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

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Abstract—*N*-Protected racemic di-*exo*- and di-*endo*-3-aminobicyclo[2.2.1]heptane-2-methanols and di-*exo*- and di-*endo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanols were resolved through lipase-catalysed *O*-acylation, using vinyl butyrate in organic solvents. Of the lipases screened most showed a preference for the (2*S*)-enantiomer. © 2001 Published by Elsevier Science Ltd.

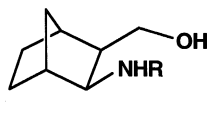
1. Introduction

1,3-Amino alcohols and their derivatives play important roles in the synthesis of various compounds with biological activity.[1–5] Enantiomerically pure **1**–**4** can be used as chiral building blocks for pharmacologically active fused saturated 1,3-heterocycles.[6] In the design of a pharmaceutical drug, enantiopurity can be crucial. A fascinating method for the preparation of pure enantiomers is to use biocatalysts, either through the enzyme-catalysed resolution of racemic mixtures, or through enzymatic enantiodifferentiation of prochiral or *meso*-compounds.[7]

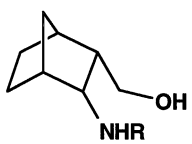
We reported recently on the enzymatic resolution of certain cyclic 1,3-amino alcohols in organic media.[8] Herein, we present results on a set of conformationally restricted bicyclic 1,3-amino alcohols. We chose to investigate the enzymatic transesterification of the primary alcohols **1**–**4**, after protection of the amino group, as we had previously found that the enzymatic *N*-acylation of 1,3-amino alcohols, in contrast with that of β-amino esters,[9] resulted in low e.e. values, as a consequence of spontaneous *N*-acylation.[8] The resolution of primary alcohols usually gives poorer results than that of secondary ones[10] (in the former the hydroxy group is not directly attached to the stereogenic centre). Nevertheless, we showed that high enantioselectivities can be achieved for 2-*N*-acylaminocyclohexane- and cyclohexenemethanols.[8] Kazlauskas et al. suggested that conformational restrictions could contribute to higher selectivities in the acylations of primary alcohols.[11] Good selectivity was therefore expected for the bicyclic 1,3-*N*-acylamino alcohols **5**–**9**.

2. Results and discussion

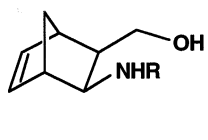
The enantioselectivity of the lipase-catalysed acyl transfer was investigated for compounds **5**–**9** in several ethereal co-solvents (Scheme 1). For *N*-protection, the easily removable *tert*-butoxycarbonyl (Boc) and benzyloxycarbonyl (Z) groups were chosen. In order to



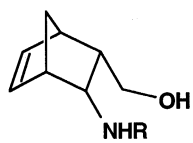
1: R=H
5: R=$COOCH_2Ph$
9: R=$COOC(CH_3)_3$



2: R=H
6: R=$COOCH_2Ph$



3: R=H
7: R=$COOCH_2Ph$



4: R=H
8: R=$COOCH_2Ph$

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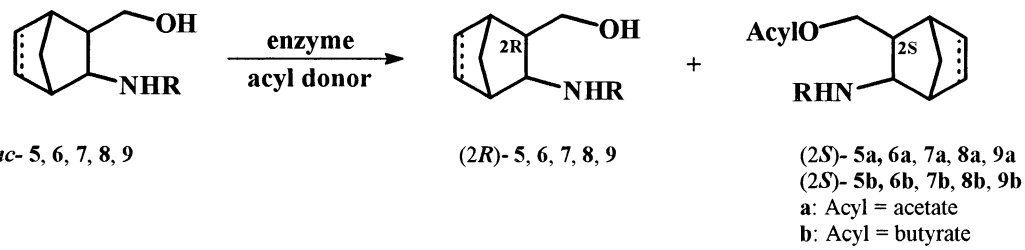

rac- **5**, **6**, **7**, **8**, **9** (2*R*)- **5**, **6**, **7**, **8**, **9** (2*S*)- **5a**, **6a**, **7a**, **8a**, **9a**
(2*S*)- **5b**, **6b**, **7b**, **8b**, **9b**
a: Acyl = acetate
b: Acyl = butyrate

Scheme 1.

ensure an irreversible acyl transfer, and on the basis of previous studies,[12–15] vinyl esters were used as acyl donors. For an irreversible acyl transfer, chromatographic techniques allow determination of the enantiomeric ratio (E)[16] at an early stage of the reaction.

In a first approach, **1** was *N*-Boc protected to give **9**, which was then acylated with vinyl acetate in the presence of one or other of seven lipases (PS, AY, F, G, M, PPL and Novozym 435). Each reaction was repeated in different ethereal solvents (Table 1). Under these conditions, only modest enantioselectivity was observed. The highest selectivity was obtained with Novozym 435 in all solvents, diethyl ether being the best. In line with our previous findings,[8] in THF much slower reactions were observed for all enzymes. For the other lipases, sluggish reactions were observed, except for lipases PS and AY, but even these resulted in very low or even no selectivity at all.

When vinyl butyrate was substituted for vinyl acetate (Table 2), markedly higher E values and higher reaction rates were obtained for Novozym 435 (except in THF), PPL and lipase M. The reactions with lipases F and G were still sluggish, while for lipases PS and AY no increase in selectivity was observed. The best results and highest reaction rates were again obtained for Novozym 435 in di-*iso*-propyl or diethyl ether.

Enantioselectivity was often enhanced at lower temperature.[17] When **9** was acylated with vinyl butyrate in diethyl ether in the presence of Novozym 435 at 0°C (Table 2),

Table 1. Lipase-catalysed (25 mg mL^{-1}) *O*-acylation of racemic **9** (10 mg mL^{-1}) with vinyl acetate (250 μL mL^{-1}) at room temperature in various ether solvents

Enzyme	*i*-Pr_2O		*t*-BuOMe		Et_2O		THF	
	E	Conv. (%)	E	Conv. (%)	E	Conv. (%)	E	Conv. (%)
Lipase PS	3	54 (21 h)	9	46 (23 h)	4	56 (28 h)	7	50 (72 h)
Lipase AY	2[a]	60 (4 h)	1	50 (23 h)	1	45 (98 h)	2	24 (261 h)
Lipase F	10	13 (237 h)		n/a[b]	5	18 (23 h)		n/a[b]
Lipase G	18	15 (237 h)	9	16 (244 h)	3	11 (145 h)		n/a[b]
Lipase M	16	22 (290 h)		n/a[b]		n/a[b]	17	19 (96 h)
PPL	4	22 (120 h)	3	31 (72 h)	5	36 (212 h)		n/a[b]
Novozym	41	58 (2 h)	19	41 (1 h)	98	54 (2 h)	58	56 (48 h)

[a] Opposite enzyme selectivity.
[b] Reaction too slow.

Table 2. Lipase-catalysed (25 mg mL^{-1}) *O*-acylation of racemic **9** (10 mg mL^{-1}) with vinyl butyrate (250 μL mL^{-1}) at room temperature in various ether solvents

Enzyme	*i*-Pr_2O		*t*-BuOMe		Et_2O		THF	
	E	Conv. (%)	E	Conv. (%)	E	Conv. (%)	E	Conv. (%)
Lipase PS	5	58 (62 h)	3	44 (24 h)	5	53 (28 h)	6	39 (215 h)
Lipase AY	2	74 (2 h)	2	79 (23 h)	1	79 (24 h)	4	16 (215 h)
Lipase F		n/a[b]	20	28 (166 h)		n/a[b]	8	22 (215 h)
Lipase G	5	27 (213 h)	13	18 (166 h)	24	23 (145 h)		n/a[b]
Lipase M	33	45 (92 h)	41	49 (99 h)	44	46 (92 h)	28	26 (144 h)
PPL	41	41 (45 h)	38	43 (99 h)	33	45 (72 h)		n/a[b]
Novozym	127	53 (1 h)	41	47 (1 h)	127	44 (0.5 h)	45	51 (48 h)
Novozym[a]					13	29 (1 h)		

[a] Reaction performed at 0°C.
[b] Reaction too slow.

Table 3. Lipase-catalysed (25 mg mL^{-1}) *O*-acylation of racemic **5** (10 mg mL^{-1}) with vinyl acetate (250 μL mL^{-1}) and vinyl butyrate (250 μL mL^{-1}) at room temperature in various ether solvents

Vinyl ester	Enzyme	*i*-Pr_2O		*t*-BuOMe		Et_2O		THF	
		E	Conv. (%)	*E*	Conv. (%)	*E*	Conv. (%)	*E*	Conv. (%)
Acetate	Lipase PS	27	62 (21 h)	12	54 (19 h)	7	58 (28 h)	13	50 (48 h)
Butyrate		15	68 (26 h)	9	57 (44 h)	10	59 (22 h)	17	54 (94 h)
Acetate	Lipase AY	2[a]	52 (1 h)	2[a]	67 (4 h)	7[a]	67 (4 h)	5[a]	21 (168 h)
Butyrate		2[a]	54 (20 min)	1[a]	59 (20 min)	2[a]	61 (0.5 h)	2	8 (287 h)
Acetate	Lipase F	4	21 (172 h)	2	25 (144 h)	3	18 (167 h)		n/a[b]
Butyrate		19	46 (79 h)	13	25 (119 h)	13	25 (119 h)		n/a[b]
Acetate	Lipase G	43	43 (75 h)	8	20 (144 h)		n/a[b]		n/a[b]
Butyrate		51	50 (168 h)	11	7 (139 h)	5	26 (119 h)		n/a[b]
Acetate	Lipase M	21	48 (172 h)	4	53 (144 h)	11	17 (23 h)		n/a[b]
Butyrate		55	56 (26 h)	34	54 (44 h)	35	50 (22 h)	50	39 (94 h)
Acetate	PPL	25	52 (45 h)	41	58 (144 h)	21	66 (52 h)	23	20 (136 h)
Butyrate		25	56 (26 h)	13	55 (19 h)	18	53 (22 h)	32	38 (287 h)
Acetate	Novozym	27	54 (10 min)	13	56 (0.5 h)	18	56 (0.5 h)	10	58 (22 h)
Butyrate		30	46 (20 min)	13	57 (20 min)	26	48 (20 min)	10	43 (22 h)

[a] Opposite enzyme selectivity.
[b] Reaction too slow.

not only was a slower reaction observed, but surprisingly the enantioselectivity decreased ($E=13$). Low-temperature experiments in other ether solvents were not attempted because of insufficient substrate solubility.

In order to improve the selectivity, the *N*-protecting group was changed and lipase-catalysed acylation of the Z derivative **5** was also investigated in the presence of vinyl acetate and vinyl butyrate using the same enzymes (Table 3).

As a general conclusion, the reactions were much slower in THF than in the other ethers examined. For lipases PS, G and Novozym 435, the reaction rates and selectivities were comparable irrespective of the acyl donor. Fair enantioselectivity (with enzymes PS or Novozym 435), to good enantioselectivity (with lipase G), was observed particularly when the reaction was performed in di-*iso*-propyl ether. However, transesterification with lipase G was too slow to be useful. For lipase AY, no selectivity was obtained with either of the acyl donors, but the rate increased with vinyl butyrate. For lipase F, increases in selectivity and reaction rate were observed on switching to vinyl butyrate, though the reaction remained sluggish. For PPL, the selectivities were comparable with both acylating agents, except in *t*-BuOMe, where a marked drop in selectivity was seen with the vinyl butyrate reaction as compared with vinyl acetate. The most marked effect was observed for lipase M, where, on switching to vinyl butyrate both the rate and the selectivity increased significantly. Again, the best result was obtained in *i*-Pr_2O.

The results for the remaining Z derivatives **6**–**8**, upon transesterification with vinyl butyrate in *i*-Pr_2O are presented in Table 4. The data on **5** (cf. Table 3) are included for comparison. It is clear that lipases PS and AY exhibited almost no selectivity for **6**–**8**. The same holds for lipase F, with the exception of its resolution of **7**, which occurred with moderate enantioselectivity. For lipase G, the exception was **6** where moderate enantioselectivity was seen.

Table 4. Effects of different enzymes (25 mg mL^{-1}) on enantioselectivity at room temperature with vinyl butyrate (250 μL mL^{-1}) in diisopropyl ether

	5		**6**		**7**		**8**	
Enzyme	*E*	Conv. (%)	*E*	Conv. (%)	*E*	Conv. (%)	*E*	Conv. (%)
Lipase PS	15	68 (26 h)	5	50 (19 h)	9	46 (24 h)	3	23 (22 h)
Lipase AY	2[a]	54 (20 min)	2[a]	62 (0.5 h)	2[a]	45 (0.5 h)	2[a]	38 (0.5 h)
Lipase F	19	46 (79 h)	6	44 (46 h)	19	49 (45 h)	3	52 (144 h)
Lipase G	51	50 (168 h)	15	28 (46 h)	5	31 (45 h)		n/a[b]
Lipase M	55	56 (26 h)	10	63 (46 h)	22	59 (24 h)		n/a[b]
Novozym	30	46 (20 min)	40	59 (0.5 h)	5	39 (1 h)	6	62 (1 h)
PPL	25	56 (26 h)	44	53 (29 h)	23	64 (24 h)	27	53 (138 h)
PPL[c]	18	51 (48 h)	36	49 (37 h)	88	44 (6 h)		n/a[b]

[a] Opposite enzyme selectivity.
[b] Reaction too slow.
[c] Reactions performed at 8°C in diethyl ether.

In contrast to **5**, moderate selectivities were noted for **6** and **7** when lipase M was used, while the reaction with **8** appeared to be too slow. For substrates **6**–**8**, PPL appears to be the lipase of choice, the highest selectivity being observed for **6** ($E=44$). In an attempt to improve the selectivity, transesterifications with PPL were performed at 8°C (at 0°C, the substrate solubility was insufficient) (Table 4, last entry). Under these conditions the selectivities observed for **5** and **6** were comparable, whereas for **7** there was a marked increase in selectivity ($E=88$). The results for **5** and **7** in the last entry suggest that the presence of the double bond is of crucial importance for the selectivity of this enzyme. The same holds for Novozym. A comparison of the results of **5** with those from reactions of **7**, and those of **6** with those of **8**, clearly demonstrated that the presence or absence of the double bond can have a dramatic influence on the enantioselectivity.

In order to conform the selectivity of the enzyme, enantiomerically pure 1,3-amino alcohols **5**–**9** were prepared from the corresponding enantiomerically pure amino esters[9] by $LiAlH_4$ reduction in THF,[20] followed by *N*-protection. The resulting enantiomerically pure **5**–**9** and the corresponding enzymatically resolved *N*-protected derivatives were co-injected onto a chiral HPLC column (Chiralcel OD, Daicel Chemical Co.) the resultant chromatograms revealed the (2*S*) selectivity of the lipases employed.

On the basis of our preliminary results, **9** was resolved on preparative scale in diethyl ether with vinyl butyrate in the presence of Novozym 435 at room temperature. In the resolution of **6**–**8**, PPL was used as the enzyme, vinyl butyrate as the acyl donor and diethyl or di-*iso*-propyl ether as the solvent. (The procedures are described fully in Section 3.)

3. Experimental

3.1. Materials and methods

The racemic amino alcohols **1**–**4** were obtained from the corresponding β-amino acids[18–20] by $LiAlH_4$ reduction.[18,21] Lipases PS (*Pseudomonas cepacia*), AY (*Candida rugosa*), F (*Rhizopus oryzae*), G (*Penicillium camembertii*) and M (*Mucor javanicus*) were obtained from Amano Enzyme Europe, Novozym 435 (immobilised *Candida antartica B*) was from Novo Nordisk, and PPL (Porcine Pancreatic Lipase) was from Fluka BioChemika. The solvents were of analytical grade from Lab-Scan. Solvents for HPLC analyses were of HPLC grade from Lab-Scan.

HPLC analyses were performed on a Kontron 422 liquid chromatograph with RI detection. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus and are uncorrected.

3.2. Preparation of Z derivatives of racemic 1, 2, 3 and 4

Racemic **1**, **2**, **3** or **4** (0.85 g, 6 mmol) was dissolved in dichloromethane (34 mL) in the presence of Na_2CO_3 (0.68 g, 6.6 mmol) and water (34 mL), and benzyl chloroformate (0.89 mL, 6 mmol) was added dropwise to the mixture with stirring at room temperature. After stirring for one night, the reaction mixture was poured onto ice-water, a few drops of NEt_3 were added and the mixture was extracted with dichloromethane (2×34 mL). The combined organic layers were washed with brine (2×40 mL), dried on $MgSO_4$, filtered and evaporated in vacuo. The oily product was purified by open column chromatography on silica gel, using gradient elution (eluent: cyclohexane:ethyl acetate from 10:0 to 6:4). Yield: 75–80%, white crystals.

Rac-**5**: $R_f=0.43$ (*n*-hexane:EtOAc, 3:2); mp: 81–83°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.11–1.53 (6H, m, 3×CH_2), 1.88–1.90 (2H, m, H-2 and O*H*), 2.13–2.18 (2H, m, H-1 and H-4), 3.57–3.59 (1H, m, H-3), 3.66–3.81 (2H, m, CH_2OH), 5.06–5.13 (2H, m, CH_2C$_6$H$_5$), 5.26 (1H, d, $J=6.56$ Hz, N*H*), 7.26–7.36 (5H, m, CH$_2$C$_6$*H*$_5$). Anal. calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.88; H, 7.13; N, 5.38%.

Rac-**6**: $R_f=0.35$ (*n*-hexane:EtOAc, 3:2); mp: 79–81°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35–1.51 (6H, m, 3×CH_2), 1.96–2.19 (2H, m, H-2 and O*H*), 2.32–2.42 (2H, m, H-1 and H-4), 3.52–4.05 (3H, m, CH_2OH and H-3), 5.07–5.14 (3H, m, CH_2C$_6$H$_5$ and N*H*), 7.31–7.37 (5H, m, CH$_2$C$_6$*H*$_5$). Anal. calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 70.06; H, 8.11; N, 4.79%.

Rac-**7**: $R_f=0.44$ (*n*-hexane:EtOAc, 3:2); mp: 78–80°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46–1.61 (2H, m, H-7), 1.78–1.87 (2H, m, H-2 and O*H*), 2.69–2.74 (2H, m, H-1 and H-4), 3.61–3.79 (3H, m, CH_2OH and H-3), 5.11–5.24 (3H, m, CH_2C$_6$H$_5$ and N*H*), 6.10–6.28 (2H, m, H-5 and H-6), 7.31–7.37 (5H, m, CH$_2$C$_6$*H*$_5$). Anal. calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 69.89; H, 7.14; N, 5.21%.

Rac-**8**: $R_f=0.27$ (*n*-hexane:EtOAc, 3:2); mp: 83–85°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.26–1.73 (3H, m, H-7 and H-2), 2.49 (1H, d, $J=6$ Hz, O*H*), 2.92–3.03 (2H, m, H-1 and H-4), 3.30–3.40 (2H, m, CH_2OH), 4.41–4.55 (2H, m, H-3 and N*H*), 5.08–5.12 (2H, m, CH_2C$_6$H$_5$), 6.12–6.34 (2H, m, H-5 and H-6), 7.30–7.38 (5H, m, CH$_2$C$_6$*H*$_5$). Anal. calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.78; H, 7.18; N, 5.12%.

3.3. Preparation of Boc derivative (±)-9

Racemic **1** (2 g, 14.2 mmol) was dissolved in dichloromethane (70 mL) in the presence of KOH (3.97 g, 71 mmol) and water (70 mL). Di-*tert*-butyl dicarbonate (3.1 g, 14.2 mmol) was added with stirring at room temperature. After stirring overnight,

the two phases were separated. The aqueous phase was extracted with dichloromethane (2×70 mL), and the organic layers were combined, washed with brine until neutral, dried on $MgSO_4$, filtered and evaporated. Purification was carried out on a silica gel column, using gradient elution (eluent: cyclohexane:ethyl acetate from 10:0 to 6:4). $R_f = 0.51$ (*n*-hexane:EtOAc, 3:2). Yield: 74%, white crystals, mp: 135–137°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.10–1.59 (15H, m, 3×CH_2 and 3×CH_3), 1.89–1.90 (1H, m, H-2), 2.10–2.15 (2H, m, H-1 and H-4), 3.54–3.70 (3H, m, H-3 and CH_2OH), 4.86 (1H, d, $J = 5.08$ Hz, NH). Anal. calcd for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.93; H, 9.87; N, 5.95%.

3.4. Typical small-scale enzymatic resolution

In a typical small-scale experiment, racemic **5**, **6**, **7**, **8** (10 mg, 36 μL) or **9** (10 mg, 41 μL) was dissolved in an appropriate solvent (0.75 mL; see Tables 1–4) and enzyme (25 mg) and acyl donor (0.25 mL) were added. The mixture was stirred at room temperature. Samples of 100 μL were removed for analysis, the enzyme was filtered off, and the solvent was evaporated. The residue was dissolved in hexane: *iso*-propanol (1:1) and injected onto a Chiralcel OD HPLC column (0.46 cm×25 cm; Daicel Chemical Co.) (eluent: *n*-hexane:*iso*-propanol 97:3; flow = 0.9 mL min^{-1}). In the cases of **6** and **8**, the unreacted alcohol in the sample was acetylated with acetic anhydride/pyridine in the presence of 4-*N*,*N*-dimethylaminopyridine before HPLC analysis. Under these conditions, both the conversion of the reaction (c) and the enantiomeric excesses of substrate (e.e.$_s$) and product (e.e.$_p$) could be determined from one run.

3.5. Preparation of enantiomerically pure *N*-protected amino alcohols 5–9 as a reference for determination of enzyme selectivity

To a slurry of $LiAlH_4$ (80 mg, 2.10 mmol) in dry THF (4 mL), ethyl di-*exo*-(1*S*,2*R*,3*S*,4*R*)- or di-*endo*-(1*R*,2*R*,3*S*,4*S*)-3-aminobicyclo[2.2.1]heptane-2-carboxylate[9] (20 mg, 0.11 mmol) or ethyl di-*exo*-(1*S*,2*R*,3*S*,4*R*)- or di-*endo*-(1*R*,2*R*,3*S*,4*S*)-3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate[9] (20 mg, 0.11 mmol) was added with stirring. After stirring the mixture for 6 h, water (3–4 drops) were added, and the mixture was filtered and evaporated to dryness. Subsequently, the corresponding Z and Boc derivatives were prepared (see Sections 3.2 and 3.3). In order to establish the retention times and resolutions of both enantiomers of each compound, the derivatised standards were co-injected with the appropriate racemic **5**, **6**, **7**, **8** or **9** on a Chiralcel OD HPLC column, as described in Section 3.4.

3.6. Preparative-scale resolution of di-*exo*-3-*tert*-butoxycarbonylaminobicyclo[2.2.1]heptane-2-methanol 9

Racemic **9** (1 g, 4.15 mmol) was dissolved in diethyl ether (95 mL), and vinyl butyrate (5 mL) and Novozym 435 (3 g) were added. The mixture was stirred at room temperature for 30 min. The enzyme was filtered off after 53% conversion had been reached, and the solvent was evaporated. The crude product was subjected to column chromatography (eluent: cyclohexane:ethyl acetate 97:3), affording the butyrate (1*R*,2*S*,3*R*,4*S*)-**9b** and the unreacted alcohol (1*S*,2*R*,3*S*,4*R*)-**9**.

Compound (1*R*,2*S*,3*R*,4*S*)-**9b** is a white crystalline compound (0.63 g, 2.11 mmol; mp: 90–91°C; $[\alpha]_D^{20} = +53.5$ ($c = 1$, MeOH); 89% e.e.). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.88 (3H, t, $J = 7.40$ Hz, $CH_2CH_2CH_3$), 1.06–1.51 (15H, m, 3×CH_2 and 3×CH_3), 1.56–1.61 (2H, m, $CH_2CH_2CH_3$), 1.91–1.99 (1H, m, H-2), 2.03–2.06 (2H, m, H-1 and H-4), 2.22 (2H, t, $J = 7.28$ Hz, $CH_2CH_2CH_3$), 3.65–3.99 (3H, m, H-3 and CH_2OCOPr, 4.45 (1H, d, $J = 5.2$ Hz, NH). Anal. calcd for $C_{17}H_{29}NO_4$: C, 65.57; H, 9.39; N, 4.50. Found: C, 65.39; H, 9.25; N, 4.36%.

The unreacted amino alcohol (1*S*,2*R*,3*S*,4*R*)-**9** was obtained as white crystals (0.33 g, 1.48 mmol; mp: 123–124°C; $[\alpha]_D^{20} = -55.1$ ($c = 1$, MeOH); 99% e.e.). The ^{1}H NMR data on (1*S*,2*R*,3*S*,4*R*)-**9** are identical to those for (±)-**9** (see Section 3.3). Anal. found: C, 64.41; H, 9.04; N, 6.20%.

3.7. Preparative-scale resolution of di-*endo*-3-benzyloxycarbonylaminobicyclo[2.2.1]heptane-2-methanol 6

Racemic **6** (0.40 g, 1.45 mmol) was dissolved in di-*iso*-propyl ether (38 mL), and vinyl butyrate (2 mL) and PPL (1 g) were added. The mixture was stirred at room temperature for 4.5 h. The reaction was stopped by filtering off the enzyme at 37% conversion. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3), affording the butyrate (1*S*,2*S*,3*R*,4*R*)-**6b** and the unreacted alcohol (1*R*,2*R*,3*S*,4*S*)-**6**.

Compound (1*S*,2*S*,3*R*,4*R*)-**6b** is a yellow oil (0.09 g, 0.26 mmol; $[\alpha]_D^{20} = +15.9$ ($c = 0.4$, MeOH); 95% e.e.). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.91 (3H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 1.40–1.51 (6H, m, 3×CH_2), 1.58–1.64 (2H, m, $CH_2CH_2CH_3$), 2.20–2.24 (2H, m, CH_2CH$_2$CH$_3$), 2.24–2.37 (3H, m, H-1, H-2, H-4), 4.03–4.05 (2H, m, CH_2COOPr), 4.11–4.19 (1H, m, H-3), 4.90 (1H, d, $J = 7.8$ Hz, NH), 5.06–5.11 (2H, m, C$H_2C_6H_5$), 7.30–7.36 (5H, m, $CH_2C_6H_5$). Anal. calcd for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.05. Found: C, 70.15; H, 7.62; N, 4.27%.

In order to obtain the unreacted alcohol (1*R*,2*R*,3*S*,4*S*)-**6** with high e.e., racemic **6** (0.40 g; 1.45 mmol) was dissolved in di-*iso*-propyl ether (38 mL), and vinyl butyrate (2 mL) and PPL (1 g) were added. The mixture was stirred at room temperature for 29 h. The reaction was stopped by filtering off the enzyme after 53% conversion had been reached. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3), affording the butyrate (1*S*,2*S*,3*R*,4*R*)-**6b** and the unreacted alcohol (1*R*,2*R*,3*S*,4*S*)-**6**.

The alcohol (1*R*,2*R*,3*S*,4*S*)-**6** thus obtained is a yellow oil (0.17 g, 0.62 mmol; $[\alpha]_D^{20} = -1.69$ ($c = 0.54$, MeOH); 96% e.e.). The ^{1}H NMR (400 MHz, $CDCl_3$) data on (1*R*,2*R*,3*S*,4*S*)-**6** are identical to those for *rac*-**6** (see Section 3.2). Anal. found: C, 69.96; H, 7.22; N, 4.92%.

3.8. Preparative-scale resolution of di-*exo*-3-benzyloxycarbonylaminobicyclo[2.2.1]hept-5-ene-2-methanol 7

Racemic **7** (1 g, 3.62 mmol) was dissolved in diethyl ether (75 mL), and vinyl butyrate (25 mL) and PPL (2.5 g) were added. The mixture was stirred at 8°C for 6 h. The reaction was stopped by filtering off the enzyme after 44% conversion had been reached. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3), affording the butyrate (1*S*,2*S*,3*R*,4*R*)-**7b** and unreacted alcohol (1*R*,2*R*,3*S*,4*S*)-**7**.

Compound (1*S*,2*S*,3*R*,4*R*)-**7b** was isolated as a yellow oil (0.493 g, 1.44 mmol; $[\alpha]_D^{20}=+52.3$ ($c=1$, MeOH); 95% e.e.). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.16 (3H, t, $J=7.4$ Hz, $CH_2CH_2CH_3$), 1.75–1.91 (4H, m, H-7 and $CH_2CH_2CH_3$), 2.24–2.26 (1H, m, H-2), 2.47–2.51 (2H, m, $CH_2CH_2CH_3$), 2.92–2.93 (2H, m, H-1 and H-4), 4.06–4.40 (3H, m, CH_2COOPr and H-3), 4.98 (1H, d, $J=8.08$ Hz, N*H*), 5.26–5.36 (2H, m, $CH_2C_6H_5$), 6.35–6.48 (2H, m, H-5 and H-6), 7.53–7.61 (5H, m, $CH_2C_6H_5$). Anal. calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 70.35; H, 7.22; N, 4.27%.

In order to obtain the unreacted alcohol (1*R*,2*R*,3*S*,4*S*)-**7** in enantiomerically pure form, racemic **7** (0.11 g; 0.62 mmol) was dissolved in diethyl ether (10 mL), and vinyl butyrate (1.1 mL) and PPL (0.275 g) were added. The mixture was stirred at room temperature for 24 h. The reaction was stopped by filtering off the enzyme after 56% conversion had been reached. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3).

The alcohol (1*R*,2*R*,3*S*,4*S*)-**7** thus obtained is a yellow oil (0.04 g, 1.44 mmol; $[\alpha]_D^{20}=-57.6$ ($c=0.96$, MeOH); 99% ee). The ^{1}H NMR (400 MHz, $CDCl_3$) data on (1*R*,2*R*,3*S*,4*S*)-**7** are identical to those for (±)-**7** (see Section 3.2). Anal. found: C, 70.89; H, 7.18; N, 5.26%.

3.9. Preparative-scale resolution of di-*endo*-3-benzyloxycarbonylaminobicyclo[2.2.1]hept-5-ene-2-methanol 8

Racemic **8** (0.40 g, 1.46 mmol) was dissolved in diisopropyl ether (38 mL), and vinyl butyrate (2 mL) and PPL (1 g) were added. The mixture was stirred at room temperature for 8 h. The reaction was stopped by filtering off the enzyme after 30% conversion had been reached. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3), yielding the butyrate (1*R*,2*S*,3*R*,4*S*)-**8b** and the unreacted alcohol (1*S*,2*R*,3*S*,4*R*)-**8**.

Compound (1*R*,2*S*,3*R*,4*S*)-**8b** is a yellow oil (0.08 g, 0.23 mmol; $[\alpha]_D^{20}=-5.5$ ($c=0.24$, MeOH); 92% ee). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (3H, t, $J=7.71$ Hz, $CH_2CH_2CH_3$), 1.40–1.64 (4H, m, $CH_2CH_2CH_3$ and CH_2), 2.20–2.25 (2H, m, $CH_2CH_2CH_3$), 2.58–2.60 (1H, m, H-2), 2.89–3.00 (2H, m, H-1 and H-4), 3.77–3.79 (2H, m, CH_2COOPr), 4.36 (1H, d, $J=10.4$ Hz, N*H*), 4.52–4.53 (1H, m, H-3), 5.01–5.11 (2H, m, $CH_2C_6H_5$), 6.16–6.34 (2H, m, C*HCH*), 7.31–7.37 (5H, m, $CH_2C_6H_5$). Anal. calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 70.23; H, 7.41; N, 4.21%.

To obtain the unreacted alcohol (1*S*,2*R*,3*S*,4*R*)-**8** in enantiomerically pure form, racemic **8** (0.40 g, 1.46 mmol) was dissolved in di-*iso*-propyl ether (38 mL), and vinyl butyrate (2 mL) and PPL (1 g) were added. The mixture was stirred at room temperature for 138 h. The reaction was stopped by filtering off the enzyme after 53% conversion had been reached. The resolved products were separated by column chromatography (eluent: cyclohexane:ethyl acetate, 97:3).

The alcohol (1*S*,2*R*,3*S*,4*R*)-**8** thus obtained is a yellow oil (0.13 g, 0.47 mmol; $[\alpha]_D^{20}=+24$ ($c=1$, MeOH); 90% ee). The ^{1}H NMR (400 MHz, $CDCl_3$) data for (1*S*,2*R*,3*S*,4*R*)-**8** are identical to those for (±)-**8** (see Section 3.2). Anal. found: C, 70.67; H, 7.13; N, 5.19%.

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