Supporting information

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 Na_2SO_4, 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: ^{1}H- and ^{13}C-NMR spectra were recorded at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR) on a Varian 400 instrument at 20°C in $CDCl_3$. 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**:

^{1}H-NMR (400MHz, $CDCl_3$): δ 7.35-7.20(*m*, 5 H, aromatic H), 4.51-4.45(*m*, 3 H, NCH_2Ph, 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)).
^{13}C-NMR (100MHz, $CDCl_3$): 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*, CH_2Ph); 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**:

^{1}H-NMR (400MHz, $CDCl_3$): δ 7.35-7.22(*m*, 5 H, aromatic H), 4.52-4.42(*m*, 3 H, NCH_2Ph, 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)).
^{13}C-NMR (100MHz, $CDCl_3$): 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*, CH_2Ph); 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**:

^{1}H-NMR (400MHz, $CDCl_3$): 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, $3CH_3$).
^{13}C-NMR (100MHz, $CDCl_3$): δ 173.72(*s*, COO); 151.05(*s*, CO); 84.25(*s*, $C(CH_3)_3$); 64.13(*d*, C-4); 56.30(*t*, C-5); 43.75(*t*, C-3); 29.03ppm(*q*, CH_3).
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**:

^{1}H-NMR (400MHz, $CDCl_3$): 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, $3CH_3$).
^{13}C-NMR (100MHz, $CDCl_3$): δ 173.59(*s*, COO); 151.06(*s*, CO); 84.26(*s*, $C(CH_3)_3$); 64.17(*d*, C-4); 56.28(*t*, C-5); 43.76(*t*, C-3); 29.05ppm(*q*, CH_3).
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**:

^{1}H-NMR (400MHz, $CDCl_3$): δ 7.37-7.22(*m*, 5 H, aromatic H), 4.62-4.34(*m*, 3 H, NCH_2Ph, 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

H, *J* = 16.8Hz, 2.8Hz, H_B-C(3)); 0.85(*s*, 9 H, $C(CH_3)_3$); 0.046(*d*, 3 H, *J* = 3.2Hz, $SiCH_3$); 0.005ppm(*d*, 3 H, *J* = 2.4Hz, $SiCH_3$).
^{13}C-NMR (100MHz, $CDCl_3$): δ 173.90(*s*, CO); 137.23(*s*), 129.73(*d*), 128.93(*d*), 128.58(*d*) (aromatic C); 66.27(*d*, C-4); 56.96(*t*, CH_2Ph); 47.07(*t*, C-5); 42.56(*t*, C-3); 26.72(*q*, CH_3-CSi); 18.98(*s*, SiC); -3.75(*q*, $SiCH_3$); -3.86ppm(*q*, $SiCH_3$).

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

^{1}H-NMR (400MHz, $CDCl_3$): 4.36(*m*, 1 H, H-C(4)), 3.84(*dd*, 1 H, *J* = 12.0Hz, 4.8Hz, H_A-C(5)), 3.60(*dd*, 1 H, *J* = 11.6Hz, 2.8Hz, H_B-C(5)), 2.69(*dd*, 1 H, *J* = 17.6Hz, 6.0Hz, H_A-C(3)), 2.50(*dd*, 1 H, *J* = 17.6Hz, 3.2Hz, H_B-C(3)), 1.51(*s*, 9 H, $3CH_3$); 0.86(*s*, 9 H, $C(CH_3)_3$); 0.057(*s*, 3 H, $SiCH_3$); 0.051ppm(*s*, 3 H, $SiCH_3$).
^{13}C-NMR (100MHz, $CDCl_3$): δ 173.23(*s*, COO); 151.10(*s*, CO); 84.05(*s*, $C(CH_3)_3$); 64.90(*d*, C-4); 56.50(*t*, C-5); 44.20(*t*, C-3); 29.00(*q*, CH_3-CO); 26.70(*q*, CH_3-CSi); 19.01(*s*, SiC); -3.75(*q*, $SiCH_3$); -3.82ppm(*q*, $SiCH_3$).

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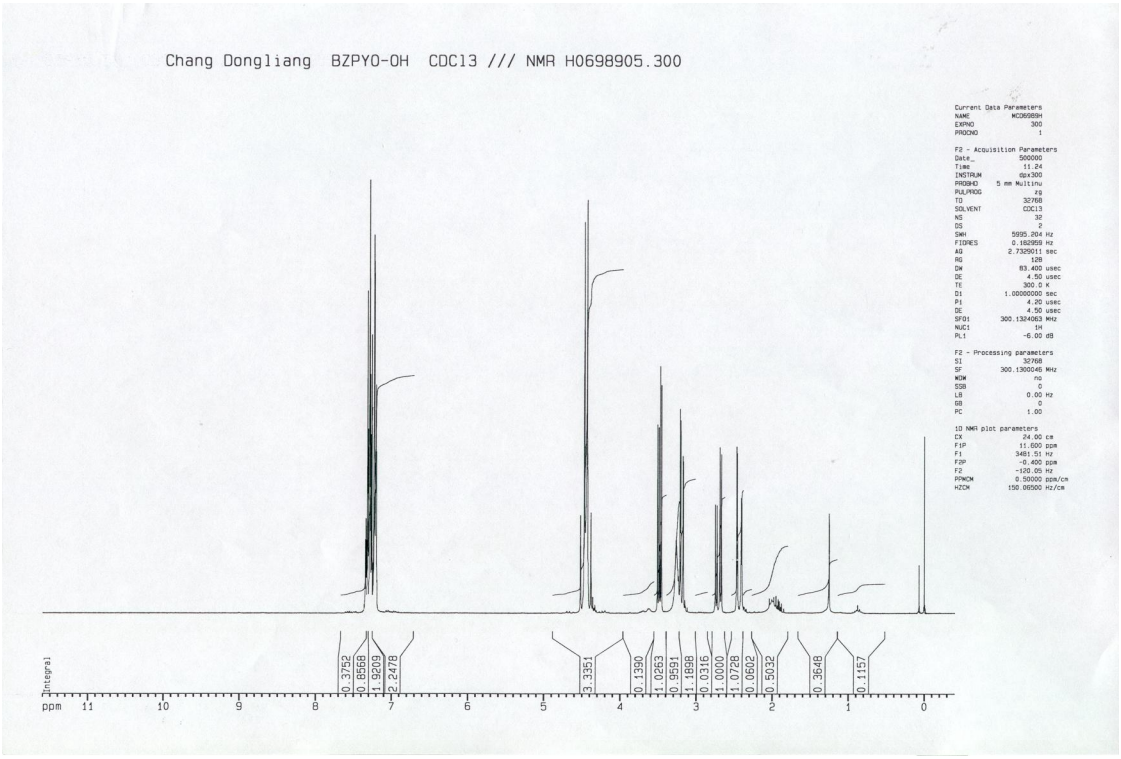
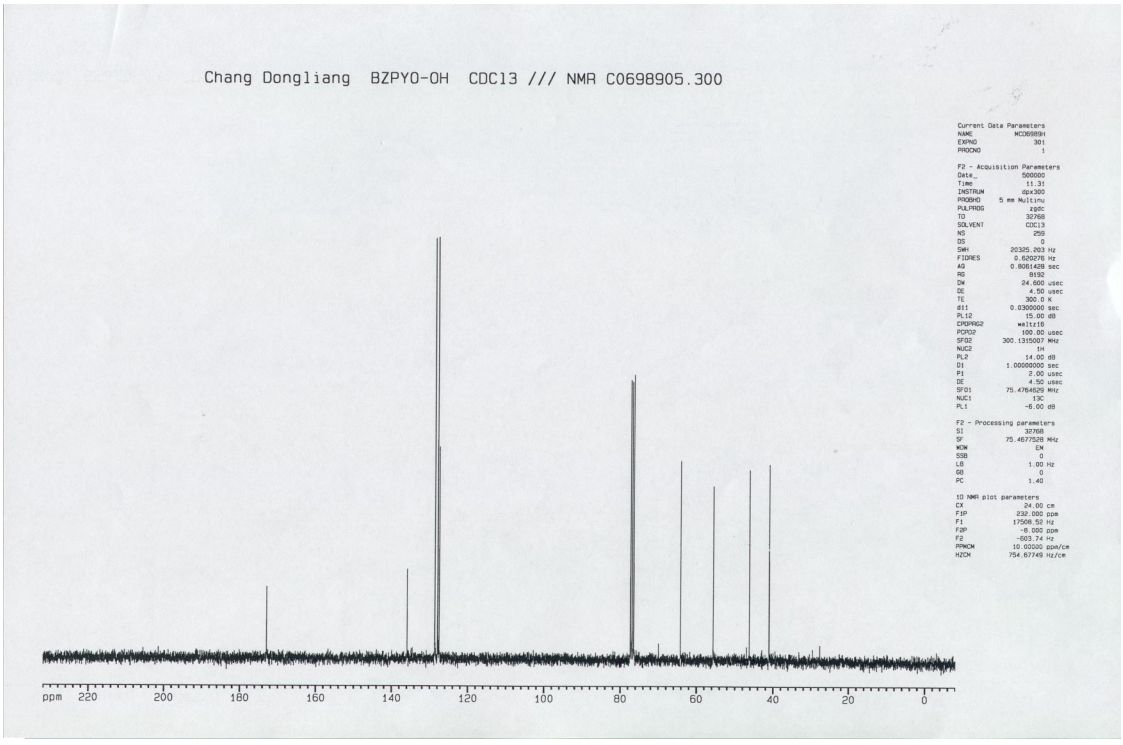
4) ^{1}H-NMR(300MHz, $CDCl_3$) and ^{13}C-NMR(75MHz, $CDCl_3$) spectra of biocatalytically synthesized *N*-benzyl-4-hydroxy-pyrrolidin-2-one **7**:





Supporting information

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