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Candida antarctica lipase A—a powerful catalyst for the resolution of heteroaromatic β-amino esters

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Abstract—Enantioselective acylations of 3-amino-3-heteroarylpropanoates ($ArCH(NH_2)CH_2CO_2Et$; Ar = 2- or 3-thienyl or -furyl) were performed in the presence of *Candida antarctica* lipase A. As a result of the excellent chemo- and enantioselectivities (E >100), gram-scale resolutions were carried out in ethyl butanoate. The hydrochloride salts of the unreacted R substrates and the butanamides of the reactive S enantiomers were thus prepared.

1. Introduction

Some β-amino acids and esters exhibit interesting pharmacological effects and many β-amino acids are of significance as constituents of natural products and pharmaceutical drug compounds.[1–6] β-Amino acids are used for the preparation of β-peptides, which adopt well-defined three-dimensional structures[1,6–10] and they also serve as precursors for β-lactams and heterocyclic compounds.[6] Consequently, enantiopure β-amino acids have become important and challenging targets in synthetic organic chemistry.

Enzymes have gained wide acceptance as asymmetric catalysts in organic chemistry, especially with the aim of the preparing enantiopure compounds. As concerns β-amino esters, our research has long been focused on the lipase-catalysed kinetic resolutions of alicyclic 2-aminocarboxylic and 3-amino-3-alkyl- or 3-amino-3-phenylpropanoic esters.[11–14] In these reactions, CAL-A (lipase A from *Candida antarctica*) has proven exceptionally effective for acylation reactions in organic solvents.[11,13] Other studies relate to catalysis with penicillin and penicillin G acylase for *N*-phenylacetylation of the amino group or hydrolysis of the *N*-phenylacetyl group in 3-aminopropanoic acid analogues containing aliphatic, aromatic and heteroaromatic substituents at position 2 or 3.[15–20] The level of enantioselectivity in the acylase methods is somewhat obscure because E values are not given and enantiomeric purities either refer to those obtained after recrystallization or are known for only one of the resolution products. Accordingly, we considered it of interest to study the CAL-A-catalysed resolution of heteroaryl-substituted β-amino esters **1**–**4** (Scheme 1). Furthermore, incorporation of an appropriate (*S*)-3-amino-3-heteroarylpropanoic acid (or ester) into nipecotic acid is known to enhance antithrombotic activity in platelet fibrinogen receptor antagonists.[19,20]

CAL-A is a calcium dependent, thermostable lipase that is reported to be highly active in a non-specific manner.[21] The enzyme is unique because of its reported S_N2 preference towards triacylglycerols.[22] Although only rarely used for enantioselective reactions, the success of CAL-A for the resolution of amino esters is conspicuous.[11,13,23,24] In this work, it was essential that CAL-A adsorbed on Celite in the presence of sucrose was used. For instance, the results for the acylation of ethyl *cis*-2-aminocyclohexyl carboxylate (the first application of CAL-A in our laboratory)[11] with 2,2,2-trifluoroethyl acetate in the presence of native ($v_o = 3$ μmol mg^{-1} min^{-1}; $E = 7$) and immobilized ($v_o = 12$ μmol mg^{-1} min^{-1}; $E = 31$) enzyme in diethyl ether show considerable reactivity and enantioselectivity enhancements for the immobilized enzyme.[25] The addition of sucrose was earlier shown to exert its own positive effect on enzymatic activity.[26] A possible explanation for the effect of sucrose lies in its highly hydrophilic nature which can help the enzyme to maintain a high water level in

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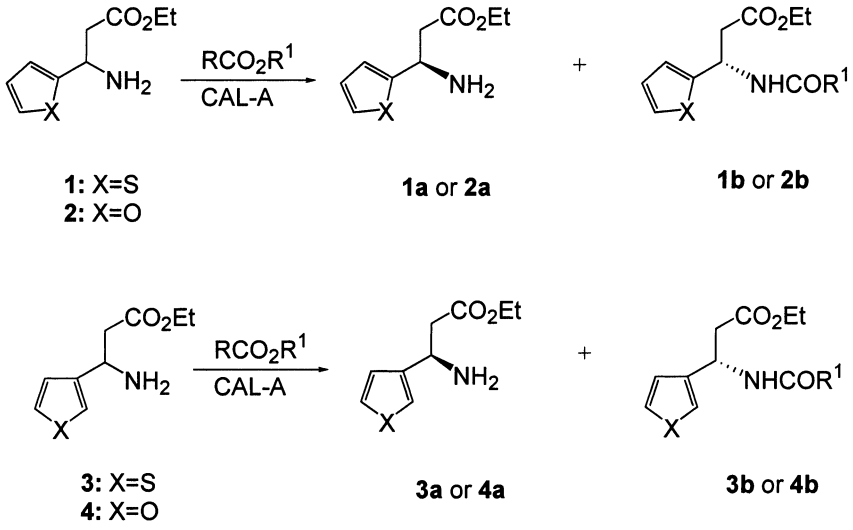
anhydrous organic solvents. On the basis of these earlier results, CAL-A on Celite when adsorbed in the presence of sucrose was used throughout the work reported herein.

2. Results and discussion

One of the most impressive properties of CAL-A is its absolute chemoselectivity in the *N*-acylation of β-amino esters under conditions where other lipases tend to lead to competition between *N*-acylation at the amino group and transesterification at the ester group, the extent of competition being controlled by the structure of the amino ester.[11–14,23,24] The absolute chemoselectivity of CAL-A is also obvious for the reactions of racemic **1–4** with the studied achiral acetate and butanoate esters and consequently, **1b–4b** are the only new products detected by GC and GC–MS (Table 1 and Scheme 1).

The acylations of **1–4** under various reaction conditions in the presence of CAL-A proceed smoothly to 50% conversion and at this point results in highly enantiopure resolution products (Table 1). The data (and more clearly the progression curves; not given here) further indicate that the reactivities (the time needed to reach a certain conversion) are practically independent of the substrate structure for given reaction conditions (Table 1, entries 2, 10, 17 and 20; entries 5, 13, 18 and 21; entries 14, 19 and 22). Substrate **1** with a 2-thienyl ring



Scheme 1. R = Me or Pr; R^1 = Et, CH_2CF_3 or Bu.

Table 1. Acylation of **1–4** (0.05 M) by CAL-A (15 mg/ml) with achiral esters (0.1 M or neat) at room temperature (25°C)

Entry	Substrate	RCO_2R^1	Solvent	Time (h)	Conv. (%)	ee_a (%)	ee_b (%)	*E*
1	**1**	$PrCO_2CH_2CF_3$	^{t}BuOMe	0.33	48	89	98	380
2	**1**	$PrCO_2CH_2CF_3$	$^i Pr_2O$	1.5	50	99	97	380
3	**1**	$PrCO_2CH_2CF_3$	MeCN	1	50	95	95	130
4	**1**	$PrCO_2CH_2CF_3$	THF	8	49	92	95	120
5	**1**	$PrCO_2Et$	$PrCO_2Et$	3	50	96	97	305
6	**1**[a]	$PrCO_2Et$	$PrCO_2Et$	6	50	97	98	410
7	**1**	$PrCO_2Bu$	$PrCO_2Bu$	0.5	45	82	99	580
8	**1**	$MeCO_2Et$	$MeCO_2Et$	60	49	88	90	60
9	**2**	$PrCO_2CH_2CF_3$	^{t}BuOMe	0.67	40	62	94	60
10	**2**	$PrCO_2CH_2CF_3$	$^i Pr_2O$	1.3	50	96	96	210
11	**2**	$PrCO_2CH_2CF_3$	MeCN	1.3	52	99	89	90
12	**2**	$PrCO_2CH_2CF_3$	THF	11	47	86	97	210
13	**2**	$PrCO_2Et$	$PrCO_2Et$	3	48	90	96	150
14	**2**[a]	$PrCO_2Et$	$PrCO_2Et$	10	50	94	92	90
15	**2**	$PrCO_2Bu$	$PrCO_2Bu$	1	49	94	97	220
16	**2**	$MeCO_2Et$	$MeCO_2Et$	66	51	97	92	100
17	**3**	$PrCO_2CH_2CF_3$	$^i Pr_2O$	1.5	47	88	98	245
18	**3**	$PrCO_2Et$	$PrCO_2Et$	4	48	90	97	220
19	**3**[a]	$PrCO_2Et$	$PrCO_2Et$	11	50	93	94	110
20	**4**	$PrCO_2CH_2CF_3$	$^i Pr_2O$	1	49	95	99	510
21	**4**	$PrCO_2Et$	$PrCO_2Et$	2	47	87	99	460
22	**4**[a]	$PrCO_2Et$	$PrCO_2Et$	11	51	>99	96	470

[a] Substrate 0.2 M.

tends to react somewhat faster than the other compounds, although this can be clearly seen only for the reaction in ethyl butanoate when more concentrated (0.1 M) substrate is used (entry 6 as compared with entries 14, 19 and 22). The reactions of **1** and **2** are faster in butyl butanoate (entries 7 and 15) and much slower in ethyl acetate (entries 8 and 16) than the corresponding reactions in ethyl butanoate (entries 5 and 13), indicating that butanoates are favoured by the enzyme. An unexpected feature of the present results is the considerable reactivity drop for the acylation of **1**–**4** in ethyl butanoate at substrate concentration of 0.1 M (entries 6, 14, 19 and 22) as compared with the generally used concentration of 0.05 M (entries 5, 13, 18 and 21). A probable reason is substrate inhibition, although this was not studied in detail in the present work.

We earlier found CAL-A to be a highly enantioselective catalyst for the acylation of various β-amino esters.[11,13] Excellent enantioselectivities in terms of the enantiomer ratio (E >100) are also observed for the reactions of **1**–**4** with various butanoate esters (Table 1). On the other hand, for the CAL-A-catalysed acylation of 3-amino-3-phenylpropanoate with 2,2,2-trifluoroethyl butanoate in diisopropyl ether ($E=75$) and with butyl butanoate in the neat ester ($E=30$), much lower enantioselectivities and reactivities have been detected.[13] It is possible that the heteroatoms in the thienyl and furyl rings of compounds **1**–**4** interact favourably with the protein structure of the enzyme, thus enhancing both the reactivity and enantioselectivity. The difference in the aromatic 3-substituents is that the polarity increases with decreasing aromatic character (i.e. in the sequence phenyl>furyl>thienyl). The accuracy of the E values in Table 1 is open to question because it is well known that for E >100 even minor errors in ee may cause a significant variation in E. However, the given values more or less correctly describe the differences in E from one substrate to another under various conditions.

Previous results relating to alicyclic β-amino esters indicated the enhanced enantioselectivity and reactivity of CAL-A with increasing carbon chain length of the acyl part of an achiral acyl donor.[11] Substrates **1** and **2** were subjected to enzymatic acylation conditions both in neat ethyl acetate and in ethyl butanoate. The reactions in the latter medium (Table 1, entries 5 and 13) were not only faster, but also more enantioselective than in the acetate ester (entries 8 and 16), structural effects being observed in addition to solvent effects. Accordingly, butanoate esters were generally used in this work.

For kinetic enzymatic resolution in organic solvents, special attention is usually paid to the nature of the achiral acyl donor in order to avoid reverse enzymatic catalysis, which can become important with the accumulation of products such as **1b**–**4b**. The reverse reaction, if it occurs, can easily be seen via the lowering of the enantiopurities of the less reactive enantiomers at higher conversions. In the present N-acylations, ee_a >99% remains unchanged after the more reactive enantiomer has reacted at somewhat over 50% conversion. The absence of the reverse reaction is in accordance with the generally accepted finding that lipases do not constrain amide bonds to react. The CAL-A-catalysed acylations of **1** and **2** with alkyl-activated 2,2,2-trifluoroethyl butanoate in organic solvents were also studied (Table 1, entries 1–4 and 9–12). Solvent effects on the reactivity and enantioselectivity are clear, the best solvents being ^{t}BuOMe and ^{i}Pr$_2$O for **1** (entries 1 and 2), and ^{i}Pr$_2$O (entries 10 and 12) for **2**. No benefit of using 2,2,2-trifluoroethyl butanoate over the more economical ethyl butanoate can be seen.

It may be concluded, that CAL-A-catalysed acylation is an excellent and highly selective method of resolution for various types of β-amino esters,[11,13] the present results adding 3-heteroaryl-substituted propanoate esters to the list. On the basis of the results, the enantiomers of **1**–**4** were resolved on a gram scale in ethyl butanoate, as shown in the experimental section (Scheme 1). In spite of the longer reaction times needed to reach 50% conversion, a substrate concentration of 0.1 M was used in the cases of **1** and **4**.

There is still a question about the absolute configurations of **1a**–**4a** and **1b**–**4b** to be solved. We herein rely on the observed enantioselectivity of CAL-A for the acylation of aliphatic β-substituted β-amino esters in the previous work[13] and expect the S absolute configuration for **1b**–**4b**. To support this, the *Candida antarctica* lipase B (CAL-B)-catalysed acylations of the present substrates were conducted in neat ethyl butanoate. The opposite selectivity observed for CAL-B relative to that for CAL-A justifies the expectation, as opposite enantiodiscrimination was observed earlier for the enzymes when aliphatic β-amino esters were used as substrates.[13,14,24] Otherwise, very slow reactions, with E values of only 4, 10, 3 and 2 for the reactions of **1**–**4**, respectively, make CAL-B useless as a catalyst for kinetic resolution of the present substrates. The absolute configurations of **1a** was also proved via reductive desulphurization by Raney nickel, resulting in 3-aminoheptanoic acid.[27] The measured specific rotation of the product in comparison with the literature value[28] indicates S absolute configuration; therefore the R absolute configuration for **1a** and S for **1b** correlate with the observed enantioselectivity of CAL-A for the acylation of aliphatic β-substituted β-amino esters.[13]

3. Materials and methods

2,2,2-Trifluoroethyl butanoate was prepared from butanoyl chloride and 2,2,2-trifluoroethanol. CAL-A (*Candida antarctica* lipase A, Chirazyme L5) and CAL-B (*Candida antarctica* lipase B, Chirazyme L2) were purchased from Boehringer–Mannheim. Before use CAL-A and sucrose (0.24 g) were dissolved in Tris–HCl buffer (pH 7.9), the solution was added on Celite (4.0 g) and thereafter left to dry at room temperature.[26] The final lipase content in the enzyme preparation was 10% (w/w). The solvents were of the highest analytical grade from Lab Scan Ltd and were dried over molecular sieves before use. The progress of the reactions and the ee values were followed by taking samples (0.1 ml) at

intervals and analysing them by gas chromatography on a Chrompack CP-Chirasil-L-Val column (25 m). For good baseline separation, the unreacted amino group in the sample was derivatized with acetic or propanoic anhydride in the presence of pyridine containing 1% 4-*N*,*N*-dimethylaminopyridine (DMAP) before injections. In a typical small-scale experiment, one or other of the substrates **1**–**4** (0.05 M if not otherwise stated) was dissolved in the reaction medium containing an acyl donor (2 equiv. in an organic solvent or the solvent itself) and the CAL-A preparation (15 mg/ml) was added. The reactions in the case of CAL-B (40 mg/ml) were performed in the same way. All reactions proceeded at room temperature (25°C). The determination of E was based on the equation $E=\ln[1-ee_a)/(1-ee_a/ee_b)]/\ln[(1-ee_a)/(1+ee_a/ee_b)]$ with $c=ee_S/(ee_S+ee_P)$ as derived from the original equations of Chen et al.[29]

^{1}H NMR spectra were recorded on solutions in $CDCl_3$ or D_2O at ambient temperature on a JEOL L400 or a Bruker AM200 spectrometer. Chemical shifts are given in δ (ppm) relative to TMS as internal standard; multiplicities were recorded as s (singlet), d (doublet), dd (double doublet), t (triplet) or m (multiplet). MS spectra were recorded on a VG Analytical 7070E instrument. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II Elemental Analyser. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

3.1. Preparation of racemic ethyl 3-amino-3-heteroaryl propanoates, 1–4

3-Amino-3-heteroarylpropanoic acids were synthesized in a modified Rodionov synthesis from the corresponding aldehyde through condensation with malonic acid (1 equiv.) in the presence of ammonium acetate (2 equiv.), the mixture being heated under reflux in ^{i}PrOH:H_2O (9:1).[30–32] The amino acids were precipitated from the mixture, filtered off and washed with ethanol. The properties of the products are as follows.

3.1.1. 3-Amino-3-(2-thienyl)propanoic acid. White crystals, mp 225–228°C, lit. mp:[33] 207–208°C, ^{1}H NMR (400 MHz, D_2O): δ 2.84–3.00 (2H, m, CH_2CO), 4.97 (H, t, $J=7.00$ Hz CHCH$_2$CO), 7.12 (1H, dd, $J=3.60$, 5.20 Hz, aromatic), 7.26 (1H, d, $J=3.60$ Hz, aromatic), 7.52 (1H, d, $J=4.80$ Hz aromatic).

3.1.2. 3-Amino-3-(2-furyl)propanoic acid. Brown crystals, mp 219–221°C, lit. mp:[34] 206°C, ^{1}H NMR (400 MHz, D_2O): δ 2.90 (2H, d, $J=7.20$ Hz, CH_2CO), 4.76 (1H, t, $J=7.20$ Hz, CHCH$_2$CO), 6.47–6.52 (1H, m, aromatic), 6.52–6.56 (1H, m, aromatic), 7.56–7.60 (1H, m, aromatic).

3.1.3. 3-Amino-3-(3-thienyl)propanoic acid. White crystals, mp 239–242°C, lit. mp:[35] 203°C, ^{1}H NMR (400 MHz, D_2O): δ 2.81–2.94 (2H, m, CH_2CO), 4.77 (1H, t, $J=7.20$ Hz, CHCH$_2$CO), 7.22 (1H, dd, $J=2.00$, 4.80 Hz, aromatic), 7.51–7.56 (2H, m, aromatic).

3.1.4. 3-Amino-3-(3-furyl)propanoic acid. Dark-brown crystals, mp >250°C, ^{1}H NMR (400 MHz, D_2O): δ 2.78–2.84 (2H, m, CH_2CO), 4.66 (H, t, $J=7.00$ Hz CHCH$_2$CO), 6.58 (1H, d, $J=1.20$ Hz, aromatic), 7.57 (1H, t, $J=1.80$ Hz, aromatic), 7.66 (1H, s, aromatic).

For the preparation of amino esters **1**–**4**, the above acids were esterified with ethanol in the presence of thionyl chloride, followed by the bubbling of ammonia through the solution in CH_2Cl_2 for 2 h. Ammonium chloride was filtered off and the filtrate was evaporated. The free base was eluted from a silica column, using CH_2Cl_2:MeOH (95:5) as eluent.

3.1.5. Ethyl 3-amino-3-(2-thienyl)propanoate, 1. ^{1}H NMR (400 MHz): δ 1.23 (3H, t, $J=7.15$ Hz, OCH$_2$CH_3), 2.70–2.90 (2H, m, CH_2CO), 4.22 (2H, q, $J=7.19$, OCH_2CH$_3$), 4.75–4.80 (1H, m, CHCH$_2$CO), 6.92 (1H, d, $J=3.42$ Hz, aromatic), 7.18 (2H, t, $J=6.41$ Hz, aromatic).

3.1.6. Ethyl 3-amino-3-(2-furyl)propanoate, 2. ^{1}H NMR (400 MHz): δ 1.21 (3H, t, $J=7.12$ Hz, OCH$_2$CH_3), 2.60–2.75 (1H, m, CH_2CO), 2.75–2.90 (1H, m, CH_2CO), 4.15 (2H, q, $J=7.12$, OCH_2CH$_3$), 4.35–4.40 (1H, m, CHCH$_2$CO), 6.10–6.15 (1H, m, aromatic), 6.25–6.30 (1H, m, aromatic), 7.30–7.35 (1H, m, aromatic).

3.1.7. Ethyl 3-amino-3-(3-thienyl)propanoate, 3. ^{1}H NMR (200 MHz): δ 1.25 (3H, t, $J=7.12$ Hz, OCH$_2$CH_3), 2.50–2.80 (2H, m, CH_2CO), 4.15 (2H, q, $J=7.15$ Hz, OCH_2CH$_3$), 4.45–4.60 (1H, m, CHCH$_2$CO), 7.07 (1H, d, $J=5.06$ Hz, aromatic), 7.17 (1H, d, $J=2.52$ Hz, aromatic), 7.25–7.35 (1H, m, aromatic).

3.1.8. Ethyl 3-amino-3-(3-furyl)propanoate, 4. ^{1}H NMR (400 MHz): δ 1.24 (3H, t, $J=7.15$ Hz, OCH$_2$CH_3), 2.50–2.75 (2H, m, CH_2CO), 4.10–4.25 (2H, m, OCH_2CH$_3$), 4.30–4.40 (1H, m, CHCH$_2$CO), 6.30–6.40 (1H, m, aromatic), 7.20–7.30 (1H, m, aromatic), 7.30–7.40 (1H, m, aromatic).

3.2. Gram-scale resolution of ethyl 3-amino-3-(2-thienyl)propanoate, 1

Racemic **1** (1.0 g, 5.03 mmol) was dissolved in ethyl butanoate (50 ml), and CAL-A (15 mg/ml) was added. The mixture was stirred at room temperature. The reaction was stopped at 51% conversion ($ee_{1a}=98\%$ and $ee_{1b}=96\%$) after 8 h by filtering off the enzyme. After evaporation, the residue was purified by column chromatography using CH_2Cl_2:MeOH (95:5) as an eluent to separate compounds **1a** and **1b**.

Dry HCl gas was bubbled through the solution of **1a** for 2–3 h and the solvent was evaporated. **1a**·HCl was crystallized from diethyl ether in the cold, the filtrate was recrystallized from a mixture of absolute ethanol and diethyl ether, and the oily product was washed with diethyl ether, resulting in (*R*)-**1a**·HCl (0.33 g, 1.40 mmol) as a slowly crystallizing light-yellow oil: $[\alpha]_D^{25}=$

+4.6 (*c* 1.0, MeOH); $M=199$ according to MS. Anal. calcd for $C_9H_{14}ClNO_2S$: C, 45.86; H, 5.99; Cl, 15.04; N, 5.94; S, 13.60. Found: C, 45.45; H, 6.30; Cl, 16.18; N, 6.30; S, 15.02%. ^{1}H NMR (200 MHz): δ 1.21 (3H, t, $J=7.13$ Hz, OCH_2CH_3), 3.15–3.35 (2H, m, $CHCH_2CO$), 4.14 (2H, q, $J=7.14$ Hz, OCH_2CH_3), 4.95–5.10 (1H, m, $CHCH_2CO$), 6.95–7.05 (1H, m, aromatic), 7.25–7.40 (2H, m, aromatic), 8.90 (3H, br s, $NH_3^+Cl^-$) ppm.

After evaporation of the eluent, (*S*)-**1b** (0.62 g, 2.30 mmol) was obtained as a yellow oil: $[\alpha]_D^{25}=-84.2$ (*c* 1.0, MeOH); $M=269$ according to MS. Anal. calcd for $C_{13}H_{19}NO_3S$: C, 57.97; H, 7.11; N, 5.20; S, 11.90. Found: C, 60.46; H, 8.01; N, 5.72; S, 13.56%. ^{1}H NMR (400 MHz): δ 0.94 (3H, t, $J=7.37$ Hz, $CH_3CH_2CH_2$), 1.19 (3H, t, $J=7.15$ Hz, OCH_2CH_3), 1.55–1.75 (2H, m, $CH_3CH_2CH_2$), 2.18 (2H, t, $J=7.48$ Hz, $CH_3CH_2CH_2$), 2.80–3.00 (2H, m, CH_2CO), 5.65–5.75 (1H, m, $CHCH_2CO$), 6.62 (1H, d, $J=8.55$ Hz, NH), 6.85–6.95 (1H, m, aromatic), 7.15–7.20 (2H, m, aromatic) ppm.

3.3. Gram-scale resolution of ethyl 3-amino-3-(2-furyl)propanoate, 2

Racemic **2** (1.0 g, 5.46 mmol) was dissolved in ethyl butanoate (109 ml), and CAL-A (15 mg/ml) was added. The reaction was stopped at 51% conversion ($ee_{2a}=96\%$ and $ee_{2b}=93\%$) after 7 h by filtering off the enzyme, followed by the work-up as above.

3.3.1. (*R*)-2a·HCl. Yield: 0.45 g, 2.05 mmol; a slowly crystallizing brown oil, $[\alpha]_D^{25}=+9.5$ (*c* 1.0, MeOH); $M=183$ according to MS. Anal. calcd for $C_9H_{14}ClNO_3$: C, 49.21; H, 6.42; Cl, 16.14; N, 6.38. Found: C, 48.16; H, 6.86; Cl, 15.42; N, 6.68%. ^{1}H NMR (200 MHz): δ 1.34 (3H, t, $J=7.13$ Hz, OCH_2CH_3), 3.15–3.50 (2H, m, CH_2CO), 4.26 (2H, q, $J=7.13$ Hz, OCH_2CH_3), 4.85–5.15 (1H, m, $CHCH_2CO$), 6.40–6.50 (1H, m, aromatic), 6.60–6.80 (1H, m, aromatic), 7.45–7.65 (1H, m, aromatic), 9.03 (3H, br s, $NH_3^+Cl^-$) ppm.

3.3.2. (*S*)-2b. Yield: 0.53 g, 2.09 mmol; an orange oil, $[\alpha]_D^{25}=-84.5$ (*c* 1.0, MeOH); $M=253$ according to MS. Anal. calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 62.43; H, 8.95; N, 6.34%. ^{1}H NMR (200 MHz): δ 0.95 (3H, t, $J=7.37$ Hz, $CH_3CH_2CH_2$), 1.23 (3H, t, $J=7.13$ Hz, OCH_2CH_3), 1.60–1.80 (2H, m, $CH_3CH_2CH_2$), 2.20 (2H, t, $J=7.20$ Hz, $CH_3CH_2CH_2$), 2.70–3.00 (2H, m, CH_2CO), 4.12 (2H, q, $J=7.07$ Hz, OCH_2CH_3), 5.45–5.65 (1H, m, $CHCH_2CO$), 6.15–6.20 (1H, m, aromatic), 6.25–6.35 (1H, m, aromatic), 6.44 (1H, d, $J=8.34$ Hz, NH), 7.30–7.35 (1H, m, aromatic) ppm.

3.4. Gram-scale resolution of ethyl 3-amino-3-(3-thienyl)propanoate, 3

Racemic **3** (1.0 g, 5.03 mmol) was dissolved in ethyl butanoate (100 ml), and CAL-A (15 mg/ml) was added. The reaction was stopped at 51% conversion ($ee_{3a}>99\%$ and $ee_{3b}=96\%$) after 5 h by filtering off the enzyme, followed by the work-up as above.

3.4.1. (*R*)-3a·HCl. Yield: 0.52 g, 2.21 mmol; white crystals, mp 106–108°C, $[\alpha]_D^{25}=+1.00$ (*c* 1.0, MeOH); $M=199$ according to MS. Anal. calcd for $C_9H_{14}ClNO_2S$: C, 45.86; H, 5.99; Cl, 15.04; N, 5.94; S, 13.60. Found: C, 48.00; H, 7.07; Cl, 14.03; N, 5.39; S, 12.42%. ^{1}H NMR (200 MHz): δ 1.20 (3H, t, $J=7.13$ Hz, OCH_2CH_3), 2.95–3.35 (2H, m, CH_2CO), 4.12 (2H, q, $J=7.13$ Hz, OCH_2CH_3), 4.75–4.90 (1H, m, $CHCH_2CO$), 7.25–7.35 (2H, m, aromatic), 7.52–7.58 (1H, m, aromatic), 8.82 (3H, br s, $NH_3^+Cl^-$) ppm.

3.4.2. (*S*)-3b. Yield: 0.69 g, 2.56 mmol; an orange oil, $[\alpha]_D^{25}=-83.1$ (*c* 1.0, MeOH); $M=269$ according to MS. Anal. calcd for $C_{13}H_{19}NO_3S$: C, 57.97; H, 7.11; N, 5.20; S, 11.90. Found: C, 56.12; H, 7.59; N, 5.75; S, 11.55%. ^{1}H NMR (200 MHz): δ 0.96 (3H, t, $J=7.33$ Hz, $CH_3CH_2CH_2$), 1.20 (3H, t, $J=6.17$ Hz, OCH_2CH_3), 1.55–1.80 (2H, m, $CH_3CH_2CH_2$), 2.20 (2H, t, $J=7.46$ Hz, $CH_3CH_2CH_2$), 2.75–3.00 (2H, m, CH_2CO), 4.10 (2H, q, $J=7.12$ Hz, OCH_2CH_3), 5.40–5.60 (1H, m, $CHCH_2CO$), 6.50–6.65 (1H, d, $J=8.16$ Hz, NH), 6.98–7.05 (1H, m, aromatic), 7.05–7.15 (1H, m, aromatic), 7.30–7.35 (1H, m, aromatic) ppm.

3.5. Gram-scale resolution of ethyl 3-amino-3-(3-furyl)propanoate, 4

Racemic **4** (0.5 g, 2.73 mmol) was dissolved in ethyl butanoate (27 ml) and CAL-A (15 mg/ml) was added. The reaction was stopped at 50% conversion ($ee_{4a}>99\%$ and $ee_{4b}=99\%$) after 4 h by filtering off the enzyme, followed by the work-up as above.

3.5.1. (*R*)-4a·HCl. Yield: 0.30 g, 1.36 mmol; a slowly crystallizing brown oil, $[\alpha]_D^{25}=+7.25$ (*c* 1.0, MeOH); $M=183$ according to MS. Anal. calcd for $C_9H_{14}ClNO_3$: C, 49.21; H, 6.42; Cl, 16.14; N, 6.38. Found: C, 49.70; H, 7.72; Cl, 13.83; N, 5.64%. ^{1}H NMR (400 MHz): δ 1.20 (3H, t, $J=7.16$ Hz, OCH_2CH_3), 2.58–3.10 (1H, m, CH_2CO), 3.10–3.25 (1H, m, CH_2CO), 4.00–4.25 (2H, m, OCH_2CH_3), 4.65–4.80 (1H, m, $CHCH_2CO$), 6.00–6.75 (1H, m, aromatic), 7.30–7.40 (1H, m, aromatic), 7.55–7.65 (1H, m, aromatic), 8.74 (3H, br s, $NH_3^+Cl^-$) ppm.

3.5.2. (*S*)-4b. Yield: 0.19 g, 0.75 mmol; a yellow oil, $[\alpha]_D^{25}=-57.1$ (*c* 1.0, MeOH); $M=253$ according to MS. Anal. calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 59.74; H, 8.65; N, 6.45%. ^{1}H NMR (200 MHz): δ 0.96 (3H, t, $J=7.35$ Hz, $CH_3CH_2CH_2$), 1.23 (3H, t, $J=7.12$ Hz, OCH_2CH_3), 1.60–1.80 (2H, m, $CH_3CH_2CH_2$), 2.19 (2H, t, $J=7.44$ Hz, $CH_3CH_2CH_2$), 2.70–2.95 (2H, m, CH_2CO), 4.13 (2H, q, $J=7.09$ Hz, OCH_2CH_3), 5.30–5.48 (1H, m, $CHCH_2CO$), 6.31–6.38 (1H, m, aromatic), 6.49 (1H, d, $J=8.49$ Hz, NH), 7.30–7.45 (2H, m, aromatic) ppm.

3.6. Reductive desulphurization of 1

Racemic **1** (0.3 g, 1.5 mmol) was dissolved in water with the addition of a few drops of ethanol and Raney nickel (4 g) was suspended. The mixture was stirred at 70°C under 40 atm H_2 pressure in an autoclave. After

4 days the catalyst was filtered off and the solvent was evaporated off. A white solid of 3-aminoheptanoic acid formed which was crystallized from acetone. ^{1}H NMR (400 MHz, D_2O): δ 0.90 (3H, t, $J=7.00$ Hz, CH_3), 1.25–1.45 (4H, m, $CH_2CH_2CH_3$), 1.55–1.75 (2H, m, CH_2CH), 2.35–2.50 (H, m, CH_2CO), 2.50–2.65 (H, m, CH_2CO), 3.43–3.52 (H, m, $CHCH_2CO$).

Reductive desulphurization of **1a** (*R*-configuration expected) was carried out under the conditions applied for the racemic compound. $[\alpha]_D^{20}=+12.4$ (*c* 0.8, H_2O) was determined for the resulting (*S*)-3-aminoheptanoic acid (the reduction product contained some 20% impurities, but the characteristic lines for 3-aminoheptanoic acid were identical with the ^{1}H NMR spectrum of racemate) in good accordance with the literature value[25] for (*S*)-3-aminoheptanoic acid: $[\alpha]_D^{22}=+33.3$ (*c* 0.91, H_2O), ee >90%.

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