Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 105–110

TETRAHEDRON: *ASYMMETRY*

Preparation of highly enantiopure β-amino esters by *Candida antarctica* lipase A

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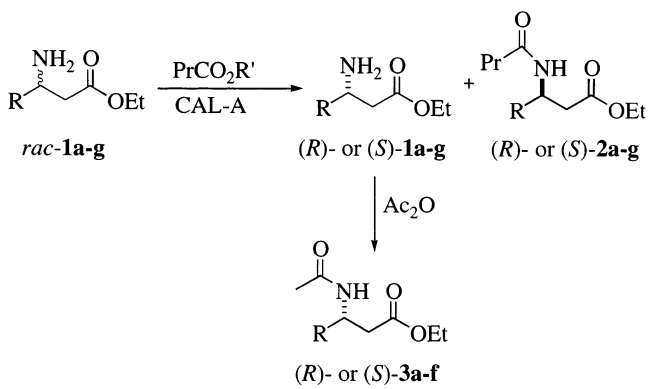
Received 27 November 2000; accepted 21 December 2000

Abstract—The enantioselectivities for the reactions of aliphatic β-substituted β-amino esters [$RCH(NH_2)CH_2CO_2Et$ with R = Me, Et, *n*-Pr, *i*-Pr, $CHEt_2$, cyclohexyl and Ph] with butyl butanoate in neat butyl butanoate and with 2,2,2-trifluoroethyl butanoate in diisopropyl ether were studied in the presence of *Candida antarctica* lipase A. Enantioselectivities ranging from good (E = 70–100) to excellent (E>100) were commonly observed, allowing gram-scale resolution of the substrates.

1. Introduction

Optically active β-amino acids are gaining ever greater significance in synthetic chemistry in connection with the design and synthesis of pharmaceutical drugs and natural products, and with the unique secondary structures and activation which they offer for peptides.[1–8] In recent years, we have focused on the preparation of enantiopure β-amino acids and their derivatives by exploiting the potential of lipases as chiral catalysts.[9–13] Special attention has been paid to the kinetic resolution of alicyclic and acyclic β-amino esters. In work with such bifunctional substrates, enzymatic chemo- and regioselectivity are of utmost importance in directing the reaction to the desired functional group. Another point is that enzymes, like other catalysts, simply catalyze the approach to equilibrium for a given reaction. The existence of an equilibrium can easily destroy the possibility of obtaining enantiopure products. For this reason, enzymatic resolution based on an acylation reaction (e.g. at the amino group) with an appropriate achiral acyl donor (activated esters, acid anhydrides, etc.) is often more applicable than a deacylation reaction at an ester group.[14,15]

By means of an enzymatic acylation strategy, ten alicyclic β-amino acids were previously resolved through the use of highly enantioselective amide formation between the amino group of racemic *cis*- and *trans*-amino esters and 2,2,2-trifluoroethyl carboxylates in diisopropyl ether in the presence of *Pseudomonas cepacia* lipase PS or *Candida antarctica* lipase A (CAL-A).[11,16] The expectation that this method would generally allow resolution of aliphatic β-amino esters proved overly optimistic, as the lipases exhibited either low chemoselectivity (lipase PS), leading to multiple products, or low enantioselectivity (CAL-A) when applied for the reaction of acyclic ethyl 3-aminobutanoate **1a**.[13] Low chemoselectivity was observed when the alkyl group of an achiral carboxylic ester differed



rac-**1a-g** (*R*)- or (*S*)-**1a-g** (*R*)- or (*S*)-**2a-g**

(*R*)- or (*S*)-**3a-f**

Scheme 1. a: R = Me; **b**: R = Et; **c**: R = *n*-Pr; **d**: R = *i*-Pr; **e**: R = $CHEt_2$; **f**: R = cyclohexyl; **g**: R = Ph, R′ = CH_2CF_3 or Bu.

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0957-4166/01/$ - see front matter
PII: S0957-4166(01)00002-7

Table 1. Enantioselectivities of CAL-A preparation (40 mg/mL) for the reactions of **1a–g** (0.1 M) in butyl butanoate and with 2,2,2-trifluoroethyl butanoate (0.2 M) in diisopropyl ether at room temperature

	R	$PrCO_2CH_2CF_3$			$PrCO_2Bu$		
		Conv. (%)	Time (h)	*E*	Conv. (%)	Time (h)	*E*
1a	Me	50	25	6±2	51	22	32±1
1b	Et	52	1.33	168±11	50	24	256±36
1c	*n*-Pr	55	1.5	72	50	9	>100
1d	*i*-Pr	49	1.5	106±8	50	130	115±9
1e	$CHEt_2$	52	4	38±3	33	16 days	2
1f	Cyclohexyl	53[a]	1	>100	23	48	9±7
1g	Ph	52	0.33	75±4	52	70	29±3

[a] Amount of enzyme preparation 30 mg/mL.

from that of the substrate. Enzyme screening revealed other lipases besides lipase PS, for example *C. antarctica* lipase B (CAL-B),[16] which displayed low chemoselectivity for the reactions of **1a**. Under optimized reaction conditions, involving either acylation of the NH_2 group followed by transesterification at the CO_2Et group or transesterification followed by acylation, the successful sequential resolution of **1a** with CAL-B was achieved.[13]

The effect of substrate structure on the enantioselectivity of CAL-A was studied in the acylation of **1a–g** with butyl butanoate in neat butyl butanoate and with 2,2,2-trifluoroethyl butanoate in diisopropyl ether (Scheme 1). Compounds **1b–g** were resolved on a gram scale.

2. Results and discussion

In accordance with the previously observed excellent chemoselectivity of CAL-A,[13] the acylation products **2a–g** were the only detectable products for the acylation of racemic **1a–g** with 2,2,2-trifluoroethyl butanoate in diisopropyl ether and with butyl butanoate in neat butyl butanoate (Table 1; Scheme 1). The reactions of **1a–g** with 2,2,2-trifluoroethyl butanoate in diisopropyl ether approach a conversion of approximately 50% faster than the reactions with butyl butanoate as acyl donor and solvent. This can readily be understood because the 2,2,2-trifluoroethyl ester as an activated alkyl ester is considerably more prone than the 'normal' esters (butyl butanoate) to ester formation with the Ser-OH of the enzyme, i.e. to the formation of a butanoyl–enzyme intermediate in the two-step catalytic mechanism.[15]

For the CAL-A-catalyzed acylation of **1b–g**, good (E= 40–100) to excellent (E>100) enantioselectivities were observed with at least one of the two acyl donors, although solvent effects and/or structural effects caused by an acyl donor can also be seen in the results. Clearly, aminopentanoate **1b** has the optimal structure for CAL-A-catalyzed enantioselective acylation. As concerns the two-step reaction mechanism, enantiodiscrimination takes place in the second step when the butanoyl–enzyme intermediate selectively reacts with one of the substrates **1a–g**, leading to formation of the corresponding optically-active amide product (one of **2a–g**). On this basis, solvent effects on enantioselectivity can be postulated as the main cause for the higher enantioselectivity observed with straight-chain amino esters such as **1a–c** in $PrCO_2Bu$ as compared with the reactions in diisopropyl ether. Similarly, diisopropyl ether is favorable when the branched-chain (including cyclic) substrates **1e–g** react. The (*R*)-configuration was assigned to the reactive enantiomers for compounds **1a** and **b**, while the (*S*)-enantiomer was the reactive enantiomer for compounds **1d** and **g**. In this way the steric arrangement of the substituents around the stereocenter is the same in the above cases (the priority sequence of the substituents changes with the structure of β-substituent R in Scheme 1). The determination of the above absolute configurations is based on the known specific rotations of the free acids or esters (see Section 3). Ion-exchange chromatography allowed the preparation of the free amino acids. In this procedure, some racemization evidently took place as seen in low values of specific rotations. On the assumption that the enzyme has straightforward stereochemical demands, it is concluded that for the CAL-A-catalyzed acylation of **1c** the (*R*)-enantiomer reacts faster, while for that of **1e** and **f** the (*S*)-enantiomer reacts faster.

The basic aim for economic enzymatic kinetic resolution should be conditions where the two enantiomers are obtained simultaneously in enantiopure form from one reaction. The enantiomer ratio, E, as a measure of the enantioselective behavior of the biocatalytic system can be used in the prediction of such conditions, E values greater than 100 being excellent. Compounds **1b–g** were successfully resolved on a gram scale through the use of CAL-A, the best method being chosen according to the E values in Table 1. The results in Table 2 clearly indicate the applicability of CAL-A for the resolution of both alicyclic β-amino acid esters[11] and acyclic compounds; **1e** is an exception in that it allows only the preparation of the less reactive enantiomer in highly enantiopure form. This was also the case for the reaction of **1a** in butyl butanoate, with E=32 (Table 1). Compound **1a** was earlier successfully resolved by using CAL-B catalysis in butyl butanoate (row 1, Table 2).[13] This is an in situ sequential resolution following both transesterification–acylation (the main route) and acylation–interesterification sequences,

where the two functional groups (NH_2 and ethyl ester) of **1a** have both reacted with the achiral ester. In this case the preparation of two highly enantiopure fractions [(*S*)-P_2 (Bu in place of Et in **1a**)+(*S*)-(**1a**) and (*R*)-P_3 (Bu in place of Et in **2a**)] at ca. 80% conversion becomes possible.

3. Materials and methods

3-Amino-4-methylpentanoic acid, 3-amino-4-ethylhexanoic acid, 3-amino-3-cyclohexylpropanoic acid, and 3-amino-3-phenylpropanoic acid were prepared by known procedures,[17–20] and transformed to the corresponding ethyl esters with thionyl chloride and dry ethanol or butanol. Acyl donors were prepared from the corresponding acid chlorides and alcohols. CAL-A (*C. antarctica* lipase A, Chirazyme L5) was purchased from Boehringer–Mannheim.[16] The enzyme was immobilized on Celite in the presence of sucrose before use, giving a final lipase content of 10% (w/w) in the lipase preparation.[21] The solvents were of the best analytical grade from Lab Scan Ltd.

As an example of a typical small-scale experiment, ethyl 3-aminobutanoate (**1a**, 0.1 M) and hexadecane (0.01 M, an internal standard) were dissolved in diisopropyl ether (2.5 mL), and an acyl donor (0.2 M) was added. The addition of the enzyme preparation (40 mg/mL) started the reaction. The reaction mixture was shaken at room temperature. The progress of the reaction and the e.e. values of the products were followed by taking samples (0.1 mL) at intervals and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). In the case of ethyl 3-amino-3-phenylpropanoate **1g**, the enantiomers were separated on a Chrompack CP-Chirasil-L-Valine column (25 m). For good baseline separation, the unreacted amino group in the sample was derivatized with acetic anhydride in the presence of pyridine containing 1% 4-*N*,*N*-dimethylaminopyridine (DMAP). The separation of the enantiomers of **2c** was unsuccessful by the GLC method so **2c** was first separated by column chromatography and then converted to the acetamide **3c** for $e.e._2$ determination as described later. Conversions were determined using $e.e._1$ and $e.e._2$ values except for the reaction of **1c**, where the conversion was determined by internal standard. The determination of *E* was based on the equation $E=\ln[(1-e.e._1)/(1-e.e._1/e.e._2)]/\ln[(1-e.e._1)/(1+e.e._1/e.e._2)]$ with $c=e.e._1/(e.e._1+e.e._2)$, as derived from the original equations of Chen et al.[22] Linear regression was applied to obtain *E* as the slope of the line.

^{1}H NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or D_2O solutions at ambient temperature on Bruker 200/Aspect 3000 or Bruker AM 400 spectrometers. Chemical shifts are given in δ (ppm) relative to TMS ($CDCl_3$, DMSO-d_6) or to TSP (D_2O) as internal standards; multiplicities were recorded as s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet) and om (overlapping multiplet). MS spectra were recorded on a VG Analytical 7070E instrument equipped with a Vaxstation 3100 M 76 computer. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II elemental analyzer. Optical rotations were measured with a JASCO DIP-360 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2/g.

3.1. Preparation of 3-aminopentanoic acid and 3-aminohexanoic acid

A mixture of (*E*)-2-pentenoic acid or (*E*)-2-hexenoic acid (0.10 mol), benzylamine (10.72 g, 0.1 mol) and dry pyridine (35 mL) was stirred at 120–130°C for 3 h. Acetone (50 mL) was then added and the mixture was cooled to ambient temperature. After standing overnight, the crystals of 3-(benzylamino)pentanoic or hexanoic acid that separated out were filtered off, washed with acetone and recrystallized from methanol–acetone.

3-(Benzylamino)pentanoic acid: 14.80 g, 0.071 mol; mp 169–170°C. Analysis calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76%. Found: C, 69.27; H, 8.04; N, 6.55%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 0.99 (3H, t, $J=7.5$, CH_3), 1.69 (1H, m, CH_2CH_3), 1.88 (1H, m, CH_2CH_3), 2.50 (1H, dd, $J=16.9$, 7.4, $CHCH_2CO$), 2.66 (1H, dd, $J=16.9$, 4.8, $CHCH_2CO$), 3.40 (1H, m, CHNH), 4.26 (2H, m, $CH_2C_6H_5$), 7.50 (5H, m, C_6H_5).

3-(Benzylamino)hexanoic acid: 14.65 g, 0.066 mol; mp 160–162°C. Analysis calcd for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33%. Found: C, 70.39; H, 8.42; N, 6.20%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 0.93 (3H, t, $J=7.3$, CH_3), 1.39 (2H, m, CH_2CH_3), 1.65 (1H, m, $CH_2CH_2CH_3$), 1.79 (1H, m, $CH_2CH_2CH_3$), 2.50 (1H, dd, $J=17.0$, 7.5, $CHCH_2CO$), 2.66 (1H, dd, $J=17.0$, 4.8, $CHCH_2CO$), 3.46 (1H, m, CHNH), 4.27 (2H, m, $CH_2C_6H_5$), 7.50 (5H, m, C_6H_5).

Table 2. Gram-scale resolutions of ethyl 3-aminocarboxylates **1a**–**g**

	R	Acyl donor	Enzyme	Time (h)	Conversion (%)	$e.e._1$ (%)	$e.e._2$ (%)
1a	Me	$PrCO_2Bu$	CAL-B	2	65	96 (*S*)	99 (*R*)[a]
1b	Et	$PrCO_2Bu$	CAL-A	15	50	97 (*S*)	97 (*R*)
1c	*n*-Pr	$PrCO_2Bu$	CAL-A	9.5	48	96 (*S*)	98 (*R*)[b]
1d	*i*-Pr	$PrCO_2CH_2CF_3$	CAL-A	2.5	53	99 (*R*)	87 (*S*)
1e	$CHEt_2$	$PrCO_2CH_2CF_3$	CAL-A	13	55	98 (*R*)	79 (*S*)
1f	Cyclohexyl	$PrCO_2CH_2CF_3$	CAL-A	1.2	51	99 (*R*)	99 (*S*)
1g	Ph	$PrCO_2CH_2CF_3$	CAL-A	1	52	98 (*R*)	90 (*S*)

[a] Product butyl 3-amino-*N*-butanoylbutanoate after sequential resolution.[13]
[b] $E.e._2$ determined after conversion to the acetamide.

3-(Benzylamino)pentanoic or hexanoic acid (0.050 mol) was dissolved in a 1:1 mixture of methanol and water (100 mL), and 20% palladium hydroxide on carbon (1.0 g) was added to the solution. The mixture was hydrogenated in an autoclave at 70°C and 90 bar for 24 h. The catalyst was removed by filtration, the filtrate was evaporated to dryness and the residue was crystallized on treatment with acetone. The crystals were filtered off, washed with acetone and recrystallized from methanol–acetone.

3-Aminopentanoic acid: 4.92 g, 0.042 mol; mp 189–191°C (mp. literature[20] 179–180°C). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 0.99 (3H, t, $J=7.5$, CH_2CH_3), 1.69 (2H, m, CH_2CH_3), 2.44 (1H, dd, $J=16.6$, 8.3, $CHCH_2CO$), 2.57 (1H, dd, $J=16.6$, 4.9, $CHCH_2CO$), 3.43 (1H, m, $CHNH_2$).

3-Aminohexanoic acid: 5.68 g, 0.043 mol; mp 210–211°C. Analysis calcd for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68%. Found: C, 55.14; H, 9.80; N, 10.45%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 0.94 (3H, t, $J=7.3$, CH_3), 1.41 (2H, m, CH_2CH_3), 1.63 (2H, m, $CH_2CH_2CH_3$), 2.43 (1H, dd, $J=16.6$, 8.3, $CHCH_2CO$), 2.57 (1H, dd, $J=16.6$, 4.9, $CHCH_2CO$), 3.51 (1H, m, $CHNH_2$).

3.2. Gram-scale resolution of ethyl 3-aminopentanoate (1b; R=Et)

Racemic **1b** (1.32 g, 9.1 mmol) was dissolved in butyl butanoate (90 mL) and CAL-A (3.6 g, 40 mg/mL) was added. The mixture was stirred at room temperature. The reaction was stopped at 50% conversion after 15 h by filtering off the enzyme. After evaporation, the residue was dissolved in diisopropyl ether (50 mL) and the unreacted substrate was derivatized with acetic anhydride (0.5 mL; 5.3 mmol) in the presence of pyridine containing 1% DMAP (0.1 mL) by stirring overnight. The products were separated on silica gel by elution with petroleum ether–propan-2-ol (100:6), the elution sequence being product **2b** before the acetamide of unreacted **1b**.

Compound (*R*)-**2b**: 0.73 g, 3.39 mmol; $[\alpha]_D^{20}$ +21.4 ($c=$ 1.00, MeOH); e.e. 97%; $M=215$ according to MS. Analysis calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51%. Found: C, 61.11; H, 9.35; N, 6.13%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 0.92 (3H, t, $J=7.4$, $CHCH_2CH_3$), 0.94 (3H, t, $J=7.3$, $CH_2CH_2CH_3$), 1.27 (3H, t, $J=7.1$, OCH_2CH_3), 1.62 (4H, m, $CHCH_2CH_3$, $CH_2CH_2CH_3$), 2.15 (2H, t, $J=7.4$, $CH_2CH_2CH_3$), 2.52 (2H, dd, $J=5.2$, 1.0, $CHCH_2CO$), 4.14 (2H, q, $J=7.1$, OCH_2CH_3), ~4.2 (1H, om, $CHNH$), 6.14 (1H, br d, $J=7.8$, NH).

Compound (*S*)-**3b**: 0.21 g, 1.12 mmol; $[\alpha]_D^{20}$ −27.5 ($c=$ 1.01, MeOH); e.e. 97%; $M=187$ according to MS. Analysis calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48%. Found: C, 57.49; H, 9.43; N, 7.63%. ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 0.92 (3H, t, $J=7.4$, $CHCH_2CH_3$), 1.27 (3H, t, $J=7.2$, OCH_2CH_3), 1.56 (2H, m, $CHCH_2CH_3$), 1.98 (3H, s, $COCH_3$), 2.52 (2H, m, $CHCH_2CO$), 4.14 (2H, q, $J=7.2$, OCH_2CH_3), ~4.18 (1H, om, $CHNH$), 6.22 (1H, br s, NH).

For determination of the absolute configuration, the acetamide of unreacted **1b** (30 mg) was boiled in 18% HCl (5 mL) for 1 h and purified by ion-exchange chromatography (Dowex 50, basic). (*S*)-3-Aminopentanoic acid {$[\alpha]_D^{25}$ +40.1 ($c=0.99$, H_2O); literature[23] value for the (*S*)-acid: $[\alpha]_D^{22}$ +39.5 ($c=0.97$, H_2O), e.e.= 91%}.

3.3. Gram-scale resolution of ethyl 3-aminohexanoate (1c; R=*n*-Pr)

Racemic **1c** (2.0 g, 12.6 mmol) was dissolved in butyl butanoate (126 mL), and Chirazyme L5 (3.78 g, 30 mg/mL) and heptadecane (0.6 g; internal standard) were added. The mixture was stirred at room temperature. The reaction was stopped at 48% conversion after 9.5 h. The unreacted substrate was derivatized with acetic anhydride, followed by purification of the products by column chromatography as above.

Compound (*R*)-**2c**: 0.46 g, 2.01 mmol; $[\alpha]_D^{20}$ +19.5 ($c=$ 1.02, MeOH); e.e. 98% (compound **2c** (0.75 g) was converted to the acetamide through acidic hydrolysis followed by esterification and derivatization with acetic anhydride); $M=229$ according to MS. Analysis calcd for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11%. Found: C, 62.47; H, 9.85; N, 6.35%. ^{1}H NMR (400 MHz ($CDCl_3$) δ (ppm): 0.91 (3H, t, $J=7.3$, $CHCH_2CH_2CH_3$), 0.94 (3H, t, $J=7.4$, $COCH_2CH_2CH_3$), 1.26 (3H, t, $J=7.1$, OCH_2CH_3), 1.35 (2H, m, $CHCH_2CH_2CH_3$), 1.51 (2H, m, $CHCH_2CH_2CH_3$), 1.65 (2H, m, $COCH_2CH_2CH_3$), 2.14 (2H, t, $J=7.5$, $COCH_2CH_2CH_3$), 2.49 (1H, dd, $J=15.8$, 5.2, $CHCH_2CO$), 2.55 (1H, dd, $J=15.8$, 5.2, $CHCH_2CO$), 4.14 (2H, m, OCH_2CH_3), 4.26 (1H, m, $CHNH$), 6.06 (1H, br d, $J=8.3$, NH).

Compound (*S*)-**3c**: 0.45 g, 2.24 mmol; $[\alpha]_D^{20}$ −19.8 ($c=$ 1.00, MeOH); e.e. 96%; $M=201$ according to MS. Analysis calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96%. Found: C, 59.49; H, 9.93; N, 6.58%. ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 0.91 (3H, t, $J=7.3$, $CH_2CH_2CH_3$), 1.27 (3H, t, $J=7.1$, OCH_2CH_3), 1.35 (2H, m, $CH_2CH_2CH_3$), 1.51 (2H, m, $CH_2CH_2CH_3$), 1.97 (3H, s, $COCH_3$), 2.48 (1H, dd, $J=15.9$, 5.1, $CHCH_2CO$), 2.55 (1H, dd, $J=15.9$, 5.1, $CHCH_2CO$), 4.14 (2H, m, OCH_2CH_3), 4.25 (1H, m, $CHNH$), 6.06 (1H, br d, $J=7.5$, NH).

3.4. Gram-scale resolution of ethyl 3-amino-4-methylpentanoate (1d; R=*i*-Pr)

Racemic **1d** (2.10 g, 13.2 mmol) was dissolved in diisopropyl ether (130 mL), and 2,2,2-trifluoroethyl butanoate (4.49 g, 26.4 mmol) and CAL-A (5.2 g, 40 mg/mL) were added. The reaction was stopped at 53% conversion after 2.5 h. The unreacted substrate was derivatized with acetic anhydride, followed by purification of the products by column chromatography as above.

Compound (*S*)-**2d**: 1.09 g, 4.75 mmol; $[\alpha]_D^{25}$ +8.0 (c= 0.99, MeOH); e.e. 87%; M=229 according to MS. Analysis calcd for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11%. Found: C, 63.14; H, 10.05; N, 6.42%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 0.75–95 (9H, om, $CH(CH_3)_2$, $CH_2CH_2CH_3$), 1.18 (3H, t, J=7.1, OCH_2CH_3), 1.47–1.85 (3H, om, $CH(CH_3)_2$, $CH_2CH_2CH_3$), 2.10 (2H, t, J=7.4, $COCH_2CH_2CH_3$), 2.44 (2H, d, J=5.5, $CHCH_2CO$), 4.00 (1H, om, CHNH), 4.06 (2H, q, J=7.1, OCH_2CH_3), 6.13 (1H, br d, J=8.3, NH).

Compound (*R*)-**3d**: 1.08 g, 5.37 mmol; $[\alpha]_D^{25}$ −11.0 (c= 0.99, MeOH); e.e. 99%; M=201 according to MS. Analysis calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96%. Found: C, 59.27; H, 9.41; N, 6.81%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 0.83 (6H, d, J=6.7, $CH(CH_3)_2$), 1.17 (3H, t, J=7.2, OCH_2CH_3), 1.72 (1H, m, $CH(CH_3)_2$), 1.89 (3H, s, $COCH_3$), 2.42 (2H, d, J=5.6, $CHCH_2CO$), 4.00 (1H, om, CHNH), 4.04 (2H, q, J=7.2, OCH_2CH_3), 6.09 (1H, br s, NH).

For determination of the absolute configuration, hydrolysis of **2d** (30 mg) was carried out in 18% HCl (5 ml) by heating under reflux for 1 h, and the product was purified by ion-exchange chromatography (Dowex 50, basic). (*S*)-3-Amino-4-methylpentanoic acid {$[\alpha]_D^{25}$ −18.1 (c=1.05, H_2O); literature[23] value for the (*R*)-acid: ($[\alpha]_D^{22}$ +40.3 (c=1.02, H_2O), e.e.=98%}.

3.5. Gram-scale resolution of ethyl 3-amino-4-ethylhexanoate (1e; R=CHEt$_2$)

Racemic **1e** (2.54 g, 13.6 mmol) was dissolved in diisopropyl ether (136 ml), and 2,2,2-trifluoroethyl butanoate (4.63 g, 27.2 mmol) and CAL-A (5.44 g, 40 mg/ml) were added. The reaction was stopped at 55% conversion after 13 h by filtering off the enzyme. The unreacted substrate was derivatized with acetic anhydride, followed by purification of the products by column chromatography as above.

Compound (*S*)-**2e**: 1.40 g, 5.44 mmol; $[\alpha]_D^{25}$ +4.3 (c= 1.02, MeOH); e.e. 79%; M=257 according to MS. Analysis calcd for $C_{14}H_{27}NO_3$: C, 65.33; H, 10.57; N, 5.44%. Found: C, 64.94; H, 10.13; N, 5.58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (3H, t, J=7.5, $CHCH_2CH_3$), 0.90 (3H, t, J=7.4, $CHCH_2CH_3$), 0.94 (3H, t, J=7.5, $CH_2CH_2CH_3$), 1.26 (3H, t, J=7.1, OCH_2CH_3), 1.28–1.48 (5H, om, $CH(CH_2CH_3)_2$), 1.65 (2H, m, $CH_2CH_2CH_3$), 2.16 (2H, t, J=7.4, $CH_2CH_2CH_3$), 2.51 (2H, d, J=5.5, $CHCH_2CO$), 4.13 (2H, m, OCH_2CH_3), 4.32 (1H, m, CHNH), 6.06 (1H, br d, J=8.9, NH).

Compound (*R*)-**3e**: 0.77 g, 3.36 mmol; $[\alpha]_D^{25}$ −8.7 (c= 1.02, MeOH); e.e. 98%; M=229 according to MS. Analysis calcd for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11%. Found: C, 63.13; H, 10.27; N, 6.36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (3H, t, J=7.3, $CHCH_2CH_3$), 0.90 (3H, t, J=7.3, $CHCH_2CH_3$), 1.26 (3H, t, J=7.1, OCH_2CH_3), 1.25–1.46 (5H, om, $CH(CH_2CH_3)_2$), 1.98 (3H, s, $COCH_3$), 2.50 (2H, m, $CHCH_2CO$), 4.14 (2H, m, OCH_2CH_3), 4.30 (1H, m, CHNH), 6.04 (1H, br d, J=8.9, NH).

3.6. Gram-scale resolution of ethyl 3-amino-3-cyclohexylpropanoate (1f; R=cyclohexyl)

Racemic **1f** (3.00 g, 15 mmol) was dissolved in diisopropyl ether (150 mL), and 2,2,2-trifluoroethyl butanoate (5.13 g, 30 mmol) and CAL-A (3.0 g, 20 mg/mL) were added. The reaction was stopped after 70 min at 51% conversion by filtering off the enzyme. The unreacted substrate was derivatized with acetic anhydride, followed by purification of the products by column chromatography as above. The eluted products were recrystallized.

Compound (*S*)-**2f**: 0.75 g, 2.78 mmol; mp 70–72°C (n-hexane); $[\alpha]_D^{25}$ −9.8 (c=1.00, MeOH); e.e. 99%; M= 269 according to MS. Analysis calcd for $C_{15}H_{27}NO_3$: C, 66.88; H, 10.10; N, 5.20%. Found: C, 66.79; H, 9.72; N, 4.84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.90–1.04 (2H, om, $CH(CH_2)_5$), 0.94 (3H, t, J=7.4, $CH_2CH_2CH_3$), 1.05–1.30 (3H, om, $CH(CH_2)_5$), 1.26 (3H, t, J=7.1, OCH_2CH_3), 1.47 (1H, m, $CH(CH_2)_5$), 1.60–1.80 (7H, om, $CH(CH_2)_5$, $CH_2CH_2CH_3$), 2.15 (2H, t, J=7.5, $CH_2CH_2CH_3$), 2.52 (2H, m, $CHCH_2CO$), 4.07 (1H, om, CHNH), 4.14 (2H, om, OCH_2CH_3), 6.02 (1H, br d, J=9.2, NH).

Compound (*R*)-**3f**: 0.61 g, 2.53 mmol; mp 103–104°C (n-hexane–EtOAc); $[\alpha]_D^{25}$ +6.3 (c=1.00, MeOH); e.e. 99%; M=241 according to MS. Analysis calcd for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80%. Found: C, 64.39; H, 9.48; N, 5.72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.97 (2H, m, $CH(CH_2)_5$), 1.05–1.30 (3H, om, $CH(CH_2)_5$), 1.26 (3H, t, J=7.1, CH_2CH_3), 1.47 (1H, m, $CH(CH_2)_5$), 1.60–1.82 (5H, om, $CH(CH_2)_5$), 1.98 (3H, s, $COCH_3$), 2.52 (2H, m, $CHCH_2CO$), 4.05 (1H, m, CHNH), 4.14 (2H, m, OCH_2CH_3), 6.05 (1H, br d, J=9.2, NH).

3.7. Gram-scale resolution of ethyl 3-amino-3-phenylpropanoate (1g; R=Ph)

Racemic **1g** (2.51 g, 13 mmol) was dissolved in diisopropyl ether (130 mL), and 2,2,2-trifluoroethyl butanoate (4.42 g, 26 mmol) and CAL-A (3.9 g, 30 mg/mL) were added. The reaction was stopped after 1 h at 52% conversion by filtering off the enzyme. The solvent was evaporated off and the residue was dissolved in diethyl ether. Gaseous hydrogen chloride bubbled through the mixture precipitated the unreacted substrate. The solvent fraction was evaporated and the residue was purified by column chromatography, with petroleum ether–propan-2-ol (10:1) as eluent.

Compound (*S*)-**2g**: 1.50 g, 5.70 mmol; $[\alpha]_D^{25}$ −60.6 (c= 1.02, MeOH); e.e. 90%; M=263 according to MS. Analysis calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32%. Found: C, 67.94; H, 8.21; N, 5.64%. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 0.93 (3H, t, J=7.1, $CH_2CH_2CH_3$), 1.16 (3H, t, J=7.1, OCH_2CH_3), 1.66 (2H, m, $CH_2CH_2CH_3$), 2.19 (3H, t, J=7.4,

$CH_2CH_2CH_3$), 2.79 (1H, dd, $J=15.5$, 6.0, $CHCH_2CO$), 2.91 (1H, dd, $J=15.5$, 6.1, $CHCH_2CO$), 4.06 (2H, q, $J=7.1$, OCH_2CH_3), 5.43 (1H, m, CHNH), 6.78 (1H, br d, $J=8.3$, NH), 7.29 (5H, m, C_6H_5).

Hydrochloride of (*R*)-**1g**: 1.24 g, 5.40 mmol; mp 115–116°C; $[\alpha]_D^{25}$ −5.9 ($c=1.01$, MeOH), literature[24] value for the hydrochloride of (*S*)-**1g**: $[\alpha]_D^{22}$ +5.8 ($c=1.0$, MeOH); e.e. 98%; $M=193$ according to MS. Analysis calcd for $C_{11}H_{16}ClNO_2$: C, 57.52; H, 7.02; N, 6.10%. Found: C, 58.09; H, 7.14; N, 6.13%. ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.06 (3H, t, $J=7.1$, CH_2CH_3), 3.01 (1H, dd, $J=15.9$, 9.5, $CHCH_2CO$), 3.27 (1H, dd, $J=15.9$, 5.3, $CHCH_2CO$), 3.97 (2H, q, $J=7.0$, CH_2CH_3), 4.55 (1H, m, CHNH$_2$), 7.39 (3H, m, C_6H_5), 7.60 (2H, m, C_6H_5), 8.92 (3H, br s, NH_2, HCl).

Acknowledgements

S.G. is grateful for a grant from the Centre for International Mobility (CIMO) in Finland. Support from the Technology Development Centre (TEKES) in Finland and from MKM (Grant No. FKFP 0535/1999) in Hungary is also gratefully acknowledged.

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