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Synthesis and Evaluation as Glycosidase Inhibitors of 2,5-Imino-D-glucitol and 1,5-Imino-D-mannitol Related Derivatives

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Abstract—Selectively functionalized 2,5-imino-D-glucitol and 1,5-imino-D-mannitol derivatives were synthesized and tested as precursors of hydrolytically resistant pseudo-disaccharides. Among them *N*-acetyl-6-amino-6-deoxy-2,5-imino-D-glucitol (**11**) and *N*-acetyl-6-amino-6-deoxy-1,5-imino-D-mannitol (**12**) were found potent and specific inhibitors against β-D-glucosidase and α-L-fucosidase, respectively.

Introduction

Glycosidases are involved in several metabolic pathways and the development of selective inhibitors is an important strategy towards the treatment of numerous diseases. The family of *N*-containing carbohydrate mimics commonly referred to as azasugars constitute a major class of transition-state analogue inhibitors of glycosidases.[1–4] Monosaccharide and azaglycoside mimics are at the moment the targets of much synthetic effort and stable aza-analogues of glycoconjugates have potential as selective inhibitors of targeted glycosidase-mediated processes.

In order to generate stable structures a few solutions have been recently proposed; in particular pseudo aza-disaccharides have been prepared, by replacing the interglycosidic oxygen atom by a non hydrolysable bond,[5–7] or by linking the aglycone directly to the intracyclic nitrogen atom.[8]

The divergent methodology we have developed to prepare enantiomerically pure functionally diverse pyrrolidines and piperidines consists of a double nucleophilic opening of C-2 symmetric bis-aziridines of L-*ido* configuration.[9–11] This strategy allows a one step access to carbon–nitrogen and carbon–sulfur linked pseudo-aza-disaccharides[12] and the preparation of valuable intermediates for coupling reactions. For instance we have recently reported the preparation of bisubstrates in which a 2,5-imino-D-glucitol is coupled at the pseudo-anomeric carbon through an ester or amide link with glucose (Scheme 1).[13]

We report here, the synthesis and evaluation as glycosidase inhibitors of various derivatives related to 6-amino-6-deoxy-2,5-imino-D-glucitol (**2**), 6-amino-6-deoxy-1,5-imino-D-mannitol (**3**) and 2,5-imino-D-glucitol (**4**). Both **2** and **3** are related to known potent glycosidases inhibitors, respectively, 2,5-imino-D-glucitol (**4**),[14] inhibitor of α- and β-glucosidases and D-1-deoxy-mannojirimycin **5**, a more potent inhibitor of α-L-fucosidase (K_i 5 μM) than of α-D-mannosidase[15] (Scheme 2).

Results and Discussion

The ring-opening of D-mannitol derived bis-aziridine **1** by nucleophiles is a versatile method for the selective synthesis of either polyhydroxylated pyrrolidines bearing a free functional group at C-1[9–11] or piperidines functionalized at C-2[11] (Scheme 3). In aprotic solvents, the ring-opening proceeds mainly with complete selectivity at the primary carbon (path m), followed by subsequent 5-*exo* cyclization leading to the pyrrolidine ring. If the ring opening of **1** proceeds with S_N1 characteristics, nucleophilic attack takes place selectively at the secondary carbon (path n) and the formation of the piperidines **3** of D-*manno* configuration is favored.

Keywords: C_2 symmetric bis-aziridine; azasugars; β-D-glucosidase inhibitor; α-L-fucosidase inhibitor.

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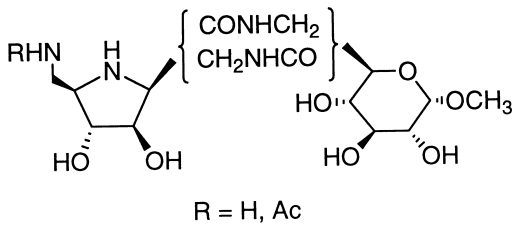


Scheme 1.

The protected 6-deoxy 6-amino iminoalditols **6**, **7** and **8** can be prepared alternatively from bis-aziridine **1** (Scheme 4). These compounds constitute suitable precursors for the construction of bisubstrates in which the unnatural sugar moiety is coupled with the aglycon either at C-1 or C-6 for pyrrolidines and either at C-2 or C-6 for piperidines. The amino group at C-6 constitutes an useful anchor group as a means of immobilization on polymer supports either for chromatographic affinity purification or solid phase synthesis.[16] We also took advantage of having easily accessible derivatives bearing a nitrogen function at C-6 to study the importance of the hydroxyl group at C-6 for the inhibitors **4** and **5**, in particular the effect of its replacement by an acetamido group.

We have tested, after total deprotection various azasugars related to **2** and **3** in order to probe the importance of the different functions for the biological activity.

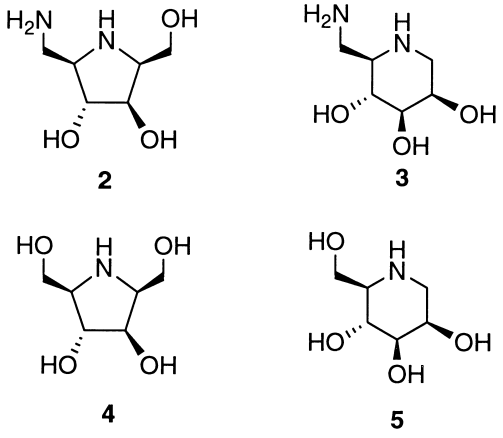
The synthesis of the cyclic carbamate-protected pyrrolidine **6** is achieved in 75% yield from **1** by Li_2NiBr_4 opening at the primary carbon (Scheme 3, path m) followed by Ag^+ promoted rearrangement.[10,11] Compound **6** could be converted, alternatively, into the known iminoglucitol **4**, the carbohydrate mimics **9a–d** and the cyclic urea **10** (Scheme 5).

Trifluoracetic acid-mediated deprotection of **6** afforded **13**, which was *N*-acetylated providing **14**. Derivatives **6**, **13** and **14** could be converted alternatively into the azasugars **9a–c** by hydrogenolysis on Pd black in acetic acid. The oxazolidinone **13** is converted into the cyclic urea **15** in basic medium and subsequent hydrogenolysis yielded **10**. Nitrous deamination of **13** using isoamylnitrite, led to a 1:1 mixture of diastereoisomeric carbamate-protected iminoglucitols **16** and **17**, both precursors of the 2,5-iminoglucitol **4**. Compounds **16** and **17** could be chromatographically separated and hydrogenolysis of **16** led to the bicyclic oxazolidone **9d**. Compounds **4** and **9d** are identical to compounds prepared previously following a different strategy.[17] The pyrrolidines **13** and **16** are useful intermediates for aglycon coupling at C-6, while the urea **15** enables coupling at C-1.

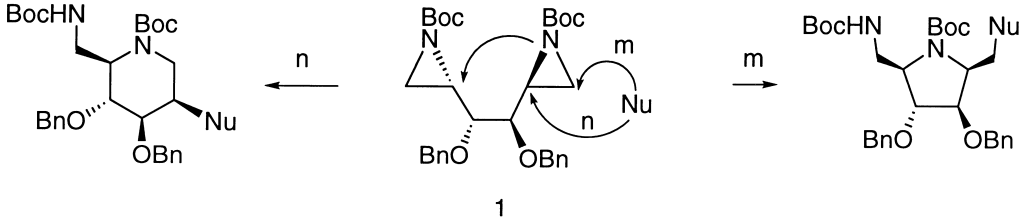
Preparation of the orthogonally protected pyrrolidines **7** and piperidines **8** has been achieved by the reaction of bis-aziridine **1** with hydroxylated reagents,[11] which regioselectivity highly depends on the catalyst. The opening of the first aziridine-ring at the primary carbon followed by 5-*exo tet* heterocyclization yields the pyrrolidines (Scheme 3, path m), while the formation of the piperidines results from the preliminary attack at the secondary carbon followed by 6-*exo tet* heterocyclization (path n).

Reaction of bis-aziridine **1** with acetic acid enabled the one step preparation of 1-*O*-acetyl pyrrolidine **7a** (precursor of **2** and **11**) in 60% yield along with 27% of **8a**, whereas the reaction of **1** with allyl alcohol, at room temperature, under ytterbium triflate catalysis, selectively yielded the piperidine **8b** (55%) precursor of **3** and **12** along with **7b** (27%) (Scheme 6). We have shown that decreasing the temperature improved the selectivity towards **8** but to the detriment of the chemical yield.

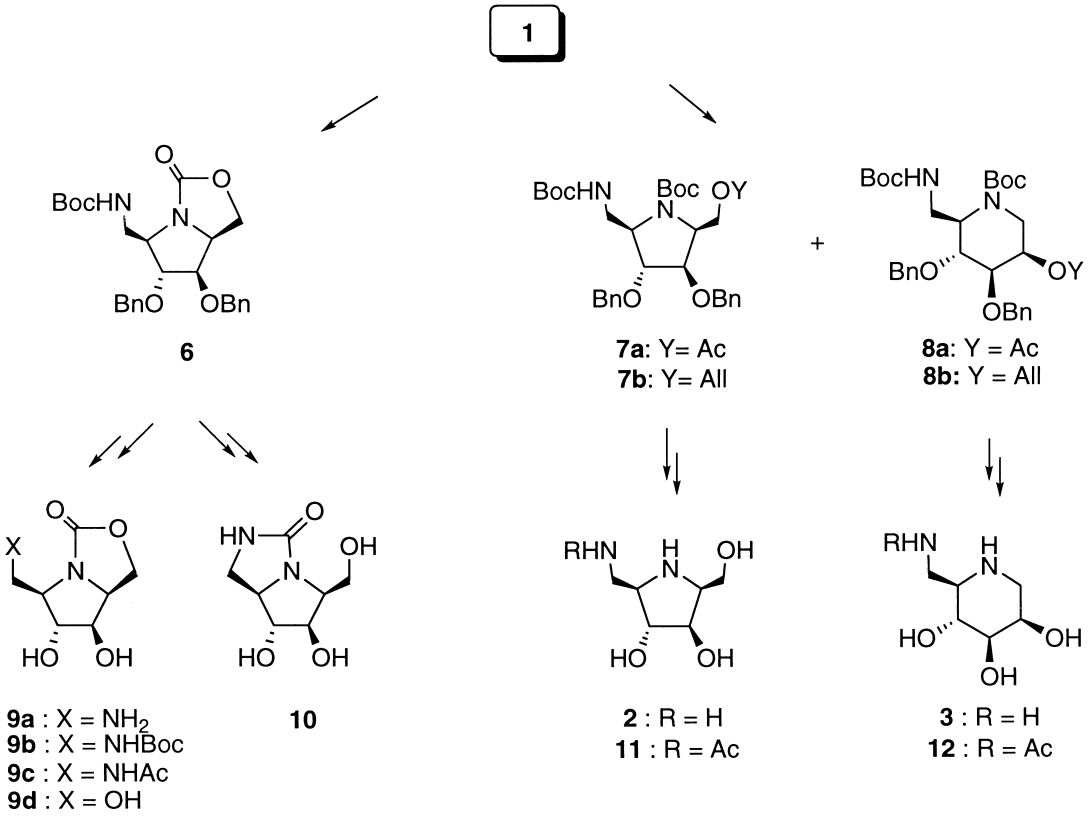
The sodium reduction followed by trifluoracetic acid-mediated deprotection of **7a** yielded **2**. We have also carried out the selective acetylation of the diamine **18** giving **19**, which presents a selectively free endocyclic amino function, in 65% yield. Total deprotection of the hydroxy groups of **19** with Na in NH_3 gave pyrrolidine **11**[18] (Scheme 6).



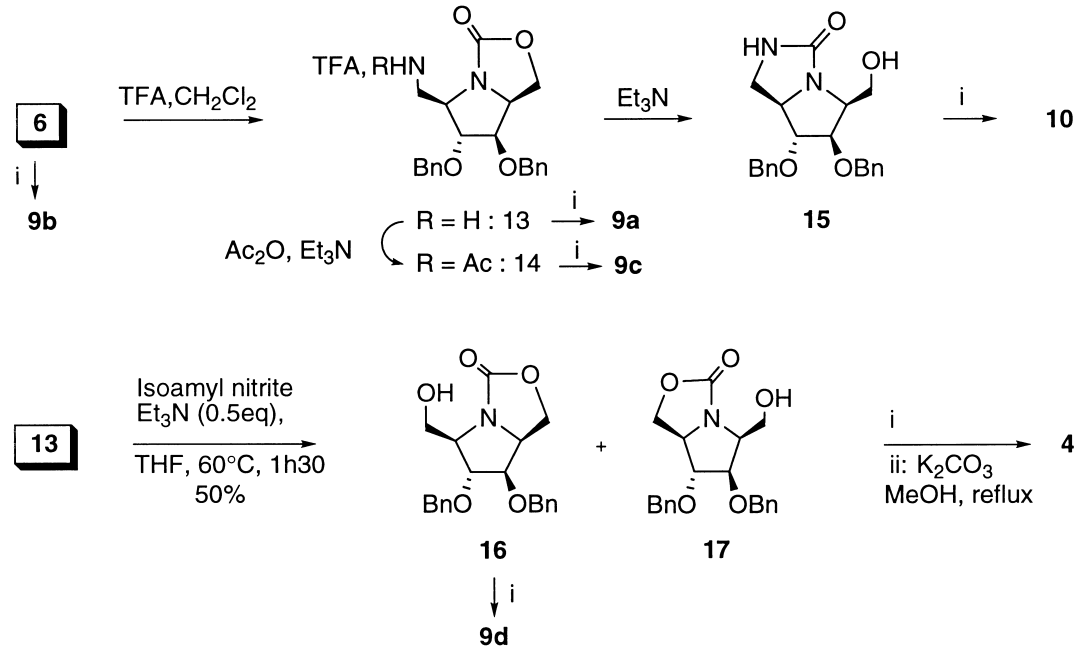
Scheme 2.



Scheme 3.

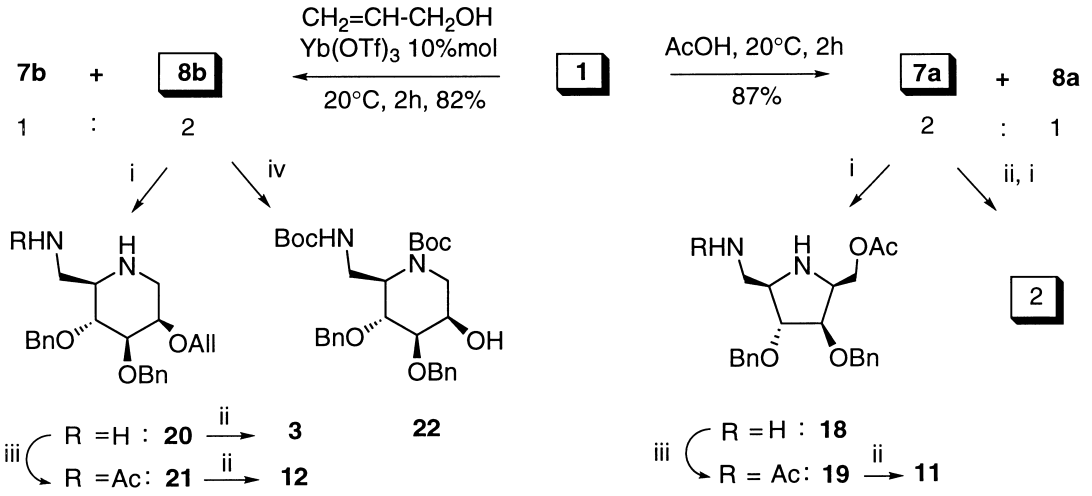


Scheme 4.



Scheme 5. i: H_2, Pd black, AcOH.

Trifluoracetic acid-mediated deprotection of **8b** gave the diamine **20** whose selective acetylation led to **21** in 76% yield, suitable for coupling at the endocyclic nitrogen. Total deprotection of the hydroxy groups of **20** and **21** using Na in NH_3 gave, respectively, piperidines **3** and **12**. On the other hand, we were able to carry out the selective reductive deprotection of the hydroxyl group at C-2 of **8b** giving **22** in 75%, using tributyltin hydride under



Scheme 6. i: TFA, CH_2Cl_2; ii: Na, NH_3; iii: Ac_2O, Et_3N; iv: Bu_3SnH, $ZnCl_2$, $Pd(Ph_3)_4$ cat.

$Pd(Ph_3)_4$ catalysis.[19] Furthermore the same piperidine **22**, a valuable intermediate for coupling reactions, was obtained by the opening of **1** with water.[11]

Inhibition Studies

Bicyclic azasugars **9a**–**9d**, **10**, azafuranoses analogues **2** and **11**, and azapyranoses **3** and **12** (Table 1) have been screened against five common glycosidases (α-glucosidase, β-glucosidase, α-galactosidase, α-mannosidase, α-L-fucosidase). Compounds **9a**, **9c**, **9d** and **10**, which do not present any positive charge either at the pseudoanomeric carbon or at the intramolecular nitrogen, showed unsurprisingly poor inhibition of α-glucosidase and were totally inactive on the other glycosidases studied. In addition to these electronic reasons, the oxazolidinone ring linking the pseudoanomeric center to the ring nitrogen of the pyrrolidine enhances the rigidity of the mimic and may sterically obstruct the access of the substrate to the active site. Therefore, bicyclic compounds **9** do not allow any SAR concerning the substituent at the C-6 position of the pseudo azasugar.

Compounds **2** and **3** displayed no inhibition towards the five enzymes tested ($K_i > 10^{-2}$ M). At the pH of the experiment (6.5), compounds **2** and **3**, positively charged at the primary amino function, are not suitable mimics of the oxocarbonium transition state. The pK_a values of the two amino functions in **2** or **3** have been calculated, using the Pallas 2.0 program file, to be = 9.8 for the primary ammonium at C-6 and respectively 5.15 and 5.92 for the intracyclic ammonium. It seems that no electrostatic interaction occurs between the positively charged ammonium at C-6 and a negatively carboxylate group in the active site.

Table 1. Inhibition study of glycosidases by azasugars **2**–**3** and **9**–**12**

Inhibitor	IC_{50}[a]	Inhibitor	K_i
9a	8 mM		
9b	NI[b]	**2**	$>10^{-3}$ M[c]
9c	6 mM	**11**	10 μM β-glucosidase
9d	7 mM	**3**	$>10^{-3}$ M[c]
10	3.5 mM	**12**	4 μM α-L-fucosidase

[a]Determined on α-glucosidase.
[b]No inhibition.
[c]On the five glycosidases assayed.

Compounds **11** and **12** showed competitive inhibition towards β-glucosidase and α-L-fucosidase, respectively. The 6-acetylamino analogue **11** of D-iminoglucitol **4**, positively charged at the intracyclic nitrogen proved to be a good ($K_i = 10$ μM) and selective inhibitor against β-glucosidase. Iminoglucitol **4**[14] inhibited α-glucosidase ($K_i \approx 1$ μM) but also β-glucosidase and α-galactosidase with one order of magnitude lower. Pyrrolidine **11** mimic of a glucosyl cation displayed no inhibition against the other enzymes assayed ($K_i > 10^{-3}$ M). The selectivity may arise from a specific interaction between the acetylamino group and the active asite of the β-glucosidase. Apparently, with the other enzymes, either this interaction is not favorable or the sterical hindrance interferes with binding to the active sites.

Due to the inherent structural similarity of α-L-fucosidase and D-mannosidase inhibitors, many compounds inhibit both groups of enzymes and therefore, due to this lack of selectivity cannot be used for practical applications. Interestingly, piperidine **12** of D-manno configuration was found to display potent and selective inhibition towards α-L-fucosidase (K_i 4 μM) and displayed no inhibition against the other enzymes assayed ($K_i > 10^{-3}$ M). A similar selectivity had been observed for the parent deoxymannojirimycin (DMJ),[15] a potent α-L-fucosidase ($K_i = 5$ μM) and a poor inhibitor of jack bean α-mannosidase (750 μM).

Conclusion

Diversely funtionalized pyrrolidines and piperidines deriving from **2** and **3** have been synthesized as precursors for the elaboration of complex glycosides and have been evaluated as glycosidase inhibitors. For the active compounds **11** and **12**, we have shown that the replacement of hydroxyl at C-6 of the known inhibitors **4** and **5** by acetamido group does not harm the activity.

This synthetic work is currently being extended to new bisubstrates using, in particular the D-mannitol **3** core.

Experimental

Chemistry

General directions. Prior to use, tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium-benzophenone and dichloromethane (CH_2Cl_2) from P_2O_5. CH_2Cl_2 and ethyl acetate (EtOAc) were filtered on K_2CO_3 prior to use. ^1H NMR (250 MHz) and ^{13}C NMR spectra were recorded in $CDCl_3$ (unless otherwise indicated) on a Brucker AM 250 (63 MHz). Chemical shifts are reported in δ (ppm) and coupling constants (*J*) are given in Hertz. Specific rotations were measured on a Perkin–Elmer 241C polarimeter with sodium (589 nm) or mercury (365 nm) lamps. UV spectra were recorded on a Uvicon 860 Spectrophotometer. Mass spectra were recorded by the Service de Spectrométrie de Masse, Université Pierre et Marie Curie, 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) unless otherwise noted; the solvent systems were given v/v. Spectroscopic (^1H and ^{13}C NMR, MS) and/or analytical data were obtained using chromatographically homogeneous samples.

General synthetic procedures

Procedure A. Hydrogenolysis of benzyl ethers. To a suspension of Pd black (50 mg) in AcOH (2.5 mL), was added the compound (0.15 mmol) to be hydrogenolyzed in AcOH (2 mL). After stirring overnight under 1 atm. hydrogen and at room temperature, the catalyst was filtered through a Celite pad and the solution was evaporated in vacuo.

Procedure B. Boc deprotection. TFA (20 equiv per *N*-*tert*-butyloxycarbonyl group) was added dropwise to a solution of Boc-protected amine in the same volume of CH_2Cl_2 at 0 °C. After stirring for 2 h at this temperature, the volatile components were evaporated and the residue was treated with dry Et_2O. If a filterable solid was obtained, the precipitate was collected, washed with dry Et_2O and dried under vacuum. Otherwise Et_2O was evaporated and the amorphous solid dried under vacuum and used directly in the subsequent step.

Procedure C. Selective acetylation. To a solution of TFA salt in CH_2Cl_2 ($9\ 10^{-2}$ M), cooled at −10 °C, were added dropwise acetic anhydride (1 equiv) and triethylamine (1 equiv). After 2 h at this temperature, the mixture was concentrated and the residue was purified by flash chromatography as specified.

Procedure D. Hydroxyl deprotection by sodium ammonia. To a solution of the compound (0.14 mmol) in liquid ammonia (25 mL) was added sodium until the blue color was persistent. The reaction mixture was stirred under ammonia reflux for 2 h. The mixture was then neutralized with NH_4Cl until disparition of the blue color. Ammonia was evaporated under a stream of argon and the residue diluted with CH_3OH or with CH_3OH and AcOH, filtered through Celite and concentrated. The crude product was further purified by column-chromatography on Kieselgel.

3,4-Di-*O*-benzyl-6-[(*tert*-butyloxycarbonyl)amino]-2,5-[(1-oxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (6). At 0 °C, to a stirred solution of **1** (569 mg, 1.08 mmol) in THF (16.4 mL) was added dropwise Li_2NiBr_4 (0.4 M in THF, 2.7 mL, 1.08 mmol). The mixture was stirred for 4 h at this temperature, then overnight at 4 °C. The mixture was then hydrolyzed with a buffer solution KH_2PO_4/K_2HPO_4 (1 M, 15 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. To the crude product containing the monobromo aziridine and the bromopyrrolidine dissolved in DMF was added $AgNO_3$ (360 mg, 2 equiv), then the mixture was heated at 70 °C for 9 h. After filtration on a Celite bed, the solvent was evaporated in vacuo. The crude product was purified by column chromatography to yield **6** as a colourless oil (380 mg, 75%); R_f 0.25 (cyclohexane:EtOAc, 7:3). $[\alpha]_D$ +14 (*c* 2.7, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H, CH_3), 3.5–3.75 (m containing brd, 3H, $J_{3,2}=3$ Hz, H-6, H-5, H-3), 3.8–4.0 (m, 1H, H-6′), 4.03 (brs, 1H, H-4), 4.20–4.70 (m, 7H, H-2, H-1, H-1′, OCH_2Ph), 5.80 (m, 1H, NH), 7.1–7.45 (m, $10H_{arom}$); ^{13}C NMR ($CDCl_3$) δ 28.4 (CH_3), 39.9 (C-6), 62.1 (C-1), 62.7, 65.3 (C-2, C-5), 71.0, 72.2 (OCH_2Ph), 79.2 (Cq_{Boc}), 79.4, 85.6 (C-3, C-4), 127.7, 127.9, 128.1, 128.6 (CH_{arom}), 137.0, 137.2 (Cq_{arom}), 156.3, 158.2 (CO). CIMS, NH_3: *m*/*z* (%) 469 (10) (MH^+), 369 (100) (MH^+-Boc).

6-[(*tert*-Butyloxycarbonyl)amino]-2,5-[(1-oxycarbonyl)-imino]-2,5,6-trideoxy-D-glucitol (9b). Compound **6** (64 mg, 0.136 mmol) was deprotected according to procedure **A**, to yield **9b** as a white solid after flash chromatography purification (33 mg, 84%); R_f 0.20 (CH_2Cl_2:MeOH, 95:5). $[\alpha]_D$ +28 lit.[18] +27.3 (*c* 0.4, $CHCl_3$), mp 45–47 °C. ^1H NMR (CD_3OD) δ 1.45 (s, 9H, CH_3), 3.40 (t, 1H, H-5), 3.64 (m, 2H, H-6, H-6′), 3.78 (brs, 1H, H-3), 4.09 (brs, 1H, H-4), 4.45 (m, 3H, H-1, H-1′, H-2), $J_{5,6}=J_{5,6'}=6$ Hz; ^{13}C NMR (CD_3OD) δ 28.7 (CH_3), 40.5 (C-6), 64.0 (C-1), 64.7, 67.1, (C-2, C-5), 80.4 (Cq_{Boc}), 74.7, 83.8 (C-3, C-4), 158.1, 160.3 (CO). HRMS calcd for $C_{12}H_{21}NO_6$ (MH^+) 289.1399, found 289.1406.

6-Amino-3,4-di-*O*-benzyl-2,5-[(1-oxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol, TFA (13). Compound **6** (475 mg, 1.014 mmol) was deprotected according to procedure **B**

to yield **13** as a white solid (406 mg, 83%), mp 151–153 °C; $[\alpha]_D$ +26 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 3.15–3.6 (m, 2H, H-6, H-6′), 3.49 (m, 1H), 3.64 (s, 1H), 3.99 (brd, 1H, *J* = 10 Hz, 1H), 4.26–4.46 (m, 5H), 4.55 (AB, *J* = 12 Hz, 1H), 4.57 (A′B′, *J* = 12 Hz, 1H), 7.10–7.40 (m, $10H_{arom}$), 8.25 (brs, 3H, NH_3^+).

6-Amino-2,5-[(1-oxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol, AcOH (9a). Compound **13** (72.38 mg, 0.15 mmol) was deprotected according to procedure **A** to yield **9a** as a very hygroscopic solid after flash chromatography purification (34 mg, 91%); R_f 0.35 (CH_2Cl_2:MeOH:H_2O:AcOH, 7:3:0.6:0.3). $[\alpha]_D$ +14 (*c* 0.5, MeOH). ^1H NMR (CD_3OD) δ 1.96 (s, 3H, CH_3), 3.48 (m, 2H), 3.64 (m, 1H), 3.83 (m, 1H), 4.09 (s, 1H), 4.4–4.55 (m, 3H); ^{13}C NMR (CD_3OD) δ 25.5 (CH_3), 42.7 (C-6), 66.4 (C-1), 66.9, 67.6 (C-2, C-5), 76.3, 86.2 (C-3, C-4), 163.2, 182.2 (CO). HRMS calcd for $C_7H_{13}N_2O_4$ (MH^+) 189.0875, found 189.0873.

6-Acetylamino-3,4-di-*O*-benzyl-2,5-[(1-oxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (14). To a solution of **13** (72.38 mg, 0.15 mmol) in DMF (375 μL) and acetic anhydride (150 μL, 1.58 mmol) was added dropwise Et_3N (21 μL, 0.15 mmol). After 1 h stirring at room temperature, the volatile components were evaporated. After addition of water (7 mL) and CH_2Cl_2 (15 mL), decantation, extraction with CH_2Cl_2 (3×15 mL), the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to yield quantitatively **14** (61.5 mg); R_f 0.12 (cyclohexane:EtOAc, 1:1), which was used in the next step without further purification. ^1H NMR ($CDCl_3$) δ 1.87 (s, 3H, CH_3), 3.45–3.7 (m containing brd, 3H, $J_{3,2}$ = 3 Hz, H-6, H-5, H-3), 4.0–4.15 (m containing brs, 2H, H-4, H-6′), 4.2–4.7 (m, 7H, H-2, H-1, H-1′, OCH_2Ph), 6.92 (m, 1H, NH), 7.1–7.45 (m, $10H_{arom}$).

6-Acetylamino-2,5-[(1-oxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (9c). Compound **14** (61.5 mg, 0.15 mmol) was deprotected according to procedure **A** to yield quantitatively **9c** (34.5 mg) as a white solid after flash chromatography; R_f 0.25 (CH_2Cl_2:MeOH, 9:1); mp 148–151 °C; $[\alpha]_D$ +67.5 (*c* 0.51, CH_3OH). ^1H NMR (CD_3OD) δ 2.0 (s, 3H, CH_3), 3.43 (t, 1H, H-5), 3.78 (m, 3H, H-3, H-6, H-6′), 4.12 (s, 1H, H-4), 4.39 (m, 3H, H-2, H-1, H-1′), $J_{5,6} = J_{5,6'}$ = 6 Hz. ^{13}C NMR (CD_3OD) δ 24.7 (CH_3), 41.0 (C-6), 65.9 (C-1), 66.5, 68.1 (C-2, C-5), 76.6, 85.6 (C-3, C-4), 162.0 (COO), 175.0 (CON). HRMS calcd for $C_9H_{15}N_2O_5$ (MH^+) 231.0981, found 231.0982.

6-Amino-3,4-di-*O*-benzyl-2,5-[(6-aminocarbonyl)imino]-2,5,6-trideoxy-D-glucitol (15). To a solution of **13** (60 mg, 0.128 mmol) in CH_2Cl_2 (0.4 mL) was added dropwise Et_3N (21.5 μL, 1.2 equiv). After 1 h stirring at room temperature the volatile components were evaporated. Compound **15** was obtained quantitatively (45.5 mg) as a colourless oil after column chromatography; R_f 0.20 (cyclohexane:EtOAc, 2:8), $[\alpha]_D$ −24.5 (*c* 0.66, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 3.28 (brt, 1H, H-6), 3.54 (t, 1H, H-6′), 3.75–3.96 (m, 4H, H-2, H-4, H-1, H-5), 4.0 (dd, 1H, *J* = 3.5 Hz, 5, H-3,), 4.20 (dd, 1H, H-1′), 4.30 (m, 1H, OH), 4.4–4.65 (m, 4H, OCH_2Ph), 4.75 (brs, 1H, NH), 7.1–7.45 (m, $10H_{arom}$), $J_{6,6'} = J_{5,6'}$ = 8.8 Hz, $J_{1,1'}$ = 12 Hz, $J_{1',2}$ = 8.8 Hz; ^{13}C NMR ($CDCl_3$) δ 44.6 (C-6), 59.3 (C-1), 62.7, 63.0 (C-2, C-5), 72.2, 72.3 (OCH_2Ph), 84.1, 86.4 (C-3, C-4), 127.6, 128.0, 128.1, 128.6 (CH_{arom}), 137.4 (Cq_{arom}), 163.3 (CO).CIMS, NH_3: *m*/*z* (%) 369 (100) (MH^+).

6-Amino-2,5-[(6-aminocarbonyl)imino]-2,5,6-trideoxy-D-glucitol (10). Compound **15** (44.3 mg, 0.12 mmol) was deprotected according to procedure **A**. Column chromatography purification (CH_2Cl_2:MeOH, 85:15 then 80:20), afforded **10** as a white solid (19 mg, 84%); R_f 0.25 (CH_2Cl_2:MeOH, 8:2), mp 142–145 °C; $[\alpha]_D$ −52.5 (*c* 0.2, MeOH). ^1H NMR (CD_3OD) δ 3.44 (dd, 1H, *J* = 7 Hz, 8.8), 3.64 (dd, 1H, *J* = 5, 10.7 Hz), 3.66 (t, 1H, *J* = 9 Hz), 3.78 (ddd, 1H, *J* = 5, 7, 9 Hz), 3.90 (t, 1H, *J* = 5 Hz), 4.01–4.13 (m, 3H), CIMS, NH_3: *m*/*z* (%) 189 (100) (MH^+).

Nitrous deamination of 13. To a solution of **13** (370 mg, 0.77 mmol) in THF (4 mL) and isoamylnitrite (533 μL, 5 equiv) was added dropwise Et_3N (50 μL, 0.5 equiv). The solution mixture was refluxed for 1 h 30 min then cooled to room temperature. After addition of water (3 mL) and CH_2Cl_2 (4 mL), decantation, extraction (3×6 mL), the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.The products were further separated by column chromatography (cyclohexane:EtOAc, 7:3) affording **16** (70 mg, 25%); R_f 0.16 and **17** (70 mg, 25%); R_f 0.12 as colourless oils.

3,4-Di-*O*-benzyl-2,5-[(1-oxycarbonyl)imino]-2,5-dideoxy-D-glucitol (16). $[\alpha]_D$ +53 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 3.7 (d, 1H, *J* = 3.5 Hz), 3.72 (dt, 1H, *J* = 2.7, 8.5 Hz), 3.8 (m, 1H), 3.89 (dt, 1H, *J* = 2.7, 12.5 Hz), 3.97 (m, 1H), 4.29 (ddd, 1H, *J* = 3.5, 5, 8.5 Hz), 4.33–4.63 (m, 7H), 7.1–7.5 (m, $10H_{arom}$); ^{13}C NMR ($CDCl_3$) δ 61.9, 62.9 (C-1, C-6), 62.9, 67.1 (C-2, C-5), 71.3, 72.0 (OCH_2Ph), 79.1, 84.6 (C-3, C-4), 127.7, 127.8, 128.3, 128.7 (CH_{arom}), 136.8, 136.9 (Cq_{arom}), 159.1 (CO). CIMS, NH_3: *m*/*z* (%) 370 (100) (MH^+).

3,4-Di-*O*-benzyl-2,5-[(6-oxycarbonyl)imino]-2,5-dideoxy-D-glucitol (17). $[\alpha]_D$ −9 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 3.9–4.2 (m, 5H), 4.43–4.74 (m, 7H), 7.15–7.35 (m, $10H_{arom}$); ^{13}C NMR ($CDCl_3$) δ 58.7, 63.1 (C-2, C-5), 64.7, 71.9, 72.4 (CH_2O), 74.4, 85.5 (C-3, C-4), 127.9, 128.4, 128.7 (CH_{arom}), 136.3 (Cq_{arom}), 160.5 (CO). CIMS, NH_3: *m*/*z* (%) 370 (100) (MH^+).

2,5-Dideoxy-2,5-[(1-oxycarbonyl)imino]-D-glucitol (9d). Compound **16** (70 mg, 0.19 mmol) was deprotected according to procedure **A**. Chromatographic purification (CH_2Cl_2:MeOH, 9:1), afforded **9d** (30 mg, 83%) as a colourless oil; R_f 0.28; $[\alpha]_D$ +34 (*c* 1.0, MeOH). ^1H NMR (CD_3OD) δ 3.49 (dt, 1H, H-5), 3.72 (brs, 1H, H-3), 3.95 (dd, 1H, H-6), 4.09 (dd, 1H, H-6′), 4.17 (brs, 1H, H-4), 4.44 (m, 3H, H-2, H-1, H-1′), $J_{2,3} = J_{4,5}$ = 1 Hz, $J_{5,6}$ = 4.8 Hz, $J_{5,6'}$ = 4.5 Hz, $J_{6,6'}$ = 11.5 Hz; ^{13}C NMR (CD_3OD) δ 59.7, 64.3 (C-1, C-6), 64.9, 68.2 (C-2, C-5), 74.4, 83.8 (C-3, C-4), 160.5 (CO). CIMS, CH_4: *m*/*z* (%) 190 (100) (MH^+).

Opening of the N-Boc bis-aziridine 1 by acetic acid. Compound **1** (920 mg, 1.75 mmol) in acetic acid (17.5 mL) was stirred for 2 h at room temperature. After concentration in vacuo, the products were further separated by column chromatography (cyclohexane:EtOAc, 4:1), affording **7a**; R_f 0.33 (612 mg, 60%) and **8a**; R_f 0.23 (275 mg, 27%) as colourless oils.

1-*O*-Acetyl-3,4-di-*O*-benzyl-6-[(*tert*-butyloxycarbonyl)-amino]-2,5-[(*tert*-butyloxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (7a). $[\alpha]_D$ −11 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.42, 1.44 (s, 18H, CH_3), 2.01 (s, 3H, OAc), 3.3–3.6 (m, 2H, H-6, H-6′), 3.75 (m, 1H, H-5), 3.98 (t, 1H, H-4), 4.07 (t, 1H, H-3), 4.14 (dd, 1H, H-1), 4.31 (m, 2H, H-1′, H-2), 4.54, 4.57 (AB, 2H, OCH_2Ph), 4.61 (s, 2H, OCH_2Ph), 5.33 (m, 1H, NH), 7.2–7.4 (m, 10H_{arom}), $J_{1,1'}$ = 10.2 Hz, $J_{1,2}$ = 4.5 Hz, $J_{3,4}$ = $J_{4,5}$ = 5.5 Hz, J_{AB} = 12 Hz; ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3CO), 28.3, 28.4 (CH_{3Boc}), 42.8 (C-6), 57.2, 61.5 (C-2, C-5), 62.4 (C-1), 72.5, 72.8 (OCH_2Ph), 79.1, 80.9 (Cq_{Boc}), 81.5, 83.0 (C-3, C-4), 127.8, 128.0, 128.4, 128.5 (CH_{arom}), 137.3, 137.8 (Cq_{arom}), 155.1, 156.1 (CO_{Boc}), 170.7 (CO_{Ac}).

2-*O*-Acetyl-6-[(*tert*-butyloxycarbonyl)amino]-3,4-di-*O*-benzyl-1,5-[(*tert*-butyloxycarbonyl)imino]-1,5,6-trideoxy-D-mannitol (8a). $[\alpha]_D$ −10 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.40, 1.42 (s, 18H, CH_3), 2.02 (s, 3H, OAc), 3.18 (brt, 2H, *J* = 12 Hz, H-1, H-6), 3.49 (dd, 1H, H-4, *J* = 1.3, 3.5 Hz), 3.6–3.8 (m, 1H, H-6′), 3.88 (brs, 1H, H-3), 4.09 (m, 1H, H-1′), 4.30–4.75 (m, 6H, OCH_2Ph, H-5, NH), 5.01 (ddd, 1H, H-2, *J* = 3, 4.7, 11 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8 (CH_3CO), 28.1, 28.3 (CH_{3Boc}), 36.9, 39.2 (C-1, C-6), 53.0 (C-5), 67.9 (C-2), 71.0, 73.3 (OCH_2Ph), 74.6 (C-3, C-4), 79.0, 80.1 (Cq_{Boc}), 127.4, 127.5, 127.7, 128.2, 128.3 (CH_{arom}), 137.5, 137.7 (Cq_{Boc}), 155.5, 155.8 (CO_{Boc}), 169.7 (CO_{Ac}).

Opening of the *N*-Boc bis-aziridine 1 by allyl alcohol. To **1** (131 mg, 0.25 mmol) in allyl alcohol (2.5 mL) was added ytterbium triflate (15 mg, 0.1 equiv) at room temperature and the mixture was stirred for 2 h at this temperature. After concentration the products were further separated by column chromatography (toluene: EtOAc, 95:5) on Kieselgel [60 and 60 H (5–40 μm), 1/1], affording **7b**; R_f 0.35 (39.3 mg, 27%) and **8b**; R_f 0.25 (80.2 mg, 55%) as colourless oils.

1-*O*-Allyl-[(*tert*-butyloxycarbonyl)amino]-3,4-di-*O*-benzyl-2,5-[(*tert*-butyloxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (7b). $[\alpha]_D$ −10.5 (*c* 0.67, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.41, 1.43 (s, 18H, CH_3), 3.38 (dt, 1H, $J_{6,6'}$ = 13.5 Hz, $J_{6,5}$ = 4.5 Hz, H-6), 3.6–3.75 (m, 4H containing H-6′ and H-5), 3.9–4.25 (m, 4H containing $OCH_2CH=CH_2$), 4.50–4.75 (m, 4H, OCH_2Ph), 5.12 (dd, 1H, *J* = 1.5, 10 Hz, $OCH_2CH=CH_2$), 5.24 (dd, 1H, *J* = 1.5, 11 Hz, $OCH_2CH=CH_2$), 5.8–5.95 (m, 2H, NH, $OCH_2CH=CH_2$), 7.2–7.4 (m, 10H_{arom}); ^{13}C NMR ($CDCl_3$) δ 28.3, 28.5 (CH_3), 42.3 (C-6), 58.0, 60.1 (C-2, C-5), 67.3 (C-1), 72.1, 72.7, 72.9 (OCH_2Ph, $OCH_2CH=CH_2$), 78.7, 80.6 (Cq_{Boc}), 81.7, 83.1 (C-3, C-4), 117.1 ($OCH_2CH=CH_2$), 127.8, 128.4 (CH_{arom}), 134.6 ($OCH_2CH=CH_2$), 137.8, 138.0 (Cq_{arom}), 155.1, 156.1 (CO). CIMS, NH_3: *m*/*z* (%) 583 (100) (MH^+).

2-*O*-Allyl-6-[(*tert*-butyloxycarbonyl)amino]-3,4-di-*O*-benzyl-1,5-[*tert*-butyloxycarbonyl)imino]-1,5,6-trideoxy-D-mannitol (8b). $[\alpha]_D$ −28.5 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.42, 1.44 (s, 18H, CH_3), 3.06 (m, 1H, H-1), 3.24 (m, 1H, H-6), 3.51 (brs, 1H, H-4), 3.63 (m, 2H), 3.82 (brs, 1H, H-3), 3.9–4.24 (m, 3H, containing $OCH_2CH=CH_2$), 4.25–4.9 (m, 6H, H-5, NH, OCH_2Ph), 5.15 (dd, 1H, *J* = 1.5, 10.5 Hz, $OCH_2CH=CH_2$), 5.26 (dd, 1H, *J* = 1.5, 16 Hz, $OCH_2CH=CH_2$), 5.89 (m, 1H, $OCH_2CH=CH_2$), 7.1–7.45 (m, 10H_{arom}); ^{13}C NMR ($CDCl_3$) δ 28.3, 28.4 (CH_3), 36.9, 39.3 (C-1, C-6), 53.2 (C-5), 70.0, 71.1 (OCH_2Ph), 73.5 ($OCH_2CH=CH_2$), 73.1, 75.1, 75.7 (C-2, C-3, C-4), 79.1, 80.0 (Cq_{Boc}), 116.5 ($OCH_2CH=CH_2$), 127.4, 127.6, 127.7, 128.3 (CH_{arom}), 134.8 ($OCH_2CH=CH_2$), 137.9, 138.3 (Cq_{arom}), 155.8 (CO). CIMS, NH_3: *m*/*z* (%) 583 (100) (MH^+).

1-*O*-Acetyl-6-amino-3,4-di-*O*-benzyl-2,5-imino-2,5,6-trideoxy-D-glucitol, TFA (18). Compound **7a** (370 mg, 0.63 mmol) was deprotected according to procedure **B** to yield quantitatively **18** (387 mg) as an amorphous solid, which was used in the next step without further purification. ^1H NMR ($CDCl_3$) δ 1.98 (s, 3H, CH_3), 3.31 (m, 1H, H-6, $J_{6,6'}$ = 13 Hz), 3.46 (brt, 1H, H-6′), 3.78 (s, 1H), 3.9–4.05 (m, 2H), 4.09 (m, 1H), 4.3–4.6 (m, 6H, containing OCH_2Ph), 7.1–7.4 (m, 10H_{arom}).

2-*O*-Allyl-6-amino-3,4-di-*O*-benzyl-1,5-imino-1,5,6-trideoxy-D-mannitol, TFA (20). Piperidine **8a** (97.4 mg, 0.17 mmol) was deprotected according to procedure **B** to yield **20** (101 mg, 100%) as an amorphous solid, which was used in the next step without further purification. ^1H NMR ($CDCl_3$) δ 3.0 (m, 1H), 3.25–3.6 (m, 3H), 3.62–3.8 (m, 2H), 3.9 (brs, 2H), 4.01, 4.08 (dd, 2H, *J* = 5.5, 12.7 Hz, $OCH_2CH=CH_2$), 4.55–4.85 (m, 4H, OCH_2Ph), 5.15–5.35 (m, 2H, $OCH_2CH=CH_2$), 5.87 (m, 1H, $OCH_2CH=CH_2$), 7.1–7.4 (m, 10H_{arom}); ^{13}C NMR ($CDCl_3$) δ 38.6 (C-6), 43.0 (C-1), 55.5 (C-5), 71.2, 72.7 (OCH_2Ph), 74.8 ($OCH_2CH=CH_2$), 69.6, 74.6, 79.0 (C-2, C-3, C-4), 118.4 ($OCH_2CH=CH_2$), 127.8, 128.0, 128.6 (CH_{arom}), 133.8 ($OCH_2CH=CH_2$), 136.8, 137.2 (Cq_{arom}).

1-*O*-Acetyl-6-*N*-acetylamino-3,4-di-*O*-benzyl-2,5-imino-2,5,6-trideoxy-D-glucitol (19). Pyrrolidine **18** (135 mg, 0.22 mmol) was selectively acetylated according to procedure **C** to yield **19** (61 mg, 65%) as a white solid after purification by flash chromatography (CH_2Cl_2:MeOH: NH_4OH, 95:5:0.1); R_f (CH_2Cl_2:MeOH, 95:5), mp 79 °C; $[\alpha]_D$ +21 (*c* 0.61, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.75 (s, 3H, CH_3CON), 2.0 (s, 3H, CH_3CO_2), 2.7 (brs, 1H, NH), 3.2–3.65 (m, 3H), 3.73 (s, 1H), 3.87 (brs, 1H), 4.2 (dd, 1H, *J* = 7.5, 11 Hz), 4.29 (dd, 1H, *J* = 5.2, 11 Hz), 4.36, 4.51 (AB, 2H, *J* = 11.7 Hz, OCH_2Ph), 4.49 (s, 2H, OCH_2Ph), 6.35 (brs, 1H, NHAc), 7.1–7.4 (m, 10H_{arom}); ^{13}C NMR ($CDCl_3$) δ 20.9, 23.0 (CH_3), 42.3 (C-6), 59.3, 62.2, 63.9 (C-1, C-2, C-5), 71.9 (OCH_2Ph), 82.1, 84.4 (C-3, C-4), 127.6, 127.8, 128.1, 128.5 (CH_{arom}), 137.3, 137.6 (Cq_{arom}), 170.6, 170.9 (CO). HRMS calcd for $C_{24}H_{31}N_2O_5$ (MH^+) 427.2232, found 427.2228.

6-*N*-Acetylamino-2-*O*-allyl-3,4-di-*O*-benzyl-1,5-imino-1,5,6-trideoxy-D-mannitol (21). The TFA salt **20** (48.8 mg, 0.8 mmol) was acetylated according to procedure **C** to

yield **21** (20 mg, 73%) as a colourless oil after purification by column chromatography (CH_2Cl_2:MeOH: NH_4OH, 95:5:1); R_f 0.5 (CH_2Cl_2:MeOH, 9:1). $[\alpha]_D$ −17.5 (*c* 0.98, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.86 (s, 3H, CH_3), 2.35–2.6 (m, 2H, H-1, H-5), 3.0–3.2 (m, 2H, H-1′, H-6), 3.5 (m, 2H, H-3, H-4), 3.6–3.75 (m containing ddd, 2H, $J=4.6$ Hz, $J_{6,6'}=13.5$ Hz, H-6′, H-2), 4.11 (m, 2H, $OCH_2CH=CH_2$), 4.61, 4.91 (AB, 2H, $J=10.8$, OCH_2Ph), 4.63, 4.7 (A′B′, 2H, $J=11.8$ Hz, OCH_2Ph), 5.15–5.30 (m, 2H, $OCH_2CH=CH_2$), 5.8 (m, 1H, NH), 5.9 (m, 1H, $OCH_2CH=CH_2$), 7.2–7.4 (m, $10H_{arom}$); ^{13}C NMR ($CDCl_3$) δ 22.7 (CH_3), 39.3 (C-6), 44.7 (C-1), 58.4 (C-5), 70.9, 72.1 (OCH_2Ph), 70.8, 75.0, 81.6 (C-2, C-3, C-4), 74.9 ($OCH_2CH=CH_2$), 117.9 ($OCH_2CH=CH_2$), 127.7, 127.8, 128.2, 128.4 (CH_{arom}), 134.2 ($OCH_2CH=CH_2$), 137.6, 137.8 (Cq_{arom}), 171.7 (CO). CIMS, NH_3: *m*/*z* (%) 425 (100) (MH^+).

6-[(*tert*-Butyloxycarbonyl)amino]-3,4-di-*O*-benzyl-1,5-[*tert*-butyloxycarbonyl)imino]-1,5,6-trideoxy-D-mannitol (22). To a solution of allyl ether **17** (26.3 mg, 0.045 mmol) in 1 mL of anhydrous THF was added anhydrous zinc chloride (16.5 mg 0.12 mmol). After 15 min stirring at 20 °C, tetrakis (triphenylphosphine) palladium (0) (10.4 mg, 0.009 mmol) was added and the stirring carried on for 15 min. Tributyltin hydride was then added dropwise (49 μL, 0.18 mmol) and reaction mixture allowed to stir for 1 h. Organic solvent was removed and the mixture was quenched by addition of water (0.5 mL), 0.1 N HCl (1 mL), and EtOAc (2.5 mL). The ethyl acetate phase was washed with 2 mL of satd $NaHCO_3$ (aq), dried ($MgSO_4$), filtered, and evaporated. Chromatographic purification (EtOAc:cyclohexane, 2:3) yielded **22** as a colourless oil (18.6 mg, 75%); R_f 0.50; $[\alpha]_D$ −23 (*c* 0.26, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.41, 1.42 (s, 18H, CH_3), 2.13 (d, 1H, OH), 2.86 (t, 1H, H-1), 3.05–3.25 (m, 1H, H-6), 3.5–3.8 (m, 3H, H-4, H-6′, H-3), 3.87 (m, 1H, H-2), 4.05 (m, 1H, H-1′); 4.3–4.75 (m, 6H, OCH_2Ph, H-5, NH), 7.1–7.3 (m, 10H, H_{arom}), $J_{1,1'}=J_{1,2}=12$ Hz, $J_{OH,2}=9.7$ Hz, $J_{2,3}=2.8$ Hz, $J_{3,4}=3$ Hz; ^{13}C NMR ($CDCl_3$) δ 28.3, 28.4 (CH_3), 38.9, 40.3 (C-1, C-6), 52.7 (C-5), 64.4 (C-2) 71.2, 73.4 (OCH_2Ph), 73.7, 77.3 (C-3, C-4), 79.3, 80.2 (Cq_{Boc}), 127.6, 127.8, 128.3, 128.4, 128.7 (CH_{arom}), 137.2, 137.7 (Cq_{arom}), 156.1, 155.9 (CO).

6-[(*tert*-Butyloxycarbonyl)amino]-2,5-[*tert*-butyloxycarbonyl)imino]-2,5,6-trideoxy-D-glucitol (23). Compound **7a** (56.5 mg, 0.097 mmol) was deprotected according to procedure **D** to yield **23** as a foam after flash chromatography purification (35 mg, 87%); R_f 0.23 (EtOAc), $[\alpha]_D$ +13.5 (*c* 2.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 1.39, 1.43 (s, 18H, CH_3), 3.44 (m, 2H), 3.59 (m, 1H), 3.7–5.0 (m, 8H containing OH), 5.45, 5.7 (brs, NH). CIMS, CH_4: *m*/*z* (%) 363 (100) (MH^+).

6-Amino-2,5-imino-2,5,6-trideoxy-D-glucitol (2). Compound **23** (30 mg, 0.08 mmol) was deprotected according to procedure **B** to yield the title compound **2** after chromatography purification; R_f 0.10 (CH_2Cl_2:MeOH: H_2O:AcOH, 5:5:1:0.5) followed by neutralization from an Amberlite IRA-400 (11 mg, 82%), $[\alpha]_D$ +12 (*c* 0.33, MeOH), $[\alpha]_{365}$ +54.5. ^1H NMR (CD_3OD) δ 2.75 (dd, 1H, H-6), 2.83 (dd, 1H, H-6′), 2.95 (m, 1H, H-5), 3.28 (m, 1H, H-2), 3.65 (dd, 1H, H-1), 3.73 (dd, 1H, H-4), 3.78 (dd, 1H, H-1′), 3.95 (dd, 1H, H-3), $J_{1,1'}=11$ Hz, $J_{1,2}=6$ Hz, $J_{1',2}=5.8$ Hz, $J_{2,3}=4.8$ Hz, $J_{3,4}=2.5$ Hz, $J_{4,5}=3.7$ Hz, $J_{5,6'}=4.5$ Hz, $J_{5,6}=6.3$ Hz, $J_{6,6'}=13$ Hz. ^{13}C NMR (CD_3OD) δ 44.7 (C-6), 62.1 (C-1), 63.0, 67.4 (C-2, C-5), 79.0, 82.0 (C-3, C-4). CIMS, NH_3: *m*/*z* (%) 163 (100) (MH^+).

6-*N*-Acetylamino-2,5-imino-2,5,6-trideoxy-D-glucitol (11). Compound **19** (60 mg, 0.14 mmol) was deprotected according to procedure **D**. Flash column chromatography using a 7:3:0.6:0.3 mixture of CH_2Cl_2:MeOH: H_2O:AcOH as eluent; R_f 0.50, gave **11** as AcOH salt (29 mg, 78%). $[\alpha]_D$ +2 (*c* 1.0, MeOH), $[\alpha]_{365}$ +12 (*c* 1.0; MeOH). A sample was neutralized from a Dowex 1X8 and characterized; $[\alpha]_D$ +26 (*c* 0.4, H_2O), lit[18]: $[\alpha]_D$ +27.2 (*c* 0.23; H_2O). ^1H NMR (D_2O) δ 2.05 (s, 3H, CH_3), 3.09 (m, 1H, H-5), 3.36 (m, 1H, H-2), 3.38 (dd, 1H, H-6), 3.49 (dd, 1H, H-6′), 3.71 (dd, 1H, H-1), 3.83 (dd, 1H, H-1′), 3.86 (dd, 1H, H-4), 4.16 (dd, 1H, H-3), $J_{1,1'}=11$ Hz, $J_{1,2}=6$ Hz, $J_{1',2}=5.5$ Hz, $J_{2,3}=5$ Hz, $J_{3,4}=3$ Hz, $J_{4,5}=5.5$ Hz, $J_{5,6'}=6.3$ Hz, $J_{5,6}=12$ Hz, $J_{6,6'}=14$ Hz. **11**· AcOH: ^{13}C NMR (CD_3OD) δ 20.7, 22.5 (CH_3), 42.1 (C-6), 61.7 (C-1), 63.0, 67.5 (C-2, C-5), 77.4, 79.7 (C-3, C-4), 172.5, 174.6 (CO). HRMS calcd for $C_8H_{17}N_2O_4$ (MH^+) 205.1188, found 205.1177.

6-Amino-1,5-imino-1,5,6-trideoxy-D-mannitol (3). Compound **20** (38.8 mg, 0.063 mmol) was deprotected according to procedure **D**. Purification was performed by flash column chromatography using a 5:5:0.5:0.5 mixture of CH_2Cl_2:MeOH:H_2O:AcOH as eluent; R_f 0.05. The residue was further eluted from a Dowex CG-400 (OH-) ion-exchange resin to afford **3** (6.6 mg, 65%). $[\alpha]_D$ −37 (*c* 0.67, MeOH). ^1H NMR (CD_3OD) δ 2.33 (m, 1H, H-5), 2.71 (m, 2H, H-1, H-6), 2.98 (m, 2H, H-1′, H-6′), 3.36 (m, 2H, H-3, H-4), 3.84 (m, 1H, H-2), $J_{1,1'}=J_{6,6'}=13$ Hz; ^{13}C NMR (CD_3OD) δ 44.1 (C-6), 50.7 (C-1), 63.1 (C-5), 70.8 (C-2), 72.3, 76.7 (C-3, C-4). CIMS, NH_3: *m*/*z* (%) 163 (100) (MH^+).

6-*N*-Acetylamino-1,5-imino-1,5,6-trideoxy-D-mannitol (12). Compound **21** (61.56 mg, 0.1405 mmol) was deprotected according to procedure **D**. After evaporation of ammonia the residue was diluted with a solution of CH_3OH: AcOH and filtered. Flash chromatography using a 7:3: 0.6:0.3 mixture of CH_2Cl_2:MeOH:H_2O:AcOH as eluent; R_f 0.30, followed by neutralization from a Dowex 1X8 gave the title compound **12** (24 mg, 81%). $[\alpha]_D$ −30 (*c* 0.8, H_2O). ^1H NMR (D_2O) 2.07 (s, 3H, CH_3) 2.6 (ddd, 1H, H-5), 2.75 (dd, 1H, H-1), 3.01 (dd, 1H, H-1′), 3.39 (dd, 1H, H-6), 3.48 (t, 1H, H-4), 3.56 (dd, 1H, H-6′), 3.6 (dd, 1H, H-3), 4.03 (m, 1H, H-2), $J_{1,1'}=J_{6,6'}=14.4$ Hz, $J_{1,2}=1.5$ Hz, $J_{1',2}=2.5$ Hz, $J_{2,3}=J_{5,6'}=3$ Hz, $J_{3,4}=J_{4,5}=9.5$ Hz, $J_{5,6}=7.3$ Hz; ^{13}C NMR (D_2O) δ 24.8 (CH_3), 43.4 (C-6), 51.3 (C-1), 62.2 (C-5), 72.0 (C-2), 73.0 (C-4), 77.1 (C-3), 177.6 (CO).

Inhibition studies

All enzymes, α-glucosidase type IV from brewers yeast (EC 3.2.1.20), β-glucosidase from almonds (EC 3.2.1.21),

α-galactosidase from *Aspergillus niger* (EC 3.2.1.22), α-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.

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 IC_{50} determinations, substrate concentrations were the K_M values and inhibitors in 10 μL MeOH were incorporated to give final concentrations ranging from 0.1 to 10 K_M. For K_i determinations, substrates, as their salt form, 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 add 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 Linearwearer–Burck method.

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