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L-Idoseptanosides: substrates of D-glucosidases?

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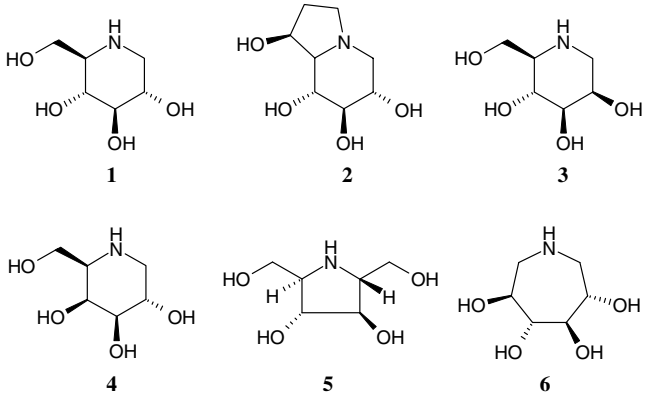
Abstract—Based on the reported glycosidase inhibitory activities of 1,6-dideoxy-1,6-imino-L-iditol, the corresponding aryl glycosides 4-nitrophenyl α- and β-L-idoseptanoside **7** and **8** were synthesised as possible glycosidase substrates. Despite their inherently larger size, these septanosides were indeed shown to be glycosidase substrates, albeit weak ones. In addition, these two substrate analogues **7** and **8** also demonstrated a remarkable degree of selectivity for β- and α-glucosidases, respectively.

1. Introduction

Iminosugars are well known and often potent inhibitors of glycosidases. Their efficacy as inhibitors has often been ascribed to their mimicry of bound protonated substrates or of reaction transition states. However, at least in the case of deoxynojirimycin and castanospermine analogues, more careful kinetic analysis with *Agrobacterium* sp. β-glucosidase has cast doubt on the hypothesis of transition state mimicry.[1] Rather, it was suggested that they act as 'fortuitous' inhibitors, binding tightly to the enzyme by virtue of the electrostatic interaction of a positively charged ammonium centre with one or more active site carboxylates as well as hydrogen bonds between active site residues and the ligand. Nonetheless, in cases where an analogy is clear, it is often the case that inhibitor configurations match that of the optimal substrate for the enzyme, consistent at least with some mimicry of ground state protonated structures. Thus, for example (Scheme 1), 1-deoxynojirimycin **1** and castanospermine **2**, which have D-*gluco*-configured hydroxyl groups around a piperidine scaffold, selectively inhibit D-glucopyranosidases. Similarly, 1-deoxymannojirimycin **3** inhibits D-mannosidases while the corresponding D-*galacto*-configured iminoalditol **4** is a strong inhibitor of D-galactosidases. Interestingly, over the past two decades, it has also been recognised that



Scheme 1.

many glycosidases are susceptible to inhibition by five- and seven-membered ring iminoalditols, as well as suitably substituted aminocyclopentanes whose structures are not as obviously suitable for the purpose as the pyranoid systems mentioned above. Such compounds include the well-known 2,5-dideoxy-2,5-imino-D-mannitol **5**, a very powerful glucosidase inhibitor[2] and 1,6-dideoxy-1,6-imino-L-iditol **6**, reported to be a good inhibitor of a wide range of various glycosidases, just to mention two examples.[3–5]

Based on the aforementioned notion that close structural relatives of good substrates are frequently good inhibitors, we envisaged that glycosides, which are structurally related to such 'unusually shaped' but efficient

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inhibitors might turn out to be suitable substrates for the respective glycosidases.

Septanoses are usually only transient and elusive tautomeric forms of hexoses and higher carbon sugars, disadvantaged in the equilibria by their inherent high free energies, which exceed those of the pyranose and furanose forms by 3–8 kcal (8–33 kJ) per mole.[6] In the case of idose, a sugar whose configuration intrinsically disfavours the pyranose form, NMR experiments revealed that the septanose was present as only 1.6% of the population.[7] Even in the case when the 5-hydroxyl was methylated, thereby preventing the formation of a pyranose tautomer, only 5.6% of the septanose tautomer was observed. Not unexpectedly in the light of these findings, O-glycosides of septanoses have not yet been found in Nature. Nonetheless, based on the fact that compound **6** was reported to be an excellent glycosidase inhibitor,[3–5] the display of the functional groups they present may allow for some suitable diastereomers of septanosides to mimic the substrates of various glycosidases. Encouragingly, molecular modelling (Fig. 1) supported this hypothesis, revealing that for a selection of low-energy conformers the structure of the β-L-idoseptanoside can be readily superimposed on the corresponding α-D-glucopyranoside. The same was true for the ring oxygen and the secondary hydroxyl groups in the corresponding α-L-idoseptanoside, which all mapped well onto the functional groups in a β-D-glucopyranoside. In both anomers, O-5 of the septanoside was found to be in close proximity to the position of O-6 of the natural substrates.

To test this concept, 4-nitrophenyl (pNP) α-**7** and β-L-idoseptanosides **8** were prepared (Scheme 2) by standard methods and probed as substrates of a range of glycosidases.

2. Results and discussion

2.1. Syntheses

2.1.1. 4-Nitrophenyl α- and β-L-idoseptanosides. Conventional reaction of L-idose,[8] (prepared from 1,2-*O*-isopropylidene-β-L-idofuranurono-6,3-lactone,[9] or from 1,2-*O*-isopropylidene-3,5,6-tri-*O*-methanesulfonyl-α-D-glucofuranose[10]) with EtSH gave known dithioacetal **9**.[11] Its 6-O-tritylation furnished the 1,6-diprotected open-chain L-idose **10** in 87% overall yield from L-idose. Per-O-benzoylation of the remaining secondary positions was found to be superior to O-acetylation in terms of stabilities of intermediates in the following steps yielding compound **11** (97%). Chemo- and regioselective deprotection of the aldehyde function in the presence of the trityl ether to give compound **12** could be achieved with $HgO/HgCl_2$ in an ice cold mixture of acetone/water/acetonitrile 2:1:2 (v/v/v) and was found to be superior to simultaneous 1,6-deprotection in terms of overall yields and reproducibility. Conventional removal of the trityl group from free aldehyde **12** gave the anomeric mixture of 2,3,4,5-O-protected L-idoseptanoses **13**α/β in 88% yield. Reaction with DAST led smoothly to a mixture of the corresponding glycosyl fluorides **14**α/β in 97% combined yield. These intermediates, in our hands, were found to be the most reliable starting materials for the following step. Acid-catalysed reaction with 4-nitrophenol gave the desired protected septanosides **15** and **16**, which were separated by chromatography. Zemplén transesterification of the benzoate esters gave free septanosides **7** (60%) and **8** (51%) as final products. As previously predicted by the modelling study (Fig. 1), the pNP group of septanoside **7** was observed to occupy an equatorial position, with an indicative $J_{1,2}$ of 6.8 Hz. In contrast, an axial orientation of the pNP group in septanoside **8** was implied by the considerably smaller $J_{1,2}$ of 2.0 Hz.

2.2. Enzymatic studies

Compounds **7** and **8** were tested as substrates for a range of α- and β-glycosidases (Table 1). Where an enzyme-catalysed hydrolysis rate was observed, Michaelis–Menten parameters were determined. The parameters obtained were compared to those for hydrolysis of the corresponding preferred pNP-pyranoside substrate for that enzyme in order to estimate the catalytic efficiency of turnover. The absence of an observed hydrolysis rate for the septanoide substrates could be interpreted in one of the two ways, either the septanoside can bind to the

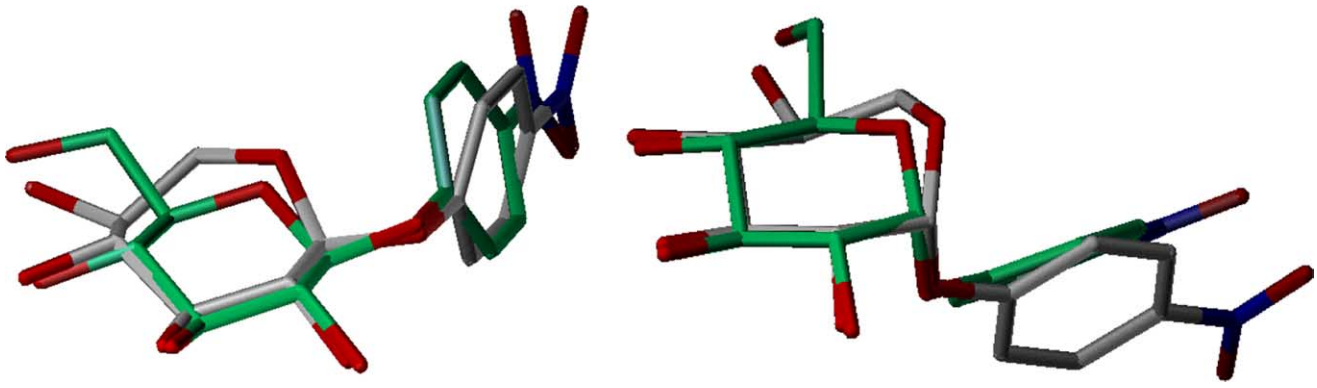
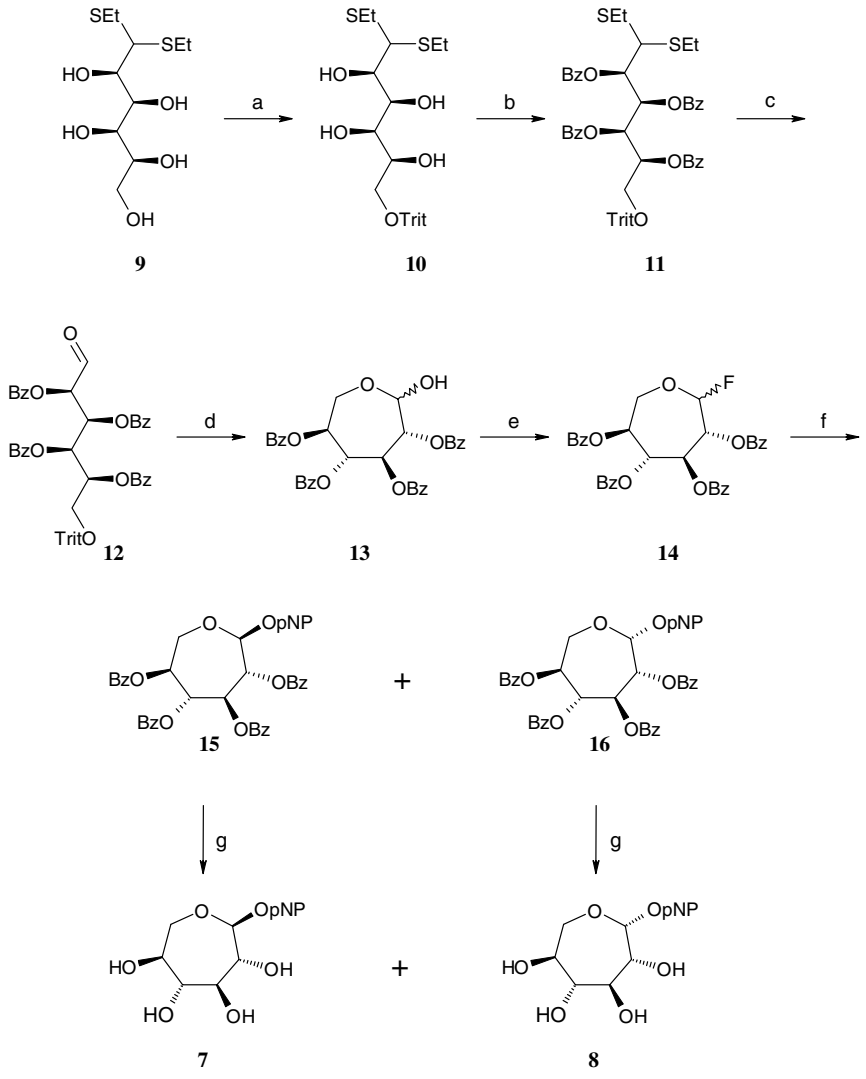


Figure 1. Left: Superposition of 4-nitrophenyl α-L-idoseptanoside **7** (grey) with 4-nitrophenyl β-D-glucopyranoside (green). Right: Superposition of 4-nitrophenyl β-L-idoseptanoside **8** (grey) with 4-nitrophenyl α-D-glucopyranoside (green).



Scheme 2. Reagents and conditions: (a) tritylCl, pyridine; (b) BzCl, pyridine; (c) HgO, $HgCl_2$, MeCN/acetone/H_2O; (d) $BF_3{\cdot}Et_2O$, Et_2O; (e) DAST, CH_2Cl_2; (f) $(4\text{-}NO_2)C_6H_4OH$, NEt_3, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (g) NaOMe, MeOH.

active site, but is unable to be turned over by the enzyme, or, the substrate may have little or no affinity for the enzyme active site. In order to distinguish between these two possibilities, each septanoside was tested as an inhibitor of the enzyme. If the outcome of this experiment were to demonstrate that septanosides were competitive inhibitors, this would imply that these compounds were bound to the active site, but were catalytically incompetent substrates. The absence of any inhibition in the presence of each septanoside would confirm that these compounds were unable to bind to the enzyme active site.

As can be seen from the molecular modelling overlay (Fig. 1), in terms of the three-dimensional spatial arrangement of hydroxyl groups, septanoside **7** has many features in common with pNP β-D-glucopyranoside. Indeed, septanoside **7** was observed to be a slow substrate for *Agrobacterium* sp. β-glucosidase (Table 1). The k_{cat}/K_M value for this substrate is approximately 10^5-fold lower than that of the equivalent pyranoside substrate. In contrast, septanoside **8**, which more closely resembles pNP-α-D-glucopyranoside (Fig. 1), was not observed to undergo enzyme-catalysed hydrolysis. No inhibition of the hydrolysis of pNP β-D-glucopyranoside was observed in the presence of 2.4 mM septanoside **8**, confirming that this anomer is not accommodated by the enzyme active site to any significant extent. When the equivalent study was performed with *S. cerevisiae* α-glucosidase, the complementary result was observed; septanoside **8** was a slow substrate with an overall 10^3-fold lower specificity constant than that for pNP α-D-glucopyranoside, whereas septanoside **7** was found not to bind to the enzyme at a concentration of 1.6 mM. While these are clearly not good substrates, the observation of significant turnover would appear to be vindication of the notions best represented in Figure 1. However, it was quite possible that these septanosides might not show any specificity, but in fact be hydrolysed by a range of glycosidases via non-specific association. In order to probe this further, studies with a wide selection of other glycosidases (α- and β-galactosidases, α- and β-xylosidases and α-mannosidase) were performed. No detectable hydrolysis of either septanoside was seen

Table 1.

Enzyme	Substrate	k_{cat} (s^{-1})[a]	K_M (mM)	k_{cat}/K_M (s^{-1} M^{-1})
β-Glucosidase	pNP-β-Glc[13]	170	0.078	2.2×10^6
Agrobacterium sp.	**7**	0.024	1.1	22
pH 7.0	**8**	<0.001	nd[b]	nd
α-Glucosidase	pNP-α-Glc[c]	7.0	0.27	2.6×10^4
S. cerevisiae	**7**	<0.001	nd	nd
pH 6.8	**8**	0.07	2.9	24
β-Galactosidase	pNP-β-Gal[17]	90	0.040	2.3×10^3
E. coli	**7**	<0.1	nd	nd
pH 7.0	**8**	<0.1	nd	nd
α-Galactosidase	pNP-α-Gal[18]	66	0.45	1.5×10^5
Green coffee bean	**7**	<0.01	nd	nd
pH 6.5	**8**	<0.01	nd	nd
β-Xylosidase	pNP-β-Xyl[14]	9.7	0.036	2.7×10^5
T. saccharolyticum	**7**	<0.01	nd	nd
pH 6.5	**8**	<0.01	nd	nd
α-Xylosidase	pNP-α-Xyl[15]	0.13	0.71	180
E. coli K12	**7**	<0.001	nd	nd
pH 7.0	**8**	<0.001	nd	nd
α-Mannosidase	pNP-α-Man[19]	130	2.0	6.5×10^4
C. ensiformis	**7**	<0.1	nd	nd
pH 4.5	**8**	<0.1	nd	nd

[a] Lower rate limit calculated at 1.6 mM **7** and 2.4 mM **8**.
[b] nd = not determined.
[c] This work.

in any case. These substrate mimetics were again tested for their ability to bind to, and inhibit, each of the enzymes tested. However, with the exception of *E. coli* α-xylosidase Yic1, no inhibition was observed for any of the enzymes studied. This clearly demonstrates that, not only do **7** and **8** not bind productively to the active sites of these other enzymes, but rather they do not bind at all. In the case of Yic1, both septanosides inhibited the hydrolysis of pNP α-D-xylopyranoside (95% at 1.6 mM and 70% at 2.4 mM for **7** and **8**, respectively). These data are in accordance with previous (unpublished) work from this laboratory, which has shown that Yic1 has a very high affinity (low μM) for the binding of β-linked glucosides in the *aglycone* subsites. In the case of *Thermoanaerobacterium saccharolyticum* β-xylosidase XynB, an increase in the rate of enzyme-catalysed cleavage of pNP β-D-xylopyranoside was observed in the presence of both septanosides (1.6 and 2.4 mM for **7** and **8**, respectively). Since neither of these compounds are substrates for XynB, this increase in rate was attributed to their ability to act as glycosyl acceptors in a transglycosylation reaction, a phenomenon previously described for this enzyme when studied with substrates possessing activated leaving groups such as *p*-nitrophenol.

3. Conclusion

In conclusion, the finding that the septanosides **7** and **8** studied are indeed substrates, albeit weak ones, for the predicted glycosidases, but not for enzymes of other specificities, supports the contention that the seven-membered ring iminoalditols are indeed binding in a 'productive' conformation. However, the low activities measured imply that those inhibitors likely are not transition state mimics. The findings also expand the known catalytic potential of glucopyranosidases in demonstrating that they are also capable of effecting the transfer of septanosides and may be useful in this regard for synthetic applications.

4. Experimental

Optical rotations were measured on a JASCO Digital Polarimeter or with a Perkin Elmer 341 with a path length of 10 cm. NMR spectra were recorded at 200 as well as 500 MHz (^{1}H), and at 50 and 125 MHz (^{13}C). $CDCl_3$ or acetone-d_6 were employed for protected compounds and MeOH-d_4 for free glycosides. Chemical shifts are listed in delta employing residual, not deuterated, solvent as the internal standard. The signals of aromatic substituents as well as of protecting groups were found in the expected regions and are not listed explicitly. Structures of crucial intermediates were unambiguously assigned by 1D-TOCSY and HSQC experiments. Electrospray mass spectra were recorded on an HP 1100 series MSD, Hewlett Packard. Samples were dissolved in acetonitrile/MeOH mixtures. The scan mode for negative ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 30 to 130 V. TLC was performed on precoated aluminium sheets (E. Merck 5554). Compounds were detected by staining with concd H_2SO_4 containing 5% vanillin.

For column chromatography Silica Gel 60 (E. Merck) was used.

Molecular modelling was performed as previously described[12] employing Sybyl versions on an Octane workstation by Silicon Graphics using the BGFS-minimiser and the Tripos Force Field.

The known L-idose and its diethyldithioacetal **9** were prepared by available methods as mentioned in Section 2.

4.1. 6-*O*-Trityl-L-idose diethyldithioacetal 10

To a solution of L-idose diethyl dithioacetal **9** (1.38 g, 4.82 mmol) in pyridine (15 mL), chlorotriphenylmethane (1.5 equiv, 2 g) was added and the mixture was kept at 40 °C until TLC indicated quantitative reaction of the starting material. The reaction mixture was concentrated under reduced pressure and the remaining residue dissolved in CH_2Cl_2. The organic layer was sequentially washed with 5% aqueous HCl, satd aqueous sodium bicarbonate and dried over Na_2SO_4. Filtration, removal of the solvent and purification on silica gel gave trityl ether **10** (2.40 g, 94%) as a faintly yellow oil. Found: C, 65.84; H, 6.91; $C_{29}H_{36}O_5S_2$ (528.7353) requires: C, 65.88; H, 6.86; Mass spectrum (API-ES): *m/z*: 527.70 [M−H]; $[\alpha]_D^{20} = -4.6$ (*c* 1.9, MeOH); δ_H (MeOH-d_4) 4.06 (d, 1H, $J_{1,2}$ 6.8 Hz, H-1), 4.04 (dd, 1H, $J_{2,3}$ 3.9 Hz, $J_{3,4}$ 4.4 Hz, H-3), 3.89 (dd, 1H, $J_{4,5}$

6.8 Hz, H-4), 3.87 (m, 1H, H-5), 3.82 (dd, 1H, H-2), 3.30 (dd, 1H, $J_{5,6}$ 4.9 Hz, $J_{6,6'}$ 9.5 Hz, H-6), 3.19 (dd, 1H, $J_{5,6'}$ 4.9 Hz, H-6′); δ_C (MeOH-d_4) 73.9, 72.2, 71.8, 71.1 (C-2, C-3, C-4, C-5), 65.2 (C-6), 54.6 (C-1).

4.2. 2,3,4,5-Tetra-*O*-benzoyl-6-*O*-trityl-L-idose diethyldithioacetal 11

To a solution of compound **10** (15.1 g, 28.6 mmol) in pyridine (100 mL), benzoyl chloride (16.2 mL, 140 mmol) was added and the mixture stirred at ambient temp for 16 h. Precipitated pyridinium hydrochloride was removed by filtration and the solution was concentrated under reduced pressure. The remaining residue was dissolved in CH_2Cl_2 and the organic layer sequentially washed with 5% aqueous HCl, 5% aqueous sodium bicarbonate and dried over Na_2SO_4. Filtration, removal of the solvent and purification on silica gel gave tetrabenzoate **11** (26.1 g, 97%) as a faintly yellow oil. Found: C, 72.35; H, 5.60; $C_{57}H_{52}O_9S_2$ (945.1726) requires: C, 72.43; H, 5.55; Mass spectrum (API-ES): *m*/*z*: 944.10 [M−H]; $[\alpha]_D^{20} = +6.2$ (*c* 1.6, CH_2Cl_2); δ_H (acetone-d_6) 6.39 (dd, 1H, $J_{3,4}$ 4.4 Hz, $J_{4,5}$ 5.9 Hz, H-4), 6.32 (dd, 1H, $J_{2,3}$ 5.9 Hz, H-3), 5.93 (dd, 1H, $J_{1,2}$ 5.4 Hz, H-2), 5.90 (m, 1H, H-5), 4.43 (d, 1H, H-1), 3.51 (dd, 1H, $J_{5,6}$ 5.6 Hz, $J_{6,6'}$ 10.0 Hz, H-6), 3.39 (dd, 1H, $J_{5,6'}$ 4.6 Hz, H-6′); δ_C (acetone-d_6) 73.0, 71.8, 70.9, 70.0 (C-2, C-3, C-4, C-5), 61.8 (C-6), 51.7 (C-1).

4.3. 2,3,4,5-Tetra-*O*-benzoyl-6-*O*-trityl-L-idose 12

To a 3% solution of compound **11** (1.61 g, 1.70 mmol) in a mixture of acetone/H_2O/CH_3CN 2:1:2 (v/v/v) HgO (590 mg, 1.6 equiv) and $HgCl_2$ (740 mg, 1.6 equiv) were added and the mixture stirred at 60 °C for 18 h until TLC indicated complete conversion of the starting material. Salts were removed by filtration and washed with acetone. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2. This solution was consecutively washed with 1 M aqueous KI solution and saturated aqueous sodium thiosulfate and dried (Na_2SO_4). Evaporation of the solvent and purification on silica gel furnished unstable free aldehyde **12** (1.39 g, 97%) as an oil, which was used immediately in the next step. Found: C, 75.82; H, 5.11; $C_{53}H_{42}O_{10}$ (838.9197) requires: C, 75.88; H, 5.05; Mass spectrum (API-ES): *m*/*z*: 837.80 [M−H]; $[\alpha]_D^{20} = -13.8$ (*c* 1.7, CH_2Cl_2); δ_H (acetone-d_6) 9.77 (m, 1H, H-1), 6.62 (m, 1H, H-4), 6.28 (m, 1H, H-3), 6.05 (m, 1H, H-2), 5.91 (m, 1H, H-5), 3.51 (m, 2H, H-6, H-6′); δ_C (acetone-d_6) 194.4 (C-1), 76.5, 71.8, 70.0, 69.8 (C-2, C-3, C-4, C-5), 62.1 (C-6).

4.4. 2,3,4,5-Tetra-*O*-benzoyl-L-idoseptanose 13

To a 3% solution of free aldehyde **12** (3.60 g, 4.29 mmol) in CH_2Cl_2, MeOH (25 mL) and $BF_3{\cdot}Et_2O$ (1.1 equiv) in Et_2O were added at ambient temp. After 15–20 min, CH_2Cl_2 (150 mL) was added and the mixture washed with 5% aqueous bicarbonate until neutral and dried over Na_2SO_4. Filtration, removal of the solvents under reduced pressure and purification of the residue on silica gel gave an anomeric mixture ($\alpha/\beta \sim 6{:}1$) of partially protected septanose **13** (2.25 g, 88%). Found: C, 68.38; H, 4.80; $C_{34}H_{28}O_{10}$ (596.5963) requires: C, 68.45; H, 4.73; Mass spectrum (API-ES): *m*/*z*: 595.60 [M−H]; α-anomer: δ_H ($CDCl_3$) 6.13 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.3 Hz, H-4), 5.96 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-3), 5.82 (dd, 1H, $J_{1,2}$ 6.8 Hz, H-2), 5.59 (ddd, 1H, $J_{5,6}$ 9.8 Hz, $J_{5,6'}$ 4.4 Hz, H-5), 5.38 (d, 1H, H-1), 4.30 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.04 (dd, 1H, H-6′); δ_C ($CDCl_3$) 96.2 (C-1), 74.0, 73.6, 72.3, 69.0 (C-2, C-3, C-4, C-5), 60.9 (C-6). β-Anomer: δ_H ($CDCl_3$) 5.91 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1); δ_C ($CDCl_3$) 92.9 (C-1), 75.2, 73.6, 72.7, 70.8 (C-2, C-3, C-4, C-5), 61.2 (C-6).

4.5. 2,3,4,5-Tetra-*O*-benzoyl-α/β-L-idoseptanosyl fluoride 14

To a 3% solution of septanose **13** (1.73 g, 2.90 mmol) in dry CH_2Cl_2, DAST (760 μL, 2 equiv) was added at −50 °C and the mixture stirred at −30 °C until all the starting materials had reacted. The reaction mixture was carefully washed with 5% aqueous bicarbonate, dried over Na_2SO_4 and filtered. Removal of the solvent under reduced pressure furnished an anomeric mixture ($\alpha/\beta \sim 1{:}10$) of the septanosyl fluorides **14**, which was pure enough for the next step (1.69 g, 97%). Found: C, 68.14; H, 4.60; $C_{34}H_{27}FO_9$ (598.5873) requires: C, 68.22; H, 4.55; Mass spectrum (API-ES): *m*/*z*: 597.50 [M−H]; β-anomer (main product) δ_H ($CDCl_3$) 6.26 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 6.8 Hz, H-4), 6.02–5.87 (m, 3H, H-1, H-2, H-3), 5.60 (m, 1H, H-5), 4.62 (dd, 1H, $J_{5,6}$ 1.7 Hz, $J_{6,6'}$ 13.8 Hz, H-6), 4.28 (m, 1H, H-6′); δ_C ($CDCl_3$) 106.6 ($J_{C,F}$ 230.1 Hz, C-1), 73.5, 71.9 (C-4, C-5), 71.9 ($J_{C,F}$ 25.4 Hz, C-2), 70.3 ($J_{C,F}$ 2.9 Hz, C-3), 61.6 ($J_{C,F}$ 4.8 Hz, C-6). α-Anomer: δ_C ($CDCl_3$) 108.8 ($J_{C,F}$ 229.3 Hz, C-1), 74.2, 71.3 (C-4, C-5), 70.8 ($J_{C,F}$ 34.6 Hz, C-2), 67.7 ($J_{C,F}$ 8.0 Hz, C-3), 61.5 (C-6).

4.6. 4-Nitrophenyl 2,3,4,5-tetra-*O*-benzoyl-α-L-idoseptanoside 15 and 4-nitrophenyl 2,3,4,5-tetra-*O*-benzoyl-β-L-idoseptanoside 16

To a 3% solution of septanosyl fluoride **14** (380 mg, 0.635 mmol) in CH_2Cl_2 containing NEt_3 (110 μL), 4-nitrophenol (115 mg, 0.83 mmol) and a few drops of $BF_3{\cdot}Et_2O$ in Et_2O (1 M) were added. After 1 h, the mixture was washed with 5% aqueous bicarbonate, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Chromatography gave pure β-**16** (190 mg, 42%) and α-septanosides (**15**, 175 mg, 38%). Found: C, 66.87; H, 4.40; $C_{40}H_{31}NO_{12}$ (717.6926) requires: C, 66.94; H, 4.35; Mass spectrum (API-ES): *m*/*z*: 716.60 [M−H]; α-anomer: $[\alpha]_D^{20} = -23.8$ (*c* 2.1, CH_2Cl_2); δ_H ($CDCl_3$) 6.19 (dd, 1H, $J_{1,2}$ 6.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 6.17 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{4,5}$ 8.8 Hz, H-4), 6.02 (dd, 1H, H-3), 5.83 (d, 1H, H-1), 5.61 (ddd, 1H, $J_{5,6}$ 10.7 Hz, $J_{5,6'}$ 4.4 Hz, H-5), 4.29 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.07 (dd, 1H, H-6); δ_C ($CDCl_3$) 99.5 (C-1), 74.2, 71.6, 71.1, 68.3 (C-2, C-3, C-4, C-5), 60.6 (C-6). β-Anomer: $[\alpha]_D^{20} = +79.0$ (*c* 2.3, CH_2Cl_2); δ_H ($CDCl_3$) 6.41 (dd, 1H, $J_{3,4}$ 5.9 Hz, $J_{2,3}$ 9.8 Hz, H-3), 6.08 (dd, 1H, $J_{1,2}$ 2.4 Hz, H-2), 5.94 (dd, 1H, $J_{4,5}$ 5.9 Hz, H-4), 5.91 (d, 1H, H-1), 5.58 (ddd, 1H, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 4.9 Hz, H-5), 4.43 (dd, 1H, $J_{6,6'}$ 13.7 Hz,

H-6), 4.17 (dd, 1H, H-6′); δ_C ($CDCl_3$) 97.2 (C-1), 73.2, 72.7, 72.0, 71.4 (C-2, C-3, C-4, C-5), 60.7 (C-6).

4.7. 4-Nitrophenyl α-L-idoseptanoside 7

To a 1% methanolic solution of compound **15** (87 mg, 0.12 mmol), methanolic NaOMe (1 M, two drops) was added at −20 °C. After completion of the reaction, the mixture was neutralised with ion exchange resin Amberlite IR 120 [H^+], filtered and concentrated under reduced pressure. Final purification was achieved by preparative TLC to give the deprotected septanoside **7** (22 mg, 60%). Found: C, 47.80; H, 5.07; $C_{12}H_{15}NO_8$ (301.2553) requires: C, 47.84; H, 5.02; Mass spectrum (API-ES): *m/z*: 300.20 [M−H]; $[\alpha]_D^{20} = -169.6$ (*c* 0.87, MeOH); δ_H (MeOH-d_4) 5.31 (d, 1H, $J_{1,2}$ 6.8 Hz, H-1), 3.86 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.71 (dd, 1H, $J_{5,6}$ 10.7 Hz, $J_{6,6'}$ 12.2 Hz, H-6), 3.57 (ddd, 1H, $J_{4,5}$ 8.8 Hz, $J_{5,6'}$ 3.9 Hz, H-5), 3.52 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.49 (dd, 1H, H-6′), 3.35 (dd, 1H, H-4); δ_C (MeOH-d_4) 102.5 (C-1), 79.3, 72.4, 71.8, 70.0 (C-2, C-3, C-4, C-5), 63.3 (C-6).

4.8. 4-Nitrophenyl β-L-idoseptanoside 8

To a 1% methanolic solution of compound **16** (116 mg, 0.16 mmol), methanolic NaOMe (1 M, two drops) was added at −20 °C. After completion of the reaction, the mixture was neutralised with ion exchange resin Amberlite IR 120 [H^+], filtered and concentrated under reduced pressure. Final purification was achieved by preparative TLC to give the deprotected septanoside **8** (25 mg, 51%). Found: C, 47.78; H, 5.06; $C_{12}H_{15}NO_8$ (301.2553) requires: C, 47.84; H, 5.02; Mass spectrum (API-ES): *m/z*: 300.20 [M−H]; $[\alpha]_D^{20} = +94.5$ (*c* 0.59, MeOH); δ_H (MeOH-d_4) 5.65 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 3.96 (dd, 1H, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 12.7 Hz, H-6), 3.91 (dd, 1H, $J_{2,3}$ 8.8 Hz, $J_{3,4}$ 8.3 Hz, H-3), 3.87 (dd, 1H, H-2), 3.75 (ddd, 1H, $J_{4,5}$ 7.3 Hz, $J_{5,6'}$ 6.5 Hz H-5), 3.61 (dd, 1H, H-6′), 3.55 (dd, 1H, H-4); δ_C (MeOH-d_4) 99.4 (C-1), 77.5, 72.7, 72.6, 71.7 (C-2, C-3, C-4, C-5), 65.5 (C-6).

4.9. Enzyme kinetics

All pNP-pyranoside substrates, *S. cerevisiae* α-glucosidase, *E. coli* β-galactosidase, Green Coffee Bean α-galactosidase and *Canavalia ensiformis* α-mannosidase were purchased from Sigma chemical company. *Agrobacterium* sp. β-glucosidase, *T. saccharolyticum* β-xylosidase and *E. coli* str. K12 α-xylosidase were expressed and purified as previously reported.[13–15] Kinetic studies were performed at 37 °C in 50 mM sodium citrate/phosphate buffer at the pH stated in Table 1. Enzyme concentrations ranging from 0.5 μg mL^{-1} to 0.1 mg mL^{-1} were used, depending on the substrate studied. As a primary screen, the hydrolysis of septanosides **7** and **8** was attempted at substrate concentrations of 1.6 and 2.4 mM, respectively. In the absence of any observed hydrolysis, inhibition of the enzyme-catalysed hydrolysis of the natural pNP pyranoside was attempted at the same septanoside concentrations with the pNP-pyranoside concentration around its K_M value. Substrate concentrations ranging from approximately $0.5 \times K_M$ to $5 \times K_M$ were employed wherever possible. Reactions were followed in a UV–vis spectrophotometer by measuring the change in absorbance of light at 400 nm. Data were analysed by direct fit of the rates observed to the Michaelis–Menten equation using the programme GraFit.[16]

For pyranoid substrates pNP β-D-glucopyranoside,[13] pNP β-D-galactopyranoside,[17] pNP α-D-galactopyranoside,[18] pNP β-D-xylopyranoside,[14] pNP α-D-xylopyranoside,[15] pNP α-D-mannopyranoside,[19] the previously reported K_m and k_{cat} values have been used.

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