

Lipase-Catalyzed Enantioselective Ring Opening of Unactivated Alicyclic-Fused γ -Lactams in an Organic Solvent

Enikő Forró, Ferenc Fülöp*

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

Supporting Information

Materials and methods. Lipolase (lipase B from *Candida antarctica*), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was from Sigma-Aldrich. Lipase PS and lipase AK were from Amano Pharmaceuticals, PPL (type II) was from Sigma, and CAL-A (lipase A from *Candida antarctica*) and Novozym 435 as an immobilized lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin were from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Before use, lipase PS, lipase AK and CAL-A (5 g) were dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). The lipase preparations thus obtained contained 20% (w/w) of lipase.

The alicyclic γ -lactams 6-azabicyclo[3.2.0]heptan-7-one, 7-azabicyclo[4.2.0]octan-8-one and 8-azabicyclo[5.2.0]nonan-9-one were prepared by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to the corresponding cycloalkene,¹ while 9-azabicyclo[6.2.0]decan-10-one was obtained by means of CSI cycloaddition to cyclooctadiene² and subsequent catalytic reduction in the presence of cyclohexene as a hydrogen donor.³ CSI, cycloalkenes and cyclooctadiene were from Aldrich. The solvents were of the highest analytical grade.

In a typical small-scale experiment, racemic 2-azetidinone (0.05 M solution) in an organic solvent (2 mL) was added to the lipase tested (20, 30, 50 or 75 mg mL⁻¹). Water (0, 1, 2, 3, 4 or 10 equiv.) was added. The mixture was shaken at 60 °C. The progress of the reaction was

(1) (a) Nativ, E.; Rona, P. *Isr. J. Chem.* **1972**, *10*, 55-58. (b) Singh, R.; Cooper, R.D.G. *Tetrahedron* **1994**, *50*, 12049-12064. (c) Furet, P.; Garcia-Echeverria, C.; Gay, B.; Scoepfer, J.; Zeller, M.; Rahuel, J. *J. Med. Chem.* **1999**, *42*, 2358-2363.

(2) Parsons, P.J.; Camp, N.P.; Underwood, J.M.; Harvey, D.M. *Tetrahedron* **1996**, *52*, 11637-11642.

(3) Forró, E.; Árva, J.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 643-649.

followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The ee values for the unreacted α -lactam enantiomers were determined by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m), while the ee values for the ring-opened amino acids produced were calculated by using hexadecane as an internal standard (Fig. 1, Eq. 6). The ee values for the isolated α -amino acid enantiomers were determined by using high-performance liquid chromatography equipped with a reversed-phase (C₁₈) column after derivatization of the sample with (1*S*,2*S*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl-isothiocyanate (DANI).⁴

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ¹H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

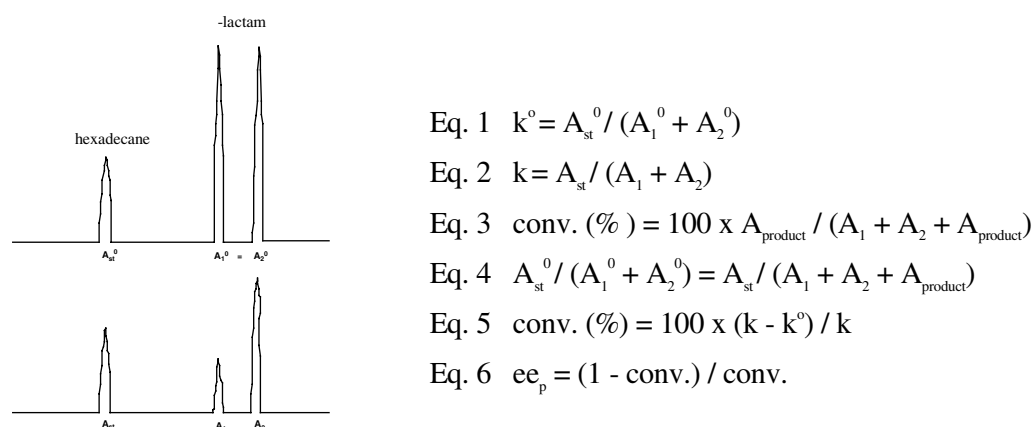
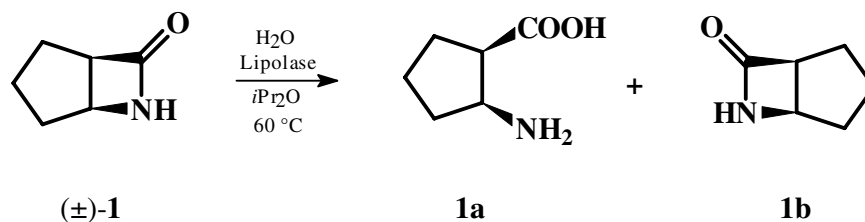


Fig. 1. Calculation of the ee values, with hexadecane as an internal standard

Gram-scale resolution of 6-azabicyclo[3.2.0]heptan-7-one, (\pm)-1



Racemic **1** (4 g, 44.98 mmol) was dissolved in diisopropyl ether (80 mL). Lipolase (4 g, 50 mg mL⁻¹) and water (0.32 mL, 17.8 mmol) were added and the mixture was shaken in an incubator shaker at 60 °C for 249 h. The reaction was stopped by filtering off the enzyme at

(4) Péter, M.; Péter, A.; Fülöp, F. *J. Chromatogr. A* **2000**, 871, 115-126.

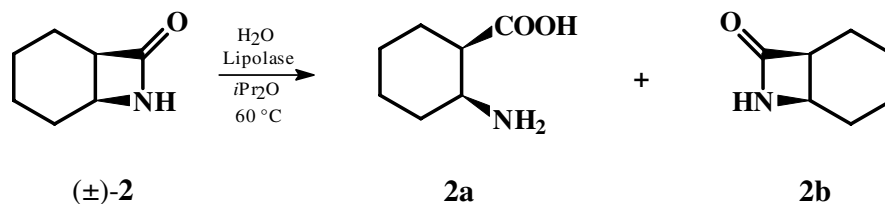
50% conversion (ee-**1b** = 93%). The solvent was evaporated off and the residue (1*S*,5*R*)-**1b** crystallized [1.68 g, 42%; recrystallized from diisopropyl ether [$[\alpha]_{\text{D}}^{25} = +35.9$ ($c = 0.5$; CHCl_3); mp 51-52 °C ee 99%]. The filtered enzyme was washed with distilled water (3x20 mL), and the water was evaporated off, yielding the crystalline α -amino acid (1*R*,2*S*)-**1a** [1.76 g, 44%; $[\alpha]_{\text{D}}^{25} = -9.1$ ($c = 0.5$; H_2O); mp 206-211 °C with sublimation (recrystallized from water:acetone), ee = 99%]. When **1a** (0.2 g) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*S*)-**1a HCl** was obtained [0.2 g, 77%; $[\alpha]_{\text{D}}^{25} = -5.1$ ($c = 0.5$; H_2O); mp 164-166 °C (recrystallized from EtOH), ee = 99%] was formed.

^1H NMR (400 MHz, D_2O) (ppm) for **1a**: 1.74-2.10 (6H, m, 3x CH_2) 2.86-2.88 (1H, m, H-1) 3.71-3.72 (1H, m, H-2). Analysis: calculated for $\text{C}_6\text{H}_{11}\text{NO}_2$: C, 55.80; H, 8.58; N, 10.84; found: C, 56.02; H, 8.58; N, 10.87.

^1H NMR (400 MHz, D_2O) (ppm) for **1a HCl**: 1.70-2.09 (6H, m, 3x CH_2) 3.04-3.06 (1H, m, H-1) 3.76-3.77 (1H, m, H-2). Analysis: calculated for $\text{C}_6\text{H}_{11}\text{NO}_2 \text{ HCl}$: C, 43.51; H, 7.30; N, 31.41; found: C, 43.28; H, 7.11; N, 31.48.

^1H NMR (400 MHz, CDCl_3) (ppm) for **1b**: 1.32-2.06 (6H, m, 3x CH_2), 3.49-3.51 (1H, m, H-1), 4.04-4.06 (1H, m, H-2), 5.82 (1H, bs, NH). Analysis: calculated for $\text{C}_6\text{H}_9\text{NO}$: C, 64.84; H, 8.16; N, 12.60; found: C, 64.57; H, 8.11; N, 12.66.

Gram-scale resolution of 7-azabicyclo[4.2.0]octan-8-one, (\pm)-**2**



Following the procedure described above, the reaction of racemic **2** (1 g, 7.98 mmol) and water (70 μL , 3.88 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg mL^{-1}) at 60 °C afforded the unreacted (1*S*,6*R*)-**2b** (0.45 g, 45%; $[\alpha]_{\text{D}}^{25} = -3.6$ ($c = 0.5$; CHCl_3); mp 73-74 °C (recrystallized from diisopropyl ether); ee = 98%] and α -amino acid (1*R*,2*S*)-**2a** [0.51 g, 45%; $[\alpha]_{\text{D}}^{25} = -19.4$ ($c = 0.5$; H_2O); lit⁵ $[\alpha]_{\text{D}}^{25} = -19.6$ ($c = 0.25$; H_2O); mp 266-267 °C with sublimation (recrystallized from water:acetone); ee = 98%] in 141 h. When

(5) Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593-1600.

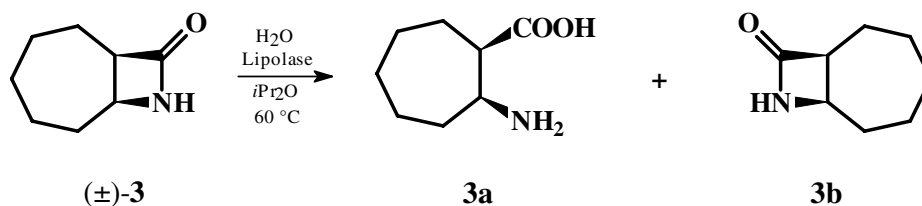
2a (0.2 g) was treated with 22% HCl/EtOH (3 mL), α -amino acid (1*R*,2*S*)-**2a** HCl [0.21 g, 83%; [α]_D²⁵ = -8.4 (*c* = 0.5; H₂O); mp 234-236 °C (recrystallized from EtOH:diethyl ether), ee = 99%] was formed.

¹H NMR (400 MHz, D₂O) (ppm) for **2a**: 1.38-1.97 (8H, m, 4xCH₂) 2.63-2.66 (1H, m, H-1) 3.45-3.49 (1H, m, H-2). Analysis: calculated for C₇H₁₃NO₂: C, 58.72; H, 9.15; N, 9.78; found: C, 58.56; H, 9.23; N, 9.77.

¹H NMR (400 MHz, D₂O) (ppm) for **2a** HCl: 1.42-2.04 (8H, m, 4xCH₂) 2.97-3.01 (1H, m, H-1) 3.56-3.57 (1H, m, H-2). Analysis: calculated for C₇H₁₃NO₂ HCl: C, 46.80; H, 7.86; N, 7.80; found: C, 46.51; H, 7.66; N, 7.89.

¹H NMR (400 MHz, CDCl₃) (ppm) data for **2b** 1.46-1.86 (8H, m, 4xCH₂), 3.22-3.23 (1H, m, H-1), 3.82-3.85 (1H, m, H-2), 5.79 (1H, bs, NH). Analysis: calculated for C₇H₁₁NO: C, 67.17; H, 8.86; N, 11.19; found: C, 67.25; H, 8.85; N, 11.10.

Gram-scale resolution of 8-azabicyclo[5.2.0]nonan-9-one, (±)-**3**



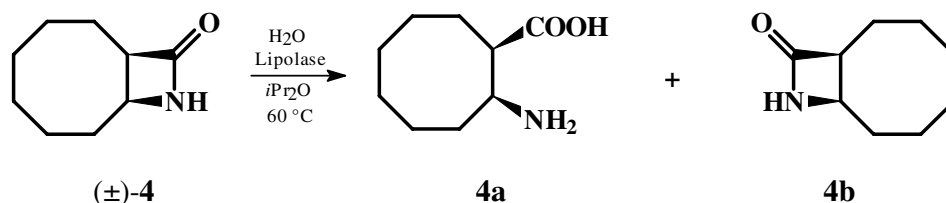
Following the procedure described above, the reaction of racemic **3** (1 g, 7.18 mmol) and water (64 μ L, 3.55 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg mL⁻¹) at 60 °C afforded the unreacted (1*S*,7*R*)-**3b** (0.41 g, 41%; [α]_D²⁵ = -5.1 (*c* = 0.5; CHCl₃); mp 95-96 °C (recrystallized from diisopropyl ether); ee = 99%) and α -amino acid (1*R*,2*S*)-**3a** [0.52 g, 47%; [α]_D²⁵ = -7.2 (*c* = 0.5; H₂O); mp 264-267 °C with sublimation (recrystallized from water:acetone); ee = 98%] in 31 h. When **3a** (0.2 g) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*S*)-**3a** HCl [0.19 g, 77%; [α]_D²⁵ = -6.1 (*c* = 0.5; H₂O); mp 209-210 °C (recrystallized from EtOH:diethyl ether), ee = 99%] was formed.

¹H NMR (400 MHz, D₂O) (ppm) for **3a**: 1.51-1.97 (10H, m, 5xCH₂) 2.76-2.78 (1H, m, H-1) 3.57-3.60 (1H, m, H-2). Analysis: calculated for C₈H₁₅NO₂: C, 61.12; H, 9.62; N, 8.91; found: C, 61.11; H, 9.52; N, 9.13.

^1H NMR (400 MHz, D_2O) (ppm) for **3a HCl**: 1.49-2.02 (10H, m, $5\times\text{CH}_2$) 3.08-3.10 (1H, m, H-1) 3.64-3.66 (1H, m, H-2). Analysis: calculated for $\text{C}_8\text{H}_{15}\text{NO}_2 \text{HCl}$: C, 49.61; H, 8.33; N, 7.23; found: C, 49.32; H, 8.00; N, 7.17.

^1H NMR (400 MHz, CDCl_3) (ppm) data for **3b** 1.21-1.93 (10H, m, $5\times\text{CH}_2$), 3.29-3.36 (1H, m, H-1), 3.79-3.86 (1H, m, H-2), 5.79 (1H, bs, NH). Analysis: calculated for $\text{C}_8\text{H}_{13}\text{NO}$: C, 69.03; H, 9.41; N, 10.06; found: C, 68.88; H, 9.44; N, 10.23.

Gram-scale resolution of 9-azabicyclo[6.2.0]decan-10-one, (\pm)-**4**



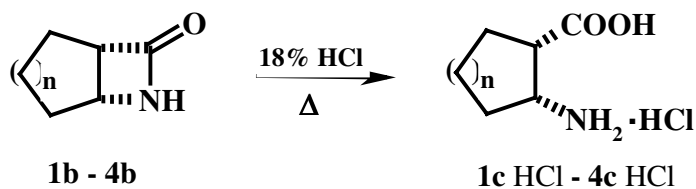
Following the procedure described above, the reaction of racemic **4** (1 g, 6.52 mmol) and water (58 μL , 3.22 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg mL^{-1}) at 60 $^\circ\text{C}$ afforded the unreacted (1*S*,8*R*)-**4b** (0.36 g, 36%; $[\alpha]_{\text{D}}^{25} = -18$ ($c = 0.5$; CHCl_3); mp 108-112 $^\circ\text{C}$ (recrystallized from diisopropyl ether); ee = 99%] and α -amino acid (1*R*,2*S*)-**4a** [0.48 g, 43%; $[\alpha]_{\text{D}}^{25} = +17.8$ ($c = 0.4$; H_2O); mp 245-248 $^\circ\text{C}$ with sublimation (recrystallized from water:acetone); ee = 95%] in 170 h. When **4a** (0.2 g) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*S*)-**4a HCl** [0.21 g, 86%; $[\alpha]_{\text{D}}^{25} = +12.2$ ($c = 0.5$; H_2O); mp 205-206 $^\circ\text{C}$ (recrystallized from EtOH :diethyl ether), ee = 99%] was formed.

^1H NMR (400 MHz, D_2O) (ppm) for **4a**: 1.51-1.94 (12H, m, $6\times\text{CH}_2$) 2.78-2.80 (1H, m, H-1) 3.60-3.63 (1H, m, H-2). Analysis: calculated for $\text{C}_9\text{H}_{17}\text{NO}_2$: C, 63.13; H, 10.01; N, 8.18; found: C, 63.01; H, 10.22; N, 8.09.

^1H NMR (400 MHz, D_2O) (ppm) for **4a HCl**: 1.46-1.86 (12H, m, $6\times\text{CH}_2$) 3.00-3.02 (1H, m, H-1) 3.71-3.73 (1H, m, H-2). Analysis: calculated for $\text{C}_9\text{H}_{17}\text{NO}_2 \text{HCl}$: C, 52.05; H, 8.87; N, 6.74; found: C, 51.89; H, 8.82; N, 6.67.

^1H NMR (400 MHz, CDCl_3) (ppm) data for **4b** 1.29-1.95 (12H, m, $4\times\text{CH}_2$), 3.02-3.06 (1H, m, H-1), 3.63-3.68 (1H, m, H-2), 5.72 (1H, bs, NH). Analysis: calculated for $\text{C}_9\text{H}_{15}\text{NO}$: C, 70.55; H, 9.87; N, 9.14; found: C, 70.58; H, 9.80; N, 9.09.

Ring opening of α -lactam enantiomers **1b** - **4b** with aqueous HCl



(1*S*,5*R*)-**1b** (0.2 g, 1.79 mmol), (1*S*,6*R*)-**2b** (0.2 g, 1.59 mmol), (1*S*,7*R*)-**3b** (0.2 g, 1.43 mmol) and (1*S*,8*R*)-**4b** (0.2 g, 1.3 mmol) were dissolved separately in 18% HCl (7 mL) and refluxed for 3 h. The solvent was evaporated off, and the product was recrystallized from water:diethyl ether, which afforded white crystals of (1*S*,2*R*)-**1c HCl** [0.25 g, 84%, $[\alpha]_{\text{D}}^{25} = +5.2$ ($c = 0.5$; H₂O); mp 166-169 °C; ee 99%], (1*S*,2*R*)-**2c HCl** [0.23 g, 81%; $[\alpha]_{\text{D}}^{25} = +8.8$ ($c = 0.3$; H₂O); mp 232-236 °C; ee 99%], (1*S*,2*R*)-**3c HCl** [0.23 g, 85%; $[\alpha]_{\text{D}}^{25} = +6.3$ ($c = 0.3$; H₂O); mp 203-206 °C; ee 99%] and (1*S*,2*R*)-**4c HCl** [0.24 g, 89%; $[\alpha]_{\text{D}}^{25} = -12.9$ ($c = 0.3$; H₂O); mp 204-206 °C; ee 99%]. The ¹H NMR (400 MHz, D₂O) (ppm) data for (1*S*,2*R*)-**1c HCl**, (1*S*,2*R*)-**2c HCl**, (1*S*,2*R*)-**3c HCl** and (1*S*,2*R*)-**4c HCl** are similar to those for (1*R*,2*S*)-**1a HCl**, (1*R*,2*S*)-**2a HCl**, (1*R*,2*S*)-**3a HCl** and (1*R*,2*S*)-**4a HCl**. Anal. found for (1*S*,2*R*)-**1c HCl**: C, 43.29; H, 7.26; N, 31.23. Anal. found for (1*S*,2*R*)-**2c HCl**: C, 46.81; H, 7.66; N, 7.80. Anal. found for (1*S*,2*R*)-**3c HCl**: C, 49.55; H, 8.30; N, 7.19. Anal. found for (1*S*,2*R*)-**4c HCl**: C, 51.78; H, 8.83; N, 6.72.

Physical data on α-amino acid hydrochloride enantiomers prepared

Amino acid hydrochloride	ee (%)	$[\alpha]_{\text{D}}^{25}$
1 <i>R</i> ,2 <i>S</i> - 1a HCl	99	-5.1 ($c = 0.5$; H ₂ O) ^a
1 <i>S</i> ,2 <i>R</i> - 1c HCl	99	+5.2 ($c = 0.5$; H ₂ O)
1 <i>R</i> ,2 <i>S</i> - 2a HCl	99	-8.4 ($c = 0.4$; H ₂ O)
1 <i>S</i> ,2 <i>R</i> - 2c HCl	99	+8.8 ($c = 0.3$; H ₂ O)
1 <i>R</i> ,2 <i>S</i> - 3a HCl	98	-6.1 ($c = 0.3$; H ₂ O)
1 <i>S</i> ,2 <i>R</i> - 3c HCl	99	+6.3 ($c = 0.3$; H ₂ O)
1 <i>R</i> ,2 <i>S</i> - 4a HCl	96	+12.2 ($c = 0.5$; H ₂ O)
1 <i>S</i> ,2 <i>R</i> - 4c HCl	99	-12.9 ($c = 0.3$; H ₂ O)

$$^{a}[\alpha]_D^{25} = -4.5 \text{ (c = 0.5, H}_2\text{O).}^{6}$$

(6) Kanerva, L.T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, 7, 1705-1716.