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Enzymatic resolution of alicyclic 1,3-amino alcohols in organic media

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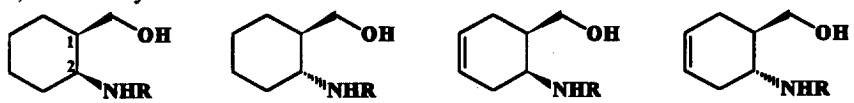
Abstract

Racemic *cis*- and *trans*-2-aminocyclohexane-1-methanol and *cis*- and *trans*-2-amino-4-cyclohexene-1-methanol were resolved via lipase-catalysed *O*-acylation of their Z derivatives, using vinyl butyrate in different ether solvents. In accordance with the empirical rule, most of the screened lipases preferred the 1*S* enantiomer.

1. Introduction

1,3-Amino alcohols **1**, **4**, **6** and **8** with two adjacent stereogenic centres find noteworthy applicability in synthetic and medicinal chemistry. Quaternary ammonium salts of (+)-**1** and (−)-**1** are used as chiral phase-transfer catalysts for the enantioselective alkylation of compounds possessing an active methylene group.[1] Compound (+)-**4** is an intermediate in the stereoselective synthesis of an orally active angiotensin converting enzyme inhibitor[2] and also in the synthesis of a novel chiral super-Lewis acid catalyst.[3] *N*-Benzyl derivatives of (+)-**1** and (−)-**1** and (+)-**4** and (−)-**4** are used for the resolution of racemic carboxylic acids.[4] Homochiral **1**, **4**, **6** and **8** can serve as building blocks of pharmacologically active fused saturated 1,3-heterocycles.[5–7]



1-3 **4, 5** **6, 7** **8, 9**

1, 4, 6, 8: R=H; **2**: R=COO*t*Bu; **3, 5, 7, 9**: R=COOCH$_2$Ph

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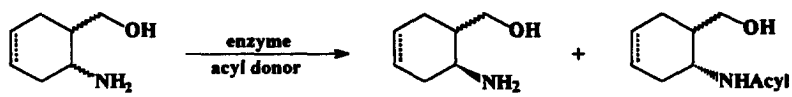
Our aim was to find appropriate conditions for the resolution of racemic **1**, **4**, **6** and **8**. As these compounds bear two functional groups, there are several possibilities for their enzymatic resolution. One of the most direct methods is resolution via acylation of the amino group[8,9] attached to the ring. Alternatively, chemical acylation of the amino function followed by enzyme-catalysed hydrolysis could be considered. Resolution via acylation of the alcohol function (after protection of the amino group) may also be successful.[10,11] There is a further possibility to achieve enantioselectivity through enzyme-catalysed *O*-deacylation of the *N,O*-diacylated compound.[12] In the latter cases, it should be emphasized that **1**, **4**, **6** and **8** are primary alcohols, possessing an extra CH_2 group between the stereogenic centre and the OH group. Flexibility along the C(1)–CH_2OH bond allows both enantiomers to adopt conformations with similar orientations of medium-sized and large substituents attached to the stereogenic centre. Weissfloch and Kazlauskas postulated that high enantioselectivity toward primary alcohols requires not only a significant difference in size of the substituents, as for secondary alcohols, but also restricted rotation along the C(1)–CH_2OH bond.[13] Discrimination between enantiomers of primary alcohols is therefore more difficult.[14]

2. Results and discussion

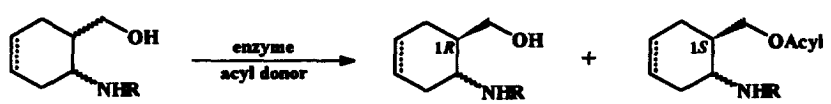
The excellent results reported earlier for the resolution of β-amino acid esters via lipase-catalysed acylation of the amino group[15] encouraged us to investigate this approach first (Scheme 1).



Scheme 1.

The irreversibility of acyl transfer is a basic demand of enzymatic kinetic resolutions. Accordingly, vinyl acetate was used as the acyl donor.[16] The enzymes screened were lipases PS, A, AY, G, F and M and proteases A, B and N (see Experimental). However, no enantioselectivity was observed, even at 0°C and with only one equivalent of the acyl donor, when diethyl ether or tetrahydrofuran was applied as cosolvent. A blank experiment revealed that the non-enzymatic process was much faster, leading to the racemic product only. The use of ethyl acetate instead of vinyl acetate as the acyl donor resulted in a slight improvement. A possible explanation for the different behaviour of β-amino alcohols as compared with β-amino esters[15] is the lower nucleophilicity of the amino function in the latter case, due to the electron-withdrawing ethoxycarbonyl group.

We therefore decided to investigate enzyme-catalysed *O*-acylation after protection of the amino group (Scheme 2). For *N*-protection, the readily removable *tert*-butyloxycarbonyl (Boc) and benzyloxycarbonyl (Z) groups were applied. The enzymatic reactions were performed in different ethers, with vinyl acetate or vinyl butyrate as the acyl donor. Enantioselectivities were considered in terms of the enantiomeric ratio (E),[17] which remains constant throughout the reaction.



2, 3, 5, 7, 9 (1*R*)-2, 3, 5, 7, 9 (1*S*)-2, 3, 5, 7, 9a and b
a: acetate
b: butyrate

Scheme 2.

Table 1
Lipase-catalysed *O*-acylation of racemic **2** in the presence of vinyl acetate

	Lipase PS		Lipase F		Lipase N		Lipase AY		PPL	
Cosolvent	E	conv. (%)	E	conv. (%)	E	conv. (%)	E	conv. (%)	E	conv. (%)
*i*Pr$_2$O	14	50 (4 h)	24	20 (40 h)	16	7 (89 h)	2.5[a]	31 (10 h)	12	31 (16 h)
*t*BuOMe	16	50 (3 h)	—	—	—	—	—	—	—	—
Et$_2$O	5	51 (15 h)	2	10 (15 h)	0	3 (15 h)	1.7	39 (15 h)	—	—
THF	16	50 (19 h)	—	—	—	—	—	—	10	22 (35 h)
None	15	49 (15 h)	—	—	—	—	—	—	—	—

[a] Opposite enzyme selectivity

Table 2
Effects of acyl donors and protective groups in enzyme-catalysed *O*-acylation of racemic **2** and **3**

			2		**3**	
Vinyl ester	Solvent	Enzyme	E	conv. (%)	E	conv. (%)
Acetate	*i*Pr$_2$O	Lipase PS	14	50 (4 h)	12	53 (2 h)
Butyrate			30	52 (2.5 h)	28	53 (1 h)
Acetate	*t*BuOMe	Lipase PS	16	50 (3 h)	—	—
Butyrate			24	53 (3 h)		
Acetate	THF	Lipase PS	16	50 (19 h)	11	50 (16 h)
Butyrate			28	54 (19 h)	35	51 (16 h)
Acetate	Et$_2$O	Lipase PS	5	51 (15 h)	22	55 (4 h)
Butyrate			50	50 (6 h)	34	54 (3 h)
Acetate	*i*Pr$_2$O	Lipase F	24	20 (40 h)	24	52 (39 h)
Butyrate			120	48 (28 h)	170	52 (16 h)
Acetate	*i*Pr$_2$O	Lipase M	—	—	104	49 (7 h)
Butyrate			40	47 (3.5 h)	>200	53 (1 h)
Butyrate	*i*Pr$_2$O	Lipase N	16	7 (89 h)	78	49 (40 h)
Butyrate	*i*Pr$_2$O	Novozym	94	53 (10 min)	38	52 (5 min)
Butyrate	*i*Pr$_2$O	PPL	17	56 (18.5 h)	8	50 (1.5 h)
Butyrate	*i*Pr$_2$O	Protease B	4	20 (84 h)	7	29 (29 h)

In a first approach, racemic **1** was protected with a Boc group **2** and the acylation was catalysed by a lipase (lipase PS, F, AY, N or PPL) in the presence of vinyl acetate. The reaction was performed in different ethers and also in vinyl acetate (Table 1). Under the given conditions, only a moderate E value was obtained.

For optimization of the enantioselectivity, the protecting group and the acyl donor were varied. In different solvents and with different enzymes, the application of vinyl butyrate instead of vinyl acetate proved favourable for the enantioselectivity, as is unequivocally demonstrated by the data in Table 2. The rate of the reaction was also higher with vinyl butyrate. Thus, the longer carbon chain of the acyl donor increases the hydrophobic character of the tetrahedral intermediate formed in the catalytic process and improves the resolution.

The effect of variation of the protecting group is not so clear-cut. It is assumed that the protective group should exert a major influence on the observed enantioselectivity, as it is directly connected to a

Table 3
Solvent-dependence of E values in the lipase PS-catalysed *O*-acylation in the presence of vinyl butyrate

	3		**5**		**7**		**9**	
Solvent	E	conv. (%)	E	conv. (%)	E	conv. (%)	E	conv. (%)
iPr_2O	28	53 (1 h)	94	50 (1 h)	25	50 (2 h)	43	50 (13 min)
*t*BuOMe	—	—	41	49 (1 h)	16	47 (2 h)	40	54 (11 min)
Et_2O	34	54 (3 h)	49	52 (3 h)	25	55 (4 h)	41	53 (45 min)
THF	35	51 (16 h)	74	54 (16 h)	30	41 (18 h)	39	48 (3.5

stereogenic centre. For secondary alcohols, it has been proved that a larger difference in steric demand of medium-sized and large substituents enhances the enantioselectivity of lipase-catalysed reactions,[18] whereas for primary alcohols this is not necessarily the case.[13] Additionally, the Boc and Z groups differ not merely sterically, but also in their electronic properties, e.g. Z could interact with aromatic amino acids present at the binding site of some lipases. Accordingly, the influence of the *N*-protective group is not predictable and can differ from enzyme to enzyme. For example, in the presence of vinyl butyrate, the corresponding E values were comparable in the lipase PS-catalysed acylation of **2** and **3**; for Novozym 435, Boc was more efficient; and for lipases M and F, the enantioselectivities were higher when Z was used. For further experiments, Z was chosen because of the superior E values in the latter two cases (Table 2).

Z Derivatives of all four racemic amino alcohols were prepared (**3**, **5**, **7** and **9**) and the solvent-dependence was investigated by using lipase PS as catalyst. With the exception of **5**, which gives a remarkably better result in di-isopropyl ether, the data in Table 3 led to the conclusion that there is no significant difference between the ether solvents as far as the enantioselectivity is concerned. However, the reaction times were the shortest in di-isopropyl ether and significantly longer in tetrahydrofuran.

Accordingly, extensive enzyme screening was carried out in di-isopropyl ether: 9 lipases and 6 proteases were tested (see Experimental). Although all the enzymes were catalytically active under the given reaction conditions, the proteases generally exhibited very low selectivities ($E<15$); only protease M satisfied the criterion required for practical purposes ($E>30$). As concerns the lipases, under the given conditions lipases PS and PPL were more selective for the *trans* compounds **5** and **9**, while lipases M, F, N and Novozym 435 were more efficient for the *cis* compounds **3** and **7** (Table 4).

In order to determine the enzyme selectivity, enantiomerically pure 1,3-amino alcohols [(1*R*,2*S*)-**1**, (1*S*,2*S*)-**4**, (1*R*,2*S*)-**6** and (1*S*,2*S*)-**8**] were prepared from the corresponding enantiomericallly pure β-amino acid esters[15] by $LiAlH_4$ reduction and used as standards for determination of the absolute configurations. These standards, the racemic compounds **1**, **4**, **6** and **8** and the *N*-deprotected unreacted alcohol enantiomers from the enzymatic reactions were derivatized with *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent).[19]

The derivatized standards and the *N*-deprotected derivatized resolved alcohol counterparts were coinjected with the appropriate racemic **1**, **4**, **6** or **8** onto a reversed phase HPLC column. The resulting chromatograms revealed the 1*S* selectivity of the enzymes, favouring the enantiomer predicted by the empirical rule for primary alcohols, formulated by Kazlauskas et al. for *Pseudomonas cepacia* lipase.[13,20] Novozym 435 displayed a very poor selectivity for **9**. The stereochemical preference of lipase AY was opposite for **3**, **5** and **9**.

At low E values, changing the solvent often results in an inverse selectivity. This occurred in the lipase AY-catalysed *O*-acylation of racemic **2** when diethyl ether was used instead of di-isopropyl ether

Table 4
Effects of different enzymes on enantioselectivity in diisopropyl ether

	3			5			7			9		
Enzyme	E	conv. (%)		E	conv. (%)		E	conv. (%)		E	conv. (%)	
Lipase PS	28	53	(1 h)	94	50	(1 h)	25	50	(2 h)	43	50	(13 min)
Lipase M	>200	53	(1 h)	3	51	(3 h)	>200	53	(1.5 h)	20	49	(1 h)
Lipase F	170	52	(16 h)	4	50	(16 h)	>200	54	(12 h)	29	52	(5 h)
Lipase N	78	49	(40 h)	6	37	(24 h)	30	41	(25 h)	24	53	(25 h)
Lipase A	25	50	(27 h)	—	—		—	—		6	34	(27 h)
Lipase AY	1.5[a]	50	(15 min)	14[a]	35	(10 min)	1.2	61	(10 min)	2[a]	57	(10 min)
Novozym	38	52	(5 min)	3	16	(10 min)	51	55	(15 min)	1	76	(30 min)
PPL	8	50	(1.5 h)	19	48	(1 h)	4	71	(2 h)	128	52	(30 min)
Protease M	46	56	(6.5 h)	—	—		—	—		—	—	

[a]Opposite enzyme selectivity

(Table 1). On comparison of the results obtained for the lipase M-catalysed *O*-acylation of racemic **3** and **5** (Table 4), the extreme difference between the E values is striking and offers an opportunity to produce a single enantiomer from a diastereomeric mixture.

In conclusion, in spite of the fact that the stereogenic centre is remote from the reaction site, racemic **1**, **4**, **6** and **8** have been successfully resolved via *O*-acylation of their Z derivatives.

3. Experimental

3.1. Materials and methods

The racemic amino alcohols **1**, **4**, **6** and **8** were obtained from esters of the corresponding β-amino acids by $LiAlH_4$ reduction.[21,22] Novozym 435 (immobilized *Candida antarctica*) was purchased from Novo Nordisk, PPL (pig pancreatic lipase) was from Fluka BioChemika, and lipases PS (*Pseudomonas cepacia*), A (*Aspergillus niger*), AY (*Candida rugosa*), F (*Rhizopus oryzae*), G (*Penicillium camembertii*), M (*Mucor javanicus*) and N (*Rhizopus niveus*), and proteases A (*Aspergillus* sp.), B (*Penicillium* sp.), M (*Aspergillus* sp.), N (*Bacillus* sp.), P (*Aspergillus* sp.) and S (*Bacillus* sp.) were from Amano Enzyme Europe. The solvents were of the best analytical grade from Lab-Scan and were used directly from the bottle. Solvents for HPLC analyses were of HPLC grade from Lab-Scan.

HPLC analyses were performed on a Kontron 422 and a Waters 600 liquid chromatograph. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^1H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Mass spectra were obtained with a Simigan TSQ 7000 spectrometer. Melting points were determined on a Kofler apparatus and are uncorrected.

3.2. Preparation of Z derivatives of racemic 1, 4, 6 and 8

A quantity (0.64 g, 5 mmol) of racemic **1**, **4**, **6** or **8** was dissolved in 25 ml dichloromethane in the presence of 0.58 g (5.5 mmol) Na_2CO_3 and 25 ml water, and 0.742 ml (5 mmol) benzyl chloroformate was then added dropwise to the mixture with stirring at room temperature. After 2–3 hours, the reaction mixture was poured onto ice–water, a few drops of TEA was added and the mixture was extracted with dichloromethane (2×25 ml). The combined organic layer was washed with brine solution (2×30 ml),

dried on $MgSO_4$ and evaporated. The oily product was purified on a silica gel column, the eluent being dichloromethane:acetone (97:3), R_f=0.25–0.28. Yield: 85–90%, white crystals, mp: 75–77°C (rac-**3**), 129–130°C (rac-**5**), 99–100°C (rac-**7**) and 88–89°C (rac-**9**). The ^1H NMR spectra were identical with the spectra of the corresponding resolved alcohols (see later).

3.3. Preparation of the Boc derivative of racemic 1

A quantity (0.39 g, 3 mmol) of *cis*-2-aminocyclohexane-1-methanol (**1**) was dissolved in 15 ml dichloromethane in the presence of KOH (0.84 g, 15 mmol) and 15 ml water, and 0.65 g (3 mmol) di-*tert*-butyl dicarbonate was then added with stirring at room temperature. After overnight stirring, the two phases were separated. The aqueous phase was extracted with dichloromethane (2×15 ml), and the combined organic layer was washed with brine solution to pH 7, dried on $MgSO_4$ and evaporated to dryness. Purification was carried out on a silica gel column, the eluent being hexane:ethyl acetate (3:1), R_f=0.21. Yield: 80%, white crystals, mp: 87–88°C.

3.4. Typical small-scale enzymatic resolution

In a typical small-scale experiment, 10 mg (38 μmol) of **2**, **3**, **5**, **7** or **9** was dissolved in 0.75 ml solvent, and 0.25 ml acyl donor and 25 mg of enzyme were added. The mixture was stirred at room temperature. Samples of 0.1 ml were taken out at intervals, the enzyme was filtered off, the solvent was evaporated off and the residue was dissolved in hexane:isopropanol (9:1) and injected onto a Chiralcel OD HPLC column (0.46 cm×25 cm; Daicel Chemical Co.), hexane:isopropanol (95:5) being used as eluent. Under these conditions, both the conversion of the reaction (c) and the enantiomeric excesses of substrate (ee_s) and product (ee_p) could be determined simultaneously from one run.

3.5. Preparation of standard, enantiomerically pure amino alcohols and determination of enzyme selectivity

To a slurry of 40 mg $LiAlH_4$ in 2 ml dry tetrahydrofuran, 10 mg ethyl (1*R*,2*S*)- or (1*S*,2*S*)-2-amino-1-cyclohexanecarboxylate or ethyl (1*R*,2*S*)- or (1*S*,2*S*)-2-amino-4-cyclohexene-1-carboxylate[15] was added with stirring. After 6 hours, the reaction was stopped by adding 3–4 drops of water. The mixture was then filtered and evaporated to dryness, and the residue was dissolved in acetonitrile and derivatized with *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent).[19]

To 5 mg (1*R*)-**3**, **5**, **7** or **9** 2–3 drops of hydrobromic acid solution in glacial acetic acid was added. After 1 hour, 50 μl TEA was added and the reaction mixture was evaporated to dryness three times with 1 ml dichloromethane. The residue was dissolved in acetonitrile and derivatized with Marfey's reagent. The conditions of determination of the enzyme selectivity are summarized in Table 5.

3.6. Gram-scale resolution of cis*-2-(benzyloxycarbonylamino)cyclohexane-1-methanol 3*

Racemic *cis*-**3** (0.8 g, 3 mmol) was dissolved in diisopropyl ether (76 ml), and vinyl butyrate (4 ml, 32 mmol) and lipase M (2 g) were added. The mixture was stirred at room temperature for 95 minutes. The enzyme was filtered off at 49% conversion and the solvent was evaporated. The crude product was subjected to column chromatography with dichloromethane:acetone (97:3) as eluent to isolate the butyrate produced (1*S*,2*R*)-**3b** and the unreacted alcohol (1*R*,2*S*)-**3**.

Table 5
Retention times and resolutions on APEX ODS (0.46 cm×25 cm, Jones Chromatography) HPLC column; eluent: methanol:water=60:40; flow rate: 0.8 ml/min; detection: 340 nm

Compound	Racemic peaks[a] t_R/min 1.	2.	R_S	Standard[b] config.	t_R/min	Major alcohol[c] t_R/min	config.	Enzyme selectivity[d]
1	13.5	29.4	5.68	1*R*,2*S*	13.5	13.5	1*R*,2*S*	*S*
4	11.3	25.7	6.55	1*S*,2*S*	11.3	25.7	1*R*,2*R*	*S*
6	12.2	21.2	3.46	1*R*,2*S*	12.2	12.2	1*R*,2*S*	*S*
8	12.9	20.6	3.06	1*S*,2*S*	12.9	20.6	1*R*,2*R*	*S*

[a] Racemic peaks separated after precolumn derivatization with Marfey's reagent. [b] Enantiomerically pure amino alcohol from the corresponding homochiral β-amino acid ester by $LiAlH_4$ reduction and derivatization with Marfey's reagent. [c] Major *N*-deprotected unreacted alcohol enantiomer derivatized with Marfey's reagent. [d] Refers to the stereogenic centre C(1) bearing the hydroxymethyl group.

Compound (1*S*,2*R*)-**3b** is a colourless oil (0.42 g, 1.26 mmol; $[\alpha]_D^{20}=-30$ (c=0.2, $CHCl_3$); 97% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (t, 3H, *J*=7.4 Hz), 1.20–2.00 (m, 11H), 2.27 (t, 2H, *J*=7.3 Hz), 3.93–3.97 (m, 1H), 4.01–4.06 (m, 2H), 4.95 (d, 1H, *J*=8.5 Hz), 5.04–5.15 (ABq, 2H, *J*=13.5 Hz), 7.29–7.38 (m, 5H). MS: 334 (M+1). $C_{19}H_{27}NO_4$ calc. 68.43% C, 8.17% H, 4.20% N; found 68.29% C, 7.79% H, 4.93% N.

For further purification, the unreacted amino alcohol (1*R*,2*S*)-**3** was dissolved in diethyl ether and extracted with Na_2CO_3 solution to remove the excess of butyric acid. Recrystallization from hexane/diisopropyl ether afforded (1*R*,2*S*)-**3** as white crystals (0.32 g, 1.22 mmol; mp: 90.5–91.5°C; $[\alpha]_D^{20}=+7$ (c=0.2, $CHCl_3$); 92% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.90–0.95 (m, 1H), 1.18–1.36 (m, 3H), 1.61–1.82 (m, 5H), 3.24–3.40 (m, 2H), 3.79–3.83 (m, 1H), 4.12–4.15 (m, 1H), 5.00 (d, 1H, *J*=8.0 Hz), 5.01–5.15 (ABq, 2H, *J*=12.5 Hz), 7.31–7.40 (m, 5H). MS: 264 (M+1). $C_{15}H_{21}NO_3$ calc. 68.40% C, 8.04% H, 5.32% N; found 68.85% C, 8.09% H, 5.36% N.

3.7. *Gram-scale resolution of* trans-*2-(benzyloxycarbonylamino)cyclohexane-1-methanol* **5**

Racemic *trans*-**5** (0.8 g, 3 mmol) was dissolved in tetrahydrofuran (76 ml), and vinyl butyrate (4 ml, 32 mmol) and lipase PS (2 g) were added. The mixture was stirred at room temperature for 20 hours. The enzyme was filtered off at 50% conversion.

The same work-up as described above afforded the ester enantiomer (1*S*,2*S*)-**5b** as a white solid; it crystallized during long standing in a refrigerator. It was recrystallized from hexane (0.37 g, 1.11 mmol; mp: 51–53°C; $[\alpha]_D^{20}=+28$ (c=0.2, $CHCl_3$); 91% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (t, 3H, *J*=7.4 Hz), 1.13–2.05 (m, 11H), 2.26 (t, 2H, *J*=7.4 Hz), 3.38–3.45 (m, 1H), 3.95–3.99 (m, 1H), 4.15–4.18 (m, 1H) 4.66 (d, 1H, *J*=8.2 Hz), 5.04–5.11 (ABq, 2H, *J*=18.0 Hz), 7.28–7.38 (m, 5H). MS: 334 (M+1). $C_{19}H_{27}NO_4$ calc. 68.43% C, 8.17% H, 4.20% N; found 68.77% C, 8.12% H, 3.98% N.

The unreacted alcohol enantiomer (1*R*,2*R*)-**5** was obtained as white crystals (0.30 g, 1.14 mmol; mp: 100–101°C; $[\alpha]_D^{20}=-2$ (c=0.2, $CHCl_3$); 92% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.12–2.00 (m, 9H), 3.19–3.23 (m, 1H), 3.35–3.40 (m, 1H), 3.45–3.50 (m, 1H), 3.72–3.76 (m, 1H), 4.66 (d, 1H, *J*=7.4 Hz), 5.08–5.14 (ABq, 2H, *J*=14.5 Hz), 7.30–7.40 (m, 5H). MS: 264 (M+1). $C_{15}H_{21}NO_3$ calc. 68.40% C, 8.04% H, 5.32% N; found 67.88% C, 7.79% H, 5.00% N.

3.8. *Gram-scale resolution of* cis-2-(benzyloxycarbonylamino)-4-cyclohexene-1-methanol 7

Racemic *cis*-**7** (0.8 g, 3 mmol) was dissolved in diethyl ether (76 ml), and vinyl butyrate (4 ml, 32 mmol) and lipase M (2 g) were added. The mixture was stirred at room temperature for 3 hours. The enzyme was filtered off at 50% conversion. The work-up was the same as for **3**.

The resulting butyrate enantiomer (1*S*,2*R*)-**7b** is a colourless oil (0.45 g, 1.36 mmol; $[\alpha]_D^{20}=-35$ (c=0.25, $CHCl_3$); 97% ee). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.94 (t, 3H, *J*=7.3 Hz), 1.61–2.41 (m, 9H), 3.96–4.18 (m, 3H), 4.94 (d, 1H, *J*=8.7 Hz), 5.04–5.12 (ABq, 2H, *J*=18.2 Hz), 5.60–5.69 (m, 2H), 7.30–7.42 (m, 5H). MS: 332 (M+1). $C_{19}H_{25}NO_4$ calc. 68.85% C, 7.61% H, 4.23% N; found 68.25% C, 7.68% H, 4.60% N.

The corresponding alcohol counterpart (1*R*,2*S*)-**7** is a white solid (0.28 g, 1.07 mmol; mp: 97–98°C; $[\alpha]_D^{20}=-20$ (c=0.2, $CHCl_3$); 98% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.56–1.62 (m, 1H), 1.95–2.13 (m, 3H), 2.43–2.48 (m, 1H), 3.24–3.31 (m, 1H), 3.46–3.52 (m, 1H), 3.65–3.69 (m, 1H), 4.24–4.27 (m, 1H), 4.99 (d, 1H, *J*=7.9 Hz), 5.03–5.11 (ABq, 2H, *J*=14.6 Hz), 5.58–5.60 (m, 1H), 5.68–5.71 (m, 1H), 7.33–7.37 (m, 5H). MS: 262 (M+1). $C_{15}H_{19}NO_3$ calc. 68.93% C, 7.33% H, 5.36% N; found 68.68% C, 7.59% H, 5.69% N.

3.9. *Gram-scale resolution of* trans-2-(benzyloxycarbonylamino)-4-cyclohexene-1-methanol 9

Racemic *trans*-**9** (0.8 g, 3 mmol) was dissolved in diisopropyl ether (76 ml), and vinyl butyrate (4 ml, 32 mmol) and PPL (2 g) were added. The mixture was stirred at room temperature for 30 minutes. The enzyme was filtered off at 49% conversion.

The same work-up as described for **3** afforded the butyrate enantiomer (1*S*,2*S*)-**9b** as a white solid, crystallized in a refrigerator (0.41 g, 1.24 mmol; mp: 30–32°C; $[\alpha]_D^{20}=+49$ (c=0.2, $CHCl_3$); 95% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (t, 3H, *J*=7.4 Hz), 1.59–1.66 (m, 2H), 1.93–2.45 (m, 7H), 3.85–3.86 (m, 1H), 4.03–4.14 (m, 2H), 4.84 (d, 1H, *J*=7.4 Hz), 5.02–5.12 (ABq, 2H, *J*=13 Hz), 5.58–5.65 (m, 2H), 7.29–7.41 (m, 5H). MS: 332 (M+1). $C_{19}H_{25}NO_4$ calc. 68.85% C, 7.61% H, 4.23% N; found 68.14% C, 7.09% H, 4.14% N.

The corresponding alcohol counterpart (1*R*,2*R*)-**9** is a white solid (0.30 g, 1.15 mmol; mp: 113–114°C; $[\alpha]_D^{20}=-14$ (c=0.3, $CHCl_3$); 92% ee). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.52–1.57 (m, 1H), 1.91–2.10 (m, 2H), 2.39–2.44 (m, 2H), 3.27–3.31 (m, 1H), 3.39–3.45 (m, 1H), 3.76–3.81 (m, 2H), 4.74 (d, 1H, *J*=8.1 Hz), 5.09–5.16 (ABq, 2H, *J*=15.1 Hz), 5.54–5.58 (m, 1H), 5.69–5.73 (m, 1H), 7.31–7.37 (m, 5H). MS: 262 (M+1). $C_{15}H_{19}NO_3$ calc. 68.93% C, 7.33% H, 5.36% N; found 69.49% C, 7.28% H, 5.19% N.

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