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Enantioselective Trans Dihydroxylation of Nonactivated C−C Double Bonds of Aliphatic Heterocycles with *Sphingomonas* sp. HXN-200

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The bacterial strain *Sphingomonas* sp. HXN-200 was used to catalyze the trans dihydroxylation of *N*-substituted 1,2,5,6-tetrahydropyridines **1** and 3-pyrrolines **4** giving the corresponding 3,4-dihydroxypiperidines **3** and 3,4-dihydroxypyrrolidines **6**, respectively, with high enantioselectivity and high activity. The trans dihydroxylation was sequentially catalyzed by a monooxygenase and an epoxide hydrolase in the strain with epoxide as intermediate. While both epoxidation and hydrolysis steps contributed to the overall enantioselectivity in trans dihydroxylation of **1**, the enantioselectivity in trans dihydroxylation of the symmetric substrate **4** was generated only in the hydrolysis of *meso*-epoxide **5**. The absolute configuration for the bioproducts (+)-**3** and (+)-**6** was established as (3*R*,4*R*) by chemical correlations. Preparative trans dihydroxylation of **1a** and **4b** with frozen/thawed cells of *Sphingomonas* sp. HXN-200 afforded the corresponding (+)-(3*R*,4*R*)-3,4-dihydroxypiperidine **3a** and (+)-(3*R*,4*R*)-3,4-dihydroxy pyrrolidine **6b** in 96% ee both and in 60% and 80% yield, respectively. These results represent first examples of enantioselective trans dihydroxylation with nonterpene substrates and with bacterial catalyst, thus significantly extending this methodology in practical synthesis of valuable and useful trans diols. Enantioselective hydrolysis of racemic epoxide **2a** with *Sphingomonas* sp. HXN-200 gave 34% of (−)-**2a** in >99% ee, which is a versatile chiral building block. Further hydrolysis of (−)-**2a** with the same strain afforded (−)-(3*S*,4*S*)-**3a** in 96% ee and 92% yield. Thus, both enantiomers of **3a** can be prepared by biotransformation with *Sphingomonas* sp. HXN-200.

Introduction

Enantiomerically pure vicinal diols are very useful intermediates in the synthesis of a number of important pharmaceuticals and biologically active molecules, and asymmetric dihydroxylation of a C−C double bond provides a simple access to these diols. While asymmetric cis dihydroxylation can be achieved with Sharpless catalyst[1] or a dioxygenase,[2] epoxidation of a C−C double bond followed by hydrolysis could give rise to the asymmetric "trans dihydroxylation". The latter can be realized by use of a biological system containing a monooxygenase and an epoxide hydrolase.[3] Successful examples are the fungus-catalyzed trans dihydroxylations of several acyclic terpenes,[4−6] limonene,[7] and α-terpinene.[7] However, enantioselective trans dihydroxylation of a nonisoprene C−C double bond has been synthetically unsuccessful: several eukaryotic systems (plants, animal, and fungi) were known to metabolize aromatic compounds transforming the C−C double bond into the corresponding *trans*-dihydrodiol with low yield;[8] bioconversion of 1-benzyl-3-methyl-1,2,5,6-tetrahydropyridine with *Cunninghamella verticillata* VKPM F-430 gave a mixture of 3,4-*trans*-diol and two monohydroxylated products.[9] Moreover, trans dihydroxylation catalysts reported so far are limited to

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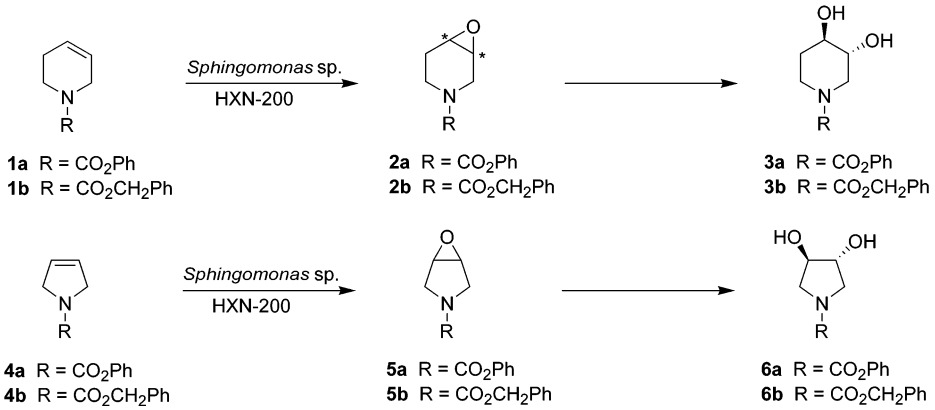
SCHEME 1. Trans Dihydroxylation of *N*-Substituted 1,2,5,6-Tetrahydropyridines 1a,b and 3-Pyrrolines 4a,b with *Sphingomonas* sp. HXN-200



the eukaryotic systems. We are interested in expanding the scope of enzymatic trans dihydroxylation in practical syntheses by developing a highly active and easy to handle bacterial catalyst with new substrate specificity. Previously, we found that *Sphingomonas* sp. HXN-200, an alkane-degrading bacterium, contains a soluble alkane monooxygenase that catalyzes the regio- and stereoselective hydroxylation of the nonactivated carbon atom of various aliphatic heterocycles.[10] Recently we also found a soluble epoxide hydrolase in this strain that catalyzes the enantioselective hydrolysis of alicyclic *meso*-epoxides.[11] Considering that the alkane monooxygenase might catalyze the epoxidation of a C−C double bond, the strain HXN-200 might be a promising catalyst for trans dihydroxylation of the nonisoprene C−C double bond of alicyclic compounds.

N-Substituted 1,2,5,6-tetrahydropyridine and 3-pyrroline are interesting substrates, since trans dihydroxylation of these compounds could give the corresponding optically active *trans*-3,4-diols. While (3*R*,4*R*)-3,4-dihydroxypiperidine is a potent inhibitor of β-D-glucuronidase[12a] and an intermediate for the preparation of xylanase inhibitor isofagomine,[12b] (3*R*,4*R*)-3,4-dihydroxypyrrolidine is a useful intermediate for the preparation of biologically active Sialyl Lewis X mimetics,[13a] new carbapenem[13b] and cephalosporin[13c] antibiotics, and novel carbohydrate mimics.[13d] The chemical synthesis of (3*R*,4*R*)-3,4-dihydroxypiperidine requires 10 steps from either D-arabinose[12a,14] or D-tartaric acid[12b,15] with low overall yield, whereas the preparation of (3*R*,4*R*)-3,4-dihydroxypyrrolidine involves a difficult reduction step from D-tartaric acid.[16,13b] Here, we report the highly enantioselective trans dihydroxylation of *N*-substituted 1,2,5,6-tetrahydropyridine and 3-pyrroline with *Sphingomonas* sp. HXN-200 as biocatalyst and the high yield preparation of (3*R*,4*R*)-3,4-dihydroxypiperidine and (3*R*,4*R*)-3,4-dihydroxypyrrolidine. In addition, we report the practical synthesis of enantiomerically pure 3,4-epoxypiperidine and (3*S*,4*S*)-3,4-dihydroxypiperidine by enantioselective hydrolysis with the same strain.

Result and Discussion

Enantioselective Trans Dihydroxylation with *Sphingomonas* sp. HXN-200. *Sphingomonas* sp. HXN-200[10c] was grown on *n*-octane in E2 medium as described,[10b] the cells were harvested, and the cell pellets were stored at −80 °C. The easy to handle frozen/thawed cells were subsequently used for trans dihydroxylation studies. Since 1,2,5,6-tetrahydropyridine and 3-pyrroline themselves were not substrates for the monooxygenase of *Sphingomonas* sp. HXN-200, docking/protecting groups[17,10] were introduced. *N*-Phenoxycarbonyl and *N*-benzyloxycarbonyl groups were shown to be excellent docking/protecting groups for the hydroxylation of pyrrolidine,[10b] thus being selected as the docking/protecting groups of 1,2,5,6-tetrahydropyridines and 3-pyrrolines for the trans dihydroxylation (Scheme 1).

Substrate **1a**[18] was synthesized in 70% yield by treatment of 1,2,5,6-tetrahydropyridine with phenyl chloroformate, and **1b** and **4a** were prepared by similar procedures.[19,20] To facilitate the analysis of the biocon-

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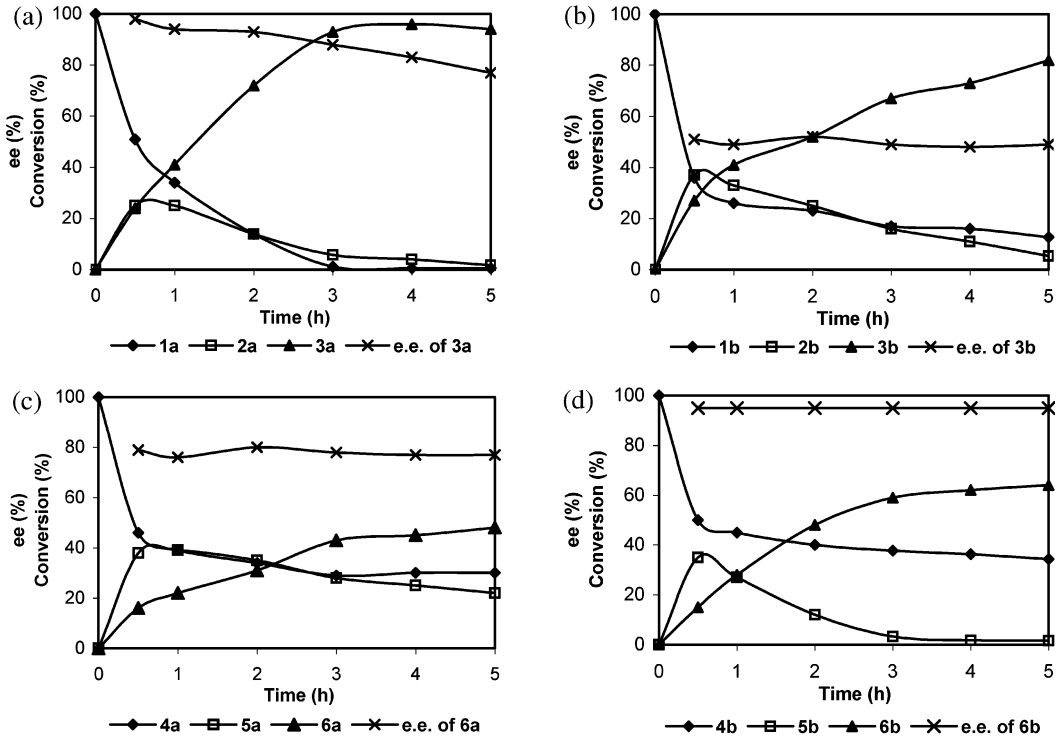

FIGURE 1. Trans dihydroxylation of **1a**,**b** and **4a**,**b** with frozen/thawed cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200: (a) **1a** (10 mM); (b) **1b** (8 mM); (c) **4a** (5 mM); and (d) **4b** (3 mM).

version, epoxides **2a**,**b** and **5a**,**b** and diols **3a**,**b** and **6a**,**b** were also prepared chemically: epoxidation of **1a** and **4a** with m-CBPA gave 79% and 67% of **2a** and **5a**, respectively; hydrolysis of **2a** and **5a** with TFA afforded the corresponding *trans*-diol **3a** and **6a** in 52% and 82% yield, respectively; similar reactions gave the epoxides **2b**[19] and **5b**[13d] and diols **3b**[12b] and **6b**.[13a,d] The structures of all synthesized compounds were confirmed by the MS, IR, ^{1}H, and ^{13}C NMR spectra.

Small-scale biotransformations were performed with frozen/thawed cells (cell concentration: 10 g cdw/L, cdw = cell dry weight; 1 g cdw corresponds to about 4 g frozen cell weight) of *Sphingomonas* sp. HXN-200 in 10 mL of 50 mM K-phosphate buffer (pH 8.0). Glucose (2%, w/v) was added to increase the yield via regeneration of the cofactor for the epoxidation, and the mixture was shaken at 200 rpm and 30 °C. Aliquots (0.1−0.2 mL) were taken from the bioconversion mixture at predetermined time points, diluted in methanol, and the cells were removed by centrifugation. The samples were analyzed by reverse-phase HPLC to follow directly the reaction in aqueous phase. The conversion was quantitated by comparing the integrated peak areas at 210 nm of the substrate and product in an analytic sample with the corresponding standards.

During the biotransformation of **1a**,**b** and **4a**,**b**, the epoxides **2a**,**b** and **5a**,**b** were formed and then hydrolyzed to the corresponding diols **3a**,**b** and **6a**,**b** (Figure 1). The formed epoxides and diols were extracted from the reaction mixture and purified, and their structures were identified by ^{1}H and ^{13}C NMR analyses. These results suggested that the alkane monooxygenase of HXN-200 is able to catalyze the epoxidation of the nonactivated C−C double bond. No hydrolysis of the chemically prepared epoxide **2a**,**b** and **5a**,**b** were observed with the cells that were boiled for 20 min; therefore, the hydrolysis during dihydroxylation must be catalyzed by the epoxide hydrolase from the strain.

As shown in Table 1, the average epoxidation activity during the first 30 min is quite high for **1a** (10 mM) and **1b** (10 mM): 16 and 18 U/g cdw (U = μmol/min), respectively. While the diol **3a** was formed in 94% at 5 h, only 65% of the diol **3b** was observed at the same time. Nevertheless, biotransformation of **1b** at 8 mM for 5 h afforded 82% of **3b.** The epoxidation activity for **4a** (5−8 mM) was also quite high (9−12 U/g cdw), but the diol **6a** was formed in only 45−48% at 5 h. In contrast, biotransformation of **4b** (2−3 mM) showed lower epoxidation activity (4.4−5.0 U/g cdw), but gave 64−97% of the diol **6b**. In fact, the diol formation depends on both epoxidation and hydrolysis. As shown in Figure 1, the concentration of the epoxides **2a**,**b** and **5a**,**b** reached maxima at 0.5−1.0 h. The epoxides **2a**,**b** and **5b** were rapidly hydrolyzed afterward, while the epoxide **5a** was hydrolyzed rather slowly resulting in low conversion to **6a**. It was also clearly shown in Figure 1 that the epoxidation rate became much slower after 0.5 h in all the cases, possibly due to the inhibition of the monooxygenase by the formed epoxides.

The alkane monooxygenase of HXN-200 showed higher epoxidation activity for the six-member ring substrate **1a**,**b** than for the five-member ring substrates **4a**,**b**. Epoxidation of 1,2,5,6-tetrahydropyridine **1a** and 3-pyrroline **4a** with a *N*-phenoxycarbonyl docking/protecting group is slightly less active than the hydroxylation of the corresponding saturated substrate piperidine and pyrrolidine,[10b,d] respectively. Epoxidation of *N*-benzyloxycarbonyl-1,2,5,6-tetrahydropyridines **1b** is more active than the hydroxylation of the corresponding piperidine,[10d]

TABLE 1. Enantioselective Trans Dihydroxylation of *N*-Substituted 1,2,5,6-Tetrahydropyridines 1a,b and 3-Pyrrolines 4a,b with Frozen/Thawed Cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200

entry	substrate (mM)	activity[a] (U/g cdw)	product	conversion and ee[b] (%)[c] 0.5 h	1 h	2 h	3 h	4 h	5 h
1	**1a** (10)	16	(+)-**2a**	25 (48)	25 (31)	14 (−22)	5.8 (−84)	4.0 (>−99)	1.7 (>−99)
			(+)-**3a**	24 (98)	41 (94)	72 (93)	93 (88)	96 (83)	94 (77)
2	**1a** (15)	14	(+)-**2a**	14 (49)	18 (37)	21 (13)	10 (−29)	7.2 (−68)	
			(+)-**3a**	14 (98)	27 (98)	54 (94)	74 (91)	82 (93)	
3	**1b** (8.0)	17	**2b**	37	33	25	16	11	5.3
			(+)-**3b**	27 (51)	41 (49)	52 (52)	67 (49)	73 (48)	82 (49)
4	**1b** (10)	18	**2b**	32	30	24	16	11	8.4
			(+)-**3b**	23 (51)	35 (51)	39 (49)	56 (51)	61 (48)	65 (50)
5	**4a** (5.0)	9.0	**5a**	38	39	35	28	25	22
			(+)-**6a**	16 (79)	22 (76)	31 (80)	43 (78)	45 (77)	48 (77)
6	**4a** (8.0)	12	**5a**	33	35	31	26	23	21
			(+)-**6a**	14 (78)	23 (77)	30 (76)	37 (75)	39 (75)	45 (78)
7	**4b** (2.0)	4.4	**5b**	40	28	5.1	0		
			(+)-**6b**	26 (95)	56	90	97 (95)		
8	**4b** (3.0)	5.0	**5b**	35	27	12	3.3	1.6	1.7
			(+)-**6b**	15 (95)	28 (95)	48 (95)	59 (95)	62 (95)	64 (95)

[a] Biotransformation was performed in a 10 mL cell suspension in 50 mM K-phosphate buffer (pH 8.0) containing glucose (2%), and activity is the epoxidation activity based on the total product formation over the first 30 min. [b] Ee is given in parentheses. [c] Conversion and ee were determined by HPLC analysis; error limit is 2% of the stated values; "−" for ee means the opposite enantiomer is in excess.

whereas epoxidation of 3-pyrroline **4b** is less active than the hydroxylation of *N*-benzyloxycarbonyl-pyrrolidine.[10b]

To investigate the enantioselectivity for the trans dihydroxylation, analytic samples were prepared by taking 0.5-mL aliquots from biotransformation mixture and extracting with equal volume of ethyl acetate. HPLC analysis with a chiral column (Chiralcel OB-H, OD-H, or Chiralpak AS) gave the ee values of the epoxide **2a** and diols **3a**,**b** and **6a**,**b**. During dihydroxylation of **1a** (10 mM), the ee of the diol (+)-**3a** was 98% at 30 min and then decreased to 77% at 5 h (Figure 1a). The ee of the epoxide (+)-**2a** was 48% at 30 min and changed to >−99% at 5 h (Table 1). These results suggested the following: (a) the enantioselective epoxidation of **1a** initially gave (+)-**2a** in about 73% ee; (b) (+)-**2a** was preferably hydrolyzed affording (+)-**3a** in very high ee at the beginning; (c) (−)-**2a** was also hydrolyzed at a slower rate resulting in a decrease of the ee of (+)-**3a** with time; and (d) pure (−)-**2a** remained at 5 h as a result of resolution. Similar results for the trans dihydroxylation of a 15 mM solution of **1a** were obtained (Table 1). Reaction for 4 h gave 82% of (+)-**3a** in 93% ee. Changing the docking/protecting group of **1** from the phenoxycarbonyl to the benzyloxycarbonyl group resulted in lower enantioselectivity for the trans dihydroxylation. As shown in Figure 1b, the ee of the diol **3b** was 51% at 30 min and remained nearly unchanged during the reaction. Although an attempt to determine the ee of the epoxide **2b** by HPLC analysis with a chiral column (Chiralcel OB-H, OD-H, OJ or Chiralpak AS) failed, the constant low ee value of **3b** during the reaction suggested that the enantioselectivity for hydrolysis of **2b** is very poor. It can be thus assumed that epoxidation of **1b** gave the epoxide **2b** in about 50% ee. The fact that HXN-200 catalyzes the enantioselective epoxidation of **1a**,**b** is remarkable, since enantioselective epoxidation of the aliphatic heterocyclic C−C double bond was proven to be difficult with microbial enzyme: only *Rhodococcus rhodochrous* 9703 (ATCC19067) was reported to oxidize **1b** giving the corresponding epoxide as the major product with no information about the ee.[21]

In the case of trans dihydroxylation of the symmetric substrate **4a**,**b**, the enantioselectivity was generated only in the hydrolysis step, since epoxidation gave *meso*-epoxide **5a**,**b**. While biotransformation of **4a** with a phenoxycarbonyl docking/protecting group gave diol **6a** in 78% ee, much higher enantioselectivity was observed in dihydroxylation of **3b** with a benzyloxycarbonyl docking/protecting group: the diol **6b** was formed in 95% ee.

To our knowledge, these are the first examples of enantioselective trans dihydroxylation with a bacterial catalyst. These are also the first enantioselective dihydroxylations of 1,2,5,6-tetrahydropyridines and 3-pyrrolines, since Osmium-catalyzed dihydroxylations of 1,2,5,6-tetrahydro pyridines and 3-prrolines resulted in racemic and *meso*-*cis*-diols, respectively.[22,23]

Enantioselective Hydrolysis of Racemic Epoxide 2a,b and *meso*-Epoxides 5a,b. To investigate the second reaction step of the trans dihydroxylation, enzymatic hydrolysis was performed with racemic epoxides **2a**,**b** and *meso*-epoxides **5a**,**b** as substrates, respectively. The biotransformation was carried out with frozen/thawed cells in 10 mL of 50 mM K-phosphate buffer (pH 8.0) and followed by HPLC analysis as described above. In each case, the expected *trans*-diol was formed without any byproduct. Hydrolysis of (±)-**2a** was examined with three different concentrations (Table 2), and the enzymatic activity reached 15−18 U/g cdw, which is nearly the same as the epoxidation activity for **1a**. As expected, (+)-**2a** was hydrolyzed faster than (−)-**2a**, giving (−)-**2a** in high ee at 3−5 h. The enantioselectivity factor E was calculated as 12.8 (for 20 mM at 5 h) from the equation $E = \ln(1 - c)(1 - ee_s)/\ln(1 - c)(1 + ee_s)$.[24]

As shown in Figure 2, **2b** was hydrolyzed as fast as **2a**. Although the ee of **2b** could not be determined, the ee of the formed diol **3b** was found to be very low. The enantioselectivity factor was calculated as 2.7 (for 10 mM

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TABLE 2. Hydrolysis of *N*-Substituted 3,4-Epoxy-piperidines 2a,b and 3,4-Epoxy-pyrrolidines 5a,b with Frozen/Thawed Cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200

entry	substrate (mM)	activity[a] (U/g cdw)	product	conversion and ee[b] (%)[c] 0.5 h	1 h	2 h	3 h	4 h	5 h
1	**2a** (10)	15	(−)-**2a**	56 (30)	46 (49)	32 (92)	27 (>99)		
			(+)-**3a**	44 (63)	54 (60)	68 (39)	73 (23)		
2	**2a** (15)	15	(−)-**2a**	71 (17)	61 (28)	46 (58)	39 (87)	32 (>99)	
			(+)-**3a**	29 (64)	39 (69)	54 (64)	61 (57)	68 (36)	
3	**2a** (20)	18	(−)-**2a**	73 (13)	63 (20)	53 (45)	45 (71)	41 (89)	35 (97)
			(+)-**3a**	27 (66)	37 (68)	47 (66)	55 (62)	59 (56)	65 (50)
4	**2b** (10)	15	**2b**	56	50	33	23	11	8.9
			(+)-**3b**	44 (10)	50 (11)	67 (14)	77 (16)	89 (11)	91 (7)
5	**2b** (20)	17	**2b**	75	68	58	48	45	39
			(+)-**3b**	25 (12)	32 (16)	42 (19)	52 (17)	55 (19)	61 (15)
6	**5a** (5.0)	6.2	(+)-**6a**	37 (68)	49	83	93	98	>99 (68)
7	**5a** (8.0)	8.1	(+)-**6a**	31 (68)	36	63	77	83	95 (66)
8	**5a** (10)	9.2	(+)-**6a**	28 (67)	36	57	70	76	91 (67)
9	**5b** (10)[d]	17	(+)-**6b**	52 (95)	72	88	98	>99	>99 (95)
10	**5b** (15)[d]	18	(+)-**6b**	37 (95)	53	70	86	96	98 (95)
11	**5b** (20)[d]	18	(+)-**6b**	27 (95)	43	56	70	79	85 (95)

[a] Biotransformation was performed in a 10 mL cell suspension in 50 mM K-phosphate buffer (pH 8.0) containing glucose (2%), and the activity was determined over the first 30 min. [b] Ee is given in parentheses. [c] Conversion and ee were determined by HPLC analysis; error limit is 2% of the stated values. [d] Data from ref 11.

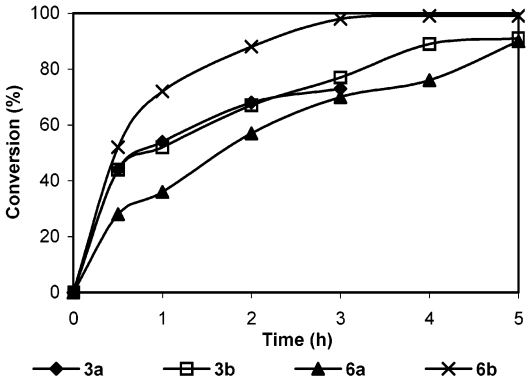


FIGURE 2. Hydrolysis of (±)-**2a**,**b** and **5a**,**b** (all 10 mM) to the corresponding **3a**,**b** and **6a**,**b** with frozen/thawed cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200.

at 4 h) according to the equation $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.[24] Hydrolysis of the *meso*-epoxide **5b** is the fastest one among all hydrolyses, and the *trans*-diol **6b** was obtained in 95% ee and >99% conversion.[11] For this asymmetric reaction, E was calculated as 39 according to the equation $E = (1 + ee_p)/(1 - ee_p)$.[24] Hydrolysis of **5a** showed the lowest activity (6−9 U/g cdw), which explains why a significant amount of **5a** remained in the trans dihydroxylation of **4a** (Figure 1c). The diol **6a** was formed in 67% ee, corresponding to an E of 5.1. Obviously, the epoxide hydrolase of HXN-200 prefers substrates with special size: *N*-phenoxycarbonyl epoxypiperidine **2a** and *N*-benzyloxycarbonyl epoxypyrrolidine **5b** have similar sizes, and both of them are excellent substrates for the hydrolysis with high activity and enantioselectivity; hydrolysis of **2b** with one carbon more or **5a** with one carbon less is significantly less enantioselective. Comparison of the retention times in chiral HPLC chromatograms suggested the same enantiomer in excess for diols **3a**,**b** and **6a**,**b** obtained from dihydroxylation of **1a**,**b** and **4a**,**b** and from hydrolysis of **2a**,**b** and **5a**,**b**, respectively.

Preparative Trans Dihydroxylation of 1a and 4b and Absolute Stereochemistry. To demonstrate the synthetic applicability of the enantioselective trans dihydroxylation, preparative biotransformations were performed with frozen/thawed cells as biocatalyst in a shaking flask (Scheme 2). Bioconversion of **1a** (10 mM) on a 100-mL scale at a cell density of 10 g cdw/L gave 91% of (+)-**3a** at 3 h. Extraction of the product with *n*-butanol/ethyl acetate (1:1) and purification by flash chromatography on silica gel afforded 77% of (+)-**3a** in 90% ee (Table 3). Reaction of **1a** at an increased concentration (15 mM) for 3 h gave (+)-**3a** in 96% ee with 83% conversion, and the pure product was isolated in 60% yield (106.9 mg) with an $[\alpha]^{25}_D$ of +4.24 (*c* 1.44 in $CHCl_3$). Similarly, biotransformation of **4b** (2.0 mM) on a 100-mL scale for 3 h gave 95% of (+)-**6b**. Workup and chromatographic purification afforded 80% (37.8 mg) of the pure product in 96% ee with an $[\alpha]^{25}_D$ of +7.56 (*c* 1.80 in $CHCl_3$).

To establish the absolute configuration, bioproduct (+)-**3a** was transformed into 3,4-dihydroxy piperidine hydrochloride **7** in 72% yield by treatment with 6 N HCl (Scheme 2). The obtained compound **7** has an $[\alpha]^{25}_D$ of −14.0 (*c* 0.50 in MeOH), while the chemically synthesized (3*R*,4*R*)-**7**[25] has an $[\alpha]^{25}_D$ of −15.0 (*c* 0.24 in MeOH) that was determined in this study. Therefore, the absolute configuration of bioproduct (+)-**3a** can be deduced as (3*R*,4*R*). Treatment of (−)-**7** with benzyl chloroformate gave (+)-(3*R*,4*R*)-**3b** in 71% yield. Comparison of the retention times of (+)-(3*R*,4*R*)-**3b** and bioproduct **3b** in chiral HPLC chromatograms suggested the (+)-(3*R*,4*R*) for the major enantiomer of bioproduct **3b**. Deprotection of bioproduct (+)-**6b** by hydrogenation gave 94% of (−)-(3*R*,4*R*)-**8** with an $[\alpha]^{25}_D$ of −18.6 (*c* 0.80, in MeOH), thus establishing the absolute configuration of bioproduct (+)-**6b** as (3*R*,4*R*).[11,26] Reaction of (−)-**8** with phenyl chloro-

(23) (a) Punniyamurthy, T.; Katsuki, T. *Tetrahedron* **1999**, *55*, 9439−9454. (b) Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martin, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, I.; Garren, K. W.; Hoffman, D. J.; Plattner, J. J. *J. Med. Chem.* **1990**, *33*, 1962−1969.

(24) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559−571.

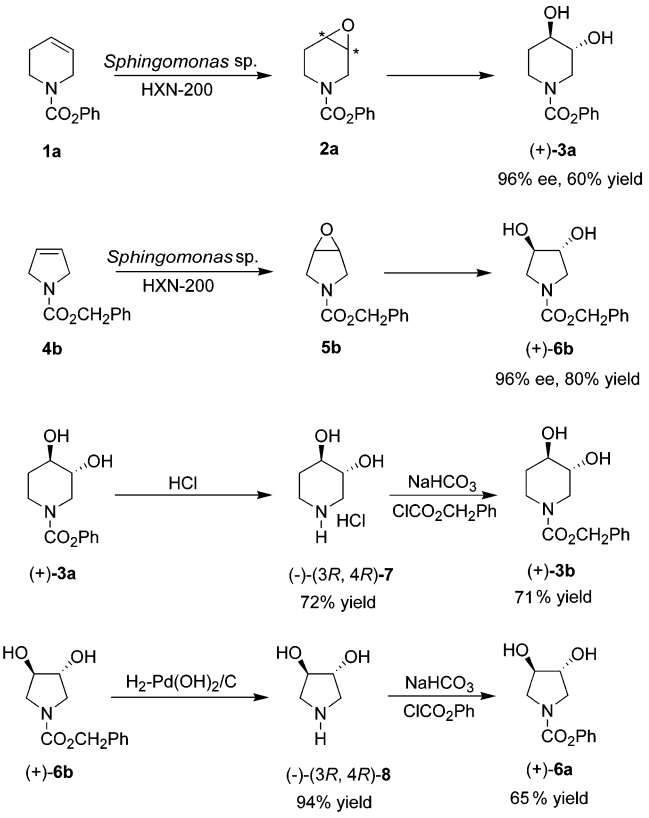
(25) An authentic sample of (3*R*,4*R*)-**7** prepared according to ref 12a was given by Prof. Y. Ichikawa at Johns Hopkins University.

TABLE 3. Preparation of (+)-3a, (+)-6b, (−)-2a, and (−)-3a by Biotransformation with Frozen/Thawed Cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200

entry	substrate (mM)	scale (mL)	activity (U/g cdw)	product	time (h)	conversion[a] (%)	yield[b] (%)	ee[a] (%)
1	**1a** (10.0)	100	27[c]	(+)-**3a**	3.0	91	77.4	90
2	**1a** (15.0)	50	37[c]	(+)-**3a**	3.0	83	60.0	96
3	**4b** (2.0)	100	6.0[c]	(+)-**6b**	3.0	95	79.8	96
4	(±)-**2a** (15.0)	50	13[d]	(−)-**2a**	3.0	66	33.5	>99
5	(−)-**2a** (5.0)	20	6.7[d]	(−)-**3a**	6.0	>99	91.6	96

[a] Conversion and ee were determined by HPLC analysis; error limit is 2% of the stated values. [b] Yield of the isolated pure product. [c] Epoxidation activity was determined form the total product formation over the first 30 min. [d] Hydrolysis activity was determined over the first 30 min.

SCHEME 2. Preparation of (+)-3a and (+)-6b by Enantioselective Trans Dihydroxylation of 1a and 4b with *Sphingomonas* sp. HXN-200 and Stereochemistry Correlation of Bioproducts 3a,b and 6a,b



formate afforded (+)-(3*R*,4*R*)-**6a** in 65% yield, and comparison of the retention times in chiral HPLC analysis established the absolute configuration as (+)-(3*R*,4*R*) for the major enantiomer of bioproduct **6a**.

Preparation of (−)-2a and (−)-3a by Hydrolysis with *Sphingomonas* sp. HXN-200. The epoxide hydrolase of HXN-200 was shown to be enantioselective in hydrolysis of racemic **2a**, thus enabling the preparation of enantiomerically pure epoxide **2a** via resolution (Figure 3). Hydrolysis of racemic **2a** (15 mM) for 3 h gave (−)-**2a** in >99.9% ee. Workup and purification afforded 34% of (−)-**2a** with an $[\alpha]^{25}_D$ of − 18.05 (*c* 1.23 in $CHCl_3$) (Table 3). This synthesis is important, since enantiopure epoxide **2a** is very difficult to prepare by either chemical

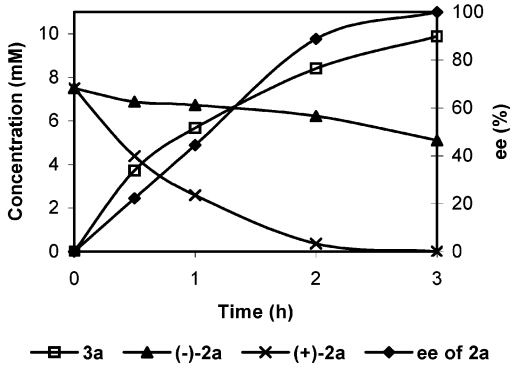


FIGURE 3. Hydrolysis of (±)-**2a** (15 mM) to **3a** with frozen/thawed cells (10 g cdw/L) of *Sphingomonas* sp. HXN-200.

or enzymatic methods and it can be a versatile chiral building block. Following the highly regioselective ring opening of racemic 3,4-epoxypiperidine with HBr,[27] (−)-**2a** could be transformed into enantiopure *trans*-4-bromo-3-hydroxy-piperidine, which can be further transformed into other enantiopure compounds such as *cis*-4-amino-3-hydroxy- and *cis*-3,4-dihydroxy-piperidine. Moreover, (−)-**2a** is a useful intermediate for the preparation of single enantiomer of antidepressant Ifoxetine sulfate[28] and prokinetic agent Cisapride hydrate.[29,30]

While (+)-**3a** was prepared from **1a** by enantioselective trans dihydroxylation, (−)-**3a** was also successfully prepared by hydrolysis of (−)-**2a** (5 mM) with HXN-200 (Scheme 3). The hydrolysis activity for (−)-**2a** is 6.6 U/g cdw, which is half of that for the racemic **2a**. Nevertheless, (−)-**2a** was totally converted to the corresponding *trans*-diol **3a** after 6 h of biotransformation (Table 3). Workup and chromatographic purification afforded (−)-**3a** in 92% yield and 96% ee with an $[\alpha]^{25}_D$ of − 4.05 (*c* 0.65 in $CHCl_3$). The high ee of the product indicates that the ring opening of (−)-**2a** is highly regioselective.

Conclusion

Sphingomonas sp. HXN-200 containing a monooxygenase and an epoxide hydrolase catalyzes the trans dihydroxylation of *N*-phenoxycarbonyl-1,2,5,6-tetrahy-

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(28) Waldmeier, P. C.; Delini-Stula, A.; Paioni, R. *Drugs Future* **1987**, *12*, 126.

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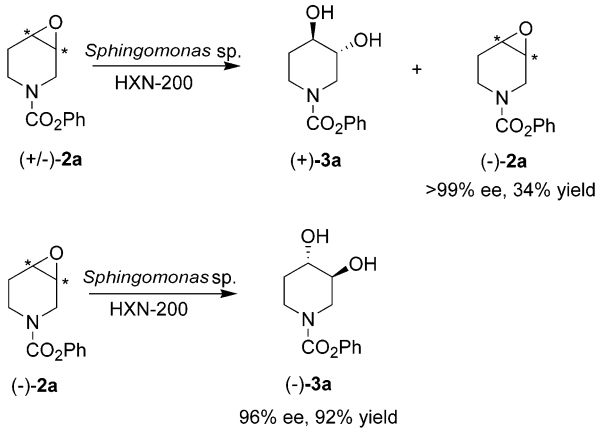
SCHEME 3. Preparation of (−)-2a and (−)-3a by Hydrolysis with *Sphingomonas* sp. HXN-200



dropyridine **1a** and *N*-benzyloxycarbonyl-3-pyrroline **4b**, respectively, with high activity and high enantioselectivity. While enantioselectivity for the trans dihydroxylation of **1a** is a combination of those for the epoxidation of **1a** and hydrolysis of **2a**, the enantioselectivity for the trans dihydroxylation of symmetric substrate **4b** is due only to the hydrolysis of *meso*-epoxide **5b**. The absolute configuration was established as (3*R*,4*R*) for both (+)-**3a** and (+)-**6b** by chemical correlations. Preparative trans dihydroxylations of **1a** and **4b** with frozen/thawed cells of *Sphingomonas* sp. HXN-200 as biocatalyst afforded the corresponding (+)-3,4-dihydroxypiperidine **3a** and (+)-3,4-dihydroxypyrrolidine **6b** in 60% and 80% yield, respectively, and both in 96% ee. These highly yielding and highly enantioselective trans dihydroxylations provide the simplest syntheses of the *trans*-diols which are useful pharmaceutical intermediates. These are the first enantioselective trans dihydroxylations of a nonisoprene C−C double bond of an aliphatic substrate and the first bacterium-catalyzed enantioselective trans dihydroxylations, thus significantly extending this useful methodology in organic synthesis.

Enantioselective hydrolysis of the racemic epoxide **2a** with the epoxide hydrolase of HXN-200 afforded (−)-**2a**, a versatile chiral building block, in >99% ee and 34% yield. Further hydrolysis of (−)-**2a** with HXN-200 showed excellent regioselectivity and gave (−)-(3*S*,4*S*)-**3a** in 96% ee and 92% yield. Thus, both enantiomers of **3a** can be prepared in high ee by biotransformation with *Sphingomonas* sp. HXN-200.

Experimental Section

***N*-Phenoxycarbonyl-1,2,5,6-tetrahydropyridine (1a).** A solution of phenyl chloroformate (1.50 mL, 12.0 mmol) in THF (5 mL) was added dropwise to a stirred mixture of 1,2,5,6-tetrahydropyridine (0.996 g, 12.0 mmol) and $NaHCO_3$ (1.30 g, 15.6 mmol) in THF−water (1:1, 12 mL) at 0 °C, and the mixture was stirred at room temperature for 3 h. $CHCl_3$ (20 mL) and 5% aqueous Na_2CO_3 (10 mL) were added, the organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phase was dried over Na_2SO_4 and filtered, and the solvent was removed by evaporation. Column chromatography on silica gel gave 1.6953 g (69.6%) of **1a**[18] as solid. Mp 55.9−56.7 °C; R_f 0.38, (*n*-hexane/ethyl acetate 8/2); purity 99.0% (GC, t_R = 8.20 min); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (t, 2 H, J = 7.4 Hz), 7.20 (t, 1 H, J = 7.2 Hz), 7.12 (d, 2 H, J = 7.2 Hz), 5.90 (s, 1 H), 5.73 (s, 1 H), 4.15 (s, 1 H), 4.04 (s, 1 H), 3.74 (s, 1 H), 3.65 (s, 1 H), 2.25 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 152.5, 130.3, 126.3, 122.9, 126.7, 126.2, 125.4, 124.8, 44.9, 42.4, 41.6, 26.4, 25.9; MS *m*/*z* 204 (100%, M + 1); IR ($CHCl_3$) ν 1713, 1425, 1238, 1204 cm^{-1}.

***N*-Phenoxycarbonyl-3,4-epoxypiperidine (2a).** *m*-CPBA (1.43 g, 8.34 mmol) was added to a solution of **1a** (740.6 mg, 3.64 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred at room temperature for 24 h. NaOH (1 N, 15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was separated and dried over Na_2SO_4, and the solvent was removed. Column chromatography on a silica gel gave 631.2 mg (79.0%) of **2a** with 99.7% purity (GC, t_R = 9.02 min). The spectroscopic data are the same as those for (−)-**2a** reported below.

***N*-Phenoxycarbonyl-3,4-dihydroxypiperidine (3a).** TFA (0.30 mL) was added to a solution of **2a** (30.0 mg, 0.137 mmol) in $CHCl_3$ (2 mL) and the mixture was stirred at reflux for 6 h. pH was adjusted to 8.0 by addition of 10% Na_2CO_3 and the mixture was extracted with $CHCl_3$ (3 × 20 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed. Column chromatography on silica gel afforded 16.7 mg (51.5%) of **3a** with a purity of 99.3% (GC, t_R = 10.30 min). The spectroscopic data are the same as those for (+)-**3a** reported below.

***N*-Benzyloxycarbonyl-3,4-dihydroxypiperidine (3b).** Similar to the preparation of **3a**, hydrolysis of **2b** (30.0 mg, 0.129 mmol) gave 19.9 mg (61.6%) of **3b** with 98.7% purity (GC, t_R = 9.53 min). The spectroscopic data agree with those reported in ref 12b.

***N*-Phenoxycarbonyl-3,4-epoxy-pyrrolidine (5a).** Similar to the preparation of **2a**, reaction of *m*-CPBA (0.52 g, 3.0 mmol) with **4a** (284 mg, 1.50 mmol) gave 205.0 mg (66.7%) of **5a** as solid. Mp 72.1−73.0 °C; R_f 0.42 (*n*-hexane/ethyl acetate 1/1); purity 99.0% (GC, t_R = 9.05 min); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (t, 2 H, J = 7.41 Hz), 7.20 (t, 1 H, J = 7.2 Hz), 7.11 (d, 2 H, J = 7.2 Hz), 4.04 (d, 1 H, J = 13.2 Hz), 3.95 (d, 1 H, J = 13.2 Hz), 3.77 (s, 2 H), 3.58 (d, 1 H, J = 13.2 Hz), 3.48 (d, 1 H, J = 13.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.7, 152.1, 130.4, 126.5, 122.8, 56.6, 56.1, 48.7; MS *m*/*z* 206 (100%, M + 1); IR ($CHCl_3$) ν 1721, 1420, 1392, 1204 cm^{-1}.

***N*-Phenoxycarbonyl-3,4-dihydroxy-pyrrolidine (6a).** Similar to the preparation of **3a**, reaction of **5a** (30.0 mg, 0.15 mmol) with TFA (0.30 mL) afforded 26.9 mg (82.4%) of **6a** with 97.6% purity (GC, t_R = 9.30 min). The spectroscopic data are the same as those for (+)-**6a** reported below.

General Procedure for Bioconversion on a Small Scale. Frozen cells of *Sphingomonas* sp. HXN-200 were thawed and suspended to a cell density of 10 g cdw/L in 10 mL of 50 mM K-phosphate buffer containing glucose (2% w/v) at pH 8.0 in a 100-mL Erlenmeyer flask. Substrates were added either directly (**1a**, **2b**, **4a**, **5a**) or as a MeOH solution (**1b**, **2a**, **4b**, **5b**) to a final concentration of 2−20 mM. The mixture was shaken at 200 rpm at 30 °C for 3−5 h and the reaction was followed by HPLC analysis of samples that were prepared by taking aliquots (0.1−0.2 mL) from a bioconversion mixture at predetermined time points and mixing with an equal volume of MeOH followed by removal of the cells by centrifugation. The ee of the products was determined by HPLC analysis on a chiral column, and the samples were prepared by taking aliquots (0.5 mL) from a biotransformation mixture, mixing with an equal volume of ethyl acetate, removing the cells, separating the organic phase after centrifugation, drying over sodium sulfate, and filtering. The results are listed in Tables 1 and 2.

(a) HPLC analysis of conversion: Hypersil BDS-C18 column (5 μm, 125 mm × 4 mm); eluent, a mixture of A (10 mM K-phosphate buffer, pH 7.0) and B (acetonitrile); flow rate, 1.0 mL/min; detection, UV at 210, 225, and 254 nm; t_R of **3a** = 1.6 min, t_R of **2a** = 3.4 min, t_R of **1a** = 9.1 min (A/B 65:35); t_R of **3b** = 1.8 min, t_R of **2b** = 4.1 min, t_R of **1b** = 10.8 min (A/B 70:30); t_R of **6a** = 1.8 min, t_R of **5a** = 3.3 min, t_R of **4a** =

7.5 min (A/B 70:30); t_R of **6b** = 2.0 min, t_R of **5b** = 4.2 min, t_R of **4b** = 9.2 min (A/B 70:30).

(b) HPLC analysis of ee: chiral column (250 mm × 4.6 mm); UV detection at 210 nm; a mixture of A (*n*-hexane) and B (2-propanol). For **2a**: Chiralcel OB-H column; flow rate, 0.5 mL/min, A/B (60/40); t_R = 49.4 and 55.4 min. For **3a**: Chiralcel OB-H column; flow rate, 0.5 mL/min, A/B (95/5); t_R = 92.7 and 110.4 min. For **3b**: Chiralpak AS column; flow rate, 1.0 mL/min, A/B (93/7); t_R = 25.9 and 29.0 min. For **6a**: Chiralcel OD-H column; flow rate, 0.5 mL/min, A/B (85/15); t_R = 18.1 and 25.5 min. For **6b**: Chiralpak AS column; flow rate, 1.0 mL/min, A/B (97/3); t_R = 114.1 and 130.4 min.

General Procedure for Biotransformation on a Preparative Scale. Substrate was added to a 20−100 mL suspension of frozen/thawed cells of *Sphingomonas sp.* HXN-200 (10.0 g cdw/L) in 50 mM K-phosphate buffer (pH 8.0) containing glucose (2%) in a 100−500 mL shaking flask. The mixture was shaken at 200 rpm and 30 °C for 3−6 h. The cells were removed by centrifugation and the supernatant was extracted with *n*-butanol/ethyl acetate (1:1) (for **3a**, **6b**) or ethyl acetate (for **2a**). 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 and the results are summarized in Table 3.

(+)-(3*R*,4*R*)-*N*-Phenoxycarbonyl-3,4-dihydroxy-piperidine (3a). Trans dihydroxylation of **1a** (152.3 mg, 0.75 mmol) on a 50-mL scale gave 106.9 mg (60.0%) of (+)-**3a** as solid. Mp 136.8−137.5 °C; ee 96% (3*R*,4*R*); $[\alpha]^{25}_D$ +4.24 (*c* 1.44, $CHCl_3$); R_f 0.32 (ethyl acetate); purity 99.3% (GC, t_R = 10.30 min); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (t, 2 H, *J* = 7.8 Hz), 7.20 (t, 1 H, *J* = 7.4 Hz), 7.08 (d, 2 H, *J* = 8.4 Hz), 4.34−4.15 (m, 2 H); 3.54−3.45 (m, 2 H), 3.12−2.77 (m, 3 H), 2.04−1.96 (m, 1 H), 1.90 (s, 1 H), 1.59−1.56 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 152.2, 130.4, 126.6, 122.8, 74.5, 73.9, 72.9, 72.5, 49.2, 49.0, 43.8, 43.5, 32.4; MS *m*/*z* 238 (9%, M + 1), 118 (100%); IR ($CHCl_3$) ν 3614, 3425, 1713, 1428, 1204 cm^{-1}.

(+)-(3*R*,4*R*)-*N*-Benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine (6b). Trans dihydroxylation of **4b** (40.6 mg, 0.20 mmol) on a 100-mL scale gave 37.8 mg (79.8%) of (+)-**6b** as a syrup: ee 96% (3*R*,4*R*); $[\alpha]^{25}_D$ +7.56 (*c* 1.80, $CHCl_3$); R_f 0.27 (ethyl acetate); purity 98.9% (GC, t_R = 10.61 min). The spectroscopic data agree with those for (3*R*,4*R*)-**6b** reported in ref 13a.

(−)-*N*-Phenoxycarbonyl-3,4-epoxy-piperidine (2a). Hydrolysis of racemic **2a** (164.3 mg, 0.75 mmol) on a 50-mL scale afforded 54.9 mg (33.5%) of (−)-**2a** as a syrup: ee >99.9%; $[\alpha]^{25}_D$ −18.05 (*c* 1.23, $CHCl_3$); R_f 0.24 (hexane/ethyl acetate 2/1); purity 99.7% (GC, t_R = 9.02 min); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (t, 2 H, *J* = 7.8 Hz), 7.20 (t, 1 H, *J* = 7.2 Hz), 7.10 (dd, 2 H, *J* = 7.2, 6.8 Hz), 4.08−3.97 (m, 2 H), 3.80 (d, 0.5 H, *J* = 17.2 Hz), 3.67 (dt, 0.5 H, *J* = 13.2, 5.2 Hz), 3.55 (dt, 0.5 H, *J* = 13.2, 5.2 Hz), 3.39−3.27 (m, 2.5 H), 2.16 (dt, 1 H, *J* = 14.8, 4.4 Hz), 2.08−1.98 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1, 154.9, 152.3, 130.4, 126.5, 122.8, 51.8, 51.6, 51.4, 51.1, 44.0, 43.8, 39.2, 38.7, 25.8, 25.2; MS *m*/*z* 220 (90%, M + 1), 118 (100%); IR ($CHCl_3$) ν 1716, 1428, 1205 cm^{-1}.

(−)-(3*S*,4*S*)-*N*-Phenoxycarbonyl-3,4-dihydroxy-piperidine (3a). Hydrolysis of (−)-**2a** (21.9 mg, 0.10 mmol) on a 20-mL scale gave 21.7 mg (91.6%) of (−)-**3a**: purity 97.9% (GC, t_R = 10.30 min); ee 96% (3*S*,4*S*); $[\alpha]^{25}_D$ −4.05 (*c* 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (t, 2 H, *J* = 7.8 Hz), 7.20 (t, 1 H, *J* = 7.5 Hz), 7.08 (d, 2 H, *J* = 7.8 Hz), 4.38−4.15 (m, 2 H), 3.56−3.47 (m, 2 H), 3.18−2.75 (m, 2 H), 2.52 (s, 2 H), 2.05−1.95 (m, 1 H), 1.60−1.55 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.6, 151.0, 129.2, 125.4, 121.6, 73.4, 72.9, 71.9, 71.5, 48.2, 48.1, 42.7, 42.4, 31.4; MS *m*/*z* 238 (100%, M + 1); IR ($CHCl_3$) ν 3621, 3457, 1713, 1428, 1209 cm^{-1}.

Absolute Stereochemistry of Bioproducts. Transformation of Bioproduct (+)-3a to (−)-(3*R*,4*R*)-3,4-Dihydroxypiperidine Hydrochloride (7). A suspension of bioproduct (+)-**3a** (50.0 mg, 0.21 mmol) in MeOH (1 mL) and 6 N HCl (5 mL) was stirred at reflux for 2 h. pH was adjusted to 10 by addition of 12 N NaOH and the solvent was evaporated to dryness. The residue was treated with MeOH and purified by column chromatography on silica gel (R_f 0.18, $CHCl_3$/MeOH/25%aq NH_3H_2O 8:3:1) to afford 20.2 mg (81.8%) of 3,4-dihydroxypiperidine. Part of this product (10.0 mg, 0.085 mmol) was dissolved in MeOH (2 mL), HCl (32%, 50 μL) was added, and the resulting mixture was evaporated to dryness giving 11.5 mg (87.7%) of (−)-**7** as a solid: $[\alpha]^{25}_D$ −14.0 (*c* 0.50, MeOH). The spectroscopic data agree with those for (3*R*,4*R*)-**7** reported in ref 12a. $[\alpha]^{25}_D$ of a synthetic sample of (3*R*,4*R*)-**7**[25] was determined as −15.0 (*c* 0.24, MeOH).

Transformation of (−)-7 to (+)-(3*R*,4*R*)-*N*-Benzyloxycarbonyl-3,4-dihydroxy-piperidine (3b). Phenyl chloroformate (0.050 mL, 0.397 mmol) in THF (2 mL) was added dropwise to a mixture of (−)-**7** (10.0 mg, 0.085 mmol) and $NaHCO_3$ (160 mg, 1.905 mmol) in THF/H_2O (1:1, 4 mL) at room temperature, the mixture was stirred for 20 h, and ethyl acetate (30 mL) was added. The organic phase was separated, dried over Na_2SO_4, and filtered, and the solvent was removed by evaporation. Column chromatography on silica gel gave 15.2 mg (71.2%; 0.061 mmol) of (3*R*,4*R*)-**3b**: $[\alpha]^{25}_D$ +3.51 (*c* 0.76, $CHCl_3$); R_f 0.38 (ethyl acetate); purity 97.9% (GC, t_R = 9.53 min). The spectroscopic data agree with those reported in ref 12b. The major enantiomer of bioproduct **3b** has the same retention time as the chemically prepared (+)-(3*R*,4*R*)-**3b**.

Transformation of Bioproduct (+)-6b to (−)-(3*R*,4*R*)-3,4-Dihydroxypyrrolidine (8). A mixture of bioproduct (+)-**6b** (100 mg, 0.42 mmol) and 20% $Pd(OH)_2$/C (55 mg) in MeOH (3.0 mL) was stirred under hydrogen for 24 h and filtered through Celite. Column chromatography on silica gel afforded **8** in 93.9% (40.8 mg) as waxy solid: R_f 0.17, (CH_2Cl_2/MeOH/25% aq NH_3H_2O 8:3:1); $[\alpha]_D^{25}$ −18.6 (*c* 0.80, MeOH); ^{1}H NMR (D_2O, 400 MHz) δ 4.03−4.01 (m, 2 H), 3.08 (dd, 1 H, *J* = 12.8, 4.4 Hz); 2.73 (d, 1 H, *J* = 12.8 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 79.6, 54.6; MS (*m*/*z*) 104 (100%, M + 1); $[\alpha]^{26}_D$ of (3*S*,4*S*)-**8** was reported to be +20.7 (*c* 0.3, MeOH).[26]

Transformation of (−)-8 to (+)-(3*R*,4*R*)-*N*-Phenoxycarbonyl-3,4-dihydroxy-pyrrolidine (6a). Similar to the transformation of (−)-**7** to (+)-**3b**, reaction of (−)-**8** (10.0 mg, 0.097 mmol) with phenyl chloroformate (0.050 mL, 0.397 mmol) in THF (2 mL) gave 14.0 mg (64.9%, 0.063 mmol) of (3*R*,4*R*)-**6a** as solid: mp 125.4−126.1 °C; $[\alpha]^{25}_D$ +17.71 (*c* 0.70, MeOH); R_f 0.22 (ethyl acetate); purity 97.8% (GC, t_R = 9.30 min); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (t, 2 H, *J* = 7.4 Hz), 7.20 (t, 1 H, *J* = 7.2 Hz), 7.12 (d, 2 H, *J* = 7.2 Hz), 4.22 (m, 1 H), 4.18 (m, 1 H), 3.86 (dd, 1 H, *J* = 12.1, 4.4 Hz), 3.76 (dd, 1 H, *J* = 12.1, 4.4 Hz), 3.58 (d, 1 H, *J* = 12.1 Hz), 3.50 (d, 1 H, *J* = 12.1 Hz), 2.13 (s, 2 H); ^{13}C NMR (CD_3OD, 75 MHz) δ 155.3, 152.5, 130.2, 126.4, 122.7, 76.2, 75.4, 53.3; MS (*m*/*z*) 224 (100%, M + 1); IR ($CHCl_3$) ν 3620, 3444, 1714, 1408 cm^{-1}. The major enantiomer of bioproduct **6a** has the same retention time as the chemically prepared (+)-(3*R*,4*R*)-**6a**.

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Supporting Information Available: General experimental details; ^{1}H and ^{13}C NMR spectra of the bioproducts (−)-**2a**, (−)-**3a**, (+)-**3a**, and (+)-**6b** and the chemically prepared compounds **1a**, **2a**, **3a**, (+)-**3b**, **5a**, **6a**, (+)-**6a**, (−)-**7**, and (−)-**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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