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Lipase-catalysed kinetic resolution in organic solvents: an approach to enantiopure α-methyl-β-alanine esters

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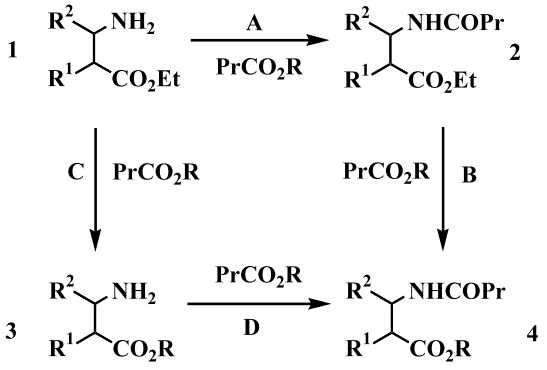
Abstract—The *Candida antarctica* lipase A (CAL-A) and B (CAL-B)-catalysed resolutions of α-methyl-β-alanine ethyl ester **1** with neat ethyl and butyl butanoates and with 2,2,2-trifluoroethyl butanoate in organic solvents were studied, as were the alcoholyses in neat butanol and with methanol (0.8 M) in diisopropyl ether. The two enzymes, which display opposite (*S* for CAL-A and *R* for CAL-B) and low enantioselectivities ($E=7$–10), allowed the preparation of the two enantiomers in a two-step resolution protocol. The *R* enantiomer (ee = 97%) was first separated as its Boc-protected derivative from the CAL-A-catalysed resolution mixture of (*R*)-**1** and the enantiomerically enriched *N*-butanoylated counterpart. The enantiopurification of the latter gave the *S* enantiomer (ee = 96%) in the following CAL-B-catalysed 'interesterification' in butyl butanoate.

1. Introduction

β-Alanine is a precursor in the biosynthesis of the vitamin, pantothenic acid. During the 1940s, the racemic α-methyl analogue (α-methyl-β-alanine, 3-amino-2-methylpropanoic acid) and α-methylpantothenic acid were synthesised with the aim of testing growth activity effects on certain bacteria and yeasts.[1] Later, it became evident that (*R*)-(−)-α-methyl-β-alanine is a non-proteogenic natural amino acid, identified for instance in human urine and in the bulbs of *Iris tingitana*.[2–5] The preparation of various β-amino acid enantiomers by chemical and enzymatic methods has subsequently received increasing attention because β-amino acids are important constituents of natural products and pharmaceuticals.[5–25] Some of the published synthetic methods include chemical steps leading to the enantiomers of α-methyl-β-alanine.[5–9] The use of lipases A (CAL-A) and B (CAL-B) from *Candida antarctica* and lipase PS from *Pseudomonas cepacia*[15–19] and furthermore the use of penicillin or penicillin G acylase[20–24] have resulted in success in the kinetic resolution of β-substituted-β-amino acid derivatives. However, enzymatic methods have not been described for the α-substituted analogues.

In the present work, the lipase-catalysed kinetic resolution of readily available racemic α-methyl-β-alanine ethyl ester (**1**; $R^1=Me$ and $R^2=H$, Scheme 1) was studied, the primary focus being on the CAL-A- and CAL-B-catalysed reactions of the substrate with achiral esters in organic solvents. This is of interest not only because of the environmentally benign and simple possibility for resolving enantiomers, but also because it affords information on other enzymatic selectivities. Thus, CAL-B was previously shown to mediate the competing *N*-acylation (formation of **2**) and 'interesterification' (formation of **3**) of **1** ($R^1=H$ and $R^2=$alkyl



Scheme 1.

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or aryl) with achiral esters such as $PrCO_2R$.[18,19] The size of R^2 and the nature of R strongly affect the chemoselective behaviour in such a way that the reaction routes A+B+C+D (R^2 = Me) and C+D (R^2 = Et) in the formation of **4**, and C (R^2 = ^{i}Pr) in the formation of **3** were earlier detected when R = Bu for example.[18] We have chosen to call steps B and C interesterifications in order to emphasise the fact that an achiral ester rather than the corresponding alcohol liberated into the reaction mixture is involved in an enzymatic reaction. In support of this, no alcoholysis was previously observed between the β-methyl analogue **1** (R^1 = H and R^2 = Me) and 2,2,2-trifluoroethanol, while **3** (R^1 = H, R^2 = Me and R = CH_2CF_3) and **2** (R^1 = H and R^2 = Me) were both detected in the reaction of **1** (R^1 = H and R^2 = Me) with 2,2,2-trifluoroethyl butanoate in the presence of CAL-B.[19] For CAL-A catalysis, on the other hand, the reactions of **1** (R^1 = H and R^2 = alkyl, aryl or heteroaryl) with achiral esters have always given the corresponding *N*-acylated products **2** (as the only detectable new products) in a highly chemoselective manner.[17] In order to solve the difficult resolution task involved in the present work, the CAL-B-catalysed interesterification and alcoholysis of racemic **2** (R^1 = Me, R^2 = H) were also studied.

2. Results and discussion

α-Methyl-β-alanine ethyl ester (**1**, R^1 = Me and R^2 = H, Scheme 1) was subjected to the CAL-A- and CAL-B-catalysed reactions with neat ethyl and butyl butanoates and with 2,2,2-trifluoroethyl butanoate in diisopropyl ether (DIPE). The butanoate esters were chosen because the lipases are known to prefer them as achiral reagents over carboxylates with shorter carbon chain lengths.[16,19] The results are shown in Table 1 and 2. As expected on the basis of the earlier results,[16,17] only the *N*-butanoylated product **2** (R^1 = Me and R^2 = H) was obtained in the case of CAL-A catalysis, allowing calculation of the enantiomer ratio, *E*. The *E* value was also obtained for the CAL-B-catalysed acylation of **1** (R^1 = Me and R^2 = H) in ethyl butanoate (R = Et, Table 1, row 1). However, for CAL-B catalysis the corresponding reaction with $PrCO_2R$ (R differs from Et) was fast through step A and was followed by the slower formation of **4** (R^1 = Me and R^2 = H) through the sequential steps A+B. Thus, the enantioselectivities of CAL-B are discussed in the terms of ee_4 values at a certain amount of product **4** formed (rows 3–6). Formation of product **3** (R^1 = Me and R^2 = H) was not detected at all.

For the reactions of **1** (R^1 = Me and R^2 = H), the results for the enzymes in Table 1 (rows 1, 3 and 5) reveal disappointingly low enantioselectivities as compared with those previously observed for the β-methyl analogue **1** (R^1 = H and R^2 = Me, rows 2, 4 and 6) under the same conditions. The observed low enantioselectivities cannot be explained by chemical reactions. With the aim of enhancing the observed *S* enantioselectivity of CAL-A, solvent effects were studied for the enzymatic acylation of **1** (R^1 = Me and R^2 = H) with 2,2,2-trifluoroethyl butanoate in organic solvents (Table 2). The results indicate smooth reactions for the formation of product **2** (R^1 = Me and R^2 = H) with little variation in the *E* value; acetonitrile (*E* = 7) was chosen for further work. Attempts to enhance the enantioselectivity of the enzymatic reaction in acetonitrile by adding water (0.01–2% w/w) or in the form of hydrated salts in order to maintain constant water activity [a_w = 0.15 for $Na_2HPO_4/Na_2HPO_4{\cdot}2H_2O$ (52 mg/ml; 1:1) and a_w = 0.76 for $Na_2SO_4/Na_2SO_4{\cdot}10H_2O$ (72 mg/ml; 1:1)] failed.[26]

In CAL-B catalysis, the total enantiomeric outcome of the *N*-butanoylation (step A) and the following in situ interesterification (step B) of **1** (R^1 = Me and R^2 = H) with neat butyl butanoate is moderate, step A being extremely fast under the conditions where the reaction through step B proceeds smoothly (Scheme 1). Step A is expected to proceed without or with only very low enantioselectivity because the amino group is situated on the primary carbon atom far from the reaction centre and product **2** (R^1 = Me and R^2 = H; Table 1, rows 3 and 5) was practically racemic up to the point

Table 1. CAL-A (40 mg/ml of the enzyme preparation) and CAL-B (75 mg/ml)-catalysed reactions of **1** (0.1 M) with $PrCO_2R$ in the neat ester or (0.2 M) in DIPE at room temperature (25°C)

Row	**1**		$PrCO_2R$/solvent	CAL-A			CAL-B		
	R^1	R^2		Time (h)	Conv.[a](%)	*E*	Time (h)	Conv.[a] (%)	*E* or (ee_4/amount[b])
1	Me	H	$PrCO_2Et/PrCO_2Et$	1	66	5	0.2	60	1
2	H	Me	$PrCO_2Et/PrCO_2Et$	–	–	–	25[d]	53[d]	100[d]
3	Me	H	$PrCO_2Bu/PrCO_2Bu$	1	52	5	0.5	100	(71%/11%)[e]
4	H	Me	$PrCO_2Bu/PrCO_2Bu$	22[c]	51[c]	32[c]	0.75[d]	50[d]	(>99.9%/33%)[d,f]
5	Me	H	$PrCO_2CH_2CF_3$/DIPE	0.3	39	5	1	100	(75%/12%)[e]
6	H	Me	$PrCO_2CH_2CF_3$/DIPE	25[c]	50[c]	6[c]	4[d]	52[d]	(>99.9%/20%)[d,f]

[a] Proportion of substrate **1** that has reacted to product **2**, independently of whether there is further reaction to **4** or not.
[b] Proportion of product **4** in the mixture.
[c] Ref. 17.
[d] Ref. 19.
[e] Sequential resolution (steps A+B).
[f] Sequential resolution (steps A+B+C+D).

Table 2. CAL-A (40 mg/ml of the enzyme preparation)-catalysed reaction of **1** (R^1 = Me and R^2 = H; 0.1 M) with 2,2,2-trifluoroethyl butanoate (0.2 M) in organic solvents at room temperature

Solvent	Time (min)	Conv. (%)	*E*
DIPE	20	39	5
THF	60	37	5
tert-Amyl alcohol	20	35	6
Acetonitrile	10	19	7
Acetonitrile (0.01% (w/w) water)	10	22	6
Acetonitrile (0.5% (w/w) water)	10	21	5
Acetonitrile (2% (w/w) water)	10	18	4
Acetonitrile[a]	30	11	7
Acetonitrile ($Na_2HPO_4/Na_2HPO_4{\cdot}2H_2O$ (1/1))[a]	30	11	5
Acetonitrile ($Na_2SO_4/Na_2SO_4{\cdot}10H_2O$ (1/1))[a]	30	12	6

[a] 20 mg/ml of the enzyme preparation.

where **1** (R^1 = Me and R^2 = H) had totally reacted. The enantioselectivity of step B was studied separately by preparing racemate **2** (R^1 = Me and R^2 = H) by chemical means and by subjecting the substrate to CAL-B-catalysed interesterification with neat butyl butanoate and for alcoholyses with neat butanol and with methanol (0.8 M) in acetonitrile. The results are shown in Table 3. Moderately *R*-enantioselective reactions in butyl butanoate, proceeded smoothly, the enantioselectivity being independent of temperature. The level of enantioselectivity in the present alcoholyses was negligible, in contrast with the alcoholysis of β-methyl-substituted **1** (R^1 = H and R^2 = Me; E >100) in neat butanol. Attempts to enhance the enantioselectivity of step B by tailoring the substrate structure failed. Thus, E = 3–13 was observed for the CAL-B-catalysed interesterifications of Boc- and Fmoc-protected substrates **1** (R^1 = Me and R^2 = H) and for the CAL-A-catalysed acylation of α-methyl-β-alanine *tert*-butyl ester under the conditions of the present work.

The above results lead to two important observations. First, substrate **1** (R^1 = Me and R^2 = H, Scheme 1) is the first β-amino ester for which the acylation–interesterification sequence (A+B) to produce **4** (R = Bu, R^1 = Me and R^2 = H) proceeds in a highly chemoselective manner on the action of CAL-B. This strongly supports the conclusion that the interesterification step C becomes more favourable as the size of the R^2 group increases.[18,19] Even more importantly, the opposite enantioselectivities of CAL-A and CAL-B paved the way for the transformation of racemic α-methyl-β-alanine ethyl ester **1** (R^1 = Me and R^2 = H) into its enantiomers.

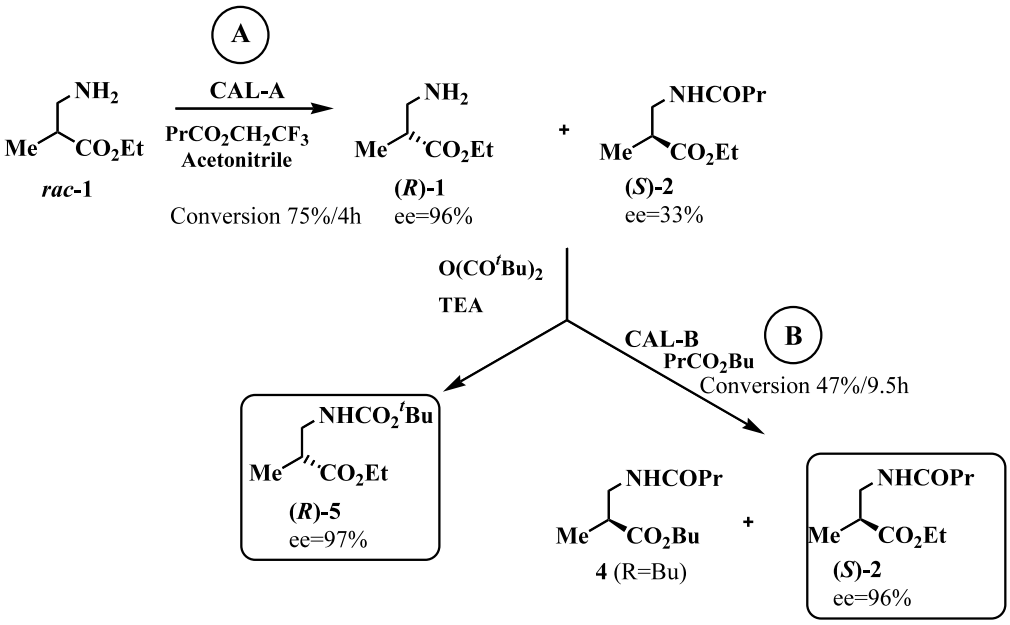
Table 3. CAL-B (75 mg/ml)-catalysed reactions of **2** (R^1 = Me and R^2 = H; 0.1 M) in neat butyl butanoate or in butanol and with methanol (0.8 M) in diisopropyl ether

Reagent	Temperature (°C)	Time (h)	Conv. (%)	*E*
$PrCO_2Bu$	25 (rt)	1	18	10
$PrCO_2Bu$	7	1	4	10
$PrCO_2Bu$	40	1	32	10
BuOH	25 (rt)	1	2	2
MeOH	25 (rt)	20	67	4

2.1. Gram-scale resolutions by CAL-A and CAL-B

In spite of the observed low enantioselectivity (E = 7) of CAL-A, the less reactive *R* enantiomer of **1** (R^1 = Me and R^2 = H) can be prepared if a low chemical yield is acceptable by acylating **1** (R^1 = Me and R^2 = H) with 2,2,2-trifluoroethyl butanoate in acetonitrile. The resolution on a gram-scale was performed in practice and the *R* enantiomer as Boc-protected derivative **5** (ee 97%) was separated from the resolution mixture (Scheme 2). A convenient feature of this method is that Boc derivatisation can be performed directly in the resolution mixture after the enzyme is filtered off. The theoretical chemical yield of the product from the initially racemic substrate is 25%. The problem is how to exploit the enantiomerically enriched resolution product (*S*)-**2**, which forms in 75% from the initial racemate **1**. One possibility is racemisation. (−)-α-Methyl-β-alanine was earlier racemised by boiling in aqueous NaOH (5N 16 h/106°C).[2] This provides a possibility for the transformation of racemic **1** totally to (*R*)-**1** by the repeated racemisation of (*S*)-**2**, esterification of the acid obtained and CAL-A-catalysed resolution of the amino ester. However, this protocol was not carried out in the present work.

Similarly, CAL-B, as an *R*-selective enzyme with moderate enantioselectivity (E = 10), is suitable for the preparation of the less reactive (*S*)-**2** (R^1 = Me and R^2 = H), either by in situ sequential resolution from **1** (R^1 = Me and R^2 = H) to the corresponding product **4** (steps A+B, Scheme 1) or by the resolution of racemic **2** (R^1 = Me and R^2 = H) (corresponding to step B, Scheme 2), both in neat butyl butanoate. For this purpose, racemic **2** (R^1 = Me and R^2 = H) was subjected to CAL-B-catalysed interesterification with butyl butanoate, leading to the formation of (*S*)-**2** (ee = 94%) and (*R*)-**4** (ee = 48%) at 66% conversion after 11 h. Thus, the theoretical yield of (*S*)-**2** (R^1 = Me and R^2 = H) from one resolution is 34%. As above, the racemisation of (*R*)-**4** (R = Bu, R^1 = Me and R^2 = H) through the use of NaOH and repeated resolutions can be used to transform racemic **1** into (*S*)-**2**. As another possibility, the enantiomerically enriched (*R*)-**4** (R = Bu, R^1 = Me and R^2 = H) can be subjected to CAL-B-catalysed interesterification in neat $PrCO_2R$, where R differs from Bu.



Scheme 2.

However, the enantioselectivity of racemic butyl ester **4** (R = Bu, R^1 = Me and R^2 = H) for the interesterification in neat ethyl butanoate (E = 5) did not tempt us to carry out the procedure on a large scale.

When both enantiomers of **1** (R^1 = Me and R^2 = H) are desired, the most elegant method is based on utilisation of the opposite enantioselectivities of CAL-A and CAL-B. Thus, the above *N*-acylated product (*S*)-**2** (R^1 = Me and R^2 = H; ee = 33%) obtained from CAL-A-catalysed acylation with 2,2,2-trifluoroethyl butanoate in acetonitrile was subjected to CAL-B-catalysed interesterification in neat butyl butanoate, leading to (*S*)-**2** (ee = 96%) as the less reactive enantiomer and to the formation of (*R*)-**4** (R = Bu, R^1 = Me and R^2 = H) as shown in Scheme 2.

3. Experimental

2,2,2-Trifluoroethyl and butyl butanoates were prepared from the corresponding acid chlorides and alcohols. CAL-A (*Candida antarctica* lipase A, Chirazyme L5) and CAL-B (*Candida antarctica* lipase B, Chirazyme L2) were purchased from Boehringer-Mannheim. CAL-A was immobilised on Celite in the presence of sucrose before use,[27] the final preparation containing 10% (w/w) of the lipase. The solvents were of the highest analytical grade from Lab Scan Ltd.

In a typical small-scale experiment, **1** (R^1 = Me and R^2 = H; 0.1 M) was dissolved in an appropriate solvent (2 ml), which also served as an achiral reagent ($PrCO_2Bu$, BuOH or MeOH (0.8 M) in acetonitrile). In another possibility, 2,2,2-trifluoroethyl butanoate (0.2 M) was added to the reaction mixture in an organic solvent. The reaction was started by addition of the CAL-A preparation (40 mg/ml) or CAL-B (75 mg/ml). If not otherwise stated, the reaction mixture was shaken at room temperature (25°C). The progress of the reactions and the ee values of the substrates and products were checked by taking samples (0.1 ml) at intervals and analysing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). For good baseline separation, the unreacted amino group in the sample was acetylated with acetic anhydride in the presence of pyridine containing 1% 4-dimethylaminopyridine. The determination of E was based on the equation $E = \ln[(1-ee_S)/(1+ee_S/ee_P)]/\ln[(1+ee_S)/(1+ee_S/ee_P)]$ with $c = ee_S/(ee_S+ee_P)$, as derived from the original equations of Chen et al.[28]

^{1}H NMR spectra were recorded in $CDCl_3$ 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), q (quartet), m (multiplet) or om (overlapping multiplet). MS spectra were recorded on a VG Analytical 7070E instrument. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II Elemental Analyzer. 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 ethyl 3-amino-2-methylpropanoate, 1

Racemic ethyl 3-benzylamino-2-methylpropanoate[29] (22.13 g, 0.1 mol) was dissolved in ethanol (75 ml), and 10% palladium on charcoal (2.0 g) was added to the solution. The mixture was hydrogenated at ambient temperature at atmospheric pressure. When the hydrogen uptake was over (ca. 20 h), the catalyst was filtered

off and washed with ethanol. The combined filtrate and washings were evaporated under reduced pressure at 40°C and the oily residue was distilled in vacuum, yielding **1** (8.92 g, 0.068 mol, bp 90–92°C/29 mm Hg (bp literature[30] 71°C/13 mm Hg). ^{1}H NMR (400 MHz): δ 1.16 (3H, d, $J=7.11$ Hz, CH_3CH), 1.20 (2H, s, NH_2), 1.27 (3H, t, $J=7.12$ Hz, CH$_2$CH_3), 2.52 (1H, m, CH$_3$CH), 2.79 (1H, dd, $J=12.90$, 7.72 Hz, CH_2NH$_2$), 2.92 (1H, dd, $J=12.90$, 5.12 Hz, CH_2NH$_2$), 4.16 (2H, q, $J=7.12$ Hz, CH_2CH$_3$).

3.2. Preparation of the enantiomers of ethyl 3-amino-2-methylpropanoate, 1

3.2.1. Resolution method 1. Racemic **1** (1.00 g, 7.76 mmol) was dissolved in acetonitrile (74 ml), and the CAL-A preparation (1.52 g) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (2.34 ml, 15.5 mmol) and stopped after 4 h at 75% conversion by filtering off the enzyme. To this solution, triethylamine (0.29 g, 2.86 mmol) and di-*tert*-butyldicarbonate (0.46 g, 2.11 mmol) were added. The reaction proceeded overnight, after which the solvent was evaporated off. Purification on silica, elution with acetone–hexane (2:8), gave (*R*)-**5** 0.31 g, 1.34 mmol; $[\alpha]_D^{20}=-26.6$ (*c* 1.0, MeOH); ee 97%; M = 231 according to MS. Anal. calcd for $C_{11}H_{21}NO_4$: C, 57.14; H, 9.15; N, 6.06. Found: C, 57.28; H, 9.46; N, 5.84%. ^{1}H NMR (400 MHz): δ 1.17 (3H, d, $J=7.20$ Hz, CH_3CH), 1.27 (3H, t, $J=7.12$ Hz, OCH$_2$CH_3), 1.43 (9H, s, (CH_3)$_3$C), 2.65 (1H, m, CH$_3$CH), 3.28–3.37 (2H, om, CH_2NH), 4.15 (2H, q, $J=7.12$ Hz, OCH_2CH$_3$), 4.92 (1H, s, NH).

3.2.2. Resolution method 2. (*S*)-**2** (0.95 g, 4.73 mmol; $[\alpha]_D^{25}=+14.0$ (*c* 1.0, MeOH); ee = 33%) from Resolution 1 above was dissolved in butyl butanoate (95 ml) and the reaction was started by the addition of CAL-B (7.1 g). The reaction was stopped after 9.5 h at 47% conversion by filtering off the enzyme. The solvent was evaporated and the residue was purified on silica by elution with acetone–hexane (1:3). The less reactive (*S*)-**2** enantiomer (0.49 g, 2.44 mmol; $[\alpha]_D^{25}=+36.4$ (*c* 1.0, MeOH); ee = 96%) was separated. Anal. calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 58.92; H, 9.27; N, 6.72%. M = 201 according to MS. ^{1}H NMR (200 MHz): δ 0.94 (3H, t, $J=7.30$ Hz, CH_3CH$_2$CH$_2$), 1.18 (3H, d, $J=7.15$ Hz, CH_3CH), 1.27 (3H, t, $J=7.16$ Hz, OCH$_2$CH_3), 1.62 (2H, m, CH$_3$CH_2CH$_2$), 2.14 (2H, m, CH$_3$CH$_2$CH_2), 2.66 (1H, m, CH$_3$CH), 3.26 (1H, m, CH_2NH), 3.52 (1H, om, CH_2NH), 4.14 (2H, m, COOCH_2CH$_3$), 6.02 (1H, s, NH).

Slightly enantiomerically enriched butyl ester **4** (0.50 g, 2.18 mmol; $[\alpha]_D^{25}=-11.0$ (*c* 1.0, MeOH); ee = 37%) was eluted from the column before (*S*)-**2**. M = 229 according to MS. Anal. calcd for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11. Found: C, 62.91; H, 10.01; N, 6.05%. ^{1}H NMR (400 MHz): δ 0.94 (6H, m, CH_3CH$_2$CH$_2$CO+ OCH$_2$CH$_2$CH$_2$CH_3), 1.18 (3H, d, $J=7.26$ Hz, CH_3CH), 1.38 (2H, m, OCH$_2$CH$_2$CH_2CH$_3$), 1.56–1.70 (4H, om, CH$_3$CH_2CH$_2$CO+OCH$_2$CH_2CH$_2$CH$_3$), 2.14 (2H, m, CH$_3$CH$_2$CH_2CO), 2.70 (1H, m, CH$_3$CH), 3.31 (1H, m, CH_2NH), 3.53 (1H, m, CH_2NH), 4.10 (2H, m, OCH_2CH$_2$CH$_2$CH$_3$), 6.02 (H, s, NH).

3.3. Determination of absolute configuration

For determination of the absolute configuration, butyramide (*S*)-**2** (30 mg, ee = 96%) was heated under reflux in 18% HCl (5 ml) for 1 h, followed by purification of the corresponding acid by ion-exchange chromatography (Amberlite IRA-401). The specific rotation for the (*S*)-3-amino-2-methylpropanoic acid obtained ($[\alpha]_D^{25}=+11.7$ (*c* 0.26, H$_2$O) confirmed the *S* absolute configuration: $[\alpha]_D^{17}=-14.2$ (*c* 0.42, H$_2$O),[5] $[\alpha]_D^{20}=-13.2$ (*c* 0.6, H$_2$O)[8] and $[\alpha]_D^{27}=-15.3$ (*c* 0.1, H$_2$O)[4] for the (*R*)-acid and $[\alpha]_D^{20}=+13.7$ (*c* 1.0, H$_2$O)[8] for the (*S*)-acid.

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