Bioorganic & Medicinal Chemistry 19 (2011) 6720–6725

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of anomeric 1,5-anhydrosugars as conformationally locked selective α-mannosidase inhibitors

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ARTICLE INFO

Article history:
Received 2 September 2011
Revised 23 September 2011
Accepted 24 September 2011
Available online 29 September 2011

Keywords:
Anomeric anhydrosugars
α-Mannosidase inhibitors
D-Glucose

ABSTRACT

Anomeric 1,5-anhydrosugar **2** was synthesized from D-glucose derived *N*-Cbz protected aminodiol **8**. The key step involves, acid catalyzed hydrolysis of 1,2-acetonide group in **8** to get hemiacetal that concomitantly undergoes formation of the pyranose ring by attack of C-3 hydroxyethyl group on anomeric C-1, leading to the formation of dioxabicyclo[3.2.1]octane skeleton which on hydrogenolyis gave **2**. The glycosidase inhibitory activities of hydroxy- and amino-substituted anomeric 1,5-anhydrosugars **1** and **2**, respectively, showed selective inhibition of α-mannosidase. These results were substantiated by molecular docking studies using WHAT IF software and AUTODOCK 4.0 program.

1. Introduction

Anomeric anhydrosugars are sugar derivatives obtained by intramolecular elimination of water molecule from arbitrary hydroxyl group and anomeric hydroxyl group of sugar.[1] Among these, the anomeric 1,6-anhydrosugar levomannosan **I** (Fig. 1) is obtained from the D-mannose wherein the $^4C_1$ conformation of the D-mannose is converted to the $^1C_4$ conformation along with unfavorable axial rich hydroxyl groups that are stabilized through hydrogen bonding. Such type of bicyclic anomeric anhydrosugars render glycosidase inhibitory activity and form a unique class of conformationally locked inhibitors. For example, Vasella and co-workers[2] reported the synthesis of polyhydroxylated isoquinuclidines **II** which found to be selective inhibitor of a retaining β-mannosidase. Similarly, Chandrasekaran and co-workers[3] demonstrated (by docking studies) that the thiolevomannosan **III** and its sulfoxide and sulfone analogs showed more potent α-mannosidase inhibitory activity than well-known inhibitor kifunensine due to the axial rich $^1C_4$ chair conformation. Thus, the conformationally locked bicyclic anhydrosugars are rationale for the design of promising α-mannosidase inhibitors that are involved in the N-glycosylation pathway[4] by trimming $Glc_3Man_9GlcNAc_2$ moieties in human ER. As a result, discovery of a locked anhydrosugar as a potent therapeutic agent is a challenge in the search for the treatment of diseases such as diabetes and cancer[5] which are associated with the glycosidic bond cleavage.

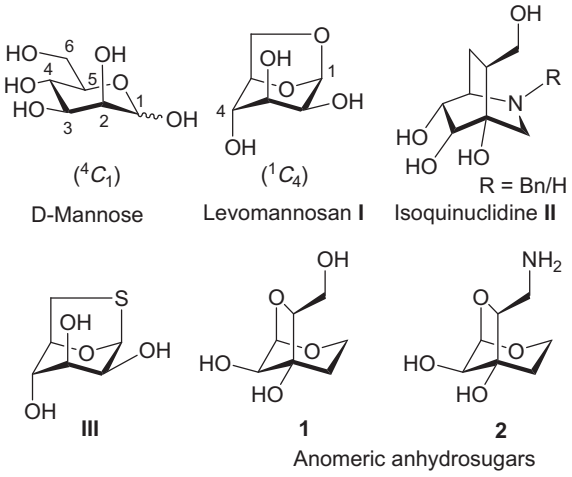

Figure 1. Potent mannosidase inhibitors (**II** and **III**) and anomeric anhydrosugars (**1** and **2**).

Recently, we have developed a synthetic strategy for the synthesis of anomeric 1,5-anhydrosugar **1** from D-glucose (Scheme 1) and demonstrated its utility for the synthesis of carbohydrate core of amipurimycin analog.[6] It was anticipated that the bicyclic and conformationally locked anomeric 1,5-anhydrosugar **1** will exhibit potent glycosidase inhibitory activity. Therefore, as a part of our continuing efforts in the sugar chemistry[7], we planned the synthesis of amino-substituted 1,5-anhydrosugar analog **2** by short and efficient route and studied glycosidase inhibitory activity

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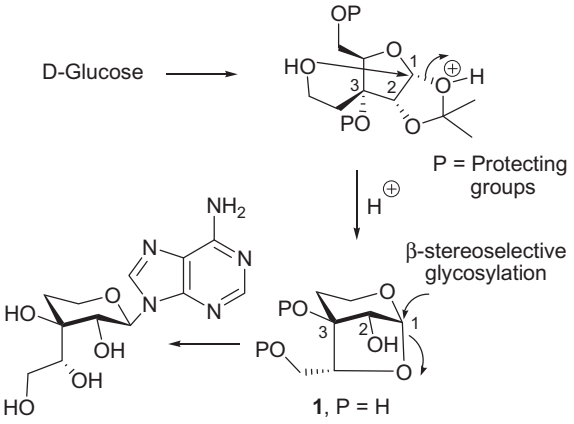
doi:10.1016/j.bmc.2011.09.046



Scheme 1. Synthesis of anomeric anhydrosugar **1**.

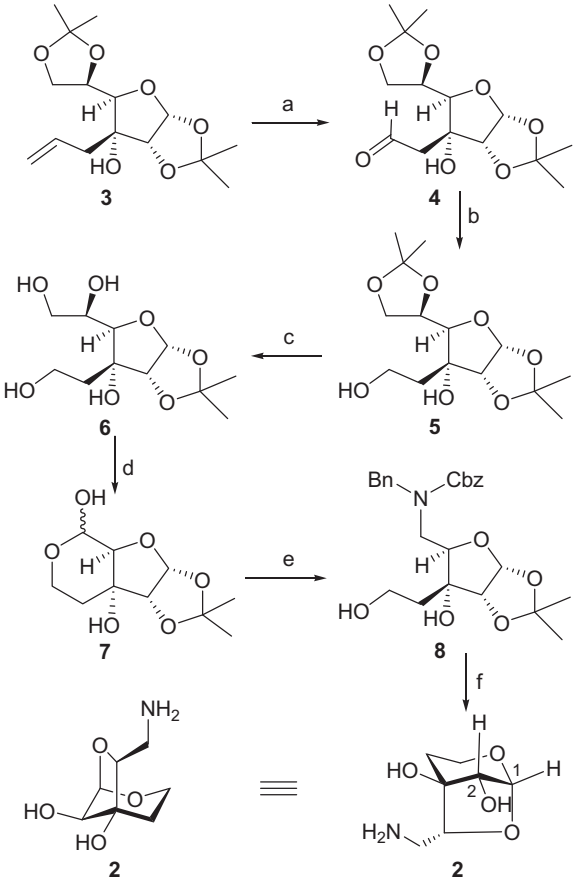
of **1** and **2**. It was found that amino-substituted 1,5-anhydrosugar analogue **2** is more potent than **1**. The results obtained are substantiated by the molecular docking studies.

2. Result and discussion

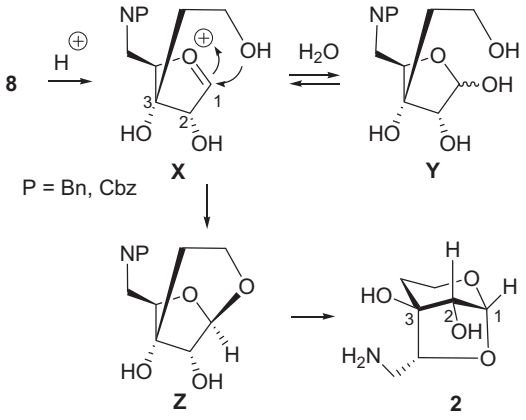
As shown in Scheme 2, the required homoallyl alcohol **3** was prepared from the D-glucose in grams quantity as reported earlier by us.[6] Dihydroxylation of the double bond in **3** using potassium osmate (5 mol %) and NMO followed by oxidative cleavage of the intermediate diol with $NaIO_4$ gave aldehyde **4**.[7a] Reduction of **4** using $NaBH_4$ gave diol **5** in 82% yield. Selective 5,6-acetonide deprotection in **5** using 30% $HClO_4$ in THF gave tetrol **6** that on treatment with sodium metaperiodate afforded **7** as a mixture of hemiacetal. Intermolecular reductive amination of **7** using benzylamine and sodium cyanoborohydride in methanol followed by treatment with benzyloxycarbonyl chloride and sodium bicarbonate in methanol–water afforded *N*-Cbz protected aminodiol **8**. Treatment of **8** with TFA–water and subsequent hydrogenation using 10% Pd/C, H_2 (80 psi) in methanol afforded amino-substituted 1,5-anomeric anhydrosugar **2**.

The formation of 1,5-anhydrosugar **2** can be explained as follows. We believe that, under acidic conditions, opening of the 1,2-acetonide functionality in **8** results in generation of an oxocarbenium ion **X**, which could be attacked either reversibly by water to give rise to a hemiacetal **Y** or irreversibly by the primary alcohol to yield 1,5-anhydrosugar **Z**. We suggest that the attack of the primary alcohol to the C-1 position of the oxocarbenium ion **X** in an intramolecular and irreversible manner gives rise to a stable pyranose ring shifting the equilibrium in favor of the bridged bicyclic system **Z** that on hydrogenolysis leads to **2**.

The structure and conformation of **2** was established by the ^1H NMR wherein, the chemical shift assignments and coupling constant values were obtained from the decoupling experiments. In the ^1H NMR spectrum of **2**, the H-1 and H-2 protons appeared as two singlets at δ 5.27 and 3.78, respectively. The absence of vicinal coupling constant between the H-1 and H-2 requires the dihedral angle between these protons to be approximately 90°. The absolute configuration (*R*), at C-2 in the substrate **8** is retained in the bicyclic system **2** with the axial orientation of the H-2 in pyranose ring therefore; the H-1 was assigned the equatorial orientation with $^4C_1$ conformation. This is in agreement with the attack of the C-3 hydroxyethyl group to the anomeric carbon C-1 (Scheme 3) forcing the H-1 into the equatorial position. The little twist in the $^4C_1$ conformation, due to the bridged system, makes the dihedral angle between the H-1 and H-2 close to 90° accounting for the absence of a vicinal coupling constant.



Scheme 2. Reagents and conditions: (a) (i) cat. $K_2OsO_4{\cdot}2H_2O$, NMO, acetone–water (8:1), rt, 24 h; (ii) $NaIO_4$, acetone–water (9:1), 0 °C–rt, 2 h, 80% for two steps; (b) NaBH4, THF–water (4:1), 0 °C, 45 min, 82%; (c) 30% $HClO_4$, THF, 0 °C, 2.5 h, 87%; (d) $NaIO_4$, acetone–water (9:1), 0 °C–rt, 2 h, 80%; (e) (i) $BnNH_2$, AcOH, $NaCNBH_3$, MeOH, −78 °C, 2 h, (ii) CbzCl, $NaHCO_3$, MeOH, 0 °C–rt, 6 h, 85% for two steps; (f) (i) TFA–H_2O (3:1), 0 °C–rt, 3 h; (ii) 10% Pd/C, H_2 (80 psi), MeOH, 24 h, 72% for two steps.



Scheme 3. Formation of anomeric anhydrosugar **2**.

2.1. Glycosidase inhibitory activity

Glycosidase inhibitory activity of **1** and **2** was studied with different glycosidases.[8] The anomeric 1,5-anhydrosugars **1** and **2** showed no inhibition against α-galactosidase (E.C. 3.2.1.22), α-glucosidase (E.C. 3.2.1.20) and α-amylase (E.C. 3.2.1.1). However, both anhydrosugars **1** and **2** were found to be selective inhibitors against jack bean α-mannosidase (E.C. 3.2.1.24) with IC_{50} =

133.18 μM and IC_{50} = 98.32 μM, respectively. The inhibitory activity of anhydrosugars **1** and **2** were found to be weak as compared to the known α-mannosidase inhibitor swainsonine[9] (IC_{50} = 0.1–0.5 μM) and thiolevomannosan **III** (IC_{50} = 1.38 μM).[3] However, compounds **1** and **2** showed stronger inhibition as compared to the isoquinuclidine (R = Bn, IC_{50} = 20 mM; R = H, IC_{50} = 9.6 mM).[2] The selective α-mannosidase activity in micromolar range prompted us to substantiate our results with molecular docking.

2.2. Molecular docking

In order to understand the interactions of compounds **1** and **2** with the amino acid residues of mannosidase, we performed the molecular docking studies. The mannosidases are lectin binding proteins and *N*-glycans of α-mannosidase have unique topologies, important functions in protein folding and oligomerization or enzyme activity.[10] Glycosidase inhibitory activity of **1** and **2** showed their efficiency as selective α-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, we have chosen human mannosidase (PDB: 1FO3) as the target structure to perform the molecular docking studies. It could be used to predict the binding efficiency of compounds **1** and **2** to the human mannosidase. The template structure of human mannosidase is used to dock the ligand **1** and **2** into the binding pocket of mannosidase. This might provide an insight into the interaction of anomeric anhydrosugars **1** and **2** with mannosidase from Jack Bean. The active site was predicted by using WHAT IF software. The AutoDock 4.0 program[11] was used to perform an automatic docking exploration for different conformations of the ligands in the model. The total binding energies for ligand **1** and **2** are given in Table 1.

As shown in Figure 2, interactions were observed between compound **1** and human mannosidase (PDB: 1FO3) with Thr394, Ser464, Glu397, Ala460, and Leu522. Ligand **1** forms total nine hydrogen bonds with the active site residues. Autodock binding free energy for ligand **1** is −7.79 kcal/mol. The interactions between ligand **2** and human mannosidase (PDB: 1FO3) are given in the Figure 3. For ligand **2**, interactions were observed with Phe329, Leu525, Thr394, Ser400, Glu397, Ala396, Thr688, Asp463, Ser464, and Glu689.

Ligand **2** forms total 15 hydrogen bonds with the active site residues. Autodock binding free energy for ligand **2** is −6.85 kcal/mol, suggesting the strong binding to the active pocket of enzyme. The higher binding affinity for ligand **2** is presumably attributed to the formation of tighter hydrogen bonds between the C-5 amino group and several amino acids at the binding site that allow the lateral protonation. Both **1** and **2** possess high potential binding affinity into the binding site of 3D macromolecule. Docked inhibitors exhibited reasonable RMSD values and the molecular docking studies were found to be in agreement with the biological activity.

Table 1
The total energy (E_{total}), Van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between active site residues of human mannosidase (PDB: 1FO3) and ligand **1/2**

Ligand	Residues	E_{vdw} (kcal mol^{-1})	E_{elec} (kcal mol^{-1})	E_{total} (kcal mol^{-1})
1	Thr394	15.007	92.33	107.337
	Ser464	10.193	75.25	85.443
	Glu397	−11.388	148.06	136.672
	Ala460	15.027	19.03	34.057
	Leu525	−15.804	48.72	32.916
2	Phe329	−11.492	73.32	61.828
	Leu525	−15.804	48.45	32.646
	Thr394	15.011	93.46	108.471
	Ser400	8.223	302.46	310.683
	Glu397	−11.393	148.46	137.067
	Ala396	−9.815	79.26	69.445
	Thr688	−0.179	10.04	9.861
	Asp463	10.629	7.92	18.549
	Ser464	12.708	108.80	121.508
	Glu689	−5.85	7.67	1.820

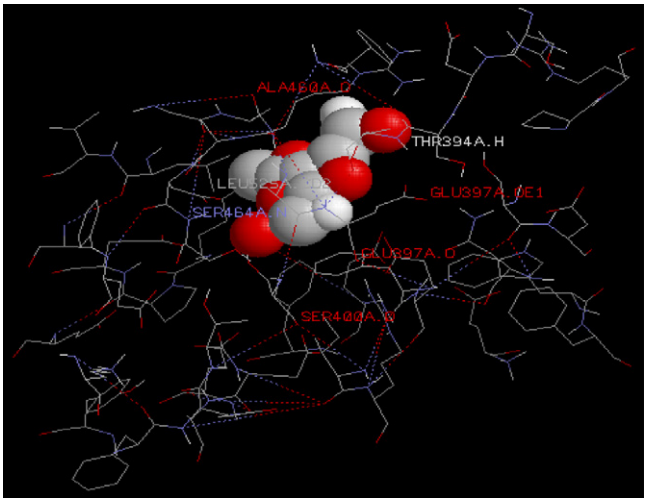


Figure 2. Binding of ligand **1** to the active pocket of apo-mannosidase (PDB: 1FO3); dotted lines represent hydrogen bonds.

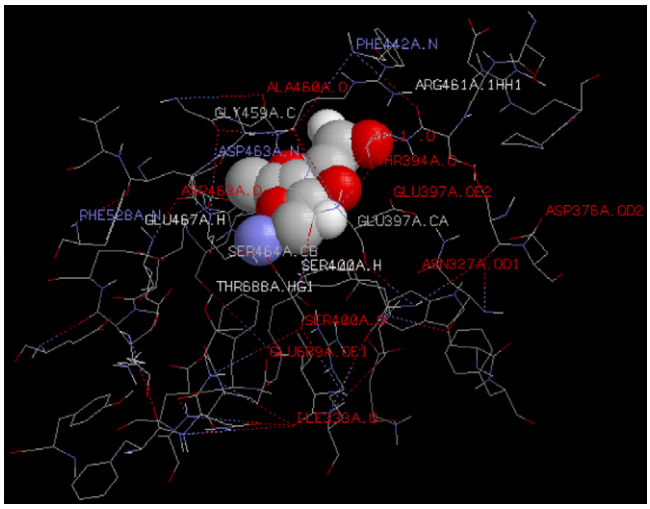


Figure 3. Binding of ligand **2** to the active pocket of apo-mannosidase (PDB: 1FO3); dotted lines represent hydrogen bonds.

3. Conclusions

We have exploited the carbon skeleton of D-glucose to construct the bicyclic and conformationally locked anomeric 1,5-anhydrosugar **1** and **2**. A unique feature of our strategy is the acid catalyzed closing of the pyran ring utilizing a hydroxyethyl residue of the sugar furanose ring in **8** to get the bicyclic ring system **2** with $^4C_1$ conformation. Both the anhydrosugars **1** and **2** were found to be selective α-mannosidase inhibitor. The amino-substituted anomeric 1,5-anhydrosugar **2** showed more potent inhibition than **1**. This fact was supported by molecular docking studies wherein **2** showed strong binding to the active pocket of human mannosidase (PDB: 1FO3) as compared to **1**. Thus, our findings support the fact that the bicyclic and conformationally locked frameworks in

anhydrosugars render a unique property to act as selective mannosidase inhibitors.

4. Experimental section

4.1. General experimental methods

Melting points were recorded with Thomas Hoover melting point apparatus. IR spectra were recorded with Shimadzu FTIR-8400 as a thin film or in nujol mull or using KBr pellets and are expressed in cm^{-1}. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded Varian Mercury 300 using $CDCl_3/D_2O$ as a solvent. Chemical shifts were reported in δ unit (ppm) with reference to TMS as an internal standard and *J* values are given in Hz. Assignments of the signals were confirmed by decoupling experiments. Elemental analyses were carried out with Elemental Analyser Flash 1112. Optical rotations were measured using JASCO P-1020 polarimeter with Sodium light (589.3 nm). Thin layer chromatography was performed on pre-coated 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. Methanol and THF were purified and dried before use. After decomposition of the reaction mixture, the work-up involves extraction with solvent, washing of combined organic layer with water, brine, drying over anhydrous sodium sulfate and evaporation of solvent under reduced pressure below 50 °C.

4.1.1. 1,2:5,6-Di-*O*-isopropylidene-3-*C*-(1′-acetaldehydo)-α-D-allo-1,4-furanose (4)[7a]

To a solution of **3** (5.00 g, 16.66 mmol) in acetone-water (40 mL, 8:1) was added NMO (5.89 g, 33.33 mmol) and potassium osmate (0.03 g, 5 mol %). The reaction mixture was stirred at room temperature for 24 h. Sodium sulphite (2.50 g) was added and stirred for 1 h. Acetone was removed under reduced pressure, residue was extracted with ethyl acetate (20 mL × 4) and concentrated to afford triol as a thick liquid. To the crude triol (4.80 g, 14.37 mmol) in acetone–water (30 mL, 9:1) was added sodium metaperiodate (4.61 g, 21.55 mmol) at 0 °C and stirred for 2 h. Ethylene glycol (1 mL) was added and acetone was evaporated under reduced pressure. Residue was extracted with chloroform (25 mL × 3) and concentrated. Purification by column chromatography (*n*-hexane/ethyl acetate = 9/1) gave **4** (4.0 g, 80% over two steps) as a white solid: mp 108–109 °C; R_f 0.60 (*n*-hexane/ethyl acetate = 1/1); $[\alpha]_D^{25}$ +40.70 (*c* 5.35, $CHCl_3$); IR ($CDCl_3$): 1724 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (3H, s, C*H*$_3$), 1.36 (3H, s, C*H*$_3$), 1.46 (3H, s, C*H*$_3$), 1.59 (3H, s, C*H*$_3$), 2.39 (1H, dd, *J* = 15.6 and 3.3 Hz, *H*-1′a), 2.98 (1H, dd, *J* = 15.6 and 1.5 Hz, *H*-1′b), 3.13 (1H, br s, exchangeable with D_2O, –O*H*), 3.77 (1H, d, *J* = 8.4 Hz, *H*-4), 3.91 (1H, dd, *J* = 8.4 and 5.7 Hz, *H*-6a), 4.02 (1H, td, *J* = 8.4 and 5.7 Hz, *H*-5), 4.19 (1H, dd, *J* = 8.4, 5.7 Hz, *H*-6b), 4.41 (1H, d, *J* = 3.6 Hz, *H*-2), 5.73 (1H, d, *J* = 3.6 Hz, *H*-1), 9.94 (1H, dd, *J* = 3.3 and 1.8 Hz, C*H*O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5 (*C*H$_3$), 26.7 (*C*H$_3$), 26.8 (*C*H$_3$), 27.0 (*C*H$_3$), 45.4 (*C*-1′), 68.3 (*C*-6), 73.5 (*C*-5), 78.8 (*C*-3), 82.0/82.1 (*C*-4/*C*-2), 103.7 (*C*-1), 110.2 (O*C*O), 113.1 (O*C*O), 201.3 (*C*HO); Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.62; H, 7.33. Found: C, 55.81; H, 7.57.

4.1.2. 1,2:5,6-Di-*O*-isopropylidene-3-*C*-(1′-hydroxyethyl)-α-D-allo-1,4-furanose (5)

To an ice-cooled solution of **4** (1.00 g, 3.31 mmol) in THF-water (10 mL, 4:1) was added sodium borohydride (0.14 g, 2.63 mmol) in two portions. Reaction mixture was stirred for 30 min and quenched by adding saturated aq NH_4Cl solution (5 mL). THF was evaporated under reduced pressure, extracted with chloroform (20 mL × 3) and concentrated. Purification by column chromatography (*n*-hexane/ethyl acetate = 3/2) gave **5** (0.82 g, 82%) as a solid: mp 91–93 °C; R_f 0.50 (*n*-hexane/ethyl acetate = 1/1); $[\alpha]_D^{25}$ +22.35 (*c* 0.4, $CHCl_3$); IR (neat): 3600–2900 (broad) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.37 (6H, s, 2 × C*H*$_3$), 1.45 (3H, s, C*H*$_3$), 1.59 (3H, s, C*H*$_3$), 1.50–1.70 (1H, m, *H*-1′a), 2.10–2.21 (1H, m, *H*-1′b), 2.10–3.06 (1H, br s, exchangeable with D_2O, –O*H*), 2.40–2.60 (1H, br s, exchangeable with D_2O, –O*H*), 3.75–3.79 (1H, m, *H*-2′a), 3.88–4.00 (3H, m, *H*-2′b, *H*-6a, *H*-6b), 4.08–4.17 (2H, m, *H*-4, *H*-5); 4.56 (1H, d, *J* = 3.9 Hz, *H*-2), 5.70 (1H, d, *J* = 3.9 Hz, *H*-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.1 (*C*H$_3$), 26.3 (*C*H$_3$), 26.5 (*C*H$_3$), 26.5 (*C*H$_3$), 32.8 (*C*-1′), 58.5 (*C*-2′), 67.7 (*C*-6), 73.0 (*C*-5), 79.8/82.1 (*C*-4/*C*-2), 81.1(*C*-3), 103.4 (*C*-1), 109.6 (O*C*O), 112.6 (O*C*O); Anal. Calcd for $C_{14}H_{24}O_7$: C, 55.25; H, 7.95. Found: C, 55.37; H, 8.04.

4.1.3. 1,2-*O*-Isopropylidene-3-*C*-(1′-hydroxyethyl)-α-D-allo-1,4-furanose (6)

To a solution of **5** (2.00 g, 6.56 mmol) in THF (10 mL) was added 30% $HClO_4$ (2.0 mL) slowly with continuous stirring at 0 °C. Reaction mixture was stirred for 1.5 h at 0 °C and quenched by adding saturated aq K_2CO_3 solution. THF was evaporated under reduced pressure and residue was extracted with $CHCl_3$ (20 mL × 3) and concentrated. Purification by column chromatography (ethyl acetate) gave tetrol **6** (1.51 g, 87%) as a thick liquid. R_f 0.14 (ethyl acetate); $[\alpha]_D^{25}$ +46.88 (*c* 0.5, H_2O); IR (neat): 3600–3000 (broad) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.39 (3H, s, C*H*$_3$), 1.59 (3H, s, C*H*$_3$), 1.76–1.83 (1H, m, *H*-1′a), 2.10–2.18 (1H, m, *H*-1′b), 3.58–3.64 (1H, m, *H*-2′a), 3.75–3.90 (5H, m, *H*-2′b, *H*-6a, *H*-6b, *H*-4, *H*-5), 4.82 (1H, br s, *H*-2), 5.86 (1H, br s, *H*-1); ^{13}C NMR (75 MHz, D_2O) δ 26.3 (*C*H$_3$), 26.4 (*C*H$_3$), 32.8 (*C*-1′), 58.1 (*C*-2′), 64.7 (*C*-6), 69.8 (*C*-5), 79.4/79.6 (*C*-4/*C*-2), 81.2 (*C*-3), 103.2 (*C*-1), 112.5 (O*C*O); Anal. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63. Found: C, 50.10; H, 7.81.

4.1.4. Hemiacetal (7)

To a solution of tetrol **6** (1.50 g, 5.68 mmol) in acetone–water (15 mL, 9:1) was added sodium metaperiodate (1.82 g, 8.52 mmol) at 0 °C and stirred for 2 h. Ethylene glycol (1 mL) was added and acetone was evaporated under reduced pressure. The residue was extracted with chloroform (20 mL × 3) and concentrated. Purification by column chromatography (*n*-hexane/ethyl acetate = 1/1) gave mixture of hemiacetal **7** (1.05 g, 80%) as a viscous oil. IR (neat): 3600–2900 (broad) cm^{-1}; ^1H and ^{13}C NMR spectra showed mixture of hemiacetal; Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.89; H, 7.13.

4.1.5. 1,2-*O*-Isopropylidene-3-*C*-(1′-hydroxyethyl)-5-*N*-benzyl-*N*-benzyloxycarbonyl-α-D-ribo-1,4-furanose (8)[12]

To a solution of benzyl amine (1.03 mL, 9.48 mmol) and glacial acetic acid (0.02 mL) in dry methanol (15 mL) was added solution of **7** (2.00 g, 8.62 mmol) in methanol (15 mL) over a period of 30 min at −20 °C and stirred for 1 h. Sodium cyanoborohydride (1.35 g, 21.55 mmol) was added in three portions (10 min) and solution was warmed to 0 °C and stirred for 2 h. Reaction mixture was quenched by adding saturated aq $NaHCO_3$ solution. Methanol was removed under reduced pressure; residue was extracted with chloroform (25 mL × 3) and concentrated to afford crude amine. To a solution of crude amine (2.78 g, 8.60 mmol) in methanol–water (25 mL, 9:1) at 0 °C was added sodium bicarbonate (2.16 g, 25.82 mmol) and benzyloxycarbonyl chloride (1.83 mL, 12.9 mmol). The reaction mixture was allowed to attain room temperature and stirred for 3 h. Methanol was evaporated under reduced pressure, residue extracted with chloroform (25 mL × 3) and concentrated. Purification by column chromatography (*n*-hexane/ethyl acetate = 1/1) gave **8** (3.35 g, 85% over two steps) as a thick liquid. R_f 0.30 (*n*-hexane/ethyl acetate = 2/3); $[\alpha]_D^{25}$ +113.90 (*c* 1.0, $CHCl_3$); IR ($CDCl_3$): 3525 (broad), 1697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25–1.66 (7H, m, 2 × C*H*$_3$, *H*-2′a), 1.79–1.96 (1H, m, *H*-2′b), 2.75 (1H, br s, exchangeable with D_2O, O*H*), 2.95–3.30 (1H, m, *H*-5a),

3.20 (1H, br s, exchangeable with D_2O, O*H*), 3.60–4.60 (6H, m, *H*-1′a, *H*-1′b, *H*-2, *H*-5b, *N*-C*H*$_2$Ph), 4.75–5.40 (3H, m, *H*-4, NCOOC*H*$_2$Ph), 5.70–5.85 (1H, m, *H*-1), 7.05–7.25 (10H, m, Ar-*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3 (s) (2 × *C*H$_3$), 31.5 (*C*-2′), 45.7 (*C*-5), 51.0 (*N*-*C*H$_2$Ph), 58.3 (NCOOCH$_2$Ph), 67.4 (*C*-1′), 79.6/81.1/82.1 (*C*-2/*C*-4/*C*-3), 103.5 (*C*-1), 112.4 (O*C*O), 127.3 (strong), 127.7, 127.8 (strong), 127.9, 128.4 (strong), 128.5 (strong), 136.3, 137.5 (strong) (Ar-*C*), 156.4 (NCOOCH$_2$Ph); Anal. Calcd for $C_{25}H_{31}NO_7$: C, 65.63; H, 6.83. Found: C, 65.79; H, 6.97.

4.1.6. (1*S*,5*R*,6*R*,8*R*)-5,8-Dihydroxy-6-aminomethyl-2,7-dioxabicyclo[3.2.1]octane (2)[13]

A solution of **8** (0.40 g, 8.74 mmol) in TFA–water (5 mL, 3:1) was stirred for 3 h at 0 °C to rt. TFA was co-evaporated with toluene afforded viscous liquid. To a solution of crude product (0.34 g, 8.51 mmol) in methanol (15 mL) was added 10% Pd/C (0.20 g) and the solution was hydrogenated at 80 psi for 12 h. The catalyst was filtered off, washed with methanol, and the filtrate was concentrated. Purification by column chromatography (methanol/chloroform = 3/2) furnished **2** (0.11 g, 72% for two steps) as a viscous liquid. R_f 0.27 (methanol); $[\alpha]_D^{25}$ +3.23 (*c* 5.0, H_2O); IR (neat): 3500–3000 (broad) cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 1.97 (1H, dd, *J* = 14.1 and 5.4 Hz, *H*-6a), 2.00–2.15 (1H, m, *H*-6b), 3.33 (1H, dd, *J* = 13.5 and 2.4 Hz, *H*-5a), 3.46 (1H, dd, *J* = 13.5 and 10.5 Hz, *H*-5b), 3.78 (1H, s, *H*-2), 3.80–4.00 (2H, m, *H*-7a, *H*-7b), 4.13 (1H, dd, *J* = 10.8 and 2.4 Hz, *H*-4), 5.27 (1H, s, *H*-1); ^{13}C NMR (75 MHz, D_2O) δ 31.3 (*C*-6), 37.5 (*C*-5), 59.0 (*C*-7), 75.6 (*C*-4), 76.2/77.7 (*C*-2/3), 102.5 (*C*-1); Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48. Found: C, 48.20; H, 7.61.

4.1.7. General procedure for glycosidase inhibition assay

Glycosidase inhibition assay of anomeric 1,5-anhydrosugars **1** and **2** was carried out by mixing 0.1 U/cm^3 each of α-galactosidase, α-mannosidase and α-glucosidase with the samples and incubated for 1 h at 37 °C. Enzyme action for α-galactosidase was initiated by addition of 10 mM *p*-nitrophenyl-α-D-galactopyranoside (*p*NPG) as a substrate in 200 mM sodium acetate buffer. The reaction was incubated at 37 °C for 10 min and stopped by adding 2 cm^3 of 200 mM borate buffer of pH 9.8. α-Mannosidase activity was initiated by addition of 10 mM *p*-nitrophenyl-α-D-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 cm^3 of 200 mM borate buffer of pH 9.8. Initiation of α-glucosidase activity was done by addition of 10 mM *p*-nitrophenyl-α-D-glucopyranoside in 100 mM phosphate buffer of pH 6.8 and stopped by adding 2 cm^3 of 0.1 M Na_2CO_3 after an incubation of 10 min at 37 °C. α-Glycosidase activity was determined by measuring absorbance of the *p*-nitrophenol released from *p*NPG at 420 nm using Shimadzu Spectrophotometer UV-1601. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 μM of *p*-nitrophenyl pyranoside per minute under assay condition.

4.1.8. General procedure for α-amylase inhibition assay

Amylase activity was assayed using a modified Bernfeld method (1955) using starch as substrate. 50 μg/cm^3 (O.D. adjusted to 0.4 at 280 nm) of porcine pancreatic α-amylase was incubated with 5 mg/cm^3 samples at 37 °C for 10 min. One percent starch was used as substrate. The samples without α-amylase were used as controls and the test reading were subtracted from the absorbance of these controls. The reducing sugar was estimated using DNSA assay at 540 nm and the enzyme units were expressed as micromolar per minute. One unit of enzyme was defined as the amount of enzyme required to liberate 1 μM of maltose under assay conditions. The final inhibition shown by different samples were compared with the standard inhibitor, acarbose.

4.2. Statistical analysis

The statistical analysis was performed using one way analysis of variance (ANOVA), with a significance (P <0.05). Results are expressed as means ± SEM.

4.2.1. Molecular docking

Interactions of compounds **1** and **2** against amino acid residues of human mannosidase (PDB: 1FO3) were found out employing molecular docking studies. The active site was predicted by using WHAT IF software. AutoDock 4.0 is one of the most widely used docking programs in computational binding studies. It offers a reasonable result in comparison with other methods in computationally intensive calculations. Prior to the AutoDock, AutoGrid was carried out for the preparation of the grid map using a grid box with a npts (number of points in *xyz*) of 60–60–60 Å box and 120–120–120 Å box, which encloses the ligand. The box spacing was 0.3 Å and grid center was designated at dimensions (*x*, *y*, *z*): −4.143, 52.376 and −7.665. A scoring grid was calculated from the ligand structure to minimize the computation time. AutoDock was run using maximum number of retries and generations of 10,000 and 27,000, respectively. The genetic algorithm with local search was used finally for calculation of the docking possibilities. The complexes obtained by AutoDock were minimized using a maximum 300 iterations and the hybrid genetic algorithm with local search runs with max of 250 cycles using different random number seeds to obtain score convergence. VMD[14] was used for visualization of the molecular models generated and for the evaluation of ligand–receptor interactions. 3D structures of ligands **1** and **2** were energetically minimized by using MOPAC 2009 (semi-empirical quantum mechanics).

Acknowledgments

R.S.M. is thankful to the UGC, New Delhi for the senior research fellowship. We are thankful to CSIR, New Delhi (Project No. 01(2343)/09/EMR-II) for the financial support.

Supplementary data

Supplementary data (copies of ^{1}H and ^{13}C NMR spectra of compounds **5**, **6**, **8** and **2**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.046.

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