Polyhydroxylated homoazepanes and 1-deoxy-homonojirimycin analogues: synthesis and glycosidase inhibition study†

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The Johnson–Claisen rearrangement of D-*gluco* and L-*ido*-derived allylic orthoesters afforded γ,δ-unsaturated ester that on ester reduction, epoxidation, regioselective oxirane opening by sodium azide and hydrogenation led to sugar amino alcohols—immediate precursors for 1-deoxy-homonojirimycin **3a**,**b** and polyhydroxylated homoazepanes **4a**,**b**. Our synthetic approach and glycosidase inhibitory activity is reported.

Introduction

Glycobiology is an emerging research field at the frontier of chemistry, enzymology and biology. Many important biological processes, wherein glycosidases play a crucial role, are being uncovered leading to the possibility of finding new therapeutic targets for the treatment of various diseases such as obesity, diabetes, viral infection including AIDS and cancer.[1] Most glycosidase inhibitors share two common structural features: (i) a basic nitrogen atom that at physiological pH mimics the positive charge formed during the hydrolysis of the glycosidic bond and (ii) an array of hydroxyl groups in a conformationally restricted motif which selectively fit into the enzyme active site.[2] The iminosugar structure is the obvious choice as their polyhydroxylated nitrogen containing ring skeleton is identical to that of sugars and thus mimics the enzyme. The parent molecule of iminosugars (also called as azasugars) namely nojirimycin **1a** (Fig. 1) is the compound in which the ring oxygen of D-glucose is replaced by a nitrogen atom,[3] whereas in 1-deoxynojirimycin **1b** (and other 1-deoxyazasugars), the hemiacetalic functionality is replaced by an aminomethylene group. The latter modification improved the glycosidase inhibitory activity and some of them are therapeutically useful.[3b,4–6] In iminosugars the absolute configuration at each stereocentre is crucial for its biological activity and therefore many stereoselective syntheses of 1-deoxynojirimycin **1b** and analogues have been described to date.[7,8]

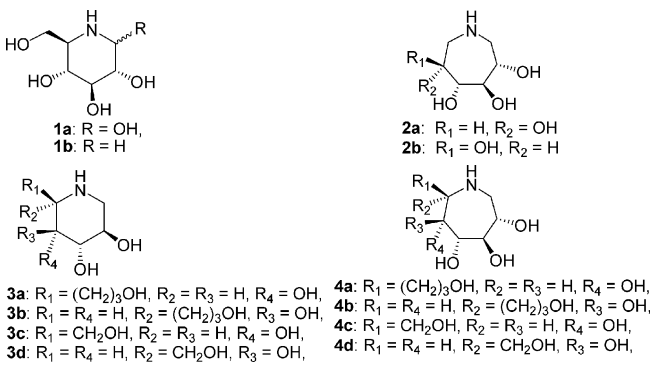
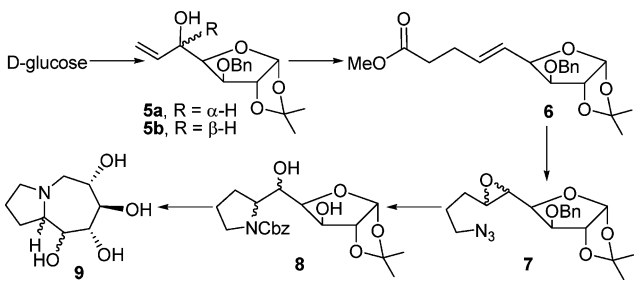


Fig. 1 Nojirimycin and azepane analogues.

The one carbon ring homologues of **1b** are polyhydroxylated azepanes **2a**,**b** and **4c**,**d** (Fig. 1)[9–11] which are glycosidase inhibitors and potential DNA minor groove binding ligands (MGBL) due to the flexibility of the seven-membered ring.[12] Thus, the development of new azasugars and azepanes opened a dynamic research field at the interface between glycobiology and synthetic organic chemistry. In this regard, we have recently reported the synthesis of tetrahydroxy perhydroazaazulenes.[13] Our approach hinges on the Johnson–Claisen rearrangement of D-glucose derived allylic alcohols **5a**,**b** using trimethyl orthoacetate and propionic acid, to give γ,δ-unsaturated ester **6** (Scheme 1). Conversion of methyl ester **6** to an azidomethyl group and epoxidation gave **7**. Reductive 5-*exo*-*tet*-cyclization of **7** under Staudinger conditions led to **8** that on opening of the 1,2-acetonide functionality followed by reductive aminocyclization afforded perhydroazaazulenes **9**.



Scheme 1 Synthesis of perhydroazaazulenes.

In continuation of our investigations in the area of iminosugars,[14] we thought of exploiting the Johnson–Claisen rearrangement product **6** in the synthesis of an altogether new 1-deoxy-D-*altro*-homonojirimycin **3a**, 1-deoxy-L-*gluco*-homonojirimycin **3b** and the corresponding ring homologated polyhydroxylated

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† Electronic supplementary information (ESI) available: The detailed experimental procedures for preparation of compounds **11**, **12a**,**b**, **13a**,**b** and **14a**,**b** and copies of ^{1}H and ^{13}C NMR spectra of compounds **4a**, **4b**, **4a**·HCl, **4b**·HCl, **3a**, **3b**, **3a**·HCl, **3b**·HCl, **11**, **12a**,**b**, **13a**,**b**, **14a**,**b** and **15a**,**b**. See DOI: 10.1039/b609000a

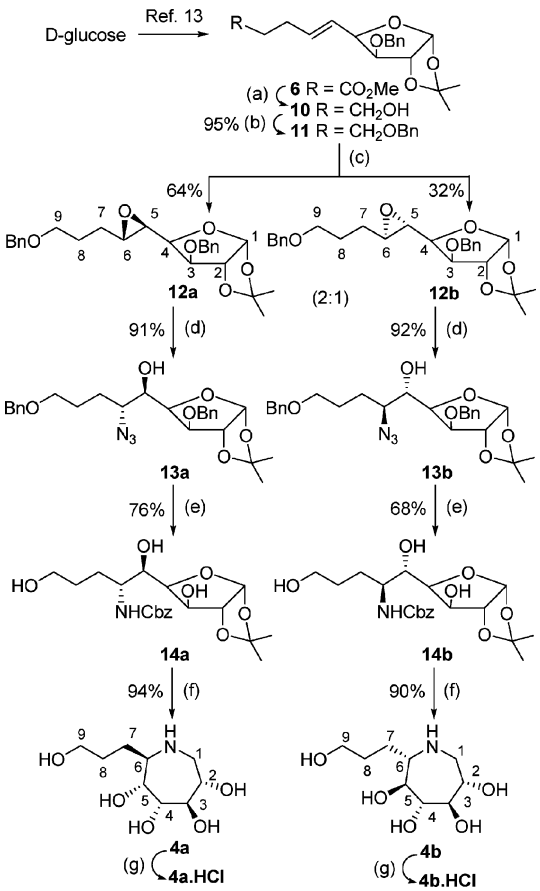
homoazepanes **4a** and **4b**, and studied their glycosidase inhibitory activity. Our results are described herein.

Results and discussion

The γ,δ-unsaturated ester **6** was obtained by the Johnson–Claisen rearrangement of D-glucose derived allylic alcohols **5a** and/or **5b** in 90% yield. Reduction of the ester functionality in **6** by LAH followed by benzylation gave benzyl ether **11** which on treatment with *m*-CPBA afforded a diastereomeric mixture of epoxides **12a** and **12b** in a 2 : 1 ratio. The appreciable difference in the R_f values allowed us to separate the epoxides by column chromatography. The formation of **12a** as a major product could be due to the six-membered hydrogen bonding of *m*-CPBA with the oxygen of C3-OBn and the delivery of oxygen from the β-face; whereas the five-membered hydrogen bonding of *m*-CPBA with the furanose oxygen and attack from the α-face gave **12b** as a minor product. Based on this the absolute configurations at the newly generated C5,C6-stereocentres were assigned as 5*R*,6*S* for **12a** and 5*S*,6*R* for **12b**.[15]

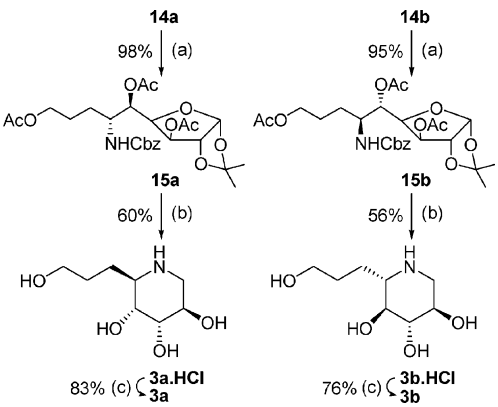
The utility of **12a**,**b** was initially demonstrated in the formation of homoazepanes **4a**,**b** respectively. Thus, regioselective epoxide ring opening of **12a** and **12b** at C6, with lithium azide in DMF, afforded exclusively 6-azido compounds **13a** and **13b**, respectively, in high yield[16] (Scheme 2). The appearance of a double doublet at ~δ 4.2 corresponding to H5 and a multiplet at δ 3.42–3.58 for H6 in **13a**/**13b** indicated the regioselective formation of 6-azido compounds. In the subsequent step, removal of –OBn groups and reduction of the azide functionality in **13a**, using ammonium formate in the presence of 10% Pd/C, followed by selective Cbz protection afforded *N*-Cbz protected amino alcohol **14a** as a white solid in 76% yield. Compound **14a** on reaction with TFA–water (to cleave the 1,2-acetonide functionality) followed by reductive aminocyclization, using ammonium formate in the presence of 10% Pd/C in methanol, followed by chromatographic purification afforded 1,6,7,8-tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*R*,6*R*)-L-*glycero*-D-*gluco*-nonitol **4a** as a thick oil. The free base was treated with MeOH–HCl to give **4a** as a hydrochloride salt. The same sequence of reactions with **12b** gave 1,6,7,8-tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*S*,6*S*)-D-*glycero*-L-*ido*-nonitol **4b** and its hydrochloride salt **4b**·HCl (Scheme 2). Compounds **13a**/**13b** and **14a**/**14b** were characterized by spectral and analytical techniques and the data were found to be in agreement with the structures.

Although the spectral data of homoazepanes **4a** and **4b** were found to be consistent with the structures, the configurational assignments at each stereocentre in **4a** and **4b** were tentatively made, stereocentres C2/C3/C4 are the same as in the D-glucose and C5/C6 by knowing the stereochemistry of epoxides **12a** and **12b**. The ^{1}H NMR spectra of **4a** and **4b** did not give any supportive information on the conformational and configurational assignment due to the flexibility in seven-membered ring systems.[9–11] As an alternative, we thought of converting **12a**/**12b** to the corresponding six-membered piperidine derivatives **3a**/**3b** (by chopping the anomeric carbon), wherein the configurational assignment at each carbon atom will be clearly evident from the six-membered cyclic chair structure, by either $^4C_1$ or $^1C_4$ conformation, and the same configurational assignments would be applicable to the homoazepanes **4a**,**b**.



Scheme 2 *Reagents and conditions*: (a) LAH, THF, 0 °C, 1 h; (b) NaH, BnBr, TBAI, THF, 0 °C to rt, 8 h; (c) *m*-CPBA, CH_2Cl_2, 0 °C to 25 °C, 24 h; (d) NaN_3, LiCl, DMF, 100 °C, 8 h; (e) (i) $HCOONH_4$, 10% Pd/C, MeOH, 80 °C, 1 h; (ii) CbzCl, $NaHCO_3$, MeOH–H_2O, 0 °C to rt, 12 h; (f) (i) TFA–H_2O (2 : 1), 0 °C to rt, 2.5 h; (ii) $HCOONH_4$, 10% Pd/C, MeOH, 80 °C, 1 h; (g) MeOH–HCl, rt, 3 h.

Thus, targeting the synthesis of 1-deoxy-homonojirimycin analogues **3a**,**b**, the free hydroxyl groups in **14a** were protected as acetates by treatment with acetic anhydride and pyridine to afford triacetyl derivative **15a** (Scheme 3). In the next step, cleavage of the 1,2-acetonide functionality in **15a** (TFA–water), treatment with



Scheme 3 *Reagents and conditions* (a) Ac_2O, Py, DMAP, 8 h; (b) (i) TFA–H_2O (2 : 1), 0 °C to rt, 2.5 h; (ii) $NaIO_4$, acetone–water (9 : 1), 0 °C, 30 min; (iii) H_2, 10% Pd/C, MeOH, 80 psi, 12 h; (iv) HCl–CH_3OH (9 : 1), 80 °C, 3 h; (c) aq. NH_3, MeOH, rt, 15 min.

sodium metaperiodate (to cleave the anomeric carbon atom) and hydrogenation in the presence of 10% Pd/C in methanol afforded a mixture of tetra-*O*-acetyl- and tri-*O*-acetyl-*O*-formyl-1-deoxy-D-*altro*-homonojirimycins.[17] One pot removal of *O*-acetyl and *O*-formyl groups using methanolic hydrogen chloride afforded the hydrochloride salt of the 1-deoxy-D-altro-homonojirimycin **3a** as a sticky gum, which on treatment with methanolic ammonia and purification afforded **3a** as a thick liquid.

Similarly, **14b** gave 1-deoxy-L-*gluco*-homonojirimycin **3b** as a thick liquid. Compound **15b** was characterized by spectral and analytical techniques and the data were found to be in agreement with the structure.

Conformational analysis

The six-membered iminosugars are known to exist in $^4C_1$ or $^1C_4$ conformations.[11b] In order to determine the conformations of **3a** and **3b**, we studied their ^{1}H NMR spectra and the coupling constant information was obtained by decoupling experiments. In the ^{1}H NMR spectrum of **3a**, the appearance of two doublets of doublets, corresponding to H1a and H1e with one large geminal coupling constant ($J_{1a,1e}$ = 13.8 Hz) and small vicinal coupling constants ($J_{1a,2e}$ = 3.6 Hz and $J_{1e,2e}$ = 2.7 Hz) indicated the equatorial orientation of H2 and this fact was confirmed by the appearance of H2 as a doublet of doublets of doublets with small coupling constants ($J_{2e,1a}$ = 4.8 Hz, $J_{2e,1e}$ = 2.7 Hz and $J_{2e,3e}$ = 3.0 Hz). The relative disposition of H2 and H3 is *trans* as in the starting compound **15a** the corresponding H3 and H4 are *trans* and the same stereochemistry is maintained in **3a**. Therefore, H3 is assumed to be equatorial and confirmed by its appearance as a doublet of doublets with small *J* values ($J_{3e,4a}$ = 4.8 Hz and $J_{3e,2e}$ = 3.0 Hz). H4, however, appeared as doublet of doublets with one large coupling constant ($J_{4a,5a}$ = 9.0 Hz) indicating a di-axial relation between H4 and H5. The doublet of triplets corresponding to H5 with a large ($J_{4a,5a}$ = 9.0 Hz) coupling constant confirmed the di-axial orientation of H4/H5. This fact clearly indicated the $^4C_1$ conformation (Fig. 2) of **3a** with 4*R*,5*R* absolute configurations at the newly generated stereocentres. In the ^{1}H NMR spectrum of **3b**, the appearance of one of the doublet of doublets corresponding to H1 with a large coupling constant of 12.6 Hz indicated the axial orientation of H2. H3 and H4 appeared as two triplets with large coupling constants ($J_{2,3} = J_{3,4} = J_{4,5}$ = 9.6 Hz) indicating the relative axial orientation of these protons and the $^1C_4$ conformation of **3b** with 4*S*,5*S* absolute configurations at the newly generated stereocentres. Thus, the stereochemistry assigned to **3a** and **3b** on the basis of the stereochemistry of **15a** and **15b** was confirmed by the ^{1}H NMR of **3a** and **3b**. Because the formation of **3a** and **3b** involves the loss of the corresponding anomeric carbon of **15a** and **15b**, the configurational assignments at each carbon atom of **3a** and **3b** provided proof for the stereochemistry of homoazepanes **4a** and **4b** at four stereocentres.

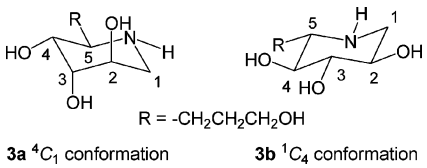


Fig. 2 Conformations of **3a**,**b**.

Glycosidase inhibitory study

The glycosidase inhibitory activity against α-glucosidase, β-glucosidase, β-galactosidase, and α-mannosidase for compounds **3a**,**b** and **4a–d** was studied and the IC_{50} values are summarized in Table 1. Compounds **3a** and **3b** were found to be selective β-glucosidase inhibitors. Asano *et al.*[18] have reported 1-deoxy-D-*altro*-nojirimycin **3c** as a non-selective glycosidase inhibitor. However, the structurally similar, two carbon homologated 1-deoxy-D-*altro*-homonojirimycin **3a** showed selective β-glucosidase inhibition in millimolar concentration. Chida *et al.*[19] have reported 1-deoxy-L-*gluco*-nojirimycin **3d** with an IC_{50} value of 74 μg mL^{-1} (0.454 mM) against β-glucosidase, while 1-deoxy-L-*gluco*-homonojirimycin **3b** showed selective β-glucosidase inhibition (IC_{50} = 45.0 μM) with a ten fold increase in the activity. This effect could be attributed to the two carbon homologation at C5. The synthesis of polyhydroxylated azepanes **4c** and **4d** was reported earlier by us.[11b] Herein we report their biological activity and compare their values with the activities of newly synthesised two carbon homologated compounds **4a** and **4b**. Compound **4a** was found to be a α-glucosidase inhibitor, whereas **4c** inhibit both β-glucosidase and β-galactosidase in millimolar concentrations. Compound **4b** inhibit α-glucosidase and β-glucosidase whereas compound **4d** is active against β-glucosidase and β-galactosidase in millimolar concentrations.

Conclusions

We have demonstrated the utility of γ,δ-unsaturated ester **6** employing Johnson–Claisen rearrangement for the synthesis of homoazepanes **4a**,**b** and 1-deoxy-homonojirimycin **3a**,**b** analogues, which involves ester reduction, benzyl ether protection, epoxidation, regioselective nucleophilic ring opening by azide, hydrogenolysis and *N*-Cbz protection protocol. The ready availability

Table 1 IC_{50} values in mM[a]

Compounds	α-Glucosidase (yeast)	β-Glucosidase (almonds)	β-Galactosidase (bovine testes)	α-Mannosidase (jack bean)
4a	10.4	602.2	NI	NI
4c	ND	21.6	44.3	ND
4b	17.3	7.3	NI	NI
4d	ND	69.1	45.8	48.4
3a	NI	7.6	NI	NI
3b	NI	0.045	NI	NI

[a] NI: No inhibition under our assay conditions. ND: Not determined. Values are the average of three sets of assays performed.

of reagents, high yielding steps and good regioselectivity in the process give easy access for the synthesis of different types of otherwise difficult azasugars required for glycosidase inhibition study. Another interesting aspect of the present route is that we have converted D-glucose to **3b**, which is an L-glucose. Thus we have converted D-glucose to L-glucose by simple organic transformations and chopping of the anomeric carbon atom, which alters the numbering. We also studied compounds for their glycosidase inhibition and found that compound **3b** is a selective β-glucosidase inhibitor in the μM range and compounds **3a**, **4a** and **4b** are selective β-glucosidase inhibitors in the mM range.

Experimental

General methods

Melting points were recorded with a melting point apparatus and are uncorrected. IR spectra were recorded with FTIR as a thin film or in nujol mull or using KBr pellets and are expressed in cm^{-1}. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded using $CDCl_3$ and/or D_2O as solvent(s). Chemical shifts are reported in δ units (ppm) with reference to TMS as an internal standard and J values are given in Hz. Decoupling and DEPT experiments confirmed the assignments of the signals. Elemental analyses were carried out with a C,H analyzer. Optical rotations were measured using a polarimeter at 25 °C. Thin layer chromatography was performed on pre-coated plates (0.25 mm, silica gel 60 F_{254}). Column chromatography was carried out with silica gel (100–200 mesh). The reactions were carried out in oven-dried glassware under dry N_2. Methanol, pyridine and THF were purified and dried before use. Distilled *n*-hexane and ethyl acetate were used for column chromatography. Sodium azide, lithium chloride, benzyl chloroformate and *m*-chloroperbenzoic acid were purchased from Merck. 10% Pd/C was purchased from Aldrich and/or Fluka. After decomposition of the reaction with water, the work-up involves washing of the combined organic layers with water, then brine, drying over anhydrous sodium sulfate and evaporation of solvent under reduced pressure.

1,6,7,8-Tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*R*,6*R*)-L-*glycero*-D-*gluco*-nonitol (4a). A solution of **14a** (0.10 g, 0.243 mmol) in TFA–H_2O (3 cm^3, 2 : 1) was stirred at 25 °C for 2.5 h. Trifluroacetic acid was co-evaporated with benzene to furnish a thick liquid. To a solution of the above product in methanol was added ammonium formate (0.092 g, 1.46 mmol) and 10% Pd/C (0.05 g) and the reaction mixture was refluxed for an hour. The catalyst was filtered through a pad of celite and washed with methanol. The filtrate was concentrated to get a thick liquid, which on column purification (chloroform–methanol–25% aqueous ammonia = 8 : 1.9 : 0.1) afforded **4a** (0.048 g, 94%) as a semisolid (Found: C, 48.88; H, 8.64. Calcd for $C_9H_{19}NO_5$: C, 48.86; H, 8.66%); R_f 0.58 (methanol); $[\alpha]_D^{25}$ +3.1 (*c* 0.65 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.62–1.81 (3H, m, *H*7a,b and *H*8a), 1.82–1.98 (2H, m, *H*8b and *H*1a), 3.30–3.38 (2H, m, *H*6 and *H*1a), 3.45 (1H, dd, *J* 6.6, 4.8 Hz, *H*4), 3.64 (2H, t, *J* 5.4 Hz, CH_2OH), 3.86–3.97 (2H, m, *H*2/*H*5), 4.11 (1H, d, *J* 5.1 Hz, *H*3); δ_C (75 MHz; D_2O) 25.6, 28.9 (*C*7/*C*8), 46.6, 62.2, 63.2 (*C*1/*C*6/*C*9), 72.1, 73.7, 75.1, 75.2 (*C*2/*C*3/*C*4/*C*5).

1,6,7,8-Tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*S*,6*S*)-D-*glycero*-L-*ido*-nonitol (4b). Compound **14b** (0.10 g, 0.243 mmol) was reacted with TFA–H_2O (3 cm^3, 2 : 1) followed by ammonium formate (0.092 g, 1.46 mmol) and 10% Pd/C (0.05 g) as described in the synthesis of **4a** to afford **4b** (0.046 g, 90%) as a sticky gum (Found: C, 48.85; H, 8.65. Calcd for $C_9H_{19}NO_5$: C, 48.86; H, 8.66%); R_f 0.52 (methanol); $[\alpha]_D^{25}$ +2.7 (*c* 0.75 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.58–1.89 (3H, m, *H*7a,b and *H*8a), 1.98–2.12 (1H, m, *H*8b), 3.27 (1H, dd, *J* 14.1 and 6.6 Hz, *H*1a) 3.35 (1H, dd, *J* 14.1 and 3.3 Hz, *H*1b), 3.24–3.36 (1H, m, *H*6), 3.65 (2H, t, *J* 6.3 Hz, CH_2OH), 3.58–3.67 (1H, m, *H*3), 3.69 (1H, t, *J* 7.5 Hz, *H*4), 3.78 (1H, t, *J* 7.5 Hz, *H*5), 4.01 (1H, ddd, *J* 9.6, 6.6 and 3.3 Hz, *H*2); δ_C (75 MHz; D_2O) 29.9, 29.7 (*C*7/*C*8), 48.8, 62.5 (*C*1/*C*6), 63.6, 70.4, 73.5, 77.7, 78.1 (*C*2/*C*3/*C*4/*C*5/*C*9).

1,6,7,8-Tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*R*,6*R*)-L-*glycero*-D-*gluco*-nonitol hydrochloride (4a·HCl). To a solution of **4a** in methanol (2 cm^3) was added a drop of concentrated hydrochloric acid and the resulting reaction mixture was stirred for 15 min at 25 °C. The solvent was evaporated on a rotary evaporator to afford **4a**·HCl as a semisolid in quantitative yield (Found: C, 41.91; H, 7.81. Calcd for $C_9H_{20}ClNO_5$: C, 41.94; H, 7.82%); $[\alpha]_D^{25}$ +46.7 (*c* 0.3 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.64–1.86 (3H, m, *H*7a,b and *H*8a), 1.88–2.02 (1H, m *H*8b), 3.67 (2H, t, *J* 4.5 Hz, CH_2OH), 3.47–3.56 (1H, m, *H*1a), 3.57–3.74 (3H, m, *H*1b, *H*5 and *H*6), 3.91–4.25 (2H, m, *H*3 and *H*2), 4.14 (1H, d, *J* 5.1 Hz, *H*4); δ_C (75 MHz; D_2O) 26.5, 28.1 (*C*7/*C*8), 44.2, 59.9, 61.0 (*C*1/*C*6/*C*9), 69.7, 71.3, 73.1 (strong) (*C*2/*C*3/*C*4/*C*5).

1,6,7,8-Tetradeoxy-1,6-imino-(2*S*,3*R*,4*R*,5*S*,6*S*)-D-*glycero*-L-*ido*-nonitol hydrochloride (4b·HCl). The reaction of compound **4b** with concentrated hydrochloric acid as described in the preparation of **4a**·HCl afforded **4b**·HCl in quantitative yield (Found: C, 41.97; H, 7.79. Calcd for $C_9H_{20}ClNO_5$: C, 41.94; H, 7.82%); $[\alpha]_D^{25}$ +4.0 (*c* 0.5 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.74–1.93 (3H, m, *H*7a,b and *H*8a), 2.01–2.14 (1H, m, *H*8b), 3.32 (1H, dd, *J* 14.4 and 6.9 Hz, *H*1a), 3.33–3.41 (1H, m, *H*6), 3.40 (1H, dd, *J* 14.4 and 3.0 Hz, *H*1b), 3.66 (2H, t, *J* 6.0 Hz, CH_2OH), 3.69 (1H, d, *J* 7.5 Hz, *H*3), 3.73 (1H, d, *J* 7.5 Hz, *H*5), 3.83 (1H, t, *J* 7.5 Hz, *H*4), 4.05 (1H, ddd, *J* 9.3, 6.9 and 3.0 Hz, *H*2); δ_C (75 MHz; D_2O) 26.7, 27.4 (*C*7/*C*8), 46.2, 60.1, 61.3 (*C*1/*C*6/*C*9), 67.6, 70.8, 75.7, 75.9 (*C*2/*C*3/*C*4/*C*5).

3,5,9-Tri-*O*-acetyl-6-(*N*-benzoxycarbonylamino)-6,7,8-trideoxy-1,2-*O*-isopropylidene-α-D-*glycero*-D-*gluco*-nona-1,4-furanose (15a). To an ice-cooled solution of **14a** (0.50 g, 1.22 mmol) in dry pyridine (1.5 cm^3) was added acetic anhydride (2.48 g, 24.33 mmol). After stirring for 8 h at room temperature, ice-water (2 cm^3) was added and the reaction mixture was extracted with chloroform (3 × 15 cm^3). Usual workup and chromatographic purification (*n*-hexane–ethyl acetate = 9 : 1) afforded triacetyl derivative **15a** (0.64 g, 98%) as a white solid (Found: C, 58.11; H, 6.59. Calcd for $C_{26}H_{35}NO_{11}$: C, 58.09; H, 6.56%); R_f 0.49 (20% ethyl acetate–*n*-hexane); $[\alpha]_D^{25}$ +20.7 (*c* 0.68 in $CHCl_3$); mp 110–112 °C (ethyl acetate–*n*-hexane = 1 : 9); $\nu_{max.}$(KBr)/cm^{-1} 3300–3400 (broad band), 1742, 1671, 1529, 1450, 1375 and 1240; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.31 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.61–1.85 (4H, m, *H*7a,b and *H*8a,b), 2.00 (3H, s, COCH_3), 2.04

(3H, s, COCH_3), 2.05 (3H, s, COCH_3), 4.04–4.16 (3H, m, *H*9a,b and *H*6), 4.32 (1H, dd, *J* 9.6 and 3.0 Hz, *H*4), 4.44 (1H, d, *J* 3.6 Hz, *H*2), 4.89 (1H, d, *J* 9.6 Hz, exchanges with D_2O, N*H*), 5.05 (1H, dd, *J* 9.6 and 2.7 Hz, *H*5), 5.09 (2H, ABq, *J* 12.3 Hz, OCH_2Ph), 5.28 (1H, d, *J* 3.0 Hz, *H*3), 5.90 (1H, d, *J* 3.6 Hz, *H*1), 7.25–7.35 (5H, m, Ar*H*'s); δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.7, 20.8, 21.0 (3 × CO*C*H_3), 25.4, 26.2 (2 × *C*H_3), 26.7, 26.8 (*C*7/*C*8), 52.5, 63.9 (*C*6/*C*9), 66.8 (*C*4), 71.3 (O*C*H_2Ph), 75.0, 77.0, 82.7 (*C*2/*C*3/*C*5), 105.0 (*C*1), 112.2 (O*C*O), 127.8 (strong), 128.3 (strong), 136.2 (Ar*C*'s), 156.0 (NH*C*O), 169.4, 169.8, 170.9 (3 × *C*OCH$_3$).

3,5,9-Tri-*O*-acetyl-6-(*N*-benzoxycarbonylamino)-6,7,8-trideoxy-1,2-*O*-isopropylidene-β-L-*glycero*-L-*ido*-nona-1,4-furanose (15b). The reaction of **14b** (0.50 g, 1.22 mmol) with acetic anhydride (2.48 g, 28.72 mmol) and dry pyridine (1.5 cm^3) as described in the synthesis of **15a** afforded **15b** (0.63 g, 95%) as a thick liquid (Found: C, 58.07; H, 6.53. Calcd for $C_{26}H_{35}NO_{11}$: C, 58.09; H, 6.56%); R_f 0.44 (20% ethyl acetate–*n*-hexane); $[\alpha]_D^{25}$ −5.0 (*c* 0.4 in $CHCl_3$); $\nu_{max.}$(neat)/cm^{-1} 3300–3500 (broad band), 1735, 1600, 1400 and 1377; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.32 (3H, s, CH_3), 1.52 (3H, s, CH_3), 1.42–1.52 (1H, m, *H*7a), 1.57–1.69 (3H, m, *H*7b and *H*8a,b), 2.05 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 3.79–3.89 (1H, m, *H*6), 4.06 (2H, t, *J* 6.3 Hz, CH_2OAc), 4.43 (1H, dd, *J* 6.0 and 3.9 Hz, *H*4), 4.52 (1H, d, *J* 3.9 Hz, *H*2), 5.06 (2H, s, OCH_2Ph), 5.11 (1H, d, *J* 9.3, exchanges with D_2O, N*H*), 5.17 (1H, dd, *J* 6.0 and 4.2 Hz, *H*5), 5.19 (1H, d, *J* 3.9 Hz, *H*3), 5.90 (1H, d, *J* 3.9 Hz, *H*1), 7.26–7.34 (5H, m, Ar*H*'s); δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.8, 21.0, 21.1 (3 × CO*C*H_3), 25.1, 26.4, 26.9 (strong) (2 × *C*H_3 and *C*7/*C*8), 51.4, 51.5, 63.7 (*C*5/*C*6/*C*9), 66.8 (*C*4), 72.3 (O*C*H_2Ph), 77.2, 83.8 (*C*2/*C*3), 104.3 (*C*1), 112.5 (O*C*O), 127.9 (strong), 128.0, 128.4 (strong), 136.3 (Ar*C*'s), 155.9 (NH*C*O), 169.9, 170.1, 170.9 (3 × *C*OCH$_3$).

1,5,6,7-Tetradeoxy-1,5-imino-(2*R*,3*S*,4*R*,5*R*)-D-*altro*-octitol hydrochloride (3a·HCl). A solution of **15a** (0.20 g, 0.37 mmol) in TFA–H_2O (2 : 1, 3 cm^3) was stirred at 25 °C for 2.5 h. Trifluroacetic acid was co-evaporated with benzene to furnish a thick liquid. To a cooled solution of hemiacetal (0.18 g, 0.36 mmol) in acetone–water (10 cm^3, 5 : 1) was added sodium metaperiodate (0.09 g, 0.43 mmol) and stirred for half an hour. The reaction mixture was quenched by adding ethylene glycol (0.1 cm^3). Acetone was removed under reduced pressure and the residue obtained was extracted with chloroform (3 × 15 cm^3). Usual workup and column purification (*n*-hexane–ethyl acetate = 8 : 2) afforded aldehyde, which was directly subjected for hydrogenation in methanol (8 cm^3) and 10% Pd/C (0.08 g) under 80 psi for 12 h. The catalyst was filtered through a pad of celite, washed with methanol and the filtrate was concentrated to get a gummy solid that was directly subjected for deacetylation using MeOH–HCl (5 cm^3, 9 : 1) under reflux conditions for 3 h. The reaction mixture was cooled to room temperature and freeze-dried to get **3a**·HCl (0.051 g, 60% overall) as a semisolid (Found: C, 42.21; H, 7.96. Calcd for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97%); R_f 0.64 (methanol); $[\alpha]_D^{25}$ +29.6 (*c* 0.68 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.64–1.85 (3H, m, *H*6a,b and *H*7a), 1.98–2.16 (1H, m, *H*7b), 3.21 (1H, dd, *J* 13.2 and 3.6 Hz, *H*1a), 3.32–3.42 (2H, m, *H*1b and *H*5), 3.68 (2H, t, *J* 5.7 Hz, CH_2OH), 3.98 (1H, dd, *J* 9.6 and 3.0 Hz, *H*4), 4.02 (1H, dd, *J* 4.5 and 3.0 Hz, *H*3), 4.14–4.21 (1H, m, *H*2); δ_C (75 MHz; D_2O) 25.7, 27.5 (*C*6/*C*7), 44.3, 55.5 (*C*1/*C*5), 61.2 (*C*9), 66.2, 67.2, 68.8 (*C*2/*C*3/*C*4).

1,5,6,7-Tetradeoxy-1,5-imino-(2*R*,3*S*,4*S*,5*S*)-L-*gluco*-octitol hydrochloride (3b·HCl). The reaction of **15b** (0.20 g, 0.37 mmol) with TFA–H_2O (2 : 1, 3 cm^3) and $NaIO_4$ (0.093 g, 0.43 mmol) followed by hydrogenation in the presence of 10% Pd/C (0.08 g), using the same reaction conditions as described in the synthesis of **3a**·HCl afforded **3b**·HCl (0.048 g, 56% overall) as a semisolid (Found: C, 42.23; H, 7.99. Calcd for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97%); R_f 0.62 (methanol); $[\alpha]_D^{25}$ −22.2 (*c* 0.45 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.56–1.80 (3H, m, *H*6a,b and *H*7a), 1.94–2.14 (1H, m, *H*7b), 2.93 (1H, t, *J* 12.0 Hz, *H*1a), 3.14 (1H, ddd, *J* 9.6, 6.9 and 3.6 Hz, *H*5), 3.45 (1H, t, *J* 9.6 Hz, *H*3), 3.47 (1H, dd, *J* 12.0 and 5.1 Hz, *H*1b), 3.48 (1H, t, *J* 9.6 Hz, *H*4), 3.62 (2H, t, *J* 5.7 Hz, CH_2OH), 3.77 (1H, ddd, *J* 12.0, 9.6 and 5.1 Hz, *H*2); δ_C (75 MHz; D_2O) 28.3, 29.5 (*C*6/*C*7), 48.6 (*C*5), 61.4, 63.5, 69.3, 73.5, 78.5 (*C*1/*C*2/*C*3/*C*4/*C*8).

1,5,6,7-Tetradeoxy-1,5-imino-(2*R*,3*S*,4*R*,5*R*)-D-*altro*-octitol (3a). A solution of **3a**·HCl (0.05 g, 0.18 mmol) in methanol and 25% aqueous ammonia (2:1, 3 cm^3) was stirred at room temperature for 15 min. The solvent was evaporated on a rotary evaporator to afford a thick liquid, which on purification by column chromatography (chloroform–methanol–25% aqueous ammonia = 8 : 1.9 : 0.1) afforded **3a** (0.035 g, 83%) as a thick liquid (Found: C, 50.27; H, 8.97. Calcd for $C_8H_{17}NO_4$: C, 50.25; H, 8.96%); R_f 0.49 (methanol); $[\alpha]_D^{25}$ +26.7 (*c* 0.68 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.48–1.82 (3H, m, *H*6a,b and *H*7a), 1.83–1.94 (1H, m, *H*7b), 2.89 (1H, dd, *J* 13.8 and 3.6 Hz, *H*1a), 3.00 (1H, dt, *J* 9.0 and 4.2 Hz, *H*5), 3.09 (1H, dd, *J* 13.8 and 2.7 Hz, *H*1b), 3.64 (2H, t, *J* 6.3 Hz, CH_2OH), 3.77 (1H, dd, *J* 9.0 and 3.0 Hz, *H*4), 3.92 (1H, dd, *J* 4.8 and 3.0 Hz, *H*3), 3.97 (1H, ddd, *J* 4.8, 3.6 and 2.7 Hz, *H*2); δ_C (75 MHz; D_2O) 26.5, 27.9 (*C*6/*C*7), 44.5, 55.2 (*C*1/*C*5), 61.6 (*C*8), 68.1, 69.1, 70.1 (*C*2/*C*3/*C*4).

1,5,6,7-Tetradeoxy-1,5-imino-(2*R*,3*S*,4*S*,5*S*)-L-*gluco*-octitol (3b). The reaction of **3b**·HCl (0.047 g, 0.21 mmol) with 25% aqueous ammonia as described for **3a** and column purification (chloroform–methanol–25% aqueous ammonia = 8 : 1.9 : 0.1) afforded **3b** (0.03 g, 76%) as a thick liquid (Found: C, 50.28; H, 8.94. Calcd for $C_8H_{17}NO_4$: C, 50.25; H, 8.96%); R_f 0.47 (methanol); $[\alpha]_D^{25}$ −22.2 (*c* 0.45 in CH_3OH); $\nu_{max.}$(nujol)/cm^{-1} 3200–3600 (broad band); δ_H (300 MHz; D_2O) 1.54–1.82 (3H, m, *H*6a,b and *H*7a), 1.98–2.12 (1H, m, *H*7b), 2.78 (1H, dd, *J* 12.6 and 11.4 Hz, *H*1a), 2.93 (1H, ddd, *J* 9.6, 7.8 and 3.9 Hz, *H*5), 3.35 (1H, t, *J* 9.6 Hz, *H*3), 3.36 (1H, dd, *J* 12.6 and 4.5 Hz, *H*1b), 3.42 (1H, t, *J* 9.6 Hz, *H*4), 3.64 (2H, t, *J* 6.0 Hz, CH_2OH), 3.70 (1H, ddd, *J* 11.4, 9.6 and 4.5 Hz, *H*2); δ_C (75 MHz; D_2O) 26.5, 27.3 (*C*6/*C*7), 47.1 (*C*5), 59.2 (*C*1), 61.4, 68.2, 72.4, 76.9 (*C*2/*C*3/*C*4/*C*8).

Procedure for inhibition assay

Inhibition potencies of **4a–d** and **3a**,**b** were determined by measuring the residual hydrolytic activities of the glycosidases. Glycosidases namely α-mannosidases (jack bean), α-glucosidase (baker's yeast), β-glucosidase (almond) and β-galactosidase (bovine testes) were purchased from Sigma Chemicals Co. USA. Substrates (purchased from Sigma Chemicals Co. USA)

p-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-α-D-glucopyranoside of 2 mM concentration, were prepared in 0.025 M citrate buffer with pH 6.0, and *p*-nitrophenyl-α-D-mannopyranoside, of 2 mM concentration, was prepared in 0.025 M citrate buffer with pH 4.5. The test compound (of various concentrations of 0.5 μM to 1 mM) was preincubated with the enzyme, buffered at its optimal pH, for 30 min at 25 °C. The enzyme reaction was initiated by the addition of 100 μL of substrate. Controls were run simultaneously in the absence of test compound. The reaction was terminated at the end of 10 min by the addition of 0.05 M borate buffer (pH 9.8) and absorbance of the liberated *p*-nitrophenol was measured at 405 nm with a Shimadzu Spectrophotometer UV-1601.[20]

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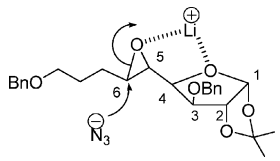
15 This assignment was further confirmed by converting epoxides **12a**,**b** to their corresponding piperidine derivatives **3a**,**b**, wherein configurational and conformational assignment was made by comparing the coupling constant values in six-membered rings of **3a**,**b**.
16 The oxirane ring opening with sodium azide in DMF was found to be unsuccessful even at 100 °C for 8 days. The probable reason for the observed regioselectivity in the presence of LiCl could be the strong chelation of the lithium ion with oxirane and furanose oxygens which favours attack at sterically less hindered C6.



17 In these particular experiments, triacetylated products were obtained as a mixture of two compounds one with free C3–OH and other with C3–OCHO. Our attempts to separate the mixture were found to be unsuccessful. Therefore, we directly converted the triacetylated products to the corresponding hydrochlorides.
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