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Chemoenzymatic approach to enantiopure piperidine-based β-amino esters in organic solvents

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Abstract—This research concentrates on the enantioselectivities of lipase-catalysed reactions with methyl esters of 2-piperidylacetic acid and 3-piperidinecarboxylic acid derivatives. N-Acetylated 2-piperidylacetic acid methyl ester displayed good enantioselectivity ($E = 66$) in a 1:1 mixture of diisopropyl ether and butyl butanoate in the presence of lipase PS-C II from *Burkholderia cepacia*. The reaction is known as interesterification with butyl butanoate rather than alcoholysis with the butanol, because butyl butanoate has to be first hydrolysed or go through alcoholysis with MeOH in order to release butanol. Other N-protective groups (Boc, Ns, Fmoc and Bzn) gave excellent enantioselectivity ($E > 200$) under the same conditions, and a gram-scale resolution was performed with *N*-Boc-2-piperidylacetic acid methyl ester. Reaction with a 3-piperidylcarboxylic acid derivative took place with disappointingly low enantioselectivity ($E = 4$), with *Candida antarctica* lipase B being the best of the lipases screened.

1. Introduction

Enantiopure N-heterocyclic amino acids are important structural units in many natural products and valuable intermediates and starting materials in the preparation of pharmaceutically important compounds.[1] Our previous work with N-heterocyclic α-amino acids opened a route to highly efficient kinetic and dynamic kinetic resolution of proline and pipecolic acid methyl esters through CAL-A-catalysed (*Candida antarctica* lipase A) N-acylation at the secondary ring nitrogen of the compounds.[2,3] Under exactly the same conditions with the same acyl donors, CAL-B (*C. antarctica* lipase B) catalysed reactions specifically at the methyl ester function in a reaction, which is known as interesterification.[2] Interesterification has recently been successfully applied in the activation of various β-amino acids as 2,2,2-trifluoroethyl esters for β-peptide synthesis by using 2,2,2-trifluoroethyl butanoate as the in situ source of the alcohol.[4] Typical to these reactions, alcoholysis with 2,2,2-trifluoroethanol did not lead to the desired 2,2,2-trifluoroethyl ester formation, suggesting more complicated mechanistic details than can be anticipated on the basis of the simple ping-pong bi-bi mechanism.[5] Our previously proposed hypothesis was that the alcohol obtained from 2,2,2-trifluoroethyl ester through enzymatic action does not leave the enzyme, but rather stays around the catalytic site.[4,6] A similar tendency for interesterification has also been observed with some other lipases, for example, with lipase PS (*Burkholderia cepacia* lipase).[5]

Inspired by these observations, we prepared piperidyl-based β-amino acid derivatives *rac*-**1a**–**f** and *rac*-**2a**–**c** and studied their lipase-catalysed kinetic resolution in organic solvents (Schemes 1–3). Various N-protective groups were used, because the nature of a group is often critical when amino acids are used for synthetic applications. N-Acylation of *rac*-**1a** and *rac*-**2a** by CAL-A (A), and aminolysis (B), alcoholysis (C) and interesterification (D) at the ester function of *rac*-**1b** (also **1c** for aminolysis) have been used as model reactions in order to determine the conditions and the best reaction type for gram-scale resolution. The highly enantioselective hydrolysis of *rac*-**1c** by lipase PS,[7] the hydrolysis of methyl *N*-benzyl-2-piperidylacetic acid by CAL-B[8] and the practically non-selective pig liver esterase-catalysed hydrolysis of *rac*-**2b**[9] have been described earlier. However, the possibilities allowed by lipase-catalysed reactions in organic solvents are not known so far. Herein, we report the potential and challenges in interesterification, when the product is

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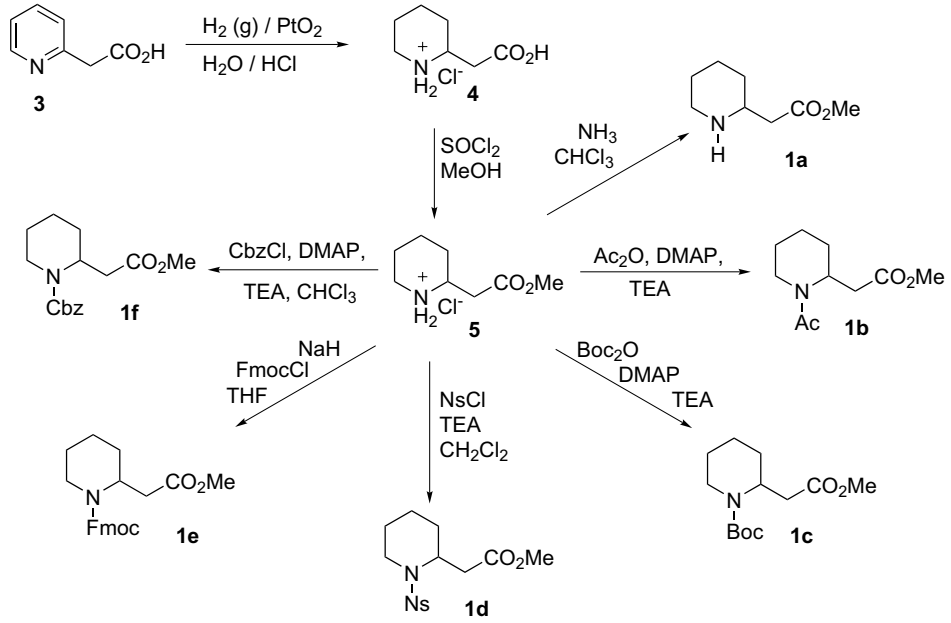
formed from a mixture of butyl butanoate and a β-amino ester in the presence of a lipase in an organic solvent. Throughout this article we distinguish the terms alcoholysis [a reaction between an ester (RCO_2R^1) and an alcohol (R^2OH)] and interesterification [a reaction whereby two esters (RCO_2R^1 and $R^3CO_2R^2$) have changed their alkyl groups in the products formed], although in both reactions, the alcohol R^2OH evidently serves as a nucleophile in the deacylation step of the bi-bi ping-pong mechanism of serine hydrolases, including lipases.[10]

2. Results and discussion

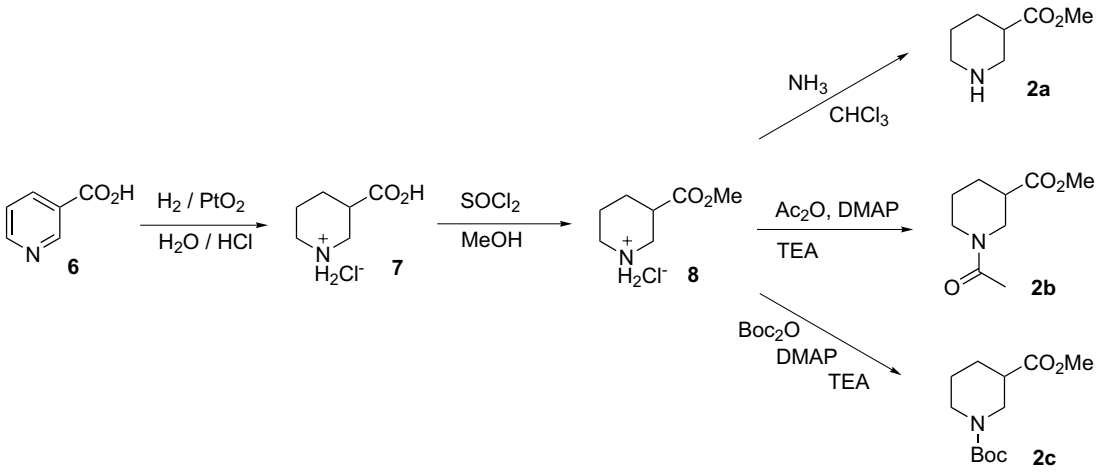
2.1. Preparation of racemic compounds

Racemic amino esters **1a**–**f** and **2a**–**c** were synthesised according to the routes shown in Schemes 1 and 2. The key step of the syntheses was the catalytic hydrogenation of 2-pyridylacetic acid **3** and nicotinic acid **6**. The acids were quantitatively transformed into the corresponding *rac*-**4** and *rac*-**7** by a known method.[9]

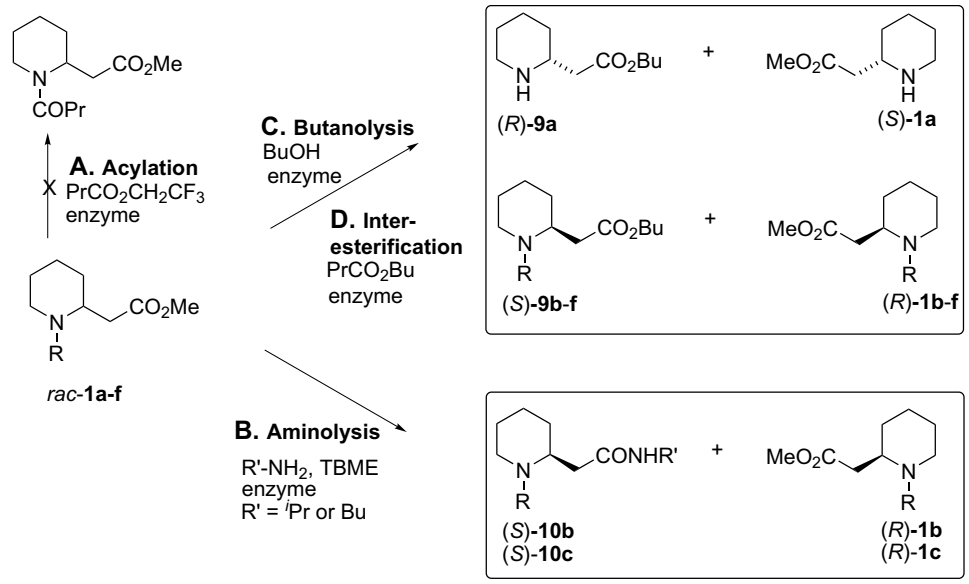
The structures of the compounds prepared were established by ^1H and ^{13}C NMR spectroscopic methods. Although compounds **1c** and **2b** are previously known, their stereochemistry has not been analysed in detail.[7–9] NMR spectroscopic studies revealed two isomers in equilibrium with each other for **1b**, **1c**, **1e**, **1f**, **2b** and **2c**. The isomers correspond to the *E*- and *Z*-isomers with respect to the partial double bond of the amide moiety (Scheme 4). For the *N*-acetyl derivatives **1b** and **2b**, the *E*- and *Z*-isomers gave separate NMR spectra at 25 °C, whereas a lower temperature (−50 °C used) was needed for the carboxamide derivatives. Thus, the rotation of the N-substituent is less restricted, indicating a lower double bond degree in the latter.



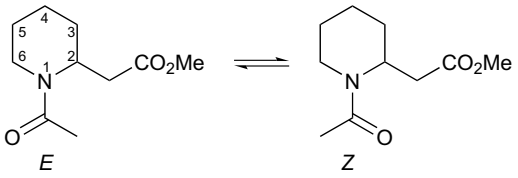
Scheme 1. Preparation of racemic 2-piperidylacetic acid derivatives **1a**–**f**.



Scheme 2. Preparation of racemic 3-piperidinecarboxylic acid derivatives **2a**–**c**.



Scheme 3. Enzymatic resolution strategies.



Scheme 4. *E*/*Z* Isomeric equilibrium of **1b**. Note, that in the carboxamide derivatives **1c**, **1e**, **1f** and **2c**, the *E*/*Z* forms are the other way round than in the acetamide derivatives **1b** and **2b**.

The molar ratios of the *E*- and *Z*-isomers are collected in Table 1. For *N*-acetyl derivatives **1b** and **2b**, the *E*- and *Z*-isomers are easily recognised by their H-2, H-6eq, C-2 and C-6 chemical shifts. In the *Z*-isomers, the equatorial H-2 is deshielded and C-2 shielded by the adjacent carbonyl group, whereas in the *E*-isomers H-6eq is deshielded and C-6 is shielded likewise as the carbonyl group is turned adjacent to them. In **1c**, **1e**, **1f** and **2c**, the characterisation of the isomers is not as unambiguous, as their N-substituent possesses two oxygens deshielding both H-2eq and H-6eq. Attempts to characterise the *E*- and *Z*-isomers of the carboxamide derivatives with NOE measurements did not result in further evidence.

Table 1. The mole ratios of the *E*- and *Z*-isomers as determined from the peak area integrals of selected, well separated ^{1}H or ^{13}C NMR signals

Compound	Time (°C)	*E*/*Z*
1b	+25	53:47
1c	−50	57:43
1e	−50	56:44
1f	−50	55:45
2b	+25	53:47
2c	−50	71:29

The piperidine ring remains in a chair conformation in each compound, as shown by the ^{3}J couplings between the hydrogens on the ring carbons. The equatorial position of H-2 in the 1,2-disubstituted compounds **1b**–**f** is shown by the lack of a large $^{3}J_{\text{H-2,H-3ax}}$ in each case and, therefore, the substituent at C-2 must remain axial. In **1a**, the methyl acetate substituent is equatorial and H-2 is axial, which is shown by the large $^{3}J_{\text{H-2,H-3ax}}$ (11.0 Hz). Similarly, the methyl carboxylate substituent in **2a**–**c** occupies the equatorial position, as shown by the large $^{3}J_{\text{H-2ax,H-3}}$ and $^{3}J_{\text{H-3,H-4ax}}$ values (9.0–10.7 Hz).

2.2. Enzymatic kinetic resolution of 1a–f

2.2.1. N-Acylation. On the basis of the previous studies,[2,3,6] the CAL-A-catalysed kinetic resolution of **1a** and **2a** was expected to be successful through N-acylation with 2,2,2-trifluoroethyl butanoate (Scheme 3, method A. Acylation). No reaction was observed, however, in the presence of CAL-A. The butanolysis of **1a** in neat butanol with the majority of the other 12 lipases or lipase preparations (shown in Section 4, method C. Butanolysis) did not proceed either. CAL-B (10 mg mL^{-1}), as an exception, gave (*R*)-selective alcoholysis (67% conversion after 23 h with $ee^{(S)\text{-}\mathbf{1a}} = 89\%$ and $ee^{(R)\text{-}\mathbf{9}} = 43\%$) in a reaction, which was significantly contaminated by a chemical background reaction (13% in 23 h in the absence of CAL-B). The background reaction was diminished and the reactivity greatly enhanced (78% conversion after 40 min) by the use of butyl butanoate as a solvent in place of butanol (method D. Interesterification). Unfortunately the change of solvent did not improve the enantioselectivity. As a consequence, our attention turned to N-protected **1b**–**f** as substrates.

2.2.2. Aminolysis. Lipase-catalysed aminolysis has successfully been used in the kinetic resolution of some carboxylic acid derivatives and in asymmetrisation of *meso*-dicarboxylic acids.[11–13] Aminolysis is a fascinating possibility for lipase catalysis, because the amide product obtained is generally stable in the presence of lipases.

Table 2. Lipase (80 mg mL^{-1})-catalysed aminolyses of **1b** and **1c** (0.1 M) with isopropyl- and butylamines (0.2 M) in TBME at room temperature; reaction time 24 h

Entry	Compound	Enzyme	Amine	ee_s (%)	ee_p (%)	Conversion (%)	*E*
1	**1b**	CAL-B	$^iPrNH_2$	2	65	3	5
2	**1b**	CAL-B	$BuNH_2$	<1	12	4	1
3	**1b**	PS-C II	$^iPrNH_2$[a]	7	99	6	192
4	**1b**	PS-C II	$^iPrNH_2$	6	>99	6	93
5	**1b**	PS-C II	$^iPrNH_2$[b]	4	>99	3	24
6	**1b**	PS-D	$^iPrNH_2$	1	99	1	167
7	**1b**	AK-C	$^iPrNH_2$	2	>99	2	>200
8	**1c**	CAL-B	$^iPrNH_2$	1	<1	69	1
9	**1c**	CAL-B	$BuNH_2$	1	69	2	6
10	**1c**	PS-C II	$^iPrNH_2$	8	>99	8	>200
11	**1c**	PS-C II	$BuNH_2$	7	>99	7	150

[a] $^iPrNH_2$ 0.1 M.
[b] $^iPrNH_2$ 0.5 M.

Herein, the aminolysis of **1b** and **1c** was studied by using butyl- and isopropylamines as nucleophiles in the presence of selected lipase preparations in TBME (Scheme 3, Table 2). CAL-B displayed both low reactivity (conversion reached after a certain time) and enantioselectivity with the substrates used (entries 1, 2, 8 and 9); aminolysis of **1b** with isopropylamine being an exception with relatively good reactivity (entry 8). Lipase PS (entries 3–6, 10 and 11) and AK (*Pseudomonas fluorescens* lipase; entry 7) preparations gave reactions with excellent enantioselectivities, but the reactions were too slow to be of practical value. Attempts to enhance the reactivity by changing the amine concentration failed (entries 3–5). As a conclusion, aminolysis as a reaction type was rejected.

2.2.3. Alcoholysis/interesterification. BuOH (alcoholysis), butyl butanoate (interesterification) and their mixtures with diisopropyl ether (DIPE) were chosen as the reaction media according to our previous studies with lipases.[2–6,14,15] N-Acetylated *rac*-**1b** was used as a substrate for optimisation experiments. From the lipases screened, only lipase PS-C II (lipase PS immobilised on Toyonite 200M)[16] catalysed the alcoholysis of *rac*-**1b** in neat BuOH (Table 3, entry 1). The reaction was extremely slow. A higher reactivity was obtained by adding DIPE into the reaction mixture (entries 2–4). This can be explained by solvent effects and by the previously observed inhibitory effect caused by BuOH.[17,18] The effect of BuOH concentration on the enantioselectivity was minimal, as measured by '*E*'. We have used '*E*' rather than *E*, because the enzymatic reaction of (*S*)-**9b** with the MeOH produced back to (*S*)-**1b** can be significant, especially at low (0.2 M) BuOH concentrations. For this reason, the '*E*'-values were all determined before 40% conversion as described in Section 4.3.

Interesterification of **1b** in neat butyl butanoate (Table 3, entry 5) with lipase PS-C II was fast, compared to that in neat BuOH (entry 1), and it was possible to roughly double the conversion at 47 °C (entry 6). The enzyme preparation started to become inactive at 80 °C (entry 7). The effect of temperature on the enantioselectivity was significant, '*E*' decreasing with increasing temperature. As a compromise between reactivity and enantioselectivity, 47 °C was used for all further interesterification optimisations. As shown in Figure 1, increasing DIPE contents in butyl butanoate positively affected enzymatic reactivity, '*E*' being highest when the 9:1 mixture of DIPE and butyl butanoate was used. Interestingly, the (*S*)-enantiomer reacted faster in the case of N-protected amino esters **1b–f**, whereas it was the (*R*)-enantiomer in the case of **1a** (Scheme 3).

For the lipase-catalysed reaction ($RCO_2R^1 + R^2OH \rightarrow RCO_2R^2 + R^1OH$), the first mechanistic step includes acylation of the serine hydroxyl of the active site with RCO_2R^1 as an acyl donor and the liberation of the first product R^1OH.[10] In the second mechanistic step, the formed acyl-enzyme intermediate reacts with R^2OH as an added nucleophile, leading to the formation of the second product and the liberation of the free enzyme. The interesterification mixture in the present work contains two acyl donors (*rac*-**1b** and butyl butanoate) without an added nucleophile. Thus, the acyl donors liberate MeOH and BuOH, respectively, in the formation of acyl-enzyme intermediates. This is possible only if the water in the seemingly

Table 3. Lipase PS-C II-catalysed (75 mg mL^{-1}) alcoholysis/interesterification of **1b** (0.1 M); reaction time 48 h

Entry	Medium	Temp (°C)	$ee^{(R)\text{-}\mathbf{1b}}$ (%)	$ee^{(S)\text{-}\mathbf{9b}}$ (%)	Conversion (%)	'*E*'
1	BuOH (neat)	23	—[a]	—[a]	1	—[a]
2	BuOH (0.6 M) in DIPE	23	41	97	30	95 ± 5
3	BuOH (0.4 M) in DIPE	23	51	97	35	92 ± 5
4	BuOH (0.2 M) in DIPE	23	56	96	37	69 ± 12
5	$PrCO_2Bu$ (neat)	23	12	99	10	138 ± 8
6	$PrCO_2Bu$ (neat)	47	23	95	19	42 ± 2
7	$PrCO_2Bu$ (neat)	80	4	55	7	3 ± 1

[a] Due to slow reaction ee and '*E*' was not obtained.

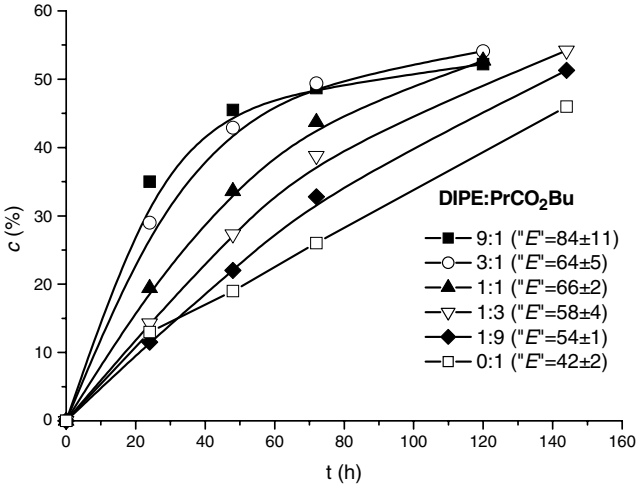


Figure 1. Progression curves for the lipase PS-C II-catalysed (75 mg mL^{-1}) interesterification of *rac*-**1b** (0.1 M) in DIPE–$PrCO_2Bu$ at 47 °C.

dry enzyme preparation acts as a nucleophile, hydrolysing the acyl-enzyme intermediates. After this initiation step, there are three competitive nucleophiles (water, MeOH and BuOH). Our hypothesis is that the liberating BuOH (and MeOH) must stay bound at the active site or its close vicinity, where it can replace water or be inserted into a possible water network. Thus, the alcohol concentration for the reaction is at least equimolar to that of the acyl-enzyme intermediate. The observed competitive inhibition by BuOH is not against this hypothesis.[17,18]

In the next step of the optimisation, *rac*-**1b** was subjected to interesterification in various mixtures of DIPE and butyl butanoate in the presence of lipase PS-C II (Fig. 1). According to the above consideration, there are enzyme molecules containing MeOH in addition to BuOH for the lipase PS-C II-catalysed interesterification of *rac*-**1b** with butyl butanoate. This explains why $ee^{(R)\text{-}\mathbf{1b}}$ did not exceed the value 86% in the 9:1 mixture of DIPE–butyl butanoate and why the reaction tended to stop near 50% conversion (Fig. 1 (■) and 2 (■ and □); Table 4, entry 1). BuOH was added into the reaction mixture in order to suppress possible methanolysis and/or hydrolysis of (*S*)-**9b** into the starting material, to lead the reaction to higher conversions and to obtain (*R*)-**1b** with better enantiopurity (Fig. 2, ● and ○; Table 4, entry 2). Finally, a 1:1 mixture of DIPE and butyl butanoate was chosen for the conditions, where the effect of MeOH seemed to be suppressed (entry 3). When BuOH was added to the reaction mixture, reactivities decreased somewhat while the enantioselectivity increased, the '*E*' values being independent of the added alcohol concentration (entries 3–6).

The N-protected substrates *rac*-**1b**–**f** were finally screened under the optimised conditions in the 1:1 mixture of DIPE and butyl butanoate at 47 °C, and the results are shown in Figure 3. The replacement of an acetyl group with *tert*-butoxycarbonyl (Boc, **1c**), *o*-nitrotoluenesulfonyl [Ns

Table 4. Effect of BuOH on the lipase PS-C II-catalysed (75 mg mL^{-1}) interesterification of **1b** (0.1 M) in the mixture of DIPE and butyl butanoate at 47 °C

Entry	DIPE–$PrCO_2Bu$	BuOH (M)	*t* (h)	$ee^{(R)\text{-}\mathbf{1b}}$ (%)	$ee^{(S)\text{-}\mathbf{9b}}$ (%)	Conversion (%)	'*E*'[a]
1	9:1	0	72	86	91	49	84 ± 11
2	9:1	0.2	144	90	87	51	89 ± 5
3	1:1	0	24	23	97	19	66 ± 2
4	1:1	0.2	24	22	98	18	99 ± 2
5	1:1	0.4	24	18	98	16	101 ± 1
6	1:1	0.6	24	14	98	12	100 ± 3

[a] *E* values calculated at conversions less than 40%.

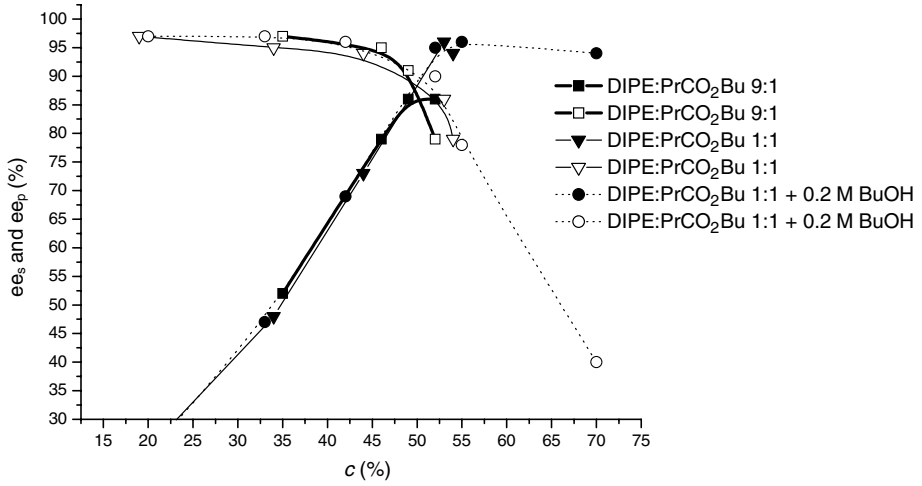


Figure 2. Enantiomeric excesses of the substrate and the product versus conversion in various DIPE–$PrCO_2Bu$–BuOH mixtures.

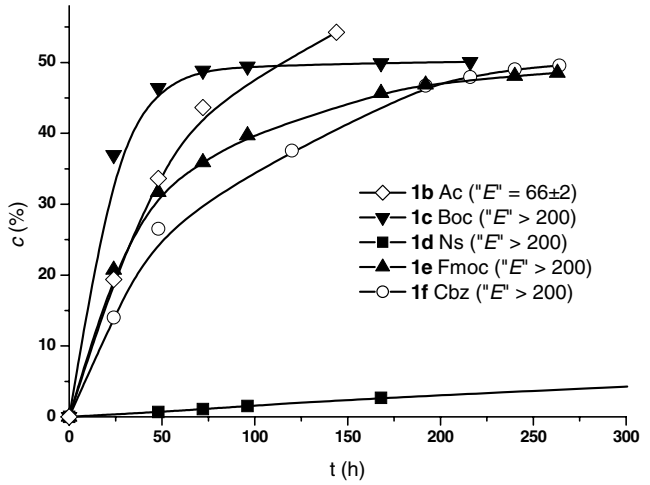


Figure 3. Lipase PS-C II-catalysed (75 mg mL^{-1}) interesterifications of 2-piperidylacetic acid methyl ester derivatives **1b–f** (0.1 M) in $PrCO_2Bu$–DIPE (1:1).

(nosyl), **1d**], 9-fluorenylmethoxycarbonyl (Fmoc, **1e**) and benzyloxycarbonyl (Cbz, **1f**) groups enhanced the enantioselectivity substantially (*E* increased from 66 for *rac*-**1b** to >200 for *rac*-**1c–f**). Enzymatic reactivities of **1b–f** differed considerably from each other. With *N*-nosyl protection (■), the reaction was too slow to be of any practical importance. *rac*-**1c** gave a relatively fast reaction to yield a mixture of (*R*)-**1c** and (*S*)-**9c** (▼).

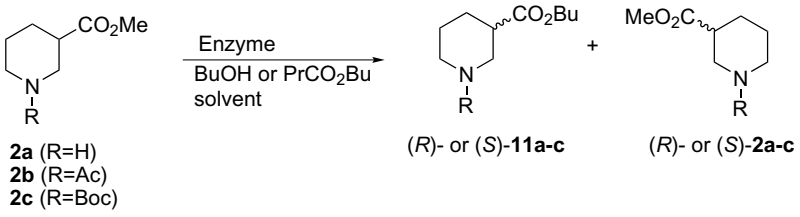
Finally, *rac*-**1c** (0.1 M; Fig. 3, ▼), as the most reactive of the present substrates, was subjected to gram-scale resolution by lipase PS-C II-catalysed (75 mg mL^{-1}) interesterification in DIPE–$PrCO_2Bu$ (1:1) in the presence of BuOH (0.2 M) at 47 °C. The reaction was stopped at 49% conversion, giving (*R*)-**1c** (ee = 94%) and (*S*)-**9c** (ee = 99%) with high chemical yields as shown in Section 4. BuOH was added to make sure that the methanolysis of (*S*)-**9c** was suppressed. The addition of BuOH (0.2 M) increased the resolution time significantly. Thus, one should be critical of the necessity of using BuOH as a conucleophile.

2.3. Enzymatic kinetic resolution of 2a–c

The lipase PS-C II-catalysed alcoholysis and interesterification methods described above were used for studies with *rac*-**2a–c** (Scheme 5, Table 5). Both reactions took place with low enantioselectivity (entries 4–6). In order to find a more usable catalyst for the kinetic resolution, enzyme screening for the alcoholysis of *rac*-**2b** in neat butanol was performed. CAL-B, as the best enzyme, gave a low '*E*' of 4 and, as a consequence, a gram-scale resolution was not performed (entries 1–3).

3. Conclusion

Research aimed at the chemoenzymatic preparation of the enantiomers of piperidyl-based β-amino acid derivatives through the lipase-catalysed kinetic resolution of *rac*-**1a–f** and *rac*-**2a–c** has been reported. The N-acylation of *rac*-**1a** and *rac*-**2a** (A), and the aminolysis (B), alcoholysis (C) and interesterification (D) at the ester function of *rac*-**1b** (also **1c** for aminolysis), as model reactions, revealed that interesterification in the presence of lipase PS-C II from *B. cepacia* is optimal in a 1:1 mixture of DIPE and butyl butanoate yielding an '*E*' = 66. Under these conditions, interesterification of *rac*-**1b–f** was highly enantioselective ('*E*' >200). It was possible to perform kinetic resolutions with all three substrates, *rac*-**1d** (*N*-nosyl protection) being an exception due to very low reactivity. Due to the highest reactivity, a gram-scale resolution was performed using *N*-Boc-protected *rac*-**1c** as the substrate, which consequently led to the unreacted (*R*)-**1c** (ee = 94%) and the interesterification product (*S*)-**9c** (ee = 99%) at 49% conversion. A method for the kinetic resolution of the nicotinic acid derived 3-piperidylacetic acid derivatives **2** was not found in this study.



2a (R=H)
2b (R=Ac)
2c (R=Boc)

Scheme 5. Enzymatic resolution of **2a–c**.

Table 5. Lipase-catalysed (75 mg mL^{-1}) reactions of *rac*-**2a–c**, (0.1 M) at 23 °C

Entry	Compound	Enzyme	Solvent	Time (h)	ee_s (%)	ee_p (%)	Conversion (%)	'*E*'
1	**2a**	CAL-B	BuOH (neat)	24	32	—[a]	41	3
2	**2b**	CAL-B	BuOH (neat)	24	89	19	83	4
3	**2b**	CAL-B	$PrCO_2Bu$–DIPE (1:1)	24	6	3	68	1
4	**2b**	PS-C II	BuOH (neat)	24	<1	6	7	1
5	**2b**	PS-C II	$PrCO_2Bu$–DIPE (1:1)	24	11	16	40	2
6	**2c**	PS-C II	$PrCO_2Bu$–DIPE (1:1)	90	31	24	56	2

[a] Not determined.

To explain why interesterification was highly enantioselective and relatively fast, we proposed a hypothesis whereby BuOH is released from butyl butanoate through the lipase-catalysed hydrolysis by water in the dry enzyme preparation, and where the released BuOH stayed at the active site either replacing active site water or being part of the water network. Accordingly, BuOH concentration is high at the active site, although its total concentration in the reaction mixture is low. This hypothesis also explains the well-known phenomenon that water in the seemingly dry enzyme preparations effectively hydrolyses various carboxylic acid substrates in dry organic solvents.

4. Experimental

4.1. Materials and methods

Reagents were purchased from Aldrich, Fluka and Acros. Solvents with the highest analytical grade were obtained from Aldrich, J. T. Baker and Lab-Scan. Amano provided lipases AH, PS, PS-C II and PS-D I from *B. cepacia*, lipases AK and AK-C I from *P. fluorescens* and lipase R from *Penicillium roquefort*. Lipases from *Thermomyces lanuginos* (Lipozyme TL IM), *Rhizomucor miehei* (Lipozyme RM IM) and *C. antarctica* A and B (CAL-A = Novozyme® 735, CAL-B = Novozyme® 435) were the products of Novo Nordisk. Lipases from porcine pancreas (PPL) and *Candida rugosa* (CRL, type VII) were obtained from Sigma. Esterase from porcine liver (Chirazyme® E-1, PLE) was the product of Roche.

MS-spectra were measured with VG 7070E (EI) or ZapSpec-oa Tof (EI, FAB) and optical rotations with PerkinElmer polarimeter. The solution state ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance 500 spectrometer equipped with BBI-5 mm-Zgrad-ATM probe operating at 500.13 and 125.77 MHz, respectively. The spectra were recorded either at +25 °C or at −50 °C with a non-spinning sample in 5 mm NMR tubes using $CDCl_3$ as a solvent, except for **5** D_2O was used. The ^{1}H spectra of the $CDCl_3$ samples were referenced internally to the residual protonated solvent signal ($CHCl_3$, 7.27 ppm) and ^{13}C spectra to the solvent signal ($CDCl_3$, 77.10 ppm). TSP was used as the reference compound in the D_2O samples (0.00 ppm for both ^{1}H and ^{13}C). In some cases, the ^{1}H spectra were analysed employing the PERCH iteration software for the extraction of δ and $J_{H,H}$.[19,20] The correct assignment of chemical shifts was confirmed by application of two-dimensional correlation measurements, including gradient selected DQF-COSY measurements (cosygpmfqf pulse program), gradient selected $^{1}H,^{13}C$ HSQC measurements (hsqcetgpsi2 pulse program optimised for 145 Hz $^{1}J_{CH}$ couplings), gradient selected NOESY measurements (noesygpph pulse program with a 0.3 s mixing time) and gradient selected $^{1}H,^{13}C$ HMBC measurements (hmbcgplpndqf pulse program optimised for 10 Hz $^{n}J_{CH}$ couplings). The chemical shifts and coupling constants are reported at +25 °C, unless otherwise mentioned.

The absolute configurations were determined by comparing the specific rotation of **1c** with a literature value.[21] The absolute configuration of the reactive enantiomer was supposed to stay the same with other protective groups.

4.2. Preparation of racemic starting materials

4.2.1. 2-Piperidylacetic acid derivatives 1a–f.

4.2.1.1. 2-Piperidylacetic acid methyl ester hydrochloride 5. The synthesis is based on known methods.[9] 2-Pyridylacetic acid **3** (9.01 g, 51.9 mmol) was dissolved in diluted hydrochloric acid (0.71 M). Hydrogenation (5 bar) took place for 50 h at room temperature in the presence of PtO_2 (200 mg, 0.88 mmol). Filtering of the catalyst and evaporation of the solvent afforded the crude oily product **4** (9.32 g, 51.9 mmol).

In the second step 2-piperidylacetic acid hydrochloride **4** (2.00 g, 11.1 mmol) was dissolved in methanol (100 mL), and thionyl chloride (1.3 mL, 18 mmol) was added dropwise in an ice bath. The reaction was mixed for half an hour in an ice bath, one hour at room temperature and 3 h by refluxing. Evaporation of the solvent yielded **5** as a white solid (2.15 g, 11.1 mmol). ^{1}H NMR (500 MHz, D_2O) δ (ppm): 3.74 (s, 3H, CH_3), 3.55 (m, 1H, *H*-2), 3.43 (dddd, $J_{H\text{-}6ax} = -12.8$, $J_{H\text{-}5ax} = 4.1$, $J_{H\text{-}5eq} = 2.2$ and $J_{H\text{-}4eq} = 2.0$ Hz, 1H, *H*-6eq), 3.04 (ddd, $J_{H\text{-}6eq} = -12.8$, $J_{H\text{-}5ax} = 12.7$ and $J_{H\text{-}5eq} = 2.5$ Hz, 1H, *H*-6ax), 2.79 (dd, 1H, CH_2CO_2), 2.78 (dd, 1H, CH_2CO_2), 1.96 (m, 1H, *H*-3eq), 1.89 (m, 1H, *H*-5eq), 1.87 (m, 1H, *H*-4eq), 1.64 (m, 1H, *H*-5ax), 1.56 (m, 1H, *H*-4ax), 1.54 (m, 1H, *H*-3ax). ^{13}C NMR (500 MHz, D_2O) δ (ppm): 175.36 (CO_2), 56.20 (*C*-2), 55.60 (CH_3), 48.00 (*C*-6), 40.14 (CH_2CO_2), 31.06 (*C*-3), 24.62 (*C*-5), 24.32 (*C*-4).

4.2.1.2. 2-Piperidylacetic acid methyl ester 1a. 2-Piperidylacetic acid hydrochloride **4** (608 mg, 3.14 mmol) was dissolved in chloroform. Gaseous ammonia was bubbled into the solution. Precipitated ammonium chloride was filtered off. Evaporation of the solvent in vacuum gave **1a** (360 mg, 2.29 mmol, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.66 (s, 3H, CH_3), 3.02 (dddd, $J_{H\text{-}6ax} = -11.9$, $J_{H\text{-}5ax} = 4.0$, $J_{H\text{-}5eq} = 2.6$ and $J_{H\text{-}4eq} = 1.7$ Hz, 1H, *H*-6eq), 2.89 (dddd, $J_{H\text{-}3ax} = 11.0$, $J_{H\text{-}7b} = 8.6$, $J_{H\text{-}7a} = 4.4$ and $J_{H\text{-}3eq} = 2.6$ Hz, 1H, *H*-2), 2.64 (ddd, $J_{H\text{-}6eq} = -11.9$, $J_{H\text{-}5ax} = 12.2$ and $J_{H\text{-}5eq} = 2.7$ Hz, 1H, *H*-6ax), 2.37 (dd, $J_{H\text{-}7b} = -15.9$ and $J_{H\text{-}2} = 4.4$ Hz, 1H, *H*-7a, CH_2CO_2), 2.35 (dd, $J_{H\text{-}7a} = -15.9$ and $J_{H\text{-}2} = 8.6$ Hz, 1H, *H*-7b, CH_2CO_2), 1.76 (dddddd, $J_{H\text{-}4ax} = -13.4$, $J_{H\text{-}5ax} = 3.9$, $J_{H\text{-}3ax} = 3.9$, $J_{H\text{-}3eq} = 3.1$, $J_{H\text{-}5eq} = 2.8$ and $J_{H\text{-}6eq} = 1.7$ Hz, 1H, *H*-4eq), 1.60 (ddddd, $J_{H\text{-}3ax} = -12.8$, $J_{H\text{-}4ax} = 3.7$, $J_{H\text{-}4eq} = 3.1$, $J_{H\text{-}2} = 2.6$ and $J_{H\text{-}5eq} = 1.5$ Hz, 1H, *H*-3eq), 1.57 (dddddd, $J_{H\text{-}5ax} = -13.1$, $J_{H\text{-}4ax} = 4.0$, $J_{H\text{-}4eq} = 2.8$, $J_{H\text{-}6ax} = 2.7$, $J_{H\text{-}6eq} = 2.6$ and $J_{H\text{-}3eq} = 1.5$ Hz, 1H, *H*-5eq), 1.39 (ddddd, $J_{H\text{-}5eq} = -13.1$, $J_{H\text{-}4ax} = 12.9$, $J_{H\text{-}6ax} = 12.2$, $J_{H\text{-}6eq} = 4.0$ and $J_{H\text{-}4eq} = 3.9$ Hz, 1H, *H*-5ax), 1.35 (ddddd, $J_{H\text{-}4eq} = -13.4$, $J_{H\text{-}5ax} = 12.9$, $J_{H\text{-}3ax} = 12.8$, $J_{H\text{-}5eq} = 4.0$ and $J_{H\text{-}3eq} = 3.7$ Hz, 1H, *H*-4ax), 1.15 (dddd, $J_{H\text{-}3eq} = -12.8$, $J_{H\text{-}4ax} = 12.8$, $J_{H\text{-}2} = 11.0$ and $J_{H\text{-}4eq} = 3.9$ Hz, 1H, *H*-3ax). ^{13}C NMR (500 MHz, $CDCl_3$) δ

(ppm): 172.85 (CO_2), 53.37 (*C*-2), 51.58 (CH_3), 46.86 (*C*-6), 41.46 (CH_2CO_2), 32.62 (*C*-3), 26.01 (*C*-5), 24.61 (*C*-4).

4.2.1.3. *N*-Acetyl-2-piperidylacetic acid methyl ester 1b. 2-Piperidylacetic acid methyl ester hydrochloride **5** (2.00 g, 10.3 mmol) was dissolved in chloroform (50 mL). The addition of triethylamine (TEA, 7.4 mL, 52 mmol), DMAP (4-*N*,*N*-dimethylaminopyridine, 64 mg, 0.52 mmol) and acetic anhydride (2.1 mL, 22 mmol) started the reaction. After 24 h, the reaction was stopped by adding methanol (20 mL). Evaporation of the solvent and purification by column chromatography with silica gel (acetone–petroleum ether 3:7–1:1) yielded oily product **1b** (1.64 g, 8.2 mmol, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): Major isomer (53%): 4.54 (br d, $J_{H\text{-}6ax} = -13.0$ Hz, 1H, *H*-6eq), 4.44 (br m, 1H, *H*-2), 3.66 (s, 3H, CO_2CH_3), 2.77 (dd, $J_{H\text{-}7b} = -15.3$ and $J_{H\text{-}2} = 8.0$ Hz, 1H, *H*-7a, CH_2CO_2), 2.61 (dd, 1H, *H*-7b, CH_2CO_2), 2.58 (m, 1H, *H*-6ax), 2.14 (s, 3H, $COCH_3$), 1.71–1.48 (m, 5H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq and *H*-5eq), 1.36 (m, 1H, *H*-5ax). Minor isomer (47%): 5.17 (br m, 1H, *H*-2), 3.63 (s, 3H, CO_2CH_3), 3.61 (br m, 1H, *H*-6eq), 3.12 (ddd, $J_{H6\text{-}eq} = -13.4$, $J_{H\text{-}5ax} = 13.4$ and $J_{H\text{-}5eq} = 2.8$ Hz, 1H, *H*-6ax), 2.59 (dd, 1H, *H*-7a, CH_2CO_2), 2.52 (dd, $J_{H\text{-}7a} = -14.0$ and $J_{H\text{-}2} = 7.8$ Hz, 1H, *H*-7b, CH_2CO_2), 2.05 (s, 3H, $COCH_3$), 1.71–1.48 (m, 5H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq and *H*-5eq), 1.41 (m, 1H, *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): Major isomer (53%): 171.52 (CO_2), 169.48 (N*C*O), 51.94 (CO_2CH_3), 50.73 (*C*-2), 36.57 (*C*-6), 35.51 (CH_2CO_2), 29.31 (*C*-3), 25.24 (*C*-5), 21.38 ($COCH_3$), 19.04 (*C*-4). Minor isomer (47%): 171.65 (CO_2), 169.48 (N*C*O), 51.78 (CO_2CH_3), 45.26 (*C*-2), 42.07 (*C*-6), 34.74 (CH_2CO_2), 27.96 (*C*-3), 25.82 (*C*-5), 21.86 ($COCH_3$), 18.83 (*C*-4). HRMS: $M^+ = 199.1208$, $C_{10}H_{17}NO_3$ requires $M^+ = 199.1212$.

4.2.1.4. *N*-Boc-2-piperidylacetic acid methyl ester 1c. The reaction was carried out as described for **1b**, except that acetic anhydride was replaced by di-*tert*-butyldicarbonate. The reaction yielded **1c** (1.2 g, 4.7 mmol, 48%). ^{1}H NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (57%): 4.62 (br m, 1H, *H*-2), 4.00 (br d, $J_{H\text{-}6ax} = -12.9$ Hz, 1H, *H*-6eq), 3.63 (s, 3H, CO_2CH_3), 2.70 (br dd, $J_{H\text{-}6eq} = -12.9$ and $J_{H\text{-}5ax} = 12.6$ Hz, 1H, *H*-6ax), 2.58 (dd, $J_{H\text{-}7b} = -13.7$ and $J_{H\text{-}2} = 7.5$ Hz, 1H, *H*-7a, CH_2CO_2), 2.47 (dd, $J_{H\text{-}7a} = -13.7$ and $J_{H\text{-}2} = 7.5$ Hz, 1H, *H*-7b, CH_2CO_2), 1.7–1.2 ppm (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax), 1.40 (s, 9H, $C(CH_3)_3$). Minor isomer (43%): 4.71 (br m, 1H, *H*-2), 3.88 (br d, $J_{H\text{-}6ax} = -12.8$ Hz, 1H, *H*-6eq), 3.60 (s, 3H, CO_2CH_3), 2.78 (br dd, $J_{H\text{-}6eq} = -12.9$ and $J_{H\text{-}5ax} = 12.9$ Hz, 1H, *H*-6ax), 2.62 (dd, $J_{H\text{-}7b} = -13.3$ and $J_{H\text{-}2} = 7.5$ Hz, 1H, *H*-7a, CH_2CO_2), 2.45 (dd, $J_{H\text{-}7a} = -13.3$ and $J_{H\text{-}2} = 7.5$ Hz, 1H, *H*-7b, CH_2CO_2), 1.7–1.2 ppm (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax), 1.39 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (57%): 172.21 (CO_2CH_3), 154.60 (N*C*O), 79.63 (CCH_3), 52.07 (CO_2CH_3), 47.85 (*C*-2), 38.14 (*C*-6), 34.74 (CH_2CO_2), 28.14 ($C(CH_3)_3$), 28.11 (*C*-3), 24.88 (*C*-5), 18.52 (*C*-4). Minor isomer (43%): 172.39 (CO_2CH_3), 154.60 (N*C*O), 79.49 (CCH_3), 52.18 (CO_2CH_3), 46.89 (*C*-2), 39.33 (*C*-6), 34.65 (CH_2CO_2), 28.14 ($C(CH_3)_3$), 28.11 (*C*-3), 25.08 (*C*-5), 18.58 (*C*-4). HRMS: $M^+ = 257.1626$, $C_{13}H_{23}NO_4$ requires $M^+ = 257.1627$.

4.2.1.5. *N*-Ns-2-piperidylacetic acid methyl ester 1d. The reaction was carried out as described for **1b**, except that acetic anhydride was replaced by nosyl chloride (1.5 equiv) and chloroform with dichloromethane. DMAP was not used as a catalyst. The reaction yielded **1d** (795 mg, 2.3 mmol, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.10 (m, $J_{H\text{-}3'} = 7.9$, $J_{H\text{-}2'} = 1.5$ and $J_{H\text{-}1'} = 0.3$ Hz, 1H, *H*-4′, $CHCNO_2$), 7.70 (m, $J_{H\text{-}1'} = 8.0$, $J_{H\text{-}3'} = 7.8$ and $J_{H\text{-}4'} = 1.5$ Hz, 1H, *H*-2′, SCCHC*H*), 7.69 (m, $J_{H\text{-}4'} = 7.9$, $J_{H\text{-}2'} = 7.8$ and $J_{H\text{-}1'} = 1.2$ Hz, 1H, *H*-3′, $CHCHCNO_2$), 7.65 (m, $J_{H\text{-}2'} = 8.0$, $J_{H\text{-}3'} = 1.2$ and $J_{H\text{-}4'} = 0.3$ Hz, 1H, *H*-1′, SCC*H*), 4.48 (m, 1H, *H*-2), 3.80 (br d, $J_{H\text{-}6ax} = -14.0$ Hz, 1H, *H*-6eq), 3.60 (s, 3H, CH_3), 3.06 (ddd, $J_{H\text{-}6eq} = -14.0$, $J_{H\text{-}5ax} = 13.0$ and $J_{H\text{-}5eq} = 2.6$ Hz, 1H, *H*-6ax), 2.73 (dd, $J_{H\text{-}7b} = -15.2$ and $J_{H\text{-}2} = 9.1$ Hz, 1H, *H*-7a, CH_2CO_2), 2.63 (dd, $J_{H\text{-}7a} = -15.2$ and $J_{H\text{-}2} = 5.7$ Hz, 1H, *H*-7b, CH_2CO_2), 1.73 (m, 1H, *H*-3ax), 1.69–1.61 (br m, 3H, *H*-3eq, *H*-4eq and *H*-5eq), 1.55 (m, 1H, *H*-4ax), 1.50 (m, 1H, *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 171.14 (CO_2), 147.72 (CNO_2), 133.86 (CSO_2), 133.49 (*C*HCHCS), 131.89 ($CHCHCNO_2$), 131.24 ($CHCNO_2$), 124.35 (*C*HCS), 51.88 (CH_3), 50.39 (*C*-2), 41.60 (*C*-6), 35.11 (CH_2CO_2), 28.10 (*C*-3), 25.18 (*C*-5), 18.28 (*C*-4). HRMS: $MH^+ = 343.0956$, $C_{14}H_{18}N_2O_6S$ requires $MH^+ = 343.0964$.

4.2.1.6. *N*-Fmoc-2-piperidylacetic acid methyl ester 1e. The synthetic procedures followed a known method.[22] 2-Piperidylacetic acid methyl ester hydrochloride **5** (0.10 g, 0.52 mmol) was dissolved in dry tetrahydrofuran (THF, 2 mL) followed by the addition of sodium hydride (62 mg, 1.6 mmol, 60%) in dry THF. The reaction was mixed for 3.5 h at room temperature, after which FmocCl (207 mg, 0.78 mmol) in THF (3 mL) was added. After taking place overnight, the reaction was stopped with methanol (5 mL). Evaporation yielded the crude product, which was dissolved in water (10 mL) and extracted in DIPE (1 × 10 mL). The organic phase was washed with water (1 × 10 mL) and dried with $MgSO_4$. Purification by column chromatography with silica gel (acetone–petroleum ether 1:9) afforded **1e** (102 mg, 0.27 mmol, 52%). ^{1}H NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (56%): 7.80 (br, 2H, Ar*H*), 7.63 (br, 2H, Ar*H*), 7.44 (br, 2H, Ar*H*), 7.36 (br, 2H, Ar*H*), 4.79 (br, 1H, *H*-2), 4.46 (br m, 2H, NCO_2CH_2), 4.27 (br, 1H, NCO_2CH_2CH), 4.10 (br, 1H, *H*-6eq), 3.70 (s, 3H, CH_3), 2.80 (br m, 1H, *H*-6ax), 2.72 (br m, 1H, CH_2CO_2), 2.46 (br m, 1H, CH_2CO_2), 1.75–1.35 (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax). Minor isomer (44%): 7.80 (br, 2H, *H*-5′, Ar*H*), 7.63 (br, 2H, *H*-2′, Ar*H*), 7.44 (br, 2H, *H*-4′, Ar*H*), 7.36 (br, 2H, *H*-3′, Ar*H*), 4.85 (br, 1H, *H*-2), 4.36 (br m, 2H, NCO_2CH_2), 4.27 (br, 1H, NCO_2CH_2CH), 4.10 (br, 1H, *H*-6eq), 3.62 (s, 3H, CH_3), 3.01 (br m, 1H, *H*-6ax), 2.72 (br m, 1H, CH_2CO_2), 2.57 (br m, 1H, CH_2CO_2), 1.75–1.35 (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (56%): 171.78 (CO_2CH_3), 155.16 (NCO_2CH_2), 143.40 (2C, *C*-1a′, Ar*C*), 141.05 (2C, *C*-5a′, Ar*C*), 127.62 (2C, *C*-4′, Ar*C*H), 126.96 (2C, *C*-3′, Ar*C*H),

125.08 (2C, *C*-2′, Ar*C*H), 120.02 (2C, *C*-5′, Ar*C*H), 67.31 (NCO_2CH_2), 52.24 (CH_3), 47.65 (*C*-2), 46.72 (*C*-1′, NCO_2CH_2CH), 39.32 (*C*-6), 34.54 (CH_2CO_2), 28.00 (*C*-3), 24.91 (*C*-5), 18.38 (*C*-4). Minor isomer (44%): 172.17 (CO_2CH_3), 155.11 (NCO_2CH_2), 143.40 (2C, *C*-1a′, Ar*C*), 141.05 (2C, *C*-5a′, Ar*C*), 127.62 (2C, *C*-4′, Ar*C*H), 126.96 (2C, *C*-3′, Ar*C*H), 125.08 (2C, *C*-2′, Ar*C*H), 120.02 (2C, *C*-5′, Ar*C*H), 67.20 (NCO_2CH_2), 52.24 (CH_3), 47.65 (*C*-2), 46.66 (*C*-1′, NCO_2CH_2CH), 39.39 (*C*-6), 34.58 (CH_2CO_2), 27.58 (*C*-3), 25.16 (*C*-5), 18.58 (*C*-4). HRMS: $M^+ = 379.1786$, $C_{23}H_{25}NO_4$ requires $M^+ = 379.1784$.

4.2.1.7. *N*-Cbz-2-piperidylacetic acid methyl ester 1f. The reaction was carried out as described for **1b**, except that acetic anhydride was replaced by benzyloxycarbonyl chloride. The reaction yielded **1f** (630 mg, 2.2 mmol, 84%). ^{1}H NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (55%): 7.41–7.31 (m, 5H, Ar*H*), 5.11 (s, 2H, CO_2CH_2Ph), 4.79 (m, 1H, *H*-2), 4.11 (br d, 1H, *H*-6eq), 3.55 (s, 3H, CH_3), 2.84 (br dd, $J_{H\text{-}6eq} = -13.4$ and $J_{H\text{-}5ax} = 13.4$ Hz, 1H, *H*-6ax), 2.67 (dd, $J_{H\text{-}7b} = -14.3$ and $J_{H\text{-}2} = 7.9$ Hz, 1H, *H*-7a, CH_2CO_2), 2.55 (dd, $J_{H\text{-}7a} = -14.3$ and $J_{H\text{-}2} = 7.4$ Hz, 1H, *H*-7b, CH_2CO_2), 1.72–1.55 (m, 4H, *H*-3ax, *H*-3eq, *H*-4eq and *H*-5eq), 1.50 (m, 1H, *H*-4ax), 1.38 (m, 1H, *H*-5ax). Minor isomer (45%): 7.41–7.31 (m, 5H, Ar*H*), 5.09 (s, 2H, CO_2CH_2Ph), 4.82 (m, 1H, *H*-2), 4.02 (br d, 1H, *H*-6eq), 3.60 (s, 3H, CH_3), 2.87 (br dd, $J_{H\text{-}6eq} = -13.4$ and $J_{H\text{-}5ax} = 13.4$ Hz, 1H, *H*-6ax), 2.66 (dd, $J_{H\text{-}7b} = -14.1$ and $J_{H\text{-}2} = 7.8$ Hz, 1H, *H*-7a, CH_2CO_2), 2.56 (dd, $J_{H\text{-}7a} = -14.1$ and $J_{H\text{-}2} = 7.4$ Hz, 1H, *H*-7b, CH_2CO_2), 1.72–1.55 (m, 4H, *H*-3ax, *H*-3eq, *H*-4eq and *H*-5eq), 1.50 (m, 1H, *H*-4ax), 1.38 (m, 1H, *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (55%): 171.95 (CO_2CH_3), 155.06 (NCO_2CH_2), 136.23 (*C*-*i*), 128.37 (2C, *C*-*m*), 127.87 (*C*-*p*), 127.55 (2C, *C*-*o*), 66.90 (CO_2CH_2Ph), 51.97 (CH_3), 47.79 (*C*-2), 38.98 (*C*-6), 34.79 (CH_2CO_2), 28.14 (*C*-3), 24.85 (*C*-5), 18.41 (*C*-4). Minor isomer (45%): 172.04 (CO_2CH_3), 155.04 (NCO_2CH_2), 136.21 (*C*-*i*), 128.37 (2C, *C*-*m*), 127.87 (*C*-*p*), 127.55 (2C, *C*-*o*), 66.90 (CO_2CH_2Ph), 52.05 (CH_3), 47.49 (*C*-2), 39.26 (*C*-6), 34.49 (CH_2CO_2), 27.82 (*C*-3), 25.03 (*C*-5), 18.46 (*C*-4). HRMS: $M^+ = 291.1469$, $C_{16}H_{21}NO_4$ requires $M^+ = 291.1471$.

4.2.2. 3-Piperidinecarboxylic acid derivatives. N-Protected 3-piperidinecarboxylic acid methyl esters were prepared by starting from nicotinic acid **6** followed by hydrogenation of the aromatic ring, esterification and N-protection (Scheme 2). The reactions were performed as described for the 2-piperidylacetic acid derivatives **1a**–**c**.

4.2.2.1. 3-Piperidylacetic acid methyl ester 2a. Yield 530 mg, 3.70 mmol, 96%.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.66 (s, 3H, CH_3), 3.14 (dd, $J_{H\text{-}2ax} = -12.4$ and $J_{H\text{-}3} = 3.5$ Hz, 1H, *H*-2eq), 2.91 (ddd, $J_{H\text{-}6ax} = -12.3$, $J_{H\text{-}5eq} = 4.8$ and $J_{H\text{-}5ax} = 3.3$ Hz, 1H, *H*-6eq), 2.80 (dd, $J_{H\text{-}2eq} = -12.4$ and $J_{H\text{-}3} = 9.3$ Hz, 1H, *H*-2ax), 2.62 (ddd, $J_{H\text{-}6eq} = -12.3$, $J_{H\text{-}5ax} = 10.3$ and $J_{H\text{-}5eq} = 3.2$ Hz, 1H, *H*-6ax), 2.44 (dddd, $J_{H\text{-}4ax} = 10.0$, $J_{H\text{-}2ax} = 9.3$, $J_{H\text{-}4eq} = 4.3$ and $J_{H\text{-}2eq} = 3.5$ Hz, 1H, *H*-3), 1.96 (dddd, $J_{H\text{-}4ax} = -13.1$, $J_{H\text{-}5eq} = 5.5$, $J_{H\text{-}3} = 4.3$ and $J_{H\text{-}5ax} = 3.4$ Hz, 1H, *H*-4eq), 1.65 (ddddd, $J_{H\text{-}5ax} = -13.6$, $J_{H\text{-}4eq} = 5.5$, $J_{H\text{-}6eq} = 4.8$, $J_{H\text{-}4ax} = 3.9$ and $J_{H\text{-}6ax} = 3.2$ Hz, 1H, *H*-5eq), 1.65 (dddd, $J_{H\text{-}4eq} = -13.1$, $J_{H\text{-}5ax} = 11.1$, $J_{H\text{-}3} = 10.0$ and $J_{H\text{-}5eq} = 3.9$ Hz, 1H, *H*-4ax), 1.44 (ddddd, $J_{H\text{-}5eq} = -13.6$, $J_{H\text{-}4ax} = 11.1$, $J_{H\text{-}6ax} = 10.3$, $J_{H\text{-}4eq} = 3.4$ and $J_{H\text{-}6eq} = 3.3$ Hz, 1H, *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 174.85 (CO_2), 51.59 (CH_3), 48.58 (*C*-2), 46.41 (*C*-6), 42.41 (*C*-3), 27.38 (*C*-4), 25.51 (*C*-5).

4.2.2.2. *N*-Acetyl-3-piperidinecarboxylic acid methyl ester 2b. Yield 1.35 g, 7.29 mmol, 66%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): Major isomer (53%): 3.98 (ddd, $J_{H\text{-}6ax} = -13.2$, $J_{H\text{-}5eq} = 4.8$ and $J_{H\text{-}5ax} = 4.6$ Hz, 1H, *H*-6eq), 3.74 (br dd, $J_{H\text{-}2ax} = -13.5$ and $J_{H\text{-}3} = 3.5$ Hz, 1H, *H*-2eq), 3.69 (s, 3H, CO_2CH_3), 3.43 (dd, $J_{H\text{-}2eq} = -13.5$ and $J_{H\text{-}3} = 9.0$ Hz, 1H, *H*-2ax), 3.06 (m, $J_{H\text{-}6eq} = -13.2$ and $J_{H\text{-}5eq} = 3.2$ Hz, 1H, *H*-6ax), 2.49 (dddd, $J_{H\text{-}2ax} = 9.2$, $J_{H\text{-}4ax} = 9.2$, $J_{H\text{-}2eq} = 4.1$ and $J_{H\text{-}4eq} = 4.1$ Hz, 1H, *H*-3), 2.11 (s, 3H, $NCOCH_3$), 2.01 (m, 1H, *H*-4eq), 1.79 (m, 1H, *H*-4ax), 1.65 (m, 1H, *H*-5eq), 1.47 (m, 1H, *H*-5ax). Minor isomer (47%): 4.60 (m, $J_{H\text{-}2ax} = -13.2$ Hz, 1H, *H*-2eq), 3.70 (m, 1H, *H*-6eq), 3.66 (s, 3H, CO_2CH_3), 3.05 (m, 1H, *H*-6ax), 2.83 (dd, $J_{H\text{-}2eq} = -13.2$ and $J_{H\text{-}3} = 10.7$ Hz, 1H, *H*-2ax), 2.44 (dddd, $J_{H\text{-}2ax} = 10.7$, $J_{H\text{-}4ax} = 10.9$, $J_{H\text{-}2eq} = 4.0$ and $J_{H\text{-}4eq} = 4.0$ Hz, 1H, *H*-3), 2.07 (s, 3H, $NCOCH_3$), 2.07 (m, 1H, *H*-4eq), 1.80 (m, 1H, *H*-5eq), 1.67 (m, 1H, *H*-4ax), 1.47 (m, 1H, *H*-5ax). ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): Major isomer (53%): 173.67 (CO_2CH_3), 169.19 ($NCOCH_3$), 51.98 (CO_2CH_3), 48.07 (*C*-2), 41.78 (*C*-6), 41.59 (*C*-3), 27.12 (*C*-4), 23.77 (*C*-5), 21.45 ($NCOCH_3$). Minor isomer (47%): 173.31 (CO_2CH_3), 169.06 ($NCOCH_3$), 51.81 (CO_2CH_3), 46.74 (*C*-6), 43.43 (*C*-2), 41.06 (*C*-3), 27.26 (*C*-4), 24.99 (*C*-5), 21.50 ($NCOCH_3$). HRMS: $M^+ = 185.1051$, $C_9H_{15}NO_3$ requires $M^+ = 185.1052$.

4.2.2.3. *N*-Boc-3-piperidinecarboxylic acid methyl ester 2c. Yield 1.54 g, 6.33 mmol, 77%. ^{1}H NMR (500 MHz, $CDCl_3$, –50 °C) δ (ppm): Major isomer (71%): 4.09 (br, 1H, *H*-2eq), 3.96 (br, 1H, *H*-6eq), 3.67 (s, 3H, CO_2CH_3) 2.93 (br, 1H, *H*-2ax), 2.70 (br dd, $J_{H\text{-}6eq} = -11.3$ and $J_{H\text{-}5ax} = 11.3$ Hz, 1H, *H*-6ax), 2.43 (br, 1H, *H*-3), 2.04 (br d, $J_{H\text{-}4ax} = -11.5$ Hz, 1H, *H*-4eq), 1.69 (br d, 1H, *H*-5eq), 1.52 (br, 1H, *H*-4ax), 1.43 (br, 1H, *H*-5ax), 1.41 (s, 9H, $C(CH_3)_3$). Minor isomer (29%): 4.27 (br, 1H, *H*-2eq), 3.96 (br, 1H, *H*-6eq), 3.67 (s, 3H, CO_2CH_3) 2.77 (br, 1H, *H*-2ax), 2.70 (br dd, $J_{H\text{-}6eq} = -11.3$ and $J_{H\text{-}5ax} = 11.3$ Hz, 1H, *H*-6ax), 2.43 (br, 1H, *H*-3), 2.04 (br d, $J_{H\text{-}4ax} = -11.5$ Hz, 1H, *H*-4eq), 1.69 (br d, 1H, *H*-5eq), 1.52 (br, 1H, *H*-4ax), 1.43 (br, 1H, *H*-5ax), 1.41 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (71%): 174.33 (CO_2CH_3), 154.52 (NCO_2), 79.79 ($NCO_2C(CH_3)_3$), 52.15 (CO_2CH_3), 45.32 (*C*-2), 42.80 (*C*-6), 40.97 (*C*-3), 28.21 ($C(CH_3)_3$), 27.21 (*C*-4), 23.92 (*C*-5). Minor isomer (29%): 174.33 (CO_2CH_3), 154.57 (NCO_2), 79.79 ($NCO_2C(CH_3)_3$), 52.15 (CO_2CH_3), 44.52 (*C*-2), 44.03 (*C*-6), 40.97 (*C*-3), 28.21 ($C(CH_3)_3$), 27.21 (*C*-4), 24.24 (*C*-5). HRMS: $M^+ = 243.1468$; $C_{12}H_{21}NO_4$ requires $M^+ = 243.1471$.

4.3. Enzymatic reactions

The conversion was mainly calculated according to the equation $c = ee_S/(ee_S + ee_P)$ at conversions lower than 40%. Due to the presence of competitive nucleophiles, MeOH and BuOH (and water), conversions were also determined from the peak areas of the substrate and the product taking into account the different responses in the FID-detector. This gave conversion values similar to those obtained from ee-values. The determination of E is based on the equation $E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)]$.[23] Using linear regression E was achieved as a slope of a line. E values may contain contributions from the competitive nucleophiles and, for this reason, 'E' is used instead of E throughout the work.

Volumes of the test reactions were 0.5–1.0 mL. In butanolyses, **1a** or **1b** (0.1 M) was dissolved in butanol or butanol/DIPE mixture. Addition of the enzyme started the reaction. The reactions were shaken at room temperature or in an incubator (47 °C). The enzyme was filtered off from the samples (50 μL), which were analysed by GC equipped with a chiral column (Varian permethylated β-cyclodextrin). In interesterifications, **1a**–**f** or **2b**–**c** (0.1 M) were dissolved in $PrCO_2Bu$ or $PrCO_2Bu$–DIPE mixture (1 or 0.5 mL). Addition of the enzyme (75 mg mL^{-1}) started the reaction. The reactions were shaken at room temperature or in an incubator (47 °C). The samples (50 μL) were taken at intervals and analysed as above or by HPLC equipped with a chiralcel OD-column. Since the enantiomers of **2c** were not separated by the chiral columns used, the reaction products were analysed by separating substrate **2c** and product **11c** with column chromatography using acetone–petroleum ether 1:9 as solvent. Boc-protection was removed by HCl in methanol (0.83 M) and the enantiomers of **2a** produced were derivatised into **2b** with acetic anhydride. In aminolyses, **1b** or **1c** (0.1 M) was dissolved in TBME (1 or 0.5 mL). Addition of an amine ($^{i}PrNH_2$ or $BuNH_2$, 0.2 M) and the enzyme (75 or 80 mg mL^{-1}) started the reaction. The reactions were shaken at room temperature. Samples (50 μL) were taken at intervals and analysed as described above.

Gram-scale reaction was performed with *N*-Boc-2-piperidylacetic acid methyl ester **1c** (750 mg, 2.92 mmol) by dissolving it into $PrCO_2Bu$–DIPE -mixture (1:1, 29.2 mL) and by adding butanol (690 μL, 0.2 M) and lipase PS-C II (2.19 mg, 75 mg mL^{-1}). The reaction was shaken for 168 h at 47 °C. The enzyme was filtered off and washed with DIPE and acetone. (*R*)-**1c** and (*S*)-**9c** were separated by column chromatography with silica gel by using acetone–petroleum ether (1:99–5:95) as an eluent. Unreacted (*R*)-**1c** [308 mg, 1.20 mmol, $ee_s = 94\%$, $[\alpha]_D^{20} = +9.9$ (*c* 4.54, $CHCl_3$)] and the product (*S*)-**9c** [339 mg, 1.13 mmol, $ee_p = 99\%$, $[\alpha]_D^{20} = -5.4$ (*c* 1, $CHCl_3$)] were separated. Literature value for (*S*)-**1c** has been reported $[\alpha]_D^{20} = -8.3$ (*c* 4.54, $CHCl_3$).[21]

Compound (*S*)-**9c**: ^{1}H NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (60%): 4.55 (br m, 1H, *H*-2), 3.93 (br d, 1H, *H*-6eq), 3.92 (br m, 2H, CO_2CH_2), 2.64 (br dd, $J_{H\text{-}6eq} = -12.7$ and $J_{H\text{-}5ax} = 12.7$ Hz, 1H, *H*-6ax), 2.43 (br m, 2H, CH_2CO_2), 1.6–1.2 (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax), 1.48 (m, 2H, $CO_2CH_2CH_2$), 1.34 (s, 9H, $C(CH_3)_3$), 1.23 (m, 2H, $CO_2CH_2CH_2CH_2$), 0.80 (m, 3H, CH_2CH_3). Minor isomer (40%): 4.66 (br m, 1H, *H*-2), 3.92 (br m, 2H, CO_2CH_2), 3.81 (br d, $J_{H\text{-}6ax} = -12.8$ Hz, 1H, *H*-6eq), 2.71 (br dd, $J_{H\text{-}6eq} = -12.8$ and $J_{H\text{-}5ax} = 13.1$ Hz, 1H, *H*-6ax), 2.46 (br m, 2H, CH_2CO_2), 1.6–1.2 (m, 6H, *H*-3ax, *H*-3eq, *H*-4ax, *H*-4eq, *H*-5eq and *H*-5ax), 1.48 (m, 2H, $CO_2CH_2CH_2$), 1.32 (s, 9H, $C(CH_3)_3$), 1.23 (m, 2H, $CO_2CH_2CH_2CH_2$), 0.80 (m, 3H, CH_2CH_3). ^{13}C NMR (500 MHz, $CDCl_3$, −50 °C) δ (ppm): Major isomer (60%): 171.68 (CO_2CH_2), 154.47 (NCO), 79.43 (CCH_3), 64.41 (CO_2CH_2), 47.78 (*C*-2), 38.06 (*C*-6), 34.84 (CH_2CO_2), 30.04 ($CO_2CH_2CH_2$), 27.97 ($C(CH_3)_3$), 27.80 (*C*-3), 24.75 (*C*-5), 18.90 ($CO_2CH_2CH_2CH_2$), 18.37 (*C*-4), 13.73 (CH_2CH_3). Minor isomer (40%): 171.68 (CO_2CH_2), 154.39 (NCO), 79.27 (CCH_3), 64.41 (CO_2CH_2), 46.60 (*C*-2), 39.27 (*C*-6), 34.53 (CH_2CO_2), 30.04 ($CO_2CH_2CH_2$), 28.01 ($C(CH_3)_3$), 27.65 (*C*-3), 25.02 (*C*-5), 18.90 ($CO_2CH_2CH_2CH_2$), 18.44 (*C*-4), 13.73 (CH_2CH_3). HRMS: $M^+ = 299.2095$, $C_{16}H_{29}NO_4$ requires $M^+ = 299.2097$.

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