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Practical Syntheses of *N*-Substituted 3-Hydroxyazetidines and 4-Hydroxypiperidines by Hydroxylation with *Sphingomonas* sp. HXN-200

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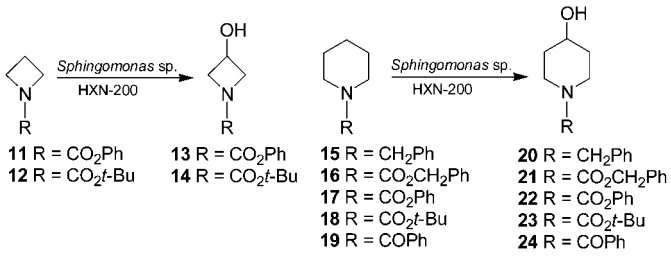
ABSTRACT



Hydroxylation of *N*-substituted azetidines 11 and 12 and piperidines 15–19 with *Sphingomonas* sp. HXN-200 gave 91–98% of the corresponding 3-hydroxyazetidines 13 and 14 and 4-hydroxypiperidines 20–24, respectively, with high activity and excellent regioselectivity. High yields and high product concentrations (2 g/L) were achieved with frozen/thawed cells as biocatalyst. For the first time, rehydrated lyophilized cells were successfully used for the biohydroxylation.

3-Hydroxyazetidine and 4-hydroxypiperidine are useful pharmaceutical intermediates. For example, 3-hydroxyazetidine is used in the synthesis of oral carbapenem antibiotics L-036 **1** and L-084 **2**, antiepileptic Dezinamide **3**, and antihypertensive Azelnidipine **4**;[1] 4-hydroxypiperidine is used for the preparation of allergic rhinitis drugs Ebastine **5** and Betotastine besilate **6**, antibacterial Nadifloxacin **7**, antiallergy/antiasthmatic Linazolast **8**, and agents for antiplatelet therapy Lamifiban **9** and Sibrafiban **10**[2] (Scheme 1). In practice it is often advantageous, if not required, to use 3-hydroxyazetidine and 4-hydroxypiperidine in their *N*-protected form.

There are synthesis routes to 3-hydroxyazetidine and its *N*-substituted derivatives, but each of them has drawbacks: amination of epichlorohydrin is limited to primary hindered amines;[3a−e] amination of 1,3-dichloro-2-propyl ether gives low yield;[3f] halogenation of 2,3-epoxyamines has only one example for *tert*-butylamine;[3g] the route via 1-azabicyclo-[1.1.0]butane requires a special reagent;[3h] and reduction of azetidinone requires difficult to obtain starting materials.[3i]

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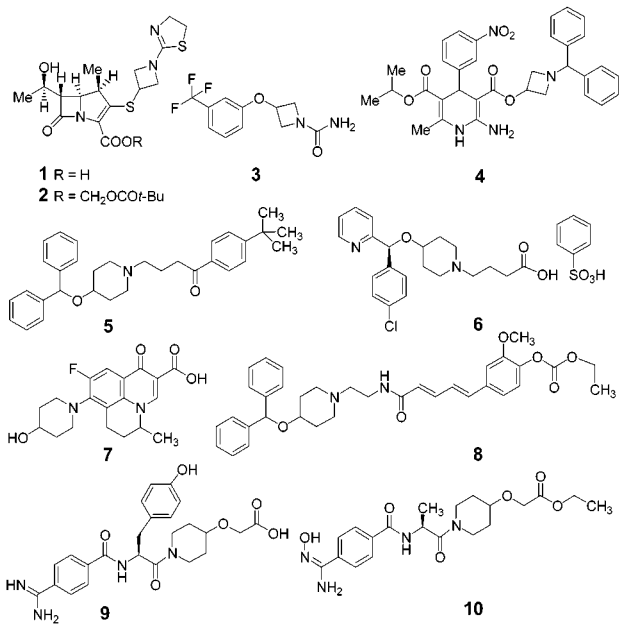
Scheme 1



Syntheses of 4-hydroxypiperidine and *N*-substituted 4-hydroxypiperidines are also not straightforward: preparations involving reduction of *N*-substituted 4-piperidones,[4a−e] hydrogenation of 4-hydroxypyridine or *N*-substituted 1*H*-pyridin-4-one,[4f−h] or hydrogenation and cyclization of 3-hydroxy-glutaronitrile[4i] give low overall yields in multisteps; syntheses via Mannich-type cyclization of formaldehyde with benzylbut-3-enyl-amine[4j] or with *N*-benzylammonium trifluoroacetate and allyl-trimethyl-silane[4k] are not practical; hydroboration of *N*-trimethylsilanyl- or *N*-benzyloxylcarbonyl-1,2,5,6-tetrahydropyridine[4l−n] gives a mixture of 3- and 4-hydroxy piperidines.

Regioselective hydroxylation of azetidine and piperidine represents one of the simplest ways for preparing the hydroxylated derivatives. While selective hydroxylation on a nonactivated carbon atom remains still a challenge in synthetic chemistry,[5] biohydroxylation can be a useful tool for this type of transformation.[6−7] However, no successful biohydroxylation of azetidine or *N*-substituted azetidines has been reported thus far. Hydroxylations of *N*-substituted piperidines with *Beauveria sulfurescens* ATCC 7159[7i,8] or *Aspergillus niger* VKM F-1119[9] are known, but the low activity, yield, and product concentration (less than 0.1 g/L) limit their synthetic applications.

We have recently found that the bacterial strain *Sphingomonas* sp. HXN-200 is an excellent biocatalyst for regio- and stereoselective hydroxylations of pyrrolidines[7a,c] and pyrrolidin-2-ones.[7b] Here, we report the hydroxylation with this strain of *N*-substituted azetidines and piperidines, four- and six-membered heterocycles, for the preparation of the corresponding 3-hydroxyazetidines and 4-hydroxypiperidines and the successful use of rehydrated lyophilized cell powder as hydroxylation catalyst.

Sphingomonas sp. HXN-200 was grown on *n*-octane vapor in 30 L of E2 medium[10] at 30 °C and 1500 rpm for 90 h to a cell density of 8.5 g/L. The cells were harvested, and the cell pellets (2.5 kg wet cells consisting of 10% dry cells) were stored at −80 °C. The frozen/thawed cells were used

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for hydroxylation of *N*-substituted azetidines **11** and **12** and piperidines **15**−**19**. Substrates **11** and **12**[11] were synthesized by reaction of azetidine with phenyl chloroformate and di-*tert*-butyldicarbonate in 67% and 83% yield, respectively.[12] Piperidines **15**,[13] **16**,[8a] and **18**[14] were prepared according to established procedures, and **17**[15] was prepared in an improved yield of 82%.

Hydroxylations were performed with frozen/thawed cells of *Sphingomonas* sp. HXN-200 on a 10-mL scale in the exploratory stage,[16] and the bioconversions were followed by HPLC analyses.[17] As shown in Table 1, hydroxylation of azetidine **11** and **12** gave the desired 3-hydroxyazetidine **13** and **14** with high activities (15−17 U/g cdw, U = μmol/min, cdw = cell dry weight) and high conversions. No byproducts were detected, indicating the excellent regioselectivity and clean biotransformation. Moreover, higher product concentrations could be achieved as follows: hydroxylation of 10 mM solutions of **11** and **12** gave 82% and 89% of 3-hydroxyazetidine **13** and **14**, respectively. This demonstrates the first successful biohydroxylation of *N*-substituted azetidines.

Similarly, hydroxylation of piperidines **15**−**19** afforded the desired 4-hydroxypiperidines **20**−**24**. As shown in Table 2, high activity was obtained in hydroxylations of **15**−**18** (12−29 U/g cdw), while moderate activity (4.5 U/g cdw) was observed for the hydroxylation of **19**, probably as a result of the steric hindrance of the *N*-benzoyl group in the substrate. No other regioisomers were detected during hydroxylation, indicating excellent regioselectivity. Hydroxylation of **15** was a clean reaction, while hydroxylation of **16**−**19** gave a small amount of the corresponding 4-ketones. Nevertheless, over 91% of 4-hydroxypiperidines **20**−**24** were formed in hydroxylation of **15** (5 mM), **16** (2 mM), **17** (8 mM), **18** (6 mM), and **19** (5 mM), respectively.

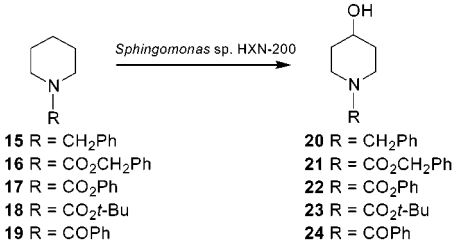
Preparative hydroxylations were carried out on scales of 60 mL to 2 L with frozen/thawed cells, as shown in Table 3. Hydroxylation of azetidine **12** (15.8 mM) on a 1-L scale gave 2.140 g (79%) of 3-hydroxyazetidine **14**.[18] Hydroxylation of **11** on a 60-mL scale gave **13**[19] in 81% yield. Similarly, hydroxylation of piperidine **15** (5.0 mM) at a cell concentration of 4.0 g cdw/L gave 4-hydroxypiperidine **20**[20]

Table 1. Hydroxylation of *N*-Substituted Azetidines **11** and **12** with Frozen/Thawed Cells of *Sphingomonas* sp. HXN-200 (4.0 g cdw/L)

substrate (mM)	product	activity[a] (U/g cdw)	conversion[b] (%) 0.5 h	1 h	2 h	3 h	5 h
11 (5.0)	**13**	15	33	61	96		
11 (7.0)	**13**	16	27	44	71	89	98
11 (10.0)	**13**	15	18	30	49	64	82
12 (5.0)	**14**	17	39	60	93		
12 (10.0)	**14**	17	20	35	60	74	89

[a] Activity was determined over the first 30 min. [b] Conversion was determined by HPLC analysis; error limit, 2% of the stated values.

Table 2. Hydroxylation of *N*-Substituted Piperidines **15**−**19** with Frozen/Thawed Cells of *Sphingomonas* sp. HXN-200 (4.0 g cdw/L)



substrate (mM)	produdct	activity[a] (U/g cdw)	conversion[b] (%) 0.5 h	1 h	2 h	3 h	5 h[c]
15 (5.0)	**20**	20	49	77	94	98	98
16 (2.0)	**21**	12	71	83	87	87	91 (1.4)
16 (3.0)	**21**	13	49	61	65	68	69
17 (7.0)	**22**	19	31	53	84	91	94 (5.1)
17 (8.0)	**22**	18	26	51	76	88	94 (4.1)
18 (5.0)	**23**	29	69	91	94	94	94 (6.3)
18 (6.0)	**23**	27	54	79	86	93	93 (6.6)
19 (2.0)	**24**	4.5	27	50	78	91	97 (1.8)
19 (3.0)	**24**	4.0	16	32	57	74	90 (1.7)

[a] Activity was determined over the first 30 min. [b] Conversion was determined by HPLC analysis; error limit, 2% of the stated values. [c] Number in bracket is the conversion to the corresponding 4-ketones at 5 h; no bracket indicates no ketones formed.

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(12) Data for **11**: R_f 0.15 (silica gel, ethyl acetate/hexane 2:8); mp 41.1−42.3 °C; ^{1}H NMR (300 MHz, 243 K) δ 7.41−7.34 (m, 2 H), 7.24−7.19 (m, 1 H), 7.14−7.10 (m, 2 H), 4.22 (t, 2 H, J = 7.8), 4.12 (t, 2 H, J = 7.8), 2.32 (quin, 2 H, J = 7.8); ^{13}C NMR (75 MHz) δ 154.19(s), 150.58 (s), 129.30 (d), 125.29 (d), 121.66 (d), 50.04 (t), 48.94 (t), 15.48 (t); MS *m*/*z* 178 (100%, M + 1); IR ($CHCl_3$) ν 1716, 1595 cm^{-1}. Two sets of signals for *N*CH_2 in the ^{1}H NMR spectrum at 243 K indicates the existence of two rotamers due to the restricted rotation of the *N*-CO bond.

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(16) **General Procedure.** Substrate was added to a 10-mL suspension of the frozen/thawed cells (0.40 g with 40 mg cdw) in 50 mM K-phosphate buffer (pH = 8.0) containing glucose (2%, w/v). The mixture was shaken at 200 rpm and 30 °C for 5 h. Aliquots (0.1−0.2 mL) were taken out at predetermined time points and diluted in MeOH, and the cells were removed by centrifugation. The samples were analyzed by HPLC.

(17) **HPLC Analysis.** Column, Hypersil BDS−C18 (5 μm, 125 mm × 4 mm); eluent, a mixture of A (10 mM K-phosphate buffer, pH 7.0) and B (acetonitrile); flow, 1.0 mL/min.; detection, UV at 210, 225, and 254 nm; retention time, 2.1 min for **13**, 5.4 min for **11** (A/B 70:30), 2.7 min for **14**, 9.2 min for **12** (A/B 75:25), 3.0 min for **20**, 5.2 min for **15** (A/B 85:15), 2.0 min for **21**, 8.8 min for **16** (A/B 55:44), 1.7 min for **22**, 6.6 min for **17** (A/B 55:45), 1.5 min for **23**, 5.6 min for **18** (A/B 50:50), 1.5 min for **24**, 5.4 min for **19** (A/B 70:30); the conversion was quantified by comparing the integrated peak areas at 210 nm of the samples with the substrate and product standards.

Table 3. Preparation of *N*-Substituted 3-Hydroxyazetidines **13** and **14** and 4-Hydroxypiperidines **20**−**24** by Hydroxylation with Frozen/Thawed Cells of *Sphingomonas* sp. HXN-200

substrate (mM)	scale (mL)	cells (g cdw/L)	time (h)	conv[a] (%)	yield[b] %	yield[b] mg
11 (4.0)	60	4.0	1.5	98	81.0	37.5
12[c] (15.8)	1000	10.2	5.0	83	79.0	2140
15 (5.0)	2000	4.0	4.0	98	82.9	1501
15 (15.0)	1000	10.2	5.2	98	76.2	2072
16 (2.0)	100	4.0	3.0	96	70.2	33.0
17 (7.0)	100	4.0	4.0	91	83.2	43.6
18 (5.0)	100	4.0	2.0	96	69.5	69.3
19 (2.0)	100	4.0	5.0	83	71.5	29.3

[a] Conversion was determined by HPLC analysis; error limit, 2% of the stated values. [b] Yield of the isolated pure product. [c] Substrate was added at different time points.[18]

in 83% yield. The product concentration was easily increased to 2.072 g/L by use of a higher cell density (10.2 g cdw/L) and higher substrate concentration (15 mM). Compounds **21**,[21] **22**,[22] **23**,[23] and **24**[9] were also prepared in good yields by hydroxylation of **16**−**19**, respectively. These results are clearly superior to those obtained with other hydroxylation systems.[7i,8,9]

To facilitate the application of this interesting biohydroxylation system in organic synthesis, we developed a lyophilized cell powder preparation of *Sphingomonas* sp. HXN-200 as a practical catalyst for use in organic synthesis. Hydroxylation of piperidine **15** (5 mM) with the rehydrated catalyst powder[24] at a density of 4.0 g cdw/L afforded 80% of 4-hydroxypiperidine **20**. Comparing with a similar hydroxylation with frozen/thawed cells, shown in Figure 1, 85% of the activity was achieved with the lyophilized powder. It has been shown that hydroxylation with *Sphingomonas* sp. HXN-200 is catalyzed by a NADH-dependent enzyme.[7a] The fact that rehydrated lyophilized cells are able to carry out such a NADH-dependent hydroxylation indicates that these cells are capable of retaining and regenerating NADH at rates equal to or exceeding the rate of hydroxylation. Although it is known that lyophilized microbial cells retain activities for hydrolytic reactions after rehydration,[25] our result is the first example of the use of lyophilized cells for a cofactor-dependent hydroxylation.

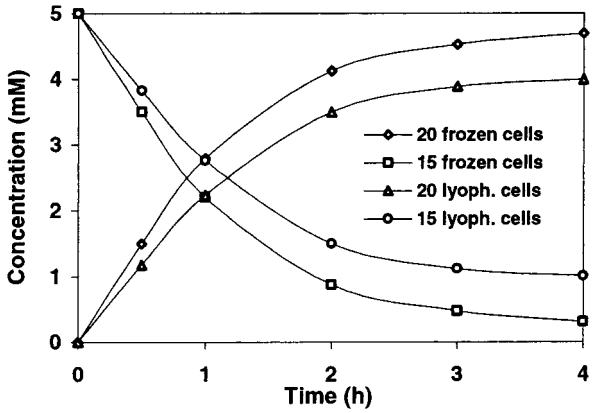

Figure 1. Biohydroxylation of *N*-benzylpiperidine **15** (5.0 mM) to **20** with rehydrated lyophilized cells and frozen/thawed cells of *Sphingomonas* sp. HXN-200 (4.0 g cdw/L) in 20 mL of 50 mM K-phosphate buffer (pH 8.0) containing glucose (2%).

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Supporting Information Available: Growth curve of *Sphingomonas* sp. HXN-200 on a 30-L scale; experimental details for the chemical preparation of **11**, **12**, and **17** and biocatalytic preparation of **13**, **14**, and **20**−**24**; ^{1}H and ^{13}C NMR spectra of bioproducts **13**, **14**, and **20**−**24** and substrates **11**, **12**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) **Preparation of 14.** A 1-L suspension of the frozen/thawed cells (102 g with 10.2 g cdw) in 50 mM K-phosphate buffer (pH 8.0) containing glucose (2%, w/v, for the intracellular regeneration of cofactors) was stirred at 1500 rpm and at 30 °C under the introduction of air at 1 L/min in a 3-L bioreactor. Substrate **12** was added at different time points: 10.1 mmol at the beginning, 2.0 mmol at 30 min, 2.0 mmol at 147 min, and 1.7 mmol at 180 min. The biotransformation was followed by analytical HPLC and stopped at 5 h by centrifugation. The pH of the supernatant was adjusted to 11−12 by addition of KOH followed by extraction with ethyl acetate. The organic phase was separated and dried over Na_2SO_4, and the solvent was removed by evaporation. The product was purified by column chromatography on silica gel (R_f 0.27, *n*-hexane/ethyl acetate 1:1) to give **14** in 79% yield (2.140 g): mp 44.8−46.8 °C; ^{1}H NMR (400 MHz) δ 4.53 (m, 1 H); 4.11−4.07 (dd, 2 H, J = 10.4, 7.2 Hz), 3.78−3.74 (dd, 2 H, J = 8.8, 4.4 Hz), 3.60 (s, 1 H); 1.38 (s, 9 H); ^{13}C NMR (100 MHz) δ 157.54 (s), 80.83 (s), 62.33 (d), 60.06 (t), 29.42 (q); MS *m*/*z* 173 (11%, M), 130 (100%), 118 (40%); IR ($CHCl_3$) ν 3400, 1683 cm^{-1}.

(19) Data for **13**: R_f 0.13 (silica gel, *n*-hexane/ethyl acetate 1:1); mp 100.8−102.6 °C; ^{1}H NMR (400 MHz) δ 7.38−7.08 (m, 5 H), 4.57 (m, br, 1 H), 4.30 (s, br, 2 H), 3.97 (s, br, 2 H), 3.14 (s, br, 1 H); ^{13}C NMR (100 MHz) δ 155.63 (s), 151.96 (s), 130.41 (d), 126.52 (d), 122.65 (d), 62.56 (d), 60.85 (t), 59.95 (t); MS *m*/*z* 194 (100%, M + 1), 113 (12%); IR ($CHCl_3$) ν 3401, 1720, 1595 cm^{-1}.

(20) Jucker, E.; Schenker, E. (Sandoz) CH 463504, 1965).

(21) Alig, L.; Edenhofer, A.; Hadvary, P.; Huerzeler, M.; Knopp, D.; Mueller, M.; Steiner, B.; Trzeciak, A.; Weller, T. *J. Med. Chem.* **1992**, *35*, 4393.

(22) Data for **22**: R_f 0.11 (silica gel, *n*-hexane/ethyl acetate 1:1); mp 116.5−118.3 °C; ^{1}H NMR (200 MHz) δ 7.40−7.06 (m, 5 H), 4.10−3.85 (m, 3 H), 3.28 (s, br., 2 H), 1.98−1.85 (m, 2 H), 1.81 (s, 1 H), 1.66−1.48 (ddt, 2 H, J = 13.0, 8.8, and 4.1); ^{13}C NMR (50 MHz) δ 153.75 (s), 151.42 (s), 129.26 (d), 125.25 (d), 121.73 (d), 67.08 (d), 41.62 (t), 33.97 (t); MS *m*/*z* 222 (100%, M + 1), 206 (24%); IR ($CHCl_3$) ν 3452, 1710, 1594 cm^{-1}.

(23) Kucznierz, R.; Grams, F.; Leinert, H.; Marzenell, K.; Engh, R.; von der Saal, W. *J. Med. Chem.* **1998**, *41*, 4983.

(24) **Hydroxylation of 15 with Rehydrated Lyophilized Cell Powder.** Frozen/thawed cells of *Sphingomonas* sp. HXN-200 were lyophilized at low temperature for 3 days. For the experiment of Figure 1, the dry powder (80 mg), which was stored at 4 °C for 2 weeks, was suspended in 20 mL of 50 mM K-phosphate buffer (pH = 8.0) containing glucose (2% w/v) in a 100 mL Erlenmeyer flask. Piperidine **15** (17.5 mg) was added to the suspension. The flask was shaken at 200 rpm at 30 °C for 4 h, and the formation of **20** was followed by HPLC analysis.[17]

(25) Mischitz M.; Faber K.; Willetts, A. *Biotechnol. Lett.* **1995**, *17*, 893.