JOC Article

Synthesis and High-Throughput Screening of *N*-Acetyl-*β*-hexosaminidase Inhibitor Libraries Targeting Osteoarthritis

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C1 Nitrogen iminocyclitols are potent inhibitors of *N*-acetyl-*β*-hexosaminidases. Given hexosaminidases' important roles in osteoarthritis, we developed two straightforward and efficient syntheses of C1 nitrogen iminocyclitols from two readily available starting materials, D-mannosamine hydrochloride and the microbial oxidation product of fructose. A diversity-oriented synthetic strategy was then performed by coupling these core structures with various aldehydes, carboxylic acids, and alkynes to generate three separate libraries. High-throughput screening of the generated libraries with human *N*-acetyl-*β*-hexosaminidases produced only moderate inhibitory activities. However, the synthetic approach and screening strategy for these compounds will be applied to develop new potent inhibitors of human *N*-acetyl-*β*-hexosaminidases, particularly when combined with the structural information of these enzymes.

Introduction

N-Acetyl-*β*-hexosaminidases (EC 3.2.1.52) belong to the group of lysosomal hydrolases. They catalyze the hydrolysis of terminal, nonreducing *N*-acetyl-*β*-D-glucosamine and *N*-acetyl-*β*-D-galactosamine residues in glycoproteins, G_{M2}-gangliosides, and glycosaminoglycans.[1] Human *N*-acetyl-*β*-hexosaminidases are dimeric enzymes composed of α and *β* subunits and hence have three isoforms: *N*-acetyl-*β*-hexosaminidase A (*αβ*, HexA), *N*-acetyl-*β*-hexosaminidase B (αα, HexB), and *N*-acetyl-*β*-hexosaminidase S (*ββ*, HexS). The *β* subunit hydrolyzes mainly neutral substrates, whereas the α subunit is able to hydrolyze charged substrates, although dimerization of the two units is a prerequisite for the enzyme's activity.[2] *N*-Acetyl-*β*-hexosaminidases are the dominant glycosaminoglycan-degrading glycosidases released by chondrocytes into the extracellular compartment, and the dominant glycosidases in synovial fluid of patients with osteoarthritis. Stimulation of chondrocytes with proinflammatory cytokine interleukin-1*β* results in a selective secretion of *N*-acetyl-*β*-hexosaminidases.[3] Of particular interest is the enzyme's direct involvement in the cartilage matrix degradation.[4]

During the course of hexosaminidase catalysis, the terminal *N*-acetyl-*β*-hexosamine of polysaccharides is generally believed to exhibit a flattened half-chair conformation with substantial oxocarbenium ion character at the anomeric position, which is stabilized by a deprotonated carboxyl group from the enzyme (Figure 1).[5] The mechanism involving the participation of the neighboring C2 acetamido group of the substrate was also suggested.[6] Iminocyclitols, whose ring nitrogen would be protonated under physiological conditions, are transition state analogues for glycosidase catalysis and therefore good inhibitors for glycosidases. The inhibition constants of iminocyclitols toward various glycosidases are usually in the nanomolar range.[7] In our previous study, C1 *N*-acetyl iminocyclitols **1**, **2**, **3**, and **4** were found to show very potent inhibition activity against *N*-acetyl-*β*-hexosaminidases from human placenta (Figure 2). In particular,

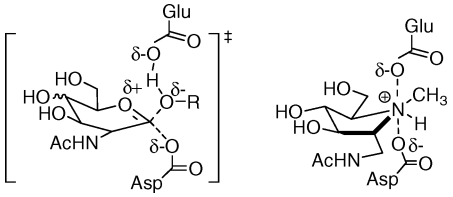

FIGURE 1. Postulated mechanism of hexosaminidases catalysis and an iminocyclitol structure.

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(1) (a) Winchester, B. G. *Subcell. Biochem.* **1996**, *27*, 191. (b) Watanabe, K. *J. Biochem. (Tokyo)* **1936**, *24*, 297.
(2) (a) Mahuran, D. J.; Lowden, J. A. *Can. J. Biochem.* **1980**, *58*, 287. (b) Hou, Y.; Tse, R.; Mahuran, D. J. *Biochemistry* **1996**, *35*, 3963. (c) Meier, E. M.; Schwarzmann, G.; Fürst, W.; Sandhoff, K. *J. Biol. Chem.* **1991**, *266*, 1897.
(3) Shikhman, A. R.; Brinson, D. C.; Lotz, M. K. *Arthritis Rheum.* **2000**, *43*, 1307.
(4) Liu, J.; Shikhman, A. R.; Lotz, M. K.; Wong, C.-H. *Chem. Biol.* **2001**, *8*, 701.
(5) (a) Rye, C. S.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 573. (b) Sinnott, M. *Chem. Rev.* **1990**, *90*, 1171.
(6) (a) Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. *Nature* **2001**, *412*, 835. (b) Knapp, S.; Vocadlo, D. J.; Gao, Z.; Kirk, B.; Lou, J.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118*, 6804.
(7) (a) Bols, M. *Acc. Chem. Res.* **1998**, *32*, 1. (b) Takayama, S.; Martin, R.; Wu, J.; Laslo, K.; Siuzdak, G.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8145.

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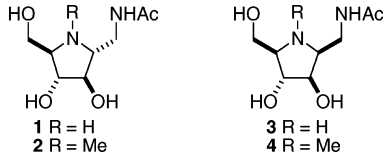
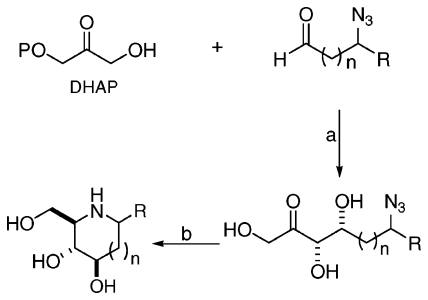


FIGURE 2. Structures of *N*-acetyl iminocyclitol inhibitors of *N*-acetyl-β-hexosaminidases.

SCHEME 1[a]

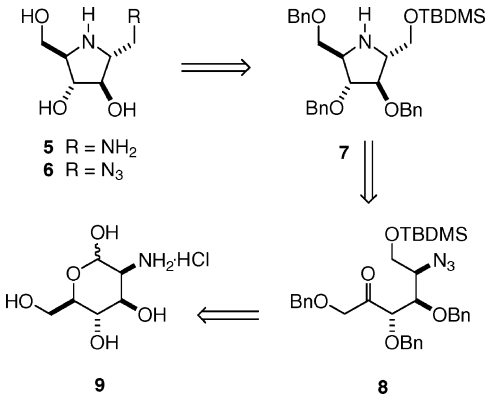


[a] Reagents and conditions: (a) (i) FDP aldolase; (ii) acid phosphatase; (b) H_2, Pd−C.

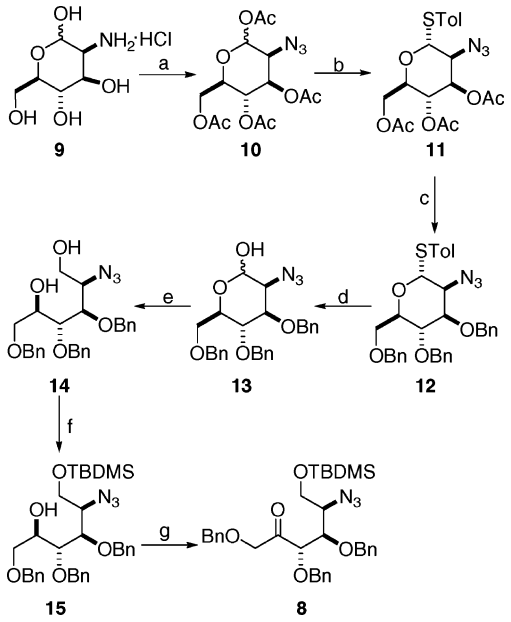
incubation of human chondrosarcoma cells with iminocyclitol **2** resulted in an accumulation of glycosaminoglycans in the cell-associated fraction and a decrease in the release of glycosaminoglycans into the culture supernatant.[4] The discovery of iminocyclitols as potential chondroprotective agents suggests a new avenue for the development of drugs to treat osteoarthritis.

In view of their interesting biological properties, considerable effort has been devoted to the development of efficient syntheses of various iminocyclitols.[8] Our group has developed a chemoenzymatic method to generate numerous iminocyclitols, which generally involves an aldol condensation catalyzed by fructose 1,6-diphosphate (FDP) aldolase (EC 4.1.1.13). Various glucose-type iminocyclitols can be easily prepared by a three-step procedure: condensation of an aldehyde and DHAP catalyzed by FDP aldolase, followed by dephosphorylation with acid phosphatase and then intramolecular reductive amination (Scheme 1).[9] FDP aldolase's high stereospecificity and broad substrate tolerance allow the straightforward preparation of a large number of iminocyclitols.[10] However, the high cost of removing the phosphate group of the aldol condensation products with acid phosphatase (EC 3.1.3.2) ($440/2,500 U) combined with FDP aldolase's low activity to certain unnatural substrates make large-scale chemoenzymatic iminocyclitol synthesis unfavorable. As an extension of our research on the effect of iminocyclitols on *N*-acetyl-β-hexosaminidases for the possible treatment of osteoarthritis, we report here two novel routes to synthesize C1 nitrogen iminocyclitol

SCHEME 2. Retrosynthetic Analysis



SCHEME 3[a]



[a] Reagents and conditions: (a) (i) TfN_3 (5 equiv), K_2CO_3, MeOH, H_2O, $CuSO_4$ (cat.); (ii) Ac_2O, Pyr, 0 °C, 89% for two steps; (b) *p*-toluene sulfide (4 equiv), $BF_3{\cdot}Et_2O$ (6 equiv), 0 °C, 90%; (c) (i) MeONa (2 equiv), MeOH, 0 °C, (ii) NaH, BnBr, DMF, 0 °C, 87%; (d) NIS (3 equiv), acetone, H_2O, 65%; (e) $NaBH_4$ (excess), MeOH, 83%; (f) TBDMSCl (1.1 equiv), Pyr, 0 °C, 71%; (g) Dess−Martin reagent (1.1 equiv), 91%, CH_2Cl_2.

structures: one starting from D-mannosamine hydrochloride and another starting with the microbial oxidation product of fructose. Reported also are the rapid generation of three libraries from three different iminocyclitol core structures and their high-throughput screening with human *N*-acetyl-β-hexosaminidases.

Results and Discussion

New Route to Synthesize *N*-Acetyl Iminocyclitols 5 and 6. The retrosynthetic pathway of compounds **5** and **6** is represented in Scheme 2. The key step is the preparation of pyrrolidine **7** by cyclization of the azidoketone **8** through intramolecular reductive amination. Compound **8** is accessible from commercially available D-mannosamine hydrochloride **9**.

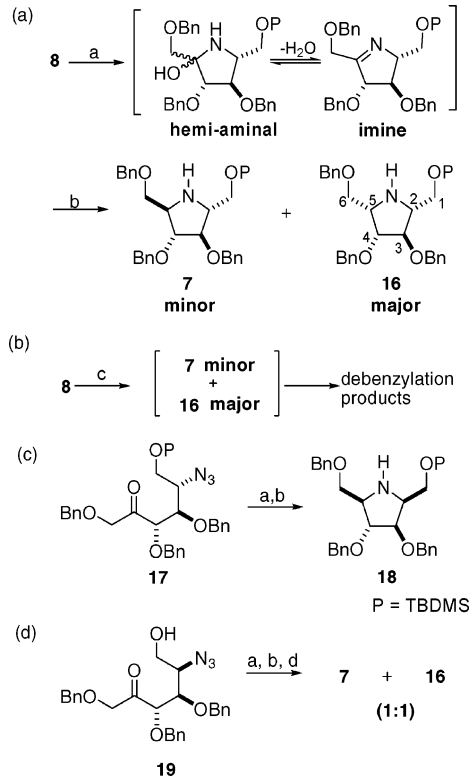
As shown in Scheme 3, the primary amine of D-mannosamine hydrochloride **9** was first converted into

(8) (a) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340. (b) Koumbis, A. E.; Gallos, J. K. *Curr. Org. Chem.* **2003**, *7*, 771. (c) Legler, G. In *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999; pp 49−56.

(9) (a) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443. (b) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier: NewYork, 1994; pp 195−210. (c) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, Y. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 412.

(10) (a) Takaoka, Y.; Kajimoto, T.; Wong, C.-H. *J. Org. Chem.* **1993**, *58*, 4809. (b) Hung, R. R.; Straub, J. A.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 3849.

SCHEME 4[a]



[a] Reagents and conditions: (a) H_2, Pd−C, Pyr, THF; (b) $NaBH_3CN$, *p*-TsOH, MeOH or $NaBH(OAc)_3$, *p*-TsOH, CH_2Cl_2; (c) H_2 (100 psi), Pd−C, MeOH; (d) TBDMSCl, Pyr, 0 °C.

an azido functionality using a diazo transfer reaction.[11] The crude product was peracetylated using acetic anhydride in pyridine to give **10** as an anomeric mixture in 89% yield for two steps. Treatment of this anomeric mixture with *p*-toluene sulfide and $BF_3{\cdot}Et_2O$ provided the hemi-thioketal **11** in 90% yield with traces of β-anomer. After removal of the acetate groups using sodium methoxide, benzylation of the crude product with NaH and BnBr in DMF provided compound **12** in 87% yield for two steps. Hydrolysis of the hemi-thioketal functionality in the presence of *N*-iodo succinimide gave **13** in 65% yield. This C2-azido-pyranose was reduced to the corresponding alcohol **14** using $NaBH_4$. After selective protection of the primary hydroxyl group using TBDMSCl in pyridine, the secondary hydroxyl group of **15** was oxidized with the Dess−Martin periodinane to provide the azido-ketone **8**.[12]

Several reductive amination conditions were tried in order to cyclize **8** to give products with the desired stereochemistry. The azido group was first partially reduced using Pd−C in the presence of pyridine under 1 atm of H_2 (Scheme 4a).[13] The hemi-aminal intermediate formed was reduced with $NaBH_3CN$ and *p*-TsOH in methanol to give the undesired epimer **16** as the predominant product, where C4 and C5 are in *cis* configuration.[14] The same reductive amination of the hemi-aminal with $NaBH(OAc)_3$ and *p*-TsOH in dichloromethane afforded **7** and the undesired **16** in a 1:3 ratio.[15] When ketone **8** was reduced with 100 psi H_2 in the presence of Pd−C in methanol, the major product was still **16** as shown by TLC after a short period of reaction time (Scheme 4b). Interestingly, reductive amination with $NaBH_3CN$ or $NaBH(OAc)_3$ performed on **17** gave the C4−C5 *trans* isomer **18** as the predominant product in 72% yield (Scheme 4c). After removal of the TBDMS of **8**, reductive amination of **19** under similar conditions produced a 1:1 mixture of the two epimers (Scheme 4d).

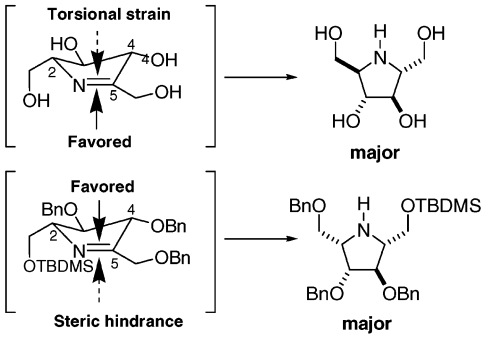


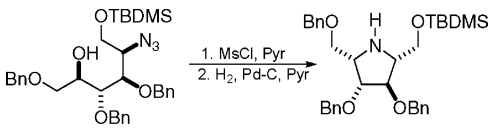
FIGURE 3. Reduction of imines.

Previous studies suggest that the intramolecular reductive amination of unprotected glucose-type azido-ketone substrates usually favors C4−C5 *trans* configuration as a result of torsional strain.[16] Our current research suggests that the steric effect caused by the bulky C1 group (TBDMS) plays a critical role in the intramolecular reductive amination of azido ketones. Figure 3 shows how the C1 TBDMS blocks the α face of the imine intermediate to favor the β face hydrogen addition.

As a result of the inability to obtain the desired isomer **7** in high stereoselectivity by imine reduction, an alternative approach was sought. Ketone **8** was reduced using $NaBH_4$ in MeOH (Scheme 5), where a Felkin−Ahn-type addition afforded predominantly the secondary alcohol **20** with the (5*S*) configuration.[17] The hydroxyl group of **20** was activated as mesylate, and after reduction of the azido group, there followed an immediate cyclization to give the desired isomer **7** as the major product (**7**/**16** = 61:26).[18]

(11) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029.
(12) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
(13) Medgyes, A.; Bajza, I.; Farkas, E.; Pozsgay, V.; Liptak, A. *J. Carbohydr. Chem.* **2000**, *3*, 285.

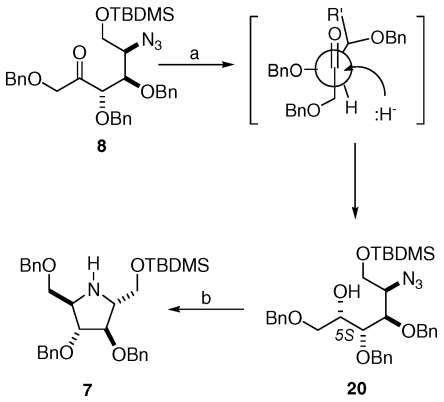
(14) The stereochemistry of **16** was ascertained by reacting **15** with MsCl and then reducing the azido group using H_2 and Pd−C in pyridine under hydrogen atmosphere (1 atm).



15 **16**

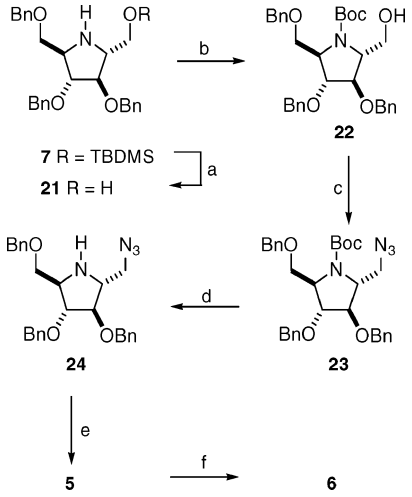
(15) (a) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849. (b) For a review on reductive amination, see: Baxter, E.; Reitz, A. B. *Org. React.* **2002**, 59, 1.
(16) (a) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280. (b) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A., Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187. (c) Wang, Y.-F.; Dumas, D. P.; Wong, C.-H. *Tetrahedron Lett.* **1993**, *34*, 403.
(17) Mengel, A.; Reiser, O. *Chem. Rev.* **1999**, *99*, 1191.
(18) Liu, J.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2002**, *41*, 1404.

SCHEME 5[a]



[a] Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C; (b) (i) MsCl, Pyr, 0 °C, (ii) H_2 (1 atm), Pd−C, Pyr, THF, 61% for 3 steps.

SCHEME 6[a]

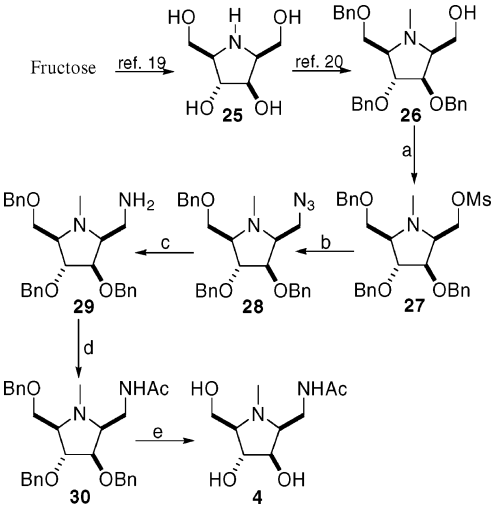


[a] Reagents and conditions: (a) TBAF, THF, 0 °C, 97%; (b) Boc_2O, Et_3N, 0 °C, 83%; (c) (i) MsCl, Et_3N, 0 °C, (ii) NaN_3, DMF, 75 °C, 53%; (d) TFA, 0 °C, 97%; (e) H_2 (70 psi), Pd−C, MeOH, aq HCl (pH 4), (quant); (f) TfN_3, K_2CO_3, MeOH, H_2O, $CuSO_4$, 85%.

The silyl protecting group of **7** was removed using TBAF to give the alcohol **21**, whose secondary amine was then protected with the treatment of Boc_2O to afford **22** in 83% yield, which existed as a mixture of atropoisomers (Scheme 6). The hydroxyl group of **22** was converted to a mesylate, followed by displacement with NaN_3 at 75 °C to give the azido compound **23** in 53% yield for two steps. The Boc group was removed using trifluoroacetic acid to provide **24** in 97% yield. Reduction of **24** using Pd−C in methanol and aqueous HCl (pH 4) under H_2 afforded **5** in quantitative yield. Amine **5** was treated with a solution of TfN_3 to afford azido iminocyclitol **6** in 85% yield.

New Route to *N*-Acetyl Iminiocyclitol 4. We also developed a new method to synthesize *N*-methyl-*β*-isomer iminocyclitol **4** from 2,5-anhydro-2,5-imino-D-glucitol **25**, which can be produced in large quantities by the microbial oxidation of fructose (Scheme 7).[19] Following the procedure described in the literature, **25** was transformed to *N*-methyl iminocyclitol **26** in four steps.[20] The hydroxyl group in **26** was activated with MsCl to afford **27**, which

SCHEME 7[a]



[a] Reagents and conditions: (a) MsCl, Pyr; (b) NaN_3, NaI, 87% from **26**; (c) Ph_3P, THF, 50 °C; (d) Ac_2O, Pyr, 87% from **28**; (e) H_2 (50 psi), Pd−C, 89%.

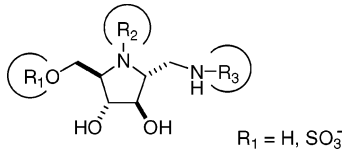


FIGURE 4. Derivatives of iminocyclitol.

was treated directly with NaN_3 in pyridine to afford azido compound **28**. Reduction of the azido group followed by acetylation of the resulting amine generated **30**, which was subjected to hydrogenation to give the final *N*-methyl-*β*-acetamido-iminocyclitol **4** in high yield.

Aromatic Derivatives of the Ring Nitrogen of Iminocyclitol 1. We have previously shown that 6-SO_3^- iminocyclitol (R_1 = SO_3^-, R_2 = Me, R_3 = Ac, Figure 4) was able to selectively inhibit *N*-acetyl-*β*-hexosaminidase A, which would cause fewer side effects.[4] In our current research, we investigated the substituent effects on the ring nitrogen (R_2) and C1 nitrogen (R_3).

It has long been recognized that aromatic moieties are major players in molecular recognition as a result of their capability to interact with both hydrophobic residues and polar substituents.[21] Accordingly, we synthesized a series of aromatic derivatives from the core iminocyclitol structure **1** (Scheme 8). The couplings of different aromatic aldehydes with **1** proceeded smoothly via reductive amination with $NaBH_3CN$ to give various aromatic derivatives in 60−70% yields.

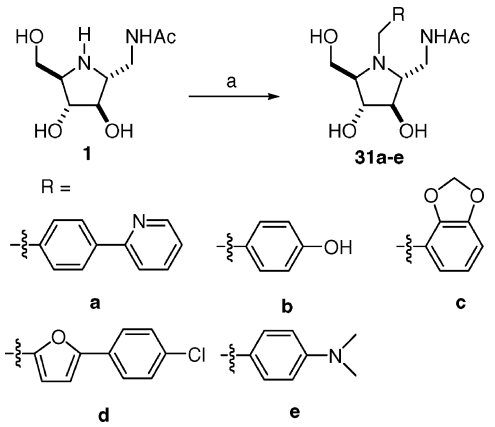
The IC_{50}'s of aromatic derivatives **31a**−**e** against human *N*-acetyl-*β*-hexosaminidases in the presence of 4-methylumbellyferyl-*N*-acetyl-*β*-D-glucosamine were all in the low micromolar range (Table 1). Compared with iminocyclitol **2**'s 24 nM, the aromatic moieties decrease the iminocyclitol's activity against *N*-acetyl-*β*-hexosaminidases. Compound **31b** is the best inhibitor in this

(19) Baxter, E. W.; Reitz, A. B. *J. Org. Chem.* **1994**, *59*, 3175.

(20) Wong, C.-H.; Provencher, L.; Porco, J. A.; Jung, S.-H.; Wang, Y.-F.; Chen, L.; Wang, R.; Steensma, D. H. *J. Org. Chem.* **1995**, *60*, 1492.

(21) Hajduk, P. J.; Bures, M.; Praestgaard, J.; Fesik, S. W. *J. Med. Chem.* **2000**, *43*, 3443.

SCHEME 8[a]

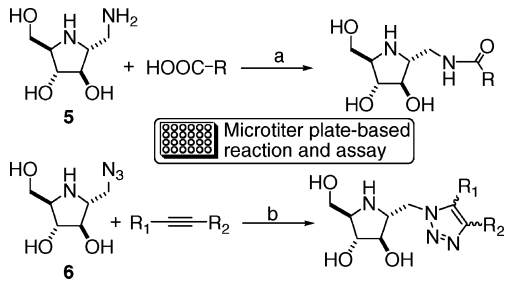


[a] Reagents and conditions: Ar−CHO, $NaBH_3CN$, MeOH, rt, 60−70%.

TABLE 1. Inhibition Activities of Aromatic Derivatives of Iminocyclitol 31a−c

	31a	31b	31c	31d	31e
$IC_{50}(\mu M)$	9.5	4.1	38	10	18

SCHEME 9[a]

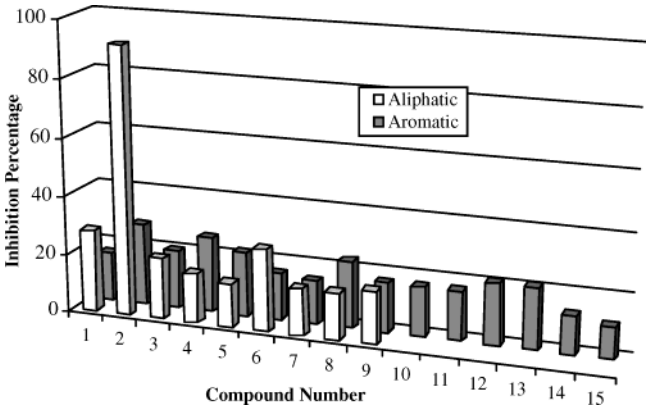


[a] Reagents and conditions: (a) HBTU, DIEA, rt; (b) 80 °C, overnight.

category, where it appears that the hydrophilic group may help to improve inhibitory activity.

Rapid Generation and High-Throughput Screening of Iminocyclitol Libraries. To investigate the effect of a substituent on the C1 nitrogen position, a rapid generation and high-throughput screening program was initiated. Two strategies were used to generate libraries

CHART 1. Amide Library Bioassay Results



from the core structures **5** and **6** (Scheme 9): one is based on amide formation, where the primary amine of **5** reacted with various carboxylic acids, a strategy that has been successfully used in the discovery of HIV protease inhibitors,[22] and the other involves triazole formation, where the azido iminocyclitol **6** was coupled with different alkynes to generate a triazole library. This azide−alkyne coupling has been the focus of recent publications because the azido group is orthogonal to most functional groups in biological systems and the azide−alkyne coupling is an extremely efficient reaction.[23] Along with the triazole formation in situ in microtiter plates, the triazole library was used to screen various lectins.[24]

The coupling reactions were carried out in 96-well microtiter plates and the crude reaction products used directly for the enzymatic assay to make the high-throughput synthesis and screening more efficient. This method entails that compound synthesis will be scaled up for characterization, once a potent inhibitor is identified.

The mixture of iminocyclitol **5**, carboxylic acid (Figure 5), diisopropyl ethylamine (DIEA), and HBTU in methanol was shaken in a 96-well microtiter plate overnight. After the starting material was consumed, the mixture was diluted and transferred to another 96-well microtiter plate for the *N*-acetyl-β-hexosaminidases inhibition assay, where the final inhibitor concentration was 25 μM (based on 100% conversion of starting materials to amides). The inhibition percentage relative to the control with no inhibitor was calculated on the basis of the

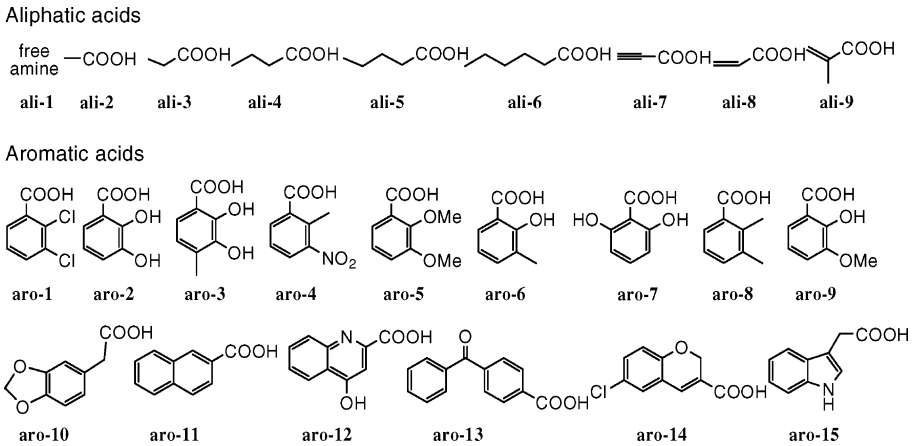
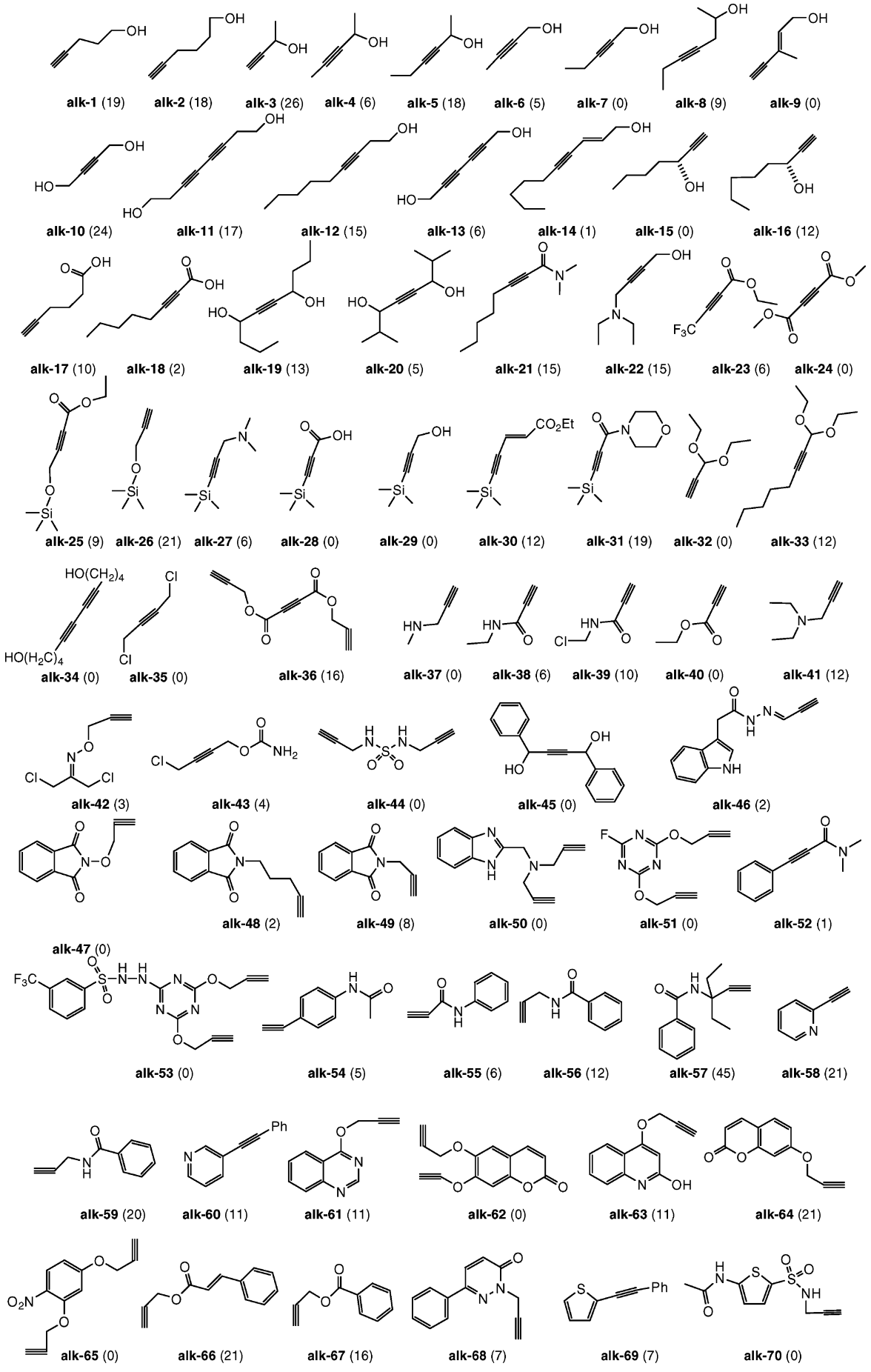


FIGURE 5. Carboxylic acid building blocks.


alk-1 (19)
alk-2 (18)
alk-3 (26)
alk-4 (6)
alk-5 (18)
alk-6 (5)
alk-7 (0)
alk-8 (9)
alk-9 (0)
alk-10 (24)
alk-11 (17)
alk-12 (15)
alk-13 (6)
alk-14 (1)
alk-15 (0)
alk-16 (12)
alk-17 (10)
alk-18 (2)
alk-19 (13)
alk-20 (5)
alk-21 (15)
alk-22 (15)
alk-23 (6)
alk-24 (0)
alk-25 (9)
alk-26 (21)
alk-27 (6)
alk-28 (0)
alk-29 (0)
alk-30 (12)
alk-31 (19)
alk-32 (0)
alk-33 (12)
alk-34 (0)
alk-35 (0)
alk-36 (16)
alk-37 (0)
alk-38 (6)
alk-39 (10)
alk-40 (0)
alk-41 (12)
alk-42 (3)
alk-43 (4)
alk-44 (0)
alk-45 (0)
alk-46 (2)
alk-47 (0)
alk-48 (2)
alk-49 (8)
alk-50 (0)
alk-51 (0)
alk-52 (1)
alk-53 (0)
alk-54 (5)
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alk-57 (45)
alk-58 (21)
alk-59 (20)
alk-60 (11)
alk-61 (11)
alk-62 (0)
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alk-65 (0)
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alk-67 (16)
alk-68 (7)
alk-69 (7)
alk-70 (0)

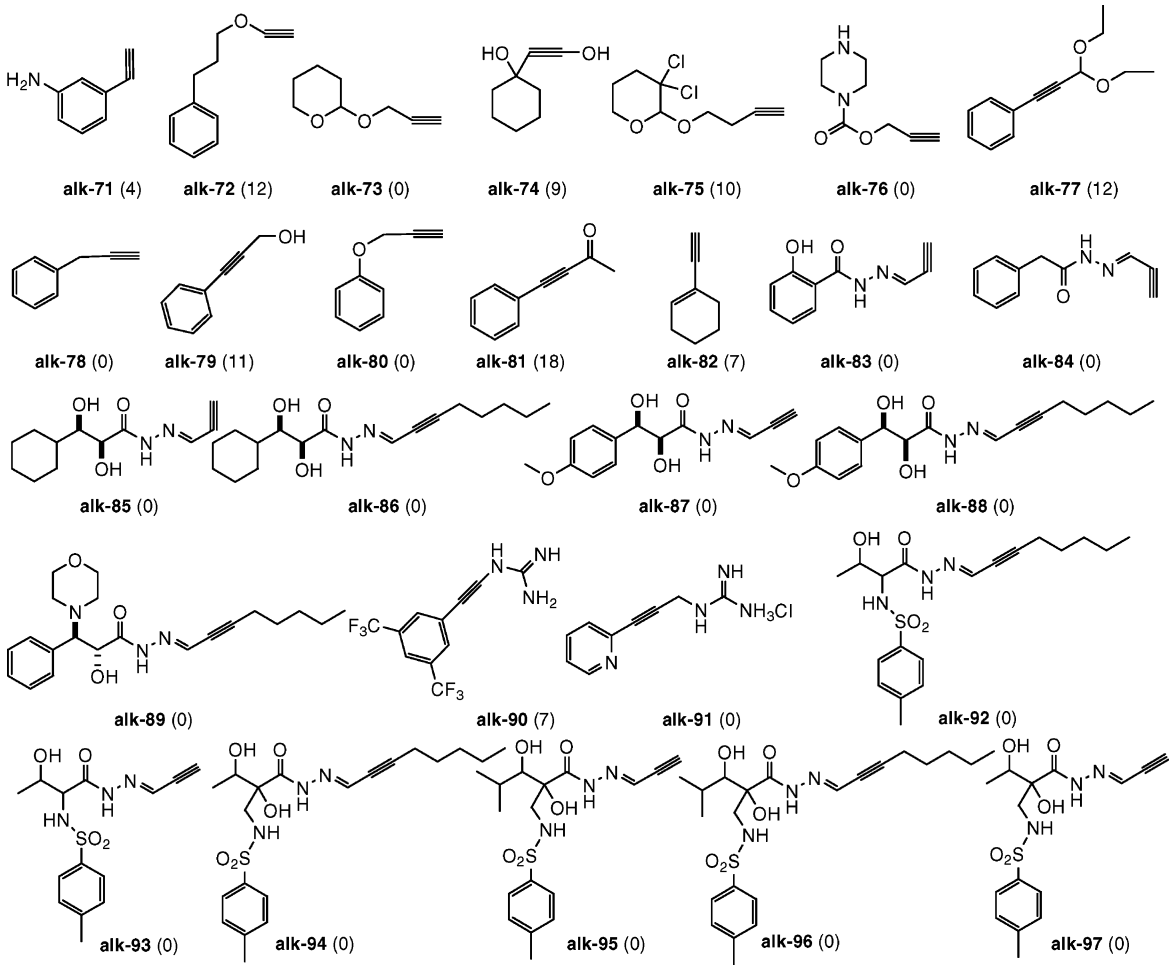

FIGURE 6. Alkyne building blocks and bioassay results (% inhibition shown in parentheses).

fluorescence readings of the released 4-methylumbelliferone from the substrate 4-methylumbellyferyl-*N*-acetyl-β-D-glucosamine (Chart 1). Acetic acid derivative (**ali-2**, Figure 5) is the best inhibitor (92% inhibition at 25 μM). It is interesting to note the large activity difference between the acetic acid derivative and other carboxylic acids derivatives, which indicates that the acetamido group is critical for the inhibitor's activity against human *N*-acetyl-β-hexosaminidases. There is no apparent activity difference between aromatic and aliphatic inhibitors, although none is as potent as the iminocyclitol **2**.

Figure 6 shows the 97 alkynes containing different functional groups that were coupled to **6** by heating at 80 °C for 24 h generating both the 1,4- and 1,5-regioismers for greater diversity, although 1,4-triazoles can be generated exclusively using copper salts.[25] In cases where two nonequivalent alkyne groups existed (**alk-62** and **alk-65**, Figure 6), four isomers could be generated. At 25 μM, the inhibitors were tested against human *N*-acetyl-β-hexosaminiadses, and the inhibition percentage was calculated as described above for the amide library. No inhibitor as potent as iminocyclitol **2** (Figure 2) has been yet discovered.

Conclusion

In summary, two new methods to prepare iminocyclitols were described, starting from D-mannosamine hydrochloride and a modification of fructose's microbial oxidation product. The substituent effects on the ring nitrogen and C1 nitrogen of α-iminocyclitol were investigated through a rapid synthesis of diversity-oriented libraries through reductive amination, amide coupling, and triazole formation reactions. The later two reactions could be carried out in 96-well microtiter plates, where the crude products were screened directly against human *N*-acetyl-β-hexosaminidases. The high efficiency of the coupling reactions meant that many iminocyclitol derivatives could be easily generated and screened in a short time. Although no significant improvement in inhibition was found, our results indicate that modifying both the ring nitrogen and C1 nitrogen causes significant activity losses; possibly these groups are directly involved in interactions within the enzymes active site. With the availability of the structure of human *N*-acetyl-β-hexosaminidase B,[26] it is possible to rationally design potent *N*-acetyl-β-hexosaminidase inhibitors using similar high-throughput approaches. Work is in progress to synthesize

(22) Brik, A.; Lin, Y.-C.; Elder, J.; Wong, C.-H. *Chem. Biol.* **2002**, *9*, 891.
(23) Breinbauer, R.; Köhn, M. *ChemBioChem.* **2003**, *4*, 1147.
(24) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397.
(25) (a) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

and screen novel iminocyclitols following the methodology described above and will be reported in due course.

Experimental Section

General Procedures. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium−benzophenone and dichloromethane (CH_2Cl_2) from calcium hydride. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

2-Deoxy-2-azido-1,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (10). To a mixture of NaN_3 (46 g, 0.71 mole) in water (93 mL) and CH_2Cl_2 (93 mL) was added dropwise Tf_2O (22 mL, 5.5 equiv) over 30 min under vigorous stirring at 0 °C. After 2 h, the organic phase was separated, and the aqueous phase was extracted twice with CH_2Cl_2 (46 mL). The organic layers were combined and washed successively with aqueous saturated $NaHCO_3$ (150 mL) and water (150 mL). The solution of TfN_3 was used immediately without drying.

D-Mannosamine hydrochloride (5 g, 23.2 mmol), K_2CO_3 (4.25 g, 30.7 mmol, 1.32 equiv), and $CuSO_4$ (53 mg, 0.33 mmol) were dissolved in water (67 mL) and MeOH (130 mL). The solution of TfN_3 previously obtained (186 mL) was added dropwise under vigorous stirring at room temperature. After the green biphasic mixture was stirred at room temperature for 2 days, the solvent was removed in vacuo. To the mixture of the crude solid in dry pyridine (150 mL) was added dropwise a solution of acetic anhydride (40 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 5 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (silica, 1:1 EtOAc/hexane) to yield compound **10** (7.18 g, 89%) as a pale-yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.11 (s, 1H, H-1 α), 5.89 (s, 0.5H, H-1 β), 5.38 (t, J = 6.1 Hz, 2H), 5.27 (t, J = 9.6 Hz, 0.5H), 5.14 (dd, J = 3.1, 9.6 Hz, 0.5H), 4.28−4.23 (m, 1.5H), 4.18 (d, J = 3.5 Hz, 0.5H), 4.11−4.03 (m, 3.5H), 3.80 (dd, J = 3.5, 6.1 Hz, 0.5H), 2.19−2.05 (8s, 18H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 169.65, 169.64, 169.60, 169.0, 168.0, 167.87, 167.85, 72.7, 71.4, 70.3, 70.1, 64.9, 64.6, 61.4, 60.7, 60.1, 20.4, 20.3, 20.29, 20.21, 20.1, 20.09; HRMS calcd for $C_{14}H_{19}N_3O_9$ [M + Na]$^{+\bullet}$ 396.1017, found 396.1013.

***p*-Methylphenyl 2-Deoxy-2-azido-3,4,6-tri-*O*-acetyl-1-thio-D-mannopyranoside (11).** Compound **10** (3.8 g, 10.2 mmol) and *p*-toluene sulfide (2.5 g, 20.2 mmol, 1.9 equiv) in dry CH_2Cl_2 (68 mL) were cooled to 0 °C. $BF_3{\cdot}Et_2O$ (2.5 mL, 20 mmol, 1.9 equiv) was added dropwise under argon and the reaction mixture was stirred for 6 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 2:7 EtOAc/hexane) to yield compound **11** (4.0 g, 90%) as a pale-yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 8.0 Hz, 1H, β), 7.37 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 5.46 (s, 1H), 5.36−5.33 (m, 2H and 1H, β), 5.13 (ddd, J = 2.2, 4.0, 9.9 Hz, 1H, β), 4.80 (s, 1H, β), 4.50 (m, 1H), 4.29 (t, J = 1.5 Hz, 1H), 4.26 (dd, J = 5.5, 12.1 Hz, 1H), 4.13−4.06 (m, 2H), 2.33 (s, 3H), 2.13−2.02 (7s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.28, 170.24, 169.7, 169.6, 169.2, 169.1, 138.3, 138.1, 132.3, 132.2, 129.8, 129.6, 129.5, 128.3, 85.9, 85.8, 76.0, 73.6, 70.8, 69.2, 65.8, 65.5, 63.2, 62.3, 62.2, 61.9, 20.8, 20.4, 20.38, 20.37, 20.34, 20.2, 20.1; ESI+ m/z 460 (M + Na$^+$), ESI− m/z 472 (M + Cl$^-$).

***p*-Methylphenyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-azido-1-thio-β-D-mannopyranoside (12).** To a solution of compound **11** (3.99 g, 9.1 mmol) in dry MeOH (200 mL) was added NaOMe (3.6 mL, 1.8 mmol) dropwise at 0 °C under argon. After 1.5 h at 0 °C and 1 h at room temperature, the solvent was removed in vacuo. The residue was dissolved in dry DMF (80 mL) and cooled to 0 °C. NaH (1.7 g, 4.7 equiv) and BnBr (8 mL, 67.1 mmol, 7.4 equiv) were successively added, and the solution was stirred under argon overnight. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:8 EtOAc/hexane) to yield compound **12** (4.61 g, 87%) as a pale-yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36−7.16 (m, 17H), 6.98 (d, J = 7.8 Hz, 2H), 5.39 (s, 1H, H-1), 4.82 (d of AB, J = 10.9 Hz, 1H, PhCH_2), 4.69 (d of AB, J = 10.9 Hz, 1H, PhCH_2), 4.60 (d of AB, J = 12.2 Hz, 1H, PhCH_2), 4.56 (d of AB, J = 11.0 Hz, 1H, PhCH_2), 4.49 (d of AB, J = 10.5 Hz, 1H, PhCH_2), 4.40 (d of AB, J = 12.2 Hz, 1H, PhCH_2), 4.30 (dd, J = 4.4, 9.6 Hz, 1H, H-6), 4.04 (m, 1H, H-4), 4.01 (dd, J = 3.5, 9.2 Hz, 1H, H-6′), 3.92 (t, J = 9.6 Hz, 1H, H-5), 3.75 (dd, J = 4.0, 10.9 Hz, 1H, H-3), 3.65 (d, J = 10.9 Hz, 1H, H-2), 2.22 (s, 3H, ArCH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.8, 137.7, 137.2, 132.1, 129.6, 129.2, 128.2, 128.07, 128.05, 127.7, 127.6, 127.5, 127.42, 127.40, 127.2, 86.2, 79.7, 74.9, 74.4, 73.0, 72.4, 72.3, 68.5, 62.3, 20.8; MALDI-TOF m/z 604 (M + Na$^+$).

2-Deoxy-2-azido-3,4,6-tri-*O*-benzyl-D-mannitol (14). To a solution of compound **12** (433 mg, 0.74 mmol) in acetone (72 mL) were successively added water (0.8 mL) and NIS (1.3 g, 5.7 mmol, 7.8 equiv). After 3 h at room temperature, the reaction was quenched by addition of aqueous $Na_2S_2O_3$ (1 M). The aqueous phase was extracted with CH_2Cl_2 (150 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash chromatography (silica, 1:3 EtOAc/hexane) to yield 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-mannopyranose **13** (544 mg, 65%) as a pale-yellow oil: HRMS calcd for $C_{27}H_{29}N_3O_5$ [M + Na]$^{+\bullet}$ 498.1999, found 498.1992.

To a solution of compound **13** (495 mg, 1.04 mmol) in dry MeOH (18 mL) was added one portion of $NaBH_4$ (79 mg, 2.08 mmol, 2 equiv) at 0 °C under argon. The solution was stirred at 0 °C for 5 min and then at room temperature for 2 h. Portions of $NaBH_4$ were added under the same conditions until total disappearance of the starting material. The reaction mixture was then cooled to 0 °C and quenched by addition of glacial AcOH (1 mL). After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:2 EtOAc/hexane) to yield compound **14** (410 mg, 83%) as a pale-yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34−7.23 (m, 15H), 4.70 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.61 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.59 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.54 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.51 (d of AB, J = 11.8 Hz, 1H, PhCH_2), 4.48 (d of AB, J = 11.4 Hz, 1H, PHCH_2), 4.10 (dd, J = 7.0, 14.0 Hz, 1H, H-3), 3.98 (ddd, J = 4.4, 8.3, 11.8 Hz, 1H, H-5), 3.89−3.86 (m, 2H, H-4, OH), 3.77−3.72 (m, 2H, H-6, OH), 3.69−3.65 (m, 2H, H-6′, OH), 3.61 (dd, J = 4.9, 14.4 Hz, 1H, H-2); ^{13}C (150 MHz, $CDCl_3$) δ 137.7, 137.6, 137.5, 128.4, 128.36, 128.34, 128.29, 128.27, 128.1, 127.96, 127.93, 127.86, 127.83, 127.7, 78.4, 77.7, 74.4, 74.1, 73.3, 70.9, 69.8, 62.9, 62.0; HRMS calcd for $C_{27}H_{31}N_3O_5$ [M + Na]$^{+\bullet}$ 500.2156, found 500.2163.

1-(*tert*-Butyl-dimethyl-sylanyloxy)-2-deoxy-2-azido-3,4,6-tri-*O*-benzyl-D-mannitol (15). To a solution of compound **14** (1.4 g, 2.93 mmol) in dry pyridine (46 mL) was added TBDMSCl (484 mg, 3.22 mmol, 1.1 equiv) at 0 °C under argon. The reaction mixture was stirred overnight. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:5 EtOAc/hexane) to yield compound **15** (1.23 g, 71%) as a pale-yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35−7.23 (m, 15H), 4.71 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.61 (d of AB, J = 11.4 Hz, 2H, PhCH_2), 4.54 (d of AB, J = 11.4 Hz, 1H, PhCH_2), 4.53 (d of AB, J = 11.8 Hz, 1H, PhCH_2), 4.50 (d of AB, J = 11.8 Hz, 1H, PhCH_2), 4.01−3.97 (m, 2H, H-3, 4), 3.89 (dd, J = 2.2, 8.7 Hz, 1H, H-6), 3.81 (dd, J = 6.1, 10.6 Hz, 1H, H-5), 3.76 (dd, J = 2.2, 8.7 Hz, 1H, H-6′), 3.68 (dd, J = 3.5, 9.6 Hz, 1H, H-1), 3.63 (dd, J = 4.8, 9.6 Hz, 1H, H-1′), 3.60−3.58 (m, 1H, H-2), 2.55 (d, J = 6.6 Hz, 1H),

(26) (a) Maier, T.; Strater, N.; Schuette, C. G.; Klingenstein, R.; Sandhoff, K.; Saenger, W. *J. Mol. Biol.* **2003**, *328*, 669. (b) Mark, B. L.; Mahuran, D. J.; Cherney, M. M.; Zhao, D.; Knapp, S.; James, M. N. G. *J. Mol. Biol.* **2003**, *327*, 1003.

0.91 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.97, 137.96, 137.7, 128.5, 128.4, 128.3, 128.12, 128.10, 128.0, 127.9, 127.8, 127.7, 78.6, 76.9, 74.5, 74.3, 73.4, 70.9, 69.6, 63.6, 62.4, 25.8, 18.1, −5.6; HRMS calcd for $C_{33}H_{45}N_3O_5Si$ $[M + Na]^{+\bullet}$ 614.3021, found 614.3029.

1-(*tert*-Butyl-dimethyl-sylanyloxy)-2-deoxy-2-azido-3,4,6-tri-*O*-benzyl-5-oxo-D-mannitol (8). To a solution of compound **15** (149 mg, 0.25 mmol) in dry CH_2Cl_2 (2.4 mL) was added Dess−Martin reagent (110 mg, 0.26 mmol, 1.04 equiv) at 0 °C under argon. The solution was stirred for 30 min at 0 °C and then overnight at room temperature. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:6 EtOAc/hexane) to yield compound **8** (135 mg, 91%) as a pale-yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.19 (m, 15H), 4.58−4.48 (m, 4H, PhCH_2, H-6, 6′), 4.45−4.39 (m, 2H, PhCH_2), 4.32 (d of AB, J = 18.3 Hz, 1H, PhCH_2), 4.27 (d, J = 2.5 Hz, 1H, H-4), 4.22 (d of AB, J = 18.7 Hz, 1H, PhCH_2), 3.99 (dd, J = 2.5, 5.1 Hz, 1H, H-3), 3.97 (t, J = 2.9 Hz, 1H, H-1), 3.80 (dd, J = 5.5, 11.0 Hz, 1H, H-1′), 3.62 (ddd, J = 2.9, 5.5, 8.8 Hz, 1H, H-2), 0.91 (s, 9H, C(CH_3)$_3$), 0.08 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.8, 137.08, 137.07, 136.5, 128.57, 128.52, 128.46, 128.43, 128.3, 128.2, 128.0, 127.98, 127.95, 83.7, 78.4, 74.8, 74.6, 73.3, 62.9, 61.9, 25.8, 18.1, −5.6; HRMS calcd for $C_{33}H_{44}N_3O_5Si$ $[M + Na]^{+\bullet}$ 612.2864, found 612.2864.

(2*R*,3*R*,4*R*,5*R*)-2-(*tert*-Butyl-dimethyl-sylanyloxy-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (7) and (2*R*,3*R*,4*R*,5*S*)-2-(*tert*-Butyl-dimethyl-sylanyloxy-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (16). Method A. Compound **8** (110 mg, 0.18 mmol) was stirred in THF/pyridine (1:1, 4 mL) in the presence of Pd−C (200 mg) under hydrogen atmosphere (1 atm) for 4 h. After the catalyst was filtered off using Celite, the solvent was evaporated in vacuo. To the solution of the crude product in dry MeOH (1.5 mL) were added $NaBH_3CN$ (17 mg, 0.27 mmol, 1.5 equiv) and *p*-TsOH (38 mg, 0.2 mmol, 1.1 equiv). The reaction mixture was stirred overnight at room temperature. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 2:5 EtOAc/hexane) to yield compounds **7** (7 mg, 7%) and **16** (70 mg, 69%).

Method B. Following the similar procedure described in method A, **7** and **16** were isolated in a 1:3 ratio when $NaBH(OAc)_3$ was used instead of $NaBH_3CN$.

Method C. To a solution of compound **8** (400 mg, 0.82 mmol) in dry MeOH (8.5 mL) was added $NaBH_4$ (31 mg, 0.82 mmol, 1 equiv) at 0 °C under argon. After 1 h, the reaction was quenched by addition of glacial AcOH (0.17 mL). After the solvent was removed in vacuo, the crude product was passed through a short silica column (1:5 EtOAc/hexane) to yield **20** (393 mg, 98%), which was then dissolved in dry pyridine (2.7 mL). To the solution was added dropwise MsCl (0.1 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 2 h. After the solvent was removed in vacuo, the residue was filtered through a pack of silica using EtOAc as eluent. After the solvent was removed in vacuo, the oily residue was dissolved in pyridine/THF (1:1 16 mL) and stirred under hydrogen atmosphere (1 atm) in the presence of Pd−C (900 mg) for 24 h. The reaction mixture was then filtered through Celite. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 2:5 to 5:5 EtOAc/hexane) to yield compound **7** (189 mg, 62%) and compound **16** (82 mg, 27%) as pale-yellow oils.

Data for compound **7**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.25 (m, 15H), 4.57−4.50 (m, 6H, PhCH_2), 3.93 (m, 2H, H-3, 4), 3.66 (d, J = 5.8 Hz, 2H, H-6, 6′), 3.55 (dd, J = 5.8, 9.5 Hz, 1H, H-1), 3.51 (dd, J = 6.2, 9.5 Hz, 1H, H-1′), 3.36 (dd, J = 5.1, 10.3 Hz, 1H, H-2), 3.21 (dd, J = 5.1, 10.3 Hz, 1H, H-5), 2.0 (bs, 2H), 0.9 (s, 9H, C(CH_3)$_3$), 0.05 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.3, 138.2, 128.37, 128.32, 128.2, 127.74, 127.70, 127.67, 127.65, 127.63, 127.5, 86.7, 86.0, 73.2, 71.8, 71.7, 71.0, 63.3, 63.1, 61.6, 25.9, 18.3, −5.4; HRMS calcd for $C_{33}H_{45}N_1O_4Si$ $[M + H]^{+\bullet}$ 548.3190, found 548.3185.

Data for compound **16**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.25 (m, 15H), 4.57−4.34 (m, 6H, PhCH_2), 3.93 (dd, J = 1.1, 4.4 Hz, 1H, H-3), 3.86 (dd, J = 1.1, 4.0 Hz, 1H, H-4), 3.74 (dd, J = 6.2, 9.1 Hz, 1H, H-6), 3.70−3.65 (m, 2H, H-1, 1′), 3.62 (dd, J = 7.0, 9.1 Hz, 1H, H-6′), 3.52 (dd, J = 6.2, 11.0 Hz, 1H, H-2), 3.18 (dd, J = 4.7, 9.1 Hz, 1H, H-5), 1.91 (bs, 2H), 0.80 (s, 9H, C(CH_3)$_3$), 0.01 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.36, 138.34, 138.33, 128.4, 128.3, 128.29, 128.28, 127.7, 127.6, 127.58, 127.56, 127.54, 84.4, 83.5, 73.4, 71.5, 71.3, 69.5, 65.5, 63.9, 60.8, 25.9, 18.2, −5.4; ESI+ m/z 548 $(M + H^+)$, 570 $(M + Na^+)$.

1-(*tert*-Butyl-dimethyl-sylanyloxy)-2-deoxy-2-azido-3,4,6-tri-*O*-benzyl-5-oxo-D-glucitol (17). Compound **17** was prepared from D-glucosammine hydrochloride following the same route as the synthesis of **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.24 (m, 15H), 4.41−4.63 (m, 6H, PhCH_2), 4.28−4.25 (m, 2H, H-6, 6′), 4.17 (d, J = 4.4 Hz, 1H, H-3), 3.90 (t, J = 4.4 Hz, 1H, H-4), 3.67−3.58 (m, 3H, H-1, 1′, 2), 0.87 (s, 9H, C(CH_3)$_3$), 0.17 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2, 137.1, 136.6, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 81.9, 78.5, 74.5, 74.3, 73.8, 73.3, 63.2, 62.9, 25.8, 18.7, −5.5; ESI+ m/z 590 $(M + H^+)$, 612 $(M + Na^+)$.

(2*S*,3*R*,4*R*,5*R*)-2-(*tert*-Butyl-dimethyl-sylanyloxy-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (18). A mixture of compound **17** (18 mg, 0.03 mmol) and Lindlar catalyst (10 mg) in toluene (0.1 mL) was stirred under hydrogen atmosphere (1 atm) for 1 h. After the catalyst was filtered off using Celite, the solvent was evaporated in vacuo. To a solution of the crude product in dry MeOH (0.25 mL) were added $NaBH_3CN$ (3 mg, 0.05 mmol, 1.5 equiv) and *p*-TsOH (6.4 mg, 0.033 mmol, 1.1 equiv) at room temperature. The reaction mixture was stirred at room temperature for 3 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 5:2 EtOAc/hexane) to afford **18** (12 mg, 72% yield): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36−7.24 (m, 15H), 4.55−4.44 (m, 6H, PhCH_2), 4.01−3.99 (m, 2H, H-3, 4), 3.75 (dd, J = 6.6, 9.6 Hz, 1H, H-6), 3.68−3.64 (m, 3H, H-6′, 1, 1′), 3.54 (t, J = 10.2 Hz, 1H, H-2), 3.49 (dd, J = 6.1, 11.8 Hz, 1H, H-5), 0.88 (s, 9H, C(CH_3)$_3$), 0.04 (s, 6H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.42, 138.40, 138.3, 128.33, 128.31, 127.7, 127.59, 127.56, 127.55, 127.50, 82.7, 82.3, 73.3, 72.3, 72.2, 69.9, 62.0, 60.3, 50.0, 25.9, 18.3, −5.3; ESI+ m/z 548 $(M + H^+)$.

(2*R*,3*R*,4*R*,5*R*)-2-(Hydroxy-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (21). To a solution of compound **7** (197 mg, 0.36 mmol) in dry THF (4.5 mL) was added TBAF (0.9 mL, 0.9 mmol, 2.5 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 5:0.3 $CHCl_3$/MeOH) to yield compound **21** (125 mg, 97%) as a white crystalline solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35−7.25 (m, 15H), 4.67−4.55 (m, 6H, PhCH_2), 3.94 (dd, J = 3.9, 5.0 Hz, 1H, H-3), 3.86 (t, J = 3.9 Hz, 1H, H-4), 3.60 (dd, J = 4.4, 11.0 Hz, 1H, H-6), 3.57−3.54 (m, 2H, H-6′, 1), 3.51 (dd, J = 5.7, 9.7 Hz, 1H, H-1′), 3.36 (m, 2H, H-2, 5), 3.06 (bs, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.85, 137.83, 137.79, 128.45, 128.41, 128.40, 128.84, 127.80, 127.77, 127.73, 85.5, 85.1, 73.2, 72.0, 71.2, 69.1, 63.1, 61.9, 61.4; HRMS calcd for $C_{27}H_{31}NO_4$ $[M + H]^{+\bullet}$ 434.2326, found 434.2323.

***N*-*tert*-Butyloxycarbonyl-(2*R*,3*R*,4*R*,5*R*)-2-(hydroxy-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (22).** To a solution of compound **21** (151 mg, 0.35 mmol) in CH_2Cl_2 (1.75 mL) in the presence of Et_3N (74 μL) was added Boc_2O (108 mg, 0.50 mmol, 1.4 equiv) at 0 °C under argon. The reaction mixture was stirred at room temperature overnight. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:3 EtOAc/hexane) to yield compound **22** (155 mg, 83%) as a pale-yellow oil. The spectra are consistent with those reported.[27]

(27) Takebayashi, M.; Hiranuma, S.; Kanie, Y.; Kajimoto, T.; Kanie, O.; Wong, C.-H. *J. Org. Chem.* **1999**, *64*, 5280.

***N*-*tert*-Butyloxycarbonyl-(2*R*,3*R*,4*R*,5*R*)-2-(azido-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (23).** To a solution of compound **22** (154 mg, 0.35 mmol) and Et_3N (56 μL) in dry CH_2Cl_2 (1.8 mL) was added dropwise MsCl (30 μL, 0.39 mmol, 1.1 equiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 2 h, diluted with EtOAc (6 mL), and washed successively with aqueous saturated $NaHSO_4$ (1.5 mL), aqueous saturated $NaHCO_3$ (1.5 mL), and water (1.5 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to yield a yellow oil. The crude product was taken up in dry DMF (3 mL) in the presence of NaN_3 (190 mg, 2.92 mmol), and the reaction mixture was stirred at 75 °C under argon for 24 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:9 EtOAc/hexane) to yield compound **23** (mixture of atropoisomers) (85 mg, 53%) as a pale-yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.20 (m, 15H), 4.65−4.39 (m, 6H, PhCH_2), 4.15−4.19 (m, 1.5H), 4.00−3.93 (m, 3H), 3.85 (dd, J = 3.7, 11.0 Hz, 0.5H), 3.74 (dd, J = 4.0, 8.8 Hz, 0.5H atropoisomer b), 3.69 (dd, J = 4.0 Hz, 11.4 Hz, 0.5H atropoisomer a), 3.51−3.45 (m, 1H), 3.28−3.35 (m, 1H), 1.47 (s, 4.5H), 1.4 (s, 4.5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.4, 138.1, 137.54, 137.51, 137.4, 128.48, 128.42, 128.41, 128.3, 128.2, 127.8, 127.79, 127.72, 127.6, 127.59, 127.58, 127.4, 83.4, 82.7, 82.0, 81.2, 73.03, 73.00, 71.3, 71.26, 71.22, 71.0, 68.3, 67.5, 63.0, 62.9, 62.7, 62.6, 50.9, 49.6, 28.4, 28.3; HRMS calcd for $C_{32}H_{38}N_4O_5$ $[M + Na]^{+\bullet}$ 581.2734, found 581.2742.

(2*R*,3*R*,4*R*,5*R*)-2-(Azido-methyl)-3,4-di-*O*-benzyl-5-(*O*-benzyl-methyl)-pyrrolidine (24). To a solution of compound **23** (59 mg, 0.10 mmol) in dry CH_2Cl_2 (0.6 mL) was added dropwise TFA (0.5 mL) at 0 °C under argon. The reaction mixture was stirred at this temperature for 2 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 1:1 EtOAc/hexane) to yield compound **24** (47 mg, 97%) as a pale-yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.26 (m, 15H), 4.55−4.49 (m, 6H, PhCH_2), 3.91 (t, J = 3.7 Hz, 1H, H-3), 3.83 (t, J = 4.4 Hz, 1H, H-4), 3.53 (dd, J = 5.5, 9.5 Hz, 1H, H-6), 3.50 (dd, J = 5.8, 9.5 Hz, 1H, H-6′), 3.41−3.30 (m, 4H, H-1, 1′, 2, 5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.0, 137.9, 137.8, 128.44, 128.41, 127.8, 127.79, 127.76, 127.72, 86.5, 85.7, 73.2, 72.00, 71.97, 70.1, 61.8, 61.4, 53.5; HRMS calcd for $C_{27}H_{30}N_4O_3$ $[M + H]^{+\bullet}$ 459.2391, found 459.2394.

(2*R*,3*R*,4*R*,5*R*)-2-(Amino-methyl)-3,4-dihydroxy-5-(hydroxy-methyl)-pyrrolidine (5). To a solution of compound **24** (32 mg, 70 μmol) in MeOH (0.6 mL) and aqueous HCl (0.15 mL, pH 4) was added Pd−C (20 mg). The reaction mixture was stirred under hydrogen atmosphere (70 psi) for 24 h before being filtered through Celite. After the solvent was removed in vacuo, the dihydrochloride of compound **5** was obtained (quant) as an oily residue: ^{1}H NMR (600 MHz, D_2O) δ 4.00−3.95 (m, 2H, H-3, 4), 3.84 (dd, J = 4.8, 14.5 Hz, 1H, H-6), 3.75 (dd, J = 7.5, 14.5 Hz, 1H, H-6′), 3.52−3.47 (m, 1H, H-5), 3.36 (dd, J = 6.0, 15.8 Hz, 1H, H-1), 3.34−3.28 (m, 1H, H-2), 3.27 (dd, J = 10.0, 15.8 Hz, 1H, H-1′); ^{13}C NMR (150 MHz, D_2O) δ 78.4, 76.1, 62.9, 60.2, 58.5, 40.9; ESI+ m/z 163 ($M + H^+$), 185 ($M + Na^+$).

(2*R*,3*R*,4*R*,5*R*)-2-(Azido-methyl)-3,4-dihydroxy-5-(hydroxy-methyl)-pyrrolidine (6). To a solution of compound **24** (40 mg, 87 μmol) in MeOH (0.8 mL) and aqueous HCl (0.2 mL, pH 4) was added Pd−C (25 mg). The reaction mixture was stirred under hydrogen atmosphere (70 psi) for 24 h and filtered through Celite, and the solvent was removed in vacuo. The crude product obtained, $CuSO_4$ (1 mg) and K_2CO_3 (35 mg, 0.25 mmol, 3 equiv), were dissolved in MeOH (0.8 mL) and H_2O (0.2 mL). TfN_3 in CH_2Cl_2 (0.7 mL) was added dropwise over 1 h at room temperature, and the reaction mixture was vigorously stirred at room temperature for 24 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography (silica, 8:2:0.2 $CHCl_3$/MeOH/H_2O) to yield compound **6** (14 mg, 85% yield) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 4.03−4.01 (m, 2H, H-3, 4), 3.88−3.83 (m, 2H, H-1, 6), 3.80 (dd, J = 5.8, 12.4 Hz, 1H, H-6′), 3.73 (dd, J = 6.6, 13.5 Hz, 1H, H-1′), 3.50−3.46 (m, 1H, H-5), 3.44−3.40 (m, 1H, H-2); ^{13}C NMR (150 MHz, D_2O) δ 86.3, 85.3, 72.6, 70.2, 69.2, 60.2; HRMS calcd for $C_6H_{12}N_4O_3$ $[M + H]^{+\bullet}$ 189.0982, found 189.0984.

(2*S*,3*R*,4*R*,5*R*)-2-(Azidomethyl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-1-methylpyrrolidine (28). To a solution of compound **26** (170 mg, 0.4 mmol) and pyridine (122 μL, 1.5 mmol) in CH_2Cl_2 (5 mL) was added MsCl (110 μL, 1.4 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h. The solvent was removed under high vacuum. Without purification, the residue was dissolved in DMF (20 mL). NaN_3 (200 mg, 3.1 mmol) and NaI (60.5 mg, 0.40 mmol) were added to this solution. After 5 h, the reaction was quenched with H_2O and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica, 1:2 EtOAc/hexane) to afford azido compound **28** (156 mg, 87%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36−7.24 (m, 15H, Ph), 4.54 (d of AB, J = 11.7 Hz, 1H, PhCH_2), 4.53 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.50 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.46 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.45 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.39 (d of AB, J = 11.7 Hz, 1H, PhCH_2), 3.90 (d, J = 4.5 Hz, 1H, H-4), 3.81 (m, 1H, H-3), 3.59 (dd, J = 8.5, 11.8 Hz, 1H, H-1), 3.54 (dd, J = 5.2, 8.6 Hz, 1H, H-6), 3.42 (dd, J = 8.2, 8.6 Hz, 1H, H-6′), 3.32 (dd, J = 4.8, 11.8 Hz, 1H, H-1′), 2.85 (dt, J = 4.8, 8.5 Hz, 1H, H-2), 2.76 (ddd, J = 2.5, 5.2, 7.7 Hz, 1H, H-5), 2.43 (s, 3H, NCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 138.0, 137.7, 128.4, 128.3, 127.9, 127.7, 127.7, 127.6, 127.6, 127.5, 82.5, 82.0, 73.1, 71.9, 71.6, 71.0, 67.5, 49.4, 41.3; HRMS calcd for $C_{28}H_{32}N_4O_3$ $[M + H]^{+\bullet}$ 473.2553, found 473.2528.

***N*-(((2*S*,3*R*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)-methyl)-1-methylpyrrolidin-2-yl)methyl)acetamide (30).** To a solution of **28** (104 mg, 0.22 mmol) in dry THF (10 mL) was added PPh_3 (118 mg, 0.45 mmol). The mixture was heated to 50 °C for 4 h and concentrated in vacuo. The residue was dissolved in pyridine (8 mL). Acetic anhydride (91 μL, 0.86 mmol) was added to the reaction mixture at 0 °C. After 12 h, the reaction was quenched with water (50 mL) and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, 60:1 CH_2Cl_2/MeOH) afforded **30** (91 mg, 87% yield from **28**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37−7.25 (m, 15H, Ph), 4.61 (d of AB, J = 11.9 Hz, 1H, PhCH_2), 4.57 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.55 (d of AB, J = 12.1 Hz, 1H, PhCH_2), 4.53 (d of AB, J = 11.9 Hz, 1H, PhCH_2), 4.52 (d of AB, J = 11.9 Hz, 1H, PhCH_2), 4.26 (d of AB, J = 11.9 Hz, 1H, PhCH_2), 3.84 (d, J = 3.3 Hz, 1H, H-4), 3.81 (d, J = 4.9 Hz, 1H, H-3), 3.60 (dd, J = 4.6, 9.7 Hz, 1H, H-6), 3.54 (ddd, J = 3.0, 5.3, 13.4 Hz, 1H, H-1), 3.48 (dd, J = 7.3, 9.7 Hz, 1H, H-6′), 3.34 (ddd, J = 5.3, 7.6, 13.4 Hz, 1H, H-1′), 2.77 (ddd, J = 3.3, 4.9, 7.6 Hz, 1H, H-2), 2.72 (dt, J = 4.3, 7.6 Hz, 1H, H-5), 2.37 (s, 3H, NCH_3), 1.69 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 138.2, 138.1, 137.7, 128.7, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.6, 83.0, 82.7, 71.4, 71.2, 71.2, 70.6, 40.2, 37.1, 23.1; HRMS calcd for $C_{30}H_{36}N_2O_4$ $[M + H]^{+\bullet}$ 489.2753, found 489.2738.

***N*-(((2*S*,3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-1-methylpyrrolidin-2-yl)methyl)acetamide (4).** Compound **30** (34 mg, 0.07 mmol) was dissolved in AcOH/THF/H_2O (4:2:1, 6 mL) and treated with Pd−C (10%) (10 mg). The reaction mixture was hydrogenated under 50 psi pressure. After 1 d, the catalyst was removed by filtration, and the filtrate was concentrated and purified by flash chromatography (silica, 6:4:0.7 $CHCl_3$/MeOH/H_2O) to yield **4** (16.6 mg, 89%) as an oil: ^{1}H NMR (500 MHz, D_2O) δ 4.06 (dd, J = 3.0, 5.5 Hz, 1H, H-4), 3.77 (dd, J = 3.0, 4.4 Hz, 1H, H-3), 3.75 (dd, J = 7.3, 11.7 Hz, 1H, H-6), 3.68 (dd, J = 4.4, 11.7 Hz, 1H, H-6′), 3.46 (dd, J = 4.1, 14.0 Hz, 1H, H-1), 3.22 (dd, J = 7.0, 14.0 Hz, 1H, H-1′), 2.73 (m, 1H, H-2), 2.45 (m, 1H, H-5), 2.35 (s, 3H, NCH_3), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, D_2O) δ 175.2, 79.4, 76.7,

71.8, 69.3, 59.3, 40.7, 40.4, 22.6; HRMS calcd for $C_9H_{18}N_2O_4$ $[M + Na]^{+\bullet}$ 241.1164, found 241.1172.

General Procedure To Prepare Aromatic Derivatives of Iminocyclitol 4. To the mixture of **4** (11 mg, 0.053 mmol) and aldehyde (0.11 mmol) in MeOH (0.7 mL) was added $NaBH_3CN$ (6.7 mg, 0.11 mmol). After the mixture was stirred at room temperature overnight, the solvent was removed in vacuo. Purification of the residue with flash chromatography (silica, 9:1 $CHCl_3$/MeOH) afforded the product.

***N*-(((2*R*,3*R*,4*R*,5*R*)-1-(4-(Pyridin-2-yl)benzyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide (31a).** Yield 62%; ^{1}H NMR (500 MHz, CD_3OD) δ 8.58 (d, J = 4.0 Hz, 1H, C*H*═N−C), 7.91−7.81 (m, 4H), 7.54 (d, J = 8.0 Hz, 2H), 7.34 (ddd, J = 1.1, 5.1, 7.3 Hz, 1H, C*H*CH═N), 4.02 (m, 3H, ArCH_2, H-4), 3.83 (t, J = 2.7, 1H, H-3), 3.77 (dd, J = 4.8, 11.4 Hz, 1H, H-6), 3.64 (dd, J = 3.0, 11.4 Hz, 1H, H-6′), 3.44 (dd, J = 3.3, 14.0 Hz, 1H, H-1), 3.31 (dd, J = 5.9, 14.0 Hz, 1H, H-1′), 3.13 (m, 1H, H-2), 3.06 (m, 1H, H-5), 1.93 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 173.5, 158.9, 150.2, 142.4, 139.1, 138.9, 129.7, 128.1, 123.6, 122.5, 81.1, 80.7, 69.9, 69.7, 61.1, 51.9, 40.2, 22.7; HRMS calcd for $C_{20}H_{25}N_3O_4$ $[M + H]^{+\bullet}$ 372.1923, found 372.1911.

***N*-(((2*R*,3*R*,4*R*,5*R*)-1-(4-Hydroxybenzyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide (31b).** Yield 62%; ^{1}H NMR (600 MHz, CD_3OD) δ 7.20 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 8.8 Hz, 2H), 3.98 (t, J = 2.2 Hz, 1H, H-4), 3.87 (d of AB, J = 13.8 Hz, 1H, ArCH_2), 3.82 (d of AB, J = 13.8 Hz, 1H, ArCH_2), 3.79 (t, J = 2.6 Hz, 1H, H-3), 3.75 (dd, J = 5.3, 11.4 Hz, 1H, H-6), 3.62 (dd, J = 3.1, 11.4 Hz, 1H, H-6′), 3.40 (dd, J = 3.5, 13.8 Hz, 1H, H-1), 3.25 (dd, J = 6.2, 13.8 Hz, 1H, H-1′), 3.07 (dt, J = 3.0, 6.2 Hz, 1H, H-2), 3.00 (dt, J = 2.2, 4.4 Hz, 1H, H-5), 1.92 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, CD_3OD) δ 173.5, 157.4, 131.3, 130.5, 116.1, 81.3, 80.7, 69.9, 69.6, 61.0, 51.8, 40.2, 22.7; HRMS calcd for $C_{15}H_{22}N_2O_5$ $[M + H]^{+\bullet}$ 311.1607, found 311.1598.

***N*-(((2*R*,3*R*,4*R*,5*R*)-1-((Benzo[*d*][1,3]dioxol-4-yl)methyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide (31c).** Yield 67%; ^{1}H NMR (600 MHz, CD_3OD) δ 6.86 (d, J = 7.9 Hz, 1H), 6.72 (t, J = 7.9 Hz, 1H), 6.64 (d, J = 7.9 Hz, 1H), 5.88 (d, J = 1.3 Hz, 1H, OCH_2O), 5.86 (d, J = 1.3 Hz, 1H, OCH_2O), 3.92 (t, J = 2.6 Hz, 1H, H-4), 3.87 (d of AB, J = 14.0 Hz, 1H, ArCH_2), 3.78 (d of AB, J = 14.0 Hz, 1H, ArCH_2), 3.75 (dd, J = 4.8, 11.4 Hz, 1H, H-6), 3.70 (t, J = 3.1 Hz, 1H, H-3), 3.59 (dd, J = 2.6, 11.4 Hz, 1H, H-6′), 3.45 (dd, J = 2.6, 14.0 Hz, 1H, H-1), 3.19 (dd, J = 6.0, 14.0 Hz, 1H, H-1′), 3.02 (dt, J = 3.6, 6.0 Hz, 1H, H-2), 2.90 (dt, J = 2.6, 4.8 Hz, 1H, H-5), 1.86 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, CD_3OD) δ 173.5, 148.8, 146.8, 123.6, 122.6, 122.4, 108.2, 102.0, 81.2, 80.6, 69.6, 69.4, 60.8, 45.7, 39.7, 22.7; HRMS calcd for $C_{16}H_{22}N_2O_6$ $[M + H]^{+\bullet}$ 339.1556, found 339.1552.

***N*-(((2*R*,3*R*,4*R*,5*R*)-1-((5-(4-Chlorophenyl)furan-2-yl)-methyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-methyl)acetamide (31d).** Yield 60%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.64 (m, 2H), 7.35 (m, 2H), 6.71 (d, J = 3.3 Hz, 1H), 6.38 (d, J = 3.3 Hz, 1H), 4.08 (d of AB, J = 15.0 Hz, 1H, ArCH_2), 3.96 (d of AB, J = 15.0 Hz, 1H, ArCH_2), 3.94 (dd, J = 4.0, 11.4 Hz, 1H, H-6), 3.86 (dd, J = 4.8, 11.4 Hz, 1H, H-6′), 3.76 (m, 2H, H-3, 4), 3.51 (dd, J = 3.0, 13.8 Hz, 1H, H-1), 3.22 (dd, J = 6.2, 13.8 Hz, 1H, H-1′), 3.14 (m, 2H, H-2, 5), 1.89 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 173.5, 154.8, 153.3, 133.7, 131.0, 129.9, 126.0, 111.1, 107.6, 81.1, 80.5, 70.5, 69.6, 61.1, 45.1, 40.2, 22.7; HRMS calcd for $C_{19}H_{23}N_2O_5Cl$ $[M + Na]^{+\bullet}$ 417.1193, found 417.1189.

***N*-(((2*R*,3*R*,4*R*,5*R*)-1-(4-(Dimethylamino)benzyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)-acetamide (31e).** Yield 70%; ^{1}H NMR (600 MHz, CD_3OD) δ 7.23 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.8 Hz, 2H), 3.98 (t, J = 2.2 Hz, 1H, H-4), 3.85 (d of AB, J = 14.0 Hz, 1H, ArCH_2), 3.82 (d of AB, J = 14.0 Hz, 1H, ArCH_2), 3.79 (t, J = 2.6 Hz, 1H, H-3), 3.75 (dd, J = 5.3, 11.4 Hz, 1H, H-6), 3.63 (dd, J = 3.1, 11.4 Hz, 1H, H-6′), 3.40 (dd, J = 3.1, 13.6 Hz, 1H, H-1), 3.25 (dd, J = 5.7, 13.6 Hz, 1H, H-1′), 3.08 (m, 1H, H-2), 3.01 (m, 1H, H-5), 2.88 (s, 6H, $N(CH_3)_2$), 1.91 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, CD_3OD) δ 177.3, 151.0, 129.6, 113.8, 80.7, 80.1, 69.4, 69.1, 60.4, 51.4, 40.7, 39.6, 22.1; HRMS calcd for $C_{17}H_{27}N_3O_4$ $[M + Na]^{+\bullet}$ 360.1899, found 360.1896.

Assay for Hexosaminidase Activity. 4-Methylumbelliferyl-*N*-acetyl-β-D-glucosamine at a final concentration of 0.1 mM in sodium citrate buffer (0.1 M, pH 4.5) was used as a substrate for hexosaminidase activity assay. Human placental hexosaminidases were diluted in sodium citrate buffer (0.1 M, pH 4.5) and incubated with the substrate and inhibitor at 37 °C for 2 h. The reaction was then stopped by the addition of sodium glycine buffer (0.5 M, pH 10.5). Hexosaminidase activity was measured by the release of 4-methylumbelliferone, utilizing fluorometry with the excitation wavelength of 360 nm and the emission wavelength of 460 nm.

General Procedure for Amide Coupling Reactions. To each 200-μL well of a microtiter plate containing diisopropyl ethylamine (10 μL, 80 mM solution in MeOH), HBTU (6 μL, 106 mM solution in MeOH), and carboxylic acid (2 μL, 400 mM solution in MeOH) was added **5** (2 μL, 400 mM solution in MeOH). The mixture was kept at room temperature overnight before being diluted with H_2O and transferred to a second 96-well microtiter plate to make a 0.25 mM solution of the inhibitor (based on 100% conversion of **5**) for the enzymatic assay.

General Procedure for Triazole Formation Reactions. To each 200-μL well of a microtiter plate containing alkynes (10 μL, 400 mM solution in toluene) was added 2 μL of **6** (400 mM solution in MeOH), except when the starting material was solid and 50 μL of toluene was used as solvent. The mixture was heated to 80 °C for 24 h before diluted with H_2O and transferred to a second 96-well microtiter plate to make a 0.25 mM solution of the inhibitor (based on 100% conversion of **6**) for the enzymatic assay.

Biological Assays of Microtiter Plate Library. The microtiter plate assay was performed on a fluorescence spectrophotometer at room temperature. Assays were run in citrate buffer (pH 4.5). Human placenta hexosaminidases stock (10 μL, 2.38 U/mL), inhibitor (10 μL, 0.25 mM), and 4-methylumbellyferyl-*N*-acetyl-β-D-glucosamine (10 μL, 0.2 mM in DMF) were used in the assay (total volume was 100 μL). The fluorescence signal was monitored by λ excitation at 335 nM and λ emission at 460 nM.

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Note Added after ASAP Posting. Several structures were missing from Figure 6 in the version posted ASAP August 7, 2004; the corrected version was posted August 19, 2004.

Supporting Information Available: NMR spectra for compounds **4**−**8**, **10**−**12**, **14**−**18**, **21**, **23**, **24**, **26**, **28**, **30**, and **31a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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