JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

α-Geminal Dihydroxymethyl Piperidine and Pyrrolidine Iminosugars: Synthesis, Conformational Analysis, Glycosidase Inhibitory Activity, and Molecular Docking Studies

Nitin J. Pawar,[†] Vijay Singh Parihar,[†] Sanjay T. Chavan,[‡] Rakesh Joshi,[§] Pranaya V. Joshi,[∥] Sushma G. Sabharwal,[‡] Vedavati G. Puranik,[∥] and Dilip D. Dhavale*,[†]

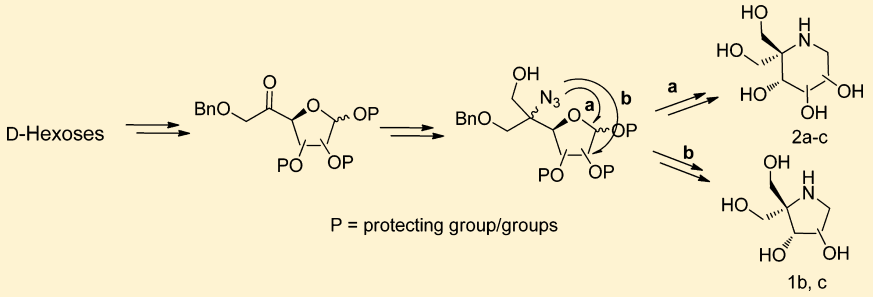
[†]Department of Chemistry, Garware Research Centre, University of Pune, Pune, 411 007, India

[‡]Division of Biochemistry, Department of Chemistry, University of Pune, Pune, 411 008, India

[§]Biochemical Sciences Division, National Chemical Laboratory, Pune, 411 008, India

[∥]Centre for Material Characterization, National Chemical Laboratory, Pune, 411 008, India

Supporting Information



ABSTRACT: The Jocic–Reeve and Corey–Link type reaction of dichloromethyllithium with suitably protected 5-keto-hexofuranoses followed by treatment with sodium azide and sodium borohydride reduction gave 5-azido-5-hydroxylmethyl substituted hexofuranoses **7a–c** with required geminal dihydroxymethyl group. Removal of protecting groups and converting the C-1 anomeric carbon into free hemiacetal followed by intramolecular reductive aminocyclization with in situ generated C5-amino functionality afforded corresponding 5C-dihydroxymethyl piperidine iminosugars **2a–c**. Alternatively, removal of protecting groups in **7b** and **7c** and chopping of C1-anomeric carbon gave C2-aldehyde that on intramolecular reductive aminocyclization with C5-amino gave 4C-dihydroxymethyl pyrrolidine iminosugars **1b** and **1c**, respectively. On the basis of the ^{1}H NMR studies, the conformations of **2a**/**2b** were assigned as $^4C_1$ and that of **2c** as $^1C_4$. The glycosidase inhibitory activities of all five iminosugars were studied with various glycosidase enzymes and compared with natural D-*gluco*-1-deoxynojirimycin (DNJ). All the five compounds were found to be potent inhibitors of rice α-glucosidase with K_i and IC_{50} values in the nanomolar concentration range. Iminosugars **2b** and **1b** were found to be more potent inhibitors than their parent iminosugar. These results were substantiated by in silico molecular docking studies.

INTRODUCTION

A number of piperidine **Ia** (Figure 1) and pyrrolidine **IIa** iminosugars, with variation in the position and orientation of hydroxyalkyl substitution, are known to be promising and selective glycosidase inhibitors.[1,2] In an attempt to know the effect of an additional hydroxymethyl group on their biological activity, the dihydroxyalkyl substituted six/five membered iminosugars were either isolated or synthesized and evaluated for glycosidase inhibitory activity.[3] For example, naturally occurring α-D-*gluco*-homonojirimycin, β-D-*manno*-homonojirimycin (general structure **Ib**)[3a] and 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) **IIb**,[1] with α,α′-dihydroxymethyl group, are potent inhibitors of α-glucosidases, α-L-fucosidase and α-glucosidase, respectively.[3b] Similarly, α,β- and α,β′-dihydroxymethyl substituted piperidine iminosugars were found to be more potent and selective inhibitors of different glycosidases.[4,5]

In a classical variation in the position of dihydroxymethyl substituents, Altenbach et al. and Estevez et al. synthesized α-geminal dihydroxymethyl substituted pyrrolidine iminosugar **1a** (Figure 1) that showed potent α-galactosidase inhibitory activity,[6] while Fleet and co-workers reported seven membered azepane iminosugars that showed loss in inhibitory activity due to conformational distortion.[7] Although five and seven membered α-geminal dihydroxymethyl iminosugars are known, analogous six membered piperidine iminosugars are not known so far. As a part of our continuing efforts in this area,[8] we are now reporting hitherto unknown α-geminal dihydroxymethyl substituted pyrrolidine iminosugars **1b**, **1c** and piperidine iminosugars **2a**, **2b**, **2c** and study of their

Received: May 24, 2012

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX–XXX

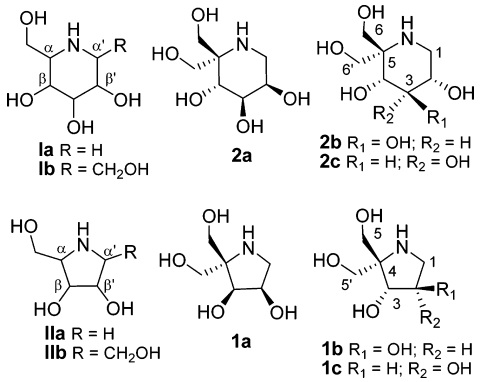
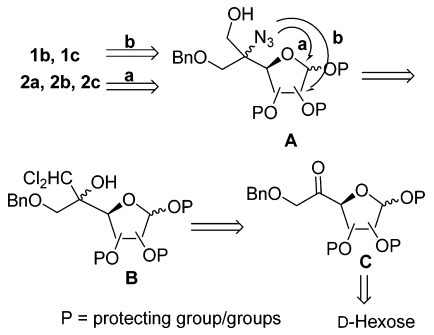


Figure 1. Piperidine and pyrrolidine iminosugars.

glycosidase inhibitory activity. A unique feature of iminosugars **1** and **2** is their dual configurational nature due to the presence of stereogenic C-4 or C-5 carbon atom, respectively, wherein diastereotopic hydroxymethyl groups in **1b** allow it to be called as either 4-*C*-hydroxymethyl-1,4-dideoxy-1,4-imino-D-arabinitol (C_1−C_5) or 4-*C*-hydroxymethyl-1,4-dideoxy-1,4-imino-L-xylitol (C_1−$C_{5'}$). Similarly, **2b** may be recognized as either D-*gluco*-deoxynojirimycin (C_1−C_6) or L-*ido*-deoxynojirimycin (C_1−$C_{6'}$).

In general, introduction of the geminal dihydroxyalkyl moiety at the α-position to the ring nitrogen atom of the piperidine/pyrrolidine ring skeleton is difficult. Altenbach et al. introduced hydroxymethyl group using the Birch reduction of methyl *N*-Boc-pyrrolecarboxylate followed by treatment with iodomethyl pivalate and a reduction of the carboxylate group to get racemic geminal dihydroxymethyl pyrrolidine **1a**.[6a] Estevez et al. introduced geminal hydroxymethyl groups in one pot by aldol condensation of protected nitrotetrose with paraformaldehyde.[6b] In our approach, we thought of utilizing a carbon skeleton of D-hexoses (D-glucose, D-allose, and D-mannose) wherein the C-6 hydroxymethyl of hexoses is retained in the target compound, and the second hydroxymethyl group is introduced by the Jocic−Reeve and Corey−Link type approach[9] with D-hexo-5-uloses.[10] As shown in retrosynthetic analysis (Scheme 1), intramolecular reductive amination of the

Scheme 1. Retrosynthesis of 1 and 2



C-5 amino functionality (formed in situ from the C-5 azido group) with the C-2 or C-1 aldehyde in azido derivative **A** will lead to the formation of pyrolidine **1** or piperidine **2** immunosugars, respectively. The azido compound **A** with required geminal dihydroxymethyl groups at the C-5 of hexoses will be obtained from the α-dichloromethyl carbinol **B** by treatment with NaN_3 (to get α-azido aldehyde) followed by reduction. The nuclophilic addition of the dichloromethyl lithium to the C-5 ketone functionality of D-hexo-5-uloses **C** (easily obtained from hexoses) will give an access to suitably protected α-dichloromethyl carbinol **B**.[10] Our results in this direction are described herein.

RESULTS AND DISCUSSION

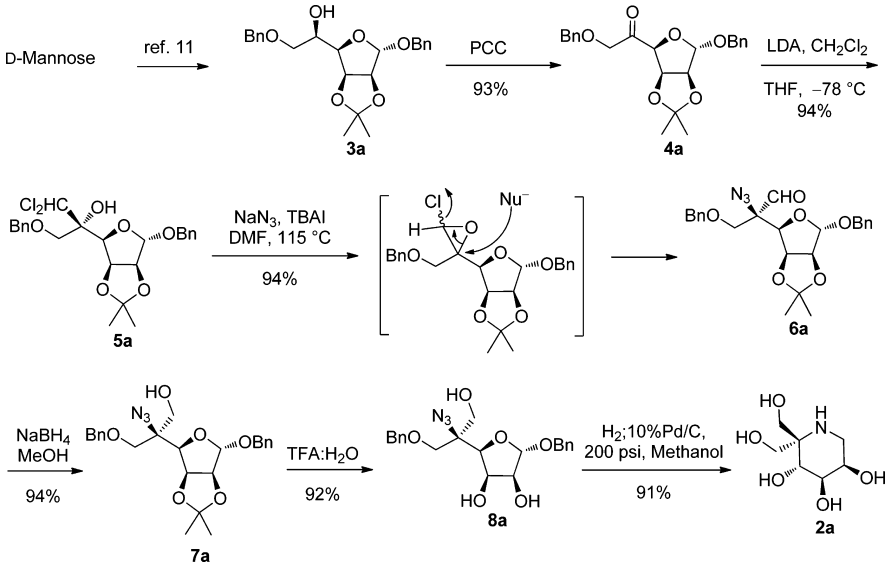
Required benzyl-2,3-*O*-isopropylidene-6-*O*-benzyl-α-D-*manno*-furanoside **3a** was prepared from D-mannose as reported earlier.[11] Oxidation of **3a** using PCC afforded ketone **4a** as a white solid (Scheme 2) in 93% yield. The condensation of ketone **4a** with dichloromethyllithium gave a diastereomeric mixture of dichloroalcohols, in the ratio of 9:1, as evident from the ^{1}H NMR spectrum of crude product. An appreciable difference in the R_f value allowed us to separate the isomers by column chromatography to get **5a** and its C5-epimer in the 85 and 9% yield, respectively. The ^{1}H NMR spectra were unable to differentiate the C5-diasteriomeres. Fortunately, the major isomer **5a** was isolated as a solid, and the X-ray crystallographic data (Figure 2) established the absolute configuration as 5*S* and confirmed the formation of L-*gulo*-isomer. The minor isomer was therefore considered as D-*manno* isomer, with 5*R* absolute configuration. Formation of **5a** as a major product could be explained by considering the preferred conformer of **4a** (Figure 3). We believe that the dipole movement in **4a** is considerably minimized by alignment of oxygen atoms in the C-5 ketone and the furanose ring on the opposite sides, wherein the *Re* face attack to the C5-keto group, from the side opposite to the 2,3-*O*-isopropylidene group, is preferred over the *Si* face attack (hindered because of the acetonide group), leading to the formation of **5a** as a major product.

In the next step, treatment of **5a** with sodium azide afforded α-azido aldehyde **6a** via formation of epoxy-chloride intermediate[10] followed by opening of the oxirane ring by S_N2 attack of the azide ion at *C*-5. Absolute configuration at the newly generated C-5 center was assigned as 5*R* on the basis of the X-ray crystallographic data of the compound obtained in the subsequent steps. Thus, reduction of the α-azido aldehyde **6a** using $NaBH_4$ afforded α-hydroxymethyl azido compound **7a** as a thick liquid that on removal of the 2,3-acetonide group with TFA−water gave triol **8a** as a white solid. The X-ray crystallographic data of **8a** (Figure 4) established the absolute configuration as 5*R*, and therefore confirm the formation of D-*manno*-isomer **6a** with 5*R* configuration. In the final step, reductive aminocyclization of **8a** using H_2, 10% Pd/C in methanol afforded 5-*C*-hydroxymethyl-D-*manno*-1-deoxynojirimycin **2a** (C_1−C_6) in 91% yield as a thick liquid. This one pot three steps process involves hydrogenolysis of C6- and C1-*O*-benzyl groups to get anomeric mixture of hemiacetals, reduction of azide functionality to primary amine, and concomitant intramolecular reductive aminocyclization of C5-amino functionality with C1-aldehyde to give α-geminal dihydroxymethyl piperidine iminosugar **2a** as a single product.

For the synthesis of piperidine iminosugar **2b**, the required 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose-5-ulose **4b** was prepared from D-glucose as reported earlier.[11] The condensation of ketone **4b** with dichloromethyllithium at −78 °C afforded an inseparable diastereomeric mixture of dichloroalcohols **5b** in 89% yield (Scheme 3).[12] In the next step, treatment of **5b** with sodium azide gave α-azido aldehydes **6b** as an inseparable mixture of diastereomers. Reduction of aldehydes in **6b** using $NaBH_4$ gave hydroxymethyl compounds **7b**, which on hydrolysis of 1,2-acetonide group with TFA−water (to get an anomeric mixture of hemiacetals) followed by

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of Iminosugar 2a



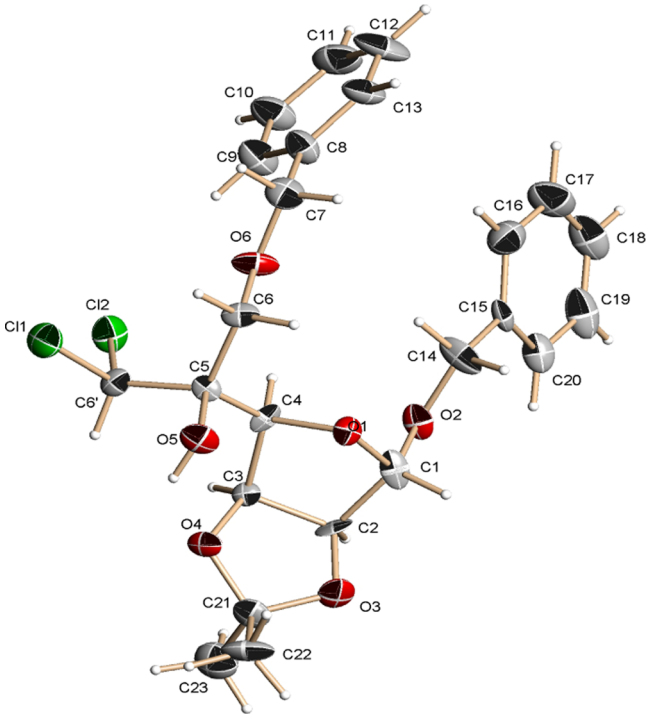


Figure 2. ORTEP diagram of compound **5a**.

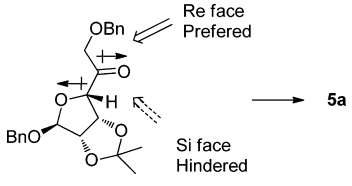


Figure 3. Explanation for diastereoselectivity.

intramolecular reductive aminocyclization using H_2,10% Pd/C in methanol at 200 psi afforded 5-C-hydroxymethyl-D-*gluco*-1-deoxynojirimycin **2b** (C_1−C_6) as a semisolid. The same sequence of reactions, as described for **2b**, was separately performed with 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose-5-ulose **4c**.[11] The reaction of **4c** with dichloromethyllithium in THF at −78 °C also afforded an inseparable mixture of diastereomers **5c**.[12]

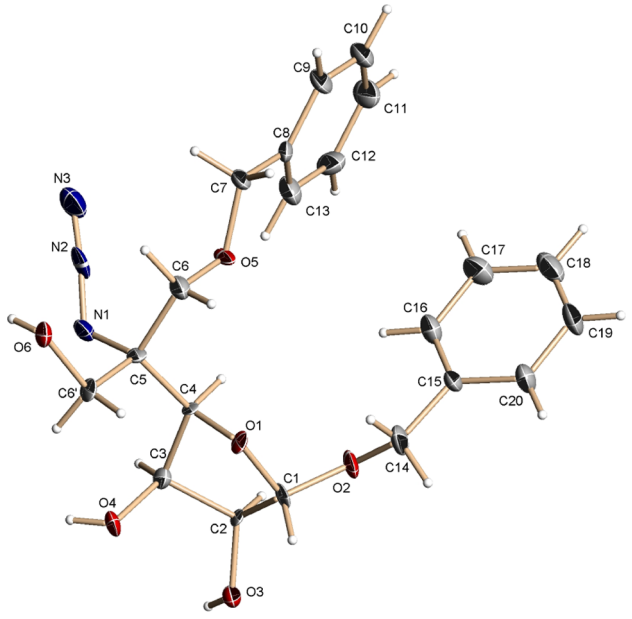


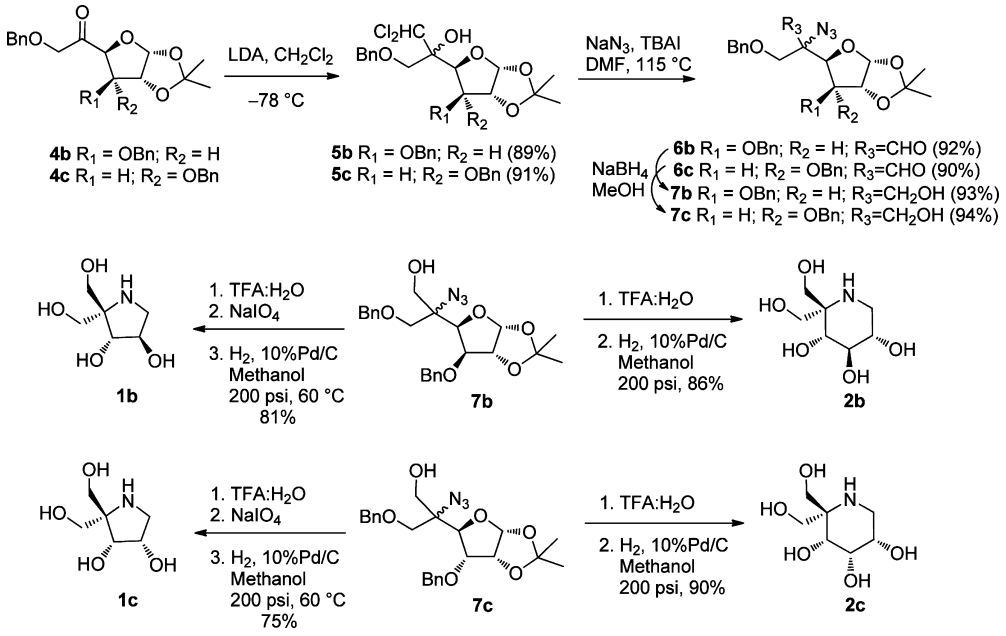
Figure 4. ORTEP diagram of compound **8a**.

Treatment of **5c** with NaN_3 gave azido aldehydes **6c** that on reduction with $NaBH_4$ gave **7c** in 77% (over three steps). Hydrolysis of the 1,2-acetonide functionality in **7c** with TFA−water followed by reductive aminocyclization (H_2, 10% Pd/C) gave 5-C-hydroxymethyl-D-*allo*-1-deoxynojirimycin **2c** (C_1−C_6) as a semisolid.

The syntheses of α-geminal dihydroxymethyl pyrrolidine iminosugars **1b** and **1c** were achieved from **7b** and **7c**, respectively (Scheme 3). Thus, hydrolysis of the 1,2-*O*-acetonide functionality in **7b**/**7c** with TFA−water gave anomeric mixture of hemiacetals that on oxidative cleavage using sodium metaperiodate in acetone−water (to cleave anomeric carbon) followed by reductive aminocyclization using H_2, 10% Pd/C in methanol afforded 4-*C*-hydroxymeth-

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis of Iminosugars 1b, 1c, 2b, 2c



yl-1,4-dideoxy-1,4-imino-D-arabinitol **1b** (C_1–C_5) and 4-*C*-hydroxymethyl-1,4-dideoxy-1,4-imino-D-ribitol **1c** (C_1–C_5) in 81 and 75% yield (over three steps), respectively.

Conformational Assignments of 2a, 2b and 2c. In general, six membered piperidine iminosugars exist in $^4C_1$ or $^1C_4$ conformation depending on orientation of hydroxyalkyl substituent in the ring.[2v,8a] A change in conformation of iminosugars has profound effect on their binding properties with glycosidases and thus affects their inhibitory potential.[2,7a] As the iminosugars **2** have two hydroxymethyl substituents at α-position to the ring nitrogen, it is interesting to find their conformations. Conformations in **2** were determined by coupling constant values between the H-1 and H-2 protons (^{1}H NMR in D_2O). The coupling constant values were obtained from decoupling experiments and are listed in Table 1. In **2a**, the lower coupling constant of the H-1 and H-2 for

Table 1. Coupling /values of 2a, 2b and 2c

	2a	2b	2c
H_{1a}	2.92 (dd)	2.63 (dd)	2.90 (dd)
	$J_{1a,1e}$ = 14.8 Hz	$J_{1a,1e}$ = 12.0 Hz	$J_{1a,1e}$ = 13.5 Hz
	$J_{1a,2e}$ = 3.0 Hz	$J_{1a,2a}$ =12.0 Hz	$J_{1a,2e}$ = 4.1 Hz
H_{1e}	3.01 (dd)	2.95 (dd)	2.98 (dd)
	$J_{1e,1a}$ = 14.8 Hz	$J_{1e,1a}$= 12.0 Hz	$J_{1e,1a}$ = 13.5 Hz
	$J_{1e,2e}$ = 1.7 Hz	$J_{1e,2a}$= 4.0 Hz	$J_{1e,2e}$ = 7.1 Hz

axial–equatorial protons (3.0 Hz) and diequatorial protons (1.7 Hz) requires equatorial orientation of the H-2. This indicated that compound **2a** exists in $^4C_1$ conformation (Figure 5).

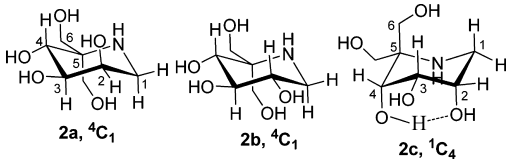


Figure 5. Conformations of **2a**, **2b** and **2c**.

Similarly, in **2b** the high coupling constant of $J_{1a,2a}$ = 12.0 Hz and small coupling constant for $J_{1e,2a}$ = 4.0 Hz indicated the $^4C_1$ for **2b**, while in **2c** the small coupling constant $J_{1a,2e}$ = 4.1 Hz and $J_{1e,2e}$ = 7.1 Hz suggested that the compound **2c** exists in $^1C_4$ conformation (Figure 5). We believe that the conformation $^1C_4$ is stabilized by intramolecular hydrogen bonding between C-2 and C-4 OH, thus resulting into little twist in the conformation wherein the dihedral angle between H_{1e}, H_{2e} is ~140°, which is in agreement with the observed $J_{1e,2e}$ = 7.1 Hz.[8j,k]

Biological Activity. Glycosidase inhibitory activity of **1b**, **1c**, **2a**, **2b**, **2c** was studied with reference to the known standard D-*gluco*-1-deoxynojiromycin (DNJ) and is summarized in Table 2. All the five compounds were found to be potent inhibitors of rice α-glucosidase with K_i and IC_{50} values in the nanomolar concentration range. The presence of hydroxymethyl group at C-5 position in **2b** resulted in a slight increase in its α-glucosidase inhibitory activity as compared to DNJ. Iminosugars **2b** and **1b** moderately inhibited the α-glucosidase from baker's yeast. Iminosugar **2c** showed moderate inhibition of β-galactosidase from bovine liver. Although DNJ did not show any inhibitory activity against β-galactosidase (bovine liver) and α-galactosidase (*Geobacillus sp.*), iminosugars **1b**, **2a**, **2b** and **2c** were found to have moderate inhibitory activity against the microbial α-galactosidase (*Geobacillus sp.*) under assay conditions. Also, all the tested iminosugars did not show any significant inhibitory activity against β-galactosidase, β-glucosidase (from almond seeds), α-mannosidase, *N*-acetyl-β-D-glucosaminidase (Jack bean seeds), α-fucosidase and *N*-acetyl-β-D-glucosaminidase (bovine kidney). The inhibition constants (K_i) were determined from Lineweaver–Burk plots (Supporting Information).

Molecular Docking Studies. Three dimensional (3D) model of yeast α-glucosidase, rice α-glucosidase and *Geobacillus* α-galactosidase were built by comparative modeling using MODELER 9 V4 crystallographic structure of related protein as a template.[13] Modeled structures were validated by Ramchandra plot analysis.[13b] Binding pockets of enzymes and docking simulation were predicted using AutoDock 4.0.[13c] This employs the preparation of receptor by adding hydrogens and assigning

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX–XXX

Table 2. IC_{50} (μM) and K_i (μM) Values for New Iminosugars and Standard DNJ[a]

	DNJ		1b		1c		2a		2b		2c	
Enzymes (Source)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)
α-glucosidase (rice)	0.036	0.066	0.028	0.083	5	4	4	3	0.032	0.066	0.052	0.083
α-glucosidase (baker's yeast)	23	22	128	25	NI	NI	NI	NI	97	20	NI	NI
β-galactosidase (bovine liver)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	40	25
α-galactosidase (*Geobacillus sp.*)	NI	NI	14	40	NI	NI	22	20	15	13	19	34

[a]NI: No inhibition at 1 mM concentration of inhibitor. Data is average of three sets of assay performed.

Kollman charges. Similarly, the ligands were prepared by assigning the Gasteiger charges and nonpolar hydrogens. Docking simulations were done with Lamarckian Genetic algorithm (LGA). LGA uses a set of 30 structurally known protein ligand complexes with experimentally determined binding constants to calibrate empirical free energy function. Grid box for docking was set around central atom of protein with dimension of 40 × 40 × 40 Å. Parameters were set to a LGA calculation of 10 000 runs, whereas energy evaluations were set to 1 500 000 and 27 000 generations (repetition of process). The obtained docked poses were then summarized and analyzed using Autodock Tool.

Ramchandra plot analysis[13b] of the rice α-glucosidase, yeast α-glucosidase and *Geobacillus* α-galactosidase structures showed that >95% residues are favored and allowed φ, ψ backbone conformational regions (Supporting Information, Figure 1A–C). All inhibitor molecules form the weak interactions (van der Walls interaction and hydrogen bonds) with binding site (Table 3 and Figure 6) of rice/yeast α-glucosidase and *Geobacillus* α-galactosidase and binding energy values of interactions were summarized in Table 3. Binding poses (orientations and conformations) and different interactions of the inhibitor in the binding site of the enzymes were shown in Figure 6. For further analysis, the hits with best binding scores were selected. The trend followed by theoretical binding energy was corroborated with experimentally observed inhibitory activity values. Compounds **1b**, **2b** and **2c** were found to be potent inhibitor of rice α-glucosidase with lowest K_i/IC_{50} and binding energy values (Table 2 and 3).

Ligands **1b**, **2b** and **2c** form ~6, 5, and 6 stable hydrogen bonds, respectively, with the binding site residues of rice α-glucosidase. Lower binding free energy for ligand **1b**, **2a** and **2c** suggests the strong binding to the binding site of enzyme. The higher binding affinity for these ligands is presumably attributed to the formation of higher number of stable hydrogen bonds between the reactive group and several amino acids at the binding site.

CONCLUSIONS

In conclusion, we have exploited the C-5 keto functionality of D-*gluco*-, D-*allo*- and D-*manno*- hexoses to introduce hydroxymethyl and amino functionalities required to achieve synthesis of geminal dihydroxymethyl substituted piperidine **2a**, **2b**, **2c** and pyrrolidine **1b**, **1c** iminosugars. These iminosugars inhibited rice α-glucosidase, yeast α-glucosidase, and the *Geobacillus* α-galactosidase, although to different extents, possibly because of their different specificities and different sources. The trend observed in docking results is in agreement with the experimental studies. The present synthetic analogy could be exploited to different keto hexoses to get a variety of iminosugars. In addition, *N*-alkylated (C_1–C_{10} chain) or *N*-hydroxyalkyl derivatives of **1** and **2** could be easily obtained to get the library of iminosugars for biological evaluation. Work in this direction is in progress.

EXPERIMENTAL SECTION

General Methods. Melting points were recorded with Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded with a FTIR as a thin film or using KBr pellets and are expressed in cm^{-1}. ^1H NMR (300 MHz/400 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using $CDCl_3$ 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 Hertz. Elemental analyses were carried out with C, H-analyzer. Optical rotations were measured using polarimeter at 25 °C. High resolution mass spectra (HRMS) were obtained in positive ion electrospray ionization (ESI) mode using TOF (time-of-flight) analyzer. Thin-layer chromatography was performed on precoated plates (0.25 mm, silica gel 60 F254). Column chromatography was carried out with silica gel (100–200 mesh). The reactions were carried out in oven-dried glassware under dry N_2 atmosphere. Methanol, DCM and THF were purified and dried with the method reported.[14] Petroleum ether (PE) that was used is a distillation fraction between 40 and 60 °C. 10% Pd/C were purchased from Aldrich or Fluka. After neutralization, workup involves washing of combined organic layer with water, brine, drying over anhydrous sodium sulfate, and evaporation of solvent under reduced pressure.

Benzyl-2,3-*O*-isopropylidene-6-*O*-benzyl-α-D-*lyxo*-hexofuranoside-5-ulose (4a). To a suspension of PCC (25.2 g, 120 mmol) and finely powdered molecular sieves 4 Å (32.0 g) in dry CH_2Cl_2 (150 mL) was added a solution of **3a** (16.0 g, 40 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred at 30 °C for 3 h. The reaction mixture was quenched using 2-propanol (10 mL), diluted with ether and filtered through Celite, and the filtrate was evaporated in vacuo. The residue was purified by column chromatography by eluting with petroleum ether/EtOAc, (9:1) to give **4a** (14.8 g, 93%) as a white solid: R_f 0.42 (petroleum ether/EtOAc, 9:1); mp 57–9 °C; $[\alpha]_D^{22}$ = −5.0 (*c* 0.69, CH_2Cl_2); IR (KBr, ν, cm^{-1}) 1730, 1381, 1111, 1089; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.33 (s, 3H), 4.33 (s, 2H), 4.48 (d, *J* = 11.4 Hz, 1H), 4.56–4.70 (m, 4H), 4.72 (d, *J* = 4.3 Hz, 1H), 5.10 (dd, *J* = 5.8, 4.3 Hz, 1H), 5.21 (s, 1H), 7.28–7.37 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 25.7, 69.3, 73.4, 74.1, 80.8, 84.2, 84.3, 105.5, 113.1, 127.9, 128.0, 128.2, 128.5, 136.8, 137.2, 203.0. Anal. Calculated for $C_{23}H_{26}O_6$: C, 69.33; H, 6.58. Found: C, 69.49; H, 6.62.

Benzyl-2,3-*O*-isopropylidene-6-*O*-benzyl-5-*C*-dichloromethyl-α-L-*gulo*-furanoside (5a). To a solution of diisopropylamine (5.32 mL, 37.68 mmol) in dry THF (80 mL) at 30 °C under nitrogen atmosphere was added 1.6 molar solution of *n*-butyllithium in hexane (23.53 mL, 37.68 mmol). After stirring for 30 min, the solution was cooled to −78 °C and ketone **4a** (5.0 g, 12.56 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise. The temperature was then allowed to raise slowly to 20 °C and then stirred at 20 °C for 4 h. Saturated solution of NH_4Cl (10 mL) was added slowly, the mixture was diluted with EtOAc, and the usual workup followed, chromatographic purificaion first elution using with (petroleum ether/EtOAc, 98:02) gave minor isomer (C-5 epimer) (0.54 g, 9%) as thick liquid: R_f 0.56 (petroleum ether/EtOAc, 9:1); $[\alpha]_D^{23}$ = +72.7 (*c* 0.52, CH_2Cl_2); IR

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX–XXX

Table 3. Total Binding Energy in kcal mol^{-1} and Interacting Amino Acid Residues between Enzymes and Tested Ligands[a]

	rice α-glucosidase		yeast α-glucosidase		*Geobacillus* α-galactosidase	
compound	ΔG_{bind}	interacting residues	ΔG_{bind}	interacting residues	ΔG_{bind}	interacting residues
DNJ	−81.49	ASP-775, PHE-776, SER-777, ASN-798, VAL-799	−59.49	LYS-155, THR-234, SER-235, PHE-310, PHE-311, ASN-412, SER-419, PHE-420	−63.6	LYS-176, GLU-337, ASN-334, ASP-366, ASP-548
1b	−79.28	LYS-470, ASP-775, ASN-798, VAL-799	−55.00	ASP-232, THR-234, PHE-310, PHE-311, ASN-412	−64.1	ASP-366, ASP-367, ASN-480, GLY-528, ASP-548
1c	−75.72	ASP-775, PHE-776, SER-777, VAL-797, ASN-798	−63.92	ASP-232, THR-234, SER-235, PHE-310, ASN-412	−70.1	LYS-176, THR-336, ASP-366, ASP-367, ASP-498, ASP-548
2a	−83.9	LYS-695, ASP-775, SER-777, VAL-797, ASN-798, VAL-799	−56.65	ASP-232, THR234, SER-235, PHE-310, PHE-311, ASN-412, ILE-416	−62.8	LYS-176, THR-336 ASP-366, ASP-367, ARG-443, LYS-476, ASN-480, GLU-604
2b	−89.7	LYS-695, ASP-775, SER-777, THP-782, VAL-797, ASN-798, VAL-799	−63.06	ASP-232, SER-235, PHE-310, PHE-311, ASN-412, ILE-416	−71.5	LYS-176, THR-336, ASP-366, ASP-367, LYS-476, ASN-480 ASP-548, GLU-604
2c	−87.3	LYS-695, ASP-775, PHE-776, SER-777, THP-796, VAL-797, ASN-798, VAL-799	−52.5	LUS-155, ASP-232, SER-235, PHE-310, PHE-311, ASN-412,	−67.8	LYS-176, THR-336, ASP-366, ASP-367, LYS-476, ASN-480, ASP-548, GLU-604

[a]AMBER'95 force field present in AUTODOCK 4.0 was used for total binding energy determination.

(neat, ν, cm^{-1}) 3362−3504, (br), 1635,1085, 871; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (s, 3H), 1.42 (s, 3H), 3.85 (d, J = 10.6 Hz, 1H), 4.05 (d, J = 10.6 Hz, 1H), 4.36 (d, J = 2.9 Hz, 1H), 4.39 (d, J = 11.2 Hz, 1H), 4.56 (d, J = 5.3 Hz, 1H), 4.57 (ABq, J = 12.0 Hz, 2H), 4.65 (d, J = 11.2 Hz, 1H), 4.94 (dd, J = 5.3, 2.9 Hz, 1H), 5.06 (s, 1H), 5.15 (s, exchangeable with D_2O, 1H), 6.10 (s, 1H), 7.19−7.34 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2, 25.7, 69.1, 71.2, 73.9, 75.2, 76.7, 78.3, 81.8, 85.2, 104.0, 112.9, 127.3, 127.6, 128.0, 128.3, 128.5, 136.8, 137.9. Anal. Calculated For $C_{24}H_{28}Cl_2O_6$; C,59.63; H, 5.84. Found: C, 59.80; H, 5.94. Further elution with (petroleum ether/EtOAc, 98:04 to 90:10) gave **5a** (5.14 g, 85%) as crystalline white solid: R_f 0.50 (petroleum ether/EtOAc, 9:1); mp 110−12 °C; $[\alpha]_D^{22}$ = +74.4 (c 0.19, CH_2Cl_2); IR (KBr, ν, cm^{-1}) 3427 (st), 1074, 731; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 3H), 1.50 (s, 3H), 3.88 (ABq, J = 9.9 Hz, 2H), 4.41 (d, J = 3.4 Hz, 1H), 4.45 (d, J = 11.9 Hz, 1H), 4.55 − 4.71 (m, 4H), 4.85 (s, exchangeable with D_2O, 1H), 5.10 − 5.20 (m, 2H), 6.17 (s, 1H), 7.22−7.37 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 25.8, 69.1, 71.5, 73.7, 75.5, 75.8, 79.3, 81.7, 85.3, 104.3, 113.1, 127.6, 127.7, 128.0, 128.2, 128.3, 128.5, 136.9, 137.8. Anal. Calculated For $C_{24}H_{28}Cl_2O_6$: C, 59.63; H, 5.84. Found: C, 60.00; H, 5.98. Diastereomeric ratio was evaluated by ^{1}H NMR analysis of crude reaction mixture, dr: 1:9.

Benzyl-5-deoxy-5-azido-5-*C*-benzyloxymethyl-2,3-*O*-isopropylidene-α-D-*manno*-furano-1,6-dialdose (6a). To a solution of **5a** (3.55 g, 7.36 mmol) in dry DMF (15 mL) was added sodium azide (2.39 g, 36.82 mmol), TBAI (1.35 g, 3.68 mmol), and the mixture was heated for 4 h at 110 °C. Reaction mixture was extracted with EtOAc (100 mL × 3) and washed with water (50 mL × 2) and brine. Organic layer was dried over anhydrous sodium sulfate, and solvent was evaporated under reduced pressure. Chromatography using petroleum ether/EtOAc, (95:05) as an eluant to gave **6a** (3.15 g, 94%) as thick liquid: R_f 0.50 (petroleum ether/EtOAc, 92:08); $[\alpha]_D^{22}$ = +64.9 (c 0.80 CH_2Cl_2); IR (neat, ν, cm^{-1}) 2125, 1735 (N_3, CHO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.43 (s, 3H), 3.84 (AB quartet, J = 10.5 Hz, 2H), 4.27 (d, J = 3.3 Hz, 1H), 4.48 (d, J = 12.0 Hz, 1H), 4.59−4.67 (m, 4H), 4.82 (dd, J = 5.7, 3.3 Hz, 1H), 5.16 (s,1H), 7.27−7.36 (m,10H), 9.72 (s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1, 25.2, 69.4, 69.8, 70.4, 73.8, 79.0, 80.3, 84.4, 105.4, 113.1, 127.5, 127.9, 128.0, 128.1, 128.5, 136.9, 137.1, 195.6. Anal. Calculated For $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.84; H, 6.18; N, 9.01.

Benzyl-5-deoxy-5-azido-5-*C*-benzyloxymethyl-2,3-*O*-isopropylidene-α-D-*manno*-furanoside (7a). To an ice-cooled solution of **6a** (2.00 g, 4.41 mmol) in MeOH (10 mL) was added sodium borohydride (326 mg, 8.83 mmol) in two portions. Reaction mixture was stirred for 3 h and quenched by adding saturated aq NH_4Cl solution (5 mL). Methanol was evaporated under reduced pressure and residue extracted with EtOAc (30 mL × 3) and concentrated. Usual workup and purification by column chromatography (petroleum ether/ethyl acetate, 9:1) gave **7a** (1.88 g, 94%) as a thick liquid: R_f 0.33 (petroleum ether/ethyl acetate, 9:1); $[\alpha]_D^{22}$ = +40.5 (c 1.73, CH_2Cl_2); IR (neat, ν, cm^{-1}) 3489 (br), 2125 (st),1080, 754; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.44 (s, 3H), 2.64 (t, J = 6.7 Hz, exchangeable with D_2O, 1H), 3.78 (ABq, J = 10.0 Hz, 2H), 3.91 (d, J = 6.7 Hz, after D_2O exchange appear as, s, 2H), 4.23 (d, J = 2.8 Hz, 1H), 4.46 (d, J = 11.5 Hz, 1H), 4.53−4.66 (m, 4H), 4.77 (dd, J = 5.8, 3.3 Hz, 1H), 5.09 (s, 1H), 7.26−7.35 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2, 25.6, 63.6, 66.5, 69.2, 70.9, 73.7, 79.5, 79.7, 85.0, 104.8, 112.9, 127.7, 127.8, 127.9, 128.1, 128.5, 137.0, 137.5. Anal. Calculated For $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.22; H, 6.57; N, 8.95.

Benzyl-5-deoxy-5-azido-5-*C*-benzyloxymethyl-α-D-*manno*-furanoside (8a). A solution of **7a** (350 mg, 0.76 mmol) in TFA−water (4 mL, 3:1) was stirred for 3 h at 0 °C. TFA was coevaporated with toluene at reduced pressure. Purification by column chromatography (petroleum ether/EtOAc, 7:3) gave **8a** (296 mg, 92%) as a white solid: R_f 0.50 (petroleum ether/EtOAc, 1:1); mp 97−99 °C; $[\alpha]_D^{22}$ = +54.2 (c 0.16, CH_2Cl_2); IR (KBr, ν, cm^{-1}) 3317 (br), 2125 (st), 1038, 1004, 732; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09−3.40 (br, exchangeable with D_2O, 3H), 3.75 (s, 2H), 3.89 (AB quartet, J = 11.9 Hz, 2H), 4.12−4.17 (m, 2H), 4.33 (t, J = 4.6 Hz, 1H), 4.45 (d, J = 11.9

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX−XXX

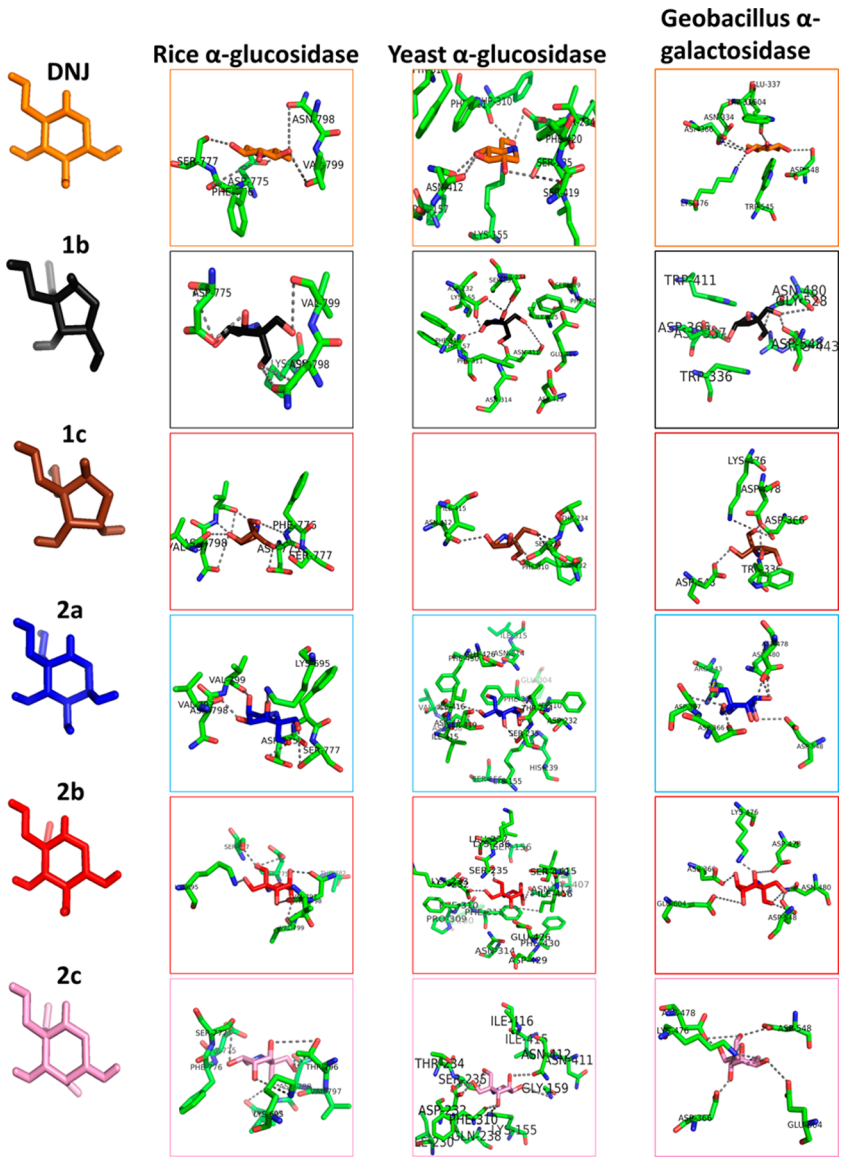


Figure 6. Binding of ligands DNJ, **2a**, **2b**, **2c**, **1b**, and **1c** with active pocket of rice α-glucosidase, yeast α-glucosidase and *Geobacillus* α-galactosidase.

Hz, 1H), 4.58 (bs, 2H), 4.67 (d, J = 11.9 Hz, 1H), 5.09 (d, J = 1.9 Hz, 1H, after opening of 2,3-*O*-isopropylidene H_1 becomes d), 7.27−7.39 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.6, 66.7, 70.2, 70.3, 71.5, 74.0, 77.0, 79.6, 107.1, 127.8, 127.9, 128.2, 128.5, 128.6, 136.8, 137.5. Anal. Calculated For $C_{21}H_{25}N_3O_6$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.45; H, 6.22; N, 9.92.

(3*R*,4*R*,5*R*)-2,2-Bis(hydroxymethyl)piperidine-3,4,5-triol (2a). To a solution of **8a** (200 mg, 0.48 mmol) in methanol (20 mL) was added 10% Pd/C (50 mg), and reaction mixture was hydrogenated at 200 psi for 24 h. Reaction mixture was filtered through Celite, washed with methanol, and solvent was evaporated at reduced pressure. Purification by column chromatography (chloroform/methanol, 8:2) gave **2a** (85 mg, 91%) as a thick liquid: R_f 0.33 (25% aq NH_4OH/MeOH, 1/99); $[\alpha]_D^{22}$ = −40.4 (c 1.09 MeOH); IR (neat, ν, cm^{-1}) 3593−3300 (br); ^{1}H NMR (300 MHz, D_2O) δ 2.92 (dd, J = 14.8, 3.0 Hz, 1H), 3.01 (dd, J = 14.8, 1.7 Hz, 1H), 3.61 (d, J = 11.5 Hz, 1H), 3.66 (d, J = 12.4 Hz, 1H), 3.77−3.86 (m, 3H),[irradiation of signal at 4.03 gives 3.81 (d, J = 11.5 Hz, 1H), 3.82 (d, J = 12.4 Hz, 1H), 3.83 (d, J = 9.8 Hz, 1H)], 3.93 (d, J = 9.8 Hz, 1H), 4.01−4.05 (narrow m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 46.2, 60.0, 63.4, 64.7, 71.0, 71.2, 73.0; MS (ESI) m/z = 194 (MH^+); HRMS calculated for $C_7H_{15}NO_5$ (MH^+) 194.1028, found 194.1029.

(3*R*,4*R*,5*S*)-2,2-Bis(hydroxymethyl)piperidine-3,4,5-triol (2b). Reaction of **7b** (300 mg, 0.65 mmol) with TFA−water (4 mL, 3:1) followed by hydrogenation as discussed for **2a** gave **2b** (110 mg, 86%) as semisolid: R_f 0.33 (25% aq NH_4OH/MeOH, 1:99); $[\alpha]_D^{24}$ = +10.0 (c 1.59, MeOH); IR (KBr, ν, cm^{-1}) 3134−3600 (br); ^{1}H NMR (400 MHz, D_2O) δ 2.63 (dd, J = 12.0, 12.0 Hz, 1H), 2.95 (dd, J = 12.0, 4.0 Hz, 1H), 3.42−3.56 (m, 3H), 3.58 (d, J = 12.0 Hz, 1H), 3.63 (d, J = 12.0 Hz, 1H), 3.68 (d, J = 12.0 Hz, 1H), 3.82 (d, J = 12.0 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 43.5, 57.4, 60.0, 63.1, 70.9, 72.4, 74.5; MS (ESI) m/z = 194 (MH^+); HRMS calculated for $C_7H_{15}NO_5$ (MH^+) 194.1028, found 194.1029;

(3*R*,4*R*)-2,2-Bis(hydroxymethyl)pyrrolidine-3,4-diol (1b). Reaction of **7b** (500 mg, 1.09 mmol) with TFA−water (6 mL, 3:1) as described for **2b** gave crude hemiacetals that were treated with sodium metaperiodate (415 mg, 1.64 mmol) in acetone−H_2O (8 mL, 3:1) at 0 °C to rt for 1 h. Reaction mixture was quenched with ethylene-diol (2 mL), acetone was evaporated under reduced pressure, and reaction mixture was filtered through Celite, washed with EtOAc, and solvent was evaporated at reduced pressure to afford a thick liquid: R_f = 0.80 (petroleum ether/EtOAc, 1:1). The above crude product was dissolved in methanol and hydrogenated with H_2-10%Pd/C (50 mg) at 200 psi at 60 °C for 24 h. The catalyst was filtered through Celite, washed with methanol, and evaporated to give thick oil. Purification by

dx.doi.org/10.1021/jo3009534 | J. Org. Chem. XXXX, XXX, XXX−XXX

column chromatography (chloroform/methanol, 8:2) gave **1b** (145 mg, 81% over three steps) as a thick oil: R_f 0.36 (25% aq NH_4OH/MeOH, 1:99); $[\alpha]_D^{24}$ = +7.3 (*c* 0.81, MeOH); IR (neat, ν, cm^{-1}) 3441−3450 (b); ^1H NMR (300 MHz, D_2O) δ 2.95 (dd, *J* = 12.0, 5.8 Hz, 1H), 3.39 (dd, *J* = 12.0, 6.5 Hz, 1H), 3.70 (s, 2H), 3.72 (ABq, *J* = 12.2 Hz, 2H), 4.09 (d, *J* = 5.0 Hz, 1H), 4.28 (ddd, *J* = 6.5, 5.8, 5.0 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 48.1, 59.8, 61.5, 68.2, 75.6, 78.4; MS (ESI) *m*/*z* = 164 (MH^+); HRMS calculated for $C_6H_{13}NO_4$ (MH^+) 164.0923, found 164.0927.

(3*R*,4*S*,5*S*)-2,2-Bis(hydroxymethyl)piperidine-3,4,5-triol (2c). Reaction of **7c** (300 mg, 0.65 mmol) with TFA−water (4 mL, 3:1) followed by hydrogenation as described for **2b** gave **2c** (115 mg, 90% over two steps) as semisolid: R_f 0.40 (25% aq NH_4OH/MeOH, 1/99); $[\alpha]_D^{27}$ = +8.0 (*c* 2058, MeOH); IR (neat, ν, cm^{-1}) 3439 (br); ^1H NMR (300 MHz, D_2O) δ 2.90 (dd, *J* = 13.5, 4.1 Hz, 1H), 2.98 (dd, *J* = 13.5, 7.1 Hz, 1H), 3.61 (d, *J* = 11.7 Hz, 1H), 3.69 (d, *J* = 11.8 Hz, 1H), 3.72 (d, *J* = 11.7 Hz, 1H), 3.81 (d, *J* = 3.0 Hz, 1H), 3.82−3.90 (m, 1H), 3.95 (d, *J* = 11.8 Hz, 1H), 4.01 (t, *J* = 3.0 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 42.0, 60.5 (2C), 60.8, 67.9, 69.1, 69.4; MS (ESI) *m*/*z* = 194 (MH^+); HRMS calculated for $C_7H_{15}NO_5$ (MH^+) 194.1028, found 194.1028.

(3*R*,4*S*)-2, 2-Bis(hydroxymethyl)pyrrolidine-3,4-diol (1c). Reaction of **7c** (500 mg, 1.09 mmol) with TFA−water (4 mL, 3:1) followed by $NaIO_4$ and hydrogenation as described for **1b** gave **1c** (135 mg, 75% over three steps) as a semisolid: R_f 0.42 (25% aq NH_4/MeOH, 1/99); $[\alpha]_D^{25}$ = +15.1 (*c* 0.30 MeOH); IR (KBr, ν, cm^{-1}) 3348 (br); ^1H NMR (300 MHz, D_2O) δ 3.37 (dd, *J* = 12.6, 3.0 Hz, 1H), 3.47 (dd, *J* = 12.6, 4.4 Hz, 1H), 3.76 (d, *J* = 12.0 Hz, 1H), 3.79 (d, *J* = 12.4 Hz, 1H), 3.87 (d, *J* = 12.0 Hz, 1H), 3.99 (d, *J* = 12.4 Hz, 1H), 4.34 (d, *J* = 4.7 Hz, 1H), 4.46−4.56 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 48.5, 58.5, 60.1, 69.6, 70.1, 72.2; MS (ESI) *m*/*z* = 164 (MH^+); HRMS calculated for $C_6H_{13}NO_4$ (MH^+) 164.0923, found 164.0927.

Synthesis of Dichloro Alcohols (5b). Reaction of **4b** (5.10 g, 12.81 mmol) with LDA as described for **5a** for 6 h gave dichloro alcohols **5b** (5.50 g, 89%) as inseparable mixture of diastereomers (dr = 2:3) as evaluated by the ^1H NMR analysis of crude reaction mixture: R_f 0.50 (petroleum ether/EtOAc, 8:2); IR (KBr, ν, cm^{-1}) 3435 (st), 1080, 736, 698; Partial interpretation of δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.50 (s, 3H), 3.80−4.20 and 4.30−4.75 (multiple signal, 9H), 4.40 (bs, exchangeable with D_2O, 1H), 5.96 (d, *J* = 3.4 Hz, 1H), 6.09 (s, 1H), 7.06−7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.6, 72.12, 73.7, 74.4, 75.2, 75.8, 79.5, 81.4, 84.8, 103.6, 111.9, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.5, 128.6, 135.8, 137.8; For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.49 (s, 3H), 3.80−4.20 and 4.30−4.75 (multiple signal, 9H), 5.29 (bs, exchangeable with D_2O, 1H), 5.98 (d, *J* = 3.8 Hz, 1H), 6.19 (s, 1H), 7.06−7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 26.9, 70.8, 72.08, 72.12, 75.0, 77.4, 78.0, 81.6, 84.7, 104.0, 112.2, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.5, 128.6, 136.1, 137; MS (ESI-, *m*/*z*) 483.01 (MH+); LCMS (MH + Na) = 507. Anal. Calculated For $C_{24}H_{28}Cl_2O_6$: C, 59.63; H, 5.84. Found: C, 59.27; H, 6.12.

Synthesis of Azido Aldehydes (6b). Reaction of **5b** (3.30 g, 6.84 mmol) with sodium azide as described for **6a** gave azido aldehydes **6b** (2.87 g, 92%) as inseparable mixture of diastereomers (dr = 2:3) as evaluated by ^1H NMR analysis of crude reaction mixture: R_f 0.50 (petroleum ether/EtOAc = 8:2); IR (neat, ν, cm^{-1}) 2928, 2125, 1730, 1074; Partial interpretation δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, s), 1.45 (3H, s), 3.68−4.06 and 4.24−4.59 (multiple signal, 9H), 5.89 (d, *J* = 3.5 Hz, 1H), 7.19−7.39 (Multiple signal, 10H), 9.66 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.8, 70.3, 72.2, 72.7, 73.6, 81.1, 81.3, 81.9, 105.2, 112.5, 127.5, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 136.3, 137.2, 195.2; For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 3H), 1.48 (s, 3H), 3.80−4.06 and 4.24−4.49 (multiple signal, 9H), 5.96 (d, *J* = 3.8 Hz, 1H), 7.19−7.39 (Multiple signal, 10H), 9.60 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 26.9, 69.7, 70.7, 73.7, 76.6, 81.7, 81.9, 82.1, 105.0, 112.3, 127.5, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 136.2, 137.1, 196.2. Anal. Calculated For $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.58; H, 6.36; N, 8.95.

Synthesis of Azido Alcohols (7b). Reaction of **6b** (2.03 g, 4.48 mmol) as described for **7a** gave azido alcohols **7b** (1.90 g, 93%) as inseparable mixture of diastereomers (dr = 2:3) as evaluated by ^1H NMR analysis of crude reaction mixture: R_f 0.33 (petroleum ether/EtOAc, 8:2); IR (neat, ν, cm^{-1}) 3454−3487 (br), 2117, 1076, 1028, 738; Partial interpretation δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, s), 1.47 (3H, s), 2.75 (t, *J* = 6.7 Hz, exchangeable with D_2O, 1H) 3.65−4.03 and 4.28−4.66 (multiple signal, 11H), 5.92 (d, *J* = 3.5 Hz, 1H), 7.25−7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.7,63.5, 65.8, 71.3, 72.0, 73.6, 79.9, 81.5, 82.1, 104.4, 111.9, 127.6, 127.8, 128.1, 128.2, 128.4, 128.6, 136.5, 137.4; For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, s), 1.50 (3H, s), 2.64 (t, *J* = 6.7 Hz, exchangeable with D_2O, 1H) 3.65−4.03 and 4.28−4.66 (multiple signal, 11H), 5.92 (d, *J* = 3.5 Hz, 1H), 7.25−7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.8, 63.8, 66.4, 71.3, 71.8, 73.6, 80.3, 81.6, 82.1, 104.3, 112.0, 127.6, 127.8, 128.1, 128.2, 128.4, 128.6, 136.5, 137.4; MS (ESI-, *m*/*z*) 455.88 (M^+); LCMS (M+Na) = 478. Anal. Calculated For $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.49; H, 6.70; N, 8.98.

Synthesis of Dichloro Alcohols (5c). Reaction of **4c** (5.50 g, 13.88 mmol) as described for **5a** gave dichloro alcohols **5c** (6.67 g, 91%) as a inseparable mixture of diastereomer (2:3) as evaluated by ^1H NMR analysis of crude reaction mixture: R_f 0.50 (petroleum ether/EtOAc, 3:1); IR (KBr, ν, cm^{-1}) 3452 (br), 1026, 1105, 738, 698; Partial interpretation of δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.57 (s, 3H), 3.47 (s, exchangeable with D_2O, 1H), 3.80 (ABq, *J* = 10.2 Hz, 2H), 4.16 (dd, J = 4.0, 4.0 Hz, 1H), 4.36−4.77 (multiple signal, 6H), 5.70 (d, *J* = 3.5 Hz, 1H), 5.95 (s, 1H), 7.28 −7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7, 26.9, 69.2, 71.9, 73.8, 75.2, 76.1, 77.5, 77.7, 78.4, 104.1, 113.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 137.1, 137.5; For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.56 (3H, s), 3.53 (s, exchangeable with D_2O, 1H), 3.81 (ABq, *J* = 10.2 Hz, 2H), 4.08 (dd, J = 4.3, 4.3 Hz, 1H), 4.36−4.77 (multiple signal, 6H), 5.74 (d, *J* = 3.6 Hz, 1H), 6.09 (s, 1H), 7.28 −7.38 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.8, 27.0, 67.0, 72.1, 73.7, 75.7, 76.0, 77.3, 77.8, 78.8, 104.0, 113.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 137.2, 137.3; LCMS (MH+Na) = 507. Anal. Calculated For $C_{24}H_{28}Cl_2O_6$: C, 59.63; H, 5.84. Found: C, 59.85; H, 6.10.

Synthesis of Azido Aldehydes (6c). Reaction of **5c** (4.00 g, 8.29 mmol) with sodium azide as described for **6a** gave azido-aldehydes **6c** (3.38 g, 90%) as inseparable mixture of diastereomers (2:3) as evaluated by ^1H NMR analysis of crude reaction mixture: R_f 0.50 (petroleum ether/EtOAc, 8:2); IR (KBr, ν, cm^{-1}) 2993, 2987, 2933, 2127, 1741; Partial interpretation δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, s), 1.56 (s, 3H), 3.60−4.12 and 4.30−4.72 (multiple signal, 9H), 5.72 (d, *J* = 3.5 Hz, 1H), 7.24−7.36 (Multiple signal, 10H), 9.61 (s,1H,); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.3, 26.8, 71.0, 72.3, 73.2, 73.7, 77.0, 77.2, 77.8, 104.6, 113.6, 127.6, 127.7, 127.9, 128.2, 128.3, 128.5, 136.7, 136.9, 195.6; For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, s), 1.56 (3H, s), 3.60−4.12 and 4.30−4.72 (multiple signal, 9H), 5.70 (d, *J* = 3.5 Hz, 1H), 7.24−7.36 (Multiple signal, 10H), 9.48 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.5, 26.8, 69.5, 72.2, 73.1, 73.7, 76.6, 77.4, 77.7, 104.3, 113.6, 127.6, 127.7, 127.9, 128.2, 128.3, 128.5, 136.7, 136.9, 196.7. Anal. Calculated For $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.87; H, 6.31; N, 9.53.

Synthesis of Azido Alcohols (7c). Reaction of **6c** (2.20 g, 4.85 mmol) with $NaBH_4$ as described for **7a** gave azido-alcohols 7c (2.08 g, 94%) as inseparable mixture of diastereomers (2:3) as evaluated by ^1H NMR analysis of crude reaction mixture: R_f 0.33 (petroleum ether/EtOAc, 8:2); IR (neat, ν, cm^{-1}) 3489−3508 (br), 2115; Partial interpretation δ_H and δ_C; For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.56 (s, 3H), 2.69 − 2.73 (m, exchangeable with D_2O, 1H), 3.51−4.19 and 4.46−4.80 (multiple signal, 11H), 5.74 (d, *J* = 3.8 Hz, 1H), 7.26−7.37 (Multiple signal,

dx.doi.org/10.1021/jo3009534 | *J. Org. Chem.* XXXX, XXX, XXX−XXX

10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 26.9, 63.9, 66.1, 70.1, 72.2, 73.6, 77.2, 77.7, 79.4, 104.0, 113.3, 127.5, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 136.9, 137.4; For minor diastereomer, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.60 (s, 3H), 2.69 − 2.73 (m, exchangeable with D_2O, 1H), 3.51−4.19 and 4.46−4.80 (multiple signal, 11H), 5.79 (d, J = 3.8 Hz, 1H), 7.26−7.37 (Multiple signal, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 26.9, 62.1, 66.5, 66.9, 72.1, 73.5, 77.1, 77.3, 79.5, 104.2, 113.3, 127.5, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 136.3, 137.6; LCMS (M+Na) = 478. Anal. Calculated For $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.46; H, 6.68; N, 9.50.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra of compounds **4a**, **5a−c**, **6a−c**, **7a−c**, **8a−c**, **2a−c**, and **1b−c** as well as crystallographic data (CIF) of compound **5a** and **8a**, glycosidase inhibition assay, Lineweaver−Burk plots and docking tables/plots. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ddd@chem.unipune.ac.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are thankful to Department of Science and Technology, New Delhi (Project File No. SR/S1/OC-20/2010) for providing financial support. Authors N.J.P. and S.T.C. are thankful to UGC and CSIR, New Delhi for providing the Senior Research Fellowship.

■ REFERENCES

(1) Asano, N.; Oseki, K.; Kiuz, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701.

(2) (a) Bols, M.; Lillelund, V. H.; Jensen, H. H.; Liang, X. *Chem. Rev.* **2002**, *102*, 515. (b) Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750. (c) Stutz, A. E. *Iminosugars as Glycosidase Inhibitors, Nojirimycin and Beyond*; Wiley-VCH: Weinheim, 1999. (d) See the recent book on iminosugars: Compain, P., Martin, O. R., Eds.; *Imunosugars: From Synthesis to Therapeutic Applications*; Wiley: New York, 2007. (e) Blanco, M. J.; Sardina, F. J. *J. Org. Chem.* **1998**, *63*, 3411. (f) Burley, I.; Hewson, A. T. *Tetrahedron Lett.* **1994**, *35*, 7099. (g) Bols, M. *Tetrahedron Lett.* **1996**, *37*, 2097. (h) Pawar, V. U.; Chavan, S. T.; Sabharwal, S. G.; Shinde, V. S. *Bioorg. Med. Chem.* **2010**, *18*, 7799. (i) Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036. (j) Simone, M. I.; Soengas, R. G.; Jenkinson, S. F.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2012**, *23*, 401. (k) Dragutan, I.; Dragutan, V.; Demonceau, A. *RSC Adv.* **2012**, *2*, 719. (l) Moreno-Clavijo, E.; Carmona, A. T.; Moreno-Vargas, A. J.; Molina, L.; Robina, I. *Curr. Org. Synth.* **2011**, *8*, 102. (m) Dragutan, I.; Dragutan, V.; Mitan, C.; Vosloo, H. C. M.; Delaude, L.; Demonceau, A. *Beilstein J. Org. Chem.* **2011**, *7*, 699. (n) Oulaidi, F.; Front-Deschamps, S.; Gallienne, E.; Lesellier, E.; Ikeda, K.; Asano, N.; Compain, P.; Martin, O. R. *ChemMedChem* **2011**, *6*, 353. (o) Wardrop, D. J.; Waidyarachchi, S. L. *Nat. Prod. Rep.* **2010**, *27*, 1431. (p) Estévez, A. M.; Soengas, R. G.; Otero, J. M.; Estévez, J. C.; Nash, R. J.; Estévez, R. J. *Tetrahedron: Asymmetry* **2010**, *21*, 21. (q) Cipolla, L; Araújo, A. C.; Bini, D.; Gabrielli, L.; Russo, L.; Shaikh, N. *Expert Opin. Drug Discovery* **2010**, *5*, 721. (r) Compain, P.; Decroocq, C.; Iehl, J.; Holler, M.; Hazelard, D.; Barragán, T. M.; Mellet, C. O.; Nierengarten, J.-F. *Angew. Chem., Int. Ed.* **2010**, *49*, 5753. (s) Davis, B. G. *Tetrahedron: Asymmetry* **2009**, *20*, 652. (t) Compain, P.; Chagnault, V.; Martin, O. R. *Tetrahedron: Asymmetry* **2009**, *20*, 672. (u) Winchester, B. G. *Tetrahedron: Asymmetry* **2009**, *20*, 645. (v) Luo, B.; Marcelo, F.; Desir, J.; Zhang, Y.; Sollogoub, M.; Kato, A.; Adachi, I.; Canada, F. J.; Jimenez-Barbero, J.; Bleriot, Y. *J. Carbohydr. Chem.* **2011**, *30*, 641.

(3) (a) Asano, N.; Yamauchi, T.; Kagamifuchi, K.; Shimizu, N.; Takahashi, S.; Takatsuka, H.; Ikeda, K.; Kizu, H.; Chuakul, W.; Kettawan, A.; Okamoto, T. *J. Nat. Prod.* **2005**, *68*, 1238. (b) Asano, N.; Nishida, M.; Kato, A.; Kizu, H.; Matsui, K.; Shimada, Y.; Itoh, T.; Baba, M.; Watson, A. A.; Nash, R. J.; Lilley, P. M.; de., Q.; Watkin, D. J.; Fleet, G. W. J. *J. Med. Chem.* **1998**, *41*, 2565. (c) Ye, X.-S.; Sun, F.; Liu, M.; Li, Q.; Wang, Y.; Zhang, G.; Zhang, L.-H.; Zhang, X.-L. *J. Med. Chem.* **2005**, *48*, 3688.

(4) (b) Ostrowski, J.; Altenbach, H.-J.; Wischnat, R.; Brauer, D. J. *Eur. J. Org. Chem.* **2003**, 1104. (b) Schuster, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 615.

(5) Gupta, P.; Vankar, Y. D. *Eur. J. Org. Chem.* **2009**, 1925.

(6) (a) Schieweck, F.; Altenbach, H. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3409. (b) Otero, J. M.; Soengas, R. G.; Estévez, J. C.; Estévez, R. J.; Watkin, D. J.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *Org. Lett.* **2007**, *9* (4), 623.

(7) (a) Otero, J. M.; Estévez, A. M.; Soengas, R. G.; Estévez, J. C.; Nash, R. J.; Fleet, G. W. J.; Estévez, R. J. *Tetrahedron: Asymmetry.* **2008**, *19*, 2443. (b) Soengas, R. G.; Estévez, A. M. *Eur. J. Org. Chem.* **2010**, 5190.

(8) (a) Dhavale, D. D.; Markad, S. D.; Karanjule, N. S.; PrakashaReddy, J. *J. Org. Chem.* **2004**, *69*, 4760. (b) Patil, N. T.; Tilekar, J. N.; Dhavale, D. D. *J. Org. Chem.* **2001**, *66*, 1065. (c) Markad, S. D.; Karanjule, N. S.; Sharma, T.; Sabharwal, S. G.; Dhavale, D. D. *Bioorg. Med. Chem.* **2006**, *14*, 5535. (d) Matin, M. M.; Sharma, T.; Sabharwal, S. G.; Dhavale, D. D. *Org. Biomol. Chem.* **2005**, *3*, 1702. (e) Dhavale, D. D.; Matin, M. M.; Sharma, T.; Sabharwal, S. G. *Bioorg. Med. Chem.* **2004**, *12*, 4039. (f) Dhavale, D. D.; Ajish Kumar, K. S.; Chaudhari, V. D.; Sharma, T.; Sabharwal, S. G.; PrakashaReddy., J. *Org. Biomol. Chem.* **2005**, *3*, 3720. (g) Ajish Kumar, K. S.; Chaudhari, V. D.; Puranik, V. G.; Dhavale, D. D. *Eur. J. Org. Chem.* **2007**, *29*, 4895. (h) Mane, R. S.; Ajish Kumar, K. S.; Dhavale, D. D. *J. Org. Chem.* **2008**, *73*, 3284. (i) Jabgunde, A. M.; Kalamkar, N. B.; Chavan, S. T.; Sabharwal, S. G.; Dhavale, D. D. *Bioorg. Med. Chem.* **2011**, *19*, 5912 and references cited therein. (j) Sanap, S. P.; Ghosh, S.; Jabgunde, A. M.; Pinjari, R. V.; Gejji, S. P.; Singh, S.; Chopade, B. A.; Dhavale, D. D. *Org. Biomol. Chem.* **2010**, *8*, 3307. (k) Karanjule, N. S.; Markad, S. D.; Sharma, T.; Sabharwal, S. G.; Puranik, V. G.; Dhavale., D. D. *J. Org. Chem.* **2005**, *70*, 1356.

(9) (a) Reeve, W. *Synthesis* **1971**, *3*, 131. (b) Corey, E. J.; Link, J. O. *Tetrahedron lett.* **1992**, *33*, 3431. (c) Corey, E. J.; Link, J. O. *J. Am. Chem. Soc.* **1992**, *114*, 1906. (d) Snowdem, T. S. *ARKIVOC* **2012**, 24.

(10) (a) Sato, K.; Kajihara, Y.; Nakamura, Y.; Yoshimura, J. *Chem. Lett.* **1991**, 1559. (b) Sato, K.; Suzuki, K.; Ueda, M.; Kajihara, Y.; Hori, H. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 225. (c) Sato, K.; Sekiguchi, T.; Hozumi, T.; Yamazaki, T.; Akai, S. *Tetrahedron Lett.* **2002**, *43*, 3087. (d) Yoshikawa, M.; Yokokawa, Y.; Okuno, Y.; Murakami, N. *Tetrahedron* **1995**, *51* (22), 6209. (e) Sarda, P.; Olesker, A.; Lukacs, G. *Tetrahedron* **1997**, *53* (15), 5493. (f) Sato, K.; Akai, S.; Shoji, H.; Sugita, N.; Yoshida, S.; Nagai, Y.; Suzuki, K.; Nakamura, Y.; Kajihara, Y.; Funabashi, M.; Yoshimura, J. *J. Org. Chem.* **2008**, *73*, 1234. (g) Cafiero, L. R.; Snowden, T. S. *Org. Lett.* **2008**, *10*, 3853. (h) Sorensen, M. H.; Nielsen, C.; Nielsen, P. *J. Org. Chem.* **2001**, *66*, 4878.

(11) (a) Yu, H.; Cao, H.; Tiwari, V. K.; Li, Y.; Chen, X. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5037. (b) Hanaya, T.; Sugiyama, K -i.; Kawamoto, H.; Yamamoto, H. *Carbohydr. Res.* **2003**, *338*, 1641. (c) Malik, S.; Dixit, V. A.; Bharatam, P. V.; Kartha, K. P. R. *Carbohydr. Res.* **2010**, *345*, 559.

(12) From the ^{1}NMR spectrum of crude product **5b**/**5c**, the diastereomeric ratio of C-5 epimeric compounds was found to be ∼2:3. Our attempts to separate diastereomeric mixture using column/flash chromatography were unsuccessful. As the C-5 stereocenter is destroyed in the final product to get geminal-dihydroxymethyl group,

we continued with the diastereomeric mixture of **5b** and **5c** for further steps.

(13) Sali, A.; Blundell, T. L. *J. Mol. Biol.* **1993**, *234*, 779. (b) Laskowski, R. A.; MacArthur, M. W.; Moss, D .S.; Thornton, J. M. *J. Appl. Crystallogr.* **1993**, *26*, 283. (c) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.

(14) Vogel, A. I., Tatchell, A. R., Furnis, B. S., Hannaford, A. J., Smith, P. W. G. Vogel's Textbook of Practical Organic Chemistry (5th Edition), Addison-Wesley, 1989.