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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3,4-Disubstituted β-lactams 3-benzoylox-4-[4-chlorophenyl]azetidin-2-one [(3S′,4R′)-(+/-)-1], 3-benzoylox-4-phenylazetidin-2-one [(3S′,4R′)-(-/-)-2] and 4-[4-chlorophenyl]-3-phenoxyazetidin-2-one [(3S′,4R′)-(-/-)-3] were resolved through immobilized CAL-B-catalysed ring-cleavage reactions. Excellent enantioselectivities (E > 200) were obtained for (3S′,4R′)-(-/-)-1 and (3S′,4R′)-(-/-)-2 when the reactions were performed with added H2O as nucleophile in tert-butyl methyl ether at 70 °C, whereas only moderate E (12) was achieved for (3S′,4R′)-(-/-)-3 under the same conditions but in diisopropyl ether. The resulting ring-opened β-amino acids [(2R,3S)-4 (ee > 98%), (2R,3S)-5 (ee > 98%) and (2R,3S)-6 (ee > 50%)] and the unreacted β-lactams [(3S,4R)-1–3] (ee > 98%) could be easily separated.

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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 V1a agonist [4] or anticanter [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 (2R,3S)-3-amino-3-phenyl-2-hydroxypropanoic acid [(2R,3S)-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 H2O in t-BuOMe at 60 °C, with immobilized CAL-B) and sequential kinetic resolution of racemic cis-3-acetocy-4-phenylazetidin-2-one (1 equiv. of H2O in iPr2O 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-benzoylox-4-(4-chlorophenyl) azetidin-2-one, 3-benzoylox-4-phenylazetidin-2-one and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(3S′,4R′)-(-/-)-1–3] (Scheme 1), and then to synthetize (2R,3S)-3-phenylisoserine (2R,3S)-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 (3S′,4R′)-(±)-1–3 were synthesized according to a literature method [21]. A mixture of p-ethoxyaniline...
and the appropriate aldehyde furnished the Schiff bases (Z)-N-(4-chlorobenzylidene)-4-ethoxybenzaldehyde (10) and (Z)-N-benzylidene-4-ethoxybenzaldehyde (11), which, through cycladditions in the presence of the appropriate acyl chlorides, 2-phenoxyacetyl chloride (8) or 2-benzoxoacyl 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 H2O in iPr2O 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 iPr2O (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.

H2O, as a nucleophile, is essential for the ring-opening reaction, through its quantity in the reaction medium can affect the enzy-
matic activity [18,22]. Experiments were therefore also performed with different quantities of added H2O (Table 1, entries 7–10 and 12–15). On increase of the amount of H2O up to 50 equiv., the reactions became faster without a drop in E (entries 8–10), but a further increase of the H2O 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 H2O in the reaction mixture (due to the H2O present in the reaction medium) [22], the quantity of H2O 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 H2O 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 H2O, 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 H2O 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 iPr2O 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 iPr2O 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 H2O in t-BuOMe at 70 °C, while the preparative-scale resolution of (±)-3 was performed with 25 equiv. of H2O in iPr2O at 70 °C. In order to obtain (2R,3S)-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).
Table 1
Effects of solvents and the quantities of H\textsubscript{2}O on the immobilized CAL-B-catalysed ring cleavage of (±)-1.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>H\textsubscript{2}O (equiv.)</th>
<th>Temperature (°C)</th>
<th>ee\textsubscript{1}(%</th>
<th>ee\textsubscript{2}(%</th>
<th>Conv. (%)</th>
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<td>35</td>
<td>95</td>
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<td>55</td>
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\textsuperscript{a} 0.015 M substrate, H\textsubscript{2}O, 30 mg mL\textsuperscript{-1} immobilized CAL-B, after 65 h.
\textsuperscript{b} According to HPLC (Section 3).
\textsuperscript{c} According to HPLC after derivatization (Section 3).

2.3. Synthesis of Taxol side-chain intermediate

To prepare (2R,3S)-3-amino-3-phenyl-2-hydroxypropanoic acid [[(2R,3S)-5], the key intermediate of Taxol, the debenzylation of (2R,3S)-5 (ee=99%) was performed in a continuous flow system (Hi-CUBE\textsuperscript{b}) 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, (2R,3S)-7 was obtained with good ee (99%) and in nearly quantitative yield (93%) after four cycles. The absolute configuration for the enantiomer 7 obtained was proved by comparing the literature \cite{20} [α] value for (2R,3S)-3-amino-3-phenyl-2-hydroxypropanoic acid ([α]c\textsubscript{25} = -7.2 (c = 0.34, H\textsubscript{2}O), ee > 99%) with the [α] value measured for enantiomer 7 ([α]c\textsubscript{25} = -7.2 (c = 0.34, H\textsubscript{2}O), ee = 99%). Thus, immobilized CAL-B catalysed the ring opening of (3S,4R\textsuperscript{*}),(±)-2 with (2R,3S) selectivity, while for (3S,4R\textsuperscript{*}),(±)-1 and (3S,4R\textsuperscript{*}),(±)-3 the analysed chromatograms indicated the same enantiomeric preference for immobilized CAL-B.

2.4. Conclusions

An efficient enzymatic method was developed for the ring opening of 3,4-disubstituted β-lactams (3S,4R\textsuperscript{*}),(±)-1–3. High enantiomeric selectivities (E > 200) were obtained for the ring-opening reactions of (3S,4R\textsuperscript{*}),(±)-1 and (3S,4R\textsuperscript{*}),(±)-2 when immobilized CAL-B was used as catalyst, with 25 equiv. of H\textsubscript{2}O as nucleophile, in t-BuOMe at 70 °C, while a relatively modest E (12) was obtained for immobilized CAL-B-catalysed ring opening of (3S,4R\textsuperscript{*}),(±)-3 in iPr\textsubscript{2}O with 25 equiv. of H\textsubscript{2}O 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 (2R,3S)-3-amino-3-phenyl-2-hydroxypropanoic acid [[(2R,3S)-7], a key intermediate for the Taxol\textsuperscript{b} side-chain.

3. Experimental

3.1. Materials and methods

Immobilized CAL-B (lipase B from \textit{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\textsubscript{2}O (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 (3S,4R\textsuperscript{*}),(±)-1 and (3S,4R\textsuperscript{*}),(±)-3 and the product β-amino acid (2R,3S)-6 after pre-column derivatization [\textsuperscript{23}] with CH\textsubscript{3}N\textsubscript{2} (Caution! derivatization with CH\textsubscript{3}N\textsubscript{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.\textsuperscript{a,b,c}

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Reaction time (h)</th>
<th>Temperature (°C)</th>
<th>H\textsubscript{2}O (equiv.)</th>
<th>ee\textsubscript{1}\textsuperscript{a}(%</th>
<th>ee\textsubscript{2}\textsuperscript{b}(%</th>
<th>Conv. (%)</th>
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\textsuperscript{a} 0.015 M substrate, H\textsubscript{2}O, 30 mg mL\textsuperscript{-1} immobilized CAL-B.
\textsuperscript{b} According to HPLC (Section 3).
\textsuperscript{c} According to HPLC after derivatization (Section 3).
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 CHCl₃ was slowly added to a solution of 4-chlorobenzylidene-4-ethoxyphenylamine (11, 0.26 g, 1.0 mmol) and Et₃N (0.42 mL, 3.0 mmol) in CH₂Cl₂ (20 mL) at −10 °C. The reaction was then allowed to warm up to room temperature, stirred for 12 h, washed with NaHCO₃ solution (20 mL) and brine (20 mL), then dried (Na₂SO₄) 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 H₂O (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 H₂O was then added and the mixture was extracted with EtoAc (3 × 20 mL) and washed with 10% aqueous NaHCO₃ (20 mL). The organic layer was combined and washed with 10% Na₂SO₄ (2 × 15 mL), 10% NaHCO₃ (10 mL), and brine (20 mL), and dried with Na₂SO₄. 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, CH); 4.29–4.35 (d, J = 11.16 Hz); 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 C₁₅H₁₃NO₂: C, 66.79; H, 4.90; N, 4.87; Analysis: found for (3S,4R)⁺(±)-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 benzylidine-4-ethoxybenzenezamine (25, 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, CH); 4.25–4.30 (d, J = 11.44 Hz, 1H, CH₂); 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 C₁₅H₁₃NO₂: C, 75.87; H, 5.97; N, 5.53; Analysis: found for (3S,4R)⁺(±)-2: C, 75.89; H, 5.95; N, 5.55.

3.4. Synthesis of 4-(4-chlorophenyl)-3-phenoxazetidin-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
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 H₂O (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%; [α]D²⁵ = −20 (c 0.3; CHCl₃); m.p. = 188–190 °C]. The filtered immobilized CAL-B was washed with distilled H₂O (3 × 15 mL), and the H₂O was evaporated off. The crystalline β-amino acid was (2R,3S)-4 (96 mg, 30%; ee = 99%; [α]D²⁵ = +38 (c 0.1; MeOH); m.p. = 238–240 °C).

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

1H NMR (400 MHz, CD₂OD, TMS) δ (ppm) for (2R,3S)-4: 4.51–4.56 (s, 2H, NH₂); 4.65–4.70 (m, 2H, CH₂, CH₃H); 6.92–7.05 (m, 3H, Ar); 7.21–7.30 (m, 2H, Ar); 7.41–7.55 (m, 4H, Ar); Analysis: calcd. For C₁₅H₁₃ClNO₂: C, 71.67; H, 4.84; N, 4.80; Analysis: found: for (2R,3S)-6: C, 71.67; H, 4.86; N, 4.82.

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

Racemic 3 (200 mg, 0.73 mmol) was dissolved in iPr₂O (30 mL), immobilized CAL-B (1.5 g, 30 mg mL⁻¹) and H₂O (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%; [α]D²⁵ = +45 (c 0.21; CHCl₃); m.p. = 194–195 °C]. The filtered immobilized CAL-B was washed with distilled H₂O (3 × 15 mL), and the H₂O was evaporated off. The crystalline β-amino acid was (2R,3S)-6 (100 mg, 47%; ee = 50%; [α]D²⁵ = +11 (c 0.2; MeOH); m.p. = 250–258 °C).

The 1H NMR (400 MHz, DMSO, TMS) δ (ppm) data for (3S,4R)-6 were the same as those for (±)-3.

1H NMR (400 MHz, CD₂OD, TMS) δ (ppm) for (2R,3S)-6: 4.31–4.37 (d, J = 5.9 Hz, 1H, CH₂(OH)CH₂COOH); 4.55–4.60 (d, J = 5.9 Hz, 1H, CH₂H₂N₂); Analysis: calcd. For C₁₅H₁₃NO₂: C, 70.81; H, 6.32; N, 5.14.

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

The debenzylation was carried out in a continuous flow system. The 2R,3S)-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. (2R,3S)-7 ([11 mg, 97%; ee = 99%; [α]D²⁵ = −7.1 (c 0.34; H₂O) these data being approximately equivalent to the literature [20] [α] data for (3S,4R)-5 (ee = 99%; [α]D²⁵ = −7.2 (c 0.34; H₂O)).

1H NMR (400 MHz, D₂O, TMS) δ (ppm) for (2R,3S)-7: 4.31–4.37 (d, J = 5.9 Hz, 1H, CH₂(OH)CH₂COOH); 4.55–4.60 (d, J = 5.9 Hz, 1H, CH₂H₂N₂); Analysis: calcd. For C₁₅H₁₃NO₂: C, 59.68; H, 6.12; N, 7.73; Analysis: found: for (2R,3S)-7: C, 59.69; H, 6.10; N, 7.73.

### Table 3

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<th>Substrate</th>
<th>Time (h)</th>
<th>Conv. (%)</th>
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<th>β-Lactam Yield (%)</th>
<th>Isomer</th>
<th>ee (%)</th>
<th>[α]D²⁵</th>
<th>β-Amino acid Yield (%)</th>
<th>Isomer</th>
<th>ee (%)</th>
<th>[α]D²⁵</th>
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<td>98</td>
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<td>99</td>
<td>+38</td>
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<tr>
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<td>&gt;200</td>
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<td>35,4R-2</td>
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<td>−15</td>
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<td>28,3S-5</td>
<td>99</td>
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<tr>
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<td>66</td>
<td>12</td>
<td>16</td>
<td>35,4R-3</td>
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<td>+45</td>
<td>61</td>
<td>28,3S-6</td>
<td>50</td>
<td>+11</td>
</tr>
</tbody>
</table>

a 0.015 M substrate, 25 equiv. of H₂O, 30 mg mL⁻¹ immobilized CAL-B in t-BuOMe at 70 °C.
b 0.015 M substrate, 25 equiv. of H₂O, 30 mg mL⁻¹ immobilized CAL-B, in iPr₂O at 70 °C.
c According to HPLC (Section 3).
d According to HPLC after derivatization (Section 3).
e c 0.30; CHCl₃.
f c 0.10; MeOH.
g c 0.21; CHCl₃.
h c 0.30; EtOH.
i c 0.21; CHCl₃.
ji c 0.20; MeOH.
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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.molcatt.2015.11.011.

References