

The First Direct Enzymatic Hydrolysis of Alicyclic β -Amino Esters: A Route to Enantiopure *cis* and *trans* β -Amino Acids

Enikő Forró and Ferenc Fülöp*^[a]

Abstract: The first direct enzymatic method is reported for the synthesis of *cis* and *trans* β -amino acid enantiomers through the lipase-catalyzed enantioselective hydrolysis of alicyclic β -amino esters in organic media. High enantioselectivities (*E* usually > 100) were observed when the *Candida antarctica* lipase B catalyzed reactions were performed with H₂O (0.5 equivalents) in *i*Pr₂O at 65 °C. The resolved products, obtained in good yields ($\geq 42\%$), could be easily separated.

Keywords: amino acids • cispentacin • enantioselectivity • enzymes • hydrolysis

Introduction

Alicyclic β -amino acids are important compounds from both pharmacological (for example, cispentacin)^[1] and chemical aspects.^[2] They can serve as building blocks for the synthesis of modified peptides with increased activity and stability^[3] and with well-defined three-dimensional structures (for example, β -peptides with possible antibiotic activity) similar to those of natural peptides.^[4] They can also be used in heterocyclic,^[5] combinatorial^[6] chemistry and in drug research.^[7]

In the past few years, several enzymatic syntheses have been developed for enantiopure alicyclic β -amino acid derivatives. As an example, the indirect enzymatic method through the lipase-catalyzed asymmetric acylation of the primary hydroxy group of N-hydroxymethylated β -lactams or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to the β -amino ester or acid, respectively, are not too efficient and relatively long procedures.^[8,9] Kanerva et al. described the enzymatic N-acylation of *cis* and *trans* alicyclic β -amino esters, but hydrolysis of the N-acylated products to the corresponding β -amino acids was not performed.^[10]

We have recently discovered a simple and efficient direct enzymatic route to alicyclic β -amino acid enantiomers through lipase-catalyzed enantioselective (*E* > 200) ring cleavage of β -lactams in an organic medium (for example,

the synthesis of cispentacin).^[10] The great advantages of this method are that the lactam ring need not necessarily be activated and the product *cis* β -amino acids are obtained in good yields.

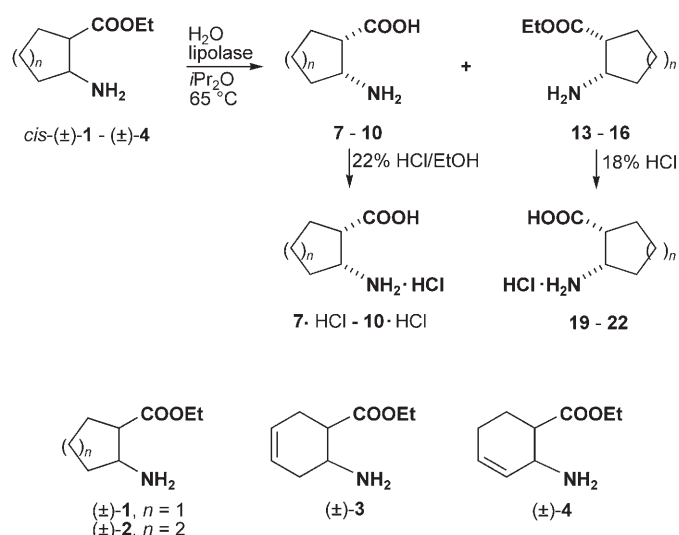
Few articles have dealt with the enzymatic hydrolysis of amino esters. For example, Katayama et al. described the enzyme-catalyzed hydrolysis of methyl 1,2,3,4-tetrahydroquinoline-1-acetate through enantioselective hydrolysis (*E* = 94) of the ester function.^[12] The substrate contains one stereogenic centre and the ring nitrogen is a secondary one. The reaction was performed in an organic solvent, with Novozym 435 as the enzyme and 5% H₂O as the nucleophile. It is also important to mention that Lloyd et al. prepared alicyclic N-Boc-protected γ -amino acids through lipase-type VII (*Candida rugosa*)-catalyzed methyl ester hydrolysis with KH₂PO₄ (pH 7) at 26 °C.^[13]

In this paper, we report a new direct enzymatic method for the enantioselective hydrolysis of both *cis* β -amino esters ($\pm$)-**1**–($\pm$)-**4** (Scheme 1) and *trans* β -amino esters ($\pm$)-**5** and ($\pm$)-**6** (Scheme 2), yielding the β -amino acids **7**–**12** and unreacted β -amino esters **13**–**18**.

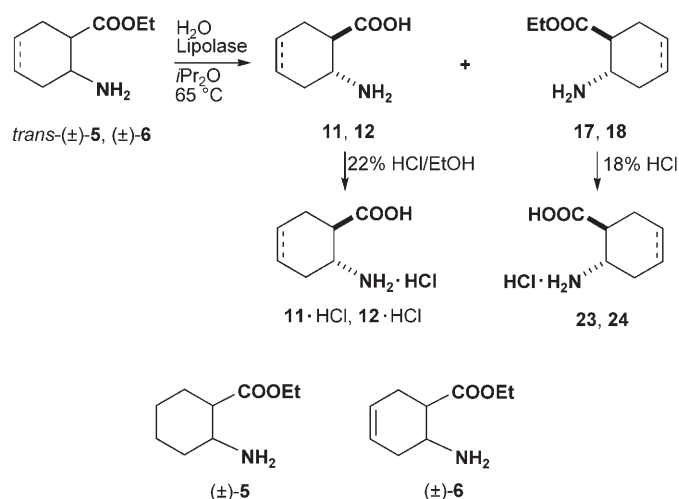
Results and Discussion

Our preliminary experiments were started with enzyme screening, including lipase PS (*Pseudomonas cepacia*), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*), Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*), Novozym 435 (lipase B from *Candida antarctica* immobilized on a macroporous polyacrylic resin), Chirazyme L-5 (lipase A from *Candida antarctica*), Lipolase (lipase B from *Candida antarctica*, produced by submerged

[a] Dr. E. Forró, Prof. Dr. F. Fülöp
Institute of Pharmaceutical Chemistry
University of Szeged, 6701 Szeged
PO Box 427 (Hungary)
Fax: (+36) 62-545-705
E-mail: fulop@pharm.u-szeged.hu



Scheme 1. Enantioselective hydrolysis of alicyclic *cis*-β-amino esters (±)-1–(±)-4.



Scheme 2. Enantioselective hydrolysis of alicyclic *trans*-β-amino esters (±)-5 and (±)-6.

fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (porcine pancreatic lipase). The reactions were performed on model compound **2** with 0.5 equivalents of H₂O

Abstract in Hungarian: Hatékony enzimész módszerrel dolgoztunk ki elsőként *cis*- és *transz* β-aminosav enantiomerek előállítására aliciklusos β-aminosav észterek lipáz-katalizálta enantioszelektív hidrolízisének keresztül. Nagy enantioszelektivitás (E általában >100) volt elérhető, amikor a reakciókat *Candida antarctica* B lipáz-katalízissel, víz (0.5 ekv.) hozzáadásával, diizopropil-éterben, 65°C-on végeztük. A jó termeléssel kapott ($\geq 42\%$) enantiomerek elválasztása egyszerűen, szerves-vizes extrakcióval történt.

in *i*Pr₂O at 65°C (Scheme 1). Lipases PS, AK and AY did not exhibit any activity at 45°C (no conversion after 48 h), while PPL even catalyzed the hydrolysis of **2** at 45°C, but the reaction rate was low (Table 1, entry 17). The Chirazyme L-5-catalyzed hydrolysis of **2** was slower and much less enantioselective (entry 3). Chirazyme L-2 (entry 1), Novozym 435 (entry 2) and Lipolase (entry 4) were all promising catalysts, directing the hydrolysis with slight differences in enantioselectivities. Lipolase was chosen as the enzyme for further studies.

Several solvents were tested to study the solvent effect in the Lipolase-catalyzed hydrolysis of (±)-**2** at 65°C. The reaction proceeded somewhat more slowly in *n*-hexane and in toluene (Table 1, entries 10 and 11) and much more slowly in dioxane, (CH₃)₂CO and THF (entries 5–7) than those in ether-type solvents (entries 4, 8 and 9), which all afforded high enantioselectivities ($E > 100$).

Next, we analyzed the effects of temperature on the enantioselectivity and reaction rate. The Lipolase-catalyzed hydrolysis of (±)-**2** with 0.5 equivalents of H₂O in *i*Pr₂O at 25°C proved to be a very slow reaction, although the enantioselectivity was high (Table 1, entry 12). When the reaction was performed at 45 or at 60°C (entries 13 and 14), the reaction rate increased and the enantioselectivity remained high. A further increase in the temperature (70°C) did not affect the enantioselectivity, and the reaction did not become faster (entry 15). Indeed, the reaction even slowed down when the reaction temperature was increased to 80°C (entry 16).

The catalytic activity of the tested Lipolase was not affected when the reaction was performed without the addition of H₂O (Table 2, entry 3): the H₂O present in the reaction medium ($<0.1\%$) or in the enzyme preparation ($<5\%$) was sufficient for the hydrolysis.

The reaction rate for the hydrolysis in the case of (±)-**2** increased slightly on increase of the amount of enzyme (Table 2, entries 1, 2 and 7–9).

Finally, we analyzed the reusability of the enzyme: the hydrolysis of (±)-**2** was tested with Lipolase that had already been used in 1, 2 or 3 cycles (Table 2, entries 4–6). The catalytic activities of the tested Lipolase were progressively slightly lowered, though the enantiomeric excess of the product was apparently not affected.

Hydrolysis of the other *cis* ((±)-**1**, (±)-**3**, and (±)-**4**) and *trans* β-amino acid esters (Scheme 2, (±)-**5** and (±)-**6**) likewise exhibited excellent enantioselectivities under these conditions.

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**–(±)-**6** were performed with 0.5 equivalents of H₂O in the presence of Lipolase in *i*Pr₂O at 65°C. The products were characterized by a relatively good enantiomeric excess next to 50% conversion. The results are reported in Table 3 and the Experimental Section.

It should be mentioned that an attempt was made to improve the enzyme/substrate mass ratio (from 1.8:1 to 1:1) in the case of (±)-**2** (Experimental Section), and similarly good results ($E > 200$) were obtained; however, a longer re-

Table 1. Conversion and enantioselectivity of hydrolysis of ($\pm$)-**2**.^[a]

Entry	Enzyme (30 mg mL ⁻¹)	T [°C]	Solvent	Reaction t [h]	Conv. [%]	<i>ee</i> _s ^[b] [%]	<i>ee</i> _p ^[c] [%]	<i>E</i>
1	Chirazyme L-2	65	<i>i</i> Pr ₂ O	26	48	89	96	147
2	Novozym 435	65	<i>i</i> Pr ₂ O	26	47	87	97	187
3	Chirazyme L-5 ^[d]	65	<i>i</i> Pr ₂ O	48	15	10	58	4
4	Lipolase	65	<i>i</i> Pr ₂ O	25	48	90	98	307
5	Lipolase	65	dioxane	28	19	23	99	249
6	Lipolase	65	(CH ₃) ₂ CO	28	6	6	99	211
7	Lipolase	65	THF	28	12	14	99	228
8	Lipolase	65	Et ₂ O	28	45	80	98	245
9	Lipolase	65	<i>t</i> BuOMe	28	49	92	96	161
10	Lipolase	65	toluene	28	29	40	99	295
11	Lipolase	65	<i>n</i> -hexane	28	38	61	98	185
12	Lipolase	25	<i>i</i> Pr ₂ O	20 (5 d)	11 (47)	12 (87)	99 (97)	223 (187)
13	Lipolase	45	<i>i</i> Pr ₂ O	20	21	26	99	256
14	Lipolase	60	<i>i</i> Pr ₂ O	19	36	55	99	346
15	Lipolase	70	<i>i</i> Pr ₂ O	19	36	55	98	172
16	Lipolase	80	<i>i</i> Pr ₂ O	19	29	40	98	146
17	PPL	45	<i>i</i> Pr ₂ O	48	6	6	99	211

[a] 0.05 M substrate, 0.5 equiv H₂O. [b] According to GC. [c] According to GC after double derivatisation. [d] Contains 20 % (w/w) of lipase adsorbed on Celite in the presence of sucrose.

chloride salts **7**·HCl–**12**·HCl. The absolute configurations were proved by comparing the $[\alpha]$ values with the literature data (Experimental Section), except in the case of **12**, for which the unsaturated β -amino acid enantiomer was reduced catalytically to (1*R*,2*R*)-**11** by transfer hydrogenation in the presence of cyclohexene as a hydrogen donor. Thus, the absolute configurations indicated Lipolase-catalyzed *S*-selective hydrolysis for the *cis* compounds, while for the *trans* compounds the opposite *R* selectivity was observed.

Conclusion

The first, simple and efficient direct enzymatic method that is equally applicable for the synthesis of *cis* and *trans* β -amino acids has been developed. The Lipolase (lipase B from *Candida antarctica*) catalyzed highly enantioselective hydrolysis of both *cis* (**1–4**) and *trans* β -amino esters (**5** and **6**) (*E* usually > 100), when H₂O (0.5 equivalents) is used in *i*Pr₂O at 65 °C, results in *cis* (1*S*,2*R*)-**7–10** and *trans* β -amino acids (1*R*,2*R*)-**11** and **12**, unreacted *cis* β -amino esters (1*R*,2*S*)-**13–16** and *trans* β -amino esters (1*S*,2*S*)-**17** and **18** in good yields (42–48 %). The products could be easily separated. Transformations of esters **13–18** with 18 % aqueous HCl resulted in the enantiomers of β -amino acid hydrochlorides **19–24** ($\geq 94\%$ *ee*, *ee* = enantiomeric excess). The present method was successfully used for the synthesis of cispentacin.

Experimental Section

Materials and methods: Lipolase (lipase B from *Candida antarctica*) produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin was obtained from Sigma-Aldrich. Lipase PS and lipase AK were obtained from

Table 2. Conversion and enantioselectivity for the hydrolysis of ($\pm$)-**2**.^[a]

Entry	Lipolase [mg mL ⁻¹]	H ₂ O [equiv]	Reaction t [h]	Conv. [%]	<i>ee</i> _s ^[b] [%]	<i>ee</i> _p ^[c] [%]	<i>E</i>
1	10	0.5	25	42	70	98	208
2	20	0.5	25	45	79	98	239
3	30	–	29	43	75	98	224
4	30 ^[d]	–	24	43	74	98	244
5	30 ^[e]	–	24	38	59	97	120
6	30 ^[f]	–	24	32	46	97	103
7	40	0.5	25	48	91	97	209
8	50	0.5	22	50	95	96	183
9	75	0.5	22	51	99	94	174

[a] 0.05 M substrate, *i*Pr₂O, 65 °C. [b] According to GC. [c] According to GC after double derivatisation. [d] Already used once. [e] Already used twice. [f] Already used three times.

action time was necessary (the conversion was 42 % after 45 h).

The transformations involving the hydrolysis of β -amino esters **13–18** with 18 % aqueous HCl resulted in the enantiomers of the β -amino acid hydrochlorides **19–24** (Schemes 1 and 2, Table 3). Compound **19** is the well-known natural cyclic β -amino acid cispentacin.^[1a] Treatment of amino acids **7–12** with 22 % HCl/EtOH resulted in enantiopure hydro-

Table 3. Lipolase-catalyzed preparative-scale hydrolysis of ($\pm$)-**1**–($\pm$)-**6**.^[a]

	t [h]	Conv. [%]	<i>E</i>	β -Amino acid-HCl (19–24)			Yield [%]	β -Amino acid (7–12)		
				Yield [%]	Isomer	<i>ee</i> ^[b] [%]		Yield [%]	Isomer	<i>ee</i> [%] ^[c]
($\pm$)- 1	87	48	74	42	1 <i>R</i> ,2 <i>S</i>	94	–5 ^[d]	42	1 <i>S</i> ,2 <i>R</i>	96
($\pm$)- 2	31	49	> 200	46	1 <i>R</i> ,2 <i>S</i>	99	–8.4 ^[e]	47	1 <i>S</i> ,2 <i>R</i>	98
($\pm$)- 3	72	50	110	45	1 <i>R</i> ,2 <i>S</i>	98	–28 ^[g]	46	1 <i>S</i> ,2 <i>R</i>	98
($\pm$)- 4	66	49	133	45	1 <i>R</i> ,2 <i>S</i>	98	+121 ^[d]	46	1 <i>S</i> ,2 <i>R</i>	99
($\pm$)- 5	68	49	> 200	46	1 <i>S</i> ,2 <i>S</i>	99	+51 ^[d]	48	1 <i>R</i> ,2 <i>R</i>	99
($\pm$)- 6	58	50	183	44	1 <i>S</i> ,2 <i>S</i>	99	+123 ^[i]	45	1 <i>R</i> ,2 <i>R</i>	99

[a] 30 mg mL⁻¹ enzyme in *i*Pr₂O, 0.5 equiv H₂O, 65 °C. [b] According to GC after double derivatisation. [c] *c* = 0.21. [d] *c* = 0.23. [e] *c* = 0.5. [f] *c* = 0.28. [g] *c* = 0.11. [h] *c* = 0.26. [i] *c* = 0.27.

Amano Pharmaceuticals, PPL (type II) was obtained from Sigma and Chirazyme L-5 (lipase A from *Candida antarctica*) and Novozym 435 as an immobilized lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Before use, lipase PS, lipase AK and CAL-A (5 g) were dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). The lipase preparations thus obtained contained 20% (w/w) of lipase. The alicyclic *cis* and *trans* β -amino esters were prepared according to the literature.^[1a,14] The solvents were of the highest analytical grade.

In a typical small-scale experiment, racemic β -amino ester (0.05 M solution) in an organic solvent (2 mL) was added to the lipase tested (10, 20, 30, 40, 50 or 75 mg mL⁻¹). H₂O (0 or 0.5 equiv) was added. The mixture was shaken at 25°C, 45°C, 60°C, 65°C, 70°C or 80°C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The *ee* values for the unreacted β -amino ester and the β -amino acid enantiomers produced were determined by gas chromatography on a Chromopak Chirasil-Dex CB column (25 m) or Chromopak L-Val (25 m) column after double derivatisation with 1) diazomethane (*Caution! The derivatisation with dichloromethane should be carried out under a well-working fume hood*) and 2) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (Chirasil-L-Val column, 120°C for 5 min $\rightarrow$ 190°C, rate of temperature rise: 20°C min⁻¹, 140 kPa, retention times [min]: **13**: 9.20 (antipode 9.40), **7**: 8.64 (antipode 8.62), **14**: 10.28 (antipode 10.42), **8**: 9.74 (antipode 9.65), **9**: 9.29 (antipode 9.21), **10**: 8.95 (antipode 8.79); CP-Chirasil-Dex CB column, 120°C for 2 min $\rightarrow$ 190°C, rate of temperature rise 20°C min⁻¹, 140 kPa, retention times [min]: **15**: 10.68 (antipode 10.77), **16**: 9.89 (antipode 10.51), **17**: 10.81 (antipode 10.93), **11**: 8.86 (antipode 8.78)). The *ee* values for **12** and **18** were determined as in the case of **11** and **17**, after catalytic reduction to the corresponding saturated compounds.^[8c]

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ¹H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

Preparative-scale resolution of ethyl *cis*-2-aminocyclopentane-1-carboxylate ($\pm$)-1**:** Lipolase (450 mg, 30 mg mL⁻¹) and H₂O (9.0 μ L, 0.50 mmol) were added to the racemic **1** (157 mg, 1.00 mmol) in *i*Pr₂O (15 mL), and the mixture was shaken in an incubator shaker at 65°C for 87 h. The reaction was stopped by filtering off the enzyme at 48% conversion (**13**: 93% *ee*). The solvent was evaporated off and the residue (**1R,2S**)-**13** was immediately hydrolyzed by refluxing (4 h) with 18% aqueous HCl solution (7 mL) into β -amino acid hydrochloride (**1R,2S**)-**19**. Yield: 69.4 mg, 42%; [α]_D²⁵ = -5 (*c* = 0.21 in H₂O), lit.^[11a] [α]_D²⁵ = -5.1 (*c* = 0.5 in H₂O); m.p. 164–166°C (recrystallised from EtOH and Et₂O); 94% *ee*.

The filtered enzyme was washed with distilled H₂O (3 $\times$ 15 mL), and the H₂O was evaporated off, yielding the crystalline β -amino acid (**1S,2R**)-**7**. Yield: 54.1 mg, 42%; [α]_D²⁵ = +8 (*c* = 0.23 in H₂O); m.p. 218–219°C with sublimation (recrystallised from H₂O and (CH₃)₂CO); 96% *ee*.

When **7** (30 mg) was treated with 22% HCl/EtOH (3 mL), (**1S,2R**)-**7**-HCl was obtained. Yield: 28 mg, 73%; [α]_D²⁵ = +5 (*c* = 0.22 in H₂O), lit.^[11a] [α]_D²⁵ = +5.2 (*c* = 0.5 in H₂O); m.p. 162–166°C (recrystallised from EtOH and Et₂O); 96% *ee*.

Data for 7: ¹H NMR (400 MHz, D₂O, 25°C, TMS): δ = 1.70–2.12 (m, 6H; 3 $\times$ CH₂), 2.84–2.89 (m, 1H; H-1), 3.72–3.73 ppm (m, 1H; H-2); elemental analysis calcd (%) for C₆H₁₁NO₂ (129.08): C 55.80, H 8.58, N 10.84; found: C 55.69, H 8.50, N 10.96.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) spectroscopic data are similar for **7**-HCl and **19**: δ = 1.75–2.17 (m, 6H; 3 $\times$ CH₂), 3.10–3.16 (m, 1H; H-1), 3.84–3.85 ppm (m, 1H; H-2); elemental analysis calcd (%) for C₆H₁₁NO₂·HCl (165.62): C 43.51, H 7.30, N 8.46; found for **7**-HCl: C 43.41, H 6.97, N 8.46; found for **19**: C 43.28, H 7.11, N 8.61.

Preparative-scale resolution of ethyl *cis*-2-aminocyclohexane-1-carboxylate ($\pm$)-2**:** Following the procedure described above, the reaction of racemic **2** (1 g, 5.83 mmol) and H₂O (52.5 μ L, 2.91 mmol) in *i*Pr₂O (60 mL) in the presence of Lipolase (1.8 g, 30 mg mL⁻¹) at 65°C afforded (**1R,2S**)-**20**

and (**1S,2R**)-**8** in 31 h. Compound (**1R,2S**)-**20**: Yield: 482 mg, 46%; [α]_D²⁵ = -8.4 (*c* = 0.5 in H₂O) (lit.^[11a] [α]_D²⁵ = -8.4 (*c* = 0.4 in H₂O); m.p. 229–231°C (recrystallised from EtOH and Et₂O)); 99% *ee*. Compound (**1S,2R**)-**8**: Yield: 393 mg, 47%; [α]_D²⁵ = +22 (*c* = 0.28 in H₂O); m.p. 264–266°C (recrystallised from H₂O and (CH₃)₂CO); 98% *ee*.

The hydrolysis of ($\pm$)-**2** with a reduced amount of Lipolase (1 g, 16.6 mg mL⁻¹) under the same conditions afforded (**1R,2S**)-**20** and (**1S,2R**)-**8** in 66 h. Compound (**1R,2S**)-**20**: Yield: 492 mg, 47%; [α]_D²⁵ = -8.1 (*c* = 0.35 in H₂O); m.p. 229–233°C; 99% *ee*. Compound (**1S,2R**)-**8**: Yield: 390 mg, 47%; [α]_D²⁵ = +21.1 (*c* = 0.3 in H₂O); m.p. 260–261°C; 99% *ee*.

When **8** (100 mg) was treated with 22% HCl/EtOH (5 mL), (**1S,2R**)-**8**-HCl was obtained. Yield: 110 mg, 88%; [α]_D²⁵ = +8.3 (*c* = 0.33 in H₂O) (lit.^[11a] [α]_D²⁵ = +8.8 (*c* = 0.3 in H₂O); m.p. 230–233°C (recrystallised from EtOH and Et₂O)); 99% *ee*.

Data for 8: ¹H NMR (400 MHz, D₂O, 25°C, TMS): δ = 1.46–2.02 (m, 8H; 4 $\times$ CH₂), 2.63–2.67 (m, 1H; H-1), 3.45–3.49 ppm (m, 1H; H-2); elemental analysis calcd (%) for C₇H₁₃NO₂ (143.18): C 58.72, H 9.15, N 9.78; found: C 58.66, H 9.21, N 9.72.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) data are similar for **8**-HCl and **20**: δ = 1.43–2.02 (m, 8H; 4 $\times$ CH₂), 2.98–3.02 (m, 1H; H-1), 3.55–3.59 ppm (m, 1H; H-2); elemental analysis calcd (%) for C₇H₁₃NO₂·HCl (179.64): C 46.80, H 7.86, N 7.80; found for **8**-HCl: C 46.57, H 7.69, N 7.75; found for **20**: C 46.59, H 7.88, N 7.91.

Preparative-scale resolution of ethyl *cis*-(2-aminocyclohex-4-ene)-1-carboxylate ($\pm$)-3**:** Following the procedure described above, the reaction of racemic **3** (1 g, 5.90 mmol) and H₂O (53.2 μ L, 2.95 mmol) in *i*Pr₂O (60 mL) in the presence of Lipolase (1.8 g, 30 mg mL⁻¹) at 65°C afforded (**1R,2S**)-**21** and (**1S,2R**)-**9** in 72 h. Compound (**1R,2S**)-**21**: Yield: 472 mg, 45%; [α]_D²⁵ = -28 (*c* = 0.11 in H₂O), lit.^[11c] [α]_D²⁵ = -26 (*c* = 0.25 in H₂O); m.p. 205–210°C (recrystallised from EtOH and Et₂O); 98% *ee*. Compound (**1S,2R**)-**9**: Yield: 383 mg, 46%; [α]_D²⁵ = +34 (*c* = 0.26 in H₂O); m.p. 234–239°C (recrystallised from H₂O and (CH₃)₂CO); 98% *ee*.

When **9** (100 mg) was treated with 22% HCl/EtOH (5 mL), (**1S,2R**)-**9**-HCl was obtained. Yield: 110 mg, 88%; [α]_D²⁵ = +28 (*c* = 0.24 in H₂O); lit.^[11c] [α]_D²⁵ = +25.8 (*c* = 0.4 in H₂O); m.p. 205–209°C (recrystallised from EtOH and Et₂O); 99% *ee*.

Data for 9: ¹H NMR (400 MHz, D₂O, 25°C, TMS): δ = 2.25–2.51 (m, 4H; 2 $\times$ CH₂), 2.75–2.77 (m, 1H; H-1), 3.78–3.79 (m, 1H; H-2), 5.64–5.84 ppm (m, 2H; CHCH); elemental analysis calcd (%) for C₇H₁₁NO₂ (141.17): C 59.56, H 7.85, N 9.92; found: C 59.76, H 7.68, N 9.89.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) spectroscopic data are similar for **9**-HCl and **21**: δ = 2.32–2.55 (m, 4H; 2 $\times$ CH₂), 3.07–3.12 (m, 1H; H-1), 3.90–3.91 (m, 1H; H-2), 5.66–5.82 ppm (m, 2H; CHCH); elemental analysis calcd (%) for C₇H₁₁NO₂·HCl (177.63): C 47.33, H 6.81, N 7.89; found for **9**-HCl: C 47.33, H 6.81, N 7.89; found for **21**: C 47.45, H 6.77, N 7.99.

Preparative-scale resolution of ethyl *cis*-(2-aminocyclohex-3-ene)-1-carboxylate ($\pm$)-4**:** Following the procedure described above, the reaction of racemic **4** (1 g, 5.90 mmol) and H₂O (53.2 μ L, 2.95 mmol) in *i*Pr₂O (60 mL) in the presence of Lipolase (1.8 g, 30 mg mL⁻¹) at 65°C afforded (**1R,2S**)-**22** and (**1S,2R**)-**10** in 66 h. Compound (**1R,2S**)-**22**: Yield: 472 mg, 45%; [α]_D²⁵ = +121 (*c* = 0.21 in H₂O) (lit.^[11d] [α]_D²⁵ = +121.7 (*c* = 0.4 in H₂O)); m.p. 204–210°C (recrystallised from EtOH and Et₂O); 98% *ee*. Compound (**1S,2R**)-**10**: Yield: 383 mg, 46%; [α]_D²⁵ = -120 (*c* = 0.25 in H₂O); m.p. 234–236°C (recrystallised from H₂O and (CH₃)₂CO); 99% *ee*. When **10** (100 mg) was treated with 22% HCl/EtOH (5 mL), (**1S,2R**)-**10**-HCl was obtained. Yield: 98 mg, 78%; [α]_D²⁵ = -120 (*c* = 0.24 in H₂O) (lit.^[11d] [α]_D²⁵ = -121.4 (*c* = 0.4 in H₂O)); m.p. 205–211°C (recrystallised from EtOH and Et₂O); 99% *ee*.

Data for 10: ¹H NMR (400 MHz, D₂O, 25°C, TMS): δ = 1.85–2.18 (m, 4H; 2 $\times$ CH₂), 2.74–2.78 (m, 1H; H-1), 3.97–3.99 (m, 1H; H-2), 5.74–6.15 ppm (m, 2H; CHCH); elemental analysis calcd (%) for C₇H₁₁NO₂ (141.17): C 59.56, H 7.85, N 9.92; found: C 59.65, H 7.70, N 10.07.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) data are similar for **10**-HCl and **22**: δ = 1.92–2.21 (m, 4H; 2 $\times$ CH₂), 3.04–3.08 (m, 1H; H-1), 4.08–4.09 (m, 1H; H-2), 5.73–6.16 ppm (m, 2H; CHCH); elemental analysis calcd

(%) for $C_7H_{11}NO_2 \cdot HCl$ (177.63): C 47.33, H 6.81, N 7.89; found for **8**·HCl: C 47.22, H 6.97, N 7.81; found for **21**: C 47.33, H 6.82, N 7.69.

Preparative-scale resolution of ethyl *trans*-2-aminocyclohexane-1-carboxylate ((±)-5**):** Following the procedure described above, the reaction of racemic **5** (1 g, 5.83 mmol) and H_2O (52.5 μ L, 2.91 mmol) in iPr_2O (60 mL) in the presence of Lipolase (1.8 g, 30 mg mL⁻¹) at 65°C afforded (1*S*,2*S*)-**23** and (1*R*,2*R*)-**11** in 68 h. Compound (1*S*,2*S*)-**23**: Yield: 482 mg, 46%; $[\alpha]_D^{25} = +51$ ($c = 0.21$ in H_2O); m.p. 198–201°C (recrystallised from EtOH and Et₂O); 99% *ee*. Compound (1*R*,2*R*)-**11**: Yield: 404 mg, 48%; $[\alpha]_D^{25} = -65$ ($c = 0.26$ in H_2O), lit.^[15] $[\alpha]_D^{25} = -57.8$ ($c = 0.5$ in H_2O); m.p. 263–265°C (recrystallised from H_2O and $(CH_3)_2CO$); 99% *ee*.

When **11** (100 mg) was treated with 22% HCl/EtOH (5 mL), (1*R*,2*R*)-**11**·HCl was obtained. Yield: 111 mg, 89%; $[\alpha]_D^{25} = -51$ ($c = 0.21$ in H_2O); m.p. 194–197°C (recrystallised from EtOH and Et₂O); 99% *ee*.

Data for **11:** ¹H NMR (400 MHz, D₂O, 25°C, TMS): $\delta = 1.29$ –2.11 (m, 8H; 4×CH₂), 2.14–2.23 (m, 1H; H-1), 3.20–3.27 ppm (m, 1H; H-2); elemental analysis calcd (%) for $C_7H_{13}NO_2$ (143.18): C 58.72, H 9.15, N 9.78; found: C 58.84, H 9.16, N 9.60.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) spectroscopic data are similar for **11**·HCl and **23**: $\delta = 1.27$ –2.18 (m, 8H, 4×CH₂), 2.49–2.54 (m, 1H; H-1), 3.35–3.42 ppm (m, 1H; H-2); elemental analysis calcd (%) for $C_7H_{13}NO_2 \cdot HCl$ (179.64): C 46.80, H 7.86, N 7.80; found for **11**·HCl: C 46.57, H 7.69, N 7.75; found for **23**: C 46.77, H 7.91, N 7.82.

Preparative-scale resolution of ethyl *trans*-(2-aminocyclohex-4-ene)-1-carboxylate ((±)-6**):** Following the procedure described above, the reaction of racemic **6** (1 g, 5.90 mmol) and H_2O (53.2 μ L, 2.95 mmol) in iPr_2O (60 mL) in the presence of Lipolase (1.8 g, 30 mg mL⁻¹) at 65°C afforded (1*S*,2*S*)-**24** and (1*R*,2*R*)-**12** in 58 h. Compound (1*S*,2*S*)-**24**: Yield: 461 mg, 44%; $[\alpha]_D^{25} = +123$ ($c = 0.27$ in H_2O); m.p. 182–184°C (recrystallised from EtOH and Et₂O); 99% *ee*. Compound (1*R*,2*R*)-**12**: Yield: 375 mg, 45%; $[\alpha]_D^{25} = -152$ ($c = 0.29$ in H_2O); m.p. 268–270°C (recrystallised from H_2O and $(CH_3)_2CO$); 99% *ee*.

When **12** (100 mg) was treated with 22% HCl/EtOH (5 mL), (1*R*,2*R*)-**12**·HCl was obtained. Yield: 105 mg, 84%; $[\alpha]_D^{25} = -121$ ($c = 0.25$ in H_2O); m.p. 182–185°C (recrystallised from EtOH and Et₂O); 99% *ee*.

Data for **12:** ¹H NMR (400 MHz, D₂O, 25°C, TMS): $\delta = 1.17$ –2.61 (m, 5H; 2×CH₂ and H-1), 3.52–3.58 (m, 1H; H-2), 5.64–6.81 ppm (m, 2H; CHCH); elemental analysis calcd (%) for $C_7H_{11}NO_2$ (141.17): C 59.56, H 7.85, N 9.92; found: C 59.70, H 7.82, N 9.91.

The ¹H NMR (400 MHz, D₂O, 25°C, TMS) spectroscopic data are similar for **12**·HCl and **24**: $\delta = 2.22$ –2.57 (m, 4H, 2×CH₂), 2.85–2.89 (m, 1H; H-1), 3.70–3.72 (m, 1H; H-2), 5.67–5.77 ppm (m, 2H; CHCH); elemental analysis calcd (%) for $C_7H_{11}NO_2 \cdot HCl$ (177.63): C 47.33, H 6.81, N 7.89; found for **8**·HCl: C 47.28, H 6.97, N 7.77; found for **21**: C 47.15, H 6.79, N 7.66.

Acknowledgements

The authors acknowledge receipt of OTKA grants T 046440 and T 049407, GVOP-3.1.1.-2004-05-0255/3.0 and a Bolyai Fellowship for E.F.

- [1] a) F. Fülöp, *Chem. Rev.* **2001**, *101*, 2181–2204; b) F. Fülöp in *Studies in Natural Product Chemistry Vol. 22* (Ed.: Atta-ur-Rahman), Elsevier Science Publishers, **2000**, pp. 273–306; c) K. Park, M. J. Kurth, *Tetrahedron* **2002**, *58*, 8629–8659; d) J. Mittendorf, J. Benet-Buchholz, P. Fey, K. H. Mohrs, *Synthesis* **2003**, 136–140; e) A. Kuhl, M. G. Hahn, M. Dumić, J. Mittendorf, *Amino Acids* **2005**, *29*, 89–100; f) F. Fülöp, T. A. Martinek, G. K. Tóth, *Chem. Soc. Rev.* **2006**, *35*, 323–334.

- [2] a) Z. Szakonyi, S. Gyónfalvi, E. Forró, A. Hetényi, F. Fülöp, *Eur. J. Org. Chem.* **2005**, 4017–4023; b) F. Fülöp, M. Palkó, E. Forró, M. Dervarics, T. Martinek, *Eur. J. Org. Chem.* **2005**, 3214–3220.
- [3] a) K. M. Brashear, C. A. Hunt, B. T. Kucer, M. E. Duggan, G. D. Hartman, G. A. Rodan, S. B. Rodan, C. Leu, T. Prueksaritanont, C. Fernandez-Metzler, A. Barrish, C. F. Homnick, J. H. Hutchinson, P. J. Coleman, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3483–3486; b) D. L. Steer, R. A. Lew, P. Perlmutter, A. I. Smith, M. I. Aguilar, *Current Med. Chem.* **2002**, *9*, 811–822; c) C. Palomo, I. Ganboa, M. Oiarbide, G. T. Sciano, J. L. Miranda, *ARKIVOC* **2002**, Part V, 8–16; d) F. Fülöp, E. Forró, G. K. Tóth, *Org. Lett.* **2004**, *6*, 4239–4241.
- [4] a) D. H. Appela, L. A. Christianson, D. A. Klein, D. R. Powell, X. L. Huang, I. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381–384; b) E. A. Porter, X. F. Wang, H. S. Lee, B. Weisblum, S. H. Gellman, *Nature* **2000**, *404*, 565–565; c) T. Martinek, G. K. Tóth, E. Vass, M. Hollósi, F. Fülöp, *Angew. Chem.* **2002**, *114*, 1794–1797; *Angew. Chem. Int. Ed.* **2002**, *41*, 1718–1721; d) T. A. Martinek, I. M. Mándity, L. Fülöp, G. K. Tóth, E. Vass, M. Hollósi, E. Forró, F. Fülöp, *J. Am. Chem. Soc.* **2006**, *128*, 13539–13544.
- [5] a) S. G. Davies, G. Bhalay, *Tetrahedron: Asymmetry* **1996**, *7*, 1595–1596; b) Z. Szakonyi, T. Martinek, A. Hetényi, F. Fülöp, *Tetrahedron: Asymmetry* **2000**, *11*, 4571–4579.
- [6] a) S. Gedey, J. van der Eycken, F. Fülöp, *Org. Lett.* **2002**, *4*, 1967–1969; b) I. Kanizsai, Z. Szakonyi, R. Sillanpää, F. Fülöp, *Tetrahedron Lett.* **2006**, *47*, 9113–9116; I. Kanizsai, S. Gyónfalvi, Z. Szakonyi, R. Sillanpää, F. Fülöp, *Green Chem.* **2007**, *9*, 357–360.
- [7] *Enantioselective Synthesis of β -Amino Acids* (Eds.: E. Juaristi, V. A. Soloshonok), Wiley-Interscience, New York, **2005**.
- [8] a) P. Csomós, L. T. Kanerva, G. Bernáth, F. Fülöp, *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796; b) J. Kámán, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600; c) E. Forró, J. Árva, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 643–649.
- [9] Reviews: a) E. Forró, F. Fülöp, *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102; b) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854.
- [10] L. T. Kanerva, P. Csomós, O. Sundholm, G. Bernáth, F. Fülöp, *Tetrahedron: Asymmetry* **1996**, *7*, 1705–1716.
- [11] a) E. Forró, F. Fülöp, *Org. Lett.* **2003**, *5*, 1209–1212; b) E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2004**, *15*, 573–575; c) E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2004**, *15*, 2875–2880; d) E. Forró, F. Fülöp, *Chem. Eur. J.* **2006**, *12*, 2587–2592.
- [12] S. Katayama, N. Ae, R. Nagata, *Tetrahedron: Asymmetry* **1998**, *9*, 4295–4299.
- [13] R. C. Lloyd, M. C. Lloyd, M. E. B. Smith, K. E. Holt, J. P. Swift, P. A. Keene, S. J. C. Taylor, R. McCague, *Tetrahedron* **2004**, *60*, 717–728.
- [14] G. Bernáth, G. Stájer, A. E. Szabó, F. Fülöp, P. Sohár, *Tetrahedron*, **1985**, *41*, 1353–1365.
- [15] J. Priego, P. Flores, C. Ortiz-Nava, J. Escalante, *Tetrahedron: Asymmetry* **2004**, *15*, 3545–3549.

Received: February 13, 2007
Published online: May 11, 2007