ARTICLE

OBC
www.rsc.org/obc

Synthesis and evaluation of the glycosidase inhibitory activity of 5-hydroxy substituted isofagomine analogues

Mohammed M. Matin,[a] Tarun Sharma,[b] Sushma G. Sabharwal[b] and Dilip D. Dhavale*[c]

[a] *Department of Chemistry, University of Chittagong, Chittagong 4331, Bangladesh*

[b] *Division of Biochemistry, Department of Chemistry, University of Pune, Pune 411 007, India*

[c] *Garware Research Centre, Department of Chemistry, University of Pune, Pune 411 007, India. E-mail: ddd@chem.unipune.ernet.in; Fax: +91-20-2569-1728*

Received 3rd December 2004, Accepted 16th February 2005
First published as an Advance Article on the web 29th March 2005

An efficient strategy for the synthesis of 5-hydroxy substituted isofagomine analogues **4a, 4b** and **4c**, having both $-CH_2OH/CH_3$ and –OH functionality at the C-5 position, and evaluation of their inhibitory potency is reported. The synthetic methodology involves the aldol–Cannizzaro reaction of easily available α-D-xylopentodialdose followed by hydrogenolysis to afford the triol **5**. Selective amidation of the α- and β-hydroxymethyl group at C-4, deprotection of the 1,2-acetonide group and hydrogenation gave the target molecules, which were found to be potent against β-glycosidases with IC_{50} values in the micro molar range. Compound **4c** showed excellent potency against glycosidases and human salivary amylase.

Introduction

A number of natural as well as synthetic analogues of aza-sugars such as nojirimycin **1a**, 1-deoxynojirimycin **1b** and 1,2-dideoxynojirimycin namely fagomine **2** (Fig. 1) are potent glycosidase inhibitors and are therefore of significant therapeutic interest in the treatment of diabetes, viral infection and influenza.[1] In the search for the design and development of the anomer-selective β-glycosidase inhibitors, Bols and co-workers noticed a subtle change in the glycosidase inhibitory activity by moving the nitrogen atom of fagomine **2** to the anomeric position C-1. This led to the development of isofagomine **3a** which showed stronger and more selective β-glucosidase inhibitory activity.[2] The 5-hydroxy isofagomine **3b** is notable and found to be an inhibitor of glycolipid biosynthesis although with little inhibitory activity against glycosidases.[3] As part of our continuing efforts in the area of azasugars,[4] we are now reporting the synthesis of hitherto unknown 5-hydroxy isofagomine analogues **4a–c** (Fig. 1) and their glycosidase inhibitory activity. Pandey and co-workers have reported the synthesis of compound **3c** however; mistakenly the structure of the compound was drawn as **4b**.[2i,5]

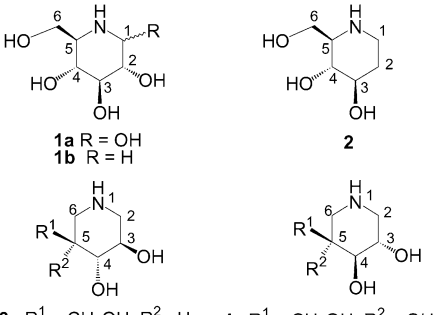
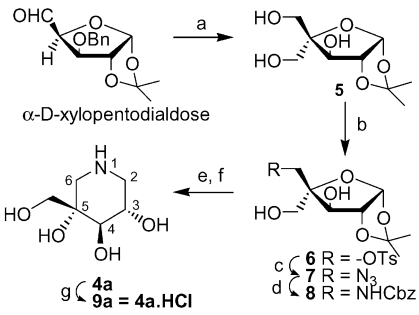


Fig. 1 Azasugar analogues.

Results and discussion

Synthesis of (3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy-5-hydroxymethylpiperidine (4a)

Our synthetic strategy begins with the known triol **5** (Scheme 1) that was easily obtained by the aldol–Cannizzaro reaction of 3-



Scheme 1 *Reagents and conditions*: (a) see ref. 4*e*; (b) TsCl, pyridine, −15 °C, 24 h, 57%; (c) NaN_3, DMF, 110 °C, 60 h, 30%; (d) i. LAH, THF, 0 °C–rt, 1.5 h; ii. CbzC1, $NaHCO_3$, EtOH–H_2O, 4 h, 83%; (e) TFA–H_2O (3 : 2), 0 °C–rt, 2.5 h, 97%; (f) $HCOONH_4$, 10% Pd/C, MeOH, reflux, 45 min, 91%; (g) dry MeOH, HCl, 25 °C, 2 h, 98%.

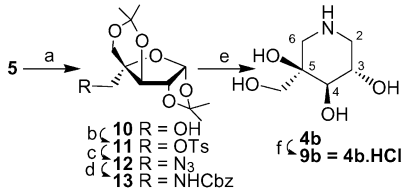
O-benzyl-1,2-*O*-isopropylidene-α-D-xylopentodialdose.[4e] Tosylation of **5** furnished the crystalline compound **6** (57%) wherein the 4β-*O*-hydroxymethylene group was selectively tosylated. The assignment of structure **6** was established by 1D-NOESY in which irradiation of a signal at δ 4.26 (H-3α) showed NOE for the methylene protons at δ 3.65 and 3.88 indicating the presence of a 4α-CH_2OH group with (4*S*) absolute configuration. The formation of **6** could be attributed to the presence of an α-oriented 1,2-acetonide functionality that hindered the approach of the bulky tosyl group from the α-face.[6] In the next step, treatment of **6** with NaN_3 in DMF afforded **7** as an amorphous solid. An interesting observation was noticed in the ^1H NMR spectrum of **7** in which the H-3 proton was found to be considerably downfield, appearing at δ 5.03, as compared to the normal position (δ ~4.00). This downfield shift could be attributed to the diamagnetic anisotropic effect of the azide group. In the next step, the reduction of the azide functionality in **7** with LAH and selective *N*-Cbz protection gave amino alcohol **8** as a syrup. Finally, hydrolysis of the 1,2-acetonide functionality and hydrogenation (ammonium formate and 10% Pd/C) afforded 5-hydroxy isofagomine **4a** as a thick oil. Treatment of **4a** with MeOH–HCl gave hydrochloride salt **9a**.

Synthesis of (3*S*, 4*R*, 5*R*)-3,4,5-trihydroxy-5-hydroxymethylpiperidine (4b)

The synthesis of 5-hydroxy isofagomine **4b**, the C-5 epimer of **4a**, requires selective tosylation of the 4α-hydroxymethylene group in **5**. Thus, treatment of triol **5** with 2,2-dimethoxy propane

DOI: 10.1039/b418283a

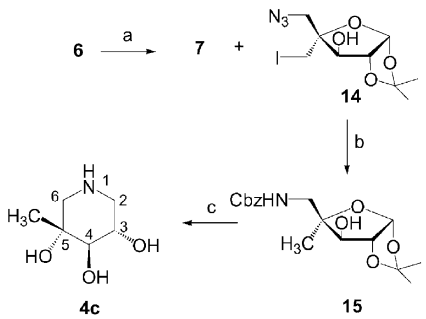
and *p*-TSA (cat.) in methanol afforded the 1,2 : 3,5-di-*O*-isopropylidene derivative **10** (Scheme 2) as the only product (57%).[4e] The tosylation of the free 4α-hydroxymethylene group in **10** gave **11** that on treatment with sodium azide afforded **12**. The reduction of **12** with LAH and selective *N*-Cbz protection furnished **13** (88%). In the final step, one pot hydrolysis of both acetonide groups in **13** followed by hydrogenation afforded **4b** as a thick oil. The reaction of **4b** with MeOH–HCl gave hydrochloride salt **9b**.[3]



Scheme 2 *Reagents and conditions*: (a) 2,2-dimethoxy propane, *p*-TSA, MeOH, 25 °C, 5 min, 97%; (b) TsCl, pyridine, 0 °C–rt, 12 h, 92%; (c) NaN_3, DMF, TBAI, MS, 110 °C, 48 h, 62%; (d) i. LAH, THF, 0 °C–rt, 1.5 h; ii. CbzCl, $NaHCO_3$, EtOH–H_2O, 0 °C–rt, 4 h, 88%; (e) i. TFA–H_2O (3 : 2), 0 °C–rt, 2.5 h, 95%; ii. $HCOONH_4$, 10% Pd/C, MeOH, reflux, 45 min, 83%; (f) dry MeOH, HCl, 25 °C, 2 h, 98%.

Synthesis of (3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy-5-methylpiperidine (4c)

During the synthesis of isofagomine **4a**, the conversion of **6** to azido derivative **7** resulted in a poor yield irrespective of a change in reaction conditions like solvent, temperature and mole ratio. In order to improve the yield, the reaction of **6** with NaN_3 was performed in the presence of TBAI (0.5 equiv) that furnished two compounds, **7** and **14**, in 22% and 19% yield, respectively (Scheme 3). The exact mechanism for the formation of **14** is not clear to us. However, we believe that the scrambling of the tosyl group between the C4-hydroxymethylene groups leads to the formation of a 4α-tosyl derivative that on S_N2 displacement with iodide afforded **14**. Our attempts to improve the yield of **14** were unsuccessful. The structure of **14** was established by 1D-NOESY wherein the irradiation of two proton singlets at δ 3.65 (CH_2I) showed NOE for the singlet at δ 5.04 (H-3α) indicating the α-orientation of the $-CH_2I$ group. In the subsequent step, the reaction of **14** with LAH in THF furnished an amino compound (as evident from the ^{1}H NMR of the crude product) that was directly converted to an *N*-Cbz protected amino alcohol **15**. The removal of the 1,2-acetonide functionality followed by hydrogenation gave isofagomine **4c** as a thick oil with –OH and $-CH_3$ groups at the C-5 position.



Scheme 3 *Reagents and conditions*: (a) NaN_3, TBAI (0.5 equiv), DMF, 110 °C, 6 h, 41%; (b) i. LAH, THF, 0 °C–rt, 1.5 h; ii. $NaHCO_3$, CbzCl, MeOH–H_2O, 0 °C–rt, 4 h, 89%; (c) i TFA–H_2O (3 : 2), 0 °C–rt, 3 h; ii. $HCOONH_4$, 10% Pd/C, MeOH, reflux, 45 min, 72%.

Conformational assignment of 4a, 4b and 4c

The isofagomine **3a** is known to exist in a $^2C_5$ conformation however; our recent studies indicated that isofagomine analogues might exist in either $^5C_2$ or $^2C_5$ conformations depending on the type and orientation of substituents at C3–C5.[4c] Therefore, the conformations of **4a–c** were studied using coupling constant information obtained from ^{1}H NMR decoupling experiments (Table 1). In **4a**, the appearances of a doublet of doublet for H-2a at δ 2.16 ($J_{2a,2e} = 11.0$ and $J_{2a,3} = 10.8$ Hz) and a ddd for H-2e at δ 2.92 ($J_{2e,2a} = 11.0$, $J_{2e,3} = 5.3$ and $J_{2e,6e} = 1.5$ Hz) were informative. The large coupling constant ($J_{2a,3} = 10.8$ Hz) for the H-2 axial proton requires a *trans*-diaxial relationship with H-3. In addition, the *trans*-diaxial disposition of the H-3 and H-4 protons was evident from the large coupling constant ($J_{3,4} = 9.4$ Hz). In the precursor **8**, the relative stereochemistry of the substituents at C-2, C-3 and C-3, C-4 is *trans* and the same stereochemistry is retained in the product. Based on this data compound **4a** was assigned the $^5C_2$ conformation with a (5*S*) configuration (Fig. 2). In this conformation, although the $-CH_2OH$ group is axially oriented with 1,3-diaxial interactions, we believe that the conformation ($^5C_2$) is stabilized by intramolecular hydrogen bonding between the $-CH_2OH$ and the ring nitrogen atom as shown in Fig. 2. This fact is supported by the ^{1}H NMR spectrum of **4a** wherein the methylene protons ($-CH_2OH$) were found to be relatively shielded (δ 2.56 and 2.72) as compared to their normal position (δ ~3.50). This upfield shift could be attributed to the hydrogen bonding that increases the electron density at the oxygen atom (of $-CH_2OH$) and thus makes it less electron withdrawing compared with the normal oxygen atom. While, in the ^{1}H NMR spectrum of the corresponding hydrochloride salt **9a** the methylene protons appeared at their normal positions [δ 3.19 (d, $J = 13.4$ Hz) and 3.44 (d, $J = 13.4$ Hz)], probably due to the loss of intramolecular hydrogen bonding. In the ^{1}H NMR spectrum of **4a**, the appearance of H-6e as a doublet of doublet at δ 2.66 ($J = 11.0$ and 1.5 Hz) was due to 'W' type coupling with H-2e. This was supported by the appearance of H-2e as a ddd with $J = 11.0$, 5.3 and 1.5 Hz. The small J of 1.5 Hz was thus due to 'W' type coupling as shown in Fig. 2.

Table 1 ^{1}H–^{1}H coupling constants in compounds **4a–c** and **9a,b**

	J/Hz						
Compound no.	$J_{2a,2e}$	$J_{2a,3a}$	$J_{2e,3a}$	$J_{2e,6e}$	$J_{3a,4a}$	$J_{6a,6e}$	$J_{7a,7b}$
4a	11.0	10.8	5.3	1.5	9.4	11.0	13.4
9a	11.6	11.0	5.4	2.0	9.1	11.6	13.4
4b	12.0	12.0	4.9	1.7	9.4	13.5	11.7
9b	11.9	11.9	5.2	1.7	9.4	13.2	11.7
4c	11.9	11.9	5.3	1.8	9.4	13.2	—

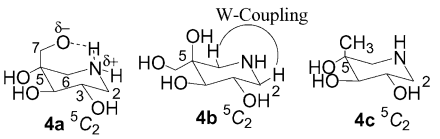
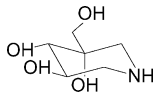
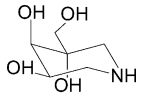


Fig. 2 Conformations of azasugars.

In the ^{1}H NMR spectrum of **4b**, the appearance of one triplet at δ 2.57 for H-2a with large coupling constants ($J_{2a,2e} = J_{2a,3} =$ 12.0 Hz) indicated a *trans*-diaxial relationship of H-2a with the H-3 proton. The H-2e appeared as a ddd at δ 3.24 ($J_{2e,2a} =$ 12.0, $J_{2e,3} = 4.9$ and $J_{2e,6} = 1.7$ Hz), wherein the small coupling constant $J_{2e,6} = 1.7$ Hz with the H-6e proton was due to 'W' coupling. The H-4 proton appeared as a doublet with $J = 9.4$ Hz; this clearly requires *trans*-diaxial orientation between the H-3 and H-4 protons. In the precursor **13**, the C-4 and C-5 –OH groups are *cis*-orientated. Therefore, **4b** was assigned the $^5C_2$ conformation with a (5*R*) configuration. The ^{1}H NMR spectra of **4a/4b** and their corresponding hydrochloride salts **9a/9b** were very similar with respect to coupling constants of protons (except the downfield shifts of the H-2 and H-6 protons). This indicated that the conformations of **4a** and **4b** remained unchanged in the presence of a piperidinium ion.[7]

Table 2 Ki*/IC_{50}# values in μM

	α-Glucosidase[a]	β-Glucosidase[b]	α-Galactosidase[c]	β-Galactosidase[d]
 **16a**	230*	4.3*	>5000#	>5000#
 **16b**	2000#	420#	610#	5.7*

[a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Green coffee bean α-galactosidase. [d] Aspergillus oryzae β-galactosidase.

In the case of **4c**, the ^{1}H NMR spectrum also showed the *trans*-diaxial relationship between H-2a and H-3 as well as H-3 and H-4 as evident from the large coupling constants ($J_{2a,3}$ = 11.9 and $J_{3,4}$ = 9.4 Hz). As the *trans* relationship between the C-4 and C-5 –OH groups in the precursor **15** should be retained in the product, the $^5C_2$ conformation with an axially oriented methyl group and (5*S*) configuration was assigned to **4c**.

Glycosidase inhibitory study

Isofagomine is known to be a potent inhibitor of β-glucosidase (IC_{50} 0.11 μM) and most of the inhibition data for isofagomine analogues is known in IC_{50} values. So the IC_{50} values were determined for compounds **4a–c** and are summarized in Table 3. The α-glucosidases however are generally more powerfully inhibited by 1-deoxynojirimycin analogues. There are several explanations available for the observed inhibitory profiles of isofagomine compared to that of the related 1-deoxynojirimycin.[8] For comparative studies, the inhibitory profile of previously known structurally related compounds **16a** and **16b** was taken into account. The IC_{50} values of **16a** and **16b** indicate that both compounds are moderate to poor inhibitors of glycosidases (Table 2).[1d] However, inhibition potential increased tremendously for compounds **4a** and **4b**, which have a C3α-OH group (Table 3). Compound **4a** showed selectivity towards β-glycosidase inhibition that is an expected notion for the isofagomine class of glycosidase inhibitors. Compound **4c**, with methyl and hydroxyl groups at C-5 and C3α–OH, strongly inhibits most of the glycosidase enzymes. This result could be attributed to the hydrophobic interaction of the CH_3 group at the active site of glycosidases. In addition, **4c** also demonstrated excellent potency against human salivary amylase.

Conclusion

Thus, we have demonstrated a convenient method for the synthesis of 5-hydroxy-5-hydroxymethyl isofagomine analogues **4a**,**4b** and 5-hydroxy-5-methyl isofagomine analogue **4c**. The glycosidase inhibitory activity of **4a–c** showed good inhibitory potency against the β-glycosidases. Isofagomine **4c** showed excellent potency against human salivary amylase.

Table 3 Inhibitory potencies of 1-azasugars **4a–c**

	IC_{50}/μM		
Enzyme	**4a**	**4b**	**4c**
α-Glucosidase[a]	NI	7.11	1.75
β-Glucosidase[b]	2.57	16.78	1.76
α-Galactosidase[b]	8.74	17.12	NI
β-Galactosidase[c]	2.18	16.85	1.89
α-Mannosidase[d]	2.20	NI	1.77
Human salivary amylase	NI	6.43	0.34

[a] Yeast. [b] Almonds. [c] Bovine testis. [d] Jack bean. NI—inhibition not observed under assay conditions. Data is average of three sets of assay performed.

Experimental

General methods

Melting points were recorded with a Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded with Shimadzu FTIR-8400 as a thin film or in nujol mull or using KBr pellets and are expressed in cm^{-1}. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded with Varian Mercury 300 using $CDCl_3$ or D_2O as a solvent. Chemical shifts were reported in δ unit (ppm) with reference to TMS as an internal standard and J values are given in Hz. Elemental analyses were carried out with Elemental Analyser Flash 1112. Optical rotations were measured using a Bellingham Stanley-ADP digital polarimeter with sodium light (589.3 nm) at 25 °C. Thin layer chromatography was performed on Merck 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, DMF and THF were purified and dried before use. Petroleum ether (PE) that was used is a distillation fraction between 40–60 °C. LAH, CbzCl, 10% Pd–C were purchased from Aldrich and/or Fluka. After decomposition of the reaction with water, the work-up involves washing of the combined organic layer with water, brine, drying over anhydrous sodium sulfate and evaporation of the solvent at reduced pressure. Triol **5** was prepared by the aldol–crossed Cannizzaro reaction of dialdose and was reported earlier.[4e] For enzyme inhibition studies, substrates were purchased from Sigma Chemicals Co. USA. α-Glucosidase from yeast, β-mannosidase from jack beans and β-galactosidase from bovine testis were purchased from Sigma Chemicals Co. USA. β-Glucosidase and β-galactosidase were extracted and purified from sweet almonds.

1,2-*O*-Isopropylidene-4-(*S*)-*C*-(hydroxymethyl)-5-*O*-*p*-toluenesulfonyl-β-L-threo-pento-1,4-furanose (6). To a cooled solution of triol **5** (2.1 g, 9.53 mmol) at −15 °C in dry pyridine (3 mL) was added *p*-toluenesulfonyl chloride (1.73 g, 9.07 mmol) and stirring was continued at −15 °C for 24 h. The reaction was decomposed by the addition of ice–water. Pyridine was co-evaporated with toluene (2 × 3 mL). Chromatographic purification (PE–ethyl acetate = 9 : 1) gave monotosyl derivative **6** (2.04 g, 57%) as a white solid, mp 132–133 °C. Found: C, 51.41; H, 6.02. Calc. for $C_{16}H_{22}SO_8$ C, 51.33; H, 5.92; R_f 0.49 (66% ethyl acetate–*n*-hexane); $[\alpha]_D$ −7.1 (*c* 0.85, $CHCl_3$); ν_{max} (nujol) 3600–3220 (br band) and 1379 cm^{-1}; δ_H (300 MHz, $CDCl_3 + D_2O$) 1.33 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.49 (3H, s,

C*H*$_3$), 3.65 (1H, d, J = 12.2 Hz, CH_2OH), 3.88 (1H, d, J = 12.2 Hz, CH_2OH), 4.19 (1H, d, J = 10.0 Hz, CH_2OTs), 4.23 (1H, d, J = 10.0 Hz, CH_2OTs), 4.26 (1H, s, *H*3), 4.66 (1H, d, J = 4.1 Hz, *H*2), 5.94 (1H, d, J = 4.1 Hz, *H*1), 7.40 (2H, d, J = 8.1 Hz, Ar-*H*), 7.84 (2H, d, J = 8.1 Hz, Ar-*H*); δ_C (75 MHz, $CDCl_3$) 21.7, 25.9, 26.6 (*C*H$_3$), 61.4 (*C*H$_2$OH), 66.7 (*C*H$_2$OTs), 76.0 (*C*-3), 87.2 (*C*-4), 88.8 (*C*-2), 105.2 (*C*-1), 112.7 (O*C*O), 128.0, 130.0, 131.9, 145.5 (Ar–*C*).

1,2-*O*-Isopropylidene-4-(*S*)-*C*-(hydroxymethyl)-5-azido-β-L-threo-pento-1,4-furanose (7). To a solution of **6** (2.2 g, 5.9 mmol) in anhydrous DMF (12 mL) was added NaN_3 (2.28 g, 35.2 mmol) and MS (1.5 g) and the reaction mixture was stirred at 110 °C for 60 h. The reaction mixture was cooled to room temperature. After adding water the reaction mixture was extracted with ethyl acetate. The organic layer was dried, concentrated and purified by chromatography (*n*-hexane–ethyl acetate = 4 : 1) to furnish azido compound **7** (0.432 g, 30%) as a white solid, mp 80–81 °C. Found: C, 44.14; H, 6.12. Calc. for $C_9H_{15}N_3O_5$ C, 44.08; H, 6.16; R_f 0.22 (33% ethyl acetate–*n*-hexane); $[\alpha]_D$ −4.0 (*c* 1, $CHCl_3$); ν_{max} (nujol) 3500–3200 (br band) and 2104 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.36 (3H, s, C*H*$_3$), 1.52 (3H, s, CH$_3$), 1.70–1.98 (2H, br s, O*H*, exchange with D_2O), 3.86 (2H, AB quartet, J = 12.0 Hz, CH_2N$_3$), 4.36 (1H, d, J = 7.5 Hz, CH_2OH), 4.63 (1H, d, J = 7.5 Hz, CH_2OH), 4.75 (1H, d, J = 2.9 Hz, *H*2), 5.03 (1H, s, *H*3), 6.26 (1H, d, J = 2.9 Hz, *H*1); δ_C (75 MHz, $CDCl_3$) 26.5, 27.5 (CH$_3$), 62.8 (*C*H$_2$N$_3$), 78.7 (*C*H$_2$OH), 84.6, 88.2, 89.6 (*C*-2/*C*-3/*C*-4), 107.8 (*C*-1), 114.3 (O*C*O).

1,2-*O*-Isopropylidene-4-(*S*)-*C*-(hydroxymethyl)-5-(*N*-benzoxycarbonylamino)-β-L-threo-pento-1,4-furanose (8). To an ice cooled suspension of LAH (0.464 g, 12.3 mmol) in dry THF (10 mL) was added a solution of **7** (0.5 g, 2.04 mmol) in dry THF (7 mL) over a period of 10 min. The reaction mixture was warmed to room temperature and stirred for 1.5 h. Ethyl acetate (10 mL) was added at 0 °C, stirred for 10 min and quenched with a saturated solution of NH_4Cl (2 mL). The solution was filtered, the residue was washed with ethyl acetate (3 × 3 mL) and worked up. The organic layer was concentrated, dried and dissolved in ethanol–water (10 mL, 1 : 1), followed by the addition of $NaHCO_3$ (0.479 g, 5.70 mmol) at 0 °C and benzyl chloroformate (0.522 g, 3.1 mmol). The mixture was stirred at room temperature for 4 h, quenched with water and extracted with ethyl acetate (3 × 10 mL). Work-up and chromatography (*n*-hexane–ethyl acetate = 4 : 1) provided **8** (0.598 g, 83%) as a syrup. Found: C, 57.83; H, 6.63. Calc. for $C_{17}H_{23}NO_7$ C, 57.78; H, 6.56; R_f 0.33 (66% ethyl acetate–*n*-hexane); $[\alpha]_D$ −24.7 (*c* 1.0, $CHCl_3$); ν_{max} (neat) 3550–3200 (br band) and 1670 cm^{-1}; δ_H (300 MHz, $CDCl_3$ + D_2O) 1.29 (3H, s, CH$_3$), 1.50 (3H, s, C*H*$_3$), 3.50 (1H, d, J = 14.0 Hz, CH_AH$_B$NH), 3.64 (2H, AB quartet, J = 13.6 Hz, CH_2OH), 3.69 (1H, d, J = 14.0 Hz, CH$_A$*H*$_B$NH), 4.16 (1H, s, *H*3), 4.61 (1H, d, J = 4.1 Hz, *H*2), 5.11 (2H, br s, *O*-CH_2Ph), 5.93 (1H, d, J = 4.1 Hz, *H*1), 7.22–7.40 (5H, m, Ar-*H*); δ_C (75 MHz, $CDCl_3$ + D_2O) 25.7, 26.7 (*C*H$_3$), 42.9 (*N*-*C*H$_2$), 61.0 (*O*-*C*H$_2$Ph), 67.3 (*C*H$_2$OH), 77.4, 87.2, 89.8 (*C*-2/*C*-3/*C*-4), 105.4 (*C*-1), 112.1 (O*C*O), 128.1, 128.5, 136.0 (Ar–*C*), 157.9 (*C*O). [The ^{13}C NMR spectrum showed doubling of signals due to rotational isomers possible because of restricted rotation around the C–N bond].

(3*S*,4*R*,5*S*)-3,4,5-Trihydroxy-5-hydroxymethylpiperidine (4a). To a solution of TFA–water (5 mL, 3 : 2) was added **8** (0.55 g, 1.6 mmol) at 0 °C, which was then stirred for 30 min. The solution was allowed to warm to 25 °C and stirred for 2.5 h. TFA–water was co-evaporated with toluene under a high vacuum to provide an anomeric mixture of hemiacetal (0.473 g, 97%), which was directly used in the next reaction. To a solution of hemiacetal in dry methanol (8 mL) was added 10% Pd/C (0.1 g) and ammonium formate (0.761 g, 12.1 mmol). The reaction mixture was refluxed for 45 min and filtered through celite, washed with methanol and concentrated. The concentrated thick liquid was purified by a silica column using $CHCl_3$–MeOH–NH_3 (25% solution) = 50 : 49 : 1 as an eluant to yield **4a** (0.231 g, 91%) as a thick oil. Found: C, 44.20; H, 8.08. Calc. for $C_6H_{13}NO_4$ C, 44.16; H, 8.03; R_f 0.09 (50% MeOH–$CHCl_3$); $[\alpha]_D$ +34.3 (*c* 0.35, MeOH); ν_{max} (neat) 3600–3200 (br band) cm^{-1}; δ_H (300 MHz, D_2O) 2.16 (1H, dd, J = 11.0 and 10.8 Hz, *H*2a), 2.39 (1H, d, J = 11.0 Hz, *H*6a), 2.56 (1H, d, J = 13.4 Hz, *O*-CH_2), 2.66 (1H, dd, J = 11.0 and 1.5 Hz, *H*6e), 2.72 (1H, d, J = 13.4 Hz, *O*-CH_2), 2.92 (1H, ddd, J = 11.0, 5.3 and 1.5 Hz, *H*2e), 3.26 (1H, d, J = 9.4 Hz, *H*4), 3.58 (1H, ddd, J = 10.8, 9.4 and 5.3 Hz, *H*3); δ_C (75 MHz, D_2O) 45.6, 49.2 (*C*-2/*C*-6), 50.4 (*C*H$_2$OH), 69.0, 73.7, 75.6 (*C*-3/*C*-4/*C*-5).

(3*S*,4*R*,5*S*)-3,4,5-Trihydroxy-5-hydroxymethylpiperidine hydrochloride (9a). To a stirred solution of compound **4a** (0.2 g, 1.3 mmol) in dry methanol was added HCl (0.2 mL) under nitrogen. The reaction mixture was stirred at 25 °C for 2 h and the solvent was evaporated under reduced pressure to give **9a** (0.24 g, 98%) as a semi-solid. Found: C, 36.12; H, 7.15. Calc. for $C_6H_{14}NO_4Cl$ C, 36.09; H, 7.07; R_f 0.02 (80% MeOH–$CHCl_3$); $[\alpha]_D$ +15.0 (*c* 0.4, MeOH); ν_{max} (nujol) 3600–3220 (br band) cm^{-1}; δ_H (300 MHz, D_2O) 2.90 (1H, dd, J = 11.6 and 11.0 Hz, *H*2a), 3.13 (1H, d, J = 11.6 Hz, *H*6a), 3.19 (1H, d, J = 13.4 Hz, *O*-CH_2), 3.40 (1H, dd, J = 11.6 and 2.0 Hz, *H*6e), 3.44 (1H, d, J = 13.4 Hz, *O*-CH_2), 3.54 (1H, ddd, J = 11.6, 5.4 and 2.0 Hz, *H*2e), 3.69 (1H, d, J = 9.1 Hz, *H*4), 4.60 (1H, ddd, J = 11.0, 9.1 and 5.4 Hz, *H*3); δ_C (75 MHz, D_2O) 44.2, 46.4 (*C*-2/*C*-6), 48.7 (*C*H$_2$OH), 65.3, 69.6, 74.8 (*C*-3/*C*-4/*C*-5).

1,2 : 3,5-Di-*O*-isopropylidene-4-(*R*)-*C*-(*p*-toluenesulfonyloxymethyl)-β-L-threo-pento-1,4-furanose (11). To a solution of **10** (1.0 g, 3.9 mmol) in anhydrous pyridine (2 mL) was added *p*-toluenesulfonyl chloride (0.88 g, 4.6 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred for 12 h. Usual work-up and chromatography (PE–ethyl acetate = 9.5 : 0.5) provided monotosylate **11** (1.47 g, 92%) as a white solid, mp 114–115 °C. Found: C, 55.03; H, 6.37. Calc. for $C_{19}H_{26}SO_8$ C, 55.06; H, 6.32; R_f 0.43 (33% ethyl acetate–*n*-hexane); $[\alpha]_D$ −21.18 (*c* 0.85, $CHCl_3$); ν_{max} (KBr) 1375 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.27 (3H, s, C*H*$_3$), 1.31 (3H, s, C*H*$_3$), 1.37 (3H, s, C*H*$_3$), 1.40 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 3.63 (1H, d, J = 12.7 Hz, CH_2), 3.94 (1H, d, J = 12.7 Hz, CH_2), 3.99 (1H, d, J = 10.0 Hz, CH_2OTs), 4.26 (1H, s, *H*3), 4.32 (1H, d, J = 10.0 Hz, CH_2OTs), 4.56 (1H, d, J = 3.8 Hz, *H*2), 5.99 (1H, d, J = 3.8 Hz, *H*1), 7.38 (2H, d, J = 8.2 Hz, Ar-*H*), 7.83 (2H, d, J = 8.2 Hz, Ar-*H*); δ_C (75 MHz, $CDCl_3$) 20.6, 21.6, 25.3, 25.8, 26.8 (*C*H$_3$), 62.1 (*C*H$_2$), 68.9 (*C*H$_2$OTs), 73.9 (*C*-3), 81.5 (*C*-4), 85.2 (*C*-2), 98.5 (O*C*O), 106.0 (*C*-1), 112.3 (O*C*O), 128.1, 129.9, 132.4, 145.1 (Ar-*C*).

1,2 : 3,5-Di-*O*-isopropylidene-4-(*R*)-*C*-(azidomethyl)-β-L-threo-pento-1,4-furanose (12). A mixture of **11** (1.2 g, 2.9 mmol), NaN_3 (1.13 g, 17.4 mmol), TBAI (0.015 g, cat) and MS (1 g) in anhydrous DMF was stirred at 110 °C for 48 h. The reaction mixture was cooled to room temperature. After adding water the reaction mixture was extracted with ethyl acetate. The organic layer was dried, concentrated and purified by chromatography (*n*-hexane–ethyl acetate = 19 : 1) to furnish azido compound **12** (0.512 g, 62%) as a pale yellow solid, mp 91–92 °C. Found: C, 50.59; H, 6.66. Calc. for $C_{12}H_{19}N_3O_5$ C, 50.52; H, 6.71); R_f 0.66 (33% ethyl acetate–*n*-hexane); $[\alpha]_D$ +2.1 (*c* 0.95, $CHCl_3$); ν_{max} (nujol) 2098 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.34 (3H, s, C*H*$_3$), 1.39 (3H, s, C*H*$_3$), 1.41 (3H, s, C*H*$_3$), 1.62 (3H, s, C*H*$_3$), 3.44 (1H, d, J = 12.6 Hz, CH_2N$_3$), 3.68 (2H, d, J = 12.6 Hz, CH_2N$_3$ and *O*-CH_2), 3.90 (1H, d, J = 12.6 Hz, *O*-CH_2), 4.15 (1H, s, *H*3), 4.63 (1H, d, J = 3.9 Hz, *H*2), 6.08 (1H, d, J = 3.9 Hz, *H*1); δ_C (75 MHz, $CDCl_3$) 21.2, 25.3, 26.2, 26.4 (*C*H$_3$), 53.2 (*C*H$_2$N$_3$), 63.0 (*O*-*C*H$_2$), 75.0, 84.5, 85.0 (*C*-2/*C*-3/*C*-4), 99.0 (O*C*O), 106.2 (*C*-1), 112.2 (O*C*O).

1,2 : 3,5-Di-*O*-isopropylidene-4-(*R*)-*C*-(*N*-benzoxycarbonylaminomethyl)-β-L-threo-pento-1,4-furanose (13). To an ice cooled suspension of LAH (0.319 g, 8.4 mmol) in dry THF (10 mL) was added a solution of **12** (0.4 g, 1.40 mmol) in dry THF (7 mL) over a period of 10 min. The reaction mixture was warmed to room temperature and stirred for 1.5 h. Ethyl acetate (10 mL) was added at 0 °C, stirred for 10 min and quenched with a saturated solution of NH_4Cl (2 mL). The solution was filtered, the residue washed with ethyl acetate (3 × 3 mL) and worked up. The organic layer was concentrated, dried and dissolved in ethanol–water (5 mL, 1 : 1) followed by the addition of $NaHCO_3$ (0.329 g, 3.92 mmol) at 0 °C and benzyl chloroformate (0.358 g, 2.1 mmol). The mixture was stirred at room temperature for 4 h, quenched with water and extracted with chloroform (3 × 10 mL). Work-up and chromatography (*n*-hexane–ethyl acetate = 25 : 1) provided **13** (0.485 g, 88%) as a solid, mp 84–85 °C. Found: C, 61.15; H, 7.00. Calc. for $C_{20}H_{27}NO_7$ C, 61.06; H, 6.92; R_f 0.63 (25% ethyl acetate–*n*-hexane); $[\alpha]_D$ −19.44 (*c* 0.72, $CHCl_3$); ν_{max} (KBr) 3360–3100 (br band), 1693 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.32 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.54 (3H, s, CH_3), 3.47 (1H, dd, *J* = 14.0 and 2.9 Hz, CH_2N$_3$), 3.64 (1H, d, *J* = 12.3 Hz, *O*-CH_2), 3.72 (1H, dd, *J* = 14.0 and 8.0 Hz, CH_2N$_3$), 3.78 (1H, d, *J* = 12.3 Hz, *O*-CH_2), 4.13 (1H, s, *H*3), 4.61 (1H, d, *J* = 3.8 Hz, *H*2), 5.14 (2H, d, *J* = 3.3 Hz, *O*-CH_2Ph), 5.26–5.29 (1H, br d, *J* = 8.0 Hz, N*H*, exchange with D_2O), 6.02 (1H, d, *J* = 3.8 Hz, *H*1), 7.30–7.45 (5H, m, Ar-*H*) [on D_2O exchange, the doublet of doublets at δ 3.47 and 3.72 became doublets with *J* = 14.0 Hz]; δ_C (75 MHz, $CDCl_3$) 21.2, 25.3, 26.1, 26.3 (*C*H_3), 44.0 (*N*-*C*H_2), 63.3 (*O*-*C*H_2Ph), 66.6 (*O*-*C*H_2), 75.3, 84.3, 85.2 (*C*-2/*C*-3/*C*-4), 98.9 (O*C*O), 105.8 (*C*-1), 112.1 (O*C*O), 127.9, 128.1, 128.3, 136.3 (Ar-*C*), 156.6 (*C*O).

(3*S*,4*R*,5*R*)-3,4,5-Trihydroxy-5-hydroxymethylpiperidine (4b). To a solution of TFA–water (4 mL, 3 : 2) was added **13** (0.39 g, 0.99 mmol) at 0 °C, which was then stirred for 30 min. The solution was allowed to warm to 25 °C and stirred for 2.5 h. TFA–water was co-evaporated with toluene under a high vacuum to provide an anomeric mixture of hemiacetal (0.304 g, 98%), which was directly used in the next reaction. To a solution of hemiacetal in dry methanol (7 mL) was added 10% Pd/C (0.08 g) and ammonium formate (0.489 g, 7.75 mmol). The reaction mixture was refluxed for 45 min and filtered through celite, washed with methanol and concentrated. The concentrated thick liquid was purified by a silica column using $CHCl_3$–MeOH–NH_3 (25% solution) = 50 : 49 : 1 as an eluant to yield **4b** (0.131 g, 83%) as a thick oil. Found: C, 44.22; H, 8.09. Calc. for $C_6H_{13}NO_4$ C, 44.16; H, 8.03; R_f 0.09 (50% MeOH–$CHCl_3$); $[\alpha]_D$ +33.68 (*c* 0.95, MeOH); ν_{max} (nujol) 3550–3200 (br band) cm^{-1}; δ_H (300 MHz, D_2O) 2.57 (1H, t, *J* = 12.0 Hz, *H*2a), 2.86 (1H, d, *J* = 13.5 Hz, *H*6a), 3.06 (1H, dd, *J* = 13.5 and 1.7 Hz, *H*6e), 3.24 (1H, ddd, *J* = 12.0, 4.9 and 1.7 Hz, *H*2e), 3.41 (1H, d, *J* = 9.4 Hz, *H*4), 3.49 (2H, AB quartet, *J* = 11.7 Hz, CH_2OH), 3.83 (1H, ddd, *J* = 12.0, 9.4 and 4.9 Hz, *H*3); δ_C (75 MHz, D_2O) 47.2, 49.2 (*C*-2/*C*-6), 63.0 (*C*H_2OH), 66.4, 72.5, 72.9 (*C*-3/*C*-4/*C*-5).

(3*S*,4*R*,5*R*)-3,4,5-Trihydroxy-5-hydroxymethylpiperidine hydrochloride (9b). To a stirred solution of compound **4b** (0.1 g, 0.613 mmol) in dry methanol was added HCl (0.1 mL) under nitrogen. The reaction mixture was stirred at 25 °C for 2 h and the solvent was evaporated under reduced pressure to give **9b** (0.12 g, 98%) as a semi-solid. Found: C, 36.12; H, 7.15. Calc. for $C_6H_{14}NO_4Cl$ C, 36.09; H, 7.07; R_f 0.02 (90% MeOH–$CHCl_3$); $[\alpha]_D$ +18.95 (*c* 0.95, MeOH); ν_{max} (nujol) 3600–3230 (br band) cm^{-1}; δ_H (300 MHz, D_2O) 2.71 (1H, t, *J* = 11.9 Hz, *H*2a), 3.02 (1H, d, *J* = 13.2 Hz, *H*6a), 3.20 (1H, dd, *J* = 13.2 and 1.7 Hz, *H*6e), 3.37 (1H, ddd, *J* = 11.9, 5.2 and 1.7 Hz, *H*2e), 3.45 (1H, d, *J* = 9.4 Hz, *H*4), 3.47 (1H, d, *J* = 11.7 Hz, CH_2OH), 3.56 (1H, d, *J* = 11.7 Hz, CH_2OH), 3.91 (1H, ddd, *J* = 11.9, 9.4 and 5.2 Hz, *H*3); δ_C (75 MHz, D_2O) 46.5, 48.9 (*C*-2/*C*-6), 62.7 (*C*H_2OH), 65.3, 71.9, 72.4 (*C*-3/*C*-4/*C*-5).

1,2-*O*-Isopropylidene-4-(*R*)-*C*-(iodomethyl)-5-azido-β-L-threo-pento-1,4-furanose (14). To a solution of **6** (2.0 g, 5.34 mmol) in anhydrous DMF (12 mL) was added NaN_3 (2.08 g, 32.0 mmol), TBAI (0.5 equiv) and MS (1.5 g) and the reaction mixture was stirred at 110 °C for 60 h. The reaction mixture was cooled to room temperature. After adding water the reaction mixture was extracted with ethyl acetate. The organic layer was dried, concentrated and purified by chromatography (*n*-hexane–ethyl acetate = 19 : 1) to furnish azido compound **14** (0.36 g, 19%) as a syrup. Found: C, 30.49; H, 4.01. Calc. for $C_9H_{14}N_3O_4I$ C, 30.44; H, 3.97; R_f 0.66 (25% ethyl acetate–*n*-hexane); $[\alpha]_D$ −49.33 (*c* 1.5, CH_2Cl_2); ν_{max} (neat) 3550–3250 (br band), 2104 and 1230 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.40 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.61–1.80 (1H, br s, O*H*, exchange with D_2O), 3.65 (2H, s, CH_2I), 4.42 (1H, d, *J* = 7.5 Hz, CH_2N$_3$), 4.67 (1H, d, *J* = 7.5 Hz, CH_2N$_3$), 4.74 (1H, d, *J* = 3.2 Hz, *H*2), 5.04 (1H, s, *H*3), 6.27 (1H, d, *J* = 3.2 Hz, *H*1); δ_C (75 MHz, $CDCl_3$) 26.6, 27.6 (*C*H_3), 53.0 (*C*H_2N$_3$), 79.4 (*C*H_2I), 84.2, 87.2 (*C*-2/*C*-3), 87.8 (*C*-4), 107.7 (*C*-1), 114.8 (O*C*O).

Further elution with *n*-hexane–ethyl acetate (4 : 1) provided the azido compound **7** (0.288 g, 22%) as a white solid, mp 80–81 °C.

1,2-*O*-Isopropylidene-4-(*R*)-*C*-(methyl)-5-(*N*-benzoxycarbonylamino)-β-L-threo-pento-1,4-furanose (15). A solution of **14** (0.35 g, 0.986 mmol) in THF (6 mL) was added to a suspension of LAH (0.224 g, 5.9 mmol) in THF over a period of 10 min at 0 °C. The reaction mixture was warmed to room temperature, stirred for 1.5 h and worked up as described for compound **7**. The residue was dissolved in ethanol–water (5 mL, 1 : 1) followed by the addition of $NaHCO_3$ (0.231 g, 2.75 mmol) at 0 °C and benzyl chloroformate (0.252 g, 1.477 mmol). The mixture was stirred at room temperature for 4 h, quenched with water and extracted with chloroform (3 × 10 mL). Work-up and chromatography (*n*-hexane–ethyl acetate = 5 : 1) provided **15** (0.296 g, 89%) as a thick oil. Found: C, 60.49; H, 6.89. Calc. for $C_{17}H_{23}NO_6$ C, 60.52; H, 6.87; R_f 0.55 (50% ethyl acetate–*n*-hexane); $[\alpha]_D$ −12.7 (*c* 1.1, $CHCl_3$); ν_{max} (neat) 3600–3190 (br band) and 1709 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.23 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.51 (3H, s, CH_3), 3.32–3.56 (2H, m, CH_2NH), 4.07 (1H, s, *H*3), 4.65 (1H, d, *J* = 4.0 Hz, *H*2), 5.14 (2H, br s, *O*-CH_2Ph), 5.30–5.40 (1H, br s, N*H*/O*H*, exchange with D_2O), 5.87 (1H, d, *J* = 4.0 Hz, *H*1), 7.31–7.40 (5H, m, Ar-*H*); δ_C (75 MHz, $CDCl_3$ + D_2O) 18.9, 26.2, 26.8 (CH_3), 46.3 (*N*-*C*H_2), 66.9, 87.5, 87.7 (*C*-2/*C*-3/*C*-4), 103.9 (*C*-1), 112.3 (O*C*O), 127.9, 128.1, 128.5, 136.3 (Ar-*C*), 157.1 (*C*O).

(3*S*,4*R*,5*S*)-3,4,5-Trihydroxy-5-methylpiperidine (4c). To a solution of TFA–water (4 mL, 3 : 2) was added **15** (0.26 g, 0.77 mmol) at 0 °C, which was then stirred for 30 min. The solution was allowed to warm to 25 °C and stirred for 2.5 h. TFA–water was co-evaporated with toluene under a high vacuum to provide an anomeric mixture of hemiacetal (0.224 g, 98%), which was directly used in the next reaction. To a solution of hemiacetal in dry methanol (8 mL) was added 10% Pd/C (0.08 g) and ammonium formate (0.38 g, 6.03 mmol). The reaction mixture was refluxed for 45 min and filtered through celite, washed with methanol and concentrated. The concentrated thick liquid was purified by a silica column using $CHCl_3$–MeOH–NH_3 (25% solution) = 55 : 44 : 1 as an eluant to yield **4c** (0.082 g, 72%) as a thick oil. Found: C, 45.02; H, 8.95. Calc. for $C_6H_{13}NO_3$ C, 48.97; H, 8.90; R_f 0.21 (50% MeOH–$CHCl_3$); $[\alpha]_D$ +10.67 (*c* 0.75, MeOH); ν_{max} (nujol) = 3540–3100 (br band) cm^{-1}; δ_H (300 MHz, D_2O) 1.33 (3H, s, CH_3), 2.85 (1H, t, *J* = 11.9 Hz, *H*2a), 3.10 (1H, d, *J* = 13.2 Hz, *H*6a), 3.26 (1H, dd, *J* = 13.2 and 1.8 Hz, *H*6e), 3.42 (1H, d, *J* = 9.4 Hz, *H*4), 3.48 (1H, ddd, *J* = 11.9, 5.3 and 1.8 Hz, *H*2e), 4.00 (1H, ddd, *J* = 5.3, 9.4 and 11.9 Hz, *H*3); δ_C (75 MHz, D_2O) 21.7 (*C*H_3), 46.7, 52.1 (*C*-2/*C*-6), 65.4 (*C*-5), 69.9, 75.9 (*C*-3/*C*-4).

General procedure for inhibition assay

Inhibition potencies of the isofagomine analogues **4a–c** were determined by measuring the residual hydrolytic activities of the glycosidases. The substrates (purchased from Sigma Chemicals Co. USA) namely *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, of 2 mM concentration were prepared in a 0.025 M citrate buffer at pH 6.0, *p*-nitrophenyl-α-D-mannopyranoside of 2 mM was prepared in a 0.025 M citrate buffer at pH 4.0. The test compound was preincubated with the respective enzyme buffered at its optimal pH, for 1 h at 25 °C. The enzyme reaction was initiated by the addition of 100 μL of substrate. Controls were run simultaneously in the absence of a test compound. The reaction was terminated at the end of 10 min by the addition of a 0.05 M borate buffer (pH 9.8) and absorbance of the liberated *p*-nitrophenol was measured at 405 nm using a Shimadzu Spectrophotometer UV-1601. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 μmole of *p*-nitrophenyl pyranoside per minute at 25 °C.[9] Inhibitory potency of the compounds against human salivary amylase (HSA) was estimated using soluble starch as a substrate.[10]

Acknowledgements

We thank the Department of Science and Technology (SP/S1/G-32/2000), New Delhi for financial support. MMM is grateful to the Indian Council for Cultural Relations (ICCR), New Delhi for a fellowship. The support of the University Grants Commission under CAS phase I (New Delhi) to procure a High Field NMR facility is gratefully acknowledged.

References

1 (*a*) T. Kajimoto, K. K. C. Liu, R. L. Pederson, Z. Zhong Jr., Y. Ichikawa, J. A. Porco and C. H. Wong, *J. Am. Chem. Soc.*, 1991, **113**, 6187; (*b*) Y. Xu and W. Zhou, *J. Chem. Soc., Perkin Trans. 1*, 1997, **1**, 741; (*c*) N. Asano, A. Kato, M. Miyauchi, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash and G. W. J. Fleet, *J. Nat. Prod.*, 1998, **61**, 625; (*d*) M. Bols, V. H. Lillelund, H. H. Jensen and X. Liang, *Chem. Rev.*, 2002, **102**, 515; (*e*) A. D. Elbein, *Annu. Rev. Biochem.*, 1987, **56**, 497; (*f*) A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob and T. W. Rademacher, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 9229; A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob and T. W. Rademacher, *FEBS Lett.*, 1988, **237**, 128; (*g*) G. W. J. Fleet, *Chem. Br.*, 1989, 287; (*h*) B. Winchester and G. W. J. Fleet, *Glycobiology*, 1992, **2**, 199; (*i*) Y. L. Merror, L. Poitout, J. Deepazy, I. Dosbaa, S. Geoffroy and M. Foglietti, *Bioorg. Med. Chem.*, 1997, **5**, 519; (*j*) A. E. Stuts, *Iminosugars as Glycosidase Inhibitors. Nojirimycin and Beyond*, Wiley-VCH, Weinheim, 1999; (*k*) T. D. Heightman and A. T. Vasella, *Angew. Chem., Int. Ed.*, 1999, **38**, 750; (*l*) M. Bols, V. H. Lillelund, H. H. Jensen and X. Liang, *Chem. Rev.*, 2002, **102**, 515 and references cited therein; (*m*) J. Y. Goujon, D. Gueyrard, C. Philippe, M. R. Olivier and N. Asano, *Tetrahedron: Asymmetry*, 2003, **14**, 1969.

2 (*a*) W. Dong, T. Jespersen, M. Bols, T. Skrydstrup and M. R. Sierks, *Biochemistry*, 1996, **35**, 2788; (*b*) T. M. Jespersen, W. Dong, M. R. Sierks, T. Skrydstrup, I. Lundt and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778; (*c*) T. M. Jespersen and M. Bols, *Tetrahedron*, 1994, **50**, 13449; (*d*) Y. Ichikawa and Y. Igarashi, *Tetrahedron Lett.*, 1995, **36**, 4585; (*e*) M. Bols, *Acc. Chem. Res.*, 1998, **31**, 1; (*f*) Y. Ichikawa, Y. Igarashi, M. Ichikawa and Y. Suhura, *J. Am. Chem. Soc.*, 1998, **120**, 5854; (*g*) S. J. Willams, R. Hoos and S. G. Withers, *J. Am. Chem. Soc.*, 2000, **122**, 2223; (*h*) Y. Nishimura, E. Shitara, H. Adachi, M. Toyoshima, M. Nakajima, Y. Okami and T. Takeuchi, *J. Org. Chem.*, 2000, **65**, 2; (*i*) G. Pandey, M. Kapur, M. I. Khan and S. M. Gaikwad, *Org. Biomol. Chem.*, 2003, **1**, 3321.

3 M. Ichikawa, Y. Igarashi and Y. Ichikawa, *Tetrahedron Lett.*, 1995, **36**, 1767. The isofagomine analogues **3b** and **4b** are enantiomers. The ^{1}H and ^{13}C NMR of the hydrochloride salt of **3b** is in consonance with **9b** (the hydrochloride salt of **4b**). The authors have not reported the specific rotation for **3b**·HCl.

4 (*a*) D. D. Dhavale, S. D. Markad, N. S. Karanjule and J. PrkashaReddy, *J. Org. Chem.*, 2004, **69**, 4760; (*b*) N. T. Patil, J. N. Tilekar and D. D. Dhavale, *J. Org. Chem.*, 2001, **66**, 1065 and references cited therein; (*c*) N. T. Patil, S. John, S. G. Sabharwal and D. D. Dhavale, *Bioorg. Med. Chem.*, 2002, **10**, 2155; (*d*) D. D. Dhavale, M. M. Matin, T. Sharma and S. G. Sabharwal, *Bioorg. Med. Chem.*, 2003, **11**, 3295; (*e*) D. D. Dhavale and M. M. Matin, *Tetrahedron*, 2004, **60**, 4275; (*f*) D. D. Dhavale, M. M. Matin, T. Sharma and S. G. Sabharwal, *Bioorg. Med. Chem.*, 2004, **12**, 4039.

5 We are thankful to Dr Ganesh Pandey for providing the spectral data of **3c**, which is different from that of compound **4b**.

6 An alternative explanation for this selectivity is the intramolecular hydrogen bonding of the C-3 oxygen atom with the hydrogen atom of the C-4β CH_2OH group, which makes this hydroxymethylene oxygen more nucleophilic for tosylation. We are grateful to one of the referees for this suggestion.

7 Mikael Bols and co-workers have reported that polyhydroxylated piperidines change their conformation with respect to hydrogen ion concentration, see: H. H. Jensen, L. Lyngbye, A. Jensen and M. Bols, *Chem. Eur. J.*, 2002, **8**, 1218.

8 (*a*) D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11; (*b*) T. D. Heightman and A. T. Vasella, *Angew. Chem., Int. Ed.*, 1999, **38**, 750; (*c*) T. M. Jespersen, W. Dong, M. R. Sierks, T. Skrydstrup, I. Lundt and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778.

9 L. Yu-The and L. Suc-Chen, in *Methods in Enzymology*, ed. Victor Ginsberg, Academic Press, 1972, vol. 28, part B, 702.

10 G. L. Miller, *Anal. Chem.*, 1972, **31**, 426.