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Probing the aglycon binding site of a β-glucosidase: a collection of C-1-modified 2,5-dideoxy-2,5-imino-D-mannitol derivatives and their structure–activity relationships as competitive inhibitors

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Dedicated to Professor Christian Pedersen

Abstract—A range of new C-1 modified derivatives of the powerful glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol has been synthesised and their biological activities probed with the β-glucosidase from *Agrobacterium* sp. K_i values are compared with those of previously prepared close relatives. Findings suggest dramatic effects exerted by the aglycon binding site on substrate/inhibitor binding.

1. Introduction

The iminoalditol 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **1**), a natural product,[1] is known as a potent reversible inhibitor of D-glucosidases and invertase rivalling the activity of 1-deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol, DNJ, **2**), the paradigmatic D-glucosidase inhibitor in the class of compounds under consideration (Fig. 1).[2]

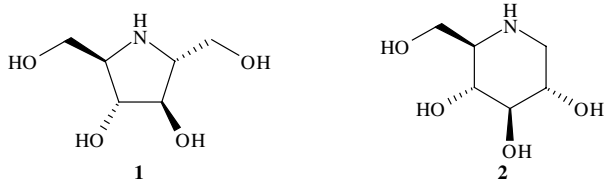

Figure 1. Structures of DMDP (**1**) and 1-DNJ (**2**).

This fact was attributed to the relatively flat five-membered ring, which was assumed to more closely mimic the putative transition state of enzymatic glycoside hydrolysis, along with its C_2-axis of symmetry. Standard modifications at C-1, such as in the 1-deoxyfluoro (**3**) as well as the 1-*O*-methyl (**4**) derivatives caused losses of inhibitory activities in the range of two orders of magnitude (Fig. 2).[3]

The same was true for **5**, the 1-aminodeoxy derivative of compound **1**, which was prepared via an Amadori rearrangement reaction of 5-azidodeoxy-D-glucofuranose.[4]

C-1 modified derivatives with extended alkyl chains such as compounds **6** as well as **8** and **9**, initially designed for immobilisation studies,[5] were synthesised and found to exhibit inhibitory properties comparable to or even better than those of parent compound **1**.[5] Similar results have been found with **10** and, by other workers,[6] with compound **7** as well as natural products featuring the iminomannitol basic structure such as the broussonetines,[7] for example, compound **12**. These results suggested that the aglycon binding site is able to exert

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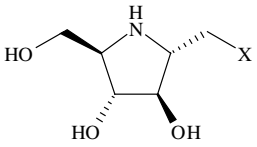


3: X = F
4: X = OMe
5: X = NH_2
6: X = $NH(CH_2)_6OH$
7: X = NHAc
8: X = $O(CH_2)_6OH$
9: X = $NHCO(CH_2)_5OH$
10: X = $NHCO(CH_2)_4CH_3$
11: X = $NHCO(CH_2)_2Ph$

Figure 2. Structures of compounds **3**–**11**.

binding forces compensating for activity losses caused by the removal of one crucial hydroxyl group as well as the loss of symmetry resulting from this formal 'deoxygenation' (Fig. 3).

Further studies revealed that mixed aliphatic–aromatic side chains exhibit similar properties, for example in compound **11**.[8] The general significance of long aliphatic substituents had also been shown by Legler and coworkers[9] with compound **13** as well as by Vasella and his group[10] with compound **14** in the iminosugar field and by Ogawa et al.[11] for examples in the aminocyclitol family of compounds such as **15**, just to mention a few relevant examples. To gain a better understanding of the structure-activity relationships applying in 'our' family of compounds, we have extended the range of derivatives by substitution with markedly lipophilic aromatic side chains such as in **16** and **17**.[8] Some of these derivatives, in particular, compounds **18** and **19** featuring dialkylamino substituents in the respective aromatic system, exhibited K_i values in the low nanomolar range exceeding the parent compound's activity by two orders of magnitude (Fig. 4).[12]

When compared to the 'simple' modifications in **3**–**5** as well as **7**, this strong contribution to the binding process by the 1-*N*-substituent resulted in a re-gain of activity of four orders of magnitude. Clearly, this observation makes the aglycon binding site a highly interesting and worthwhile target to explore.

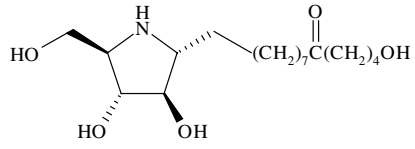
Unfortunately and despite all efforts made thus far, for the β-glucosidase from *Agrobacterium* sp. there is no XRD structure available, as yet.

Consequently, we continued to resort to the conventional means of exploration taking advantage of pieces of information provided by structure–activity considerations based on results with selected new inhibitors.

In addition, novel chain-extended 1-*O*-alkyl derivatives of 2,5-dideoxy-2,5-imino-D-glucitol, the epimer at C-2 of compound **1**, were synthesised and their activities compared with the parent compounds.

2. Results and discussion

To address the respective influence of various parameters such as the nature of the hetero atom linking side chain and sugar, the nature and type of hybridisation of the attached carbon or heteroatom, the size of the aromatic system as well as the presence and positioning of the aromatic amine substituent, derivatives **20**–**27** were synthesised. Compound **37**, the known epimer of **1**, as well as its derivatives **32** and **33** were also expected to improve our understanding of the structural prerequisites necessary for optimal inhibition of the enzyme under scrutiny.



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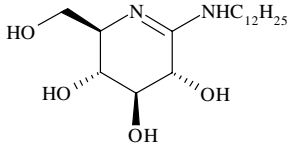

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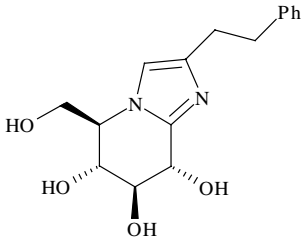

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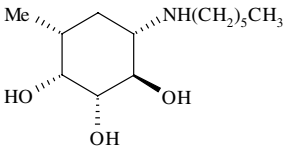

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Figure 3. Structures of compounds **12**–**15**.

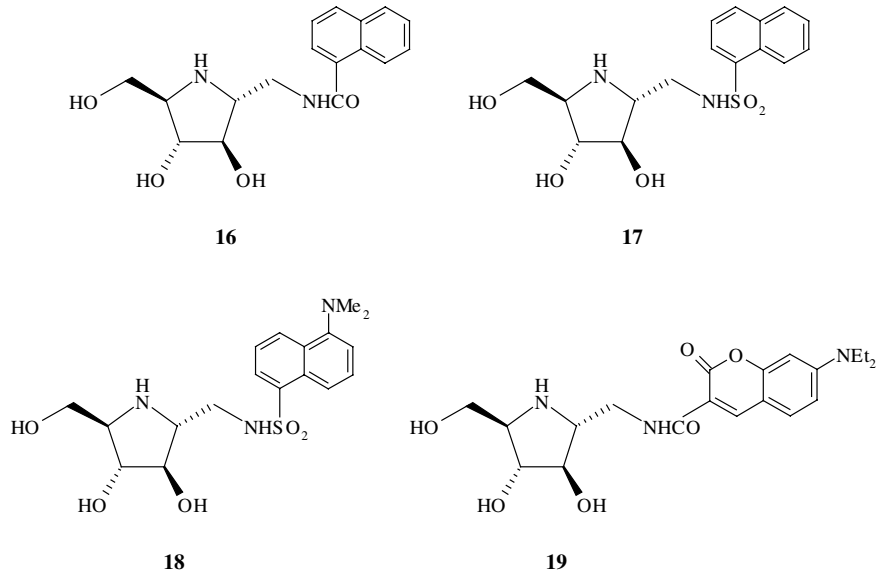
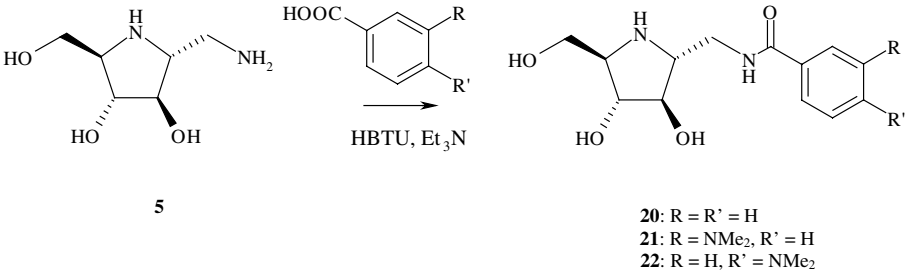


Figure 4. Structures of compounds **16**–**19**.

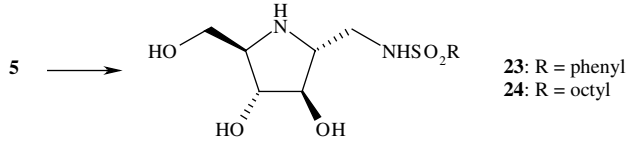


Scheme 1. Synthesis of compounds **20**–**22**.

Compounds **20**–**22** were accessible by the same reaction sequence, starting from known 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol (**5**).[4] The different phenyl substituents were introduced by coupling of the corresponding benzoic acid derivatives employing *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoroborate (HBTU) as the coupling reagent (Scheme 1).

Yields ranged from 75% for the simple benzamide compound **20** to 14% for the 3-dimethylaminobenzoyl derivative **21** and 16% for the 4-substituted compound **22**.

To address the influence of the carboxamide versus sulfonamide linkage on biological activities, sulfonamides **23** and **24** were prepared by reaction of compound **5** with the respective sulfonyl chloride (Scheme 2).

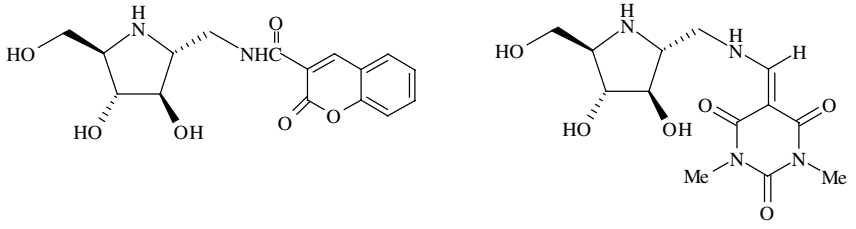


Scheme 2. Synthesis of compounds **23** and **24**.

To investigate the contributions of the diethylamino group and the aromatic system of the strong inhibitor **19**, the corresponding derivative **25** was synthesised (Fig. 5) by treatment of compound **5** with coumarin-3-carboxylic acid in the presence of HBTU (63% yield).

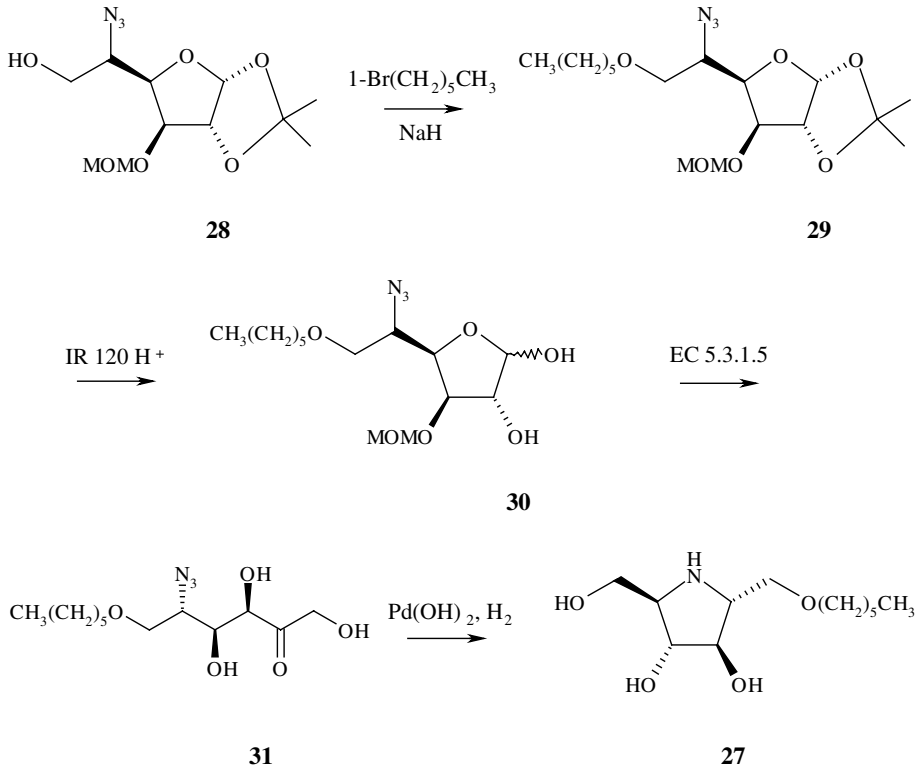
Inhibitor **26** containing a vinylogous amide was prepared from **5** in practically quantitative yield by reaction with 1,3-dimethyl-5-[(dimethylamino)methylene]-2,4,6-(1H, 3H, 5H)-trioxopyrimidine (DTPM reagent)[13] in methanol in the presence of Et_3N (Fig. 5).

For the synthesis of 2,5-dideoxy-6-*O*-hexyl-2,5-imino-D-mannitol **27**, 5-azido-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-α-D-glucofuranose[5] **28** was employed as the starting material (Scheme 3). Compound **28** was treated with sodium hydride and 1-bromohexane in THF to obtain **29**, followed by removal of the 1,2-*O*-isopropylidene group with ion exchange resin Amberlite IR 120 [H^+] to yield free glucofuranose derivative **30**. Its conversion to the corresponding open chain 5-azido-6-*O*-hexyl fructose derivative **31** was achieved exploiting glucose isomerase (EC 5.3.1.5).[14] The equilibrium of the isomerisation reaction was found to be on the aldose side. Consequently, remaining starting material had to be oxidised with Br_2 and $BaCO_3$ to the corresponding



25 **26**

Figure 5. Structures of compounds **25** and **26**.



28 **29** **30** **31** **27**

Scheme 3. Synthesis of compounds **27**.

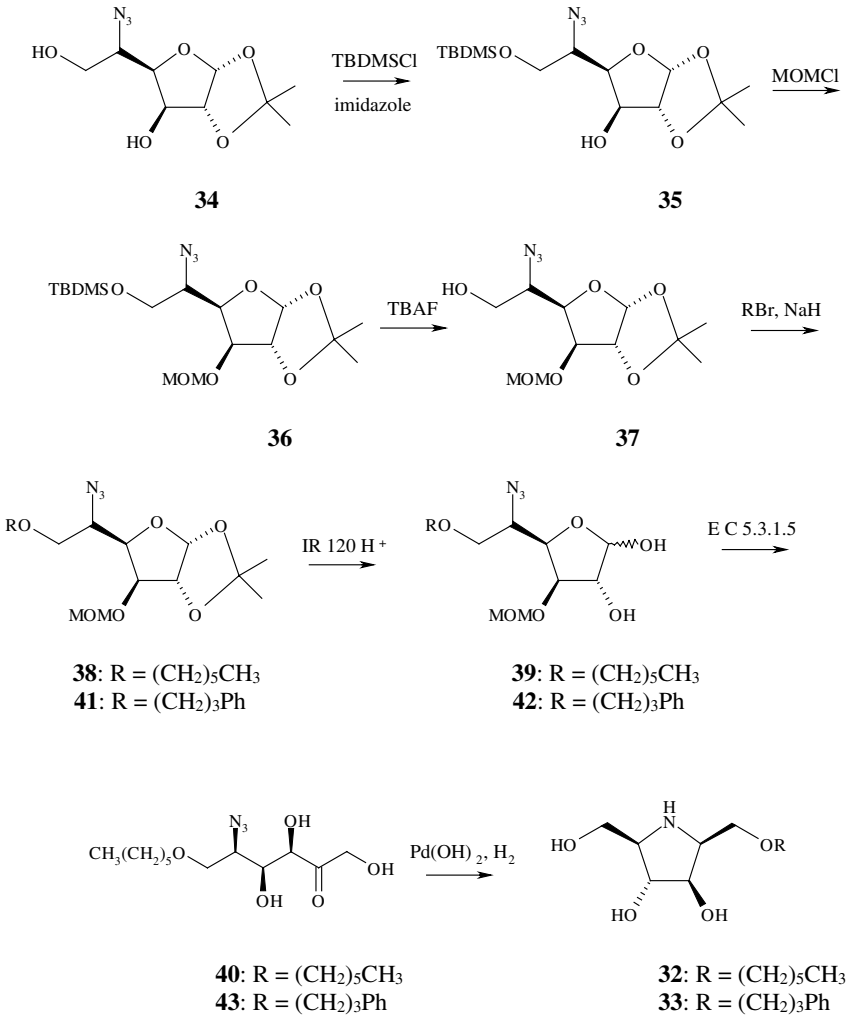
gluconolactone, which could be separated from **31** by chromatography. Hydrogenation of **31** with concomitant intramolecular reductive amination with $Pd(OH)_2$/C gave the desired 2,5-dideoxy-6-*O*-hexyl-2,5-imino-D-mannitol **27**.

To investigate the influence of the stereochemistry at position C-2, two 2,5-imino-2,5-dideoxy-D-glucitol derivatives **32** and **33** were synthesised. Starting from 5-azido-5-deoxy-1,2-*O*-isopropylidene-L-idofuranose[15] **34** protection of position *O*-6 was achieved as the *t*-butyldimethylsilyl ether **35**. Protection of the remaining free hydroxyl group at position 3 with chloromethyl methyl ether gave **36** and deprotection of the silyl ether employing tetrabutylammonium fluoride furnished L-idofuranose derivative **37**. Reaction of the liberated hydroxyl group with bromohexane in the presence of sodium hydride gave **38**. Treatment with acidic ion exchange resin Amberlite IR 120 [H^+] in acetonitrile–water furnished 5-azido-5-deoxy-6-*O*-hexyl-L-idofuranose **39**. Subsequent isomerisation with glucose isomerase to the corresponding L-sorbose derivative **40** followed by oxidation of the remaining aldose allowed access to pure **40**. Its hydrogenation gave the desired 1-*O*-hexyl-iminoalditol **32** (Scheme 4).

The 6-*O*-phenylpropyl derivative **33** was synthesised accordingly. Following the above sequence employing 1-bromophenylpropane in the reaction of L-idofuranose (**37**) to derivative **41**, acidic deprotection (**42**), enzymatic isomerisation to the L-sorbose derivative **43** and subsequent intramolecular reductive amination gave 2,5-dideoxy-2,5-imino-6-*O*-(1-phenylpropyl)-D-glucitol **33** in fair overall yields.

3. Biological activities

K_i values were measured with β-glucosidase from *Agrobacterium* sp. in the presence of the corresponding



Scheme 4. Synthesis of compounds **32** and **33**.

inhibitors using *para*-nitrophenyl-β-glucoside as substrate ([S]~1.5 K_m) in 50 mM NaPi buffer containing 0.1% BSA at pH 7.

From the data obtained for the compounds in Table 1, the following conclusions can be drawn: All compounds screened are competitive inhibitors of the enzyme under scrutiny. Amides as well as sulfonamides at the 1-position (**9**, **10**, **24**) are generally better inhibitors than the corresponding 1-ethers (**8**, **27**). There are no significant differences in activity between structurally equivalent amides and sulfonamides such as between **20** and **23**, **10** and **24** as well as **16** and **17**.

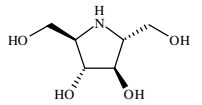
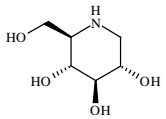
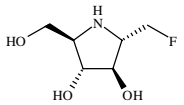
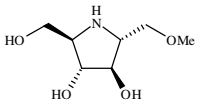
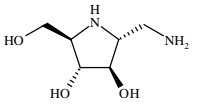
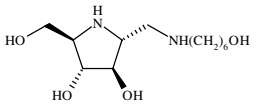
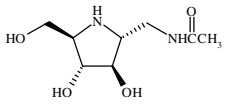
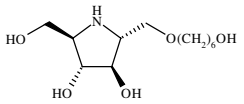
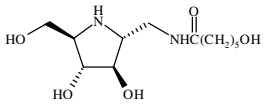
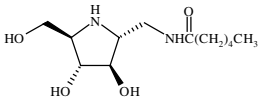
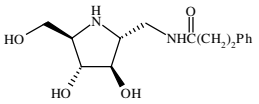
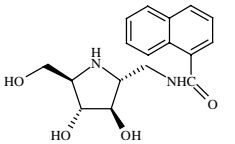
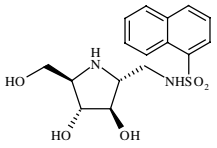
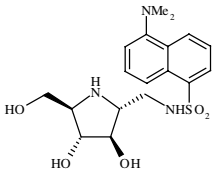
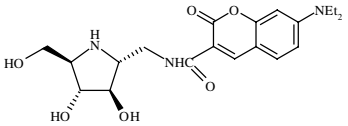
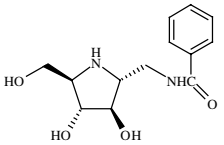
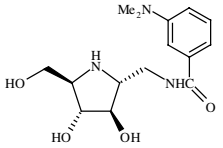
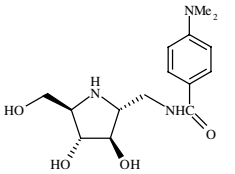
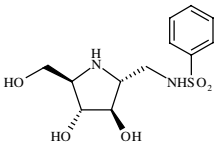
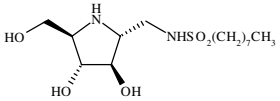
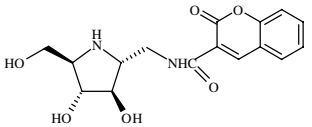
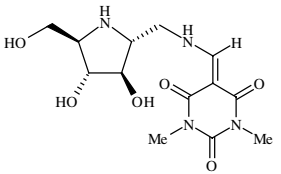
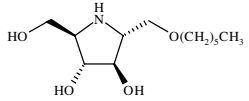
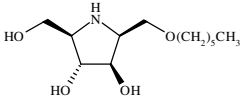
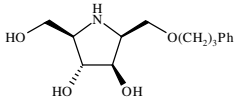
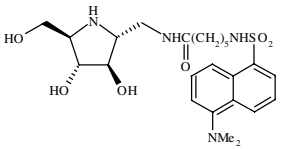
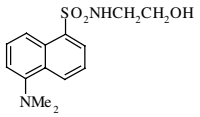
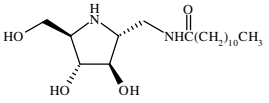
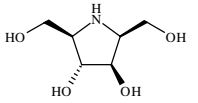
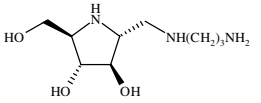
Medium length, simple unsubstituted aliphatic substituents (e.g., **10** and **27**) are distinctly better than short chains (**4**, **7**). They are slightly better than terminally functionalised relatives (**8** and **9**) with no additional effect of increased chain length (**12**), and are comparable to the simple unsubstituted (**20**) and amine substituted monocyclic aromatic systems (**21** and **22**) as well as the unsubstituted napthyl derivatives (**16**, **17**) probed in this study. Small improvements were detected with amine substituted benzamides (**21** and **22**) over the unsubstituted compound (**20**). Interestingly, comparing two bicyclic aromatic substituents, the unsubstituted napthoyl (**16**) and coumarin carboxamide (**25**) compounds, the heterocyclic coumarin system offers an advantage of one order of magnitude in inhibitory power.

Bicyclic aromatic systems featuring tertiary amine substituents in the distal ring such as **18** and **19** have been found most powerful, thus far, with improvements of two orders of magnitude when compared to parent compound **1** as well as similarly strong improvements over their corresponding unsubstituted relatives (**17** and **25**, respectively). They compare favourably with the most active compounds known to date.

The distance of the amine substituted aromatic system from the inhibitor moiety was not found crucial in the one example (**18** vs **34**) probed.

Compound **26** is half as active as the parent compound **1**, but still in the sub-micromolar range. This type of substituent may provide a new entry to a group of compounds in which additional hydrogen bonding between the inhibitor and the enzyme may strengthen the sugar–protein interaction.

Table 1. Activities (K_i, μM, pH = 7.0) of C-1 derivatives of DMDP against *Agrobacterium* sp. β-glucosidase

					
1[3] $K_i = 0.2$	**2**[12] $K_i = 12$	**3**[3] $K_i = 30$	**4**[3] $K_i = 10$	**5**[5] $K_i = 25$	**6**[5] $K_i = 10$
					
7[5] $K_i = 25$	**8**[5] $K_i = 1.2$	**9**[5] $K_i = 0.3$	**10**[5] $K_i = 0.1$	**11**[8] $K_i = 0.15$	**16**[8] $K_i = 0.55$
					
17[8] $K_i = 0.10$	**18**[12] $K_i = 0.0024$	**19**[8] $K_i = 0.0012$	**20** $K_i = 0.88$	**21** $K_i = 0.39$	**22** $K_i = 0.27$
					
23 $K_i = 1.0$	**24** $K_i = 0.2$	**25** $K_i = 0.037$	**26** $K_i = 0.44$	**27** $K_i = 0.74$	**32** $K_i = 120$
					
33 $K_i = 50$	**34**[12] $K_i = 0.0021$	**35**[12] $K_i = 420$	**36**[8] $K_i = 0.1$	**37** $K_i = 19$	**38**[5] $K_i = 15$

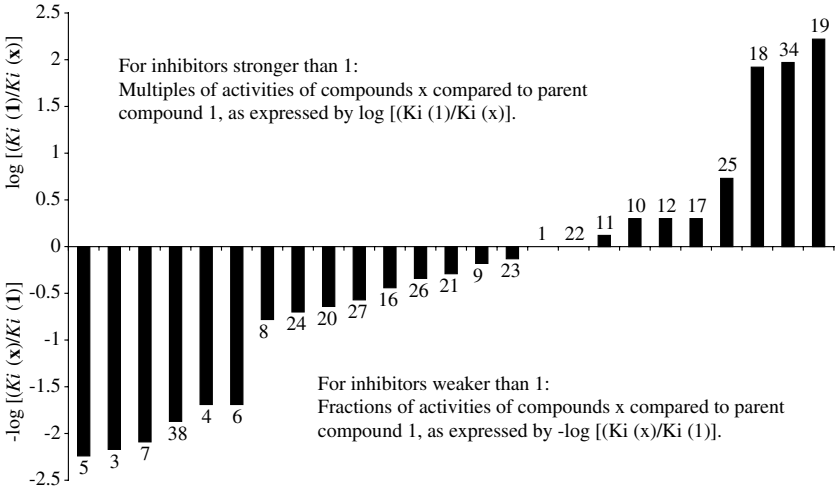


Figure 6. Comparison of K_i values of compounds as multiples, or fractions, respectively, of K_i (**1**).

Two *gluco*-configured derivatives, **32** and **33**, epimeric to compound **1** at position C-2 were synthesised, carrying a hexyl and a propylphenyl ether substituent, respectively, at C-1. Not unexpectedly, compared to their C-2 epimers, both compounds showed only weak interaction with β-glucosidase from *Agrobacterium* sp (Fig. 6).

4. Conclusion

We have synthesised a range of new derivatives of the powerful glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol as well as selected relatives of the corresponding D-*gluco* epimer with different substituents at positions C-1 and C-6, respectively. These compounds were found to be competitive inhibitors of the *Agrobacterium* sp. β-glucosidase employed in this study. Clearly, it could be shown that various lipophilic substituents generally increased the activity and that an additional alkylamino group attached to aromatic systems further improved the interaction between the iminosugar and the enzyme. The size of the aromatic system was also found to be very important for strong inhibitory activity.

5. Experimental

Thin layer chromatography (TLC) was performed on precoated aluminium 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. ^{1}H NMR spectra were recorded on a Varian INOVA 500 operating at 499.925 MHz. ^{13}C NMR spectra were recorded at 75.47 or 125 MHz. Residual nondeuterated solvent was used as internal standard for determination of chemical shifts. Signals of aromatic as well as alkyl substituents including protecting groups were found in the expected regions and are not listed explicitly. Mass spectroscopy was conducted on a HP 1100 series MSD, Hewlett Packard. The scan mode for positive ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 50 to 250 V with best molecular peaks observed at 150 V.

Agrobacterium sp. β-glucosidase was purified and assayed as described.[16] Kinetic studies were performed at 37 °C in pH 7.0 sodium phosphate buffer (50 mM) containing 0.1 % bovine serum albumin, using 7.2×10^{-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 = $1.5\times K_m$) 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 ($1/v$ vs [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 0.015–0.6 mM. Data were analysed by direct fit to the Michaelis Menten equation describing reaction in the presence of inhibitors using the program GraFit.[17]

5.1. 1-Benzoylamino-1,2,5-trideoxy-2,5-imino-D-mannitol (20)

Compound **5**[4] (98 mg, 0.6 mmol) was dissolved in 10 mL methanol, to the cooled (0 °C) solution HBTU (230 mg, 0.6 mmol), Et_3N (0.1 mL, 0.71 mmol) and benzoic acid (41 mg, 0.3 mmol) were added and the reaction was stirred at room temperature for 1 h. During this period 3 mL DMF were slowly added for solubility reasons. The solvents were removed under reduced pressure and the crude product was purified by silica gel chromatography using $CHCl_3$/MeOH (v/v 5/1 containing 1% NH_4OH) to give 120 mg (74.5%). $[\alpha]_D^{20}$ +22.0 (*c* 0.5, H_2O). ^{13}C NMR (D_2O): δ 1712 (C=O), 79.3, 77.6 (C-3, C-4), 61.7, 61.6, 59.9 (C-2, C-5, C-6), 42.2 (C-1). ^{1}H NMR (D_2O): δ 3,80–3.73 (2d, 2H, $J_{2,3=4,5}$ 3.8, H-3, H-4),

3.61 (dd, 1H, $J_{6,6'} = 11.8$, $J_{6',5} = 4.4$, H-6′), 3.53 (dd, 1H, $J_{5,6} = 6.2$, H-6), 3.49–3.39 (m, 2H, H-2, H-5), 3.13 (dd, 1H, $J_{1,1'} = 12.2$, $J_{1,2} = 5.9$, H-1), 2.98 (dd, 1H, $J_{1',2} = 4.8$, H-1′).

5.2. 1-(3-Dimethylamino)benzoylamino-1,2,5-trideoxy-2,5-imino-D-mannitol (21)

Compound **5** (90 mg, 0.56 mmol) and 3-dimethylaminobenzoic acid (91.6 mg, 0.55 mmol), HBTU (209 mg, 0.55 mmol) and Et_3N (0.1 mL, 0.71 mmol) were reacted to obtain product **21** (24 mg, 14%): $[\alpha]_D^{20}$ +24.4 (*c* 0.6, H_2O). ^{13}C NMR (CD_3OD): δ 171.3 (C=O), 77.9, 76.4 (C-3, C-4), 64.6 (C-5), 63.4 (C-2), 59.7 (C-6), 40.8 (C-1), 39.6 (2 C, $2\times NCH_3$). ^1H NMR: 3.82 (m, 2H, $J_{3,4} = 5.8$, H-3, H-4), 3.71 (dd, 1H, $J_{5,6} = 3.9$, $J_{6,6'} = 11.2$, H-6), 3.63 (dd, 1H, $J_{5,6'} = 2.4$, H -6′), 3.61 (m, 1H, $J_{1',2} = 5.4$, H-1′), 3.54 (dd, 1H, $J_{1,2} = 7.3$, $J_{1,1'} = 13.7$, H-1), 3.24 (m, 1H, H-2), 3.08 (m, 1H, H-5), 2.99 (2s, 6H, $2CH_3$). MS ([MH^+] *m*/*z*) Calcd for $C_{15}H_{23}O_4N_3$: 309.41; Found: $[M+H]^+$ 310.35.

5.3. 1-(4-Dimethylamino)-benzoylamino-1,2,5-trideoxy-2,5-imino-D-mannitol (22)

A 5% solution of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol **5** (53 mg, 0.327 mmol) was dissolved in DMF (5 mL) and the mixture was cooled to 0 °C. 4-Dimethylaminobenzoic acid (55 mg, 0.33 mmol), HBTU (131 mg, 0.35 mmol) and Et_3N (71 μL, 0.50 mmol) were added and the mixture was stirred at 25 °C. After 4.5 h, TLC indicated completed reaction. The mixture was concentrated under reduced pressure and purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 100:500:6). Product **22** was obtained as white semicrystalline solid (16 mg, 16%): $[\alpha]_D^{20}$ +22.5 (*c* 0.8, H_2O). ^{13}C NMR (D_2O): δ 170.3 (C=O), 75.8, 74.3 (C-3, C-4), 62.9 (C-5), 61.4 (C-6), 58.1 (C-2), 46.4 (2C, CH_3), 39.4 (C-1). ^1H NMR: δ 4.08 (m, 2H, H-3, H-4), 3.90 (dd, 1H, $J_{5,6} = 3.7$, $J_{6,6'} = 12.7$, H-6); 3.83 (m, 3H, H-1, H-1′, H-6′), 3.72 (m, 1H, H-5), 3.62 (m, 1H, H-2), 3.21 (2s, 6H, $2CH_3$). MS ([MH^+] *m*/*z*) Calcd for $C_{15}H_{24}O_4N_3$: 310.41; Found: $[M+H]^+$ 310.34.

5.4. 1-(Benzenesulfonyl)amino-1,2,5-trideoxy-2,5-imino-D-mannitol (23)

To a 5% solution of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol **5** (39 mg, 0.24 mmol) in MeOH (1.2 mL), benzenesulfonic chloride (31.0 μL, 0.24 mmol) was added and the pH 7–8 was adjusted by addition of Et_3N (24.7 μL, 0.18 mmol). The reaction mixture was stirred at room temperature for 2 h, when TLC ($CHCl_3$/MeOH/NH_4OH 1:2:1) indicated completed conversion. The mixture was concentrated under reduced pressure and purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH 400:100:1). Product **23** was obtained as white glass (30.2 mg, 42%): $[\alpha]_D^{20}$ +23.9 (*c* 0.6, MeOH). ^{13}C NMR (D_2O): δ 76.1, 75.0 (C-3, C-4), 62.9, 61.0, 58.8 (C-2, C-5, C-6), 41.8 (C-1). ^1H NMR: δ 3.90 (dd, 1H, $J_{3,4} = 6.3$, $J_{2,3} = 7.3$, H-3), 3.82 (dd, 1H, $J_{4,5} = 7.8$, H-4), 3.76 (dd, 1H, $J_{6,6'} = 12.6$, $J_{5,6} = 3.9$, H-6), 3.68 (dd, 1H, $J_{5,6'} = 5.9$, H-6′), 3.41–3.36 (m, 2H, H-2, H-5) 3.13 (d, 2H, $J_{1',2} = 5.9$, H-1, H-1′).

5.5. 1-(*n*-Octylsulfonyl)amino-1,2,5-trideoxy-2,5-imino-D-mannitol (24)

To a 5% solution of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol **5** (39 mg, 0.24 mmol) in DMF (1.2 mL) octyl sulfonic chloride (50 μL, 0.24 mmol) and Et_3N (three drops) were added and the reaction stirred at room temperature for 3 h. TLC ($CHCl_3$/MeOH/NH_4OH 1:2:1) indicated completed reaction. The mixture was concentrated under reduced pressure and purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH 500:100:1). Product **23** was obtained as white semicrystalline solid (30.2 mg, 42%): $[\alpha]_D^{20}$ +23.0 (*c* 0.2, MeOH). ^{13}C NMR (CD_3OD): δ 78.0, 76,9 (C-3, C-4), 64.5, 62.8, 60.4 (C-2, C-5, C-6), 51.5 (C-7), 43.3 (C-1), 31.8 (C-8), 29.1, 29.0, 28.2, 23.5, 22,5 (C-9, C-10, C-11, C-12, C-13), 13.2 (C-14). ^1H NMR: δ 3.91 (dd, 1H, $J_{2,3} = 5.9$, $J_{3,4} = 5.40$, H-3), 3.87 (dd, 1H, $J_{3,4} = J_{4,5} = 5.4$, H-4), 3.79 (dd, 1H, $J_{6,6'} = 11.2$, $J_{6,5} = 3.9$, H-6), 3.70 (dd, 1H, $J_{6',5} = 6.3$, H-6′), 3.40–3.26 (m, 4H, H-2, H-5, H-1, H-1′), 3.11 (t, 2H, J = 7.8, H-7), 1.78 (m, 2H, H-8), 1.45 (m, 2H, H-9), 1.40–1.28 (m, 8H, H-10, H-11, H-12, H-13), 0.92 (t, 3H, J = 6.8, H-14).

5.6. 1-Amino-*N*-(coumarin-3-oyl)-1,2,5-trideoxy-2,5-imino-D-mannitol (25)

A 10% solution of 1-amino-1,2,5-trideoxy-D-mannitol **5** (72 mg, 0.44 mmol) in DMF was cooled to 0 °C and consecutively coumarin-3-carboxylic acid (84 mg, 0.44 mmol), HBTU (167,4 mg, 0.45 mmol) and Et_3N (0.1 mL, 0.71 mmol) were added and the reaction was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and chromatographed on silica gel ($CHCl_3$/MeOH/NH_4OH 100:600:7). Product **25** was obtained as white glass (92.5 mg, 63%): $[\alpha]_D^{20}$ +20.7 (*c* 0.8, H_2O). ^{13}C NMR (CD_3OD): δ 79.9, 77.9 (C-3, C-4), 63.7, 61.6, 61.3 (C-2, C-5, C-6), 42.4 (C-1). ^1H NMR: δ 3.80 (2 dd, 2H, $J_{6,6'} = 13.2$, $J_{5,6} = J_{5,6'} = 6.7$, H-6, H-6′), 3.70 (dd, 1H, $J_{4,5} = 3.9$, $J_{3,4} = 11.2$, H-4), 3.67 (dd, 1H, $J_{1,1'} = 13.2$, $J_{1',2} = 5.4$, H-1′), 3.62 (dd, 1H, $J_{2,3} = 5.9$, H-3), 3.56 (dd, 1H, $J_{1,2} = 6.8$, H-1), 3.21 (m, 1H, H-2), 3.03 (m, 1H, H-5). MS([MH^+] *m*/*z*) Calcd for $C_{16}H_{18}O_6N_2$: 334.4; Found: $[M+H]^+$ 335.1.

5.7. 1-[*N*-(1,3-Dimethyl-2,4,6 (1H, 3H, 5H)-trioxopyrimidine-5-ylidene)methyl]amino-1,2,5-trideoxy-2,5-imino-D-mannitol (26)

The starting material **5** (57.2 mg, 0.32 mmol) was dissolved in 2 mL methanol, Et_3N (88 μL, 0.36 mmol) and 1,3-dimethyl-5-[(dimethylamino)methylene] 2,4,6 (1H, 3H, 5H)-trioxopyrimidine (DTPM reagent)[13] (75 mg, 0.36 mmol) dissolved in 200 μL MeOH were added in

one portion and the reaction was stirred at room temperature for 1 h to give **26** (108.6 mg, 99 %) as a white precipitate.^{13}C NMR (D_2O): δ 179.5 (C=O), 164.5, 162.5, 160.8, 153.0 (DTPM), 90.5 (DTPM), 78.6, 76.8 (C-3, C-4), 61.8, 61.0, 59.7 (C-2, C-5, C-6), 52.6 (C-1).^{1}H NMR (D_2O): δ 3.80 (m, 2H, $J_{2,3} = J_{3,4} = 3.8$, H-3, H-4), 3.68 (dd, 1H, $J_{6,6'} = 10.8$, $J_{5,6'} = 4.7$, H-6′), 3.60 (dd, 1H, $J_{5,6} = 5.5$, H-6), 3.58–3.52 (m, 2H, H-2, H-5), 3.24–3.22 (m, 1H, H-1′), 3.06 (s, 6H, $2CH_3$), 2.99–296 (m, 1H, H-1).

5.8. 5-Azido-5-deoxy-6-*O*-hexyl-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-α-D-glucofuranose (29)

To a 5% solution of **28**[5] (630 mg, 2.18 mmol) in THF containing 20% DMF, sodium hydride (55% dispersion in oil, 638.2 mg, 15.2 mmol) and 1-bromohexane (770.0 μL, 5.5 mmol) were added. The mixture was stirred at room temperature until the starting material was no longer detectable by TLC (4 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, washed with 5% aqueous HCl, satd aqueous $NaHCO_3$ 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 **29** (595 g, 76%): $[\alpha]_D^{20}$ −17.0 (*c* 1.0, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 112.3, 105.4 (C-1), 96.9, 82.9 (C-2), 80.3 (C-3), 78.5 (C-4), 71.8, 71.7 (C-6), 58.9 (C-5), 56.3, 31.9, 29.8, 27.0, 26.5, 25.9, 22.8, 14.2. ^{1}H NMR: δ 5.86 (d, 1H, $J_{1,2} = 3.5$, H-1), 4.74 (dd, 2H, $J = 6.6$), 4.6 (d, 1H, H-2), 4.15 (m, 1H, $J_{3,4} = 2.6$, H-3), 4.03 (dd, 1H, $J_{4,5} = 9.7$, H-4), 3.83 (m, 1H, $J_{5,6} = 2.2$, $J_{5,6'} = 6.2$, H-5), 3.59 (dd, 1H, $J_{6,6'} = 10.1$, H-6), 3.48 (dd, 1H, H-6′), 3.44 (s, 2H), 1.56 (m, 2H), 1.46 (2s, 2×3H),1.29 (m, 6H), 0.87 (m, 3H).

5.9. 5-Azido-5-deoxy-6-*O*-hexyl-α-D-glucofuranose (30)

A 5% solution of intermediate **29** (1.0 g, 2.68 mmol) in 50% aqueous CH_3CN was stirred with 15 mL of ion-exchange resin Amberlite IR-120 [H^+] at 45 °C until TLC showed quantitative conversion into a single, more polar 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 **30** (500 mg, 71%): $[\alpha]_D^{20}$ −27.5 (*c* 0.4, CH_2Cl_2); β-Anomer: ^{13}C NMR ($CDCl_3$): δ 103.5 (C-1), 81.1 (C-4), 77.2 (C-2), 76.2 (C-3), 72.1 (C-7), 70.6 (C-6), 60.6 (C-5), 31.8, 29.7, 25.8, 22.8, 14.2. α-Anomer: ^{13}C NMR ($CDCl_3$): δ 97.1 (C-1), 79.9 (C-4), 77.9 (C-2), 76.6 (C-3), 72.2, 70.9 (C-6), 60.1 (C-5), 31.8, 29.7, 25.8, 22.8, 14.2. ^{1}H NMR: δ 5.55 (d, 1H, $J_{1,2} = 2.4$, H-1β), 5.20 (s, 1H, H-1α), 4.29 (s, 1H, H-2α), 4.22–4.17 (m, 2H, $J_{3,4} = 3.4$, $J_{4,5} = 8.8$, H-3α, H-3β, H-4α, H-4β), 4.11 (m, 1H, H-2β), 3.93 (m, 1H, $J_{5,6} = 2.9$, $J_{5,6'} = 6.1$, H-5α), 3.88–3.83 (m, 2H, $J_{6,6'} = 10.3$, H-6α, H-5β), 3.79 (dd, 1H, H-6β), 3.67 (dd, 1H, H-6′α), 3.62 (dd, 1H, H-6′β), 3.51 (m, 4H), 1.60 (m, 4H), 1.32 (m, 12H), 0.89 (t, 6H).

5.10. 5-Azido-5-deoxy-6-*O*-hexyl-D-fructose (31)

A 5% aqueous solution of the free aldose **30** was spun on the rotary evaporator in the presence of immobilised glucose isomerase (Sweetzyme T, EC 5.3.1.5, 1.5 g) at 60 °C for 12 h. The solids were filtered off, $BaCO_3$ (643 mg, 3.3 mmol) and bromine (150 mg, 0.94 mmol) were added and the solution was stirred at ambient temperature until the remaining aldose was converted into the less polar D-gluconotactone. Air was bubbled through the solution to remove the excess of bromine. After filtration, the solution was concentrated under reduced pressure and the crude product was purified by silica gel chromatography (cyclohexane/ethyl acetate 4/1 v/v) to give pure ketose **31** (30 mg, 8%): $[\alpha]_D^{20}$ −40.8 (*c* 0.4, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 212.4 (C-2), 75.6 (C-3), 72.7 (C-4), 72.4 (C-7), 71.0 (C-6), 66.7 (C-1), 60.8 (C-5), 31.8, 29.7, 25.9, 21.3, 14.2. ^{1}H NMR: δ 4.60 (d, 1H, $J = 19.8$, H-1), 4.49–4.41 (m, 2H, H-1′, H-3), 4.00 (m, 1H, H-4), 3.72–3.56 (m, 3H, H-5, H-6, H-6′), 3.52 (m, 2H), 1.52 (m, 2H), 1.35 (m, 6H), 0.89 (t, 3H).

5.11. 2,5,6-Trideoxy-1-*O*-hexyl-2,5-imino-D-mannitol (27)

Hydrogenation and concomitant intramolecular reductive amination of ketose **31** (30 mg, 0.1 mmol) was conducted in dry methanol in the presence of a catalytic amount of $Pd(OH)_2$/C (20%) under hydrogen atmosphere at ambient pressure. After 2 h, TLC ($CHCl_3$/MeOH/NH_4OH 2:2:1) showed no further starting material. The catalyst was removed by filtration, the solution was concentrated and the resulting yellow syrup was purified by silica gel chromatography(CCl_3/MeOH/NH_4OH 120/10/1) to furnish inhibitor **27** (8 mg, 31%) as a colourless oil: $[\alpha]_D^{20}$ +16.9 (*c* 0.1, MeOH); ^{13}C NMR (CD_3OD): δ 78.5, 78.4 (C-3, C-4), 71.4 (C-1), 70.9 (C-6), 63.5 (C-7), 61.8 (C-5), 61.8 (C-2), 34.2 (C-10), 31.7 (C-9), 25.8 (C-8), 22.5 (C-11), 13.2 (C-12). ^{1}H NMR: δ 3.79–3.78 (m, 2H, H-3, H-4), 3.69–3.42 (m, 2H, H-1, H-1′, H-2, H-6, H-6′), 3.33 (m, 2H, H-7), 3.09 (m, 1H, H-5), 1.59 (m, 2H, H-8), 1.37 (m, 6H, H-9, H-10, H-11), 0.91 (t, 3H, H-12).

5.12. 5-Azido-6-*O*-*t*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (35)

To a 20% solution of 5-azido-5-deoxy-1,2-*O*-isopropylidene-L-idofuranose (**34**)[15] (3.0 g, 12.2 mmol) in dry DMF imidazole (2.1 g, 30.6 mmol) and *t*-butyl-dimethylsilyl chloride (2.2 g, 14.7 mmol) were added. The reaction was stirred at 40 °C until TLC (cyclohexane/ethyl acetate 1/1) confirmed quantitative conversion (2 h). The mixture was diluted with water, extracted with CH_2Cl_2 twice, the organic phase was dried and concentrated under reduced pressure. Silica gel chromatography (cyclohexane/ethyl acetate 5/1 v/v) gave silylated compound **35** (4.40 g, 100%) as a colourless syrup: $[\alpha]_D^{20}$ −11.5 (*c* 0.8, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 112.0 (C-7), 104.8 (C-1), 85.2, 82.6 (C-2, C-4), 75.5 (C-3), 63.5, 62.2 (C-5, C-6). ^{1}H NMR: δ 5.85 (d, 1H, $J_{1,2} = 3.5$, H-1), 4.42 (d, 1H,

H-2), 4.07 (s, 1H, H-3), 3.99 (dd, 1H, $J_{4,5} = 7.0$, H-4), 3.67 (m, 1H, $J_{5,6} = 5.7$, $J_{5,5'} = 9.2$, H-5), 3.59 (m, 1H, $J_{6,6'} = 11.0$, H-6), 3.54 (m, 1H, H-6′).

5.13. 5-Azido-6-*O*-*t*-butyldimethylsilyl-5-deoxy-1,2-*O*-isopropylidene-3-methyloxymethyl-β-L-idofuranose (36)

A 5% solution of compound **35** (2.7 g, 7.5 mmol) in dry DMF was treated with ethyl-*N*,*N*-diisopropylamine (5.1 mL, 30 mmol) and freshly prepared MOM chloride[18] (50% in MeOH, 3.6 mL, 22 mmol). The mixture was stirred at room temperature until the starting material was no longer detectable by TLC (30 h). Solvents were evaporated under reduced pressure, the remaining residue was diluted with CH_2Cl_2, consecutively washed with 5% aqueous HCl, satd aqueous $NaHCO_3$ 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 6:1 v/v) to yield compound **36** (2.46 g, 81%): $[\alpha]_D^{20}$ −38.5 (*c* 0.8, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 112.3 (C-7), 104.8 (C-1), 96.5 (C-14), 83.3, 80.8 (C-2, C-4), 78.8 (C-3), 63.4, 62.3 (C-5, C-6). ^{1}H NMR: δ 5.85 (d, 1H, $J_{1,2} = 4.0$, H-1), 4.63 (m, 2H, H-14), 4.52 (d, 1H, H-2), 4.16 (dd, 1H, $J_{4,5} = 7.9$, H-4), 3.97 (d, 1H, $J_{3,4} = 3.1$, H-3), 3.70 (m, 1H, $J_{5,6} = 5.2$, $J_{5,5'} = 8.0$, H-5), 3.64 (m, 1H, H-6), 3.59 (m, 1H, H-6′).

5.14. 5-Azido-5-deoxy-1,2-*O*-isopropylidene-3-methyloxymethyl-β-L-idofuranose (37)

To a 5% solution of compound **36** (2.7 g, 5.6 mmol) in dry THF, tetrabutylammonium fluoride trihydrate (2.7 g, 8.5 mmol) was added and the reaction was stirred at 45 °C until TLC (cyclohexane/ethyl acetate 1:1) indicated completion (1 h). The solution was concentrated under reduced pressure and the crude product was purified by silica gel chromatography (cyclohexane/ethyl acetate 3:1 v/v) to give compound **37** in quantitative yield (1.86 g): $[\alpha]_D^{20}$ −85.1 (*c* 1.3, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 112.3 (C-7), 104.8 (C-1), 96.6 (C-10), 83.3, 80.3 (C-2, C-4), 80.2 (C-3), 63.2, 62.2 (C-5, C-6). ^{1}H NMR: δ 5.98 (d, 1H, $J_{1,2} = 4.0$, H-1), 4.72 (m, 2H, $J = 6.6$, H-10), 4.63 (d, 1H, H-2), 4.25 (dd, 1H, $J_{4,5} = 8.8$, H-4), 4.08 (d, 1H, $J_{3,4} = 3.1$, H-3), 3.87 (m, 1H, $J_{5,6} = 3.5$, $J_{5,5'} = 6.2$, H-5), 3.73 (m, 1H, $J_{6,6'} = 11.4$, H-6), 3.56 (m, 1H, H-6′).

5.15. 5-Azido-5-deoxy-6-*O*-hexyl-1,2-*O*-isopropylidene-3-methyloxymethyl-β-L-idofuranose (38)

Applying the same procedure as for compound **29** to compound **37** (750 mg, 2.60 mmol), employing 1-bromohexane (900 μL, 6.5 mmol) and NaH (55% dispersion in oil, 0.8 g, 19.0 mmol) product **38** (818 mg, 85%) was be obtained: $[\alpha]_D^{20}$ −40.9 (*c* 1.1, CH_2Cl_2); ^{13}C NMR: ($CDCl_3$) δ 112.3 (C-7), 104.8 (C-1), 96.7 (C-10), 83.3, 81.0 (C-2, C-4), 79.7 (C-3), 72.1 (C-12), 70.4 (C-6), 60.8 (C-5). ^{1}H NMR: δ 5.93 (d, 1H, $J_{1,2} = 3.5$, H-1), 4.71 (dd, 2H, $J = 7.0$, H-10), 4.61 (d, 1H, H-2), 4.22 (dd, 1H, $J_{4,5} = 8.8$, H-4), 4.05 (d, 1H, $J_{3,4} = 3.1$, H-3), 3.85 (m, 1H, $J_{5,6} = 3.5$, $J_{5,6'} = 6.2$, H-5), 3.56 (m, 1H, $J_{6,6'} = 11.6$, H-6), 3.49 (m, 1H, H-6′).

5.16. 5-Azido-5-deoxy-6-*O*-hexyl-β-L-idofuranose (39)

Deprotection of compound **38** (780 mg, 2.09 mmol) employing excess ion exchange resin Amberlite IR-120 [H^+] in 50% aqueous acetonitrile gave compound **39** (404 mg, 67%): $[\alpha]_D^{20}$ + 35.1 (*c* 1.1 CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ β-Anomer: 103.2 (C-1), 84.6 (C-4), 80.0 (C-2), 77.0 (C-3), 72.4, 70.4 (C-6), 62.0 (C-5), 31.7, 29.6, 25.8, 22.8, 14.2. α-Anomer: 96.6 (C-1), 81.2 (C-4), 77.9 (C-2), 76.2 (C-3), 72.4, 70.3 (C-6), 60.7 (C-5), 31.7, 29.6, 25.8, 22. 8, 14.2. ^{1}H NMR: δ 5.58 (m, 1H, H-1α), 5.22 (s, 1H, H-1β), 4-58–4.26 (m, 5H, H-2α, H-2β, H-3β, H-4α, H-4β), 4.30 (d, 1H, H-3α), 3.92 (m, 1H, $J_{5,6} = 4.3$, $J_{5,6'} = 8.3$, H-5β), 3.82 (m, 1H, H-5α), 3.62 (dd, 2H, $J_{6,6'} = 10.8$, H-6α, H-6β), 3.52–3.45 (m, 6H, H-6′α, H-6′β, H-7), 1.58 (m, 4H), 1.30 (m, 12H), 0.88 (t, 6H).

5.17. 5-Azido-5-deoxy-6-*O*-hexyl-L-sorbose (40)

Employing immobilised glucose isomerase (Sweetzyme T, EC 5.3.1.5, 1.5 g) to 5-azido-5-deoxy-6-*O*-hexyl-β-L-idofuranose**39** (400 mg, 1.38 mmol) at 60 °C gave product **40** (94 mg, 24%): $[\alpha]_D^{20}$ +30.0 (*c* 0.2 CH_2Cl_2). ^{13}C NMR ($CDCl_3$): δ 211.2 (C-2), 75.6 (C-3), 73.1 (C-4), 72.5 (C-1), 67.3 (C-6), 61.6 (C-5), 31.8, 29.6, 25.9, 22.8, 14.2. ^{1}H NMR: δ 4.50 (d, 1H, *J* 17.6 Hz, H-1), 4.34 (d, 1H, H-1′), 4.08 (m, 1H, H-3), 3.71 (m, 3H, H-4, H-6, H-6′), 3.45 (m, 3H, H-5, H-7), 1.53 (m, 2H), 1.20 (m, 6H), 0.83 (t, 3H).

5.18. 2,5-Dideoxy-6-*O*-hexyl-2,5-imino-D-glucitol (32)

The same procedure as for compound **27** applied to 5-azido-5-deoxy-6-*O*-hexyl-L-sorbose **40** (75 mg, 0.26 mmol) employing $Pd(OH)_2$/C under hydrogen atmosphere at ambient pressure gave product **32** (29 mg, 44%): $[\alpha]_D^{20}$ +12.5 (*c* 0.1, MeOH). ^{13}C NMR (CD_3OD): δ 79.4 (C-3), 77.9 (C-4), 71.4 (C-7), 69.5 (C-6), 66.3 (C-2), 62.3 (C-1), 60.1 (C-5), 31.7 (C-10), 29.6 (C-9), 25.8 (C-8), 22.5 (C-11), 13.2 (C-12). ^{1}H NMR: 3.94 (dd, 1H, $J_{3,4} = 4.4$, H-3), 3.82 (dd, 1H, $J_{4,5} = 4.8$, H-4), 3.67 (m, 2H, H-1, H-1′), 3.58–3.43 (m, 3H, H-5, H-6, H-6′), 3.33 (m, 2H, H-7), 2.97 (m, 1H, $J_{5,6} = 9.2$, H-2), 1.57 (m, 2H, H-8), 1.32 (m, 6H, H-9, H-10, H-11), 0.91 (t, 3H, H-12).

5.19. 5-Azido-5-deoxy-6-*O*-(1-phenylpropyl)-1,2-*O*-isopropylidene-3-methyloxymethyl-β-L-idofuranose (41)

To a 5% solution of compound **37** (750 mg, 2.6 mmol) in THF containing 20% DMF 1-bromophenylpropane (1 mL, 6.6 mmol) and NaH (55% dispersion in oil, 760 mg, 18.2 mmol) were added to obtain product **41** in quantitative yield (1.06 g): $[\alpha]_D^{20}$ −36.9 (*c* 0.4, CH_2Cl_2). ^{13}C NMR ($CDCl_3$): δ 142.0, 128.7, 128.6, 126.1 (phenyl), 112.4, 104.8 (C-1), 96.7 (MOM), 83.4, 80.9 (C-2, C-4),

79.6 (C-3), 70.9, 70.5 (C-6), 60.8 (C-5), 56.5, 32.4, 31.4. ^{1}H NMR: δ 7.31–7.17 (m, 5H, phenyl), 5.95 (d, 1H, $J_{1,2} = 3.5$, H-1), 4.71 (dd, 2H, $J = 7.0$), 4.61 (d, 1H, H-2), 4.26 (dd, 1H, $J_{4,5} = 8.8$, H-4), 4.06 (d, 1H, $J_{3,4} = 3.1$, H-3), 3.88 (m, 1H, $J_{5,6} = 3.5$, $J_{5,6'} = 6.2$, H-5), 3.59 (m, 1H, $J_{6,6'} = 10.5$, H-6), 3.44 (m, 2H), 3.49 (m, 1H, H-6′), 3.40 (s, 3H), 2.70 (m, 2H), 1.91 (m, 2H).

5.20. 5-Azido-5-deoxy-6-*O*-(1-phenylpropyl)-L-idofuranose (42)

Deprotection of compound **41** (100 mg, 0.25 mmol) employing ion exchange resin Amberlite IR-120 [H^+] gave product **42** (45 mg, 57%): $[\alpha]_D^{20}$ −17.84 (*c* 1.1, CH_2Cl_2). ^{13}C NMR ($CDCl_3$): δ β-Anomer: 141.7, 128.7, 128.6, 126.3 (phenyl), 103.2 (C-1), 84.4 (C-4), 80.1 (C-2), 77.1 (C-3), 71.4 (C-7), 70.4 (C-6), 62.0 (C-5), 32.4 (C-8), 31.2 (C-9). α-Anomer: 141.7, 128.7, 128.6, 126.3 (phenyl), 96.6 (C-1), 80.9 (C-4), 77.9 (C-2), 76.2 (C-3), 71.5 (C-7), 70.5 (C-6), 60.6 (C-5), 32.4 (C-8), 31.2 (C-9). ^{1}H NMR: δ 7.19–7.06 (m, 10H, phenyl), 5.60 (d, 1H, $J_{1,2} = 3.52$, H-1α), 5.22 (s, 1H, H-1β), 4-58–4.26 (m, 5H, H-2α, H-2β, H-3β, H-4α, H-4β), 4.30 (d, 1H, H-3α), 3.92–3.62 (m, 6H, H-5β, H-5α, H-6α, H-6βH-6′α, H-6′β), 3.52–3.45 (m, 4H, H-7, H-9), 1.98 (m, 4H, H-8).

5.21. 5-Azido-5-deoxy-6-*O*-(1-phenylpropyl)-L-sorbose (43)

Isomerisation of compound **42** (600 mg, 1.86 mmol) with immobilised glucose isomerase (Sweetzyme T, EC 5.3.1.5, 1.5 g) at 60 °C gave product **43** (106 mg, 18%): $[\alpha]_D^{20}$ +46.0 (*c* 0.1, CH_2Cl_2). ^{13}C NMR ($CDCl_3$): δ 210.8 (C-2), 141.6, 128.7, 128.7, 126.2 (phenyl), 75.7 (C-3), 72.9 (C-4), 71.4 (C-6), 71.2 (C-7), 67.3 (C-1), 61.7 (C-5), 32.4 (C-9), 31.2 (C-8). ^{1}H NMR: δ 4.97–4.19 (m, 5H, phenyl), 4.55 (d, 1H, $J = 18.0$, H-1), 4.39 (d, 1H, H-1′), 4.15 (m, 1H, H-3), 3.60 (m, 3H, H-4, H-6, H-6′), 3.53 (m, 3H, H-5, H-7, H-7′), 2.70 (m, 2H, H-9), 1.94 (m, 2H, H-8).

5.22. 2,5-Dideoxy-6-*O*-(1-phenylpropyl)-2,5-imino-D-glucitol (33)

Hydrogenation of compound **43** (25 mg, 0.16 mmol) employing $Pd(OH)_2$/C (20%) under hydrogen atmosphere at ambient pressure gave product **33** (29 mg, 67%): $[\alpha]_D^{20}$ +7.1 (*c* 0.1, MeOH). ^{13}C NMR (CD_3OD): δ 142.1, 128.3, 128.2, 125.6 (phenyl), 79.4 (C-3), 77.8 (C-4), 70.4 (C-7), 69.5 (C-6), 66.3 (C-2), 62.1 (C-1), 60.2 (C-5), 32.1 (C-9), 31.5 (C-8). ^{1}H NMR: δ 7.29–7.13 (m, 5H, phenyl), 3.95 (dd, 1H, $J_{3,4} = 4.83$, H-3), 3.84 (dd, 1H, $J_{4,5} = 7.0$, H-4), 3.74–3.58 (m, 3H, H-1, H-1′, H-5), 3.57–3.26 (m, 4H, H-6, H-6′, H-7), 2.98 (dd, 1H, $J_{2,3} = 2.2$, H-2), 2.69 (t, 2H, H-9), 1.79 (m, 2H, H-8).

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