. Since substrate binding is required to induce homolysis (8) and as a next step H-transfer from the substrate to the adenosyl radical should occur, we can assume that the corresponding portion of the substrate should fit between the $Co^{II}$ and the 5'-adenosyl radical, but the room between these and the substrate should be less than 2.0–2.5 Å, which is enough for insertion of a water molecule.

Using approximate "space requirements" of substrates for several $CoB_{12}$-dependent enzymes (for propanediol and ethanolamine roughly 3–4 Å, while for the methylmalonyl moiety of methylmalonyl-CoA an approximately 6-Å "diameter" has been calculated) a value of about 6–7 Å between the radicals formed by the homolysis of $CoB_{12}$ can be predicted in the case of enzymes acting on relatively small substrates (e.g., diol dehydratase or ethanolamine ammonia lyase), while a value of about 9–10 Å is likely for methylmalonyl-CoA mutase, which catalyzes the rearrangement of a larger substrate. These considerations agree well with data obtained from ESR spectroscopy: a value of about 6 Å was proposed for diol dehydratase (9, 10), whereas a value of about 10–12 Å was postulated for ribonucleotide reductase (11).

For testing the above hypothesis posthomolysis intermediate analogues of $CoB_{12}$ carrying an oligomethylene chain ($C_3$–$C_7$) between the central Co atom and the 5'-*O* atom of adenosine were synthesized (13) and investigated by examining their inhibitory properties on methylmalonyl-CoA mutase from *P. shermanii*.




**SCHEME 1.**

### *Inhibition of Methylmalonyl-CoA Mutase Reaction by $C_3$–$C_7$ Analogues*

According to our preliminary investigations the $C_3$–$C_7$ analogues were found to be competitive inhibitors of the methylmalonyl-CoA mutase reaction with respect of $CoB_{12}$. Competitive inhibition by $CoB_{12}$ analogues may be rationalized by the simplified kinetic model depicted by Scheme 1.

Considering the small $K_m$ value of $CoB_{12}$ (for determination of $K_m$ under our experimental conditions see Fig. 2) and the rate of the S–P rearrangement it is concluded that once $CoB_{12}$ is bound to the apoprotein, it takes part in numerous catalytic cycles (i.e., $k_{-1} \ll k_1$, $k_2$, $k_3$, or $k_4$). Classical inhibition equations are developed for the true substrates; thus, they may only be used for determination of kinetic parameters for inhibition by $CoB_{12}$ analogues if $CoB_{12}$ and its competitor meet with the apoprotein coincidentally. Preincubation of the free enzyme

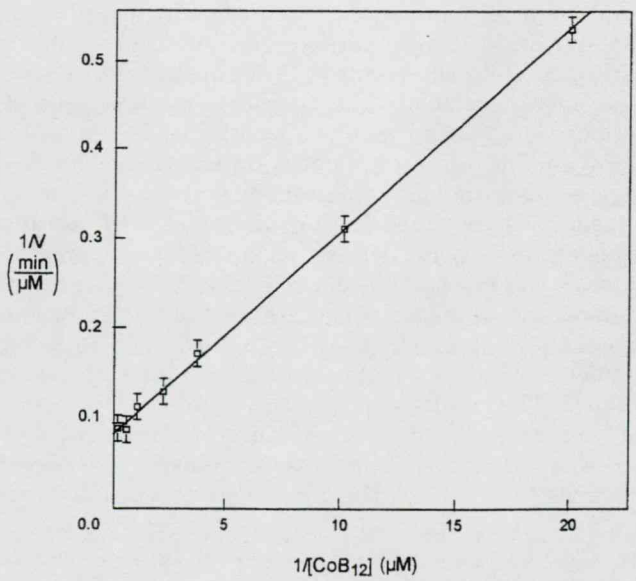



**FIG. 2.** Linweaver–Burk plot for the dependence of methylmalonyl-CoA mutase-catalyzed succinyl-CoA–methylmalonyl-CoA rearrangement on coenzyme $B_{12}$ concentration at 30°C. Vertical bars denote SE of the mean for three separate determinations. (Regression data: $R = 0.997$, standard error = 0.012; kinetic constants obtained from linearized data: $K_m = 0.238$ $\mu$M; $V_{max} = 11.3$ $\mu$M·min$^{-1}$.)

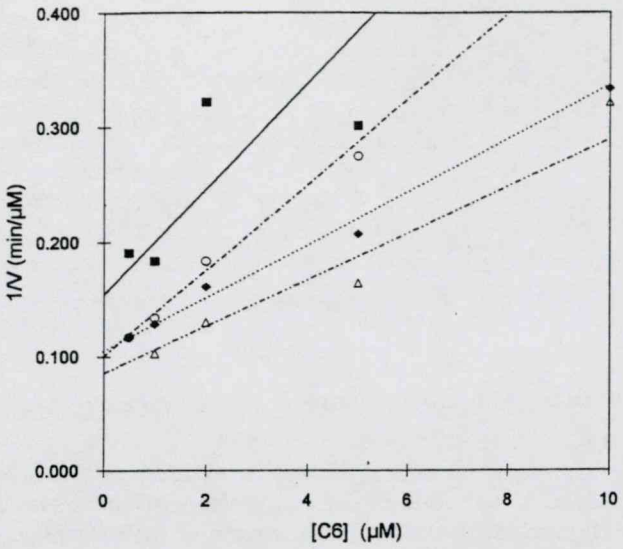



**FIG. 3.** Dixon linearization of data obtained by the "parallel" addition method for determination of the $K_i$ value of analogue $C_6$. Rate measurements were started with synchronous addition of $CoB_{12}$ and analogue $C_6$ to the assay mixture [—, ■: 0.5 μM $CoB_{12}$ ($R$ = 0.47); – · –, ○: 1.0 μM $CoB_{12}$ ($R$ = 0.89); · · ·, ◆: 2.5 μM $CoB_{12}$ ($R$ = 0.91); · · – · ·, △: 5.0 μM $CoB_{12}$ ($R$ = 0.96)]. Individual $K_i$ values were calculated for each line ($K_i$ = (slope · [$CoB_{12}$] · $V_{max}$)/$K_m$) by using the kinetic constants of $CoB_{12}$ obtained under the same conditions (cf. Fig. 2). $K_i$ of analogue $C_6$ determined by this method is the mean ($K_i$ = 0.77 ± 0.18 μM) of these four individual values.

with the inhibitor results in a decreased value for the apparent inhibition constants, whereas addition of the inhibitor after pretreatment with the coenzyme leads to their overestimation. In contrast, the time of substrate addition did not influence the rate of reaction, indicating that our $CoB_{12}$ analogues are not competitive inhibitors with respect to the succinyl-CoA substrate. In our kinetic investigations by the "parallel" method assay reactions were started with the simultaneous addition of $CoB_{12}$ and the inhibitor. Velocity data measured by varying the coenzyme and inhibitor concentrations were analyzed by Dixon linearization. In the case of the analogue $C_6$, details for determination of the apparent $K_i$ value by this method are shown in Fig. 3.

Apparent inhibition constants were obtained for analogues $C_3$–$C_5$ and $C_7$ by the same procedure. The $K_i$ values determined by the parallel method are collected in Table I. The results indicate that all of the $C_3$–$C_7$ analogues are strong competitive inhibitors of the succinyl-CoA–methylmalonyl-CoA rearrangement. Moreover, analogue $C_6$, in which the distance in the zig-zag chain conformation is about 10 Å between the central Co atom and the 5′ carbon of the adenosine (13), proved to be the strongest inhibitor. This is in agreement with the postulated distance between the $Co^{II}$ and 5′-methylene radicals in the activated complex.

The kinetic model depicted in Scheme 1 suggested another possibility for evaluation of the inhibition constants for analogues $C_3$–$C_7$. Since $K_i$ values for the analogues are nearly as small as $K_m$ of $CoB_{12}$, and so $k_i \gg k_{-i}$, releasing an inhibitor when once bound to the enzyme is slow even if compared to the time necessary for the velocity–assay reaction. Accordingly, if the apoprotein is preincubated with one of our inhibitors and then the reaction is started with addition of $CoB_{12}$, in the first moment reaction takes place only with that fraction of the enzyme which remained free. By varying the inhibitor concentration and adding only a minimum amount of $CoB_{12}$, which provides rates corresponding to $V_{max}$ in uninhibited reactions, the concentration of the inhibitor that leads to a rate of $V_{max}/2$ (i.e., $K_i$) can be determined. Thus, analogues $C_3$–$C_7$ were also investigated by this "direct" method. Assay mixtures containing the methylmalonyl-CoA mutase had been preincubated with the inhibitor, and the reaction was started by addition of $CoB_{12}$ and succinyl-CoA. Details of $K_i$ determination by the "direct" method in the case of the analogue $C_6$ are given in Fig. 4. Inhibition constants for analogues $C_3$–$C_5$ and $C_7$ were obtained by the same method. The $K_i$ values determined by the direct method are collected in the second column of Table I. Again, the predicted trend was found: all of the analogues were strong inhibitors and $K_i$ values versus chain length showed a minimum at the $C_6$ analogue.

The fact that all of our inhibitors have $K_i$ values of the same order of magnitude may be rationalized by the conformational flexibility both of the apoprotein and the oligomethylene chain in the posthomolysis intermediate mimics. A dramatic difference between the inhibitory properties of the $CoB_{12}$ analogues cannot be expected since these analogues lie on the reaction path leading from intact $CoB_{12}$ to the completely homolysed and separated biradical intermediate. Nevertheless, the maximum inhibition was found at the $C_6$ analogue, in which the allowed distance between Co and 5′-C of the adenosyl moiety is closest to the postulated one in the posthomolysis intermediate, irrespective of the method of determination.

TABLE I
Inhibition Constants ($K_i$) of Analogues $C_3$–$C_7$ Obtained by "Parallel" and "Direct" Methods[a]

| Analogue | Parallel $K_i$ (μM) | Direct $K_i$ (N.P., $R$)[b] (μM) |
|---|---|---|
| $C_3$ | 2.48 ± 0.58 | 0.97 ± 0.15 (9, 0.99) |
| $C_4$ | 1.45 ± 0.29 | 0.77 ± 0.17 (8, 0.98) |
| $C_5$ | 1.13 ± 0.56 | 0.62 ± 0.10 (8, 0.99) |
| $C_6$ | 0.77 ± 0.18 | 0.48 ± 0.12 (8, 0.96) |
| $C_7$ | 2.10 ± 0.81 | 0.65 ± 0.19 (9, 0.93) |

[a] $K_i$ values of analogues $C_3$–$C_7$ were obtained in a manner similar to that described for analogue $C_6$ (c.f. Fig. 3. and Fig. 4. for parallel and direct methods for determination of $K_i$, respectively).

[b] In parentheses: N.P., number of experimental data points; $R$, regression coefficient in Dixon linearization.

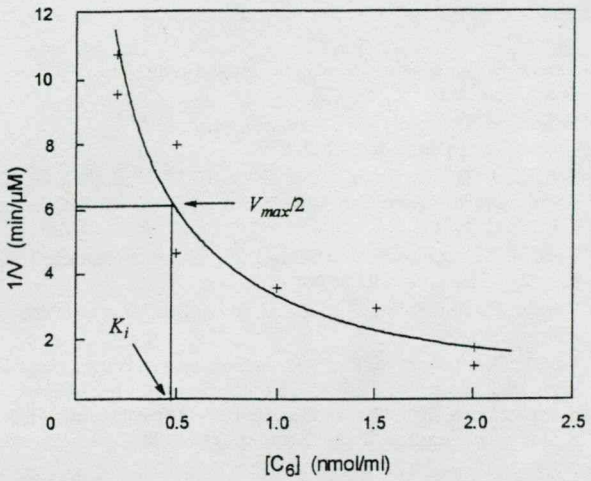



**FIG. 4.** Determination of the $K_i$ value for analogue $C_6$ by the "direct" method. Symbols (+) represents experimental values obtained by preincubation of the assay system with analogue $C_6$ for 60 s and starting the reaction by addition of $CoB_{12}$ (2.5 nmol). Curve (—) was calculated by using the parameters of line fitted ($R$ = 0.963) to the experimental points by Dixon linearization. The curve projected to the [$C_6$] axis at the rate corresponding to $V_{max}/2$ (6.2 ml/μM) gave a value of the inhibition constant ($K_i$) of 0.48 ± 0.12.

Differences in the $K_i$ values obtained by the two methods may arise from several factors. The real kinetic model may be more complicated than that depicted in Scheme 1. The apoprotein which is built up from two nonidentical subunits (31, 32) may contain more than one binding region for $CoB_{12}$ with different binding properties toward the individual posthomolysis intermediate analogues and $CoB_{12}$. This assumption is supported by the results of titration of $CoB_{12}$ bound to mutase from *P. shermanii* by circular dichroism measurements indicating binding of more than 1 mol $CoB_{12}$ to 1 mol mutase (33).

The strong inhibition of the mutase reaction by all of the analogues, however, indicates availability of the active site also for the larger posthomolysis intermediate mimicking analogues. Thus, the active site is probably located near to the protein surface and its size should be changed dynamically during a catalytic cycle. If the direct method is used, binding of the $CoB_{12}$ analogue to the enzyme protein is static (none of the enzyme molecules take part in enzymic reaction) resulting in "frozen" inhibitor–protein complexes. In contrast, by using the "parallel" method binding to the protein is a dynamic process; the inhibitor kinetically competes with the smaller $CoB_{12}$.

### *Inhibition Experiments with Hydroxycobalamin, Adenosine, and CoASH*

Exchange experiments carried out with the analogues $C_3$–$C_7$ and $CoB_{12}$ indicated (parts of the results are compiled in Table II) that once a transition state analogue is bound to the enzyme protein, $CoB_{12}$ cannot replace the inhibitor even after several minutes. Similarly, when the enzyme was preincubated with $CoB_{12}$, the inhibition level of the parallel addition was reached only after several minutes. Data indicate that molecules in which both the corrin and adenosyl moieties are present bind tightly to the enzyme protein. In addition to the binding regions for the corrin and adenosyl moieties, a third one for the CoA substrate is necessary for the enzyme action as indicated by the fact that homolysis of the Co–C bond in the enzyme–$CoB_{12}$ complex occurs only upon binding of the substrate (8).

We thought it worthwhile to investigate the inhibitory properties of molecules having binding ability to only one of the above mentioned three binding areas. Thus, inhibition of the mutase reaction was investigated by addition of CoASH (possible competitor for the CoA-substrate-binding site), hydroxycobalamin (competitor for the corrin-binding area), and adenosine (competitor for the adenosyl-binding region) as single inhibitors and also in admixtures. The results (Table II) indicate that none of these compounds have a dramatic inhibitory effect even at concentrations four times higher than that necessary to reach about 50% inhibition with the analogues $C_3$–$C_7$. Interestingly, after a short inhibition period HOCbl seemed to be a slight activator, even in admixture with CoASH and adenosine at high concentration [it is interesting to mention here that diol dehydratase, in contrast to the mutase, is strongly inhibited by methyl- and cyano- (17) or hydroxycobalamin (18)]. In the case of methylmalonyl-CoA mutase only one of the two $CoB_{12}$-binding sites is likely to be catalyt-

TABLE II

Effect of Analogues $C_3$–$C_7$, Hydroxycobalamin, Adenosine, and CoASH on the Reaction of Mutase from *P. shermanii*[a]

| | $V_i/V_{max}$ (%)[b] | |
|---|---|---|
| Inhibitor (concentration, μM) | 2 min | 4 min |
| $C_3$ (0.5) | 48 | 65 |
| $C_4$ (0.5) | 51 | 61 |
| $C_5$ (0.5) | 52 | 67 |
| $C_6$ (0.5) | 51 | 61 |
| $C_7$ (0.5) | 51 | 61 |
| CoASH (2) | 74 | 100 |
| Adenosine (2) | 93 | 100 |
| HOCbl (2) | 87 | 105 |
| HOCbl, CoASH, adenosine (1, each) | 84 | 110 |
| HOCbl, adenosine (20, each) | 88 | 105 |

[a] Rates of reactions were measured by using the assay described under Materials and Methods but at 37°C. In each case 2.0 μM of $CoB_{12}$ was added to the assay mixture preincubated with the compounds given below for 60s.

[b] Percentage rate of the "inhibited" reaction compared to the rate of the noninhibited reaction at the same moment.

ically active, but binding a second $CoB_{12}$ molecule to the catalytically inactive site may increase the catalytic ability of the active site. This would allow a slight activation by HOCbl when it remained bound to the catalytically inactive site but was removed from the active site.

The above results indicate that for a tight and long-term binding to the mutase protein simultaneous occupation of corrin and adenosyl binding areas is not enough, but a functional relation should also exist between the two binding parts.

## ACKNOWLEDGMENT

The authors are grateful to Ms. I. Merkler for the preparation of the enzymes from *P. shermanii*.

## REFERENCES

1. Lehnert, P. G., and Hodgkin, D. C. (1962) *Nature* **192**, 937–940.
2. Zagalak, B., and Friedrich, W. (Eds.) (1979) Vitamin $B_{12}$, de Gruyter Verlag, Berlin/New York.
3. Babior, B. M. (1988) *BioFactors* **1**, 21–26.
4. Rétey, J. (1990) *in* Chemical Aspects of Enzyme Biotechnology (Baldwin, T. O., Ed.), pp. 223–234, Plenum, New York.
5. Rétey, J. (1990) *Angew. Chem.* **102**, 373–379; *Angew. Chem. Int. Ed. Engl.* **29**, 355–361.
6. Babior, B. M., and Krouwer, J. S. (1979) *CRC Crit. Rev. Biochem.* **6**, 35–102.
7. Krouwer, J. S., Holmquist, B., Kipnes, R. S., and Babior, B. M. (1980) *Biochim. Biophys. Acta* **612**, 153–159.
8. Zhao, Y., Such, J., and Rétey, J. (1992) *Angew. Chem.* **31**, 215–221; *Angew. Chem. Int. Ed. Engl.* **31**, 215–216.
9. Babior, B. M., Moos, T. H., Orme-Johnson, W. H., and Beinert, H. (1974) *J. Biol. Chem.* **249**, 4537–4544.
10. Schepler, K. L., Dunham, W. R., Sands, R. H., Fee, J. A., and Abeles, R. H. (1975) *Biochim. Biophys. Acta* **397**, 510–518.
11. Buettner, G. R., and Coffmann, R. E. (1977) *Biochim. Biophys. Acta* **480**, 495–505.
12. Boas, J. F., Hicks, P. R., and Pilbrow, J. R. (1978) *J. Chem. Soc. Faraday Trans. II* **74**, 417–423.
13. Poppe, L., Hull, W. E., and Rétey, J. (1993) *Helv. Chim. Acta* **76**, 2367–2383.
14. Sando, G. N., Blakley, L. R., and Hogenkamp, H. P. C. (1975) *J. Biol. Chem.* **250**, 8774–8779.
15. Sando, G. N., Grant, M. E., and Hogenkamp, H. P. C. (1976) *Biochim. Biophys. Acta* **428**, 228–232.
16. Schrauzer, G. N., Katz, R. N., Grate, J. H., and Vickrey, T. M. (1976) *Angew. Chem.* **88**, 186–187; *Angew. Chem. Int. Ed. Engl.* **15**, 170–171.
17. Toraya, T., Kazutoshi, U., Saburo, F., and Hogenkamp, H. P. C. (1977) *J. Biol. Chem.* **252**, 963–970.
18. Toraya, T., and Fukui, S. (1982) *J. Nutr. Sci. Vitaminol.* **28**, 225–236.
19. Fukui, S., and Toraya, T. (1984) *in* Progress in Bioorganic Chemistry and Molecular Biology, Proceedings of the International Symposium on Frontiers of Bioorganic Chemistry and Molecular Biology (Ovchinikov, Y. A., Ed.), pp. 487–493, Elsevier, Amsterdam.
20. Ushio, K., Fukui, S., and Toraya, T. (1984) *Biochim. Biophys. Acta* **428**, 228–232.
21. Toraya, T., Matsumoto, T., Ichikawa, M., Itoh, T., Sugawara, T., and Mizuno, Y. (1986) *J. Biol. Chem.* **261**, 9289–9293.
22. Toraya, T., Watanabe, N., Ichikawa, M., Matsumoto, T., and Ushio, K. (1987) *J. Biol. Chem.* **262**, 8544–8550.
23. Toraya, T., and Ishida, A. (1988) *Biochemistry* **27**, 7677–7681.
24. Ichikawa, M., and Toraya, T. (1988) *Biochim. Biophys. Acta* **952**, 191–200.
25. Yakusheva, M. I., Gurevich, V. M., and Poznanskaya, A. A. (1982) *Biokhimiya* **47**, 1471–1475.
26. Toraya, T., and Ishida, A. (1991) *J. Biol. Chem.* **266**, 5430–5437.
27. Eberhard, G., Schlayer, H., Heinz, J. Fridrich, E., Utz, B., and Müller, O. (1988) *Biol. Chem. Hoppe-Seyler* **369**, 1091–1098.
28. Kellermeyer, R. W., Allen, S. H. G., Stjernholm, R., and Wood, H. G. (1964) *J. Biol. Chem.* **239**, 2562–2569.
29. Kellermeyer, R. W., and Wood, H. G. (1969) *Methods Enzymol.* **13**, 207–215.
30. Zagalak, B., Rétey, J., and Sund, H. (1974) *Eur. J. Biochem.* **44**, 529–535.
31. Rétey, J. (1982) *in* $B_{12}$ (Dolphin, D., Ed.), Vol. 2, pp. 357–379, Wiley, New York.
32. Francalanci, F., Davis, N. K., Fuller, J. R., Mertitt, D., and Leadlay, P. F. (1986) *Biochem. J.* **236**, 489–495.
33. Edelmann, L. (1991) Ph.D. Thesis, Universität Karlsruhe.

# XVI. melléklet

**POPPE, L.**, RÉTEY, J.:

Kinetic Investigations using Inhibitors Mimicking the Posthomolysis Intermediate in the Coenzyme-$B_{12}$ Dependent Glycerol Dehydratase and Diol Dehydratase Reactions,

*Eur. J. Biochem.*, **1997**, *245*, 398.

Eur. J. Biochem. 245, 398–401 (1997)


# Kinetic investigations with inhibitors that mimic the posthomolysis intermediate in the reactions of coenzyme-$B_{12}$-dependent glycerol dehydratase and diol dehydratase

László POPPE[1,2] and János RÉTEY[1]

[1] Department of Biochemistry, Institute of Organic Chemistry, University of Karlsruhe, Karlsruhe, Germany
[2] Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary

(Received 13 January 1997) – EJB 97 0042/4

Kinetic investigations were performed on the coenzyme-$B_{12}$-dependent glycerol dehydratase and diol dehydratase reactions using 1,2-propanediol as substrate and [ω-(adenosin-5′-*O*-yl)alkyl]cobalamins as mimics of the posthomolysis intermediate state of the coenzyme. All the coenzyme-$B_{12}$ analogues with oligomethylene chains ($C_3$–$C_7$) inserted between the central Co atom and the 5′ O of the adenosine moiety were competitive inhibitors with respect to coenzyme $B_{12}$. The apparent inhibition constants ($K_i$) of the shorter-chain inhibitors, especially the $C_5$ inhibitor, were smaller for both enzymes than those of the longer-chain ($C_6$, $C_7$) compounds. These results are in agreement with the expected (0.6–0.9 nm) distance between the Co and 5′-methylene paramagnetic centers in the posthomolysis intermediate state of coenzyme $B_{12}$ in these reactions.

*Keywords:* [ω-(adenosin-5′-*O*-yl)alkyl]cobalamin; analogues of coenzyme $B_{12}$; glycerol dehydratase; propanediol dehydratase; kinetic investigation.

Coenzyme $B_{12}$ ($CoB_{12}$) is an essential cofactor for several enzymes that catalyze rearrangement reactions [1–4]. This molecule contains a unique covalent Co-C bond, which is stable in aqueous solution but easily undergoes homolytic cleavage in $CoB_{12}$-dependent enzymatic reactions. This homolysis is the essential initial step in the catalytic cycles of all $CoB_{12}$-dependent enzymatic processes. Binding of $CoB_{12}$ to the apoprotein provides energy for the Co-C bond cleavage and protects the highly reactive intermediates from the environment [5]. $CoB_{12}$-dependent enzymes promote cobalt-carbon bond cleavage by application of a stretching force by interaction with the adenosyl moiety and corrin part [6]. The energy required for this stretching is provided by a conformational change of the apoprotein, which is probably triggered by binding the substrate. In the posthomolysis complex, however, the protein should have in a catalytically active, relaxed conformation.

Therefore, coenzyme-$B_{12}$ analogues that mimic the geometry of the posthomolysis intermediate, i.e. containing both the corrin part and the adenosyl moiety at a distance that corresponds to the distance between these moieties in the $CoB_{12}$ · apoenzyme complex after the homolytic Co-C bond cleavage, may provide valuable information on the structure of the activated complex. For example, the best fitting analogue is expected to be the strongest inhibitor of a given $CoB_{12}$-dependent reaction. A series of such posthomolysis-intermediate analogues (Fig. 1), in which the distance between the corrin part and the adenosyl moiety were varied by insertion of an oligomethylene chain ($C_3$–$C_7$) between the 5′ O of adenosine and the Co of corrin, has been therefore synthesized [7].

Based on the principle of 'negative catalysis' [5] and the space requirement of the substrate, we have postulated a distance of approximately 1 nm between CoII and the 5′ C radical in the posthomolysis intermediate of the (*R*)-methylmalonyl-CoA mutase from *Propionibacterium shermanii*, and we have tested our hypothesis by studying the inhibitory properties of these analogues [8]. Apparent inhibition constants for the $C_3$–$C_7$ analogues showed the predicted trend: the strongest inhibition was found with the $C_6$ analogue, in which, assuming a zig-zag oligomethylene-chain conformation, the 5′C–Co distance is about 1 nm. We postulated a smaller (about 600–700 pm) 5′-C radical–CoII distance for $CoB_{12}$-dependent enzymes acting on relatively small substrates, such as ethanolamine or 1,2-propanediol [8]. Our postulated distances agree well with experimental data. A range of 0.6–1.2 nm between the CoII and 5′-$CH_2$ paramagnetic centers has been estimated on the basis of EPR studies for several $CoB_{12}$-dependent enzymes [9–12]: a value of about 0.6 nm was proposed for diol dehydratase [9, 10], whereas a value of about 1.0–1.2 nm was calculated for ribonucleotide reductase [11].

To test our hypothesis on the Co–5′-$CH_2$ distance in the posthomolysis complex of an enzyme acting on a small substrate, inhibitory properties of the $C_3$–$C_7$ analogues have been investigated with glycerol dehydratase from *Citrobacter freundii* (overexpressed in *Escherichia coli*) and diol dehydratase from *Salmonella typhimurium* (overexpressed in *E. coli*) with racemic 1,2-propanediol as substrate.

## MATERIALS AND METHODS

**Materials.** Yeast alcohol dehydrogenase and β-NADH $Li_3$ were products of Boehringer Mannheim. Racemic 1,2-propane-

*Correspondence to* J. Rétey, Lehrstuhl Biochemie am Institut für Org. Chemie der Universität Karlsruhe, Kaiserstraße 12, D-76128 Karlsruhe, Germany
*Fax:* +49 72 6084823.

*Abbreviations.* $CoB_{12}$, coenzyme $B_{12}$; HO-Cbl, aquacobalamin (vitamin $B_{12a}$); CN-Cbl, cyanocobalamin (vitamin $B_{12}$).

*Enzyme.* Glycerol dehydratase (EC 4.2.1.30); propanediol dehydratase (EC 4.2.1.28); methylmalonyl-CoA mutase (EC 5.4.99.2); alcohol dehydrogenase (EC 1.1.1.1).

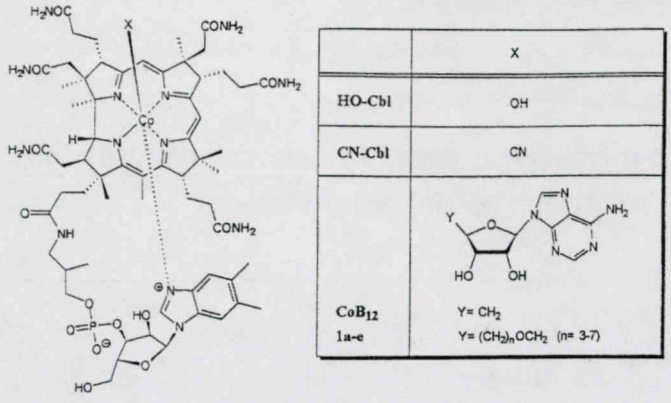



**Fig. 1. Structure of corrinoids used in this study.**

diol was supplied by Aldrich. $CoB_{12}$, vitamin $B_{12}$ (CN-Cbl) and vitamin $B_{12a}$ (HO-Cbl) were from Fluka. $C_3-C_7$ analogues (1a–e) were prepared by alkylation of vitamin $B_{12}$ with the corresponding tosylates [7]. Purities of all cobalamin derivatives were checked by HPLC [7] prior to use (all >98% pure). For kinetic measurements $CoB_{12}$ and the inhibitors were dissolved in bidistilled water and kept at 0–4°C. All operations with $CoB_{12}$, CN-Cbl and the $C_3-C_7$ analogues were carried out in the dark.

**Bacterial strains, cell cultivation and enzyme isolation.** *Glycerol dehydratase.* Overexpressing *E. coli* cells [13] containing the genomic DNA of glycerol dehydratase from *C. freundii* were used. The glycerol dehydratase gene was inserted into a pM52 plasmid harboring *Hin*dIII and *Bst*EII fragments in a bluescript vector $pSK^+$.

*Overexpressing media.* 6.0 g $KH_2PO_4$, 14.0 g $K_2HPO_4$, 3.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$ and trace element solution (1 ml 5.0 g/l EDTA, 2.0 g/l $FeSO_4 \cdot 7H_2O$, 0.1 g/l $ZnSO_4 \cdot 7H_2O$, 25 mg/l $MnSO_4$, 0.3 g/l $H_3BO_3$, 0.2 g/l $CoCl_2 \cdot 6H_2O$, 10 mg/l $CuCl_2 \cdot 2H_2O$, 20 mg/l $NiCl_2 \cdot 6H_2O$, 30 mg/l $NaMoO_4 \cdot 2H_2O$, pH 6.7) were dissolved in 950 ml bidistilled water. The pH was adjusted to 7.0, followed by addition of 10.0 g tryptone, 10.0 g NaCl and 5.0 g yeast extract to the solution. The solution was made up to 1 l with bidistilled water and sterilized in an autoclave. After sterilization, 9.2 g glycerol, 100 mg ampicillin and 75 mg kanamycin in 20 ml sterile bidistilled water was added and the volume was made up to 950 ml with sterile bidistilled water.

*Cell cultivation and harvesting.* Luria-Bertani-agar plates containing ampicillin and kanamycin were inoculated with glycerol-dehydratase-overexpressing *E. coli* and incubated at 30°C for 20 h. Several (3–5) colonies, taken from different positions of the agar plate, were transferred into 50 ml Luria-Bertani media and incubated at 30°C, with shaking, for 16 h. This culture was added to 950 ml overexpressing media and incubated at 30°C for 12 h, with shaking at 250 rpm (until the $A_{600}$ reached 1.0–1.5). The cell culture was heated to 42°C within 5 min, and shaken at this temperature for 0.5 h. Then, the culture was shaken at 37°C for 3 h. The cells were harvested by centrifugation at 5000 rpm (4400) for 10 min and stored at −80°C until use.

*Enzyme isolation.* Cells (2.1 g, wet mass) were suspended in 15 ml 25 mM potassium phosphate, pH 7.8, 2% (by vol.) racemic 1,2-propanediol, and the cells were disrupted by sonication at 4–8°C (Branson sonifier, full power, three times, 1 min each). After centrifugation of the resulting slurry at 23000×*g* and 0°C for 30 min, the supernatant was centrifuged at 23000×*g* and 4°C for 20 min. 10 ml of the final supernatant was filtered through a 0.45-µm filter and applied to a HiLoad 26/60 Superdex 200 (Pharmacia) column. The column was eluted at 4°C with 25 mM sodium phosphate, pH 7.5, 0.1 M KCl, 2% (by vol.) racemic 1,2-propanediol, at 2.5 ml/min. The fractions with significant enzyme activity (73–78 min) were unified and concentrated to 3.8 ml. A specific activity of 120 U/mg on racemic 1,2-propanediol substrate was determined for this preparation. The protein concentration was calculated from 1.45 $A_{280}$ − 0.74 $A_{260}$.

*Diol dehydratase.* Overexpressing *E. coli* cells containing genes of *S. typhimurium* LT2 diol dehydratase (three ORF, *pduCDE*) which were cloned in vector p*lacI*$^q$PO-bgIII. Expression of these genes was under the control of a wild-type *lac* promoter and *lacI*$^q$. The genotypes of the strains used were TA828 *met*E205 *ara*-9 DEL299 pXY18 and TA830 *met*E205 *ara*-9 DEL299 pXY39.

*Overexpressing media.* 6.0 g $Na_2HPO_4$, 3.0 g $KH_2PO_4$, 1.0 g $NH_4Cl$, 0.5 g NaCl in 100 ml bidistilled water (pH 7.4) were sterilized in an autoclave. To this solution 2 ml 1 M $MgSO_4$ (sterilized), 1 ml 0.1 M $CaCl_2$ (sterilized), 10 g sodium succinate, 25 mg histidine, 25 mg methionine, 85 mg ampicillin were added, and the solution was made up to 1000 ml with sterilized bidistilled water.

*Cell cultivation.* Cells from standard Luria-Bertani-agar plates containing ampicillin were transferred to 20 ml media and incubated at 30°C, with shaking, for 24 h. This culture was added to 1000 ml media and incubated with shaking at 250 rpm at 30°C for 10 h (until the $A_{600}$ reached about 1.0). 1 mM isopropylthio-β-D-galactoside was added to the culture, and shaking was continued at 30°C for 5 h ($A_{600}$ icreased to 1.4–1.5).

*Enzyme isolation.* After harvesting by centrifugation at 4000×*g* for 10 min, the cells were suspended in 20 ml of 50 mM sodium phosphate, pH 8.0, 1% *rac*-1,2-propanediol, and disrupted by sonication at 4–8°C (Branson sonifier, full power, three times, 1 min). The resulting slurry was centrifuged at 23000×*g* at 0°C for 30 min. After decanting the supernatant, the pellet was suspended in 20 ml of the above buffer and subjected to a second, similar centrifugation. The pellet from the second centrifugation was suspended in 20 ml 50 mM sodium phosphate, pH 8.0, 1% *rac*-1,2-propanediol, 1% Triton X-100, 1% sodium cholate, and stored in an ice bath for 30 min. This suspension was centrifuged (23000×*g* at 0°C for 20 min). After concentration (Centricon cell with 10-kDa membrane), the supernatant was filtered through a 0.45-µm filter and applied to a HiLoad 26/60 Superdex 200 (Pharmacia) column [4°C; elution with 25 mM sodium phosphate, pH 7.5, 0.1 M KCl and 2% (by vol.) *rac*-1,2-propanediol; flow rate, 2.5 ml/min]. The fractions showing enzyme activity (55–63 min) were unified and concentrated to 5 ml.

**Enzyme assay.** Alcohol dehydrogenase/β-NADH coupled assays of glycerol dehydratase or diol dehydratase activity on racemic 1,2-propanediol were based on the method of Bachovchin et al. [14] with minor modifications. In a plastic cuvette at 37°C, 990 µl 40 mM potassium phosphate, pH 8.0, 0.2 M racemic 1,2-propanediol, 0.2 mM β-NADH, 2.5 µl yeast alcohol dehydrogenase (15 U) and 2.5–5 µl glycerol dehydratase or diol dehydratase (0.2–0.3 U) were mixed and the resulting solution was incubated at 37°C (standard assay, 3 min). A control value that was used for correction was determined without addition of coenzyme-$B_{12}$. The enzymic reaction was started by addition of coenzyme $B_{12}$ [standard assay, 2.5 µl 1 mM (glycerol dehydratase assay), 5 µl, 1 mM (diol dehydratase assay)], and the decrease of absorbance at 340 nm was recorded for several minutes at 37°C. The rate of 1,2-propanediol/propionaldehyde transformation was calculated from the change of absorption at 340 nm due to the consumption of NADH in the coupled enzyme assay. The following equation was used:

$$V = 1000[(\Delta A - \Delta A_0)/\varepsilon l]$$

where $V$ = rate of reaction (nmol · min$^{-1}$ · cm$^{-3}$), $\Delta A$ = change of the absorption (min$^{-1}$), $\Delta A_0$ = change of the absorp-

tion without enzymatic reaction ($min^{-1}$). $\varepsilon$ = absorption coefficient of $\beta$-NADH at 340 nm (6.22 $cm^2 \cdot mmol^{-1}$). $l$ = cell length (1 cm). and 1000 is a conversion factor from μmol to nmol.

**Kinetic investigations.** Mean rate values of the enzyme assays measured between 1−2 min (glycerol dehydratase assay) or 2−3 min (dioldehydratase assay) were used for calculation of the kinetic constants.

Kinetic constants ($K_m$, $V_{max}$) for $CoB_{12}$ were determined by means of standard linearization methods (1−5 μl 0.001−1 mM $CoB_{12}$. 9−12 data points; linearization by Lineweaver-Burk. Hanes and Eadie-Hofstee: average values of kinetic constants were obtained by these three methods).

Apparent inhibition constants ($K_i$) for $C_3$−$C_7$ analogues. CN-Cbl and HO-Cbl were determined by our previously established parallel method [8]. $CoB_{12}$ (1−5 μl 0.1−1 mM) and inhibitor (1−5 μl 0.01−1 mM) were added simultaneously to the assay mixture. At three or four $CoB_{12}$ concentrations (0.1−2.5 μM). inhibitor concentrations were varied (8−10 data points for each). Inhibition constants were calculated from $K_i$ =(slope · $[CoB_{12}] \cdot V_{max})/K_m$ where $K_i$, $[CoB_{12}]$ and $K_m$ were in nmol, and $V_{max}$ was in nmol/min. Linear regression was performed in a Dixon plot. and the final $K_i$ taken as an average of the particular $K_i$ values of the data sets at different $[CoB_{12}]$ values.

**Table 1. Kinetic properties of $CoB_{12}$, the $C_3$−$C_7$ $CoB_{12}$ analogues (1a−e), CN-Cbl and HO-Cbl with glycerol dehydratase (from *C. freundii* overexpressed in *E. coli*) and diol dehydratase (from *S. typhimurium* overexpressed in *E. coli*).** Kinetic investigations were performed as described in Materials and Methods. $K_m$, apparent Michaelis-Menten constant; $K_i$, apparent inhibition constant. n.d., no inhibition detected up to 5 μM.

| Compound | Type of constant | Values of $K_m$ and $K_i$ for | |
|---|---|---|---|
| | | glycerol dehydratase | did dehydratase |
| | | nM | |
| $CoB_{12}$ | $K_m$ | 12.6 ± 2.2 | 750 ± 110 |
| 1a ($C_3$) | $K_i$ | 10.5 ± 1.5 | 770 ± 120 |
| 1b ($C_4$) | $K_i$ | 9.7 ± 1.2 | 860 ± 150 |
| 1c ($C_5$) | $K_i$ | 5.9 ± 1.0 | 500 ± 80 |
| 1d ($C_6$) | $K_i$ | 15.1 ± 3.0 | 830 ± 120 |
| 1e ($C_7$) | $K_i$ | 11.7 ± 1.6 | 630 ± 100 |
| HO-Cbl | $K_i$ | 8.6 ± 1.4 | 680 ± 110 |
| CN-Cbl | $K_i$ | 21.6 ± 2.7 | 1420 ± 200 |
| Adenosine | $K_i$ | n.d. | 7500 ± 1400 |

## RESULTS AND DISCUSSION

The distance between the paramagnetic centers (CoII and 5′-adenosyl radical) in the posthomolysis intermediate of coenzyme-$B_{12}$-dependent enzymes can be estimated if we consider the size of substrate together with the principle of negative catalysis [5], i.e. one important role of the apoprotein is to protect the highly reactive intermediates from the environment. Consequently, the substrate should fit between the CoII and 5′-adenosyl radical, but the distance between these and the substrate should be less than 200−250 pm, which is the space requirement of a water molecule. Based on these considerations, a value of about 0.9−1.0 nm was calculated for methylmalonyl-CoA mutase, while a value of about 0.6−0.8 nm between the radicals formed by the homolysis of $CoB_{12}$ was predicted for enzymes acting on relatively small substrates (e.g. diol dehydratase or glycerol dehydratase) [8]. The inhibition pattern of the posthomolysis-intermediate analogues on (*R*)-methylmalonyl-CoA mutase from *P. shermanii* was in agreement with the prediction that the $C_6$ analogue (1d; ~1 nm between Co and 5′-C. assuming a zig-zag conformation) would be the strongest inhibitor [8].

As examples of enzymes acting on a small substrate, we chose glycerol dehydratase and diol dehydratase for our kinetic studies. Since glycerol dehydratase is known to accept glycerol and 1,2-propanediol as substrates [15], racemic 1,2-propanediol was applied as substrate throughout in our work, enabling us to apply a convenient ultraviolet-based alcohol dehydrogenase/NADH coupled enzyme assay [14]. The inhibitory behavior of the $C_5$−$C_7$ analogues of coenzyme $B_{12}$, CN-Cbl, HO-Cbl and adenosine were investigated with glycerol dehydratase and diol dehydratase. The $C_5$−$C_7$ analogues were tested as mimics of the posthomolysis intermediate, having affinity for the corrin-binding and the adenosyl-binding sites of the apoprotein. Inhibition by HO-Cbl or CN-Cbl was considered to be caused by blocking the corrin-binding site, whereas adenosine was used as a possible block of the adenosyl-binding site. The results of our kinetic investigations are summarized in Table 1.

Inhibition by the $C_3$−$C_7$ analogues of glycerol dehydratase and diol dehydratase showed some interesting features. These analogues are competitive inhibitors with respect to $CoB_{12}$. The binding process, however, is not a fast equilibrium [8], so that several catalytic cycles occur before $CoB_{12}$ is replaced by the inhibitor. Although the kinetic constants for glycerol dehydratase and diol dehydratase differ by two orders of magnitude (≈10 nM for glycerol dehydratase and ≈1 μM for diol dehydratase), which is remarkable considering the high sequence similarity of the two enzymes, the relative inhibition patterns of the inhibitors on the two enzymes are quite similar. In agreement with our expectations, the shorter-chain analogues, especially the $C_5$ analogue (1c), were found to be slightly stronger inhibitors than the $C_6$ or $C_7$ analogues. The $K_i$ values of the $C_3$−$C_7$ $CoB_{12}$ analogues with glycerol dehydratase and diol dehydratase were of the same order of magnitude as the corresponding $K_m$ value of $CoB_{12}$. The strongest inhibitor, the $C_5$ analogue, exhibited significantly smaller $K_i$values than the corresponding $K_m$ of $CoB_{12}$ with glycerol dehydratase and diol dehydratase. The $C_3$−$C_7$ analogues are therefore considered to not be transition-state analogues, but analogues of the posthomolysis intermediates. Homolysis of the Co-C bond of $CoB_{12}$ is induced by a conformational change of the apoprotein upon binding of the substrate to the enzyme · coenzyme complex, pulling apart the 5′-deoxyadenosyl moiety from the Co. A similar conformational change is expected upon binding of the posthomolysis-state analogues to the apoenzyme, but because of the extensibility of the oligomethylene chains, no homolysis occurs. The relatively small but significant differences in the $K_i$ values can be explained by the fact that all the distances between the 5′-deoxyadenosyl moiety and the Co defined by the oligomethylene chains in these analogues lie on the reaction pathway of the conformationally induced homolysis, the optimum distance being the length of the pentamethylene chain. It may seem that the flexibility of the longer chains ($C_6$ and $C_7$) would also allow the optimum distance, but this may not be true at the enzyme's active site, which restricts the chain's conformational freedom by steric encumbrance.

The inhibitory potency of the [ω-(adenosin-5′-*O*-yl)alkyl]cobalamins with diol dehydratase is of the same order of magnitude as has been published for that of the adeninylalkylcobalamins [16]. Although the dioldehydratases assayed were from different sources, one can state that the ribose position does not contribute much of the binding affinity of the cobalamin analogues, while it may be important for the homolysis of the Co-C bond of coenzyme $B_{12}$.

While the $K_i$ values of the strongest inhibitor, the $C_5$ analogue, are smaller than the corresponding $K_m$ of $CoB_{12}$ with glycerol dehydratase or diol dehydratase, for (*R*)-methylmalonyl-CoA mutase, the strongest inhibitor, the $C_6$ analogue (1d), had a $K_i$ value about threefold higher than the $K_m$ of $CoB_{12}$ [8]. This phenomena can be rationalized by taking into account the 'base-on' or 'base-off' nature (i.e. depending on whether the lower ligand of the central Co of $CoB_{12}$ in the enzyme · coenzyme complex is the dimethylbenzimidazole moiety or a histidine residue of the apoenzyme) of the $CoB_{12}$ binding by the corresponding enzymes. The recently published crystal structure of (*R*)-methylmalonyl-CoA mutase from *P. shermanii* showed the 'base-off' nature of the enzyme [17]. Consequently, the loop carrying the dimethylbenzimidazole ligand of the $C_6$ inhibitor should be removed and replaced by the protein histidine imidazole while binding to the active center, which results in looser binding. In contrast, glycerol dehydratase and diol dehydratase seem to belong to the 'base-on' family. No similarity with the characteristic sequence pattern [18] of known 'base-off' enzymes, considering the conserved residues that participate in $CoB_{12}$ binding, was found in the sequences of several glycerol dehydratase and diol dehydratase enzymes of different origin [Daniel, R., personal communication]; and investigation of the EPR spectra of a 2-methyl-1,2-propanediol–inactivated diol dehydratase holoenzyme obtained with [$^{15}$N]apoenzyme and [$^{14}$N]coenzyme [19] also supports the 'base-on' nature of diol dehydratase. Accordingly, fitting the strongest $C_5$ inhibitor does not require a substantial change in the Co-ligand sphere during binding to the post-homolysis conformation state of the glycerol dehydratase or diol dehydratase apoenzymes.

The inhibition patterns of $C_3$–$C_7$ analogues on glycerol dehydratase and diol dehydratase alternate in a similar manner: analogues with odd numbers of inserted methylene units seem to be slightly stronger inhibitors than those with even numbers of methylenes. Such alternation was found in the orientation of adenosyl moiety relative to the corrin part in the calculated conformations of $C_3$–$C_7$ compounds, for $^1$H-NMR chemical shifts of several signals of these analogues, or for their solubilities [8].

Inhibition kinetic data for HO-Cbl and CN-Cbl with glycerol dehydratase and diol dehydratase have interesting features. Although both of these molecules consist of a corrin part bearing only a small axial ligand, there is a remarkable difference between their inhibitory properties. While CN-Cbl, bearing an apolar ligand, was a moderate inhibitor, having a $K_i$ value similar to those of $C_6$ and $C_7$ analogues, HO-Cbl, bearing a polar hydrophilic ligand, was found to be a rather strong inhibitor, with slightly smaller apparent $K_i$ values than the corresponding $K_m$ constants for $CoB_{12}$ in the glycerol dehydratase and diol dehydratase reactions.

Adenosine, however, showed different inhibitory properties toward glycerol dehydratase and diol dehydratase. While in the diol dehydratase reaction, the apparent $K_i$ value for adenosine was about tenfold higher than the $K_m$ of $CoB_{12}$, no significant inhibition of the glycerol dehydratase reaction was found up to 5 μM (≈400 $K_m$ of $CoB_{12}$) adenosine.

The generous gifts of an *E. coli* strain overexpressing glycerol dehydratase from *C. freundii* (by Dr R. Daniel and Prof. G. Gottschalk, University of Göttingen, Germany) and an *E. coli* strain overexpressing diol dehydratase from *S. typhimurium* (by T. A. Bobik, University of Florida, Gainesville, USA) are gratefully acknowledged. We are grateful to R. Nitsche (University of Karlsruhe, Germany) for isolating the diol dehydratase enzyme. We thank the Humboldt Foundation and the Federation of European Biochemical Societies for short-term postdoctoral fellowships to *L. P.*, as well as the European Union and the *Fonds der Chemischen Industrie* for financial support.

## REFERENCES

1. Zagalak, B. & Friedrich, W. (1979) Vitamin $B_{12}$, Walter de Gruyter & Co., Berlin, New York.
2. Golding, B. T. (1982) Mechanism of action of the $B_{12}$ coenzyme: theory and models in $B_{12}$ (Dolphin, D., ed.) vol. 1, pp. 543–582, John Wiley & Sons, New York.
3. Babior, B. M. (1988) The mechanism of adenosylcobalamin-dependent rearrangements. *Biofactors 1*, 21–26.
4. Rétey, J. (1990) Steric course and mechanism of coenzyme $B_{12}$-dependent rearrangements, in *Chemical aspects of enzyme biotechnology* (Baldwin, T. O., ed.) pp. 223–234, Plenum Press, New York.
5. Rétey, J. (1990) Enzymic reaction selectivity by negative catalysis or how do enzymes deal with highly reactive intermediates? *Angew. Chem. Int. Ed. Engl. 29*, 355–361.
6. Babior, B. M. & Krouwer, J. S. (1979) The mechanism of adenosylcobalamin-dependent reactions. *CRC Crit. Rev. Biochem. 6*, 35–102.
7. Poppe, L., Hull, W. E. & Rétey, J. (1993) Synthesis and characterization of (5′-deoxyadenosin-5′yl)cobalamin (= 'adenosylcobalamin') analogues mimicking the transition-state geometry of coenzyme-$B_{12}$-dependent rearrangements. *Helv. Chim. Acta 76*, 2367–2383.
8. Poppe, L. & Rétey, J. (1995) [ω-(Adenosin-5′-*O*-yl)alkyl]cobalamins mimicking the posthomolysis intermediate of coenzyme $B_{12}$-dependent rearrangements: kinetic investigations on methylmalonyl-CoA mutase. *Arch. Biochem. Biophys. 316*, 541–546.
9. Babior, B. M., Moos, T. H., Orme-Johnson, W. H. & Beinert, H. (1974) Mechanism of action of ethanolamine ammonia-lyase, a $B_{12}$-dependent enzyme. 13. Participation of paramagnetic species in the catalytic deamination of 2-aminopropanol, *J. Biol. Chem. 249*, 4537–4544.
10. Schepler, K. L., Dunham, W. R., Sands, R. H., Fee, J. A. & Abeles, R. H. (1975) Physical explanation of the EPR spectrum observed during catalysis by enzymes utilizing coenzyme $B_{12}$, *Biochim. Biophys. Acta 397*, 510–518.
11. Buettner, G. R. & Coffmann, R. E. (1977) EPR determination of the cobalt(II)-free radical magnetic geometry of the doublet species arising in a coenzyme $B_{12}$-enzyme reaction, *Biochim. Biophys. Acta 480*, 495–505.
12. Boas, J. F., Hicks, P. R., Pilbrow, J. R. & Smith, T. D. (1978) Interpretation of electron spin resonance spectra due to some $B_{12}$-dependent enzyme reactions. *J. Chem. Soc. Faraday Trans. II 74*, 417–423.
13. Daniel, R. & G. Gottschalk (1992) Growth temperature-dependent activity of glycerol dehydratase in *Escherichia coli* expressing the *Citrobacter freundii dha* Regulon. *FEMS Microbiol. Lett. 100*, 281–286.
14. Bachovchin, W. W., Eagar, R. G., Moore, K. W. & Richards J. H. (1977) Mechanism of action of adenosylcobalamin: glycerol and other substrate analogues as substrates and inactivators for propanediol dehydratase – kinetics, stereospecificity, and mechanism, *Biochemistry 16*, 1082–1092.
15. Forage, R. G. & Foster, M. A. (1979) Resolution of the coenzyme $B_{12}$-dependent dehydratases of *Klebsiella* sp. and *Citrobacter freundii*. *Biochim. Biophys. Acta 569*, 249–258.
16. Toraya, T., Ushio, K., Fukui, S. & Hogenkamp, H. P. C. (1977) Studies on the mechanism of the adenosylcobalamin-dependent dioldehydratase reaction by the use of the analogs of the coenzyme, *J. Biol. Chem. 252*, 963–970.
17. Mancia, F., Keep, N. H., Nakagawa, A., Leadlay, P. F., McSweeney, S., Rasmussen, B., Bösecke, P., Diat, O. & Evans, P. R. (1996) How coenzyme $B_{12}$ radicals are generated: the crystal structure of methylmalonyl-coenzyme A mutase at 2 Å resolution, *Structure (Lond.) 4*, 339–350.
18. Marsh, E. N. G. & Holloway, D. E. (1992) Cloning and sequencing of glutamate mutase component S from *Clostridium tetanomorphum*. Homologies with other cobalamin-dependent enzymes, *FEBS Lett. 310*, 167–170.
19. Toraya, T. (1996) Recent structure-function studies of $B_{12}$ coenzymes in diol dehydratase and some of other $B_{12}$-proteins, *4th European Symposium on Vitamin $B_{12}$* and $B_{12}$-Proteins, 2–6 September, 1996, Innsbruck, Austria.

# XVII. melléklet

SUTO, R. K., **POPPE, L.**, RÉTEY, J., FINKE, R. G.:

Ribonucleoside triphosphate reductase from *Lactobacillus leichmannii*: kinetic evaluation of a series of adenosylcobalamin competitive inhibitors, [ω-(adenosin-5′-O-yl)alkyl]cobalamins, which mimic the post Co-C homolysis intermediate,

*Bioorg. Chem.* **1999**, *27*, 451.

Bioorganic Chemistry 27, 451–462 (1999)
Article ID bioo.1999.1149, available online at http://www.idealibrary.com on IDEAL®

# Ribonucleoside Triphosphate Reductase from *Lactobacillus leichmannii:* Kinetic Evaluation of a Series of Adenosylcobalamin Competitive Inhibitors, [ω-(Adenosin-5′-*O*-*yl*)alkyl]cobalamins, Which Mimic the Post Co-C Homolysis Intermediate

Robert K. Suto,* László Poppe,†‡ János Rétey,† and Richard G. Finke§

**Department of Biochemistry and §Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523; the †Lehrstuhl für Biochemie im Institut für Organische Chemie, Universität Karlsruhe, Richard-Willstätter-Allee, D-76128, Karlsruhe, Germany; and the ‡Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025 Budapest, Hungary*

*Received January 29, 1999*

A series of [ω-(adenosin-5′-O-*yl*)alkyl]cobalamins were examined for their inhibitory properties of ribonucleoside triphosphate reductase (RTPR) from *Lactobacillus leichmannii* in the presence of 5′-deoxyadenosylcobalamin (AdoCbl, Coenzyme $B_{12}$). These AdoCbl analogs, in which oligomethylene chains ($C_3$–$C_7$) were inserted between the corrin Co-atom and a 5′-O-atom of the adenosine moiety, were designed to probe the Co-C bond posthomolysis state in AdoCbl-dependent enzymes, a state in which the Co and 5′-C distance is believed to be significantly increased. Experimentally, all five analogs were competitive inhibitors, with $K_i$ in the range of 8–56 μM. The [ω-(adenosin-5′-O-*yl*)alkyl]cobalamin analog with $C_5$ methylene carbons was the strongest inhibitor. This same pattern of inhibition, in which the $C_5$-analog is the strongest inhibitor, was previously observed in the AdoCbl-dependent *eliminase* enzyme systems, diol dehydratase and glycerol dehydratase. However, in methylmalonyl CoA *mutase*, the strongest inhibition is by the $C_6$-analog. This supports the hypothesis that the cobalamin posthomolysis intermediate in the eliminase enzymes differs from that in the mutase enzymes. These findings led, in turn, to an examination of the visible spectra of enzyme-bound cob(II)alamin in these two subclasses of AdoCbl-dependent enzymes. The results reveal an additional insight into the difference between the two classes: in the *eliminases*, the γ-band of bound cob(II)alamin is shifted from the 473 nm for free cob(II)alamin to *longer* wavelengths, 475–480 nm. However, in *mutases*, the γ-band of bound cob(II)alamin is shifted to shorter wavelengths, 465–470 nm. Overall, the results (a) provide strong evidence that two subclasses of AdoCbl-dependent enzymes exist, (b) give insights into the probable posthomolysis state in RTPR and other eliminases, and (c) identifies the $C_5$-analog as the tightest-binding analog for crystallization and other biophysical studies. 

## INTRODUCTION

An essential process in the catalytic cycle of 5′-deoxyadenosylcobalamin-dependent (AdoCbl; Coenzyme $B_{12}$) enzymatic reactions is the reversible homolytic cleavage



0045-2068/99 $30.00

of the AdoCbl Co-C bond to produce cob(II)alamin and a 5′-deoxyadenosyl radical or a cysteinyl thiyl radical (*1–3*). The mechanisms by which AdoCbl-dependent enzymes activate, cleave, and reform the Co-C bond during catalysis have been the focus of a wide variety of research efforts (*4–8*), especially since chemical precedent studies of AdoCbl Co-C bond cleavage revealed the $10^{12\pm1}$ enzymic acceleration of this step throughout the AdoCbl-dependent enzymes (*9–12*).

In order to better understand the structure of the intermediate species and the mechanisms involved, a series of five novel adenosylcobalamin analogs, [ω-(Adenosin-5′-O-*yl*)alkyl]cobalamins, were designed and previously synthesized with the goal of probing the posthomolysis state geometry in AdoCbl-catalyzed reactions (*13*). These [ω-(adenosin-5′-O-*yl*)alkyl]cobalamins have oligomethylene chains ($C_3$–$C_7$) inserted between the corrin Co-atom and the 5′-O-atom of adenosine (Fig. 1). Depending on the length of the oligomethylene chain, these novel analogs are expected to act as stronger or weaker inhibitors of AdoCbl-dependent reactions via their stronger or weaker binding to the posthomolysis conformation of the enzyme. The tightest binding of these AdoCbl analogs also allows an estimate of the separation distance in the enzyme between cob(II)alamin and 5′-deoxyadenosine following Co-C bond homolysis. This series of [ω-(adenosin-5′-O-*yl*)alkyl]cobalamin analogs has also been

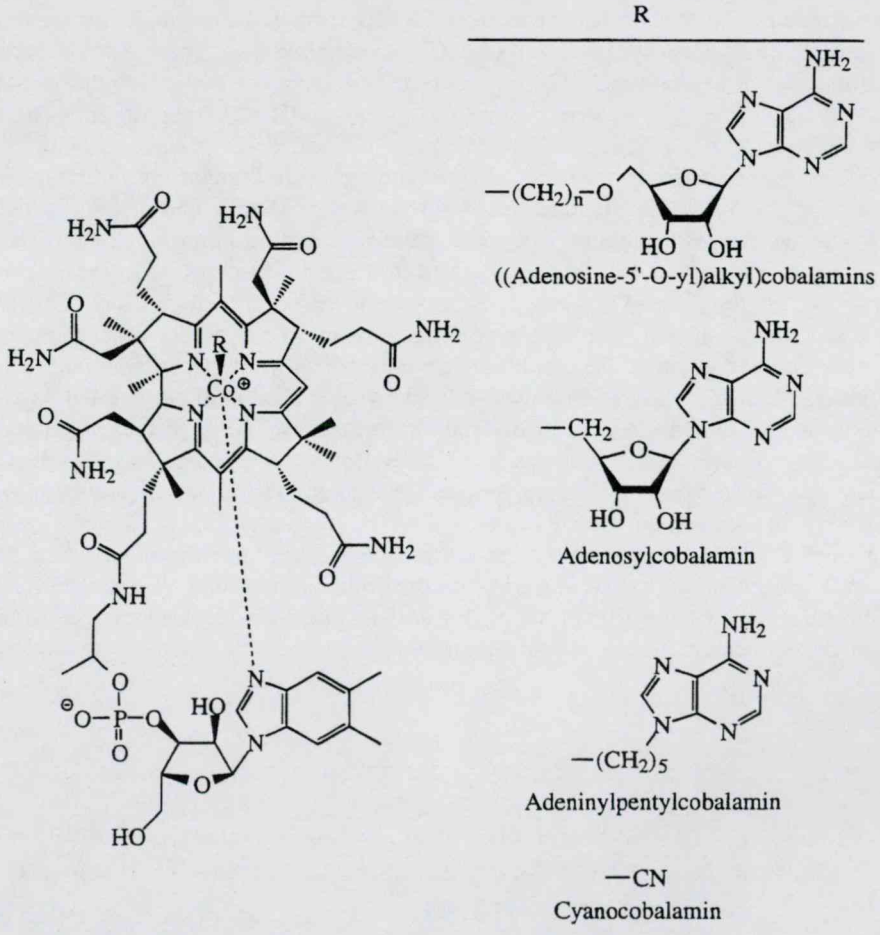



**FIG. 1.** Schematic structures of the AdoCbl analogs used in the present studies.

examined in the AdoCbl-dependent enzyme systems of methylmalonyl-CoA mutase from *Propionibacterium shermanii* (*14*), glycerol dehydratase from *Citrobacter freundii* (*15*), and diol dehydratase from *Salmonella typhimurium* (*15*).

Herein we extend the analysis of the inhibitory properties of this series of [ω-(adenosin-5′-O-*yl*)alkyl]cobalamins by examining them with RTPR and in competition against AdoCbl. The resulting competitive inhibition pattern is compared to that observed for the three other, previously studied AdoCbl-dependent enzymes. Our results, in combination with the literature (*14*), provide the clearest evidence to date in support of the hypothesis that the posthomolysis intermediates found in the AdoCbl-dependent eliminase enzymes are different than those in the mutase enzymes. The results also yield an estimate for the Co–C separation distance in the posthomolysis intermediate and identify the $C_5$-analog as the tightest-binding analog for biophysical studies of the AdoCbl(analogs)•RTPR holoenzyme complex.

## EXPERIMENTAL PROCEDURES

*Materials.* The [ω-(adenosin-5′-O-*yl*)alkyl]cobalamins were synthesized, purified, and characterized according to Poppe *et al.* (*13*). The *L. leichmannii* ribonucleotide triphosphate reductase was isolated from overexpressing *Escherichia coli* cells (*16*) and purified using our recent shortened protocol (*17*), one including a dGTP-Sepharose affinity column (*18*). AdoCbl, hydroxycobalamin, adenosine, ATP, dithiothreitol, diphenylamine, and all other materials were obtained from Sigma and used without further purification. Adeninylpentylcobalamin (AdePeCbl) was synthesized by published procedures (*19–21*), with the following modification (full details are available elsewhere (*12*)): since adeninylpentylchloride was initially too impure to crystallize from aqueous methanol as reported (*21*), adeninylpentylchloride was purified using a Chromatotron (Harrison Research Co.) with a 4-mm silica gel disc (Merck, 7749) using dichloromethane/methanol (13:1, v/v) as the developing solvent. The resultant adeninylpentylcobalamin was 97% pure by HPLC (Alltech Versapack $C_{18}$ column, 4.1 × 300 mm, 10-μm particle size, 60 Å pore size, eluted at 0.5 ml/min for 30 min with a linear gradient of 10–44% $CH_3CN$ in 0.085 M $H_3PO_4$, adjusted to pH 3 with triethylamine). A single $^{31}P$ resonance was observed at −0.5 ppm vs a $H_3PO_4$ standard. The expected molecular weight was observed by mass spectroscopy (FAB-MS, glycerol matrix: found, M + H = 1533.6 Da; calculated, M + H = 1532.7 Da).

*Methods.* The analogs were tested as inhibitors of RTPR's ability to convert ATP to dATP in the presence of various AdoCbl concentrations. The concentration of each stock cobalamin solution (~2 mM) was determined using the literature extinction coefficients for [ω-(adenosin-5′-O-*yl*)alkyl]cobalamins (*13*), adeninylpentylcobalamin (*20*), or aquocobalamin (*23*). The activity of RTPR was assayed using our modification of the diphenylamine procedure (*17*), an assay which measures the amount of dATP produced from ATP.

In a typical kinetic experiment, all procedures were done under dim red light to avoid photolysis of AdoCbl's sensitive Co-C bond. The pH of the stock assay reaction solution (containing 83.3 mM potassium phosphate buffer (pH 7.3), 1.67 M sodium acetate, 16.7 mM ATP, 50 mM DTT) was adjusted to pH 7.3 with NaOH. The final reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.3), 1 M sodium acetate, 10 mM ATP, 30 mM DTT, prechosen amounts of AdoCbl (1, 2, 3, 8 μM)

and analogs (1, 5, 10, 15, 20 $\mu$M), and 0.34 $\mu$M ribonucleotide reductase (7 $\mu$g) in a total volume of 0.25 ml. All of the components, except for the RTPR, were mixed in a 13 × 100-mm glass test tube on ice. A series of deoxyribose standards were prepared by adding dAMP (150–500 $\mu$M) as the deoxyribose source. RTPR was added to all of the tubes except those containing the dAMP standard, and the reduction of ATP by RTPR was initiated by incubation at 37°C in a circulating water bath. Following a 10-min incubation, the reaction mixtures were placed on ice and 0.2 ml of 0.5 M 2-chloroacetamide in 0.25 M potassium phosphate (pH 7.5) was added (to derivatize free thiols which interfere with the assay's color development (*24*)). Each tube was then vortex mixed, capped with a marble, and heated at 100 °C in a heat block for 30 min. After cooling briefly on ice, 1 ml of diphenylamine reagent (*25,26*) was added. The diphenylamine reagent (made by mixing 0.5 g diphenylamine, 25 g glacial acetic acid, 750 $\mu$l conc. $H_2SO_4$, and 250 $\mu$l 50 mM cupric acetate), which contains 0.5 mmol cupric acetate to accelerate color development, was freshly prepared, since its storage causes a precipitate to form in the samples. The absorbance at 594 nm was measured after incubation at 37 °C for 2 h, during which the clear colorless solutions change to purple and then blue. The amount of deoxyribose generated was calculated from the dAMP standard point calibration curve (*27*). For the determination of the $K_m$ and $V_{max}$ of AdoCbl with RTPR, the above assay was run using only AdoCbl (i.e., no inhibitors) at various concentrations (0.1, 0.3, 0.5, 1, 1.5, 2, 3, 8 $\mu$M).

To generate cob(II)alamin, aquocobalamin was reduced by an excess of thiol to cob(II)alamin (*28–30*) under the assay conditions (30 mM DTT, pH 7.3); aquocobalamin was completely converted to cob(II)alamin by dithiothreitol within 1 min, and the resultant cob(II)alamin was stable for the duration of the assay period. Cob(II)alamin inhibition was evaluated in the absence and presence of an equivalent amount of adenosine in the assay solution, entries 6 and 7 in Table 1, respectively.

The $K_m$ and $V_{max}$ for AdoCbl were estimated by the preferred direct linear-plot method (*31*). At each inhibitor concentration, a direct linear plot was used to determine $K_m^{app}$ and $V^{app}$, and the inhibition type (competitive, pure noncompetitive, mixed, or

TABLE 1

Apparent Inhibition Constants, $K_i^{app}$, for AdoCbl Analogs in RTPR Determined by the Direct Linear Method

| Cobalamin Analog | $K_i$ ($\mu$M) | Inhibition type |
|---|---|---|
| [3-(Adenosin-5′-*O*-*yl*)propyl]cobalamin ($C_3$) | 55.8 ± 0.4 | Competitive |
| [4-(Adenosin-5′-*O*-*yl*)butyl]cobalamin ($C_4$) | 18.9 ± 0.4 | Competitive |
| [5-(Adenosin-5′-*O*-*yl*)pentyl]cobalamin ($C_5$) | 7.7 ± 0.2 | Competitive |
| [6-(Adenosin-5′-*O*-*yl*)hexyl]cobalamin ($C_6$) | 24.6 ± 0.5 | Competitive |
| [7-(Adenosin-5′-*O*-*yl*)heptyl]cobalamin ($C_7$) | 12.8 ± 0.4 | Competitive |
| Cob(II)alamin | 20.8 ± 0.2 | Competitive |
| Cob(II)alamin + Adenosine | 14.3 ± 0.2 | Competitive |
| Cyanocobalamin (CNCbl) | 42.6 ± 0.6 | Competitive |
| Adeninylpentylcobalamin (AdePeCbl) | 1.3 ± 0.9 | Mixed |

uncompetitive) was evaluated (*31,32*). The inhibition constants ($K_i$) were then determined in the usual fashion (*31–33*) from plotting $K_m^{app}/V^{app}$ against the inhibitor concentration in the competitive inhibitor cases ([ω-(adenosin-5′-O-*yl*)alkyl]cobalamins, cob(II)alamin, and cyanocobalamin), and from plotting $1/V^{app}$ against the inhibitor concentration in the pure noncompetitive inhibition case (adenylpentylcobalamin).

*Supporting information available.* The following additional control and other experiments are available in the PhD Thesis of Robert K. Suto (*22*): synthesis and characterization of adeninylpentylcobalamin; kinetic analysis of AdoCbl interaction with RTPR; all the direct linear plots for each of the nine AdoCbl analogs and determination of apparent inhibition constants, $K_i^{app}$, in RTPR.

## RESULTS

*[ω-(Adenosin-5′-O-yl)alkyl]cobalamin inhibition kinetic studies.* Under the conditions used, the kinetic values determined for AdoCbl interacting with RTPR are: $K_m$ = 1.5 μM, and $V_{max}$ = 1.8 μM, as determined by the direct linear-plot method (Fig. 2). The $K_m$ value is consistent with the range of values observed previously by others ($K_m$ = 1.3 μM (*16*), 1.1 μM (*34*), 1.59 μM (*34*), and 8.3 μM (*35*)).

The abilities of the individual [ω-(adenosin-5′-O-*yl*)alkyl]cobalamin analogs to inhibit RTPRs interaction with AdoCbl were measured and compared with several other cobalamins (Table 1). The $K_i$ constants were calculated and reported using the method of direct linear plots (*31–33*); analysis of the data using the classical Dixon linearization (*36,37*) and Lineweaver–Burk linearization (*38*) methods resulted in values similar to the direct linear plot (available elsewhere (*22*)). The direct linear-plot data for AdoCbl shown in Fig. 2 is a representative example of the data (*22*). Figure 3 shows the plot used to determine $K_i$ for the $C_5$ analog, again as a representative plot.

All of the [ω-(adenosin-5′-O-*yl*)alkyl]cobalamin analogs affected the $K_m^{app}$ but not the $V^{app}$; hence, they are competitive inhibitors (*31–33*). In RTPR, an odd/even pattern in the oligomethylene chain number and in the observed $K_i$ is observed in which the odd-numbered chain analogs, $C_5$ and $C_7$, are stronger inhibitors than the even-numbered chain analogs, $C_4$ and $C_6$. The $C_5$ analog is the strongest inhibitor of the series. The $C_3$-analog was a relatively poor inhibitor, suggesting that, in this analog, the 3-(adenosin-5′-O-*yl*)propyl group does not contribute significantly to binding.

As expected, none of the [ω-(adenosin-5′-O-*yl*)alkyl]cobalamin analogs were active coenzymes of RTPR when examined in the absence of AdoCbl. All of the analogs were stable in the presence of RTPR; in no case was Co-C homolysis observed (i.e., no detectable cob(II)alamin was produced).

*Control experiments examining cob(II)alamin, cyanocobalamin, and adenylpentylcobalamin inhibition kinetic studies.* Previously, Yamada *et al.* (*29*) found cob(II)alamin to be a linear competitive inhibitor, with an apparent $K_i$ of 37 ± 2 μM in the absence of 5′-deoxyadenosine, and an apparent $K_i$ of 3.0 ± 2 μM in the presence of 50 μM 5′-deoxyadenosine (and under their exact conditions). They also found that 50 μM adenosine significantly increased the binding of cob(II)alamin, but decreased the activity to roughly half. Since cob(II)alamin can also mimic the posthomolysis state, but now without the formation of the Co-C cleavage hydrogen abstraction product 5′-deoxyadenosine, and thus without generation of the cysteine radical (*39,40*), we too have examined cob(II)alamin's inhibition properties (and with our more highly

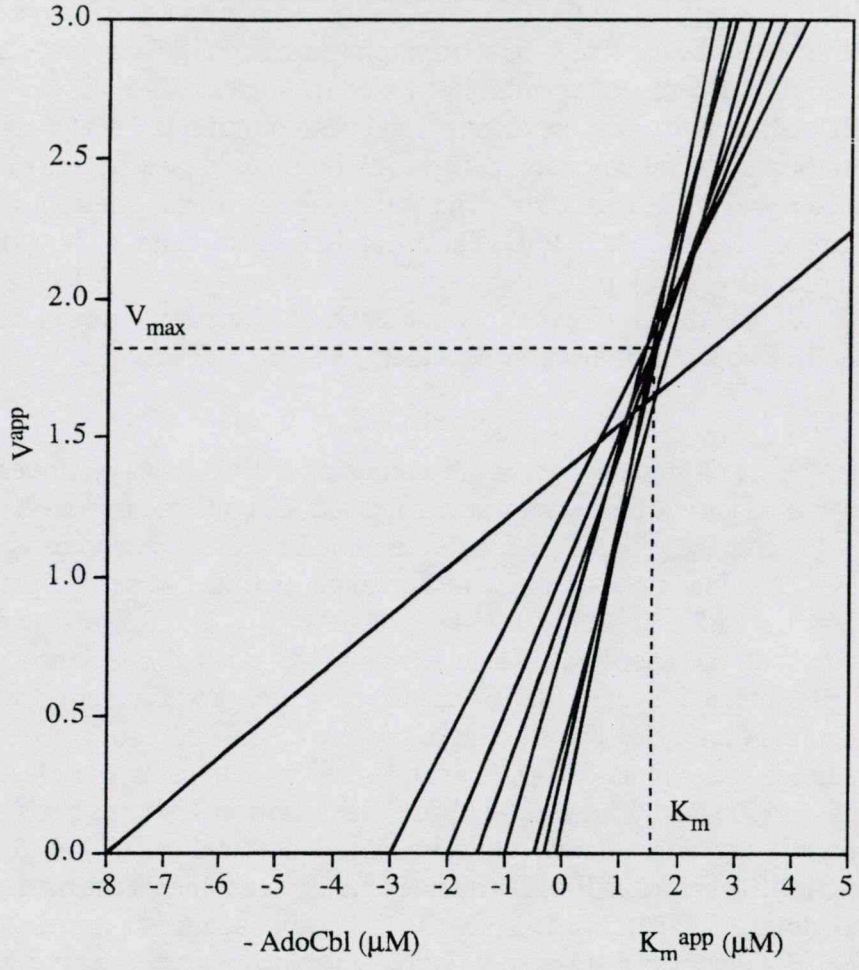



**FIG. 2.** Direct linear plot of the $K_m$ and $V_{max}$ for AdoCbl with RTPR showing the data (solid lines) and $K_m$ and $V_{max}$ (dotted lines).

purified, dGTP-based affinity column purified RTPR (*17*)). Cob(II)alamin proved to be a competitive inhibitor in our hands as well, with apparent $K_i$ of 14.3 ± 0.2 $\mu$M (in the presence of an equivalent amount of adenosine) and 20.8 ± 0.2 $\mu$M (in the absence of adenosine). Adenosine was chosen for this present work, rather than 5′-deoxyadenosine, due to the fact that a 5′ oxygen is already present in the (adenosin-5′-O-*yl*)alkylcobalamin series (see Fig. 1). Thus, the presence of a stoichiometric amount of adenosine, at least at the concentrations of adenosine used (1–20 $\mu$M), lowered the $K_i$ of the cob(II)alamin inhibition. The result that cob(II)alamin binds tightly is fully consistent with the knowledge that the corrin half of the posthomolysis intermediate is cob(II)alamin, and the idea that tight binding of cob(II)alamin and 5′-deoxyadenosine provides an important part of the driving force for homolytic cleavage of the Co-C bond (*12,30,41*).

The use of cyanocobalamin (CNCbl, Vitamin $B_{12}$, where a cyano group is the R group in Fig. 1) resulted in weaker competitive inhibition. CNCbl has also been found to be a moderate inhibitor in diol dehydratase and glycerol dehydratase (*15*).

Last, we also examined adeninylpentylcobalamin (AdePeCbl; Fig. 1), an analog

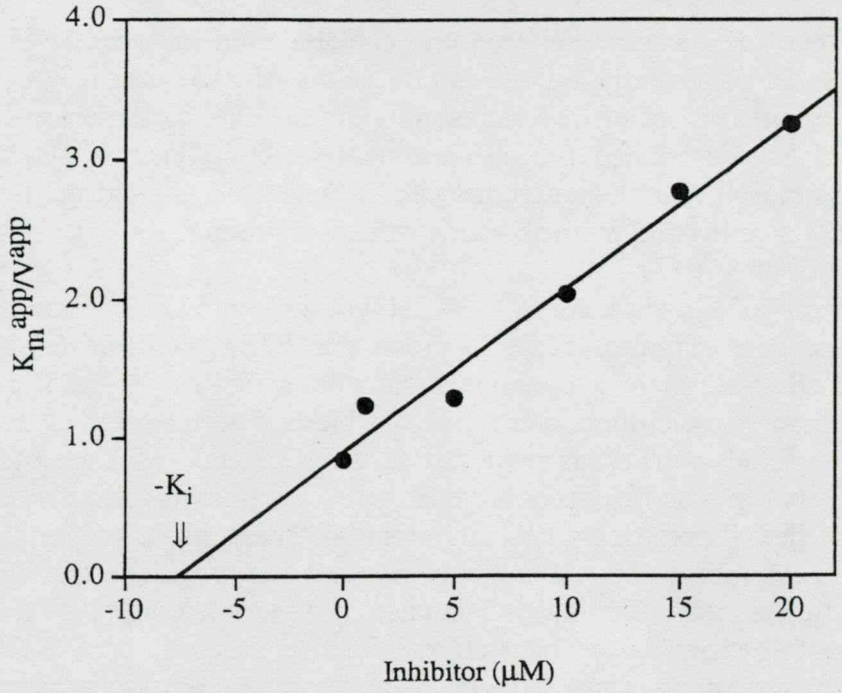



**FIG. 3.** Determination of [5-(adenosin-5′-*O*-yl)pentyl]cobalamin's $K_i$ value by plotting $K_m^{app}/V^{app}$ against inhibitor concentration. Each of the five sets of $K_m^{app}$ and $V^{app}$ value were determined by direct linear plots for each inhibitor concentration (*22*).

that was first synthesized in 1976 by Sando *et al.* (*20*) as part of a series of AdoCbl analogs, (adeninylalkyl)cobalamins. In these analogs, the ribose moiety of the 5′-deoxyadenosine is replaced by two to six methylene carbons. This series of analogs have previously been shown to be inhibitors of AdoCbl binding in ribonucleoside triphosphate reductase (RTPR) from *Lactobacillus leichmannii* (*20*), diol dehydratase from *Klebsiella pneumoniae* (*Aerobacter aerogenes*) (*42*), and ethanolamine ammonia-lyase from *Clostridium* sp (*43*). The AdePeCbl analog is of specific interest since it was (and still is, vide infra) the best (tightest binding) inhibitor known for RTPR.

AdePeCbl, unlike all the other analogs examined, was *not* a competitive inhibitor (Table 1). Rather, $V^{app}$ and $K_m^{app}$ changed as a function of the inhibitor concentration and therefore fits the criteria for a mixed inhibitor (*32*). The observed $K_i$ of 1.3 $\mu$M (for pure noncompetitive inhibition) is within experimental error of those previously observed (*20*), a finding which adds credence to both our data, and the previous data, consistent with AdePeCbl mixed inhibition.

## DISCUSSION

We anticipated that the series of AdoCbl analogs, [$\omega$-(adenosin-5′-O-*yl*)alkyl]cobalamins, in which the distance between the corrin and the adenosyl moiety was lengthened by insertion of oligomethylene chains ($C_3$–$C_7$), would be strong competitive inhibitors of RTPR (*14*). The strongest inhibitor of the $C_3$–$C_7$ series, the $C_5$-analog ($K_i$ = 7.7 ± 0.2 $\mu$M), binds tighter than even cob(II)alamin alone ($K_i$ = 20.8 ± 0.2 $\mu$M), suggesting that the $C_5$-analog closely mimics the cob(II)alamin posthomolysis state. *Assuming* that the $C_5$-analog binds to RTPR with its oligomethylene chain fully

extended, then the distance between the cob(II)alamin and the 5′-$CH_2$ group of adenosine in the posthomolysis state can be estimated to be near 9.3 Å (the distance previously calculated, see (*13*)). Interestingly, recent EPR measurements with RTPR and AdoCbl have estimated the distance between cob(II)alamin and the cysteine (C408) thiyl radical intermediate, produced following Co-C cleavage by abstraction of the cys408's -S-H bond by the 5′-deoxyadenosyl group (*39,44*), to be 5–7 Å, with an upper limit of 8 Å (*3*).

Comparison of [*ω*-(adenosin-5′-O-*yl*)alkyl]cobalamin interaction among the AdoCbl-dependent enzymes (Table 2) shows that *RTPR, glycerol dehydratase, and diol dehydratase* all share a common pattern in which *the $C_5$-analog is the best inhibitor.* Among these eliminase enzymes, the binding affinity of the AdoCbl analogs to RTPR is generally weaker (as is the case for AdoCbl itself (*15*)), and the differences between the analogs is more pronounced. However, contrasting this, in methylmalonyl-CoA mutase the $C_6$-analog is the best inhibitor (*14*). These results support the suggested separation of AdoCbl-dependent enzymes into two classifications according to either their catalytic reactions (eliminase vs mutase) (*2,45*), or according to their AdoCbl-binding motif (benzimidazole base-on vs histidine base-on) (*46*). RTPR and diol dehydratase are eliminases that have been shown to belong to the structural class in which the dimethylbenzimidazole base retains its coordination to cobalt upon binding to the enzyme (*47–49*). X-ray crystallography (*50*) demonstrates that methylmalonyl-CoA *mutase* displaces the dimethylbenzimidazole base with a histidine sidechain imidazole base coordinating to the cobalt. Other mutases are also predicted to be in this structural class due to conservation of a structure-based sequence motif (*46*) and independent EPR evidence (*51,52*). The difference between the two classes has also

TABLE 2

Summary of Competitive Inhibition by [*ω*-(Adenosin-5′-*O*-*yl*)alkyl]cobalamins in AdoCbl-Dependent Enzymes[a]

| Mechanistic class | Eliminase | | | Mutase |
|---|---|---|---|---|
| Structural class | Benzimidazole base-on | | | Histidine base-on |
| No. of methylene groups | Ribonucleotide reductase $K_i$ (μM) | Glycerol dehydratase[b] $K_i$ (× $10^{-3}$ μM) | Diol dehydratase[b] $K_i$ (μM) | Methylmalonyl-CoA mutase[c] $K_i$ (μM) |
| 3 | 55.8 ± 0.4 | 10.5 ± 1.5 | 0.77 ± 0.12 | 2.48 ± 0.58 |
| 4 | 18.9 ± 0.4 | 9.7 ± 1.2 | 0.86 ± 0.15 | 1.45 ± 0.29 |
| 5 | 7.7 ± 0.2 | 5.9 ± 1.0 | 0.50 ± 0.08[d] | 1.13 ± 0.56 |
| 6 | 24.6 ± 0.5 | 15.1 ± 3.0 | 0.83 ± 0.12[d] | 0.77 ± 0.18 |
| 7 | 12.8 ± 0.4 | 11.7 ± 1.6 | 0.63 ± 0.10 | 2.10 ± 0.81 |

[a] Obtained by the direct linear plot method. Dixon and Lineweaver–Burk analysis are available elsewhere (*22*).

[b] Data from Poppe and Rétey (*15*).

[c] Data from Poppe and Rétey (*14*).

[d] In answer to a referee's query, the difference, and propagated error bars (at 1$\sigma$), between the $C_2$ and $C_6$ analogs for dioldehydratase, are 0.33 ± 0.14; hence, the difference is larger than the 1$\sigma$, but not the 3$\sigma$, error bars.

been demonstrated by a base-off analog of AdoCbl, one which served as a coenzyme for methylmalonyl-CoA mutase reaction, yet one which is an inhibitor for diol dehydratase and glycerol dehydratase (*53*).

The series of results with the [$\omega$-(adenosin-5′-O-*yl*)alkyl]cobalamin analogs provided herein (Table 2) suggests that the posthomolysis cob(II)alamin and adenosine intermediates may be in different conformations in the mutases vs the eliminases. This hypothesis, in turn, led us to tabulate the literature spectra of cob(II)alamin when bound to AdoCbl-dependent enzymes (Table 3). The results reveal an additional difference in the posthomolysis state between the two classes. In the *eliminases*, the $\gamma$-band of bound cob(II)alamin is shifted from the 473 nm for free cob(II)alamin in neutral $H_2O$ to *longer* wavelengths, 475–480 nm. However, in *mutases*, the $\gamma$-band of bound cob(II)alamin is shifted to shorter wavelengths, 465–470 nm. (Note that free cob(II)inamide, which does not contain an axial base, has a 470-nm maxima, and that the X-ray crystal structure of methylmalonyl-CoA mutase contains bound cob(II)alamin with a long Co-histidine bond length of ~2.5 Å (*50*).) The absorption spectra of corrinoids above 300 nm correspond to $\pi$–$\pi^*$ transitions within the corrin ring. Unfortunately, and although there is a fair understanding of the relationship between corrin structure and the corresponding absorption spectra of Co(III) corrins (e.g., the intensities, position, and number of bands are generally sensitive to the number and nature of the axial ligand (*54,55*)), very little is known about how to interpret the absorption spectra of cob(II)alamins. In any event, the local environment of the enzyme bound cob(II)alamin appears to be different between the two classes of AdoCbl-dependent enzymes and compared to that of free cob(II)alamin. One known

TABLE 3

Cob(II)alamin Bound to AdoCbl-Dependent Enzymes

| Enzyme | Enzyme bound Cob(II)alamin maxima | Reference for enzyme bound Cob(II)alamin Spectra | Cob(II)alamin axial ligand (references) |
|---|---|---|---|
| RTPR | 480 | (*22*) | Benzimidazole |
| | 475 | (*29*) | (*47*) |
| Diol dehydratase | 478 | (*62*) | Benzimidazole (*48,49*) |
| Glycerol dehydratase | 480 | (*63*) | Benzimidazole[a] |
| Ethanolamine ammonia-lyase | 475 | (*64*) | Benzimidazole[a] |
| Methylmalonyl-CoA mutase | 467 | (*65*) | Histidine (*50,52*) |
| Glutamate mutase | 470 | (*66*) | Histidine (*51*) |
| 2-Methyleneglutarate mutase | 465 | (*67*) | Histidine[a] |
| | 470 | (*68*) | |
| $\beta$-Lysine-5,6-aminomutase | 468 | (*69,70*) | Histidine[a] |
| Free cob(II)alamin | 473 | (*54*) | Benzimidazole |
| Free cob(II)inamide | 470 | (*54*) | — |

[a] Predicted cobalt axial ligand.

difference is in the nitrogenous base coordinated to the cobalt within these two subclasses of AdoCbl-dependent enzymes, an intermolecular histidine imidazole vs. an intramolecular 5,6-dimethylbenzimidazole.[1] Another conceivable difference is that different corrin ring conformations are present in the bound cob(II)alamin product.

The finding that the [$\omega$-(adenosin-5′-O-*yl*)alkyl]cobalamins are competitive inhibitors, that is, bind at the active site in competition with AdoCbl, whereas the AdePeCbl analog is apparently a mixed inhibitor (i.e., is clearly a different type of inhibition), merits comment. Possible different types of, or sites for, binding of the AdePeCbl analog include (a) that only the adenine, or only the cobalamin, components of AdePeCbl bind at the active site (i.e., but *not both*); or (b) that only the adenine moeity binds at either the substrate binding part of the active site or at the separate allosteric site within RTPR (*56*)—that is, that the adenine part of AdePeCbl may be behaving as a partial mimic of ATP. Additional studies—and ideally at least one X-ray crystallographic structural study of RTPR—will be required to distinguish these possibilities, however.

In conclusion, the present kinetic studies of RTPR with the [$\omega$-(adenosin-5′-O-*yl*)alkyl]cobalamins analog series (i) provide the best evidence to date, when combined with some of our earlier work (*14*), that the AdoCbl-dependent enzymes break into two subclasses, (ii) demonstrate that the $C_5$-analog is the strongest inhibitor for RTPR (rather than the longer chain, $C_6$-analog as seen for methylmalonyl-CoA mutase), (iii) show that the UV-visible spectra of bound cob(II)alamin differ for these two subclasses of AdoCbl-dependent enzymes as well, and (iv) identify, therefore, the $C_5$ analog as the preferred one for crystallization trials and certain biophysical studies.

## ACKNOWLEDGMENTS

We thank Wesley T. White for the synthesis of adeninylpentylcobalamin. Financial support was provided by the NIH (Grant DK 26214), the European Commission, and the Fonds der Chemischen Industrie. L.P. thanks the Alexander von Humboldt Foundation for a fellowship.

## REFERENCES

1. Marsh, E. N. G. (1995) *BioEssays* **17**, 431–441.
2. Buckel, W., and Golding, B. T. (1996) *Chem. Soc. Rev.* 329–337.
3. Gerfen, G. J., Licht, S., Willems, J.-P., Hoffman, B. M., and Stubbe, J. (1996) *J. Am. Chem. Soc.* **118**, 8192–8197.
4. Kräutler, B., Arigoni, D., and Golding, B. T. (Eds.) (1998) Vitamin $B_{12}$ and $B_{12}$ Proteins: Lectures presented at the 4th European Symposium on Vitamin $B_{12}$ and $B_{12}$ Proteins, Wiley–VCH, Weinheim.
5. Hay, B. P., and Finke, R. G. (1987) *J. Am. Chem. Soc.* **109**, 8012–8018.

[1] The exact role, in the AdoCbl-dependent enzymes, of the axial base coordinated to cobalt is presently unknown (*41,57*). One possibility is that the axial base is involved in influencing the AdoCbl stability and thus the thermodynamics of the Co-C bond reformation step (*45,58*). Of interest here is that, in the mutase systems, the adenosine (Ado-H) plus product radical intermediate (P•) (*59*) to Ado• plus product (P-H) step is thermoneutral (*60*). However, in RTPR, which has been demonstrated to involve a cysteine thiyl radical intermediate (*3,39,40*), the AdoCbl Co-C bond reformation step, protein-S• plus Ado-H to protein-H plus Ado•, is ca. 7 kcal uphill (*58,60*). Hence, the structural differences of the axial base appears to correlate with the catalytic reaction, the cob(II)alamin intermediate, and the involvement of a protein-radical intermediate. A second possible function of the Co (protein histidine imidazole) axial base, discussed elsewhere (*61*), is that the imidazole axial base may be crucial in controlling *possible* electron transfer reactions to the radical substrate.

6. Hay, B. P., and Finke, R. G. (1986) *J. Am. Chem. Soc.* **108,** 4820–4829.
7. Glusker, J. P. (1995) *Vitamins Hormones* **50,** 1–70.
8. Ludwig, M. L., and Matthews, R. G. (1997) *Annu. Rev. Biochem.* **66,** 269–313.
9. Finke, R. G., and Hay, B. P. (1984) *Inorg. Chem.* **23,** 3041–3043.
10. Hay, B. P., and Finke, R. G. (1988) *Polyhedron* **7,** 1469–1481.
11. Waddington, M. W., and Finke, R. G. (1993) *J. Am. Chem. Soc.* **115,** 4629–4640.
12. Finke, R. G. (1998) *in* Vitamin $B_{12}$ and $B_{12}$ Proteins: Lectures presented at the 4th European Symposium on Vitamin $B_{12}$ and $B_{12}$ Proteins (Krautler, B., Arigoni, D., and Golding, B. T., Eds), pp. 383–402, Wiley–VCH, Weinheim.
13. Poppe, L., Hull, W. E., and Rétey, J. (1993) *Helv. Chim. Acta* **76,** 2367–2383.
14. Poppe, L., and Rétey, J. (1995) *Arch. Biochem. Biophys.* **316,** 541–546.
15. Poppe, L., and Rétey, J. (1997) *Eur. J. Biochem.* **245,** 398–401.
16. Booker, S., and Stubbe, J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8352–8356.
17. Suto, R. K., Whalen, M. A., and Finke, R. G. (1999) *Prep. Biochem. Biotechnol.* **29,** 273–309.
18. Suto, R. K., Whalen, M. A., Bender, B. R., and Finke, R. G. (1998) *Nucleosides Nucleotides* **17,** 1453–1471.
19. Hogenkamp, H. P. C. (1974) *Biochemistry* **13,** 2736–2740.
20. Sando, G. N., Grant, M. E., and Hogenkamp, H. P. C. (1976) *Biochim. Biophys. Acta* **428,** 228–232.
21. Carraway, K. L., Huang, P. C., and Scott, T. G. (1968) Synthetic Procedures in Nucleic Acid Chemistry (Zorbach, W. W., and Tipson, R. S., Eds.), Vol. 1, Interscience, New York.
22. Suto, R. K. (1998) Ph.D. Thesis, Colorado State University, Fort Collins, CO.
23. Schneider and Stroinski (1987) Comprehensive $B_{12}$, de Gruyter, Berlin.
24. Blakley, R. L. (1966) *J. Biol. Chem.* **241,** 176–179.
25. Jacobsen, D. W., DiGirolamo, P. M., and Huennekens, F. M. (1975) *Mol. Pharmacol.* **11,** 174–184.
26. Giles, K. W., and Myers, A. (1965) *Nature* **206,** 93.
27. Blakley, R. L. (1978) *Methods Enzymol.* **LI,** 246–259.
28. Alder, N., Medwick, T., and Poznanski, T. J. (1966) *J. Am. Chem. Soc.* **88,** 5018–5020.
29. Yamada, R., Tomao, Y., and Blakley, R. L. (1971) *Biochemistry* **10,** 3959–3968.
30. Hamilton, J. A., Yamada, R., Blakley, R. L., Hogenkamp, H. P. C., Looney, F. D., and Winfield, M. E. (1971) *Biochemistry* **10,** 347–355.
31. Eisenthal, R., and Cornish-Bowden, A. (1974) *Biochem. J.* **139,** 715–720.
32. Cornish-Bowden, A. (1988) Principles of Enzyme Kinetics, Butterworths, London.
33. Cornish-Bowden, A., and Eisenthal, R. (1974) *Biochem. J.* **139,** 721–730.
34. Booker, S. J. (1994) Ph.D. Thesis, Massachusetts Institute of Technology, Boston, MA.
35. Blakley, R. L. (1965) *J. Biol. Chem.* **240,** 2173–2180.
36. Dixon, M. (1953) *Biochem. J.* **55,** 170–171.
37. Purich, D. L., and Fromm, H. J. (1972) *Biochim. Biophys. Acta* **268,** 1–3.
38. Lineweaver, H., and Burk, D. (1934) *J. Am. Chem. Soc.* **56,** 658–666.
39. Silva, D. J., Stubbe, J., Samano, V., and Robins, M. J. (1998) *Biochemistry* **37,** 5528–5535.
40. Licht, S., Gerfen, G. J., and Stubbe, J. (1996) *Science* **271,** 477–481.
41. Garr, C. D., Sirovatka, J. M., and Finke, R. G. (1996) *Inorg. Chem.* **35,** 5912–5922.
42. Toraya, T., Ushio, K., Fukui, S., and Hogenkamp, H. P. C. (1977) *J. Biol. Chem.* **252,** 963–970.
43. Krouwer, J. S., Holmquist, B., Kipnes, R. S., and Babior, B. M. (1980) *Biochim. Biophys. Acta* **612,** 153–159.
44. Booker, S., Licht, S., Broderick, J., and Stubbe, J. (1994) *Biochemistry* **33,** 12676–12685.
45. Marsh, E. N. G. (1995) *Biochemisty* **34,** 7542–7547.
46. Ludwig, M. L., Drennan, C. L., and Matthews, R. G. (1996) *Structure* **4,** 505–512.
47. Lawrence, C. C., Gerfen, G. J., Samano, V., Nitsche, R., Robins, M. J., Rétey, J. and Stubbe, J., (1999) *J. Biol. Chem.* **274,** 7039–7042.
48. Yamanishi, M., Yamada, S., Muguruma, H., Murakami, Y., Tobimatsu, T., Ishida, A., Yamauchi, J., and Toraya, T. (1998) *Biochemistry* **37,** 4799–4803.
49. Abend, A., Nitsche, R., Bandarian, V., Stupperich, E., and Rétey, J. (1998) *Angew. Chem. Int. Ed.* **37,** 625–627.
50. Mancia, F., Keep, N. H., Nakagawa, A., Leadlay, P. F., McSweeney, S., Rasmussen, B., Bosecke, P., Diat, O., and Evans, P. R. (1996) *Structure* **4,** 339–350.

51. Zelder, O., Beatrix, B., Kroll, F., and Buckel, W. (1995) *FEBS Lett.* **369,** 252–254.
52. Padmakumar, R., Taoka, S., Padmakumar, R., and Banerjee, R. (1995) *J. Am. Chem. Soc.* **117,** 7033–7034.
53. Poppe, L., Stupperich, E., Hull, W., E., Buckel, T., and Rétey, J. (1997) *Eur. J. Biochem.* **250,** 303–307.
54. Pratt, J. M. (1972) Inorganic Chemistry of Vitamin B12, Academic Press, London.
55. Giannotti, C. (1982) *in* B12 (Dolphin, D., Ed.), Vol. 1, pp. 393–430, Wiley, New York.
56. Ludwig, W., and Follmann, H. (1978) *Eur. J. Biochem.* **82,** 393–403.
57. Sirovatka, J. M., and Finke, R. G. (1997) *J. Am. Chem. Soc.* **119,** 3057–3067.
58. Stubbe, J., and van der Donk, W. A. (1998) *Chem. Rev.* **98,** 705–762.
59. Padmakumar, R., Padmakumar, R., and Banerjee, R. (1997) *Biochemistry* **36,** 3713–3718.
60. McMillen, D. F., and Golden, D. M. (1982) *Annu. Rev. Phys. Chem.* **33,** 493–532.
61. Sirovatka, J. M., and Finke, R. G. (1999) *Inorg. Chem.* **38,** 1697–1707.
62. Toraya, T. (1985) *Arch. Biochem. Biophys.* **242,** 470–477.
63. Schneider, Z., Larsen, E. G., Jacobson, G., Johnson, B. C., and Pawelkiewicz, J. (1970) *J. Biol. Chem.* **245,** 3388–3396.
64. Hollaway, M. R., White, H. A., Joblin, K. N., Johnson, A. W., Lappert, M. F., and Wallis, O. C. (1978) *Eur. J. Biochem.* **82,** 143–154.
65. Keep, N., Smith, G. A., Evans, M. C. W., Diakun, G. P., and Leadlay, P. F. (1993) *Biochem. J.* **295,** 387–392.
66. Leutbecher, U., Bocher, R., Linder, D., and Buckel, W. (1992) *Eur. J. Biochem.* **205,** 759–765.
67. Zelder, O., and Buckel, W. (1993) *Biol. Chem. Hoppe-Seyler* **374,** 85–90.
68. Michel, C., Albracht, S. P. J., and Buckel, W. (1992) *Eur. J. Biochem.* **205,** 767–773.
69. Baker, J. J., van der Drift, C., and Stadtman, T. C. (1973) *Biochemistry* **12,** 1054–1063.
70. Baker, J. J., and Stadtman, T. C. (1982) *in* B12 (Dolphin, D., Ed.), Vol. 2, pp. 203–232, Wiley, New York.

# XVIII. melléklet

**POPPE, L.**, STUPPERICH, E., HULL, W. E., BUCKEL, T., RÉTEY, J.:

A New 'Base-off' Analogue of Coenzyme- $B_{12}$ with a Modified Nucleotide Loop: $^1$H-NMR Structure Analysis and Kinetic Studies with (*R*)-Methylmalonyl-CoA Mutase, Glycerol Dehydratase and Diol Dehydratase,

*Eur. J. Biochem.*, **1997**, *250*, 303.

Eur. J. Biochem. 250. 303–307 (1997)


# A base-off analogue of coenzyme-$B_{12}$ with a modified nucleotide loop

## $^1$H-NMR structure analysis and kinetic studies with (*R*)-methylmalonyl-CoA mutase, glycerol dehydratase, and diol dehydratase

László POPPE[1,2], Erhard STUPPERICH[3], William E. HULL[4], Thomas BUCKEL[1] and János RÉTEY[1]

[1] Department of Biochemistry, Institute of Organic Chemistry, University of Karlsruhe, Karlsruhe, Germany
[2] Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary
[3] Applied Microbiology, University of Ulm, Ulm, Germany
[4] Central Spectroscopy Department, German Cancer Research Center, Heidelberg, Germany

(Received 8 August 1997) – EJB 97 1140/4

(Co$\beta$-5′-Deoxyadenosin-5′-yl)-(*p*-cresolyl)cobamide (Ado-PCC), an analogue of the base-off form of coenzyme-$B_{12}$ ($CoB_{12}$), was prepared by alkylation of (Co$\alpha$/$\beta$-cyano/aqua)-(*p*-cresolyl)cobamide (PCC) with 5′-chloro-5′-deoxyadenosine. The 500 MHz $^1$H-NMR spectrum of Ado-PCC in $D_2O$ at pH 7.4 was completely analyzed using COSY and NOESY two-dimensional experiments. The coenzyme and inhibitory activities of Ado-PCC were tested with three coenzyme-$B_{12}$-dependent enzymes: (*R*)-methylmalonyl-CoA mutase, glycerol dehydratase, and diol dehydratase. Ado-PCC showed strong coenzyme activity with methylmalonyl-CoA mutase, which is known to bind the base-off form of $CoB_{12}$. In contrast, Ado-PCC had no coenzyme activity but acted instead as a competitive inhibitor with glycerol dehydratase and diol dehydratase, which are likely to prefer the base-on form of $CoB_{12}$. These results indicate that Ado-PCC, whose structure is analogous to the base-off form of $CoB_{12}$, can be used for probing the mode of coenzyme binding by coenzyme-$B_{12}$-dependent enzymes.

*Keywords:* (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresolyl)cobamide; base-off analog of coenzyme-$B_{12}$; (*R*)-methylmalonyl-CoA mutase (*Propionibacterium shermanii*); glycerol dehydratase (*Citrobacter freundii* gene overexpressed in *Escherichia coli*); diol dehydratase (*Salmonella typhimurium* gene overexpressed in *E. coli*).

Coenzyme-$B_{12}$, or adenosylcobalamin ($CoB_{12}$) is an essential cofactor for several enzymes catalyzing rearrangement reactions [1–5]. The homolysis of the unique covalent bond between cobalt and C5′ of the adenosyl ligand in this fascinating molecule leads to a 5′-adenosyl radical and a pentacoordinated Co(II) atom and is the common initial step in the catalytic cycle of all $CoB_{12}$-dependent enzymic reactions. It has been assumed [5] that a stretching force between these moieties caused by binding to the protein plays a crucial role in homolysis. The energy required for this homolysis originates from a conformational change in the protein induced by binding of the substrate. In the homolysis of the cobalt-carbon bond, the nature of the $\alpha$ ligand attached to the central cobalt and the mode of binding of $CoB_{12}$ to the protein may play a crucial role [6, 7]. $CoB_{12}$ may bind to the apoenzyme in two different ways. In the base-off binding mode, the 5,6-dimethylbenzimidazolyl moiety of the nucleotide loop of $CoB_{12}$ is displaced from the cobalt and replaced by a histidine residue of the protein. In the base-on mode, the original 5,6-dimethylbenzimidazolyl moiety of $CoB_{12}$ remains as the $\alpha$ ligand of cobalt in the enzyme-coenzyme complex.

The X-ray crystal structure of (2*R*)-methylmalonyl-CoA mutase from *Propionibacterium shermanii*, in a ternary complex with $CoB_{12}$ and desulpho-CoA (a truncated substrate), proved that the base-off binding mode operates for this enzyme [8]. Sequence comparison of (2*R*)-methylmalonyl-CoA mutase with other base-off enzymes [9] reveals some conserved residues in the $CoB_{12}$-binding region. Moreover, extensive structural similarities were found between the cobalamin-binding domain of this mutase and methionine synthase [10, 11], which catalyzes the transfer of a methyl group from its methylcobalamin cofactor to the substrate homocysteine to form methionine.

In contrast, glycerol dehydratase and diol dehydratase appear to be base-on enzymes. This is supported by a preliminary EPR study of the complex between [$^{14}$N]coenzyme and diol dehydratase [$^{15}$N]apoenzyme which had been inactivated with 2-methyl-2,2-propanediol [12]. Furthermore, sequence analysis of several glycerol dehydratase and diol dehydratase enzymes from various sources [13] indicated that in the $CoB_{12}$-binding domain there are no matches with the sequence patterns of the conserved residues of the known base-off enzymes [9].

Although several base-off analogues of $CoB_{12}$ have been synthesized for chemical characterization and model studies [6, 7, 14], to our knowledge none of them has been tested with $CoB_{12}$-dependent enzymes. Therefore, we took advantage of a new base-off analogue of $CoB_{12}$, in which the nucleotide loop is

*Correspondence to* J. Rétey, Lehrstuhl Biochemie am Institut für Organische Chemie der Universität Karlsruhe, Kaiserstraße 12, D-76128 Karlsruhe, Germany
*Fax:* +49 721 608-4823.
*E-mail:* biochem@ochhades.chemie.uni-karlsruhe.de

*Abbreviations.* $CoB_{12}$, coenzyme $B_{12}$; HO-Cbl, aquacobalamin (vitamin $B_{12a}$); CN-Cbl, cyanocobalamin (vitamin $B_{12}$); PCC, (CN/aq)-*p*-cresolyl-Cba; Ado-PCC, (CN/aq)-*p*-cresolyl-AdoCba.

*Enzymes.* Glycerol dehydratase (EC 4.2.1.30); propanediol dehydratase (EC 4.2.1.28); methylmalonyl-CoA mutase (EC 5.4.99.2); alcohol dehydrogenase (EC 1.1.1.1); dehydrogenase (EC 1.1.1.37); methylmalonyl-CoA epimerase (EC 5.1.99.1); methylmalonyl-CoA carboxyltransferase (EC 2.1.3.1).

not coordinated to the cobalt of the corrin moiety, and used it for kinetic studies with three $CoB_{12}$-dependent enzymes. The coordination of the cobalt of $CoB_{12}$ by a basic nitrogen atom from the $\alpha$ side is required to allow homolysis of the Co-carbon bond on the $\beta$ side [6, 7]. Therefore, the base-off enzymes provide a histidine residue of the protein as the essential $\alpha$ nitrogen ligand, and our base-off $CoB_{12}$ analogue is expected to act as a fully functional coenzyme with these enzymes. On the other hand, our base-off analogue should not exhibit coenzyme activity with base-on enzymes which do not provide the essential nitrogen ligand and cannot promote the homolysis of the Co-carbon bond.

Here, we report in detail the synthesis, the $^1$H-NMR data, and the enzyme-kinetic properties of (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresolyl)cobamide (Ado-PCC), a base-off analogue of $CoB_{12}$.

## MATERIALS AND METHODS

**Materials.** Adenosine, coenzyme-$B_{12}$, sodium tetrahydroborate, and succinic anhydride were obtained from Fluka Chemie AG. Racemic 1,2-propanediol was supplied by Aldrich. (Co$\alpha$/$\beta$-cyano/aqua)-(*p*-cresolyl)cobamide (PCC) was isolated from *Sporomusa ovata* cells in its cyano form by extraction with potassium-cyanide-containing acetic acid, pH 5, followed by centrifugation at 15000 *g* for 15 min, treatment of the supernatant by neutral aluminum oxide, desalting, chromatography on a XAD-2 column, and RP18-HPLC as previously described [15–17]. Yeast alcohol dehydrogenase, L-malate:NAD oxidoreductase (malate dehydrogenase), $\beta$-NADH $Li_3$ (NADH), and coenzyme-A (CoA) were from Boehringer Mannheim. (2*R*)-Methylmalonyl-CoA mutase, methylmalonyl-CoA epimerase, and methylmalonyl-CoA carboxyltransferase from *Propionibacterium shermanii* were isolated and assayed according to previously published methods [18–20]. Glycerol dehydratase was isolated as described previously [21] from overexpressing *Escherichia coli* cells containing the genomic DNA of glycerol dehydratase from *Citrobacter freundii* [22, 23]. Diol dehydratase was isolated as previously published [21] from overexpressing *E. coli* cells containing genes of diol dehydratase from *Salmonella typhimurium* LT2.

**Synthesis and characterization of (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresolyl)cobamide (Ado-PCC).** To a solution of (Co$\alpha$/$\beta$-cyano/aqua)-(*p*-cresolyl)cobamide (7.5 mg, 5.6 μmol) in 0.5 ml deoxygenated water, a solution of sodium tetrahydroborate (5 mg) in 0.4 ml deoxygenated water was added under argon atmosphere at room temperature, and the resulting solution was stirred for 30 min. A solution of 5′-chloro-5′-deoxyadenosine [24] (10 mg, 28 μmol) in 0.4 ml deoxygenated acetonitrile was subsequently added, and the mixture was stirred at room temperature in the dark for 1 h. The reaction mixture was filtered under sterile conditions through a 30-kDa nitrocellulose membrane and applied onto a preparative HPLC column (Macherey & Nagel, 250 mm×20 mm Nucleosil-7-$C_{18}$). The column was eluted with a water/methanol gradient (20–85% methanol over 35 min at a flow rate of 5 ml/min, diode array detection by monitoring at 240 nm and 280 nm, ultraviolet spectra between 200 nm and 600 nm from the peaks). The fractions containing the product were concentrated in a SpeedVac to yield 5.7 mg Ado-PCC (yield 64%, purity 98%). Analytical HPLC indicated a retention time $R_t$ of 4.5 min [Macherey & Nagel, 125 mm×4 mm, 5 μm LiChrospher 100 RP-18 column, linear gradient: 40–90% B in A over 12 min (solvent A: 0.02% trifluoroacetic acid in water; solvent B: 0.02% trifluoroacetic acid in methanol), flow rate 1 ml/min, diode array detection]. Peaks in the ultraviolet region were detected at the following wavelengths: 268, 305, 381, 456 nm.

**$^1$H-NMR spectroscopy.** $^1$H-NMR spectra of Ado-PCC (1.1 mg in 0.4 ml 20 mM sodium phosphate/$D_2O$ buffer, pH meter reading 7.45, 5-mm sample tube) were recorded at 10°C on a Bruker AM-500 spectrometer using conventional Fourier transform methods as in our previous studies [25]. The residual HDO resonance was suppressed by selective presaturation. Parameters for the one-dimensional spectrum were spectral width 4762 Hz, 32 K time-domain points, presaturation for 4 s, 50° flip angle, acquisition time 3.44 s, 512 transients. Data processing was performed with Lorentz-Gauss resolution enhancement at a digital resolution of 0.29 Hz. For well-resolved resonances, chemical shifts (relative to internal trimethylsilyl propionic acid) and coupling constants were derived from the peak-picking output (cubic interpolation); chemical shifts given to only two decimal places were estimated from cross-peaks in the two-dimensional COSY spectrum. A total of 79 nonexchangeable protons was confirmed by integration and the complete assignments are given in Table 1.

Two-dimensional magnitude-mode COSY-$\beta$ and NOESY spectra were obtained using conventional pulse sequences and the following parameters. For COSY, spectral width 4348 Hz, 2 K time-domain points in $t_2$, acquisition time 0.236 s, 512 $t_1$ increments with 48 transients each, 20 ms initial delay in each time domain and $\beta$ = 60° read pulse, relaxation delay with presaturation = 3 s, repetition time sine-bell window functions, zero-filling to 1 K in $t_1$, digital resolution 4.35 Hz/pt. For NOESY, as for COSY except relaxation delay with presaturation 2.5 s, 64 transients per increment, 90° read pulse, mixing time 600 ms with maximum 15% random variation. The COSY spectrum could be analyzed for the complete assignment of all coupled spins; the NOESY spectrum provided the additional correlations needed to completely assign all corrin methyl groups.

**Enzyme kinetics.** Kinetic studies were performed using the NADH-coupled, ultraviolet-based assay systems developed for methylmalonyl-CoA mutase [26], glycerol dehydratase, and diol dehydratase [21]. A mean rate for the enzyme reaction, measured over the interval $t$ = 2–3 min of the assay, was used for the calculation of kinetic parameters.

Apparent Michaelis-Menten constants ($K_m$, $V_{max}$) for $CoB_{12}$ and Ado-PCC were determined using standard methods (1–5 μl of 0.001–1 mM $CoB_{12}$ solutions, 12 data points; linearized plots according to Lineweaver-Burk, Hanes, and Eadie-Hofstee). Kinetic constants were calculated as the average of the values obtained with these three methods.

Apparent inhibition constants ($K_i$) for Ado-PCC were determined by the so-called parallel method [26]. $CoB_{12}$ (1–5 μl 0.1–1 mM solutions) and inhibitor (1–5 μl 0.01–1 mM solutions) were added simultaneously to the assay mixture. At three different $CoB_{12}$ concentrations (0.1–1 μM), inhibitor concentrations were varied (5–7 data points for each coenzyme concentration). Inhibition constants were calculated from the slope of the lines obtained by linear regression of these data sets in a Dixon plot using the following equation:

$$K_i = \text{slope} \times [CoB_{12}]\ V_{max}/K_m ,$$

where $K_i$, $[CoB_{12}]$, $K_m$ concentrations are nM and $V_{max}$ in units of nmol/min. Finally, $K_i$ was computed as the average of the three experimental $K_i$ values obtained at different $CoB_{12}$ concentrations.

## RESULTS

A unique corrinoid, (Co$\alpha$/$\beta$-cyano/aqua)-(*p*-cresolyl)cobamide (PCC), which differs from vitamin $B_{12}$ in the moiety attached

**Table 1. 500-MHz $^1$H-NMR data for $CoB_{12}$ and the analogue Ado-PCC in $D_2O$.** Assignment nomenclature is analogous to [25]. Chemical shifts for $CoB_{12}$ are from [28] and [29]. *J* coupling partners and assigned constants are given where these could be resolved. Detected partners giving NOESY cross-peaks are given; bold-face print indicates the strongest effect. d, doublet; q, quadruplet; s, singlet.

| Assignment | | Signal type | Chemical shifts | | | *J* couplings (Ado-PCC) | NOEs (Ado-PCC) |
|---|---|---|---|---|---|---|---|
| | | | Ado-PCC (pH 7.45, 10 °C) | base-off $CoB_{12}$ (pH 2.1, 20 °C) | base-on $CoB_{12}$ (pH 7.0, 20 °C) | | |
| | | | ppm | | | Hz | |
| Corrin methyl | M1 | s | 0.704 | 0.81 | 0.47 | | M2, M3 |
| | M2 | s | 1.405 | 1.48 | 1.36 | | M1, **M5**, H3 |
| | M5 | s | 2.389 | 2.43 | 1.45 | | M2, **H3**, M7, 7'a, b |
| | M7 | s | 1.819 | 1.82 | 1.70 | | **M5** |
| | M12$\beta$ | s | 0.845 | 1.00 | 0.87 | | M12$\alpha$, M15, H13 |
| | M12$\alpha$ | s | 1.585 | 1.67 | 1.32 | | M12$\beta$ |
| | M15 | s | 2.336 | 2.46 | 2.43 | | H13, M12, **M17** |
| | M17 | s | 1.141 | 1.40 | 1.36 | | **M15**, H19 |
| Corrin CH | H3 | dd | 4.217 | 4.23 | 4.10 | 3'a, b:2, 9.5 | M2, **M5** |
| | H8 | dd | 3.784 | 3.73 | 3.29 | 8'a, b:8.7, 4.5 | H10 |
| | H10 | s | 6.994 | 6.97 | 5.93 | | H8, M12$\beta$, **M12$\alpha$** |
| | H13 | dd | 3.308 | 3.43 | 2.89 | 131a, b:4, 6 | **M15**, M12$\beta$ |
| | H18 | ddd | 2.787 | 2.85 | 2.65 | 18'a, b:9, 3 | |
| | H19 | d | 4.653 | 4.70 | 4.24 | 18:10.7 | M17, 2'a, b |
| Corrin $CH_2$ side chain | 2'a, b | d | 2.66, 2.285 | 2.60, 2.46 | 2.41 | 2'a, b: −13.5 | |
| | 3'a, b | m | 1.99, 1.89 | 2.11, 1.97 | 2.06, 1.96 | | |
| | $3^2$a, b | ddd | 2.50, 2.43 | 2.55 | 2.50 | | |
| | 7'a, b | d | 2.275, 1.73 | 2.61, 2.14 | 2.19, 1.72 | 7'a, b: −13.5 | M5 |
| | 8'a, b | m | 2.25, 1.82 | 2.21, 1.75 | 1.75, 0.81 | | |
| | $8^2$a, b | ddd | 2.43, 2.31 | 2.35, 2.35 | 1.73, 0.88 | | |
| | 13'a, b | m | 2.18, 1.88 | 2.21, 1.92 | 2.22, 2.00 | | |
| | $13^2$a, b | ddd | 2.15, 1.78 | 2.21, 1.86 | 2.54 | | |
| | 17'a, b | ddd | 2.42, 1.83 | 2.51, 1.85 | 2.45, 2.06 | | |
| | $17^2$a, b | ddd | 2.36, 1.76 | 2.31, 1.85 | 2.45, 1.78 | | |
| | 18'a, b | dd | 2.68, 2.49 | 2.78 | 2.65 | | |
| Aminopropan-2-ol side chain | $CH_2$ (Apr-1a, b) | dd | 3.470, 3.395 | 3.38, 3.27 | 3.54, 3.16 | 1a, b: −14.1 | |
| | CH (Apr-2) | dddq | 4.42 | 4.36 | 4.33 | 1a, b: 3.7, 5.0 | |
| | $CH_3$ (Apr-3) | d | 1.238 | 1.23 | 1.21 | 2: 6.4 | |
| Loop ribose | 1' | d | 5.664 | 6.56 | 6.26 | 2': 4.6 | 2', Cr2,6 |
| | 2' | dd | 4.331 | 4.97 | 4.23 | 3': 6.3, P: 1.0 | 3' |
| | 3' | dd | 4.551 | 4.83 | 4.72 | 4': 2.5, P: 8.1 | 5' |
| | 4' | dd | 4.38 | 4.79 | 4.10 | 5'a, b: 3.0, 3.5 | 5' |
| | 5'a, b | m | 3.72, 3.70 | 3.94, 3.84 | 3.88, 3.74 | 5'a, b: −13 | 4' |
| Cresolyl | Cr2,6 | d | 6.873 | | | ortho: 8.6 | 1' |
| | Cr3,5 | d | 7.026 | | | | Cr4-Me |
| | Cr4-Me | s | 2.128 | | | | Cr3,5 |
| Adenosyl | H2 | s | 8.233 | 8.43 | 8.19 | | M15 (?) |
| | H8 | s | 8.037 | 8.21 | 8.00 | | 1', 2', 3' |
| | 1' | d | 5.615 | 5.61 | 5.56 | 2': 3.8 | 2', M12, M17 |
| | 2' | dd | 4.40 | 4.34 | 4.54 | 3': 6.2 | 3' |
| | 3' | dd | 3.754 | 3.90 | 3.74 | 4': 6.2 | 2' |
| | 4' | d | 2.002 | 1.98 | 2.54 | 5'a, b: 1.0, 9.0 | |
| | 5'a, b | dd, dd | 0.602, 0.312 | 1.46, 0.38 | 1.55, 0.57 | 5'a, b: −8.5 | |

to the ribose C1' of the nucleotide loop, is the cofactor used by the corrinoid-dependent methyl transfer enzymes of *Sporomusa ovata* [15−17]. The $\alpha$ side of the central cobalt in this cofactor cannot be coordinated by the *p*-cresolyl moiety of the nucleotide loop. Accordingly, PCC is bound to corrinoid-containing enzymes via a histidine residue that serves as $\alpha$ ligand [27]. The reductive alkylation of vitamin $B_{12a}$ is an established method for obtaining alkylated corrinoids [25]. Since PCC was expected to be alkylated analogously, this corrinoid was chosen as the starting compound for the preparation of a new base-off analogue of $CoB_{12}$ (Fig. 1). In accordance with our expectations, alkylation of PCC by 5'-chloro-5'-deoxyadenosine proceeded exclusively from the $\beta$ side, providing Ado-PCC in good yield.

The 500-MHz $^1$H-NMR spectrum of Ado-PCC in $D_2O$ was completely analyzed with the help of two-dimensional COSY and NOESY experiments. The data for Ado-PCC and,

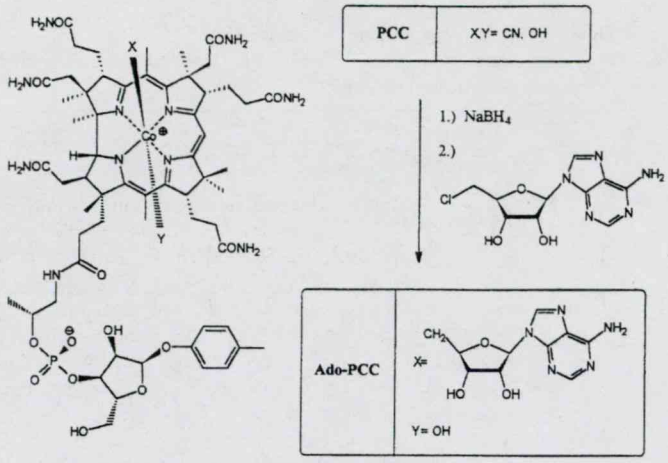



**Fig. 1. Synthesis of (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresolyl)cobamide (Ado-PCC) from (Co$\alpha\beta$-cyano/aqua)-(*p*-cresolyl)cobamide (PCC).** Ligands X and Y are on the $\beta$ and $\alpha$ side of the corrin ring, respectively.

**Table 2. Kinetic properties of Ado-PCC with (2*R*)-methylmalonyl-CoA mutase, glycerol dehydratase, and diol dehydratase.** Apparent $K_m$ values were measured according to the Enzyme kinetics section in Materials and Methods. Apparent inhibition constants were estimated by the 'parallel' method described in this section. (*R*)-Methylmalonyl-CoA mutase was isolated from *Propionibacterium shermanii* ($V_{max}$ = 23 ± 3 nmol/min with $CoB_{12}$). Glycerol dehydratase was isolated from overexpressing *E. coli* containing the gene from *Citrobacter freundii* ($V_{max}$ = 48 ± 4 nmol/min with $CoB_{12}$). Diol dehydratase was isolated from overexpressing *Escherichia coli* containing the gene from *Salmonella typhimurium* ($V_{max}$ = 51 ± 6 nmol/min with $CoB_{12}$). n.i., no inhibition. n.a., no coenzyme activity was found up to 25 μM Ado-PCC.

| Enzyme | $K_m$ ($CoB_{12}$) | $K'_m$ (Ado-PCC) | $K_i$ (Ado-PCC) |
|---|---|---|---|
| | nM | | |
| (*R*)-Methylmalonyl-CoA mutase | 354 ± 87 | 64 ± 21 | n.i. |
| Glycerol dehydratase | 14 ± 3 | n.a. | 160 ± 37 |
| Diol dehydratase | 750 ± 120 | n.a. | 9200 ± 1700 |

for comparison, data obtained by Bax and coworkers for the base-on [28] and base-off [29] forms of $CoB_{12}$ are summarized in Table 1.

Three $CoB_{12}$-dependent enzymes were tested in kinetic studies using the resulting base-off analogue. (2*R*)-Methylmalonyl-CoA mutase was chosen as a reference enzyme since the base-off mode of $CoB_{12}$ binding had been unambiguously established by X-ray crystallography [8]. Glycerol dehydratase and diol dehydratase are considered to be base-on enzymes, and these were investigated for both coenzyme activity and enzyme inhibition. The results of these kinetic investigations are summarized in Table 2.

## DISCUSSION

Comparison of the $^1$H-NMR data for Ado-PCC with those for $CoB_{12}$ confirm that the analogue has the base-off structure. The corrin methyl groups at positions 1, 5, 7, and 12$\alpha$ as well as the six methine protons are particularly sensitive to the presence or absence of 5,6-dimethylbenzimidazolyl as $\alpha$ ligand in $CoB_{12}$, and the chemical shifts for these protons in Ado-PCC agree well with the values for base-off $CoB_{12}$. The NMR parameters for the aminopropanol part of the nucleotide loop in Ado-PCC are also consistent with the base-off structure; in particular, the vicinal coupling constants of 3.7 Hz and 5.0 Hz for Apr-2 agree well with the values of 3.9 Hz and 5.2 Hz found for base-off $CoB_{12}$ [29]. The $^3J_{PH}$ coupling of phosphorus to the loop ribose H3′ is the same (8.1 Hz) for Ado-PCC and base-off $CoB_{12}$, but other couplings and shifts for this ribose are significantly different due to the influence of the cresolyl moiety. For the adenosyl $\beta$ ligand, the greatest sensitivity to base attachment is found for the chemical shift of H4′, which is nearly the same in Ado-PCC and base-off $CoB_{12}$. Interestingly, the chemical shifts for adenosyl H5′a, corrin M12$\beta$ and M17 are, respectively, about 0.86, 0.15, and 0.36 ppm upfield of the positions observed for base-off $CoB_{12}$. This may reflect a different orientation of the adenosyl group or specific effects of the cresolyl group in Ado-PCC.

As expected, Ado-PCC proved to be a fully functional coenzyme for methylmalonyl-CoA mutase. Surprisingly, the $K'_m$ value for Ado-PCC is even smaller than that of the natural coenzyme. This may be rationalized by considering that the binding of $CoB_{12}$ to the enzyme requires first the displacement of the 5,6-dimethylbenzimidazolyl moiety bound to cobalt before the imidazole side chain of the essential histidine in the protein can bind to the cobalt. In contrast, a Co-N bond need not be broken before binding of Ado-PCC, whose *p*-cresolyl moiety may in fact fit into the hydrophobic pocket normally occupied by the dimethylbenzimidazole of $CoB_{12}$. No coenzyme activity was found with glycerol dehydratase and diol dehydratase at concentrations two or even three orders of magnitude higher than the $K_m$ of $CoB_{12}$, providing further evidence for the base-on mode of cofactor binding for these enzymes.

The kinetic studies with Ado-PCC, however, revealed significant competitive inhibition with respect to $CoB_{12}$ for both glycerol dehydratase and diol dehydratase, with the $K_i$ for Ado-PCC being about a factor of ten higher than the $K_m$ of $CoB_{12}$. Cyanocobalamin or aquacobalamin with 5,6-dimethylbenzimidazolyl as $\alpha$ ligand and CN or OH as $\beta$ ligand are strong inhibitors of glycerol dehydratase and diol dehydratase, with $K_i$ of 21.6 or 8.6 and 1420 or 680 nM, respectively [21]. This indicates that a base-on analogue can occupy the corrin-binding site and can compete with $CoB_{12}$ binding. The fact that the base-off corrinoid PCC with OH or CN as $\beta$ ligand on cobalt proved not to be inhibitor for these enzymes indicates that the adenosyl moiety as $\beta$ ligand is necessary for the binding of Ado-PCC to these enzymes and the resulting inhibition. Thus, Ado-PCC is apparently anchored by its adenosyl moiety to the holoenzyme, but the (*p*-cresolyl)cobamide moiety may still fit into the corrinoid binding region by adopting a base-on conformation. Consequently, the absence of coenzyme activity for Ado-PCC in glycerol dehydratase and diol dehydratase can be best rationalized by the absence of a basic nitrogen as $\alpha$ ligand for the central cobalt; such a ligand is required to facilitate the homolysis of the Co-C bond in Ado-PCC complexed to enzymes.

In summary, Ado-PCC has a structure that is completely analogous to the base-off form of $CoB_{12}$. Ado-PCC proved to be a fully functional coenzyme with methylmalonyl-CoA mutase, which is known to bind the base-off form of $CoB_{12}$. Ado-PCC had no coenzyme activity with glycerol dehydratase and diol dehydratase, but inhibited these enzymes in a competitive manner, implying that these enzymes require a coenzyme in the base-on form. These results indicate that this base-off analogue of $CoB_{12}$ can be used as a general probe for testing the coenzyme-binding mode of coenzyme-$B_{12}$-dependent enzymes.

We gratefully acknowledge the generous gifts of an *E. coli* strain overexpressing glycerol dehydratase from *Citrobacter freundii* (by Dr R. Daniel and Prof. G. Gottschalk; Göttingen, Germany) and an *E. coli* strain overexpressing diol dehydratase from *Salmonella typhimurium* (by T. A. Bobik, Gainesville, USA). We also thank R. Nitsche and I. Merkler for isolating diol dehydratase and methylmalonyl-CoA mutase, respectively. Finally, we thank the Humboldt Foundation for a short-term postdoctoral fellowship to L. P., as well as the European Union and the *Fonds der Chemischen Industrie* for financial support.

## REFERENCES

1. *Vitamin $B_{12}$* (1979) W. de Gruyter Verlag, Berlin-New York.
2. Golding, B. T. (1982) Mechanism of action of the $B_{12}$ coenzyme. Theory and models. in *$B_{12}$* (Dolphin, D., ed.) vol. 1, pp. 543–582. John Wiley & Sons, New York.
3. Babior, B. M. (1988) The mechanism of adenosylcobalamin-dependent rearrangements, *Biofactors 1*, 21–26.
4. Rétey, J. (1990) Steric course and mechanism of coenzyme $B_{12}$-dependent rearrangements, in *Chemical aspects of enzyme biotechnology* (Baldwin, T. O., ed.) pp. 223–234, Plenum Press, New York.
5. Babior, B. M. & Krouwer, J. S. (1979) The mechanism of adenosylcobalamin-dependent reactions, *CRC Crit. Rev. Biochem. 6*, 35–102.
6. Garr, C. D., Sirovatka, J. M. & Finke, R. G. (1996) Adocobinamide, the base-off analog of coenzyme-$B_{12}$ (adocobalamin). 2. Probing the base-on effect in coenzyme-$B_{12}$ via cobalt-carbon bond thermolysis product and kinetic studies as a function of exogenous pyridine bases, *J. Am. Chem. Soc. 118*, 11 142–11 155.
7. Garr, C. D. & Finke, R. G. (1996) Adenosylcobinamide, the base-free analog of coenzyme-$B_{12}$ (adenosylcobalamin). I. Probing the role of the axial 5,6-dimethylbenzimidazole base in coenzyme-$B_{12}$ via exogenous axial base $K_{association}$, $\Delta H$, and $\Delta S$ measurements plus a critical review on the relevant biochemical literature. *Inorg. Chem. 35*, 5912–5923.
8. Mancia, F., Keep, N. H., Nakagawa, A., Leadlay, P. F., McSweeney, S., Rasmussen, B., Bösecke, P., Diat, O. & Evans, P. R. (1996) How coenzyme $B_{12}$ radicals are generated: the crystal structure of methylmalonyl-coenzyme A mutase at 2 Å resolution. *Structure 4*, 339–350.
9. Marsh, E. N. G. & Holloway, D. E. (1992) Cloning and sequencing of glutamate mutase components from *Clostridium tetanomorphum*. Homologies with other cobalamin-dependent enzymes. *FEBS Lett. 310*, 167–170.
10. Jarrett, J. T., Amaratunga, M., Drennan, C. L., Scholten, J. D., Sands, R. H., Ludwig, M. L. & Matthews, R. G. (1996) Mutations in the $B_{12}$-binding region of methionine synthase: how the protein controls methylcobalamin reactivity. *Biochemistry 35*, 2464–2675.
11. Ludwig, M. L., Drennan, C. L. & Matthews, R. G. (1996) The reactivity of $B_{12}$-cofactors: the proteins make a difference. *Structure 4*, 505–512.
12. Toraya, T. (1996) Recent structure-function studies of $B_{12}$ coenzymes in diol dehydratase and some of other $B_{12}$-proteins. *4th European Symposium on vitamin $B_{12}$* and $B_{12}$-proteins, September 2–6, 1996, Innsbruck, Austria, p. 13.
13. Daniel, R., Seyfried, M. & Gottschalk, G. (1996) Cloning, sequencing and overexpression of the genes encoding coenzyme-$B_{12}$-dependent glycerol dehydratase of *Citrobacter freundii*. *4th European Symposium on vitamin $B_{12}$ and $B_{12}$-proteins*, September 2–6, 1996, Innsbruck, Austria, p. 2.
14. Luo, L. B., Chen, H. L., Wu, Z., Yan, H. & Tang, W. X. (1996) Synthesis and characterization of coenzyme-$B_{12}$ base-off form analogs-2′-deoxynucleosidylcobinamides, *J. Inorg. Biochem. 61*, 15–23.
15. Stupperich, E. & Eisinger, H. J. (1989) Biosynthesis of *para*-cresolyl cobamide in *Sporomusa ovata*, *Arch. Microbiol. 151*, 372–377.
16. Stupperich, E. (1993) Recent advances in elucidation of biological corrinoid functions, *FEMS Microbiol. Rev. 12*, 349–366.
17. Bykhovskii, V. Y., Santander, P. J., Stupperich, E., Zaitseva, N. I., Pusheva, M. A., Detkova, E. N., Valyushok, D. S. & Scott, A. I. (1996) Biosynthesis of corrinoids in obligatory anaerobic microorganisms: isolation and identification of intermediates of biogenesis of vitamin-$B_{12}$ and its analogs accumulated by growing *Clostridium thermoaceticum*, *Sporomusa ovata*, and *Acetohalobium arabaticum*, *Appl. Biochem. Microbiol. 32*, 170–178.
18. Kellermeyer, R. W., Allen, S. H. G., Stjernholm, R. & Wood, H. G. (1964) Purification and properties of methylmalonyl-CoA isomerase from *Propionibacteria*, *J. Biol. Chem. 239*, 2562–2569.
19. Kellermeyer, R. W. & Wood, H. G. (1969) 2-Methylmalonyl-CoA mutase from *Propionibacterium shermanii* (methylmalonyl-CoA isomerase), *Methods Enzymol. 13*, 207–215.
20. Zagalak, B., Rétey, J. & Sund, H. (1974) Methylmalonyl-CoA mutase from *Propionibacterium shermanii*, *Eur. J. Biochem. 44*, 529–535.
21. Poppe, L. & Rétey, J. (1997) Kinetic investigations with inhibitors that mimic the posthomolysis intermediate in the reactions of coenzyme-$B_{12}$-dependent glycerol dehydratase and diol dehydratase, *Eur. J. Biochem. 245*, 398–401.
22. Daniel, R. & Gottschalk, G. (1992) Growth temperature-dependent activity of glycerol dehydratase in *Escherichia coli* expressing the *Citrobacter freundii* dha regulon, *FEMS Microbiol. Lett. 100*, 281–286.
23. Seyfried, M., Daniel, R. & Gottschalk, G. (1996) Cloning, sequencing and overexpression of the genes encoding coenzyme-$B_{12}$-dependent glycerol dehydratase of *Citrobacter freundii*, *J. Bacteriol. 178*, 5793–5796.
24. Robins, M. J., Hansske, F., Wnuk, S. F. & Kanai, T. (1991) Nucleic acid related compounds. 66. Improved syntheses of 5′-chloro-5′deoxy- and 5′-*S*-aryl(or alkyl)-5′-thionucleosides, *Can. J. Chem. 69*, 1469–1474.
25. Poppe, L., Hull, W. E. & Rétey, J. (1993) Synthesis and characterization of (5′-deoxyadenosin-5′-yl)cobalamin (= 'adenosylcobalamin') analogues mimicking the transition-state geometry of coenzyme-$B_{12}$-dependent rearrangements, *Helv. Chim. Acta 76*, 2367–2383.
26. Poppe, L. & Rétey, J. (1995) [ω-(Adenosin-5′-*O*-yl)-alkyl]cobalamins mimicking the posthomolysis intermediate of coenzyme $B_{12}$-dependent rearrangements: kinetic investigations on methylmalonyl-CoA mutase, *Arch. Biochem. Biophys. 316*, 541–546.
27. Stupperich, E. (1994) Corrinoid-dependent mechanism of acetogenesis from methanol, in *Acetogenesis* (Drake, H., ed.) pp. 180–194, Chapman & Hall, New York.
28. Summers, M. F., Marzilli, L. G. & Bax, A. (1986) Complete proton and carbon-13 assignments of coenzyme-$B_{12}$ through the use of new two-dimensional NMR experiments, *J. Am. Chem. Soc. 108*, 4285–4294.
29. Bax, A., Marzilli, L. G. & Summers, M. F. (1987) New insights into the solution behavior of cobalamins. Studies of the base-off form of coenzyme-$B_{12}$ using modern two-dimensional NMR techniques, *J. Am. Chem. Soc. 109*, 566–574.

# XIX. melléklet

**POPPE, L.**, BOTHE, H., BRÖKER, G., BUCKEL, W., STUPPERICH. E., RÉTEY, J.:

Elucidation of the coenzyme binding mode of further $B_{12}$-dependent enzymes using a base-off analogue of coenzyme $B_{12}$,

*J. Mol. Catal. B: Enzymatic*, **1999**, elfogadva.

# ELUCIDATION OF THE COENZYME BINDING MODE OF FURTHER $B_{12}$-DEPENDENT ENZYMES USING A BASE-OFF ANALOGUE OF COENZYME $B_{12}$

László Poppe [1,2], Harald Bothe [3], Gerd Bröker [3], Wolfgang Buckel [3*], Erhard Stupperich [4] and János Rétey [1*]

[1] Department of Biochemistry, Institute of Organic Chemistry, University of Karlsruhe, Richard-Willstätter-Allee 2, D-76128 Karlsruhe, Germany.

[2] Chemical Research Centre, Institute for Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025 Budapest, Hungary.

[3] Laboratorium für Mikrobiologie, FB Biologie, Philipps-Universität, Karl-von-Frisch Strasse, D-35032 Marburg, Germany.

[4] Applied Microbiology, University of Ulm, Albert-Einstein-Allee 11, D-89070 Ulm, Germany.

* To Whom Correspondence should be addressed: J. Rétey, Lehrstuhl Biochemie am Institut für Organishe Chemie der Universität Karlsruhe, Richard-Willstätter-Allee 2, D-76128 Karlsruhe, Germany; Phone: +49-721-6082092, Fax: +49-721-6084823, E-mail: biochem@ochhades.chemie.uni-karlsruhe.de; W. Buckel, Laboratorium für Mikrobiologie, Philipps-Universität Marburg, Karl-von-Frisch Strasse, D-35032 Marburg, Germany, Phone: +49-6421-281528, Fax: +49-6421-288979, E-mail: buckel@mailer.uni-marburg.de.

**ABSTRACT**

(Co$\beta$-5′-Deoxyadenosin-5′-yl)-(*p*-cresyl)cobamide (Ado-PCC) a base-off analogue of coenzyme-$B_{12}$ (Ado-Cbl) was used to elucidate the coenzyme $B_{12}$ binding mode of glutamate mutase, 2-methyleneglutarate mutase and ethanolamine ammonia-lyase. Ado-PCC functions as excellent coenzyme for the carbon skeleton rearrangements with apparent $K_m$ values of 200 and 10 nM for glutamate and 2-methyleneglutarate mutases, respectively. The corresponding values for Ado-Cbl are 60 and 54 nM, respectively.

In contrast, Ado-PCC showed no coenzyme activity with ethanolamine ammonia-lyase but was a competitive inhibitor with respect to Ado-Cbl. The $K_i$ value for Ado-PCC was 26, the apparent $K_m$ value for Ado-Cbl was 30 nM.

These results are in agreement with the notion that in glutamate and 2-methyleneglutarate mutases a conserved histidine residue of the protein is coordinated to the cobalt atom of coenzyme $B_{12}$, whereas in ethanolamine ammonia-lyase the dimethylbenzimidazole residue of the coenzyme itself serves as ligand.

***Keywords:***

(Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresyl)cobamide; base-off analogue of coenzyme-$B_{12}$; ethanolamine ammonia-lyase (*Salmonella typhimurium*), glutamate mutase (*Clostridium cochlearium*); 2-methyleneglutarate mutase (*Clostridium barkeri*).

*Abbreviations.* HO-Cbl, aquacobalamin (vitamin $B_{12a}$); Ado-PCC, (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresyl)cobamide.

## INTRODUCTION

Coenzyme $B_{12}$-dependent enzymes, as are methyltransferases and carbon skeleton mutases, bind their cofactor via a conserved histidine residue of the protein, which is coordinated to the cobalt atom of coenzyme $B_{12}$, and the dimethylbenzimidazole residue of the coenzyme is attached to another site. On the other hand, it has been shown for dioldehydratases, that upon binding of the coenzyme to the apoenzyme, the dimethylbenzimidazole ligand remains coordinated to the cobalt and is not exchanged by histidine residue from the protein. This difference in coenzyme binding has been termed "base off" and "base on", respectively. Since the imidazole part of histidine is also a base, the designation "base exchange" rather than "base off" is more appropriate.

Until now three methods have been published to distinguish between these binding modes: X-ray crystallography [1,2], EPR spectroscopy using $^{15}N$-labelled enzymes and/or coenzymes [3-7] and using the base-off analogue of coenzyme-$B_{12}$, Ado-PCC, which behaves either as excellent coenzyme or an inhibitor [8] depending on the binding mode.

The first X-ray structure showing a cobalamin coordinated to a histidine residue of the protein was elucidated by Drennan et al [1] with the methylcobalamin-dependnt methionine synthase. More recently, Mancia et al. [2] and Reitzer et al. [9] identified the same kind of binding in the coenzyme-$B_{12}$-dependent methylmalonyl CoA-mutase and glutamate mutase, respectively.

The EPR method is based on the superhyperfine splitting of the spectrum of cob(II)alamin by an axial nitrogen ligand. Interaction of unpaired electron with the $^{59}Co$ nucleus [$I(^{59}Co)=7/2$] [10, 11] leads to an octet (hyperfine splitting) while each member thereof is further split to triplets reflecting additional interaction with the axial nitrogen ligand. Substitution of the latter by the isotope $^{15}N$ [$I(^{15}N)=1/2$] results in an octet of doublets. On the

basis of this phenomenon it has been shown using $^{15}$N-labelled enzymes that those rearranging carbon skeletons replace the original dimethylbenzimidazole ligand by the imidazole of a histidine residue of the protein.

By contrast, using Ado-Cbl labelled with $^{15}$N in the dimethylbenzimidazole ligand, it has been shown that diol dehydratase [6] and ribonucleotide reductase [12] bind the cofactor without base exchange. The same conclusion has been drawn using an $^{15}$N-labelled artificial coenzyme $B_{12}$ analogue [7].

The third method to differentiate between the two binding modes is the use of base-off analogues of coenzyme $B_{12}$. In a previous paper we have shown that Ado-PCC served as a coenzyme in the methylmalonyl-CoA mutase reaction, while it was an inhibitor for propanediol dehydratase and glycerol dehydratase [8].

Here we present further results confirming the usefulness of Ado-PCC (Fig. 1) for differentiating between the two coenzyme binding modes.

## EXPERIMENTAL

**Materials:** Adenosine, coenzyme-$B_{12}$ and sodium tetrahydroborate were obtained from Fluka Chemie A.G. Other other biochemicals were purchased from Boehringer Mannheim. (*Coβ*-5′-Deoxyadenosin-5′-yl)-(p-cresyl)cobamide (Ado-PCC) was synthesized and purified as previously described [8].

**Enzymes:** Alcohol dehydrogenase from yeast (EC 1.1.1.1) was obtained from Boehringer Mannheim. Glutamate mutase (EC 5.4.99.1) from *Clostridium cochlearium* [13] and 2-methyleneglutarate mutase (EC 5.4.99.4) from *Clostridium barkeri* [14] were overproduced in *E. coli* and isolated as previously described. Methylaspartate ammonia lyase (EC 4.3.1.2) and 3-methylitaconate isomerase (EC 5.3.3.6) were isolated from *Clostridium barkeri* [15-17]. The *E. coli* strain CAG626, which overexpresses the genes coding for ethanolamine ammonia lyase

(EC 4.3.1.7) from *Salmonella typhimurium* (plasmid pE AL31/50) [18], was generously provided by Drs. B. Babior [18] and C. B. Grissom [19].

***Isolation of ethanolamine ammonia lyase (EAL):***

*Cell cultivation and harvesting.* Luria-Bertani-agar plates (containing ampicillin, 60 mg/L) were inoculated with the *E. coli* strain and incubated at 37 °C with shaking at 250 rpm for 16 h. This culture was added to 1 L of the above LB media and incubated at 37 °C with shaking at 250 rpm for 3 h (until the $A_{600}$ reached about 1.0) and then (130 mg of isopropyl β-D-thiogalactoside was added. The culture was shaken at 37 °C for further 4 h. Finally the cells were harvested by centrifugation at 4500 × g for 10 min and the pellet was washed with potassium phosphate buffer (20 mM, pH 7.5).

*Enzyme Isolation.* The pellet (3.5 g wet paste) was suspended in 10 mL 20 mM potassium phosphate, pH 7.5, which contained 40 U benzonase (Merck), 5 mM benzamidine and 0.5 mM phenylmethylsulfonyl fluoride, and the cells were disrupted by sonification (Branson, Model 450, 70% power setting) at 4-8 °C for 10 min. The resulting slurry was centrifuged at 30,000 × g and 0°C for 30 min. To the supernatant 16.4 % ammonium sulfate was added and the solution was stirred at 4 °C for 30 min and centrifuged at 30,000 × g and 0 °C for 30 min. The pellet was dissolved in 30 mL 10 mM potassium phosphate, pH 7.4, 1 mM ethanolamine hydrochloride, 10 mM potassium chloride, 5 mM dithiotreitol and 10 % (by vol.) glycerol. The solution was clarified by dialysis against the same buffer at 4 °C overnight and applied to a 30 x 16 mm Resource Q column (Pharmacia) equipped with a 25 x 16 mm HiTrap Q (Pharmacia) precolumn 20 °C, 6 mL/min. The enzyme was eluted with a linear gradient of solvent B in solvent A; solvent A: potassium 10 mM phosphate, pH 7.4, 10 mM potassium chloride, 10 mM ethanolamine hydrochloride and 5 mM dithiotreitol; solvent B: 10 mM potassium phosphate, pH 7.4, 1.5 M potassium chloride, 10 mM ethanolamine hydrochloride and 5 mM dithiotreitol. The active fractions of ethanolamine ammonia lyase were eluted between 20 and

30 % of solvent B. The active fractions were combined and applied to a 600 × 26 mm HiLoad 26/600 Superdex 200 column (Pharmacia) (4 °C, 1.5 mL/min, elution with 20 mM potassium phosphate, pH 7.5, 0.5 M potassium chloride, 10 mM ethanolamine hydrochloride and 5 mM dithiotreitol). The fractions showing enzyme activity (100-130 min) were combined to yield 34.6 mg of protein with a specific activity of 36 U/mg. After the solution was kept at 4 °C overnight, glycerol was added to a final concentration of 50 % (by vol.) and the mixture was frozen at -20 °C. Under these conditions the specific activity of 28.8 U/mg remained unchanged after storage for six months.

**Enzyme assays:**

*Glutamate mutase*: The assay was based on the monitoring of the absorption of mesaconate ($\lambda_{max}$= 240 nm) [15, 16]. The assay mixture (total volume 1 ml) contained: 10 mM glutamate, 1 mM mercaptoethanol, 47 mM Tris/HCl pH 8.3, 9.4 mM KCl, 0.94 mM $MgCl_2$, 3 U methylaspartase, 0.25 μM component E and 3.1 μM component S of glutamate mutase. Component S was incubated with mercaptoethanol for 3 min at 37°C. The reaction was started by addition of the coenzyme or coenzyme analogue [20].

*2-Methyleneglutarate mutase*: The assay was based on the monitoring of the absorption of dimethylmaleinate at 256 nm [17]. The assay mixture (total volume 1 ml) contained: 10 mM 2-methyleneglutarate, 100 mM potassium phosphate buffer (pH 7.4), 0.3 U 3-methylitaconate isomerase, and 0.3 μM 2-methyleneglutarate mutase. After incubation of the assay mixture at 37 °C for 5 min, the reaction was started by addition of the coenzyme or coenzyme analogue.

*Ethanolamine ammonia lyase*: The assay was based on established yeast alcohol dehydrogenase - NADH coupled methods [3, 4] with minor modifications. In a cuvette 50 mM potassium phosphate buffer, pH 7.5, 10 mM ethanolamine hydrochloride and 0.2 mM of β-NADH, 5 U yeast alcohol dehydrogenase and 20 μl ethanolamine ammonia lyase solution (1:3 dilution from the above glycerol stock with 50 mM potassium phosphate, pH 7.5) were mixed

and the resulting solution was incubated at 37 °C for 3 min. A blank was determined without addition of coenzyme-$B_{12}$. The enzymatic reaction was started by addition of 0.001-1 mM coenzyme-$B_{12}$ solution and the decrease of absorbance at 340 nm was recorded for several min at 37 °C. The rate of reaction was calculated from the change of absorption at 340 nm.

**Kinetic investigations:**

The kinetic constants for coenzyme $B_{12}$ and Ado-PCC with the three enzymes were measured using the spectrophotometric assays described above. The $K_m$ and $V_{max}$ values were determined with 1 nM - 1 mM coenzyme $B_{12}$ or Ado-PCC at 9 data points. The standard linearisation methods by Lineweaver-Burk, Hanes or Eadie-Hofstee were used.

The apparent Michaelis constants for coenzyme $B_{12}$ and Ado-PCC are given in Table 1. It should be noted that the determined apparent $K_m$ values for the cobamides are dependent on the concentration of the enzyme in the assay. In the simple Michealis-Menten equation used in this work, a basic assumption is a great excess of substrate over enzyme. Since here the enzyme and coenzyme concentrations were in the same range, these constants should be regarded as apparent relative values.

The apparent inhibition constant ($K_i$) for Ado-PCC was determined by the so-called 'parallel' method [21]. To the assay mixture coenzyme $B_{12}$ and Ado-PCC were added simultaneously. The inhibitor concentrations were varied (6-8 data points) at three different coenzyme $B_{12}$ concentrations (0.5 - 2.5 μM). Inhibition constants were calculated from the equation: $K_i = (\text{slope} \times [\text{coenzyme } B_{12}] \times V_{max})/K_m$, where $K_i$, $K_m$ and [coenzyme $B_{12}$] are given in nM, $V_{max}$ in nmol/min; the slopes were taken from the linear regressions in Dixon plots (1/V *vs.* [Ado-PCC]); the final was $K_i$ taken as an average of the particular $K_i$ values at different coenzyme $B_{12}$ concentrations.

## RESULTS AND DISCUSSION

Ado-PCC a base-off analogue of coenzyme $B_{12}$ [8] was probed as a coenzyme and/or inhibitor of the coenzyme $B_{12}$ dependent recombinant enzymes, ethanolamine ammonia lyase, 2-methyleneglutarate mutase and glutamate mutase. The kinetic constants of Ado-PCC with the three enzymes are listed in Table 1. For ethanolamine ammonia lyase Ado-PCC acts only as an inhibitor and the corresponding inhibition constant ($K_i$) is similar to the relative $K_m$ for coenzyme $B_{12}$ (30 nM).

For the two carbon skeleton rearranging enzymes 2-methyleneglutarate mutase and glutamate mutase Ado-PCC serves as coenzyme. In the case of 2-methyleneglutarate mutase the relative $K_m$ value for the Ado-PCC was found about five times lower than that for coenzyme $B_{12}$. Moreover, while there was a lag phase of 1 – 2 min when the reaction was started with the natural coenzyme, this was not observed with Ado-PCC. On the other hand, the $K_m$ value for glutamate mutase was about twice as high with Ado-PCC as with coenzyme $B_{12}$. At higher concentrations (>300 nM) Ado-PCC caused some inhibition.

Ado-PCC, a base-off analogue of coenzyme-$B_{12}$, turned out to be an excellent probe for testing the binding mode of Ado-Cbl dependent enzymes. For those enzymes binding the coenzyme in the base-exchange mode, Ado-PCC is an excellent coenzyme, whereas for the others it acts as an inhibitor [8]. Recently, we suggested that the planar hydrophobic *p*-cresyl group may occupy the same hydrophobic binding pocket of the base-off enzymes as the dimethylbenzimidazolyl group of coenzyme $B_{12}$ [8]. This idea is supported by the high apparent affinity, i.e. low $K_m$ value, of Ado-PCC for these enzymes. In the case of Ado-PCC, the removal of the original base from the co-ordination sphere of the cobalt is not necessary, which may facilitate the binding process. Support for this interpretation comes from kinetic results with 2-methyleneglutarate mutase. As mentioned above, the lag phase upon initiating the reaction with coenzyme $B_{12}$ is abolished when Ado-PCC is used as coenzyme. In other

words, the energy barrier to remove the dimethylbenzimidazole base from the co-ordination sphere of the cobalt may cause the lag phase observed with the natural coenzyme.

## CONCLUSIONS

The kinetic results with three further coenzyme-$B_{12}$ dependent enzymes confirmed the utility of Ado-PCC to demonstrate the binding mode of the coenzyme to the protein. For glutamate mutase the base-exchange binding mode has been shown by EPR spectroscopy using $^{15}N$-labelled enzymes [4]. Glutamate mutase, 2-methyleneglutarate mutase and methylmalonyl-CoA mutase possess in the $B_{12}$-binding region a consensus sequence with a conserved histidine which substitutes for the displaced dimethylbenzimidazole as axial cobalt ligand [2, 13, 14, 22-25]. Such a consensus sequence is lacking in propanediol and glycerol dehydratases as well as in ethanolamine ammonia-lyase [18, 26, 27]. Our results with Ado-PCC are consistent with expectations derived from the amino acid sequences of these enzymes.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the generous gift of an *E. coli* strain overproducing ethanolamine ammonia lyase from Drs. B. Babior and C. B. Grissom. We thank the European Union and the Fonds der Chemischen Industrie for financial support. *L. P.* also thanks the Alexander von Humboldt Foundation for a short term postdoctoral fellowship (1998) and the Hungarian OTKA (T023162) and OMFB (01789/98) for financial support.

## REFERENCES

[1] C. Luschinsky-Drennan, S. Huang, J. T. Drummond, R. G. Matthews, M. L. Ludwig, *Science 266* (1994)1669.

[2] F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. Mc Sweeney, B. Rasmussen, P. Bösecke, O. Diat, P. R. Evans, *Structure 4* (1996) 339.

[3] E. Stupperich, H. J. Eisinger, S. P. J. Albracht, *Eur. J. Biochem. 193* (1990) 105.

[4] O. Zelder, B. Beatrix, F. Kroll, W. Buckel, *FEBS Lett. 369* (1995) 252.

[5] R. Padmakumar, S. Taoka, R. Padmakumar, R. Banerjee, *J. Am. Chem. Soc. 117* (1995) 7033.

[6] A. Abend, R. Nitsche, V. Bandarian, E. Stupperich, J. Rétey, *Angew. Chem . 110* (1998) 643, *Angew. Chem. Int. Ed. Engl. 37* (1998) 625.

[7] M. Yamanishi, S. Yamada, H. Muguruma, Y. Murakami, T. Tobimatsu, A. Ishida, J. Yamauchi, T. Toraya, *Biochemistry 37* (1998) 4799.

[8] L. Poppe, E. Stupperich, W. E. Hull, T. Buckel, J. Rétey, *Eur. J. Biochem. 250* (1998) 303.

[9] R. Reitzer, K. Gruber, G. Jogl, U. G. Wagner, H. Bothe, W. Buckel, C. Kratky, *Structure* (1999) in press.

[10] J. R. Pilbrow, in D. Dolphin, (Ed.), $B_{12}$, Vol. 1., Wiley Interscience, New-York, 1982, p. 431.

[11] J. R. Pilbrow, M. E. Winfield, *Mol. Phys. 25* (1973) 1073.

[12] C. C. Lawrence, G. J. Gerfen, V. Samano, R. Nitsche, M. J. Robins, J. Rétey, J. Stubbe, *J. Biol. Chem. 274* (1999) 7739.

[13] O. Zelder, B. Beatrix, U. Leutbecher, W. Buckel, *Eur. J. Biochem. 226* (1994) 577.

[14] B. Beatrix, O. Zelder, W. Buckel, *Eur. J. Biochem. 221* (1994) 101.

[15] H. A. Barker, V. Rooze, F. Suzuki, A. A. Iodice, *J. Biol. Chem. 239* (1964) 3260.

[16] U. Leutbecher, *Ph.D. Thesis*, Philipps-Universität, Marburg, 1992.

[17] C. Michel, G. Hartrampf, W. Buckel, *Eur. J. Biochem. 184* (1989) 103.

[18] L. P. Faust, B. M. Babior, *Arch. Biochem. Biophys. 294* (1992) 50.

[19] T. T. Harkins,C. B. Grissom, *J. Am. Chem. Soc. 117* (1995) 566.

[20] H. A.Barker, V. Rooze, F. Suzuki, A. A. Iodice, *J. Biol. Chem. 239* (1964) 3260.

[21] L. Poppe, J. Rétey, *Arch. Biochem. Biophys. 316* (1995) 541.

[22] E. N. Marsh, N. McKie, N. K. Davis, P. F. Leadlay, *Biochem. J. 260* (1989) 345.

[23] R. Jansen, F. Kalousek, W. A. Fenton, L. E. Rosenberg, F. D. Ledley, *Genomics 4* (1989) 198.

[24] E. Andrews, R. Jansen, A. M. Crane, S. Cholin, D. McDonnell, F. D. Ledley, *Biochem. Med. & Metabol. Biol. 50* (1993) 135.

[25] E. N. G. Marsh, D. E. Holloway, *FEBS Lett. 310* (1992) 167.

[26] T. Tobimatsu, T. Hara, M. Sakaguchi, Y. Kishimoto, Y. Wada, M. Isoda, T. Sakai, T. Toraya, *J. Biol. Chem. 270* (1995) 7142.

[27] M. Seyfried, R. Daniel, G. Gottschalk, *J. Bacteriol. 178* (1996) 5793.

**FIGURE 1.** Structure of (Co$\beta$-5′-deoxyadenosin-5′-yl)-(*p*-cresyl)cobamide (Ado-PCC)

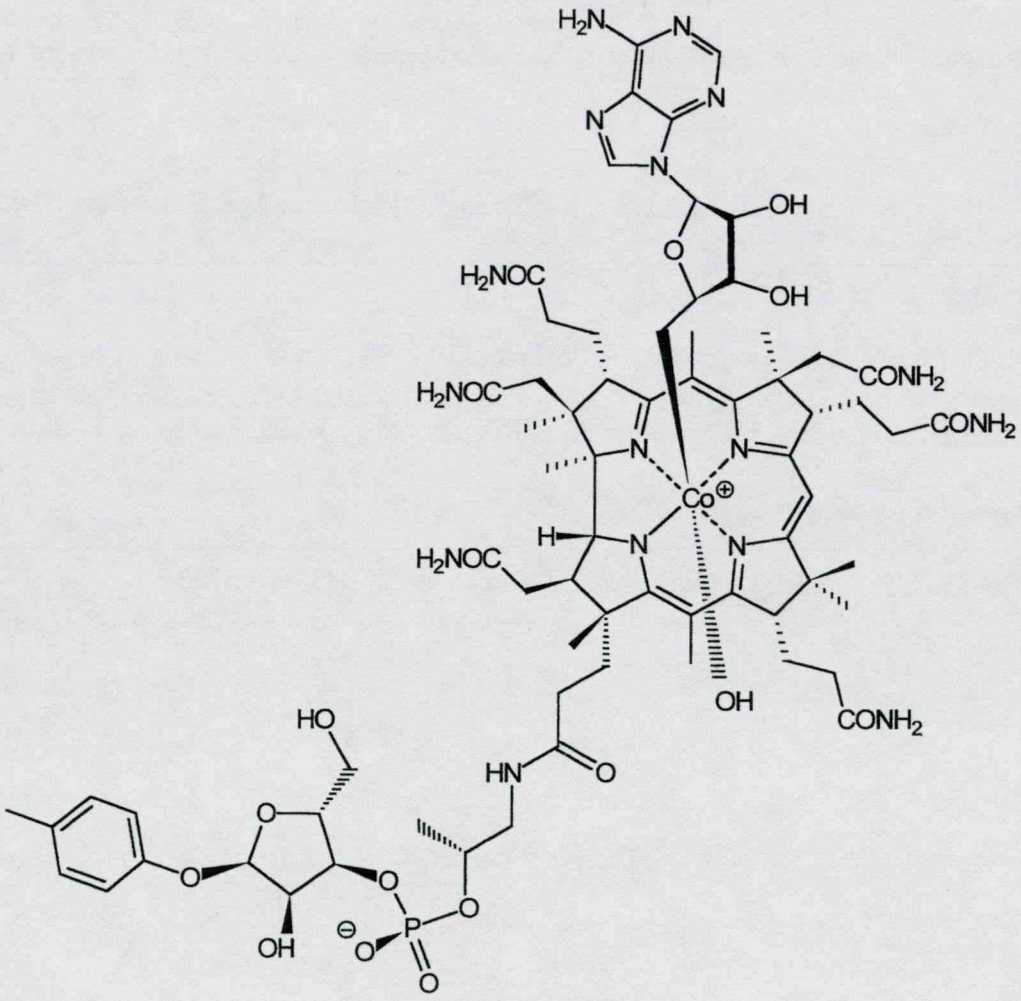


**TABLE 1.** *Kinetic properties of Ado-PCC with 2-methyleneglutarate mutase, glutamate mutase and ethanolamine ammonia lyase*

| Enzyme | Coenzyme $B_{12}$ | | Ado-PCC | | |
|---|---|---|---|---|---|
| | $K_m$ | $k_{cat}$ | $K_m$ | $k_{cat}$ | $K_i$ |
| | [nM] | [$s^{-1}$] | [nM] | [$s^{-1}$] | [nM] |
| Ethanolamine ammonia lyase | 30 ± 6 | | no activity | | 25 ± 6 |
| 2-Methyleneglutarate mutase | 54 ± 4 | 2.9 ± 0.2 | 10 ± 1 | 2.2 ± 0.2 | no inhibition |
| Glutamate mutase | 100 ± 20 | 9.0 ± 1 | 200 ± 20 | 0.9 ± 0.1 | no inhibition* |

Apparent $K_m$ constants were measured as described under 'Kinetic investigations'. Apparent inhibition constant $K_i$ was estimated by the 'parallel' method [21]. Enzymes: ethanolamine ammonia-lyase of *Salmonella typhimurium* overproduced in *E. coli* ($V_{max}$ = 35 ± 3 nmol/min with coenzyme $B_{12}$); 2-methyleneglutarate mutase from *Clostridium barkeri* overproduced in *E. coli* ($V_{max}$ = 129 ± 7 nmol/min with coenzyme $B_{12}$); glutamate mutase from *Clostridium cochlearium* overproduced in *E. coli* ($V_{max}$ = 3760 ± 120 nmol/min with coenzyme $B_{12}$). *At higher concentrations (>300 nM) Ado-PCC caused some inhibition.

# XX. melléklet

**POPPE, L.**, HULL, W. E., NITSCHE, R., GRAF, T., STUPPERICH, E., RÉTEY, J.:

Hydroxyalkylcobalamins as Competitive Inhibitors in Coenzyme $B_{12}$-dependent Enzymic Reactions: $^1$H-NMR Structure Analysis and Kinetic Studies with Glycerol Dehydratase and Diol Dehydratase,

*Helv. Chim. Acta*, **1999**, *82*, 1250.

# (Hydroxyalkyl)cob(III)alamins as Competitive Inhibitors in Coenzyme $B_{12}$-Dependent Enzymic Reactions: $^1$H-NMR Structure Analysis and Kinetic Studies with Glycerol Dehydratase and Diol Dehydratase

by **László Poppe**[a][b], **William E. Hull**[c], **Rainer Nitsche**[a], **Torsten Graf**[a], **Erhard Stupperich**[d], and **János Rétey**[a]*

[a]) Department of Biochemistry, Institute of Organic Chemistry, University of Karlsruhe, Richard-Willstätter-Allee 2, D-76128 Karlsruhe; fax: +49-721-6084823; e-mail: biochem@ochhades.chemie.uni-karlsruhe.de

[b]) Chemical Research Center, Institute for Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59–67, H-1025 Budapest

[c]) Central Spectroscopy Department, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg

[d]) Applied Microbiology, University of Ulm, Albert-Einstein-Allee 11, D-89070 Ulm

A series of (hydroxyalkyl)cobalamins, *i.e.*, **1a–d**, (HO–$(CH_2)_n$–Cbl, $n=2-5$), two diastereoisomeric (2,3-dihydroxypropyl)cobalamins, *i.e.*, **2a,b** ([(*R*)- and (*S*)-[$(HO)_2$pr]-Cbl) and their diastereoisomeric 'base-off' analogues, the (*Coβ*-2,3-dihydroxypropyl-[1'-*O*-(*p*-tolyl)cobamides]) **3a,b** ([(*R*)- and (*S*)-$(HO)_2$pr]-PTC) were prepared and characterized by their 500-MHz $^1$H-NMR spectra. The inhibitory activities of these compounds and of hydroxocobalamin (HO–Cbl) and (Co*α*-cyano)(Co*β*-hydroxo)[1'-*O*-(*p*-tolyl)cobamide] (HO–PTC) were tested with two coenzyme-$B_{12}$-dependent enzymes: glycerol dehydratase (GDH) and propane-1,2-diol dehydratase (DDH) (*Table 4*). The hydroxyalkyl and dihydroxypropyl derivatives of cobalamin acted as strong competitive inhibitors of coenzyme $B_{12}$ (5'-deoxy-5'-adenosylcobalamin, Ado–Cbl) for both enzymes, with $K_i$ values falling within the range defined by HO–Cbl (best inhibitor) and CN–Cbl ($K_i/K_m$ ratio of *ca.* 2). The short-chain HO–$(CH_2)_n$–Cbl (**1a,b**; $n=2$ or 3) exhibited $K_i$ equal to the $K_m$ for Ado–Cbl. The [(*R*)- and (*S*)-$(HO)_2$pr]–Cbl (**2a,b**) and the long-chain HO–$(CH_2)_n$–Cbl (**1c,d**; $n=4, 5$) were less efficient inhibitors, with [(*S*)-$(HO)_2$pr]–Cbl (**2a**) performing slightly better than the (*R*)-diastereoisomer **2b** for both enzymes. The 'base-off' analogues, Ado–PTC and [(*R*)- and (*S*)-$(HO)_2$pr]–PTC (**3a,b**), were moderate inhibitors with $K_i/K_m$ ratios of 4.5–28 for GDH or 7–13 for DDH. [(*S*)-$(HO)_2$pr]–PTC (**3a**) was the best inhibitor in this group. The non-alkylated analogue (HO,CN)–PTC proved to be a very poor inhibitor. These results confirm that the 'base-on' binding mode of coenzyme $B_{12}$ is preferred for GDH and DDH. The increase in $K_i$ for PTC- *vs.* Cbl-type inhibitors may result from the entropic penalty required for folding of the PTC nucleotide chain into a Cbl-like loop conformation. Hydrophilic interactions between the *β*-ligand of the inhibitor and ribosyl- or substrate-binding sites may make an important contribution to the formation or stabilization of the apoenzyme-inhibitor complex, especially for the PTC derivative.

**Introduction.** – In several enzyme-catalyzed rearrangements, coenzyme $B_{12}$ (Ado–Cbl; Ado = 5'-deoxy-5'-adenosyl) plays an essential role as a cofactor [1–5]. The common initial step in these reactions is the homolysis of the bond between the Co-atom and C(5') of the adenosyl ligand on the *β*-face of the cofactor, leading to highly reactive radical intermediates which initiate the rearrangement of the substrate. It has been assumed that the energy required for this homolysis originates from a conformational change in the protein induced by binding of the substrate.

Coenzyme-$B_{12}$-dependent enzymes may bind their cofactor in two different ways. In the 'base-on' mode, the original 5,6-dimethylbenzimidazolyl moiety of the lower

nucleotide loop of Ado–Cbl remains as the $\alpha$-ligand of the Co-atom in the enzyme-coenzyme complex. In the 'base-off' binding mode, the 5,6-dimethylbenzimidazolyl moiety is displaced from the Co-atom and a histidine residue of the protein coordinates to the Co-atom as $\alpha$-ligand.

In the homolysis of the Co–C bond, the nature of the $\alpha$-ligand attached to the Co-center and the mode of binding of Ado–Cbl to the protein may play a crucial role. It was concluded that for homolysis of the Co–C bond on the $\beta$-side, the central Co-atom of the corrin ring should be coordinated by a basic N-atom from the $\alpha$-side [6][7]; *i.e.*, a histidine N-atom from the protein for the 'base-off' enzymes or a 5,6-dimethylbenzimidazole N-atom from Ado–Cbl for the 'base-on' enzymes. On the basis of X-ray crystallography [8][9], EPR spectroscopy using $^{15}$N-labeled enzymes and/or coenzymes [10–15], and enzyme kinetics with Ado–PTC[1]) as a 'base-off' analogue of coenzyme $B_{12}$ [16][17], the binding mode for several coenzyme-$B_{12}$-dependent enzymes has been determined. In the coenzyme-$B_{12}$-binding region of the 'base-off' enzymes, there is a consensus sequence with a conserved histidine [18–23], but such a sequence similarity is lacking in propanediol and glycerol dehydratases [24][25].

In a previous study, we investigated the interaction of glycerol dehydratase (GDH) and propanediol dehydratase (DDH) with a series of [$\omega$-(adenosin-5′-*O*-yl)alkyl]cobalamins (Ado–O$(CH_2)_m$–Cbl; $m=3-7$) as possible models or mimics of the posthomolysis intermediate state of coenzyme $B_{12}$ [26]. The hypothesis is that, following bond homolysis, the separation between the Co and adenosyl C(5′) atoms increases due to a conformational change in the enzyme, possibly triggered by substrate binding. Therefore, coenzyme-$B_{12}$ analogues with an increased spacing between the Co-atom and the adenosyl moiety may bind to and stabilize the protein in the posthomolysis conformation. The 'optimal' spacing may depend on the size of the substrate and the geometric relationship between its binding site and the corrin binding site. In agreement with our expectations based on the small size of the substrate for GDH and DDH, the short-chain coenzyme-$B_{12}$ analogues ($m=3-5$), especially the $C_5$ analogue ($K_i=5.9$ and 500 nM for GDH and DDH, resp.), were found to be slightly stronger inhibitors than those with longer chains ($m=6$ and 7; $K_i=11.7$ and 15.1 nM resp., for GDH, and 630 and 830 nM, resp. for DDH). In our previous study, the dehydratase-inhibition results obtained with hydroxocobalamin (HO–Cbl) and cyanocobalamin (CN–Cbl) showed interesting features [26]. Although both HO–Cbl and CN–Cbl consist of a cobalamin bearing only a $\beta$-ligand of minimal size, their inhibition properties differed significantly. While CN–Cbl with the apolar $\beta$-CN ligand proved to be a moderate inhibitor, similar to the long-chain ($m=6$ and 7) Ado–O$(CH_2)_m$–Cbl posthomolysis-state analogues, HO–Cbl bearing the polar, hydrophilic $\beta$-OH ligand was found to be the strongest inhibitor tested for both dehydratases. The ratio of $K_i$ values for HO–Cbl *vs.* CN–Cbl was 0.40 for GDH and 0.48 for DDH, which translates into a binding free energy difference $\Delta\Delta G$ of *ca.* 2.4 kJ/mol in both cases (assuming $\Delta G_{bound} = +RT \ln K_i$).

We rationalized the difference in $K_i$ for HO–Cbl *vs.* CN–Cbl by proposing that HO–Cbl with its hydrophilic $\beta$-OH ligand may interact *via* a protein-associated $H_2O$

[1]) The abbreviation PCC (for (*p*-cresolyl)cobamide) was used previously [10][16] to represent the same moiety abbreviated in this work as PTC for [1′-*O*-(*p*-tolyl)cobamide].

molecule with the substrate binding site in addition to the corrin binding site in each dehydratase. The estimated −2.4 kJ/mol difference in $\Delta G$ for the binding of HO−Cbl *vs.* CN−Cbl is consistent with the formation of one additional H-bond in the complex. Another possibility is that solvent $H_2O$ tightly associated with the OH ligand of HO−Cbl is displaced upon binding of the inhibitor to the protein, leading to a favorable positive entropy change not available for CN−Cbl.

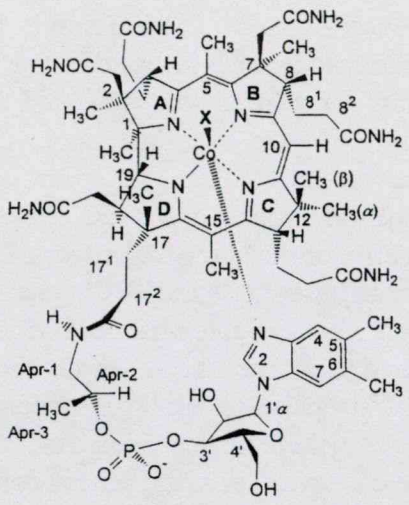
For the dehydratases, the binding of small, hydrophilic substrates apparently provides an important contribution to the energetics of the catalytic process. Only after addition of substrate to the enzyme-coenzyme-$B_{12}$ complex, EPR signals can be detected (Co−C bond homolysis); these signals also indicate that a conformational change in the ternary complex results in increased separation between the free radical centers [13][14]. The primary OH moiety of propane-1,2-diol or glycerol is not involved in the enzymic rearrangement process; therefore, we assume that it is important for the binding of the substrate of the coenzyme-enzyme binary complex *via* hydrophilic interactions.

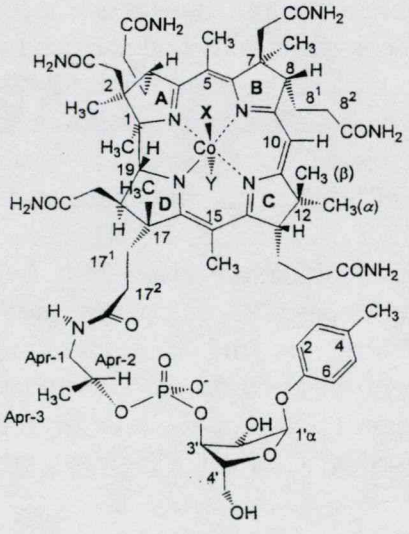
Based on these considerations, we decided to investigate several coenzyme-$B_{12}$ analogues with small hydroxyalkyl groups as hydrophilic $\beta$-ligands. Our hypothesis was that such analogues may interact with the protein at both the corrin and substrate binding sites and serve as models for a substrate-corrin binding mode, which may mimic a posthomolysis substrate-enzyme-coenzyme complex. Another possibility is that the hydroxyalkyl group may interact with a putative ribose (adenosyl) binding site, which may be important in binding of the natural coenzyme.

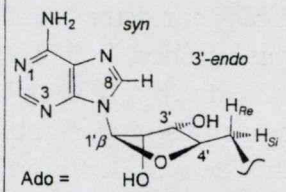
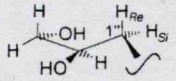
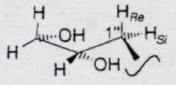
In this study, we report the synthesis, the $^1$H-NMR data, and the enzyme-kinetic behavior of a series of (hydroxyalkyl)cobalamins, *i.e.* **1a**–**d** (HO−$(CH_2)_n$−Cbl, $n=2$–5), two diastereoisomeric (2,3-dihydroxypropyl)cobalamins, *i.e.*, **2a**,**b** ([(*R*)- and (*S*)−$(HO)_2$pr]−Cbl) and their diastereoisomeric 'base-off' analogues, the (Co$\beta$-2,3-dihydroxypropyl)[1′-*O*-(*p*-tolyl)cobamides] **3a**,**b** [(*R*)- and (*S*)-$(HO)_2$pr]−PTC[1]) (*Fig.*) in the glycerol dehydratase and diol dehydratase reactions. The preparation of [(*R*)- and (*S*)−$(HO)_2$pr]−Cbl (**2a**,**b**) and partial $^1$H-NMR data have been described earlier by *Dixon et al.* [27], and the X-ray crystal structures [28] are available in the *Cambridge Crystallographic Data Files.*

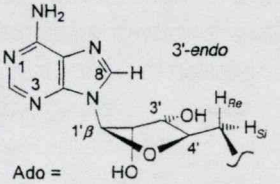
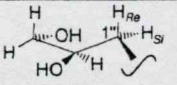
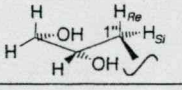
**Results.** – The desired (hydroxyalkyl)cobalamins were synthesized from HO−Cbl or its 'base-off' analogue (Co$\alpha$-cyano)(Co$\beta$-hydroxo)[1′-*O*-(*p*-tolyl)cobamide] (HO−PTC), which differs from vitamin $B_{12a}$ by only the presence of a (*p*-tosyl)oxy moiety instead of the 5,6-dimethylbenzimidazole within the nucleotide loop. The established reductive alkylation method for obtaining alkylated corrinoids from vitamin $B_{12a}$ [29] was used in these reactions as well, and conveniently provided the $\beta$-alkylated products (*Fig.*) in good yields.

One- and two-dimensional $^1$H-NMR spectra were obtained in $D_2O$ at 500 MHz for the two short-chain (hydroxyalkyl)cobalamins **1a**,**b** (HO$(CH_2)_n$−Cbl, $n=2$ and 3), the two diastereoisomeric 'base-on' dihydroxypropyl derivatives **2a**,**b** and their diastereoisomeric 'base-off' analogues **3a**,**b**. The geminal, vicinal, and long-range coupling information provided by the COSY-$\beta$ 2D experiment and the spatial information provided by the 2D-NOESY data were sufficient for the unambiguous assignment of all proton signals, with the possible exception of methylene protons at positions C(17[1])





| Cbl Derivatives | X |
|---|---|
| Ado-Cbl | Ado =  |
| AdoO$(CH_2)_m$-Cbl | AdoO$(CH_2)_m$ ($m$ = 3-7) |
| CN-Cbl | CN |
| HO-Cbl | OH |
| HO$(CH_2)_n$-Cbl (**1a-d**) | HO$(CH_2)_n$ ($n$ = 2-5) |
| [(*S*)-(HO)$_2$pr]-Cbl (**2a**) |  |
| [(*R*)-(HO)$_2$pr]-Cbl (**2b**) |  |

| PTC[1] Derivatives | X[a] |
|---|---|
| Ado-PTC | Ado =  |
| HO-PCC | HO |
| [(*S*)-(HO)$_2$pr]-PTC (**3a**) |  |
| [(*R*)-(HO)$_2$pr]-PTC (**3b**) |  |

[a]) Ado-PTC: Y = HO; HO–PCC: Y = CN; **3**: Y = HO.

Figure. *Cobalamins* **1a–d** *and* **2a,b** ((*Coβ*-X)–Cbl) *and 1′-*O (p-*tolyl)cobamides* **3a,b** (Co*β*-X)–PTC) *discussed in this work*. Ligands X and Y are on the *β*- and *α*-side of the corrin ring, respectively. The numbering scheme is that used in the NMR tables. For X–Cbl, the configurations and conformations of the corrin substituents, the nucleotide loop, and the adenosyl group are shown as accurately as possible, according to the crystal structure of coenzyme $B_{12}$ (Ado–Cbl) and in agreement with the NMR data. Bonds drawn close to vertical or horizontal directions represent axial or equatorial substituents, respectively. The dimethylbenzimidazole (Dbi) group of the nucleotide loop in Cbl derivatives lies essentially in the perpendicular plane containing the corrin C(5) and C(15) atoms, with the imidazole proton H–C(2) pointing in the direction of the corrin C(15) atom. The ribose conformation of the adenosyl moiety and nucleotide loop of Cbl derivatives is predominantly '*C*(3′)-*endo*' (H–C(3′)/H–C(4′) *trans* diaxial), but it is predominantly '*C*(2′)-*endo*' (H–C(2′) axial) in the 'base-off' loop region of the PTC derivatives. As X ligand, the adenosyl moiety is oriented with H–C(4′) pointing toward H–C(19) and the *trans* H–C(5′) pointing toward H–C(10). The ribose ring is roughly perpendicular to the corrin ring with the ring O-atom pointing toward C(14)–C(15). The adenine group in '*syn*' orientation is roughly parallel to the corrin and lies over H–C(13) and $Me_\beta$–C(12) with the adenine H–C(8) pointing toward the corrin C(7) atom. The dihydroxypropyl moieties used as X ligands are shown in conformations most closely matching the adenosyl ribose, which has the (*S*)-configuration at C(4′).

The *pro-S* protons at C(1″) and C(3″) in the dihydroxypropyl ligands are drawn with vertical bonds.

and C(17[2]). *Table 1* summarizes the chemical-shift data for four 'base-on' cobalamin compounds in comparison with the data for Ado–Cbl [30][31]. *Table 2* compares the chemical-shift data for the 'base-off' analogues **3a,b** with the published data for the 'base-off' form of Ado–Cbl (Ado–'Cbl') [32] and the 'base-off' analogue Ado–PTC[1]) [16].

A large number of individual geminal and vicinal coupling constants were evaluated in the resolution-enhanced 1D spectra. For the four Cbl analogues **1a,b** and **2a,b** examined in this study, all resolved couplings for the corrin-ring protons and side chains, as well as the couplings in the aminopropyl and ribose segments of the nucleotide loop, show only minor variations as a function of the $\beta$-ligand and agree well with the corresponding values for Ado–Cbl and the 'base-on' Ado–$O(CH_2)_m$–Cbl analogues [29]. As expected, for the two $(HO)_2$pr–PTC 'base-off' derivatives **3a,b** those couplings which could be determined deviate in several cases from the couplings observed for 'base-on' Ado–Cbl, especially in the nucleotide loop, but agree well with the corresponding values measured for Ado–PTC [16]. The coupling constants for the hydroxyalkyl $\beta$-ligands in the new Cbl and PTC derivatives are unique and are, therefore, summarized in *Table 3*, together with coupling constants for the ribose moieties.

The synthesized compounds were tested as inhibitors in the glycerol dehydratase and diol dehydratase reactions, and the results are summarized in *Table 4*, with the inhibitors arranged roughly in the order of decreasing effectiveness. Assuming that the relative $K_i$ values reflect the relative dissociation constants $K_d$ for the binary inhibitor-enzyme complexes, then a relative free energy for inhibitor binding can be calculated as shown in *Table 4*. All of the (hydroxyalkyl)cobalamins, *i.e.*, those analogues which can be used to model the substrate-enzyme-coenzyme complex that forms with the 'base-on' form of coenzyme $B_{12}$ (Ado–Cbl), proved to be potent competitive inhibitors ($K_i$ = 13–25 nM for GDH, 760–1250 nM for DDH) with respect to Ado–Cbl ($K_m$ = 12.6 nM for GDH, 720 nM for DDH). For both enzymes, the short-chain $HO(CH_2)_n$–Cbl analogues **1a,b** ($n$ = 2 and 3) showed the strongest inhibition with $K_i$ equal within experimental error to the $K_m$ of Ado–Cbl. Introduction of a second OH group to the $C_3$ alkyl chain reduced the effectiveness of the inhibitors somewhat, *i.e.*, [(*R*)- and (*S*)-$(HO)_2$pr]–Cbl (**2a** and **2b**, resp.) exhibited $K_i$ values of 17.6 and 14.8 nM, respectively, for GDH, and 1250 and 1080 nM, respectively, for DDH. An increase in the chain length as in the monohydroxyalkyl analogues **1c,d** ($n$ = 3 and 4) also reduced the effectiveness, resulting in $K_i$ values of 17.0 and 25.2 nM, respectively, for GDH, and 1060 and 950 nM, respectively, for DDH.

On the other hand, the dihydroxypropyl derivatives of the 'base-off' corrinato complex PTC were significantly less potent as inhibitors and comparable to Ado–PTC [16], *i.e.*, [(*R*)- and (*S*)-$(HO)_2$pr]–PTC (**3a** and **3b**, resp.) had $K_i$ values of 348 and 57 nM, respectively, for GDH, and 6400 and 5100 nM, respectively, for DDH.

For comparison, the 'base-on' analogue HO–Cbl proved to be the strongest inhibitor with $K_i$ values slightly lower than the $K_m$ for coenzyme $B_{12}$ (Ado–Cbl), while CN–Cbl was about as potent as the poorest hydroxyalkyl-Cbl (*Table 4*). Finally, the parent 'base-off' corrinato complex HO–PTC, bearing a $\beta$-hydroxo ligand, showed only very weak or no detectable inhibition in the GDH or DDH reactions, respectively.

Table 1. *500-MHz $^1$H-NMR Chemical-Shift Data* ($D_2O$, pH 7.4, 10°) *for Coenzyme $B_{12}$ (Ado–Cbl) and Hydroxyalkyl Derivatives* **1a,b** *and* **2a,b**[a]). Chemical shifts relative to TSP (=sodium 3-(trimethylsilyl) ($D_4$)propanoate).

| | Signal type | Chemical shifts [ppm] | | | | |
|---|---|---|---|---|---|---|
| | | Ado–Cbl[b]) | [(*R*)-(HO)$_2$pr]–Cbl (**2b**) | [(*S*)-(HO)$_2$pr]–Cbl (**2a**) | HO(CH$_2$)$_3$–Cbl (**1b**) | HO(CH$_2$)$_2$–Cbl (**1a**) |
| *Corrin Me* | | | | | | |
| Me–C(1)(a) | br. *s* | 0.47 | 0.537 | 0.454 | 0.540 | 0.510 |
| Me–C(2)(e) | br. *s* | 1.36 | 1.420 | 1.382 | 1.411 | 1.409 |
| Me–C(5) | *s* | 2.45[c]) | 2.514 | 2.519 | 2.524 | 2.537 |
| Me–C(7)(e) | br. *s* | 1.70 | 1.798 | 1.864 | 1.805 | 1.823 |
| Me$_\alpha$–C(12)(e) | br. *s* | 1.32 | 1.443 | 1.398 | 1.468 | 1.458 |
| Me$_\beta$–C(12)(a) | br. *s* | 0.87 | 1.178 | 1.260 | 1.115 | 1.143 |
| Me–C(15) | br. *s* | 2.43 | 2.506 | 2.523 | 2.504 | 2.512 |
| Me–C(17)(a) | *s* | 1.36 | 1.466 | 1.441 | 1.426 | 1.430 |
| *Corrin CH* | | | | | | |
| H–C(3)(e) | *dd* | 4.10 | 4.070 | 4.066 | 4.076 | 4.101 |
| H–C(8)(e) | *dd* | 3.29 | 3.401 | 3.278 | 3.403 | 3.403 |
| H–C(10) | *s* | 5.93 | 6.059 | 6.045 | 6.072 | 6.069 |
| H–C(13)(e) | *dd* | 2.89 | 3.238 | 3.248 | 3.240 | 3.260 |
| H–C(18)(a) | *ddd* | 2.65 | 2.67 | 2.67 | 2.68 | 2.672 |
| H–C(19)(a) | *d* | 4.24 | 4.638 | 4.183 | 4.157 | 4.138 |
| *Corrin CH$_2$* | | | | | | |
| CH$_2$(2$^1$)$_{a,b}$ | *d* | 2.41 | 2.582, 2.381 | 2.422, 2.378 | 2.43, 2.42 | 2.452, 2.389 |
| CH$_2$(3$^1$)$_{a,b}$ | *m* | 2.06, 1.96 | 2.154, 2.025 | 2.103, 1.970 | 2.135, 2.030 | 2.120, 2.015 |
| CH$_2$(3$^2$)$_{a,b}$ | *ddd* | 2.50 | 2.56, 2.486 | 2.535, 2.460 | 2.55, 2.48 | 2.55, 2.48 |
| CH$_2$(7$^1$)$_{a,b}$ | *d* | 2.19, 1.72 | 2.547, 1.976 | 2.452, 2.232 | 2.5375, 2.023 | 2.550, 2.031 |
| CH$_2$(8$^1$)$_{a,b}$ | *m* | 1.75, 0.81 | 1.815, 0.805 | 1.895, 0.950 | 1.84, 0.830 | 1.860, 0.845 |
| CH$_2$(8$^2$)$_{a,b}$ | *ddd* | 1.73, 0.88 | 1.815, 0.908 | 1.83, 0.910 | 1.80, 0.970 | 1.82, 0.954 |
| CH$_2$(13$^1$)$_{a,b}$ | *m* | 2.22, 2.00 | 2.16, 2.11 | 2.22, 2.145 | 2.115, 2.08 | 2.10, 2.07 |
| CH$_2$(13$^2$)$_{a,b}$ | *ddd* | 2.54 | 2.64, 2.61 | 2.65, 2.62 | 2.65, 2.625 | 2.65, 2.63 |
| CH$_2$(17$^1$)$_{a,b}$ [d]) | *ddd* | 1.78 | 2.545, 1.836 | 2.555, 1.825 | 2.543, 1.830 | 2.552, 1.820 |
| CH$_2$(17$^2$)$_{a,b}$ [d]) | *ddd* | 2.45, 2.06 | 2.477, 2.115 | 2.49, 2.120 | 2.47, 2.117 | 2.47, 2.10 |
| CH$_2$(18$^1$)$_{a,b}$ | *dd* | 2.65 | 2.751, 2.662 | 2.751, 2.655 | 2.767, 2.68 | 2.769, 2.675 |
| *1-Aminopropan-2-ol* | | | | | | |
| CH$_2$(1)(Apr)$_{a,b}$ | *dd* | 3.54, 3.16 | 3.538, 3.213 | 3.553, 3.191 | 3.540, 3.204 | 3.547, 3.168 |
| CH(2)(Apr) | *dddq* | 4.33 | 4.354 | 4.345 | 4.355 | 4.345 |
| Me(3)(Apr) | *d* | 1.21 | 1.207 | 1.215 | 1.209 | 1.214 |
| *Loop ribose (C(3')-endo)* | | | | | | |
| H$_\alpha$–C(1')(Rib) | *d* | 6.26 | 6.285 | 6.294 | 6.279 | 6.287 |
| H–C(2')(Rib)(e) | *dd* | 4.23 | 4.241 | 4.236 | 4.241 | 4.235 |
| H–C(3')(Rib)(a) | *dd* | 4.72 | 4.753 | 4.756 | 4.748 | 4.743 |
| H–C(4')(Rib)(a) | *dddd* | 4.10 | 4.131 | 4.116 | 4.132 | 4.117 |
| CH$_2$(5')(Rib)$_{a,b}$(*g,g*) | *dd* | 3.88, 3.74 | 3.913, 3.755 | 3.914, 3.754 | 3.915, 3.755 | 3.915, 3.751 |
| *Dimethylbenzimidazol* | | | | | | |
| H–C(2)(Dbi) | *s* | 6.95 | 6.989 | 6.983 | 6.989 | 6.969 |
| H–C(4)(Dbi) | br. *s* | 6.24 | 6.252 | 6.282 | 6.269 | 6.289 |
| H–C(7)(Dbi) | br. *s* | 7.16 | 7.189 | 7.197 | 7.186 | 7.195 |
| Me–C(5)(Dbi) | *s* | 2.19 | 2.242 | 2.242 | 2.244 | 2.248 |
| Me–C(6)(Dbi) | *s* | 2.19 | 2.230 | 2.234 | 2.233 | 2.236 |
| *Alkyl-Co* | | | | | | |
| CH$_2$(1'')$_{a,b}$ | *dd*(*d*) | 1.55, 0.57[e]) | 1.118, 0.507 | 1.589, 0.843 | 1.375, 0.535 | 1.361, 0.597 |
| H–C(2'') or CH$_2$(2'')$_{a,b}$ | *m* | 2.54[e]) | 1.681 | 1.647 | 0.50, –0.13 | 2.54, 1.960 |
| CH$_2$(3'')$_{a,b}$ | *dd*(*d*) | | 2.885, 2.778 | 2.836, 2.730 | 3.10, 3.07 | |

[a]) Numbering scheme according to the *Figure*; configuration codes in parentheses: a = axial, e = equatorial, *t* = *trans*, *g* = *gauche*, superscripts 1 or 2 refer to positions in corrin side chains; subscripts a and b refer to the high-frequency and low-frequency proton of CH$_2$ groups: Apr = 1-aminopropan-2-ol, Dbi = 5,6-dimethyl-1*H*-benzimidazole, Ade = adenine, Tol = *p*-tolyl; Rib and Ade–Rib refer to loop ribose ($\alpha$-side) and adenosyl ribose ($\beta$-side), resp. All assignments were confirmed by COSY and NOESY data, with the exception (see *Footnote d*) of the assignments for CH$_2$(17$^1$) and CH$_2$(17$^2$) which may be reversed (coupling or NOE with Me–C(17) not detected). [b]) From [30], pH 7.0, 20°. [c]) Cited as 1.45 (typographical error) in [16] and [29]; in [29], the shift for Me–C(5) of compound **1a** should be 2.439 ppm. [d]) The original assignments for CH$_2$(17$^1$) and CH$_2$(17$^2$) in Ado–Cbl [30] are given here as corrected (reversed) by *Pagano et al.* [31]; our assignments are made by analogy, considering the H–C(17$^1$)$_b$ and H–C(17$^2$)$_b$ shifts; our previous assignments for AdoO(CH$_2$)$_n$–Cbl derivatives [29] should also be reversed. [e]) For Ado–Cbl, CH$_2$(1'')$_{a,b}$ correspond to CH$_2$(5')(Ade–Rib)$_{a,b}$ and H–C(2'') to H–C(4')(Ade–Rib).

Table 2. *500-MHz $^1$H-NMR Chemical-Shift Data* ($D_2O$, pH 7.4, 10°) *for 'Base-off' Coenzyme $B_{12}$* ('base-off' form of Ado–Cbl) *and (Coβ-X)–PTC Derivatives* **3a,b** [a]). Chemical shifts relative to TSP (=sodium 3-(trimethylsilyl) ($D_4$)propanoate).

| | Signal type | Chemical shifts [ppm] | | | |
|---|---|---|---|---|---|
| | | Ado–'Cbl' ('base-off') [b]) | Ado–PTC [c]) | [(*R*)-(HO)$_2$pr]–PTC (**3b**) | [(*S*)-(HO)$_2$pr]–PTC (**3a**) |
| *Corrin Me* | | | | | |
| Me–C(1) (a) | br. *s* | 0.81 | 0.704 | 0.788 | 0.786 |
| Me–C(2) (e) | br. *s* | 1.48 | 1.405 | 1.511 | 1.469 |
| Me–C(5) | *s* | 2.43 | 2.389 | 2.374 | 2.399 |
| Me–C(7) (e) | br. *s* | 1.82 | 1.819 | 1.854 | 1.923 |
| $Me_\alpha$–C(12) (e) | br. *s* | 1.67 | 1.585 | 1.648 | 1.640 |
| $Me_\beta$–C(12) (a) | br. *s* | 1.00 | 0.845 | 1.037 | 1.094 |
| Me–C(15) | br. *s* | 2.46 | 2.336 | 2.483 | 2.484 |
| Me–C(17) (a) | *s* | 1.40 | 1.141 | 1.498 | 1.469 |
| *Corrin CH* | | | | | |
| H–C(3) (e) | *dd* | 4.23 | 4.217 | 4.047 | 4.128 |
| H–C(8) (e) | *dd* | 3.73 | 3.784 | 3.797 | 3.797 |
| H–C(10) | *s* | 6.97 | 6.994 | 7.025 | 7.036 |
| H–C(13) (e) | *dd* | 3.43 | 3.308 | 3.543 | 3.557 |
| H–C(18) (a) | *ddd* | 2.85 | 2.787 | 2.870 | 2.906 |
| H–C(19) (a) | *d* | 4.70 | 4.653 | 5.106 | 4.733 |
| *Corrin $CH_2$* | | | | | |
| $CH_2(2^1)_{a,b}$ | *d* | 2.60, 2.46 | 2.66, 2.285 | 2.665, 2.495 | 2.599, 2.480 |
| $CH_2(3^1)_{a,b}$ | *m* | 2.11, 1.97 | 1.99, 1.89 | 2.05, 1.925 | 2.012, 1.91 |
| $CH_2(3^2)_{a,b}$ | *ddd* | 2.55 | 2.507, 2.475 | 2.532, 2.49 | 2.520, 2.50 |
| $CH_2(7^1)_{a,b}$ | *d* | 2.61, 2.14 | 2.275, 1.731 | 2.440, 1.983 | 2.460, 2.130 |
| $CH_2(8^1)_{a,b}$ | *m* | 2.21, 1.75 | 2.250, 1.81 | 2.295, 1.835 | 2.32, 1.88 |
| $CH_2(8^2)_{a,b}$ | *ddd* | 2.35, 2.35 | 2.41, 2.31 | 2.445, 2.36 | 2.43, 2.315 |
| $CH_2(13^1)_{a,b}$ | *m* | 2.21, 1.92 | 2.185, 1.885 | 2.25, 1.960 | 2.24, 1.95 |
| $CH_2(13^2)_{a,b}$ | *ddd* | 2.21, 1.86 | 2.148, 1.788 | 2.185, 1.830 | 2.174, 1.809 |
| $CH_2(17^1)_{a,b}$ [d]) | *ddd* | 2.51, 1.85 | 2.425, 1.76 | 2.525, 1.835 | 2.55, 1.86 |
| $CH_2(17^2)_{a,b}$ [d]) | *ddd* | 2.31, 1.85 | 2.365, 1.833 | 2.39, 1.86 | 2.45, 1.86 |
| $CH_2(18^1)_{a,b}$ | *dd* | 2.78 | 2.68, 2.49 | 2.823 | 2.85, 2.80 |
| *1-Aminopropan-2-ol* (Apr) | | | | | |
| $CH_2$(1)(Apr)$_{a,b}$ | *dd* | 3.38, 3.27 | 3.470, 3.395 | 3.45, 3.42 | 3.475, 3.426 |
| CH(Apr) | *dddq* | 4.36 | 4.42 | 4.416 | 4.423 |
| Me(3)(Apr) | *d* | 1.23 | 1.238 | 1.248 | 1.255 |
| *Loop ribose* (C(2')-*endo*) | | | | | |
| $H_\alpha$–C(1')(Rib) (e) | *d* | 6.56 | 5.664 | 5.660 | 5.670 |
| H–C(2')(Rib) (a) | *dd* | 4.97 | 4.331 | 4.333 | 4.339 |
| H–C(3')(Rib) (e) | *dd* | 4.83 | 4.551 | 4.550 | 4.557 |
| H–C(4')(Rib) (a) | *dd* | 4.79 | 4.38 | 4.389 | 4.398 |
| $CH_2$(5')(Rib)$_{a,b}$(*g,g*) | *dd* | 3.94, 3.84 | 3.72, 3.70 | 3.71, 3.70 | 3.725, 3.710 |
| *Tolyl* | | | | | |
| H–C(2)/H–C(6)(Tol) | *d* | | 6.873 | 6.878 | 6.890 |
| H–C(3)/H–C(5)(Tol) | *d* | | 7.026 | 7.033 | 7.044 |
| Me–C(4)(Tol) | *s* | | 2.128 | 2.120 | 2.136 |
| *Adenosyl* | | | | | |
| H–C(2)(Ade) | *s* | 8.43 | 8.234 | | |
| H–C(8)(Ade) | *s* | 8.21 | 8.037 | | |
| $H_\beta$–C(1')(Ade–Rib) | *d* | 5.61 | 5.615 | | |
| H–C(2')(Ade–Rib) (e) | *dd* | 4.34 | 4.40 | | |
| H–C(3')(Ade–Rib) (a) | *dd* | 3.90 | 3.754 | | |
| H–C(4')(Ade–Rib) (a) | *ddd* | 1.98 | 2.002 | | |
| $CH_2$(5')(Ade–Rib)$_{a,b}$(*g,t*) | *dd* | 1.46, 0.38 | 0.602, 0.312 | | |
| *Dihydroxypropyl* | | | | | |
| $CH_2(1'')_{a,b}$(*t,g*) | *dd* | | | 0.953, –0.134 | 1.064, 0.372 |
| H–C(2'') | *m* | | | 1.04 | 1.006 |
| $CH_2(3'')_{a,b}$(*t,g*) | *dd* | | | 2.712, 2.642 | 2.670, 2.534 |

[a]) See *Footnote a* in *Table 1*. [b]) From [32], pH 2.1. [c]) From [29], with minor revisions for some of the corrin $CH_2$ groups. [d]) Our assignments for $CH_2(17^1)$ and $CH_2(17^2)$ are not definitive (NOEs with Me–C(17) not detected) but are made by analogy with those for 'base-off' Ado–'Cbl'.

Table 3. *$^1H,^1H$-Coupling Constants J [Hz] for Ribose Moieties or Hydroxyalkyl Chains in Coβ-X Derivatives*

| | $J$[Hz][a]) |
|---|---|
| Ado–Cbl[b]) Ade–Rib | $^2J$(5'a,5'b) = –9.2, $^3J$(4',5'a) = < 2, $^3J$(4',5'b) = 9.2, $^3J$(3',4') = 6.7, $^3J$(2',3') = 5.8, $^3J$(1',2') = 3.3 |
| loop Rib | $^2J$(5'a,5'b) = –13.0, $^3J$(4',5'a) = 2.7, $^3J$(4',5'b) = 3.9, $^3J$(3',4') = 8.9, $^3J$(2',3') = 4.3, $^3J$(1',2') = 3.0 |
| Ado–PTC Ade–Rib | $^2J$(5'a,5'b) = –8.5, $^3J$(4',5'a) = 1.0, $^3J$(4',5'b) = 9.0, $^3J$(3',4') = 6.2, $^3J$(2',3') = 6.2, $^3J$(1',2') = 3.8 |
| loop Rib | $^2J$(5'a,5'b) = –13.0, $^3J$(4',5'a) = 3.0, $^3J$(4',5'b) = 3.5, $^3J$(3',4') = 2.5, $^3J$(2',3') = 6.3, $^3J$(1',2') = 4.6 |
| [(*R*)-(HO)$_2$pr]–Cbl (**2b**) | $^2J$(1a,1b) = –9.5, $^3J$(1a,2) = 6.9, $^3J$(1b,2) = 1.8, $^3J$(2,3a) = 7.3, $^3J$(2,3b) = 4.8, $^2J$(3a,3b) = –11.4 |
| [(*R*)-(HO)$_2$pr]–PTC (**3b**) | $^2J$(1a,1b) = –7.7, $^3J$(1a,2) = 7.7, $^3J$(1b,2) = 1.0, $^3J$(2,3a) = 6.5, $^3J$(2,3b) = 5.6, $^2J$(3a,3b) = –11.4 |
| [(*S*)-(HO)$_2$pr]–Cbl (**2a**) | $^2J$(1a,1b) = –8.9, $^3J$(1a,2) = 5.5, $^3J$(1b,2) = 4.2, $^3J$(2,3a) = 6.9, $^3J$(2,3b) = 4.1, $^2J$(3a,3b) = –11.4 |
| [(*S*)-(HO)$_2$pr]–PTC (**3a**) | $^2J$(1a,1b) = –7.5, $^3J$(1a,2) = 4.0, $^3J$(1b,2) = 5.3, $^3J$(2,3a) = 6.9, $^3J$(2,3b) = 4.9, $^2J$(3a,3b) = –11.4 |
| HO(CH$_2$)$_3$–Cbl (**1b**[c]) | $^3J$(2,3a) = 6.9 and 6.3, $^3J$(2,3b) = 6.4 and 4.2, $^2J$(3a,3b) = –10.7 |
| HO(CH$_2$)$_2$–Cbl (**1a**[c]) | $^2J$(1a,1b) = –7.0, $^3J$(1a,2a) = 6.0, $^3J$(1a,2b) = 12.6, $^3J$(1b,2a) = 12.5, $^3J$(1b,2b) = 4.8, $^2J$(2a,2b) = –10.5 |

[a]) Alkyl atoms C(1), C(2), and C(3) (" symbol omitted) are numbered starting with the Co-bound atoms and correspond to adenosyl C-atoms C(5'), C(4'), C(3'). [b]) From [30]. [c]) Tentative assignments; complete determination of all coupling constants was not possible.

Table 4. *Kinetic Properties of Hydroxyalkyl Derivatives* **1a–d** *and* **2a,b** *((Coβ-X)–Cbl) and* **3a,b** *((Coβ-X)–PTC) with Glycerol Dehydratase and Diol Dehydratase*

| | $K_i$ [nM][a]) | | Relative $\Delta G$ [kJ/mol][d]) | |
|---|---|---|---|---|
| Inhibitor | GDH[b]) | DDH[c]) | GDH[b]) | DDH[c]) |
| HO–Cbl[e]) | 8.6 ± 1.4 | 680 ± 110 | –0.98 | –0.15 |
| Ado–Cbl ($K_m$ data) | 12.6 ± 2.2 | 720 ± 80 | 0.0 | 0.0 |
| HO(CH$_2$)$_2$–Cbl (**1a**) | 13.4 ± 3.2 | 770 ± 70 | 0.16 | 0.17 |
| HO(CH$_2$)$_3$–Cbl (**1b**) | 13.3 ± 3.1 | 760 ± 70 | 0.14 | 0.14 |
| [(*S*)-(HO)$_2$pr]–Cbl (**2a**) | 14.8 ± 4.1 | 1080 ± 100 | 0.41 | 1.04 |
| [(*R*)-(HO)$_2$pr]–Cbl (**2b**) | 17.6 ± 4.8 | 1250 ± 110 | 0.86 | 1.42 |
| HO(CH$_2$)$_4$–Cbl (**1c**) | 17.0 ± 4.5 | 1060 ± 90 | 0.77 | 0.99 |
| HO(CH$_2$)$_5$–Cbl (**1d**) | 25.2 ± 7.8 | 950 ± 80 | 1.78 | 0.71 |
| CN–Cbl[e]) | 21.6 ± 2.7 | 1420 ± 200 | 1.38 | 1.74 |
| [(*S*)-(HO)$_2$pr]–PTC (**3a**) | 57 ± 7 | 5100 ± 490 | 3.87 | 5.02 |
| [(*R*)-(HO)$_2$pr]–PTC (**3b**) | 348 ± 31 | 6400 ± 580 | 8.52 | 5.61 |
| Ado–PTC | 160 ± 37 | 9200 ± 1700 | 6.52 | 6.54 |
| HO–PTC | 9960 ± 100 | > 25000[f]) | 17.13 | > 9.1 |

[a]) Apparent inhibition constants at 37°, estimated by the 'parallel' method [41]; apparent $K_m$ constants for Ado–Cbl were measured as described previously [26][41]. [b]) Glycerol dehydratase from overexpressing *Escherichia coli* containing the gene from *Citrobacter freundii* ($V_{max}$ = 48 ± 4 nmol/min with Ado–Cbl). [c]) Diol dehydratase from overexpressing *Escherichia coli* containing the gene from *Salmonella typhimurium* ($V_{max}$ = 51 ± 6 nmol/min with Ado–Cbl). [e]) From [16]. [f]) No significant inhibition was found for up to 25 μM HO–PTC. [d]) Estimated free energy of inhibitor binding relative to Ado–Cbl; calculated as $RT \ln(K_i/K_m)$ at 37°.

**Discussion.** – *Structural Properties of the Coenzyme-$B_{12}$ Analogues.* Molecular-modeling studies for Ado–Cbl and the [(*R*)- and (*S*)–(HO)$_2$pr]–Cbl (**2a,b**) gave low-energy conformations which faithfully reproduced the geometries of the crystal structures, including the non-planar characteristics of the corrin ring, the axial/equatorial orientation of side chains, the loop and ribose conformations, and the orientations of adenosyl and dimethylbenzimidazole (Dbi) groups. These features are accurately represented in the diagrams of the *Figure*. It should be noted, however, that

good agreement between modeled and crystal structures was only obtained after adding appropriate parameters to the HyperChem force field for bonds to the Co- and P-atom.

The dihedral angles for protons in the adenosyl ribose (Ade–Rib) of Ado–Cbl in the crystal structure differed by $<20°$ from those in the energy-minimized modeled structure. The C(3')-*endo* ribose conformation (H–C(3') axial) in the crystal must predominate in solution for both Ado–Cbl and Ado–PTC since the observed vicinal coupling constants $J(1',2')$, $J(2',3')$, and $J(3',4')$ were 3.3, 5.8, and 6.7 Hz for Ado–Cbl [30] and 3.8, 6.2, 6.2 Hz for Ado–PTC [16], consistent with the modeled torsional angles of *ca.* 105, 38, and $-168°$ obtained for Ado–Cbl. The modeled torsional angles for the C(4')–C(5') bond in Ado–Cbl were 69 and $-174°$, consistent with $J(4',5'a)$ and $J(4',5'b)$ of $< 2$ and 9.2 Hz, respectively, in Ado–Cbl and 1.0 and 9.0 Hz, respectively, in Ado–PTC. Thus, $H_b$–C(5) is *trans* to H–C(4') and oriented towards the corrin C(10) atom while $H_a$–C(5') points toward the corrin-ring A. These orientations result in a *ca.* 1-ppm upfield shift for $H_b$–C(5') relative to $H_a$–C(5') in both Ado–Cbl and Ado–PTC. The ribose ring lies nearly perpendicular to the corrin ring with the ribose-ring O-atom pointing towards the corrin C(14) atom. The adenine ring is in the *syn* conformation, nearly parallel to the corrin ring and positioned over the corrin-ring C. This results in the characteristic upfield shift of *ca.* 0.3 ppm for $Me_\beta$–C(12) of the Ado derivatives relative to the hydroxyalkyl derivatives.

In the nucleotide loop of the Cbl derivatives, the $\alpha$-ribose conformation is also C(3')-*endo* (dihedral angles of $-26°$ for protons H–C(1'), H–C(2'), $40°$ for H–C(2'), H–C(3'), and $-167°$ for H–C(3'), H–C(4')), resulting in a coupling-constant pattern similar to that for the Ade–Rib. The predominance of the C(4')–C(5') rotamer with OH *trans* and both H–C(5') *gauche* to H–C(4') is confirmed by the small values of both coupling constants. In PTC derivatives, the nucleotide loop is open and flexible. In this case the loop ribose adopts predominantly a C(2')-*endo* (H–C(2') axial) conformation (dihedral angles of $42°$ for H–C(1'), H–C(2'), $-37°$ for H–C(2'), H–C(3'), and $-106°$ for H–C(3'), H–C(4') result in vicinal coupling constants of 4.6, 6.3, and 2.5 Hz, resp.). Modeling indicates that the C(2')-*endo* and C(3')-*endo* ribose conformations have only a small energy difference ($< 4$ kJ/mol) for an open nucleotide loop, but that the C(3')-*endo* conformer is preferred when the loop is closed and Dbi is attached to the Co-atom.

The loss of the Dbi group as $\alpha$-ligand at the Co-atom perpendicular to the corrin plane in the 'base-off' derivatives removes Dbi as a source of upfield aromatic-ring shift effects. Therefore, corrin groups pointing downwards and normally oriented over the plane of Dbi (*i.e.*, Me–C(1), Me–C(2), $Me_\alpha$–C(12), $CH_2(8^1)$, and $CH_2(8^2)$) move downfield in the 'base-off' analogues. In the modeling studies, it was found that the cobalamin conformational energy is lowered by several kJ/mol when the side chain located at C(8) and pointing axially downwards is oriented to place the $CH_2$ groups very close to the face of Dbi (hydrophobic stabilization); this intramolecular interaction is responsible for the unique and abnormally large shielding of these $CH_2$ groups relative to other side chains. This effect is lost in the PTC derivatives. In addition, all of the corrin-ring protons are shifted downfield in the PTC analogues (by as much as 1 ppm for H–C(10); this probably reflects a significant change in electron density at the Co-atom (and the corrin ring system) in the 'base-off' form.

Some interesting conformational properties of the hydroxyalkyl ligands in the coenzyme-$B_{12}$ analogues discussed here can be derived from the analysis of chemical shifts and couplings. The crystal structure of [(*R*)-(HO)$_2$pr–Cbl (**2b**) [28] shows that the Co–C(1″) bond has the same angular orientation as the Co–C(5′) bond in Ado–Cbl with the *pro-S* proton ($H_a$–C(1″) or $H_a$–C(5′)) pointing towards the corrin-ring A and the *pro-R* proton ($H_b$–C(1″) or $H_b$–C(5′)) pointing toward C(10). In contrast, C(1″) is rotated by *ca.* 90° counterclockwise in the (*S*)-(HO)$_2$pr derivative **2a** so that $H_{Si}$–C(1″) points towards H–C(19) and $H_{Re}$–C(1″) towards Me–C(5). Here, we distinguish $CH_2$ protons by the subscripts a and b to denote low- and high-field shifts (high and low frequencies), respectively, and *Re* and *Si* to designate the *pro-R* and *pro-S* positions. This conformational difference between the two dihydroxypropyl derivatives (confirmed as energy minima in modeling studies) is reflected in the chemical shifts of the $CH_2$(1″) protons. For [(*R*)-(HO)$_2$pr]–Cbl (**2b**) $H_b$–C(1″) has nearly the same strongly shielded chemical shift as $H_b$–C(5′) in Ado–Cbl (*pro-R* position) while $H_a$–C(1″) ($H_a$–C(5)) resonates downfield by 0.61 (0.98) ppm (*pro-S* position). For [(*S*)-(HO)$_2$pr]–Cbl (**2a**), both chemical shifts are less shielded by *ca.* 0.4 ppm, consistent with a rotation away from the central part of the delocalized corrin double-bond system. This trend is also observed for the (HO)$_2$pr–PTC analogues, although there is a general increase in the local shielding effects when the Co-atom is in the 'base-off' form.

Another diagnostic feature is the chemical shift of the corrin H–C(19) which is shifted downfield by *ca.* 0.4 ppm in the (*R*)-(HO)$_2$pr *vs.* the (*S*)-(HO)$_2$pr or Ado derivative for both the Cbl and PTC analogues. The crystal structure shows that for [(*R*)-(HO)$_2$pr]–Cbl (**2b**), the O-atom of OH–C(2″) is relatively close to H–C(19) (2.6 Å) and is probably the source of the downfield shift [27]. This conformation corresponds to the lowest-energy C(1″)–C(2″) rotamer in the modeling studies (shown in the *Figure*); C(3″) is *trans* to Co and H–C(2″) is approximately *trans* to $H_{Si}$–C(1″) (*ca.* 145°) and at an angle of near −100° to $H_{Re}$–C(1″), consistent with the observed vicinal coupling constants for **2b** (*Table 3*; $J$(1″a,2″) = 6.9 and $J$(1″b,2″) = 1.8 Hz). Similar coupling constants are observed for [(*R*)-(HO)$_2$pr]–PTC (**3b**). On the other hand, the C(2″)–C(3″) bond is nearly vertical relative to the corrin plane, so that all three rotamers for the orientation of OH–C(3″) are possible. Thus, the difference between the two $J$(2″,3″) in the (*R*)-(HO)$_2$pr derivatives is smaller.

In contrast, for [(*S*)-(HO)$_2$pr]–Cbl (**2a**), $H_{Si}$–C(1″) points towards H–C(19). Modeling indicates that two C(1″)–C(2″) rotamers are probably populated. One of these has OH–C(2″) *trans* to the Co-atom, as in the crystal structure, while the other has C(3″) *trans*, as shown in the *Figure*. Thus, each H–C(1″) may spend time in either a *trans* or a *gauche* position relative to H–C(2″), consistent with the observed $J$(1″,2″) of 4–5 Hz. In the crystal structure, OH–C(3″) is *gauche* to OH–C(2″) and oriented towards Me–C(17), corresponding to the rotamer with H–C(2″) *gauche* to both H–C(3″). For the other C(1″)–C(2″) rotamer (C(3″) *trans* to Co), all three C(2″)–C(3″) rotamers should be possible, but the conformation with *trans*-OH groups and $H_{Re}$–C(3″) *trans* to H–C(2″) (*Figure*) may be favored, resulting in a larger coupling constant for H–C(2″) to $H_{Re}$–C(3″) (tentatively assigned as $H_a$–C(3″)). These observations and arguments apply to [(*S*)-(HO)$_2$pr]–PTC (**3a**) as well.

Another interesting feature of the spectra is the geminal coupling of the $CH_2$ group attached to the Co-atom. The magnitude of this coupling constant in the Ado– and $(HO)_2$pr–Cbl analogues ranges from 8.9 to 9.5 Hz but is reduced to 7.5 to 8.5 Hz in the corresponding PTC derivatives and to 7.0 for $HO(CH_2)_2$–Cbl (**1a**). A reduction in this coupling constant is consistent with an increase in the electron-withdrawing power of the Co-atom in its 'base-off' form or of OH–C(2″) when the C(3″) atom is absent. For $HO(CH_2)_2$–Cbl (**1a**), large *trans* vicinal coupling constants (12.5 Hz) were observed ($J$(1″a,2″b) and $J$(1″b,2″a)). This indicates that the hydroxyethyl group adopts exclusively a staggered conformation with Co and OH in a *trans* ('*anti*') relationship and with $H_a$–C(1″) and $H_a$–C(2″) pointing toward the 'west' side of the corrin ring and the more strongly shielded $H_b$–C(1″) and $H_b$–C(2″) pointing toward the 'east'. For $HO(CH_2)_3$–Cbl (**1b**), a detailed analysis of the alkyl coupling constants could not be made, but the $CH_2$(1″) chemical shifts and the shift difference for $CH_2$(2″) were very similar to the values observed for $HO(CH_2)_2$–Cbl (**1a**), indicating similar conformational properties.

In summary, we find that the upper ($\beta$) and lower ($\alpha$) regions of the corrin ring system in coenzyme-$B_{12}$ analogues are largely independent in their conformational properties. The $\beta$-ligands (Ado, hydroxyalkyl) behave the same in the 'base-on' Cbl and 'base-off' PTC derivatives. These ligands are restricted in their conformational freedom by the various Me groups and side chains at the corrin ring. The crystal structures of Ado–Cbl and the $(HO)_2$pr derivatives appear to provide good representations for the (predominant) conformation in solution. It is interesting to note that C(4′) of the adenosyl ligand has the same ($S$)-configuration as C(2″) of the ($S$)-$(HO)_2$pr derivative (*Fig.*). Surprisingly, the ($S$)-$(HO)_2$pr derivative has a significantly different conformation and orientation of the $C_3$ chain compared to the Ado (C(3′)–C(4′)–C(5′)) and ($R$)-$(HO)_2$pr ligands, which show highly similar conformations. However, in the modeled structures, the orientations of the OH groups of the ($R$)-$(HO)_2$pr derivative poorly match those of Ado, while OH–C(3″) of the ($S$)-$(HO)_2$pr derivative and OH–C(2′) of Ado both point toward the corrin-ring D and are located 4.5–5 Å above the corrin C(16) atom. Thus, the ($S$)-$(HO)_2$pr derivative may interact more favorably than the ($R$)-form with a putative ribose binding site or with a substrate binding site (see below), resulting in the observed difference between the inhibition constants $K_i$.

*Inhibitor Properties of the Coenzyme-$B_{12}$ Analogues.* From the data in *Table 4*, we note that the absolute values of $K_i$ of inhibitors and $K_m$ of coenzyme $B_{12}$ differ by a factor of 20 or more for glycerol dehydratase and for diol dehydratase. However, there are crude similarities in the order or ranking of inhibitor potencies (relative $K_i$ values) for the two enzymes. Such a similarity may be a result of the homology found in the amino-acid sequences of several representatives of these two kinds of dehydratases [24][25]. Furthermore, both dehydratases accept either glycerol or racemic propane-1,2-diol as substrate [29][33].

As expected, all of the 'base-on' hydroxyalkyl analogues were efficient inhibitors of both enzymes with a narrow range of apparent $K_i$. The $K_i$ values differed by, at most, a factor of two from the apparent $K_m$ for coenzyme $B_{12}$ (Ado–Cbl). It should be noted that such a range of $K_i$ corresponds to differences in a binding free energy $\Delta G = \Delta H - T\Delta S$ of less than 2 kJ/mol, which is less than the $\Delta G$ associated with a single H-bond

(*ca.* 5 kJ/mol), and certainly within the range of possible variations due to entropy effects alone [34]. For both enzymes, the two short-chain monohydroxy analogues **1a,b**, *i.e.* $OH(CH_2)_n$–Cbl ($n=2$ and 3), had lower $K_i$ relative to the long-chain derivatives **1c,d** ($n=4$ and 5). One possible interpretation of this result is that a hydrophilic area of interaction between the $\beta$-ligand and the protein must lie relatively close to the Co-center. However, when one compares ligands with different chain lengths and, therefore, different degrees of freedom for internal motions, the loss of motional entropy upon binding to a protein must be taken into account. At room temperature, this effect on $\Delta G$ for binding has been estimated to be *ca.* + 1.4 kJ/mol for each single bond whose motion is 'frozen' in the complex [34]. Thus, for alkyl groups with two to five C-atoms, interaction of a terminal OH group with a binding pocket in the protein is expected to result in an entropy penalty which increases with chain length and which may compensate for or even exceed any negative enthalpy changes due to increasing hydrophobic interactions with increasing alkyl chain length, for example.

The kinetic results obtained with the 'base-on' dihydroxypropyl analogues **2a,b** indicate that a second OH group in the $\beta$-ligand provides no significant increase in binding affinity. This is consistent with the hypothesis that the ligand interacts with the substrate binding site and that only the terminal (primary) OH group of the substrate (or ligand) can be involved in binding (see *Introduction*). Alternatively, the $\beta$-ligand may interact with a ribose binding site which is possibly utilized by the natural coenzyme. Theoretically, two ribose OH groups are available for H-bonding, but our NMR and modeling studies indicate that in the favored conformations of the dihydroxypropyl ligands, only one OH group can adopt an orientation similar to the adenosyl OH–C(2'), for example. This 'match' in orientation was better for the [(*S*)-$(HO)_2$pr]–Cbl (**2a**), and this analogue was indeed a slightly better inhibitor compared to the [(*R*)-$(HO)_2$pr]–Cbl (**2b**). However, the monohydroxyalkyl ligands may have a small advantage of greater conformational flexibility for positioning the terminal OH group in the appropriate orientation for binding.

It is interesting and perhaps surprising to note that the simple hydroxyethyl and hydroxypropyl groups as $\beta$-ligands in **1a,b** result in inhibitors with $K_i$ equal to the $K_m$ for coenzyme $B_{12}$ (Ado–Cbl). One possible explanation is that the binding stabilization provided by the adenosyl moiety is simply limited to the interaction of a single OH group with the protein, an interaction which can be generated also by the small hydroxyalkyl group. Another and perhaps more likely explanation is that the bulky adenosyl group introduces positive contributions to $\Delta G$ (steric repulsions, 'induced fit' energy, *etc.*) which largely compensate the available negative, stabilizing contributions. In general, however, it is quite difficult to describe binding interactions and to interpret the small differences in *Table 4* in an *a priori* manner without knowledge of binding-site geometry and the residues involved (X-ray structures are not available for the enzymes discussed here). A further difficulty arises when binding *via* H-bonds is concerned. Not only the number of putative H-bonds in the complex is important, but also the net change in the total number of H-bonds after complexation and the net change in the number of free solvent $H_2O$ molecules. For all of the Cbl derivatives presented here, the small range of $\Delta G$ values represents small differences in the sums over a series of much larger numbers (enthalpies and entropies with positive and negative contributions). The differences in $\Delta G$ are smaller than the free energy we can assign to any single

discrete interaction such as one H-bond. Thus, it is probably reasonable to consider all Cbl derivatives examined here as *essentially* equally good inhibitors for GDH and DDH.

In contrast, the 'base-off' PTC analogues **3a,b** are significantly poorer inhibitors and exhibit a much wider range of potencies and larger differences in relative $K_i$ values for the two enzymes. One expects that the 'unattached' nucleotide loop at the PTC derivatives can, in principle, be folded so as to fit into the presumed binding pocket utilized by the natural coenzyme and its Cbl analogues [16]. Thus, essentially the same enthalpy of binding should be achievable for Ado–PTC as for Ado–Cbl, but this requires a significant entropy penalty since several degrees of freedom in the 'open' loop will be reduced, if not frozen, in the final 'closed' or 'base-on' conformation upon binding. As many as ten single bonds are involved whose mobilities will be reduced in the complex. For an entropic contribution of 1.4 kJ/mol per bond, one predicts an increase in $\Delta G$ of up to 14 kJ/mol, which covers the range of 4–8 kJ/mol calculated for Ado–PTC and the dihydroxypropyl derivatives **3a,b** (*Table 4*). The differences *between* these three inhibitors are larger than for the Cbl analogues, but still no larger than the free-energy contribution for a single H-bond. As observed for the Cbl derivatives, the [(*S*)-(HO)$_2$pr]–PTC (**3a**) is a better inhibitor than the (*R*)-isomer **3b**; it is, in fact, even better than the Ado analogue. This is consistent with the concept that the small (*S*)-(HO)$_2$pr ligand can enter into favorable interactions with either a substrate or ribose site. These interactions may be similar to those pursued by the Ado ligand, but perhaps without unfavorable compensating effects (*e.g.*, protein conformational changes) caused by the adenosyl's bulk, which may be more important when a closed nucleotide loop is not already present.

It is surprising that HO–PTC itself (with a $\beta$-hydroxo ligand) proved to be a very poor inhibitor, while the analogue HO–Cbl is the best inhibitor. This is clearly not explainable with simple entropy effects. One possibility is that the binding of coenzyme-$B_{12}$ analogues occurs in a stepwise manner and that stabilizing interactions between an appropriate $\beta$-ligand and the protein are first necessary to give the PTC nucleotide loop the opportunity to adequately fold to occupy the normal Dib-loop pocket. For HO–PTC, the initial complex may not be stable enough to promote this folding process or induction of the corresponding optimal protein conformation. For HO–Cbl the corrin moiety and the nucleotide loop presumably already have the optimal, relatively rigid conformation, so that binding can proceed directly without the need for a prestabilizing complex. As is well-known in drug design, if an inhibitor can be produced with a rigid conformation matching the bound or active conformation of an otherwise flexible substrate, then binding affinity may be enhanced by several orders of magnitude.

In conclusion, the inhibition kinetic data presented here confirm that both glycerol dehydratase and diol dehydratase reactions utilize the 'base-on' binding mode of coenzyme $B_{12}$. A series of 'base-on' cobalamin derivatives with hydroxyalkyl ligands on the $\beta$-side, *i.e.*, **1a–d** and **2a,b**, proved to be strong competitive inhibitors of the dehydratases with a narrow range of $K_i$; the best inhibitor had $K_i$ equal to $K_m$ of coenzyme $B_{12}$. The 'base-off' corrinato complexes (PTC analogues) with adenosyl or hydroxyalkyl ligands on the $\beta$-side, *i.e.*, **3a,b**, exhibited significant but poorer potency as inhibitors. The parent compound HO–PTC, with only a $\beta$-hydroxo ligand, was a very

poor inhibitor while HO–Cbl was the best for both enzymes. These results indicate that for the 'base-off' analogues interactions between the $\beta$-ligand and a hydrophilic binding site near the Co-center (for substrate or possibly for the adenosyl ribose) are important for stabilizing an initial complex and facilitating the folding of the nucleotide loop into a 'base-on' conformation in the final inhibitor-apoenzyme complex. All Cbl analogues have the 'ideal' 'base-on' conformation of the nucleotide loop, and their potencies as inhibitors, judged by $K_i$, are much less dependent upon the nature of the $\beta$-ligand.

## Experimental Part

*Materials.* Coenzyme $B_{12}$, vitamin $B_{12a}$ $\omega$-haloalkanols, and sodium tetrahydroborate ($NaBH_4$) were obtained from *Fluka Chemie AG*. The enantiomeric (*R*)- and (*S*)-3-chloropropane-1,2-diols were obtained by enzymic resolution [35]. Racemic propane-1,2-diol was supplied by *Aldrich*. (Co$\alpha$-Cyano)(Co$\beta$-hydroxo)[1'-*O*-(*p*-tolyl)cobamide] (HO–PTC) was isolated from *Sporomusa ovata* cells by extraction with KCN-containing AcOH buffer at pH 5 followed by centrifugation, treatment of the supernatant by neutral aluminum oxide, desalting, chromatography on a *XAD-2* column, and reversed-phase HPLC (*RP18*) as previously described [36–38]. Yeast alcohol dehydrogenase and $\beta$-NADH $Li_3$ (NADH) were products of *Boehringer Mannheim GmbH*. Glycerol dehydratase (GDH) was isolated as described previously [26] from overexpressing *Escherichia coli* cells containing the genomic DNA for glycerol dehydratase from *Citrobacter freundii* [25][39]. Propanediol dehydratase (DDH) was isolated as reported previously [26] from overexpressing *E. coli* cells containing genes for diol dehydratase from *Salmonella typhimurium* LT2.

*[(2-Hydroxyethyl)-, (3-Hydroxypropyl)-, (4-Hydroxybutyl)-, and (5-Hydroxypentyl)]cob(III)alamin* (**1a**,**1b**,**1c**, and **1d**, resp.). *[(S)-2,3-Dihydroxypropyl]- and [(R)-2,3-Dihydroxypropyl]cob(III)alamin* (**2a** and **2b**, resp.), *and [Co$\beta$-[(S)-2,3-Dihydroxypropyl]]- and [Co$\beta$-[(R)-2,3-Dihydroxypropyl]][1'-O-(p-tolyl)cobamide]* (**3a** and **3b**, resp.). To a soln. of HO–Cbl or HO–PTC (5 µmol) in deoxygenated $H_2O$ (0.5 ml), a soln. of $NaBH_4$ (5 mg) in deoxygenated $H_2O$ (0.4 ml) was added under Ar at r.t., and the resulting soln. was stirred for 30 min. Then a soln. of the corresponding $\omega$-haloalkanol or -diol (50–100 µmol) in deoxygenated MeCN (0.4 ml) was added, and the mixture was stirred in the dark at r.t. for 1 h. After sterile filtration through a 30-kD nitrocellulose membrane, the mixture was submitted to prep. HPLC (*Macherey & Nagel*, 250 mm × 1", *Nucleosil-7-$C_{18}$* column; 20–85% MeOH/$H_2O$ gradient over 35 min, flow rate 5 ml/min; diode array detection, monitoring at 240 and 280 nm; UV spectra obtained between 200 and 600 nm from the HPLC peaks). The fractions containing the product were concentrated in a *SpeedVac* to give the desired (Co$\beta$-X)–Cbl **1a–d** or **2a**,**b**, or (Co$\beta$-X)–PTC **3a**,**b** in over 60% yields. Purities of the products were over 95% by anal. HPLC (*Macherey & Nagel*, 125 × 4 mm, 5 µm *LiChrospher 100 RP-18* column, linear gradient of 40–70% *B* in *A* over 12 min (*A*: 0.02% $CF_3COOH$ in $H_2O$; *B*: 0.02% $CF_3COOH$ in MeOH), flow rate 1 ml/min; diode-array detection).

*$^1$H-NMR Spectroscopy.* Soln. of (Co$\beta$-X)–Cbl **1a**,**b** or **2a**,**b** (2.6–3.1 mg) or (Co$\beta$-X)–PTC **3a**,**b** (0.7–0.9 mg) in 0.4 ml of a 20 mM sodium phosphate/$D_2O$ buffer (pH-meter reading 7.4, 5-mm sample tube) were measured at 10° by means of a *Bruker-AM-500* spectrometer using conventional *Fourier* transform methods as in our previous studies [16][29]. The residual HDO resonance was suppressed by selective presaturation. Parameters for the 1D spectra of **1a**,**b** or **2a**,**b** were: spectral width 4310 Hz, 32 K time-domain points, presaturation for 3.1 s, 50° flip angle, acquisition time 3.8 s, 400 transients. Data processing was performed with zero-filling to 64 K data, *Lorentz-Gauss* resolution enhancement, and a digital resolution of 0.13 Hz. Similar parameters were used for **3a**,**b**. For well-resolved resonances, chemical shifts (given in three decimal places, rel. to internal sodium 3-(trimethylsilyl)propanoate (TSP)) and coupling constants were derived from the peak-picking output (cubic interpolation); chemical shifts, given in only two decimal places, were estimated from cross-peaks in the 2D COSY experiment. The total number of nonexchangeable protons was confirmed by integration, and the complete assignments are given in *Tables 1* and *2*.

Two-dimensional magnitude-mode COSY-$\beta$ and NOESY data were obtained from conventional pulse sequences and the following parameters for **1a**,**b** or **2a**,**b**. COSY: spectral width 3906 Hz, 2 K time-domain points in $t_2$, acquisition time 0.261 s, 512 $t_1$ increments with 32 transients each, 40 ms initial delay in the $t_1$ time domain to enhance the effects of long-range couplings, $\beta = 50°$ as read pulse to improve the detection of cross-peaks close to the diagonal and provide some discrimination between vicinal and geminal couplings, relaxation delay with presaturation 2.25 s, sine-bell window functions, zero-filling to 1 K in $t_1$, digital resolution 3.8 Hz/pt.

NOESY: as for COSY, except 48 or 64 transients per $t_1$ increment, 90° read pulse, mixing time 500 ms with max. ±15% random variation. For **3a,b**, similar parameters were used except: presaturation delay 2.3 or 2.7 s, 30 ms initial $t_1$ delay for COSY, 600 ms mixing time for NOESY.

*Molecular Modeling.* As an aid to interpretation of the NMR data, modeling of Ado–Cbl and several of the analogues discussed here was performed using the MM+ force field of HyperChem 4.5 (*HyperCube, Inc.*), with additional parameters added for bond lengths and angles for Co–C, Co–N, Co–O, and P–O bonds. These parameters were derived from the X-ray structure of Ado–Cbl [40] (atomic-coordinate file DADCBL in the *Cambridge Crystallographic Data Files*) and from parameters in the Alchemy 3 force field. In addition, the atomic coordinate files CUVCIF and CUVCOL for [(*R*)- and (*S*)-(HO)$_2$pr]–Cbl (**2a,b**), resp., [28] were also available.

*Enzyme Assays.* Assays for glycerol-dehydratase and diol-dehydratase activity with racemic propane-1,2-diol as substrate were performed using a yeast alcohol dehydrogenase/NADH-coupled, UV-based assay system at 37° [26]. A mean rate for the enzyme reaction, measured over the interval $t = 2-3$ min of the assay, was used for the calculation of kinetic constants.

*Kinetic Investigations.* Apparent inhibition constants ($K_i$) for **1a–d, 2a,b**, and **3a,b** were determined by the method applied for the inhibition kinetics of posthomolysis analogues of coenzyme $B_{12}$ (= Ado–Cbl) [26][41]. Ado–Cbl and various amounts of inhibitor were added simultaneously to the assay mixture, and the experiments were repeated at several (usually three) concentrations of Ado–Cbl. Inhibition constants were calculated from linearized data sets in a *Dixon* plot. $K_i$ was computed as the average of the experimental $K_i$ values obtained at different Ado–Cbl concentrations.

We gratefully acknowledge the generous gifts of an *E. coli* strain overexpressing glycerol dehydratase from *Citrobacter freundii* (from Dr. *R. Daniel* and Prof. *G. Gottschalk*, Göttingen, Germany) and a plasmid overexpressing diol dehydratase from *Salmonella typhimurium* (from *T. A. Bobik*, Gainesville, FL, USA). We thank the *European Union* and the *Fonds der Chemischen Industrie* for financial support. *L. P.* thanks the *Alexander von Humboldt Foundation* for short-term postdoctoral fellowships (1996, 1997) and the *Hungarian OTKA* (T023162) and *OMFB* (01789/98) for financial support.

## REFERENCES

[1] 'Vitamin B12', Eds. B. Zagalak, W. Friedrich, W. de Gruyter Verlag, Berlin-New York, 1979.
[2] B. M. Babior, J. S. Krouwer, *CRC Crit. Rev. Biochem.* **1979**, *6*, 35.
[3] B. T. Golding, in 'B12', Ed. D. Dolphin, John Wiley & Sons, New York, 1982, Vol. 1, pp. 543–582.
[4] B. M. Babior, *BioFactors* **1988**, *1*, 21.
[5] J. Rétey, in 'Chemical Aspects of Enzyme Biotechnology', Ed. T. O. Baldwin, Plenum Press, New York, 1990, p. 223.
[6] C. D. Garr, J. M. Sirovatka, R. G. Finke, *J. Am. Chem. Soc.* **1996**, *118*, 11142.
[7] C. D. Garr, R. G. Finke, *Inorg. Chem.* **1996**, *35*, 5912.
[8] C. Luschinsky-Drennan, S. Huang, J. T. Drummond, R. G. Matthews, M. L. Ludwig, *Science (Washington, D.C.)* **1994**, *266*, 1669.
[9] F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. McSweeney, B. Rasmussen, P. Bösecke, O. Diat, P. R. Evans, *Structure* **1996**, *4*, 339.
[10] E. Stupperich, H. J. Eisinger, S. P. J. Albracht, *Eur. J. Biochem.* **1990**, *193*, 105.
[11] O. Zelder, B. Beatrix, F. Kroll, W. Buckel, *FEBS Lett.* **1995**, *369*, 252.
[12] R. Padmakumar, S. Taoka, R. Padmakumar, R. Banerjee, *J. Am. Chem. Soc.* **1995**, *117*, 7033.
[13] A. Abend, R. Nitsche, V. Bandarian, E. Stupperich, J. Rétey, *Angew. Chem.* **1998**, *110*, 643; *ibid. Int. Ed.* **1998**, *37*, 625.
[14] M. Yamanishi, S. Yamada, H. Muguruma, Y. Murakami, T. Tobimatsu, A. Ishida, J. Yamauchi, T. Toraya, *Biochemistry* **1998**, *37*, 4799.
[15] C. C. Lawrence, G. J. Gerfen, V. Samano, R. Nitsche, M. J. Robins, J. Rétey, J. Stubbe, *J. Biol. Chem.* **1999**, *274*, 7039.
[16] L. Poppe, E. Stupperich, W. E. Hull, T. Buckel, J. Rétey, *Eur. J. Biochem.* **1997**, *250*, 303.
[17] L. Poppe, H. Bothe, G. Broeker, W. Buckel, E. Stupperich, J. Rétey, *J. Mol. Catal. B*, submitted.
[18] O. Zelder, B. Beatrix, U. Leutbecher, W. Buckel, *Eur. J. Biochem.* **1994**, *226*, 577.
[19] B. Beatrix, O. Zelder, W. Buckel, *Eur. J. Biochem.* **1994**, *221*, 101.

[20] E. N. Marsh, N. McKie, N. K. Davis, P. F. Leadlay, *Biochem. J.* **1989**, *260*, 345.
[21] R. Jansen, F. Kalousek, W. A. Fenton, L. E. Rosenberg, F. D. Ledley, *Genomics* **1989**, *4*, 198.
[22] E. Andrews, R. Jansen, A. M. Crane, S. Cholin, D. McDonnell, F. D. Ledley, *Biochem. Med. Metabol. Biol.* **1993**, *50*, 135.
[23] E. N. G. Marsh, D. E. Holloway, *FEBS Lett.* **1992**, *310*, 167.
[24] T. Tobimatsu, T. Hara, M. Sakaguchi, Y. Kishimoto, Y. Wada, M. Isoda, T. Sakai, T. Toraya, *J. Biol. Chem.* **1995**, *270*, 7142.
[25] M. Seyfried, R. Daniel, G. Gottschalk, *J. Bacteriol.* **1996**, *178*, 5793.
[26] L. Poppe, J. Rétey, *Eur. J. Biochem.* **1997**, *245*, 398.
[27] R. M. Dixon, B. T. Golding, O. W. Howarth, J. L. Murphy, *J. Chem. Soc., Chem. Commun.* **1983**, 243.
[28] N. W. Alcock, R. M. Dixon, B. T. Golding, *J. Chem. Soc., Chem. Commun.* **1985**, 603.
[29] L. Poppe, W. E. Hull, J. Rétey, *Helv. Chim. Acta* **1993**, *76*, 2367.
[30] M. F. Summers, L. G. Marzilli, A. Bax, *J. Am. Chem. Soc.* **1986**, *108*, 4285.
[31] T. G. Pagano, L. G. Marzilli, M. M. Flocco, C. Tsai, H. L. Carrel, J. P. Glusker, *J. Am. Chem. Soc.* **1991**, *113*, 531.
[32] A. Bax, L. G. Marzilli, M. F. Summers, *J. Am. Chem. Soc.* **1987**, *109*, 566.
[33] W. W. Bachovchin, R. G. Eagar, K. W. Moore, J. H. Richards, *Biochemistry* **1977**, *16*, 1082.
[34] H. J. Böhm, G. Klebe, *Angew. Chem.* **1996**, *108*, 2750; *ibid., Int. Ed. Engl.* **1996**, *35*, 2588.
[35] L. Poppe, L. Novák, M. Kajtár-Peredy, C. Szántay, *Tetrahedron: Asymmetry* **1993**, *4*, 2211.
[36] E. Stupperich, H. Eisinger, *Arch. Microbiol.* **1989**, *151*, 372.
[37] E. Stupperich, *FEMS Microbiol. Rev.* **1993**, *12*, 349.
[38] V. Ya Bykhovskii, P. J. Santander, E. Stupperich, N. I. Zaitseva, M. A. Pusheva, E. N. Detkov, D. S. Valyushok, I. A. Scott, *Appl. Biochem. Microbiol.* **1996**, *32*, 179.
[39] R. Daniel, G. Gottschalk, *FEMS Microbiol. Lett.* **1992**, *100*, 218.
[40] P. G. Lenhert, *Proc. Royal Soc. London, Ser. A* **1968**, *303*, 45.
[41] L. Poppe, J. Rétey, *Arch. Biochem. Biophys.* **1995**, *316*, 541.

*Received April 21, 1999*

# A. melléklet

**POPPE, L.**, NOVÁK, L.:

Hidrolítikus enzimek a szerves kémiában: új lehetőség optikailag aktív vegyületek előállítására,

*Magy. Kém. Lapja*, **1988**, *43*, 237.

magyar kémikusok lapja

XLIII. ÉVFOLYAM 7. SZÁM

# Hidrolítikus enzimek a szerves kémiában: új lehetőség optikailag aktív vegyületek előállítására

POPPE LÁSZLÓ*
NOVÁK LAJOS*

## Bevezetés

A gyógyszer- és finomvegyszergyártás fejlődésével egyre nő az igény a hatékony, optikailag aktív vegyületek előállítására alkalmas módszerek iránt. Mikrobiológiai, fermentációs módszereket és eljárásokat már viszonylag régóta alkalmaznak ezen iparágakban bonyolult vegyületek totálszintézisére ill. azok részbeni módosítására. E módszerek többsége optikailag aktív termékeket eredményez, de problémákat okozhat a folyamat szabályozása, hozama, a termékek elkülönítése és a speciális alkalmazhatóság.

Tisztított enzimek alkalmazása e problémák nagy részét megoldja, és olyan vegyületek előállítására nyújt lehetőséget, melyek hagyományos kémiai módszerekkel nem, vagy csak nehezen állíthatóak elő hatékonyan. Napjainkban már ipari méretekben is alkalmaznak enzimeket, elsősorban oxidációs-redukciós folyamatok katalízisére [1], ezekkel enyhe körülmények között nagy optikai tisztaságú termékek állíthatók elő. Az enzimeket néhány tulajdonságuk igen hasznossá és értékessé teheti a szerves kémiában. Különösen fontos lehet a szintetikus alkalmazás szempontjából a nagy reakciósebesség (az enzim aktív centrumában az elemi átalakulások sűrűsége akár $10^6$—$10^8$ $s^{-1}$ is lehet), a szelektivitás (specifikus átalakulások katalízise igen nagy sztereoszelektivitással) és az enyhe körülmények (gyakorlatilag semleges pH és szobahőmérséklet). Felhasználhatóságukat tovább növeli a hosszú ideig eltartható, könnyen kezelhető, többször felhasználható, rögzített enzimek előállítása [1, 2, 21, 22, 42, 43].

Az utóbbi években a szerves kémikusok is mind nagyobb érdeklődéssel fordulnak a mikrobiológiai ill. enzimatikus úton előállítható optikailag aktív intermedierek felé [3—5]. Számos ilyen vegyület előállítását oldották meg az utóbbi években mikrobiológiai módszerekkel [6—11], ill. lómájból kivonható alkohol dehidrogenáz (HLADH) enzimrendszerrel [12—18]. Napjainkban a legnagyobb fejlődés azonban a hidrolítikus enzimek (EC. 3) alkalmazásában mutatkozik [18—21]. Ennek oka az, hogy míg a fermentációs technikák mikrobiológiai ismereteket, speciális eszközöket és viszonylag híg oldatokat igényelnek, vagy az oxidoreduktázok (EC. 1.) felhasználásának koenzimigényük szabhat határt, addig a hidrolázok különösebb biokémiai vagy mikrobiológiai ismeret nélkül, egyszerű körülmények között használhatók. Vizes oldatban az alábbi, igen egyszerű reakciót katalizálják:

$$R^1-CO-X-R^2+H_2O \underset{}{\overset{\text{Hidrolízis}}{\rightleftharpoons}} R^1-COOH+HX-R^2$$

$$X=O(S, NH)$$

A reakció a szubsztrát szerkezetétől függően vezethet mind optikailag aktív karbonsavakhoz, mind optikailag aktív alkoholokhoz (ill. X=S, NH esetében tiolokhoz, aminokhoz). Megfelelő körülmények között (apoláris közeg, sav, alkohol vagy észter felesleg) azonban a hidrolázok katalizálhatnak észteresítési vagy átészterezési reakciókat is [23—26], melyek szintén eredményezhetnek optikailag aktív vegyületeket. Ezen tulajdonságaik a hidrolítikus enzimeket különösen alkalmassá teszik optikailag aktív szintézisintermedierek előállítására, elvileg két úton: prokirális, ill. mezo vegyületek átalakításával, vagy racém vegyületpárok rezolválásával.

## Szintetikusan alkalmazott hidrolítikus enzimek

### *α-Kimotripszin (CTR) (EC. 3.4.4.5.)*

Az egyik legrégebben ismert és legtöbbet tanulmányozott enzim, az emlősök hasnyálmirigyében termelődik és a bélcsatornában aktiválódik. Az emésztés során a fehérjék bontását végzi, elsősorban az aromás L-amino-karbonsavak karboxilja melletti peptidkötéseket hasítja.

Ezzel az enzimmel végezték az első olyan vizsgálatokat, melyek során prokirális vegyületeket teljes egészében optikailag aktív származékká alakítottak enzimatikus úton [27—30]. A különböző mesterséges szubsztrátokkal végzett összehasonlító kinetikai vizsgálatok [31, 32] eredménye szerint más hidrolázokhoz viszonyítva lassú, kis aktivitású, de igen nagyfokú szelektivitást mutató enzim. Aktivitását az N-acetil-L-tirozin-etilészterrel mérhető hidrolízis-sebességgel definiálták [33]. Vízoldható formában kereskedelmi forgalomból beszerezhető (SIGMA, REANAL), rögzített formában [21, 42] sokáig tárolható és többször felhasználható.

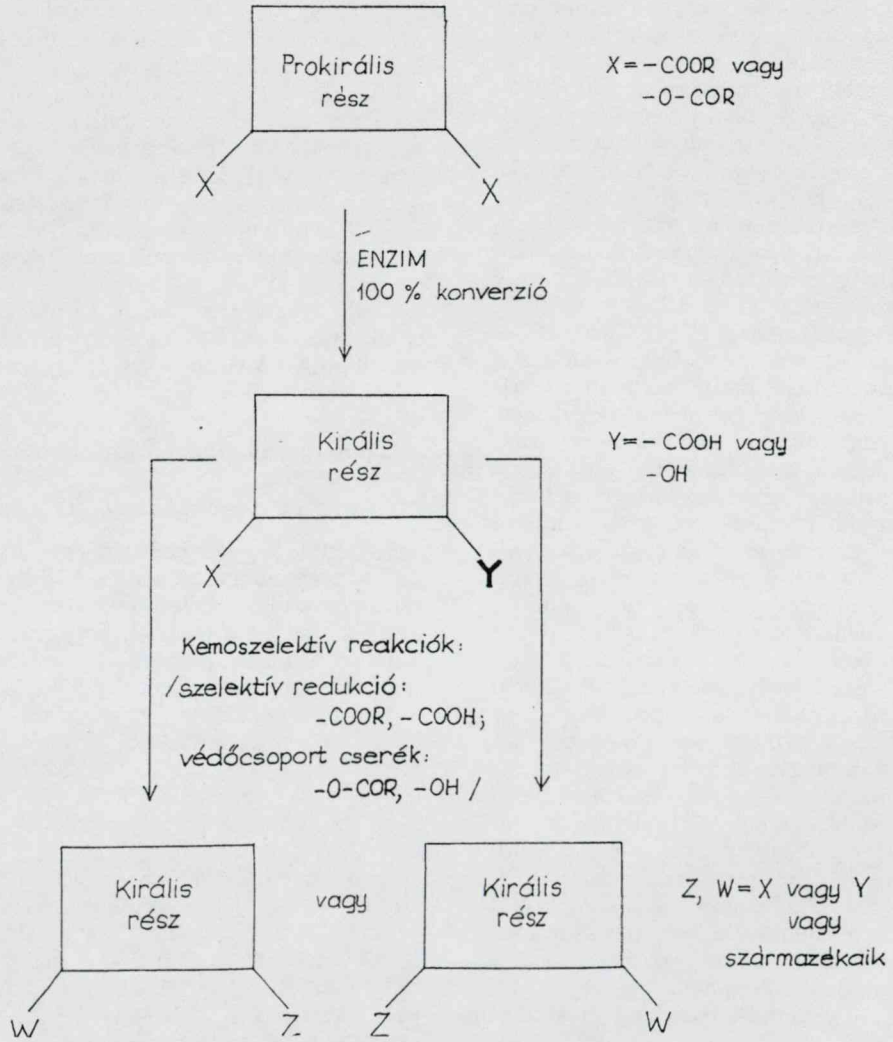
* Budapesti Műszaki Egyetem, Szerves Kémiai Tanszék.

*Disznómáj karboxil-észteráz (PLE) (EC. 3.1.1.1.)* [34—36]

A karboxil-észterázok az egész élővilágban elterjedt, széles specificitással rendelkező enzimek, karbonsav észterek hidrolízisét katalizálják. Szintetikusan leginkább a disznómájból kivont karboxil-észterázt (PLE) alkalmazzák. Ez az enzim a legtöbb mesterséges szubsztrát esetében nagyságrendekkel aktívabb az α-kimotripszinnél és sokszor szelektivitása is megközelíti, vagy eléri azét. Tisztasági fokától függetlenül lineáris reakciólefutást ad, szerves oldószerek (pl. benzol) kis koncentrációban aktiválják [32, 35]. Mesterséges szubsztrátjai elágazó láncú karbonsavak vagy elágazó láncú alkoholok észterei is lehetnek. Aktivitását etil-butiráttal mérve definiálták [32, 35]. Tiszta, vízoldható formában kereskedelmi forgalomból beszerezhető (SIGMA, SERVA), rögzített formában is ismert [2, 21]. A *Horgan* és mtsai tisztítási módszerének [34] módosított első két lépésével általunk előállított nyers enzimkészítményt is sikerrel alkalmaztuk [37, 38].

*Disznó-hasnyálmirigy lipáz (PPL) (EC. 3.1.1.3.)* [39—41]

A lipáz enzimek a zsírok emésztésében fontos szerepet játszanak, a trigliceridek észterkötéseinek hidrolítikus hatását katalizálják. Több állati és növényi szövetben megtalálhatóak [41]. A hosszabb szénláncú zsírsavak észtereinek hasítását gyorsabban végzik, mint a rövidekét, a di- és monogliceridek hidrolízisét is katalizálják egyre csökkenő mértékben. Mesterséges szubsztrátjaik az egyenes szénláncú karbonsavak elágazó láncú alkoholokkal képzett észterei lehetnek. A lipázok aktivitását trigliceridek [39, 41], újabban triacetin hidrolízisének segítségével definiálják. A szerves preparatív kutatásokban elsősorban a kereskedelemből (SIGMA) olcsón beszerezhető vízoldható disznóhasnyálmirigy lipázt (PPL) használják, de emellett mikroszervezetek által termelt lipázokat is alkalmaznak [21—26]. Rögzített lipáz is ismeretes [22, 42].




*1. ábra. Prokirális vagy mezo vegyületek hidrolízise*



mkl

## A hidrolítikus enzimek alkalmazási lehetőségei

A hidrolítikus enzimeket három módon lehet hasznosítani a preparatív szerves kémiában: prokirális vagy mezo vegyületek átalakítására, racém vegyületpárok rezolválására illetve regio- vagy diasztereoszelektív átalakításokra. Elvileg mindhárom alkalmazási lehetőség során az enzimkatalizált folyamat jellege szerint az átalakítás lehet hidrolízis, észteresítés ill. átészteresítés.

### *Prokirális vagy mezo vegyületek átalakítása*

A hidrolítikus enzimek királis katalízise e folyamatban használható ki a leghatékonyabban *(1. ábra)*. Ezeknek a szimmetriaelemmel rendelkező vegyületeknek két, kémiailag egyenértékű csoportja eltérő sebességgel reagál, mivel annak az enantiotóp csoportnak az átalakítása gyorsabb, amelyik az enzim meghatározott térbeli szerkezetű, királis felületű aktív centrumába energetikailag kedvezőbb módon illeszkedik.

| Termék (szubsztrát) | | $R^1$ | $R^2$ | Term. % | Opt. t. %ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|---|
| Termék /szubsztrát/ | a | Me | Et | >90 | 73 | PLE | [44] |
| | b | $(CH_2)_5CH_3$ | Me | >90 | 87 | PLE | [44] |
| | c | $(CH_2)_6CH_3$ | Me | >90 | 88 | PLE | [44] |
| | d | Bz | Me | >90 | 100 | CTR | [45] |
| | | | | >90 | 45 | PLE | [45] |
| | e | Et | Ph | >90 | 84 | PLE | [46] |
| | f | Me | OH | 82 | 45 | PLE | [19] |
| | g | OCOPh | Me | >90 | 94 | PLE | [21] |
| | h | OPh | Me | >90 | 97 | PLE | [21] |
| | i | OBz | Me | >90 | 86 | PLE | [21] |
| | j | Me | Ph | >90 | 81 | PLE | [21] |
| | a | Me | H | 98 | >99 | PLE | [47, 19, 52—55] |
| | b | Et | H | 77 | >99 | PLE | [47] |
| | c | Pr | H | 90 | >99 | PLE | [47] |
| | d | Pr | H | 61 | >99 | PLE | [47] |
| | e | $H^i$ | H | 90 | >99 | PLE | [47] |
| | f | Ph | H | 91 | >99 | PLE | [47] |
| | g | Bz | H | 90 | >99 | PLE | [47] |
| | h | Me | OH | 62 | >99 | PLE, CTR | [48] |
| | i | H | OH | 88 | >99 | CTR | [30] |
| | j | H | OH | 95 | 12 | PLE | [19] |
| | k | $OA_c$ | H | 45 | 87 | PLE | [49] |
| | l | $NH_2$ | H | 93 | 40 | PLE | [50] |
| | m | H | NHCOBz | 94 | >96 | PLE | [50] |
| /AcO/ III | | | | 34 | 95 | PPL | [51] |

*2. ábra. Prokirális diészterek hidrolízise*

| Termék (szubsztrát) | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|
| /MeOOC/ IV | 92 | 48 | PLE | [19] |
| /MeOOC/ V | 85 | 64 | PLE | [57] |
| | 48 | 100 | CTR | [19] |
| /MeOOC/ OH VI | 95 | 98 | PLE | [19] |
| /AcO/ VII a | | 90 | PPL | [58] |
| VII b /OAc/ | 36 | 80 | PLE | [58] |
| | 15 | 95 | PLE | [58] |

*3. ábra. Nyíltláncú mezo vegyületek hidrolízise*

Az enzimreakció után az addig megegyező X csoportok egyike megváltozik, így a szimmetrikus kiindulási vegyület elvileg teljes mennyisége optikailag aktív termékké alakul át. Ebben, a kémiailag már különböző X és Y csoportokat megfelelő módszerekkel szelektíven továbbalakítva, ugyanazon anyagból a származék tetszés szerinti enantiomerjéhez juthatunk.

Ez a módszer elvileg mind enzimkatalizált hidrolízis, mind enzimatikus észteresítési vagy átészteresítési folyamatok során alkalmazható, a mai napig azonban a két utóbbi alkalmazásra prokirális vagy mezo vegyületek esetében még nincs példa.

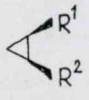
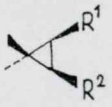
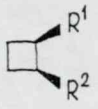
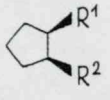
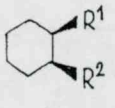
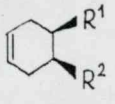
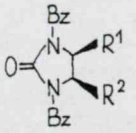
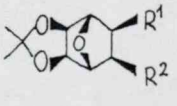
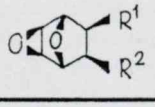
*Prokirális vegyületek hidrolízise (2. ábra).* A szubsztrátok lehetnek prokirális dikarbonsavak vagy diolok észterszármazékai. Diszubsztituált malonészterek (2. ábra, I) hidrolízise során megfelelő szubsztituensek esetében jó szelektivitást észleltek PLE enzimmel. Dietil észterekkel végzett hidrolízisek során a szelektivitás csökkenése tapasztalható [44]. A CTR enzim kizárólag a benzillel szubsztituált vegyületeket hidrolizálja, igen jó optikai tisztasággal [44, 45].

A $\beta$-szubsztituált glutársav észterek (2. ábra, II) hidrolízise PLE hatására apoláris szubsztituensek esetében (II: a—g) gyorsan és jó optikai tisztasággal (%ee > 80) játszódik le. Dietil észterek hidrolízisekor a szelektivitás nagymértékben csökken [52, 53, 56]. Poláris szubsztituensek esetében (II: j, 1) az ellentétes oldal hidrolízise gyorsabb, alacsony optikai tisztaságú (%ee < 40) termékek képződnek. E vegyületek hidrolízise jó szelektivitással CTR enzimmel valósítható meg, illetve az enzimhidrolízis védett származékokon (II: k, m) PLE enzimmel is jól elvégezhető.

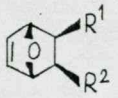
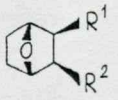
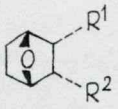
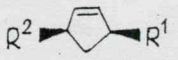
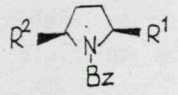
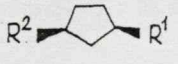
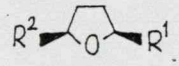
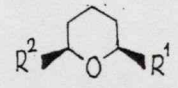
Prokirális diolok észterszármazékainak (III) átalakítására eddig csak a PPL enzimet alkalmazták.

*Nyíltláncú mezo vegyületek hidrolízise (3. ábra).* E vegyületek enzimatikus hidrolízisével egy lépésben, elvileg teljes mennyiségben képezhetőek olyan optikailag aktív anyagok, melyek egynél több aszimmetriacentrumot tartalmaznak. A VII vegyületpár példáján azt figyelhetjük meg, hogy adott esetben másik enzim alkalmazásával ellentétes szelektivitás érhető el [58].

*Gyűrűs mezo vegyületek hidrolízise (4/a és 4/b ábra).* E vegyületcsoport mindhárom ismertetett hidrolitikus enzim alkalmazására lehetőséget ad, sok

| Termék | | $R^1$ | $R^2$ | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|---|
|  | VIII | COOMe | COOH | 90 | >94 | PLE | [19, 59, 60] |
| | | $CH_2OAc$ | $CH_2OH$ | 74 | 93 | PPL | [61, 62] |
|  | IX | COOMe | COOH | 42 | 80 | PLE | [19, 59] |
| | | $CH_2OAc$ | $CH_2OH$ | 75 | 40 | PPL | [62] |
|  | X | COOMe | COOH | 98 | 90 | PLE | [19, 59, 60] |
| | | $CH_2OAc$ | $CH_2OH$ | 78 | 96 | PPL | [61, 62] |
|  | XI | COOH | COOMe | 80 | 9 | PLE | [19, 59, 60] |
| | | $CH_2OAc$ | $CH_2OH$ | 90 | 89 | PPL | [61, 62] |
|  | XII | COOH | COOMe | 75 | 80 | PLE | [19, 59, 60] |
| | | $CH_2OAc$ | $CH_2OH$ | 67 | 87 | PPL | [61, 62] |
|  | XIII | COOH | COOMe | 94 | >94 | PLE | [19, 59, 60] |
| | | $CH_2OAc$ | $CH_2OH$ | 96 | >99 | PPL | [62] |
|  | XIV | COOMe | COOH | 71 | 38 | PLE | [63] |
| | | $CH_2OH$ | $CH_2OAc$ | 70 | 92 | PLE | [51] |
|  | XV | COOH | COOMe | 96 | 77 | PLE | [64] |
|  | XVI | COOH | COOMe | 100 | 77 | PLE | [64] |

*4/a ábra. Gyűrűs mezo vegyületek hidrolízise*

| Termék | | $R^1$ | $R^2$ | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|---|
|  | XVII | COOH | COOMe | 86 | 75 | PLE | [65] |
|  | XVIII | COOH | COOMe | 82 | 98 | PLE | [65] |
|  | XIX | COOH | COOMe | 87 | 64 | PLE | [65] |
|  | XX | OH | OAc | 83 | 81 | PLE | [2, 58] |
|  | XXI | COOMe | COOH | 85 | 80 | PLE | [66] |
|  | XXII | COOH<br>COOMe | COOMe<br>COOH | | 60<br>80 | PLE<br>CTR | [21]<br>[21] |
|  | XXIII | COOMe<br>COOH<br>$CH_2OAc$<br>$CH_2OH$ | COOH<br>COOMe<br>$CH_2OH$<br>$CH_2OAc$ | | 42<br>91<br>76<br>22 | PLE<br>PPL<br>PLE<br>PPL | [18]<br>[18]<br>[18]<br>[18] |
|  | XXIV | COOMe<br>COOH | COOH<br>COOMe | | 71<br>53 | PLE<br>PPL | [18]<br>[18] |

*4/b ábra. Gyűrűs mezo vegyületek hidrolízise*

esetben egymást kiegészítő módon. A legáltalánosabb a PLE enzim alkalmazása, ennél a termék szerkezete és optikai tisztasága a gyűrű merevségétől, rögzített konformációjától erősen függ [19]. Ha a PLE enzim dikarbonsav észterek esetében nem alkalmazható megfelelő szelektivitással (**XI**), a megfelelő diolszármazék PPL enzimmel végzett hidrolízise eredményre vezethet. CTR enzim alkalmazásával esetenként jobb optikai tisztaság érhető el, mint a PLE enzimmel megvalósítható (**XXII**).

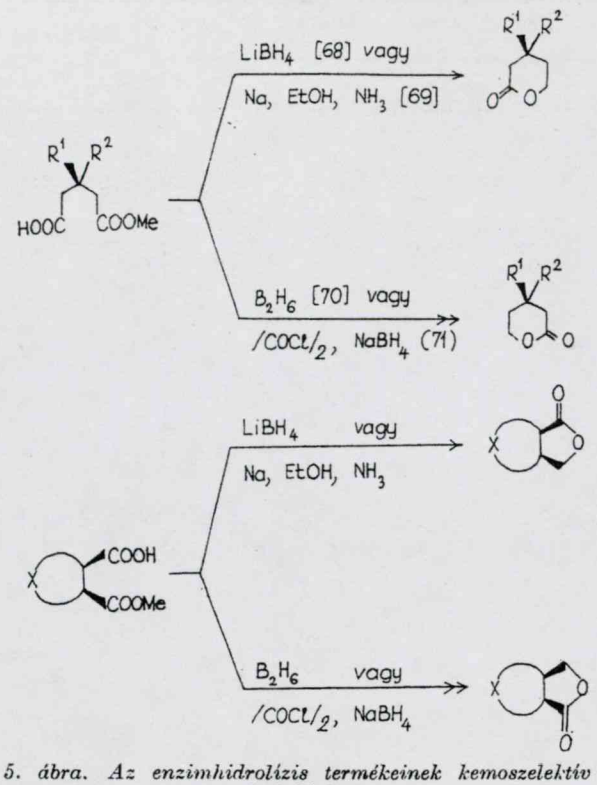
A prokirális vagy mezo vegyületek enzimhidrolízise után kemoszelektív átalakításokkal *(5. ábra)* a kívánt enantiomer állítható elő.

*Racém vegyületpárok kinetikus rezolválása*

A hidrolítikus enzimek felhasználhatóak enantiomer keverékek elválasztására *(6. ábra)* [23—26, 72—83]. A módszer azon alapul, hogy az egyes enantiomerek különböző sebességgel alakulnak át az enzim királis felületű aktív centrumán. Ha ez a sebességkülönbség elegendően nagy, akkor a racém vegyületpár 50% konverzió után optikailag aktív, kémiailag eltérő X és Y csoportokat tartalmazó anyagok elegyévé alakítható. E vegyületek elválasztása már egyszerű fizikai módszerekkel megoldható, elválasztás és átalakítások után a két enantiomer elvileg 50—50% termeléssel nyerhető. A racém vegyületek enzimatikus rezolválását mind hidrolízissel, mind észteresítéssel vagy átészteresítéssel meg lehet valósítani [26].

*Racém vegyületpárok elválasztása enzimhidrolízissel (7. ábra).* Vizes oldatokban enzimatikus hidrolízis segítségével a racém karbonsavak vagy alkoholok észterszármazékai kinetikusan rezolválhatóak. A konverziót változtatva a kívánt enantiomer optikai tisztasága növelhető [72, 75], kis konverzió esetén a reagáló enantiomer tisztasága nő, nagy konverziónál a nem reagálóé.

*Racém vegyületek elválasztása enzimkatalizált észteresítéssel vagy átészteresítéssel (8/a és 8/b ábra).* Meg-

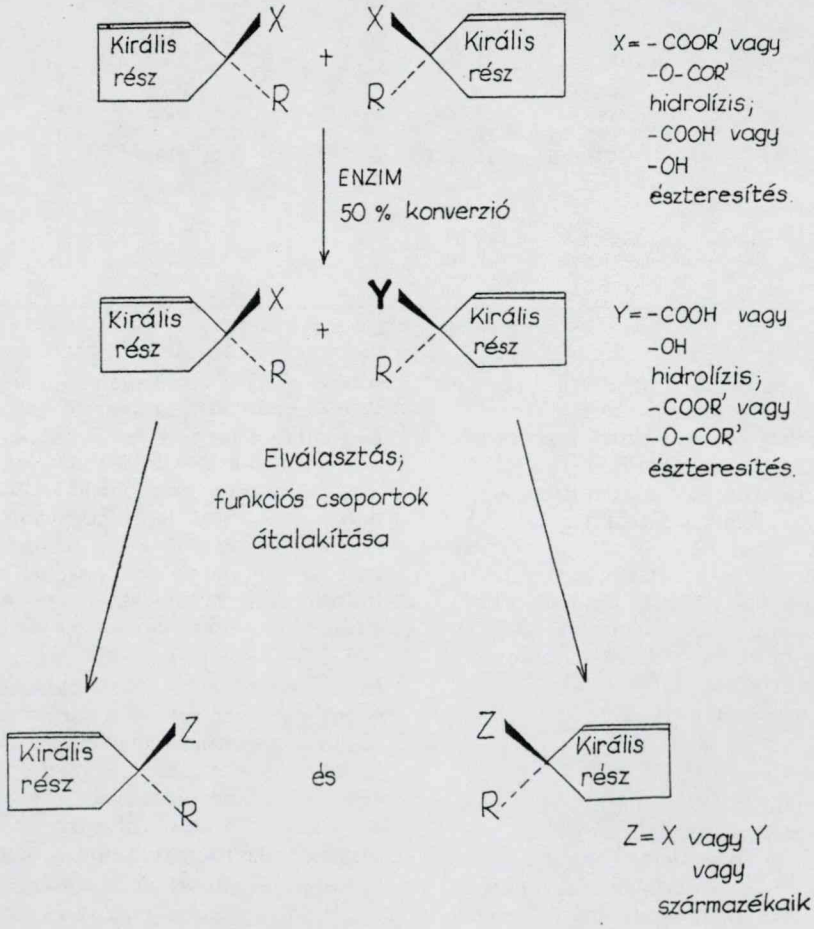



*5. ábra. Az enzimhidrolízis termékeinek kemoszelektív átalakítása*

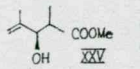
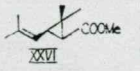
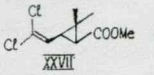
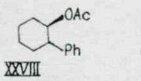
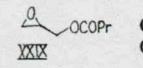
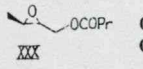
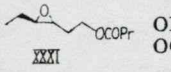
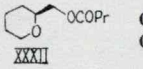
felelő körülmények között a hidrolázok katalizálhatnak észterképzési vagy átészteresítési reakciókat. Ezek a folyamatok egyensúlyi okokból vizes oldatokban nem valósíthatóak meg, ezért az ilyen típusú átalakításokat szerves oldószer-víz emulzióban [23] vagy aprotikus szerves oldószerekben [25, 26, 74] végzik. Az átalakításokat ugyanazon szubsztráton, ugyanazon enzimmel végezve megfigyelhető, hogy a termékek optikai tisztasága jelentős mértékben megnövekedhet, ha vizet tartalmazó rendszerről, folyamatról vízmentesre (pl. hidrolízisről észteresítésre vagy szerves oldószer-víz emulzióról vízmentes oldószerre) térnek át [26, 74]. A jelenség magyarázata az lehet [26], hogy a kétféle közegben az enzim szolvatációja nagymértékben eltér, aprotikus, szerves oldószerekben az enzim merevebb, meghatározottabb konformációt vesz fel.

*Enzimkatalizált diasztereoszelektív átalakítások*

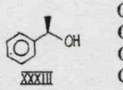
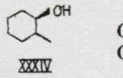
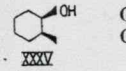
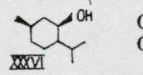
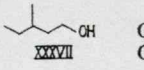
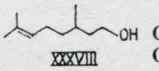
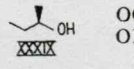
A hidrolítikus enzimek szelektivitása kihasználható diasztereomer vegyületek elválasztására [75, 84], vagy egy molekulán belüli diasztereotóp csoportok [85] közötti különbségtételre *(9. ábra)* olyan esetekben is, mikor az kémiailag nehezen megvalósítható, vagy fontos, hogy a reakció enyhe körülmények között játszódjon le.




*6. ábra. Racém vegyületek kinetikus rezolválása hidrolítikus enzimekkel*

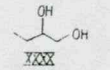
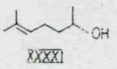
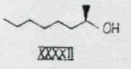
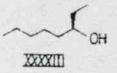
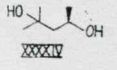
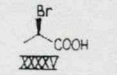
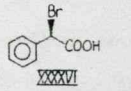
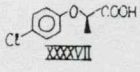
| Hidrolizáló enantiomer- | Termék[b] | Konv. % | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|
|  XXV | COOH<br>COOMe | 50 | | 64<br>63 | PLE | [56] |
|  XXVI | COOH<br>COOMe | 50 | 43<br>40 | 46<br>40 | PLE | [75] |
|  XXVII | COOH<br>COOMe | | 45 | 80 | PLE | [75] |
|  XXVIII | OH<br>OAc | 50 | | <99<br><99 | PLE | [73] |
|  XXIX | OH<br>OCOPr | 60 | 36 | <92 | PPL | [72] |
|  XXX | OH<br>OCOPr | 60 | | 95 | PPL | [72] |
|  XXXI | OH<br>OCOPr | 60 | | 81 | PPL | [72]] |
|  XXXII | OH<br>OCOPr | 60 | | 95 | PPL | [72] |

*7. ábra. Kinetikus rezolválás enzimhidrolízissel*

| Átalakuló enantiomer- | Termék[b] | Konv. % | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|
|  XXXIII | OCOPr<br>OH<br>OCOPr<br>OH | 50<br>45 | 41<br>39<br>34<br>31 | 85<br>88<br>95<br>90 | CCL[c]<br>PPL | [23]<br>[74] |
|  XXXIV | OCOR[d]<br>OH | 44 | | 98<br>80 | CCL[c] | [25] |
|  XXXV | OCOR[d]<br>OH | 52 | | 80<br>86 | CCL[c] | [25] |
|  XXXVI | OCOR[d]<br>OH | 57(45) | | 69(95)<br>88 | CCL[c] | [25, 26] |
|  XXXVII | OCOEt<br>OH | 50 | 38<br>22 | 94 | PLE | [23] |
|  XXXVIII | OCOEt<br>OH | 50 | 35<br>29 | 96<br>92 | PLE | [23] |
|  XXXIX | OCOPr<br>OH | 50 | 35<br>35 | 93<br>89 | CCL[c] | [23] |

*8/a ábra. Kinetikus rezolválás enzimatikus észteresítéssel és átészteresítéssel*

*a* az átalakuló enantiomernek megfelelő konfiguráció; *b* a második sorban feltüntetett reagálatlan vegyület ellentétes konfigurációjú; *c* Candida cylidracea mikroorganizmusból kivont lipáz; *d* $R = (CH_2)_{11}CH_3$

| Átalakuló enantiomer- | Termék[b] | Konv. % | Term. % | Opt. t. % ee | Enzim | Irodalom |
|---|---|---|---|---|---|---|
|  XXXX | OCOPr<br>OH | 50 | 39<br>36 | 90<br>89 | CCL[c] | [23] |
|  XXXXI | OCOPr<br>OH | 50 | 39<br>42 | 85<br>91 | CCL[c] | [23] |
|  XXXXII | OCOPr<br>OH | 47 | 33<br>32 | 95<br>90 | PPL | [74] |
|  XXXXIII | OCOPr<br>OH | 45 | 34<br>31 | 70<br>57 | PPL | [74] |
|  XXXXIV | OCOPr<br>OH | 48 | 29<br>27 | 87<br>92 | PPL | [74] |
|  XXXXV | COOBu<br>COOH | 45<br>78 | 40<br>21 | 96<br>99 | CCL[c] | [74] |
|  XXXXVI | COOBu<br>COOH | 20 | 16 | 99 | CCL[c] | [74] |
|  XXXXVII | COOBu<br>COOH | 45<br>65 | 36<br>34 | 70<br>65 | CCL[c] | [74] |

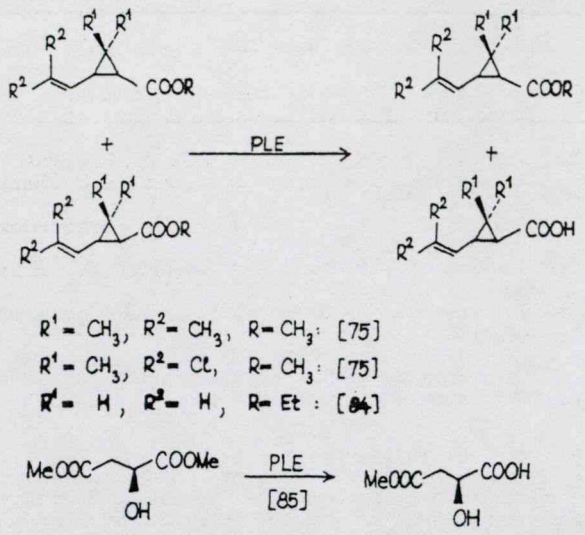
*8/b ábra. Kinetikus rezolválás enzimatikus észteresítéssel és átészteresítéssel*

## Alkalmazhatóság, felhasználás

Az előzőekben ismertetett enzimatikus reakciókkal olyan királis építőelemek széles köre állítható elő, melyek általánosan alkalmazhatóak a szintetikus kémia igen sok területén. A módszer sok esetben összemérhető mind árban, mind hatékonyságban a „tisztán" kémiai aszimmetrikus szintézismódszerekkel, egyes esetekben alkalmazása kifejezetten előnyös. Egyszerű laboratóriumi körülmények között ezek a reakciók alkalmasak lehetnek többmólnyi anyag előállítására is [2]. Az utóbbi években számos biológiailag aktív vegyület előállítása során alkalmaztak hidrolítikus enzimeket, illetve sok szintézisintermedier hatékony előállítását oldották meg azok segítségével: pl. barbiturátok [20, 21, 46], $\beta$-laktám antibiotikumok [50, 66], S-$\alpha$-metil-DOPA [45], piretroidok [20, 75], prosztanoidok [2, 21, 67], biológiailag aktív makrociklusos vegyületek [52—54], feromonok [84] és még sok más biológiailag aktív vegyület szintézise valósítható meg segítségükkel.

Kutatócsoportunk is sikerrel alkalmazta a nyers PLE enzimet a fáraóhangya optikailag aktív nyomjelző feromonjának szintézise során [37, 38] többmólnyi IIa előállítására, piretrin észterek kinetikus rezolválására, valamint a 3-acetoxiglutársav dimetilészter (IIk) hidrolízisére [49].

*Az anyag beérkezett:* 1986. márc. 18.




*9. ábra. Enzimkatalizált diasztereoszelektív átalakítások*

## IRODALOM


[1] *Bowen, R.—Pugh, S.:* Chemistry and Industry, 323 (1985).

[2] *Laumen, K.—Reimerdes, E. H.—Schneider, M.:* Tetrahedron Lett., *26*, 407 (1985).

[3] *Jones, J. B.—Sih, C. J.—Perlmann, D.:* Applications of Biochemical Systems in Organic Chemistry. New York, J. Wiley and Sons 1976.

[4] *Fischli, A.:* Modern Synthetic Methods (szerk.: Scheffold, R.). Frankfurt am Main, Salle and Sauerländer 1980. 269. old.

[5] *Whitesides, G. M.—Wong, C. H.:* Aldrichimica Acta, *16*, 27 (1983); Angew. Chem., *97*, 617 (1985).

[6] *Sih, C. J.—Chen, C. S.:* Angew. Chem., *96*, 556 (1984).

[7] *Ohta, H.—Tetsukawa, H.—Noto, N.:* J. Org. Chem., *47*, 2400 (1982).

[8] *Brooks, D. W.—Hasdiyasni, H.—Chakrabarti, S.:* Tetrahedron Lett., *25*, 1241 (1984).

[9] *Gopalan, A. S.—Sih, C. J.:* Tetrahedron Lett., *25*, 5235 (1984).

[10] *Kitazume, T.—Sato, T. Ishikawa, N.:* Chem. Lett., 1811 (1984).

[11] *Kotani, H.—Kuze, Y.—Uchida, S.—Miyabe, S.—Iimori, T.—Okano, K.—Kobayashi, S.—Ohno, M.:* Agric. Biol. Chem., *47*, 1363 (1983).

[12] *Irwin, A. J.—Jones, J. B.:* J. Am. Chem. Soc., *98*, 8476 (1976).

[13] *Davies, J.—Jones, J. B.:* J. Am. Chem. Soc., *101*, 5405 (1979).

[14] *Jones, J. B.—Jakovac, I. J.:* Can. J. Chem., *60*, 19 (1982).

[15] *Irwin, A. J.—Jones, J. B.:* J. Am. Chem. Soc., 99, 556 (1977).

[16] *Bridges, A. J.—Raman, P. S.—Ng, G. S. Y.—Jones, J. B.:* J. Am. Chem. Soc., *106*, 1461 (1984).

[17] *Ng, G. S. Y.—Yuan, L. C.—Jakovac, I. J.—Jones, J. B.:* Tetrahedron, *40*, 1235 (1984).

[18] *Jones, J. B.:* FECS International Conference on Chemistry and Biotechnology of Biologically Active Natural Products, Szófia, *1.* kötet, 18. old., 1985.

[19] *Mohr, P.—Waespe—Saracević, N.—Tamm, C.—Gawronska, K.—Gawronski, J. K.:* Helv. Chim. Acta, *66*, 2501 (1983).

[20] *Schneider, M.—Engel, N.:* FECS International Conference on Chemistry and Biotechnology of Biologically Active Natural Products, Budapest, 203. old., 1983.

[21] *Breitgoff, D.—Essert, T.—Laumen, K.—Schneider, M.:* FECS International Conference on Chemistry and Biotechnology of Biologically Active Natural Products, Szófia, *3.* kötet, 127. old., 1985.

[22] *Marlot, C.—Langrand, G.—Triantaphylides, C.—Baratti, J.:* Biotech. Lett., 9, 647 (1985).

[23] *Cambou, B.—Klibanov, A. M.:* J. Am. Chem. Soc., *106*, 2687 (1984).

[24] *Cambou, B.—Klibanov, A. M.:* Biotech. Bioengineer., *26*, 1449 (1984).

[25] *Langrand, G.—Secchi, M.—Buono, G.—Baratti, J.—Triantaphylides, C.:* Tetrahedron Lett., *26*, 1857 (1985).

[26] *Langrand, G.—Baratti, J.—Buono, G.—Triantaphylides, C.:* Tetrahedron Lett., *27*, 29 (1986).

[27] *Cohen, S. G.—Klee, L. H.:* J. Am. Chem. Soc., *82*, 6038 (1960).

[28] *Cohen, S. G.—Khedouri, E.:* J. Am. Chem. Soc., *83*, 1093 (1961).

[29] *Cohen, S. G.—Spriznak, Y.—Khedouri, E.:* J. Am. Chem. Soc., *83*, 4225 (1961).

[30] *Cohen, S. G.—Khedouri, E.:* J. Am. Chem. Soc., *83*, 4228 (1961).

[31] *Horgan, D. J.—Webb, E. C.—Zerner, B.:* Biochem Biophys. Res. Comm., *23*, 23 (1966).

[32] *Stoops, J. K.—Horgan, D. J.—Runnegar, M. T. C.—Jersey, J.—Webb, E. C.—Zerner, B.:* Biochemistry, *8*, 2026 (1969).

[33] *Schonbaum, G. R.—Zerner, B.—Bender, M. L.:* J. Biol. Chem., *236*, 2930 (1961).

[34] *Horgan, D. J.—Stoops, J. K.—Webb, E. C.—Zerner, B.:* Biochemistry, *8*, 2000 (1969).

[35] *Dudman, N. P. B.—Zerner, B.:* Methods in Enzymology, New York, Academic Press 1975, *35*, 191 (1975).

[36] *Krish, K.:* The Enzymes. 3. kiadás, New York, Academic Press 1971, 5. kötet, 3. fejezet.

[37] *Poppe, L.—Novák, L.—Kolonits, P.—Bata, Á.—Szabó, É.:* Előadás az MTA Izoprenoidkémiai Munkabizottság Alakuló Ülésén, Balatonfüred, 1985.

[38] *Poppe, L.—Novák, L.—Szántay, Cs.—Kolonits, P.—Bata, Á.:* Tetrahedron Lett., közlés alatt.

[39] *Verger, R.—De Haas, G. H.—Sarda, L.—Desnuelle, P.:* Biochim. Biophys. Acta, *188*, 272 (1969).

[40] *Singer, T. P.:* J. Biol. Chem., *174*, 11 (1948).

[41] *Desnuelle, P.:* The Enzymes, 3. kiadás, New York, Academic Press 1972, 7. kötet, 575. oldal.

[42] *Barker, S. A.—Kennedy, J. F.:* Data on Techniques for Enzyme Immobilization in Handbook of Enzyme Biotechnology (szerk. Wiesemann, A.). New York, Ellis Horwood Ltd. 1975.

[43] *Goldstein, L.—Manecke, G.:* The Chemistry of Enzyme Immobilization in Applied Biochemistry and Bioengineering Vol. 1, Immobilized Enzyme Principles. New York, Academic Press 1976.

[44] *Björkling, F.—Boutelje, J.—Gatenbeck, S.—Hult, K.—Norin, T.—Szmulik, P.:* Tetrahedron, *41*, 1347 (1985).

[45] *Björkling, F.—Boutelje, J.—Gatenbeck, S.—Hult, K.—Norin, T.:* Tetrahedron Lett., *26*, 4957 (1985).

[46] *Schneider, M.—Engel, N.—Boensmann, H.:* Angew. Chem., *96*, 54 (1984).

[47] *Francis, C. J.—Jones, J. B.:* J. Chem. Soc., Chem. Comm., 579 (1984).

[48] *Huang, F. C.—Hsu Lee, L. F.—Mittal, R. S. D.—Ravikumar, R. P.—Chan, J. A.—Sih, C. J.—Caspi, E.—Eck, C. R.:* J. Am. Chem. Soc., *97*, 4144 (1975).

[49] *Poppe L.:* Nem közölt eredmények.

[50] *Ohno, M.—Kobayashi, S.—Iimori, T.—Wang, Y. F.—Izawa, T.:* J. Am. Chem. Soc., *103*, 2405 (1981).

[51] *Wang, Y. F.—Sih, C. J.:* Tetrahedron Lett., *25*, 4999 (1984).

[52] *Mohr, P.—Tori, M.—Grossen, P.—Herold, P.—Tamm, G.:* Helv. Chim. Acta, *65*, 1412 (1982).

[53] *Herold, P.—Mohr, P.—Tamm, C.:* Helv. Chim. Acta, *66*, 744 (1983).

[54] *Ackermann, J.—Waespe—Saracevic, N.—Tamm, C.:* Helv. Chim. Acta, *67*, 254 (1984).

[55] *Van Middlesworth, F.—Wang, Y. F.—Zhou, B. N.—Di Tullio, D.—Sih, C. J.:* Tetrahedron Lett., *26*, 961 (1985).
[56] *Chen, C. S.—Fujimoto, Y.—Girdaukas, G.—Sih, C. J.:* J. Am. Chem. Soc., *104*, 7294 (1982).
[57] *Chen, C. S.—Fujimoto, Y.—Sih, C. J.:* J. Am. Chem. Soc., *103*, 3580 (1981).
[58] *Wang, Y. F.—Chen, C. S.—Girdaukas, G.—Sih, C. J.:* J. Am. Chem. Soc., *106*, 3695 (1984).
[59] *Schneider, M.—Engel, N.—Hönicke, P.—Heinemann, G.—Görisch, H.:* Angew. Chem., *96*, 55 (1984).
[60] *Sabbioni, G.—Shea, M. L.—Jones, J. B.:* J. Chem. Soc., Chem. Comm., 236 (1984).
[61] *Kasel, W.—Hultin, P. G.—Jones, J. B.:* J. Chem. Soc., Chem. Comm., 1563 (1985).
[62] *Laumen, K.—Schneider, M.:* Tetrahedron Lett., *26*, 2073 (1985).
[63] *Iruchijama, S.—Hasegawa, K.—Tsuchihashi, G.:* Agric. Biol. Chem., *46*, 1907 (1982).
[64] *Ohno, M.—Ito, Y.—Arita, M.—Shibata, T.—Adachi, K.—Sawai, H.:* Tetrahedron, *40*, 145 (1984).
[65] *Bloch, R.—Guibe-Jampel, E.—Girard, C.:* Tetrahedron Lett., *26*, 4087 (1985).
[66] *Kurihara, M.—Kamiyama, K.—Kobayashi, S.—Ohno, M.:* Tetrahedron Lett., *26*, 5831 (1985).
[67] *Gais, H. J.—Lukas, K. L.:* Angew. Chem., *96*, 140 (1984).
[68] *Cornforth, J. W.—Cornforth, R. H.—Popjak, G.—Yengoyan, L. S.:* J. Biol. Chem., *241*, 3970 (1966).
[69] *Paquette, L. A.—Nelson, N. A.:* J. Org. Chem., *27*, 2272 (1962).
[70] *Yoon, N. M.—Pak, C. S.—Brown, H. C.—Krishnamurthy, S.—Stocky, T. P.:* J. Org. Chem., *38*, 2786 (1973).
[71] *Fujisawa, T.—Mori, T.—Sato, T.:* Chem. Lett., 835 (1983).
[72] *Ladner, W. E.—Whitesides, G. M.:* J. Am. Chem. Soc., *106*, 7250 (1984).
[73] *Whitesell, J. K.—Chen, H. H.—Lawrence, R. M.:* J. Org. Chem., *50*, 4663 (1985).
[74] *Kirchner, G.—Scollar, M. P.—Klibanov, A. M.:* J. Am. Chem. Soc., *107*, 7072 (1985).
[75] *Schneider, M.—Engel, N.—Boensmann, H.:* Angew. Chem., *96*, 52 (1984).
[76] *Lavayre, J.—Verrier, J.—Baratti, J.:* Biotech. Bioeng., *24*, 2175 (1982).
[77] *Iriuchijima, S.—Kojima, N.:* Agric. Biol. Chem., *46*, 1153 (1982).
[78] *Iriuchijima, S.—Keiyu, A.—Kojima, N.:* Agric. Biol. Chem., *46*, 1593 (1982).
[79] *Oritani, T.—Yamashita, K.:* Agric. Biol. Chem., *38*, 1965 (1974).
[80] *McGahren, W. J.—Sax, K. J.—Kunstmann, M. P.—Ellestad, G. A.:* J. Org. Chem., *42*, 1659 (1977).
[81] *Mori, K.—Akao, H.:* Tetrahedron, *36*, 91 (1980).
[82] *Iriuchijima, S.—Keyiu, A.:* Agric. Biol. Chem., *45*, 1389 (1981).
[83] *Kawai, K. Imuta, M.—Ziffer, H.:* Tetrahedron Lett., *22*, 2527 (1981).
[84] *Schotten, T.—Boland, W.—Jaenicke, L.:* Helv. Chim. Acta, *68*, 1186 (1985).
[85] *Papageorgiou, C.—Benezra, C.:* J. Org. Chem., *50*, 1145 (1985).

(Lezárva az 1986 januárjáig összegyűjtött forrásmunkák alapján.)

## SUMMARY

This paper illustrates the scope of synthetic applicabilities of hydrolytic enzymes in organic chemistry. The enzymatically produced optically active compounds can be used as building blocks for a wide range of asymetric synthesis.

Costs and efficiency of this method are comparable with that of chemical asymetric procedures. Enzymic reactions have advantages over chemical methods in certain cases.

This enzymes are readily available and easily handled with standard laboratory equipment and are capable catalyzing transformations of prochiral or meso as well as racemic substrates into optically active compounds.

## РЕЗЮМЕ

В статье показано применение гидролитических энзимов в препаративной органической химии. Оптически активные соединения, полученные с помощью энзимов, могут быть широко использованы в качестве структурных элементов для дальнейшего синтеза асимметричных соединений. Эффективность и стоимость энзимного метода синтеза сравнима с химическими асимметричными методами синтеза, а в определенных случаях даже является преимущественным. Используемые энзимы легко доступны, хорошо обрабатываются на обычных лабораторных установках и способны катализировать превращения прохиральных, мезосоединений и рацемических веществ до оптически активных соединений.

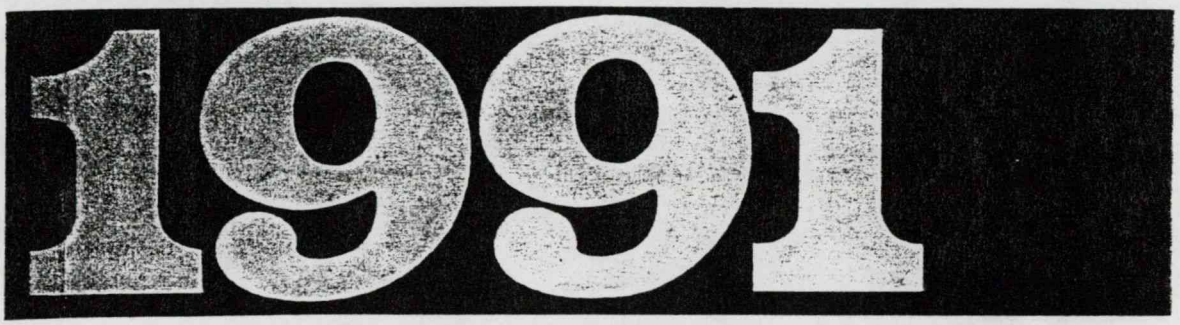
# B. melléklet

**POPPE, L.**, NOVÁK, L.:

*Biokatalízis a szintetikus kémiában*

A kémia újabb eredményei, **73**, Budapest: Akadémiai Kiadó, **1991**

# A kémia újabb eredményei




Poppe László • Novák Lajos
Biokatalízis a szintetikus kémiában

Majoros Béla
Inverz reaktivitás, szintézistervezés

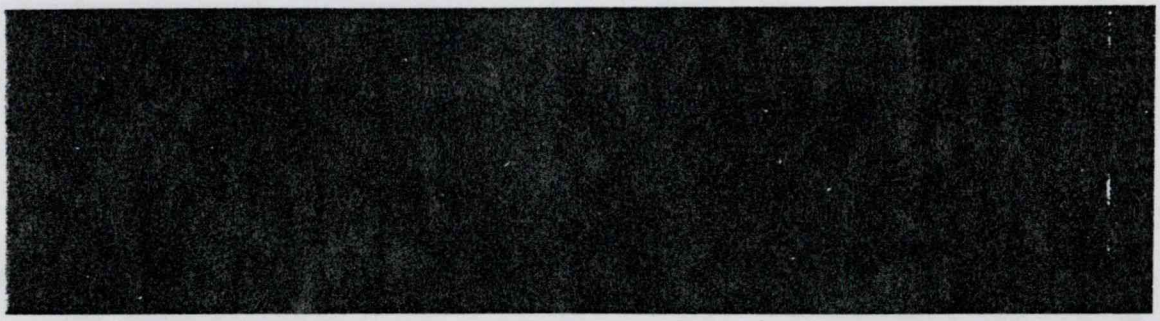
Akadémiai Kiadó • Budapest

Ára: 168,– Ft

# A KÉMIA ÚJABB EREDMÉNYEI

## 73. kötet

SZERKESZTI
CSÁKVÁRI BÉLA



E kötet két, elméleti és gyakorlati szempontból jelentős szerves kémiai témakörrel foglalkozik.

*Poppe László* és *Novák Lajos* Biokatalízis a szintetikus kémiában c. monográfiája a különféle enzimek és egyszerű mikroorganizmusok preparatív kémiai alkalmazási lehetőségeit ismerteti több mint ötszáz szakcikk alapján. A biokatalizátorok felhasználása — elsősorban optikailag aktív szintézis-intermedierek előállítására — a modern szintetikus kémia gyorsan fejlődő területe.

*Majoros Béla* Inverz reaktivitás, szintézistervezés c. munkája egy viszonylag új és ma is fejlődő rendszerezési móddal foglalkozik, amely elsősorban a számítógépes szintézistervezés elméletéből és gyakorlatából ered. Az inverz reaktivitáson alapuló reakciók széles körben és hatásosan alkalmazottak különféle szintézisek retroszintetikus analízisében.

ISBN 963 05 5865 3

# A KÉMIA ÚJABB EREDMÉNYEI

73. kötet



AKADÉMIAI KIADÓ · BUDAPEST 1991

# BIOKATALÍZIS
# A SZINTETIKUS KÉMIÁBAN

POPPE LÁSZLÓ
a kémiai tudomány kandidátusa

NOVÁK LAJOS
a kémiai tudomány doktora

# TARTALOM

# C. melléklet

**POPPE, L.**, NOVÁK, L.:

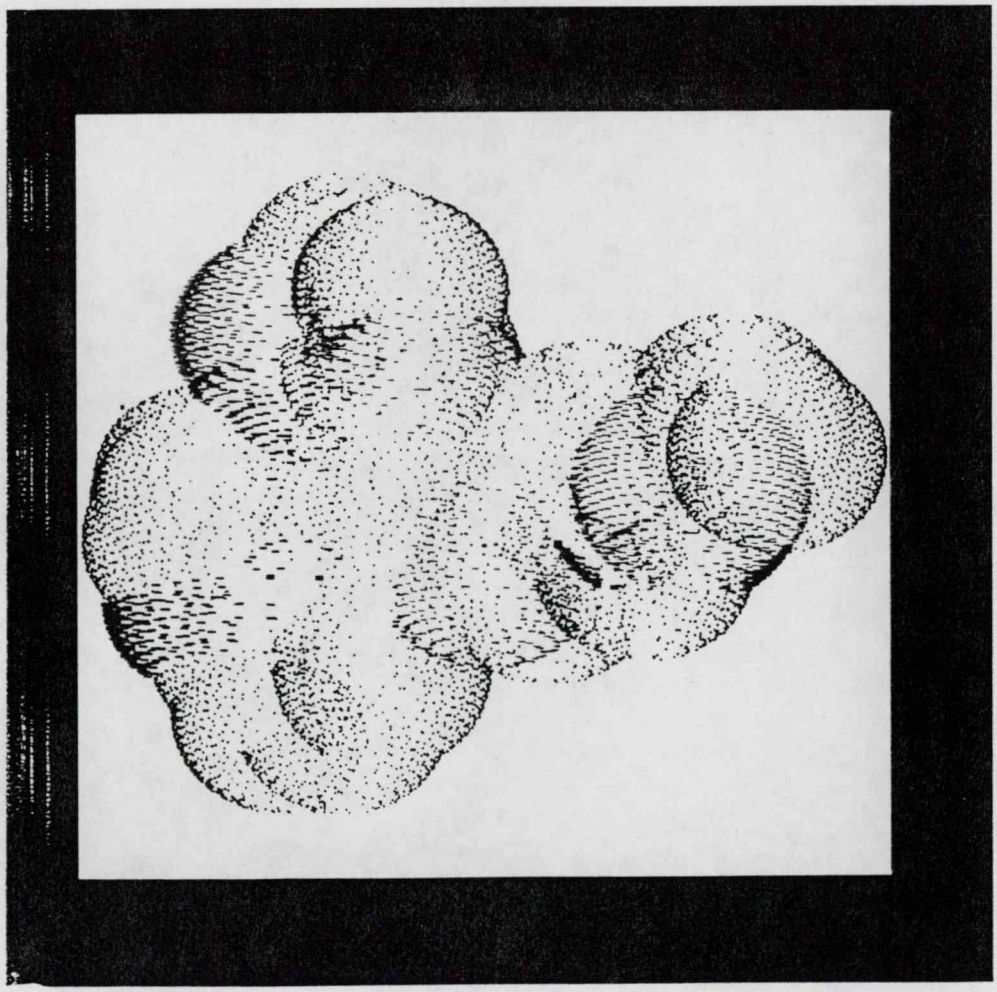
*Selective Biocatalysis: A Synthetic Approach,*

Weinheim-New York: Verlag Chemie, **1992.**

László Poppe, Lajos Novák

# Selective Biocatalysis

## A Synthetic Approach



VCH

László Poppe, Lajos Novák

# Selective Biocatalysis

## A Synthetic Approach

This stimulating monograph is a much needed response to the increasing use of biocatalysts in organic synthesis. In over 300 well-referenced pages, it

- reviews in detail applications and other aspects of the most widely used enzymes (e.g. hydrolases, oxido-reductases, and baker's yeast)
- illustrates with examples the potential in biocatalysis of other, less frequently used enzymes and microorganisms
- discusses general problems of biocatalysis (e.g. stereo-chemistry of transformations, methods of influencing selectivity)
- indicates future trends in biocatalysis

Researchers and students in preparative organic chemistry, biotechnology and biochemistry will find this up-to-date monograph highly valuable in their daily work.

**ISBN 3-527-28372-2 (VCH, Weinheim)**
**ISBN 1-56081-188-9 (VCH, New York)**

VCH

L. Poppe, L. Novák

# Selective Biocatalysis

## A Synthetic Approach

VCH
Weinheim
New York
Cambridge
Basel

Dr. László Poppe
Central Research Institute for Chemistry,
Hungarian Academy of Sciences
H-1025 Budapest
Pusztaszeri út 59-67
Hungary

Prof. Lajos Novák
Institute of Organic Chemistry
Technical University Budapest
H-1521 Budapest
XI., Gellért tér 4
Hungary



Published jointly by
VCH Verlagsgesellschaft mbH, Weinheim (Federal Republic of Germany)
VCH Publishers, Inc., New York, NY (USA)

Editorial Director: Dr. Christina Dyllick-Brenzinger
Production Manager: Dipl.-Ing. (FH) Hans Jörg Maier

Library of Congress Card No.: 92-9284

British Library Cataloguing-in-Publication Data: A catalogue record for this book is available from the British Library.

Deutsche Bibliothek Cataloguing-in-Publication Data:
**Poppe, László:**
Selective biocatalysis: a synthetic approach / L. Poppe; L. Novák. - Weinheim; New York; Cambridge; Basel: VCH, 1992
ISBN 3-527-28372-2
NE: Novák, Lajos:



Composition: Ulrich Hellinger, D-6901 Heiligkreuzsteinach. Printing: betz-druck gmbh, D-6100 Darmstadt 12. Bookbinding: J. Schäffer GmbH & Co.KG, D-6718 Grünstadt.
Printed in the Federal Republic of Germany.

# Contents

Distribution:
VCH. P.O. Box 10 11 61. D-6940 Weinheim (Federal Republic of Germany)
Switzerland: VCH. P.O. Box. CH-4020 Basel (Switzerland)
United Kingdom and Ireland: VCH (UK) Ltd.. 8 Wellington Court, Wellington Street. Cambridge CB1 1HZ (England)
USA and Canada: VCH. Suite 909. 220 East 23rd Street. New York. NY 10010-4606 (USA)

ISBN 3-527-28372-2 (VCH. Weinheim) ISBN 1-56081-188-9 (VCH. New York)