A Short and Flexible Route to Aza-β-(1→6)-*C*-disaccharides: Selective α-Glycosidase Inhibitors

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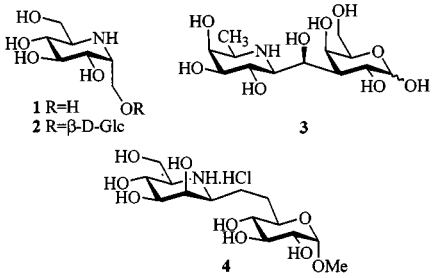
The syntheses of azaMan-β-(1→6)-*C*-Glc (**4**), azaGlc-β-(1→6)-*C*-Glc (**5**), and azaGal-β-(1→6)-*C*-Glc (**6**) based upon double reductive amination of acetylenic carbohydrate-derived diketones is described. The required diketones are obtained by addition of the acetylenic sugar anion derived from dibromoolefin **7** to benzyl-protected mannopyranolactone, glucopyranolactone, or galactopyranolactone, followed by reduction of the ketose and oxidation of the resulting diol. Ensuing double reductive amination and hydrogenolysis affords the target compounds in reasonable to good yields. Enzyme inhibition tests show that neither of the three compounds **4**, **5**, and **6** inhibit β-glycosidases, while moderate to good inhibitory activities were found on α-glycosidases, the most active being **6** (α-galactosidase: K_i = 0.092 μM).

Introduction

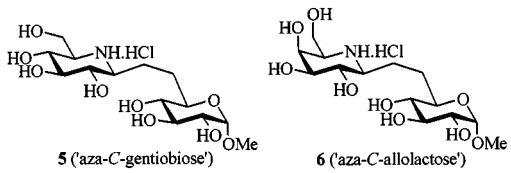
Azasugars (polyhydroxylated pyrrolidines and piperidines) represent an interesting class of glycosidase inhibitors and are widely recognised as potential therapeutics for the treatment of inter alia diabetes,[1] cancer,[2] and viral infections.[3] The biological activity of these glycomimetics may be explained, apart from their conformational resemblance to natural sugars, by the protonation of the ring nitrogen at physiological pH, mimicking the developing charge of an intermediate oxocarbonium ion during glycosidic bond cleavage.[4]

Aza-*C*-glycosides possess an additional carbon substituent at the pseudo-anomeric center, which is thought to mimic the aglycon part of an oligosaccharide. In this respect, it has been stated[5] that glycosidase inhibitors which permit interaction with the aglycon binding site are much more potent than azasugars lacking this feature. For example, α-homonojirimycin (**1**) shows stronger and more selective inhibition of α- versus β-*exo*-glycosidases than, for example, 1-deoxynojirimycin.[6]



An extension of the latter type of glycomimetics is represented by aza-*C*-disaccharides, in which an azasugar is linked to a second monosaccharide unit by a *C*-glycosidic bond. Several examples of aza-*C*-disaccharides showing significant biological activities have been reported recently (e.g. **2**[7] and **3**[8]). Nevertheless, the only examples of true aza-*C*-analogues of parent disaccharides are those reported by Johnson et al. (e.g. **4**).[9] However, these analogues were restricted to aza-*manno* configurations. In order to gain more insight into the mechanism of glycosidase inhibition and structure-activity relationship, it is of major importance to have access to a large variety of aza-*C*-analogues of natural disaccharides.

As part of an ongoing study on the design and preparation of oligosaccharide analogues,[10] we here report the synthesis of known[9] aza-*C*-mannoside **4** as well as the new aza-*C*-analogues (**5** and **6**) of gentiobiose and allolactose.



Results and Discussion

Recently, Reitz et al. reported a synthetic route to azasugars employing reductive amination of dicarbonyl sugars.[11] According to this procedure, a wide range of azasugar derivatives including β-homonojirimycin[12] and castanospermine[13] have been constructed. It occurred to us that this reductive amination protocol could be adopted for the construction of the target aza-β-(1→6)-*C*-disaccharides **4**, **5**, and **6**. In order to assess the viability of this concept, the preparation of **4** was undertaken (see Scheme 1). The required diketone **10a** was synthesised by performing the following sequence of reactions. Conversion of known[14]

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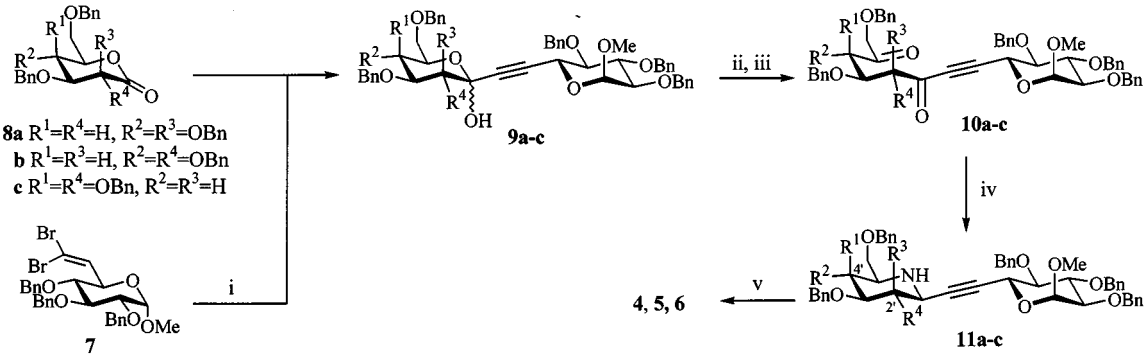

Scheme 1. Synthesis of aza-β-(1→6)-*C*-disaccharides **4**, **5**, and **6**. Reagents and conditions: (i) *n*BuLi, THF, −50 °C, then **8a−c**, (90% **9a**, 93% **9b**, 88% **9c**); (ii) $NaBH_4$, $MeOH/CH_2Cl_2$; (iii) TFAA, DMSO, Et_3N, CH_2Cl_2, −70 °C to room temp. (64% **10a**, 61% **10b**, 66% **10c** over 2 steps); (iv) $NH4^+HCO2^-$, $NaCNBH_3$, mol. sieves (3 Å), $MeOH/CH_2Cl_2$ (61% **11a**, 63% **11b**, 58% **11c**); (v) H_2, Pd/C, EtOAc/EtOH/1 M HCl (88% **4**, 80% **5**, 85% **6**).

dibromoolefin **7** with an excess of *n*-butyllithium at −50 °C into the corresponding acetylenic anion was followed by the addition of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranolactone (**8a**)[15] to give ketose **9a** in 90% yield. Reduction of **9a** with sodium borohydride followed by oxidation (DMSO-trifluoroacetic anhydride)[12][16] of the newly generated hydroxyl groups afforded diketone **10a**. Double reductive amination under the influence of ammonium formate and sodium cyanoborohydride[12] yielded fully protected aza-*C*-disaccharide **11a** as a single isomer, the β-D-*manno* configuration of which was firmly established by NMR spectroscopy (e.g. $J_{1',2'} < 1$ Hz, $J_{4',5'} = 9.8$ Hz). Hydrogenolysis of the benzyl groups and concomitant reduction of the triple bond in **11a** with hydrogen and Pd on carbon led to the isolation of the known[9] azaMan-β-*C*-(1→6)-D-Glc **4**.

The syntheses of aza-*C*-glucoside **5** and aza-*C*-galactoside **6** were accomplished in a similar fashion. Thus, the acetylenic anion derived from **7** was treated in situ with lactones **8b** and **8c**,[15] to give the respective *gluco*- and *galacto*-ketoses **9b** and **9c**, in excellent yields. The ensuing reduction/oxidation sequence, as mentioned for the conversion of **9a** into **10a**, gave the expected diketones **10b** and **10c** in comparable yields. Double reductive amination of **10b** and **10c** afforded the corresponding aza-*C*-disaccharides **11b** and **11c**. The magnitudes of $J_{1',2'}$ and $J_{4',5'}$ confirmed the β-D-configurations of the disaccharides. It is of interest to note that reductive amination of the diketones **10a−c** in all three cases led to the isolation of β-D-azasugars in comparable yields, indicating that the orientation at either the 2′ or 4′ position of the diketone moiety does not influence the stereochemical outcome of the double reductive amination step. Subsequent debenzylation and reduction of the triple bond in **11b** and **11c** yielded azaGlc-β-*C*-(1→6)-Glc (**5**) and azaGal-β-*C*-(1→6)-Glc (**6**), respectively.

The inhibitory potential of aza-*C*-disaccharides **4**, **5**, and **6** on a range of glycosidases is summarised in Table 1. Johnson and co-workers[9] already demonstrated that **4** inhibits neither common α- nor β-mannosidases. This result is further endorsed by the finding[20] that the same compound does not show any activity towards α-mannosidase (jack beans and almonds) and β-mannosidase from *Helix pomatia*. AzaMan-β-*C*-Glc **4** did show activity towards amyloglucosidase, an α-glucosidase that is also inhibited by a range of other aza-*C*-mannosides.[9] The latter observation, together with the inhibitory activities of (+)-lentiginosine and its analogues,[17] indicates that this enzyme is not very demanding in terms of steric requirements of the inhibitor. Surprisingly, rice α-glucosidase is inhibited by azaGlc-β-*C*-Glc **5** (see Table 1, entry 6), whereas β-glucosidase from almonds (entry 10) was totally unaffected. This inhibitory action deviates from that observed for β-homonojirimycin,[18] which inhibits both α-glucosidase (yeast, $K_i = 900$ μM) and β-glucosidase (almonds, $K_i = 430$ μM). Similarly, azaGal-β-*C*-Glc **6** shows strong inhibition of α-galactosidases from coffee beans and *E. coli* (see entries 1 and 3). These values are comparable to those reported for 1-deoxygalactostatin ($K_i = 0.0016$ and 0.24 μM for the aforementioned enzymes),[19] indicating that the aglycon moiety in **6** does not have a profound influence on the magnitude of inhibition. On the other hand, compound **6** is a very weak inhibitor of β-galactosidases (13−38% inhibition at 1 mM), while 1-deoxygalactostatin does inhibit β-galactosidases significantly (K_i′s vary from 0.16 to 12.5 μM). The enzyme specificity of **6** is further demonstrated by the fact that other glycosidases,[20] with the exception of α-*N*-acetylgalactosaminidase (entry 11), were virtually unaffected by compound **6**.

Conclusion

In conclusion, we have described a straightforward route towards the synthesis of aza-β-(1→6)-*C*-disaccharides, which offers flexibility in the incorporation of the azasugar moiety by varying the sugar lactone starting compound. To the best of our knowledge, the novel "aza-*C*-gentiobiose" **5** and "aza-*C*-allolactose" **6** are the first examples of aza-*C*-analogues of natural disaccharides. Furthermore, it has been shown that these compounds (in contrast to **4**) are selective inhibitors of α-glycosidases, azaGal-Glc **6** being the most potent. This remarkable α-glycosidase selectivity is probably due to the presence of an additional bulky aglycon

Table 1. Enzyme inhibitory activities of azaMan-Glc **4**, azaGlc-Glc **5**, and azaGal-Glc **6**

Entry	**4**		**5**		**6**	
	IC_{50} [a]	K_i [a]	IC_{50}	K_i	IC_{50}	K_i
α-galactosidase						
1. coffee beans	n.i.	n.d.	n.i.	n.d.	0.77	0.092
2. *Aspergillus niger*	n.i.	n.d.	n.i.	n.d.	10	2
3. *Escherichia coli*	228	630[b]	310	200[b]	0.205	0.22[b]
β-galactosidase						
4. *Aspergillus niger*	n.i.	n.d.	n.i.	n.d.	n.i.	n.d.
α-glucosidase (maltase)						
5. yeast	n.i.	n.d.	500	n.d.	n.i.	n.d.
6. rice	n.i.	n.d.	85	107	n.i.	n.d.
α-glucosidase (isomaltase)						
7. baker's yeast	n.i.	n.d.	500	n.d.	n.i.	n.d.
amyloglucosidase						
8. *Aspergillus niger*	41	21	5	5	n.i.	n.d.
9. *Rhizopus* mold	17	10	5	4	n.i.	n.d.
β-glucosidase						
10. almonds	n.i.	n.d.	n.i.	n.d.	n.i.	n.d.
α-*N*-Ac-galactosaminidase						
11. chicken liver	n.i.	n.d.	n.i.	n.d.	16	9.8

[a] Values are given in μM, n.i.: less than 50% or no inhibition at 1 mM, n.d.: not determined. – [b] Non-competitive inhibitor.

moiety. More insight into the mechanism of this selectivity may be attained by varying the aglycon moiety. The latter aspect is presently under investigation and will be reported in due course.

Experimental Section

^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol JNM-FX-200 (200 and 50 MHz, respectively). ^{1}H-^{1}H-COSY and ^{13}C-^{1}H-COSY spectra were recorded on a Bruker DPX-300 (300 and 75 MHz). NMR shifts are reported in ppm (δ) relative to tetramethylsilane. – Optical rotations were determined at 20 °C by means of a Propol polarimeter. – Mass spectrometry was performed on a PE/SCIEX API 165 equipped with an electrospray interface. – Elemental analysis was performed on a Perkin–Elmer CHNS Analyzer 2400. – Dichloromethane (Baker, p.a.), 1,2-dichloroethane (Rathburn, HPLC), and tetrahydrofuran (THF, Baker, p.a.) were stored over molecular sieves (4 Å). Methanol (Baker, p.a.) was stored over molecular sieves (3 Å). Dimethyl sulfoxide (DMSO) was refluxed with CaH_2 for 16 h, distilled, and stored over molecular sieves (4 Å). Triethylamine (TEA) was refluxed with CaH_2 for 4 h, distilled, and stored over CaH_2. *n*-Butyllithium (*n*BuLi, Aldrich), trifluoroacetic anhydride (TFAA, Aldrich), sodium borohydride, sodium cyanoborohydride, and ammonium formate (Acros) were used as received. – Column chromatography was performed on Baker silica gel (0.063–0.200 mm). Gel filtration chromatography was performed in an aqueous 0.15 M triethylammonium bicarbonate buffer on HW-40 S material (Toyopearl). TLC analysis was conducted on DC-Fertigfolien (Schleicher & Schuell, F1500, LS254), with detection by UV absorption (254 nm) where applicable and charring with 20% H_2SO_4 in ethanol. – Reactions were run at ambient temperature unless stated otherwise.

General Procedure for the Condensation of Dibromoolefin 7 with Sugar Lactones 8a–c: Dibromoolefin **7**[14] was dissolved in dry THF (5 mL/mmol) and cooled to −50 °C. Under a constant stream of nitrogen, *n*BuLi (1.6 M in hexanes, 2.5 equiv.) was added dropwise and the mixture was stirred for 0.5 h at −50 °C. Next, a solution of the lactone (2 equiv.) in THF was added and stirring at −50 °C was continued until TLC analysis (25% EtOAc/light petroleum) showed complete disappearance of the acetylenic component (0.5 to 1 h). The mixture was poured into sat. NH_4Cl and extracted with Et_2O. The organic layer was washed with sat. NH_4Cl, dried ($MgSO_4$), and concentrated. Silica gel chromatography (10 to 40% EtOAc/light petroleum) yielded the ketose as a colourless glass.

***manno*-Ketose 9a:** 1.77 g (1.78 mmol), 90% based upon 1.22 g (1.98 mmol) of **7**. – ^{13}C NMR (50 MHz, $CDCl_3$): 138.2–137.7, 128.3–127.2, 97.9, 92.5, 92.1, 85.0, 84.4, 81.4, 80.5, 78.9, 78.5, 77.9, 77.7, 75.5, 75.0, 74.6, 74.2, 73.0, 72.8, 71.9, 69.1, 61.3, 55.2. – $C_{63}H_{64}O_{11}$: calc. C 75.88; H 6.47; found: C 75.45; H 6.73.

***gluco*-Ketose 9b:** 1.52 g (1.53 mmol), 93% based upon 1.01 g (1.64 mmol) of **7**. – ^{13}C NMR (50 MHz, $CDCl_3$): 138.5–137.9, 128.3–127.2, 98.3, 95.4, 91.5, 85.2, 84.3, 84.0, 83.4, 82.2, 82.0, 81.8, 81.3, 81.1, 80.7, 79.1, 79.0, 77.3, 77.1, 75.7, 75.5, 75.3, 75.1, 74.9, 74.6, 73.9, 73.4, 73.2, 71.7, 68.3, 61.5, 55.7. – $C_{63}H_{64}O_{11}$: calc. C 75.88; H 6.47; found: C 76.13; H 6.66.

***galacto*-Ketose 9c:** 2.17 g (2.18 mmol), 88% based upon 1.53 g (2.47 mmol) of **7**. – ^{13}C NMR (50 MHz, $CDCl_3$): 138.3–137.7, 128.0–127.1, 97.9, 96.8, 91.8, 84.6, 81.6, 81.4, 80.9, 80.5, 80.1, 79.0, 78.9, 75.4, 74.7, 74.4, 73.0, 72.3, 70.2, 68.2, 67.4, 65.4, 61.3, 55.1. – $C_{63}H_{64}O_{11}$: calc. C 75.88; H 6.47; found: C 75.37; H 6.41.

General Procedure for the Conversion of Ketoses 9a–c into Diketones 10a–c: $NaBH_4$ (5 equiv.) was added to a solution of the ketose in CH_2Cl_2/MeOH (ca. 1:5 v/v). Stirring at room temperature for 0.5 to 1 h showed complete conversion of the starting compound into a lower running component (TLC: 50% EtOAc/light petroleum). Acetone was added, the solvents were removed in vacuo and the residue was separated between EtOAc and water. The aqueous layer was extracted twice with EtOAc and the combined organic layers were washed with sat. NH_4Cl. Drying ($MgSO_4$) and evaporation of the solvent yielded the crude diol, which was used without further purification. To a cooled (−78 °C) solution of DMSO (6 equiv. relative to the diol) in dry CH_2Cl_2 (5 mL per mmol of the diol) under nitrogen atmosphere was added a solution of TFAA (4.5 equiv. relative to the diol) in CH_2Cl_2 (3 mL per mmol diol). After stirring at −78 °C for 20 min, a solution of the diol (previously dried by coevaporation with dry DCE) in CH_2Cl_2 (2 mL per mmol) was added. Stirring at −78 °C was continued for 1.5 h and TEA was added (8 equiv.). The mixture was allowed to warm up to room temperature and after 15 min diluted with Et_2O. The organic layer was washed with water, sat. $NaHCO_3$ and brine, then dried ($MgSO_4$) and concentrated. Silica gel flash chromatography of the product mixture (toluene/EtOAc/MeOH, 19:0.9:0.1, containing 0.5% TEA) yielded the desired diketone as a colourless oil.

***manno*-Diketone 10a:** 1.13 g (1.13 mmol), 64% from **9a**. – ^{13}C NMR (50 MHz, $CDCl_3$): 207.7, 186.3, 138.4–136.6, 128.5–127.8, 98.5, 92.8, 83.3, 82.9, 81.2, 80.8, 79.9, 78.9, 75.9, 75.6, 74.4, 74.2, 73.6, 73.1, 72.5, 61.6, 55.8. – MS (ESI): calc. for $C_{63}H_{62}O_{11}$ 994.4, found *m/z* 995 [M + H].

***gluco*-Diketone 10b:** 0.93 g (0.93 mmol), 61% from **9b**. – ^{13}C NMR (50 MHz, $CDCl_3$): 206.0, 186.1, 138.3–136.5, 128.4–127.6, 98.3, 91.6, 82.8, 82.7, 81.0, 80.5, 80.3, 78.6, 75.5, 75.3, 74.1, 73.9, 73.4, 73.2, 72.8, 61.5, 55.5. – MS (ESI): calc. for $C_{63}H_{62}O_{11}$ 994.4, found *m/z* 995 [M + H], 1017 [M + Na].

***galacto*-Diketone 10c:** 1.43 g (1.44 mmol), 66% from **9c**. – ^{13}C NMR (50 MHz, $CDCl_3$): 206.4, 185.8, 138.2–136.4, 128.0–127.4, 98.1, 91.7, 84.0, 82.5, 81.5, 80.7, 80.5, 80.2, 79.4, 78.6, 75.4, 75.1, 74.2, 73.9, 73.1, 72.6, 72.2, 61.3, 55.4. – MS (ESI): calc. for $C_{63}H_{62}O_{11}$ 994.4, found *m/z* 995 [M + H], 1017 [M + Na].

General Procedure for the Double Reductive Amination of 10a−c to the Protected aza-*C*-Disaccharides 11a−c: To a solution of the diketone in dry CH_2Cl_2/MeOH (1:5, 10 mL/mmol) were added ammonium formate (3 equiv.), $NaCNBH_3$ (3 equiv.), and molecular sieves (3 Å, 1 g/mmol). The resulting suspension was stirred overnight at ambient temperature. TLC analysis (toluene/EtOAc/MeOH, 9:0.9:0.1, containing 1% TEA) indicated complete consumption of the starting compound and formation of one major lower running spot. The molecular sieves were filtered off and the mixture was extracted with EtOAc, followed by washing with sat. $NaHCO_3$ (2×). After extraction of the aqueous layer with EtOAc, the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. Silica gel chromatography (toluene/EtOAc/MeOH, 19:0.9:0.1 to 9:0.9:0.1, with 0.5% TEA) yielded the desired protected aza-*C*-alkynyl-disaccharides.

11a: Yellowish oil, 0.67 g (0.69 mmol), 61%. − ^{13}C NMR (75 MHz, $CDCl_3$): 138.7−138.0, 128.4−127.3, 98.3, 84.6, 84.2, 82.1, 81.0, 80.5, 79.2, 76.3, 76.0, 75.9, 75.5, 75.3, 74.2, 73.5, 73.1, 71.9, 68.9, 61.7, 59.6, 55.7, 50.7. − ^{1}H-COSY (300 MHz, C_6D_6): 7.56−7.07 (m, 35 H), 5.22−4.84 (m, 7 H), 4.73 (br. d, 1 H, J = 9.8 Hz), 4.62 (d, 1 H, J = 3.5 Hz), 4.56−4.09 (m, 7 H), 3.81 (br. d, 1 H, J = 1.4 Hz), 3.76 (dd, 1 H, J_1 = 9.0 Hz, J_2 = 9.8 Hz), 3.71−3.52 (m, 5 H), 3.38 (bs, 1 H, 1′-H, β), 3.22 (dd, 1 H, J_1 = 2.4 Hz, J_2 = 9.5 Hz), 3.10 (s, 3 H), 2.56 (br. d, 1 H, J = 9.8 Hz, 5′-H, D-configuration). − MS (ESI): calc. for $C_{63}H_{65}NO_9$ 979.5, found *m/z* 980 [M + H], 1002 [M + Na]. − $C_{63}H_{65}NO_9$: C 77.20; H 6.68; N 1.43; found: C 76.53; H 6.88; N 1.56.

11b: White solid, 0.57 g (0.59 mmol), 63%. − ^{13}C NMR (50 MHz, $CDCl_3$): 138.5−138.0, 128.2−127.4, 98.3, 86.7, 84.4, 83.9, 82.1, 80.9, 80.8, 79.6, 79.1, 75.7, 75.3, 75.0, 73.3, 69.0, 61.6, 58.7, 55.4, 51.6. − ^{1}H-COSY (300 MHz, $CDCl_3$): 7.38−7.12 (m, 35 H), 4.98−4.62 (m, 12 H), 4.53 (d, 1 H, J = 3.6 Hz), 4.44 (m, 2 H), 4.40 (m, 1 H), 3.86 (t, 1 H, J = 9.3 Hz, 2′-H, β), 3.66 (dd, 1 H, J_1 = 2.6 Hz, J_2 = 9.0 Hz), 3.51−3.42 (m, 5 H), 3.36 (s, 3 H), 2.74 (ddd, 1 H, J_1 = 2.6 Hz, J_2 = 6.2 Hz, J_3 = 9.3 Hz, 5′-H, D-configuration). − MS (ESI): calc. for $C_{63}H_{65}NO_9$ 979.5, found *m/z* 980.1 [M + H], 1002.1 [M + Na], 1019.1 [M + K]. − $C_{63}H_{65}NO_9$: C 77.20; H 6.68; N 1.43; found: C 77.73; H 6.92; N 1.39.

11c: Colourless oil, 0.82 g (0.84 mmol), 58%. − ^{13}C NMR (75 MHz, $CDCl_3$): 138.7−137.7, 128.4−127.4, 98.2, 84.8, 84.5, 82.2, 81.0, 80.9, 80.3, 79.1, 75.8, 75.7, 75.4, 74.5, 74.1, 73.7, 73.4, 72.5, 69.4, 61.7, 57.4, 55.5, 52.4. − ^{1}H-COSY (300 MHz, $CDCl_3$): 7.38−7.16 (m, 35 H), 5.05−4.36 (m, 16 H), 3.97 (br. d, 1 H, J = 1.4 Hz, 4′-H, D-configuration), 3.86 (t, 1 H, J = 9.6 Hz, 2′-H, β), 3.85 (t, 1 H, J = 9.3 Hz), 3.50−3.40 (m, 5 H), 3.35 (s, 3 H), 3.31 (t, 1 H), 2.77 (br. t, 1 H). − MS (ESI): $C_{63}H_{65}NO_9$ 979.5, found *m/z* 980.8 [M + H], 1002.6 [M + Na], 1983.2 [M + M + Na]. − $C_{63}H_{65}NO_9$: C 77.20; H 6.68; N 1.43; found: C 77.02; H 6.74; N 1.68.

General Procedure for the Reduction/Deprotection of 11a−c to 4, 5, and 6: The protected aza-*C*-disaccharide was dissolved in EtOAc/EtOH/1 M HCl (0.5:1:1, 15 mL/mmol) after which 5% Pd/C was added (300 mg/mmol). After a nitrogen flush of the reaction vessel, hydrogen gas was bubbled through the mixture for 30 min after which stirring was continued under an atmospheric pressure of hydrogen for 6 h (**11a**) or 24 h (**11b-c**). TLC analysis (EtOAc/MeOH/H_2O, 7:2:1, containing 1% TEA) indicated completion of the reaction. The catalyst was filtered off and rinsed thoroughly with MeOH and H_2O. The solvents were removed and the resulting oil was taken up in a minimal amount of MeOH and precipitated with Et_2O to give the crude target compound as a powder. Pure samples of the aza-*C*-disaccharides were obtained by HW-40 gel filtration and lyophilisation.

azaMan-β-(1→6)-*C*-Glc · HCl (4): White solid, 80 mg (0.21 mmol), 88% based on 0.23 g of **11a**. − ^{13}C NMR (50 MHz, CD_3OD): 101.3, 75.3, 75.0, 74.8, 73.5, 71.9, 68.8, 67.4, 62.3, 60.3, 59.7, 55.8, 28.0, 25.3. − MS (ESI): calc. for $C_{14}H_{27}NO_9$: 353.2; found: *m/z* 354 [M + H]. − α_D^{20} +16.6 (*c* 0.30, MeOH). − $C_{14}H_{28}ClNO_9$: C 43.14, H 7.24, N 3.59; found: C 42.48, H 7.16, N 3.39.

azaGlc-β-(1→6)-*C*-Glc · HCl (5): White solid, 59 mg (0.15 mmol), 80% based on 0.19 g of **11b**. − ^{13}C NMR (50 MHz, CD_3OD): 101.4, 78.3, 74.9, 74.7, 73.4, 73.0, 72.3, 69.4, 62.0, 61.0, 58.6, 56.0, 28.4, 26.5. − MS (ESI): calc. for $C_{14}H_{27}NO_9$: 353.2; found: *m/z* 354 [M + H]. − α_D^{20} +26.6 (*c* 0.58, MeOH). − $C_{14}H_{28}ClNO_9$: C 43.14, H 7.24, N 3.59; found: C 43.16, H 7.44, N 3.41.

azaGal-β-(1→6)-*C*-Glc · HCl (6): White solid, 96 mg (0.25 mmol), 85% based on 0.28 g of **11c**. − ^{13}C NMR (50 MHz, CD_3OD): 101.3, 74.9, 74.8, 74.5, 73.2, 72.3, 71.0, 68.1, 61.6, 61.1, 60.1, 56.1, 28.3, 26.8. MS (ESI): calc. for $C_{14}H_{27}NO_9$: 353.2; found: *m/z* 354 [M + H]. − α_D^{20} = +52.0 (*c* 0.58, MeOH). − $C_{14}H_{28}ClNO_9$: C 43.14, H 7.24, N 3.59; found: C 43.00, H 7.39, N 3.66.

Enzymatic Assays: The inhibition tests were performed on 25 commercially available glycosidases following the method of Saul et al.[21] with appropriate *p*-nitrophenyl glycoside substrates (Sigma). The following glycosidases were purchased from Oxford Glycosystem or from Sigma Chemical Co.: bovine epididymis α-L-fucosidase (EC 3.2.1.51), human placenta α-L-fucosidase, coffee beans, *Aspergillus niger* and *Escherichia coli* α- and β-galactosidases (EC 3.2.1.22 and 3.2.1.23), bovine liver and *Aspergillus orizae* β-galactosidases (EC 3.2.1.23), yeast and rice maltases (EC 3.2.1.20), isomaltase from baker yeast (EC 3.2.1.10), *Aspergillus niger* and *Rhizopus* mold amyloglucosidases (EC 3.2.1.3), almonds and *Caldocellum saccharolyticum* β-glucosidases (EC 3.2.1.21), jack beans and almonds α-mannosidases (EC 3.2.1.24), *Helix pomatia* β-mannosidase (EC 3.2.1.25), *Aspergillus niger* β-xylosidase (EC 3.2.1.37), chicken liver α-*N*-acetylgalactosaminidase and jack bean, bovine A and B β-*N*-acetyl-glucosaminidases.

In preliminary screenings, enzymatic activity was determined in the presence of two different high concentrations of the inhibitors (1 mM and 2 mM). For inhibition superior to 50% at 1 mM, the IC_{50} values were calculated by measuring glycosidase activity in the presence of various concentrations of inhibitor. The inhibition constants (K_i) and the nature of inhibition were determined from Lineweaver-Burk plots (only for compounds displaying an IC_{50} lower than 500 μM). A typical enzymatic assay (final volume 120 μL), contains 0.01 to 0.5 U/mL of the enzyme and 5 mM aqueous solution of the appropriate *p*-nitrophenyl glycoside substrate buffered to the optimum pH of the enzyme. Enzyme and inhibitor were incubated in a 96 wells polystyrene microplate for 5 min at 20 °C and the reaction started by addition of the substrate. After 20 min incubation at 37 °C, the reaction was stopped by addition of 0.2 mL sodium borate buffer (0.2 M, pH 9.8). The *p*-nitrophenolate formed was quantified by measuring the absorption at 405 nm on a microplate reader (Digiscan Asys Hightech). Under the conditions of the assay, the *p*-nitrophenolate released led to optical densities linear with both time of the reaction and concentration of the enzyme.

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