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Sequential resolution of ethyl 3-aminobutyrate with carboxylic acid esters by *Candida antarctica* lipase B

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Abstract

The reactions of ethyl 3-aminobutyrate **1** with carboxylic acid esters, catalyzed by lipases from *Candida antarctica*, *Pseudomonas cepacia* and *Pseudomonas fluorescens*, have been studied. The reactions take place on the amino and ester functions of the substrate provided that the alkyl group of the achiral ester differs from ethyl. This property has been exploited for the *Candida antarctica* lipase B-catalyzed resolution of **1** in butyl butyrate, leading to the unreacted enantiomer (*S*)-**1** and butyl 3-aminobutyrate, and to the butanamide of butyl (*R*)-3-aminobutyrate.

1. Introduction

β-Amino acids are important constituents of many natural products, and their free forms and derivatives exhibit interesting pharmacological effects.[1] β-Amino acids are also useful tools in the synthesis of modified peptides. A number of syntheses and transformations leading to β-amino acids in diastereomerically and enantiomerically enriched forms have been reviewed.[1,2] The potential of lipases as chiral catalysts is widely exploited for the kinetic resolution of racemic mixtures.[3,4] Use was made previously of *Pseudomonas cepacia* (lipase PS) and *Candida antarctica* lipase A (Chirazyme L5)-catalyzed asymmetric amide formation to prepare the enantiomers of various alicyclic β-amino acids from the racemic amino acid esters with the aid of an appropriate 2,2,2-trifluoroethyl carboxylate in diisopropyl ether.[5,6] Additionally, the lipase-catalyzed acylation of *N*-hydroxymethylated β-lactams afforded optically active precursors for the preparation of alicyclic β-amino acids.[7]

Good enantioselectivity (with an enantiomeric ratio of E=74) was also described for the preferential acetylation of the ethyl (*R*)-3-aminobutyrate **1** in the presence of *Candida antarctica* lipase B (Chirazyme

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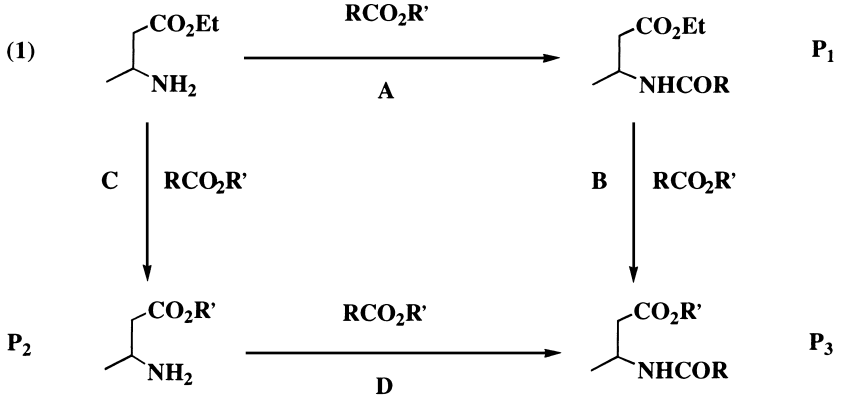
L2) in ethyl acetate.[6,8] For an E=74, the highest theoretical enantiomeric excess (ee_P) value of the (*R*)-amide produced (P_1, R=Me) was 97% at the very beginning of the reaction.[9] The ee value gradually decreased with increasing conversion and resulted in an ee_P=87% when the unreacted substrate reached a value of ee_S=99% at 53% conversion, i.e. the method allows the preparation of the less reactive (*S*)-enantiomer in a reasonable chemical yield. As synthons or intermediates for the synthesis of natural products, e.g. for the synthesis of indolizine alkaloids, the stereochemical structure of β-amino acids often corresponds to the structure of the (*R*)-enantiomer.[10]

The primary aim of this work was to study the lipase-catalyzed resolution of racemic **1**, focusing on enzymatic transformations which produce the (*R*)-enantiomer in particular. Compound **1** can be seen as a simple structural analogue of alicyclic β-amino esters such as ethyl *cis*- and *trans*-β-aminocyclopentanecarboxylates. Consequently, the resolution of **1** in the presence of lipase PS or Chirazyme L5 was expected to proceed in a highly chemo- and enantioselective manner.[5] Surprisingly, the resolution tended to lead to low chemoselectivity with the formation of multiple products. This opened up possibilities for studies of sequential resolution and for the enantiopurity enhancement of the enzymatically obtained product. The conventional in situ sequential resolutions involve compounds containing two identical reactive functional groups (e.g. the acylation–acylation sequence of diols or the esterification–esterification sequence of carboxylic acid derivatives) or compounds containing one functional group which twice visits the active site of an enzyme (e.g. an ester transforming to another ester through a hydrolysis–esterification sequence).[11–13] In the present work, the resolution exploits the two different functional groups of **1** through routes A → B or C → D (Scheme 1). The reactions in the presence of various achiral esters have been studied in order to optimize the resolution conditions.



Scheme 1. R=Me, Et, Pr or CH_2Cl and R′=Et, Bu or CH_2CF_3

2. Results and discussion

2.1. Enzymes and products

An expectation according to the previous work[5] was that in the presence of 2,2,2-trifluoroethyl butyrate or chloroacetate lipase PS leads to the highly (*R*)-selective transformation of **1** to the corresponding (*R*)-butyramide (P_1, R=Pr) or (*R*)-chloroacetamide (P_1, R=CH_2Cl) as the only detectable product, respectively (Scheme 1, route A). However, in the present work the products P_1 (Table 1, rows 1 and 2) were contaminated by racemic amide, resulting from the simultaneous chemical reaction, which became especially important during long reaction times. Moreover, 2,2,2-trifluoroethyl butyrate as the

acyl donor led to a mixture of products (row 1). In the case of Chirazyme L5 catalysis, the formation of (*R*)-butyramide (P_1, R=Pr; row 7) proceeded with low enantioselectivity. For the above reasons, the reaction of racemic **1** with 2,2,2-trifluoroethyl butyrate was further screened using various immobilized *Pseudomonas* lipases and Chirazyme L2 in diisopropyl ether. The tendency for the formation of multiple products according to Scheme 1 is obvious (Table 1).

Table 1
Enzyme screening for the reaction of **1** (0.1 M) with 2,2,2-trifluoroethyl butyrate (0.2 M) in diisopropylether at room temperature within 25 h

		Conv.	Reactivity/Enantiopurity						
Enzyme	(mg/ml)	(%)	P_1[a]	ee_{P1}	P_2[a]	ee_{P2}	P_3[a]	ee_{P3}	$ee_{(1)}$
Lipase PS	(75)	20	++	69	+	rac	—		20
Lipase PS[b]	(75)	60	+++	52	—		—		89
Lipase PSC-I	(75)	20	++	60	+	rac	negligible		15
Lipase PSC-II	(75)	55	+++	75	++	76	+	40	88
Lipase AKC-I	(75)	30	++	52	+	rac	negligible		22
Lipase AK	(75)	10	+	43	—		—		5
Chirazyme L5	(40)	50	+++	76	—		—		75
Chirazyme L2[c]	(30)	52	++	87	+	5	++	>99.9	78

[a] +, ++ and +++ refer to relative product contents according to the areas in the chromatogram. [b] 2,2,2-Trifluoroethyl chloroacetate (R=CH_2Cl) as substrate. [c] Reaction time 4 h.

A GLC–MS method was used to determine the structures of products P_1–P_3. For P_1 (R=Pr), P_2 (R′=Bu) and P_3 (R=Pr, R′=Bu) the identification was confirmed by preparing the corresponding racemates. Efforts to prepare pure products P_2 (R′=CH_2CF_3) and P_3 (R=Me or Pr, R′=CH_2CF_3) were less successful. Good baseline separations for each stereoisomer in Scheme 1 were achieved when **1** and P_2 (R′=Bu or CH_2CF_3) were chemically acylated before the chiral GLC analysis. Attention was paid to the fact that, in Chirazyme L2 catalysis, product (*R*)-P_3 (R=Pr, R′=CH_2CF_3) was obtained in an enantiopure form under the given screening conditions. Accordingly, this enzyme was chosen for further studies.

2.2. *Structural effects for* Candida antarctica *lipase B*

The structure of an achiral ester RCO_2R' may affect the course and the enantioselectivity and reactivity of Chirazyme L2-catalyzed transformations (Scheme 1, Table 2, Figs. 1–3). The esters RCO_2Et serve as achiral reagents and the reaction media. The reaction route is A (Scheme 1). Accordingly, the solvent effects of RCO_2Et (R=Me, Et or Pr) are inextricably included in the results. If this fact is accepted, it can be concluded that the time needed to reach a certain conversion increases with increasing length of the carbon chain R in RCO_2R' (R′=Et or CH_2CF_3; Table 2, rows 1–4 and rows 7 and 8). The enantioselectivity of Chirazyme L2, on the other hand, favours long-chains (R=Pr) rather than short-chains or chloro-substituted ethyl carboxylates, as is seen by comparing the *E* values. For the 2,2,2-

Table 2
Chirazyme L2 (40 mg/ml)-catalyzed reactions of **1** (0.1 M) with esters in the ester as solvent at room temperature

RCO_2R'	Time (h)	Conv. (%)	$ee_{(1)}$ (%) $(S)^e$	ee_{P1} (%) $(R)^e$	ee_{P2} (%) $(R)^e$	ee_{P3} (%) $(R)^e$	E
CH_3CO_2Et	4	53	94	84	—	—	45 ± 5
CH_3CO_2Et [a]	6	52	62	95	—	—	74
$EtCO_2Et$	25	53	96	83	—	—	38 ± 7
$PrCO_2Et$	25	53	98	88	—	—	100 ± 15
$ClCH_2CO_2Et$	4	52	87	79	—	—	18 ± 3
$PrCO_2Bu$ [b]	0.75	50	75	78	34	>99.9	—
$CH_3CO_2CH_2CF_3$ [c]	0.5	57	47	91	23	>99.9	—
$PrCO_2CH_2CF_3$ [c]	4	52	78	87	5	>99.9	—
$ClCH_2CO_2CH_2CF_3$ [d]	0,5	56	72	56	50	>99.9	—

[a] See ref. 8. [b] Enzyme content 30 mg/ml. [c] Enzyme content 30 mg/ml in di-isopropyl ether. [d] Considerable chemical reaction. [e] Absolute configuration.

trifluoroethyl esters (R′=CH_2CF_3, rows 79), it is not possible to discuss enantioselectivity in terms of the *E* values because R′ now differs from ethyl in **1**. The structural effects caused by R are seen in the proportions of the various products and in the ways in which the ee values for each are affected by the conversion. The results in Table 2 demonstrate that butyrates in general and 2,2,2-trifluoroethyl and butyl butyrates in particular are the most promising candidates for further studies.

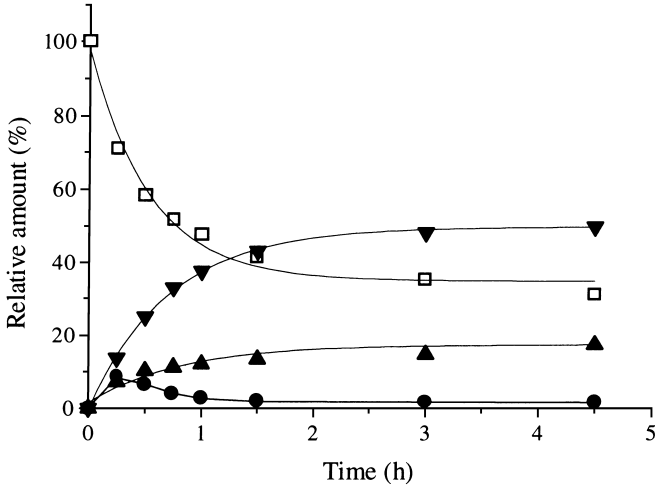


Figure 1. Relative amounts of various products vs time for the Chirazyme L2 (30 mg/ml)-catalyzed reaction of **1** (0.1 M) in butyl butyrate: **1** (□); P_1 (R=Pr) (●); P_2 (R′=Bu) (▲) and P_3 (R=Pr, R′=Bu) (▼)

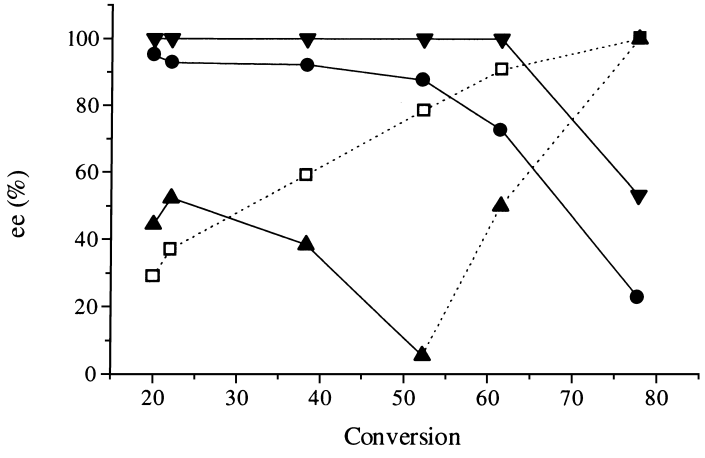


Figure 2. Enantiomeric excess vs conversion (%) for the Chirazyme L2 (30 mg/ml)-catalyzed reaction of **1** (0.1 M) with 2,2,2-trifluoroethyl butyrate (0.2 M) in diisopropyl ether: **1** (□); P_1 (R=Pr) (●); P_2 (R′=CH_2CF_3) (▲) and P_3 (R=Pr, R′=CH_2CF_3) (▼)

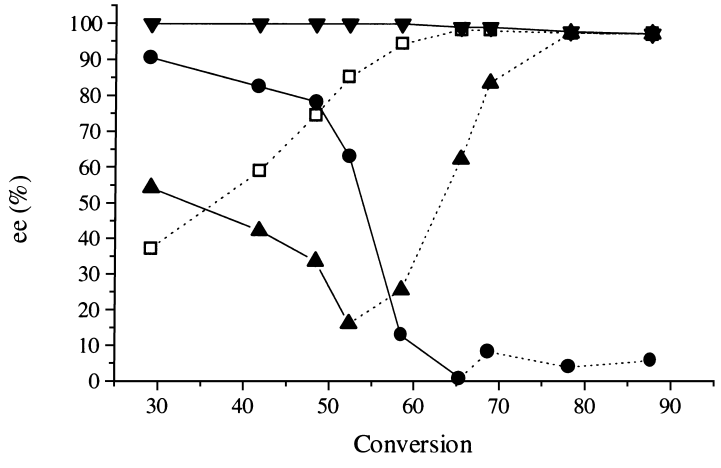


Figure 3. Enantiomeric excess vs conversion (%) for the Chriazyme L2 (30 mg/ml)-catalyzed reaction of **1** (0.1 M) in butyl butyrate: **1** (□); P_1 (R=Pr) (●); P_2 (R′=Bu) (▲) and P_3 (R=Pr, R′=Bu) (▼)

2.3. Sequential resolution by Candida antarctica *lipase B*

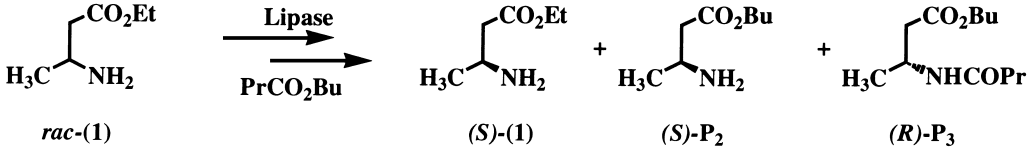
Chirazyme L2 is a commonly used lipase for the enantioselective acylation of amines.[14] Accordingly, the formation of product P_1 (R=Pr; Scheme 1, route A; Tables 1 and 2) is possible. On the other hand, the enzyme also catalyzes enantioselective reactions of carboxylic acid derivatives. With dicarboxylic acids and esters, such as *N*-protected D-glutamic acid diesters, the regioselectivity of Chirazyme L2 can be directed by adding nucleophiles to the less hindered carbonyl groups.[12,15,16] Clearly, the ester group in **1** corresponds to such a less hindered position, allowing the enzymatic reaction to occur at the ester function and the formation of product P_2 (R′=CH_2CF_3 or Bu; route C). Since routes A and C are possible, it is natural that the further reactions through routes B and D can lead to the formation of product P_3.

For sequential resolution of **1** by Chirazyme L2, the two functional groups are simultaneously susceptible to enzymatic transformation, the activation energy differences for their reactions with 2,2,2-trifluoroethyl or butyl butyrate determining regioselectivity/chemoselectivity. For 2,2,2-trifluoroethyl butyrate, the observed product distribution $P_1>P_3>P_2$ [e.g., the area ratio **1**:P_1:P_2:P_3=4.0:1.5:0.3:1.0

(R=Pr and R′=CH_2CF_3) at 60% conversion] differs significantly from that for butyl butyrate where P_3 (R=Pr, R′=Bu) is the major and P_1 (R=Pr) the minor product (Fig. 1). These observations indicate that the activated 2,2,2-trifluoroethyl butyrate favours butanamide formation (product P_1, R=Pr), followed by the formation of product P_3 (R=Pr, R′=CH_2CF_3) through route A+B (Scheme 1). In support of this, the concentration of P_2 (R′=CH_2CF_3; route C) is low and that of P_1 (R=Pr, route A) relatively high all the way up to 80% conversion. The separate reaction of racemic P_1 (R=Pr) with 2,2,2-trifluoroethyl butyrate (route B) was relatively fast and highly enantioselective (27% conversion in 40 min, ee_{P3}=98%), further indicating that the formation of P_1 must be favourable when **1** reacts with (*R*)-P_3 (R=Pr, R′=CH_2CF_3).

On the other hand, for sequential resolution of **1** with butyl butyrate the formation of product P_2 (R′=Bu) (route C) is favoured (Fig. 1). In order to confirm this, racemic P_1 (R=Pr) and P_2 (R′=Bu) were separately subjected to reactions with butyl butyrate under the resolution conditions. The formation of P_3 (R=Pr, R′=Bu; route B) from racemic P_1 (R=Pr) in butyl butyrate proceeded extremely slowly (2–3% conversion in 6 h). Because the concentration of P_1 stayed negligible throughout the reaction route A+B cannot be significant. In contrast, amide formation from racemic P_2 (R′=Bu; route D) was favourable and 50% conversion was reached in 6 h with ee_{P2}/ee_{P3}=97%/95%.

The ee values vs the corresponding total conversion for the Chirazyme L2-catalyzed reactions of **1** with 2,2,2-trifluoroethyl butyrate and with butyl butyrate are shown in Figs. 2 and 3, respectively. In accordance with the previously observed (*R*)-selectivity of Chirazyme L2,[8] the (*R*)-enantiomer reacts faster in the formation of product P_1 (route A) or P_2 (route C). According to the chiral GLC method, these (*R*)-enantiomers are also the reactive enantiomers in the formation of product P_3 through route B or D. This means that the products (*S*)-P_1 and (*S*)-P_2 start to accumulate with time. This is seen as the change of the prevailing (*R*)-enantiomer to the (*S*) one at higher conversions and is shown by the change of the solid lines to the dotted ones in Figs. 2 and 3. With 2,2,2-trifluoroethyl butyrate as achiral reagent (route A+B favoured), the favourable lipase-catalyzed ethanolysis of the activated ester P_3 (R=Pr, R′=CH_2CF_3) back to P_1 (R=Pr) and the consequent equilibrium nature of step B can explain the racemization of P_3 after about 60% conversion (Fig. 2). This information related to the above-mentioned product distribution (P_1>P_3>P_2) suggests that the resolution of **1** in butyl butyrate is more favourable. Thus, in butyl butyrate, both product P_2 (R′=Bu) and the unreacted substrate fraction have an (*S*)-stereocentre at conversion close to 80%, whereas product P_3 (R=Pr and R′=Bu) at this point represents the corresponding antipode at 97% ee (Scheme 2, Fig. 3). By stopping the reaction before 70% conversion ee_{P3} >97% is obtained. The main aim of this work was the preparation of the (*R*)-enantiomer. To this end, a successful gram-scale resolution of **1** in butyl butyrate was performed, allowing the preparation of (*R*)-P_3 (R=Pr, R′=Bu) (ee >99%) when the reaction was stopped at 65% conversion, as is shown in the Experimental.



Scheme 2.

An interesting point concerning the Chirazyme L2-catalyzed reactions of **1** with 2,2,2-trifluoroethyl and butyl butyrates is the process leading to the observed products P_1–P_3 (Scheme 1). Routes A and D are simple amide formations (aminolyses, $CH_3CH(NH_2)CH_2CO_2X$+RCO_2R'), whereas the lipase-catalyzed transesterifications ($CH_3CH(NHX)CH_2CO_2Et$+RCO_2R') need more attention. With 2,2,2-trifluoroethyl butyrate as achiral reagent, the formation of products P_2 (R′=CH_2CF_3; route C) or P_3 (R=Pr, R′=CH_2CF_3; route B) was not expected because of the poorly nucleophilic nature of 2,2,2-trifluoroethanol, liberated when 2,2,2-trifluoroethyl butyrate forms a butyryl–enzyme intermediate. In-

deed, no reaction took place between **1** or P_1 (R=Pr) and 2,2,2-trifluoroethanol (0.1 M) in diisopropyl ether or in neat 2,2,2-trifluoroethanol within 2–3 days in the presence of Chirazyme L2. However, as already mentioned, product P_3 (R=Pr, R′′=CH_2CF_3) was smoothly produced from P_1 (R=Pr) and 2,2,2-trifluoroethyl butyrate. We propose that intramolecular hydrogen bonding (N–H···O=C) and the liberated alcohol already situated at or in the proximity of the active site aid the present transesterifications. In the case of butyl butyrate, on the other hand, the Chirazyme L2-catalyzed butanolysis (0.01–0.1 M) of **1** and P_1 (R=Pr) in diisopropyl ether proceeded efficiently and accordingly enzymatic butanolysis by liberated butanol is more natural.

3. Conclusions

Chirazyme L2 has proved its potential for the resolution of racemic amino ester **1** with an appropriate achiral carboxylic acid ester (RCO_2R', with R′ differing from the alkyl group of the racemic ester) exploiting sequential resolution. One of the enantiomers is then obtained as product (*R*)-P_3 through route A+B or C+D (Scheme 1). With butyl butyrate as RCO_2R' and as solvent (route C+D), the concentration of P_1 (R=Pr) is negligible, allowing the preparation of two highly enantiopure fractions [(*S*)-P_2 (R′=Bu)+(*S*)-**1** and (*R*)-P_3 (R=Pr, R′=Bu)] at ca. 80% total conversion. In order to understand the reasons for the observed enantioselectivities and reactivities (Figs. 1–3), the enzyme-catalyzed butyrylation of racemic butyl 3-aminobutyrate (route D) and the transesterification/butanolysis of butyramide P_1 (route B) in butyl butyrate were separately investigated. Excellent enantioselectivities (E>100) were generally observed, indicating that these reactions can serve as tools for resolution as well. Route D corresponds to the previous Chirazyme L2-catalyzed resolution of ethyl 3-aminobutyrate in ethyl acetate (E=74[8] or 38; Table 2) and in connection with the present results indicates the importance of structural and solvent effects on enantioselectivity.

4. Experimental

4.1. Materials and methods

Chirazyme L2 and L5 were purchased from Boehringer Mannheim.[6] Lipase PS, PSC-I and PSC-II from *Pseudomonas cepacia* and lipase AK and AKC-I from *Pseudomonas fluorescens* were purchased or were generous gifts from Amano Pharmaceuticals. Lipase preparations from lipases PS, AK and Chirazyme L5 were adsorbed on Celite in the presence of sucrose.[5] The final preparations contained 20% (lipases PS and AK) or 10% (w/w) (Chirazyme L5) of the lipase. 3-Aminobutyric acid was prepared by known methods.[17] Racemic ethyl and butyl 3-aminobutyrates were prepared from the amino acid by standard procedures, using thionyl chloride and the corresponding dry alcohol. Achiral esters were prepared from the corresponding acid chlorides and alcohols. The solvents were of the best analytical grade from Lab Scan.

In a typical small-scale experiment, ethyl 3-aminobutyrate (0.1 M) and hexadecane (0.01 M, an internal standard) were dissolved in 2.5 ml of diisopropyl ether, and the acyl donor (0.2 M) was added, or the solution was made in alkyl carboxylate, which served as acyl donor and solvent. The enzyme preparation (30–75 mg/ml) was added in order to start the reaction. The reaction mixture was shaken at room temperature. The progress of the reaction and the ee values of the products were followed by taking samples (0.1 ml) at intervals and analyzing them by gas chromatography on a Chrompack CP-

cyclodextrin-β 2,3,6-M-9 column (25 m). Before the analysis, the unreacted ethyl ester **1** in the sample was derivatized with acetic or butyric anhydride in the presence of 4-*N*,*N*-dimethylaminopyridine and pyridine.

^{1}H NMR spectra were measured on a Bruker 200/Aspect 3000 spectrometer in $CDCl_3$ (tetramethylsilane as internal standard). Mass 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^{-1}.

4.2. Gram-scale resolution of ethyl 3-aminobutyrate

Racemic ethyl 3-aminobutyrate (2.33 g, 17.7 mmol) was dissolved in butyl butyrate (178 mmol) and Chirazyme L2 (5.3 g) was added. The mixture was stirred at room temperature for 2 h. The reaction was stopped by filtering off the enzyme at 65% conversion. In order to facilitate the separation in the column, the unreacted **1** and product P_2 (R′=Bu) were transformed to the corresponding acetamides with acetic anhydride (2 ml, 0.021 mol) in the presence of 4-*N*,*N*-dimethylaminopyridine and pyridine (1 ml) by stirring the reaction mixture overnight. After evaporation, the products were separated on silica gel by elution with petroleum ether:propan-1-ol (100:6), the elution sequence being P_3 (R=Pr, R′=Bu) before the acetamide of P_2 (R′=Bu) and P_1 (R=Pr). After the three products had eluted, the eluent was changed to propanol and the acetamide of unreacted (*S*)-**1** was obtained. Evaporation of the solvent gave the products and the unreacted substrate, except that the amount of P_1 (R=Pr) was too small to be isolated.

4.2.1. (R)-P_3 (R=Pr, R′=Bu)

1.32 g, 5.77 mmol; $[\alpha]_D^{20}$ +16.1 (c=1.0, MeOH); ee 99%. M=229 according to MS. ^{1}H NMR (200 MHz) δ (ppm): 0.9 (6H, m, 2×CH_2CH_3), 1.2 (3H, d, *J*=6.6, $CHCH_3$), 1.3 (2H, m, $CH_2CH_2CH_2$), 1.6 (4H, m, $CH_2CH_2CH_2$, $CH_2CH_2CH_3$), 2.1 (2H, t, *J*=7.4, $COCH_2$), 2.5 (2H, d, *J*=5.3, CH_2CO_2), 4.0 (2H, t, *J*=6.5, CO_2CH_2), 4.3 (1H, m, CH). Analysis: calculated for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11; found: C, 62.49; H, 10.41; N, 6.11.

4.2.2. Acetamide of (S)-P_2 (R′=Bu)

0.46 g, 2.28 mmol; contains 10% of the unreacted substrate **1** and 4% of the produced P_3. M=201 according to GLC–MS. ^{1}H NMR (200 MHz) δ (ppm): 0.9 (3H, t, *J*=7.25, CH_2CH_3), 1.2 (3H, d, *J*=6.72, $CHCH_3$), 1.3–1.7 (4H, m, $2CH_2$), 1.9 (3H, s, $COCH_3$), 2.5 (2H, d, *J*=5.16, CH_2CO_2), 4.0 (2H, t, *J*=6.7, CO_2CH_2), 4.3 (1H, m, CH).

4.2.3. Acetamide of (S)-*1*

0.83 g, 4.82 mmol; $[\alpha]_D^{20}$ −22.5 (c=1.0, MeOH); ee 96%. M=173 according to MS. ^{1}H NMR (200 MHz) δ (ppm) 1.1–1.3 (6H, m, $2CH_3$), 1.9 (3H, s, $COCH_3$), 2.5 (2H, d, *J*=5.4, CH_2CO_2), 4.1 (2H, m, *J*=7.0, CO_2CH_2), 4.3 (1H, m, CH). Analysis: calculated for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09; found: C, 54.06; H, 8.74; N, 7.54.

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