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Two isosteric fluorinated derivatives of the powerful glucosidase inhibitors, 1-deoxynojirimycin and 2,5-dideoxy-2,5-imino-D-mannitol: Syntheses and glycosidase-inhibitory activities of 1,2,5-trideoxy-2-fluoro-1,5-imino-D-glucitol and of 1,2,5-trideoxy-1-fluoro-2,5-imino-D-mannitol [1]

Sören M. Andersen [a], Michael Ebner [b], Christian W. Ekhart [b], Günther Gradnig [b], Günter Legler [c], Inge Lundt [a], Arnold E. Stütz [b,*], Stephen G. Withers [d], Tanja Wrodnigg [b]

[a] *Department of Organic Chemistry, Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark*

[b] *Institut für Organische Chemie der Technischen Universität Graz, Stremayrgasse 16, A-8010 Graz, Austria*

[c] *Institut für Biochemie der Universität Köln, Otto-Fischer-Str. 12–14, D-50674 Köln, Germany*

[d] *Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1*

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Abstract

1,2,5-Trideoxy-2-fluoro-1,5-imino-D-glucitol, the 2-deoxyfluoro derivative of 1-deoxynojirimycin, as well as 1,2,5-trideoxy-1-fluoro-2,5-imino-D-mannitol and 2,5-dideoxy-2,5-imino-1-*O*-methyl-D-mannitol, two new analogues of the natural product and powerful glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol, were synthesised featuring glucose isomerase-catalysed aldose–ketose interconversion reactions as the key steps of the syntheses. Results of inhibition studies conducted with these compounds and previously obtained deoxyfluoro derivatives of 1-deoxynojirimycin, employing glucosidases from various sources, showed that the replacement of a hydroxyl function by fluorine caused an impairment of the

* Corresponding author. Fax: +43-316-873-8740; e-mail:stuetz@orgc.tu-graz.ac.at.

[1] Dedicated to Professor Hans Paulsen on the happy occasion of his 75th birthday.

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inhibitory potency. This effect was smallest for the hydroxyl group at C-6 and up to four orders of magnitude larger for replacements at C-2 and C-3. © 1997 Elsevier Science Ltd.

Keywords: Deoxyfluoro sugars; Glucose isomerase; Glucosidase inhibitors; Inhibitory activity

1. 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].

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. Compounds **1** and **2** are stereochemically closely related to D-glucose, the hydroxyl group at C-1 in castanospermine being equivalent to OH-6 in 1-deoxynojirimycin and, in contrast to the latter, being locked in its position by the bicyclic system. Furthermore, inspection of Dreiding models as well as computer-aided molecular modelling have revealed that 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 (Fig. 1). This finding is also supported by the biological activities of compound **3** against D-glucosidases [2] as well as its unnatural 1-acetamido-1-deoxy derivative with D-hexosaminidases [3]. The higher activity of pyrrolidine **3** as compared to piperidine **1** was attributed to a closer relationship of the flatter five-membered ring to the oxo-carbenium ion in the proposed transition state of enzymatic glucoside hydrolysis [2].

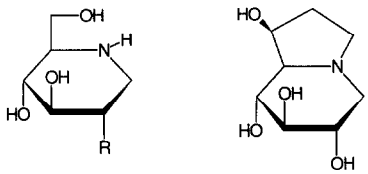
In context with our interest in structure–activity relationships of various non-natural derivatives of the natural products under consideration, we have become interested in the close structural relationship of compounds **1** and **3** on the one hand and the effect of non-natural substituents displacing hydroxyl groups in such systems on the other.

2. Results and discussion

Syntheses.—Based on previous successful approaches [4], 1,2-dideoxy-2-fluoronojirimycin [5] (1,2,5-trideoxy-2-fluoro-1,5-imino-D-glucitol, **4**) and its five-membered ring analogue (1,2,5-trideoxy-1-fluoro-2,5-imino-D-mannitol, **5**) were synthesised according to Scheme 1 involving glucose isomerase (EC 5.3.1.5) catalysed key steps. In order to cross-examine the influence of the strongly electronegative fluoro substituent on the biological activity of the five-membered ring system, 2,5-dideoxy-2,5-imino-1-*O*-methyl-D-mannitol (**6**) was prepared for comparison.

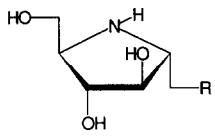
For the preparation of the 1-deoxynojirimycin derivative **4**, 5-deoxy-5-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose (**7**), easily available [6] in four simple steps from 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone, served as the starting material. Conventional 6-*O*-tritylation with chlorotriphenylmethane in pyridine, followed by addition of acetic anhydride, gave 3-*O*-acetyl-5-deoxy-5-fluoro-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-β-L-idofuranose (**8**) in a simple one-pot procedure. This compound was, without further purification, regioselectively deprotected at O-6 with the aid of $BF_3 \cdot$ diethyl ether complex in chloroform [7], to give 3-*O*-acetyl-5-deoxy-5-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose (**9**). Formation of the triflate at position-6 employing trifluoromethanesulfonic anhydride in dichloromethane–pyridine and subsequent displacement of the leaving group with the aid of sodium azide in acetone or *N*,*N*-dimethylformamide (Me_2NCHO) furnished the desired intermediate, 6-azido-5,6-dideoxy-5-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose (**10**), in good yield. Deprotection by acidic hydrolysis of the isopropylidene acetal, employing the ion-exchange resin, Amberlite IR-120 [H^+], in a mixture of acetonitrile and water, led to the corresponding free L-idofuranose derivative **11**. In the key step of this sequence, compound **11** was reacted with Sweetzyme T, an immobilised glucose isomerase (EC 5.3.1.5), to give the corresponding open-chain L-sorbose derivative **12**. The ratio at equilibrium was found to be around 3.5:1 in favour of the ketose under the conditions previously employed for the conversion of various C-5-modified D-glucofuranose as well as L-idofuranose derivatives into the corresponding pyranoid D-fructose and L-sorbose analogues [8]. Isolated yields ranged from 60 to 70%. Unreacted aldose can

be recycled to improve the overall turnover. It seems noteworthy to emphasise that the enzyme is not only able to convert this L-sugar but, moreover, that the equilibrium is distinctly favouring the open-chain ketose over the cyclic aldofuranose hemiacetal. This has previously been observed for a range of furanoid derivatives of D-glucose, modified at positions-5 and -6, which could be smoothly converted into the corresponding open-chain D-fructose analogues [4]. In all cases probed to date, the ketose adopts the open-chain state. NMR studies provided no evidence for dimerisation.



1: R = OH
4: R = F

2



3: R = OH
5: R = F
6: R = OMe

Desired ketose **12** can be separated from the starting material by chromatography, but, more conveniently, the aqueous mixture is treated with bromine to oxidise [9] the starting material to the corresponding aldonolactone to allow easier separation. Aldonolactone **13**, thus obtained, can easily be recycled by reduction with sodium boronate in aqueous methanol [10]. Conventional cyclisation of the azidoketose by catalytic hydrogenation over palladium-on-charcoal in dry methanol and concomitant intramolecular reductive amination gave the desired inhibitor **4** in good yields. Small amounts of the epimer at C-5, also formed during the reaction, could be removed by chromatography on silica gel, followed by crystallisation of the main product from ether–methanol.

Compound **4** was also conveniently made available by a sucrose-based approach, previously successful [11] in the synthesis of the powerful hexosaminidase

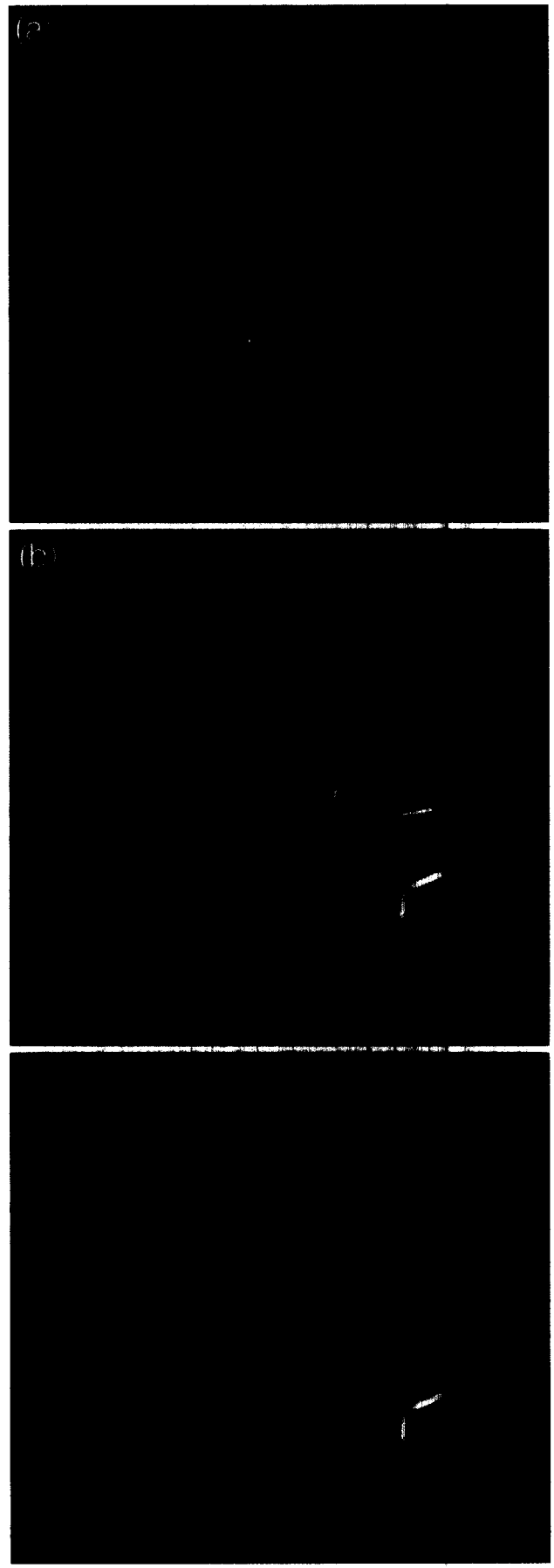
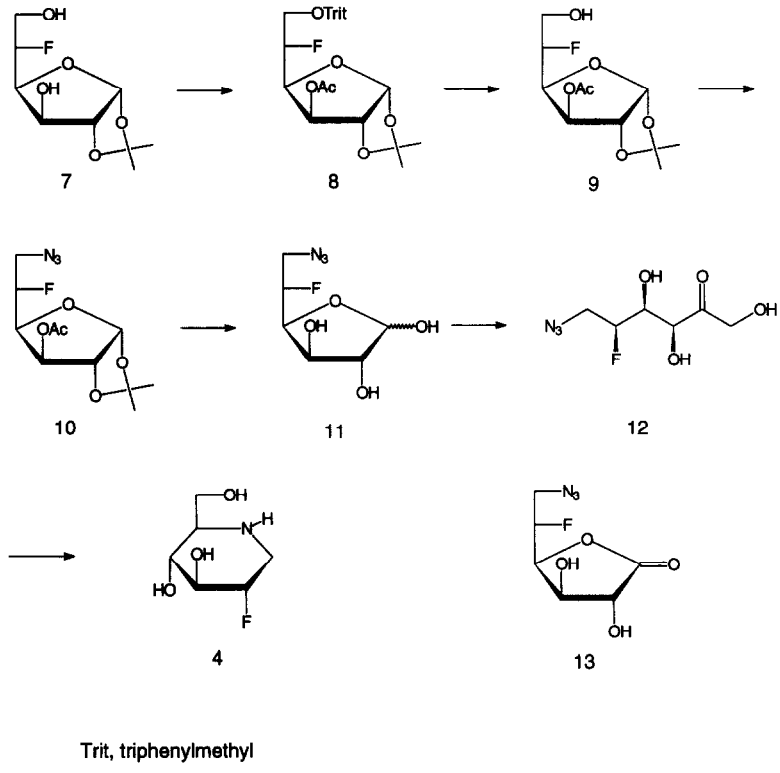


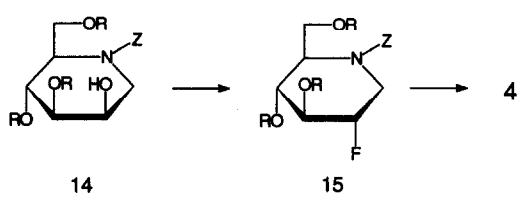
Fig. 1. Molecular modelling presentation: structural similarities of compounds **1** and **3**. (a) 1-Deoxynojirimycin (**1**). (b) Superposition of compounds **1** and **3**. (c) 2,5-Dideoxy-2,5-imino-D-mannitol (**3**).



Trit, triphenylmethyl

Scheme 1.

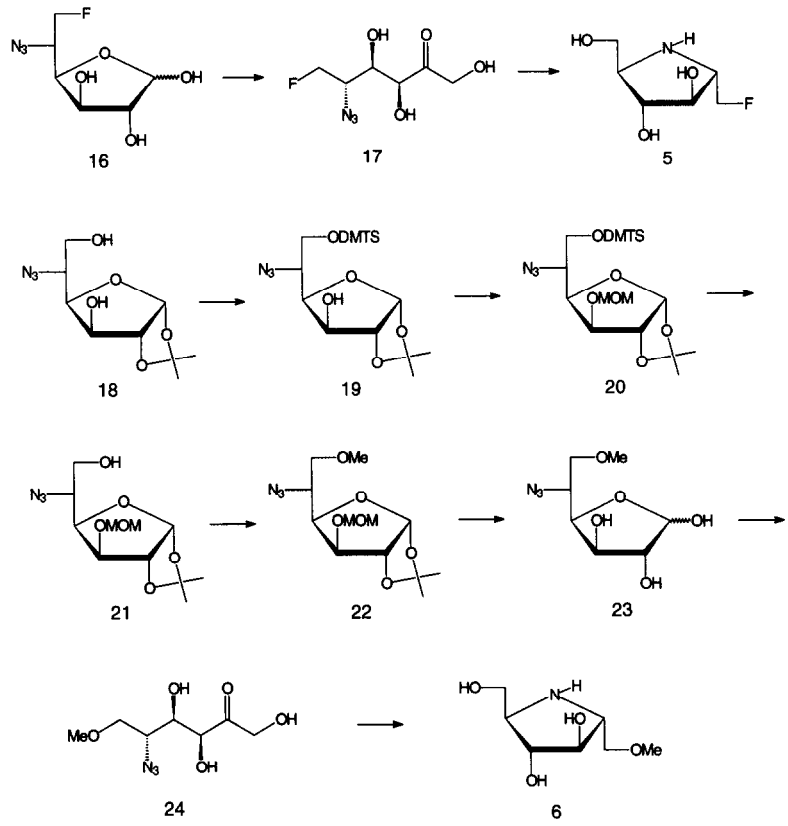
inhibitor 2-acetamido-1,2-dideoxynojirimycin (2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol) and various *N*-2-modified analogues (Scheme 2). Starting with partially protected 1,5-dideoxy-1,5-imino-D-mannitol (**14**), accessible from sucrose in six steps [11], treatment with diethylaminosulfur trifluoride (DAST) at −30 °C in dichloromethane in the presence of pyridine gave, with clean inversion of configuration at C-2, the corresponding protected 2-deoxy-2-fluoro derivative of 1,5-dideoxy-1,5-imino-D-glucitol (**15**) in good yield. In the absence of pyridine, a mixture of D-*gluco* and D-*manno* epimers in about 1:1 ratio was found to have formed under otherwise identical conditions. In keeping with previous observations [11], fully *N*,*O*-protected 1-deoxynojirimycin derivatives exhibit small vicinal ^{1}H–^{1}H-coupling constants, indicating conformational deviation from the usually preferred $^4C_1$ chair.



R, benzyl
Z, benzyloxycarbonyl

Scheme 2.

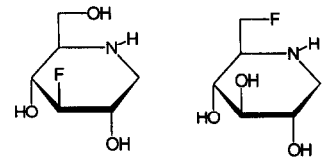
Hydrogenolytic deprotection of intermediate **15** gave product **4** in good yield. The 1-deoxyfluoro derivative of 2,5-dideoxy-2,5-imino-D-mannitol, compound **5**, was synthesised in two simple steps (Scheme 3) from known [12] 5-azido-5,6-dideoxy-6-fluoro-D-glucofuranose (**16**) (easily prepared from D-glucofuranurono-6,3-lactone) by enzymatic isomerisation into the open-chain fructose derivative **17**, followed by conventional catalytic hydrogenation of this intermediate over palladium-on-charcoal in anhydrous methanol. The corresponding 1-*O*-methyl derivative of **3**, compound **6**, was prepared from the known [13] 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**18**). Reaction of compound **18** with chlorodimethyl(1,1,2-trimethylpropyl)silane employing conventional O-silylation conditions, afforded compound **19**. This was followed by O-methoxymethylation at position-3 leading to fully protected intermediate **20**. Subsequent desilylation of O-6 using tetrabutylammonium fluoride in tetrahydrofuran (THF) furnished primary alcohol **21**. Conventional treatment of this compound with iodomethane in the presence of sodium hydride in THF furnished methyl ether **22**. Acidic deprotection gave free D-glucofuranose **23**, which could successfully be isomerised to



Scheme 3.

give open-chain D-fructose analogue **24**, which upon catalytic hydrogenation employing standard conditions was cyclised to yield inhibitor **6**.

Inhibition of glycosidases.—Activities of competitive inhibitors **4**, **5**, and **6** as well as of their parent compounds **1** and **3**, respectively, against a range of glucosidases are reported in Table 1. For comparison, data obtained from two previously reported [14,15] deoxyfluoro derivatives of 1-deoxynojirimycin, namely 1,3,5-trideoxy-3-fluoro-1,5-imino-D-glucitol (**25**) and 1,5,6-trideoxy-6-fluoro-1,5-imino-D-glucitol (**26**), are included. The most pronounced effect on enzyme–ligand interactions caused by the replacement of a hydroxyl group by fluorine [16] is the loss of a hydrogen-bond donor from the ligand. The ability of the ligand to accept hydrogen-bonds is also strongly impaired, but may not be completely extinguished. Dipole interactions with the fluorine substituent, on the other hand, are expected to be stronger, whereas van der Waals forces will be weaker because of its smaller size and polarisability.



Comparison of the inhibition constants (Table 1) for the fluorinated compounds with their hydroxylated counterparts shows, with the exception of compound **26** with yeast α-glucosidase, a general but widely differing impairment of the inhibitory potency in the deoxyfluoro derivatives. The importance of individual hydroxyl groups for enzyme–ligand interactions may be judged from ratios of K_I (fluorinated)/K_i (hydroxylated). On this basis, the

Table 1
Glycosidase-inhibitory activities: K_i values (μM)

Glucosidases	pH	Inhibitors [a]						
		1	**3**	**4**	**5**	**6**	**25**	**26**
α (Yeast)	6.0	25	n.d	2000	n.d.	n.d.	2500	19
	6.5	n.d.	0.73	n.d.	57	n.d.	n.d.	n.d.
α (Rice)	6.0	0.01	n.d.	n.d.	n.d.	n.d.	0.35	0.4
β (Almonds)	5.0	300	1.7	n.d.	260	n.d.	n.d.	n.d.
	6.0	38	n.d.	> 10,000	n.d.	n.d.	> 10,000	600
β (*Agrobacterium faecalis*)	7.0	12	0.2	350	30	10	n.d.	n.d.
β (*Asp. wentii*)	5.0	0.3	57	n.d.	190	n.d.	380	250
	6.0	0.06	n.d.	2800	n.d.	n.d.	160	n.d.
β (Bovine kidney, lysosomal)	5.0	21	44	180	n.d.	n.d.	130	n.d.
	6.0	6.0	n.d.	n.d.	n.d.	n.d.	29	n.d.
β-D-Fructofuranosidase	5.0	> 5000	6.8	n.d.	n.d.			
(invertase, yeast)	6.0	n.d.	3.5	n.d.	8.5			
	7.0	n.d.	1.1	n.d.	n.d.			

[a] n.d. = Not determined.

interactions appear to be weakest with the hydroxyl groups at C-6 and strongest with those at C-2 and C-3. Strong inhibition by basic sugar analogues involves formation of an ion pair from the protonated inhibitor and a closely positioned carboxylate group within the active site [17]. This can either occur by proton transfer to the basic form of the inhibitor or, more rarely, by binding the protonated inhibitor. A detailed interpretation of the K_I ratios therefore requires information on the ionisation state of the inhibitors. Potentiometric titrations gave pK_a 5.85 for compound **4** and pK_a 5.75 for derivative **25**. Because of a similar distance of the fluorine substituent from the basic (cationic) centre in **5** and **26**, their pK_a values will be shifted to lower figures as compared to the parent compounds **1** (pK_a 6.3) and **3** (pK_a 7.2), respectively. Corrections based on K_i values calculated with the concentration of the preferentially inhibiting species are significant only for the enzyme from *Asp. wentii* where K_I(**4**)/K_I(**1**) is 47,000 (from Table 1) and 27,000 (concentration of the cationic form) and for the almond enzyme where K_I(**4**)/K_I(**1**) > 260 (from Table 1) and > 500 (concentration of free base). Interestingly, the largest effects of the replacement of a hydroxyl group by fluorine were found with the β-glucosidases from *Asp. wentii* and almonds where the importance of the natural substituent at C-2 had been demonstrated with 2-deoxy-β-D-glucosides [18,19]. With these enzymes the replacement of the hydroxyl group by a hydrogen atom caused a 10^5- and 10^3-fold reduction of the bond cleavage rates, respectively. Substrate binding as expressed by K_m was much less effected by this structural alteration.

In the case of *Agrobacterium faecalis* β-glucosidase, a 30-fold reduction in affinity is seen upon substitution of the 2-hydroxyl of deoxynojirimycin by fluorine. Interestingly, the equivalent replacement in a substrate resulted in a 2-fold *increase* in true affinity, as shown through relative K_d values determined by pre-steady-state kinetics [20]. However, effects on catalysis were certainly deleterious. Replacement by hydrogen reduced the rate over 1000-fold. Replacement by fluorine reduced it 4000-fold if the substrate incorporated an excellent leaving group (2,4-dinitrophenolate) and at least 10^8-fold with a poorer leaving group (4-nitrophenolate) [20]. Consequently, the effects on binding of deoxynojirimycin lie between these two extremes.

2,5-Dideoxy-2,5-imino-D-mannitol (K_i = 200 nM) is one of the best inhibitors yet discovered for *A. faecalis* β-glucosidase rivalling the inhibition seen with the *N*-aryl carbamate derivatives of deoxynojirimycin (K_i = 150–1200 nM) [21]. Why this should bind so well is not obvious, but it is apparent that interactions at the 1-position are somewhat important

as O-methylation and deoxyfluorination both reduce the affinity by some 50- and 150-fold, respectively. The similar reductions in affinity might suggest that the cause of the reduction is not steric, but rather the removal of a hydrogen-bond in which the hydroxyl group at that position ordinarily acts as a hydrogen-bond donor. Neither the methoxy nor the fluoro substituent can fulfill that role. However, the actual mode of binding of compound **3** and its analogues is not all clear, despite the structural similarities of inhibitors **1** and **3** as mentioned in the Introduction.

The relatively small effect of the change from 1-OH to 1-F in compound **5** on the inhibition of the *Asp. wentii* enzyme should be seen in relation to the different effects of formally converting 1-deoxynojirimycin (**1**) into its five-membered ring analogue **3** on the inhibition of the glucosidases under consideration. Whereas this structural alteration was detrimental to the inhibition of the enzyme from *Asp. wentii*, it caused a considerable improvement of the inhibition of the other enzymes probed. The small effect caused by the replacement of the primary alcohol with the fluorine atom on the inhibition of yeast invertase would point to very weak interactions of this enzyme with the hydroxymethyl group on C-2 or C-5 of the substrate or structurally related inhibitors. Its magnitude is that expected from the two-fold reduction of binding modes caused by abolishing the C_2-symmetry of compound **3**. The two modes of binding the symmetrical compound **3** do not exist for the glucosidases because its hydroxymethyl group on C-2 is probably required for interactions with that part of the glycon binding site complementary to the C-2 hydroxyl group of glucosides.

3. Experimental

General methods.—Melting points were recorded on a Tottoli apparatus and are uncorrected. Optical rotations were measured on a JASCO Digital Polarimeter with a path length of 10 cm. NMR spectra were recorded at 200 as well as 300 MHz (^{1}H), and at 50.29 and 75.47 MHz (^{13}C). $CDCl_3$ was employed for protected compounds and D_2O or CD_3OD for free sugars and inhibitors. Chemical shifts are listed in δ employing residual, not deuterated, solvent as the internal standard. The signals of the protecting groups were found in the expected regions and are not listed explicitly. TLC was performed on precoated aluminum sheets (E. Merck 5554). TLC plates were stained with concd H_2SO_4 containing 5% vanillin. For column chromatography Silica Gel 60 (E. Merck) was used.

3-O-Acetyl-5-deoxy-5-fluoro-1,2-O-isopropylidene-6-O-triphenylmethyl-β-L-idofuranose (**8**).—To a soln of 5-deoxy-5-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose (**7**, Ref. [6], 1.48 g, 6.66 mmol) in CH_2Cl_2 (100 mL) containing 5% pyridine, chlorotriphenylmethane (4.0 g, 2.1 equiv) was added, and the soln was stirred until TLC indicated the quantitative conversion of the starting material into a faster moving compound. Pyridine (12 mL) and Ac_2O (6 mL) were added, and the mixture was kept at ambient temperature until the reaction was complete. Methanol was added, and the reaction mixture was concd under reduced pressure. The dark-coloured syrupy residue was partitioned between CH_2Cl_2 and 5% aq HCl, the organic layer was washed with satd aq $NaHCO_3$ and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave crude product that was purified by chromatography to give 3.07 g (91%) of compound **8** as white crystals: mp 155–157 °C; $[\alpha]_D^{20}$ −27.5° (*c* 1.3, EtOAc); ^{1}H NMR: δ 5.98 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.05 (1 H, d, $J_{3,4}$ 3.2 Hz, H-3), 4.85 (m, 1 H, $J_{5,F}$ 50 Hz, H-5), 4.77 (m, 1 H, $J_{4,5}$ 6.2, $J_{4,F}$ 17 Hz, H-4), 4.53 (dd, 1 H, $J_{2,F}$ 2.3 Hz, H-2), 3.55 (ddd, 1 H, $J_{5,6}$ 2.8, $J_{6,6'}$ 11, $J_{6,F}$ 20.5 Hz, H-6), 3.10 (ddd, 1 H, $J_{6',F}$ 29.7 Hz, H-6'); ^{13}C NMR: δ 104.7 (C-1), 83.5 (C-2), 91.0 ($J_{5,F}$ 177.3 Hz, C-5), 77.8 ($J_{4,F}$ 20.2 Hz, C-4), 77.1 ($J_{3,F}$ 6.8 Hz, C-3), 63.1 ($J_{6,F}$ 20.1 Hz, C-6). MS: found *m/z* 506.210, required 506.576. Anal. Calcd for $C_{30}H_{31}FO_6$: C, 71.1; H, 6.17. Found: C, 70.85; H, 6.20.

3-O-Acetyl-5-deoxy-5-fluoro-1,2-O-isopropylidene-β-L-idofuranose (**9**).—To a soln of compound **8** (2.84 g, 5.61 mmol) in CH_2Cl_2 (100 mL) boron trifluoride etherate (1 mL) and MeOH (20 mL) were added, and the mixture was kept at ambient temperature until TLC indicated the completion of the reaction. Dichloromethane (100 mL) was added, and the reaction mixture was washed with aq satd $NaHCO_3$, filtered, and concd under reduced pressure. Chromatography of the oily residue gave pure compound **9** (1.40 g, 94%): mp. 98–99 °C; $[\alpha]_D^{20}$ −17.6° (*c* 1.0, EtOAc); ^{1}H NMR: δ 5.95 (d, $J_{1,2}$ 3.9 Hz, H-1), 5.25 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.73 (dddd, 1 H, $J_{4,5}$ 5.9, $J_{5,6}$ 3.1, $J_{5,6'}$ 4.9, $J_{5,F}$ 48.4 Hz, H-5), 4.52 (dd, 1 H, $J_{2,F}$ 2.2 Hz, H-2), 4.42 (ddd, 1 H, $J_{4,F}$ 18.3 Hz, H-4), 3.82 (ddd, 1 H, $J_{6,6'}$ 12, $J_{6,F}$ 23.1 Hz, H-6), 3.74 (ddd, $J_{6',F}$ 24.1 Hz, H-6'); ^{13}C NMR: δ 104.6 (C-1), 91.6 ($J_{5,F}$ 175.4 Hz, C-5), 83.4 (C-2), 77.5 ($J_{4,F}$ 18.9

Hz, C-4), 76.4 ($J_{3,F}$ 5.7 Hz, C-3), 62.1 ($J_{6,F}$ 22.9 Hz, C-6). MS: found *m/z* 249.077 ($M - CH_3$), required 264.243. Anal. Calcd for $C_{11}H_{17}FO_6$: C, 50.0; H, 6.48. Found: C, 49.7; H, 6.54.

3-O-Acetyl-6-azido-5,6-dideoxy-5-fluoro-1,2-O-isopropylidene-β-L-idofuranose (**10**).—To a soln of compound **9** (1.01 g, 3.82 mmol) in CH_2Cl_2 (100 mL) containing 5% pyridine, trifluoromethanesulfonic anhydride (1.25 mL, 2 equiv) was added at 0 °C, and the reaction was monitored by TLC. After 30 min CH_2Cl_2 (100 mL) was added, and the reaction mixture was washed consecutively with 5% aq HCl and satd aq $NaHCO_3$ and dried (Na_2SO_4). After filtration and removal of the solvent under reduced pressure, the oily residue was dissolved in Me_2NCHO (35 mL), NaN_3 (10 equiv) was added, and the mixture was stirred at ambient temperature until TLC indicated quantitative conversion of the triflate into a single, slower moving compound. After addition of CH_2Cl_2 (150 mL), solids were filtered off. The organic layer was washed with water, dried (Na_2SO_4), and concd under reduced pressure. Chromatography of the remaining oil gave 990 mg (90%) of syrupy azidodeoxy sugar **10**: $[\alpha]_D^{20}$ −29.2° (*c* 2.7, EtOAc); ^{1}H NMR: δ 5.97 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.25 (bd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 4.80 (dddd, 1 H, $J_{4,5}$ 5.1, $J_{5,6}$ 6.2, $J_{5,6'}$ 4, $J_{5,F}$ 47.8 Hz, H-5), 4.55 (dd, 1 H, $J_{2,F}$ 1.8 Hz, H-2), 4.36 (ddd, 1 H, $J_{4,F}$ 19.5 Hz, H-4), 3.56 (ddd, 1 H, $J_{6,6'}$ 13.7, $J_{6,F}$ 21.4 Hz, H-6), 3.45 (ddd, 1 H, $J_{6',F}$ 24.4 Hz, H-6′); ^{13}C NMR: δ 104.7 (C-1), 90.0 ($J_{5,F}$ 185.4 Hz, C-5), 83.5 (C-2), 77.7 ($J_{3,F}$ 2.4 Hz, C-3), 77.2 ($J_{4,F}$ 11 Hz, C-4), 51.7 ($J_{6,F}$ 23.1 Hz, C-6). Anal. Calcd for $C_{11}H_{16}FN_3O_5$: C, 45.68; H, 5.58: Found: C, 45.60; H, 5.62.

6-Azido-5,6-dideoxy-5-fluoro-L-idofuranose (**11**).—A soln of intermediate **10** (960 mg, 3.32 mmol) in a 50% aq soln of acetonitrile (35 mL) was stirred with Amberlite IR-120 [H^+] ion-exchange resin at 60 °C for 14 h. After removal of the resin by filtration, the cloudy soln was concd under reduced pressure, and the oily yellow residue was chromatographed to give free L-idofuranose derivative **11** (630 mg, 91%) as a semicrystalline unstable solid that was immediately used in the next step. ^{1}H NMR indicated a 1:1 mixture of anomers; ^{13}C NMR: δ 104.4 (C-1α), 97.9 (c-1β), 94.4, 93.2 ($J_{5,F}$ 174.3/175.9 Hz, C-5α/C-5β), 82.0, 78.6 ($J_{4,F}$ 17.6/17.5 Hz, C-4α/C-4β), 82.1, 77.7 ($J_{2,F}$ 0/1.9 Hz, H-2α/H-2β), 76.7, 76.6 ($J_{3,F}$ 5.2/6.8 Hz, C-3α/C-3β), 52.9, 52.5 ($J_{6,F}$ 20.2/21.2 Hz, C-6α/C-6β).

6-Azido-5,6-dideoxy-5-fluoro-L-sorbose (**12**).—To a 2% soln of L-idofuranose **11** (600 mg, 2.90 mmol) in distilled water containing $MgSO_4$ (25 mg), immobilised glucose isomerase (Sweetzyme T, 1.5 g) was added, and the mixture was spun on a rotary evaporator at 60 °C for 8 h, at the end of which time TLC indicated no further change in the compound distribution. The enzyme was removed by filtration, the filtrate was concd under reduced pressure, and the residue was chromatographed on silica gel to yield 330 mg (55%) of desired product **12**, unchanged starting material **11** (145 mg, 24%), as well as 100 mg (16.7%) of a mixture of both. Compound **12** was a syrup: $[\alpha]_D^{20}$ −55.5° (*c* 1.7, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.74 (dddd, 1 H, $J_{4,5} = J_{5,6} = 5.8$, $J_{5,6'}$ 3.7, $J_{5,F}$ 48.4 Hz, H-5), 4.51 (dd, 2 H, $J_{1,1'}$ 5 Hz, H-1, H-1′), 4.30 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.12 (ddd, 1 H, $J_{4,F}$ 17 Hz, H-4), 3.66 (ddd, 1 H, $J_{6,6'}$ 14.3, $J_{6,F}$ 23.9 Hz, H-6), 3.57 (ddd, 1 H, $J_{6',F}$ 26.8 Hz, H-6′); ^{13}C NMR (MeOH-d_4): δ 212.4 (C-2), 94.4 ($J_{5,F}$ 175.4 Hz, C-5), 76.6 ($J_{3,F}$ 5.4 Hz, C-3), 72.5 ($J_{4,F}$ 20.3 Hz, C-4), 67.8 (C-1), 52.6 ($J_{6,F}$ 21.3 Hz, C-6). Anal. Calcd for $C_6H_{10}FN_3O_4$: C, 34.8; H, 4.87. Found: C, 34.6; H, 4.95.

1,2,5-Trideoxy-2-fluoro-1,5-imino-D-glucitol (**4**) *from* **12**.—Conventional catalytic hydrogenation of L-sorbose derivative **12** (220 mg, 1.1 mmol) in dry MeOH (30 mL) over Pd-on-charcoal (10%) (Caution! Extreme fire hazard) at ambient pressure for 14 h gave iminoalditol **4** as the main product, together with small amounts of the corresponding epimer at C-5. Removal of the catalyst by filtration, and evaporation of the solvent gave crude compound **4**, which was purified by crystallisation from dry MeOH–diethyl ether to yield 135 mg (77%) of pure material as a colourless wax: $[\alpha]_D^{20}$ +44.0° (*c* 0.7, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.30 (dddd, 1 H, $J_{1a,2}$ 10.7, $J_{1e,2}$ 5.5, $J_{2,3}$ 8.7, $J_{2,F}$ 50.8 Hz, H-2), 3.86 (dd, 1 H, $J_{5,6}$ 3.1, $J_{6,6'}$ 11 Hz, H-6), 3.64 (dd, 1 H, $J_{5,6'}$ 6.4 Hz, H-6′), 3.46 (ddd, 1 H, $J_{3,4}$ 9, $J_{3,F}$ 14.7 Hz, H-3), 3.28 (ddd, 1 H, $J_{1a,1e}$ 11.9, $J_{1e,F}$ 1.7 Hz, H-1e), 3.22 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 2.65 (ddd, 1 H, $J_{1a,F}$ 4.7 Hz, H-1a), 2.50 (ddd, 1 H, H-5); ^{13}C NMR (MeOH-d_4): δ 92.5 ($J_{2,F}$ 179 Hz, C-2), 78.8 ($J_{3,F}$ 16.2 Hz, C-3), 73.1 ($J_{4,F}$ 8.6 Hz, C-4), 63.0 (C-6), 62.7 (C-5). Anal. Calcd for $C_6H_{13}FNO_3$: C, 43.37; H, 7.89. Found: C, 43.10; H, 7.70.

3,4,6-Tri-O-benzyl-N-benzoxycarbonyl-1,2,5-trideoxy-2-fluoro-1,5-imino-D-glucitol (**15**).—To a soln of compound **14** (Ref. [11], 130 mg, 0.23 mmol) in dry CH_2Cl_2 (25 mL) containing 2% of pyridine, diethylaminosulfur trifluoride (DAST, 0.15 mL, 5 equiv) was added at 0 °C, and the mixture was allowed to react at ambient temperature for 48 h,

after which time no remaining starting material could be detected by TLC. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and consecutively washed with 5% aq HCl and satd aq $NaHCO_3$. After drying (Na_2SO_4) and filtration, the soln was concd under reduced pressure. Chromatography of the residue gave compound **15** (130 mg, 53%) as a colourless oil: $[\alpha]_D^{20}$ −27.0° (*c* 1.6, $CHCl_3$); ^{1}H NMR: δ 4.70 (m, 1 H, $J_{1e,2}$ 3.3, $J_{2,3}$ 3.7, $J_{2,F}$ 49.0 Hz, H-2), 4.35 (m, 2 H, $J_{1e,F}$ 18.1 Hz, H-1e, H-5), 3.90–3.77 (m, 2 H, $J_{3,4}$ 6.4 Hz, H-3, H-4), 3.72–3.61 (m, 2 H, H-6, H-6′), 3.44 (ddd, 1 H, $J_{1a,1e}$ 15.3, $J_{1a,2}$ 2.8, $J_{1a,F}$ 39.6 Hz, H-1a); ^{13}C NMR: δ 91.0 ($J_{2,F}$ 182 Hz, C-2), 79.4 ($J_{3,F}$ 24 Hz, C-3), 72.7 ($J_{4,F}$ 5 Hz, C-4), 68.1 (C-6), 55.3 (C-5), 42.9 ($J_{1,F}$ 24 Hz, C-1). Anal. Calcd for $C_{35}H_{36}FNO_5$: C, 73.79; H, 6.37. Found: C, 74.01; H, 6.44.

*1,2,5-Trideoxy-2-fluoro-1,5-imino-*D*-glucitol* (**4**) *from* **15**.—To a soln of compound **15** (90 mg, 0.16 mmol) in EtOH (20 mL), $Pd(OH)_2$-on-charcoal (Pearlman's catalyst, 10%, 105 mg) was added, and the mixture was stirred under an atmosphere of hydrogen at ambient pressure for 40 h. After removal of the catalyst by filtration, the residue was chromatographed on silica gel to give 23 mg (88%) of **4** as a semicrystalline solid: $[\alpha]_D^{20}$ +44.3° (*c* 0.7, MeOH). NMR data as above.

*5-Azido-5,6-dideoxy-6-fluoro-*D*-fructose* (**17**).—To a soln of 5-azido-5,6-dideoxy-6-fluoro-D-glucofuranose (**16**, Ref. [12], 230 mg, 1.11 mmol) in distilled water (50 mL) containing $MgSO_4$ (50 mg) was added immobilised glucose isomerase (Sweetzyme T, 1.3 g), and the mixture was spun on a rotary evaporator at 65 °C for 7 h. Removal of the enzyme by filtration, and concn of the aq soln under reduced pressure furnished a yellow oil that was dissolved in EtOAc. This cloudy soln was filtered over a short plug of silica gel to remove baseline material, and the soln was again concd under reduced pressure. The remaining material was dissolved in distilled water, and bromine (0.5 g, 3 mmol) and $BaCO_3$ (1 g) were added. After 20 min excess bromine was removed by bubbling air through the reaction mixture. Solids were removed by filtration, the soln was concd under reduced pressure, and the residue was chromatographed on silica gel to give 145 mg (63%) of pure fructose derivative **17**: $[\alpha]_D^{20}$ −33.5° (*c* 0.6, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.84 (ddd, 1 H, $J_{5,6}$ 2.1, $J_{6,6'}$ 10, $J_{6,F}$ 47.1 Hz, H-6), 4.69 (ddd, 1 H, $J_{5,6'}$ 6.0, $J_{6'F}$ 47.4 Hz, H-6′), 4.50 (d, 2 H, $J_{1,1'}$ 5 Hz, H-1, H-1′), 4.43 (d, 1 H, $J_{3,4}$ 1.6 Hz, H-3), 3.96–3.68 (m, 2 H, H-4, H-5); ^{13}C NMR (MeOH-d_4): δ 213.6 (C-2), 84.8 (d, $J_{6,F}$ 170 Hz, C-6), 76.9 (C-3), 71.1 (d, $J_{4,F}$ 6.6 Hz, C-4), 68.0 (C-1), 63.1 (d, $J_{5,F}$ 17.4 Hz, C-5). Anal. Calcd for $C_6H_{10}FN_3O_4$: C, 34.8; H, 4.87. Found: C, 34.65; H, 4.96.

*1,2,5-Trideoxy-1-fluoro-2,5-imino-*D*-mannitol* (**5**).—A 2% soln of compound **17** (95 mg, 0.46 mmol) in dry MeOH was stirred with Pd-on-charcoal (10%, 0.5 equiv by weight) under an atmosphere of hydrogen at ambient pressure until TLC indicated quantitative conversion of the starting material into a comparably very polar product. The catalyst was removed by filtration, the filtrate was concd under reduced pressure, and the residue was dissolved in distilled water and briefly treated with a strongly basic ion-exchange resin (E. Merck III). After filtration, water was removed under reduced pressure to yield a colourless foam (63 mg, 83%) that failed to crystallise from MeOH–Et_2O: $[\alpha]_D^{20}$ +21.7° (*c* 0.65, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.51 (ddd, 1 H, $J_{1,1'}$ 9.7, $J_{1,2}$ 3.2, $J_{1,F}$ 47.7 Hz, H-1), 4.44 (ddd, 1 H, $J_{1',2}$ 6, $J_{1',F}$ 47.7 Hz, H-1′), 3.82 (m, 2 H, H-3, H-4), 3.69 (dd, 1 H, $J_{5,6}$ 4.2, $J_{6,6'}$ 11.3 Hz, H-6), 3.61 (dd, 1 H, $J_{5,6'}$ 5.4 Hz, H-6′), 3.18 (bm, $J_{2,F}$ 22 Hz, H-2), 2.97 (m, 1 H, H-5); ^{13}C NMR: δ 84.9 (d, $J_{1,F}$ 169 Hz, C-1), 79.2 (d, $J_{4,F}$ 1.4 Hz, C-4), 78.7 (d, $J_{3,F}$ 6.5 Hz, C-3), 64.4 (C-5), 62.9 (d, $J_{2,F}$ 18.5 Hz, C-2), 62.85 (C-6). Anal. Calcd for $C_6H_{13}FNO_3$: C, 43.4, H, 7.89. Found: C, 43.5; H, 7.59.

*5-Azido-5-deoxy-6-*O*-dimethyl(1,1,2-trimethylpropyl)silyl-1,2-*O*-isopropylidene-α-*D*-glucofuranose* (**19**).—To a 5% soln of 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**18**, Ref. [14], 2.50 g, 10.2 mmol) in Me_2NCHO, imidazole (2.1 g, 3 equiv) and chlorodimethyl(1,1,2-trimethylpropyl)silane (4.0 mL, 2 equiv) were added consecutively, and the mixture was kept at ambient temperature until TLC indicated complete conversion of the starting material into a single, considerably faster moving product. The reaction mixture was concd under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (250 mL), and the soln was washed with 5% aq HCl and satd aq $NaHCO_3$. The organic layer was dried (Na_2SO_4), the solids were removed by filtration, and the filtrate was concd under reduced pressure. The residue was passed over a short column of silica gel to give 3.16 g (94%) pure syrupy compound **19**: $[\alpha]_D^{20}$ −28.8° (*c* 1.8, $CHCl_3$); ^{1}H NMR: δ 5.90 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.47 (d, 1 H, H-2), 4.20 (d, 1 H, $J_{3,4}$ 2.6 Hz, H-3), 4.07 (dd, 1 H, $J_{4,5}$ 8.8 Hz, H-4), 3.95 (dd, 1 H, $J_{5,6}$ 2.7, $J_{6,6'}$ 10.6 Hz, H-6), 3.77 (dd, 1 H, $J_{5,6'}$ 6.1 Hz, H-6′), 3.65 (ddd, 1 H, H-5), 3.20 (bs, 1 H, 3-OH); ^{13}C NMR: δ 105.2

(C-1), 85.2, 77.7, 75.0 (C-2, C-3, C-4), 63.7 (C-6), 61.0 (C-5).

5-Azido-5-deoxy-6-O-dimethyl(1,1,2-trimethylpropyl)silyl-1,2-O-isopropylidene-3-O-(methoxymethyl)-α-D-glucofuranose (**20**).—To a 10% soln of the starting material **18** (3.0 g, 12.2 mmol) in Me_2NCHO, imidazole (2.5 g, 3 equiv) and chlorodimethyl(1,1,2-trimethylpropyl)silane (3.6 mL, 1.5 equiv) were added, and the mixture was stirred at ambient temperature until TLC confirmed quantitative conversion of the starting material into a faster moving product. The reaction mixture was partitioned between CH_2Cl_2 and 5% aq HCl, and the organic layer was washed with satd aq $NaHCO_3$. After drying (Na_2SO_4) and removal of the drying agent by filtration, Et_3N (5 mL, 3 equiv) and chloromethyl methyl ether [22] (2 equiv) were added to the organic layer, which was kept under reflux until TLC indicated quantitative formation of a less polar main product. The reaction mixture was concd under reduced pressure, and the resulting yellow syrup was chromatographed to furnish compound **20** (4.4 g, 95%) as a colourless syrup: $[\alpha]_D^{20}$ −25.2° (*c* 1.6, CH_2Cl_2); ^1H NMR: δ 5.88 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.66 (d, 1 H, H-2), 4.16 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-3), 4.05 (m, 2 H, H-4, H-6), 3.78 (dd, 1 H, $J_{5,6'}$ 6.7, $J_{6,6'}$ 10.5 Hz, H-6'), 3.65 (ddd, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 2.5 Hz, H-5); ^{13}C NMR: δ 105.2 (C-1), 82.8, 80.2, 77.9 (C-2, C-3, C-4), 64.1 (C-6), 60.4 (C-5). Anal. Calcd for $C_{17}H_{31}N_3O_6$: C, 54.7; H, 8.37. Found: C, 54.5; H, 8.41.

5-Azido-5-deoxy-1,2-O-isopropylidene-3-O-(methoxymethyl)-α-D-glucofuranose (**21**).—To a 5% soln of the starting material **20** (4.0 g, 10.7 mmol) in THF, tetrabutylammonium fluoride trihydrate (5.1 g, 1.5 equiv) was added, and the mixture was kept at 50 °C until no unreacted starting material could be detected by TLC. The soln was concd under reduced pressure and subjected to column chromatography to give compound **21** (2.80 g, 90%) as a white foam: $[\alpha]_D^{20}$ −6.2° (*c* 1.2, $CHCl_3$); ^1H NMR: δ 5.89 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.65 (d, 1 H, H-2), 4.17 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.10 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.97 (m, 1 H, H-5), 3.86–3.70 (m, 2 H, H-6, H-6'), 2.65 (bs, 1 H, disappears with D_2O, 6-OH); ^{13}C NMR: δ 105 1 (C-1), 82.7, 80.5, 78.7 (C-2, C-3, C-4), 63.4, 60.4 (C-5, C-6). Anal. Calcd for $C_{11}H_{19}N_3O_6$: C, 45.7; H, 6.62. Found: C, 45.9; H, 6.72.

5-Azido-5-deoxy-1,2-O-isopropylidene-6-O-methyl-3-O-(methoxymethyl)-α-D-glucofuranose (**22**).—To a 5% soln of compound **21** (2.55 g, 8.81 mmol) in THF containing 20% Me_2NCHO, sodium hydride (80% dispersion in oil, 0.8 g, 3 equiv) and iodomethane (1.1 mL, 2 equiv) were added, and the mixture was stirred at ambient temperature until the starting material was no longer detectable by TLC. Excess MeOH was carefully added, and the heterogenous mixture was concd under reduced pressure. The residue was dissolved in CH_2Cl_2 (150 mL) and sequentially extracted with 5% aq HCl and satd aq $NaHCO_3$. The organic layer was dried, Na_2SO_4 was removed by filtration, and the filtrate was concd under reduced pressure. Chromatographic purification of the residue furnished methyl ether **22** (2.38 g, 89%) as a colourless oil: $[\alpha]_D^{20}$ −15.3° (*c* 1.7, CH_2Cl_2); ^1H NMR: δ 5.86 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.60 (d, 1 H, H-2), 4.14 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.03 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 3.78–3.87 (m, 2 H, H-5, H-6), 3.59 (dd, $J_{5,6'}$ 7.6, $J_{6,6'}$ 10.5 Hz, H-6'), 3.41 (s, 3 H, 3-OMe); ^{13}C NMR: δ 105.2 (C-1), 82.7, 80.3, 78.3 (C-2, C-2, C-4), 73.3 (C-6), 59.2 (C-5), 56.1 (3-OMe). Anal. Calcd for $C_{12}H_{21}N_3O_6$: C, 57.5; H, 6.98. Found: C, 57.65; H, 7.02.

5-Azido-5-deoxy-6-O-methyl-D-glucofuranose (**23**).—A 3% soln of protected methyl ether **22** (2.30 g, 7.58 mmol) in 50% aq CH_3CN was stirred with 7 mL of Amberlite IR-120 [H^+] ion-exchange resin at 45 °C until TLC showed quantitative conversion into a single, distinctly slower moving product. The resin was removed by filtration, the filtrate was concd under reduced pressure, and the remaining residue was purified by chromatography to give free D-glucofuranose **23** (1.28 g, 77%) as a 1:1 mixture of anomers (as determined by NMR spectroscopy): $[\alpha]_D^{20}$ −23.2° (*c* 0.9, CH_2Cl_2); ^{13}C NMR: δ 103.3 (C-1α), 96.9 (C-1β), 80.8, 79.7, 76.8, 76.75, 75.95, 75.9 (C-2α/β, C-3α/β, C-4α/β), 72.6, 72.3 (C-6α/β), 60.4, 59.8, 59.4, 59.3 (C-5α/β, 3-OMe α/β).

5-Azido-5-deoxy-6-O-methyl-D-fructose (**24**).—A 3% aq soln of free aldose **23** (360 mg, 1.64 mmol) containing $MgSO_4$ (15 mg) was spun on a rotary evaporator in the presence of immobilised glucose isomerase (Sweetzyme T, EC 5.3.1.5, 1.2 g) at 65 °C for 5 h. The solids were filtered off, and $BaCO_3$ (1 g) was added to the cloudy soln. After the addition of bromine (0.5 g, 3 mmol), the mixture was stirred at room temperature until all aldose was converted into a faster moving product. Air was bubbled through the soln to remove excess bromine, the solids were filtered off, and the filtrate was concd under reduced pressure. Chromatography of the brown, oily residue gave D-fructose derivative **24** (220 mg, 61%) as a colourless glass: $[\alpha]_D^{20}$ −39.5° (*c* 0.9, CH_2Cl_2); ^{13}C

NMR (D_2O): δ 213.1 (C-2), 75.4, 71.4, 70.1, 66.1 (C-3, C-4, C-5, C-6), 60.9 (C-1), 58.5 (6-OMe). Anal. Calcd for $C_7H_{13}N_3O_5$: C, 38.35; H, 5.98. Found: C, 38.20; H, 6.04.

2,5-Dideoxy-2,5-imino-1-O-methyl-D-mannitol (**6**). —A 2% soln of ketose **24** (76 mg, 0.35 mmol) in dry MeOH was stirred in the presence of Pd-on-charcoal (10%, 0.3 equiv by weight) under an atmosphere of hydrogen at ambient pressure for 14 h. The catalyst was removed by filtration, and the filtrate was concd under reduced pressure. The residue was dissolved in a minimum amount of distilled water and subjected to ion-pair chromatography on Amberlite CG-50 [H^+] employing 0.05 M aq ammonia as the mobile phase. After removal of the solvent under reduced pressure, crystallisation of the slightly yellow glassy residue from MeOH–Et_2O gave pure inhibitor **6** (39.9 mg, 65%) as an off-white powder: $[\alpha]_D^{20}$ +15.2° (*c* 0.4, MeOH); ^1H NMR (MeOH-d_4): δ 3.83–3.77 (m, 2 H, H-3, H-4), 3.68 (dd, 1 H, $J_{1,1'}$ 11.2, $J_{1,2}$ 4.2 Hz, H-1), 3.57 (dd, 1 H, $J_{1',2}$ 5.9 Hz, H-1'), 3.52 (dd, 1 H, $J_{5,6}$ 4.1, $J_{6,6'}$ 9.6 Hz, H-6), 3.45 (dd, 1 H, $J_{5,6'}$ 6.4 Hz, H-6'), 3.11–2.95 (bm, 2 H, H-2, H-5); ^{13}C NMR (MeOH-d_4): δ 80.0, 79.6 (C-3, C-4), 74.7 (C-1), 64.5, 63.4, 62.6 (C-2, C-5, C-6), 59.3 (1-OMe). Anal. Calcd for $C_7H_{15}NO_4$: C, 47.4; H, 8.53. Found: C, 47.35; H, 8.55.

Enzyme inhibition studies and determination of K_i *values* were carried out as previously described [2,23].

Molecular modelling.—All calculations were carried out using Tripos Sybyl, versions 6.25 and 6.3, on a Silicon Graphics Iris Indigo workstation. In the superposition studies, root mean square values of 0.257 for all oxygen atoms matched and 0.293 for all oxygen and nitrogen atoms matched were found. The conformations were derived from docking studies of inhibitors **1** and **3** with a glucoamylase (pdb: 1dog, EC 3.2.1.3) [24]. The enzyme–inhibitor complexes were relaxed using means of molecular dynamics and minimised with Powell (conjugate gradient) and BFGS (quasi Newton) minimisers, a maximum iterations counter of $1 \cdot 10^6$ using the Tripos force field, and Gasteiger–Hückel charges until a gradient of 0.05 kcal · mol^{-1} · $Å^{-1}$ was reached.

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