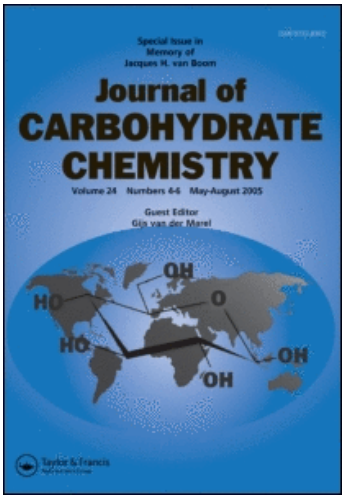

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BIOLOGICALLY ACTIVE 1-AMINODEOXY AND 1-*O*-ALKYL DERIVATIVES OF THE POWERFUL D-GLUCOSIDASE INHIBITOR 2,5-DIDEOXY-2,5-IMINO-D-MANNITOL

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ABSTRACT

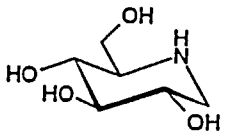
By an Amadori rearrangement of easily available 5-azido-5-deoxy-D-glucofuranose with dibenzylamine and subsequent catalytic hydrogenation of the resulting 5-azido-1-(*N*,*N*-dibenzyl)amino-1,5-dideoxy-D-fructopyranose, 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol was obtained in only two steps and in excellent overall yield. Likewise, other amines were employed to introduce extended side chains ultimately suitable for attachment of the inhibitor to solid supports. The reported rearrangement reaction is a high yielding, convenient and apparently general entry to 1-aminodeoxyketopyranoses modified at C-5, facilitated by the ring enlargement of the aldofuranose to the ketopyranose as an additional driving force. A range of selected chain extended analogues was prepared by acylation of N-1. Inhibitors obtained exhibit K_i-values with D-glucosidases in the micromolar range. Interestingly, 1-*N*-acylation resulted in superior inhibitory activities, as did the addition of a hexyl chain.

INTRODUCTION

Sugar analogues with basic nitrogen instead of oxygen in the ring have been discovered as natural products and have attracted considerable attention due to their glycosidase inhibitory properties, leading to notable biological effects.[1] Various powerful analogues and derivatives have been synthesized by chemical as well as enzymatic methods.[2]

Regarding glucosidase inhibitory activities, some of the most important representatives in this series of compounds are the six-membered ring 1-deoxynojirimycin (**1**), the structurally closely related bicyclic system castanospermine (**2**), as well as the five-membered ring 2,5-dideoxy-2,5-imino-D-mannitol (**3**), the latter molecule featuring a C_2-axis of symmetry (Figure 1). Compounds **1** and **2** are stereochemically closely related to D-glucose, the hydroxyl group at C-1 in castanospermine probably being related to OH-6 in 1-deoxynojirimycin and, in contrast to the latter, being locked in its position by the bicyclic system. Furthermore, compounds **1** and **3** exhibit practically isosteric arrangements of their functional groups, the primary hydroxyl group OH-1 of the five-membered ring being superimposable with OH-2 of the 1-deoxynojirimycin system. This fact finds additional support in the biological activities of compound **3** against D-glucosidases[3] as well as its unnatural 1-acetamido-1-deoxy derivative **4** with D-hexosaminidases.[4] The higher inhibitory activity of pyrrolidine **3** as compared to piperidine **1** was attributed to a closer relationship of the flatter five-membered ring to the oxocarbenium ion in the proposed transition state of enzymatic glucoside hydrolysis.[3]

In the context of our interest in structure-activity relationships of various non-natural derivatives of the natural products under consideration, we have been interested in the close structural relationship of compounds **1** and **3** on the one hand and the effect of non-natural substituents displacing hydroxyl groups in such systems on the other. Recently, we found that modifications at C-1 of pyrrolidine **3** such as deoxyfluorination or *O*-methylation do not dramatically reduce its inhibitory power.[5] Aiming at a more refined picture of structure-activity relationships in this series of compounds as well as targeting potential applications of C-1 modified derivatives of iminoalditol **3**, this finding encouraged us to investigate the properties of various chain-extended analogues. Examples of such derivatives have recently been found in nature and have been reported to be potent glycosidase inhibitors.[6] Consequently, suitably functionalised extended side



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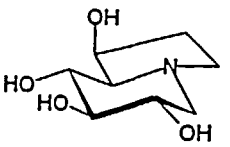

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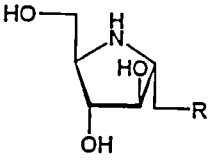


3: R = OH
4: R = NHAc

Figure 1

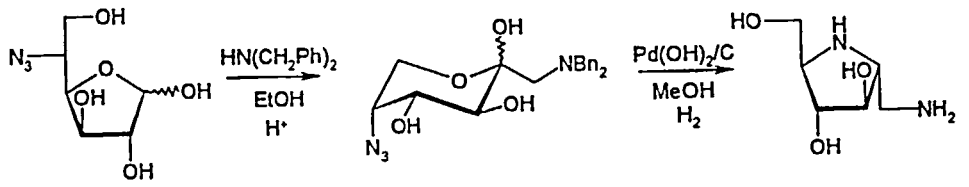
chains were assumed to be interesting spacer arms for immobilisation studies provided they would not cause significant loss of inhibitory activities.

Immobilised glycosidase inhibitors such as the *N*-(6-carboxy)hexyl derivative of 1-deoxynojirimycin (**1**) attached to aminohexylsepharose have found application as ligands for the purification of glycosidases by affinity chromatography.[7] Traditionally, the ring nitrogen has been employed as the anchor functionality to attach spacer arms which in turn have been linked to suitable solid supports. In a similar fashion, free amine moieties could offer useful anchor group properties as an alternative means of immobilisation on polymer supports without compromising interactions with the key endocylic nitrogen atom.

RESULTS AND DISCUSSION

In the course of a project concerned with the synthesis of such novel glycosidase inhibitor analogues, we became interested in a simple, reasonably versatile, and efficient synthetic route to 1-aminodeoxy derivatives of 2,5-dideoxy-2,5-imino-D-mannitol (**3**), a powerful inhibitor of glucosidases and invertase,[3] for example, compound **5** (Scheme 1).

5-Azido-5-deoxy-D-glucofuranose (**6**, Scheme 1), the direct precursor of compound **3**, is available in multi-gramme quantities[8] through a six-step synthesis from D-glucofuranurono-6,3-lactone. Bearing in mind that the release of ring strain in 5-membered ring **6** is a strong driving force for the quantitative isomerisation[9] into the corresponding D-fructopyranose isomer, we expected that an Amadori rearrangement[10] reaction would introduce the desired amino group at C-1 with concomitant formation of



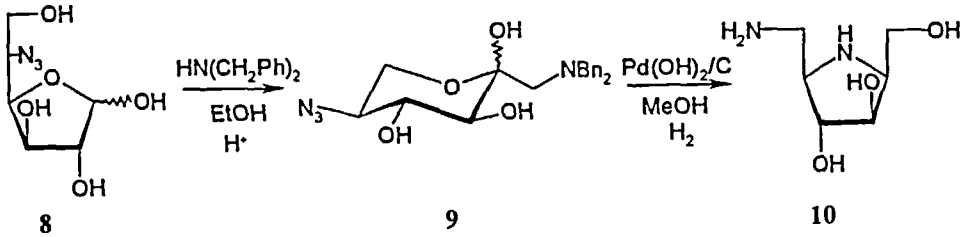
6 7 5

Scheme 1

the D-fructopyranose derivative **7** which, in turn, could be cyclized by intramolecular reductive amination to furnish inhibitor **5**. Gratifyingly, by reaction of glucofuranose **6** with dibenzylamine in EtOH in the presence of glacial acetic acid at 40 °C, compound **7** was obtained in over 90% yield.[11] Ring closure to pyrrolidine derivative **5** and removal of the *N*-benzyl groups were achieved by hydrogenolysis of compound **7** in dry MeOH at ambient pressure in the presence of $Pd(OH)_2$-on-carbon (20%). The corresponding 1-acetamido derivative **4**, previously synthesised by a chemo-enzymatic approach and reported to be a potent hexosaminidase inhibitor,[4] was prepared (80% yield) by treatment with acetic anhydride in DMSO or, more conveniently, employing MeOH as the solvent system.[11] Under these conditions, the primary amine was highly chemo- and regioselectively *N*-acetylated in the presence of the unprotected imine function of the pyrrolidine ring.

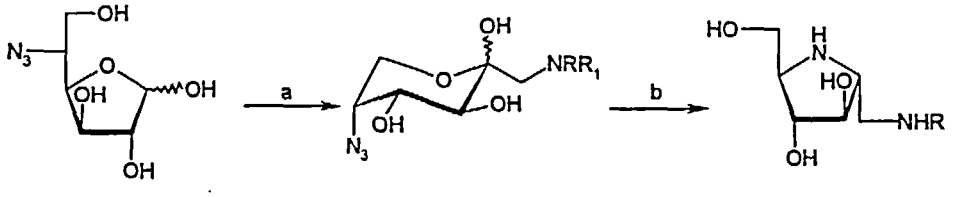
When 5-azido-5-deoxy-L-idofuranose[9] (**8**), the epimer of **5** at C-5, was subjected to the same reaction conditions, the corresponding 1-aminodeoxy derivative of L-sorbopyranose (**9**) could be isolated in 80% yield. From compound **8**, the new 6-amino-2,5,6-trideoxy-2,5-imino-D-glucitol (**10**), recently also prepared by Duréault and co-workers,[12] was readily available[11] (Scheme 2).

Compound **6** was successfully reacted with terminally functionalised amines such as 6-aminohexanol to give D-fructose derivative **11** (over 90% isolated yield) and 3-(benzylamino)propionitrile to furnish compound **12** (80% yield after chromatography). From these, inhibitors **13** and **14** were obtained by conventional catalytic hydrogenation (Scheme 3). Interestingly, these aminodeoxy derivatives of parent compound **3** were found to exhibit comparable or even better inhibitory activities with β-glucosidase from



8 9 10

Scheme 2



6

11: R = $(CH_2)_6OH$, R_1 = H
12: R = $(CH_2)_2CN$, R_1 = Bn

13: R = $(CH_2)_6OH$
14: R = $(CH_2)_3NH_2$

a) $HNRR_1$, MeOH, AcOH, 40 °C; b) H_2, $Pd(OH)_2/C$, MeOH

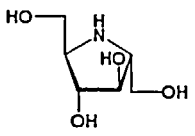
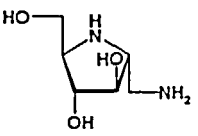
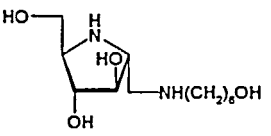
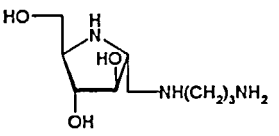
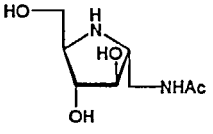
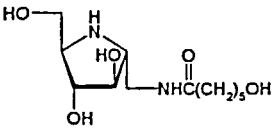
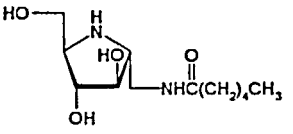
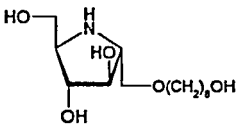
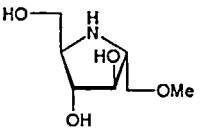
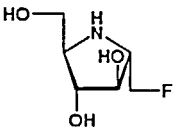
Scheme 3

Agrobacterium sp. than the simple deoxyfluoro and 1-*O*-methyl analogues previously investigated (Table). This result encouraged us to address various structural aspects and their potential influence on inhibitory activities.

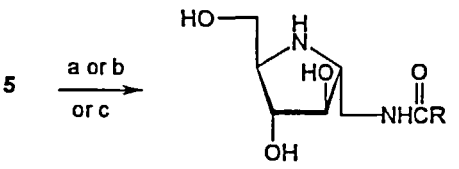
The significance of the basicity of the 1-amino group for inhibitory activity was addressed with the 6-hydroxyhexanoyl amide **15** which was prepared from compound **5** by reaction with 6-hydroxyhexanoic lactone. Much to our surprise **15** was found to be considerably more potent than compound **13**.

The importance of the terminal hydroxyl group in compounds **13** and **15** for inhibitory activity was probed with the unfunctionalised hexanoic amide **16** which was synthesised by reaction of primary amine **5** with hexanoic anhydride in methanol. At this stage, compound **4** was also included in the study (Scheme 4).

Table. K_i-values of compounds with β-glucosidase from *Agrobacterium* sp. at pH 6

			
3	5	13	14
0.2 μM	25 μM	10 μM	15 μM
			
4	15	16	
6.5 μM	0.3 μM	0.1 μM	
			
17	18	19	
1.2 μM	10 μM	30 μM	

To gain additional information, 6'-hydroxyhexyl ether derivative **17** was prepared by analogy to a previously[5] established approach according to Scheme 5. Partially protected azidodeoxysugar **18** was reacted with *O*-methoxymethyl protected 6-bromohexanol in the presence of sodium hydride in a mixture of DMF/THF 1:4. Subsequent deprotection of intermediate **19** with acidic ion exchange resin Amberlite IR 120 in aqueous acetonitrile furnished free azidodeoxyglucose derivative **20** as a 1:1 mixture of anomers. Treatment of compound **20** with glucose isomerase (Sweetzyme T from Novo) at 70 °C led to a 3:1 mixture of open-chain fructose derivative **21** and



15: R = $(CH_2)_5OH$

16: R = $(CH_2)_4CH_3$

4: R = CH_3

a) 6-hydroxyhexanoic lactone, MeOH, b) hexanoic anhydride, MeOH, c) Ac_2O, MeOH

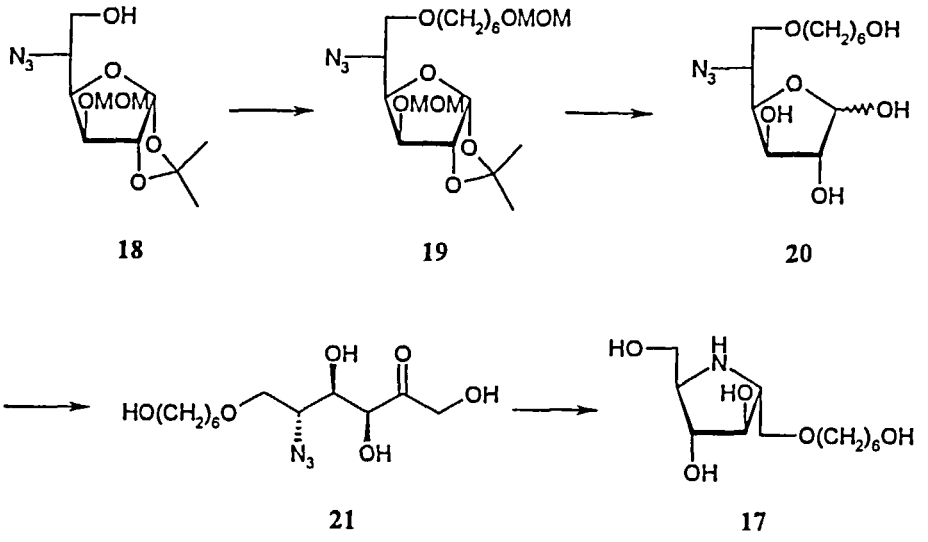
Scheme 4

unreacted starting material. Conventional chromatographic separation failed in this particular case but after bromine oxidation of aldose **20** to the corresponding lactone, the desired ketose could easily be isolated. Conventional ring closure by hydrogenation and concomitant intramolecular reductive amination gave inhibitor **17** in 30% yield after chromatography.

Inhibitory activities (IC_{50} at pH 6.2) of compound **5** were probed with β-glucosidase from bitter almonds (32 μM), α-glucosidase from rice (49 μM), α-glucosidase from mouse small intestine (102 μM) as well as maltase from bakers yeast (215 μM).

Inhibition constants (K_i) with β-glucosidase from *Agrobacterium* sp. of pyrrolidines **4** and **5** as well as chain extended analogues **13 - 17** are summarized in the Table. For comparison, data of previously reported[5] 1-*O*-methyl (**18**) and 1-deoxyfluoro (**19**) derivatives of parent compound **3** are included.

Most reversible glycosidase inhibitors are basic sugar analogues which presumably bind tightly in the active site through electrostatic interactions with carboxylate residues therein. It seemed possible, therefore, that the introduction of a second amino group into the substrate could provide yet better inhibition. The results in the Table clearly show this not to be the case, at least for the *Agrobacterium* sp. β-glucosidase. Interactions around C-1 of the inhibitor appear to be important, since simple modification of the hydroxyl



18 19 20

21 17

Scheme 5

group (methylation, deoxyfluorination or deoxyamination) decrease inhibitory activity approximately 100 fold. This decrease can be compensated for by the introduction of an alkyl chain onto C-1 of the inhibitor, presumably as a consequence of hydrophobic association of the alkyl chain with the protein surface. Indeed, the best inhibition (K_i = 0.1 μM) is seen with the compound terminating in a simple methyl group. These results clearly indicate that chain-extended derivatives **13**, **15**, and **17** of inhibitor **3** are eminently suitable for immobilisation studies.

EXPERIMENTAL

General Methods. Melting points were determined on a Tottoli apparatus (Büchi 300) and are uncorrected. Optical rotations were measured with a PERKIN ELMER 341 Polarimeter at 589 nm at ambient temp. NMR spectra were recorded at 300.13 or 200 MHz (^{1}H) as well as 75.47 or 50.29 MHz (^{13}C). Residual nondeuterated solvent was used as internal standard for determination of chemical shifts. TLC was performed on precoated aluminum plates (Merck 5554) employing 5% vanillin/sulfuric acid as well as ceric ammonium molybdate as staining agents. For column chromatography, silica gel 60,

230-400 mesh (Merck 9385), was used.

General Procedure for Amadori Reactions with 5-Azido-5-deoxyfuranoses. To a 10% solution of 5-azido-5-deoxy-D-glucose (**6**) or 5-azido-5-deoxy-L-idofuranose (**8**) in dry ethanol, the respective amine (1 eq) and glacial acetic acid (1 eq) were added and the mixture was stirred at 40 °C until TLC indicated completion of the reaction (45 min). The solvents were removed under reduced pressure and the residue was chromatographed on silica gel.

1-(*N*,*N*-Dibenzyl)amino-5-azido-1,5-dideoxy-D-fructopyranose (7). Following the general procedure for Amadori reactions, 5-azidodeoxy-D-glucose (**6**, 0.82 g, 4.00 mmol) was reacted with commercial (colourless) dibenzylamine to give a faster moving product (ethyl acetate, R_f 0.8). The reaction was completed after 45 min at 40 °C. Product **7** could be isolated (1.54 g, 99%) by silica gel chromatography (ethyl acetate/cyclohexane 1:1, v/v): $[\alpha]_D^{20}$ -83.9° (*c* 3.1, CH_2Cl_2); ^{13}C NMR ($CDCl_3$) δ 138.2, 129.4, 128.9, 128.7, 128.6, 127.5 (phenyl), 97.0 (C-2), 71.6, 71.0 (C-3, C-4), 61.8, 61.1 (C-5, C-6), 59.1 (2 C, 2 NCH_2Ph), 56.2 (C-1); ^1H NMR δ 7.41 - 7.20 (m, 10 H, 2 phenyl), 4.09 - 3.97 (m, 3 H, H-6, NCH_2Ph), 3.92 (dd, 1 H, $J_{3,4}$ 9.48 Hz, $J_{4,5}$ 3.8 Hz, H-4), 3.86 (m, 1 H, H-5), 3.65 (dd, 1 H, $J_{5,6'}$ 1.1 Hz, $J_{6,6'}$ 12.6 Hz, H-6'), 3.50 (d, 2 H, J 13.3 Hz, NCH_2Ph), 3.39 (d, 1 H, H-3), 3.07 (d, 1 H, $J_{1,1'}$ 13.6 Hz, H-1), 2.73 (d, 1 H, H-1').

Anal. Calcd for $C_{20}H_{24}N_4O_4$ (384.44): C, 62.49; H, 6.29. Found: C, 62.37; H, 6.33.

1-Amino-1,2,5-trideoxy-2,5-imino-D-mannitol (5). To a 5% solution of 1-(*N*,*N*-dibenzyl)amino-5-azido-1,5-dideoxy-D-fructopyranose (**7**, 2.01 g, 5.23 mmol) in dry methanol, $Pd(OH)_2/C$ (20%, 0.2 g) was added and the reaction was stirred under an atmosphere of hydrogen at ambient pressure until TLC ($CHCl_3$/MeOH/NH_4OH 1:2:1, v/v/v, R_f 0.4) indicated its completion (4 d). The catalyst was removed by filtration, the solution was concentrated under reduced pressure and the crude product was purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 6.5:5:1, v/v/v) to give compound **5** (442 mg, 52%): $[\alpha]_D^{20}$ (free base) +49.4° (*c* 0.9, H_2O); ^{13}C NMR (free base in D_2O) δ 79.5, 77.8 (C-3, C-4), 62.0, 61.9, 61.6 (C-2, C-5, C-6), 34.6 (C-1); ^{13}C NMR (D_2O/HCl, pH 1) δ 76.3, 73.7 (C-3, C-4), 63.3, 58.3, 58.0 (C-2, C-5, C-6), 38.6 (C-1); ^1H NMR (free base in D_2O) δ 4.27 - 3.93 (m, 2 H, H-3, H-4), 3.81 (dd, 1 H, $J_{6,6'}$ 12.5 Hz, $J_{5,6}$ 4.1 Hz, H-6),

3.69 (dd, 1 H, $J_{5,6'}$ 6.2 Hz, H-6'), 3.68 - 3.48 (m, 2 H, H-2, H-5), 3.39 (d, 2 H, $J_{1,2}$ 6.8 Hz, H-1, H-1').

Anal. Calcd for $C_6H_{14}N_2O_3$ (162.19): C, 44.44; H, 8.70. Found: C, 44.37; H, 8.85.

1-Acetamido-1,2,5-trideoxy-2,5-imino-D-mannitol (4). To a 5% solution of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol (**5**, 48.8 mg, 0.3 mmol) in dry methanol, acetic anhydride (1 eq) was added at 0 °C. After 30 min, TLC revealed that the reaction was completed ($CHCl_3$/MeOH/NH_4OH 2:2:1, v/v/v, R_f 0.6). Compound **4** (37 mg, 60%) was isolated by silica gel chromatography ($CHCl_3$/MeOH 5:1, v/v, containing 1% of conc NH_4OH): $[\alpha]_D^{20}$ +36.3° (*c* 1.1, H_2O); ^{13}C NMR (free base, D_2O) δ: 175.4 (C=O), 80.2, 78.6 (C-3, C-4), 62.7, 62.4, 60.5 (C-2, C-5, C-6), 42.7 (C-1), 22.7 (CH_3); ^{13}C NMR (D_2O/HCl, pH 1) δ 176.6 (C=O), 76.3, 74.9 (C-3, C-4), 63.4, 62.1, 58.7 (C-2, C-5, C-6), 39.4 (C-1), 22.5 (CH_3); ^1H NMR (free base, D_2O) δ 3.92 - 3.79 (m, 2 H, H-3, H-4), 3.76 (dd, 1 H, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 11.6 Hz, H-6), 3.67 (dd, $J_{5,6'}$ 6.1 Hz, H-6'), 3.46 (dd, 1 H, $J_{1,1'}$ 14.0 Hz, $J_{1,2}$ 5.1 Hz, H-1), 3.34 (dd, 1 H, $J_{1',2}$ 7.2 Hz, H-1'), 3.18-3.01 (m, 2 H, H-2, H-5), 2.05 (s, 3 H, CH_3).

Anal. Calcd for $C_8H_{16}N_2O_4$ (204.23): C, 47.05; H, 7.90. Found: C, 47.10; H, 7.98.

5-Azido-1,5-dideoxy-1-(6-hydroxyhexyl)amino-D-fructopyranose (11). Applying the general procedure to 5-azidodeoxyglucose **6** (490 mg, 2.39 mmol) employing 6-aminohexanol (1.1 eq) gave product **11** (700 mg, 96%) after purification by silica gel chromatography (ethyl acetate/MeOH 10:1, v/v, containing 1% of triethylamine): $[\alpha]_D^{20}$ -53.3° (*c* 1.8, MeOH); ^{13}C NMR (MeOH-d_4) δ 96.8 (C-2), 72.2, 71.3 (C-3, C-4), 64.2, 62.7 (2 C), (C-5, C-6, C-6'), 54.4 (C-1), 47.6 (C-1'), 33.4, 27.5, 26.9, 26.5 (C-2', C-3', C-4', C-5'); ^1H NMR δ 4.07 (dd, 1 H, $J_{3,4}$ 9.5 Hz, $J_{4,5}$ 3.8 Hz, H-4), 4.04 (dd, 1 H, $J_{5,6}$ 1.7 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 3.91 (m, 1 H, H-5), 3.69 (dd, 1 H, $J_{5,6'}$ 1.7 Hz, H-6'), 3.65 (d, 1 H, H-3), 3.56 (t, 2 H, J 6.3 Hz, 2 H-6"), 3.28 - 3.11 (m, 2 H, H-1, H-1'), 3.05 - 2.93 (m, 2 H, 2 H-1"), 1.80 - 1.35 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5").

5-Azido-1-(*N*-benzyl-*N*-cyanoethylene)amino-1,5-dideoxy-D-fructopyranose (12). Compound **6** (2.2 g 10.7 mmol) was treated with 3-(benzylamino)propionitrile (1.1 eq) according to the general procedure. After chromatography (ethyl acetate/cyclohexane 1:3, v/v) product **12** (3.52 g, 95%) was obtained as a faintly yellow oil: $[\alpha]_D^{20}$ -87.9° (*c* 2.9, CH_2Cl_2); ^{13}C NMR ($CDCl_3$) δ:137.4, 129.1, 128.8, 127.9 (Phenyl), 118.9 (CN), 97.2

(C-2), 71.2, 70.8 (C-3, C-4), 62.2, 61.1, 60.4, 57.8 (C-1, C-5, C-6, $N\underline{C}H_2Ph$), 50.3 ($N\underline{C}H_2CH_2CN$), 17.0 ($NCH_2\underline{C}H_2CN$). ^1H NMR δ 7.40 - 7.20 (m, 5 H, Phenyl), 4.16 - 3.92 (m, 4 H, H-4, H-5, H-6, H-6'), 3.64 (d, 2 H, CH_2Ph), 3.49 (d, 1 H, $J_{3,4}$ 9.6Hz, H-3), 3.31 (d, 1 H. $J_{1,1'}$ 13.8 Hz, H-1), 3.02 (m, 1 H, 1 $NC\underline{H}_2CH_2CN$), 2.79 (m, 1 H, 1 $NC\underline{H}_2CH_2CN$), 2.75 (d, 1 H, H-1'), 2.43 (t, 2 H, 2 $NCH_2C\underline{H}_2CN$).

1,2,5-Trideoxy-1-(6-hydroxyhexyl)amino-2,5-imino-D-mannitol (13). To a 5% solution of compound **11** (135 mg, 0.44 mmol) in dry methanol, $Pd(OH)_2/C$ (20%, 100 mg) was added and the mixture was stirred under an atmosphere of hydrogen at ambient pressure until TLC ($CHCl_3$/MeOH/NH_4OH 1:2:1, v/v/v) indicated the completion of the hydrogenation (2 h). The product (30 mg 26%) could be obtained by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 2:2:1, v/v/v) as a white foam. $[\alpha]_D^{20}$+22.3° (*c* 1.7, H_2O); ^{13}C NMR (D_2O) δ 76.2, 73.6 (C-3, C-4), 63.4, 61.5 (C-6, C-6'), 58.0, 57.6 (C-2, C-5), 48.5. 46.2 (C-1, C-1'), 30.7 (C-5'), 25.1 (2 C) 24.2 (C-2', C-3', C-4'). ^1H NMR δ 3.87 - 3.50 (m, 6 H, H-3, H-4, H-6, H-6', 2 H-6"), 3.13 - 2.92 (m, 2 H, H-2, H-5), 2.85 - 2.52 (m, 4 H, H-1, H-1', 2 H-1"), 1.69 - 1.20 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5"). ^1H NMR (D_2O/HCl, pH 1): δ 3.89 - 3.73 (m, 2 H, H-3, H-4), 3.70 - 3.17 (m, 8 H, H-1, H-1', H-2, H-5, H-6, H-6', 2 H-6"), 2.82 (t, 2 H, 2 H-1"), 1.51 - 0.94 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5").

Anal. Calcd for $C_{12}H_{26}N_2O_4$ (262.43): C, 54.92; H, 9.99. Found: C, 55.01; H, 10.11.

1-(1-Aminopropyl)amino-1,2,5-trideoxy-2,5-imino-D-mannitol (14). The hydrogenolysis of D-fructose derivative **12** (216 mg, 0.62 mmol) was carried out in a 5% solution in dry methanol with $Pd(OH)_2/C$ (20%, 100 mg) under an atmosphere of hydrogen at ambient pressure. When TLC ($CHCl_3$/MeOH/NH_4OH 1:2:1, v/v) indicated complete conversion of the starting material (24 h), the catalyst was removed by filtration and the product was purified by silica gel chromatography ($CHCl_3$/MeOH 2:1, v/v, containing 5% NH_4OH). The product **14** [42.0 mg (31%)] was obtained: $[\alpha]_D^{20}$ +2° (*c* 0.9, H_2O); ^{13}C NMR (D_2O/HCl, pH 1) δ 76.5, 72.7, 72.4, 66.1 (C-2, C-3, C-4, C-5), 56.2, 51.2, 46.6, 43.7 (C-1, C-6, C-1', C-3'), 22.0 (C-2'). ^1H NMR δ 4.16 - 3.68 (m, 8 H, H-1, H-1', H-2, H-3, H-4, H-5, H-6, H-6'), 3.58 - 3.41 (m, 3 H, H-1', 2 H-3'), 3.30 - 2.96 (m, 1 H, H-1'), 2.45 - 1.98 (m, 2 H, 2 H-2').

Anal. Calcd for $C_9H_{21}N_3O_3$ (219.3): C, 49.29; H, 9.65. Found: C, 49.05; H, 9.71.

1-Amino-1,2,5-trideoxy-*N*-(6-hydroxyhexanoyl)-2,5-imino-D-mannitol (15). Iminomannitol **5** (41 mg, 0.25 mmol) was dissolved in dry methanol (7 mL) and excess 6-caprolactone was added. The mixture was stirred at 70 °C for 2 h. After quantitative conversion of compound **5** into a faster moving main product, the reaction mixture was diluted with distilled water and washed with ethyl acetate. The aqueous phase was separated and concentrated under reduced pressure. Purification by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 6:2:1 v/v/v) of the resulting crude product gave inhibitor **15** (20.3 mg, 29%) as a white glassy material: $[\alpha]_D^{20}$ +26.5° (*c* 0.3, H_2O); ^{13}C NMR (D_2O/HCl pH 1) δ 179.7 (CO), 76.7, 75.2 (C-3, C-4), 63.8, 62.5 (2 C), 62.4, 58.9 (C-1, C-2, C-5, C-6, C-6'), 39.7 (C-2'), 36.3, 31.8, 25.6 (C-3', C-4', C-5'). ^1H NMR δ 4.10 - 3.45 (m, 10 H, H-1, H-1', H-2, H-3, H-4, H-5, H-6, H-6', 2 H-6"), 2.37 - 2.15 (m, 2 H, 2 H-2"), 1.65 - 1. 15 (m, 6 H, 2 H-3", 2 H-4", 2 H-5").

Anal. Calcd for $C_{12}H_{24}N_2O_5$ (276.33): C, 52.16; H, 8.75. Found: C, 52.07; H, 8.93.

1-Amino-1,2,5-trideoxy-1-*N*-hexanoyl-2,5-imino-D-mannitol (16). To a 5% solution of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol (**5**, 45.6 mg, 0.3 mmol) in dry methanol, hexanoic anhydride (1 eq) was added at 0 °C. After 30 min, TLC (MeOH/$CHCl_3$/NH_4OH 2:2:1, v/v/v, R_f 0.6) revealed that the reaction was completed. Compound **16** (71 mg, 97%) was isolated by silica gel chromatography ($CHCl_3$/ MeOH 4:1, v/v, containing 1% of concd NH_4OH): $[\alpha]_D^{20}$ +40° (*c* 0.9, H_2O); ^{13}C NMR (free base in D_2O) δ 178.6 (C-1'), 80.2, 78.5 (C-3, C-4), 62.7, 62.4, 60.6 (C-2, C-5, C-6), 42.5 (C-1), 36.6, 31.3, 25.9, 22.5, 14.0 (C-2', C-3', C-4', C-5', C-6'). ^1H NMR δ 3.77 - 3.69 (m, 2 H, H-3, H-4), 3.62 (dd, 1 H, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 11.6 Hz, H-6), 3.52 (dd, $J_{5,6'}$ 6.0 Hz, H-6'), 3.33 (dd, 1 H, $J_{1,1'}$13.8 Hz, $J_{1,2}$ 6.7 Hz, H-1), 3.22 (dd, 1 H, $J_{1',2}$ 5.4 Hz, H-1'), 2.89 - 2.88 (m, 2 H, H-2, H-5) 2.18 (t, 2 H, J 7.4 Hz, H-2"), 1.51 (m, 2 H, H-3"), 1.22 (m, 4 H, H-4", H-5"), 0.78 (t, 3 H, J 6.7 Hz, H-6").

Anal: Calcd for $C_{12}H_{24}N_2O_4$ (260.33): C, 55.37; H, 9.29. Found: C, 55.28; H, 9.37.

6-Bromo-1-*O*-methoxymethylhexanol. To a 10% solution of 6-bromo-1-hexanol (8.0 g, 44.2 mmol) in CH_2Cl_2, freshly prepared chloromethyl methyl ether (4 eq) and *N*-

ethyldiisopropylamine (5 eq) were added and the reaction mixture was stirred at room temperature until TLC (cyclohexane/ethyl acetate 2:1, v/v) indicated completion of the reaction. The reaction mixture was washed with 5% aq HCl and sat aq $NaHCO_3$ and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure. Chromatography (cyclohexane/ethyl acetate 50:1, v/v) of the oily residue gave pure product (9.90 g, 99%). ^{13}C NMR ($CDCl_3$) δ 96.6 (MOM), 67.8 (C-6), 55.3 (MOM), 34.0, 32.9, 29.8, 28.2, 25.7 (C-1, C-2, C-3, C-4, C-5). ^1H NMR δ 4.6 (s, 2 H, MOM), 3.60 - 3.35 (m, 4 H, 2 H-1, 2 H-6), 3.33 (s, 3 H, MOM), 1.95 - 1.30 (m, 8 H, 2 H-2, 2 H-3, 2 H-4, 2 H-5).

5-Azido-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-6-*O*-(6′-*O*-methoxymethylhexyl)-α-D-glucofuranose (19). To a 5% solution of intermediate **18**[5] (3.57 g, 12.34 mmol) in THF containing 20% of DMF, sodium hydride (80% dispersion in oil, 3 eq) and 6-bromo-1-*O*-methoxymethylhexanol (1.5 eq) were added and the mixture was stirred at room temperature until the starting material was no longer detectable by TLC (6 h). The heterogeneous mixture was quenched by addition of excess MeOH. Solvents were evaporated under reduced pressure, the remaining residue was diluted with CH_2Cl_2, consecutively washed with 5% aq HCl and with sat aq $NaHCO_3$ and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane/ethyl acetate 10:1 v/v) to yield pure compound **19** (4.61 g, 87%). $[\alpha]_D^{20}$ -18.6° (*c* 1.3 $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 112.1 (Isopr), 105.2 (C-1), 96.7, 96.4 (2 MOM), 82.7, 80.1, 87.3 71.6, 71.5, 67.8 (C-2, C-3, C-4, C-6, C-1', C-6'), 58.7 (C-5), 56.1, 55.1 (2 MOM), 29.7, 29.6, 26.8, 26.3, 26.1, 25.9 (C-2', C-3', C-4', C-5', Isopr). ^1H NMR δ 5.86 (d, 1 H, $J_{1,2}$ 3.6, H-1), 4.74, 4.60 (2 s, 4 H, 2 MOM), 4.58 (d, 1 H, H-2), 4.15 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-3), 4.02 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.85 (m, 2 H, H-5, H-6), 3.60 (m, 1 H, H-6'), 3.58 - 3.40 (m, 4 H, 2 H-1", 2 H-6"), 3.41, 3.32 (2 s, 6 H, 2 MOM), 1.70 - 1.25 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5"), 1.46, 1.30 (2 s, 6 H, Isopr).

Anal. Calcd for $C_{19}H_{35}N_3O_8$ (433.13): C, 52.69; H, 8.14. Found: C, 52.72; H, 8.20.

5-Azido-5-deoxy-6-*O*-(6'-hydroxy)hexyl-D-glucofuranose (20). A 5% solution of protected intermediate **19** (1.74 g, 4.01 mmol) in 50% aq CH_3CN was stirred with 15 mL of ion-exchange resin Amberlite IR-120 [H^+] at 45 °C until TLC showed quantitative

conversion (3 d) into a single, slower moving product. The resin was removed by filtration, the filtrate was concentrated under reduced pressure, and the crude product was purified by silica gel chromatography (ethyl acetate) to give free D-glucofuranose derivative **20** (1.18 g, 96.4%). $[\alpha]_D^{20}$ -19.7° (*c* 1.8, acetone) ^{13}C NMR (acetone-d_6) δ 104.7, 98.6 (C-1 α/β), 81.3, 81.2 (C-4 α/β), 78.4, 77.3, 77.0, 76.5, 72.8, 72.7, 72.0 (2 C), 67.9, 62.6, 61.7, 60.9 (C-2, C-3, C-5, C-6, C-1', C-6', α/β), 30.8, 30.6, 30.4, 30.0, 29.6, 26.9, 26.8, 26.6 (C-2', C-3', C-4', C-5', α/β); α/β 1:1.

Anal. Calcd for $C_{12}H_{23}N_3O_6$ (305.33): C, 47.21; H, 7.58. Found: C, 47.15; H, 7.69.

5-Azido-5-deoxy-6-*O*-(6'-hydroxy)hexyl-D-fructose (21). A 3% aq solution of free aldose **20** (2.15 g, 7.04 mmol) containing $MgSO_4$ (30 mg) was spun on a rotary evaporator in the presence of immobilised glucose isomerase (Sweetzyme T, EC 5.3.1.5, 3 g) at 60°C for 12 h. The solids were filtered off, $BaCO_3$ (3.86 g, 19.6 mmol) and bromine (1.04 g, 6.5 mmol) were added and the cloudy solution was stirred at ambient temperature until all aldose was converted into a faster moving compound (R_f 0.8). Air was bubbled through the solution to remove excess of bromine, solids were filtered off. The solution was concentrated under reduced pressure and the crude product was purified by silica gel chromatography (cyclohexane/ethyl acetate 1:4, v/v) to give pure ketose **21** (0.51 g, 23.7%): $[\alpha]_D^{20}$ -40.1° (*c* 1.2, MeOH); ^{13}C NMR (MeOH-d_4) δ 213.9 (C-2), 77.1 (C-3), 72.4, 72.3, 72.1 (C-4, C-6, C-1'), 68.1 (C-1), 63.0, 62.7 (C-6', C-5), 33.7, 30.8 (C-2', C-5'), 27.0, 26.8 (C-3', C-4'). ^{1}H NMR δ 4.57 (d, 1 H, $J_{1,1'}$ 19 Hz, H-1), 4.46 (d, 1 H, H-1'), 3.87 (t, 1 H, J 6.7 Hz, 2 H-1"), 3.78 - 3.45 (m, 7 H, H-3, H-4, H-5, H-6, H-6', 2 H-6"), 1.73 - 1.30 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5").

Anal. Calcd for $C_{12}H_{23}N_3O_6$ (305.33): C, 47.21; H, 7.58. Found: C, 47.25; H, 7.70.

2,5-Dideoxy-1-*O*-(6'-hydroxyhexyl)-2,5-imino-D-mannitol (17). Hydrogenation and concomitant intramolecular reductive amination of ketose **21** (58.6 mg, 0.19 mmol) was conducted in dry methanol in the presence of $Pd(OH)_2/C$ (20%, 100 mg) under an atmosphere of hydrogen at ambient pressure until TLC ($CHCl_3$/MeOH/NH_4OH 2:2:1) confirmed quantitative conversion (3 h). The catalyst was removed by filtration, the solution was concentrated and the resulting yellow syrup was chromatographed on silica

gel ($CHCl_3$/MeOH 10:1, v/v, containing 1% NH_4OH) to furnish inhibitor **17** (32 mg, 63%) as a colourless oil: $[\alpha]_D^{20}$ +30.1° (*c* 0.4, MeOH); ^{13}C NMR (MeOH-d_4) δ 79.9, 79.6 (C-4, C-3), 72.5, 72.4 (C-2, C-5), 64.8, 63.3, 63.0 (C-1, C-6, C-1', C-6'), 33.7 (C-5'), 30.7, 27.2, 26.8 (C-2', C-3', C-4'); ^1H NMR δ 3.77 (m, 2 H, H-3, H-4), 3.68 (dd, 1 H, $J_{6,6'}$ 11.2 Hz, $J_{5,6}$ 4.2 Hz, H-6), 3.62 - 3.40 (m, 7 H, H-6', H-1, H-1', 2 H-1", 2 H-6"), 3.15 - 2.95 (m, 2 H, H-2, H-5), 1.70 - 1.33 (m, 8 H, 2 H-2", 2 H-3", 2 H-4", 2 H-5").

Anal. Calcd for $C_{12}H_{25}NO_5$ (263.33): C, 54.73; H, 9.57. Found: C, 54.64; H, 9.65.

Enzymatic Studies. *Agrobacterium* sp. β-glucosidase was purified and assayed as described previously.[13] Kinetic studies were performed at 37 °C in pH 7.0 sodium phosphate buffer (50 mM) containing 0.1 % bovine serum albumin, using 7.2 x 10^{-5} mg/mL enzyme. Approximate values of K_i were determined using a fixed concentration of substrate, 4-nitrophenyl β-D-glucopyranoside (0.11 mM = 1.5 x K_m) and inhibitor concentrations ranging from 0.2 times to 5 times the K_i value ultimately determined. A horizontal line drawn through $1/V_{max}$ in a Dixon plot of this data (1/v versus [S]) intersects the experimental line at an inhibitor concentration equal to K_i. Full K_i determinations were performed using the same range of inhibitor concentrations while also varying substrate (4-nitrophenyl glucoside) concentrations from approximately 0.015 mM to 0.6 mM. Data were analysed by direct fit to the Michaelis Menten equation describing reaction in the presence of inhibitors using the program GraFit.[14]

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