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Synthesis of new six- and seven-membered 1-*N*-iminosugars as promising glycosidase inhibitors

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ABSTRACT

New six- and seven-membered 1-*N*-iminosugars were prepared from D-glucose by the stereoselective Michael addition of nitromethane to D-glucose derived α,β-unsaturated ester **A** followed by one pot reduction of nitro/ester functionality and subsequent amine protection to get *N*-Cbz protected aminol **6**. Hydrolysis of 1,2-acetonide and reductive aminocyclization gave seven membered 1-*N*-iminosugar **5b**. While, hydrolysis of 1,2-acetonide followed by $NaIO_4$ oxidative cleavage and hydrogenation using 10% $Pd(OH)_2$/C, H_2 gave six membered 1-*N*-iminosugar **4a**; the hydrogenation using 10% Pd/C–H_2 however, gave *N*-methyl substituted 1-*N*-iminosugar **4b**. The hydrochloride salts of **4a**/**4b** and **5b** were found to be specific α-galactosidase and moderate α-glucosidae inhibitors, respectively, in micro molar range.

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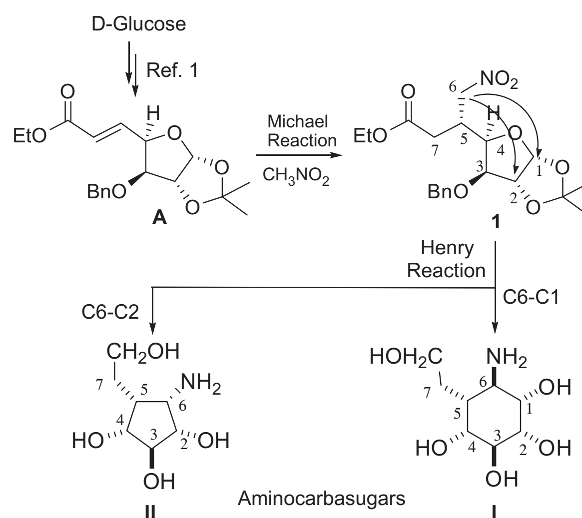
1. Introduction

Recently, we have reported a synthetic route[1] to the five- and six-membered aminocarbasugars **I** and **II**, respectively, using a chiral synthon **1** which is obtained by the stereoselective Michael addition of nitromethane to D-glucose derived α,β-unsaturated ester **A** followed by the intramolecular Henry reaction (after hydrolysis of 1,2-acetonide) as key steps (Scheme 1). In the continuation of our interest in iminosugars,[2] we anticipated that the Michael adduct **1** could be exploited for the preparation of new analogues of six- and seven-membered 1-*N*-iminosugars.

This class of compounds, also known as azasugars, are sugar mimics in which the ring oxygen of monosaccharide is replaced by the basic nitrogen atom.[3] Nojirimycin (NJ) **2a** (Fig. 1) and 1-deoxynojirimycin (DNJ) **2b** are the early studied examples of iminosugars which were found to be potential inhibitors of carbohydrate processing enzymes namely glycosidases and glycosyl transferases.[4] The study of iminosugars subsequently led to the discovery of Migliol™ (*N*-hydroxyethyl DNJ) and Zavesca,™ (*N*-butyl DNJ) an iminosugar based drugs, commercialized for the treatment of type II diabetes and Gaucher's disease, respectively.[5] In the search for more potent glycosidase inhibitors, a new class of iminosugars wherein an anomeric carbon of sugar is replaced by the basic nitrogen atom, known as 1-*N*-iminosugars (or 1-azasugars),[6] has emerged as potent and highly selective glycosidase inhibitors. Bols and co-workers reported the first molecule of this family and named as isofagomine **3a** which was found to be selective inhibitor of β-glucosidase (K_i = 0.11 μM, sweet almonds).[6a]



Scheme 1. Synthesis of aminocarbasugars.

Due to the pronounced and selective inhibition activities of 1-*N*-iminosugars, several other analogues with variation in the ring size along with position and the nature of hydroxy alkyl side chain have been prepared. For example, Takahata and co-workers[8a] as well as Mehta et al.[8b] reported the C5 hydroxyethyl side chain analogues of 1-*N*-iminosugars known as homoisofagomine **3b** and **3c**,

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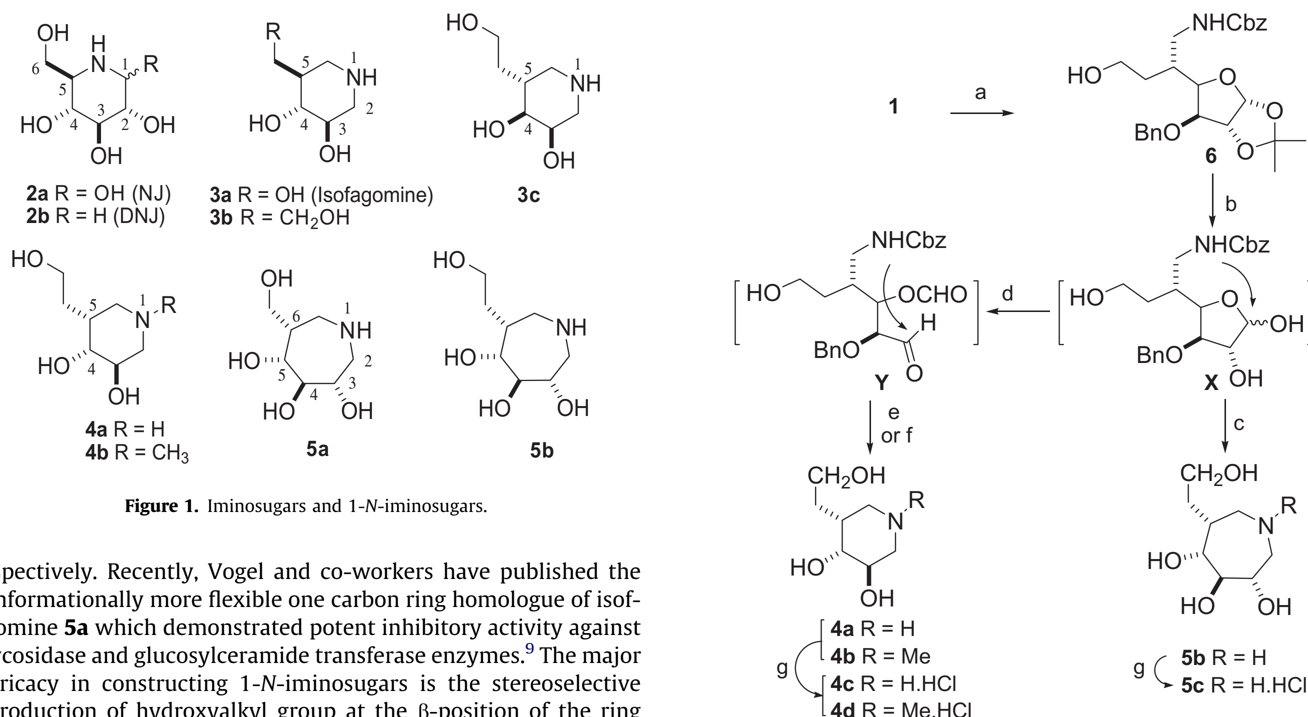

Figure 1. Iminosugars and 1-*N*-iminosugars.

Scheme 2. Reagents and conditions: (a) (i) $LiAlH_4$, THF, 0 °C to reflux, 10 h; (ii) Cbz–Cl, aq $NaHCO_3$, MeOH, 4 h, 65%; (b) TFA–water (3:2), rt, 2 h; (c) H_2, 10% $Pd/(OH)_2/C$, MeOH–H_2O, 80 psi, rt, 36 h, 80%; (d) $NaIO_4$, acetone–water, rt, 1 h; (e) H_2, 10% $Pd(OH)_2/C$, MeOH–H_2O, 80 psi, rt, 36 h, 75%; (f) H_2, 10% Pd/C, dry MeOH, 80 psi, 40 h 59%; (g) HCl, MeOH, rt, 4 h.

respectively. Recently, Vogel and co-workers have published the conformationally more flexible one carbon ring homologue of isofagomine **5a** which demonstrated potent inhibitory activity against glycosidase and glucosylceramide transferase enzymes.[9] The major intricacy in constructing 1-*N*-iminosugars is the stereoselective introduction of hydroxyalkyl group at the β-position of the ring nitrogen. Despite this difficulty, a number of asymmetric as well as chiron approaches to a variety of 1-*N*-iminosugars are reported in the literature.[7–10] We now report here synthesis of six- and seven-membered 1-*N*-iminosugars with elongated hydroxyethyl side chain at C5 **4a**/**4b** and **5b,** respectively, and glycosidase inhibitory activity of their hydrochloride salts.

2. Result and discussion

The requisite sugar appended γ-nitroester **1** with L-*ido*-configuration was prepared in high stereoselective manner from D-glucose in six steps as described earlier.[1] In the subsequent step, one-pot reduction of nitro and ester functionality in **1** using LAH in THF at reflux followed by reaction with benzylchloroformate and $NaHCO_3$ in methanol/water afforded *N*-Cbz protected aminol **6** in 65% yield (Scheme 2).

In the next step, treatment of **6** with TFA–water (3:2) at room temperature gave anomeric mixture of hemiacetal **X** (as evident from the ^{1}H NMR of the crude product) that was directly subjected for the reductive aminocyclization under hydrogenation conditions using catalytic 10% $Pd(OH)_2/C$ in methanol to afford seven membered 1-*N*-iminosugar **5b** in 80% yield. Treatment of **5b** with MeOH–HCl afforded hydrochloride salt **5c.** While targeting the synthesis of six membered 1-*N*-iminosugar **4a**, the hemiacetal **X** was treated with sodium metaperiodate in acetone–water to furnish intermediate **Y** that on reaction with 10% $Pd(OH)_2/C$ in methanol/water under hydrogen pressure afforded 5-*epi*-homoisofagomine **4a** in 75% yield. Reaction of **4a** with MeOH–HCl gave hydrochloride salt **4c**. An interesting observation was noticed when hydrogenation of **Y** was performed with 10% Pd/C in dry methanol. After 40 h N-methylation of 5-*epi*-homoisofagomine was observed to afford *N*-methyl-5-*epi*-homoisofagimine **4b** as the only product in 59% yield,[11] that was converted to the hydrochloride salt **4d** by treatment with MeOH–HCl.

2.1. Inhibitory activity evaluation

The glycosidase inhibitory activity of hydrochloride salt **4c**, **4d** and **5c** was evaluated against following glycosidases: β-galactosidase and β-glucosidase (isolated from almond seeds), α-galactosidase (isolated from *Geobacillus toebii* BK1), α-mannosidase and *N*-acetyl-β-D-glucosaminidase (isolated from Jack bean seeds) and α-glucosidase (procured from Sigma Chemical Co). The IC_{50} and K_i values obtained are summarised in Table 1. The isofagomine analogues **4c** and **4d** were found to be specific competitive inhibitors of α-galactosidase with K_i = 66 μM and K_i = 54 μM, respectively. However, **4c** and **4d** showed weak inhibition against α-glucosidase with 30% and 44% inhibition, respectively. On the other hand seven membered iminosugar **5c** was noticed to be moderate competitive inhibitor of α-glucosidase with K_i = 131 μM. Comparison of K_i and IC_{50} values of **4c** and **4d** suggested **4d** is more potent inhibitor of α-galactosidase under the assay condition. This may be attributed to the *N*-alkylation of **4c**.

Conclusion

We have demonstrated a short and an efficient methodology for the synthesis of six- and seven-membered 1-*N*-iminosugars **4a**, **4b**, and **5b** and corresponding hydrochloride salts **4c**, **4d**, and **5c**. Glycosidase inhibitory activity study indicated that compounds **4c** and **4d** have higher inhibitory activity against α-galactosidase, whereas α-glucosidase was inhibited moderately by **5c**. The increased potency of alkyl derivative **4d** towards α-galactosidase encourage further research on biological studies of modified alkyl amino derivatives of **4d**.

3. Experimental

3.1. General methods

Melting points were recorded with 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

Table 1
Inhibitory potencies of **4c**, **4d** and **5c**

Enzymes	**4c**		**4d**		**5c**	
	IC_{50}[a] (μM)	K_i[b] (μM)	IC_{50}[a] (μM)	K_i[b] (μM)	IC_{50}[a] (μM)	K_i[b] (μM)
α-Galactosidase (*Geobacillus toebii* BK 1)	65	66	50	54	NI	NI
β-Galactosidase (Almond Seeds)	NI	NI	NI	NI	NI	NI
α-Glucosidase (Baker's yeast)	NI	NI	NI	NI	675	131
β-Glucosidase (Almond Seeds)	NI	NI	NI	NI	NI	NI
α-Mannosidases (Jack bean)	NI	NI	NI	NI	NI	NI
N-Acetyl-β-D-glucosaminidase (Jack bean)	NI	NI	NI	NI	NI	NI

NI: no inhibition.
[a] Less than 50% inhibition at 1 mM concentration of inhibitor.
[b] No inhibition at 1 mM concentration of inhibitor. Data is average of three sets of assay performed.

KBr pellets and are expressed in cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with Varian Mercury instrument using $CDCl_3$ and/or D_2O as solvent(s). Chemical shifts were reported in δ unit (parts per million) with reference to TMS as an internal standard and *J* values are given in Hz. Elemental analysis were carried out with Thermo-Electron Corporation CHNS analyzer Flash-EA 1112 at Department of Chemistry, University of Pune, Pune. Optical rotations were measured using JASCO P1020 digital polarimeter with sodium light (589.3 nm) at 25 C. Thin-layer chromatography was performed on pre-coated plates (0.25 mm, Silica gel 60 F254). Visualization was made by absorption of UV light or by thermal development after spraying with 3.5% solution of 2,4-dinitrophenylhydrazine in ethanol/H_2SO_4 and with basic aqueous potassium permanganate solution. Column chromatography was carried out with silica gel (100–200 mesh). Unless not mention the reactions were carried out in an oven-dried glassware's under dry N_2. Acetone, Methanol, DMF, and THF were purified and dried before use. Distilled ethyl acetate, CH_2Cl_2, and methanol were used for column chromatography. Petroleum ether that was used is a distillation fraction between 40 and 60 °C. After decomposition of the reaction with water, work-up involves washing of combined organic layer with water, brine, drying over anhydrous sodium sulfate, and evaporation of solvent under reduced pressure.

3.1.1. 13-*O*-Benzyl-5-(*N*-benzyloxyccarbonyl)-aminomethyl-5,6-dideoxy-1,2-*O*-isopropylidine-β-L-*ido*-hepto-1,4-furanose (6)

To an ice-cold suspension of LAH (1.90 g, 50.07 mmol) in anhydrous THF (20 mL), a solution of **1** (4.1 g, 10.02 mmol) in THF (15 mL) was added over 10 min. The resulting mixture was stirred at room temperature for 15 min and then refluxed. After 10 h, the reaction mixture was cooled to room temperature and quenched with ethyl acetate (20 mL) followed by saturated solution of aq ammonium chloride. The residue was filtered through Celite, solvent was dried (Na_2SO_4) and concentrated to afford viscous oil. To the above crude product in methanol–water (9:1) was added sodium bicarbonate (2.25 g, 30.0 mmol) and benzylchloroformate (50% solution in toluene) (5.1 mL, 15.0 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 4 h. Methanol was removed on rotary evaporator under reduced pressure and the product was extracted with EtOAc (3 × 40 mL). The combined organic layer was dried (Na_2SO_4) and concentrated. Column purification of crude product using pet ether/ethyl acetate = 4:1 gave **6** as a viscous oil (3.07 g, 65%). R_f 0.42 (ethyl acetate/pet ether = 1:4); $[\alpha]_D^{25}$ −35.4 (*c* 4.8, $CHCl_3$); IR (neat): 3650–3200 (broad), 2982, 1712, 1518, 1255, 1074, 1024 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22–1.44 (5H, m, CH_3, *H*-6), 1.48 (3H, s, CH_3), 2.12–2.25 (1H, m, *H*-5), 2.45 (1H, br s, –O*H*, exchangeable with D_2O), 3.30 (1H, dt, *J* = 11.8 and 5.9 Hz, CH_2NH), 3.46–3.59 (1H, m, CH_2NH), 3.62–3.78 (2H, m, *H*-7), 3.87 (1H, d, *J* = 3.0 Hz, *H*-3), 3.95 (1H, dd, *J* = 10.0 and 3.0 Hz, *H*-4), 4.45 (1H, d, *J* = 11.7 Hz, OCH_2Ph), 4.62 (1H, d, *J* = 3.8 Hz, *H*-2), 4.69 (1H, d, *J* = 11.7 Hz, OCH_2Ph), 5.07 (2H, ABq, *J* = 12.3 Hz, NCO_2CH_2Ph), 5.51 (1H, b t, *J* = 5.8 Hz, N*H*CO, exchangeable with D_2O), 5.90 (1H, d, *J* = 3.8 Hz, *H*-1), 7.20–7.42 (10H, m, Ar-*H*); ^{13}C NMR ($CDCl_3$) 25.9 (CH_3), 26.5 (CH_3), 31.1 (*C*-6), 35.1 (*C*-5), 42.1 (CH_2NH), 60.4 (*C*-7), 66.5 (OCH_2Ph), 71.7 (NCO_2CH_2Ph), 81.1, 81.3, 83.0 (*C*-4/*C*-3/*C*-2), 104.5 (*C*-1), 111.3 (OCO), 127.7, 127.9 (strong), 128.0 (strong), 128.3, 128.4 (strong), 136.5, 136.9 (Ar), 157.1 (*C*O). Anal. Calcd. for $C_{26}H_{33}NO_7$: C 66.22, H 7.05. Found C 66.15, H 6.99.

3.1.2. 2(3*R*,4*R*,5*R*)-3, 4-Dihydroxy-5-(hydroxyethyl) piperidine (4a) and its hydrochloride salt (4c)

Solution of **6** (0.4 g, 0.845 mmol) in TFA–H_2O (4 mL, 3:2) was stirred at 0 °C for 20 min and at room temperature for 2 h. TFA was co-evaporated with toluene to furnish hemiacetal. To the stirred solution of hemiacetal in acetone–water (6 mL, 4:1) was added $NaIO_4$ (0.271 g, 1.268 mmol) at 0 °C. The resulting mixture was stirred at 15 °C for 1 h. Acetone was removed on rotary evaporater at 20 °C and product was extracted with dichloromethane CH_2Cl_2 (3 × 20 mL). The combined organic layer was concentrated, dried (Na_2SO_4) and purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:1) to afforded viscous oil. The crude product was dissolved in methanol/water (9:1) and hydrogenated at 80 psi using 10% $Pd(OH)_2/C$ at 30 °C for 36 h. The reaction mixture was filtered through Celite, concentrated and purified using column chromatography (Methanol/Chloroform, 1:4) to afford **4a** as viscous oil (0.102 g, 75%) R_f 0.20 (1%, NH_4OH/Methanol); $[\alpha]_D^{22}$ 15.5 (*c* 0.18, MeOH); IR: 3600–2900 (broad) cm^{-1}; ^1H NMR (D_2O): δ 1.48–1.61 (m, 1H, *H*-6), 1.62–1.78 (1H, m, *H*-6), 2.28–2.42 (1H, m, *H*-4*a*), 2.93 (1H, t, *J* = 12.3 Hz, *H*-5*a*), 3.08–3.36 (3H, m, *H*-1*a*, *H*-1*e*, *H*-5*e*), 3.67 (2H, t, *J* = 6.6 Hz, *H*-7), 3.87 (1H, br s, *H*-3), 4.03 (1H, br s, *H*-2); ^{13}C NMR (D_2O): δ 29.9 (*C*-6), 30.2 (*C*-4), 43.1, 44.5 (*C*-1/*C*-5), 58.6 (*C*-7), 65.9 (*C*-2), 67.4 (*C*-3). Anal. calcd. for $C_7H_{15}NO_3$: C 52.16, H 9.38; Found C 52.20, H 9.31. Solution of **4a** (0.04 g, 0.248 mmol) in methanol/HCl (2 mL, 0.5 M) was stirred at 25 °C for 4 h. The solvent was evaporated under reduced pressure and the residue was washed with ethyl acetate, dry ether and dried under vacuum to get **4c** (0.04 g, 81% yield) as a semisolid. R_f 0.12 (1%, NH_4OH/methanol) (elongated tail observed on TLC plate); $[\alpha]_D^{23}$ −5.4 (*c* 0.2, MeOH); IR: 3600–2900 (broad) cm^{-1}; ^1H NMR (300 MHz, D_2O); δ 1.48–1.62 (1H, m, *H*-6), 1.62–1.78 (1H, m, *H*-6), 2.28–2.44 (1H, m, *H*-4*a*), 2.95 (1H, t, *J* = 12.3 Hz, *H*-5*a*), 3.12–3.38 (3H, m, *H*-1*a*, *H*-1*e*, *H*-5*e*), 3.67 (2H, t, *J* = 6.6 Hz, *H*-7), 3.88 (1H, br s, *H*-3), 4.04 (1H, br s, *H*-2); ^{13}C NMR (D_2O): δ 29.3 (*C*-6), 30.3 (*C*-4), 42.7, 44.5 (*C*-1/*C*-5), 58.5 (*C*-7), 65.5 (*C*-2), 66.5 (*C*-3). Anal. calcd. for $C_7H_{16}ClNO_3$: C 42.54, H 8.16; Found C 42.39, H 8.02.

3.1.3. 31,5,6-Trideoxy-5-hydroxyethyl-1-6-imino-(2*S*,3*R*,4*R*,5*R*)-D-glucitol (5b) and its hydrochloride salt (5c)

Reaction of **6** (0.198 g, 0.421 mmol) with TFA–H_2O (3 mL, 3:2) at 0 °C gave hemiacetal as a viscous oil. That on hydrogenation in

methanol using 10% $Pd(OH)_2$/C (0.060 g) at 80 psi using at 30 °C for 36 h. Work up of reaction afforded **5b** as viscous oil (0.064 g, 80%).

R_f 0.15 (1%, NH_4OH /Methanol); $[\alpha]_D^{27}$ −3.3593 (*c* 0.16, MeOH); IR (neat): 3650–2900 (broad) cm^{-1}; ^1H NMR (D_2O) δ 1.52–1.84 (2H, m, *H*-7), 2.17–2.32 (1H, m, *H*-5), 2.89 (1H, dd, *J* = 13.6 3.9 Hz, H-1a) 3.02–3.28 (3H, m, *H*-1*e*, *H*-6), 3.69 (2H, t, *J* = 6.6 Hz, *H*-8), 3.82–4.02 (3H, m, *H*-2, *H*-3, *H*-4); ^{13}C NMR (D_2O) δ 33.1 (*C*-7), 33.7 (*C*-5), 45.9, 47.5 (*C*-1/*C*-6), 59.5 (*C*-8), 72.1, 75.1, 76.3 (*C*-2)/(*C*-3)/(*C*-4). Reaction of compound **5b** (0.043 g, 0.225 mmol) with methanol–HCl (2 mL, 0.5 M) gave hydrochloride salt (following the same procedure as **4c)** as semisolid **5c** (0.047 g, 93% yield): R_f 0.11 (1% NH_4OH/methanol); $[\alpha]_D^{22}$ −16.5 (*c* 0.3, MeOH); IR (neat): 3650–2900 (broad) cm^{-1}; ^1H NMR (D_2O) δ 1.60–1.82 (2H, m, *H*-7), 2.36–2.49 (1H, m, *H*-5), 3.21 (1H, dd, *J* = 13.2, 4.7 Hz, H-6) 3.25–3.48 (3H, m, *H*-1*a*, *H*-1*e*, *H*-6), 3.68 (2H, t, *J* = 6.5 Hz, *H*-8), 3.94–4.04 (2H, narrow m, *H*-3, *H*-4), 4.40–4.53 (1H, m, *H*-2); ^{13}C NMR (D_2O) δ 30.2 (*C*-7), 33.1 (*C*-5), 44.9, 45.0 (*C*-1/*C*-6), 58.9 (*C*-8), 69.8, 73.9, 74.2 (*C*-2)/(*C*-3)/(*C*-4).

3.1.4. 4(3*R*,4R,5R)-3,4-Dihydroxy-5-(hydroxyethyl)-*N*-methylpiperidine (4b) and its hydrochloride salt (4d)

Reaction of **6** (0.164 g, 0.348 mmol) with TFA–H_2O (3 mL, 3:2) followed by treatment with $NaIO_4$ (0.112 g, 0.522 mmol). Following the same reaction procedure as in **4a**, afforded intermediate **Y**. That on hydrogenation in dry methanol at 80 psi using 10% Pd-C (0.050 g) at 30 °C for 40 h and work up afforded **4b** as a viscous oil (0.036 g, 59%). Treatment of **4b** (0.036 g, 0.205 mmol) with methanol–HCl (2 mL, 0.5 M) as in case of **4a**, afforded hydrochloride salt **4d** (0.038 g, 89% yield) as a semisolid. R_f 0.22 (1%, NH_4OH /methanol) (elongated tail observed on TLC plate); $[\alpha]_D^{22}$ 6.5 (*c* 1, MeOH); IR (neat): 3650–2900 (broad) cm^{-1}; ^1H NMR (D_2O): δ 1.47–1.62 (1H, m, *H*-6), 1.63–1.78 (1H, m, *H*-6), 2.36–2.48 (1H, m, *H*-4*a*), 2.86 (3H, s, NCH_3), 2.96 (1H, t, *J* = 12.6 Hz, *H*-5*a*), 3.25 (1H, dd, *J* = 12.6, 3.9 Hz, *H*-5*e*), 3.32 (2H, br s, *H*-1*a*, *H*-1*e*, accidental equivalence), 3.68 (2H, t, *J* = 6.5 Hz, *H*-7), 3.85 (1H, apparent triplet, *J* = 2.7 Hz, *H*-5a), 4.03–4.12 (1H, narrow multiplet, *J* = 1.7 Hz, *H*-2e); ^{13}C NMR (D_2O): δ 29.9, 30.1 (*C*-6)/(*C*-4), 43.2 (NCH_3), 53.1 (*C*-5) 54.1 (*C*-1), 58.2 (*C*-7), 65.2 (*C*-2), 66.2 (*C*-3). Anal. calcd. for $C_8H_{18}ClNO_3$: C 45.39, H 8.57, Found C 45.36, H 8.62.

3.2. General procedure for inhibition assay

The substrates *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside and *p*-nitrophenyl-α-D-mannopyranoside were procured from Sigma Chemicals Co., USA.

Inhibition activities of 5-*epi*-homoisofagomine hydrochloride **4c**, *N*-methyl-5-*epi*-homoisofagomine hydrochloride **4d** and trihydroxyazepane hydrochloride **5c** were determined by measuring the residual hydrolytic activities of the glycosidases with 2 mM concentration of *p*-nitrophenyl-glycopyranoside prepared in citrate buffer (0.025 M, pH 4.0) and used for assay. The test compound (of various concentrations from 10 μM to 1000 μM) was pre-incubated with the enzyme, buffered at its optimal pH, for 1 h at 37 °C (for α-galactosidase at 60 °C). The enzyme reaction was initiated by the addition of 100 μL of substrate. Reaction was terminated with 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 UV–visible Spectrophotometer. Controls were run simultaneously in the absence of test compound. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 μmol of *p*-nitrophenol per minute under assay condition. The inhibition constants (K_i) and the nature of the inhibition were determined from Lineweaver Burk plots.

Acknowledgments

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Supplementary data

Supplementary data (general experimental methods. ^1H and ^{13}C NMR spectra of compounds **6**, **4a**, **4c**, **4d**, **5b**, and **5c**. Lineweaver–Burk plot of **4c**, **4d**, with α-galactosidase and **5c** with α-glucosidase) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.059.

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11. In ^{13}C NMR spectrum of compound **4d** along with N–CH_3 signal at δ 45.8 Hz, the C2 and C6 (–N–CH_2) appeared at δ 56.7 and 55.6 Hz as a downfield signals compared to the corresponding piperidine **4c**. This is due to the β-substituent (N-substitution) effect.