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Preparation of enantiomerically pure *N*-heterocyclic amino alcohols by enzymatic kinetic resolution

Giorgio Tofani [a], Antonella Petri [a,*], Oreste Piccolo [b,*]

[a] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Giuseppe Moruzzi 3, 56124 Pisa, Italy*
[b] *Studio di Consulenza Scientifica (SCSOP), Via Bornò 5, 23896 Sirtori (LC), Italy*

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ABSTRACT

The synthesis of both enantiomers of *N*-benzyl-3-hydroxypyrrolidine and *N*-benzyl-3-hydroxypiperidine via enzymatic kinetic resolution of the corresponding racemic esters is described. Various commercially available hydrolases were studied as biocatalysts in native and immobilized form. The best results were obtained with lipases PS, AK, CAL-B and with protease Alcalase, which were active and selective for the kinetic resolutions of racemic esters ($E > 100$). Under optimized reaction conditions, highly enantiomerically enriched (up to 99.5% ee) resolution products were obtained. Lipase and protease showed opposite enantiopreference on the esters, allowing the preparation of both enantiomers of the target compounds. Semi-continuous reactions in column reactors with immobilized biocatalysts were also performed with high enantioselectivities. Inversion of the configuration at C(3) of *N*-benzyl-3-hydroxypyrrolidine was quantitatively effected in a short number of steps.

1. Introduction

N-Heterocyclic chiral molecules have been of considerable interest for industry and academic research.[1,2] In particular, enantiomerically pure 3-hydroxypyrrolidine **1** and 3-hydroxypiperidine **2** (Fig. 1) have proven very useful in a wide range of applications either as valuable intermediates for the synthesis of biologically active compounds[3–6] or as versatile chiral ligands and promoters in organocatalysis.[7,8]

A number of synthetic[5,9] as well as biotransformation[10] approaches for the preparation of amino alcohols **1** and **2** have already been described. However, most of the reported procedures show one or more drawbacks from the viewpoint of economical efficiency. Among the available biocatalytic routes, enzymatic kinetic resolution of racemic alcohols via hydrolysis or acylation has been extensively studied.[11] Indeed, biocatalytic processes have often proved to be greener, less hazardous and less polluting than conventional chemical transformations.[12] Several examples have been reported in the literature for the kinetic resolution of racemic amino alcohols **1** and **2** and of their corresponding esters.[13–16] Recently, an efficient chemoenzymatic dynamic kinetic resolution using various lipases in combination with a ruthenium based catalyst has also been described.[17] This efficient approach however involves an expensive catalyst, making it unsuitable for large scale synthesis.

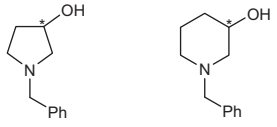

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Figure 1. Structure of *N*-benzyl-3-hydroxypyrrolidine **1** and *N*-benzyl-3-hydroxypiperidine **2**.

Herein, the kinetic resolution of derivatives of *N*-benzyl-3-hydroxypyrrolidine and *N*-benzyl-3-hydroxypiperidine alcohols by using several commercially available hydrolytic enzymes in their native and immobilized form is described. The resulting optimized conditions for the analytical scale reactions were successfully applied in semi-continuous processes in column reactors with immobilized biocatalysts to facilitate the enzymatic resolution.

2. Results and discussion

Initially, the kinetic resolution of racemic acetate *rac*-**3** was studied using different commercially available enzymes (Scheme 1).

* Corresponding authors. Tel.: +39 050 2219279 (A.P.).
E-mail addresses: antonella.petri@unipi.it (A. Petri), orestepiccolo@tin.it (O. Piccolo).

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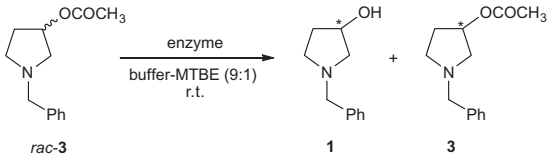


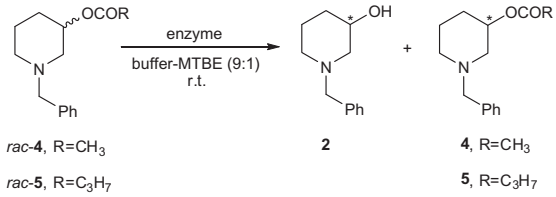
Scheme 1. Enzymatic kinetic resolution of *rac*-**3**.

Since the addition of an organic solvent was reported to favour the biocatalysed hydrolysis and to improve enantioselectivity in some cases, we decided to perform the reaction in a 9:1 v/v mixture of aqueous phosphate buffer (50 mM, pH 7) and MTBE. Among the previously examined co-solvents, the choice of MTBE was determined by the following considerations: (i) good ability to solubilize substrates and extract the amino alcohols compounds from the buffer solution; (ii) limited miscibility with the aqueous phase to prevent the inactivation of the enzyme and facilitate the work-up of the reaction mixture; (iii) suitability for industrial use and low cost. In addition, unlike dioxane which has been previously used in this reaction by Tomori et al.,[13] MTBE has not been classified as a carcinogen. *n*-Butanol, which has a log*P* value similar to that of MTBE, has been considered as a possible alternative but in this solvent the enzyme catalysed hydrolysis proceeded with low activity. The reactions were monitored by HPLC. Data on reaction times, conversions and ee values are presented in Table 1.

It was found that the best results in terms of activity and selectivity were obtained using lipase enzymes PS and AK and with protease Alcalase. All reactions were carried out by using an enzyme/substrate ratio of 1:5. It is interesting to note that Lipase PS was already reported[13] as an efficient enzyme but the described reaction required a large amount of the biocatalyst, making it unsuitable for a large scale synthesis. Lipase AK and Alcalase were also used in the present research in 100% buffer as the reaction solvent (entries 6 and 9). It was found that under these conditions, the rate of hydrolysis was higher but the enantioselectivity was lower.

In order to optimize the productivity, the kinetic resolution was carried out at a higher concentration of the substrate. It was found that lipase AK was quite effective up to 70 g/L. The selectivity remained high ($E > 100$); however, as expected, the rate of hydrolysis decreased with higher concentrations (25 h vs 8.5 h). This result is particularly promising since it was possible to carry out the kinetic resolution in 25 h, with excellent enantioselectivities both of alcohol **1** and acetate **3**, by using a lower amount of biocatalyst (<1 wt %) with respect to the values reported in literature.[13–17]

Using the aforementioned optimal experimental conditions for the resolution of *rac*-**3**, the same screening procedure was further extended to the racemic 3-hydroxypiperidine derivative *rac*-**4** (Scheme 2, Table 2).



Scheme 2. Enzymatic kinetic resolution of *rac*-**4** and *rac*-**5**.

The best results were obtained with the enzymes PS and CAL-B. However it is apparent from data in Table 2 that six-member ring compounds are less reactive than the corresponding compounds with a five-member ring (Table 1). This behaviour was already reported in a previous study.[13] The butanoate ester *rac*-**5** was also tested as substrate (Scheme 2). As presented in Table 3, Palatase and Alcalase were found to be the most active and selective enzymes. However, these data indicated that the enantioselectivity was only slightly affected by changing the acyl chain length.

We next investigated the use of immobilized enzymes in view of a possible improvement of the process. Indeed, immobilized biocatalysts are easily separable from the reaction mixture and potentially recyclable. The screening process involved immobilized CAL-B, PS, AK, Palatase and Alcalase which proved to be most efficient enzymes in their native form for the kinetic resolution of *rac*-**3**–**5**. The results are reported in Table 4.

AK-IM and PS-IM allowed to obtain (*R*)-**1** with high enantioselectivity (ee >99.5%, $E > 100$). Both enantiomers of amino alcohol **2** were obtained with 98% ee from the hydrolysis of the corresponding acetate and butanoate.

Encouraged by these results, we next examined the possibility of using the immobilized enzymes for semi-continuous kinetic resolution reactions. For this purpose, a microreactor consisting of a PEEK column filled with AK-IM was prepared and the reaction mixture containing *rac*-**3** was re-circulated inside by using a suitable pump. The reaction was monitored by HPLC and stopped at an approx. 50% conversion, removing the reaction mixture. The column was washed with phosphate buffer (50 mM, pH 7). The eluted solution and washings were combined to quantitatively recover

Table 1
Screening of different enzymes for the hydrolysis of *rac*-**3**

Entry	Enzyme	Time (h)	conv[a](%)	ee_P[a] (%)	ee_S[a] (%)	E[b]
1	AS	23	62	12 (*S*)	20 (*R*)	2
2	AY30	1	68	24 (*R*)	50 (*S*)	3
3	CAL-A	5	39	62 (*R*)	40 (*S*)	6
4	Palatase	6	52	82 (*R*)	90 (*S*)	31
5	CAL-B	1	49	96 (*R*)	92 (*S*)	>100
6[c]	Alcalase	5	42	90 (*S*)	66 (*R*)	38
7	Alcalase	15	44	>99.5 (*S*)	78 (*R*)	>100
8	PS	0.5	50	>99.5 (*R*)	>99.5 (*S*)	>100
9[c]	AK	2	48	98 (*R*)	90 (*S*)	>100
10	AK	8.5	50	>99.5 (*R*)	>99.5 (*S*)	>100
11[d]	AK	25	50	>99.5 (*R*)	98 (*S*)	>100

All reactions were carried out at room temperature. Enzyme/Substrate 1:5 (w/w). 10% MTBE (v/v) co-solvent used with respect to buffer.

[a] Conversion and ee were determined from HPLC. The absolute configurations of the substrate and the product were assigned by a comparison of the elution orders on chiral HPLC (cfr. Experimental).

[b] E was evaluated by using $E = \ln[1- conv(1 + ee_P)]/\ln[1-conv(1 - ee_P)]$.[18]

[c] 100% buffer as reaction solvent.

[d] Substrate concentration 70 g/L.

Table 2
Screening of different enzymes for the hydrolysis of *rac*-**4**

Entry	Enzyme	Time (h)	conv[a](%)	ee_P[a] (%)	ee_S[a] (%)	E[b]
1	AS	32	33	20 (*S*)	19 (*R*)	2
2	CAL-A	30	42	38 (*R*)	28 (*S*)	3
3	AY30	0.25	53	56 (*S*)	64 (*R*)	7
4	Alcalase	33	20	88 (*S*)	22 (*R*)	19
5	AK	72	33	94 (*R*)	46 (*S*)	51
6	Palatase	16	35	94 (*R*)	50 (*S*)	53
7	PS	2	50	96 (*R*)	96 (*S*)	>100
8	CAL-B	6	33	>99.5 (*R*)	50 (*S*)	>100

All reactions were carried out at room temperature. Enzyme/Substrate 1:5 (w/w). 10% MTBE co-solvent used with respect to buffer.

[a] Conversion and ee were determined using HPLC. The absolute configurations of the substrate and the product were assigned by a comparison of the elution orders on chiral HPLC (cfr. Experimental).

[b] E was evaluated by using $E = \ln[1- conv(1 + ee_P)]/\ln[1-conv(1 - ee_P)]$.[18]

Table 3
Screening of different enzymes for the hydrolysis of *rac*-**5**

Entry	Enzyme	Time (h)	conv[a](%)	ee_P[a] (%)	ee_S[a] (%)	E[b]
1	AK	72	36	18 (*R*)	10 (*S*)	2
2[c]	AY30	0.2	39	20 (*S*)	13 (*R*)	2
3	CAL-A	0.5	33	36 (*R*)	18 (*S*)	3
4	AS	25	52	66 (*S*)	72 (*R*)	10
5	CAL-B	4	33	94 (*R*)	46 (*S*)	51
6	PS	4	49	92 (*R*)	88 (*S*)	70
7	Palatase	9	40	96 (*R*)	64 (*S*)	95
8	Alcalase	3	42	98 (*S*)	72 (*R*)	>100

All reactions were carried out at room temperature. Enzyme/Substrate 1:5 (w/w). 10% MTBE co-solvent used with respect to buffer.

[a] Conversion and ee were determined using HPLC. The absolute configurations of the substrate and the product were assigned by a comparison of the elution orders on chiral HPLC (cfr. Experimental).

[b] E was evaluated by using $E = \ln[1- conv(1 + ee_P)]/\ln[1-conv(1 - ee_P)]$.[18]

[c] Enzyme/Substrate 1:50 (w/w).

Table 4
Hydrolysis of *rac*-**3**–**5** with immobilized enzymes

Entry	Substrate	Enzyme	Time (h)	conv[a](%)	ee_P[a] (%)	ee_S[a] (%)	E[b]
1	rac-**3**	CAL-B-IM	5	49	90 (*R*)	86 (*S*)	53
2		Alcalase-IM	7	53	82 (*S*)	94 (*R*)	35
3[c]		AK-IM	7	46	>99.5 (*R*)	84 (*S*)	>100
4		PS-IM	24	50	>99.5 (*R*)	>99.5 (*S*)	>100
5	rac-**4**	CAL-B-IM	72	32	92 (*R*)	44 (*S*)	37
6		PS-IM	48	42	98 (*R*)	70 (*S*)	>100
7	rac-**5**	Palatase-IM	48	49	86 (*R*)	82 (*S*)	33
8		Alcalase-IM	5	49	98 (*S*)	94 (*R*)	>100

All reactions were carried out at room temperature. Substrate/Immobilized enzyme1:20 (w/w). 10% MTBE co-solvent used with respect to buffer.

[a] Conversion and ee were determined using HPLC. The absolute configurations of the substrate and the product were assigned by a comparison of the elution orders on chiral HPLC (cfr. Experimental).

[b] E was evaluated by using $E = \ln[1- conv(1 + ee_P)]/\ln[1-conv(1 - ee_P)]$.[18]

[c] Substrate/Immobilized enzyme 1:10 (w/w).

the amino alcohol (*R*)-**1** and the unreacted acetate (*S*)-**3** which exhibited the same ee values reported for the batch reaction. The column was used for five reaction cycles to evaluate the recycling of the biocatalyst. The activity and enantioselectivity values remained comparable with the data obtained in the first reaction.

The enzyme Alcalase-IM was then used for the kinetic resolution of substrate *rac*-**5** in a microreactor prepared as previously described for the hydrolysis of *rac*-**3**. (*S*)-**2** and the unreacted (*R*)-**5** were quantitatively recovered from the eluted solution and washings, obtaining the same ee values reported for the batch reaction. The column was used five times, without loss of activity or enantioselectivity.

The inversion of configuration at C(3) of alcohol **1** was finally investigated in order to evaluate the possibility of reusing the unwanted enantiomer, thus obtaining a quantitative yield after the kinetic resolution. One of the possible approaches is the transformation of the hydroxyl group into a suitable leaving group which allows the attack by a nucleophile and the resulting inversion of the configuration of the alcohol. If this reaction is carried out on the alcohol/acetate mixture obtained at the end of the enzymatic reaction, it would be possible to obtain the enantiomerically pure acetate with a theoretical yield of 100%. Inversion of configuration of alcohol **1** via the corresponding mesylate has already been reported,[16] but with partial loss of ee. We therefore envisaged using the tosylate in the same reaction sequence (Scheme 3).

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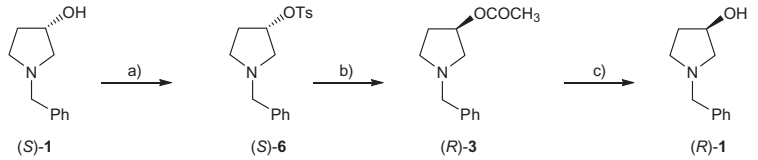

Scheme 3. Inversion of configuration at C(3) of (*S*)-**1**. Reagents and conditions: (a) TsCl, DABCO, MTBE, rt; (b) AcONa, DMSO, 120 °C; (c) NaOH 2 M, rt.

Tosylation of the alcohol (*S*)-**1** (ee >99.5%) afforded the key intermediate **6** with quantitative yield. Subsequent displacement of the tosylate with sodium acetate gave the *R* enantiomer of acetate **3** (ee 95%). Finally, amino alcohol (*R*)-**1** was obtained with quantitative yield and 95% ee by alkaline hydrolysis. This result illustrates the viability of this procedure for the preparation of the enantiopure alcohols with quantitative yield starting from alcohol/acetate mixtures obtained at the end of the enzymatic reaction without any purification. The same reaction sequence was performed on the substrate (*S*)-**2**. The tosylate was obtained in good yield, but subsequent reaction with sodium acetate afforded only the elimination product.

3. Conclusions

In conclusion, an efficient enzymatic kinetic resolution of racemic esters *rac*-**3**–**5** has been achieved to provide a practical preparation of both enantiomers of *N*-benzyl-3-hydroxypyrrolidine **1** and *N*-benzyl-3-hydroxypiperidine **2**. The target compounds were obtained with high enantiomeric excess under mild reaction conditions by the proper selection of the biocatalyst and the use of a suitable co-solvent. The use of immobilized enzymes was performed with excellent enantioselectivity in both batch and column reactions. Finally, inversion of the stereochemistry at C(3) of alcohol **1** was achieved without racemization.

4. Experimental

4.1. Reagents and solvents

N-Benzyl-3-hydroxypyrrolidine, *N*-benzyl-3-hydroxypiperidine, (*S*)-*N*-benzyl-3-hydroxypyrrolidine, (*R*)-*N*-benzyl-3-hydroxypiperidine and all other inorganic and organic reagents and solvents were purchased from Sigma–Aldrich. All solvents were dried and purified by standard methods as required. Lipase enzymes CAL-B from *Candida antarctica B* (from Novozymes, protein concentration 3.5 mg/ml, activity 8000 TBU/ml), Palatase from *Rhizomucor miehei* recombinant in *Aspergillus oryzae* (from Novozymes, 5.5 mg/ml, 19,000 TBU/ml), CAL-A from *Candida antarctica A* (from Novozymes, 13.5 mg/ml, 10,000 TBU/ml), PS from *Burkholderia cepacia* (from Amano, 5.4 mg/g, 24,000 TBU/g), AK from *Pseudomonas fluorescens* (from Amano, 142 mg/g, 20,000 TBU/g), AY30 from *Candida rugosa* (from Amano, 5.3 mg/g, 6500 TBU/g), AS from *Aspergillus niger* (from Amano, 71.9 mg/g, 12,000 TBU/g) and protease Alcalase from *Bacillus licheniformis* (from Novozymes, 50 mg/ml, 4500 ELU/ml) were all obtained from BiCT srl (Lodi, Italy). Immobilized enzymes CALB-IM from *Candida antarctica B* (3200 TBU/g), PS-IM *Burkholderia cepacia* (3211 TBU/g), Palatase-IM from *Rhizomucor miehei* recombinant in *Aspergillus oryzae* (5100 TBU/g), AK-IM from *Pseudomonas fluorescens* (2583 TBU/g) and Alcalase-IM from *Bacillus licheniformis* (850 ELU/g) were also purchased from BiCT srl.

4.2. Analytical methods

The ^{1}H NMR spectra were recorded on a Varian 300 spectrometer at 300 MHz. Spectra were recorded at 25 °C in $CDCl_3$. Chemical shifts on the δ scale are expressed in ppm values. Thin-layer chromatography (TLC) was carried out using with Macherey-Nagel Alugram® SIL G/UV254 plates by using ethyl acetate/triethylamine (100:1, v/v) as eluent.

High performance liquid chromatography (HPLC) analyses were conducted with a Jasco instrument using a Gemini C18 column (150 × 4.6 mm) and a mixture of acetonitrile/water/diethylamine 65:35:0.1 as eluent [flow rate: 1 ml/min; detection wavelength: 256 nm; retention times: 2.0 min for **1**, 3.2 min for **3**, 2.3 min for **2**, 4.5 min for **4** and 8.5 min for **5**]. The identification of peaks in the chromatograms was made by spiking with commercially available substrates or references prepared during this investigation. The enantiomeric excess (ee %) was determined by HPLC with chiral columns. For separation of *rac*-**1** and *rac*-**3**: Chiralcel OJ (Daicel), 250 × 4.6 mm; eluent hexane/ethanol/diethylamine, 100:1:0.1; flow rate: 1 ml/min; detection wavelength: 254 nm; retention times: 27.5 min for (*R*)-**1**, 30.5 min for (*S*)-**1**, 12.4 min for (*R*)-**3** and 13.3 min for (*S*)-**3**. For separation of *rac*-**2** and *rac*-**4**: Chiralcel OJ (Daicel), 250 × 4.6 mm; eluent hexane/ethanol/diethylamine, 98:2:0.1; flow rate: 0.5 ml/min; detection wavelength: 254 nm; retention times: 26.8 min for (*R*)-**2**, 29.4 min for (*S*)-**2**, 14.4 min for (*R*)-**4** and 22 min for (*S*)-**4**. For separation of *rac*-**2** and *rac*-**5**: Lux Cellulose-3 (Phenomenex), 250 × 4.6 mm; eluent hexane/isopropanol/diethylamine, 95:5:0.1; flow rate: 0.5 ml/min; detection wavelength: 254 nm; retention times: 16.9 min for (*R*)-**2**, 19.0 min for (*S*)-**2**, 9.8 min for (*R*)-**5**, 11.5 min for (*S*)-**5**. The absolute configuration of substrates and products was assigned by a comparison of the elution orders with those of authentic samples of known configuration. Optical rotations were determined on a Jasco Dip-360 polarimeter at 20 °C using sodium D light.

4.3. Synthesis of racemic esters *rac*-3–5

4.3.1. Synthesis of racemic *N*-benzyl-3-pyrrolidinyl acetate *rac*-3

To a solution of 0.50 g (2.82 mmol) of *rac*-**1** in 1 ml of dry pyridine cooled to 0 °C, 0.35 ml (3.70 mmol) of acetic anhydride was added under nitrogen. The resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with toluene (3 × 3 ml) and then concentrated under reduced pressure to give 0.61 g (2.79 mmol) of *rac*-**3** as a reddish oil (99% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.80 (s, 1H); 2.03 (s, 3H); 2.21–2.43 (m, 2H); 2.65–2.83 (m, 3H); 3.58 (d, 1H, J = 12.9 Hz); 3.68 (d, 1H, J = 12.9 Hz); 5.14–5.20 (m, 1H); 7.22–7.40 (m, 5H); TLC R_f = 0.52.

4.3.2. Synthesis of racemic *N*-benzyl-3-piperidinyl acetate *rac*-4

To a solution of 0.50 g (2.62 mmol) of *rac*-**2** in 1 ml of dry pyridine cooled to 0 °C, 0.35 ml (3.70 mmol) of acetic anhydride was added under nitrogen. The resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with toluene (3 × 3 ml) and concentrated under reduced pressure to give 0.57 g (2.45 mmol) of *rac*-**4** as a pale yellow oil (94% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.46–1.94 (m, 4H); 2.04 (s, 3H); 2.10–2.21 (m, 2H); 2.45–2.79 (m, 2H); 3.57 (s, 2H); 4.84 (s, 1H); 7.22–7.45 (m, 5H); TLC R_f = 0.59.

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4.3.3. Synthesis of racemic *N*-benzyl-3-piperidinyl butanoate *rac*-5

To a mixture of 0.50 g (2.62 mmol) of *rac*-**2** and 1 ml of dry pyridine 0.57 ml (3.50 mmol) of butanoic anhydride was added at 0 °C under nitrogen. The resulting mixture was stirred over night at room temperature. The reaction mixture was diluted with dichloromethane and then extracted with a saturated sodium carbonate solution (2 × 4 ml) and with 4 ml of a saturated sodium chloride solution. The organic phase was dried with sodium sulfate and then concentrated at reduced pressure to give 0.65 g (2.49 mmol) of *rac*-**5** as a pale yellow oil (95% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 0.98 (t, 3H, J = 5.9 Hz); 1.43–1.45 (m, 1H); 1.63–1.90 (m, 3H); 2.31–2.40 (m, 4H); 2.46 (m, 2H), 2.67–2.83 (m, 2H); 3.58–3.64 (m, 2H); 4.85–4.89 (m, 1H); 7.22–7.31 (m, 5H); TLC R_f = 0.60.

4.4. Analytical scale enzymatic hydrolysis of *rac*-3–5

The reactions were conducted at room temperature in suitable vessels, using a vortex mixer. In a typical small scale experiment, to a solution of racemic ester (10 mg) in potassium phosphate buffer (50 mM, pH 7) or in a potassium phosphate buffer/organic solvent mixture (1 mL), native (2 mg) or immobilized (200 mg) enzymes were added. The reactions were monitored by HPLC by taking samples at different intervals of time. The reaction mixture was extracted with dichloromethane (2 × 0.5 mL). The organic layer was dried over sodium sulfate, concentrated at reduced pressure and analysed by HPLC as described in Section 4.2. Data on reaction times, conversion and enantiomeric excess of the substrates and the products are presented in Tables 1–4.

4.5. Preparative scale procedure

4.5.1. Preparative scale hydrolysis of *rac*-3 by using a native enzyme

Into a stirred solution of 250 mg (1.14 mmol) of *rac*-**3** in a 9:1 mixture (3.5 mL) of potassium phosphate buffer (50 mM, pH 7) and MTBE, 2 mg of lipase AK were added. The reaction was monitored by HPLC by taking samples at different intervals of time and stopped at an approximately 50% conversion. The reaction mixture was extracted with dichloromethane. The organic layer was dried over sodium sulfate, concentrated at reduced pressure and subjected to preparative TLC to give (*R*)-**1** with >99.5% ee (83 mg; 41% yield) and (*S*)-**3** with 98% ee (122 mg; 49% yield) (conv = 50, E > 100). (*R*)-**1**: colourless oil; $[\alpha]_D^{20}$ = +3.9 (*c* 0.5, MeOH) {lit.[13] $[\alpha]_D^{20}$ = +3.9 (*c* 0.5, MeOH)}. The spectroscopic data for this compound were in agreement with those of its racemate.

4.5.2. Preparative scale hydrolysis of *rac*-3 by using an immobilized enzyme in a semi-continuous flow system

0.5 g of lipase AK-IM was packed into a PEEK column which was then connected to a pump. Phosphate buffer solution (50 mM, pH 7) was circulated through the column at a flow rate of 4 mL/min prior to use. A solution of *rac*-**3** (0.20 g, 0.91 mmol) in a 9:1 mixture (4 mL) of phosphate buffer (50 mM, pH 7) and MTBE was passed through the column reactor at 25 °C. The reaction was monitored by HPLC by taking samples at different intervals of time and stopped at an approximately 50% conversion. After 24 h, the column was washed with 5 ml of a 9:1 mixture of potassium phosphate buffer (50 mM, pH 7) and MTBE and then with 5 ml of potassium phosphate buffer. The eluted solution and washings were extracted with dichloromethane. The organic layer was dried over sodium sulfate, concentrated at reduced pressure and analysed by HPLC as described in Section 4.2. Purification of the residue by silica gel chromatography gave (*R*)-**1** (40% yield) with >99.5% ee and (*S*)-**3** (47% yield) with 98% ee.

(*R*)-**1**: colourless oil; $[\alpha]_D^{20}$ = +3.9 (*c* 0.5, MeOH) {lit.[13] $[\alpha]_D^{20}$ = +3.9 (*c* 0.5, MeOH)}. The spectroscopic data for this compound were in agreement with those of its racemate.

The column was reused in five subsequent reactions under identical experimental conditions without loss in activity and enantioselectivity.

4.5.3. Preparative scale hydrolysis of *rac*-5 by using an immobilized enzyme in a semi-continuous flow system

0.65 g of protease Alcalase-IM was packed into a PEEK column which was then connected to a suitable pump. Phosphate buffer solution (50 mM, pH 7) was circulated through the column at a flow rate of 4 mL/min prior to use. A solution of *rac*-**5** (0.20 g, 0.77 mmol) in a 9:1 mixture (4 mL) of phosphate buffer (50 mM, pH 7) and MTBE was passed through the column reactor at 25 °C. The reaction was monitored by HPLC by taking samples at different intervals of time and stopped at an approximately 50% conversion. After 8 h, the column was washed with 5 ml of a 9:1 mixture of potassium phosphate buffer (50 mM, pH 7) and MTBE and then with 5 ml of potassium phosphate buffer. The eluted solution and washings were extracted with dichloromethane. The organic layer was dried over sodium sulfate, concentrated at reduced pressure and analysed by HPLC as described in Section 4.2. Purification of the residue by silica gel chromatography gave (*S*)-**2** (43% yield) with 98% ee and (*R*)-**5** (47% yield) with 94% ee.

(*S*)-**2**: colourless oil; $[\alpha]_D^{20}$ = +13.5 (*c* 0.5, MeOH) {lit.[13] $[\alpha]_D^{20}$ of (*R*)-**2** = −13.3 (*c* 0.22, MeOH)}. The spectral data for this compound were in agreement with those of its racemate.

The column was reused in five subsequent reactions under identical experimental conditions without loss in activity and enantioselectivity.

4.6. Inversion at C-3 of (3*S*)-*N*-benzyl-3-hydroxypyrrolidine (*S*)-1

To a solution of 0.50 g (2.82 mmol) of (*S*)-**1** (ee >99.5%) and 0.40 g (3.57 mmol) of 1,4-diazabicyclo[2.2.2]octane (DABCO) in 3 ml of MTBE cooled to 0 °C, 0.65 g (3.14 mmol) of 4-toluenesulfonyl chloride was added under nitrogen. The mixture was stirred overnight at room temperature. Then 2 ml of ethyl acetate and 3 ml of saturated sodium bicarbonate solution were added and the mixture was stirred for 15 min. The organic layer was separated and extracted with 3 ml of saturated sodium bicarbonate solution, 3 ml of saturated ammonium chloride solution and 3 ml of saturated sodium chloride solution. The organic layer was dried over sodium sulfate and concentrated at reduced pressure to give 0.93 g (2.81 mmol) of (*S*)-**6** as a reddish oil (99% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.60–2.41 (m, 6H); 2.78–3.10 (m, 3H); 3.65 (s, 2H); 4.9 (m, 1H); 7.32–7.58 (m, 9H); TLC R_f = 0.54.

To a solution of 0.50 g (1.51 mmol) of (*S*)-**6** in 2 ml of dry dimethyl sulfoxide, 0.25 g (3.05 mmol) of anhydrous sodium acetate was added. The mixture was stirred at 120 °C for 2 h. The reaction was monitored by HPLC. After cooling to room temperature, the mixture was diluted with water. The aqueous layer was extracted with dichloromethane (3 × 0.5 mL). The organic layers were combined, dried over sodium sulfate and concentrated at reduced pressure to give 0.31 g (1.42 mmol) of (*R*)-**3**, (94% yield, 95% ee). To this residue, 1 ml (80 mmol) of 2 M sodium hydroxide solution was added. This mixture was stirred at room temperature for 1 h and then the aqueous layer was extracted with dichloromethane (3 × 0.5 mL). The organic layers were combined, dried over sodium sulfate and concentrated at reduced pressure to give 0.20 g of (*R*)-*N*-benzyl-3-hydroxypyrrolidine (*R*)-**1** (quantitative yield, 95% ee).

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