ORGANIC LETTERS

2000
Vol. 2, No. 24
3949−3952

Preparation of (*S*)-*N*-Substituted 4-Hydroxy-pyrrolidin-2-ones by Regio- and Stereoselective Hydroxylation with *Sphingomonas* sp. HXN-200

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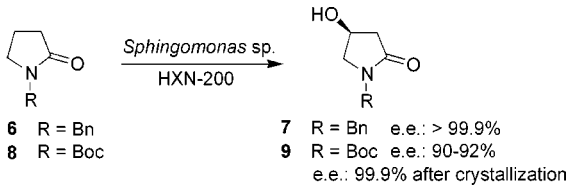
Received October 16, 2000

ABSTRACT



Enantiopure (*S*)-*N*-substituted 4-hydroxy-pyrrolidin-2-ones have been prepared for the first time by regio- and stereoselective hydroxylation of the corresponding pyrrolidin-2-ones by use of a biocatalyst. Hydroxylation of 6 and 8 with *Sphingomonas* sp. HXN-200 afforded 68% of (*S*)-7 in >99.9% ee and 46% of (*S*)-9 in 92% ee, respectively. Simple crystallization increased the ee of (*S*)-9 to 99.9% in 82% yield.

Optically active 4-hydroxy-pyrrolidin-2-one and its *N*-substituted derivatives are useful intermediates for the preparation of several pharmaceuticals. The (*S*)-enantiomers, for example, can be used in the synthesis of an oral carbapenem antibiotic CS-834[1] **1** and nootropic drug (*S*)-Oxiracetame[2] **2**; the (*R*)-enantiomers can be used in the preparation of an antidepressant agent (*R*)-Rolipram[3] **3**, anticonvulsant (*R*)-γ-amino-β-hydroxybutyric acid (GABOB)[4,5] **4**, and antihyperlipoproteinemic L-Carnitine (vitamin B_T)[5] **5**.

Several methods for synthesis of optically active 4-hydroxy-pyrrolidin-2-one and its *N*-substituted derivatives have been developed, but each has one or more drawbacks: (1) Syntheses via direct cyclization[4c,6] or cyclization with ammonia[7] or with alkyl- or aralkylamine[8] need optically active precursors that cannot be prepared easily. (2) Preparation involving reduction of (*S*)-*N*-benzyl-4-hydroxy-pyrrolidin-2,5-dione[9] is multistep and requires special reagents. (3) Synthesis from (2*S*,4*R*)-4-hydroxyproline[10] requires expen-

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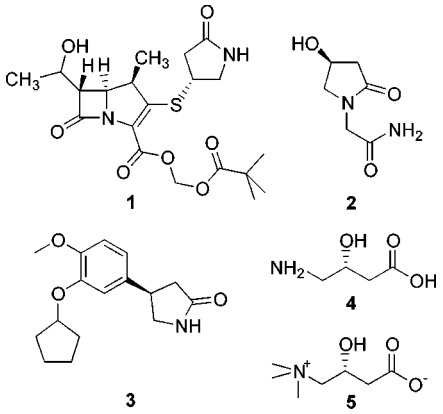
10.1021/ol006735q CCC: $19.00
Published on Web 11/10/2000

sive starting material. (4) Synthesis via photochemical rearrangement of special oxaziridines[5] occurs with low yield. (5) Resolution of racemic 4-hydroxy-pyrrolidin-2-ones with stereoselective esterase[11] is a low-yield process and requires the preparation of the racemates.



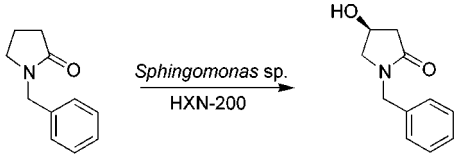
Regio- and stereoselective hydroxylation of pyrrolidin-2-ones is the simplest route for preparing optically active 4-hydroxy-pyrrolidin-2-one and its *N*-substituted derivatives. However, regio- and stereoselective hydroxylation on non-activated carbon atom remains a challenge in synthetic chemistry.[12] On the other hand, biohydroxylation can be a useful tool for this type of transformation.[13,14] However, selective biohydroxylation of pyrrolidin-2-ones has proven to be very difficult. Hydroxylation of *N*-benzoyl- and *N*-phenylacetyl-pyrrolidin-2-one with *Beauveria sulfurescens* (ATCC 7159), a well-known fungus for hydroxylation, gave only 21% of *N*-benzoyl-4-hydroxy-pyrrolidin-2-one and 5% of *N*-phenylacetyl-4-hydroxy-pyrrolidin-2-one, respectively, in very low ee.[6b] Moreover, several byproducts were formed in each case.

In our previous study on biohydroxylation of pyrrolidines,[14a] we found that *Sphingomonas* sp. HXN-200[15] is an excellent biocatalyst for regio- and stereoselective hydroxylation of *N*-substituted pyrrolidines, giving the corresponding optically active 3-hydroxypyrrolidines. Here, we report a simple and practical synthesis of (*S*)-*N*-substituted 4-hydroxy-pyrrolidin-2-ones by hydroxylation of the corresponding pyrrolidin-2-ones with *Sphingomonas* sp. HXN-200 as biocatalyst.

Hydroxylation of **6** and **8** was performed with resting cells of *Sphingomonas* sp. HXN-200 on a 10-mL scale in the exploratory stage.[16] The reaction was followed by analytical HPLC.[17] Hydroxylation of **6** and **8** afforded the desired 4-hydroxy products **7** and **9**, respectively. Comparison of the retention time and the UV absorption area at 210 nm with the standards of **6**−**9** suggested the conversion to the products.

As shown in Table 1, hydroxylation of a 2 mM solution of *N*-benzyl-pyrrolidin-2-one **6** with resting cells (4.0 g/L) of *Sphingomonas* sp. HXN-200 that had been prepared by using octane vapor as sole carbon source[15b] gave 70% of the desired *N*-benzyl-4-hydroxy-pyrrolidin-2-one **7** as main product[18] in the presence of glucose (2%, w/v) for 5 h. The addition of glucose increased the conversion significantly. This is because the biohydroxylation is cofactor-dependent and the addition of glucose contributed to the intracellular regeneration of cofactors. This effect was also observed in

Table 1. Hydroxylation of **6** to **7** with Resting Cells (4.0 g/L) of *Sphingomonas* sp. HXN-200



6 → **7**, e.e. > 99.9% (*S*)

6 (mM)	glucose (%)	activity[a] (U/g CDW)	**7** (%) 0.5 h	1 h	2 h	3 h	5 h
2.0	0	2.6	15	19	22	22	23
2.0	2	4.4	26	41	62	69	70
3.0	2	4.6	18	29	49	58	65
4.0	2	4.1	12	19	36	47	57
5.0	0	3.0	7.0	8.0	9.0	10	10
5.0	2	4.3	10	14	24	36	47

[a] Activity was determined over the first 30 min.

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(15) (a) *Sphingomonas* sp. HXN-200 was isolated from a waste air filter by Plaggemeier, Th.; Schmid, A.; Engesser, K. at University of Stuttgart. (b) For growth conditions, see ref 14a. (c) This strain is available for scientific researches from the culture collection at Institute of Biotechnology, ETH Zurich, Switzerland.

(16) **General Procedure.** Substrate **6** or **8** (2−16 mM) was added to 10 mL of cell suspension (4.0 g/L) of *Sphingomonas* sp. HXN-200 in 50 mM potassium phosphate buffer (pH 8.0) containing glucose (0−2%, w/v) in a 100 mL shaking flask. The mixture was shaken at 200 rpm and 30 °C for 5 h. Samples (100 μL) were taken out at different times and mixed with methanol (100 μL), and the cells were removed by centrifugation. The supernatant was analyzed by HPLC.

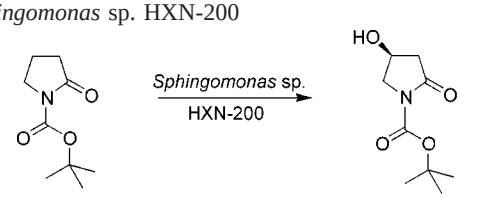
(17) HPLC analysis: Hypersil BDS-C18 column (125 mm × 4 mm); UV detection at 210 nm; acetonitrile/10mM potassium phosphate buffer (pH 7.0) 20/80 as eluent; flow at 1 mL/min; retention time 2.7 min for **7**, 8.1 min for **6**, 2.7 min for **9**, and 6.7 min for **8**.

(18) *N*-Benzyl-3-hydroxy-pyrrolidin-2-one was formed as byproduct. Ratio of **7**/byproduct is about 5/1.

hydroxylation of a 5 mM solution of **6**; the conversion to **7** at 5 h was increased from 10% to 47% by addition of 2% of glucose. Hydroxylation of 3 and 4 mM solutions of **6** for 5 h gave 65% and 57% of **7**, respectively, with activity of 4.6 and 4.1 U/g CDW (U = μmol/min, CDW = cell dry weight) in the first 30 min.

Higher activity was observed for hydroxylation of *N*-*tert*-butoxycarbonyl-pyrrolidin-2-one **8** with resting cells (4.0 g/L) of *Sphingomonas* sp. HXN-200. As shown in Table 2,

Table 2. Hydroxylation of **8** to **9** with Resting Cells (4.0 g/L) of *Sphingomonas* sp. HXN-200



8 **9**
e.e. 90-92% (*S*)

8[a] (mM)	activity[b] (U/g CDW)	**9** (%) 0.5 h	1 h	2 h	3h	5h
2.0	4.4	26	47	67	73	80
5.0	6.8	16	34	54	66	76
8.0	8.9	13	28	48	61	71
10	8.5	10	22	39	52	65
14	11	9.0	22	40	51	63
16	9.5	7.0	19	34	45	57

[a] Bioconversion was performed in the presence of glucose (2%). [b] Activity was determined over the first 30 min.

hydroxylation of a 14 mM solution of **8** gave an activity of 11 U/g CDW and 63% conversion to **9** at 5 h. Interestingly, both conversion and activity are not very much dependent on the starting concentration of substrate, which is advantageous for practical bioconversions; 57−80% of **9** were formed in hydroxylations of 2−16 mM solutions of **8** for 5 h. No byproduct was formed in biohydroxylation of **8**, demonstrating the excellent regioselectivity of the biocatalyst.

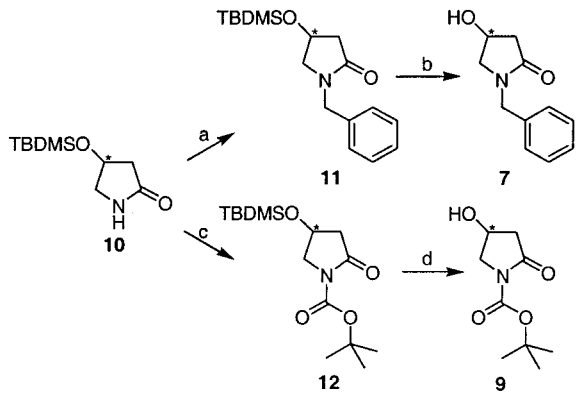
Preparation of **7** and **9** were performed on a 50-mL scale.[19] As shown in Table 3, biohydroxylation of **6** (3 mM) with resting cells (4.0 g/L) of *Sphingomonas* sp. HXN-200 for 5 h formed 66% of **7**; 55% (0.32 g/L) of pure product[20a] was isolated. For a practical bioconversion, the product concentration has to be increased. This can be easily achieved by using higher starting concentration of **6** and higher cell density. Hydroxylation of a 6 mM solution of **6** with 8.0 and 10 g/L of resting cells of *Sphingomonas* sp. HXN-200 afforded 42% (0.48 g/L) and 68% (0.78 g/L) of **7**, respectively.

Biohydroxylation of **8** (14 mM) with 4.0 g/L of resting cells of *Sphingomonas* sp. HXN-200 afforded **9**[20b] in 39% yield (1.10 g/L). Similarly, increase of cell density to 8.0 g/L improved the yield to 1.29 g/L (46%). Further improvement was achieved by use of more substrate and more cells: hydroxylation of a 20 mM solution of **8** with 10 g/L of resting cells of *Sphingomonas* sp. HXN-200 gave the pure product **9** in 42% yield (1.69 g/L).

On the basis of our experience, the yield of **7** and **9** can be further improved by performing the hydroxylation in a bioreactor.

For determination of the ee of the biohydroxylation products **7** and **9**, standard (*R*)- and (*S*)-**7** and **9** were synthesized from the corresponding known compounds (*R*)- and (*S*)-**10**[21]. As shown in Scheme 1, benzylation of (*R*)-

Scheme 1



a. NaH, THF, 0°C, BnCl, 19% of (*R*)-**11**; 23% of (*S*)-**11**. b. Bu_4NF, THF, 41% of (*R*)-**7**; 34% of (*S*)-**7**. c. $(Boc)_2O$, DMAP, Et_3N, 89% of (*R*)-**12**; 92% of (*S*)-**12**. d. Bu_4NF, THF, 0°C, acetic acid, 8.1% of (*R*)-**9**; 16% of (*S*)-**9**.

and (*S*)-**10** afforded the corresponding (*R*)- and (*S*)-**11** in 19% and 23% yield, respectively. Deprotection of **11** gave (*R*)- and (*S*)-**7** in 41% and 34% yield, respectively. Similarly, treatment of (*R*)- and (*S*)-**10** with Boc_2O, DMAP, and Et_3N

(19) **Preparation of 7.** To 50 mL of cell suspension (4.0 g/L) of *Sphingomonas* sp. HXN-200 in 50 mM potassium phosphate buffer (pH 8.0) containing glucose (2%, w/v) in a 500 mL shaking flask was added **6** (26.3 mg, 0.15 mmol). The mixture was stirred at 200 rpm and 30 °C. The reaction was followed by analytical HPLC and was stopped at 5 h with 66% conversion. 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 of **7** = 0.13 and R_f of **6** = 0.50; ethyl acetate/methanol 9:1) afforded 15.8 mg (55%) of **6** as white powder.

(20) (a) **Data for 7.** Mp 107.3−108.0 °C. $[\alpha]^{25}_D$ −34.1° (*c* 1.00, $CHCl_3$).[9] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35−7.19 (m, 5 H, aromatic H), 4.52−4.38 (m, 3 H, NCH_2Ph, H-C(4)), 3.48 (dd, 1 H, J = 10.9 and 5.6 Hz, H_A-C(5)), 3.26 (s, br., 1 H, OH), 3.18 (dd, 1 H, J = 10.8 and 2.0 Hz, H_B-C(5)), 2.70 (dd, 1 H, J = 17.4 and 6.6 Hz, H_A-C(3)), 2.43 ppm (dd, 1 H, J = 17.3 and 2.5 Hz, H_B-C(3)). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.94 (s, CO); 135.97 (s), 128.70 (d), 127.98 (d), 127.60 (d, aromatic C); 64.27 (d, C-4); 55.71 (t, CH_2Ph); 46.32 (t, C-5); 41.14 ppm (t, C-3). MS: *m*/*z* 192.1-(M+1, 100), 174.1(9). IR (cm^{-1}): 3401, 3007, 2928, 1682, 1483, 1435, 1262, 1082. (b) **Data for 9.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.47 (*s*, 1 H, H-C(4)), 3.88 (dd, 1 H, J = 11.9 and 5.1 Hz, H_A-C(5)), 3.77 (d, 1 H, J = 11.8 Hz, H_B-C(5)), 3.15 (s, 1 H, OH), 2.77 (dd, 1 H, J = 17.7 and 6.1 Hz, H_A-C(3)), 2.43 (d, 1 H, J = 17.7 Hz, H_B-C(3)), 1.52 ppm (s, 9 H, 3 CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8 (s, COO); 150.05 (s, CO); 83.19 (s, $C(CH_3)_3$); 63.03 (d, C-4); 55.31 (t, C-5); 42.71 (t, C-3); 28.03 ppm (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. (c) **Data for 9** (after crystallization): mp 133.5−134.6 °C. $[\alpha]^{25}_D$ +2.1° (*c* 1.86, $CHCl_3$).

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afforded 89% and 92% of (*R*)- and (*S*)-**12**, respectively. Deprotection of **12** gave the corresponding (*R*)- and (*S*)-**9** in 8.1% and 16% yield, respectively. Here, the yields were not optimized. The structures of (*R*)- and (*S*)-**7**, **9**, **11**, and **12** were identified by ^{1}H and ^{13}C NMR and MS spectra.

The ee of the biohydroxylation products **7** and **9** were determined by HPLC with a chiral column:[22] >99.9% ee (*S*) for **7** and 90−92% ee (*S*) for **9**, as shown in Table 3.

Table 3. Preparation of **7** and **9** by Hydroxylation of **6** and **8**, Respectively, with Resting Cells of *Sphingomonas* sp. HXN-200

substrate (mM)	cells (g/L)	product	conversion (%)	yield (%)	ee (*S*) (%)
6 (3.0)	4.0	**7**	66	55	>99.9
6 (6.0)	8.0	**7**	59	42	>99.9
6 (6.0)	10	**7**	75	68	>99.9
8 (14)	4.0	**9**	48	39	90
8 (14)	8.0	**9**	66	46	92
8 (20)	10	**9**	49	42	90

The ee of **9** was increased from 92% to 99.9% (*S*)[20c] in 82% yield by simple crystallization from *n*-hexane/ethyl acetate (2:1).

In summary, we have developed a simple and practical synthesis of (*S*)-*N*-substituted 4-hydroxy-pyrrolidin-2-ones by hydroxylation of the corresponding pyrrolidin-2-ones with *Sphingomonas* sp. HXN-200.

Acknowledgment. We are indebted to Mr. H.-J. Feiten (ETH Zurich) for preparing cells of *Sphingomonas* sp. HXN-200. We thank Dr. J. B. van Beilen (ETH Zurich) for helpful discussion and Mr. Th. Plaggemeier and Prof. K. Engesser (University of Stuttgart) for supplying us with the HXN-200 strain.

Supporting Information Available: Experimental details for biocatalytic preparation of **9**; data of chemically prepared compounds **7**, **9**, **11**, and **12**; ^{1}H and ^{13}C NMR spectra of biohydroxylation products **7** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL006735Q

(22) (a) The ee of **7** was determined by analytical HPLC: column, Chiralpak AS.; eluent, *n*-hexane/2-propanol 4:1; flow, 1.0 mL/min; T_R (*S*) = 20.3 min; T_R (*R*) = 30.5 min. (b) The ee of **9** was determined by analytical HPLC: column, Chiralcel OB-H; eluent, *n*-hexane/2-propanol 7:1; flow, 0.5 mL/min; T_R (*R*) = 17.9 min; T_R (*S*) = 22.6 min.