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Synthesis and Kinetic Analysis of the *N*-Acetylhexosaminidase Inhibitor XylNAc-Isofagomine

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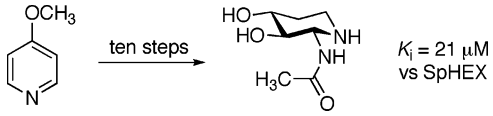

An efficient 10-step preparation from 4-methoxypyridine of (2*R*,3*R*,4*R*)-2-acetamido-3,4-dihydroxypiperidine ("XylNAc-isofagomine") in optically active form is described. Key steps include an enantioselective reduction with catecholborane/(*S*)-2-methyl-CBS-oxazaborolidine, and a stereoselective *pseudo*-glycosylation of lithium azide by a cyclic sulfite ester. The title compound showed a $K_i = 21$ μM when evaluated against the *N*-acetyl-β-hexosaminidase from *Streptomyces plicatus*.

Introduction

Retaining *N*-acetyl-β-hexosaminidases, which promote the hydrolysis of terminal *N*-acetylglucosamine and *N*-acetylgalactosamine residues from glycoconjugates, are found widely among organisms from bacteria to mammals. Their importance is exemplified by two human enzymes of this type, hexosaminidase A (HEX A) and hexosaminidase B (HEX B), whose malfunction can lead to Tay-Sachs and Sandhoff diseases, respectively.[1] Studies of the action and mode of binding of various *N*-acetylhexosaminidase substrates and inhibitors has enabled the formulation of two distinct double-inversion mechanistic possibilities.[2] Either an enzyme carboxylate participates intermolecularly at the anomeric carbon[3] or that role is taken by the substrate acetamido carbonyl in the intramolecular sense. In the first case (found among family[4] 3 enzymes), a covalently bound *O*-glycosyl carboxylate intermediate is formed, whereas in the second case, found for *N*-acetylhexosaminidases of families 20 and 84[5] (and likewise for family 18 chitinases and family 56 hyaluronidases), there is an intermediate oxazolinium ion held noncovalently in the enzyme active site (Figure 1).

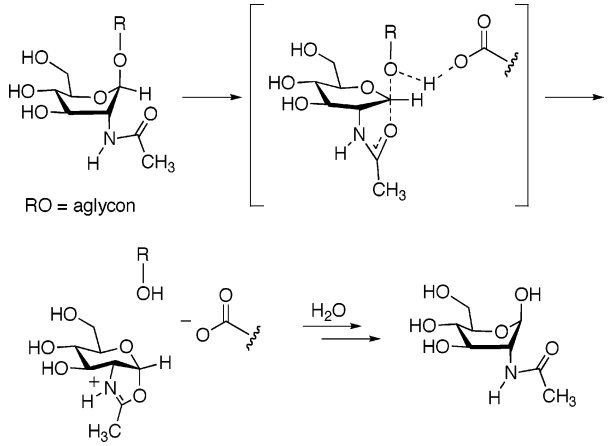

FIGURE 1. Retaining*N*-acetyl-β-hexosaminidase mechanism featuring participation by the 2-acetamido carbonyl oxygen at C-1 and an oxazolinium intermediate.

Both GlcNAc-isofagomine (**1**)[6] and GalNAc-isofagomine (**2**),[7–9] in their N-protonated forms, show good inhibition against various mammalian *N*-acetyl-β-hexosaminidases.

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(1) Gravel, R. A.; Clark, J. T. R.; Kaback, M. M.; Mahuran, D.; Sandhoff, K.; Suzuki, K. In *The Metabolic Basis of Inherited Disease*; Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds; McGraw-Hill: New York, 1995; pp 1807–1839.

(2) Mark, B. L.; James, M. N. G. *Can. J. Chem.* **2002**, *80*, 1064–1074.

(3) Vocadlo, D. J.; Mayer, C.; He, S.; Withers, S. G. *Biochemistry* **2000**, *39*, 117–126.

(4) Classification of glycosyl hydrolases into families: (a) Henrissat, B.; Bairoch, A. *Biochem. J.* **1996**, *316*, 695–696. (b) http://afmb.cnrs-mrs.fr/CAZY (accessed May 1, 2005).

(5) Macauley, M. S.; Whitworth, G. E.; Debowski, A.; Chin, D.; Vocadlo, D. J. *J. Biol. Chem.* **2005**, *280*, 25313–25322.

(6) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem.* **1999**, *7*, 1241–1246.

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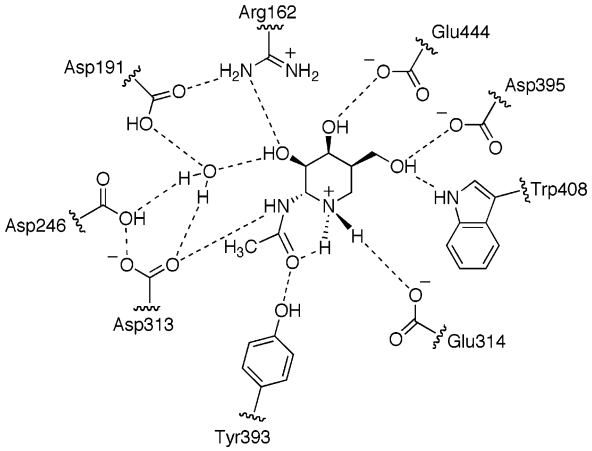
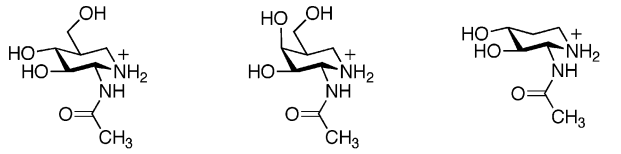


FIGURE 2. Schematic representation of the important active-site H-bonding interactions between **2** and SpHEX (data taken from ref 10).

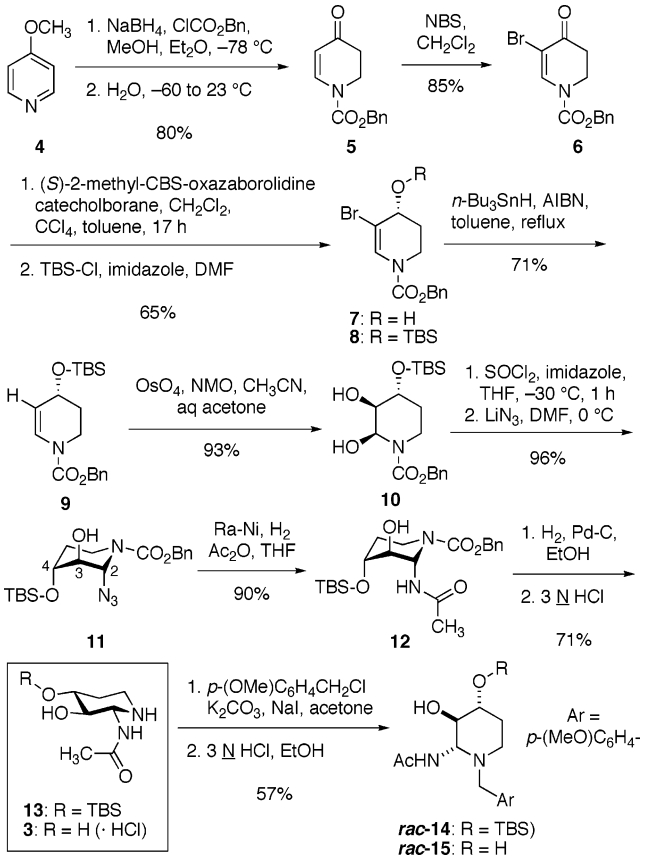
The inhibitor GalNAc-isofagomine (**2**) exhibits a K_i of 2.7 μM against the family 20 *N*-acetylhexosaminidase from *Streptomyces plicatus* (SpHEX), a bacterial enzyme that employs the acetamido-participating mechanism of Figure 1 and processes both gluco- and galacto-configured substrates.[10]



1: GlcNAc-isofagomine **2**: GalNAc-isofagomine **3**: XylNAc-isofagomine

Crystallographic studies of GalNAc-isofagomine bound in the SpHEX active site (Figure 2) indicate that protonated **2** is held in a slightly flattened (at the endocyclic nitrogen) chair conformation, with notable hydrogen bonds between the axial N(1)−H and the amide carbonyl and between the equatorial N−H and the general acid residue Glu314. With its amide carbonyl oxygen positioned under the ring and close to N-1, the bound form of **2** resembles the transition state that precedes the oxazolinium intermediate of Figure 1. Hydrogen bonds involving the inhibitor hydroxyls (two at O-3, one at O-4, and two at O-6) contribute further to the binding. Although **2** has the galacto configuration at C-4, related crystallographic studies on SpHEX[11] indicate that an inhibitor of the gluco configuration enjoys an additional H-bond interaction with Arg162 (a total of two H-bonds at O-4). Thus, modifying the configuration of **2** to gluco might increase the affinity of the inhibitor (i.e., GlcNAc-isofagomine, **1**) by an amount that reflects this additional hydrogen bond. On the other hand, if the C-6 hydroxymethyl group were deleted, the binding implicit in two hydrogen bonds at O-6 would theoretically be lost. And yet, in studies of another inhibitor/enzyme system, deletion of the hydroxylmethyl substituent of 1-deoxy-nojirimycin revealed interactions at that position to be "relatively unimportant for inhibitor binding" to sweet almond β-glucosidase.[12] It might also be useful to add aglycon mimics at N-1 or C-6 or groups that bind more tightly to the general acid Glu314. Structure **3**, "XylNAc-isofagomine," embodies the hydroxyl modifications, and is expected to be more easily synthesized than **1**, and thus also more easily converted to N-, C-, and O-substituted analogues[13] as a means to improve and understand the inhibition. We report here a short, enantioselective synthesis of **3** from 4-methoxypyridine.[14]

SCHEME 1

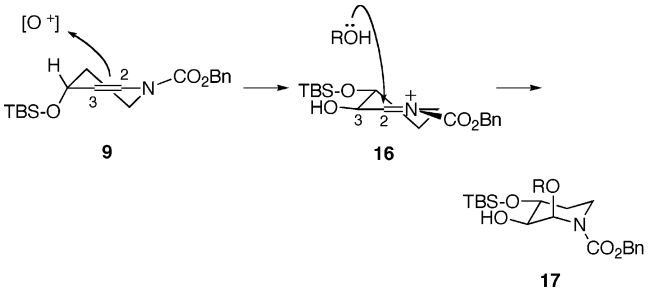


Results and Discussion

The synthetic route for the conversion of 4-methoxypyridine (**4**) to XylNAc-isofagomine **3** is shown in Scheme 1. Borohydride reduction of the benzyl chloroformate adduct of **4**, and then hydroysis, led to the N-protected dihydropiperidinone **5**. Reduction of **5** under the Luche conditions[15] ($CeCl_3$, $NaBH_4$) and then protection of the

(7) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *J. Antibiot.* **1999**, *52*, 348−350.
(8) Nishimura, Y.; Satoh, T.; Kudo, T.; Kondo, S.; Takeuchi, T. *Bioorg. Med. Chem.* **1996**, *4*, 91−96.
(9) Mark, B. L.; Mahuran, D. J.; Cherney, M. M.; Zhao, D.; Knapp, S.; James, M. N. G. *J. Mol. Biol.* **2003**, *327*, 1093−1109.
(10) Mark, B. L.; Vocadlo, D. J.; Zhao, D.; Knapp, S.; Withers, S. G.; James, M. N. G. *J. Biol. Chem.* **2001**, *276*, 42131−42137.
(11) Mark, B. L.; Vocadlo, D. J.; Knapp, S.; Triggs-Raine, B. L.; Withers, S. G.; James, M. N. G. *J. Biol. Chem.* **2001**, *276*, 10330−10337.
(12) Bernotas, R. C.; Papandreou, G.; Urbach, J.; Ganem, B. *Tetrahedron Lett.* **1990**, *31*, 3393−3396.
(13) Kondo, K.-I.; Adachi, H.; Nishimura, Y.; Takeuchi, T. *Nat. Prod. Lett.* **2001**, *15*, 371−375.
(14) Recent review of synthetic glycosidase inhibitors: Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. *Chem. Rev.* **2002**, *102*, 515−553.
(15) Comins, D. L.; Chung, G.; Foley, M. A. *Heterocycles* **1994**, *37*, 1121−1140.

SCHEME 2



hydroxyl afforded the racemic alkene ***rac*-9** in 92% overall yield. Alternatively, *enantioselective* reduction[16] of **5** with the CBS reagent (catecholborane, various solvents, −78 °C) gave an allylic alcohol with enantiomeric excess (ee) as high as 74%. The ee could be improved further by first brominating **5** to provide a ketone substrate **6** with more sterically differentiated flanking groups.[17] Reduction of **6** with (*S*)-2-methyl-CBS-oxazaborolidine and catecholborane now provided an allylic alcohol **7** whose ee measured 88.7%, as determined by chiral HPLC analysis. Protection of the hydroxyl gave the *tert*-butyldimethylsilyl ether **8**, and then reductive removal of the bromo substituent led to **9** in optically enriched form. Both forms of **9**, racemic and optically enriched, were taken through the remaining steps.

Trans functionalization of the alkene bond of **9** proved quite challenging. Although alkene oxidants (*m*-CPBA, OsO_4) generally approached from the less hindered β face, leading to the desired hydroxyl stereochemistry at C-3, subsequent *N*-acyliminium additions at C-2 with oxygen and nitrogen nucleophiles occurred primarily in an axial fashion, cis to the C-3 hydroxyl, and thus strongly favored β stereochemistry there as well (**16** → **17**, Scheme 2). For example, osmylation of **9** gave the cis diol **10**, and osmylation in the presence of *tert*-butyl alcohol or methanol gave similarly the respective 2-*S*-alkoxy adducts. Attempts to introduce a nucleophile α at C-2 by changing the piperidine ring conformation with an *O*-silyl protecting group at C-3,[18] or by incorporating an acetoxy participating group at C-3 of **10**, or by Mitsunobu reaction,[19] were largely unsuccessful.

Because the ring nitrogen activates C-2 toward substitution in a manner not unlike that of a pyranosyl anomeric carbon, substitutions at C-2 with relatively poor leaving groups seemed feasible. Furthermore, if the substitution were of the S_N2 type, then inversion at C-2 with a β-situated leaving group ought to deliver a product α-substituted at C-2. Cyclic 1,2-*O*-sulfites have been used to activate the anomeric position of glycosyl donors;[20] thus, the corresponding cyclic sulfite of diol **10** might lead to the desired 2,3-trans product. Treatment of **10** at −30 °C with 1,1′-thionyldiimidazole (generated in situ from thionyl chloride and imidazole), followed by lithium azide, resulted in a rapid and stereoselective substitution reaction to afford the trans azido alcohol **11**. Temperature control was critical in this reaction to avoid the formation of imidazole adducts at C-2. The azido, hydroxyl, and silyloxy substituents occupy axial positions on the piperidine ring of **11** according to ^{1}H NMR analysis. That the azido is axial in the most stable conformation reflects its avoidance of a probable steric interaction with the piperidine *N*-acyl substituent[21] and may also reflect a favorable dipole interaction with the 3-hydroxyl analogous to the Δ2 effect in mannopyranosides.[22] A sample of **11** recrystallized from ether had ee > 99% according to chiral HPLC analysis.

Reductive acetylation of the hindered axial azido group of **11** is slowed somewhat by its inaccessibility. Treatment of **11** with excess thioacetic acid, which normally converts azido to acetamido quite rapidly,[23] led to no reaction in refluxing chloroform or methanol solution. Selenoacetic acid, which is more nucleophilic, can be generated in toluene solution by treating acetic acid with Woolins' reagent.[24] This solution converted **11** to the acetamide **12** over 12 h at 70 °C in the presence of 2,6-lutidine, although the yield was only 75%. A more efficient method was to hydrogenate with Raney nickel in ethanolic acetic anhydride. Provided the nickel was pretreated with hydrogen gas for 30 min, the reaction was complete within 4 h. The most stable conformation of **12** also featured the C-2, -3, and -4 substituents in axial positions.

Standard *N*-carbobenzoxy removal by hydrogenolysis, followed by acidic hydrolysis of the *tert*-butyldimethylsilyl protecting group, gave the target piperidine **3** as its hydrochloric acid salt. With the N-1 protecting group removed, the piperidine ring of **3** returned to the all-equatorial conformation, according to vicinal coupling constants.

Analysis[11] of **3** as an inhibitor of SpHEX revealed it to be a competitive inhibitor with a K_i value of 21±2 μM. This value is indeed 2-fold lower than that of racemic **3** (K_i = 38±2 μM), prepared from *rac*-**9** as noted above, indicating that the "unnatural" enantiomer does not bind significantly. XylNAc-isofagomine binds some 8-fold more weakly than does the GalNAc-isofagomine version **2** (K_i = 2.7 μM), this difference arising from the two structural modifications in **3**: deletion of the hydroxymethyl and inversion of the hydroxyl at C-4. Inversion of configuration at C-4 in another inhibitor series (the NAG-thiazolines) with SpHEX revealed a 5-fold higher affinity for the gluco- (vs galacto-) configured inhibitor.[25] This is also reflected in transition state binding as seen in the relative k_{cat}/K_m values for the gluco- and galacto-configured *p*-

(16) Corey, E. J.; Bakshi, R. K. *Tetrahedron Lett.* **1990**, *31*, 611−614.
(17) Corey, E. J.; Link, J. O. *Tetrahedron Lett.* **1089**, *30*, 6275−6278.
(18) Marzabadi, C. H.; Anderson, J. E.; Gonzalez-Outeirino, J.; Gaffney, P. R. J.; White, C. G. H.; Tocher, D. A.; Todaro, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 15163−15173, and references therein.
(19) Yu, C.; Hu, L. *Org. Lett.* **2000**, *2*, 1959−1961.
(20) (a) Gagnieu, C. H.; Guiller, A.; Pacheco, H. *Carbohydr. Res.* **1988**, *180*, 233−242. (b) Meslouti, A. El; Beaupere, D.; Demailly, G.; Uzan, R. *Tetrahedron Lett.* **1994**, *35*, 3913−3916. (c) Sanders, W. J.; Kiessling, L. L. *Tetrahedron Lett.* **1994**, *35*, 7335−7338. (d) Manning, D. D.; Bertozzi, C. R.; Pohl, N. L.; Rosen, S. D.; Kiessling, L. L. *J. Org. Chem.* **1995**, *60*, 6254−6255. (e) Beaupere, D.; Meslouti, A. El; Lelievre, Ph.; Uzan, R. *Tetrahedron Lett.* **1995**, *36*, 5347−5348. (f) Sanders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 16391−16422.
(21) Knapp, S.; Hale, J. J. *J. Org. Chem.* **1993**, *58*, 2650−2651.
(22) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A.; *Conformational Analysis*; American Chemical Society: New York, 1965 (reprinted 1981); p 377.
(23) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754−7755.
(24) Knapp, S.; Darout, E. *Org. Lett.* **2005**, *7*, 203−206.
(25) Vocadlo, D.; Ph.D. Thesis; The University of British Columbia, Vancouver, BC, Canada, 2002.

nitrophenyl 2-deoxy-2-acetamido glycosides (37.0×10^5 M^{-1} s^{-1} vs 6.70×10^5 M^{-1} s^{-1}, respectively). Application of the same ratio to the isofagomines suggests that **1** should bind with a K_i of about 0.5 μM, leading to the inference that the additional hydroxymethyl group of **1** (vs **3**) contributes approximately 40-fold (~2.3 kcal/mol) to binding. Although it would be advantageous to realize this entire binding energy, the results with **3** show that good inhibition can nonetheless be realized with the simpler structure.

To illustrate the introduction of ring nitrogen substituents, ***rac*-13** was *N*-alkylated with *p*-methoxylbenzyl iodide (generated in situ) to give ***rac*-14** (Scheme 1). Removal of the TBS group led to the *N*-substituted XylNAc-isofagomine derivative ***rac*-15**. This compound proved to have no inhibitory activity against SpHEX, possibly because the *N*-*p*-methoxybenzyl group blocks the interaction of the equatorial N(1)−H with the general acid residue Glu314 (Figure 2). While N-alkylated versions of 2-deoxynojirimycin show dramatically improved inhibition of digestive glycosidases,[26] simple N-alkylation would appear to be an inappropriate strategy for generating improved inhibitors related to **3**. A similar conclusion has been reached by Kondo et al. in their studies of the interaction of N-alkylated derivatives of **2** with, for example, bovine epididymus β-*N*-acetylglucosaminidase,[27] and by Jakobsen et al.[28] and Ichikawa et al.[29] when looking at inhibition of liver glycogen phosphorylase and sweet almond β-glucosidase, respectively, by N-alkylated isofagomines.

Experimental Section

1-*N*-(Benzyloxycarbonyl)-1,4,5,6-tetrahydro-4-pyridone (5).[30] A solution of 2.5 mL (25 mmol) of 4-methoxypyridine in 50 mL of methanol was stirred with 1.04 g (27 mmol) of $NaBH_4$ at −78 °C for 15 min. A solution of 4.2 mL (27.5 mmol) of benzyl chloroformate in 5 mL of ether was added dropwise over a 30 min period. The reaction mixture was stirred for 1 h at −78 °C and then quenched with 40 mL of water at −60 °C. The solution was warmed to room temperature and extracted with ethyl acetate (3 × 60 mL). The combined organic layer was washed with water (2 × 60 mL) and then brine (30 mL). The organic layer was concentrated, and then the crude product was purified by silica gel chromatography with 1:9 ethyl acetate/petroleum ether as the eluant to afford 4.3 g (80%) of **5** as a white solid, mp 72−73 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.83 (br d, J = 6.6 Hz, 1 H), 7.37 (br s, 5 H), 5.31 (br d, J = 8.1 Hz, 1 H), 5.24 (s, 2 H), 4.02 (t, J = 7.3 Hz, 2 H), 2.53 (t, J = 7.2 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.0, 152.5, 143.2, 134.8, 128.7, 128.6, 128.3, 107.6, 69.0, 42.6, 35.7. ESI-MS *m/z*: 232 MH^+.

1-*N*-(Benzyloxycarbonyl)-3-bromo-4-oxo-1,4,5,6-tetrahydropyridine (6). A solution of 2.20 g (9.5 mmol) of **5** in 60 mL of anhydrous dichloromethane was stirred with 2.54 g (14.2 mmol) of freshly recrystallized *N*-bromosuccinimide for 5 h at room temperature. The reaction mixture was concentrated and then purified by column chromatography (12% ethyl acetate/petroleum ether) to give 2.50 g (85%) of **6** as a light yellow green syrupy liquid. ^{1}HNMR ($CDCl_3$, 300 MHz) δ: 8.25 (br s, 1 H), 7.40 (br s, 5 H), 5.28 (s, 2 H), 4.09 (t, J = 7.2 Hz, 2 H), 2.74 (t, J = 7.2 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 185.9, 151.6, 143.8, 134.6, 128.9, 128.7, 128.6, 101.5, 69.5, 42.7, 35.3. ESI-MS *m/z*: 310 MH^+.

(4*R*)-1-*N*-(Benzyloxycarbonyl)-5-bromo-4-hydroxy-1,4,5,6-tetrahydropyridine (7). A solution of 2.20 g (7.10 mmol) of **6** in 28 mL of 1:1 dichloromethane/carbon tetrachloride was treated with 7.10 mL (7.10 mmol of a 1 M solution in toluene) of (*S*)-2-methyl-CBS-oxazaborolidine, and the mixture was cooled to −78 °C. Catecholborane (9.93 mL, 9.93 mmol of a 1 M solution in tetrahydrofuran) was added dropwise. The reaction mixture was stirred at −78 °C for 17 h, warmed to 0 °C, quenched with 5 mL of saturated aqueous sodium bicarbonate, and then concentrated. The residue was dissolved in 50 mL of dichloromethane, and the organic layer was washed with water (2 × 10 mL), dried over anhydrous sodium sulfate, concentrated, and then chromatographed with 12% ethyl acetate/petroleum ether as the eluant to afford 1.50 g (68%) of **7** as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (br s, 5 H), 7.22 (br s, 1 H), 5.20 (s, 2 H), 4.24 (t, J = 3.4 Hz, 1 H), 4.00 (dd, J = 12.2, 33.2 Hz, 1 H), 3.35 (app q, J = 11.4 Hz, 1 H), 2.42 (br s, 1 H), 2.05 (app t, J = 11.4 Hz, 1 H), and 1.94 (app t, J = 13.2 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 152.2, 135.7, 128.8, 128.6, 128.4, 127.9, 104.4, 68.4, 66.7, 37.1, 30.5. ESI-MS *m/z*: 334 MNa^+. The product was determined to have ee = 88.7% by chiral SFC HPLC with an AD column (200 bar, 4−40% gradient methanol at 2% per min, 1.5 mL/min flow rate, 35 °C).

(4*R*)-1-*N*-(Benzyloxycarbonyl)-5-bromo-4-(*tert*-butyldimethylsilyloxy)-1,4,5,6-tetrahydropyridine (8). A solution of 1.60 g (5.10 mmol) of **7** and 1.31 g (19.3 mmol) of imidazole in 40 mL of dry DMF was treated with 2.20 g (14.5 mmol) of *tert*-butyldimethylsilyl chloride at 0 °C, and then the solution was allowed to warm to room temperature. After 15 h, 20 mL of methanol and 50 mL of brine was added, and then the mixture was extracted with diethyl ether (3 × 50 mL). The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated. Chromatography (6% ethyl acetate/petroleum ether) on silica afforded 2.10 g (95%) of **8** as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.37 (br s, 5 H), 7.19 (br s, 1 H), 5.18 (s, 2 H), 4.21 (t, J = 3.0 Hz, 1 H), 4.10−3.90 (m, 1 H), 3.45−3.30 (m, 1 H), 1.91−1.84 (m, 2 H), 0.90 (s, 9 H), 0.17 (s, 3 H), 0.11 (s, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 152.1, 135.8, 128.7, 128.4, 128.3, 127.2, 104.5, 68.3, 67.5, 37.2, 32.2, 26.0, 18.3, −4.0, −4.4. ESI-MS *m/z*: 448 MNa^+.

(4*R*)-1-*N*-(Benzyloxycarbonyl)-4-(*tert*-butyldimethylsilyloxy)-1,4,5,6-tetrahydropyridine (9). A solution of 2.10 g (4.92 mmol) of **8** in 6 mL of toluene was heated at reflux. A solution of 2.80 g (9.62 mmol) of tri-*n*-butyltin hydride and 5 mg of 2,2′-azo-bis(isobutyronitrile) in 3 mL of toluene was added dropwise over a 30 min period. The reaction mixture was heated at reflux for 14 h, by which time complete consumption of the starting material was indicated by TLC analysis. The solution was concentrated, and the crude product was chromatographed on silica (5% ethyl acetate/petroleum ether) to afford 1.20 g (71%) of **9** as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.42−7.30 (m, 5 H), 6.96 and 6.86 (ABq, J = 8.4 Hz, 1 H), 5.19 (s, 2 H), 5.01 (dd, J = 4.4, 8.0 Hz, 1 H), 4.89 (dd, J = 4.4, 8.4 Hz, 1 H), 4.21 (app q, J = 4.0 Hz, 1 H), 3.84 (app septet, J = 5.6 Hz, 1 H), 3.52 (ddd, J = 5.6, 8.8, 12.8 Hz, 1H), 1.86−1.75 (m, 2 H), 0.89 (s, 9 H), 0.08 (br s, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 153.2, 136.4, 128.8, 128.4, 128.3, 126.3, 109.1, 67.9, 61.8, 38.4, 31.4, 26.1, 18.4, −4.4, −5.1. ESI-MS *m/z*: 348 MH^+.

1-*N*-(Benzyloxycarbonyl)-4-(*tert*-butyldimethylsilyloxy)-1,4,5,6-tetrahydropyridine (*rac*-9). A solution of 2.5 g (11.6 mmol) of **5** and 3.9 g (10.5 mmol) of cerium(III) chloride heptahydrate in 120 mL of methanol was stirred at 0 °C. After 30 min, 0.58 g (15.3 mmol) of sodium borohydride was added in portions. After 30 min, the reaction temperature was

(26) A recent review: Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645−1680.
(27) Kondo, K.-I.; Adachi, H.; Nishimura, Y.; Takeuchi, T. *Nat. Prod. Lett.* **2001**, *15*, 371−375.
(28) Jakobsen, P.; Lundbeck, J. M.; Kristiansen, M.; Brianholt, J.; Demuth, H.; Pawlas, J.; Candela, M. P. T.; Andersen, B.; Westergaard, N.; Lundgren, K.; Asano, N. *Bioorg. Med. Chem.* **2001**, *9*, 733−744.
(29) Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007−3018.
(30) Yamada, H.; Aoyagi, S.; Kibayashi, C. *Tetrahedron. Lett.* **1996**, *37*, 8787−8790.

allowed to rise to 23 °C, and after an additional 30 min the reaction was quenched with 50 mL of water. Extraction with dichloromethane (3 × 100 mL), drying with magnesium sulfate, and then concentration gave 2.4 g (96%) of allylic alcohol that was used directly in the next step. ESI-MS: 234 MH^+.

A solution of 2.4 g (11.0 mmol) of the crude allylic alcohol and 2.7 g (40 mmol) of imidazole in 45 mL of dry DMF was stirred at 0 °C and treated with 3.4 g (19.2 mmol) of *tert*-butyldimethylsilyl chloride. The reaction was allowed to warm to 23 °C and after 6 h was quenched by the addition of 20 mL of methanol and 50 mL of water. The reaction mixture was extracted with ether (2 × 50 mL), and the combined organic extract was washed sequentially with water (3 × 50 mL) and brine (30 mL). Concentration followed by chromatography as described above gave 3.5 g (96%) of *rac*-**9** as a colorless oil with spectroscopic properties matching those of **9** detailed above.

(2*S*,3*R*,4*R*)-1-*N*-(Benzyloxycarbonyl)-4-(*tert*-butyldimethylsilyloxy)-2,3-dihydroxypiperidine (10). A solution of 0.74 g (2.1 mmol) of **9**, 0.027 g (0.1 mmol) of osmium tetroxide, and 0.37 g (3.1 mmol) of *N*-methylmorpholine *N*-oxide in 3.75 mL of 1:1:1 acetonitrile/acetone/water was stirred at room temperature for 14 h. Saturated aqueous sodium sulfite was added to quench, and after 30 min the reaction mixture was concentrated, and the residue was extracted with ethyl acetate. The organic layer was washed with water and then brine, dried over anhydrous sodium sulfate, and then concentrated. Chromatography (20% ethyl acetate/petroleum ether) on silica afforded 0.73 g (93%) of **10** as a white solid, mp 99−101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35 (br s, 5 H), 5.85 (br, s, 1 H), 5.14 (s, 2 H), 3.98−3.89 (m, 1 H), 3.87 (ddd, J = 4.8, 8.8, 11.2, 2 H), 3.40 (dd, J = 3.6, 9.2 Hz, 1 H), 3.19 (br app t, J = 12.8 Hz, 1 H), 2.49 (br s, 1 H), 1.85 (br d, J = 12.8 Hz, 1 H), 1.62 (br s, 1 H), 1.53 (tdd, J = 13.2, 11.2, 4.8 Hz, 1 H), 0.89 (s, 9 H), 0.11 (s, 3 H), 0.09 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.1, 136.3, 128.8, 128.4, 128.2, 74.7, 70.2, 69.7, 67.9, 37.9, 32.9, 26.0, 18.2, −4.1, −4.4. ESI-MS *m/z*: 380 $(M-H)^+$.

(2*R*,3*R*,4*R*)-2-Azido-1-*N*-(benzyloxycarbonyl)-4-(*tert*-butyldimethylsilyloxy)-3-hydroxypiperidine (11). A solution of 1.59 g (23.3 mmol) of imidazole in 34 mL of dry THF was cooled to 0 °C. Thionyl chloride (0.43 mL, 5.9 mmol) was added over a 5 min period. The reaction mixture was stirred at 0 °C for 30 min and then filtered to remove the imidazole hydrochloride precipitate. The filtrate was slowly added to a stirred suspension of 800 mg (2.1 mmol) of **10** in 10 mL of dry DMF at −60 °C. The reaction mixture was allowed to stir at −30 °C for 1 h, and then a solution of 420 mg (8.5 mmol) of lithium azide in 35 mL of DMF was added over a 30 min period. The reaction mixture was allowed to warm to 0 °C over 2 h and then was quenched by the addition of 10 mL of water. The reaction mixture was concentrated and then partitioned between 50 mL of ether and 30 mL of additional water. The organic layer was dried with anhydrous sodium sulfate, concentrated, and then chromatographed (15% ethyl acetate/petroleum ether) on silica to afford 819 mg (96%) of **11** as an amorphous solid, mp 98−100 °C. A sample crystallized from ether had a mp of 99−101 °C and had an ee measured at 99+% with a Chiralcel OD-H column (250 × 4.6 mm), isocratic 6% methanol/carbon dioxide at 1.5 mL/min, 200 bar, 35 °C, 215 nm detection, 15 min run time. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (br s, 5 H), 5.67 (br s, 1 H), 5.19 and 5.15 (ABq, J = 12.0 Hz, 2 H), 3.91 (br d, J = 12.8, 1 H), 3.87 (app q, J = 4.0 Hz, 1 H), 3.72 (br s, 1 H), 3.39 (dt, J = 3.2, 12.9 Hz, 1 H), 2.01 (dddd, J = 3.5, 5.6, 12.8, 14.0 Hz, 1 H), 1.56 (dq, J = 14.0, 3.2 Hz, 1 H), 0.91 (s, 9 H), 0.09 (s, 3 H), 0.06 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.3, 136.0, 128.8, 128.5, 128.3, 71.7, 70.2, 68.3, 67.8, 35.3, 27.7, 25.8, 18.2, −4.7, −4.8. ESI-MS *m/z*: 407 MH^+.

(2*S*,3*R*,4*R*)-2-Acetamido-1-*N*-(benzyloxycarbonyl)-4-(*tert*-butyldimethylsilyloxy)-3-hydroxypiperidine (12). A mixture of 100 mg (0.25 mmol) of **11**, 80 mg of Raney nickel (pretreated as an ethanol slurry for 30 min with hydrogen gas), 0.4 mL (4.2 mmol) of acetic anhydride, and 2 mL of ethanol was stirred for 4 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and then concentrated. The residue was diluted with 25 mL of ethyl acetate, which was washed in turn with water (2 × 20 mL) and brine (10 mL). The organic layer was concentrated and then chromatographed (25% ethyl acetate/petroleum ether) on silica to afford 94.8 mg (90%) of **12** as an amorphous solid, mp 112−114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42−7.25 (m, 5 H), 6.26 (d, 1H, J = 8.7 Hz, 1 H), 5.16 (s, 2 H), 4.02 (d, J = 2.1 Hz, 1 H), 3.93 (br d, J = 11.1 Hz, 1 H), 3.76 (br s, 1 H), 3.22 (br t, J = 12.0 Hz, 1 H), 2.16−2.05 (m, 1 H), 1.90 (s, 3 H), 1.44 (br d, J = 13.8 Hz, 1 H), 0.93 (s, 9 H), 0.12 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.3, 155.8, 136.9, 128.5, 127.9, 127.8, 69.6, 68.9, 67.5, 61.4, 34.0, 27.5, 26.0, 23.5, 18.1, −4.8, −4.9. ESI-MS *m/z*: 423 MH^+.

(2*R*,3*R*,4*R*)-2-Acetamido-4-(*tert*-butyldimethylsilyloxy)-3-hydroxypiperidine (13). A solution of 0.12 g (0.28 mmol) of **12** in 5 mL of ethanol was treated with 0.04 g of 10% Pd−C and purged with hydrogen three times. The suspension was allowed to stir under a hydrogen atmosphere for 18 h. The reaction mixture was filtered through Celite, and the solid was washed with 3 mL of ethanol. The combined organic extract was concentrated to provide 0.08 g (98%) of **13** as an off-white solid, mp 107−108 °C, that was directly used in the next step. ^{1}H NMR (400 MHz, CD_3OD) δ: 4.31 (d, J = 8.0 Hz, 1 H), 3.58 (ddd, J = 4.8, 8.0, 10.0 Hz, 1 H), 3.17 (t, J = 8.0 Hz, 1 H), 2.92 (dt, J = 4.0, 12.4 Hz, 1 H), 2.64 (dt, J = 2.4, 12.0 Hz, 1 H), 1.98 (s, 3 H), 1.90 (qd, 4.0, 14.0 Hz, 1 H), 1.50 (dddd, 4.4, 10.4, 11.6, 14.0 Hz, 1 H), 0.92 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.9, 75.9, 75.3, 67.6, 41.5, 34.7, 26.5, 22.9, 19.1, −4.1, −4.4. ESI-MS *m/z*: 289 MH^+.

(2*R*,3*R*,4*R*)-2-Acetamido-3,4-dihydroxypiperidine (XylNAc-isofagomine, 3). A solution of 0.08 g (0.27 mmol) of **12** in 5 mL of ethanol was treated with 5 mL of 3 N hydrochloric acid at 0 °C. After 5 h, the reaction mixture was concentrated and water was removed by azeotropic codistillation with toluene. The residue was dissolved in several μL of methanol (containing 1% concentrated aq ammonia) and applied to a small silica column. Elution with 10% methanol/dichloromethane containing 1% ammonia afforded **3** as the free base, 0.035 g (73%). R_f 0.2 (20% methanol/dichloromethane). ^{1}H NMR (400 MHz, CD_3OD) δ: 4.54 (d, J = 8.4 Hz, 1 H), 3.65−3.55 (ddd, J = 4.4, 8.4, 10.0 Hz, 1 H), 3.40 (t, J = 8.4 Hz, 1 H), 3.13 (dt, J = 4.4, 12.8 Hz, 1 H), 2.87 (ddd, J = 3.0, 11.4, 12.8 Hz, 1 H), 2.10−2.05 (obsc d, 2 H), 2.01 (s, 3 H),1.62 (dddd, J = 4.4, 10.4, 11.6, 14.4 Hz, 1 H). ^{13}CNMR (100 MHz, CD_3OD) δ: 167.5, 74.3, 72.4, 66.7, 41.3, 31.8, 22.8. ESI-MS *m/z*: 175 MH^+. $[\alpha]_D$ 12.5° (c = 0.01, MeOH).

(2*R,3*R**,4*R**)-2-Acetamido-4-(*tert*-butyldimethylsilyloxy)-3-hydroxy-1-*N*-(*p*-methoxybenzyl)-piperidine (*rac*-14).** A mixture of 0.15 g (0.52 mmol) of **3**, 0.14 g (1.03 mmol) of potassium carbonate, 0.15 g (1.0 mmol) of sodium iodide, 98 μL (0.61 mmol) of *p*-methoxybenzyl chloride, and 4 mL of acetone was stirred for 1 h. The reaction mixture was concentrated, and then the residue was partitioned between 10 mL of ethyl acetate and 10 mL of water. The organic layer was washed sequentially with water (2 × 10 mL) and brine (10 mL) and then concentrated. Chromatography on silica afforded 0.16 g (70%) of *rac*-**14** as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.44 (d, J = 9.3 Hz, 1 H), 7.20 (d, J = 8.4 Hz, 2 H), 6.83 (d, J = 8.4 Hz, 2 H), 5.07 (dd, J = 3.0, 9.0 Hz, 1 H), 3.93 (d, J = 3.0 Hz, 1 H), 3.83 (d, J = 13.5 Hz, 1 H), 3.78 (s, 3 H), 3.53 (br s 1 H), 3.27 (d, J = 13.5 Hz, 1 H), 2.82 (br d, J = 8 Hz, 1 H), 2.60 (dt, J = 2.7, 12 Hz, 1 H), 2.31 (qd, J = 2.8, 7.2 Hz, 1 H), 2.02 (s, 3 H), 1.97−1.92 (m, 1 H), 1.46 (dd, J = 2.1, 14.4 Hz, 1 H), 0.93 (s, 9 H), 0.11 (s, 3 H), 0.10 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 158.8, 130.1, 113.8, 70.0, 69.6, 67.9, 58.1, 55.4, 39.4, 28.6, 25.9, 23.7, 18.1, −4.8, −4.9. ESI-MS *m/z*: 409 MH^+.

(2*R,3*R**,4*R**)-2-Acetamido-3,4-dihydroxy-1-*N*-(*p*-methoxybenzyl)-piperidine (*rac*-15).** A solution of 0.031 g of *rac*-**14** in 1.5 mL of ethanol was treated with 1.5 mL of 3 N hydrochloric acid. The reaction was kept at 0 °C for 1 h and then at 20 °C for 2 h. The solution was concentrated and azeotropically dried with toluene (3 × 5 mL). The residue was dissolved in a small amount of methanol containing 1% concentrated aqueous ammonia and applied to a small silica column. Elution with 10% methanol/dichloromethane (containing 1% ammonia) afforded 0.021 g (81%) of *rac*-**15** as the free base. R_f 0.4 (20% methanol/dichloromethane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22 (d, J = 8.4 Hz, 2 H), 6.84 (d, J = 8.4 Hz, 2 H), 4.20 (d, J = 7.5 Hz, 1 H), 3.82 (d, J = 12.9 Hz, 1 H), 3.76 (s, 3 H), 3.52−3.42 (m, 1 H), 3.23 (t, J = 7.8 Hz, 1 H) 3.10 (d, J = 13.4 Hz, 1 H), 2.75 (dt, J = 3.9, 12.0 Hz, 1 H), 2.12−2.03 (m, 1 H), 2.00 (s, 3 H), 1.88−1.78 (m, 1 H), 1.51−1.39 (m, 1 H). ^{13}C NMR (100 MHz, CD_3OD) δ: 174.0, 160.2, 132.1, 131.1, 114.6, 77.0, 73.2, 72.1, 57.7, 55.8, 47.9, 32.0, 23.1. ESI-MS *m/z*: 295 MH^+.

Enzyme assays. *Sp. Hex*. was cloned and overexpressed in *E. coli* as described previously.[11] Continuous spectrophotometric assays were performed on a Varian Cary 300 UV−vis spectrophotometer at 360 nm and at 37 °C, by using *p*-nitrophenyl α-D-*N*-acetylglucosaminide (pNP-GlcNAc) as substrate. The buffer used for kinetic studies was 25/50 mM sodium citrate/sodium phosphate, 75 mM NaCl, pH 5. The concentration of enzyme used was 2.50 nM. Michaelis−Menten kinetic parameters were determined by using GraFit version 4.0.19. Inhibition values were obtained from the nonlinear fitting of the rate data into GraFit as a competitive inhibitor.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for new compounds in Scheme 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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