

# Lipase-Catalyzed Enantioselective Ring Opening of Unactivated Alicyclic-Fused $\gamma$ -Lactams in an Organic Solvent

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## 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  $\gamma$ -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,<sup>1</sup> while 9-azabicyclo[6.2.0]decan-10-one was obtained by means of CSI cycloaddition to cyclooctadiene<sup>2</sup> and subsequent catalytic reduction in the presence of cyclohexene as a hydrogen donor.<sup>3</sup> 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<sup>-1</sup>). 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  $\alpha$ -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  $\alpha$ -amino acid enantiomers were determined by using high-performance liquid chromatography equipped with a reversed-phase (C<sub>18</sub>) column after derivatization of the sample with (1S,2S)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl-isothiocyanate (DANI).<sup>4</sup>

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

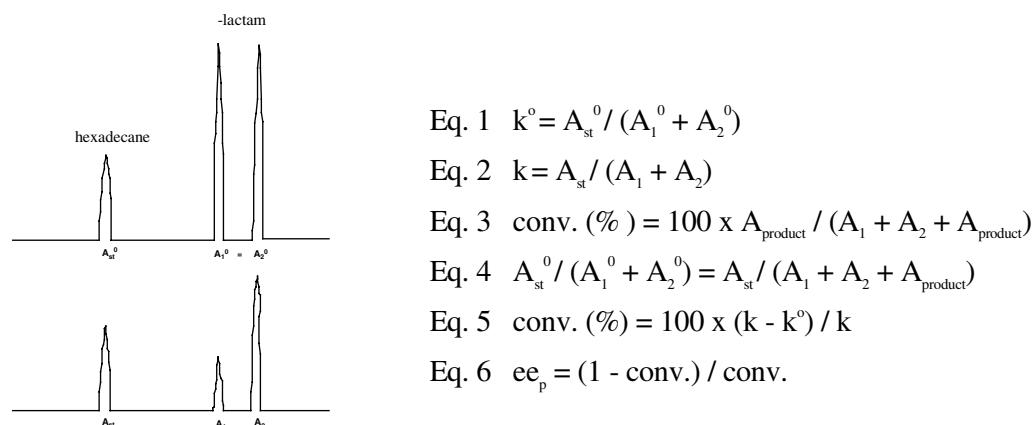
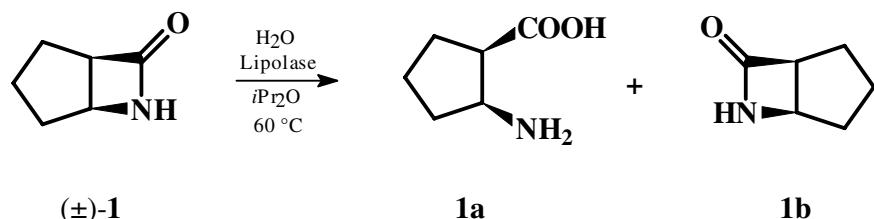


Fig. 1. Calculation of the ee values, with hexadecane as an internal standard

### 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<sup>-1</sup>) 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.

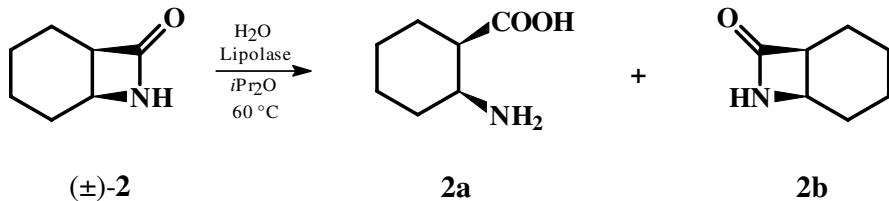
50% conversion (ee-**1b** = 93%). The solvent was evaporated off and the residue (1*S*,5*R*)-**1b** crystallized [1.68 g, 42%; recrystallized from diisopropyl ether [ $[\alpha]_D^{25} = +35.9$  ( $c = 0.5$ ;  $\text{CHCl}_3$ ); mp 51-52  $^\circ\text{C}$  ee 99%]. The filtered enzyme was washed with distilled water (3x20 mL), and the water was evaporated off, yielding the crystalline -amino acid (1*R*,2*S*)-**1a** [1.76 g, 44%;  $[\alpha]_D^{25} = -9.1$  ( $c = 0.5$ ;  $\text{H}_2\text{O}$ ); mp 206-211  $^\circ\text{C}$  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%;  $[\alpha]_D^{25} = -5.1$  ( $c = 0.5$ ;  $\text{H}_2\text{O}$ ); mp 164-166  $^\circ\text{C}$  (recrystallized from EtOH), ee = 99%] was formed.

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ) (ppm) for **1a**: 1.74-2.10 (6H, m, 3x $\text{CH}_2$ ) 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.

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ) (ppm) for **1a HCl**: 1.70-2.09 (6H, m, 3x $\text{CH}_2$ ) 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.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) (ppm) for **1b**: 1.32-2.06 (6H, m, 3x $\text{CH}_2$ ), 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  $\mu\text{L}$ , 3.88 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg  $\text{mL}^{-1}$ ) at 60  $^\circ\text{C}$  afforded the unreacted (1*S*,6*R*)-**2b** (0.45 g, 45%;  $[\alpha]_D^{25} = -3.6$  ( $c = 0.5$ ;  $\text{CHCl}_3$ ); mp 73-74  $^\circ\text{C}$  (recrystallized from diisopropyl ether); ee = 98%) and -amino acid (1*R*,2*S*)-**2a** [0.51 g, 45%;  $[\alpha]_D^{25} = -19.4$  ( $c = 0.5$ ;  $\text{H}_2\text{O}$ ); lit<sup>5</sup>  $[\alpha]_D^{25} = -19.6$  ( $c = 0.25$ ;  $\text{H}_2\text{O}$ ); mp 266-267  $^\circ\text{C}$  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.

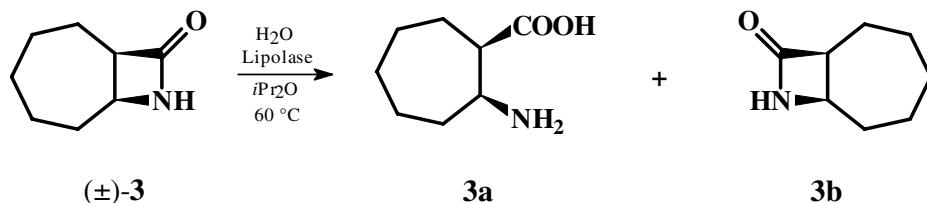
**2a** (0.2 g) was treated with 22% HCl/EtOH (3 mL), -amino acid (*1R,2S*)-**2a HCl** [0.21 g, 83%;  $[\alpha]_D^{25} = -8.4$  ( $c = 0.5$ ; H<sub>2</sub>O); mp 234-236 °C (recrystallized from EtOH:diethyl ether), ee = 99%] was formed.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **2a**: 1.38-1.97 (8H, m, 4xCH<sub>2</sub>) 2.63-2.66 (1H, m, H-1) 3.45-3.49 (1H, m, H-2). Analysis: calculated for C<sub>7</sub>H<sub>13</sub>NO<sub>2</sub>: C, 58.72; H, 9.15; N, 9.78; found: C, 58.56; H, 9.23; N, 9.77.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **2a HCl**: 1.42-2.04 (8H, m, 4xCH<sub>2</sub>) 2.97-3.01 (1H, m, H-1) 3.56-3.57 (1H, m, H-2). Analysis: calculated for C<sub>7</sub>H<sub>13</sub>NO<sub>2</sub> HCl: C, 46.80; H, 7.86; N, 7.80; found: C, 46.51; H, 7.66; N, 7.89.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) (ppm) data for **2b** 1.46-1.86 (8H, m, 4xCH<sub>2</sub>), 3.22-3.23 (1H, m, H-1), 3.82-3.85 (1H, m, H-2), 5.79 (1H, bs, NH). Analysis: calculated for C<sub>7</sub>H<sub>11</sub>NO: 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, ( $\pm$ )-**3**



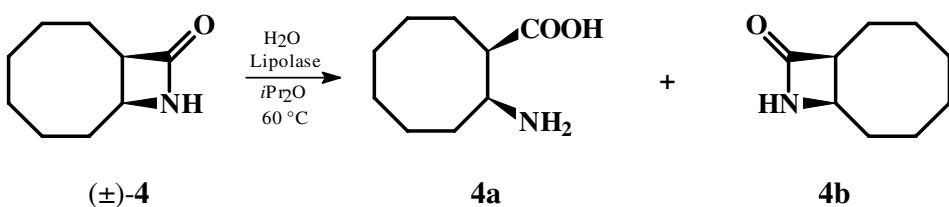
Following the procedure described above, the reaction of racemic **3** (1 g, 7.18 mmol) and water (64  $\mu$ L, 3.55 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg mL<sup>-1</sup>) at 60 °C afforded the unreacted (*1S,7R*)-**3b** (0.41 g, 41%;  $[\alpha]_D^{25} = -5.1$  ( $c = 0.5$ ; CHCl<sub>3</sub>); mp 95-96 °C (recrystallized from diisopropyl ether); ee = 99%) and -amino acid (*1R,2S*)-**3a** [0.52 g, 47%;  $[\alpha]_D^{25} = -7.2$  ( $c = 0.5$ ; H<sub>2</sub>O); 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), (*1R,2S*)-**3a HCl** [0.19 g, 77%;  $[\alpha]_D^{25} = -6.1$  ( $c = 0.5$ ; H<sub>2</sub>O); mp 209-210 °C (recrystallized from EtOH:diethyl ether), ee = 99%] was formed.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **3a**: 1.51-1.97 (10H, m, 5xCH<sub>2</sub>) 2.76-2.78 (1H, m, H-1) 3.57-3.60 (1H, m, H-2). Analysis: calculated for C<sub>8</sub>H<sub>15</sub>NO<sub>2</sub>: C, 61.12; H, 9.62; N, 8.91; found: C, 61.11; H, 9.52; N, 9.13.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **3a** HCl: 1.49-2.02 (10H, m, 5xCH<sub>2</sub>) 3.08-3.10 (1H, m, H-1) 3.64-3.66 (1H, m, H-2). Analysis: calculated for C<sub>8</sub>H<sub>15</sub>NO<sub>2</sub> HCl: C, 49.61; H, 8.33; N, 7.23; found: C, 49.32; H, 8.00; N, 7.17.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) (ppm) data for **3b** 1.21-1.93 (10H, m, 5xCH<sub>2</sub>), 3.29-3.36 (1H, m, H-1), 3.79-3.86 (1H, m, H-2), 5.79 (1H, bs, NH). Analysis: calculated for C<sub>8</sub>H<sub>13</sub>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



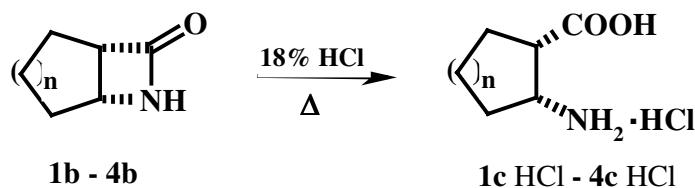
Following the procedure described above, the reaction of racemic **4** (1 g, 6.52 mmol) and water (58  $\mu$ L, 3.22 mmol) in diisopropyl ether (40 mL) in the presence of Lipolase (2 g, 50 mg  $\text{mL}^{-1}$ ) at 60  $^{\circ}\text{C}$  afforded the unreacted (1*S*,8*R*)-**4b** (0.36 g, 36%;  $[\alpha]_{\text{D}}^{25} = -18$  ( $c = 0.5$ ;  $\text{CHCl}_3$ ); mp 108-112  $^{\circ}\text{C}$  (recrystallized from diisopropyl ether); ee = 99%) and -amino acid (1*R*,2*S*)-**4a** [0.48 g, 43%;  $[\alpha]_{\text{D}}^{25} = +17.8$  ( $c = 0.4$ ;  $\text{H}_2\text{O}$ ); 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%;  $[\alpha]_{\text{D}}^{25} = +12.2$  ( $c = 0.5$ ;  $\text{H}_2\text{O}$ ); mp 205-206  $^{\circ}\text{C}$  (recrystallized from EtOH:diethyl ether), ee = 99%] was formed.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **4a**: 1.51-1.94 (12H, m, 6xCH<sub>2</sub>) 2.78-2.80 (1H, m, H-1) 3.60-3.63 (1H, m, H-2). Analysis: calculated for C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub>: C, 63.13; H, 10.01; N, 8.18; found: C, 63.01; H, 10.22; N, 8.09.

<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) for **4a** HCl: 1.46-1.86 (12H, m, 6xCH<sub>2</sub>) 3.00-3.02 (1H, m, H-1) 3.71-3.73 (1H, m, H-2). Analysis: calculated for C<sub>9</sub>H<sub>17</sub>NO<sub>2</sub> HCl: C, 52.05; H, 8.87; N, 6.74; found: C, 51.89; H, 8.82; N, 6.67.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) (ppm) data for **4b** 1.29-1.95 (12H, m, 4xCH<sub>2</sub>), 3.02-3.06 (1H, m, H-1), 3.63-3.68 (1H, m, H-2), 5.72 (1H, bs, NH). Analysis: calculated for C<sub>9</sub>H<sub>15</sub>NO: C, 70.55; H, 9.87; N, 9.14; found: C, 70.58; H, 9.80; N, 9.09.

### Ring opening of $\beta$ -lactam enantiomers 1b - 4b with aqueous HCl



*(1S,5R)-1b* (0.2 g, 1.79 mmol), *(1S,6R)-2b* (0.2 g, 1.59 mmol), *(1S,7R)-3b* (0.2 g, 1.43 mmol) and *(1S,8R)-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 *(1S,2R)-1c HCl* [0.25 g, 84%,  $[\alpha]_D^{25} = +5.2$  ( $c = 0.5$ ; H<sub>2</sub>O); mp 166-169 °C; ee 99%], *(1S,2R)-2c HCl* [0.23 g, 81%;  $[\alpha]_D^{25} = +8.8$  ( $c = 0.3$ ; H<sub>2</sub>O); mp 232-236 °C; ee 99%], *(1S,2R)-3c HCl* [0.23 g, 85%;  $[\alpha]_D^{25} = +6.3$  ( $c = 0.3$ ; H<sub>2</sub>O); mp 203-206 °C; ee 99%] and *(1S,2R)-4c HCl* [0.24 g, 89%;  $[\alpha]_D^{25} = -12.9$  ( $c = 0.3$ ; H<sub>2</sub>O); mp 204-206 °C; ee 99%]. The <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) (ppm) data for *(1S,2R)-1c HCl*, *(1S,2R)-2c HCl*, *(1S,2R)-3c HCl* and *(1S,2R)-4c HCl* are similar to those for *(1R,2S)-1a HCl*, *(1R,2S)-2a HCl*, *(1R,2S)-3a HCl* and *(1R,2S)-4a HCl*. Anal. found for *(1S,2R)-1c HCl*: C, 43.29; H, 7.26; N, 31.23. Anal. found for *(1S,2R)-2c HCl*: C, 46.81; H, 7.66; N, 7.80. Anal. found for *(1S,2R)-3c HCl*: C, 49.55; H, 8.30; N, 7.19. Anal. found for *(1S,2R)-4c HCl*: C, 51.78; H, 8.83; N, 6.72.

#### Physical data on -amino acid hydrochloride enantiomers prepared

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

<sup>a</sup>[ ]<sub>D</sub><sup>25</sup> = -4.5 (c = 0.5, H<sub>2</sub>O).<sup>6</sup>

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