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Synthesis and glycosidase inhibitory activity of pseudo-di-(or tri-)saccharides

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Abstract—The synthesis of pseudo-di-(or tri-)saccharides with a D-mannoazepane or a L-gulopiperidine skeleton either *N*-linked to a D-*gluco*-*C*-furanoside or to D-mannitol, and evaluation of their inhibitory activities against α- or β-D-glucosidase, α-D-mannosidase and α-L-fucosidase are described.

1. Introduction

The implication of interactions between cell surface carbohydrates and protein receptors in many important biological events has focused attention on carbohydrate mimics in recent years.[1,2] Glycosidases are enzymes involved in the biosynthesis of such glycoproteins, as well as several other important processes such as the catabolism of glycoconjugates and digestion. Inhibitors of these enzymes have potential in the treatment of viral infections, cancer, diabetes and other metabolic disorders.[3] Interesting naturally occurring glycosidase inhibitors are iminosugar analogs (Fig. 1) such as DMDP (2,5-bis(hydroxymethyl)-3,4-dihydroxy-pyrrolidine) and DNJ (1-deoxynojirimycin).[3] However, a significant deterrent to the use of iminosugars as therapeutic tools is the fact that they often inhibit a broad range of glycosidases leading to detrimental side effects. Recent research has shown that the affinity of an iminosugar can be significantly increased by inclusion of an appropriate aglycon moiety,[4] for example MDL 25,637[5] is a potent specific glycosidase inhibitor and an antidiabetic drug lead.[6]

In order to expand on the newer generation of stable inhibitors[7–10] with increased specificity for selected glycosidases we wished to explore *N*-substituted iminosugars linked to a sugar analog aglycon moiety by a non-hydrolyzable bond.[11] Modulation of the aglycon part was carried out using the 1,6-dideoxy-1,6-imino-D-mannitol **1** and 1,5-dideoxy-1,5-imino-L-gulitol **2**, parent iminosugars, which have moderate selectivities and binding affinities for α-L-fucosidase with K_i values of 28[12] and 22 μM, respectively.[13] Simple affinity measurements would allow us to identify any increased selectivity obtained by the addition of an aglycon substituent. In that context, we have focused on the azadisaccharides **3**, **4** and their trisaccharides analogs **5**, **6** with a D-*gluco*-*C*-furanoside or a D-mannitol derived unit as aglycon parts, respectively (Fig. 2). To our knowledge, neither azepane nor tetrahydrofuran have been reported in the elaboration to pseudosaccharides. We report here full results concerning the synthesis of these compounds and evaluation as glycosidase inhibitors.

2. Results and discussion

Recently, we have delineated a convenient access to polyhydroxylated heterocycles from C_2-symmetrical bis-epoxides **A** (3,4-*O*-acetonide) and **B** (3,4-di-*O*-benzyl)

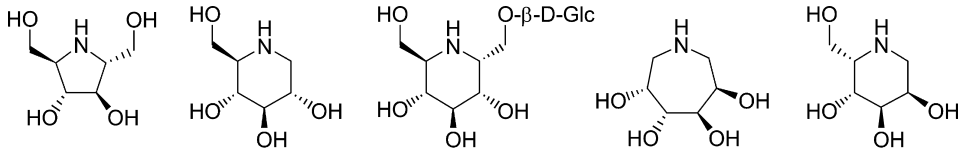

Figure 1.

Keywords: pseudo-saccharides; glycosidases; mannitol; azepane; piperidine; *C*-furanoside.

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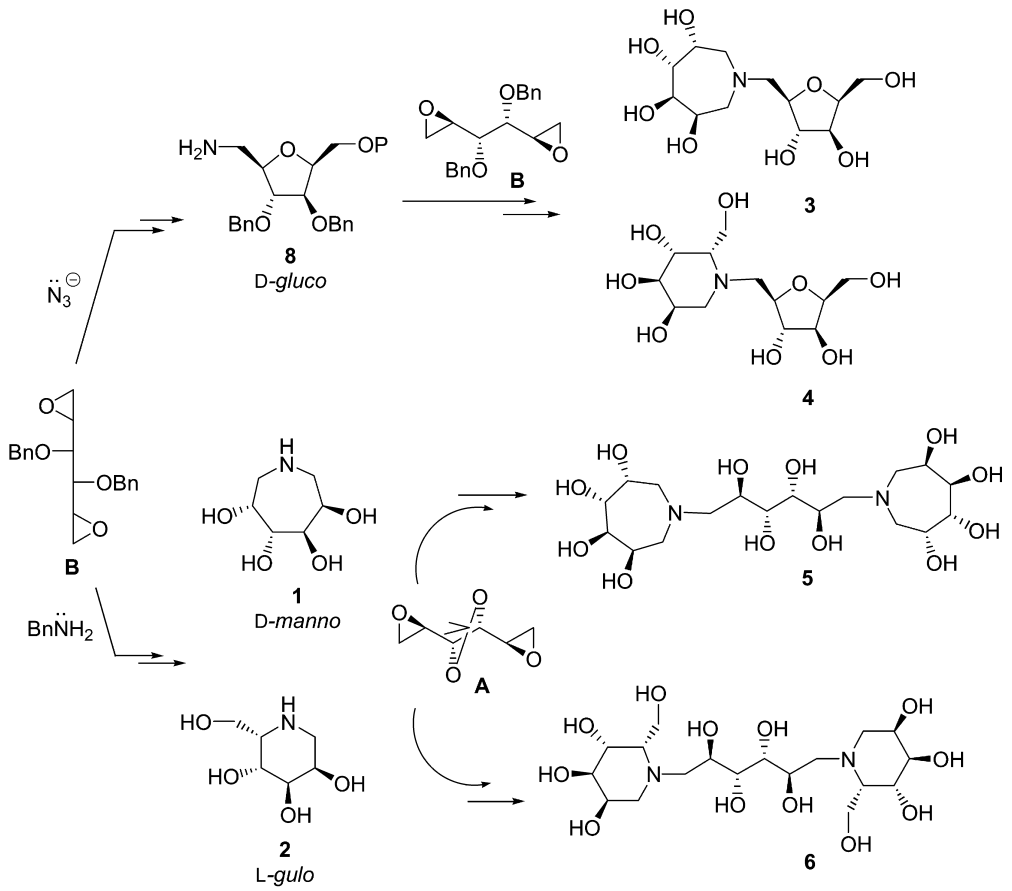
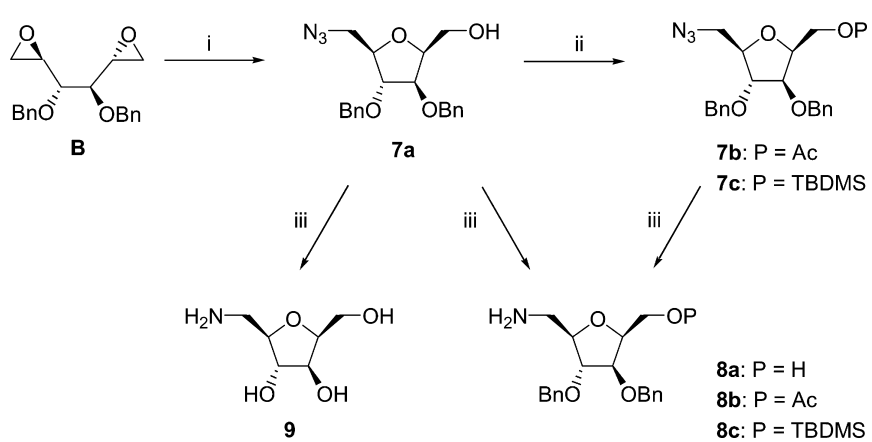


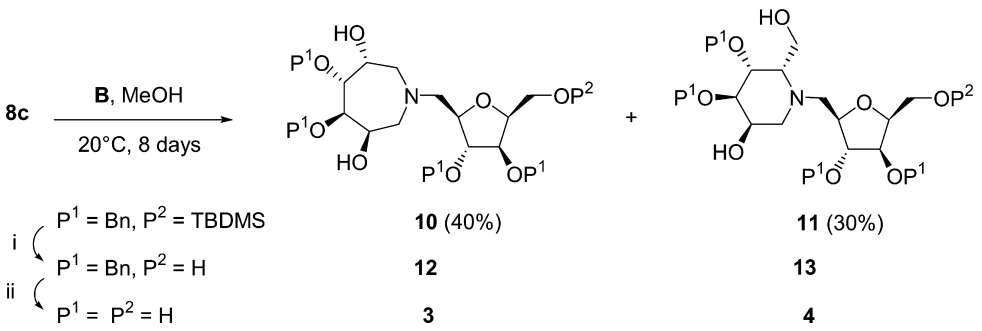
Figure 2.

derived from D-mannitol. From these compounds, after opening of a first epoxide moiety at the least substituted site, three different evolutions can occur: (i) opening at the second epoxy function of **A** by an other equivalent of nucleophile resulting in a C_2-symmetric acyclic compound; (ii) *O*-cyclization according to a *5-exo-tet* process mainly with bis-epoxide **B** giving tetrahydrofuran skeleton; (iii) nucleophile-cyclization after acid–base exchange between the alkoxide and the introduced nucleophile affording a 6- and 7-membered ring according to the regioselectivity of the second epoxide opening (**A** or **B**). Taking advantage of this flexible strategy which allows access to a great variety of carbohydrate mimics, such as imino-,[13] thio-,[14] guanidino-[15] and aminothiazolino[16] -sugars and amino-methyl-*C*-furanoside[17] derivatives, displaying various configurations, our efforts were now directed towards the synthesis of the pseudo-aza-di-(or tri-)-saccharides (Fig. 2).

On one hand, elaboration to the azadisaccharides **3** and **4** required the *N*-cyclization of the flexible 3,4-di-*O*-benzyl D-*manno* bis-epoxide **B** by the primary amino function of the aminomethyl-*C*-furanoside **8**. The latter resulted from the *O*-cyclization of the bis-epoxide **B** by the azide ion followed by reduction. On the other hand, elaboration to azatrisaccharides **5** and **6** involved the bis-nucleophile



Scheme 1. *Reagents and conditions*: (i) NaN_3, SiO_2, CH_3CN, △, 48 h, 95%; (ii) Ac_2O, DMAP, 2 h for **7b**, 91% or TBDMSCl, imidazole, DMF, 15 h for **7c**, 95%; (iii) H_2, Pd black in EtOAc for **8a** (94%), **8b** (65%), **8c** (95%), or in AcOH for **9** (90%).



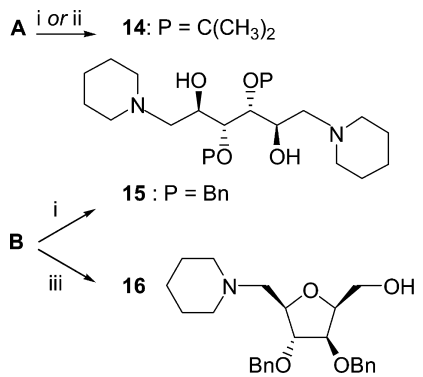
Scheme 2. *Reagents and conditions*: (i) Bu_4NF, THF, rt, 10 h, for **12** (85%), **13** (80%); (ii) H_2, Pd black, AcOH, for **3** (70%), for **4** (75%).

opening of the more rigid 3,4-*O*-acetonide D-*manno* bis-epoxide **A** by the secondary heterocyclic amine having an azepane or a piperidine skeleton **1** and **2**, respectively. These heterocyclic amines **1** and **2** are the result of the *N*-cyclization of the bis-epoxide **B** by benzylamine and subsequent hydrogenolytic removal of both *N*,*O*-benzyl protecting groups.

Synthesis of pseudo-azadisaccharides were achieved as outlined in Schemes 1 and 2. Reaction of the bis-epoxide **B** with sodium azide and silica gel under heating enabled the one step preparation of the azidomethyl-D-*gluco*-*C*-furanoside **7a** in 95% yield (Scheme 1). The azide function was cleanly reduced to an amine with dihydrogen in the presence of palladium black in ethyl acetate to afford compound **8a** in which the primary alcohol function is free (94% yield).

Although the presence of this hydroxyl group does not prevent the aminocyclization, we chose to protect the primary alcohol function because we assume that the separation of the mixture of 6 and 7-membered bicycles could be difficult.

Protection of the primary alcohol function of **7a** by an acetate group (acetic anhydride in the presence of DMAP), or by silyl ether group (*tert*-butyldimethylsilyl chloride and imidazole) led to **7b** or **7c** in high yields. The azide function was then reduced in the same conditions as above to give amine **8b** or **8c** with 65 and 95% yields, respectively. Furthermore, total reduction of **7a**, by using acetic acid instead of ethyl acetate as solvent, furnished the aminosugar **9** (90% yield).



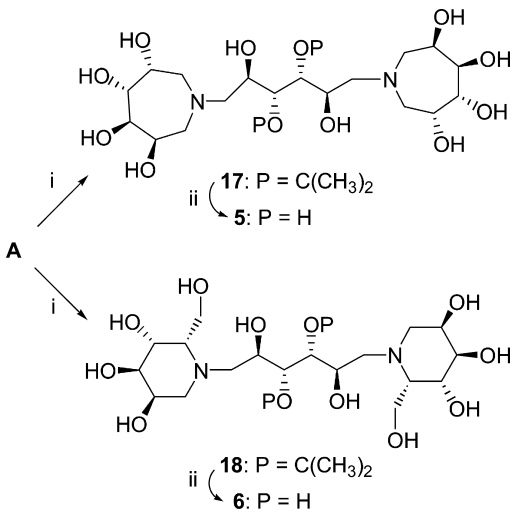
Scheme 3. *Reagents and conditions*: (i) neat piperidine, 4 days; (ii) piperidine (2 equiv.), MeOH, 20°C. (iii) piperidine (2 equiv.), CH_3CN, △.

Aminocyclization of a second bis-epoxide **B** with the primary amine function of **8a**, **8b** or **8c** has been studied in methanol at room temperature. Although aminocyclization cleanly occurred with all the amines **8**, in the case of amino derivatives **8a** or **8b**, we were not able to separate the mixture of the corresponding adducts by flash chromatography, even after peracetylation of the mixture. With the silyl derivative **8c** a mixture of *N*-methylfurano azepane **10** and *N*-methylfurano piperidine **11** was obtained, which could be easily separated by flash chromatography in 40 and 30% yields, respectively (Scheme 2). Removal of the silyl group of **10** or **11** with tetrabutylammonium fluoride (TBAF) furnished **12** or **13** in 85 and 80% yields, respectively. Finally, total hydrogenolysis of benzyl protecting groups in the presence of palladium black in acetic acid gave the desired azadisaccharides **3** and **4**, which were isolated as their acetate salts after purification by flash chromatography in 70 and 75% yields, respectively.

Elaboration to the azatrisaccharides **5** and **6** required the bis-epoxide opening with the sterically hindered heterocyclic amines **1** and **2**. For this purpose, the reactivity of piperidine, as a model, was evaluated on the 3,4-*O*-acetonide- and 3,4-di-*O*-benzyl-D-*manno*-bis-epoxides **A** and **B**, respectively (Scheme 3).

Treatment of the *O*-acetonide bis-epoxide **A**, with piperidine as solvent or with 2 equiv. of piperidine in methanol at room temperature, furnished the acyclic C_2-symmetric 1,6-bis-*N*-piperidino-1,6-dideoxy-D-mannitol derivative **14** in 90% yield. The flexible di-*O*-benzyl-bis-epoxide **B** with piperidine, as reagent and solvent, at room temperature for 4 days led to the acyclic C_2-symmetric derivative **15** (80%), while with 2 equiv. of piperidine in refluxing acetonitrile the cyclic *N*-piperidinomethyl-tetrahydrofuran **16** was isolated in 85% yield. Interestingly, after nucleophilic opening of bis-epoxide **B** by a secondary amine, *O*-cyclization occurred only by a *5-exo-tet* process: no tetrahydropyran (*6-endo-tet* cyclization) was isolated after flash chromatography. From bis-epoxide **B**, secondary amine induced regioselective *O*-cyclization whereas primary amines (benzylamine,[18] tryptamine[19] and aminomethyl-*C*-furanoside **8**) always led to a 1:1 mixture of *N*-cyclization by *6-exo-tet* and *7-endo-tet* processes.

Taking advantage of the mild experimental conditions with 2 equiv. of secondary amine in methanol at room temperature, we have carried out the nucleophilic opening of the 3,4-*O*-acetonide-D-*manno*-bis-epoxide **A** by the



Scheme 4. *Reagents and conditions*: (i) **1** or **2** (2 equiv.) MeOH, 12 h, 20°C for **17** or **18**; (ii) CF_3CO_2H/H_2O 4:1, 20°C for **5** or **6**.

polyhydroxylated azepane **1** and piperidine **2** (Scheme 4). Under the previous conditions, each of these heterocyclic secondary amines **1** or **2** cleanly reacted with the bis-epoxide **A** to afford the protected azatrisaccharides **17** or **18**, respectively. Finally, hydrolysis of the acetonide protecting group in the presence of trifluoroacetic acid at room temperature, followed by purification by flash chromatography gave access to the pseudo-trisaccharides **5** and **6** in 50% and 75% overall yields, respectively.

3. Inhibition studies

The new aza-di-(or tri-)saccharides **3**–**6** (Fig. 2) were screened against four common glycosidases (α-D-glucosidase, β-D-glucosidase, α-D-mannosidase and α-L-fucosidase) following the protocol as previously reported.[20]

The pseudo-disaccharides **3** and **4** were totally inactive on all the glycosidases studied (K_i=10^{-3} M) and consequently less potent inhibitors than their parent iminosugars **1** and **2**,[12,13] respectively.

Whereas pseudo-trisaccharide **6** showed no inhibition against all the glycosidases, the 1,6-dideoxy-1,6-bis-*N*-(1′,6′-dideoxy-1′,6′-imino-D-mannitol)-D-mannitol **5** displayed competitive and selective inhibition towards α-L-fucosidase with a K_i=15 μM; no inhibition was found at 1 mM on the other enzymes assayed.

Thus, compound **5** has a K_i value in the same order of magnitude of the azepane **1** of D-*manno* configuration but the selectivity was increased towards α-L-fucosidase since α-D-glucosidase was not inhibited, as by **1**.[12]

4. Conclusion

We have synthesized azadi-(and tri-)saccharides which have been evaluated as glycosidase inhibitors and shown that the azatrisaccharide **5** displayed complete selectivity towards α-L-fucosidase.

It should be noted on one hand, that the efficient strategy described here could also be carried out from the L-ido bis-epoxide as well as from other polyhydroxylated amines and iminosugars in order to obtain a larger family of polysaccharide analogs to study the glycosidase active sites. On the other hand, the synthesis of pseudo-azatrisaccharides could be achieved following the described strategy for pseudo-azadisaccharides by repeating the process after transforming the primary hydroxy group of the tetrahydrofuran skeleton into a primary amine. Furthermore, a straightforward preparation of pseudo-azadisaccharides could be obtained after nucleophilic opening of the 3,4-di-*O*-benzyl-D-*manno*-bis-epoxide **B** by secondary amines thanks to a preference of *O*-cyclization (5-membered ring) over *N*-cyclization (7-membered ring).

5. Experimental

5.1. General directions

Prior to use, tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium–benzophenone and dichloromethane (CH_2Cl_2) from CaH_2. CH_2Cl_2 and ethyl acetate (EtOAc) were filtered on K_2CO_3 prior to use. ^{1}H NMR (250 MHz) and ^{13}C NMR (63 MHz) spectra were recorded on a Bruker AM 250. Chemical shifts (δ) are reported in ppm. IR spectra were recorded on a Perkin–Elmer 783 Infrared Spectrophotometer. Specific rotations were measured on a Perkin–Elmer 241C polarimeter with sodium (589 nm) or mercury (365 nm) lamp. Mass spectra were recorded by the Service de Spectrométrie de Masse, Ecole Normale Supérieure, Paris. All reactions were carried out under argon atmosphere, and were monitored by thin-layer chromatography with Merck 60F-254 precoated silica (0.2 mm) on glass. Flash chromatography was performed with Merck Kieselgel 60 (200–500 μm); the solvent systems were given v/v. Spectroscopic (^{1}H and ^{13}C NMR, MS) and/or analytical data were obtained using chromatographically homogeneous samples.

5.2. 2,5-Anhydro-6-azido-3,4-di-*O*-benzyl-6-deoxy-D-glucitol (7a)

To a solution of 1,2:5,6-dianhydro-3,4-di-*O*-benzyl-D-mannitol **B** (1.0 g, 3.06 mmol) in acetonitrile (15 mL) were successively added sodium azide (2.98 g, 45.8 mmol) and silica gel (3 g). After 48 h at reflux, the mixture was cooled and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/acetone, 97:3, R_f 0.3) yielded the azidomethyl-D-*gluco*-*C*-furanoside **7a** (1.07 g, 95%) as an oil. $[\alpha]_D^{20}$=+68 (*c* 0.9, $CHCl_3$). IR (neat) ν_{OH}=3440 cm^{-1}; ν_{N3}=2120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.37 (dd, $^2J_{6a,6b}$=12.8 Hz, $^3J_{6a,5}$=5.8 Hz, 1H, H-6a), 3.42 (dd, $^2J_{6b,6a}$=12.8 Hz, $^3J_{6b,5}$=5.1 Hz, 1H, H-6b), 3.84 (dd, $^2J_{1a,1b}$=12 Hz, $^3J_{1a,2}$=4.7 Hz, 1H, H-1a), 3.90 (dd, $^2J_{1b,1a}$=12 Hz, $^3J_{1b,2}$=5.5 Hz, 1H, H-1b), 3.95 (dd, $^3J_{4,3}$=1.8 Hz, $^3J_{4,5}$=3.5 Hz, 1H, H-4), 4.0–4.2 (m, 3H, H-5, H-3, H-2), 4.45, 4.59 (AB, $^2J_{AB}$=11.8 Hz, 2H, CH_2Ph), 4.52, 4.55 (AB, $^2J_{AB}$=11.9 Hz, 2H, CH_2Ph), 7.2–7.4 (m, 10H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 52.5 (C-6), 61.7 (C-1), 72.1 (OCH_2Ph), 80.9, 82.1, 83.6, 83.8 (C-2, C-3, C-4, C-5), 127.7, 128.2, 128.6, 128.7, 137.2, 137.4 (C_{arom}). Anal. calcd for

$C_{20}H_{23}N_3O_4$: C, 65.03; H, 6.28; N, 11.37; found: C, 64.95; H, 6.35; N, 11.53.

5.3. Hydroxyl group protection of 7a

5.3.1. 1-*O*-Acetyl-2,5-anhydro-6-azido-3,4-di-*O*-benzyl-6-deoxy-D-glucitol (7b). To a solution of **7a** (100 mg, 0.271 mmol) in anhydrous CH_2Cl_2 (2 mL) was added successively 4-dimethylaminopyridine (233 mg, 1.91 mmol) and acetic anhydride (177.6 μL, 1.88 mmol). After stirring 2 h at room temperature, the mixture was hydrolyzed, extracted with CH_2Cl_2 and dried ($MgSO_4$). After evaporation to dryness and purification by column chromatography (cyclohexane/EtOAc, 8:2, R_f 0.2) compound **7b** (102 mg, 91%) was obtained as an oil. $[\alpha]_D^{20}=+67$ (*c* 1.4, CH_2Cl_2). IR (neat) $\nu_{N3}=2100\ cm^{-1}$, $\nu_{CO}=1740\ cm^{-1}$; ^1H NMR ($CDCl_3$) δ 2.05 (s, 3H, $COCH_3$), 3.37 (d, $^3J_{6,5}=5.8$ Hz, 2H, H-6), 3.90 (dd, $^3J_{4,3}=1.2$ Hz, $^3J_{4,5}=2.9$ Hz, 1H, H4), 3.98 (dd, $^3J_{3,2}=3.5$ Hz, $^3J_{3,4}=1.2$ Hz, 1H, H-3), 4.07 (dt, $^3J_{5,6a}=^3J_{5,6b}=5.8$ Hz, $^3J_{5,4}=2.9$ Hz, 1H, H-5), 4.2–4.3 (m, 2H, H-2, H-1a), 4.41 (dd, $^2J_{1b,1a}=14.7$ Hz, $^3J_{1b,2}=7.3$ Hz, 1H, H-1b), 4.45 (s, 2H, CH_2Ph), 4.43, 4.56 (AB, $^2J_{AB}=11.9$ Hz, 2H, CH_2Ph), 7.2–7.4 (m, 10H, H_{arom}); CIMS (NH_3) *m/z*: 429 (M^++18).

5.3.2. 2,5-Anhydro-6-azido-3,4-di-*O*-benzyl-6-deoxy-1-*O*-*tert*-butyldimethylsilyl-D-glucitol (7c). To a solution of **7a** (470 mg, 1.27 mmol) in dimethylformamide (6 mL) was added successively imidazole (340 mg, 2.54 mmol) and *tert*-butyldimethylsilyl chloride (385 mg, 2.54 mmol). After stirring 15 h at room temperature, the mixture was hydrolyzed with brine, extracted with CH_2Cl_2 and dried ($MgSO_4$). After evaporation to dryness and purification by column chromatography (cyclohexane/EtOAc, 97:3, R_f 0.3) compound **7c** (584 mg, 95%) was obtained as an oil. $[\alpha]_D^{20}=+55$ (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 0.1 (s, 6H, $Si(CH_3)_2$), 0.9 (s, 9H, $Si(CH_3)_3$), 3.32 (dd, $^2J_{6a,6b}=12.6$ Hz, $^3J_{6a,5}=5.6$ Hz, 1H, H-6a), 3.35 (dd, $^2J_{6b,6a}=12.6$ Hz, $^3J_{6b,5}=6.2$ Hz, 1H, H-6b), 3.81 (dd, $^2J_{1a,1b}=10.2$ Hz, $^3J_{1a,2}=5.5$ Hz, 1H, H-1a), 3.84 (m, 1H, H-4), 3.90 (dd, $^2J_{1b,1a}=10.2$ Hz, $^3J_{1b,2}=6.7$ Hz, 1H, H-1b), 3.95–4.05 (m, 2H, H-3, H-5), 4.09 (ddd, $^3J_{2,1b}=6.6$ Hz, $^3J_{2,1a}=5.6$ Hz, $^3J_{2,3}=3.9$ Hz, 1H, H-2), 4.44, 4.47 (AB, $^2J_{AB}=12$ Hz, 2H, CH_2Ph), 4.55 (s, 2H, CH_2Ph), 7.2–7.4 (m, 10H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ −5.5 ($SiCH_3$), 18.1 ($SiC(CH_3)_3$), 25.7 ($SiC(CH_3)_3$), 52.5 (C-6), 60.7 (C-1), 71.4, 71.9 (OCH_2Ph), 81.8, 82.0, 83.9, 84.0 (C-2, C-3, C-4, C-5), 127.4, 127.6, 127.7, 128.2, 137.4 (C_{arom}); CIMS (NH_3) *m/z*: 501 (M^++18, 100%), 484 (M^++1, 30%).

5.4. General procedure for azido group reduction

To a suspension of Pd black (38 mg) in EtOAc (1 mL), was added the compound (0.12 mmol) to be reduced in EtOAc (1 mL). After stirring 1 h under hydrogen (1 atm) at room temperature, the catalyst was filtered through a celite pad and the solution was concentrated in vacuo. When acetic acid was used instead of EtOAc, the benzyl ethers were hydrogenolyzed.

5.4.1. 6-Amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-glucitol (8a). Obtained with 94% yield after purification by column chromatography (CH_2Cl_2/MeOH, 9:1 then 8:2, R_f 0.3). $[\alpha]_D^{20}=+38$ (*c* 0.98, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 2.0–2.3 (brs, 3H, NH_2, OH exchangeable), 2.82 (dd, $^2J_{6a,6b}=13.4$ Hz, $^3J_{6a,5}=5.6$ Hz, 1H, H-6a), 2.93 (dd, $^2J_{6b,6a}=13.4$ Hz, $^3J_{6b,5}=3.9$ Hz, 1H, H-6b), 3.8–3.9 (m, 3H), 3.95 (dd, $^3J=4.8$, 2.3 Hz, 1H), 4.05–4.15 (m, 2H) 4.48, 4.60 (AB, $^2J_{AB}=11.9$ Hz, 2H, CH_2Ph), 4.54, 4.60 (AB, $^2J_{AB}=12.1$ Hz, 2H, CH_2Ph), 7.2–7.4 (m, 10H, H_{arom}).

5.4.2. 1-*O*-Acetyl-6-amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-glucitol (8b). Obtained with 65% yield after purification by column chromatography (CH_2Cl_2/MeOH, 97:3, R_f 0.3). $[\alpha]_D^{20}=+6$ (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.7–2.0 (brs, 2H, NH_2 exchangeable), 2.05 (s, 3H, $COCH_3$), 2.75–3.0 (m, 2H, H-6), 3.8–4.7 (m, 10H, H-1, H-2, H-3, H-4, H-5, CH_2Ph), 7.1–7.4 (m, 10H, H_{arom}).

5.4.3. 6-Amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-1-*O*-*tert*-butyldimethylsilyl-D-glucitol (8c). Obtained with 95% yield after purification by column chromatography (CH_2Cl_2/MeOH, 97:3, R_f 0.3). $[\alpha]_D^{20}=+55$ (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 0.07 (s, 6H, $Si(CH_3)_2$), 0.9 (s, 9H, $Si(CH_3)_3$) 2.85 (m, 2H, H-6), 3.75–4.05 (m, 6H, H-1, H-2, H-3, H-4, H-5), 4.44, 4.48 (AB, $^2J_{AB}=12$ Hz, 2H, CH_2Ph), 4.54, 4.56 (AB, $^2J_{AB}=12.2$ Hz, 2H, CH_2Ph), 7.2–7.4 (m, 10H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ −5.5 ($SiCH_3$), 18.6 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 31.9 (C-6), 60.9 (C-1), 71.7, 72.0 (OCH_2Ph), 81.8, 82.2, 83.6, 84.7 (C-2, C-3, C-4, C-5), 126.5, 127.6, 127.7, 128.4, 129.5, 137.7, 138.0 (C_{arom}); MS (CI, NH_3) 458 (M^++1).

5.4.4. 6-Amino-2,5-anhydro-6-deoxy-D-glucitol (9). Obtained with 90% yield after purification by column chromatography (CH_2Cl_2/MeOH/H_2O/AcOH, 7:3:0.6:0.3, R_f 0.35). $[\alpha]_D^{20}=+25$ (*c* 1.0, H_2O). ^1H NMR (D_2O) δ 3.15 (dd, $^2J_{6a,6b}=13$ Hz, $^3J_{6a,5}=3.5$ Hz, 1H, H-6a), 3.24 (dd, $^2J_{6b,6a}=13$ Hz, $^3J_{6b,5}=6.5$ Hz, 1H, H-6b), 3.85 (dd, $^2J_{1a,1b}=12$ Hz, $^3J_{1a,2}=4$ Hz, 1H, H-1a), 3.94 (dd, $^2J_{1b,1a}=12$ Hz, $^3J_{1b,2}=7.5$ Hz, 1H, H-1b), 4.0 (dd, $^3J_{5,6a}=3.5$ Hz, $^3J_{5,6b}=6.5$ Hz, 1H, H-5), 4.1–4.3 (m, 3H, H-2, H-3, H-4); ^{13}C NMR (D_2O) δ 44.5 (C-6), 63.0 (C-1), 79.0, 81.7, 84.0, 84.3 (C-2, C-3, C-4, C-5); MS (CI, NH_3) 164 (M^++1).

5.5. Preparation of pseudo-azadisaccharides 3 and 4

5.5.1. *N*-[6′-(2′,5′-Anhydro-3′,4′-di-*O*-benzyl-6′-deoxy-1′-*O*-*tert*-butyldimethylsilyl-D-glucitol)]-3,4-di-*O*-benzyl-1,6-dideoxy-1,6-imino-D-mannitol (10) and *N*-[6′-(2′,5′-anhydro-3′,4′-di-*O*-benzyl-6′-deoxy-1′-*O*-*tert*-butyldimethylsilyl-D-glucitol)]-3,4-di-*O*-benzyl-1,5-dideoxy-1,5-imino-L-gulitol (11). To a solution of **8c** (165 mg, 0.361 mmol) in methanol (2 mL) was added bis-epoxide **B** (118 mg, 0.361 mmol). After stirring 8 days at room temperature, evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH, 97:3) compounds **10** (85 mg, 40% yield, R_f 0.35) and **11** (113 mg, 30% yield, R_f 0.3) were obtained.

Compound **10**. $[\alpha]_D^{20}=+13$ (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 0.06 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $Si(CH_3)_3$), 2.77 (dd, $^2J=11.2$ Hz, $^3J=6$ Hz, 4H), 2.93 (dd, $^2J=13.4$ Hz, $^3J=3.5$ Hz, 2H), 3.7–4.0 (m, 10H, H-1′, H-2′, H-3′, H-4′,

H-5′, H-2, H-3), 4.42 (s, 2H, CH_2Ph), 4.55 (m, 2H, CH_2Ph), 4.62, 4.75 (AB, $^2J_{AB}$=11.6 Hz, 4H, CH_2Ph), 7.1–7.4 (m, 20H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ −5.4 ($SiCH_3$), 18.2 ($SiC(CH_3)_3$), 25.9 ($SiC(CH_3)_3$), 57.1 (C-1), 60.6, 62.0 (C-1′, C-6′), 68.6 (C-2), 71.7, 72.2, 73.4 (OCH_2Ph), 80.7, 81.7, 81.8, 85.6 (C-3, C-2′, C-3′, C-4′, C-5′), 127.7, 127.9, 128.4, 137.7, 138.0, 138.4 (C_{arom}); MS (CI, NH_3) 784 (M^++1).

Compound **11**. $[\alpha]_D^{20}$=+8 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 0.04 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $Si(CH_3)_3$), 2.83 (m, 2H, H-1) 2.91 (dd, $^2J_{6'a,6'b}$=14.1 Hz, $^3J_{6'a,5'}$= 6.5 Hz, 1H, H-6′a), 3.11 (dd, $^2J_{6'b,6'a}$=14.1 Hz, $^3J_{6'b,5'}$= 5.6 Hz, 1H, H-6′b), 3.22 (dt, $^3J_{5,6a}$=$^3J_{5,6b}$=7.7 Hz, $^3J_{5,4}$=5.6 Hz, 1H, H-5), 3.5–3.65 (m, 2H, H-3, H-6), 3.7–4.05 (m, 9H, H-1′, H-2′, H-3′, H-4′, H-5′, H-2, H-4, H-6), 4.42 (s, 2H, CH_2Ph), 4.55 (s, 2H, CH_2Ph), 4.58, 4.64 (AB, $^2J_{AB}$=11.7 Hz, 2H, CH_2Ph), 4.63, 4.68 (AB, $^2J_{AB}$=11.8 Hz, 2H, CH_2Ph), 7.2–7.4 (m, 20H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ −5.4 ($SiCH_3$), 18.3 ($SiC(CH_3)_3$), 25.9 ($SiC(CH_3)_3$), 50.3 (C-1), 57.8, 60.7 (C-6, C-6′, C-1′), 61.5 (C-5), 67.7 (C-2), 71.6, 72.1, 72.4, 73.2 (OCH_2Ph), 75.5, 78.2 (C-3, C-4), 81.9, 82.8, 85.7 (C-2′, C-3′, C-4′, C-5′), 127.7, 128.4, 137.7, 138.1 (C_{arom}); MS (CI, NH_3) 784 (M^++1).

5.5.2. General procedure for desilylation. Silyl ether **10** or **11** (0.13 mmol) was stirred during 10 h at room temperature with a solution of tetrabutylammonium fluoride (1.5 equiv.) in THF (2 mL, 1 M). After evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH, 96:4), compound **12** (85% yield, R_f 0.35) or **13** (80% yield, R_f 0.4) was obtained.

5.5.3. *N*-[6′-(2′,5′-Anhydro-3′,4′-di-*O*-benzyl-6′-deoxy-D-glucitol)]-3,4-di-*O*-benzyl-1,6-dideoxy-1,6-imino-D-mannitol (12). $[\alpha]_D^{20}$=+9 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 2.7–3.25 (m, 6H, H-1, H-6′), 3.7–4.2 (m, 10H, H-2, H-3, H-1′, H-2′, H-3′, H-4′, H-5′), 4.4–4.8 (m, 8H, CH_2Ph), 7.1–7.4 (m, 20H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 57.8 (C-1), 61.6, 62.0 (C-6′, C-1′), 61.9 (C-2), 71.8, 71.9, 73.5 (OCH_2Ph), 80.6, 81.8, 83.4, 85.1 (C-2′, C-3′, C-4′, C-5′, C-3), 127.6, 127.8, 128.2, 128.4, 137.3, 137.5, 138.4 (C_{arom}); MS (CI, NH_3) 670 (M^++1).

5.5.4. *N*-[6′-(2′,5′-Anhydro-3′,4′-di-*O*-benzyl-6′-deoxy-D-glucitol)]-3,4-di-*O*-benzyl-1,5-dideoxy-1,5-imino-L-gulitol (13). $[\alpha]_D^{20}$=+5 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 2.83 (m, 2H, H-1), 2.91 (dd, $^2J_{6'a,6'b}$=14.3 Hz, $^3J_{6'a,5'}$= 6.5 Hz, 1H, H-6′a), 3.1–3.25 (m, 2H, H-6′b, H-5), 3.5–3.65 (m, 2H, H-3, H-6a), 3.7–4.15 (m, 9H, H-1′, H-2′, H-3′, H-4′, H-5′, H-2, H-4, H-6b), 4.40–4.75 (m, 8H, CH_2Ph), 7.15–7.4 (m, 20H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 50.5 (C-1), 57.6, 58.2 (C-6′, C-6), 61.9 (C-5), 67.7 (C-2), 71.9, 72.0, 72.5, 73.1 (OCH_2Ph), 75.5, 78.0 (C-3, C-4), 80.5, 82.3, 83.7, 84.7 (C-2′, C-3′, C-4′, C-5′), 127.8, 128.5, 137.4, 137.5, 138.1 (C_{arom}); MS (CI, NH_3) 670 (M^++1); HRMS for $C_{40}H_{48}O_8N$ calcd 670.3379; found 670.3372.

5.5.5. General procedure for benzyl deprotection. To a suspension of Pd black in acetic acid was added the compound **12** or **13** to be reduced in acetic acid (w/w). After stirring 3 days under hydrogen (1 atm) at room temperature, the catalyst was filtered through a celite pad and the solution was concentrated in vacuo. Purification was performed by flash chromatography (CH_2Cl_2/MeOH/H_2O/AcOH, 7:3:0.6:0.3, R_f 0.35, 70% yield) for compound **3** or (CH_2Cl_2/MeOH/H_2O/AcOH, 5:5:1:0.5, R_f 0.4, 75% yield) for compound **4**.

5.5.6. *N*-[6′-(2′,5′-Anhydro-6′-deoxy-D-glucitol)]-1,6-dideoxy-1,6-imino-D-mannitol, acetate (3). $[\alpha]_D^{20}$=+2 (*c* 1.0, D_2O), $[\alpha]_{Hg}^{20}$=+4. ^1H NMR (D_2O) δ 2.18 (s, 3H, CH_3), 2.37 (d, *J*=11.3 Hz, 1H), 2.42 (dd, *J*=11.7, 2.1 Hz, 1H), 2.55–2.75 (m, 2H), 2.98 (brd, *J*=10.8 Hz, 1H), 3.10 (d, *J*=11.1 Hz, 1H), 3.7–4.0 (m, 6H, containing at 3.77 (dd, ^2J=11.9 Hz, ^3J=6.8 Hz, 1H)), 4.1–4.2 (m, 2H), 4.2–4.3 (m, 2H). ^{13}C NMR (D_2O) δ 26.2 (CH_3), 54.4, 59.1, 61.9, 63.9 (CH_2), 79.9, 82.4, 83.2, 83.5, 83.6, 84.0, 87.3 (CH); MS (FAB) 309 (M^++1).

5.5.7. *N*-[6′-(2′,5′-Anhydro-6′-deoxy-D-glucitol)]-1,5-dideoxy-1,5-imino-L-gulitol, acetate (4). $[\alpha]_D^{20}$=+5 (*c* 1.0, D_2O). ^1H NMR (D_2O) δ 1.98 (s, 3H, CH_3), 3.28 (m, 1H), 3.4–4.4 (m, 15H), containing at 3.64 (dd, ^2J=14.3 Hz, ^3J=7.8 Hz, 1H)); MS (FAB) 309 (M^++1).

5.6. Nucleophilic opening of the bis-epoxide by piperidine

5.6.1. 1,6-Dideoxy-1,6-di-*N*-piperidino-3,4-*O*-methylethylidene-D-mannitol (14). D-*manno*-Bis-epoxide **A** (0.16 mmol) in 2 mL of piperidine was stirred during 4 days at room temperature. After evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH/ NH_3, 8:2:0.3, R_f 0.5) compound **14** was obtained with 90% yield. $[\alpha]_D^{20}$=+24 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.2–1.7 (m, 18H, containing at 1.31 (s, $C(CH_3)_2$), CH_2–CH_2–CH_2N), 2.2–2.7 (m, 12H, H-1, CH_2–CH_2N), 3.6–4.2 (m, 6H, H-2, H-3, OH); MS (CI, NH_3) 357 (M^++1).

5.6.2. 3,4-Di-*O*-benzyl-1,6-dideoxy-1,6-di-*N*-piperidino-D-mannitol (15). D-*manno*-Bis-epoxide **B** (0.16 mmol) in 2 mL of piperidine was stirred during 4 days at room temperature. After evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH/NH_3, 95:5:0.15, R_f 0.35) compound **15** was obtained with 80% yield. $[\alpha]_D^{20}$=+10.5 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.3–1.7 (m, 12H, CH_2–CH_2–CH_2N), 2.2–2.7 (m, 12H, H-1, CH_2–CH_2N), 3.69 (d, $^3J_{3,2}$=6.1 Hz, 2H, H-3), 3.8–4.2 (m, 4H, H-2, OH), 4.66, 4.75 (AB, $^2J_{AB}$=11.4 Hz, 4H, CH_2Ph), 7.15–7.4 (m, 10H, H_{arom}); MS (CI, NH_3) 497 (M^++1).

5.6.3. 2,5-Anhydro-3,4-di-*O*-benzyl-6-deoxy-6-*N*-piperidino-D-glucitol (16). A solution of D-*manno*-bis-epoxide **B** (32.6 mg, 0.1 mmol) and piperidine (7 μL, 2 equiv.) in acetonitrile (0.2 mL) was refluxed for 2 h. After concentration in vacuo and purification by column chromatography (CH_2Cl_2/MeOH/NH_3, 98:2:1, R_f 0.3), compound **16** was obtained with 85% yield. ^1H NMR ($CDCl_3$) δ 1.2–1.7 (m, 6H, CH_2–CH_2–CH_2N), 2.2–2.7 (m, 6H, CH_2N, H-6), 3.7–4.4 (m, 6H, H-1, H-2, H-3, H-4, H-5) 4.5–4.7 (m, 4H, CH_2Ph); ^{13}C NMR ($CDCl_3$) δ 23.9 (N–$(CH_2)_2$–CH_2), 25.7 (N–CH_2–CH_2), 55.9 (N–CH_2–CH_2), 60.8, 61.6 (C-6,

C-1), 71.8, 71.2 (OCH_2Ph), 79.8, 80.4, 84.0, 84.1 (C-2, C-3, C-4, C-5), 127.4, 127.8, 128.1, 128.4, 137.6, 138.0 (C_{arom}); MS (CI, NH_3) 412 (M^++1).

5.7. Preparation of pseudo-azatrisaccharides 5 and 6

5.7.1. General procedure for nucleophilic opening of A. To a solution of bis-epoxide **A** (0.215 mmol) in methanol (1 mL) was added azepane **1** or piperidine **2** (2 equiv.), and the mixture was stirred during 12 h at room temperature. After evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH/NH_3, 8:3:1) compounds **17** or **18** were obtained in 70 or 95% yields, respectively.

5.7.1.1. 1,6-Dideoxy-1,6-di-*N*-(1′,6′-dideoxy-1′,6′-imino-D-mannitol)-3,4-*O*-methylethylidene-D-mannitol (17). $[\alpha]_D^{20}$= +22 (*c* 1.0, H_2O). ^1H NMR (D_2O) δ 1.49 (s, 6H, $C(CH_3)_2$), 2.6–3.15 (m, 12H, containing at 2.71 (dd, ^2J=13.4 Hz, ^3J=9.4 Hz, 2H) and at 2.87 (dd, ^2J=13.6 Hz, ^3J=6.8 Hz, 4H), H-1, H-1′), 3.9–4.25 (m, 12H, H-2, H-3, H-2′, H-3′); ^{13}C NMR (D_2O) δ 29.1 (CH_3), 59.4 (C-1′), 63.9 (C-1), 71.9, 72.2, 75.4 (C-2, C-2′, C-3′), 82.9 (C-3), 113.5 ($C(CH_3)_2$); MS (CI, NH_3) 513 (M^++1).

5.7.1.2. 1,6-Dideoxy-1,6-di-*N*-(1′,5′-dideoxy-1′,5′-imino-L-gulitol)-3,4-*O*-methylethylidene-D-mannitol (18). $[\alpha]_D^{20}$= +38 (*c* 1.0, D_2O). ^1H NMR (D_2O) δ 1.48 (s, 6H, $C(CH_3)_2$), 2.7–3.1 (m, 10H, containing at 2.75 (dd, ^2J=12.1 Hz, ^3J=8.2 Hz, 2H) and at 2.88 (dd, ^2J=13.9 Hz, ^3J=8.3 Hz, 2H), H-1, H-1′, H-5′), 3.8–4.15 (m, 14H, H-2, H-3, H-2′, H-3′, H-4′, H-6′); ^{13}C NMR (D_2O) δ 29.1 (CH_3), 54.5, 58.2, 61.5 (C-1, C-1′, C-6′), 63.4, 69.5, 71.5, 73.0 (C-2, C-2′, C-3′, C-4′, C-5′), 83.2 (C-3), 113.4 ($C(CH_3)_2$); MS (CI, NH_3) 513 (M^++1).

5.7.2. General procedure for acetonide hydrolysis. Compounds **17** or **18** were stirred during 12 h in an aqueous solution of trifluoroacetic acid (1/4 v/v) at room temperature. After evaporation to dryness and purification by column chromatography (CH_2Cl_2/MeOH/NH_3, 8:7:2, compounds **5** (R_f 0.2) or **6** (R_f 0.15) were obtained with 70 or 80% yields, respectively.

5.7.2.1. 1,6-Dideoxy-1,6-di-*N*-(1′,6′-dideoxy-1′,6′-imino-D-mannitol)-D-mannitol (5). $[\alpha]_D^{20}$=+6 (*c* 1.0, D_2O). ^1H NMR (D_2O) δ 2.80 (dd, ^2J=13 Hz, ^3J=8.4 Hz, 2H), 2.95–3.15 (m, 10H), 3.85–4.05 (m, 6H, containing at 3.98 (brs, 4H)), 4.19 (m, 4H); ^{13}C NMR (D_2O) δ 59.5 (C-1′), 65.0 (C-1), 70.3, 74.8 (C-2, C-3), 71.7, 75.4 (C-2′, C-3′);. MS (CI, NH_3) 473 (M^++1).

5.7.2.2. 1,6-Dideoxy-1,6-di-*N*-(1′,5′-dideoxy-1′,5′-imino-L-gulitol)-D-mannitol (6). $[\alpha]_D^{20}$=+28 (*c* 1.0, D_2O). ^1H NMR (D_2O) δ 2.82 (dd, ^2J=12.3 Hz, ^3J=7.7 Hz, 2H), 2.9–3.1 (m, 8H), 3.75–4.05 (m, 10H), 4.05–4.15 (m, 4H); ^{13}C NMR (D_2O) δ 56.9, 57.8, 62.8 (C-1, C-1′, C-6′), 60.8 (C-5′), 64.9, 68.1, 68.4, 72.0, 72.8 (C-2, C-3, C-2′, C-3′, C-4′); MS (CI, NH_3) 473 (M^++1).

5.8. Inhibition studies

All enzymes, α-D-glucosidase from *Bacillus stearothermophilus* (EC 3.2.1.20), β-D-glucosidase from almonds (EC 3.2.1.21), α-D-mannosidase from jack beans (EC 3.2.1.24) and α-L-fucosidase from bovine kidney (EC 3.2.1.51) and all substrates were purchased from Sigma.

Inhibition studies were displayed as previously reported.[20] Briefly, assays were run in 50 mM citrate-phosphate buffer, pH 6.5, at 37°C, using the corresponding *p*-nitrophenyl-α-glycosides in a total volume of 0.2 mL. The amount of enzyme in each assay was adjusted so that less than 10% of the substrate would be consumed.

For K_i determinations, substrates were at a concentration range of 0.2–5 K_M and inhibitors were added to final concentration between 10^{-3} and 10^{-8} M. The inhibition studies were performed by prewarming the solutions 5 min prior to the addition of enzyme to initiate the reaction. Each enzymatic reaction was quenched with 3 mL of 0.2 M Na_2CO_3 after an incubation time of 5 min. The concentration of liberated *p*-nitrophenoxide was determined by measuring the optical absorbance at 400 nm. Dissociation constants for inhibitors were calculated in the absence or presence of inhibitors according to the Lineweaver–Burk method.

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