

Preparation of (S)-N-substituted 4-hydroxy-pyrrolidin-2-ones by regio- and stereoselective hydroxylation with *Sphingomonas* sp. HXN-200

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1) Preparation of (S)-N-*tert*-butoxycarbonyl-4-hydroxy-pyrrolidin-2-one **9** by hydroxylation of N-*tert*-butoxycarbonyl-pyrrolidin-2-one **8** with resting cells of *Sphingomonas* sp. HXN-200

N-*tert*-butoxycarbonyl-2-pyrrolidinone **8** (129.5mg, 0.70mmol) was added to 50 ml of cell suspension (8.0 g/L) of *Sphingomonas* sp. HXN-200 in 50 mM K-phosphate buffer (pH=8.0) containing glucose (2%) in a 500 ml shaking flask. The mixture was shaken at 200 rpm and 30°C and the bioconversion was followed by HPLC analysis of samples that were taken out at different times (6 x 0.10 ml). The reaction was stopped at 5 h with 66% conversion to **9**. The cells were removed by centrifugation and the product was extracted into ethyl acetate. The organic phase was dried over Na₂SO₄, filtered, and evaporated. Purification by column chromatography on silica gel (R_f = 0.28, ethyl acetate) afforded 46% (63.3 mg) of the pure product. e.e. of **9**: 92% (S).

2) Increase of e.e. of (S)-**9** by crystallization

The biohydroxylation product (S)-**9** (92% e.e., 26.3mg) was dissolved in 1.5ml of *n*-hexane/ethyl acetate (2/1) and crystallized at 0°C for 24 h. 21.6mg (82%) of the crystals of (S)-**9** were obtained in 99.9% e.e.

3) Data of chemically synthesized compound **7**, **9**, **11**, and **12**

General information: ¹H- and ¹³C-NMR spectra were recorded at 400 MHz (¹H-NMR) and 100 MHz (¹³C-NMR) on a Varian 400 instrument at 20°C in CDCl₃. MS analyses were carried out on HPLC-MS Hewlett Packard 1100. IR spectra were recorded at PERKIN-ELMER 1600 Series FTIR.

Data of chemically synthesized (S)-N-benzyl-4-hydroxy-pyrrolidin-2-one **7**:

Supporting information

¹H-NMR (400MHz, CDCl₃): δ 7.35-7.20(*m*, 5 H, aromatic H), 4.51-4.45(*m*, 3 H, NCH₂Ph, H-C(4)), 3.49(*dd*, 1 H, *J* = 10.8Hz, 5.6Hz, H_A-C(5)), 3.19(*dd*, 1 H, *J* = 10.8Hz, 1.6Hz, H_B-C(5)), 2.74(*dd*, 1 H, *J* = 17.6Hz, 6.8Hz, H_A-C(3)), 2.43ppm(*dd*, 1 H, *J* = 17.6Hz, 2.4Hz, H_B-C(3)).

¹³C-NMR (100MHz, CDCl₃): δ 174.08(*s*, CO); 137.09(*s*), 129.84(*d*), 129.13(*d*), 128.75(*d*) (aromatic C); 65.59(*d*, C-4); 56.67(*t*, CH₂Ph); 47.38(*t*, C-5); 42.27ppm(*t*, C-3).
MS: *m/z* 192.1(*M*+1, 100), 174.1(9).

IR (cm⁻¹): 3400, 3007, 2928, 1681, 1483, 1435, 1262, 1082.

Data of chemically synthesized (*R*)-*N*-benzyl-4-hydroxy-pyrrolidin-2-one **7**:

¹H-NMR (400MHz, CDCl₃): δ 7.35-7.22(*m*, 5 H, aromatic H), 4.52-4.42(*m*, 3 H, NCH₂Ph, H-C(4)), 3.49(*dd*, 1 H, *J* = 11.2Hz, 6.0Hz, H_A-C(5)), 3.19(*dd*, 1 H, *J* = 11.2Hz, 1.2Hz, H_B-C(5)), 2.73(*dd*, 1 H, *J* = 17.6Hz, 6.8Hz, H_A-C(3)), 2.45ppm(*dd*, 1 H, *J* = 17.6Hz, 2.4Hz, H_B-C(3)).

¹³C-NMR (100MHz, CDCl₃): δ 174.04(*s*, CO); 137.05(*s*), 129.86(*d*), 129.13(*d*), 128.76(*d*) (aromatic C); 65.50(*d*, C-4); 56.78(*t*, CH₂Ph); 47.43(*t*, C-5); 42.29ppm(*t*, C-3).
MS: *m/z* 192.1(*M*+1, 100), 174.1(9).

IR (cm⁻¹): 3400, 3007, 2928, 1681, 1483, 1435, 1262, 1082.

Data of chemically synthesized (*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxy-pyrrolidin-2-one **9**:

¹H-NMR (400MHz, CDCl₃): 4.43(*s*, 1 H, H-C(4)), 3.86(*dd*, 1 H, *J* = 12.0Hz, 6.0Hz, H_A-C(5)), 3.77(*d*, 1 H, *J* = 11.6Hz, H_B-C(5)), 2.76(*dd*, 1 H, *J* = 17.6Hz, 6.0Hz, H_A-C(3)), 2.49(*d*, 1 H, *J* = 17.6Hz, H_B-C(3)), 1.48ppm(*s*, 9 H, 3CH₃).

¹³C-NMR (100MHz, CDCl₃): δ 173.72(*s*, COO); 151.05(*s*, CO); 84.25(*s*, C(CH₃)₃); 64.13(*d*, C-4); 56.30(*t*, C-5); 43.75(*t*, C-3); 29.03ppm(*q*, CH₃).

MS: *m/z* 202 (*M*+1, 2), 146.0 (100), 128.0 (13), 113.0 (12), 102.1 (81).

IR (cm⁻¹): 3399, 2983, 1782, 1747, 1715, 1370, 1308, 1152, 1078, 1022, 848.

Data of chemically synthesized (*R*)-*N*-*tert*-butoxycarbonyl-4-hydroxy-pyrrolidin-2-one **9**:

¹H-NMR (400MHz, CDCl₃): 4.47(*d*, *J* = 3.6Hz, 1 H, H-C(4)), 3.86(*dd*, 1 H, *J* = 11.6Hz, 4.4Hz, H_A-C(5)), 3.75(*d*, 1 H, *J* = 12.0Hz, H_B-C(5)), 2.75(*dd*, 1 H, *J* = 17.6Hz, 6.0Hz, H_A-C(3)), 2.50(*d*, 1 H, *J* = 17.6Hz, H_B-C(3)), 1.50ppm(*s*, 9 H, 3CH₃).

¹³C-NMR (100MHz, CDCl₃): δ 173.59(*s*, COO); 151.06(*s*, CO); 84.26(*s*, C(CH₃)₃); 64.17(*d*, C-4); 56.28(*t*, C-5); 43.76(*t*, C-3); 29.05ppm(*q*, CH₃).

MS: *m/z* 202 (*M*+1, 2), 146.0 (100), 128.0 (13), 113.0 (12), 102.1 (81).

IR (cm⁻¹): 3399, 2983, 1782, 1747, 1715, 1370, 1308, 1152, 1078, 1022, 848.

Data of chemically synthesized (*R*)-*N*-benzyl-4-*tert*-butyldimethylsilyloxy-pyrrolidin-2-one **11**:

¹H-NMR (400MHz, CDCl₃): δ 7.37-7.22(*m*, 5 H, aromatic H), 4.62-4.34(*m*, 3 H, NCH₂Ph, H-C(4)), 3.45(*ddd*, 1 H, *J* = 10.0Hz, 5.6Hz, 2.0Hz, H_A-C(5)), 3.12(*dd*, 1 H, *J* = 10.4Hz, 3.2Hz, H_B-C(5)), 2.66(*ddd*, 1 H, *J* = 16.8Hz, 6.8Hz, 2.0Hz, H_A-C(3)), 2.40(*dd*, 1

Supporting information

H, $J = 16.8\text{Hz}$, 2.8Hz , $\text{H}_\text{B}\text{-C}(3)$); $0.85(\text{s}, 9\text{ H}, \text{C}(\text{CH}_3)_3)$; $0.046(\text{d}, 3\text{ H}, J = 3.2\text{Hz}, \text{SiCH}_3)$; $0.005\text{ppm}(\text{d}, 3\text{ H}, J = 2.4\text{Hz}, \text{SiCH}_3)$.

^{13}C -NMR (100MHz, CDCl_3): δ $173.90(\text{s}, \text{CO})$; $137.23(\text{s})$, $129.73(\text{d})$, $128.93(\text{d})$, $128.58(\text{d})$ (aromatic C); $66.27(\text{d}, \text{C-4})$; $56.96(\text{t}, \text{CH}_2\text{Ph})$; $47.07(\text{t}, \text{C-5})$; $42.56(\text{t}, \text{C-3})$; $26.72(\text{q}, \text{CH}_3\text{-CSi})$; $18.98(\text{s}, \text{SiC})$; $-3.75(\text{q}, \text{SiCH}_3)$; $-3.86\text{ppm}(\text{q}, \text{SiCH}_3)$.

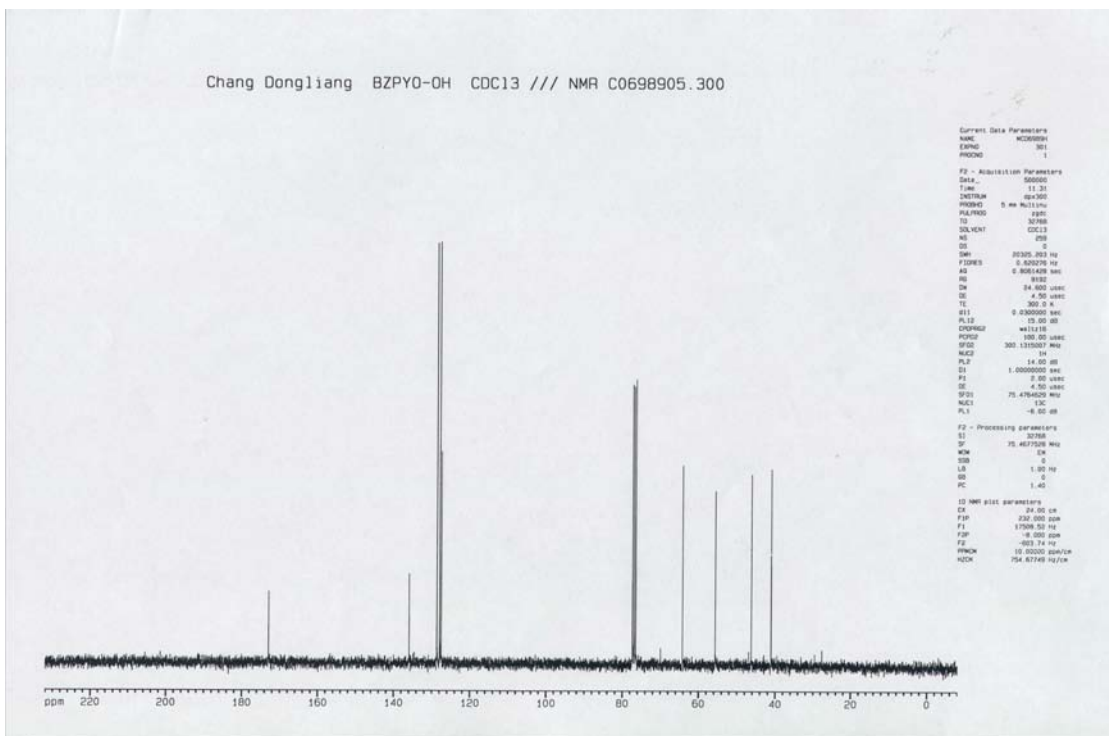
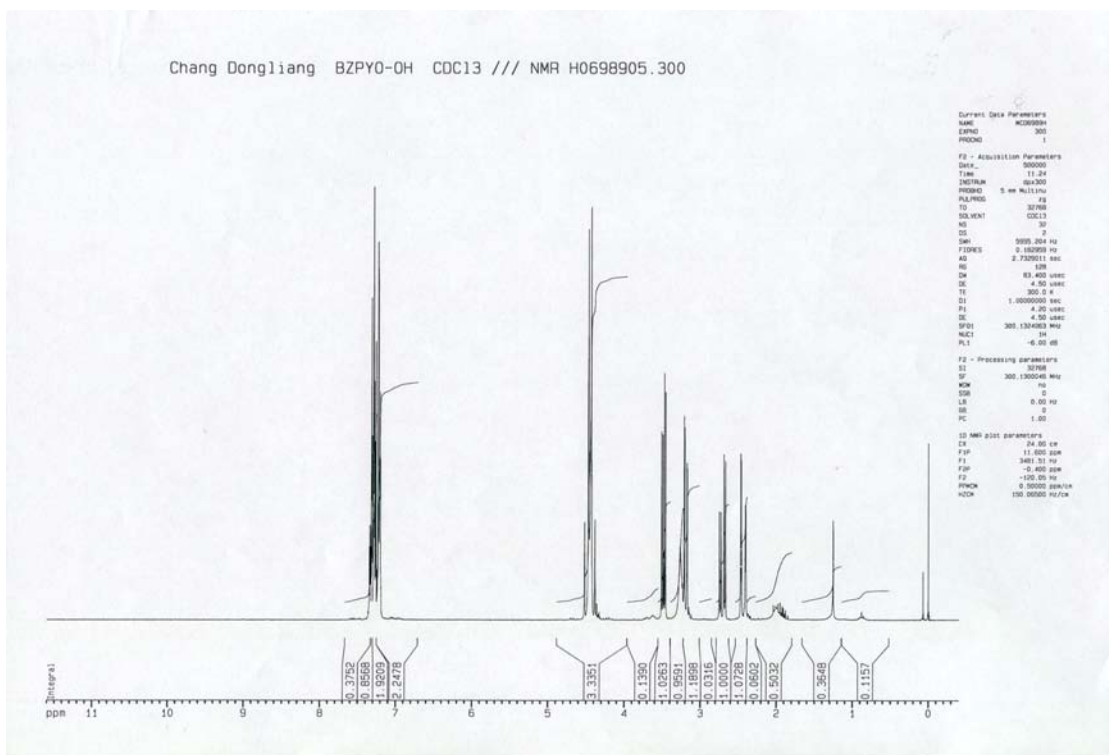
Data of chemically synthesized (*R*)-*N*-*tert*-butoxycarbonyl-4- *tert*-butyldimethylsilyloxy-pyrrolidin-2-one **12**:

^1H -NMR (400MHz, CDCl_3): $4.36(\text{m}, 1\text{ H}, \text{H-C}(4))$, $3.84(\text{dd}, 1\text{ H}, J = 12.0\text{Hz}, 4.8\text{Hz}, \text{H}_\text{A}\text{-C}(5))$, $3.60(\text{dd}, 1\text{ H}, J = 11.6\text{Hz}, 2.8\text{Hz}, \text{H}_\text{B}\text{-C}(5))$, $2.69(\text{dd}, 1\text{ H}, J = 17.6\text{Hz}, 6.0\text{Hz}, \text{H}_\text{A}\text{-C}(3))$, $2.50(\text{dd}, 1\text{ H}, J = 17.6\text{Hz}, 3.2\text{Hz}, \text{H}_\text{B}\text{-C}(3))$, $1.51(\text{s}, 9\text{ H}, 3\text{CH}_3)$; $0.86(\text{s}, 9\text{ H}, \text{C}(\text{CH}_3)_3)$; $0.057(\text{s}, 3\text{ H}, \text{SiCH}_3)$; $0.051\text{ppm}(\text{s}, 3\text{ H}, \text{SiCH}_3)$.

^{13}C -NMR (100MHz, CDCl_3): δ $173.23(\text{s}, \text{COO})$; $151.10(\text{s}, \text{CO})$; $84.05(\text{s}, \text{C}(\text{CH}_3)_3)$; $64.90(\text{d}, \text{C-4})$; $56.50(\text{t}, \text{C-5})$; $44.20(\text{t}, \text{C-3})$; $29.00(\text{q}, \text{CH}_3\text{-CO})$; $26.70(\text{q}, \text{CH}_3\text{-CSi})$; $19.01(\text{s}, \text{SiC})$; $-3.75(\text{q}, \text{SiCH}_3)$; $-3.82\text{ppm}(\text{q}, \text{SiCH}_3)$.

Supporting information

4) ^1H -NMR(300MHz, CDCl_3) and ^{13}C -NMR(75MHz, CDCl_3) spectra of biocatalytically synthesized *N*-benzyl-4-hydroxy-pyrrolidin-2-one **7**:



Supporting information

5) ^1H -NMR(300MHz, CDCl_3) and ^{13}C -NMR(75MHz, CDCl_3) spectra of biocatalytically synthesized *N*-*tert*-butoxycarbonyl-4-hydroxy-pyrrolidin-2-one **9**:

