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Pyrrolidine and Hexahydro-1*H*-Azepine Mimics of the 'Flap Up' Mannosyl Cation

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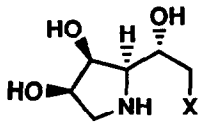
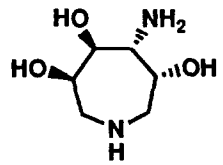
Abstract: The pyrrolidine **4**, 6-amino-1,4,6-trideoxy-1,4-imino-*D*-mannitol dihydrochloride, and the hexahydro-1*H*-azepine **5**, 4-amino-1,4,6-trideoxy-1,6-imino-*D*-mannitol dihydrochloride, were synthesized as potential inhibitors of α-mannosidase.

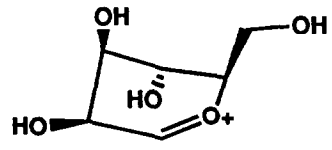
Fleet and Ganem have reported that 1,4-dideoxy- and 1,4,6-trideoxy-1,4-imino-*D*-mannitol (**1**[1] and **2**[2]) are potent inhibitors of α-mannosidase; more recently, 1,4,6-trideoxy-6-fluoro-1,4-imino-*D*-mannitol (**3**) has been shown to be an even more potent inhibitor of α-mannosidase[3]. Since there are two purported carboxylic acid moieties in the active site of α-mannosidase[4], we decided to prepare the corresponding amino analog **4** of these compounds and examine its activity against α-mannosidase. Our synthetic strategy was to prepare protected pyrrolidine **10** by intramolecular reductive amination of diamine **9**, a route analogous to that developed by Fleet for the synthesis of **1**[5]. Although we expected the pyrrolidine to be formed exclusively, we were intrigued by the potential α-mannosidase activity of the deprotected alternative cyclization product, hexahydro-1*H*-azepine **5**, and decided to model this compound against the lowest energy "flap up" half chair form of the mannosyl cation[6], the putative intermediate in the hydrolysis of mannopyranosides by α-mannosidase. We assumed the ring nitrogen of hexahydro-1*H*-azepine **5** would be protonated by the active site carboxylic acid and the exocyclic amine might stabilize the indicated chair conformation by intramolecular hydrogen bonding (Figure 1). There is an excellent overlap of the heteroatoms of the minimized structure of the hexahydro-1*H*-azepine and the mannosyl cation which suggested that the former might be an inhibitor of α-mannosidase. Especially important are the close proximities of the crucial ring nitrogen atom of **5** with the



1 X = OH
2 X = H
3 X = F
4 X = NH_2



5



mannosyl cation

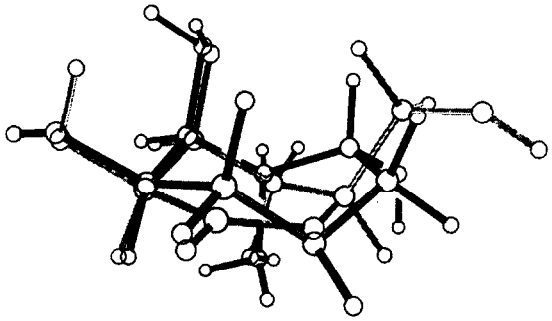
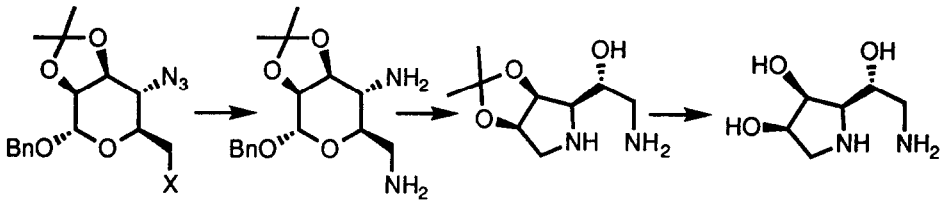


Figure 1
Hexahydro-1*H*-Azepine 5 (black)
Mannosyl cation (grey)

putative oxonium ion and the C-2 and C-3 hydroxyl groups of both structures. Both pyrrolidine **4** and hexahydro-1*H*-azepine **5** seemed readily accessible from Fleet's azido alcohol **6**[5].

For our initial synthesis of the pyrrolidine **4**, azido alcohol **6** was converted to bisazide **8** via the azido mesylate **7** (Scheme 1). Although the bisazide **8** was prepared and even recrystallized without mishap, during scaleup a small portion of the material detonated in a ground glass joint. However, we were able to prepare sufficient bisazide **8** to reduce to the corresponding diamine **9** with Pd black/C in CH_3OH. Reductive debenzylation and intramolecular reductive amination of diamine **9** gave exclusively pyrrolidine **10**. The mass spectrum of pyrrolidine **10** was characterized by a large peak at *m/z* 142, arising from cleavage of the ethanolamine sidechain [M^+- $CH(OH)CH_2NH_2$].

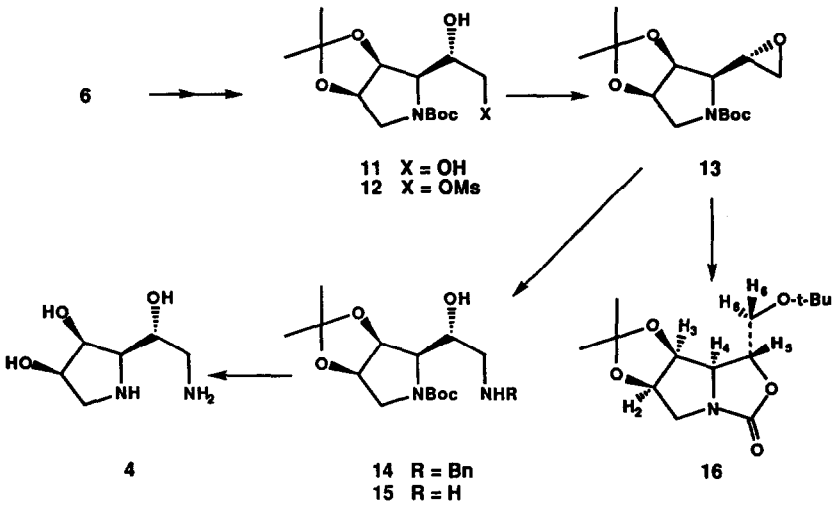


6 X = OH
7 X = OMs
8 X = N_3

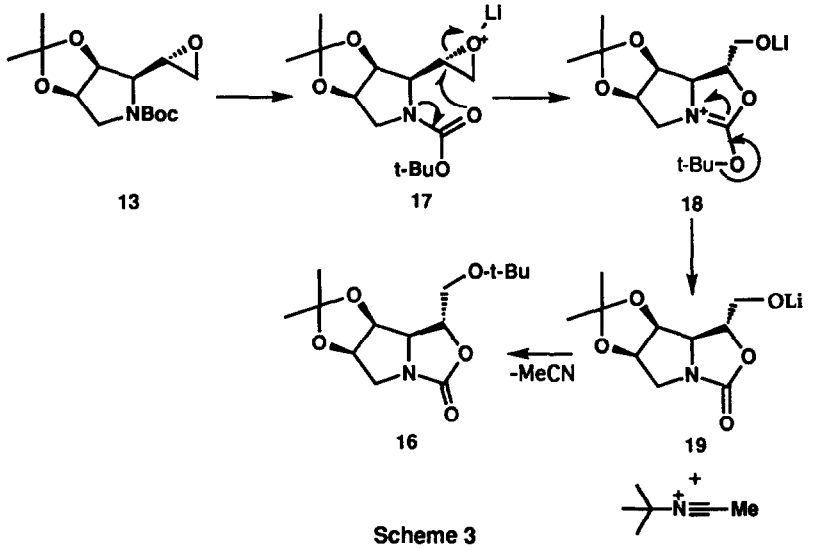
9 **10** **4**

Scheme 1

To avoid the hazards of the bisazide route and also develop a synthesis which would allow for the facile preparation of analogues, we utilized diol **11**, available from azido alcohol **6** as described by Fleet[5], as our starting material (Scheme 2). Diol **11** was converted to the monomesylate **12** with CH_3SO_2Cl in pyridine at 0 °C. Reaction of mesylate with CH_3ONa/CH_3OH gave the epoxide **13** in 92% yield. Addition of $LiClO_4$ to a solution of epoxide **13** and benzylamine in acetonitrile[7] gave amino alcohol **14** in 79% yield. However, if the $LiClO_4$ and epoxide were mixed before addition of the amine, carbamate **16** was also isolated. In fact, epoxide **13** was converted to carbamate **16** in 53% yield with $LiClO_4$ in the absence of added amine. An upfield shift of the *tert*-butoxy group from δ 1.46 in the epoxide **13** to δ 1.20 in the



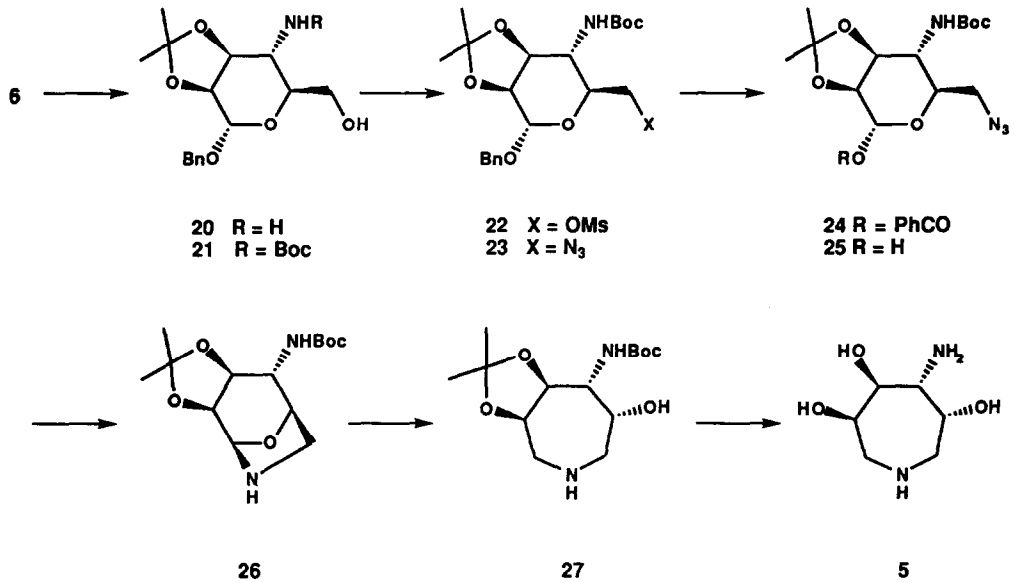
Scheme 2



Scheme 3

carbamate **16** suggested that the Boc group had been converted to a *tert*-butyl ether. A carbonyl stretch at 1750 cm^{-1} in the infrared spectrum of **16** was indicative of a cyclic carbamate. The relative stereochemistry of **16** was determined on the basis of 2D NOE data. NOE correlations were observed between the bridgehead methine, H-4, and methylene protons H-6 and methine proton H-3, indicative of a *cis* relationship between these protons. A weak correlation was also observed between methine proton H-5 and the downfield isopropylidine methyl, which is

consistent with the indicated stereochemistry. Other important NOE correlations observed were between methines H-2 and H-3 and the upfield isopropylidine methyl, and methylene protons H-6 to the *tert*-butyl methyl signals, consistent with the ether linkage. Examination of molecular models shows the Boc carbonyl group is ideally situated for a Lewis acid promoted intramolecular epoxide opening (Scheme 3, **17**→**18**). Loss of a *tert*-butyl carbonium ion from **18**, perhaps to acetonitrile to produce a transient nitrilium ion as in the Ritter reaction gives **19**, which is converted to the *tert*-butyl carbamate **16** with regeneration of acetonitrile.[8] Participation of the *tert*-butoxycarbonyl group in intramolecular reactions has been previously reported[9]. Finally, debenzylation of amino alcohol **14** gave pyrrolidine **15**, which was converted to the desired, deprotected pyrrolidine **4** with ethanolic HCl.



Scheme 4

For the synthesis of hexahydro-1*H*-azepine **5**, the azido alcohol **6** was hydrogenated with Pd/C and the resulting amine **20** was protected as its Boc derivative (Scheme 4). Mesylation (CH_3SO_2Cl, DMAP, pyridine) of the resulting alcohol **21** and displacement of the mesylate with sodium azide in DMF gave the azido ether **23**. Oxidative removal of the benzyl ether with $NaIO_4/RuO_2 \cdot xH_2O$[10] followed by saponification of resulting benzoate with CH_3ONa/CH_3OH gave lactol **25**. Surprisingly, catalytic hydrogenation of **25** with Pd black gave the stable bicyclic hemiaminal **26**. This same hemiaminal **26** was prepared directly from azido ether **23** by catalytic hydrogenation in HOAc using Pd/C, but the yield of this conversion was low. Reductive ring opening of hemiaminal **26** with $NaBH_3CN$ in HOAc gave the protected hexahydro-1*H*-azepine **27** in 93% yield. Deprotection with methanolic HCl gave the hexahydro-1*H*-azepine **5** as the

dihydrochloride salt. The NMR of **5** showed the hexahydro-1*H*-azepine adopted a half-chair conformation with the amino group *equatorial*, as H-4 appeared as a doublet of doublets with a large axial-axial coupling to H-3 (J = 10.2 Hz) and a smaller axial-equatorial coupling to H-5 (J = 2.6 Hz). Conversion to the free base by the addition of several drops of 30% NaOD led to no significant change in the solution conformation (the same coupling pattern was observed). Subsequent calculations using the SPARTAN electronic structure program have confirmed that either as the free base or as the monohydrochloride, the conformation with the 4-amino group equatorial is calculated to be 1.4-1.6 kcal/moL more stable than the modeled conformation with the 4-amino group axial.

Pyrrolidine **4** dihydrochloride did exhibit very weak inhibition of jack-bean α-mannosidase[11], with an IC_{50} value of 3-25 μg/mL. Unfortunately, hexahydro-1*H*-azepine **5** (either as the dihydrochloride or the free base) was inactive against jack-bean α-mannosidase, as the IC_{50} value was >200 μg/mL. The lack of activity of hexahydro-1*H*-azepine **5** may be a result of its solution conformation which is not recognized by the enzyme, even though the ring could flip into the modeled conformation in the enzyme active site. Alternatively, the lack of perfect correlation of the 6-OH of the mannosyl cation and the corresponding 5-OH of hexahydro-1*H*-azepine **5** may be more crucial. In their modeling study of α-mannosidase inhibitors, Winkler and Holan concluded that the "equivalent of the 6-OH appears to assist in binding of inhibitors into the active site, but it is not essential for activity."[6] We have found both here and in other studies[12] that the position of the equivalent of the 6-OH group of the mannosyl cation in a potential inhibitor is a critical determinant of activity and that a better predictor of α-mannosidase activity is the close correlation of this 6-OH group with the 8-OH group of the very potent α-mannosidase inhibitor swainsonine.[13]

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Silica Gel 60 (230-400 mesh ASTM, EM Science) was used for all flash chromatographies. Nuclear magnetic resonance spectra were recorded on Varian VXR-300, Unity-400, or Gemini-300 NMR spectrometers. Chemical shifts are reported in parts per million (ppm) versus tetramethylsilane (TMS). Coupling constants are reported in Hertz (Hz). As appropriate, ^{1}H-1*H* shift correlation spectroscopy (COSY) and 2D nuclear Overhauser effect (NOESY) experiments were performed to aid in spectral interpretation and assignments. Mass spectra were recorded on either a Finnigan MAT 4600, Finnigan MAT TSQ-700, or a VG Analytical Limited ZAB2-SE mass spectrometer using chemical ionization with CH_4 as the reagent gas. IR spectra were recorded on a Perkin-Elmer Model 1800 or Mattson Galaxy 5020 FT-IR spectrophotometer.

Benzyl 4,6-Diazido-4,6-dideoxy-2,3-*O*-isopropylidene-*D*-mannopyranoside (8). To a stirred solution of 5.84 g (17.4 mmol) of azido alcohol **6**[14] and 3.05 mL (21.9 mmol) of Et_3N in 70 mL of CH_2Cl_2 at 0°C under nitrogen was added dropwise 1.66 mL (21.4 mmol) of CH_3SO_2Cl. After 45 min, the solution was diluted with ether, and washed with cold water, brine, and dried ($MgSO_4$).

Concentration in vacuo gave mesylate **7** as a colorless oil which was dissolved in 70 mL of DMF; 3.92 g (60.3 mmol) of NaN_3 was added and the mixture was heated at 43-47 °C with stirring for 65 h. The cooled reaction mixture was poured into water and extracted with three portions of ether. The combined extracts were washed twice with water, brine, and dried ($MgSO_4$). Concentration in vacuo gave 6.20 g (99%) of bisazide **8** as a white solid. Recrystallization of a small portion from hexane gave **8** as white crystals: mp 60.5-62 °C; IR $(KBr)_{max}$ 2110, 1280, 1245, 1222, 1140, 1080, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.41-7.29 (m, 5 H), 5.16 (s, 1 H), 4.74 (d, 1 H, J = 11.7 Hz), 4.56 (d, 1 H, J = 11.7 Hz), 4.27 (dd, 1 H, J = 7.9, 5.4 Hz), 4.15 (d, 1 H, J = 5.4 Hz), 3.66 (dt, 1 H, J = 10.6, 4.5 Hz), 3.47 (dd, 1 H, J = 10.6, 8.0 Hz), 3.43 (apparent d, 2 H, J = 4.2 Hz), 1.57 (s, 3 H), 1.37 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 136.46, 128.58, 128.38, 128.21, 110.19, 96.06, 76.71, 74.96, 69.53, 67.80, 61.62, 51.67, 28.16, 26.20; $[\alpha]_D^{20}$ +65.0° (*c* 1.13, $CHCl_3$). Anal. Calcd for $C_{16}H_{20}N_6O_4$: C, 53.33; H, 5.59; N, 23.32. Found: C, 53.40; H, 5.65; N, 23.42.

6-Amino-1,4,6-trideoxy-1,4-imino-*D*-mannitol Dihydrochloride (4). A solution of 6.10 g (16.9 mmol) of bisazide **8** in CH_3OH (90 mL) containing 0.52 g Pd black was shaken in a Parr hydrogenation apparatus for 3 h, then allowed to stand overnight under 45 psi of H_2. The catalyst was removed by filtration and washed with CH_3OH. The filtrate and washings were concentrated in vacuo to give 5.34 g of oily diamine **9** which was dissolved in HOAc (90 mL) containing 0.51 g of Pd black, and hydrogenation continued for 96 h. The catalyst was removed by filtration and washed with HOAc. Concentration in vacuo gave 9 g of light amber oil which was dissolved in water and washed with EtOAc. Concentration in vacuo of the aqueous layer and flash chromatography of the residue (24:3:73 CH_3OH/conc NH_4OH/CH_2Cl_2) gave 4.5 g of the acetic acid salt of **10**; 10 g of 50% KOH was added, and the mixture extracted with four portions of EtOAc. The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to give 2.56 g (75%) of 6-amino-1,4,6-trideoxy-1,4-imino-2,3-*O*-isopropylidene-*D*-mannitol (**10**) as a pale yellow glass: ^1H NMR ($CDCl_3$) 1.45 (s, 3H), 1.32 (s, 3H). Gaseous HCl was bubbled into a chilled, stirred solution of amino pyrrolidine **10** (2.25 g, 11.1 mmol) in CH_3OH (100 mL) for 20 min. The mixture was purged with nitrogen overnight. The solution was concentrated in vacuo and the residue washed with several portions of CH_3CN/CH_3OH. Recrystallization from CH_3OH/CH_3CN gave 1.10 g (42%) of pyrrolidine **4** as a white powder: mp 225-227 °C (dec); IR $(KBr)_{max}$ 3491, 3325, 3037, 2957, 1325, 1120, 1094, 1030, 899 cm^{-1}; ^1H NMR (D_2O) δ 4.50 (m, H-2), 4.44 (t, H-3, J = 3.6 Hz), 4.33 (m, H-5), 3.67 (dd, H-4, J = 7.8, 3.6 Hz), 3.62 (dd, H-1', J = 12.2, 8.0 Hz), 3.29 (dd, H-6, J = 13.4, 2.9 Hz), 3.23 (dd, H-1, J = 12.2, 7.8 Hz), 3.14 (dd, H-6', J = 13.4, 10.4 Hz); ^{13}C NMR (D_2O) δ 72.63, 72.61, 67.11, 64.93, 50.25, 44.67; mass spectrum, *m/z* 163 (M^+ + 1, 100), 146, 145, 102; $[\alpha]_D^{20}$ +0.2° (*c* 0.5, CH_3OH). Anal. Calcd for $C_6H_{14}N_2O_3 \cdot 2HCl$: C, 30.65; H, 6.86; N, 11.91. Found: C, 30.92; H, 6.98; N, 11.86.

5,6-Anhydro-*N*-(*tert*-Butoxycarbonyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-*D*-mannitol (13). To a stirred solution of diol **11** (7.3 g, 24 mmol) in pyridine (35 mL) at 0°C was added CH_3SO_2Cl (2.24 mL, 28.7 mmol). The solution was placed in the freezer at -20 °C for 20.5

h. Ice-cold 1*N* HCl was added and the mixture extracted with EtOAc. The extracts were combined, washed with water, brine, and dried ($MgSO_4$). Concentration in vacuo gave 8.72 g (95%) of monomesylate **12** as a white solid. This labile intermediate was used without further purification. Freshly prepared CH_3ONa/CH_3OH (23.2 mmol, 23 mL) was added to vacuum-dried monomesylate **12** (8.72 g, 22.9 mmol) under nitrogen and the resulting solution was allowed to stir for 16 h. The reaction mixture was partitioned between EtOAc/water. The aqueous layer was removed, extracted with EtOAc, and the combined extracts washed with water, brine, and dried ($MgSO_4$). Concentration in vacuo gave 5.98 g (92%) of epoxide **13** as a white solid: ^1H NMR ($CDCl_3$) δ 4.85 (t, 1 H, *J* = 6.2 Hz), 4.76 (td, 1 H, *J* = 6.4, 3.2 Hz), 3.73 (dd, 1 H, *J* = 12.5, 6.3 Hz), 3.52 (dd, 1 H, *J* = 12.5, 3.2 Hz), 3.36 (dd, 1 H, *J* = 8.0, 6.4 Hz), 3.15 (ddd, 1 H, *J* = 8.0, 3.7, 2.6 Hz), 2.92 (dd, 1 H, *J* = 5.0, 3.8 Hz), 2.80 (bs, 1 H), 1.57 (s, 3 H), 1.46 (s, 9 H),1.38 (s, 3 H) ; mass spectrum, *m/z* 286 (M^++1), 230 (100), 186.

1,4,6-Trideoxy-1,4-(*tert*-butoxycarbonyl)imino-2,3-*O*-isopropylidene-6-[(phenylmethyl)amino]-*D*-mannitol (14). To a stirred solution of epoxide **13** (3.57g, 12.5 mmol) and $BnNH_2$ (5.5 mL, 50 mmol) in CH_3CN (28.5 mL) was added $LiClO_4$ (1.46 g, 13.8 mmole) and the mixture heated at 48 °C for 4.5 h. The cooled reaction mixture was poured into dilute aqueous NaOH/NaCl and extracted with diethyl ether. The combined extracts were washed with water, brine, and dried ($MgSO_4$). Concentration in vacuo gave a mixture of amino alcohol **14** and recovered epoxide **13.** The mixture was resubmitted to the reaction conditions using $BnNH_2$ (1.65 mL, 15.0 mmol) and $LiClO_4$ (439 mg, 4.13 mmole) in CH_3CN (28 mL) at 52 °C for 3.5 h. An additional portion of $LiClO_4$ (120 mg, 1.13 mmole) was added, and heating was continued for 2.5 h. The reaction mixture was allowed to stir at room temperature overnight before being worked up as above to give 5.08 g of crude amino alcohol **14.** Flash chromatography (5% CH_3OH in EtOAc) gave 3.88 g (79%) of amino alcohol **14** as a colorless oil: ^1H NMR (C_6D_6) δ 7.49 (d, 2 H, *J* = 7.3 Hz), 7.24-7.05 (m, 5 H), 4.36 (m, 1 H), 4.15 (t, 1 H, *J* = 6.6 Hz), 3.96 (m, 2 H), 3.78 (s, 2 H), 3.57 (bs, 1 H), 3.15 (dd, 1 H, *J* = 12.1, 4.7 Hz), 2.96-2.87 (m, 2 H), 1.36 (s, 9 H), 1.22 (s, 3 H), 0.95 (s, 3 H); ^{13}C NMR (C_6D_6) δ 141.47, 128.40, 128.30, 127.98, 127.84, 127.66, 126.78, 112.89, 80.50, 79.87, 77.63, 70.38, 53.98, 52.59, 27.92, 26.00, 24.17; mass spectrum, *m/z* 393 (M^+ + 1, 100), 337; exact mass calcd for $C_{21}H_{33}N_2O_5$ 393.2389, found 393.2368.

Gaseous HCl was slowly bubbled into a cold ethanolic solution of amino alcohol **14** (351 mg, 0.895 mmol) for 25 min. A heavy, white preciptate formed. The mixture was purged with nitrogen for 1.5 h the solvent, then concentrated in vacuo and the residue recrystallized from hot EtOH to give 114 mg (39%) of **1,4,6-trideoxy-1,4-imino-6-[(phenylmethyl)amino]-*D*-mannitol dihydrochloride** as a white crystalline solid: m.p. 234-235 °C (dec); ^1H NMR (CD_3OD) δ 7.43-7.29 (m, 5 H), 4.31-4.22 (m, 2 H), 4.21 (t, 1 H, *J* = 3.9 Hz), 4.16 (d, 1 H, *J* = 13.6 Hz), 4.12 (d, 1 H, *J* = 13.6 Hz), 3.53 (dd, 1 H, *J* = 6.8, 4.1 Hz), 3.32 (dd, 1 H, 11.8, 7.0 Hz), 3.23 (dd, 1 H, *J* = 12.9, 3.5 Hz), 3.06 (dd, 1 H, *J* = 12.8, 9.6 Hz), 3.04 (dd, 1 H, *J* = 11.8, 6.9 Hz); ^{13}C NMR (CD_3OD) δ 132.19, 131.23, 130.79, 130.34, 71.53, 71.48, 64.89, 64.19, 52.36, 50.25, 49.11; mass spectrum, *m/z* 293

(M$^+$+ 41), 281 (M$^+$+ 29), 254, 253 (M$^+$+ 1, 100); $[\alpha]_D^{20}$ +4.46° (*c* 0.75, CH_3OH). Anal. Calcd for $C_{13}H_{20}N_2O_3$•2HCl: C, 48.00; H, 6.82; N, 8.62. Found: C, 48.11; H, 6.97; N, 8.55.

6-Amino-1,4,6-trideoxy-1,4-(*tert*-butoxycarbonyl)imino-2,3-*O*-isopropylidene-*D*-mannitol (15). A solution of 5.18 g (13.2 mmol) of amino alcohol **14** in EtOH (60 mL) containing 386 mg $Pd(OH)_2$/C was shaken in a Parr hydrogenation apparatus under 45 psi of H_2 for 7.5 h. The catalyst was removed by filtration and washed with EtOH. The filtrate and washings were concentrated in vacuo to give 3.83 g (96%) of **15** as a white solid: ^{1}H NMR ($CDCl_3$) δ 4.89 (t, 1 H, *J* = 6.8 Hz), 4.76 (dd, 1 H, *J* = 11.9, 7.0 Hz), 4.05 (t, 1 H, *J* = 6.7 Hz), 3.94 (bs, 1 H), 3.81 (td, 1 H, *J* = 6.9, 3.0 Hz), 3.24 (dd, 1 H, *J* = 12.3, 4.0 Hz), 2.89 (bd, 1 H, *J* = 12.3 Hz), 2.84-2.54 (m, 4 H), 1.56 (s, 3 H), 1.48 (s, 9 H), 1.38 (s, 3 H). Deprotection with HCl as described above gave pyrrolidine **4** dihydrochloride identical in all respects with **4** dihydrochloride prepared from the bisazide **8**.

Tetrahydro-4-(*tert*-butoxymethyl)-2,2-dimethyl-4*H*,6*H*-1,3-dioxolo[3,4]pyrrolo[1,2-c]oxazol-6-one (16). To a stirred solution of epoxide **13** (500 mg, 1.75 mmol) in CH_3CN (4 mL) was added $LiClO_4$ (205 mg, 1.93 mmol). The reaction was allowed to stir at rt for 26.5 h, then heated at 40 °C for 2.5 h. The mixture was partitioned between EtOAc/water. The organic layer was concentrated in vacuo to give 266 mg (53%) of carbamate **16** as a white solid: mp 101-102 °C; IR (KBr) $_{max}$ 2965, 1750, 1377, 1210, 1095, 1085 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.83 (t, H-2, *J* = 4.8 Hz), 4.73 (m, H-5), 4.64 (t, H-3, *J* = 5.0 Hz), 3.92 (d, H-1β, *J* = 13.4 Hz), 3.75 (dd, H-4, *J* = 4.5, 2.4 Hz), 3.63 (dd, H-6, *J* = 9.6, 5.0 Hz), 3.51 (dd, H-6, *J* = 9.6, 6.6 Hz), 3.10 (dd, H-1α, *J* = 13.4, 4.3 Hz), 1.44 (s, 3 H), 1.31 (s, 3 H), 1.20 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 160.78, 112.22, 81.67, 79.66, 73.34, 72.98, 64.21, 62.28, 51.30, 27.23, 26.03, 23.91; mass spectrum, *m/z* 326 (M$^+$+ 41), 314 (M$^+$+ 29), 286 (M$^+$+ 1, 100), 230. Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.77; H, 8.12; N, 4.91.

Benzyl 4-Amino-4-deoxy-2,3-*O*-isopropylidene-*D*-mannopyranoside (20). Amine **20** was prepared according to Fleet's published procedure[4]; however, we were able to isolate **20** as white needles: mp 73-75 °C; IR (KBr) $_{max}$ 3011, 2991, 2937, 1384, 1376, 1245, 1140, 1077, 1028, 1001 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40-7.27 (m, 5 H), 5.13 (s, 1 H), 4.72 (d, 1 H, *J* = 11.6 Hz), 4.51 (d, 1 H, *J* = 11.6 Hz), 4.12 (d, 1 H, *J* = 5.4 Hz), 3.94 (dd, 1 H, *J* = 8.4, 5.4 Hz), 3.83 (dd, 1 H, *J* = 11.6, 5.0 Hz), 3.79 (dd, 1 H, *J* = 11.6, 4.4 Hz), 3.60-3.51 (m, 1 H), 2.88 (dd, 1 H, *J* = 10.1, 8.4 Hz), 2.05 (bs, 3 H), 1.51 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 136.90, 128.51, 128.21, 128.02, 109.38, 96.45, 79.61, 74.88, 69.77, 69.27, 63.76, 53.28, 28.16, 26.29; mass spectrum, *m/z* 350 (M$^+$+41), 338 (M$^+$+29), 311, 310 (M$^+$+1,100), 230, 202, 144; $[\alpha]_D^{20}$ +57.8° (*c* 1.07, $CHCl_3$). Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 61.89; H, 7.58; N, 4.53.

Benzyl 4-[N-(*Tert*-butoxycarbonyl)amino]-4-deoxy-2,3-*O*-isopropylidene-*D*-mannopyranoside (21). To a solution of amino alcohol **20** (3.62 g, 11.71 mmol) in THF (45 mL) was added di-*tert*-butyl dicarbonate (2.65 mL, 11.6 mmol) and the resulting solution was allowed to stir

at room temperature for 16.5 h. The solution was concentrated in vacuo and the residue purified by flash chromatography (35% EtOAc/cyclohexane) and recrystallized from CH_2Cl_2/hexane to give 3.24 g (68%) of alcohol **21** as flocculent white crystals: mp 144-145 °C; IR (KBr) $_{max}$ 3437, 3013, 2985, 1698, 1507, 1385, 1370, 1245, 1166, 1143, 1073, 1039, 1026, 996 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40-7.27 (m, 5 H), 5.19 (s, 1 H), 4.71 (d, 1 H, *J* = 11.7 Hz), 4.66 (s, 1 H), 4.54 (d, 1 H, *J* = 11.7 Hz), 4.17 (d, 1 H, *J* = 5.4 Hz), 4.08 (dd, 1 H, *J* = 8.1, 5.5 Hz), 3.81-3.62 (m, 3 H), 3.50 (bd, 1 H, *J* = 9.8 Hz), 1.54 (s, 3 H), 1.44 (s, 9 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 136.90, 128.53, 128.22, 128.06, 109.74, 96.60, 80.64, 75.45, 74.90, 70.73, 70.69, 69.45, 61.66, 49.83, 28.35, 28.24, 27.90, 26.17; mass spectrum, *m/z* 410 (M$^+$+1), 354, 338, 310, 274, 246 (100), 202, 188, 91; $[\alpha]_D^{20}$ +15.7°, (*c* 1.01, $CHCl_3$). Anal. Calcd for $C_{21}H_{31}NO_7$•0.25 H_2O: C, 60.92; H, 7.67; N, 3.38. Found: C, 61.05; H, 7.58; N, 3.46.

Benzyl 4-[*N*-(*Tert*-butoxycarbonyl)amino]-4-deoxy-2,3-*O*-isopropylidene-6-*O*-methane-sulfonyl-*D*-mannopyranoside (22). To a solution of alcohol **21** (6.83 g, 16.7 mmol) in CH_2Cl_2 (54 mL) was added pyridine (2.76 mL, 34.1 mmol), CH_3SO_2Cl (2.58 mL, 33.4 mmol), and 4-dimethylaminopyridine (205 mg, 1.68 mmol). After stirring for 20 h, the reaction was acidified with ice-cold 1 *N* HCl, extracted with EtOAc, washed with water and brine, and dried ($MgSO_4$). Concentration in vacuo and flash chromatography (3:1 EtOAc/cyclohexane) gave 7.05 g of mesylate **22** (87%) as a white foam: IR (KBr) $_{max}$ 3403, 2984, 2936, 1715, 1366, 1177, 1144, 1082 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.41-7.28 (m, 5 H), 5.09 (s, 1 H), 4.85 (bd, 1 H, *J* = 8.4 Hz), 4.78 (d, 1 H, *J* = 11.6 Hz), 4.55 (d, 1 H, *J* = 11.6 Hz), 4.38 (d, 2 H, *J* = 4.7 Hz), 4.26-4.19 (m, 1 H), 4.16 (d, 1 H, *J* = 5.5 Hz), 4.07 (bs, 1 H), 3.61 (q, 1 H, *J* = 8.5 Hz), 3.07 (s, 3 H), 1.52 (s, 3 H), 1.43 (s, 9 H), 1.34 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 136.46, 128.59, 128.38, 128.20, 109.78, 96.23, 74.85, 74.83, 74.58, 69.69, 69.58, 68.97, 49.73, 37.56, 28.25, 27.66, 25.98; mass spectrum, *m/z* 488 (M+ + 1), 432, 388, 336, 324(100), 280; $[\alpha]_D^{20}$ +33.9° (*c* 1.02, $CHCl_3$). Anal. Calcd for $C_{22}H_{33}NO_9S$•0.1 H_2O: C, 54.00; H, 6.84; N, 2.86. Found: C, 53.81; H, 6.70; N, 2.87.

Benzyl 6-Azido-4-[N-(*tert*-butoxycarbonyl)amino]-4,6-dideoxy-2,3-*O*-isopropylidene-*D*-mannopyranoside (23). A mixture of mesylate **22** (8.03 g, 16.5 mmol) and NaN_3 (7.49 g, 115 mmol) in DMF (125 mL) was heated with stirring at 80 °C for 6.5 h, allowed to stir at room temperature for 22 h, then heated at 80 °C for 5 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined extracts were washed with water, brine, dried ($MgSO_4$), and concentrated to give 6.98 g (98%) of azido ether **23** as a colorless oil. The material was of sufficient purity for further use, but flash chromatography (85:15 cyclohexane/EtOAc) gave an analytical sample of azido ether **23** as a tacky solid: mp 43-45 °C; IR (KBr) $_{max}$ 3436, 3018, 2985, 2931, 2103, 1714, 1502, 1369, 1245, 1165, 1085 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40-7.28 (m, 5 H), 5.11 (s, 1 H), 4.79 (d, 1 H, *J* = 11.6 Hz), 4.67 (bd, 1 H, *J* = 7.1 Hz), 4.58 (d, 1 H, *J* = 11.6 Hz), 4.18-4.07 (m, 2 H), 3.87 (bt, 1 H, *J* = 7.9 Hz), 3.65-3.47 (m, 2 H), 3.32 (dd, 1 H, *J* = 13.0, 2.0 Hz), 1.54 (s, 3 H), 1.43 (s, 9 H), 1.34 (s 3 H); mass spectrum, *m/z* 435 (M+ + 1), 379, 336, 271, 227,

185(100), 142, 141, 96, 91; $[\alpha]_D^{20}$ +19.4° (*c* 0.74, $CHCl_3$). Anal. Calcd for $C_{21}H_{30}N_4O_6 \cdot 0.1\ C_6H_{12}$: C, 58.58; H, 7.10; N, 12.65. Found. C, 58.67; H, 7.24; N, 12.43. TG: 1.7% loss of cyclohexane.

Benzoyl 6-Azido-4-[*N*-(*tert*-butoxycarbonyl)amino]-4,6-dideoxy-2,3-*O*-isopropylidene-*D*-mannopyranoside (24). $NaIO_4$ (28.5 g, 133 mmol) was suspended in 0.1 M Na_2HPO_4 (75 mL) and the pH adjusted to 9 with NaOH. The suspension was added to a vigorously stirred solution of azido ether **23** (11.6 g, 26.7 mmol) in 1:1 CH_3CN/CCl_4 (100 mL), and $RuO_2 \cdot H_2O$ (156 mg, 1.17 mmol) was added. After 5 h and again after 22.5 h, additional $NaIO_4$ (5.70 g, 26.7 mmol) was added. After 50 h total, the solids were filtered off and the layers separated. The aqueous layer was extracted with several portions of CH_2Cl_2,and the combined extracts were dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography of the residue (15% EtOAc in cyclohexane) gave 2.85 g (35%) of recovered azido ether **23**,and trituration with hexane gave 2.35 g (28%) of benzoate **24** as a white foam: IR (film from $CDCl_3$) $_{max}$ 3372, 2984, 2104, 1717, 1267, 1248, 1223, 1167, 1092, 1067, 968, 949, 733, 714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (d, 2 H, *J* = 7.3 Hz), 7.65-7.58 (m, 1 H), 7.51-7.44 (m, 2 H), 6.62 (s, 1 H), 4.80 (bd, 1 H, *J* = 6.0 Hz), 4.44 (bs, 1 H), 4.28 (d, 1 H, *J* = 5.2 Hz), 4.19 (bs, 1 H), 3.66-3.38 (m, 3 H), 1.59 (s, 3 H), 1.45 (s, 9 H), 1.39 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 164.06, 155.24, 133.67, 130.05, 129.89, 129.18, 128.69, 128.58, 110.18, 91.61, 74.57, 74.52, 74.31, 71.51, 51.66, 28.27, 28.20, 27.90, 26.22; mass spectrum, *m/z* 449 (M^+ + 1), 327, 271(100), 241, 185, 96; $[\alpha]_D^{20}$ -15.6° (*c* 0.80, $CHCl_3$). Anal. Calcd for $C_{21}H_{28}N_4O_7$: C, 56.24; H, 6.29; N, 12.49. Found: C, 56.06; H, 6.31; N, 12.33.

6-Azido-4-[*N*-(*tert*-butoxycarbonyl)amino]-4,6-dideoxy-2,3-*O*-isopropylidene-*D*-mannose (25). To a stirred solution of benzoate **24** (2.45g, 5.45 mmol) in CH_3OH (30 mL) was added CH_3ONa (295 mg, 5.45 mmol). After 18 h, the solution was concentrated in vacuo and the residue purified by flash chromatography (3:1 hexane/EtOAc) to give 1.23 g (66%) of lactol **25** as a white solid upon recrystallization from EtOAc/hexane: mp 72-73 °C; ^1H NMR ($CDCl_3$) δ 5.44 (d, 1 H, *J* = 3.4 Hz), 4.89 (bd, 1 H, *J* = 6.3 Hz), 4.24 (bt, 1 H, *J* = 4.5 Hz), 4.16 (d, 1 H, *J* = 5.5 Hz), 4.09-4.06 (m, 1 H), 3.68-3.35 (m, 4 H), 1.53 (s, 3 H), 1.43 (s, 9 H), 1.35 (s, 3 H). The material was used without further purification.

6-Amino-4-[*N*-(*tert*-butoxycarbonyl)amino]-1,4,6-trideoxy-1,6-imino-2,3-*O*-isopropylidene-*D*-mannopyranoside (26). To a slurry of Pd black in EtOH was added a solution of lactol **25** (1.23 g, 3.575 mmol) in EtOH (50 mL). Hydrogenation in a Parr shaker for 25 h gave hemiaminal **26** containing small amounts of hexahydro-1*H*-azepine **27** which were separated by flash chromatography (1:1 hexane/EtOAc, then EtOAc) to give 807 mg (88%) of bicyclic hemiaminal **26** as a white foam: IR (KBr) $_{max}$ 3445, 3422, 3354, 2980, 1711, 1368, 1250, 1217, 1169, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.17 (bd, 1 H, *J* = 4.3 Hz), 4.91 (d, 1 H, *J* = 4.2 Hz), 4.32 (dd, 1 H, *J* = 7.3, 1.5 Hz), 4.14-4.04 (m, 2 H), 3.97 (d, 1 H, *J* = 9.5 Hz), 3.20 (d, 1 H, *J* = 10.4 Hz), 3.07 (dd, 1 H, *J* = 10.1, 7.3 Hz), 2.04 (bs, 1 H), 1.62 (s, 3 H), 1.46 (s, 9 H), 1.31 (s, 3 H); ^{13}C NMR

($CDCl_3$) δ 109.93, 87.61, 79.89, 77.20, 76.92, 75.58, 74.63, 71.87, 52.01, 45.84, 28.49, 28.36, 26.07, 25.61; mass spectrum, *m/z* 329 (M+ + 29), 301 (M+ + 1), 245(100), 187; $[\alpha]_D^{20}$ +21.0° (*c* 0.158, $CHCl_3$). Anal. Calcd for $C_{14}H_{24}N_2O_5$: C, 55.98; H, 8.05; N, 9.33. Found: C, 55.61; H, 7.98; N, 9.04. Further elution with 9:1 EtOAc/CH_3OH gave trace variable amounts of protected hexahydro-1*H*-azepine **27** (*vide infra*).

4-[*N*-(*Tert*-butoxycarbonyl)amino]-1,4,6-trideoxy-1,6-imino-2,3-*O*-isopropylidene-*D*-mannitol (27). A solution of $NaBH_3CN$ (179 mg, 2.84 mmol) in HOAc (10 mL) was added to hemiaminal **26** (724 mg, 2.41 mmol) and the resulting solution was allowed to stir at room temperature for 3 h. The reaction mixture was chilled in an ice bath and 50% NaOH added until the aqueous layer was strongly basic. Water was added and the solution was extracted with EtOAc. The extracts were combined, dried ($MgSO_4$), and concentrated in vacuo to give 676 mg (93%) of protected hexahydro-1*H*-azepine **27** as a white foam: IR (film from $CDCl_3$) $_{max}$ 3335, 2986, 2938, 1684, 1528, 1456, 1385, 1371, 1327, 1310, 1256, 1217, 1169, 1113, 1063, 758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.26 (d, 1 H, *J* = ~6.5 Hz), 4.43 (dd, 1 H, *J* = 8.8, 7.2 Hz), 4.31-4.24 (m, 1 H), 4.00 (d, 1 H, *J* = 3.9 Hz), 3.69 (apparent dd, 1 H, *J* =8.0, 7.4 Hz), 3.22-3.08 (m, 2 H), 2.85 (d, 1 H, *J* = 13.0 Hz), 2.63-2.54 (m, 1 H), 1.45 (s, 9 H), 1.42 (s, 3 H), 1.33 (s, 3 H); mass spectrum, *m/z* 303 (M+ + 1), 247(100), 189; $[\alpha]_D^{20}$ -28.6° (*c* 0.86, $CHCl_3$). Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.61; H, 8.67; N, 9.27. Found: C, 55.21; H, 8.55; N, 9.07.

4-Amino-1,4,6-trideoxy-1,6-imino-*D*-mannitol Dihydrochloride (5). Gaseous HCl was bubbled through an ice-cold solution of protected hexahydro-1*H*-azepine **27** (804 mg, 2.66 mmol) in CH_3OH (50 mL) for 0.5 h. Nitrogen was then bubbled through the solution for 1 h. Concentration in vacuo and trituration of the residue with CH_3OH gave 435 mg (70%) of hexahydro-1*H*-azepine **5** dihydrochloride as an ivory solid: mp 229 °C (dec); IR (KBr) $_{max}$ 3374, 3339, 3241, 3190, 3144, 3044, 2961, 2845, 1599, 1582, 1489, 1458, 1271, 1177, 1140, 1117, 1065, 1055 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.46 (bs, 1 H), 9.05 (bs, 1 H), 8.19 (bs, 3 H), 6.31 (d, 1 H, *J* = 4.3 Hz), 6.02 (bs, 1 H), 5.85 (bs, 1 H), 4.33 (bs, 1 H), 4.13 (bs, 1 H), 3.77 (d, 1 H, *J* = 9.8 Hz), 3.33-3.11 (m, 5 H); ^1H NMR (D_2O) 4.48 (dt, H-5, *J* = 5.7, 2.4 Hz), 4.38 (m, H-2), 4.04 (dd, H-3, *J* = 10.2, 2.0 Hz), 3.63 (dd, H-4, *J* = 10.2, 2.6 Hz), 3.59-3.41 (m, H-1 and H-6); ^1H NMR (D_2O + 30% NaOD) 4.03-3.95 (m, H-2 and H-5), 3.76 (dd, H-3, *J* = 8.2, 3.0 Hz), 3.06 (dd, H-4, *J* = 8.2, 2.8 Hz), 2.94-2.73 (m, H-1 and H-6); ^{13}C NMR (D_2O) δ 72.45 (C-3), 71.02 (C-2), 65.94 (C-5), 58.22 (C-4), 48.80 (C-6), 48.23 (C-1); mass spectrum, *m/z* 163 (M+ + 1), 146(100); $[\alpha]_D^{20}$ -63.9° (*c* 0.84, CH_3OH). Anal. Calcd for $C_6H_{14}N_2O_3 \cdot 2HCl$: C, 30.65; H, 6.86; N, 11.92. Found: C, 30.97; H, 6.92; N, 12.05.

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14. For **6**: mp 79.5-80.5 °C; $[\alpha]_D^{20}$ +75.0 ° (*c* 1.71, $CHCl_3$); lit.[5]: mp 80-82 °C; $[\alpha]_D^{20}$ +75.0° (*c* 1.7, $CHCl_3$).

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