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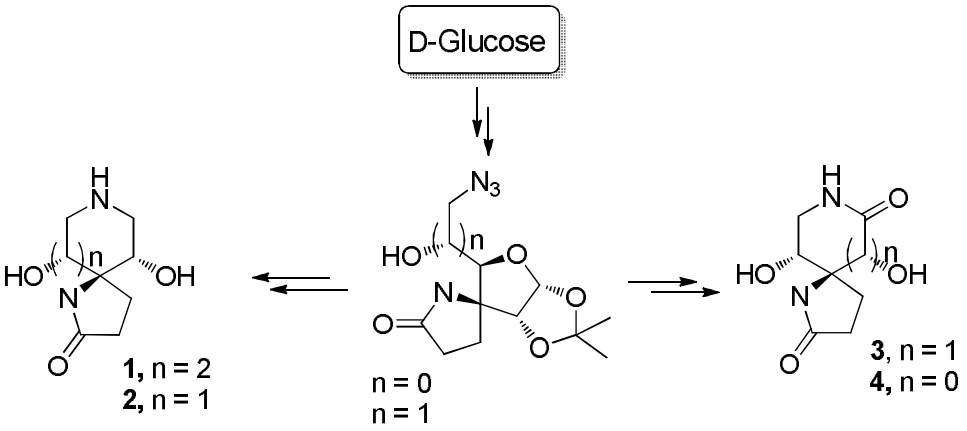
Abstract



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ARTICLE

Diazaspiro-iminosugars and Polyhydroxylated Spiro-bislactams: Synthesis, Glycosidase Inhibitory and Molecular Docking Studies

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Synthesis of a new class of iminosugars **1**-**4** has been reported. The Jocic-Reeve and Corey-Link approach with α-D-glucofuranos-3-ulose 5 afforded 3-azidoaldehyde 7 that was converted to the γ-lactam 9. Reductive aminocyclisation and Schmidt-Boyer reactions were used to get spiro-iminosugars 1-4 which showed selective and potent glycosidase inhibitory activities. Molecular docking studies support the activity data.

Introduction

Iminosugars are being studied for their synthetic modifications and glycosidase inhibitory activity, a process that plays vital role in glycoconjugate, lysosomal catabolism and glycoprotein biosynthesis/degradation within the endoplasmic reticulum.[1] While designing new iminosugars, the efforts were focused on the development of a variety of monocyclic- (azetidine, pyrrolidine, piperidine, azepine and azocane) and bicyclic- (indolizidine, pyrrolizidine, quinolizidine, conidine) iminosugars. The second renaissance in this field was centred on the discovery of conformationally constrained bridged iminosugars.[2] The class of spiro-iminosugars, however, received limited attention and only two reports are available so far. In the first report, the Royon and co-worker synthesized the spiro-pyrrolidine [4,2] iminosugar (I) as a selective α-L-fucosidase inhibitor[3] (Fig. 1). Recently, Vankar and co-workers reported the oxaazaspiro [6,5] iminosugars (II) as highly selective α-mannosidase inhibitors.[4] In both the reports, the nitrogen atom is present in one of the rings. However the class of diazaspiro-iminosugars, in which the nitrogen atoms are part of both the rings, is remained to be unexplored.[5] As a part of our efforts in the field of iminosugars,[6] we are now reporting synthesis of a new class of diazaspiro-iminosugars 1,2 and hydroxylated spiro-bislactams 3,4. These compounds showed selective and potent glycosidase inhibitory activities in nanomolar concentration.

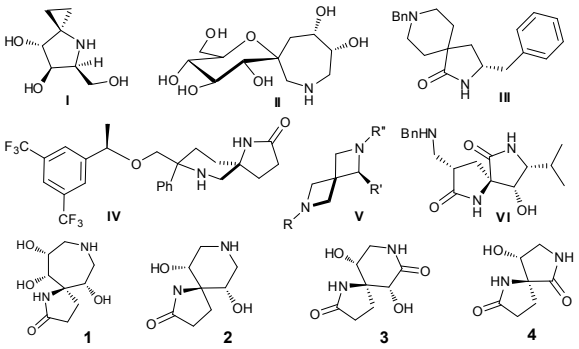

Fig. 1: Drug leads containing aza-spiro core

The highly constrained and structurally rigid azaspiro framework is a part of a number of biologically active natural products[7] such as cephalotaxine, stemonamine, halichlorine, histrionicotoxin , nankakurine A and TAN1251A.[8] While, diazaspiro skeleton is an important building block in drug leads[9] namely spirocyclic pyrrolidones III (HIV-1 protease Inhibitor)[10] and rolapitant IV (neurokinin NK1 receptor antagonist),[11] and spiro[3.3]heptanes V which can be easily grafted in to frameworks of drug like structures[12] (Fig. 1). In addition, spiro-bislactam compounds of type VI act as folded materials,[13] peptidomimetic[14] and also known to act as spiroligomer catalysts[15]

In general, the main synthetic challenges for building diazaspiro framework are construction of a quaternary carbon centre bearing the nitrogen atom and the formation of a spirocyclic ring system. Two main approaches have been used to construct the azaspirocycles. One approach involves a priory generation of a quaternary centre with the nitrogen atom followed by the formation of a spirocycle in two different steps. In another approach, generation of a quaternary centre with the nitrogen atom and formation of spirocyclic ring are achieved in cascade fashion in one pot. A number of elegant approaches of both types are known in the literature [16] In the present approach (Scheme 1), we thought of utilizing the Jocic-Reeve and Corey-Link approach[17] with D-glucofuranos-3-ulose for the generation of a tertiary carbon

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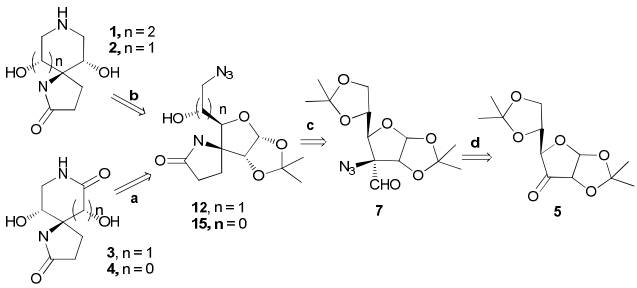
† Footnotes relating to the title and/or authors should appear here. Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

centre bearing the nitrogen atom and the formyl group that could be elaborated further by two carbon Wittig olefination and lactamization to construct the spiro ring at the C-3 position of the glucose. The part of the glucose framework is suitably placed to give an access for the formation of five/six/seven nitrogen ring structures utilizing intramolecular cyclisation of the C5/C6 azido group with the C1/C2-hemiacetal. This could be achieved either by using reductive aminocyclisation protocol or by the Schmidt Boyer rearrangement to get the diazaspiro-iminosugars 1/2 or spiro-bislactams 3/4, respectively. We anticipated that the spirocyclic three dimensional rigid conformations, in the diazaspiro-iminosugars, will provide specific interactions with aminoacid residues of glycosidase enzymes thus rendering selectivity and potency in inhibition. Our results in this direction are described herein.

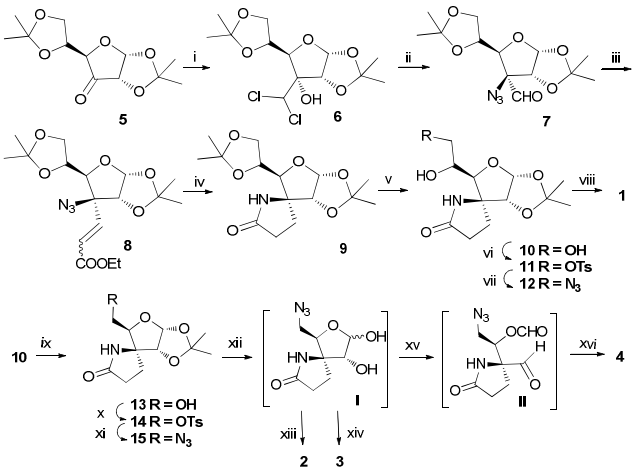


Scheme 1: Retro synthetic analysis

Results and discussion

As shown in scheme 2, the required 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-ulose **5** was prepared from D-glucose as reported earlier.[18] Reaction of **5** with dichloromethyllithium, derived from the dichloromethane and LDA at −78°C, afforded 3-dichloromethyl carbinol derivative **6** that on treatment with sodium azide afforded (3*R*)-α-azidoaldehyde **7** with a quaternary centre. This reaction involves first formation of a chlorooxirane intermediate that on opening of the oxirane ring by the S_N2 attack of the azide ion, from the β face, gave **7**.[19] The absolute configuration (3*R*) was confirmed by matching the data with reported one.[20] In the next step, the Wittig reaction of **7** using Ph_3P=CHCOOEt gave α,β-unsaturated ester **8** (*E*:*Z* = 9:1). Subsequently, intramolecular lactamisation of **8** using H_2, 10% Pd/C in methanol afforded spiro-γ-lactam core **9**. Hydrolysis of the 5,6-*O*-acetonide in **9** with 80% acetic acid provided diol **10** that on selective tosylation of primary hydroxyl group (to get **11)**, followed by the S_N2 displacement of the tosyl group using sodium azide in DMF afforded azido alcohol **12**. Finally, cleavage of the 1,2-*O*-acetonide functionality in **12** with TFA-water (3:1) and reductive aminocyclisation using H_2, 10% Pd/C gave trihydroxy-1,8-diazaspiro[4.6]undecan-2-one **1** as a semisolid.

In order to achieve the diazaspiro [4,5] iminosugar **2**, we used diol **10** that on oxidative cleavage using sodium metaperiodate in acetone-water followed by $NaBH_4$ reduction yielded primary alcohol **13** (scheme 2). Tosylation of hydroxyl group in **13** gave tosyl derivative **14** that on treatment with sodium azide gave **15**. In the final step, hydrolysis of the 1,2-*O*-acetonide and intramolecular reductive aminocyclisation (H_2, 10% pd/c,) afforded the (5r,6R,10S)-6,10-dihydroxy-1,8-diazaspiro[4.5]decan-2-one (**2**) as a sticky solid.



Scheme 2. *Reagents and conditions;* i, LDA, THF,CH_2Cl_2,−78 °C to rt, 60%; ii, NaN_3, cat. TBAI, dry DMF, 60 °C, 8h, 82% ; iii, PPh_3=CH-CO_2Et, dry DCM, reflux, 2h, 92%; iv, H_2, Pd/C, MeOH, rt, balloon pressure, 12h, 86%; v, 80 % aq.AcOH, 12h, 94%; vi, TsCl, Pyridine, cat DMAP, 0 °C to rt, 12h, 90% ; vii, NaN_3, dry-DMF, 100 °C, 6h, 91%; viii, a) TFA:H_2O (3:1), 0 °C to rt 3h; b) H_2, Pd/C, MeOH , 200 psi, 48h, 72%.; ix a) $NaIO_4$, acetone:water, 0 °C to rt 4h, b), $NaBH_4$, MeOH-H_2O, 0 °C 4h, 87%; x, TsCl, Pyridine, cat. DMAP, 0 °C to rt, 12h, 92%; xi, NaN_3, dry-DMF, 100 °C, 6h, 93%; xii, TFA:H_2O (3:1), 0 °C to rt; xiii, H_2, Pd/C, MeOH, 100 psi, 24h, 84%; xiv, TFA:H_2O (5:1),0 °C to rt, 24h, 87%; xv, $NaIO_4$, acetone:water,0 °C to rt 4h; xvi, TFA:H_2O (5:1), 0 °C to rt, 24h, 73%.

The ^{1}H-NMR of **2** showed equivalence of protons at C_6/C_{10} and C_7/C_9 due to the plane of symmetry. The H_7/H_9 axial protons (equivalent) showed *dd* with large coupling constants of 12.9 and 11.4 Hz. While; equatorial protons H_7/H_9 showed *dd* with coupling constants of 12.9 and 5.1 Hz. The relatively higher coupling constant of 5.1 Hz between 7e/9e and 6a/10a protons indicated dihedral angle of ~40° (and not usual 60°) between these protons. This fact suggests distortion of six-memberd piperidine ring from normal $^{10}C_7$ conformation **A** to the half chair conformation **B**, to release the 1,3-diaxial strain (Fig. 2).

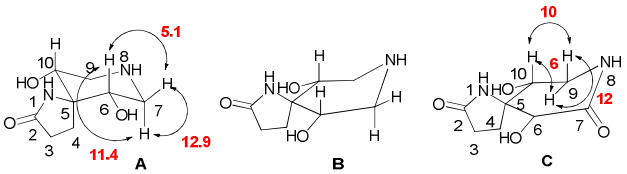


Fig. 2 Conformational assignments of compounds **2** and **3**.

Targeting towards the synthesis of spiro-bislactams, the azido compound **15** was treated with TFA:H_2O (5:1) at 0 °C to rt for 24 h that afforded γ–δ-spiro-[5,4]-bis-lactam **3** as a white solid in 87% yield. This reaction involves *in situ* hydrolysis of the 1,2-acetonide functionality and the concomitant Schmidt Boyer rearrangement[21] to give **3** in one pot. The ^{1}H-NMR spectrum of compound **3** showed 9a as *dd* with coupling constants of 12.0 and 10.0 Hz while; 9e showed *dd* with coupling constants of 12.0 and 6.0 Hz. The high value of axial-equatorial coupling constant indicated dihedral angle between these protons ~ 30°suggesting the half chair conformation **C** for 3 (Fig. 2). In order to synthesize highly constrained γ,γ-spiro[4,4]bis-lactam **4** (scheme 2), the

azido-compound **15** was treated with TFA: water (3:1) at 0 °C to rt for 4 hours that selectively gave acetonide hydrolyzed product (I) as an anomeric mixture (as evident from the IR and ^{1}H-NMR). Intermediate (I) on oxidative cleavage with $NaIO_4$ in acetone:water afforded (II) that was filtered through celite and concentrated. The crude product thus obtained was treated with TFA:H_2O (5:1) at 0 °C to rt for 24 h that underwent the Schmidt-Boyer reaction to give (5*S*,9*R*)-9-hydroxy-1,7-diazaspiro[4.4]nonane-2,6-dione **4** as a sticky white solid.

Our following attempts were unsuccessful under variety of reactions condition (fig. 3): (A) Synthesis of diazaspiro[4,5] iminosugar X-the C-5 hydroxy group in **12** (Scheme 2) was protected with benzyl group that on treatment with TFA:water (3:1) and NaIO4 (to cleave C1) followed by the intramolecular reductive amino- cyclisation (H_2, 10% Pd-C) failed to give **X** (B) Synthesis of diazaspiro[4,4]iminosugar Y-Intermediate (II) was treated with 10%Pd/C under hydrogenation condition that afforded complex mixture of products but not compound **Y** (C) Synthesis of spiro[4,6]bislactam Z-the Schmidt Boyer reaction of **12** (Scheme 2) with TFA-H20 (5:1) at 25°C for 48h failed to give **Z**. (Fig. 3).

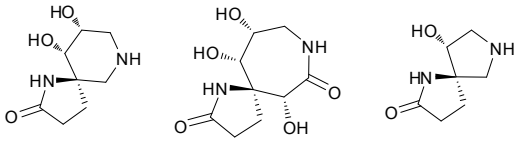


Fig. 3: Attempted diaza-spiroiminosugars X, Y and Z

Glycosidase inhibitory studies

Glycosidase inhibitory activity of **1-4**, was studied with reference to the known standard miglitol and is summarised in **Table 1**. All the four compounds were found to be potent and selective inhibitors and IC_{50} values are in the nanomolar concentration range.

Table 1 Glycosidase inhibitory activity (IC_{50} in µM) for new diaza-spiroiminosugars and standard Miglitol

Compounds	α-mannosidase	α -galactosidase	α -glucosidase
1	0.247	0.029	3.45
2	0.094	3.608	0.061
3	0.107	0.127	0.487
4	0.08	3.312	0.177
Miglitol	0.29	1.83	0.16

The diazaspiro-piminosugars **1** moderately inhibited the *α*-glucosidase (*yeast*) and α-mannosidase (*jack bean*) but found to have strong inhibitory activity against the *α*-galactosidase (IC_{50}=0.029) under assay conditions. Spiroiminosugar **2** showed potent inhibition against α-glucosidase and α-mannosidase but moderate inhibition against α-galactosidase (*green coffee bean*). Spiro-bislactam **4** was found to be strong and selective inhibitor of α-mannosidase (IC_{50}=0.08) however, it showed moderate inhibition against α-galactosidase and α-glucosidase.

Molecular docking studies

The molecular docking studies were performed in order to explore the mechanism of catalytic inhibition of α-galactosidase, α-glucosidase and α-mannosidase enzymes by iminosugars **1**, **2** and **4**, respectively. Each hundred docked conformations obtained from molecular docking studies were analyzed for binding, intermolecular and internal energies. The energetically stable docked complexes of α-galactosidase with 1, α-glucosidase with 2 and α-mannosidase with 4 possesses least energy with highest binding affinity were selected and subjected for hydrogen bonding and hydrophobic interactions analysis (Fig. 4, Table 2).

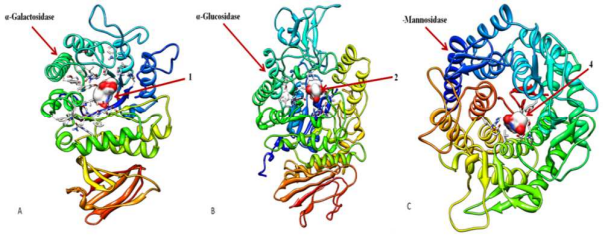


Fig. 4: Positional coordination of iminosugars: A) α-galactosidase with 1, B) α-glucosidase with 2, C) α-mannosidase with 4.

Table 2: Intermolecular hydrogen bonding and hydrophobic interactions from docked complexes of α-galactosidase with iminosugar 1, α-glucosidase with iminosugar 2 and α-mannosidase with iminosugar 4.

Sr. No.	Atoms Involved 1-2-3	Distance in Å
α-Galactosidase (3HG2.pdb)		
7	Asp92-OD2.....O2-1	2.65
8	Lys168-NZ.....O3-1	2.41
9	Asp170-OD1....O2-1	2.38
10	Glu203-OE2.....O3-1	2.93
11	Tyr207-OH.....O2-1	3.34
12	Asp231-OD2.....O4-1	2.79
13	Asp231-OD2.....N2-1	2.74
14	Arg227-NH2.....O3-1	2,71
15	Arg227-NE.....O3-1	3.00
16	Trp47,Asp93,Tyr134,Leu206,Ala230	Hydrophobic interactions
α-Glucosidase (3AJ7.pdb)		
17	Asp69-OD2…N1-2	2.61
18	Asp215-OD2.....O2-2	2.69
19	Glu277-OE1.....O2-2	2.91
20	Asp352-OD1.....O3-2	2.60
21	Arg442-NH1.....O3-2	2.56
22	Arg442-NH2.....O1-2	2.80
23	Tyr72, Phe159, Phe178, Val216	Hydrophobic Interactions
α-Mannosidase (1X9D)		
1	Arg334-NH1.....O1-4	3.02
2	Arg334-NH2.....O1-4	2.83
3	Arg597-NH1.....O3-4	284
4	Glu599-OE2.....O3-4	3.08
5	Glu689-OE1.....N2-4	2.88
6	Glu330, Ile333, Phe659, Pro598,Glu633	hydrophobic interactions

The Van der waals, electrostatics and hydrogen bonding energies for interacting residues from favorable docked complexes have been calculated and mentioned in Table 3. Figure 4A depicted the stable docked complex of α-galactosidase with 1 having -12.88 Kcal/mol least energy with highest binding affinity. Adopted stable conformation facilitate the significant interactions between the hydroxyl groups (O2, O3 and O4) of 1 with Lys168, Asp170, Glu203, Tyr207, Arg227 and amino groups (N1 and N2) of 1 with Asp92 and Asp231 residues of α-galactosidase (Fig. 5A, Table 2). The involvement of carboxylic residues such as Asp92, Asp170, Glu203 and Asp231 in catalytic activity profile has been reported in crystallographic study of substrate bound α-galactosidase[22] . The observed catalytic interactions efficiently coordinated 1 in active site cleft of α-galactosidase in order to inhibit its catalytic potential (Fig 4A, 5A).

Similarly, figure 4B depicted energetically (-10.94 Kcal/mol) favored docked complex between α-glucosidase with **2**. The iminosugar 2 was bound to the bottom of active site pocket of α-glucosidase by forming hydrogen bonding and hydrophobic interactions (Figs. 4B, 5B, Table 2). The hydrogen bonding interactions of hydroxyl groups (O1, O2 and O3) of **2** with reported catalytic carboxylic residues such as Asp215, Asp352, Glu277 and Arg442 have been found in docked complex (Fig. 5B) [23] Additional stabilization of docked complex was expected from Tyr72, Phe178 and Val216 residues which were established hydrophobic contact with **2**.

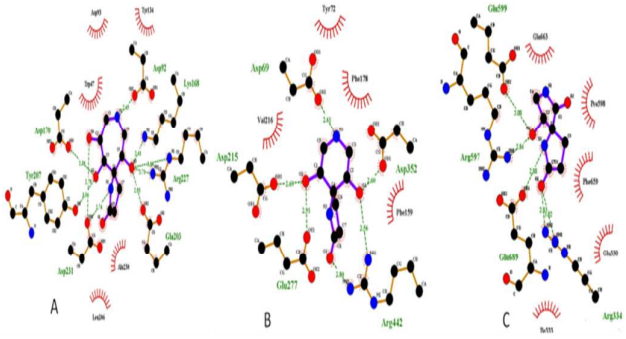


Fig. 5 Molecular interactions with enzymes: **A**-compound 1 with α-galactosidase, **B**- 2 with α-glucosidase and **C**- 4 with α-mannosidase

Further, least energy (-5.89 Kcal/mol) stable complex of α-mannosidase with **4** have shown in fig. 4C. This favoured conformation allows the strong hydrogen bonding interactions between hydroxyl (O3) and carbonyl (O1) oxygen's of **4** with Arg334, Arg597 and Glu599 resides of α-mannosidase (Fig. 5C, Table 2). The amino nitrogen (N2) of **4** was formed hydrogen bonding interaction with Glu689. These observed interactions have been reported to be essential for the activity of α-mannosidase and are in close agreement with earlier crystallographic study [24]. This kind of hydrogen bond network could stabilize the obtained α-mannosidase-**4** enzyme-inhibitor complex. Further, stability of docked complex was expected from hydrophobic interactions between Glu330, Ile333, Phe659, Pro598 and Glu633 with iminosugar **4** (Fig. 5C, Table 2).

Table 3: Calculated hydrogen bond, Van der Waals (E_{vdw}), Electrostatic (E_{ele}) and total energies (E_{total}) between interacting residues of α-galactosidase (3HG2.pdb) with 1 and α-glucosidase (3AJ7.pdb) with 2 and α-mannosidase (1X9D.pdb) with 4.

Residue No.	H-Bond energy kcal/mol	E_{vdw} kcal/mol	E_{elec} kcal/mol	E_{Total} kcal/mol
α-Galactosidase (3HG2.pdb)				
Asp92	-6.985	-0.81	0.60	-0.21
Lys168	-0.619	-0.46	-1.15	-1.61
Asp170	-3.430	-1.25	0.41	-0.84
Glu203	-1.472	-0.24	0.96	0.72
Tyr207	-0.197	-2.46	0.08	-2.38
Asp231	-0.386	-0.17	0.86	0.69
Arg227	-2.62	-1.19	-2.06	-3.25
α-Glucosidase (3AJ7.pdb)				
Asp69	-1.262	-0.49	1.24	0.75
Asp215	-0.192	-0.40	1.13	0.73
Glu277	-1.437	-1.34	0.69	-0.65
Asp352	-4.227	-0.10	0.99	0.89
Arg442	-3.80	-0.60	-1.25	-1.85
α-Mannosidase (1X9D.pdb)				
Arg334	-7.595	-0.98	-0.89	-1.87
Arg597	-2.527	-0.81	-1.59	-2.40
Glu599	-1.794	-0.94	0.84	-0.10
Glu689	-4.590	-1.23	0.56	-0.67

Moreover, calculated hydrogen bonding, Van der waals and electrostatic energies for interacting residues from favorable docked complexes of α-galactosidase with **1**, α-glucosidase with **2**, α-mannosidase with **4** evidences its catalytic inhibition (Table 3). The in-vitro inhibition efficiency (Table 1) of α-galactosidase, α-glucosidase and α-mannosidase by **1**, **2** and **4** were justified by respective least energies stable docking complexes (-12.88 Kcal/mol, -10.94 Kcal/mol and -5.89 Kcal/mol). Therefore, discussed docking results are in line with experimental outcomes and emphasized the catalytic inhibition mechanism of glycosidases by iminosugars (Figs. 4-5 and Tables 2-3).

Conclusions

In summary, we have synthesized a new class of constrained diazaspiro-iminosugars **1**,**2** and spiro-bislactam **3**,**4** from D-glucose-derived *α*-azidoaldehyde. This work demonstrates the applicability of Corey-Link reaction and Schmidt-Boyer rearrangement for the synthesis of the target molecules. The compound **1** and **2** were found to be selective and potent inhibitors of α-galactosidase and α-glucosidase, respectively, while; compound **4** was found to be potent and selective inhibitor of α-mannosidase in the nanomolar concentration range. These results are substantiated by molecular docking studies and may be of help to explore inhibition mechanism of glycosidases with iminosugars.

Experimental

General procedure

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 / 500 MHz) and ^{13}C NMR (75 MHz/125 MHz) spectra were recorded using $CDCl_3$, DMSO 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. 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 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 atmosphere. Methanol, DCM and THF were purified and dried before use. 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, work-up involves washing of combined organic layer with water, brine, drying over anhydrous sodium sulfate, and evaporation of solvent under reduced pressure.

General procedure for glycosidase inhibition assay

All enzymes namely, α-mannosidase from jack bean (*Canavalia ensiformis*), α –galactosidase from green coffee bean (*Coffea arabica*) and α –glucosidase from yeast (*Saccharomyces cerevisiae*) were procured from Sigma Aldrich, USA. Glycosidase inhibition assay of **1-4,** was carried out by mixing 0.1 unit/ml each of *α*-galactosidase, *α*-mannosidase and *α*-glucosidase with the samples and incubated for 1 hour at 37 ^oC. Initiation of enzyme action for *α*-galactosidase and *α*-mannosidase was carried out by addition of 10mM p-nitrophenyl-*α*-D-galactopyranoside (pNPG) and 10mM p-nitrophenyl-*α*-D-mannopyranoside respectively as substrates followed by incubation at 37 ^oC for 10 min. The reaction was stopped by adding 2 mL of 200 mM borate buffer of pH 9.8. *α*-glucosidase activity was initiated by addition of 10mM p-nitrophenyl-*α*-D-glucopyranoside in 100 mM phosphate buffer of pH 6.8 and stopped by adding 2 mL of 0.1 M Na_2CO_3 after an incubation of 10 minutes at 37 ^oC. *α*-glycosidase activity was determined by measuring absorbance of the p-nitrophenol released from pNPG at 420nm using spectrophotometer (SpectraMax M5, Molecular Devices Corporation, Sunnyvale, CA). 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.

Computational methodology for molecular docking studies

To gain insight into the structural aspect of the observed potent inhibition patterns of α-galactosidase, α-glucosidase and α-mannosidase by different iminosugars such as 1, 2 and 4 (Table1), molecular docking studies were performed using Autodock 4.2 software.[25] Molecular docking study is widely used to infer selectivity profile and critical interactions between active site pocket of receptors and inhibitor molecules. The crystal structure of α-Glucosidase (3AJ7.pdb) from yeast was used for docking study[24]. For α-galactosidase (*Coffea arabica*) and α-mannosidase (*Canavalia ensiformis*) the crystal structure data is not available, therefore crystal structure of α-galactosidase (3HG2.pdb) and α-mannosidase (1X9D.pdb) from human having high resolutions were selected for docking studies.[23,25] Crystal structures were obtained from protein data bank (www.rcsb.org) and prepared using Autodock wizard. Docking procedure was adopted similar to earlier docking studies.[26] Molecular structure of iminosugars 1, 2 and 4 were generated using *Spartan'14* software (Wavefunction, Inc.). Automated complete geometry optimizations have been performed on iminosugars 1, 2 and 4 using Density Functional Theory (DFT) method by employing B3LYP/6-31G** basis set.[27] Prior to docking studies the crystal structures of receptors (α-galactosidase, α-glucosidase and α-mannosidase) were minimized for each 5000 steps of steepest descent method using Swiss-PdbViewer software to remove internal strain. Further, receptor structures were refined by removing non-polar hydrogen atoms, adding kollman united atom charges and polar hydrogen atoms using Autodock wizard. Gasteiger charges and hydrogen atoms were added to the iminosugars. AutoGrid module was used to calculate the grid map and centered on iminosugars binding site of receptors, in such a way that it would totally cover the ligand iminosugars. The grid size was set to 40 A° × 40 A° × 40 A° for α-galactosidase, 44 A° × 44 A° × 44 A° for α-glucosidase and 50 A° × 54 A° × 60 A° for α-mannosidase with a grid spacing 0.375 A°. The step size of 1Å for translation and the maximum number of energy evaluation was set to 2,500,000. The 100 runs were performed for each receptor-ligand molecules. 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). Docked complexes were analyzed using Autodock wizard, while pictorial presentation of docked conformations and molecular interactions have been made using Chimera and LigPlot software's.[28]

1,2:5,6-Di-*O*-isopropylidene-3-*C*-(dichloromethyl)-α-D-allofuranose (**6**)

To a stirred solution of diisopropylamine (6.78 mL, 48.39 mmol) in dry THF (60 mL) at 30 °C under nitrogen atmosphere was added 1.6 M solution of n-butyllithium in hexane (7.3 mL, 48.39 mmol) and stirred for 30 min at room temperature. The solution was cooled to −78 °C and ketone **5** (5.00 g, 19.35 mmol) in dry CH_2Cl_2 (40 mL) was added dropwise. The temperature was then allowed to rise slowly to 20 °C and stirred. TLC analysis (Hexane/EtOAc: 3/2) after 3 h indicated no starting material. Reaction was quenched by adding saturated aq. NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (100 mL × 2) and concentrated. Purification by column chromatography (hexane/ ethyl acetate, 9.5:0.5) afforded **6** (3.97 g, 60%) as a white solid: R_f = 0.56 (hexane/ EtOAc, 9:1); m.p 133-135 °C; $[\alpha]_D^{24}$ = + 27.7 (*c,* 1.0, $CHCl_3$); IR ($CHCl_3$, ν, cm^{-1}) 3482 (br), 1220, 1079,789 ; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 6.23 (s, 1H, H-7, -$CHCl_2$), 5.93 (d, *J* = 4.3 Hz, 1H, H-1), 4.81 (d, *J* = 4.3 Hz, 1H, H-2), 4.41 (ddd, *J* = 9.8, 5.8, 4.1 Hz, 1H, H-5), 4.14 (dd, *J* = 8.8, 5.9 Hz, 1H, H-4), 4.06 (d, *J* = 9.8 Hz, 1H, H-6a), 3.96 (dd, *J* = 8.8, 4.1 Hz, 1H, H-6b), 3.51 (s, 1H, exchangeable with D_2O, -OH), 1.61 (s, 3H, CH_3), 1.59 (s, 3H, -CH_3), 1.47 (s, 3H, CH_3), 1.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 112.9 (acetonide, O-C-O) 110.2 (acetonide, O-C-O) 105.4 (C-1) 84.7 (C-2), 81.5 (C-4), 79.4 (C-5), 73.2 (C-3), 73.1 (C-6), 68.5 (C-7, C-Cl_2), 27.0 (-CH_3, 26.8 (-CH_3), 26.5 (-CH_3), 25.4 (CH_3); HRMS (ESI-TOF) m/z calculated for $C_{13}H_{20}Cl_2O_6Na$ [M + Na]: 365.0529; found: 365.0526.

3-Azido-3-deoxy-3-*C*-(formyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (7)

To a stirred solution of **6** (4.00 g, 11.68 mmol) in dry DMF (20 mL) was added sodium azide (2.28 gm, 35.06 mmol), TBAI (2.16 gm, 5.84 mmol), and the mixture was heated at 110 °C. TLC analysis (Hexane/EtOAc: 9/1) after 4 h indicated no starting material, DMF was evaporated at reduced pressure and the residue was extracted with EtOAc (30 mL × 3) and concentrated. Purification by column chromatography (hexane/ ethyl acetate: 9/1) afforded **7** (3.00 g, 82%) as a thick liquid: R_f = 0.5 (hexane/ EtOAc: 4/1); $[\alpha]_D^{25}$ = + 75.70 (*c,* 0.38, $CHCl_3$); IR ($CHCl_3$, ν, cm^{-1}) 2123, 1734, 1456, 1379, 844, 758; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.64 (s, 1H, H-1, H-CO), 5.94 (d, *J* = 3.6 Hz, 1H, H-1), 4.63 (d, *J* = 3.6 Hz, 1H, H-2), 4.55 (d, *J* = 8.8 Hz, 1H, H-4), 4.23 (ddd, *J* = 3.8, 6.1, 8.8 Hz, 1H, H-5), 4.12 (dd, *J* = 3.8, 6.1 Hz, 1H, H-6a), 4.06 (dd, *J* = 3.8, 8.8 Hz, 1H, H-6b), 1.61 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 194.5 (C-7, -CHO), 114.1 (acetonide, O-C-O), 109.9 (acetonide, O-C-O), 105.7 (C-1), 86.2 (C-2), 81.0 (C-3), 76.5 (C-4), 72.5 (C-5), 67.2 (C-6), 26.7 (-CH_3), 26.6 (-CH_3), 26.1 (-CH_3) 24.6 (-CH_3); HRMS (ESI-TOF) m/z calculated for $C_{13}H_{20}N_3O_6$ $[M + H]^+$: 314.1352; found: 314.1348.

3-Azido-3-deoxy-3-C-(ethyl-prop-2-(E)-ene-oate)-1,2:5,6-di-O-isopropyliene-α-D-glucofuranose (8)

To a solution of azidoaldehyde **7** (5.00 g, 15.95 mmol) in dry dichloromethane (50 mL) was added triphenylethoxycarbonylmethylene phosphorane (7.22 g, 20.74 mmol). The reaction mixture was refluxed for 2 h and concentrated on rotavap to give thick liquid. Chromatography of the crude reaction mixture with 95:5 pet ether/Ethyl acetate as an eluant to give **8** (5.62 g, 92%) as a thick liquid. R_f 0.50 (pet ether/ethyl acetate, 9:1). $[\alpha]_D^{25}$ +85.4 (c, 0.24 CH_2Cl_2); IR (neat, ν, cm-1): 2987, 2937, 2899, 2114, 1726, 1658, 1456 (COOR, N_3). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.0 (d, *J* = 15.8 Hz, 1H, H-7, -CH=CH-COO); 6.23 (d, *J* = 15.8 Hz, 1H, H-8, -CH=CH-COO); 5.92 (d, *J* = 3.5 Hz, 1H, H-1); 4.53 (d, *J* = 3.5 Hz, 1H, H-2); 4.18-4.30 (m, 3H, H-4, H-10, -CH_2OCO); 4.06-4.16 (m, 2H, H-5, H-6b); 4.02 (dd, *J* = 8.8, 4.7 Hz, 1H, H-6a); 1.56 (s, 3H, -CH_3); 1.38 (s, 3H, CH_3); 1.28-1.36 (m, 9H, 3CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 165.4 C-9, --COO), 139.6 (C-7, -CH=CH-COO), 124.6 (C-8, CH=CH-COO), 113.3 (acetonide, O-C-O), 109.6(acetonide, O-C-O), 104.6 (C-1), 85.6 (C-10, -CH_2-COO), 82.4 (C-2), 73.5 (C-3, -C-N_3), 73.0 (C-5/4), 67.1 (C-5/4, 60.7 (C-6, -CH_2-O), 26.8 (-CH_3), 26.6 (CH_3), 26.3 (-CH_3), 25.1 (-CH_3), 14.1 (C-10, -CH_3); Anal. calculated For $C_{17}H_{25}N_3O_7$; C, 53.26; H, 6.57; N, 10.96 Found: C, 53.27; H, 6.59; N, 11.07.

(5*R*,6*S*,8*R*,9*R*,4'*R*)-6-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)-8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (9)

To a solution of **8** (4.50 g, 11.70 mmol) in methanol was added 10% Pd/C (0.15 g). The solution was hydrogenated at balloon pressure for 12 hrs. The catalyst was filtered through Celite and washed with methanol and filtrate was concentrated to obtain a semisolid and purified by column chromatography on silica with 7:3 pet ether/Ethyl acetate as an eluant to give **9** (3.15 g, 86%) as a off-white solid. R_f 0.50 (pet ether/ethyl acetate, 1:1). mp 158-160 °C $[\alpha]_D^{25}$ – 3.5 (c, 0.21 CH_2Cl_2) IR (neat, ν, cm^{-1}): 3099-3198 (br), 1719 (CONH), 1665; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm); 7.06 (1H, s, exchangable with D_2O, -NH); 5.823(d, *J* = 3.6 Hz, 1H, H-8*);* 4.37 (d, *J* = 3.6 Hz, 1H, H-9); 4.04-4.18 (m, 2H, H-1'a,H-2'); 4.00 (dd, *J* = 7.7, 4.4 Hz, 1H, H-1'b); 3.84 (d, *J* = 8.5 Hz, 1H, H-6); 2.47-2.64 (m, 1H, H-3); 2.22-2.46 (m, 3H, H-3, H-4); 1.55 (s, 3H, CH_3); 1.44 (s, 3H, CH_3); 1.34 (s, 3H, CH_3); 1.33 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 179.5 (C-2, -CONH), 112.4(O-C-O), 109.6 (O-C-O), 103.8 (C-8), 86.7 (C-2'), 82.1 (C-9), 73.0 (C-6), 69.6 (C-5), 67.9 (C-1'), 29.5 (C-3, -CH_2-CONH), 26.7 (strong 2CH_3), 26.2 (CH_3), 25.1 (CH_3), 22.7 (C-4, -CH_2); Anal. calculated For $C_{15}H_{23}NO_6$; C, 57.50; H, 7.40; N, 4.47; Found: C, 57.50; H, 7.40; N, 4.53.

(5*R*,6*S*,8*R*,9*R*,2'*R*)-6-(1',2'-dihydroxyethane)-8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (10)

To a solution of compound **9** (3.00 g, 9.57 mmol) in acetic acid/water (20 mL, 4:1) was stirred at rt for 12 h. The mixture was then co-evaporated to dryness with toluene *in vacuo* and was dried under vacuum to afford a solid residue. The crude solid was purified by column chromatography over silica gel ($CHCl_3$/methanol 85:15) gave **10** as white solid (2.45 g, 94%) as a white solid: R_f 0.51 ($CHCl_3$/methanol 4:1). mp 213-214 °C, $[\alpha]_D^{22}$ + 40.27 (c,0.15 MeOH). IR (KBr, ν, cm^{-1}): 3490-3200 (br) (OH), 1674 (CONH). ^{1}H NMR (500 MHz, DMSO-d^6) δ (ppm); 8.06 (s, 1H, exchangable with D_2O, -NH*);* 5.69 (d, *J* = 3.5 Hz, 1H, H-8); 4.77 (d, *J* = 6.2 Hz, 1H, exchangable with D_2O, -OH); 4.49 (t, *J* = 5.6, 5.5 Hz, 1H, exchangable with D_2O, -OH); 4.28 (d, *J* = 3.5 Hz, 1H, H-9); 3.76 (d, *J* = 9.15 Hz, 1H, H-6); 3.53-3.60 (m, 1H, H1'a);3.43-3.5 (m, 1H, H-2'); 3.36-3.41 (m, 1H, H-1'b); 2.24-2.34 (m, 1H, H-3); 2.15-2.22 (m, 2H, H-3/4); 2.03-2.14 (m, 1H, H-4); 1.42 (s, 3H, CH_3); 1.28 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d^6) δ (ppm); 177.6 (C-2, -CONH), 111.6 (O-C-O), 103.5 (C-8), 87.0(C-9), 80.9 (C-2'), 70.3 (C-6), 69.2 (C-1'), 64.47 (C-5), 29.7 (C-3, -CH_2CONH), 27.3 (CH_3), 26.8 (CH_3), 23.3 (C-4 -CH_2). Anal. calculated For $C_{12}H_{19}NO_6$; C, 52.74; H, 7.01; N, 5.13; Found: C, 52.73; H, 7.03; N, 5.15;

(5*R*,6*S*,8*R*,9*R*,2'*R*)-6-(1'-tosyl-,2'-hydroxyethane)-8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (11)

To the solution of diol **10** (2.20 g, 8.05 mmol) in pyridine (15 mL) was added *p*-tolune sulphonyl chloride (2.30 g, 12.07 mmol) portion at 0 °C, catlytic DMAP (0.10 g, 0.8 mmol) and stirred for 12 h at rt. The reaction mixture was concentrated under vacum and extrated with dichloromethane (40 mL X 3). The combined organic layers were dried and evaporated under reduced pressure, which was purified by column chromatography on silica 2:3 pet ether/Ethyl acetate as an eluant to gave **11** (3.06 g, 90%) as a thick liquid. R_f 0.49 (pet ether/Ethyl acetate, 1:9). $[\alpha]_D^{22}$ + 54.2 (c, 0.26 CH_2Cl_2); IR (neat, ν, cm^{-1}): 3200-3600 (br). 2990, 1692; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm); 7.81 (d, *J* = 8.3 Hz, 2H, Ar,-CH); 7.36 (d, *J* = 8.3 Hz, 2H, Ar, -CH); 5.84 (d, *J* = 3.5 Hz, 1H, H-8); 4.38 (d, *J* = 3.5 Hz, 1H, H-9); 4.30-4.37 (m, 1H, H-2'); 3.92-4.02 (m, 2H, H-1'); 3.83 (d, *J* = 8.4 Hz, 1H, H-6); 3.00-3.70 (bs, 2H, exchangable with D_2O, -NH, -OH); 2.54-2.67 (m, 1H, H-3); 2.47 (s, 3H, Ar-CH_3); 2.28-2.45 (m, 3H, H-3, H-4); 1.48 (s, 3H, -CH_3); 1.30 (s, 3H, -CH_3) ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm); 180.4 (C-2, -CONH), 145.2 (Ar, -C-S-O), 132.0 (Ar, -C-CH_3), 130.0 (Ar, -CH-strong); 128.1 (Ar, -CH-strong); 112.3(acetonide, O-C-O); 103.7 (C-8, O-C-O), 86.4 (C-9, -C-O), 80.3 (C-6/1'), 74.2 (C-6/1'); 70.1 (C-5), 68.1 (C-2', -CH-OH), 29.4 (C-3, -CH_2CONH), 26.6 (CH_3), 26.2 (CH_3), 23.1 (CH_3), 21.7 (C-4, -CH_2). Anal. calculated For $C_{19}H_{25}NO_8S$; C, 53.38; H, 5.89; N, 3.28; S, 7.50; Found: C, 53.36; H, 5.86; N, 3.28; S, 7.51.

(5*R*,6*S*,8*R*,9*R*,2'*R*)-6-(1'-azido-,2'-hydroxyethane)-8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (12)

To a solution of tosylate **11** (1.50 g, 3.50 mmol) in dry DMF (15 mL) was added sodium azide (1.14 g, 17.54 mmol), TBAI (0.13g, 35 mmol) and the mixture was heated for 6h at 100 °C. DMF was removed at reduced pressure and Purification of crude reaction mass by column chromatography (pet.ether: ethyl acetate c, 3:7) gave **12** (0.95g, 91%) as a yellow solid: R_f 0.48 (pet ether :ethyl acetate, 1:9) mp 162-164 °C; $[\alpha]_D^{25}$ + 3.88 (c, 0.24 CH_2Cl_2); IR (KBr, ν, cm^{-1}): 3389-3170(br), 2091, 1688 (OH, N_3, CO). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm); 7.83 (s, 1H, exchangable with D_2O, H-1, -NH); 5.81 (d, *J* = 3.5 Hz, 1H, H-8); 4.40 (d, *J* = 4.5 Hz, 1H, exchangable with D_2O, -OH); 4.34 (d, *J* = 3.5 Hz, 1H, H-9); 3.88 (d, *J* = 9.0 Hz, 1H, H-6); 3.73-3.81 (m, 1H, H-2'); 3.56 (dd, *J* = 12.0, 2.5 Hz, 1H, H-1'a); 3.41 (dd, *J* = 12.0, 7.0 Hz, 1H, H-1'b); 2.40-2.57 (m, 2H, H-3, -CH_2-CONH); 2.28-2.39 (2H, m, H-4, -CH_2); 1.51 (s, 3H, -CH_3); 1.31 (s, 3H, -CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm); 180.6 (C-2, -CONH). 112.5 (O-C-O), 103.6 (C-8, O-C-O), 86.6 (C-9, -CH-O), 80.9 (C-6, -CH-O) 70.2 (C-2', -CH-OH), 69.2 (C-5, -C-NH), 56.0 (C-1', -CH_2-N_3), 29.6 (C-3, -CH_2CONH), 26.7 (CH_3), 26.3 (CH_3), 22.9 (C-4, -CH_2); Anal. calculated For $C_{12}H_{18}N_4O_5$; C, 48.32; H, 6.08; N, 18.78;; Found: C, 48.32; H, 6.09; N, 18.77.

(5*R*,6*S*,10*R*,11*S*)-6,10,11-trihydroxy-1,8-diazaspiro[4.6]undecan-2-one (1)

A solution of **12** (350 mg, 1.17 mmol) in TFA-water (8 mL, 3:1) was stirred for 3 h at 0 °C. TFA was co-evaporated with toluene at reduced pressure to furnish a thick liquid. To a solution of the above product in methanol was added 10% Pd/C (0.05 g). The solution was hydrogenated at 100 psi for 48 h. The catalyst was filtered through Celite and washed with methanol and filtrate was concentrated to obtain a semisolid. Purification by column chromatography (CH_2Cl_2/MeOH 7:3) gave **1** (0.18 g, 72%) as a sticky white solid: R_f 0.57 (CH_2Cl_2/MeOH: 1:1); $[\alpha]_D^{25}$ – 17 (c, 0.2 MeOH). IR (KBr, ν, cm^{-1}): 3200-3600 (br), 1689, ^{1}H NMR (300 MHz, D_2O) δ (ppm); 4.12-4.22 (m, 1H, H-10); 3.83 (dd, *J* = 8.1, 3.9 Hz, 1H, H-6); 3.73 (d, *J* = 3 Hz, 1H, H-11); 2.72-3.02 (m, 3H, H-7/9); 2.33-2.54 (m, 4H, H-7/9/3/4); 2.03-2.21 (m, 1H, H-4); ^{13}C NMR (75 MHz, D_2O) δ (ppm); 181.4 (C-2, -CH_2CO); 75.3 (C-6, -CHOH); 71.5 (C-11; -CHOH), 69.0 (C-10, -CHOH), 58.0 (C-5, -C-NH); 57.2 (C-9, -CH_2NH), 46.1(C-7, -CH_2NH), 30.9 (C-3, -CH_2CO); 21.6 (C-4, -CH_2); MS (ESI) *m/z* = 216.11 ; HRMS calculated for $C_9H_{17}N_2O_4$ $[M+H]^+$: 217.1188. Found: 217.1291.

(5*R*,6*S*,8*R*,9*R*)-6-hydroxymethyl,8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (13)

To a solution of compound diol **10** (2.80 g, 10.25 mmol) in acetone (15 ml) and water (3ml) was cooled to 0 °C. Sodium metaperiodate (3.27 g, 15.38 mmol) was added in portions to the cooled solution and stirred for 2 hours. Ethylene glycol (2 ml) was added to the reaction mixture and extracted with chloroform (40 ml x 3). The chloroform layer dried and evaporated to afford sticky solid: R_f 0.48 (CH_2Cl_2; MeOH: 9:1). To an ice-cooled solution of crude aldehyde in MeOH (10mL) was added sodium borohydride (1.16g, 30.76 mmol) in two portions. Reaction mixture was stirred for 3 hrs and quenched by adding saturated aq NH_4Cl solution (5 mL). MeOH was evaporated under reduced pressure, extracted with ethyl acetate (30 mL X 3) and concentrated. Purification by column chromatography (pet ether/ethyl acetate 1:9) gave **13** (2.16 g, 87%) as a sticky solid: R_f 4.9 (CH_2Cl_2/MeOH: 9:1). $[\alpha]_D^{22}$ + 41.14 (c, 0.26 MeOH); IR (KBr, ν, cm^{-1}): 3120-3 (br), 2990, 1678 (OH,CONH); ^{1}H NMR (300 MHz, D_2O+DMSO-d^6) δ (ppm); 5.82 (d, *J* = 3.9 Hz, 1H, H-8);. 4.39 (d, *J* = 3.9 Hz, 1H, H-9); 4.03 (at, *J* = 6, Hz, 1H, H-6); 3.58-3.74 (m, 2H, -CH_2OH); 2.30-2.52 (m, 3H, H-3/4); 2.02-2.18 (m, 1H, H-4); 1.49 (s, 3H, -CH_3); 1.32 (s, 3H, CH_3); ^{13}C NMR (75 MHz, D_2O+DMSO-d^6) δ (ppm); 171.3 (C-2, -*C*ONH). 103.8 (O-C-O), 95.8 (C-8, O-C-O) 78.0 (C-9/6), 73.8(C-9/6), 61.2 (C-5, -C-NH), 51.3 (CH_2OH), 20.8 (C-3, -CH_2), 17.4 (CH_3), 17.0 (CH_3), 14.0 (C-4, -CH_2); Anal. calculated For $C_{11}H_{17}NO_5$; C, 54.31; H, 7.04; N, 5.76; Found C, 54.31; H, 7.04; N, 5.74;

(5*R*,6*S*,8*R*,9*R*)-6-*O*-tosylmethyl,8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (14)

To the solution of alcohol **13** (2.00 g, 8.22 mmol) in pyridine (15 mL) was added p-tolune sulphonyl chloride (2.35 g, 12.33 mmol) in portion at 0 °C, catalytic DMAP (0.10 g, 0.82 mmol) and stirred for 12 h at rt. The reaction mixture was concentrated under vacum and extrated with dichloromethane (40 mL X 3). The combined organic layers were dried and evaporated under reduced pressure, which was purified by column chromatography on silica 3:2 pet.ether : Ethyl acetate as an eluant to afforded **14** (2.94 g, 92%), as a thick liquid. R_f 0.52 (pet ether/ethyl acetate, 1:9); $[\alpha]_D^{25}$ +54.7 (c, 0.26 CH_2Cl_2). IR (KBr, ν, cm^{-1}):2990, 1679; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm); 7.77 (d, *J* = 8.7 Hz, 2H, Ar); 7.46 (s, 1H, exchangable with D_2O); 7.33 (d, *J* = 8.7 Hz, 2H, Ar); 5.83 (d, *J* = 3.3 Hz, 1H, H-8); 4.32 (d, *J* = 3.3, Hz, 1H, H-9); 4.08-4.20 (m, 3H), 2.32-2.52 (m, 6H, H4/3, -CH_3); 1.95-2.08 (m, 1H, H-4); 1.47 (s, 3H, -CH_3); 1.29 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm); 178.6 (C-2, -CONH), 145.2 (C-S,-Ar), 132.2 (C-q, -Ar), 130.0 (strong, CH-Ar), 128.0 (strong, CH-Ar), 112.7 (C-8, O-C-O), 103.8 (O-C-O), 85.6 (-CH_2-O-), 78.3 (C-6/9), 69.5 (C-5), 66.5(C-6/9) , 29.1 (C-3, -CH_2CO), 26.6 –CH_3), 26.2 (-CH_3), 22.0 (CH_3-Ph), 21.6 (C-4, -CH_2) ; Anal. calculated For $C_{18}H_{23}NO_7S$; C, 54.40; H, 5.83; N, 3.52; S, 8.07 Found C, 54.40; H, 5.82; N, 3.51; S, 8.05.

(5*R*,6*S*,8*R*,9*R*)-6-azido-methyl,8-9-isopropylidendioxy-1-aza-7-oxaspiro[4.4]non-2-one (15)

To a solution of **14** (1.80 g, 4.52 mmol) in dry DMF (15 mL) was added sodium azide (1.47 g, 22.64 mmol), TBAI (0.17g,0.45 mmol) and the mixture was heated for 6h at 100 °C. Reaction mixture was extracted with EtOAc (50 mL X 3) and washed with water (10 mL X 2) and brine. Organic layer was dried over anhydrous sodium sulfate, and solvent was evaporated under reduced pressure. Chromatography using petroleum ether / EtOAc, (75:25) as an eluant to gave **15** (626 mg, 92.87%) as yellow solid; R_f 0.55 (petroleum ether / EtOAc, 3:7). mp 135-136 °C; $[\alpha]_D^{25}$ +33.00 (c, 0.26 CH_2Cl_2). IR (KBr, ν, cm^{-1}):3086-3162, 2880, 2095, 1711, 1665; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm); 6.76 (bs, 1H, exchangable with D_2O, H-1, -NH); 5.88 (d, *J* = 3.9 Hz, 1H, H-8); 4.37 (d, *J* = 3.9 Hz, 1H, H-9); 4.08 (t, *J* = 5.3, 6.2 Hz, 1H, H-6); 3.55 (dd, *J* = 12.8,6.2 Hz, 1H, --CH_2-N_3); 3.40(dd, *J* = 12.8, 5.3, Hz, 1H, -CH_2N_3); 2.37-2.58 (m, 3H, H-3/4a); 1.94-2.08 (m, 1H, H-4b) ; 1.53 (s, 3H, -CH_3); 1.33 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm); 177.8 (C-2, -CONH), 112.7 (-O-C-O), 103.7 (C-8, O-C-O), 85.7 (C-9, -C-O), 79.4 (C-6), 69.2 (C-5, -C-NH), 49.0 (-CH_2-N_3), 29.1 (C-3, -CH_2CONH), 26.6 (-CH_3), 26.3 (-CH_3), 22.2 (C-4, -CH_2): Anal. calculated For $C_{11}H_{16}N_4O_4$; C, 49.25; H, 6.01; N, 20.88, Found C, 49.25; H, 6.00; N, 20.87

(5r,6R,10S)-6,10-dihydroxy-1,8-diazaspiro[4.5]decan-2-one (2)

To a solution of **15** (0.300 g, 1.11 mmol) in TFA-water (4 mL, 3:1) was stirred for 3 h at 0 °C. TFA was co-evaporated with toluene at reduced pressure to furnish a thick liquid. To a solution of the above product in methanol was added 10% Pd/C (0.05 g). The solution was

hydrogenated at 100 psi for 24 h. The catalyst was filtered through Celite and washed with methanol and filtrate was concentrated to obtain a semisolid. Purification by column chromatography (CH_2Cl_2:MeOH 4:1) gave **2** (0.175 g, 84.13%) as a sticky white solid: R_f 0.45 (CH_2Cl_2; MeOH: 3:2); $[\alpha]_D^{25}$ 0.001(c, 0.23 MeOH); IR (KBr, ν, cm^{-1}): 3200-3600 (br),1687, ^{1}H NMR (300 MHz, D_2O) δ (ppm); 3.66 (dd, *J* = 11.5, 5.1 Hz, 2H, H-6/10, -CHOH); 3.00 (dd, *J* = 12.3, 5.1 Hz, 2H, H-7/9e, -CH_2NH) ; 2.50 (dd, *J* = 12.3, 11.5 Hz, 2H, H-7/9a, -CH_2NH); 2.44 (t, *J* = 8.4 Hz, 2H, H-3); 2.09 (t, *J* = 8.4 Hz, 2H, H-4, -CH_2); ^{13}C NMR (75 MHz, D_2O) δ (ppm); 181.2 (C-2, -CONH), 70.6 (C-6/10, -CHOH), 68.9 (C-5, -C-N), 46.4 (C-7/9, -CH_2NH), 30.7 (C-3, -CH_2CO), 17.4 (C-4, -CH_2); MS (ESI) *m/z* = 186.1004; HRMS calculated for $C_8H_{15}N_2O_3$ $[M+H]^+$: 187.1082. Found: 187.1085.

(5S,6R,10R)-6,10-dihydroxy-1,8-diazaspiro[4.5]decane-2,7-dione (3)

Compound **15** (0.20 g) was dissolved in (5:1) TFA: water (6 ml) and stirred at 0 °C for 30 min. the reaction mixture was allowed to attain rt and stirred for 2 h. Then additional amount of TFA (1 ml) was added, and stirred the reaction mixture at rt for 24 h. The TFA was removed under reduced pressure by co-evaporation with toluene. Compound was purified by column chromatography using $CHCl_3$/MeOH(7:3) afforded **3** (0.13 g, 87 %) as a sticky white solid : R_f 0.50 ($CHCl_3$/ MeOH : 1:1); $[\alpha]_D^{25}$ +28.6 (c, 0.12 MeOH); IR (KBr, ν, cm^{-1}): 3092-3580 (br),1686,1667; ^{1}H NMR (200 MHz, D_2O) δ (ppm); 4.12 (s, 1H, H-6, -CHOH); 4.14 (dd, *J* = 10, 6 Hz, 1H, H-10, -CHOH); 3.48 (dd, *J* = 12, 6 Hz, 1H, H-9a, -CH_2NH); 3.08 (dd, *J* = 12, 10 Hz, 1H, H-9b, -CH_2NH); 2.25-2.60 (m, 2H, H-3, -CH_2CO); 2.15 (ddd, *J* = 6, 10, 16 Hz, 1H, H-4a, -CH_2); 1.85 (ddd, *J* = 6, 10, 16 Hz, 1H, H-4b, -CH_2); ^{13}C NMR (75 MHz, D_2O) δ (ppm); 178.7 (C-2, -CONH), 169.5 (C-7, -CONH), 68.5 (C-6-CHOH), 63.6 (C-5, -C-N), 63.0 (C-10, -CHOH), 40.0 (C-9, CH_2-NH), 28.0 (C-3, -CH_2, 16.0 (C-4, -CH_2); MS (ESI) *m/z* = 200.0797; HRMS calculated for $C_8H_{13}N_2O_4$ $[M+H]^+$: 201.0875. Found: 201.08779.

(5S,9R)-9-hydroxy-1,7-diazaspiro[4.4]nonane-2,6-dione (4)

A solution of **15** (0.35 g,) in TFA-water (4 mL, 3:1) was stirred for 3 h at 0 °C. TFA was co-evaporated with toluene at reduced pressure furnish a thick liquid crude hemiacetal that were treated with sodium metaperiodate (0.42 g, 1.95 mmol) in acetone-H_2O (8 mL, 3:1) at 0 °C to rt for 2 h. Reaction mixture was quenched with ethylene-diol (1mL) 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 0.50 ($CHCl_3$; MeOH : 1:1). The above crude product was again treated with TFA: water (5:1) (6 ml) and stirred at 0 °C for 30 min. the reaction mixture was allowed to attain rt and stirred for 2 h. Then additional amount of TFA (1 ml) was added, and stirred the reaction mixture at rt for 24 h. The TFA was removed under reduced pressure by co-evaporation with toluene. Compound was purified by column chromatography using $CHCl_3$/MeOH(4:1) afforded **4** (0.16 g, 73%) as sticky white solid : R_f 0.48 ($CHCl_3$; MeOH : 3:2). **$[\alpha]_D^{25}$** –74.4 (c, 0.3 MeOH). (KBr, ν, cm^{-1}): 3100-3470 (br),1686,1690; ^{1}H NMR (500 MHz, D_2O) δ (ppm); 4.48 (t, *J* = 8.1 Hz, 1H, H-9); 3.62 (dd, *J* = 9.9, 8.1 Hz, 1H, H'-8); 3.17 (dd, *J* = 9.9, 8.1 Hz, 1H, H-8); 2.40-2.72 (m, 3H, H-3, H-4); 1.90-2.1 (m, 1H, H-4); ^{13}C NMR (125 MHz, D_2O) δ (ppm); 181.5 (-*CONH*), 176.5 (-*CONH*), 71.6 (C-9, CH-OH), 67.4 (C-5, C-N), 43.5 (C-8, CH_2-N), 28.8 (C-3), 21.6 (C-4). MS (ESI) *m/z* = 170.0691; HRMS calculated for $C_7H_{11}N_2O_3$ $[M+H]^+$: 171.0770. Found: 171.0772.

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