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Vapour-assisted enzymatic hydrolysis of β-lactams in a solvent-free system

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Dedicated to Professor Laszlo Toke on the occasion of his 75th birthday

Abstract—A new, solvent-free, vapour-assisted method, developed for the synthesis of carbocyclic *cis* β-amino acid enantiomers through the *Candida antarctica* lipase B-catalysed enantioselective (E >200) hydrolysis of β-lactams with 0.5 equiv of H_2O at 70 °C, has been demonstrated to be applicable on a preparative scale to produce (±)-**2** (the starting racemate for cispentacin), (±)-**3** (the starting racemate for 4-*tert*-butylcispentacin, a new cispentacin analogue) and (±)-**7** (the starting racemate for 1,4-ethylene-bridged cispentacin).

1. Introduction

There are ever more stringent requirements for the development of environmentally friendly, economical routes to enantiomerically pure products. Over the past few years, the synthesis[1] of enantiopure carbocyclic β-amino acids has received considerable attention, due to their wide-ranging use in peptide,[2] heterocyclic[3] and combinatorial[4] chemistry and drug research.[5] For example, nitrile-hydrolysing strains of *Rhodococcus* sp. have been introduced as novel catalysts for the preparation of β-amino acid enantiomers.[6] Kanerva et al. described the synthesis of several enantiopure carbocyclic β-amino acid derivatives, including ethyl (1*R*,2*S*,4*R*)-2-amino-4-*tert*-butylcyclopentanecarboxylate hydrochloride, through the lipase SP 526-catalysed enantioselective N-acylation of racemic β-amino esters with 2,2,2-trifluoroethyl hexanoate. A new direct enzymatic method that affords enantiopure β-amino acids through the lipase-catalysed ring cleavage of carbocyclic β-lactams has been reported,[7] as has a very efficient enzymatic technique that yields enantiopure carbocyclic *cis*- and *trans*-β-amino acids through the lipase-catalysed hydrolysis of the corresponding β-amino esters.[8] Some of the non-conventional media currently used and for lipase-catalysed reactions include organic solvents, ionic liquids and supercritical fluids, in addition to solvent-free systems.[9]

In accordance with efforts to develop environmentally friendly routes to enantiopure products, our present aim is to find an economical, new β-lactam ring-opening technique for the preparation of β-amino acid enantiomers (Scheme 1), the enzymatic reactions being performed in a solvent-free, vapour-assisted system.

2. Results and discussion

We had previously described the enzymatic resolutions of carbocyclic β-lactams (±)-**1**, (±)-**2** and (±)-**4**–(±)-**9** (Scheme 1) through the Lipolase (lipase B from *Candida antarctica*)-catalysed ring opening of β-lactams in an organic medium (E >200).[10] It was noted that the presence of H_2O at the surface of the enzyme was of key importance in the hydrolysis of the lactam; the hydrolyses of **2**, **5** and **9** in the presence of Lipolase in iPr$_2$O, for instance, were complete even without the addition of any H_2O, since the H_2O present in the enzyme preparation was sufficient enough for the β-lactam ring opening. In the light of this important observation, we decided to perform a reaction under solvent-free conditions and, if possible, to devise the enantioselective ring opening of model compounds with H_2O as a nucleophile, on the principle that 'the best solvent is no solvent'.

Since the ring cleavage of the unsaturated (±)-**2** in an organic solvent reached 50% conversion in a much shorter

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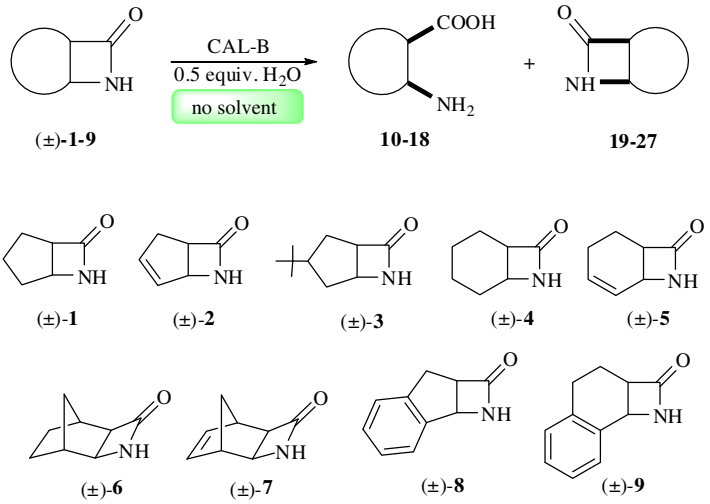


Scheme 1. Enantioselective ring cleavage of β-lactams.

reaction time (5 h[10a]) than that for its saturated analogue (±)-**1** (~10 days[7]), (±)-**2** was chosen for preliminary experiments. Evans et al. reported that ENZA 1 (*Rhodococcus equi NCIMB 40213*) catalysed the hydrolysis of (±)-**2** in phosphate buffer, in two steps at 20 °C, affording the products in high enantiomeric excess (ee >96%), but exhibited poor activity towards the saturated β-lactam (±)-**1**.[11]

We first performed the hydrolysis of (±)-**2** in an incubator shaker (210 rpm) with 0.5 equiv of H_2O in the presence of Lipolase at 50 °C without solvent (Table 1, entry 1). Lipolase displayed activity commensurable with that observed for the ring cleavage of (±)-**2** in an organic solvent (reaction in iPr_2O at 60 °C: conversion 50% after 5 h).[10a]

Temperature is a factor, which in certain cases affects the enantioselectivity and the rate of enzymatic reactions. Consequently, we investigated the influence of temperature on the Lipolase-catalysed ring cleavage of (±)-**2** (Table 1). Increasing the temperature had a positive effect: the reaction became faster, while the enantioselectivity remained unchanged (Table 1, entries 2–5). Since the enzyme became darker at 80 °C, 70 °C was chosen as the optimum temperature for the preparative-scale resolution.

The hydrolysis of (±)-**2** was also performed in an ultrasonic bath in a solvent-free system at 70 °C (Table 1, entry 4) and the same good *E* and reaction rate were obtained as when the reaction was performed in an incubator shaker under the same conditions (Table 1, entry 3).

It should be mentioned that an attempt was made to improve the enzyme:substrate mass ratio (from 4:1 to 2:1) in the case of (±)-**2**; a similarly good result (*E* >200) was obtained, but a longer reaction time was required (conversion: 25% after 3 h).

Since an improvement of the enzyme:substrate mass ratio (4:1) proved to be quite unlikely, special attention was paid to the possibility of reusing the enzyme: the hydrolysis of (±)-**2** was performed with Lipolase that had already been used in 1, 2, 3 or 4 cycles (Table 2). The catalytic activity of the Lipolase progressively became slightly lower, although the enantiomeric excess of the product was apparently not affected. The possibility of reusing the enzyme therefore makes the process an economical one.

The hydrolyses of the other β-lactams (±)-**1** and (±)-**3**–(±)-**9** likewise exhibited excellent enantioselectivities under these conditions (Table 3). Unexpectedly good results (*E* >200) were observed for (±)-**3**, which contains a very lipophilic and bulky *tert*-butyl substituent at the 4-position.

The preliminary experiments clearly demonstrated that this solvent-free method is applicable on a preparative scale for the enantioselective ring cleavage of (±)-**2**, (±)-**3** and (±)-**7** (Scheme 2), starting racemates for cispentacin and

Table 1. Effects of temperature on the ring cleavage of (±)-**2**[a]

Entry	Temperature (°C)	Time (h)	Conversion (%)	ee_s[b] (%)	ee_p[c] (%)	*E*
1	50	3	31	44	>99	>200
2	60	3	40	66	>99	>200
3	70	3	47	88	>99	>200
4	70[d]	2.5	45	83	>99	>200
5	80	3	49	94	>99	>200

[a] 0.1 mmol substrate, Lipolase (50 mg), 0.5 equiv H_2O, after 3 h.
[b] According to GC.[10a]
[c] According to GC after double derivatisation.[10a]
[d] Ultrasonic bath (35 kHz).

Table 2. Conversion and enantioselectivity of the ring opening of (±)-**2**[a] with the recycled enzyme

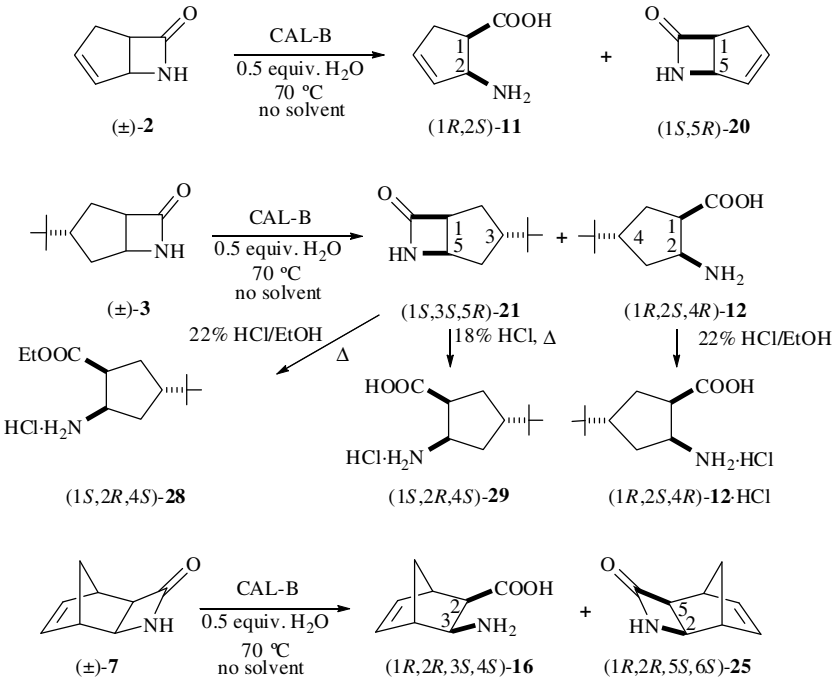
Lipolase (50 mg)	Conversion (%)	ee_s[b] (%)	ee_p[c] (%)	*E*
Used once	44	78	>99	>200
Twice used	39	63	>99	>200
3 times used	35	53	>99	>200
4 times used	29	40	>99	>200

[a] 0.1 mmol substrate, 0.5 equiv H_2O, 70 °C, after 3 h.
[b] According to GC.[10a]
[c] According to GC after double derivatisation.[10a]

Table 3. Conversion and enantioselectivity of the ring opening of (±)-**1** and (±)-**3**-(±)-**8**[a]

Substrate	Time	Conversion (%)	ee_s[b] (%)	ee_p[c] (%)	*E*
(±)-**1**	19 h (5 days)	21 (50)	27 (98)	>99 (>99)	>200 (>200)
(±)-**3**	19 h	42	72	>99	>200
(±)-**4**	19 h (5 days)	25 (50)	33 (98)	>99 (>99)	>200 (>200)
(±)-**5**	5 h	49	95	>99	>200
(±)-**6**	5 days	40	65	>99	>200
(±)-**7**	4 days	50	>99	>99	>200
(±)-**8**	5 days	46	83	>99	>200
(±)-**9**	4 days	49	94	>99	>200

[a] 0.1 mmol substrate, Lipolase (50 mg), 0.5 equiv H_2O, 70 °C.
[b] According to GC.[10]
[c] According to GC after double derivatisation.[10]



Scheme 2. Ring opening of β-lactams in a solvent-free system.

its 4-*tert*-butyl and 1,4-ethylene-bridged analogues. The results are reported in Table 4 and in Section 4.

The transformations involving the ring opening of β-lactam **21** with 18% aqueous HCl or 22% ethanolic HCl

Table 4. Lipolase-catalysed ring cleavage of (±)-**2**, (±)-**3** and (±)-**7**[a]

	Time	Conversion (%)	*E*	β-Lactam (**20**, **21** and **25**)				β-Amino acid (**11**, **12** and **16**)			
				Yield (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$	Yield (%)	Isomer	ee[c] (%)	$[\alpha]_D^{25}$
(±)-**2**	4 h	50	>200	47	1*S*,5*R*	97	−33.2[d]	41	1*R*,2*S*	>99	+99.6[e,f]
(±)-**3**	40 h	50	>200	44	1*S*,3S,5*R*	96	+54[g]	43	1*R*,2*S*,4*R*	>99	−6[h]
(±)-**7**	6 days	50	>200	44	1*R*,2*R*,5*S*,6*S*	98	+122.1[d]	41	1*R*,2*R*,3*S*,4*S*	>99	−12.3[e,i]

[a] Enzyme:substrate mass ratio 4:1, 0.5 equiv H_2O, 70 °C.
[b] According to GC.
[c] According to GC after double derivatisation.
[d] *c* 0.35, $CHCl_3$.
[e] *c* 0.3, H_2O.
[f] $[\alpha]_D^{25} = +96.7$ (*c* 0.3, H_2O).[10a]
[g] *c* 0.25, EtOH.
[h] *c* 0.2, H_2O.
[i] $[\alpha]_D^{25} = -12.2$ (*c* 0.4, H_2O).[10b]

resulted in the corresponding enantiomer of the β-amino acid hydrochloride **29** (ee = 96%) or β-amino ester hydrochloride **28** (ee = 95%), respectively. Treatment of amino acid **12** with 22% HCl/EtOH resulted in enantiopure **12**·HCl (ee >99%) (Scheme 2).

The chromatograms analysed indicated that CAL-B displays the same enantiopreference for all these β-lactams in the solvent-free system and in iPr$_2$O.[7,8,10] The value of $[\alpha]_D^{25} = +4$ (c 0.14, EtOH) for **28** and the literature value[12] for ethyl (1*R*,2*S*,4*R*)-2-amino-4-*tert*-butylcyclopentanecarboxylate hydrochloride, $[\alpha]_D^{20} = -2.4$ (c 1, EtOH), are in accordance with this.

3. Conclusions

An economical, facile and solvent-free enzymatic method has been developed for the preparation of enantiopure carbocyclic β-amino acids. The method is equally applicable for the enantioselective ring opening of mono- and bicyclic saturated and unsaturated, and tricyclic β-lactams, with 0.5 equiv of H_2O as the only reagent. Excellent enantioselectivities (E >200) were observed for the ring cleavage of (±)-**1**–(±)-**9** at 70 °C, even with Lipolase that had already been used four times. The hydrolysis of (±)-**3** resulted in (1*R*,2*S*,4*R*)-β-amino acid (a new cispentacin analogue; ee >99%) and (1*S*,3*S*,5*R*)-β-lactam (ee = 96%). The products can be easily separated.

4. Experimental

4.1. Materials and methods

Lipolase (lipase B from *C. antarctica*), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was purchased from Sigma–Aldrich. The carbocyclic β-lactams were prepared according to the literature.[7,10,13]

In a typical small-scale experiment, a racemic β-lactam (0.1 mmol) and Lipolase (25 or 50 mg of a new charge, or material that had already been used in 1, 2, 3 or 4 cycles) were mixed well, and H_2O (0.5 equiv) was then added. The mixture was shaken at 50 °C, 60 °C, 70 °C or 80 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by gas chromatography. The ee values for the unreacted β-lactam and the β-amino acid enantiomers produced were determined by gas chromatography on Chromopak Chiralsil-Dex CB or Chirasil-L-Val columns, as described earlier.[8,10] The ee value for the new enantiomeric lactam **21** was determined on a Chromopak Chiralsil-Dex CB column (190 °C isothermal; 100 kPa; retention times (min): **21**: 5.91, antipode: 5.63), while the ee value for amino acid **12** produced was determined by using the same chiral column after double derivatisation with (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (120 °C for 10 min → 180 °C (rate of temperature rise 10 °C/min; 100 kPa); retention times (min): **12**: 21.26, antipode: 21.12). The ee value for amino ester **27** was determined by using the same chiral column after derivatisation with acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (190 °C isothermal; 100 kPa; retention times (min): **27**: 9.82, antipode: 9.62). Enantioselectivity (E) was calculated using the equation $E = \{\ln[(1 - ee_s)/(1 + ee_s/ee_p)]\}/\{\ln[(1 + ee_s)/(1 + ee_s/ee_p)]\}$.[14] Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

4.2. Preparative-scale resolution of 6-azabicyclo[3.2.0]hept-3-en-7-one, (±)-2

Crystalline racemic **1** (500 mg, 4.58 mmol) was mixed well with Lipolase (2 g), after which water (41 μL, 2.29 mmol) was added, and the mixture was shaken in an incubator shaker at 70 °C for 4 h. First the unreacted β-lactam was washed off the surface of the enzyme with EtOAc (3 × 15 mL), and this was followed by the washing off of β-amino acid with distilled water (3 × 15 mL): (1*S*,5*R*)-**20** {235 mg, 47%; $[\alpha]_D^{25} = -33.2$ (c 0.35, $CHCl_3$); mp 74–75 °C, ee = 97%} and (1*R*,2*S*)-**11** {237 mg, 41%; $[\alpha]_D^{25} = +99.6$ (c 0.3, H_2O); mp >240 °C; ee >99%}. The ^{1}H NMR data on the products were identical with those given in the literature.[10a]

4.3. Small-scale resolution of (1*S**,3*S**,5*R**)-3-*tert*-butyl-6-azabicyclo[3.2.0]heptan-7-one, (±)-3

Following the procedure described above, the reaction of racemic **3** (100 mg, 0.59 mmol) and H_2O (6 μL, 0.33 mmol) in the presence of Lipolase (400 mg) at 70 °C afforded (1*S*,3*S*,5*R*)-**21** {44 mg, 44%; $[\alpha]_D^{25} = +54$ (c 0.25, EtOH); mp 136–138 °C; ee = 96%} and (1*R*,2*S*,4*R*)-**12** {47 mg, 43%; $[\alpha]_D^{25} = -6$ (c 0.2, H_2O); mp 220–222 °C; ee >99%} in 40 h. When (1*R*,2*S*,4*R*)-**12** (20 mg) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*S*,4*R*)-**12** HCl [21 mg, 78%; $[\alpha]_D^{25} = -5$ (c 0.15, H_2O); mp 226–229 °C, ee >99%] was formed. When (1*S*,3*S*,5*R*)-**21** (20 mg) was refluxed in 18% HCl or 22% HCl/EtOH (5 mL) for 3 h, white crystals of (1*S*,2*R*, 4*S*)-**29** [20 mg, 67%; $[\alpha]_D^{25} = +5$ (c 0.25, H_2O); mp 225–227 °C; ee = 96%] or (1*S*,2*R*,4*S*)-**28** [19 mg, 63%; $[\alpha]_D^{25} = +4$ (c 0.14, EtOH); ee = 95%], were obtained.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **12**: 0.78 (9H, s, 3 × CH_3) 1.75–2.05 (5H, m, 2 × CH_2 and C*H*C(CH_3)$_3$) 2.77–2.82 (1H, m, H-1) 3.62–3.68 (1H, m, H-2). Anal. Calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.99; H, 10.30; N, 7.56.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **12**·HCl and for **29**: the data for the two compounds were identical: 0.91 (9H, s, 3 × CH_3) 1.32–1.35 (3H, t, $J = 7.1$, CH_2CH_3) 1.85–2.15 (5H, m, 2 × CH_2 and C*H*C(CH_3)$_3$) 3.22–3.28 (1H, m, H-1) 3.90–3.95 (1H, m, H-2) 4.28 (2H, m, CH_2CH_3). Anal. Calcd for $C_{10}H_{19}NO_2$·HCl: C, 54.17; H, 9.09; N, 6.32. Found: C, 54.04; H, 9.00; N, 6.41.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **21**: 0.91 (9H, s, 3 × CH_3), 1.28–1.34 and 2.00–2.09 (4H, m, 2 × CH_2), 1.80–1.85 (1H, dd, $J = 13.4$, 5.6, C*H*C(CH_3)$_3$), 3.53–3.56

(1H, m, H-1), 4.06–4.08 (1H, m, H-2), 5.54 (1H, br s, NH). Anal. Calcd for $C_{10}H_{17}NO$: C, 71.81; H, 10.25; N, 8.37. Found: C, 71.71; H, 10.18; N, 8.32.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **28**: 0.91 (9H, s, $3 \times CH_3$) 1.33 (3H, t, $J = 7.1$ Hz, CH_2CH_3) 1.94–2.26 (5H, m, $2 \times CH_2$ and $CHC(CH_3)_3$) 3.22–3.28 (1H, m, H-1) 3.90–3.95 (1H, m, H-2) 4.24–4.31 (2H, m, CH_2CH_3). Anal. Calcd for $C_{12}H_{23}NO_2 \cdot HCl$: C, 57.70; H, 9.68; N, 5.61. Found: C, 57.60; H, 9.64; N, 5.56.

4.4. Preparative-scale resolution of *exo*-3-azatricyclo-[4.2.1.0[2.5]]non-7-en-4-one, (±)-7

Following the procedure described above, the reaction of racemic **7** (500 mg, 3.7 mmol) and H_2O (34 μL, 1.88 mmol) in the presence of Lipolase (2 g) at 70 °C afforded (1*R*,2*R*,5*S*,6*S*)-**25** {220 mg, 44%; $[\alpha]_D^{25} = +122.1$ (*c* 0.35, $CHCl_3$); mp 90–93 °C; ee = 98%} and (1*R*,2*R*,3*S*,4*S*)-**14** {232 mg, 41%; $[\alpha]_D^{25} = -12.3$ (*c* 0.3, H_2O); mp >260 °C; ee >99%} in 6 days. The ^{1}H NMR data on the products were identical with those given in the literature.[10b]

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References

1. (a) Fülöp, F. *Chem. Rev.* **2001**, *101*, 2181; (b) Park, K. H.; Kurth, M. J. *Tetrahedron* **2002**, *58*, 8629; (c) Fülöp, F.; Martinek, T. A.; Tóth, G. K. *Chem. Soc. Rev.* **2006**, *35*, 323; (d) Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831; (e) Forró, E.; Fülöp, F. *Mini Rev. Org. Chem.* **2004**, *1*, 931.
2. (a) Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 6376; (b) Martinek, T. A.; Mándity, I. M.; Fülöp, L.; Tóth, G. K.; Vass, E.; Hollósi, M.; Forró, E.; Fülöp, F. *J. Am. Chem. Soc.* **2006**, *128*, 13539.
3. (a) Carter, P. H. U.S. Patent 2005,277,666; *Chem. Abstr.* **2005**, *144*, 51452; (b) Gyónfalvi, S.; Szakonyi, Z.; Fülöp, F. *Tetrahedron: Asymmetry* **2003**, *14*, 3965; (c) Kapferer, P.; Vasella, A. *Helv. Chim. Acta* **2004**, *87*, 2764.
4. (a) Kanizsai, I.; Gyónfalvi, S.; Szakonyi, Z.; Sillanpää, R.; Fülöp, F. *Green Chem.* **2007**, *9*, 357; (b) Kiss, L.; Forró, E.; Sillanpää, R.; Fülöp, F. *J. Org. Chem.* **2007**, *72*, 8786.
5. (a) *Enantioselective Synthesis of β-Amino Acids*; Juaristi, E., Soloshonok, V. A., Eds.; Wiley-Interscience: New York, 2005; (b) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 15474; (c) Davies, S. G.; Smith, A. D.; Price, P. D. *Tetrahedron: Asymmetry* **2005**, *16*, 2833.
6. Winkler, M.; Martinková, L.; Knall, A. C.; Krahulec, S.; Klempier, N. *Tetrahedron* **2005**, *61*, 4249.
7. Forró, E.; Fülöp, F. *Org. Lett.* **2003**, *5*, 1273.
8. Forró, E.; Fülöp, F. *Chem. Eur. J.* **2007**, *22*, 6397.
9. (a) Sheldon, R. A. *Green Chem.* **2007**, *9*, 357; (b) Foresti, M. I.; Pedernera, M.; Bucalá, V.; Ferreira, M. L. *Enzyme Microb. Technol.* **2007**, *41*, 62.
10. (a) Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 2875; (b) Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 573; (c) Forró, E.; Fülöp, F. *Chem. Eur. J.* **2006**, *12*, 2587.
11. Evans, C.; McCague, R.; Roberts, S. M.; Sutherland, A. G.; Wisdom, R. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2276.
12. Kanerva, L. T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1705.
13. Fülöp, F.; Bernáth, G.; Spitzner, R.; Mattinen, J.; Pihlaja, K. *Acta Chim. Hung. Models Chem.* **1994**, *131*, 435.
14. Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. J. *Enzyme Microb. Technol.* **1993**, *15*, 1051.