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Enzymatic/Chemical Synthesis and Biological Evaluation of Seven-Membered Iminocyclitols

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Abstract: Several polyhydroxyperhydroazepines have been obtained either by chemoenzymatic or chemical synthesis. Condensation of ($\pm$)-3-azido-2-hydroxypropanaldehyde and dihydroxyacetone phosphate (DHAP) in the presence of a DHAP dependent aldolase followed by treatment with acid phosphatase and an isomerase gave a 6-azido-6-deoxyaldopyranose, which upon reductive amination afforded the title compound. The iminocyclitols can also be obtained by chemical manipulations of aldopyranoses, protected as benzyl glycosides or diisopropylidene ethers. Thus, D-galactose leads to a *meso*-3,4,5,6-tetrahydroxyperhydroazepine, D-mannose to a derivative with a C_2 symmetry axis, and *N*-acetylglucosamine to a 6-acetamidoiminocyclitol. Asymmetrization of the *meso* azasugar was carried out by chemical means, to yield a 3-methoxy-4,5,6-trihydroxyazepane. An attempted enzymatic synthesis of the methoxy derivatives of these azasugars was unsuccessful, leading, however, to both enantiomers of 1-deoxy-2-*O*-methylmannojirimycin. Some of these compounds display significant activity as glycosidase inhibitors, with K_i values from moderate to low micromolar range. Though all these iminocyclitols do not inhibit the mechanistically related HIV protease, the 3,6-dibenzyl derivative **30** showed moderate inhibition. The X-ray structure of **7** indicates a *pseudochair* conformation.

Glycosidases and glycosyltransferases are targets of inhibition as these enzymes are involved in the processing and synthesis of complex carbohydrates which are essential for various biological recognition processes.^{1,2} Much work has been devoted to the study of five- and six-membered azasugar families, as they are thought to mimic the transition state of these enzymatic reactions.^{3,4}

We describe here new efficient methods for the synthesis of 3,4,5,6-tetrahydroxyperhydroazepines **1–8** and evaluation of this class of seven-membered iminocyclitols as inhibitors of glycosidases. These seven-membered ring species are regarded to be conformationally more flexible than the five- and six-membered counterparts and hence may adopt a quasi-flattened conformation with minimum energetic demand, which could lead to a favorable binding in the enzyme active site. These heterocycles have been described in the literature,^{5,6} but little

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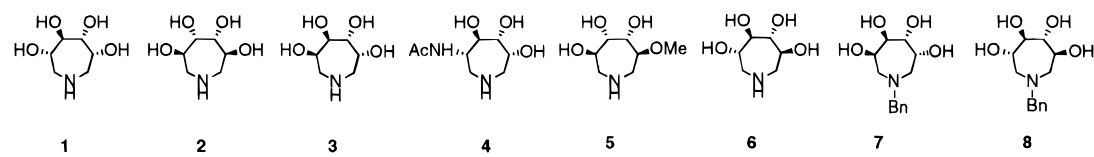
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Table 1. Inhibition of Glycosidases with Various Seven-Membered Iminocyclitols^a

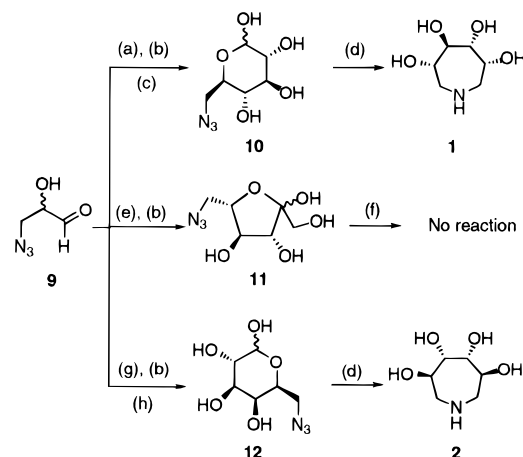
								
compd no.	[I], μM	α -mannosidase from jack beans	α -galactosidase from green coffee beans	β -galactosidase from <i>Aspergillus niger</i>	α -glucosidase from yeast	β -glucosidase from sweet almonds	β -N-acetylglucosaminidase from jack beans	α -fucosidase from bovine kidney
1	120	31 (187 $\pm$ 26)	87 (5.4 $\pm$ 0.56)	9	13	16	NI	95 (4.6 $\pm$ 0.7)
2	160	18	25 (216 $\pm$ 19)	49 (50 $\pm$ 4)	NI	14	35	57
3	240	NI	NI	NI	21	NI	94 (4.6 $\pm$ 0.4)	16
4	240	30 (364 $\pm$ 49)	NI	15	15	NI	22	89 (10.6 $\pm$ 0.7)
5	224	14	NI	NI	NI	13	41 (269 $\pm$ 20)	42
6 ^b	200	81 (25.7 $\pm$ 1.3)	65 (67 $\pm$ 4.5)	95 (6.5 $\pm$ 1.2)	78 (29.4 $\pm$ 2.2)	92 (12.8 $\pm$ 0.7)	89 (22.7 $\pm$ 2.6)	44
7 ^{b,c}	160	11	NI	NI	6	14	6	88 (23.4 $\pm$ 3.8)
8 ^{b,c}	200	48	31	3	NI	78 (30.5 $\pm$ 2.5)	NI	5

^a % inhibition at specified [I]; all show competitive inhibition, and K_i values (μM) are given in parentheses. NI stands for no inhibition. ^b Prepared through epoxide opening according to the procedure described previously.² ^c See refs 10 and 11 for spectral and physical data.

is known regarding their biological activities, with the exception that one related compound was reported to have no inhibition activity against α -mannosidase.⁶ We have found, however, that many of these iminocyclitols are potent inhibitors of glycosidases and some even exhibit higher inhibition potencies than the five- and six-membered counterparts (Table 1).

As shown in Table 1, for each of the seven glycosidases investigated, there is at least one seven-membered iminocyclitol which exhibits potent inhibition of the enzyme with K_i in the low micromolar range. Interestingly, compound **3** ($K_i = 4.6 \mu\text{M}$) is better than 1-deoxy-*N*-acetylglucosaminidase, **6** ($K_i = 6.5 \mu\text{M}$)^{1c} as an inhibitor of β -N-acetylglucosaminidase, **6** ($K_i = 25.7 \mu\text{M}$) is better than 1-deoxygalactosidase, and **6** ($K_i = 150 \mu\text{M}$)⁸ as an inhibitor of α -mannosidase. Compound **1** ($K_i = 9.4 \mu\text{M}$) is, however, weaker than 1-deoxygalactosidase ($K_i = 1.5 \text{ nM}$) as an inhibitor of α -galactosidase⁷ and also weaker ($K_i = 4.6 \mu\text{M}$) than 1-deoxyfucosidase ($K_i = 5 \text{ nM}$)⁹ as an inhibitor of α -fucosidase. A similar situation was observed for five-membered iminocyclitols.^{1c} It is also interesting that, of the iminocyclitols examined, one of them with a C_2 symmetry (**6**) inhibits all glycosidases, including α -mannosidase. Benzylation of the nitrogen group of **3** and **6** (resulting in compounds **7** and **8**) does not improve inhibition activity except in the case of α -fucosidase.

The enzymatic syntheses of iminocyclitols involve the combined use of aldolases and isomerases (Scheme 1).^{12–14} Condensation of ($\pm$)-3-azido-2-hydroxypropanaldehyde (**9**) and dihydroxyacetone phosphate (DHAP) in the presence of an aldolase followed by treatment with acid phosphatase (Pase) and an isomerase gave 6-azido-6-deoxyaldopyranoses, which upon reductive amination¹⁵ afforded the corresponding 3,4,5,6-tetrahydroazepane. Isomerization of the ketose to aldose was performed with the use of glucose and fucose isomerases (GlcI and FucI), giving the equilibrium favoring aldose, but rhamnose isomerase (RhaI) was unable to isomerize azidoketose **11**. The

Scheme 1^a

^a Conditions: (a) pH 6.7, DHAP, FDPA; (b) pH 4.5, Pase; (c) pH 7.2, TAKASWEET (GlcI), 26% for **10**; (d) H_2 (50 psi), Pd/C, H_2O , 2 d, 91–54%; (e) pH 6.7, DHAP, RhaA; 30% for **11**; (f) pH 7.2, RhaI; (g) pH 6.7, DHAP, FucA; (h) pH 7.2, FucI 21% for **12**.

products 6-azido-6-deoxy-D-glucopyranose (**10**) and 6-azido-6-deoxy-L-galactopyranose (**12**) obtained were subjected to Pd-mediated reductive amination¹⁵ in aqueous solution in the presence of H_2 (50 psi) to give 3(*R*),4(*R*),5(*R*),6(*S*)-tetrahydroxyperhydroazepine (**1**) and the *meso*-iminocyclitol 3(*S*),4(*R*),5(*S*),6(*R*)-tetrahydroxyperhydroazepine (**2**), respectively.

The chemical synthesis of iminocyclitols starts with 6-azido-6-deoxysugars prepared from readily available protected monosaccharides (diisopropylidene sugars or benzyl pyranosides), the key step being again the reductive amination of a 6-azido-6-deoxyaldohexopyranose. Thus, 6-azido-6-deoxy-D-galactopyranose (**14**) (enantiomer of compound **12**) undergoes ring expansion *via* Pd-mediated reductive amination in water to give

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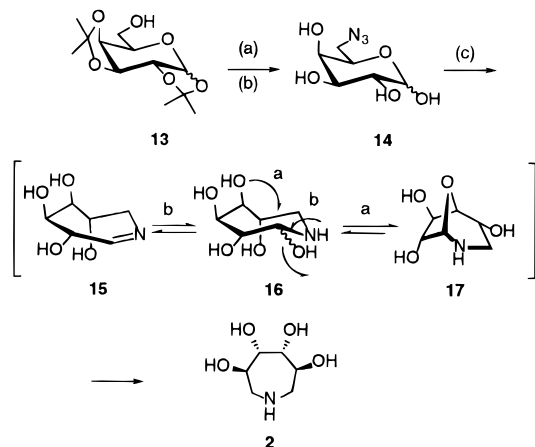
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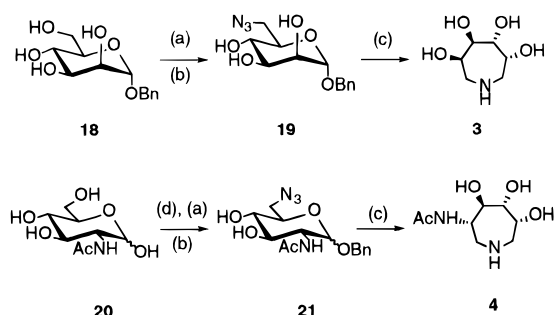
(13) FucI prefers (2*S*,3*R*)- and RhaI prefers (2*R*,3*R*)-aldose as substrate. Glucose isomerase prefers (2*R*,3*S*)-aldose as substrate. For synthetic work using isomerases see: (a) Fessner, W.-D.; Badia, J.; Eyrich, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, 33, 5231. (b) Alajarin, R.; Garcia-Junceda, E.; Wong, C.-H. *J. Org. Chem.* **1995**, 60, 4294. (c) Wong, C.-H.; Alajarin, R.; Moris-Varas, F.; Blanco, O.; Wong, C.-H. *J. Org. Chem.* **1995**, 60, 7360. (d) Page, P.; Blonski, C.; Périé, J. *Tetrahedron*, **1996**, 52, 1557.

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(15) (a) Paulsen, H.; Sangster, I.; Heyns, K. *Chem. Ber.* **1967**, 100, 802. In an attempt to prepare seven-membered iminocyclitols from an azido ketose catalyzed by Pd on C in the presence of H_2 (50 psi) the only product obtained was the aminohexopyranose: (b) Liu, K. C.; Pederson, R. L.; Wong, C. H. *J. Chem. Soc., Perkin Trans 1* **1991**, 1991, 2669.

Scheme 2^a

^a Conditions: (a) (i) Ph_3P , DEAD, THF, 0 °C; (ii) $(\text{EtO})_2\text{P}(\text{O})\text{N}_3$, 80%; (b) 80% AcOH, 70 °C, 95%; (c) H_2 (50 psi), Pd/C, H_2O , 2 d, 90%.

Scheme 3^a

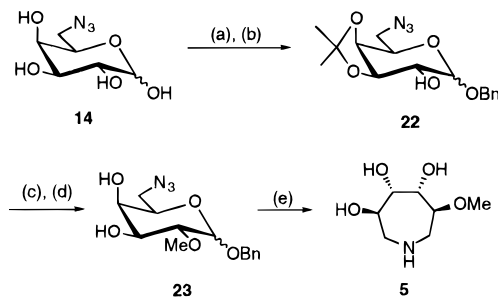
^a Conditions: (a) TsCl, Py, 0 °C, 12 h, 61–68%; (b) NaN_3 (5 equiv), NH_4Cl (5 equiv), EtOH/ H_2O , 9:1, reflux, 12 h, 70%; (c) H_2 (1 atm), Pd/C, H_2O , 12 h, 85%; (d) BnOH, HCl, 80 °C, 30%.

2 (Scheme 2). During the hydrogenation, compounds **2** and the intermediate **17** were observed by NMR in 12 h. After 2 days, **17** disappeared and only **2** was obtained. It appears that **17** is formed via intramolecular dehydration of the intermediate **16**.

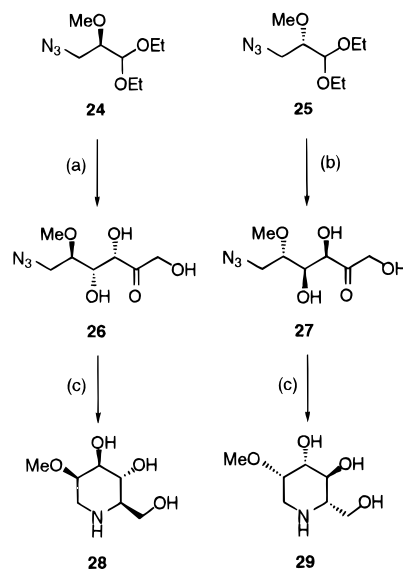
Benzyl pyranosides are easily prepared from aldopyranoses and represent a convenient entry point to these seven-membered ring heterocycles. We illustrate this approach by using benzyl mannopyranoside and benzyl *N*-acetylglucosamine as starting materials (Scheme 3). This approach is much more convenient than the bisepoxide opening described previously, since mixtures of six- and seven-membered rings are completely avoided. Tosylation of the primary hydroxyl group of **18** and benzyl pyranoside of **20** followed by azide displacement affords the azides **19** and **21**. Hydrogenolysis of both compounds afforded the desired iminocyclitols **3** and **4**.

The *meso*-iminocyclitol **2** was asymmetrized to (3*S*,4*R*,5*S*,6*R*)-3-methoxy-4,5,6-trihydroxyazepine (**5**) from 6-azido-6-deoxy- D -galactopyranoside (**14**) via benzyl glycosilation, isopropylidene protection, methylation, and reductive amination of the 2-*O*-methyl glycoside **23**, as shown in Scheme 4.

In an attempt to prepare **5** enzymatically, ($\pm$)-3-azido-2-hydroxy-propanaldehyde dimethyl acetal was acetylated and kinetically resolved by lipase PS800 mediated hydrolysis.¹⁶ The products from this enzymatic reaction were methylated (MeI, NaH, THF) to give both enantiomers of 3-azido-2-*O*-methylpropanaldehyde diethyl acetal (**24** and **25**), which were hydrolyzed and reacted with DHAP in the presence of fructose 1,6-

Scheme 4^a

^a Conditions: (a) BnOH, 80 °C, $\text{Et}_2\text{O}\cdot\text{BF}_3$, 75%; (b) 2,2-dimethoxypropane, *p*-TSA, DMF, rt, 95%; (c) MeI, NaH, THF, rt, 93%; (d) 80% AcOH, 80 °C, 95%; (e) H_2 (50 psi), Pd/C, H_2O , 2 d, 90%.

Scheme 5^a

^a Conditions: (a) (i) pH 6.7, DHAP, FDPA; (ii) pH 4.5, acid Pase; (b) (i) pH 6.7, DHAP, RhaA; (ii) pH 4.5, acid Pase; (c) H_2 , 50 psi, rt, MeOH, 3 h, 90–95%.

diphosphate aldolase (FDPA) and rhamnulose 1-phosphate aldolase (RhaA), respectively. In both cases the methylated hydroxy aldehydes were substrates for the aldolases and (3*S*,4*R*,5*R*)-6-azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (**26**) and (3*R*,4*S*,5*S*)-6-azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (**27**) were obtained. These ketoses were, however, not accepted as substrates for GlcI and RhaI, respectively. These observations are in agreement with the conclusion made by our group¹⁷ and others¹⁸ that fructose analogues modified at carbon 5 (inversion, deoxygenation, or blocked hydroxyl) are not isomerized by GlcI. Similarly, (3*R*,4*R*,5*S*)-6-azido-5-methoxy-1,3,4-trihydroxyhexan-2-one was not accepted by FucI. The ketoses **26** and **27** were then converted to (2*R*,3*R*,4*S*,5*S*)-2-hydroxyethyl-3,4-dihydroxy-5-methoxypiperidine (2-*O*-methyl-1-deoxymannojirimycin) (**28**) and the enantiomer **29**, respectively, under the reductive amination condition at 50 psi for 3 h in MeOH. None of these two azasugars exhibited significant inhibition when tested for the glycosidases mentioned above.

At this stage it is not clear how these seven-membered iminocyclitols act as inhibitors of glycosidases. The X-ray crystal structure of a representative compound (**7**) was determined¹¹ and shown to adopt a pseudochair conformation (Figure 1 and Table 2).

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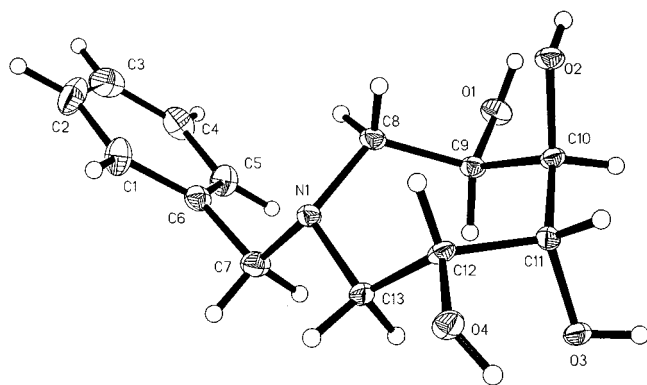
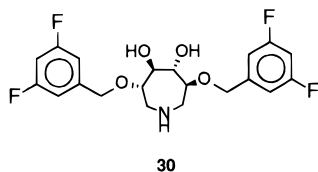


Figure 1. X-ray crystal structure of 7.

Table 2. Bond Lengths and Angles for Compound 7

Bond Lengths (Å) (Standard Deviation $\pm 10^{-3}$ Å)			
O(1)–C(9)	1.432(4)	O(2)–C(10)	1.438(4)
O(3)–C(11)	1.423(5)	O(4)–C(12)	1.441(4)
N(1)–C(7)	1.474(5)	N(1)–C(13)	1.479(5)
N(1)–C(8)	1.484(5)	C(1)–C(6)	1.377(7)
C(1)–C(2)	1.386(8)	C(2)–C(3)	1.385(9)
C(3)–C(4)	1.358(10)	C(4)–C(5)	1.388(7)
C(5)–C(6)	1.379(6)	C(6)–C(7)	1.511(6)
C(8)–C(9)	1.534(6)	C(9)–C(10)	1.516(6)
C(10)–C(11)	1.536(5)	C(11)–C(12)	1.507(6)
C(12)–C(13)	1.531(6)		
Bond Angles (deg) (Standard Deviation $\pm 10^{-1}$ deg)			
C(7)–N(1)–C(13)	108.0(3)	C(7)–N(1)–C(8)	112.9(3)
C(13)–N(1)–C(8)	114.1(3)	C(6)–C(1)–C(2)	120.8(5)
C(3)–C(2)–C(1)	119.4(6)	C(4)–C(3)–C(2)	120.4(5)
C(3)–C(4)–C(5)	119.9(5)	C(6)–C(5)–C(4)	120.8(5)
C(1)–C(6)–C(5)	118.7(4)	C(1)–C(6)–C(7)	120.9(4)
C(5)–C(6)–C(7)	120.4(5)	N(1)–C(7)–C(6)	113.1(3)
N(1)–C(8)–C(9)	117.7(3)	O(1)–C(9)–C(10)	109.8(3)
O(1)–C(9)–C(8)	109.4(3)	C(10)–C(9)–C(8)	112.6(3)
O(2)–C(10)–C(9)	108.0(3)	O(2)–C(10)–C(11)	109.7(3)
C(9)–C(10)–C(11)	114.5(3)	O(3)–C(11)–C(12)	108.0(3)
O(3)–C(11)–C(10)	109.6(3)	C(12)–C(11)–C(10)	115.9(3)
O(4)–C(12)–C(11)	109.9(3)	O(4)–C(12)–C(13)	109.3(3)
C(11)–C(12)–C(13)	114.6(3)	N(1)–C(13)–C(12)	114.1(3)

These iminocyclitols were also tested as inhibitors of a mechanistically related enzyme, i.e., the HIV protease, but no inhibition was observed. The 3,6-dibenzyl derivatives, especially **30**, are, however, moderate inhibitor of the HIV protease



with K_i around 350 μM .¹⁰ Interestingly these benzyl derivatives are not inhibitors of the glycosidases in this study.

In summary, we have demonstrated new and effective syntheses of various seven-membered iminocyclitols which have been shown to be a new class of glycosidase inhibitors and may be useful as new templates for the development of HIV protease inhibitors.

Experimental Section

Materials and Methods. Rabbit muscle aldolase (E.C. 4.1.2.13) and acid phosphatase (E.C. 3.1.3.2) were purchased from Sigma. The enzymes fucose-1-phosphate aldolase, rhamnulose 1-phosphate aldolase, fucose isomerase, and rhamnose isomerase were prepared in our laboratory as described previously.^{19,20} Benzyl α -D-mannopyra-

noside was purchased from Toronto Research Chemicals Inc. and diphenylphosphoryl azide from Fluka, and the rest of the chemicals and solvents were purchased from Aldrich and used without further treatment. Dowex 50W-X8 (Biorad, 200–400 mesh, H^+ form) was converted to NH_4^+ form by passing 2 N ammonium hydroxide and thoroughly washed with purified water prior to use. Aldol condensation was monitored enzymatically by DHAP consumption.²¹ The phosphatase-catalyzed hydrolysis was monitored by TLC (silica gel 60 from Merck). Isomerization was monitored by ^{13}C -NMR (100 MHz) analysis of the anomeric center in the cyclized form. Nuclear magnetic resonance (^1H , 400 MHz; ^{13}C , 100 MHz) spectra were obtained using D_2O ($\delta = 4.65$ ppm) or CD_3OD ($\delta = 3.5$ ppm for ^1H and 49.9 for ^{13}C). Flash chromatography was carried out with silica gel 60 (230–400 mesh). Inhibitory analysis were performed on a Beckman DU-70 spectrophotometer at 400 nm.

3(R),4(R),5(R),6(S)-Tetrahydroxyazepane (1). 3-Azido-2-hydroxypropanaldehyde (**9**) was formed *in situ* as follows: the diethyl acetal of **9** (1.512 g, 8 mmol) was dissolved in water (3 mL), and Dowex 50W-X8 (H^+ form, 200–400 mesh) was added until pH < 2. The mixture was heated at 50 $^\circ\text{C}$ for 8 h, and then the resin was filtered off and washed with water. A solution of DHAP (250 mM, 15 mL, 3.8 mmol) was added, and the mixture was adjusted to pH 6.8 with 6 N NaOH. Rabbit muscle aldolase, RAMA (840 μL , 300 units), was added, the mixture was stirred gently at room temperature until DHAP analysis indicated > 90% conversion, and the pH was adjusted to 4.7 with HCl. Acid phosphatase (780 μL , 300 units) was added and the mixture heated at 37 $^\circ\text{C}$ until the organic phosphate was hydrolyzed completely as indicated by TLC analysis ($\text{EtOH}/\text{NH}_4\text{OH}$, 1:1). 6-Azido-6-deoxyfructose was purified by silica gel chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 6:1) to yield 465 mg (61%) of a product with data in accordance with those reported previously.²² A 70 mg portion of this product was dissolved in 2 mL of Tris buffer (50 mM, 2 mM Mn^{2+} , pH 7.7), and 200 mg of immobilized glucose isomerase (TAKASWEET, Miles Labs) was added. The mixture was shaken at 37 $^\circ\text{C}$ for 24 h. ^1H - and ^{13}C -NMR analysis indicated the presence of a mixture of aldose **10** and ketose (~65:35). The enzyme was filtered off, solvent evaporated under reduced pressure, and the residue chromatographed carefully on silica gel ($\text{CHCl}_3/\text{MeOH}$, 6:1) to yield 30 mg (42%, 26% overall) of 6-azido-6-deoxy-D-glucopyranose (**10**). Spectral data are in agreement with those reported previously.²³ Azidoaldose **10** was hydrogenated at 50 psi in water using Pd/C as catalyst. The reaction was monitored by NMR and was complete after 48 h. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. 3(R),4(R),5(R),6(S)-tetrahydroxyazepane (**1**) was obtained in 94% yield (24% overall): ^1H -NMR (D_2O , δ , ppm) 3.85 (1H, ddd, $J = 1.7, 3.6, 5.4$ Hz, H3 or H6); 3.59 (2H, m, overlapped H4 + H5); 3.48 (1H, q, $J = 5.5$ Hz, H3 or H6); 2.7–2.8 (4H, dd, $J = 14.5, 5.4$ Hz, and d, $J = 5.4$ Hz, overlapped 2H2 + 2H7); ^{13}C -NMR (D_2O , δ , ppm), 75.18, 74.62, 73.28, 71.21, 69.30, 49.46, 49.20; MS (FAB^+) ($M + \text{H}$) expected 164.0923, observed 164.0920.

6-Azido-6-deoxyrhamnulose (11). 3-Azido-2-hydroxypropanaldehyde (**9**) generated as before (diethyl acetal; 284 mg, 1.5 mmol) was mixed with a solution of DHAP (230 mM, 4.34 mL, 1 mmol), and the mixture was adjusted to pH 6.8 with 6 N NaOH, followed by addition of rhamnulose 1-phosphate aldolase (270 μL , 10 units). After 24 h DHAP analysis indicated 95% conversion, and the pH was adjusted to 4.7 with HCl. Acid phosphatase (200 μL , 100 units) was added and the mixture heated at 37 $^\circ\text{C}$ until the organic phosphate was hydrolyzed completely as indicated by TLC analysis ($\text{EtOH}/\text{NH}_4\text{OH}$, 1:1). 6-Azido-6-deoxyrhamnulose (**11**) was purified by silica gel chromatography ($\text{CHCl}_3/\text{MeOH}$, 6:1) to yield 60 mg (30%). Spectral data are the same as for the enantiomeric fructose analog²² with opposite sign for the optical rotation, $[\alpha]_{\text{D}}^{25} = -8.9^\circ$ ($c = 1.95$, MeOH). Several attempts to isomerize this product in 2 mL of Tris buffer (50 mM, 2 mM Mn^{2+} , pH 7.7) and in the presence of variable amounts of rhamnose isomerase

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were unsuccessful. The mixture was shaken at 37 °C and the reaction monitored by ^1H - and ^{13}C -NMR; no aldose peaks were detected even after 3 days.

3(S),4(R),5(S),6(R)-Tetrahydrozazepane (2). Enzymatic Synthesis. 3-azido-2-hydroxypropanaldehyde (**9**) prepared as described previously (diethyl acetal; 756 mg, 4 mmol) was mixed with a solution of DHAP (262 mM, 7.6 mL, 2 mmol) at pH 6.5. Fuculose 1-phosphate aldolase was added (640 μL , 10 units), and the mixture was stirred at room temperature. After 18 h DHAP analysis indicated 91% conversion, and the pH was adjusted to 4.7 with HCl. After deprotection of the phosphate, 6-azido-6-deoxyfucose was isolated by Dowex-50W (Ba^{2+} form, 3×20 cm) chromatography^{15b} using water as eluent to yield 99 mg (25%): ^1H -NMR (D_2O , δ , ppm, major anomer only) 4.15 (1H, t, $J = 4.5$ Hz, H4); 4.05 (1H, d, $J = 4.5$ Hz, H3); 4.00 (1H, m, $J = 2, 4.5, 8.5$ Hz, H5); 3.3–3.5 (4H, m, overlapped signals, 2H1 and 2H6); ^{13}C -NMR (D_2O , δ , ppm) 102.78, 78.43, 70.96, 70.43, 62.45, 50.99 (major), 105.16, 77.65, 76.88, 71.29, 62.22, 50.32 (minor); MS (FAB^+) (M^+) expected 205.0699, observed 205.0691. This product was dissolved in 2 mL of Tris buffer (50 mM, 2 mM Mn^{2+} , pH 7.7), and 1200 units (2 mL) of fucose isomerase were added. The mixture was stirred at room temperature for 24 h. ^1H - and ^{13}C -NMR analysis indicated the consumption of the ketose and the appearance of a new compound, aldose **12** (~95:5). The enzyme was precipitated with acetone and solvent evaporated under reduced pressure, and the residue was chromatographed on Dowex-50W (Ba^{2+} form, 3×20 cm) using $\text{EtOH}/\text{H}_2\text{O}$, 1:1, as eluent to yield 82 mg (82%, 21% overall) of 6-azido-6-deoxy-L-fucopyranose (**12**). The NMR data were the same as those reported previously.^{13c} To a solution ($\text{H}_2\text{O}/\text{THF}$, 3:1, 4 mL) of **12** (60 mg) was added a catalytic amount of Pd/C, and the mixture was hydrogenated at 50 psi. After 20 h of reaction azasugar **2** was obtained as the major product, but NMR analysis also showed another minor compound, characterized as 8-oxa-2-aza-4,6,7-trihydroxybicyclo[3.2.1]-heptane (**17**): ^1H -NMR (CD_3OD , δ , ppm) 4.92 (1H, d, $J = 6$ Hz); 4.36 (1H, d, $J = 2.5$ Hz); 4.32 (1H, ddd, $J = 1, 2.5, 6$ Hz); 4.09 (1H, d, $J = 4.3$ Hz); 3.91 (1H, m); 3.18 (1H, ddd, $J = 1, 8.8, 13.5$ Hz); 2.87 (1H, dd, $J = 13.5, 10.8$ Hz); ^{13}C -NMR (CD_3OD , δ , ppm) 89.03, 87.18, 82.43, 79.05, 66.64, 46.67. After an additional period of time (~2 d) the bicyclic compound disappeared completely and 3(S),4(R),5(S),6(R)-tetrahydrozazepane (**2**) was the only product detectable (55 mg, 91%, 19% overall): ^1H -NMR (D_2O , δ , ppm) 3.82 (2H, d, $J = 6.5$ Hz, H4 + H5); 3.66 (2H, dd, $J = 4.5, 6.1$ Hz, H3 + H6); 2.77–2.67 (4H, dd, $J = 14.8, 4.5$ Hz, 2H2 + 2H7); ^{13}C -NMR (D_2O , δ , ppm) 74.28, 70.90, 51.43; MS (FAB^+), ($M + \text{H}$) expected 164.0923, observed 164.0918.

3(S),4(R),5(S),6(R)-Tetrahydrozazepane (2). Chemical Synthesis. To a solution of 1,2:3,4-diisopropylidene-D-galactose (**13**) (3.67 g, 14.1 mmol) in THF (30 mL) at 0 °C were added DEAD (2.26 mL, 14.1 mmol) and PPh_3 (3.79 g, 14.1 mmol), and the mixture was stirred for 15 min. Then, diphenylphosphoryl azide (3.04 mL, 14.1 mmol) was added dropwise and the reaction mixture stirred overnight. The solvent was evaporated under reduced pressure, and the product was purified by silica gel chromatography (CH_2Cl_2) to yield 3.25 g (80%) of 6-azido-6-deoxy-1,2:3,4-diisopropylidene-D-galactose: ^1H -NMR (CDCl_3 , δ , ppm) 5.51 (d, $J = 5$ Hz, H1); 4.60 (dd, $J = 1.5, 8$ Hz, H3); 4.30 (dd, $J = 1.5, 5$ Hz, H2); 4.15 (dd, $J = 2, 8$ Hz, H4); 3.90 (ddd, $J = 2, 5, 7.5$ Hz, H5); 3.50 (dd, $J = 12.5, 7$ Hz, H6); 3.35 (dd, $J = 12.5, 5$ Hz, H6'); ^{13}C -NMR (CDCl_3 , δ , ppm): 110.23, 109.43, 26.13, 26.04, 24.98, 24.51 (isopropylidene), 96.88, 71.53, 71.16, 70.74, 67.36, 50.91 (sugar). This compound was treated with 80% AcOH at 70 °C for 3 h (TLC showed completion, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 4:1). The solvent was removed in vacuo to yield 6-azido-6-deoxy-D-galactose (**14**) (2.15 g, 95%); the spectral data are the same as those reported for the enantiomer.^{13c} This product (60 mg) was hydrogenated at 50 psi in $\text{H}_2\text{O}/\text{THF}$, 3:1, for 2 d to give 3(S),4(R),5(S),6(R)-tetrahydrozazepane (**2**) (50 mg, 90%).

3(R),4(R),5(R),6(R)-Tetrahydrozazepane (3). To a solution of benzyl mannopyranoside (**18**) (500 mg, 1.9 mmol) in dry pyridine (5 mL) at 0 °C was added tosyl chloride (381 mg, 2 mmol) dissolved in CH_2Cl_2 , and the reaction was monitored by TLC (EtOAc). After 4 h the starting material was consumed completely. The reaction mixture was extracted with CH_2Cl_2 and washed with 1 N HCl, saturated NaHCO_3 , and brine, and purified by flash chromatography using EtOAc

as eluent to yield 531 mg (68%) of benzyl 6-tosyl-6-deoxy-D-mannopyranoside. To a solution of this compound (120 mg, 0.29 mmol) in $\text{EtOH}/\text{H}_2\text{O}$, 9:1, were added 5 equiv of NaN_3 and 5 equiv of NH_4Cl , and this mixture was refluxed overnight, the solvent removed in vacuo, and the residue chromatographed in SiO_2 using EtOAc as eluent to yield benzyl 6-azido-6-deoxy-D-mannopyranoside (**19**) (60 mg, 70%): ^{13}C -NMR (CDCl_3 , δ , ppm) 136.61, 128.43, 128.10, 127.99, 69.27 (benzyl group), 98.82, 71.53 (double intensity), 70.65, 68.14, 51.25. A solution of this product (26 mg, 0.09 mmol) in water (3 mL) was added to 20 mg of Pd/C and hydrogenated at 50 psi for 24 h. The catalyst was removed by filtration and the filtrate purified by ion-exchange chromatography (Dowex-50W, NH_4^+ form, 1×20 cm) eluted first with water and then with a NH_4OH gradient, 0 $\rightarrow$ 1 N. The fractions containing the product were pooled, and HCl was added to form the hydrochloric acid salt of 3(R),4(R),5(R),6(R)-tetrahydrozazepane (**3**) (16 mg, 85%); ^1H -NMR (D_2O , δ , ppm) 4.15 (2H, m, H4 + H5); 3.70 (2H, s, H3 + H6); 3.25 (4H, dd, $J = 14.4, 6.4$ Hz, 2H2 + 2H7); ^{13}C -NMR (D_2O , δ , ppm) 73.26, 67.03, 45.13.

6(R)-Acetamido-3(S),4(R),5(S)-trihydrozazepane (4). To 11 g of *N*-acetylglucopyranose **20** was added 68 mL of benzyl alcohol, and HCl gas was passed for 3 min. The mixture was allowed to react for 3 h, and the precipitate was collected and washed with cold water, cold ether, and hexane (yield 30%). This benzyl glucopyranoside (500 mg, 1.67 mmol) was subjected to the same sequence as that of compound **18** above, to yield benzyl 2-acetamido 6-azido-2,6-dideoxyglucopyranoside (241 mg, 43% overall yield) (**21**): ^{13}C -NMR (CD_3OD , δ , ppm, α -anomer only) 175.68, 23.68 (acetamide); 140.61, 131.27, 131.15, 130.78, 71.89 (benzyl group); 99.09, 74.64 (double intensity), 73.89, 56.62, 54.03 (sugar moiety). A sample of this compound (34 mg, 0.1 mmol) was hydrogenated over Pd/C in $\text{H}_2\text{O}/\text{THF}$, 4:1, overnight and the azasugar **4** purified by ion-exchange chromatography (Dowex-50W, NH_4^+ form, 1×20 cm) eluted first with water and then with a NH_4OH gradient, 0 $\rightarrow$ 1 N. The fractions containing the product were pooled, and HCl was added to form the hydrochloric acid salt of 6(R)-acetamido-3(S),4(R),5(S)-trihydrozazepane (**4**) (14 mg, 60%): ^1H -NMR (D_2O , δ , ppm) 4.15 (1H, dt, $J = 2, 6.5$ Hz, H3); 3.87 (1H, dt, $J = 3, 8$ Hz, H6); 3.68 (1H, t, $J = 8$ Hz, H5); 3.59 (dd, $J = 2, 8$ Hz, H5); 3.32–3.12 (4H, m, 2H2 + 2H7); ^{13}C -NMR (D_2O , δ , ppm) 75.23, 72.05, 66.83, 49.28, 45.73, 45.18, 21.86.

3(S),4(R),5(S),6(R)-3-Methoxy-4,5,6-trihydrozazepane (5). A suspension of 6-azido-6-deoxy-D-galactose (**14**) (1.63 g, 7.9 mmol) obtained as described above in benzyl alcohol (5 mL) was heated to 80 °C, and $\text{BF}_3 \cdot \text{OEt}_2$ (984 μL , 8 mmol) was added dropwise. After 20 min the suspension became transparent. The solution was allowed to cool. The reaction mixture was passed through a SiO_2 column. Benzyl alcohol came out first using $\text{EtOAc}/\text{hexane}$, 1:5, and then benzyl 6-azido-6-deoxy-D-galactopyranoside was separated from the furanose byproduct with EtOAc as eluent (1.21 g, 75%, α/β , 60:40): ^1H -NMR (CD_3OD , δ , ppm, α -anomer only) 7.4–7.6 (5H, benzyl group); 5.3 (H1, overlapped by HDO signal); 4.98 and 4.78 (2H, AB system, $J = 12$ Hz, $\text{CH}_2\text{-OBn}$); 4.18 (1H, dd, $J = 4.2, 8.7$ Hz, H5); 4.0 (3H, overlapped, H2, H3, H4); 3.75 (1H, dd, $J = 13, 8.7$ Hz, H6); 3.49 (1H, dd, $J = 13, 4.2$ Hz, H6'); ^{13}C -NMR (CD_3OD , δ , ppm, α -anomer only) 139.76, 131.64, 130.31, 100.28, 72.54, 72.36, 72.10, 71.36, 70.89, 53.62. To a solution of this product (400 mg, 1.35 mmol) in DMF (4 mL) were added 2,2-dimethoxypropane (1 mL, large excess) and a catalytic amount of *p*-tosylic acid. The reaction was driven overnight under argon at room temperature and then extracted with Et_2O . Further purification by flash chromatography ($\text{EtOAc}/\text{hexanes}$, 1:1) yielded 429 mg (95%) of benzyl 6-azido-6-deoxy-3,4-isopropylidene-D-galactopyranoside (**22**): ^{13}C -NMR (CDCl_3 , δ , ppm, α -anomer only) 136.81, 128.43, 128.32, 128.11, 69.68 (benzyl group); 109.73, 27.33, 25.62 (isopropylidene); 96.15, 75.49, 73.06 (doublet), 68.65, 68.12, 51.13 (sugar). To a solution of this latter product (204 mg, 0.6 mmol) in dry THF (5 mL) were added MeI (38.6 μL , 0.62 mmol) and NaH (16 mg, 0.66 mmol), and the mixture was reacted at room temperature for 2 h and then was extracted with CH_2Cl_2 and the resulting syrup treated with 80% AcOH at 70 °C for 3 h. Evaporation of the solvent with added water (three times) yielded 163 mg (88% from **22**) of benzyl 6-azido-6-deoxy-2-O-methyl-D-galactopyranoside (**23**): ^1H -NMR (CD_3OD , δ , ppm, α -anomer only) 7.4–7.6 (5H, benzyl group); 5.28 (1H, d, $J = 3.7$ Hz, H1); 4.95 and 4.75 (2H, AB system, $J = 12$ Hz, CH_2 -

OBn); 4.15 (1H, dd, $J = 4, 8.8$ Hz, H5); 4.05 (1H, dd, $J = 3.4, 10$ Hz, H3); 3.85 (1H, d, $J = 3.4$ Hz, Hr); 3.75 (1H, dd, $J = 13, 8.7$ Hz, H6); 3.45 (1H, dd, $J = 13, 4$ Hz, H6'); 3.68 (1H, dd, $J = 10, 3.7$ Hz, H2); 3.60 (3H, s, MeO-); ^{13}C -NMR (CD_3OD , δ , ppm, α -anomer only) 140.51, 131.22, 130.73, 97.87, 80.66, 72.87, 72.78, 71.67, 71.59, 59.69, 53.98. A sample of this product (105 mg, 0.34 mmol) was hydrogenated at 50 psi over Pd/C in water for 2 d to yield (3*S*,4*R*,5*S*,6*R*)-3-methoxy-4,5,6-trihydroxyazepane (**5**) (54 mg, 90%): ^1H -NMR (D_2O , δ , ppm) 4.29 (1H, dd, $J = 1.3, 6.3$ Hz, H4 or H5); 4.09 (1H, dd, $J = 1.3, 7.1$ Hz, H5 or H4); 3.97 (1H, dt, $J = 4.5, 7.1$ Hz, H3 or H6); 3.5 (4H, Me and H6 or H3 overlapped by Me); 3.27 (1H, dd, $J = 14.3, 4.5$ Hz, H2 or H7); 3.27 and 2.03 (1H each, dd, $J = 14.3, 4.4$ Hz, 2 H2 or 2H7); 3.7 and 4.1 (1H each, dd, $J = 14.3, 4.4$ Hz, 2H2' or 2H7'); ^{13}C -NMR (D_2O , δ , ppm) 83.99, 77.04, 75.33, 73.66, 58.67(OMe), 54.78, 50.85; MS (FAB⁺), ($M + \text{H}$) expected 178.1079, observed 178.1072.

3(S),4(R),5(S)-6-Azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (26). A solution of 3-azido-2(*R*)-*O*-methylpropanal diethyl acetal (**24**) (934 mg, 4.6 mmol) prepared by methylation of 3-azido-2(*R*)-hydroxypropanal diethyl acetal obtained enzymatically¹⁶ was dissolved in water (5 mL), and Dowex 50W-X8 (H^+ form, 200–400 mesh) was added until pH < 2. The mixture was heated at 50 °C for 8 h, and then the resin was filtered off and washed with a minimum amount of water. A solution of DHAP (262 mM, 7.6 mL, 2 mmol) was added, and the mixture was adjusted to pH 6.5 with 6 N NaOH. Fructose 1,6-diphosphate aldolase was added (1.4 mL, 500 units), and the mixture was stirred at room temperature. After 4 h the pH was adjusted to 4.7 with HCl. Acid phosphatase (500 μL , 400 units) was added and the mixture heated at 37 °C. The enzyme was precipitated by adding acetone and the residue evaporated and chromatographed on SiO_2 ($\text{CH}_2\text{-Cl}_2/\text{MeOH}$, 6:1), to give 400 mg (40%) of 3(*S*),4(*R*),5(*S*)-6-azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (**26**): ^1H -NMR (D_2O , δ , ppm) 4.48 and 4.35 (1H each, $J = 19.8$ Hz, H1 and H1'); 4.38 (1H, d, $J = 3$ Hz, H3); 3.85 (1H, dd, $J = 9.2, 1.9$ Hz, H4), 3.7 (1H, dd, $J = 13.6, 2.8$ Hz, H6); 3.45, (1H, ddd, $J = 9.2, 2.8, 3.7$ Hz, H5); 3.33 (3H, s, Me); 3.30 (1H, dd, $J = 13.5, 3.7$ Hz, H6'); ^{13}C -NMR (D_2O , δ , ppm) 213.22, 78.55, 74.85, 70.26, 65.98, 57.36, 48.97.

3(R),4(S),5(S)-6-Azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (27). A solution of 3-azido-2(*S*)-*O*-methylpropanal diethyl acetal (**25**) (832 mg, 4.1 mmol) prepared in the same way as above from the

S-enantiomer was treated in the same manner as described for the *R*-enantiomer. In this case rhamnulose 1-phosphate aldolase was added (0.5 mL, 80 units), and after usual monitoring and workup, 380 mg (38%) of (3*R*,4*S*,5*S*)-6-azido-5-methoxy-1,3,4-trihydroxyhexan-2-one (**27**) was obtained. The NMR data were identical to those of **26**.

2-O-Methyl-1-deoxy-D-mannoirimycin (28). A sample of **26** (39 mg, 0.17 mmol) was hydrogenated in MeOH (2 mL) over Pd/C at 50 psi for 3 h. After the catalyst was filtered off and the solvent evaporated, 29 mg (95%) of 2-*O*-methyl-1-deoxy-D-mannoirimycin (**28**) was obtained: ^1H -NMR (CD_3OD , δ , ppm) 3.91 (1H, dd, $J = 4.5, 11.1$ Hz, H6); 3.96 (1H, $J = 11.1, 3$ Hz, H6'); 3.75 (1H, t, $J = 9.6$ Hz, H4); 3.65 (1H, ddd, $J = 2.7, 2.8$ Hz, 1.2 Hz, H2); 3.60 (4H, Me and H3 overlapped by Me); 3.40 (1H, dd, $J = 14, 2.7$ Hz, H1); 2.75 (1H, dd, $J = 14, 1.2$ Hz, H1'); 2.55 (1H, ddd, $J = 9.7, 4.4$ Hz, 3.2 Hz, H5); ^{13}C -NMR (CD_3OD , δ , ppm) 178.58, 74.50, 68.08, 60.52, 59.88, 55.44, 43.71.

2-O-Methyl-1-deoxy-L-mannoirimycin (29). By using the same procedure as above for the D-enantiomer, 120 mg of **27** gave 88 mg (90%) of 2-*O*-methyl-1-deoxy-L-mannoirimycin (**29**), with NMR data identical to those of **28**.

Inhibition Analysis. Inhibition analyses were performed at 37 °C in 0.1 M HEPES buffer, pH 6.8, except for α -fucosidase (assayed at 37 °C in 50 mM sodium acetate buffer, pH 6.0). The amount of enzyme added in each assay was 0.05 unit (0.1 unit for β -galactosidase and α -fucosidase). For each inhibitor, four inhibitor concentrations, ranging from 0 to 3 times K_m , were used to obtain a set of data. *p*-(Nitrophenyl) glycosides were used as substrates, and the release of *p*-nitrophenol was monitored at 400 nm for 2 min. Data were collected and fitted to a Michaelis–Menten curve by using the program HyperCleland for enzyme kinetics analysis.²⁴

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