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Enantioselective hydrolysis of 3,4-disubstituted β-lactams. An efficient enzymatic method for the preparation of a key Taxol side-chain intermediate

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ABSTRACT

3,4-Disubstituted β-lactams 3-benzyloxy-4-(4-chlorophenyl)azetidin-2-one [(3*S**,4*R**)-(±)-**1**], 3-benzyloxy-4-phenylazetidin-2-one [(3*S**,4*R**)-(±)-**2**] and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(3*S**,4*R**)-(±)-**3**] were resolved through immobilized CAL-B-catalysed ring-cleavage reactions. Excellent enantioselectivities ($E > 200$) were obtained for (3*S**,4*R**)-(±)-**1** and (3*S**,4*R**)-(±)-**2** when the reactions were performed with added H_2O as nucleophile in *tert*-butyl methyl ether at 70 °C, whereas only moderate E (12) was achieved for (3*S**,4*R**)-(±)-**3** under the same conditions but in diisopropyl ether. The resulting ring-opened β-amino acids [(2*R*,3*S*)-**4** (*ee* > 98%), (2*R*,3*S*)-**5** (*ee* > 98%) and (2*R*,3*S*)-**6** (*ee* = 50%)] and the unreacted β-lactams [(3*S*,4*R*)-**1**–**3**] (*ee* > 98%) could be easily separated.

1. Introduction

A large number of recent published articles and reviews have stressed the biological and chemical importance of β-lactams and β-amino acids [1]. Molecules containing a 2-azetidinone ring may possess antibacterial activity, e.g., carumonam is a β-lactamase-resistant monobactam antibiotic [2], while others containing a *cis* 3,4-disubstituted β-lactam ring may display PPAR α/γ agonist [3], vasopressin VIa agonist [4] or anticancer [5,6] activity. β-Amino acids and some of their derivatives are widely used in combinatorial, peptide, organic and medicinal chemistry [7–9]. Numerous non-proteinogenic amino acids are available can serve as relevant components of fibrinogen receptor antagonists [10]. Taxol®, one of the most efficient anticancer agents of the past decade [11,12], contains (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid [(2*R*,3*S*)-**7**] in its side-chain. Since the total synthesis of Taxol is a very lengthy and expensive process [13,14], chemists are continuously working on the development of semi-synthetic methods which involve coupling of the C(13)-O of baccatin III derivatives [15] to the corresponding side-chain.

Earlier enzymatic studies on the ring opening of a set of cyclic and acyclic β-lactams [16–19] were continued with successful enzymatic syntheses of a Taxol side-chain key intermediate through the enantioselective ring opening of racemic *cis*-3-hydroxy-4-phenylazetidin-2-one (0.5 equiv. of H_2O in *t*-BuOMe at 60 °C, with immobilized CAL-B) and sequential kinetic resolution of racemic *cis*-3-acetoxy-4-phenylazetidin-2-one (1 equiv. of H_2O in iPr_2O at 60 °C, with immobilized CAL-B) [20]. To extend the substrate scope, and also to analyse how different-sized substituents on C3 or C4 influence the ring cleavage of β-lactams, in the present work we set out to develop immobilized CAL-B-catalysed methods for the enzymatic ring opening of racemic 3,4-disubstituted β-lactams, such as 3-benzyloxy-4-(4-chlorophenyl) azetidin-2-one, 3-benzyloxy-4-phenylazetidin-2-one and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(3*S**,4*R**)-(±)-**1**–**3**] (Scheme 1), and then to synthetize (2*R*,3*S*)-3-phenylisoserine (2*R*,3*S*)-**7**, the key intermediate of the Taxol side-chain, from the corresponding enantiomeric compound.

2. Results and discussion

2.1. Synthesis of (3S*,4R*)-(±)-1–3

Racemic β-lactams (3*S**,4*R**)-(±)-**1**–**3** were synthesized according to a literature method [21]. A mixture of *p*-ethoxyaniline

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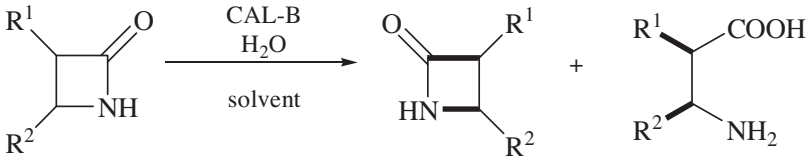


1, 4: R^1 = BnO, R^2 = *p*-ClPh
2, 5: R^1 = BnO, R^2 = Ph
3, 6: R^1 = PhO, R^2 = *p*-ClPh

Scheme 1. Immobilized CAL-B-catalysed hydrolysis of (±)-**1**–**3**.

and the appropriate aldehyde furnished the Schiff bases (Z)-*N*-(4-chlorobenzylidene)-4-ethoxybenzenamine (**10**) and (Z)-*N*-benzylidene-4-ethoxybenzenamine (**11**), which, through cycloadditions in the presence of the appropriate acyl chlorides, 2-phenoxyacetyl chloride (**8**) or 2-benzyloxyacetyl chloride (**9**), resulted in the *N*-protected *β*-lactams **12**–**14**. CAN-mediated oxidative removal of the 4-ethoxyphenyl groups gave the desired β-lactams **1**–**3** (Scheme 2).

2.2. Immobilized CAL-B-catalysed ring-opening of (3S*,4R*)-(±)-1–3

In earlier studies, immobilized CAL-B proved to be applicable for the enantioselective ($E > 200$) ring opening of both 4-aryl-substituted [17] and carbocyclic β-lactams [22], and we therefore carried out the ring opening of model compound (3S*,4R*)-(±)-**1** with 1 equiv. of H_2O in *i*Pr_2O at 60 °C, with immobilized CAL-B as catalyst (Table 1, entry 1).

In order to find the optimum conditions for the gram-scale resolution of (3S*,4R*)-(±)-**1**, solvent screening (Table 1, entries 1–6) was first performed in order to determine the effects on *E* and the reaction rate. Practically, no reaction was detected during 65 h when the reactions were performed in THF (entry 4) or 2-Me-THF (entry 5). The reactions proceeded enantioselectively ($E > 200$), but slowly in *t*-BuOMe and *i*Pr_2O (conv. = 5–8% after 65 h) (entries 1 and 6) and with somewhat higher conversions in toluene (conv. = 15% after 65 h, $E = 32$) (entry 2) or *n*-hexane (conv. = 17% after 65 h, $E = 39$) (entry 3). In view of the results, *t*-BuOMe was chosen for further preliminary experiments.

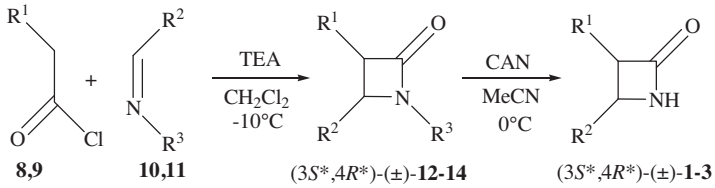
H_2O, as a nucleophile, is essential for the ring-opening reaction, through its quantity in the reaction medium can affect the enzymatic activity [18,22]. Experiments were therefore also performed with different quantities of added H_2O (Table 1, entries 7–10 and 12–15). On increase of the amount of H_2O up to 50 equiv., the reactions became faster without a drop in *E* (entries 8–10), but a further increase of the H_2O content resulted in considerably decreases in both reaction rate and *E* (entries 12–15). It is noteworthy that, in accordance with our earlier observation that a hydrolytic reaction proceeded even without added H_2O in the reaction mixture (due to the H_2O present in the reaction medium) [22], the quantity of H_2O present in the reaction medium (<0.1%) or at the surface of the immobilized CAL-B (2–5%) was sufficient for the ring cleavage of (±)-**1** (entry 7). Finally, 25 equiv. of H_2O was chosen as the optimum quantity.

On increase of the temperature of the ring-opening reaction from 60 °C (Table 1, entry 10) to 70 °C, the reaction rate increased without any decrease in enantioselectivity (Table 1, entry 11). Accordingly, 70 °C was chosen as the reaction temperature.

The above-optimized reaction conditions (25 equiv. of H_2O, *t*-BuOMe, 70 °C) were next applied for the ring cleavage of (±)-**2** and (±)-**3**. Excellent results were observed for (±)-**2** ($E > 200$), but a very poor *E* (5) for (±)-**3** (Table 2, entry 1). We therefore continued the optimizations for (±)-**3** with a new solvent screening, changing the amount of added H_2O and also the temperature of the reaction (Table 2).

The reactions in toluene and *n*-hexane proceeded relatively slowly, with low *E* (entries 2 and 3) while in MeCN and THF the enzyme did not display activity during 65 h (entries 6 and 7). A slightly increased *E* (8) was noted in *i*Pr_2O vs. *t*BuOMe ($E = 2$) (entries 4 and 5). Variation of the quantity of water (from 2 to 100 equiv., entries 8–11) and temperature (50 and 70 °C, entries 12 and 13) led to the same results as observed earlier for (±)-**1**. In summary, *E* was increased slightly ($E = 14$, entry 13) when the reaction was carried out with 25 equiv. of water in *i*Pr_2O at 70 °C (Scheme 3).

On the basis of the preliminary results, the immobilized CAL-B-catalysed preparative-scale ring-opening reactions of (±)-**1** and (±)-**2** were performed with 25 equiv. of H_2O in *t*-BuOMe at 70 °C, while the preparative-scale resolution of (±)-**3** was performed with 25 equiv. of H_2O in *i*Pr_2O at 70 °C. In order to obtain (2*R*,3*S*)-**6** with a good *ee* value, the reaction was overrun to 66% conversion. The results are reported in Table 3 and in Section 3 (Experimental part).



8: R^1 = BnO
9: R^1 = PhO
10: R^2 = Ph, R^3 = *p*-EtOPh
11 R^2 = *p*-ClPh, R^3 = *p*-EtOPh
12: R^1 = BnO, R^2 = *p*-ClPh, R^3 = *p*-EtOPh
13: R^1 = BnO, R^2 = Ph, R^3 = *p*-EtOPh
14: R^1 = PhO, R^2 = *p*-ClPh, R^3 = *p*-EtOPh
1: R^1 = BnO, R^2 = *p*-ClPh
2: R^1 = BnO, R^2 = Ph
3: R^1 = PhO, R^2 = *p*-ClPh

Scheme 2. Synthesis of (±)-**1**–**3**.

Table 1
Effects of solvents and the quantities of H_2O on the immobilized CAL-B-catalysed ring cleavage of (±)-**1**.[a]

Entry	Solvent	H_2O (equiv.)	Temperature (°C)	ee_s[b](%)	ee_p[c](%)	Conv. (%)	*E*
1	*i*Pr_2O	1	60	5	99	5	>200
2	toluene	1	60	16	93	15	32
3	*n*-hexane	1	60	20	94	17	39
4	THF	1	60	No reaction			
5	2-Me-THF	1	60	No reaction			
6	*t*-BuOMe	1	60	9	99	8	>200
7	*t*-BuOMe	0	60	5	99	5	>200
8	*t*-BuOMe	2	60	10	99	9	>200
9	*t*-BuOMe	10	60	21	99	18	>200
10	*t*-BuOMe	25	60	36	99	27	>200
11	*t*-BuOMe	25	70	54	99	35	>200
12	*t*-BuOMe	50	60	67	96	41	133
13	*t*-BuOMe	100	60	41	96	30	73
14	*t*-BuOMe	1850	60	45	95	32	61
15	H_2O	-	60	35	95	27	55

[a] 0.015 M substrate, H_2O, 30 mg mL^{-1} immobilized CAL-B, after 65 h.
[b] According to HPLC (Section 3).
[c] According to HPLC after derivatization (Section 3).

2.3. Synthesis of Taxol side-chain intermediate

To prepare (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid [(2*R*,3*S*)-**7**], the key intermediate of Taxol, the debenzylation of (2*R*,3*S*)-**5** (*ee* = 99%) was performed in a continuous flow system (H-CUBE®) by using a CatCart® filled with 10% Pd/C, operating at a flow rate of 0.1 mL/min, 50 bar, 40 °C (Scheme 4). Thus, (2*R*,3*S*)-**7** was obtained with good *ee* (99%) and in nearly quantitative yield (93%) after four cycles. The absolute configuration for the enantiomeric **7** obtained was proved by comparing the literature [20] [α] value for (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid $\{[\alpha]_D^{25} = -7.2$ ($c = 0.34$, H_2O), *ee* > 99%} with the [α] value measured for enantiomeric **7** $\{[\alpha]_D^{25} = -7.2$ ($c = 0.34$, H_2O), *ee* = 99%}. Thus, immobilized CAL-B catalysed the ring opening of (3*S* *,4*R* *)-(±)-**2** with (2*R*,3*S*) selectivity, while for (3*S**,4*R**)-(±)-**1** and (3*S**,4*R**)-(±)-**3** the analysed chromatograms indicated the same enantiopreference for immobilized CAL-B.

2.4. Conclusions

An efficient enzymatic method was developed for the ring opening of 3,4-disubstituted β-lactams (3*S**,4*R**)-(±)-**1–3**. High enantioselectivities (*E* > 200) were obtained for the ring-opening reactions of (3*S**,4*R**)-(±)-**1** and (3*S**,4*R**)-(±)-**2** when immobilized CAL-B was used as catalyst, with 25 equiv. of H_2O as nucleophile, in *t*-BuOMe at 70 °C, while a relatively modest *E* (12) was obtained for immobilized CAL-B-catalysed ring opening of (3*S**,4*R**)-(±)-**3** in *i*Pr_2O with 25 equiv. of H_2O at 70 °C. The great differences in *E* for (±)-**1** and (±)-**2** vs. (±)-**3** are presumably consequences of the very different steric hindrance of BzO vs. PhO, which influences the accommodation for the enantiomers in the active site of immobilized CAL-B. The products could be easily separated. The present enzymatic method proved suitable for the preparation of (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid [(2*R*,3*S*)-**7**], a key intermediate for the Taxol® side-chain.

3. Experimental

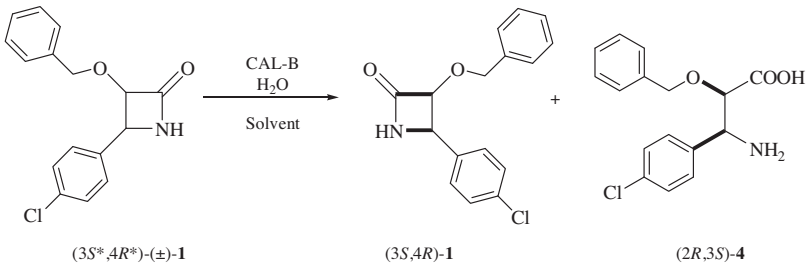
3.1. Materials and methods

Immobilized CAL-B (lipase B from *Candida antarctica*) immobilized on acrylic resin (L4777) was purchased from Sigma. All solvents were of the highest analytical grade. In a typical small-scale experiment, immobilized CAL-B (30 mg), then H_2O (1, 2, 10, 25, 50, 100 or 1850 equiv.) were added to the racemic substrate (0.015 M solution) in an organic solvent (1 mL). The mixture was shaken (167 rpm) at 50, 60 or 70 °C. The progress of the reactions was followed by taking samples from the reaction mixtures and analysing them by HPLC with a chiral column. The *ee* values for the unreacted β-lactams (3*S*,4*R*)-**1** and (3*S*,4*R*)-**3** and the product β-amino acid (2*R*,3*S*)-**6** [after pre-column derivatization [23] with CH_2N_2 (Caution! derivatization with CH_2N_2 should be performed under a well-working hood)] were determined on a Chiralpak IA column (4.6 × 250 mm);

Table 2
Immobilized CAL-B-catalysed ring-opening of (±)-**3**.[a]

Entry	Solvent	Reaction time (h)	Temperature (°C)	H_2O (equiv.)	ee_s[b](%)	ee_p[c](%)	Conv. (%)	*E*
1	*t*-BuOMe	120	70	25	42	55	43	5
2	toluene	65	60	1	7	38	15	2
3	*n*-hexane	65	60	1	6	53	10	3
4	*i*Pr_2O	65	60	1	8	75	10	8
5	*t*-BuOMe	65	60	1	5	37	12	2
6	MeCN	65	60	1	No reaction			
7	THF	65	60	1	No reaction			
8	*i*Pr_2O	65	60	2	8	75	10	8
9	*i*Pr_2O	65	60	10	38	73	34	9
10	*i*Pr_2O	65	60	25	46	72	39	10
11	*i*Pr_2O	65	60	100	70	21	76	3
12	*i*Pr_2O	65	50	25	14	78	15	9
13	*i*Pr_2O	65	70	25	81	70	54	14

[a] 0.015 M substrate, H_2O, 30 mg mL^{-1} immobilized CAL-B.
[b] According to HPLC (Section 3).
[c] According to HPLC after derivatization (Section 3).



(3S*,4R*)-(±)-**1** (3S,4R)-**1** (2R,3S)-**4**

Scheme 3. Immobilized CAL-B-catalysed ring opening of (±)-**1**.

detection at 228 nm; eluent: *n*-hexane/Et_2N/*i*PA (90/0.1/10); flow rate: 0.5 mL min^{-1}; retention times (min) for (3*S*,4*R*)-**1**: 27.86 (antipode: 25.51), (3*S*,4*R*)-**3**: 25.33 (antipode: 22.55), (2*R*,3*S*)-**6**: 32.43 (antipode: 27.08). (2*R*,3*S*)-**4** and (2*R*,3*S*)-**5** [after pre-column derivatization with CH_2N_2]; Chiralpak IA column (4.6 × 250 mm); detection at 228 nm; eluent: *n*-hexane/Et_2N/*i*PA (50/0.1/50); flow rate: 0.5 mL min^{-1}; retention times (min) for (2*R*,3*S*)-**4**: 13.80 (antipode: 11.50), (2*R*,3*S*)-**5**: 12.36 (antipode: 10.61). (3*S*,4*R*)-**2**: Chiralpak IA column (4.6 × 250 mm); detection at 228 nm; eluent: *n*-hexane/Et_2N/*i*PA (50/0.1/50); flow rate: 0.5 mL min^{-1}; retention times (min) for (3*S*,4*R*)-**2**: 9.09 (antipode: 9.79). The *ee* value for the Taxol key intermediate (2*R*,3*S*)-**7** prepared was determined by a GC method on a Chrompack Chirasil-Dex CB column after double derivatization [23] with (i) CH_2N_2; (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine [140 °C for 7 min → 190 °C (temperature rise 10 °C min^{-1}; 100 kPa; retention times (min), (2*R*,3*S*)-**7**: 19.01 (antipode: 18.70)] (Supporting Information S1–S7).

All melting points were measured on an X-4 melting-point apparatus with a microscope. ^1H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer in $CDCl_3$ D_2O and CD_3OD. 10% Pd/C CatCart® was from ThalesNano (3,378 and the product ID: THS 1,111). Optical rotations [α] were measured with a PerkinElmer 341 polarimeter.

3.2. Synthesis of 3-benzyloxy-4-(4-chlorophenyl) azetidin-2-one [(±)-1]

A solution of benzyloxyacetyl chloride (**8**, 0.23 mL, 1.5 mmol) in dry CH_2Cl_2 was slowly added to a solution of 4-chlorobenzylidene-4-ethoxyphenylamine (**11**, 0.26 g, 1.0 mmol) and Et_3N (0.42 mL, 3.0 mmol) in CH_2Cl_2 (20 mL) at −10 °C. The reaction was then allowed to warm up to room heat, stirred for 12 h, washed with $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried (Na_2SO_4) and evaporated. The product 3-benzyloxy-4-(4-chlorophenyl)-1-(4-ethoxyphenyl) azetidin-2-one (**12**) was recrystallized from EtOAc [265 mg, 65%; m.p. 166–168 °C]. A solution of CAN (0.75 g, 1.4 mmol) in H_2O (15 mL) was added dropwise to the β-lactam solution (**12**, 0.2 g, 0.5 mmol) in MeCN (15 mL) at 0 °C. The reaction was stirred at 0 °C for 30 min, 15 mL H_2O was then added and the mixture was extracted with EtOAc (3 × 20 mL) and washed with 10% aqueous $NaHCO_3$ (20 mL). The organic layer was combined and washed with 10% Na_2SO_3 (2 × 15 mL), 10% $NaHCO_3$ (10 mL), and brine (20 mL), and dried with Na_2SO_4. After filtration, the solvent was evaporated off, and the product 3-benzyloxy-4-(4-chlorophenyl) azetidin-2-one (**1**) was recrystallized from EtOAc [76 mg, 53%; m.p. 199–201 °C]. This product was described in 1998, but no ^1H NMR data and m.p. were then reported [24].

^1H NMR (400 MHz, DMSO, TMS) δ (ppm) for (±)-**1**: 4.11–4.17 (d, *J* = 11.64 Hz, 1H, C3H); 4.29–4.35 (d, *J* = 11.16); 4.86–4.90 (d, *J* = 4.64 Hz, 1H, CH2); 4.93–4.98 (d, *J* = 4.2 Hz, 1H, CH2); 6.88–6.95 (m, 2H, Ar); 4.19–4.26 (m, 3H, Ar); 7.35–7.48 (dd, *J* = 8.46 Hz, 4H, Ar); 8.63–8.69 (bs, 1H, NH). Analysis: calcd. For $C_{16}H_{14}ClNO_2$: C, 66.79; H, 4.90; N, 4.87; Analysis: found for (3*S**,4*R**)-(±)-**1**: C, 66.81; H, 4.87; N, 4.89.

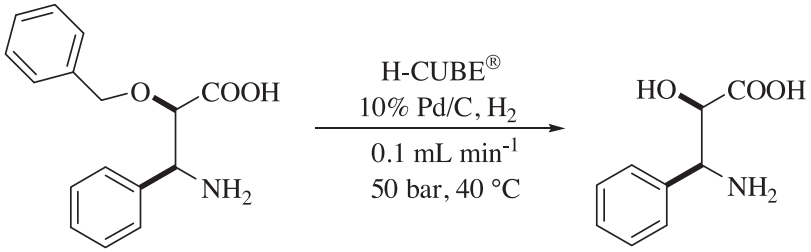
3.3. Synthesis of 3-benzyloxy-4-phenylazetidin-2-one [(±)-2]

Compound **13** was prepared from benzyloxyacetyl chloride (**8**, 0.23 mL, 1.5 mmol) and benzylidene-4-ethoxybenzenzamine (**10**, 0.23 g, 1.0 mmol) according to the procedure described in Section 3.2. [254 mg, 68%; m.p. 145–147 °C]. Removal of the 4-ethoxyphenyl group gave the desired β-lactam (±)-**2** [73 mg, 58%; m.p. 202–204 °C {lit [25]: m.p. = 188–189 °C}].

^1H NMR (400 MHz, DMSO, TMS) δ (ppm) for (±)-**2**: 4.09–4.14 (d, *J* = 11,06 Hz, 1H, C3H); 4.25–4.30 (d, *J* = 11.44 Hz, 1H, C4H); 4.86–4.89 (d, *J* = 4.44 Hz, 1H, CH2); 4.93–4.96 (m, 1H, CH2); 6.84–6.89 (m, 2H, Ar); 6.85–6.89 (m, 3H, Ar); 7.33–7.41 (m, 5H, Ar); 8.61–8.67 (bs, 1H, NH). Analysis: calcd. For $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53; Analysis: found for (3*S**,4*R**)-(±)-**2**: C, 75.89; H, 5.95; N, 5.55.

3.4. Synthesis of 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(±)-3]

Compound **14** was prepared from phenoxyacetyl chloride (**9**, 2.07 mL, 15 mmol) and 4-chlorobenzylidene-4-ethoxyphenylamine (**11**, 2.6 g, 10 mmol) according to the



(2*R*,3*S*)-**5** (2*R*,3*S*)-**7**

Scheme 4. Debenzylation of (2*R*,3*S*)-**5**.

Table 3
Preparative-scale resolution of (±)-**1**[a], (±)-**2**[a] and (±)-**3**[b].

Substrate	Time (h)	Conv.(%)	E	β-Lactam				β-Amino acid			
				Yield (%)	Isomer	ee[c](%)	$[\alpha]_D^{25}$	Yield (%)	Isomer	ee[d](%)	$[\alpha]_D^{25}$
(±)-**1**	144	50	>200	35	3*S*,4*R*-**1**	98	−20[e]	30	2*R*,3*S*-**4**	99	+38[f]
(±)-**2**	24	50	>200	48	3*S*,4*R*-**2**	98	−15[g]	47	2*R*,3*S*-**5**	99	+70[h]
(±)-**3**	336	66	12	16	3*S*,4*R*-**3**	98	+45[i]	61	2*R*,3*S*-**6**	50	+11[j]

[a] 0.015 M substrate, 25 equiv. of H_2O, 30 mg mL^{-1} immobilized CAL-B in *t*-BuOMe at 70 °C.
[b] 0.015 M substrate, 25 equiv. of H_2O, 30 mg mL^{-1} immobilized CAL-B, in iPr_2O at 70 °C.
[c] According to HPLC (Section 3).
[d] According to HPLC after derivatization (Section 3).
[e] *c* 0.30; $CHCl_3$.
[f] *c* 0.10; MeOH.
[g] *c* 0.21; $CHCl_3$.
[h] *c* 0.30; EtOH.
[i] *c* 0.21; $CHCl_3$.
[j] *c* 0.20; MeOH.

procedure described in Section 3.2. [2.88 g, 80%; m.p. 170k172 °C]. Removal of the 4-ethoxyphenyl group gave the desired β-lactam (±)-**3** [434 mg, 53%; m.p. 192–193 °C {lit [21]: m.p. = 188–190 °C}].

^{1}H NMR (400 MHz, DMSO, TMS) δ (ppm) for (±)-**3**: 5.10–5.14 (d, *J* = 4.6 Hz, 1H, C3H); 5.59–5.65 (dd, *J* = 2.12 & 4.54, 1H, C4H); 6.76–6.83 (d, *J* = 8 Hz, 2H, Ar); 6.86–6.93 (m, 1H, Ar); 7.12–7.22 (m, 2H Ar); 7.29–7.37 (m, 4H, Ar); 8.83–8.91 (bs, 1H, NH). Analysis: calcd. For $C_{15}H_{12}ClNO_2$: C, 65.82; H, 4.42; N, 5.12; Analysis: found for (3*S**,4*R**)-(±)-**3**: C, 65.83; H, 4.40; N, 5.15.

3.5. Preparative-scale resolution of racemic 3-benzyloxy-4-(4-chlorophenyl) azetidin-2-one [(±)-1]

Racemic **1** (300 mg, 1.05 mmol) was dissolved in *t*-BuOMe (40 mL), immobilized CAL-B (2.0 g, 30 mg mL^{-1}) and H_2O (375 μL, 20.83 mmol) were added, and the mixture was stirred at 70 °C for 144 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off, affording the unreacted β-lactam (3 *S*,4 *R*)-**1** {105 mg, 35%, 0.37 mmol, *ee* = 98%; $[\alpha]_D^{25}$ = −20 (*c* 0.3; $CHCl_3$); m.p. = 188–190 °C}. The filtered immobilized CAL-B was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off. The crystalline β-amino acid was (2*R*,3*S*)-**4** {96 mg, 30%; *ee* = 99%; $[\alpha]_D^{25}$ = +38 (*c* 0.1; MeOH); m.p = 238–240 °C}.

The ^{1}H NMR (400 MHz, DMSO, TMS) δ (ppm) data for (3*S*,4*R*)-**1** were the same as those for (±)-1.

^{1}H NMR (400 MHz, CD_3OD, TMS) δ (ppm) for (2*R*,3*S*)-**4**: 3.96–4.01 (d, *J* = 5.08 Hz, 1H, C2H); 4.41–4.46 (d, *J* = 11.6 Hz, 1H, C3H); 4.49–4.62 (d, *J* = 4.88 Hz, 1H, CH2) overlapping with 4.52–4.55 (bs, 2H, NH2); 4.77–4.79 (s, 1H, CH2); 7.25–7.33 (m, 4H, Ar); 7.37–7.46 (m, 5H, Ar). Analysis: calcd. For $C_{16}H_{16}ClNO_3$: C, 62.85; H, 5.27; N, 4.58; Analysis: found for (2*R*,3*S*)-**4**: C, 62.87; H, 5.29; N, 4.55.

3.6. Preparative-scale resolution of racemic 3-benzyloxy-4-phenylazetidin-2-one [(±)-2]

Racemic **2** (200 mg, 0.79 mmol) was dissolved in *t*-BuOMe (30 mL), immobilized CAL-B (1.5 g, 30 mg/mL) and H_2O (356 μL, 19.78 mmol) were added, and the mixture was stirred at 70 °C for 24 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off, affording the unreacted β-lactam (3*S*,4*R*)-**2** {96 mg, 48%; 0.37 mmol, *ee* = 98%; $[\alpha]_D^{25}$ = −15 (*c* 0.21; $CHCl_3$); m.p. = 192–193 °C}. The filtered immobilized CAL-B was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off. The crystalline β-amino acid was (2*R*,3*S*)-**5** {101 mg, 47%; *ee* = 99%; $[\alpha]_D^{25}$ = +70 (*c* 0.3; EtOH); m.p. = 218–222 °C}.

The ^{1}H NMR (400 MHz, DMSO, TMS) δ (ppm) data for (3 *S*,4 *R*)-**2** were the same as those for (±)-**2**.

^{1}H NMR (400 MHz, CD_3OD, TMS) δ (ppm) for (2 *R*,3 *S*)-**5**: 4.02–4.05 (d, *J* = 5.2 Hz, 1H, C2H); 4.41–4.46 (d, *J* = 11.52 Hz, 1H, C3H); 4.49–4.52 (d, *J* = 8.0 Hz, 1H, CH2) overlapping with 4.51–4.54 (bs, 2H, NH2); 4.76–4.78 (s, 1H, CH2); 7.24–7.32 (m, 5H, Ar); 7.39–7.49 (m, 5H, Ar). Analysis: calcd. For $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16; Analysi;1;;1;s: found for (2*R*,3*S*)-**5**: C, 70.81; H, 6.32; N, 5.14.

3.7. Preparative-scale resolution of racemic 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(±)-3]

Racemic **3** (200 mg, 0.73 mmol) was dissolved in iPr_2O (30 mL), immobilized CAL-B (1.5 g, 30 mg/mL) and H_2O (328.5 μL, 18.25 mmol) were added and the mixture was stirred at 70 °C for 336 h. The reaction was stopped by filtering off the enzyme at 66% conversion. The solvent was evaporated off, affording the unreacted β-lactam (3 *S*,4 *R*)-**3** {32 mg, 16%; 0.12 mmol, *ee* = 98%; $[\alpha]_D^{25}$ = +45 (*c* 0.21; $CHCl_3$); m.p. = 194–195 °C}. The filtered immobilized CAL-B was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off. The crystalline β-amino acid was (2*R*,3*S*)-**6** {100 mg, 47%; *ee* = 50%; $[\alpha]_D^{25}$ = +11 (*c* 0.2; MeOH); m.p. = 250–258 °C}.

The ^{1}H NMR (400 MHz, DMSO, TMS) δ (ppm) data for (3*S*,4*R*)-**3** were the same as those for (±)-**3**.

^{1}H NMR (400 MHz, CD_3OD, TMS) δ (ppm) for (2*R*,3*S*)-**6**: 4.51–4.56 (s, 2H, NH2); 4.65–4.70 (m, 2H, C3H, C4H); 6.92–7.05 (m, 3H, Ar); 7.21–7.30 (m, 2H, Ar); 7.41–7.55 (m, 4H, Ar). Analysis: calcd. For $C_{15}H_{14}ClNO_3$: C, 61.76; H, 4.84; N, 4.80; Analysis: found for (2*R*,3*S*)-**6**: C, 61.76; H, 4.86; N, 4.82.

3.8. Debenzylation of (2 R, 3 S)-5

The debenzylation was carried out in a continuous flow system. (2*R*,3*S*)-**5** (17 mg) was dissolved in MeOH (20 mL), and the solution was pumped through the compressed and heated 10% Pd/C cartridge at a flow rate of 0.1 mL min^{-1}. The pressure was 50 bar, the temperature 40 °C and the H-CUBE system was in 'Hydrogen' mode. After four cycles, the solvent was evaporated off. (2*R*,3*S*)-**7** {11 mg, 97%; *ee* = 99%; $[\alpha]_D^{25}$ = −7.1 (*c* 0.34; H_2O)} these data being approximately equivalent to the literature [20] [α] data for (3*S*,4*R*)-**5** {*ee* = 99%; $[\alpha]_D^{25}$ = −7.2 (*c* 0.34; H_2O)}.

^{1}H NMR (400 MHz, D_2O, TMS) δ (ppm) for (2*R*,3*S*)-**7**: 4.31–4.37 [d, *J* = 5.9 Hz, 1H, C*H* (OH)(COOH)], 4.55–4.60 (d, *J* = 5.9 Hz, 1H, $CHNH_2$), 7.40–7.55 (m, 5H, C_6H_5). Analysis: calcd. For $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73; Analysis: found for (2*R*,3*S*)-**7**: C, 59.69; H, 6.10; N, 7.73.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2015.11.011.

References

[1] E. Forró, F. Fülöp, Curr. Med. Chem. 19 (2012) 6178–6187.
[2] U. Vurma-Rapp, F.-H. Kayser, Eur. J. Clin. 5 (1986) 292–296.
[3] W. Wang, P. Devasthale, D. Farrelly, L. Gu, T. Harrity, M. Cap, C. Chu, L. Kunselman, N. Morgan, R. Ponticiello, R. Zebo, L. Zhang, K. Locke, J. Lippy, K. O'Malley, V. Hosagrahara, L. Zhang, P. Kadiyala, C. Chang, J. Muckelbauer, A.M. Doweyko, R. Zahler, D. Ryono, N. Hariharan, P.T.W. Chenga, Bioorg. Med. Chem. Lett. 18 (2008) 1939–1944.
[4] C.D. Guillon, G.A. Koppel, M.J. Brownstein, M.O. Chaney, C.F. Ferris, S. Lu, K.M. Fabio, M.J. Miller, N.D. Heindel, D.C. Hunden, R.D.G. Cooper, S.W. Kaldor, J.J. Skelton, B.A. Dressman, M.P. Clay, M.I. Steinberg, R.F. Brunsf, N.G. Simon, Bioorg. Med. Chem 15 (2007) 2054–2080.
[5] G. Veinberg, R. Bokaldere, K. Dikovskaya, M. Vorona, I. Kanepe, I. Shestakova, E. Yashchenko, E. Lukevics, Chem. Heterocycl. Comp. 5 (2003) 587–593.
[6] P. Singh, S.A. Williams, M.H. Shah, T. Lectka, G.J. Pritchard, J.T. Isaacs, S.R. Denmeade, Proteins: Struct. Funct. Bioinf. 70 (2008) 1416–1428.
[7] F. Fülöp, L. Kiss, Chem. Rev. 114 (2014) 1116–1169.
[8] S. Chandrasekhar, A. Sudhaka, M.U. Kiran, B.N. Babu, B. Jagadeesh, Tetrahedron Lett. 49 (2008) 7368–7371.
[9] T. Martinek, F. Fülöp, Eur. J. Biochem. 270 (2003) 3657–3666.
[10] M. Miyashita, M. Akamatsu, Y. Hayashi, T. Uenoa, Bioorg. Med. Chem. Lett. 10 (2000) 859–863.
[11] I. Ojima, S.D. Kuduk, S. Chakravarty, Adv. Med. Chem. 4 (1999) 69–124.
[12] H. Oettle, Cancer Treat. Rev. 40 (2014) 1039–1047.
[13] R.A. Holton, C. Somoza, H.B. Kim, F. Liang, R.J. Biediger, P.D. Boatman, M. Shindo, C.C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K.K. Murthi, L.N. Gentile, J.H. Liu, J. Am. Chem. Soc. 116 (1994) 1597–1598.
[14] R.A. Holton, C. Somoza, H.B. Kim, F. Liang, R.J. Biediger, P.D. Boatman, M. Shindo, C.C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K.K. Murthi, L.N. Gentile, J.H. Liu, J. Am. Chem. Soc. 116 (1994) 1599–1600.
[15] J.C. Borah, J. Boruva, N.C. Barua, Curr. Org. Synth. 4 (2007) 175–199.
[16] E. Forró, F. Fülöp, Tetrahedron: Asymmetry 21 (2010) 637–639.
[17] E. Forró, T. Paál, G. Tasnádi, F. Fülöp, Adv. Synth. Catal. 348 (2006) 917–923.
[18] E. Forró, F. Fülöp, Chem. Eur. J. 12 (2006) 2587–2592.
[19] E. Forró, F. Fülöp, Tetrahedron: Asymmetry 15 (2004) 2875–2880.
[20] E. Forró, F. Fülöp, Eur. J. Org. Chem. 16 (2010) 3074–3079.
[21] A. Jarrahpour, M. Zarei, Molecules 12 (2007) 2364–2379.
[22] E. Forró, F. Fülöp, Org. Lett. 5 (2003) 1209–1212.
[23] E. Forró, J. Chromatogr. A 1216 (2009) 1025–1029.
[24] S. Bacchi, A. Bongini, M. Panunzio, M. Villa, Synlett 8 (1998) 843–844.
[25] K. Karupaiyan, V. Srirajan, A.R.A.S. Deshmukh, B.M. Bhawal, Tetrahedron 54 (1998) 4375–4386.