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Synthesis of enantiopure 1,4-ethyl- and 1,4-ethylene-bridged cispentacin by lipase-catalyzed enantioselective ring opening of β-lactams

Enikő Forró and Ferenc Fülöp*

Institute of Pharmaceutical Chemistry, University of Szeged, H-6701 Szeged, PO Box 121, Hungary

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Abstract—1,4-Ethyl- and 1,4-ethylene-bridged cispentacin enantiomers **1a**, **1c** and **2a**, **2c** were prepared through the lipase-catalyzed enantioselective ring opening of racemic *exo*-3-azatricyclo[4.2.1.0^{2,5}]nonan-4-one, (±)-**1**, and *exo*-3-azatricyclo[4.2.1.0^{2,5}]non-7-en-4-one, (±)-**2**. High enantioselectivity (E >200) was observed when the Lipolase-catalyzed reactions were performed with 1 equiv of H_2O in diisopropyl ether at 70 °C. The resolved β-amino acids **1a** and **2a** (yield 46%) and β-lactams **1b** and **2b** (yield ⩾40%) could be easily separated. The ring opening of lactam enantiomers with 18% HCl afforded the corresponding β-amino acid hydrochloride enantiomers (ee ⩾98%).

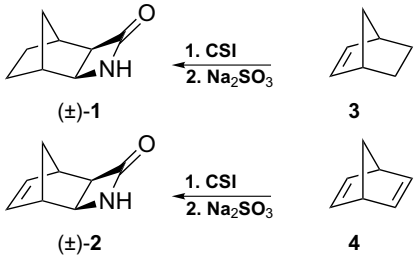
1. Introduction

A number of new enzymatic and asymmetric syntheses of β-amino acids or their derivatives and β-lactams in enantiomerically pure form have been elaborated over recent years, and most of them have been reviewed.[1–3] An important indirect enzymatic route to enantiopure β-amino acids or esters proceeds through the lipase-catalyzed asymmetric acylation of the primary *OH* group of *N*-hydroxymethylated β-lactams, or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to the corresponding β-amino acid or ester.[4–8] We have recently published a direct enzymatic method to enantiopure valuable β-amino acids (e.g., cispentacin with strong antibiotic and antifungal activities) through the lipase-catalyzed enantioselective hydrolysis of β-lactams in an organic solvent.[9,10]

Herein, we report the enantioselective ring opening of racemic *exo*-3-azatricyclo[4.2.1.0^{2,5}]nonan-4-one, (±)-**1**, and *exo*-3-azatricyclo[4.2.1.0^{2,5}]non-7-en-4-one, (±)-**2**.

2. Results and discussion

The racemic β-lactams **1** and **2** were prepared from bicyclo[2.2.1]hept-2-ene **3** and bicyclo[2.2.1]hepta-2,5-diene **4** by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (O=C=N–SO_2Cl; CSI) (Scheme 1).[11]



Scheme 1.

In an earlier study, Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) proved to be applicable for the enantioselective ring opening of 6-azabicyclo[3.2.0]heptan-7-one, 7-azabicyclo[4.2.0]octan-8-one, 8-azabicyclo[5.2.0]nonan-9-one and 9-azabicyclo[6.2.0]decan-10-one.[10] High enantioselectivities (E >200) were observed when the reactions were performed with H_2O (1 equiv) in diisopropyl ether at 60 °C. These results[10] on the lipase-catalyzed enantioselective hydrolysis of β-lactams suggested the possibility of the enantioselective ring opening of (±)-**1** and (±)-**2** (Scheme 2).

* Corresponding author. Tel.: +36-62-545564; fax: +36-62-545705; e-mail: fulop@pharma.szote.u-szeged.hu

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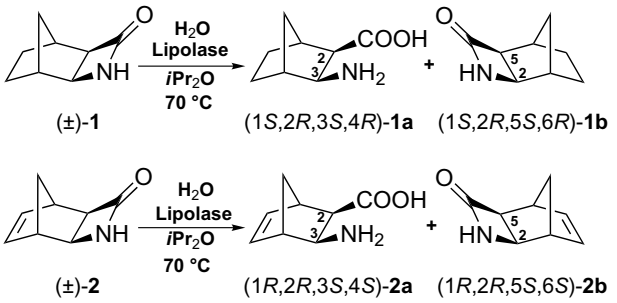
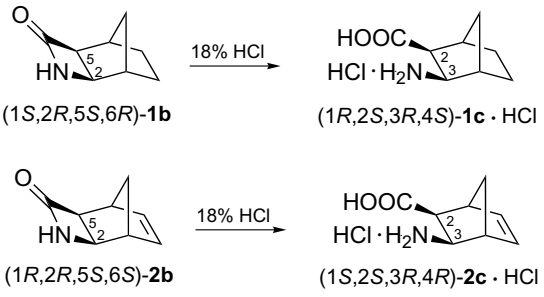


Scheme 2.



Scheme 3.

Even though the *E* value in the Lipolase-catalyzed ring opening is excellent, the reaction rate for the ring opening in the case of **2** clearly increases with increasing temperature (after 96 h, 1 equiv of H_2O, 50 mg mL^{-1} Lipolase, 60 °C: conv. = 22%, ee_s = 27%, ee_p >95%; after 96 h, 1 equiv of H_2O, 50 mg mL^{-1} Lipolase, 65 °C: conv. = 26%, ee_s = 33%, ee_p >95%; after 93 h, 1 equiv of H_2O, 50 mg mL^{-1} Lipolase, 70 °C: conv. = 30%, ee_s = 41%, ee_p >95%).[12]

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1** and (±)-**2** were performed with 1 equiv of water in the presence of Lipolase in diisopropyl ether at 70 °C. In spite of the long reaction times, the products had an excellent enantiomeric excess at 50% conversion. The results are reported in Table 1.

The transformations involving the ring opening of β-lactams **1b** and **2b** with 18% aqueous HCl resulted in the enantiomers of the β-amino acid hydrochlorides **1c**·HCl and **2c**·HCl (Scheme 3). Treatment of amino acids **1a** and **2a** with 18% aqueous HCl resulted in enantiopure hydrochlorides **1a**·HCl and **2a**·HCl. The specific rotations for the enantiomers prepared are reported in Table 2.

The absolute configuration in the case of **1** was proved by comparing the specific rotation values with the literature data (Tables 1 and 2). When (1*R*,2*R*,5*S*,6*S*)-3-azatricyclo[4.2.1.0$^{2.5}$]non-7-en-4-one **2b** was reduced catalytically in the presence of cyclohexene as a hydrogen donor,[7] the analyzed chromatogram peaks indicated that the 1*S*,2*R*,5*S*,6*R*-3-azatricyclo[4.2.1.0$^{2.5}$]nonan-4-one **1b** was formed. The specific rotation value for the isolated crude product: $[\alpha]_D^{25} = +61.2$ (*c* 0.3, $CHCl_3$) is close to the $[\alpha_D^{25} = +64.1$ (*c* 0.5, $CHCl_3$) reported for **1b**. Therefore, the absolute configuration for **2b** is (1*R*,2*R*,5*S*,6*S*) and that for **2a** is (1*R*,2*R*,3*S*,4*S*).

In conclusion, a very efficient and simple direct enzymatic synthesis of 1,4-ethyl- and 1,4-ethylene-bridged cispentacin enantiomers in an organic medium has been developed. The products (β-lactam and β-amino acid) could be easily separated. Transformations by ring opening of the β-lactams **1b** and **2b** with 18% aqueous HCl gave the enantiomers of the β-amino acid hydrochlorides **1c**·HCl and **2c**·HCl (ee >99%).

Table 2. Specific rotation of the enantiomers prepared

Amino acid hydrochloride	Ee (%)	$[\alpha]_D^{25}$[a]
1*S*,2*R*,3*S*,4*R*-**1a**·HCl[16]	99	+3.8 (*c* = 0.4, MeOH)[b]
1*R*,2*S*,3*R*,4*S*-**1c**·HCl[17]	99	−3.8 (*c* = 0.3, MeOH)
1*R*,2*R*,3*S*,4*S*-**2a**·HCl[16]	99	−13.2 (*c* = 0.5; MeOH)
		−10.8 (*c* = 0.5; H_2O)
1*S*,2*S*,3*R*,4*R*-**2c**·HCl[17]	99	+13.4 (*c* = 0.4; MeOH)
		+10.6 (*c* = 0.4; H_2O)

[a] Optical rotations were measured with a Perkin–Elmer 341 polarimeter.
[b] $[\alpha]_D^{20} = +3.9$ (*c* 2, MeOH).[18]

Table 1. Lipolase-catalyzed ring opening of (±)-**1**[13] and (±)-**2**[14]

	Time (day)	Conversion (%)	*E*	β-Lactam(**1b**–**2b**)				β-Amino acid(**1a**–**2a**)			
				Yield[a] (%)	Isomer	Ee[b] (%)	$[\alpha]_D^{25}$[c]	Yield[a] (%)	Isomer	Ee[d] (%)	$[\alpha]_D^{25}$[d]
(±)-**1**	11	50	>200	40	1*S*,2*R*,5*S*,6*R*	99	+64.1[e]	46	1*S*,2*R*,3*S*,4*R*	99	+9.1[f,g]
(±)-**2**	8	50	>200	47	1*R*,2*R*,5*S*,6*S*	99	+123.7[e]	46	1*R*,2*R*,3*S*,4*S*	98	−12.2[h]

[a] Yield 100% at 50% conversion.
[b] According to GC [CP-Chirasil-Dex CB column, 120 °C for 4 min → 170 °C (temperature rise 10 °C/min; 140 kPa) retention times (min): **1b**: 10.95 (antipode: 10.77); **2b**: 10.23 (antipode: 10.09)].
[c] Optical rotations were measured with a Perkin–Elmer 341 polarimeter.
[d] Determined by GC [after double derivatization (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (CP-Chirasil-Dex CB column, 120 °C for 5 min → 170 °C (temperature rise 10 °C/min; 70 kPa) retention times (min): **1a**: 14.01 (antipode: 14.20); **2a**: 12.96 (antipode: 13.11)].
[e] *c* = 0.5; $CHCl_3$.
[f] *c* = 0.5; H_2O.
[g] $[\alpha]_D^{20} = -8$ (*c* 1.4, H_2O) for (1*R*,2*S*,3*R*,4*S*)-**1c**[15].
[h] *c* = 0.4; H_2O.

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12. In a typical small-scale experiment, racemic β-lactam (0.05 M solution) in iPr$_2$O (2 mL) was added to Lipolase (50 mg mL^{-1}). Water (1 equiv) was added. The mixture was shaken at 60, 65 or 70 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The ee values for the unreacted β-lactam enantiomers were determined by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m), while the ees for the ring-opened amino acids produced (during preliminary experiments) were calculated by using hexadecane [CP-Chirasil-Dex CB column, 120 °C for 4 min → 170 °C (temperature rise 10 °C/min; 140 kPa) retention time 8.19 min] as an internal standard.[10]
13. Racemic **1**[11] (1 g, 7.28 mmol) was dissolved in iPr$_2$O (40 mL). Lipolase (2 g, 50 mg mL^{-1}) and water (0.13 mL, 7.28 mmol) were added and the mixture was shaken in an incubator shaker at 70 °C for 11 days. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated and the residue of (1*S*,2*R*,5*S*,6*R*)-**1b** crystallized [0.4 g, 40%; $[\alpha]_D^{25} = +64.1$ (c 0.5; $CHCl_3$); mp 58–60 °C (recrystallized from diisopropyl ether); ee 99%]. The filtered enzyme was washed with distilled water (3×20 mL), and the water was evaporated, yielding the crystalline β-amino acid (1*S*,2*R*,3*S*,4*R*)-**1a** [0.52 g, 46%; $[\alpha]_D^{25} = +9.1$ (c 0.5; H_2O); slow melting (229–242 °C, recrystallized from water/acetone), ee = 99%].
^{1}H NMR (400 MHz, D_2O) δ (ppm) for **1a**: 1.26–1.76 (6H, m, 3×CH_2), 2.4, 2.51 (2H, br s, 2×CH), 2.59 (1H, d, J = 8.1, H-2), 3.38 (1H, d, J = 8.1, H-3). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 26.2, 28.5, 33.9, 41.6, 42.4, 52.1, 55.1, 180.1. Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 62.06; H, 8.51; N, 10.14.
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **1b**: 1.02–1.69 (6H, m, 3×CH_2), 2.35, 2.41 (2H, br s, 2×CH), 2.97 (1H, d, J = 1.4, H-5), 3.38 (1H, d, J = 3.1, H-2), 5.85 (1H, br s, NH). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm) 25.3, 27.2, 31.2, 34.2, 38.5, 53.8, 58.9, 170.7. Anal. Calcd for $C_8H_{11}NO$: C, 70.04; H, 8.08; N, 10.21. Found: C, 69.89; H, 8.15; N, 10.46.
14. With the procedure described above, the reaction of racemic **2**[11] (1 g, 7.39 mmol) in iPr$_2$O (40 mL) in the presence of Lipolase (2 g, 50 mg mL^{-1}) and water (0.13 mL, 7.39 mmol) afforded the β-lactam (1*R*,2*R*,5*S*,6*S*)-**2b** [0.47 g, 47%; $[\alpha]_D^{25} = +123.7$ (c 0.5; $CHCl_3$); mp 89–91 °C (recrystallized from diisopropyl ether); ee 99%] and β-amino acid (1*R*,2*R*,3*S*,4*S*)-**2a** [0.52 g, 46%; $[\alpha]_D^{25} = -12.2$ (c 0.4; H_2O); mp >260 °C (recrystallized from water/acetone); ee = 98%] in 8 days.
^{1}H NMR (400 MHz, D_2O) δ (ppm) for **2a**: 1.6 (1H, d, J = 7.8, CH_AH$_B$), 1.87 (1H, d, J = 7.8, CH$_A$$H_B$), 2.49 (1H, d, J = 6.3, H-2) 3.01, 3.07 (2H, br s, 2×CH), 3.29 (1H, d, J = 6.2, H-3) 6.19–6.39 (2H, m, CH=CH). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 43.6, 46.7, 46.9, 47.6, 51.9, 135.1, 140.5, 180.2. Anal. Calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.6; H, 7.19; N, 9.14.
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **2b**: 1.65 (1H, d, J = 9.7, CH_AH$_B$), 1.81 (1H, d, J = 9.7, CH$_A$$H_B$), 2.88, 2.93 (2H, br s, 2×CH), 3.05 (1H, d, J = 1.6, H-2), 3.51 (1H, d, J = 3.4, H-3), 5.96 (1H, br s, NH), 6.13–6.25 (2H, m, CH=CH).^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 39.3, 41.4, 44.3, 53.7, 58.8, 136.6, 138.8, 171.4. Anal. Calcd for C_8H_9NO: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.98; H, 6.69; N, 10.36.
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16. When **1a** or **2a** (0.2 g) was treated with 18% HCl (7 mL), (1*S*,2*R*,3*S*,4*R*)-**1a**·HCl [0.21 g, 85%; $[\alpha]_D^{25} = +3.8$ (c 0.4; MeOH); mp 196–199 °C (H_2O); ee = 99%] and (1*R*,2*R*,3*S*,4*S*)-**2a**·HCl [0.23 g, 96%; $[\alpha]_D^{25} = -10.8$ (c 0.5; H_2O), −13.2 (c = 0.5; MeOH); mp 206–210 °C (H_2O); ee = 99%] were obtained.
^{1}H NMR (400 MHz, D_2O) δ (ppm) for **1a**·HCl: 1.29–1.82 (6H, m, 3×CH_2), 2.42, 2.62 (2H, br s, 2×CH), 2.85 (1H, d, J = 8.1, H-2), 3.47 (1H, d, J = 8.1, H-3). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 26.2, 28.3, 33.9, 41.4, 42.4, 50.7, 55.3, 177.7. Anal. Calcd for $C_8H_{13}NO_2$·HCl: C, 50.14; H, 7.36; N, 7.31. Found: C, 50.16; H, 7.52; N, 7.48.
^{1}H NMR (400 MHz, D_2O) δ (ppm) for **2a**·HCl: 1.66 (1H, d, J = 9.2, CH_AH$_B$), 1.92 (1H, d, J = 9.4, CH$_A$$H_B$), 2.56 (1H, d, J = 5.9, H-2) 3.04, 3.21 (2H, br s, 2×CH), 3.39 (1H, d, J = 5.8, H-3) 6.24–6.39 (2H, m, CH=CH). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 43.6, 44.7, 46.7, 47.5, 52.8, 136.1, 139.6, 176.3. Anal. Calcd for $C_8H_{11}NO_2$·HCl: C, 50.67; H, 6.38; N, 7.39. Found: C, 50.76; H, 6.16; N, 7.24.
17. **1b** or **2b** (0.2 g) was refluxed in 18% HCl (7 mL) for 3 h. The solvent was evaporated off, and the products were recrystallized from water, which afforded white crystals of (1*R*,2*S*,3*R*,4*S*)-**1c**·HCl [0.21 g, 77%, $[\alpha]_D^{25} = -3.8$ (c 0.3; MeOH); mp 198–207 °C (H_2O); ee 99%] or (1*S*,2*S*,3*R*,4*R*)-**2c**·HCl [0.23 g, 84%, $[\alpha]_D^{25} = +10.6$ (c 0.4; H_2O), +13.4 (c = 0.4; MeOH); mp 196–210 °C (H_2O); ee 99%], respectively.
The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for (1*R*,2*S*,3*R*,4*S*)-**1c**·HCl or (1*S*,2*S*,3*R*,4*R*)-**2c**·HCl are similar to those for (1*S*,2*R*,3*S*,4*R*)-**1a**·HCl and (1*R*,2*R*,3*S*,4*S*)-**2a**·HCl. Anal. found for (1*R*,2*S*,3*R*,4*S*)-**1c**·HCl: C, 49.88; H, 7.33; N, 7.24. Anal. found for (1*S*,2*S*,3*R*,4*R*)-**2c**·HCl: C, 50.67; H, 6.22; N, 7.45.
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