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Non-natural aldofuranosides as substrates of a β-glucosidase

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Abstract—Based on glycosidase inhibitory activities of some known 1,4-iminoalditols, four aldofuranosides, (4-nitro)phenyl β-D-glucofuranoside, -β-D-galactofuranoside, -α-L-idofuranoside and -α-L-altrofuranoside, were identified as possible substrates of glucosidases and galactosidases. Three of them were found to be accepted by the β-glucosidase from *Agrobacterium* sp.
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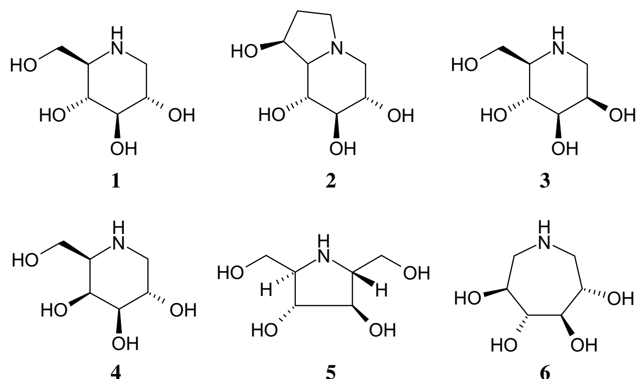
1. Introduction

Iminosugars and related structures, including iminoalditols, are powerful reversible inhibitors of glycosidases. Glycosidases, a class of enzymes well known for their high specificity for the respective natural substrate, are inhibited by these close structural relatives of their substrates/products. For example (Scheme 1), 1-deoxynojirimycin, **1**, and castanospermine **2** are powerful inhibitors of D-glucopyranosidases, 1-deoxymannojirimycin **3** inhibits D-mannosidases and the corresponding *galacto*-configured iminoalditol **4** is a strong inhibitor of galactosidases. Interestingly, over the past two decades, it has been found that many glycosidases are also 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. Such compounds include well-known 2,5-dideoxy-2,5-imino-D-mannitol **5**, a very powerful glucosidase inhibitor[1] and 1,6-dideoxy-1,6-imino-L-iditol **6**, a good inhibitor of a wide range of various glycosidases.[2,3]



Scheme 1.

Other examples (Scheme 2), just to mention a few, are 1,4-dideoxy-1,4-imino-L-allitol **7** and its epimer at C-5, 1,4-dideoxy-1,4-imino-D-talitol **8**, both of which are potent inhibitors of human liver lysosomal as well as Golgi II α-mannosidases[4] and 1,4,6-trideoxy-6-fluoro-1,4-imino-D-mannitol **9**, a powerful inhibitor of human liver mannosidases.[5] 1,4-Dideoxy-1,4-imino-D-glucitol **10** inhibits[6] β-glucosidase from sweet almonds with a K_i of 125 μM (pH 5.2) being about as potent as 1-deoxynojirimycin **1** with this enzyme at the same pH value. The corresponding epimer at C-5, 1,4-dideoxy-1,4-imino-L-iditol **11** was found to be a potent inhibitor of human liver lysosomal α-galactosidase.[7] 2-Acetamido-1,2,4-trideoxy-1,4-imino-D-galactitol **12** is a good inhibitor of hexosaminidases.[8]

Based on the traditional and common conclusion that close relatives of good substrates are frequently good inhibitors, we envisaged that glycosides, which are structurally related to such 'unusually shaped' inhibitors might turn out to be suitable substrates for glycosidases.

To probe this hypothesis, with the aid of Dreiding models as well as computer assisted molecular modelling

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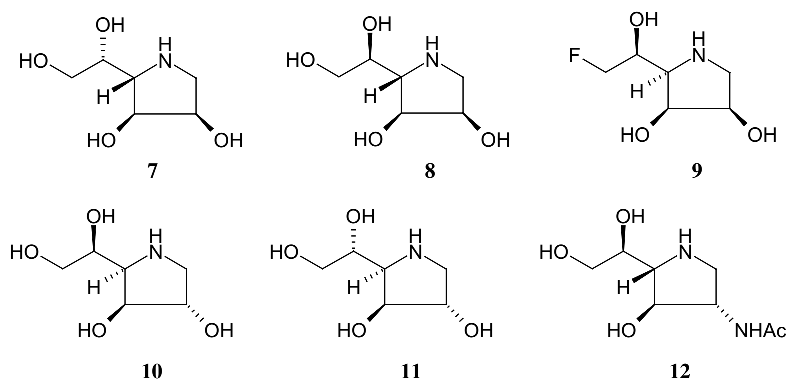


Scheme 2.

(Fig. 1, Scheme 3), we identified β-D-*gluco*-**13** and α-L-idofuranosides **14** as potential substrates for galactosidases and β-D-galacto-**15** as well as α-L-altrofuranosides **16** as likely suitable substrates for β-glucosidases.

The concept was further supported by the work of Yoshida and Nobuko[9] in 1960s, who had found that almond emulsin (but not *E. coli* K 12 and bovine liver β-galactosidases) solvolysed phenyl D-*gluco*- as well as D-galactofuranosides.

The β-glucosidase from *Agrobacterium* sp. has substantial β-galactosidase activity and its inhibition by a variety of iminosugars has been probed. Consequently

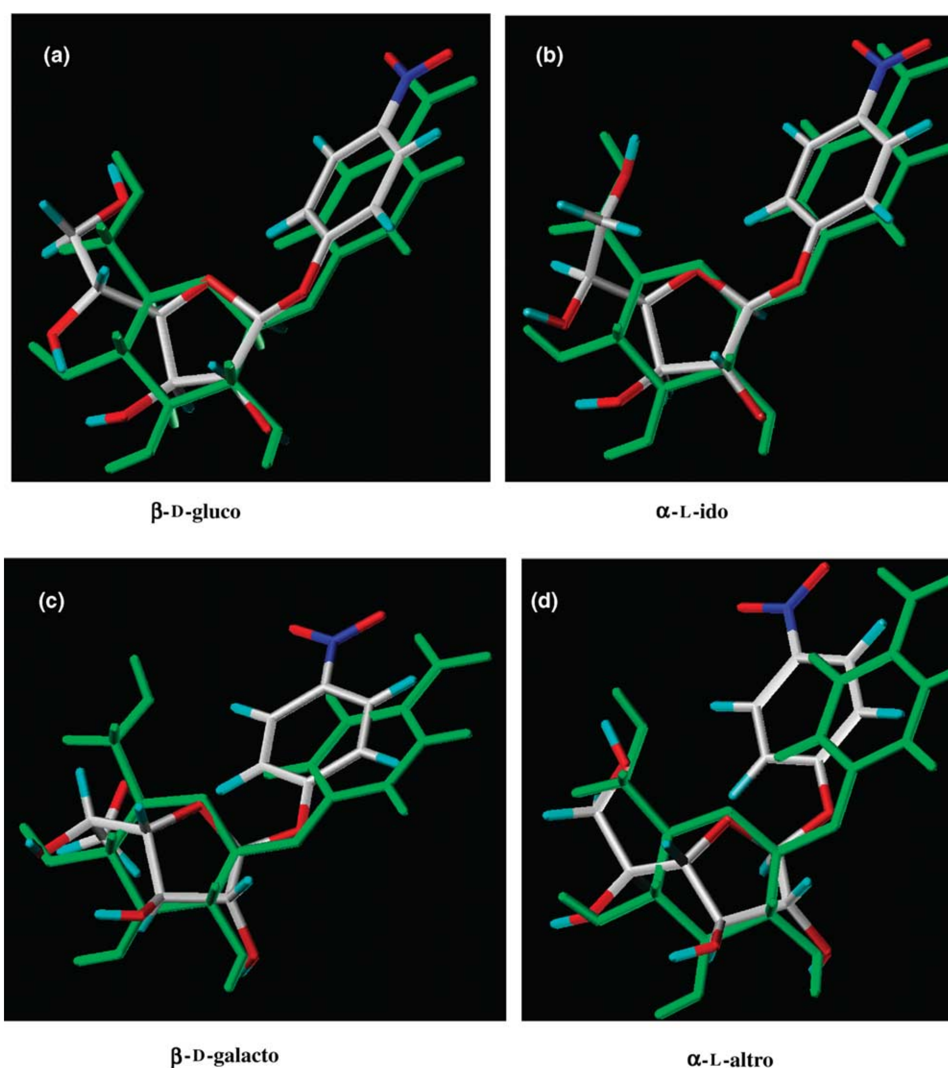
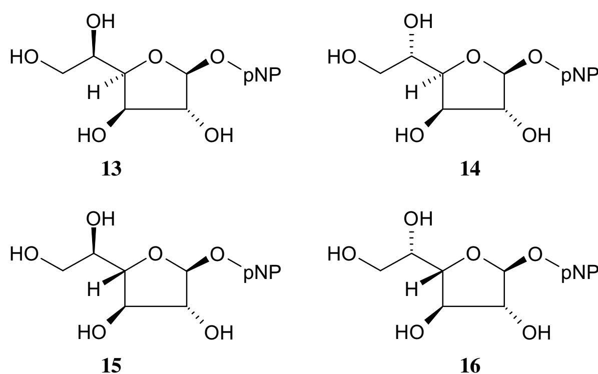


Figure 1. Superpositions of (a) pNP β-D-glucofuranoside, (b) pNP α-L-idofuranoside, (c) pNP β-D-galactofuranoside and (d) pNP α-L-altrofuranoside with pNP β-D-glucopyranoside (green).



Scheme 3.

it was chosen as the initial enzyme to test for the concept.

Whereas L-*ido*- as well as L-altrofuranosides have not been discovered as natural products, as yet, glucofuranosides have frequently been isolated from plants, for example as constituents of glycosylated flavonoids,[10] and galactofuranosides have been found in bacterial, protozoan as well as fungal polysaccharides.[11]

2. Results and discussion

2.1. Syntheses

2.1.1. (4-Nitro)phenyl α- and β-D-glucofuranosides. (4-Nitro)phenyl β- **13** and α-D-glucofuranosides **17** are known[12] and were prepared (Scheme 4) via known 1,2,3,5,6-penta-*O*-acetyl-α/β-D-glucofuranose[13] **18**, itself prepared via acetolysis[14] of 3,5,6-tri-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose **19**.

2.1.2. (4-Nitro)phenyl α- and β-L-idofuranosides. Conventional 3,5,6-tri-O-acetylation of 1,2-*O*-isopropylidene-β-L-idofuranose,[15] **20** (prepared by $NaBH_4$ reduction of 1,2-*O*-isopropylidene-β-L-idofuranurono-6,3-lactone,[16] **21**, or from 1,2-*O*-isopropylidene-3,5,6-tri-*O*-methanesulfonyl-α-D-glucofuranose,[17] **22**) to give triacetate **23** followed by acetolysis gave an anomeric mixture of 1,2,3,5,6-penta-*O*-acetyl-L-idofuranoses **24** and **25**, which was converted to the corresponding mixture of per-O-acetylated (4-nitro)phenyl furanosides, from which the pure compounds **26** and **27** were isolated. Individual treatment with NaOMe in methanol furnished pure samples of free idofuranosides **14** and **28** (Scheme 5).

2.1.3. (4-Nitro)phenyl β- and α-D-galactofuranosides. Known compounds **15** and **29** were prepared from the corresponding known per-O-benzoylated galactofuranoses **30** and **31** which were isolated from the reaction mixture obtained by per-O-benzoylation of D-galactose following available protocols (Scheme 6).[18,19]

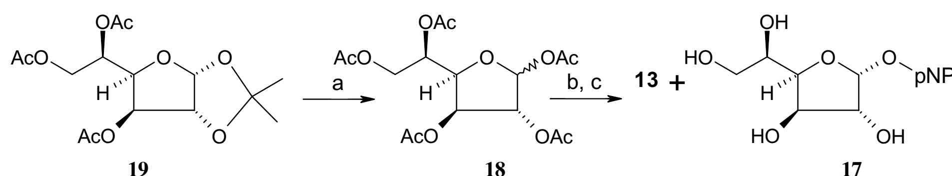
2.1.4. (4-Nitro)phenyl α- and β-L-altrofuranosides. Di-O-mesylation of 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-galactofuranose,[20] **32** (available in three steps from D-galactose by acid-catalysed reaction with acetone, 3-O-acetylation of the resulting 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose[21] and standard 5,6-O-deprotection) led to the corresponding 5,6-di-*O*-methanesulfonyl sugar **33**. Its reaction with sodium acetate in glacial acetic acid furnished 3,5,6-tri-*O*-acetyl-1,2-*O*-isopropylidene-β-L-altrofuranose **34** which by acetolysis was converted into an anomeric mixture of per-O-acetylated altrofuranoses **35** and **36**, the latter present only in very small proportions. Conventional reaction gave, after chromatography, the corresponding protected (4-nitro)phenyl α-L-altrofuranoside **37** as a single product from which pure **16** was obtained by Zemplén saponification followed by chromatography (Scheme 7).

2.2. Enzymatic studies

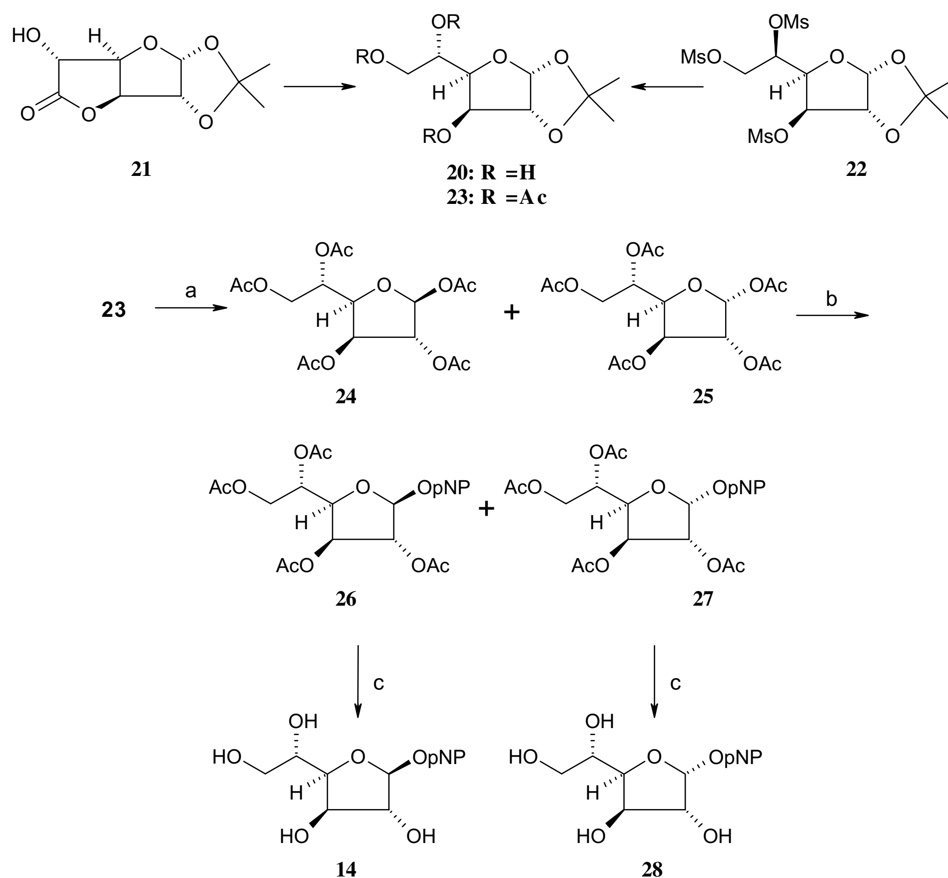
Compounds **13**–**16** were screened as substrates for *Agrobacterium* sp. β-glucosidase employing (4-nitro)phenyl β-D-glucopyranoside as the standard ($K_m = 0.072$ mM, $k_{cat} = 141\ s^{-1}$).

The best substrate turned out to be the α-L-altrofuranoside **16** with $K_m = 1.69$ mM and a $k_{cat} = 81\ s^{-1}$, followed by the β-D-glucofuranoside **13** ($K_m = 1.33$ mM, $k_{cat} = 12\ s^{-1}$). Significantly less accepted was the α-L-idofuranoside **14** ($K_m = 0.92$ mM, $k_{cat} = 0.9\ s^{-1}$). Interestingly, β-D-galactofuranoside **15** was found not to be a substrate at all.

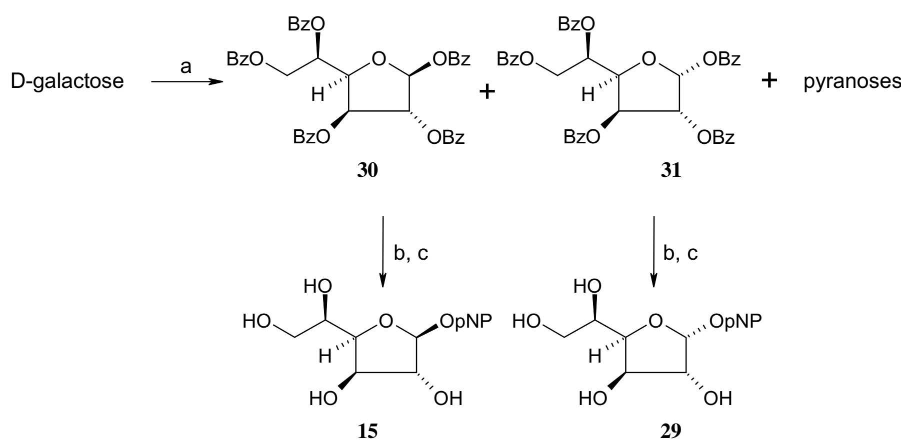
Previous kinetic studies on *Agrobacterium* sp. β-glucosidase[22] have revealed that the most important interactions between the enzyme and the substrate at the transition state are those formed with OH-2 and OH-3, contributing at least 18 and 7 $kJ\,mol^{-1}$, respectively, to transition state stabilisation. The modelling depicted in Figure 1, therefore, sought to optimise these interactions, as well as those with the ring oxygen, which is known to contribute substantially. Interactions at OH-4 and OH-6 are relatively less important, contributing 2.5–3 $kJ\,mol^{-1}$. Interestingly, of the four compounds



Scheme 4. Reagents and conditions: (a) Ac_2O, HOAc, H_2SO_4; (b) $(4\text{-}NO_2)PhOH$, PTSA, PhMe; (c) NaOMe, MeOH.



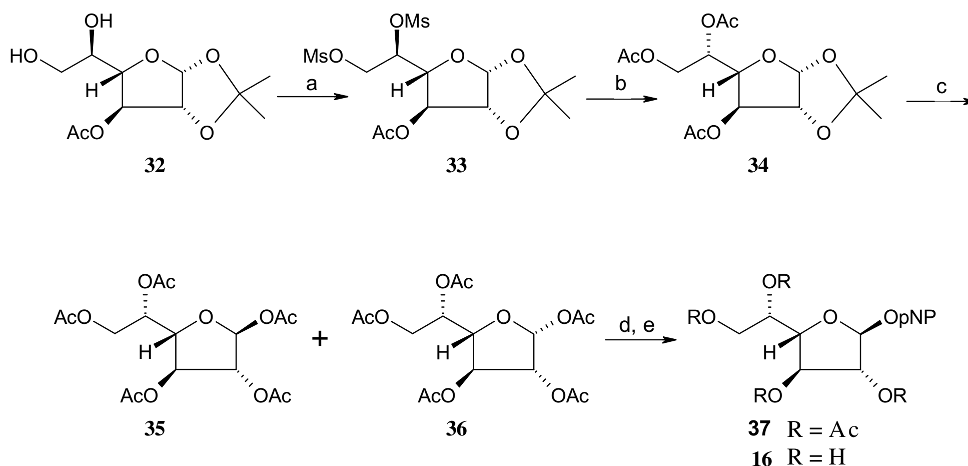
Scheme 5. Reagents and conditions: (a) Ac_2O, HOAc, H_2SO_4; (b) (4-NO_2)PhOH, PTSA, PhMe; (c) NaOMe, MeOH.



Scheme 6. Reagents and conditions: (a) BzCl, pyridine, (b) (4-NO_2)PhOH, PTSA, PhMe; (c) NaOMe, MeOH.

modelled in Figure 1, the α-L-altrofuranoside arguably provided the best overlay of interactions at the 4 and 6-positions, with O-5 of the *altro*-sugar overlaying nicely with O-4 of the glucopyranoside and O-6 of each sugar overlaying well. This is nicely consistent with the fact that the α-L-altrofuranoside was indeed the best substrate. At the level of modelling conducted it was not really possible to rank the relative resemblances of the other sugars, especially when it is considered that the most important interactions are those formed at the transition state, in which the sugar likely adopts a half chair conformation.

None of the furanosides was accepted by the β-galactosidase from *Xanthomonas manihotis*, a Family 35 enzyme, which is quite specific for β-D-galactopyranosides. This is consistent with the fact that this enzyme is more specific with respect to the interactions formed



Scheme 7. (a) MesCl, pyridine, CH_2Cl_2; (b) NaOAc, HOAc, DMF; (c) Ac_2O, HOAc, H_2SO_4; (d) $(4\text{-}NO_2)PhOH$, PTSA, PhMe; (e) NaOMe, MeOH.

at O-4 and O-6 in the pyranoside substrates. Earlier studies had shown that interactions at the 6-position contribute at least 20 $kJ\,mol^{-1}$, with similar values being likely for the 4-position, based upon rates with the *gluco*-substrate.[23] As expected, considering the 'wrong' anomeric configurations, compounds **13–16** were not hydrolysed by the α-galactosidase from green coffee beans (Family 27).

3. Conclusion

In conclusion, some of the furanosides tested, especially the altrofuranoside with the *Agrobacterium* sp. β-glucosidase, were remarkably good substrates. However, this does not appear to be a completely general phenomenon, with success possibly being related more to the promiscuity of the enzyme tested.

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). $CDCl_3$ was employed for protected compounds and D_2O as well as MeOH-d_4 for free sugars. Chemical shifts are listed in delta employing residual, not deuterated, solvent as the internal standard. The signals of aromatic substituents as well as 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. 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[24] employing Sybyl versions on an Octane workstation by Silicon Graphics using the BGFS-minimiser and the Tripos Force Field.

4.1. (4-Nitro)phenyl α- and β-D-glucofuranosides

Compounds **17** and **13**, as well as (4-nitro)phenyl α- and β-D-galactofuranosides **15** and **29** were prepared by available methods as mentioned in Results and discussion.

4.2. (4-Nitro)phenyl 2,3,5,6-tetra-*O*-acetyl-α-L-idofuranoside 26 and (4-nitro)phenyl 2,3,5,6-tetra-*O*-acetyl-β-L-idofuranoside 27

To a solution of 1,2,3,5,6-penta-*O*-acetyl-α/β-L-idofuranose **24** and **25**[25] (1.56 g, 4.0 mmol) in toluene (40 mL), (4-nitro)phenol (2.60 g, 18.7 mmol) and (4-toluene)sulfonic acid (75 mg) were added and the mixture was kept under reflux in a Dean–Stark apparatus for 3 h. After cooling to ambient temperature, the solution was washed with 5% aqueous bicarbonate, dried (Na_2SO_4), the solvent was removed under reduced pressure and the oily residue was chromatographed on silica gel (cyclohexane/ethyl acetate 4:1, v/v) to give, as the nonpolar component, syrupy β-anomer **27** (263 mg, 14%). Found: C, 51.22; H, 4.99; $C_{20}H_{23}NO_{12}$ requires: C, 51.18; H, 4.94; $[\alpha]_D^{20} = +67.9$ (c 4.6, CH_2Cl_2); δ_H ($CDCl_3$) 6.05 (d, 1H, $J_{1,2}$ 4.6 Hz, H-1), 5.68 (dd, 1H, $J_{2,3}$ 8.3 Hz, $J_{3,4}$ 8 Hz, H-3), 5.16 (ddd, 1H, $J_{4,5}$ 2.0 Hz, $J_{5,6}$ 5.1 Hz, $J_{5,6'}$ 7.5 Hz, H-5), 5.14 (dd, 1H, H-2), 4.64 (dd, 1H, H-4), 4.24 (dd, 1H, $J_{6,6'}$ 11.5 Hz, H-6), 4.15 (dd, 1H, H-6′); δ_C ($CDCl_3$) 96.5 (C-1), 75.1, 74.9, 73.3, 68.0 (C-2, C-3, C-4, C-5), 62.4 (C-6).

Next eluted was syrupy α-anomer **26**, (480 mg, 25.6%), Found: C, 51.11; H, 5.00; $C_{20}H_{23}NO_{12}$ requires: C, 51.18; H, 4.94; $[\alpha]_D^{20} = -153.1$ (c 4.6, CH_2Cl_2); δ_H ($CDCl_3$) 5.65 (s, 1H, H-1), 5.48 (n.r., 1H, H-3), 5.24 (br s, 1H, H-2), 5.24 (ddd, 1H, $J_{4,5}$ 5.3 Hz, $J_{5,6}$ 4.3 Hz, $J_{5,6'}$ 6.8 Hz, H-5), 4.68 (dd, 1H, H-4), 4.21 (dd, 1H, $J_{6,6'}$ 11.8 Hz, H-6), 3.95 (dd, 1H, H-6′); δ_C ($CDCl_3$) 103.2 (C-1), 80.3, 79.7, 74.4, 68.7 (C-2, C-3, C-4, C-5), 62.6 (C-6).

4.3. (4-Nitro)phenyl α-L-idofuranoside 14

To a 5% methanolic solution of **26** (500 mg, 1.07 mmol), a 1 M NaOMe solution (three drops) was added at 0 °C. After completion of the deprotection (TLC, ethyl acetate), the solution was neutralised with ion exchange resin Amberlite IR 120 [H^+], filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and filtered over a short plug of silica gel to give pure **14** (290 mg, 90%). Found: C, 47.89; H, 5.05; $C_{12}H_{15}NO_8$ requires: C, 47.84; H, 5.02; $[\alpha]_D^{20} = -17.1$ (*c* 2.1, MeOH); δ_H (MeOH-d_4) 5.65 (s, 1H, H-1), 4.39 (m, 1H, H-4), 4.37 (br s, 1H, H-2), 4.22 (d, 1H, $J_{3,4}$ 4.4 Hz, H-3), 3.96 (ddd, 1H, $J_{5,6}$ 4.8 Hz, $J_{5,6'}$ 5.9 Hz, H-5), 3.67 (dd, 1H, $J_{6,6'}$ 11.2 Hz, H-6), 3.58 (dd, 1H, H-6′); δ_C (MeOH-d_4) 107.6 (C-1), 85.1, 82.7, 77.4, 72.7 (C-2, C-3, C-4, C-5), 64.3 (C-6).

4.4. (4-Nitro)phenyl β-L-idofuranoside 28

To a 2% methanolic solution of **27** (200 mg, 0.426 mmol), a 1 M NaOMe solution (three drops) was added at 0 °C. After completion of the deprotection (TLC, ethyl acetate), the solution was neutralised with ion exchange resin Amberlite IR 120 [H^+], filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and filtered over a short plug of silica gel to give pure **28** (52 mg, 40%). Found: C, 47.78; H, 5.00; $C_{12}H_{15}NO_8$ requires: C, 47.84; H, 5.02; $[\alpha]_D^{20} = +49.7$ (*c* 1.8, MeOH); δ_H (MeOH-d_4) 5.79 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 4.44 (dd, 1H, $J_{2,3}$ 6.3 Hz, $J_{3,4}$ 6.6 Hz, H-3), 4.36 (dd, 1H, H-2), 4.29 (dd, 1H, $J_{4,5}$ 3.0 Hz, H-4), 3.93 (ddd, 1H, $J_{5,6}$ 6.4 Hz, $J_{5,6'}$ 6.2 Hz, H-5), 3.62 (m, 2H, H-6, H-6′); δ_C (MeOH-d_4) 101.0 (C-1), 80.1, 78.7, 76.5, 71.5 (C-2, C-3, C-4, C-5), 63.8 (C-6).

4.5. 3-*O*-Acetyl-1,2-*O*-isopropylidene-α-D-galactofuranose 32

A solution of 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose[20] (6.71 g, 22.2 mmol) in 50% aqueous AcOH was stirred at ambient temp for 10 h. The solvents were removed under reduced pressure and the residue was brought to pH 7 with 3% aqueous bicarbonate. The aqueous phase was extracted with ethyl acetate to give oily **32** (5.20 g, 89.3%). Found: C, 50.31; H, 7.01; $C_{11}H_{18}O_7$ requires: C, 50.38; H, 6.92; $[\alpha]_D^{20} = +42.6$ (*c* 1.5, $CHCl_3$); δ_H ($CDCl_3$) 5.93 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.03 (d, 1H, $J_{3,4}$ 1.5 Hz, H-3), 4.63 (d, 1H, H-2), 4.32 (m, 1H, H-4), 3.84 (m, 1H, H-5), 3.81–3.60 (m, 2H, H-6, H-6′), 3.15 (br s, 1H, OH), 2.85 (br s, 1H, OH); δ_C ($CDCl_3$) 105.7 (C-1), 86.6, 84.8, 78.0, 70.8 (C-2, C-3, C-4, C-5), 63.5 (C-6).

4.6. 3-*O*-Acetyl-1,2-*O*-isopropylidene-5,6-di-*O*-methanesulfonyl-α-D-galactofuranose 33

To a solution of **32** (4.2 g, 16.0 mmol) in pyridine (30 mL), methanesulfonyl chloride (4.2 g, 2.3 equiv) was added at 0 °C and the mixture was kept at ambient temp for 10 h. MeOH was added and the solution was concentrated under reduced pressure. The resulting syrupy material can immediately be used in the next step. An analytical sample was obtained by chromatography. Found: C, 37.28; H, 5.35; $C_{13}H_{22}O_{11}S_2$ requires: C, 37.32; H, 5.30; $[\alpha]_D^{20} = -22.8$ (*c* 3.8, $CHCl_3$); δ_H ($CDCl_3$) 5.99 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.12 (d, 1H, $J_{2,3}$ 1.5 Hz, H-3), 5.05 (ddd, 1H, $J_{4,5}$ 8.8 Hz, $J_{5,6}$ 3.7 Hz, $J_{5,6'}$ 5.5 Hz, H-5), 4.62 (d, 1H, H-2), 4.60 (dd, 1H, $J_{6,6'}$ 12.1 Hz, H-6), 4.24 (dd, 1H, H-4), 3.42 (dd, 1H, H-6′); δ_C ($CDCl_3$) 106.2 (C-1), 84.3, 83.9, 78.9, 76.6 (C-2, C-3, C-4, C-5), 67.9 (C-6).

4.7. 3,5,6-Tri-*O*-acetyl-1,2-*O*-isopropylidene-β-L-altrofuranose 34

To a 5% solution of **33** (5.10 g, 12.2 mmol) in DMF, NaOAc (25 g) and Ac_2O (75 mL) were added and the mixture was stirred at 130 °C until TLC showed completed conversion of the starting material. The mixture was allowed to reach ambient temp, solids were removed by filtration and the filtrate was concentrated under reduced pressure. CH_2Cl_2 was added to the residue and the organic layer was washed consecutively with 5% aqueous HCl and satd aqueous bicarbonate, dried (Na_2SO_4) and filtered. Solvents were removed under reduced pressure and the residue was purified on silica gel to give **34** (3.80 g, 90%). Found: C, 51.95; H, 6.47; $C_{15}H_{22}O_9$ requires: C, 52.02; H, 6.40; $[\alpha]_D^{20} = +54.3$ (*c* 1.2, $CHCl_3$); δ_H ($CDCl_3$) 5.92 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.32 (ddd, 1H, $J_{4,5}$ 7.3 Hz, $J_{5,6}$ 2.6 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 5.12 (s, 1H, H-3), 4.56 (d, 1H, H-2), 4.17 (dd, 1H, $J_{6,6'}$ 12.1 Hz, H-6), 4.09 (dd, 1H, H-6′), 4.08 (dd, 1H, H-4); δ_C ($CDCl_3$) 106.4 (C-1), 84.5, 83.4, 77.3, 69.9 (C-2, C-3, C-4, C-5), 62.5 (C-6).

4.8. 1,2,3,5,6-Penta-*O*-acetyl-α-L-altrofuranose 35

A mixture of **34** (2.36 g, 6.81 mmol), glacial acetic acid (3.6 mL), Ac_2O (0.5 mL), and H_2SO_4 (0.21 mL) was kept at ambient temp until TLC indicated completed conversion of the starting material. The mixture was poured on ice and extracted with dichloromethane. The organic layer was consecutively washed with satd aqueous bicarbonate and brine and dried (Na_2SO_4). By chromatography, pure **35** (2.30 g, 86%) was obtained as a syrup. Found: C, 49.15; H, 5.75; $C_{16}H_{22}O_{11}$ requires: C, 49.23; H, 5.68; $[\alpha]_D^{20} = -53.5$ (*c* 1.9,$CHCl_3$); δ_H ($CDCl_3$) 6.63 (s, 1H, H-1), 5.21 (ddd, 1H, $J_{4,5}$ 6.5 Hz, $J_{5,6}$ 3.3 Hz, $J_{5,6'}$ 6.1 Hz, H-5), 5.15 (d, $J_{3,4}$ 6.3 Hz, H-3), 5.06 (s, 1H, H-2), 4.39 (dd, 1H, $J_{6,6'}$ 12.5 Hz, H-6), 4.26 (dd, 1H, H-4), 4.05 (dd, 1H, H-6′); δ_C ($CDCl_3$) 99.5 (C-1), 83.4, 80.5, 76.3, 70.1 (C-2, C-3, C-4, C-5), 62.4 (C-6).

4.9. (4-Nitro)phenyl 2,3,5,6-tetra-*O*-acetyl-α-L-altrofuranoside 37

To a solution of **35** (2.0 g, 5.1 mmol) in toluene (70 mL), (4-nitro)phenol (3.2 g, 23 mmol) and (4-toluene)sulfonic acid (20 mg) were added and the mixture was kept under reflux in a Dean–Stark apparatus for 3 h. After cooling to ambient temperature, the solution was washed with 5% aqueous bicarbonate, dried (Na_2SO_4), the solvent

was removed under reduced pressure and the oily residue was chromatographed on silica gel (cyclohexane/ethyl acetate 4:1, v/v) to give syrupy **37** (1.25 g, 52%). Found: C, 51.22; H, 4.98; $C_{20}H_{23}NO_{12}$ requires: C, 51.18; H, 4.94; $[\alpha]_D^{20} = -65.3$ (*c* 1.8, $CHCl_3$); δ_H ($CDCl_3$) 5.76 (s, 1H, H-1), 5.32 (ddd, 1H, $J_{5,6}$ 2.6 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 5.33–5.28 (m, 3H, H-2, H-3, H-4), 4.17 (dd, 1H, $J_{6,6'}$ 12.1 Hz, H-6), 4.09 (dd, 1H, H-6′); δ_C ($CDCl_3$) 104.0 (C-1), 82.0, 81.2, 76.3, 70.1 (C-2, C-3, C-4, C-5), 62.5 (C-6).

4.10. (4-Nitro)phenyl α-L-altrofuranoside 16

To a 1% solution of **37** (320 mg, 0.68 mmol) in dry MeOH, 1 M NaOMe (three drops) was added at 0 °C. After completion of the deprotection (TLC, ethyl acetate), the solution was neutralised with ion exchange resin Amberlite IR 120 [H^+], filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and filtered over a short plug of silica gel to give pure **16** (180 mg, 87.6%). Found: C, 47.78; H, 5.05; $C_{12}H_{15}NO_8$ requires: C, 47.84; H, 5.02; $[\alpha]_D^{20} = -132.1$ (*c* 1.4, MeOH); δ_H (MeOH-d_4) 5.69 (s, 1H, H-1), 5.32 (ddd, 1H, $J_{5,6}$ 4.0 Hz, $J_{5,6'}$ 6.2 Hz, H-5), 4.90–4.80 (m, 3H, H-2, H-3, H-4), 3.65 (dd, 1H, $J_{6,6'}$ 11.4 Hz, H-6), 3.59 (dd, 1H, H-6′); δ_C (MeOH-d_4) 106.5 (C-1), 86.3, 82.2, 76.8, 71.9 (C-2, C-3, C-4, C-5), 63.0 (C-6).

4.11. Enzyme kinetics

All kinetic studies were performed essentially as described previously.[22] Kinetic studies were performed at 37 °C in 45 mM sodium phosphate buffer, pH 7.0 containing 0.1% bovine serum albumin. Enzyme concentrations ranging from 0.00019 to 0.35 $mg mL^{-1}$ were used, depending on the substrate hydrolysis rates studied. 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.

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