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# $\gamma$ -Hydroxyethyl piperidine iminosugar and N-alkylated derivatives: A study of their activity as glycosidase inhibitors and as immunosuppressive agents



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#### ABSTRACT

An efficient and practical strategy for the synthesis of (3R,4s,5S)-4-(2-hydroxyethyl) piperidine-3,4,5-triol and its *N*-alkyl derivatives **8a–f**, starting from the *D*-glucose, is reported. The chiral pool methodology involves preparation of the C-3-allyl- $\alpha$ -*D*-ribofuranodialdose 10, which was converted to the C-5-amino derivative **11** by reductive amination. The presence of C-3-allyl group gives an easy access to the requisite hydroxyethyl substituted compound **13**. Intramolecular reductive aminocyclization of C-5 amino group with C-1 aldehyde provided the  $\gamma$ -hydroxyethyl substituted piperidine iminosugar **8a** that was *N*-alkylated to get *N*-alkyl derivatives **8b–f**. Iminosugars **8a–f** were screened against glycosidase enzymes. Amongst synthetic *N*-alkylated iminosugars, **8b** and **8c** were found to be  $\alpha$ -galactosidase inhibitors while **8d** and **8e** were selective and moderate  $\alpha$ -mannosidase inhibitors. In addition, immunomodulatory activity of compounds **8a–f** was examined. These results were substantiated by molecular docking studies using AUTODOCK 4.2 programme.

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# 1. Introduction

Hydrolysis of the glycosidic bond, commonly called as glycosidase process, assists wide range of metabolic processes such as digestion, lysosomal glycoconjugate catabolism and glycoprotein biosynthesis degradation within the endoplasmic reticulum.<sup>1</sup> Abnormalities caused in these processes result in several pathological complications and disorders. For example, the  $\alpha$ -mannosidase is important for regulating the biosynthesis of several glycoproteins. as, it controls the N-glycosylation pathway of oligosaccharides, that are partially trimmed from Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> moieties involved in *N*-linked glycoprotein biosynthesis,<sup>2</sup> which plays a pivotal role in cancer metastasis, carbohydrate metabolic disorders, viral infection and immune response.<sup>3,4</sup> Whereas, the  $\alpha$ -glucosidase reduces postprandial hyperglycemia in the treatment of type II diabetic patients<sup>4</sup> and  $\alpha$ -galactosidase regulates lysosomal glycoconjugate catabolism and any disorder in this process or mutation in enzyme leads to lysosomal storage disorder (LSD)<sup>5</sup> and Fabry's disease.<sup>6</sup> The malfunctioning in all these processes is controlled

\* Corresponding author. *E-mail address:* ddd@chem.unipune.ac.in (D.D. Dhavale). by the use of competitive/non-competitive inhibitors. In this aspect, different analogues of iminosugars<sup>7</sup> were synthesized as selective and potent inhibitors of mannosidase, glucosidase and galactosidase enzymes. Among piperidine iminosugars, the Nalkylated iminosugars demonstrate higher and selective inhibitory activity compared to their parent iminosugars.<sup>8</sup> Asano et al. showed that the N-alkylation of deoxynojirimycin (DNJ) induces a shift in specific inhibition of glucosidases from  $\alpha$ -glucosidase II to  $\alpha$ -glucosidase I and in cell culture, the N-alkylated derivatives of DNI were found to be more effective against  $\alpha$ -glucosidase I than the DNJ.<sup>8a</sup> This fact led to the discovery of *N*-hydroxyethyl DNJ (**1a**; Fig. 1) and N-butyl DNJ 1b as promising clinical candidates for the treatment of type II diabetes and Gaucher disease, respectively. Apart from *N*-alkylation, other type of modification that has been sought is the variation of position and stereochemistry for the hydroxyalkyl substituent in the six-membered iminosugar.<sup>9</sup> This modification resulted into isofagomine 2 and related hydroxyalkyl substituted iminosugars.<sup>10</sup> The bioactivity data of these compounds indicated that the position and orientation of the hydroxyalkyl group in the piperidine iminosugar affects the efficiency and selectivity of glycosidases.<sup>6</sup> For example, isofagomine **2a** is a better inhibitor of  $\beta$ -glucosidases<sup>9k</sup> however, it's (5S)-hydroxy substituted



Figure 1. Piperidine iminosugars and related compounds.

analogue **2b** is a better inhibitor towards both  $\alpha$ -and  $\beta$ -glucosidases while; (5*R*)-hydroxy isofagomine 2c is a mild  $\beta$ -mannosidase inhibitor.<sup>7c,15d</sup> We have reported earlier the synthesis of piperidine **4** with the hydroxyethyl group at the  $\gamma$ -position of piperidine, which was found to be a selective and potent inhibitor of  $\beta$ -glucosidase.<sup>11</sup> Other analogues such as **5a** and **5b**, with  $\alpha$ -gem-dihydroxymethyl substitution, were found to be selective inhibitors of  $\alpha$ -glucosidases in the nanomolar range<sup>12</sup> while; introduction of the hydroxyethyl group at the  $\beta$ -position of the piperidine iminosugar **3** resulted in the loss of activity with very weak β-galactosidase inhibition. In addition to the glycosidase inhibition study. iminosugars have been evaluated as immunomodulatory agents. Wang et al. reported hydroxyalkyl- and N-alkyl iminosugars such as **6**, **7** as potent immunosuppressive agents.<sup>13</sup> A report from Our group demonstrated that the indolizidine iminosugars are also acting as immunomodulating agents.<sup>14</sup>

Encouraged by these results and as a part of our continuing efforts in this area,<sup>15</sup> we report herein the synthesis of (3*R*,4*s*,5*S*)-4-(2-hydroxyethyl) piperidine-3,4,5-triol **8a** and of a series of *N*-alkyl derivatives **8b–f** and the evaluation of their glycosidase inhibition as well as immunosuppressive activity. Amongst these iminosugars, compounds **8b** and **8c** showed  $\alpha$ -galactosidase inhibition while **8d** and **8e** showed selective and moderate inhibitior of the IgG secretion by CpG oligonucleotide stimulated B-cells.

# 2. Result and discussion

As shown in Scheme 1, the synthesis of target molecules started from aldehyde **10** which was derived from p-glucose in five steps as reported by us.<sup>15a</sup> In the subsequent steps, the reductive amination of C-5 aldehyde group in compound **10** with benzylamine and sodium cyanoborohydride in methanol followed by reaction with benzyloxycarbonyl chloride and sodium bicarbonate in methanol–water afforded the *N*-Cbz protected compound **11**. Dihydroxylation of **11** with catalytic amount of K<sub>2</sub>OsO<sub>4</sub>·2H<sub>2</sub>O (5 mol %) and



(a) (i) BnNH<sub>2</sub>, MeOH, NaCNBH<sub>4</sub>, -20 °C to rt (ii) CBz-CI, NaHCO<sub>3</sub> MeOH:H<sub>2</sub>O, rt, 85 % two steps (b) (i) K<sub>2</sub>OsO<sub>4</sub> .2H<sub>2</sub>O, NMO, Acetone:H<sub>2</sub>O, 0 °C to rt (ii) NaIO<sub>4</sub>, Acetone:H<sub>2</sub>O,0 °C to rt, 79 % (c) NaBH<sub>4</sub>,THF:H<sub>2</sub>O,0 °C to rt, 87 % (d) Ac<sub>2</sub>O, Pyridine,0 °C to rt, 94 % (e) (i)TFA:H<sub>2</sub>O, 0 °C to rt, (ii) Pd / C, H<sub>2</sub>, 200 psi, rt, 81 % (iii) R-Br, K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C.

Scheme 1. Synthesis of iminosugars 8a-f.

NMO gave triol that was directly subjected to oxidative cleavage using sodium metaperiodate to give aldehyde 12. Reduction of 12 using sodium borohydride in THF-water afforded diol 13 that on selective protection of primary hydroxy group, using acetic anhydride in pyridine, gave acetyl derivative **14**. In the next steps, removal of the 1,2 acetonide group using TFA-water afforded hemiacetal (as evident from the <sup>1</sup>H NMR of the crude product) that on reductive aminocyclization using 10% Pd/C in methanol under hydrogen pressure afforded 3,4,5 trihydroxy-4-hydroxyethyl piperidine 8a as a white solid. This one pot four step process involves hydrogenolysis of N-benzyl and N-Cbz groups to give primary amine that concomitantly underwent reductive aminocyclization with C-1 aldehyde to give six membered cyclic iminium ion which was in situ reduced to afford piperidine iminosugar. In the same process, we noticed that the de-acetylation of primary acetate group took place to give iminosugar 8a in overall 44% yield from **10**.<sup>16</sup> The high yields at each step allowed us to provide **8a** in good amount.

Having iminosugar **8a** in hand, *N*-alkylation was performed using appropriate alkyl bromides in DMF-K<sub>2</sub>CO<sub>3</sub> as reported earlier in the literature.<sup>17</sup> Thus, reaction of **8a**, separately with *n*-butyl, *n*hexyl, *n*-octyl, *n*-decyl, and hydroxyethyl bromide using K<sub>2</sub>CO<sub>3</sub> in DMF at 80 °C afforded corresponding *N*-alkylated iminosugars **8b–f** in good yields (60–70%).

# 2.1. Conformational assignments of 8a-f

An axial/equatorial orientation of the hydroxyalkyl substituent in the six membered piperidine iminosugars is the decisive factor for either  ${}^{4}C_{1}$  or  ${}^{1}C_{4}$  conformation<sup>9,18</sup> that plays a crucial role in the binding properties of iminosugars with different enzymes and thus affecting the inhibitory activity.<sup>6,19</sup> Therefore, conformations of **8a–f** were determined using the <sup>1</sup>H NMR data based on the coupling constant values between the H-2 and H-3 protons. In case of parent compound **8a**, due to the presence of the plane of symmetry, the C-2/C-6 axial and equatorial protons and C-3/C-5 protons were found to be magnetically equivalent. This led to the five sets of protons in the <sup>1</sup>H NMR spectra. The C-2/C-6 axial protons were appeared as an apparent triplet with large coupling constant of 10.7 Hz ( $J_{2a,2e} = J_{2a,3a} = 10.7$  Hz). In analogy with this, the H-3/H-5 appeared as a doublet of doublet (dd) with coupling constant of 10.7 and 4.5 Hz ( $J_{2a,3a} = 10.7$  Hz and  $J_{2e,3a} = 4.5$ ). This requires an axial orientation of the H-3/H-5 confirming the  ${}^{5}C_{2}$  conformation of the compound as shown in Figure 2. An analogous behaviour was noticed for *N*-alkylated iminosugars **8b–f** (Table 1), wherein H-3/H-5 appeared as a dd with large coupling constant of 10–11 Hz and equatorial coupling constant of 4–5 Hz indicating their  ${}^{5}C_{2}$  conformation (Fig. 2).

# 2.2. Biological activity

#### 2.2.1. Glycosidase inhibition

Glycosidase inhibitory activity of 8a-f was studied against  $\alpha$ -glucosidase (E.C. 3.2.1.20),  $\alpha$ -galactosidase (E.C. 3.2.1.22) and  $\alpha$ -mannosidase (E.C. 3.2.1.24) with reference to known standard *N*-hydroxyethyl DNJ (trade name Miglitol). The IC<sub>50</sub> values are summarized in Table 2. The parent iminosugar 8a showed no inhibition of  $\alpha$ -galactosidase but found to be a weak inhibitor of  $\alpha$ -glucosidase and  $\alpha$ -mannosidase while; compounds **8b** and **8c** in which the ring nitrogen is substituted with the *n*-butyl and *n*-hexyl groups, respectively, showed good inhibition of  $\alpha$ -galactosidase and  $\alpha$ -mannosidase as compared to miglitol. Among these compounds, **8b** was found to be a better  $\alpha$ -galactosidase inhibitor than 8c while compound 8c was noticed to be better inhibitor of  $\alpha$ -mannosidase than **8b**. The *n*-octyl **8d** and *n*-decyl **8e** nitrogen derivatives were found to be selective and moderate inhibitor of  $\alpha\text{-mannosidase}$  showing IC\_{50} values of 143  $\mu\text{M}$  and 112  $\mu\text{M},$ respectively. The N-hydroxyethyl substituted compound 8f (miglitol derivative) showed weaker  $\alpha$ -glucosidase activity than miglitol. The comparative study of  $IC_{50}$  values in Table 2 indicated an increase in the hydrophobicity of the molecule with increase in the N-alkyl chain length resulting into improved inhibition and selectivity towards  $\alpha$ -mannosidase. The selective  $\alpha$ -mannosidase activity in the micromolar range prompted us to substantiate our results with molecular docking studies.



Figure 2. Conformational analysis of 8a-f.

Table 1	
Chemical shift ( $\delta$ , ppm) and coupling constant (J, Hz) values for <b>8a</b> -	f

#### Table 2

 $IC_{50}\ (mM)$  values for iminosugars 8a-f and standard Miglitol (NI: no inhibition at 1 mM)

Enzyme	8a	8b	8c	8d	8e	8f	Miglitol
α-Glucosidase	0.418	0.360	NI	NI	NI	0.252	0.171
α-Galactosidase	NI	0.167	0.315	NI	NI	NI	1.850
α-Mannosidase	1.826	0.252	0.183	0.143	0.112	0.873	0.379

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$IC_{50}$ ( $\mu$ M) value	s for iminosugars <b>8</b>	la-f
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$IC_{50}$ ( $\mu M$ )	8a	8b	8c	8d	8e	8f
MLR	>10	>10	>10	>10	>10	>10
B-cell assay	>50	>50	>50	>50	15	>50
Jurkat	>10	>10	>10	>10	>10	>10

#### 2.2.2. Immunosuppressive activity

The immunosuppressive activity of newly synthesized compounds was evaluated in vitro by investigating their ability to inhibit the proliferation of human lymphocytes in the Mixed Lymphocyte Reaction (MLR) assay and to inhibit the production of IgG by human B cells stimulated with a CpG oligonucleotide (B-cell assay). These two tests target the two major components of the immune response, the T-cells and B-cell respectively. A cytotoxicity counter screen was performed on Jurkat cells and results are summarized in Table 3. Amongst the compounds studied, iminosugar **Se** showed weak activity (IC<sub>50</sub> = 15  $\mu$ M) in the B-cell assay, while the other compounds were found to be inactive in both assays.

# 2.2.3. Molecular docking

In order to understand the interactions of synthesized iminosugars with the amino acid residues of the mannosidase, molecular docking studies were performed. Since compound 8e showed highest  $\alpha$ -mannosidase activity, it was considered for the docking studies. Glycosidase inhibitory activity of 8e showed its efficiency as selective  $\alpha$ -mannosidase inhibitors (isolated from jack bean mannosidase). The complete sequence of jack bean mannosidase is unknown; hence it is difficult to perform the docking studies with the amino acid residues in the active site. Therefore, crystal structures for human  $\alpha$ -mannosidase were downloaded from the protein databank (http://www.rcsb.org) and structure (PDB: 1X9D) having high resolution was chosen as the target structure to perform the molecular docking studies and predict the binding efficiency of compound 8e to the human mannosidase (PDB: 1X9D). Molecular docking was performed using AUTODOCK 4.2 wizard.<sup>20</sup> The molecular docking results of  $\alpha$ -mannosidase (PDB: 1X9D) with compound **8e** is depicted in Figure 3. The AUTODOCK binding energy for the compound 8e is found to be 8.11 kcal/mol. The observed intermolecular hydrogen bonding and hydrophobic interactions for human mannosidase-8e complex were determined and

	, 10	<b>.</b> ,				
Compd	8a	8b	8c	8d	8e	8f
H-2a/H-6a	3.10 (t)	2.23 (t)	2.31 (t)	2.35 (t)	2.29 (t)	2.81 (t)
	$J_{2a,2e} = 11.5$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 10.8$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 11.0$
	$J_{2a,3a} = 11.5$	$J_{2a,3a} = 10.7$	$J_{2a,3a} = 10.7$	$J_{2a,3a} = 10.8$	$J_{2a,3a} = 10.7$	$J_{2a,3a} = 11.0$
H-2e/H-6e	3.25 (dd)	2.68 (dd)	2.73 (dd)	2.81 (dd)	2.72 (dd)	3.10 (dd)
	$J_{2a,2e} = 11.5$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 10.8$	$J_{2a,2e} = 10.7$	$J_{2a,2e} = 11.0$
	$J_{2e,3a} = 4.1$	$J_{2e,3a} = 4.5$	$I_{2e,3a} = 4.5$	$J_{2e,3a} = 4.1$	$J_{2e,3a} = 4.3$	$J_{2e,3a} = 4.2$
H-3/H-5	3.85 (dd)	3.47 (dd)	3.49 (dd)	3.59 (dd)	3.49 (dd)	3.65-3.85
	$J_{3a2a} = 11.5$	$J_{3a,2a} = 10.7$	$J_{3a,2a} = 10.7$	$J_{3a,2a} = 10.8$	$J_{3a,2a} = 10.7$	(m)
	$J_{3a,2e} = 4.1$	$J_{3a,2e} = 4.5$	$J_{3a,2e} = 4.5$	$J_{3a,2e} = 4.1$	$J_{3a,2e} = 4.3$	. ,



Figure 3. Molecular docking study of α-mannosidase with 8e compound.

depicted in Figure 4 and Table 4. As shown in Figure 4, iminosugar **8e** was noticed to be strongly interacting with catalytic residues such as Glu330, Arg334, Arg597, Glu599 and Glu689 with intermolecular hydrogen bonding interactions (Table 4). Besides this, the large hydrophobic side chain of **8e** enabled to establish a

hydrophobic environment with Asp463, Glu397, Phe329 and Phe659 residues of human mannosidase. Compound **8e** possesses high potential binding affinity into the binding site of 3D macromolecule and these molecular docking studies were found to be in agreement with the biological activity.

# 3. Conclusion

We have exploited carbon skeleton of D-glucose to introduce otherwise difficult hydroxyethyl functionality at  $\gamma$ -position of piperidine to obtain new  $\gamma$ -hydroxyethyl substituted iminosugar **8a**. The *N*-alkylations of **8a** afforded *N*-alkyl substituted iminosugars **8b–f**. Amongst *N*-alkylated compounds, **8b** and **8c** showed  $\alpha$ -galactosidase inhibition while; **8d** and **8e** were found to be selective as well as potent inhibitors of  $\alpha$ -mannosidase. Compound **8e** was found to be a weak inhibitor of the IgG secretion by CpG oligonucleotide stimulated B-cells. The moderate  $\alpha$ -mannosidase inhibition of **8e** was further supported by the molecular docking studies.

## 4. Experimental section

# 4.1. General experimental methods

Melting points were recorded with melting point apparatus and are uncorrected. IR spectra were recorded with an FTIR as a thin film or using KBr pellets and are expressed in cm.<sup>-1</sup> <sup>1</sup>H (300 MHz)



Figure 4. Intermolecular hydrogen bonding and hydrophobic interactions between α-mannosidase and 8e enzyme-inhibitor complex.

Table 4

The total energy ( $E_{total}$ ), Van der Waals energy ( $E_{vdw}$ ) and electrostatic energy ( $E_{elec}$ ) between active site residues of human mannosidase (PDB: 1X9D) and ligand **8e** 

Residues	H-bond energy kcal/mol	E <sub>vdw</sub> /kcal/mol	E <sub>elec</sub> /kcal/mol	E <sub>total</sub> /kcal/mol
Glu330	-0.203	-0.27	1.06	0.79
Arg334	-0.144	-1.52	-1.39	-2.91
Arg597	-0.151	-0.59	-1.40	-1.99
Glu599	-2.564	-0.85	1.01	0.15
Glu689	-2.480	-0.48	2.06	1.58

and  ${}^{13}$ C (75 MHz) NMR spectra were recorded using CDCl<sub>3</sub>, MeOD or D<sub>2</sub>O as solvent. Chemical shifts were reported in  $\delta$  units (ppm) with reference to TMS as an internal standard and *J* values are given in Hz. 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. Column chromatography was carried out with silica gel (100–200 mesh). The reactions were carried out in oven-dried glassware under dry N<sub>2</sub>. Methanol and THF were purified and dried before use. Distilled *n*-hexane and ethyl acetate were used for column chromatography. After quenching of the reaction with water, the work-up involves washing of combined organic layers with water, brine, drying over anhydrous sodium sulfate and evaporation of the solvent at reduced pressure.

# 4.1.1. 1,2-O-Isopropylidene-3-C-(2'-propenyl)-5-*N*-benzyl-*N*-benzyloxycarbonyl-α-D-ribo-1,4-furanose (11)

To a solution of benzyl amine (0.42 ml, 3.85 mmol) in dry methanol (10 ml) was added a drop of glacial acetic acid, this reaction mixture was cooled to -20 °C. At this cooled conditions then starting **10** (0.8 g, 3.50 mmol) dissolved in dry methanol (5 ml) was added drop wise over period of 10–15 min. Stir the reaction mixture at same temperature for 60 min. After this period sodium cyanoborohydride (0.602 g, 8.75 mmol) was added in two portions and reaction mixture was stirred at the same temperature for 30 min and then for 2 h at 0 C. After completion of reaction all solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate (20 ml × 2) and washed with saturated solution of sodium bicarbonate and concentrated. The crude obtained was used without purification for the next step.

The crude obtained in above step was dissolved in methanol/ water (10 ml, 9:1) and cooled to 0 °C. To this cooled solution then sodium bicarbonate (0.79 g, 9.42 mmol) was added followed by slow drop wise addition of carbobenzyloxy chloride 50% sol<sup>n</sup> (0.74 ml, 4.7 mmol) and resulting reaction mixture was stirred for 2 h while allowing to come to rt. After completion of reaction and evaporation of all solvent under reduced pressure, resulting residue was extracted with chloroform  $(20 \text{ ml} \times 2)$  and concentrated. Purification by column chromatography (*n*-hexane/ethyl acetate = 9:1) gave **11** (1.35 g, 85.4%) as viscous oil:  $R_f 0.55$  (*n*-hexane/ethyl acetate = 7:3);  $[\alpha]_{D}^{27.5}$  +33.7 (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>) 1693.5 cm<sup>-1</sup>, 1637.6 cm<sup>-1</sup>, 3443.0 cm<sup>-1</sup> (broad). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O)  $\delta$  1.32 (s, 3H), 1.52 (s, 3H), 1.82-2.12 (m, 1H), 2.20-2.42 (m, 1H), 3.0-3.40 (m, 1H), 3.70-4.10 (m, 2H), 4.20-4.45 (m, 2H), 4.80-5.40 (m, 5H), 5.62-6.0 (m, 2H), 7.10-7.37 (m, 10H);  $^{13}$ C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  26.3, 35.3, 45.5, 51.0, 67.15, 78.3, 79.93, 80.19, 103.4, 112.1, 116.8, 127.2, 127.7, 127.6, 128.3, 136.3, 137.5, 132.2, 156.2. Anal. Calcd for C<sub>26</sub>H<sub>31</sub>NO<sub>6</sub>: C, 68.86; H, 6.89. Found: C, 68.94; H, 6.68.

# 4.1.2. 1,2-O-Isopropylidene-3-C-(2-oxoethyl)-5-N-benzyl-N-benzyloxycarbonyl- $\alpha$ -D-ribo-1,4-furanose (12)

To a solution of **11** (1.3 g, 2.53 mmol) in acetone/water (15 ml, 8:2) was added NMO (0.59 g, 5.07 mmol) and potassium osmate (0.01 g, 5 mol %). The reaction mixture was stirred at rt for 24 h. Sodium sulfite (0.7 g) was added and stirred for 1 h. Acetone was removed at reduced pressure, residue obtained extracted with ethyl acetate and concentrated to afford diol as thick liquid. To a crude diol in acetone/water (20 ml, 9:1) was added sodium metaperiodate (0.71 g, 3.32 mmol) at 0 °C and stirred for 2 h. Ethylene glycol (0.5 ml) was added and acetone was evaporated under reduced pressure. The residue obtained was extracted with chloroform and concentrated. Purification using column chromatography (*n*-hexane/ethyl acetate = 9:1) gave **12** (0.9 g, 69.2% over two step) as viscous oil:  $R_f$  0.5 (*n*-hexane/ethyl acetate = 6:4);  $[\alpha]_D^{26.6}$  +50.71 (*c* 0.9, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.33

(s, 3H), 1.52 (s, 3H), 2.16–2.32 (m, 1H), 2.54–2.82 (m, 1H), 2.86–3.10 (m, 1H), 3.12–3.26 (br s, 1H, –OH), 3.75–4.50 (m, 5H), 4.75–5.20 (m, 2H), 5.56 (br s, 1H), 7.10–7.50 (m, 10H), 9.80 (br s, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  26.3, 44.6, 45.4, 51.0, 67.5, 78.2, 80.0, 80.7, 103.4, 112.7, 127.3, 127.9, 128.0, 128.5, 135.0, 137.3, 156.4, 200.9. Anal. Calcd for C<sub>25</sub>H<sub>29</sub>NO<sub>7</sub>: C, 65.92; H, 6.42. Found: C, 66.03; H, 6.22.

# 4.1.3. 1,2-O-Isopropylidene-3-C-(2-hydroxyethyl)-5-N-benzyl-N-benzyloxycarbonyl- $\alpha$ -p-ribo-1,4-furanose (13)

To a cooled solution of 12 (0.9 g, 2.89 mmol) in THF/water (10 ml, 4:1) was added sodium borohydride in portions slowly at 0 °C and then the reaction mixture was stirred at the same temperature for 1.5 h. After completion of the reaction, saturated solution of ammonium chloride (2 ml) was added carefully. Solvents was then evaporated under reduced pressure, residue obtained was extracted using ethyl acetate (10 ml  $\times$  3) and concentrated. Purification using column chromatography (n-hexane/ethyl acetate = 7:3) gave **13** (0.78 g, 86.6% over two step) as viscous oil:  $R_f$ 0.2 (*n*-hexane/ethyl acetate = 6:4);  $[\alpha]_D^{26.6}$  +42.16 (*c* 1.0, CHCl<sub>3</sub>): IR (CHCl<sub>3</sub>) 1678.1 cm<sup>-1</sup>, 3100–3600 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O)  $\delta$  1.32 (s, 3H), 1.51 (s, 3H), 1.5–1.85 (m, 2H), 3.0– 3.09 (m, 1H), 3.60-4.10 (m, 4H), 4.20-4.35 (m, 2H), 4.35-5.18 (m, 3H), 5.36 (br s, 1H), 7.16–7.37 (10H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O)  $\delta$  26.2, 31.7, 45.7, 50.8, 58.2, 67.3, 79.5, 80.0, 80.9, 103.4, 112.2, 127.1, 127.6, 128.4, 137.4, 146.2, 156.2. Anal. Calcd for C<sub>25</sub>H<sub>31</sub>NO<sub>7</sub>: C, 65.63; H, 6.83. Found: C, 65.64; H, 6.88.

# 4.1.4. 1,2-O-Isopropylidene-3-C-((2-acetoxy)ethyl)-5-*N*-benzyl-*N*-benzyloxycarbonyl-α-D-ribo-1,4-furanose (14)

To a cooled solution of **13** (0.75 g, 1.63 mmol) in dry pyridine (3 ml) was added acetic anhydride (0.17 ml, 1.80 mmol) slowly at 0 °C and the reaction mixture was stirred for 4 h while allowing reaction to come to rt. After completion of reaction extracted with chloroform (20 ml) and organic layer was washed with 2 N HCl solution (20 ml) and then concentrated. Purification using column chromatography (*n*-hexane/ethyl acetate = 1.5:8.5) gave **14** (0.76 g, 93.82%) as viscous liquid:  $R_f$  0.6 (*n*-hexane/ethyl acetate = 6:4);  $[\alpha]_{D}^{27.7}$  +40.37 (c 0.43, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>) 1699.34 cm<sup>-1</sup>, 1737.92 cm<sup>-1</sup>, 3439.19 cm<sup>-1</sup> (broad). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>+ D<sub>2</sub>O  $\delta$  1.12 (s, 3H), 1.52 (s, 3H), 1.40–2.0 (m, 2H), 2.00 (br s, 3H), 3.03-3.07 (m, 1H), 3.72-4.47 (m, 5H), 4.88-5.33 (m, 4H), 7.16-7.38 (m, 10H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>+D<sub>2</sub>O)  $\delta$  20.8, 26.3, 29.1, 45.5, 50.8, 59.9, 67.3, 78.1, 79.9, 81.2, 103.4, 112.3, 127.2, 127.7, 128.3, 128.4, 137.5, 156.3, 170.7. Anal. Calcd for C<sub>27</sub>H<sub>33</sub>NO<sub>8</sub>: C, 64.92; H, 6.66. Found: C, 64.98; H, 6.60.

### 4.1.5. (3R,4s,5S)-4-(2-Hydroxyethyl) piperidine-3,4,5-triol (8a)

Cold TFA/water (3:1, 6 ml) was added drop wise to the compound 14 (0.75 g) at 0 °C, and reaction mixture was stirred at this temperature for 2 h. After completion of reaction TFA was co-evaporated with toluene at reduced pressure. The compound obtained was dissolved in methanol then charged in parr high pressure reactor along with 10% Pd/C (0.050 g) and reaction was carried out at 150 psi of H<sub>2</sub> pressure for 24 h. After completion of reaction the catalyst was filter off over Celite bed, filtrate was evaporated at reduced pressure. Purification using column chromatography (MeOH) gave compound 8a (0.164 g, 80.8%) as white solid; mp 186–188 °C; Rf 0.2 (25% aq NH<sub>4</sub>OH/MeOH = 1:9), IR (KBr) 3500– 2600 (br) cm<sup>-1</sup>, <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  2.04 (t, J = 6.5 Hz, 2H), 3.10 (t, J = 11.5 Hz, 2H, H-2a & H-6a), 3.25 (dd, J = 11.5, 4.1 Hz, 2H, H-2e & H-6e), 3.76 (t, *J* = 6.5, Hz, 2H, H-8), 3.85 (dd, *J* = 11.5, 4.7 Hz, 2H, H-3 & H-5); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 35.7 (C-7), 44.9 (C-2 & C-6), 57.0 (C-8), 69.5 (C3 & C5) 74.3 (C-4). Anal. Calcd for C<sub>7</sub>H<sub>15</sub>NO<sub>4</sub>: C, 47.45; H, 8.53. Found: C, 47.52; H, 8.33.

#### 4.1.6. General procedure for alkylation of 8a

To the solution of compound **8a** (1 mmol) in dry DMF (1 ml) was added bromo alkane (1.5 mmol) followed by addition of the  $K_2CO_3$  (3 mmol) under  $N_2$  atmosphere, resulting mixture was then heated at 80 °C for 10–14 h. After completion of reaction solvent was evaporated under reduced pressure. Purification using column chromatography (chloroform/MeOH) gave the desired compound.

## 4.1.7. Compound (8b)

Compound obtained as off white solid with 66.5%: mp 114– 116 °C;  $R_f$  0.3 (MeOH/CHCl<sub>3</sub> = 1:9), IR (KBr) 3500–2600 (br) cm<sup>-1</sup>, <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  0.91 (t, J = 7.2 Hz, 3H, CH<sub>3</sub>), 1.24– 1.40 (m, 2H, H-11), 1.40–1.56 (m, 2H, C-10), 1.94 (t, J = 6.2 Hz, 2H, H-7), 2.23 (t, J = 10.7 Hz, 2H, H-2a & H-6a), 2.37–2.44 (m, 2H, H-9), 2.68 (dd, J = 10.7, 4.5 Hz, 2H, H-2a & H-6e), 3.47 (dd, J = 10.7, 4.5 2H, H-3 & H-5), 3.72 (t, J = 6.2, Hz, 2H, H-8); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  14.4 (C12), 21.8 (C11), 29.8 (C1), 38.9 (C7), 55.6 (C2 & C6), 58.6 (C9), 59.0 (C8), 71.3 (C3 & C5), 74.6 (C4). Anal. Calcd for C<sub>11</sub>H<sub>23</sub>NO<sub>4</sub>: C, 56.63; H, 9.94. Found: C, 56.61; H, 10.10.

# 4.1.8. Compound (8c)

Compound obtained as off white solid with 61.05%: mp 107–109 °C;  $R_f$  0.4 (MeOH/CHCl<sub>3</sub> = 1:9); IR (KBr) 3500–2600 (br) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  0.87 (t, J = 6.7 Hz, 3H, H-14), 1.18–1.38 (m, 6H, H-11, H-12, H-13), 1.42–1.60 (m, 2H, H-10), 1.94 (t, J = 6.2 Hz, 2H, H-7), 2.31 (t, J = 10.7 Hz, 2H, H-2a & H-6a), 2.40–2.52 (m, 2H, H-9), 2.73 (dd, J = 4.5, 10.7 Hz, 2H, H-2a & H-6e), 3.49 (dd, J = 4.5, 10.7 2H, H-3 & H-5), 3.73 (t, J = 6.2, Hz, 2H, H-8); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  14.4 (C-14), 23.7 (C13), 27.3 (C12), 28.2 (C11), 32.9 (C-10), 38.7 (C-7), 55.3 (C-2 & C-6), 58.5 (C-9), 59.2 (C-8), 70.9 (C-3 & C-5), 74.5 (C-4). Anal. Calcd for C<sub>13</sub>H<sub>27</sub>NO<sub>4</sub>: C, 59.74; H, 10.41. Found: C, 59.64; H, 10.47.

# 4.1.9. Compound (8d)

Compound obtained as solid with 67.82%: mp 112–115 °C;  $R_f$  0.45 (MeOH/CHCl<sub>3</sub> = 1:9); IR (KBr) 3500–2600 (br) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  0.89 (t, J = 6.0 Hz, 3H, H-16), 1.20–1.40 (m, 10H), 1.45 (m, 2H, H-10), 1.96 (t, J = 5.5 Hz, 2H, H-7), 2.35 (t, J = 10.8 Hz, 2H, H-2a & H6a), 2.42–2.56 (m, 2H, H-9), 2.81 (dd, J = 10.8, 4.1 Hz, 2H, C-2e & C-6e), 3.59 (dd, J = 10.8, 4.1 Hz, 2H, H-3 & H-6), 3.77 (t, J = 5.5, Hz, 2H, H-8); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  14.2 (C16), 23.0 (C15), 26.5 (C14), 27.8 (C13), 29.6 (C12), 29.8 (C11), 32.2 (C10), 38.1 (C7), 54.4 (C2 & C6), 57.7 (C9), 58.5 (C8), 70.3 (C3 & C5), 73.7 (C4). Anal. Calcd for C<sub>15</sub>H<sub>31</sub>NO<sub>4</sub>: C, 62.25; H, 10.80. Found: C, 62.32; H, 10.91.

#### 4.1.10. Compound (8e)

Compound obtained as colourless solid with 63.5%: mp 109–111 °C;  $R_f$  0.5 (MeOH/CHCl<sub>3</sub> = 1:9); IR (KBr) 3500–2600 (br) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  0.86 (t, J = 6.3 Hz, 3H, H-18), 1.18–1.38 (m, 14H), 1.42–1.58 (m, 2H, H-10), 1.94 (t, J = 6.1 Hz, 2H, H-7), 2.29 (t, J = 10.7 Hz, 2H, H-2a & H-6a), 2.43 (apperent t, J = 7.7, Hz, 2H, H9), 2.72 (dd, J = 10.7, 4.3 Hz, 2H, H-2e & H-6e), 3.49 (dd, J = 10.7, 4.3, 2H, H-3 & H-5), 3.71 (t, J = 6.1, Hz, 2H, H-8); <sup>13</sup>C NMR(75 MHz, CDCl<sub>3</sub>)  $\delta$  14.5 (C18), 23.8 (C17), 27.4 (C16), 28.5 (C15), 30.5 (C14), 30.6 (C13), 30.7 (C12), 33.1 (C10), 38.7 (C7), 55.3 (C2 & C6), 58.6 (C9), 59.2 (C8), 71.0 (C3 & C5), 74.57 (C4). Anal. Calcd for C<sub>17</sub>H<sub>35</sub>NO<sub>4</sub>: C, 64.32; H, 11.11. Found: C, 64.20; H, 11.23.

### 4.1.11. Compound (8f)

Compound obtained as thick liquid with 60.38%;  $R_f$  0.8 (MeOH); IR (KBr) 3500–2600 (br) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  1.98 (t, J = 6.2 Hz, 2H, H-7), 2.81 (t, J = 11 Hz, 2H, H-2a & H-6a), 3.0 (t, J = 5.2, Hz, 2H, H-9), 3.1 (dd, J = 11.0, 4.2 Hz, 2H, H-2e & H-6e), 3.65–3.85 (m, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  37.9 (C7), 54.1 (C2 & C6), 57.8 (C9) 58.4 (C8), 60.2 (C10), 69.4 (C3 & C5), 74.1 (C4). Anal. Calcd for  $C_9H_{19}NO_5$ : C, 48.86; H, 8.66. Found: C, 48.52; H, 8.33.

#### 4.2. Glycosidase inhibition assay

Glycosidase inhibition assay of derivatives was carried out by mixing 0.1 unit/ml each of  $\alpha$ -galactosidase,  $\alpha$ -mannosidase and  $\alpha$ -glucosidase with the samples and incubated for 1 h at 37 °C. Enzyme action for  $\alpha$ -galactosidase was initiated by addition of 10 mM *p*-nitrophenyl- $\alpha$ -p-galactopyranoside (*p*NPG) as a substrate in 200 mM sodium acetate buffer followed by an incubation for 10 min at 37 °C and stopped by adding 2 ml of 200 mM borate buffer of pH 9.8.  $\alpha$ -Mannosidase activity was initiated by addition of 10 mM *p*-nitrophenyl- $\alpha$ -*p*-mannopyranoside as a substrate in 100 mM citrate buffer of pH 4.5. The reaction was incubated at 37 °C for 10 min and stopped by adding 2 ml of 200 mM borate buffer of pH 9.8. Initiation of  $\alpha$ -glucosidase activity was carried out by addition of 10 mM *p*-nitrophenyl- $\alpha$ -p-glucopyranoside in 100 mM phosphate buffer of pH 6.8 and stopped by adding 2 ml of 0.1 M Na<sub>2</sub>CO<sub>3</sub> after an incubation of 10 min at 37 °C. α-Glycosidase activity was determined by measuring absorbance of the p-nitrophenol released from pNPG at 420 nm using Shimadzu Spectrophotometer UV-1601.

#### 4.3. Immunosuppressive assay

#### 4.3.1. MLR assay

The MLR assay has been described previously.<sup>21</sup> Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of healthy donors by density-gradient centrifugation over Lymphoprep. PBMCs were resuspended at a concentration of  $1.2 \times 10^6$  cells/ml in complete medium (RPMI-1640 containing 10% heat-inactivated fetal calf serum (FCS) and antibiotics). RPMI1788 cells were treated with 30 µg/ml mitomycin C for 20 min at 37 °C, washed with medium and finally suspended in complete RPMI medium to a density of  $0.45 \times 10^6$  cells/ml. An amount of 100 µl of each cell suspension was mixed with 20 µl of diluted compound. The mixed cells were cultured at 37 °C for 6 days in 5% CO<sub>2</sub>. DNA synthesis was assayed by the addition of 1  $\mu$  Ci (methyl-<sup>3</sup>H)thymidine per well during the last 18 h in culture. Thereafter, the cells were harvested on glass filter paper and the counts per minute (cpm) determined in a liquid scintillation counter.

# 4.3.2. IgG production by CpG oligonucleotide stimulated B-cells

B-cells were isolated from PBMC's of healthy donors by positive magnetic selection using CD19 MicroBeads technology (Miltenyi Biotec) according the manufacturer's protocol. Twenty five thousand cells were incubated in 50  $\mu$ l of culture medium in the presence of 0.1  $\mu$ M of ODN2006 (Invivogen) and 5  $\mu$ l of compound dilution. After 7 days incubation at 37 °C in 5% CO<sub>2</sub>, supernatants were collected and IgG concentration was determined using the Human IgG immunoassay kit (PerkinElmer) according to the manufacturer's protocol.

# 4.3.3. Cytotoxic assay

Exponential growing Jurkat cells (ATCC, TIB-152) were seeded at  $50 \times 10^3$  per 200 µl of complete medium. Twenty µL of test compound dilution were added to each well and the plates were incubated for 48 h at 37 °C, 5% CO<sub>2</sub>. Untreated cells and positive control (1% triton X-100, for the last 15 min) served as reference for maximum and minimum viability. At the end of incubation 100 µl of supernatant were removed and replaced by 10 µl of WST-1 solution (Cell Proliferation Reagent WST-1, Roche Applied Science). After 3 h incubation at 37 °C, 5% CO $_2$ , optical density were measured at 450 nm.

# 4.4. Molecular docking

# 4.4.1. Computational methodology and Molecular docking parameters

Crystal structures for human  $\alpha$ -mannosidase were downloaded from protein databank (http://www.rcsb.org) and structure (1X9D.pdb) having high resolution was selected for docking studies. Molecular docking was performed using AUTODOCK 4.2 wizard.<sup>22</sup> From receptor molecule, non-polar hydrogen atoms were replaced with polar hydrogen atoms by employing Kollman united atom charges. The obtained receptor structure of  $\alpha$ -mannosidase was minimized for 5000 steps of steepest descent method using SpdbViewer<sup>22a</sup> to remove internal stain. Minimized structure was finally subjected to docking studies with **8e** compound.

Molecular structure of **8e** synthetic ligand was constructed using '*Spartan*'14' Parallel suit for Windows<sup>22b</sup> and geometrically optimized by quantum chemical semi-empirical RM1 method.<sup>22e</sup> The Gasteiger charges and hydrogen atoms were added to the ligand molecule using AUTODOCK 4.2 wizard. Whereas, AutoGrid was used for calculating the grid maps and centered on the ligand binding site of human  $\alpha$ -mannosidase, in such a way that it would totally cover the ligand molecule.<sup>22</sup> The grid size was set to 68 A × 70 A × 68 A with a grid spacing 0.375 A. The step size of 1 Å for translation and the maximum number of energy evaluation was set to 2,500,000. The 100 runs were performed. For each of the 100 independent runs, a maximum number of 2,70,000 LGA operations were generated on a single population of 150 individuals. The operator weights for crossover, mutation and elitism were maintained as default parameters (0.80, 0.02, and 1, respectively).

The resulting 100 docked conformations were analyzed for binding energy, intermolecular energy and internal energy using AUTODOCK 4.2 wizard. Mannosidase–inhibitor complex with lowest binding energy was subjected for geometrical parameters analysis. Chimera software was used to generate pictorial presentation of selected docked conformation.<sup>22c</sup> LigPlot program was used for analysis of hydrogen bonding and hydrophobic interactions from mannosidase–**8e** complex.<sup>22d</sup>

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