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Tetrahedron: *Asymmetry* 17 (2006) 1129–1134

Lipase-catalyzed kinetic resolution of 2-aminocyclopentane- and 2-aminocyclohexanecarboxamides

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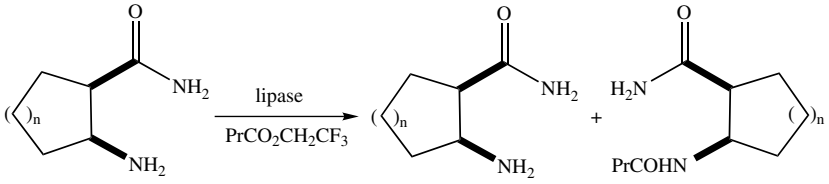
Received 20 March 2006; accepted 29 March 2006
Available online 27 April 2006

Abstract—*Candida antarctica* lipase B (CAL-B)-catalyzed *N*-acylation with 2,2,2-trifluoroethyl butanoate in solvent mixtures of *tert*-butyl methyl ether and *tert*-amyl alcohol was used to prepare all the enantiomers of *cis*- and *trans*-2-aminocyclopentane- and -cyclohexanecarboxamides. An unexpected change in enantiopreference, accompanied by low enantioselectivity, was observed when *Pseudomonas cepacia* lipase (*cis*-cyclohexane substrate) or *C. antarctica* lipase A (*cis*-cyclopentane and -cyclohexane substrates) replaced CAL-B.

1. Introduction

Over the past few years, interest in the enantiomers of alicyclic β-amino acids has increased greatly from both pharmaceutical and chemical aspects.[1–3] The natural β-amino acid cispentacin [(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid] and some of its synthetic derivatives, in particular the 4-methylene derivative (BAY 1-8888/PLD-118), have been shown to exert strong antifungal activity against *Candida albicans*.[4–6] On the other hand, self-organizing β-peptides, and *cis*- and *trans*-2-aminocyclopentane- and -cyclohexanecarboxylic acid enantiomers have been shown to form interesting, stable secondary structure motifs depending on which enantiomers the peptide is made from.[1–3]

The enantiomers of several alicyclic $\beta^{2,3}$-amino acids have previously been resolved by lipases; examples are amino esters, β-lactams, *N*-hydroxymethylated β-lactam intermediates and β-aminonitriles.[7–11] In the present work, we introduce lipase catalysis for the asymmetric *N*-acylation of alicyclic β-amino amides *rac*-**1**–**4** (Schemes 1 and 2). The motivation was to create new chemoenzymatic possibilities for the preparation of β-amino acid enantiomers, and to learn more about the enantioselective behaviour of lipases towards small structural variations in their



rac-**1** (n=1)
rac-**2** (n=2)
(1*R*,2*S*)-**1** (n=1)
(1*R*,2*S*)-**2** (n=2)
(1*S*,2*R*)-**5** (n=1)
(1*S*,2*R*)-**6** (n=2)

Scheme 1. Transformations into the enantiomers of *cis*-β-aminocarboxamides **1** and **2**.

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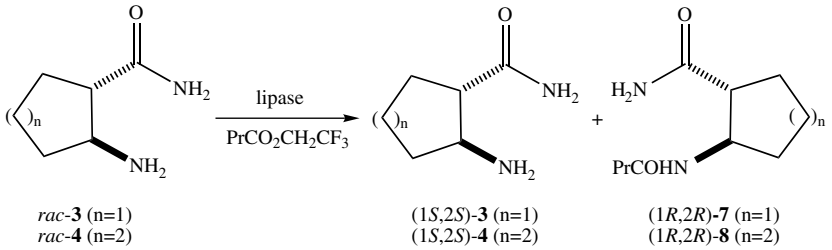
0957-4166/$ - see front matter
doi:10.1016/j.tetasy.2006.03.029



rac-**3** (n=1)
rac-**4** (n=2)

(1*S*,2*S*)-**3** (n=1)
(1*S*,2*S*)-**4** (n=2)

(1*R*,2*R*)-**7** (n=1)
(1*R*,2*R*)-**8** (n=2)

Scheme 2. Transformations into the enantiomers of *trans*-β-aminocarboxamides **3** and **4**.

substrates. Previously reported biological effects of various aminoamide derivatives in racemic and the enantiomerically pure forms furnished further motivation for the present work.[12–14] Thus, (1*S*,2*R*)-2-aminocyclohexanecarboxamide is needed as a terminal amino acid residue in small phosphotyrosine-containing peptides and in its mono-charged phosphinate isosters, prepared in order to inhibit tyrosine kinase in the treatment of cancer.[12]

Amino amides have not served as substrates for lipases previously, although the preparation of aminoamides as product enantiomers through the ammonolysis of amino esters was first described in 1993 by the groups of Gotor and Sheldon.[15,16] In the present work, ammonolysis could not be used because ethyl *cis*-2-aminocyclopentanecarboxylate is known to give a mixture of *cis*-2-aminocyclopentanecarboxamide **1** and the corresponding *trans* isomer **3**, under amidation conditions.[17] Thus, the unreacted amino ester enantiomer could not be prepared by ammonolysis without isomerization.

2. Results and discussion

On the basis of our earlier work, three lipases were selected in order to optimize the asymmetric *N*-acylation of amino amides *rac*-**1**–**4** (Schemes 1 and 2). The results are shown in Tables 1–3. Lipase PS from *Burkholderia cepacia* (adsorbed on Celite[18] or as commercial PS-C II) was selected because it has been effectively used for the (*R*)-selective *N*-acylation of the alicyclic β-amino ester and β-aminonitrile analogues of **1**–**4** in ether [Et_2O, $^iPr_2O$ (DIPE) and ^{t}BuOMe (TBME)] solutions.[7,11] Lipases A (CAL-A) and B (CAL-B) from *Candida antarctica* were also selected. CAL-A is known to display excellent (2*R*) enantioselectivity for the *N*-acylation of sterically hindered alicyclic racemic *cis*-β-amino

Table 1. *N*-Acylation of *rac*-**1** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in solvent mixtures at 48 °C; reaction time 1 h

Entry	Enzyme	Solvent	Conversion (%)	ee$^{(1R,2S)\text{-}\mathbf{1}}$ (%)	ee$^{(1S,2R)\text{-}\mathbf{5}}$ (%)	*E*
1	CAL-B	TBME/TAA (1:1)	46	83	98	>200
2	CAL-B	TBME/TAA (3:1)	50	96	98	>200
3	CAL-B	TBME/TAA (4:1)	50	97	97	>200
4	CAL-B	TBME/MeCN (1:1)	47	85	95	108 ± 17
5	Lipase PS	TBME/TAA (1:1)	28	30	76	11 ± 0.3
6	Lipase PS-C II	TBME/TAA (1:1)	34	35	67	7 ± 0.1
				ee$^{(1S,2R)\text{-}\mathbf{1}}$	ee$^{(1R,2S)\text{-}\mathbf{5}}$	
7	CAL-A	TBME/TAA (1:1)	58	69	50	4 ± 0.3

Table 2. *N*-Acylation of *rac*-**2** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in organic solvent mixtures at 48 °C

Entry	Enzyme	Solvent	Time (h)	Conversion (%)	ee$^{(1R,2S)\text{-}\mathbf{2}}$ (%)	ee$^{(1S,2R)\text{-}\mathbf{6}}$ (%)	*E*
1	CAL-B	TAA	24	30	41	95	61 ± 4
2	CAL-B	TBME/TAA (3:1)	6	31	42	91	37[c]
3	CAL-B	TBME/TAA (4:1)	6	42	66	91	40 ± 3
4	CAL-B	TBME/TAA (4:1)[a]	6	9	84	82	20 ± 5
5	CAL-B	TBME/THF (4:1)	6	59	79	56	8 ± 1
6	CAL-B	TBME/MeCN (4:1)	24	51	81	79	21 ± 1
					ee$^{(1S,2R)\text{-}\mathbf{2}}$	ee$^{(1R,2S)\text{-}\mathbf{6}}$	
7	CAL-A	TBME/MeCN (4:1)[b]	4	99	40	1	1 ± 0.2
8	Lipase PS-C II	TBME/MeCN (4:1)	24	50	19	19	2 ± 0.1
9	Lipase PS	TBME/TAA (4:1)	6	16	6	32	0.5 ± 0.1

[a] Reaction at room temperature (23 °C).
[b] An unknown side-reaction consumes the enantiomer product obtained.
[c] Calculated on the basis of one sample.

Table 3. *N*-Acylation of *rac*-**3** and *rac*-**4** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in TBME/TAA (1:1) at 48 °C

Entry	Substrate	Enzyme	Time (h)	Conversion (%)	ee$^{(1S,2S)\text{-}3 \text{ or } \text{-}4}$ (%)	ee$^{(1R,2R)\text{-}7 \text{ or } \text{-}8}$ (%)	*E*
1	**3**	CAL-B	5	50	99	>99	≫200
2	**3**	CAL-B[a]	5	43	76	>99	≫200
3	**3**	Lipase PS	2	38	50	81	16 ± 2
4	**3**	Lipase PS-C II	2	49	83	85	25 ± 8
5	**4**	CAL-B	2	47	88	>99	≫200
6	**4**	Lipase PS	2	56	5	4	1 ± 0.1
7	**4**	Lipase PS-C II	2	3	2	73	10 ± 0.4
8	**4**	CAL-A	2	1	—	66	6 ± 0.1

[a] CAL-B from Sigma under the trade name Lipolase.

esters, including the amino ester analogues of **1** and **2**.[7,19,20] Only moderate enantioselectivity of CAL-A has been observed with the corresponding aminonitrile substrates.[11] CAL-B, on the other hand, is known to display relatively high enantioselectivity in the case of racemic *cis*-2-aminocyclopentane and 2-aminocyclohexane carbonitriles.[11]

N-Acylation of *rac*-**1**–**4**, with the selected lipases, was expected to proceed with (2*R*) enantiopreference at the reaction centre as was observed for the corresponding amino esters and aminonitriles.[7,11] CAL-B-catalyzed reactions all fulfill this expectation. The specific rotation of the diamine obtained after $LiAlH_4$ reduction of (1*R*,2*S*)-**2** was consistent with the previously reported value.[11,22] However, the peaks in the GC chromatograms clearly reveal a change in enantiopreference for the CAL-A-catalyzed *N*-acylation of *rac*-**1** and for the CAL-A and lipases PS and PS-C II-catalyzed *N*-acylations of *rac*-**2**, where a slight (2*S*) enantiopreference is observed (Table 1, entry 7, and Table 2, entries 7–9, respectively).

The low solubilities of aminoamides *rac*-**1**–**4** in organic solvents other than alcohols, acetonitrile (MeCN) and tetrahydrofuran (THF) at first seemed to restrict the present enzymatic work. Primary or secondary alcohols as substrates for lipases could not be used as solvents. In the presence of CAL-B in *tert*-amyl alcohol (TAA), the *N*-acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate proceeded slowly at 48 °C (Table 2, entry 1). Lipases react faster and are more selective for reactions in ether solutions (DIPE and TBME in particular) compared to reactions in many other solvents.[7] Accordingly, the studies were continued in solvent mixtures of TBME with TAA, acetonitrile and tetrahydrofuran. Screening of the lipases for the acylation of *rac*-**1**–**4** in these solvent mixtures indicated high enantioselectivity for CAL-B catalysis (Table 1, entries 1–4; Table 2, entries 1–6; Table 3, entries 1, 2 and 5). On the other hand, lipases PS, PS-C II and CAL-A could not be used for the present studies, because of the low enantioselectivities, the *E* values now varying between 1 and 25.

As it proved more demanding to resolve alicyclic *cis*-β-amino amides than the corresponding *trans* species, the optimization was continued by using *rac*-**1** and *rac*-**2** as substrates for CAL-B catalysis. The nature of the solvent mixture had a clear effect on the *N*-acylation (Tables 1 and 2). Thus, there was a drop in enantioselectivity for *rac*-**2**, when the mixture of TBME with TAA (4:1) was replaced by a mixture of TBME with THF or MeCN, as shown in Table 2 (entries 3, 4 and 5). The enantioselectivity for *rac*-**1** similarly dropped when TBME/TAA (1:1) was replaced by TBME/MeCN (1:1) (Table 1, entries 1 and 4). CAL-B-catalyzed *N*-acylation of *rac*-**1** proceeded with excellent enantioselectivity in various mixtures (1:1, 3:1 and 4:1) of TBME/TAA (Table 1, entries 1–3), while the enantioselectivity for *rac*-**2** in neat TAA was higher than that in TBME/TAA (3:1 and 4:1) (Table 2, entry 1, as compared with 2 and 3). On this basis, it was concluded that, for a practical resolution, the amount of TBME with respect to TAA should be as low as good reactivity allows.

Finally, the gram-scale resolutions of *rac*-**1**, *rac*-**3** and *rac*-**4** allowed the preparation of the enantiomers with CAL-B in a mixture of TBME/TAA (1:1) at 50% conversion. The kinetic resolution of *rac*-**2** was performed in TBME/TAA (4:1). As a consequence of the relatively moderate enantioselectivity, the kinetic resolution was performed in two stages. In the first stage, enantiopure (1*S*,2*R*)-**6** was isolated when the reaction was stopped at ca. 30% conversion. In the second enzymatic stage, the less reactive enantiomer (1*R*,2*S*)-**2** was purified enantiomerically. The reactions were performed at 45 °C, as dramatic decreases in reactivity and enantioselectivity were evident when carried out at room temperature (23 °C) (Table 2, entry 3, as compared with 4).

3. Conclusion

In conclusion, CAL-A, CAL-B and lipase PS and PS-C II-catalyzed *N*-acylations have been studied. Only CAL-B allowed the preparation of all enantiomers of *rac*-**1**–**4**. Interestingly, CAL-A and lipase PS were the most suitable lipases for the *N*-acylation of the corresponding amino esters,[7] while lipase PS and PS-C II were applicable for the corresponding aminonitriles.[11] This work demonstrates unexpected enantiopreference differences between CAL-A and lipase PS and PS-C II as compared with CAL-B for the *cis* substrates **1** and **2**.

4. Experimental

Lipases A (CAL-A) and B (CAL-B; Novozym 435) from *C. antarctica* were purchased from Novozyme. Novozym

435, known by the trade name Lipolase, was obtained from Sigma Aldrich. Lipases PS and PS-C II from *B. cepacia* (formerly *Pseudomonas cepacia*) were products of Amano Europe, England. Before use, CAL-A and lipase PS were adsorbed on Celite in the presence of sucrose, as described previously,[18] the final lipase preparation containing 20% (w/w) of the lipase. The solvents were of the highest analytical grade. 2,2,2-Trifluoroethyl butanoate was prepared from trifluoro ethanol and butanoyl chloride. Compounds *rac*-**1**–**4** were prepared as reported earlier.[11,17,21,22]

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at ambient temperature on a JEOL L400 or a Bruker AM400 spectrometer. Chemical shifts are given in δ (ppm); multiplicities were recorded as s (singlet), d (doublet), t (triplet), m (multiplet) or om (overlapping multiplet). Optical rotations were measured with a Perkin Elmer 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

In a typical small-scale experiment, one of the compounds *rac*-**1**–**4** (0.05 M) was dissolved in TAA or an appropriate solvent mixture (1 mL), followed by addition of the enzyme preparation (50 mg/mL). The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.1 M). The reaction mixture was shaken at 48 °C unless otherwise stated. The progress of the reaction and the ee values were determined by taking samples (0.1 mL) at intervals, filtering off the enzyme and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). For good baseline separation, the unreacted amino group in the sample was acylated with acetic anhydride in the presence of pyridine containing 1% 4,4-dimethylaminopyridine. The determination of E was based on the equation $E = \ln[(1 - ee_S)/(1 + ee_S/ee_P)]/\ln[(1 + ee_S)/(1 + ee_S/ee_P)]$, where $c = ee_S/(ee_S + ee_P)$, with the use of linear regression, E being the slope of the line $\ln[(1 - c)(1 - ee_S)]$ versus $\ln[(1 - c)(1 + ee_S)]$, the subscripts referring to the less reactive substrate (S) and to the product formed (P).[23]

4.1. Gram-scale resolution of *cis*-2-aminocyclopentanecarboxamide, *rac*-1

Compound *rac*-**1** (0.3 g, 2.34 mmol) was dissolved in a mixture (47 mL) of TBME and TAA (1:1), and CAL-B (2.34 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.7 mL, 4.7 mmol). The reaction mixture was then shaken at 45 °C. The reaction was stopped after 1.25 h, at 50% conversion ($ee^{(1R,2S)\text{-}\mathbf{1}} = 97\%$, $ee^{(1S,2R)\text{-}\mathbf{5}} = 98\%$) by filtering off the enzyme. After evaporation of the solvent, the residue was purified by column chromatography, using CH_2Cl_2/MeOH (9:1, then 1:1) to separate the resolution products.

Compound (1*R*,2*S*)-**1** (0.121 g, 0.94 mmol) was obtained as white crystals: mp 135–137 °C, ee = 97%, $[\alpha]_D^{20} = -70.4$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15–2.10 (6H, om, $3 \times CH_2$), 1.93 (2H, s, CHNH_2), 2.24 (1H, m, $J = 9.30$ Hz, H-1), 3.22 (1H, m, $J = 7.04$ Hz, H-2), 5.25 (1H, s, $CONH_2$), 6.95 (1H, s, $CONH_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5 (C4), 23.3 (C5), 30.4 (C3), 45.6 (C2), 53.4 (C1), 181.7 ($CONH_2$) ppm. Anal. Calcd for $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N 21.86. Found: C, 56.02; H, 9.11; N, 21.47.

Compound (1*S*,2*R*)-**5** (0.177 g, 0.90 mmol) was obtained as white crystals: mp 176–178 °C, ee = 99%, $[\alpha]_D^{20} = +92.2$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (3H, t, $J = 7.4$ Hz, CH_3), 1.41–1.86 (6H, om, $3 \times CH_2$), 1.70 (2H, m, $CH_3CH_2CH_2$), 2.00 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, C*H*CONH$_2$), 4.24 (1H, m, C*H*NHCOPr), 6.72 (1H, s, NH_2), 7.10 (1H, s, NH_2), 7.46 (1H, d, $J = 8.0$ Hz, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1 (CH_3), 19.2 ($CH_2CH_2CH_3$), 22.6 (C4), 27.8 (C5), 32.4 (C3), 37.9 ($CH_2CH_2CH_3$), 47.1 (C2), 52.3 (C1), 172.0 (NHCO), 175.3 ($CONH_2$) ppm. Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N 14.13. Found: C, 60.31; H, 8.95; N, 13.88.

4.2. Gram-scale resolution of *cis*-2-aminocyclohexanecarboxamide, *rac*-2

Compound *rac*-**2** (0.5 g, 3.5 mmol) was dissolved in a mixture (70 mL) of TBME and TAA (4:1), after which CAL-B (3.5 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (1 mL, 7 mmol). The mixture was then shaken at 45 °C. The reaction was stopped after 5.75 h, at 27% conversion ($ee^{(1R,2S)\text{-}\mathbf{2}} = 34\%$, $ee^{(1S,2R)\text{-}\mathbf{6}} = 93\%$) by filtering off the enzyme. After evaporation, the residue was purified by a short column, using CH_2Cl_2/MeOH (9:1, then 1:1) as an eluent to separate the products.

In order to obtain the enantiomer substrate in an enantiopure form, the enantiomerically enriched substrate (0.347 g, $ee^{(1R,2S)\text{-}\mathbf{2}} = 34\%$) was subjected to further enzymatic acylation under the above reaction conditions. The reaction was stopped after 150 h ($ee^{(1R,2S)\text{-}\mathbf{2}} = 99\%$, $ee^{(1S,2R)\text{-}\mathbf{6}} = 47\%$) by filtering off the enzyme and evaporation of the solvent. The substrate and the product were separated as described above.

Compound (1*R*,2*S*)-**2** (0.091 g, 0.64 mmol) was obtained as white crystals: mp 123–124 °C, ee = 99%, $[\alpha]_D^{20} = +10.1$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25–1.80 (8H, om, $4 \times CH_2$), 1.53 (2H, s, CHN*H*2), 2.21 (1H, m, $J = 10.16$ Hz, H-1), 3.12 (1H, m, $J = 4.46$ Hz, H-2), 6.69 (2H, s, $CONH_2$), 7.64 (1H, s, $CONH_2$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.2 (C4), 24.5 (C6), 25.2 (C5), 33.4 (C3), 47.6 (C2), 49.2 (C1), 177.2 ($CONH_2$) ppm. Anal. Calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N 19.70. Found: C, 58.98; H, 9.65; N, 19.38.

Compound (1*S*,2*R*)-**6** (0.170 g, 0.80 mmol) was obtained as white crystals: mp 196.5–198 °C, ee = 95%, $[\alpha]_D^{20} = +14.6$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (3H, t, $J = 7.38$ Hz, CH_3), 1.22–1.88 (8H, om, $4 \times CH_2$), 1.46 (2H, m, $CH_2CH_2CH_3$), 2.04 (2H, t, $J = 7.26$ Hz, $CH_2CH_2CH_3$), 2.41 (1H, m, C*H*CONH$_2$), 4.09 (1H, m, C*H*NHCOPr), 6.70 (1H, s, NH_2), 7.14 (1H, s, NH_2), 7.41 (1H, d, $J = 8.8$ Hz, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (CH_3), 19.4 ($CH_2CH_2CH_3$), 22.0 (C4), 23.7 (C6), 25.1 (C5), 30.2 (C3), 37.8 ($CH_2CH_2CH_3$), 44.7 (C2), 47.3 (C1), 171.9 (NHCO), 175.6 ($CONH_2$) ppm.

Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.24; H, 9.50; N 13.02. Found: C, 61.95; H, 9.31; N, 12.73.

4.3. Gram-scale resolution of *trans*-2-aminocyclopentanecarboxamide, *rac*-3

Compound *rac*-**3** (0.098 mg, 0.76 mmol) was dissolved in a mixture (15.4 mL) of TBME and TAA (1:1), after which CAL-B (0.765 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.23 mL, 1.52 mmol). The mixture was shaken at 45 °C. After 10 h, the enzyme was filtered off, at 50% conversion ($ee^{(1S,2S)\text{-}3} = 99\%$, $ee^{(1R,2R)\text{-}7} > 99\%$). The substrate and the product were separated as described above.

Compound (1*S*,2*S*)-**3** (0.023mg, 0.18 mmol) was obtained as white crystals: mp 123–124 °C, ee = 98%, $[\alpha]_D^{20} = +38.0$ (*c* 0.25, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.95–1.90 (6H, om, $3 \times CH_2$), 1.65 (2H, s, CHNH_2), 2.63 (2H, m, H-1 and H-2), 6.67 (1H, s, $CONH_2$), 7.28 (1H, s, $CONH_2$) ppm. Anal. Calcd for $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N 21.86. Found: C, 55.97; H, 9.21; N, 21.79.

Compound (1*R*,2*R*)-**7** (0.031 mg, 0.16 mmol) was obtained as white crystals: mp 174–175 °C, ee >99%, $[\alpha]_D^{20} = -22$ (*c* 0.25, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (3H, t, $J = 7.37$ Hz, CH_3), 1.25–1.90 (6H, om, $3 \times CH_2$), 1.45 (2H, m, $CH_3CH_2CH_2$), 2.01 (2H, t, $J = 7.30$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, C*H*$CONH_2$), 4.10 (1H, m, C*H*NHCOPr), 6.72 (1H, s, NH_2), 7.28 (1H, s, NH_2), 7.78 (1H, d, $J = 7.28$ Hz, NH) ppm. Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N 14.13. Found: C, 60.14; H, 8.79; N, 14.00.

4.4. Gram-scale resolution of *trans*-2-aminocyclohexanecarboxamide, *rac*-4

Compound *rac*-**4** (0.4 g, 2.82 mmol) was dissolved in a mixture (56 mL) of TBME and TAA (1:1), after which CAL-B (2.8 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.85 mL, 5.64 mmol). The mixture was shaken at 45 °C. The reaction was stopped after 21 h, at 50% conversion ($ee^{(1S,2S)\text{-}4} = 98\%$ and $ee^{(1R,2R)\text{-}8} > 99\%$) by filtering off the enzyme. After evaporation, the residue was purified by column chromatography, using CH_2Cl_2/MeOH (1:1).

Compound (1*S*,2*S*)-**4** (0.170 g, 1.2 mmol) was obtained as white crystals: mp 125–126.5 °C, ee = 98% $[\alpha]_D^{20} = +47.2$ (*c* 0.1, MeOH), in accordance with the literature[22] value given for (1*S*,2*S*)-(+)-2-aminocyclohexanecarboxamide ($[\alpha]_D^{20} = +70.1$ (*c* 0.107, MeOH)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98–1.83 (8H, om, $4 \times CH_2$), 1.64 (2H, s, CHNH_2), 2.60–2.65 (2H, om, H-1 and H-2), 6.68 (1H, s, $CONH_2$), 7.29 (1H, s, $CONH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8 (C4), 25.1 (C6), 29.0 (C5), 34.9 (C3), 51.26 (C2), 52.8 (C1), 176.6 ($CONH_2$) ppm. Anal. Calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N 19.70. Found: C, 59.07; H, 9.60; N, 19.51.

Compound (1*R*,2*R*)-**8** (0.219 g; 1.04 mmol) was obtained as white crystals: mp 274–276 °C, ee >99% $[\alpha]_D^{20} = -12.0$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.81 (3H, t, $J = 7.2$ Hz, CH_3), 1.04–1.75 (8H, om, $4 \times CH_2$), 1.46 (2H, m, $CH_3CH_2CH_2$), 1.95 (2H, t, $J = 7.14$ Hz, $CH_2CH_2CH_3$), 2.09 (1H, m, C*H*$CONH_2$), 3.71 (1H, m, C*H*NHCOPr), 6.70 (1H, s, NH_2), 6.93 (1H, s, NH_2), 7.49 (1H, d, $J = 8.4$ Hz, N*H*COPr) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.9 (CH_3), 19.3 ($CH_2CH_2CH_3$), 25.0 (C4), 29.6 (C6), 33.1 (C5), 39.4 (C3), 40.0 ($CH_2CH_2CH_3$), 49.2 (C2), 49.6 (C1), 171.3 (NHCO), 175.8 ($CONH_2$) ppm. Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.24; H, 9.50; N 13.02. Found: C, 62.13; H, 9.27; N, 13.46.

4.5. Absolute configuration of (1*R*,2*S*)-2

Compound (1*R*,2*S*)-**2** (30 mg) was reduced with lithium aluminium hydride, to yield the corresponding diamine (17 mg) as a light-yellow oil: ee = 97%, $[\alpha]_D^{20} = +16.8$ (*c* 0.5, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22–2.05 (9H, om, H-2-6), 1.43 (2H, s, CH_2NH_2), 2.27 (2H, s, CH_2NH_2), 3.70 (1H, m, H-1), 5.00 (2H, s, CHNH_2) ppm. Rotation data correspond to the literature value for (1*S*,2*S*)-(+)-2-aminomethylcyclohexylamine {$[\alpha]_D^{20} = +21.8$ (*c* 2.18, EtOH) and +20.5 (*c* 1.0, EtOH)}.[11,22] The less reactive enantiomer thus has the (1*R*,2*S*) absolute configuration.

Acknowledgements

M.F. is grateful for a grant from the Centre for International Mobility (CIMO) in Finland. The authors also acknowledge the receipt of Hungarian Research Foundation (OTKA) grant T 049407 and the Academy of Finland grant to L.K. (grant 210263).

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