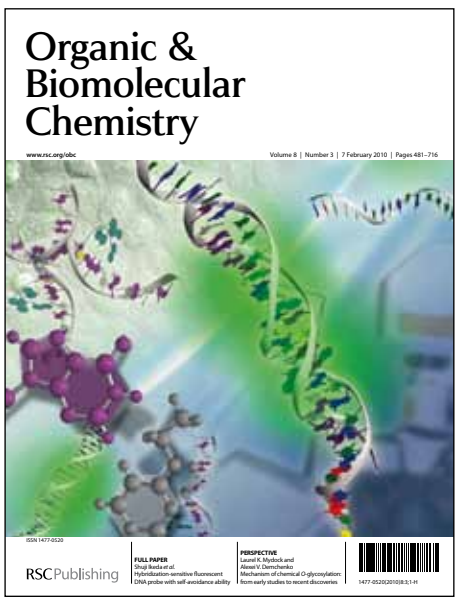
Organic & Biomolecular Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Protecting Group Directed Diversity During Mitsunobu Cyclization of a Carbohydrate Derived Diamino Triol. Synthesis of Novel Bridged Bicyclic and Six-membered Iminocyclitols.†

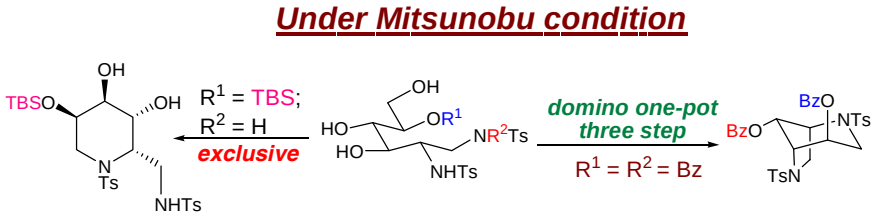
Authors: Muthupandian Ganesan, Rahul Vilas Salunke, Nem Singh and Namakkal G. Ramesh*

Department of Chemistry, Indian Institute of Technology-Delhi, Hauz Khas, New Delhi-110016, India.

*Corresponding author. E-mail: ramesh@chemistry.iitd.ac.in, Tel.: +91-11-26596584; fax: +91-11-26581102.

Graphical contents entry:

A novel and an unprecedented protecting group directed diversity leading to the synthesis of bridged bicyclic and six-membered iminocyclitols from a common carbohydrate derived diamino triol under Mitsunobu condition is reported.



Summary:

A novel protecting group directed diversity leading to the synthesis of bridged bicyclic and six-membered iminocyclitols from a common carbohydrate derived diamino triol under Mitsunobu condition is reported. When the intramolecular cyclization of benzoyl derivative **16** was carried out under Mitsunobu condition, an unprecedented one-pot domino intramolecular "cyclization – *N*→*O* benzoyl migration – cyclization" reaction sequence occurred resulting in the formation of a chiral 2,6-diazabicylo[3.2.1]octane-4,8-diol **21** in high yield. The structure of this novel bridged bicyclic compound was established through detailed NMR studies and single crystal X-ray analysis as well. On the other hand, the *tert*-butyldimethylsilyl derivative of the

same substrate afforded protected 6-amino-1,6-didieoxy-L-gulonojirimycin **32** as the sole product under identical condition. An attempt has been made to explain this difference in their reactivity through conformational analysis. The glycosidase inhibition studies of new compounds reported in this manuscript revealed that these molecules display moderate but selective inhibition against β-*N*-acetylhexosaminidase.

Introduction:

Iminocyclitols (azasugars) represent a unique class of molecules having structural and stereochemical resemblance to natural carbohydrates but with properties that are quite distinct from them. Due to their ability to inhibit various glycosidases through mimicry of the glycosidase oxo-carbenium-ion transition state, they have been found to be of medicinal value in the treatment of diseases due to viral infections and metabolic disorders including HIV and diabetes.[1,2] Their low-molecular weight, high water solubility, stability and ability to access selective targets make them attractive drug candidates especially against carbohydrate-mediated disorders.[3] Since the successful development of drugs, such as Zavesca® **1** and Glyset® **2** (Fig. 1),[4] through synthetic modifications of natural azasugars, research focus in this area has been greatly devoted toward such functional group modifications with a view to identifying better and specific glycosidase inhibitors. In this context, amino derivatives of naturally occurring five-membered iminocyclitol DMDP **4**, such as **5** and its stereo-analogues have received extensive attention in recent years[5–13] and some of these compounds have been identified as novel structures for antivirals and osteoarthritis.[14] On the other hand, literature reports on the synthesis of amino derivatives of six-membered azasugar 1-deoxynojirimycin **3**, such as 6-amino-1,6-dideoxynojirimycin **6** and its other stereo analogues, although available, are scattered, despite the fact that these compounds not only display improved inhibition against glycosidases but also serve as convenient precursors for the synthesis of other azasugars.[10,15–18] Though the amino-modified five-membered and six-membered azasugars (**5** and **6**) are structural isomers, the available methodologies are limited toward the synthesis of either one of them only. Synthetic approaches toward both of them from a common intermediate are still at large, the lone exception being due to McCort et al.[10] Even in this case, compounds 2-*epi*-**5** and 2-*epi*-**6** were obtained as a mixture, during a Lewis-acid catalyzed ring opening of sugar derived bis-aziridine with allyl alcohol, amongst which the latter was the major product (55%). To the best of our knowledge, methodology that leads to *exclusive* formation of amino-modified five-membered

azasugar and its six-membered analogue, from a common intermediate, through a divergent approach, has not been reported so far.

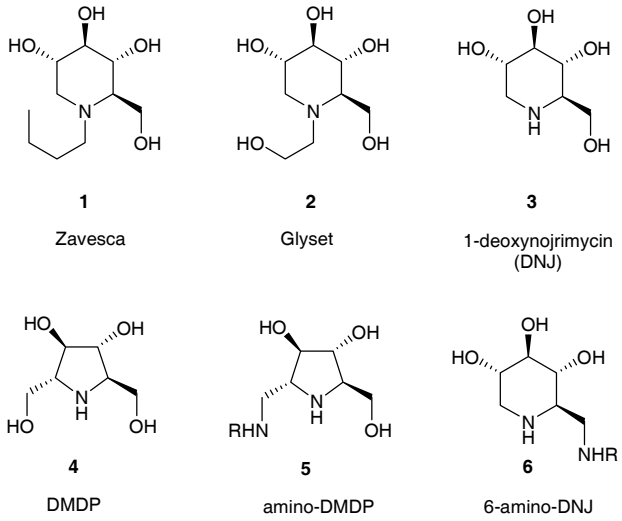
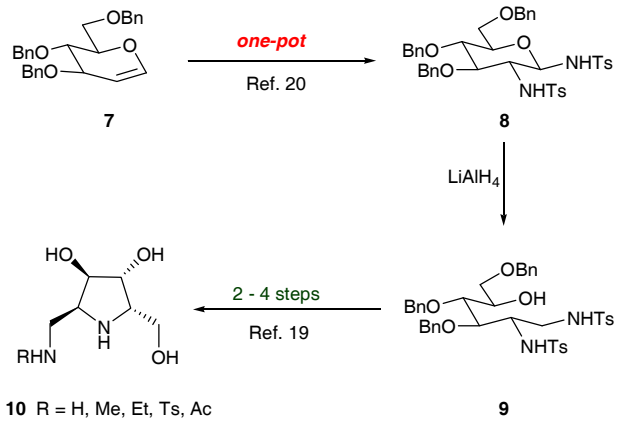


Figure 1 Representative example of natural and synthetic iminocyclitols.

Recently, we had reported a very short synthesis of a new stereo analogue of amino-DMDP and its derivatives **10** starting from diamino alcohol **9**, which in turn was synthesized in two steps from tri-*O*-benzyl-D-glucal **7** through a direct diamination followed by reduction (Scheme 1).[19,20] While further exploring the versatility of **9** towards the synthesis of amino-modified 1-deoxynojirimycin analogue **13**, serendipitous formation of a novel bridged bicyclic diaza derivative **21**, through a domino process, under Mitsunobu condition, was observed. Quite interestingly, a change in the protecting group of the same substrate exclusively afforded 6-amino-1,6-dideoxy-L-gulonojrimycin derivative **32** without any domino reaction. The new

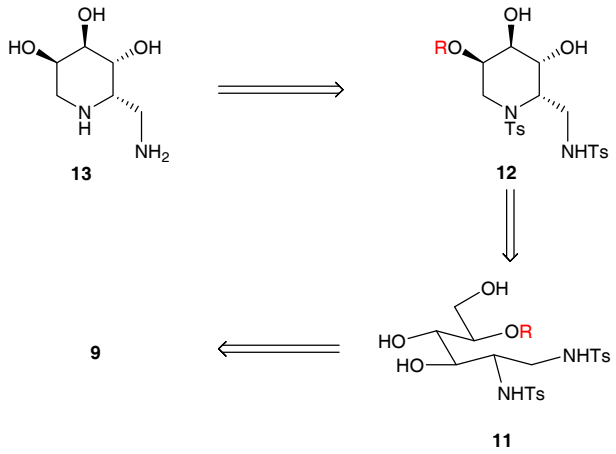


Scheme 1 Synthesis of amino-modified five-membered iminocyclitols **10** from tri-*O*-benzyl-D-glucal **7**.

compounds synthesized, after final deprotection, displayed moderate but selective inhibition against β-*N*-acetylhexosaminidase.

Results and Discussion:

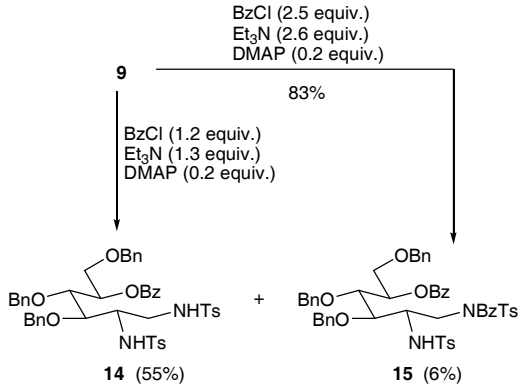
The retrosynthetic pathway of our proposed synthesis of 6-amino-1,6-dideoxy-L-gulonojirimycin **13** is depicted in Scheme 2. Protection of the hydroxyl group of **9**[19] followed by debenzylation would lead to the formation of triol **11**. Intramolecular cyclization of **11** was expected to preferably afford the polyhydroxylated piperidine **12**. Subsequent deprotection of all the protecting groups would then deliver the hitherto unreported 6-amino-1,6-dideoxy-L-gulonojirimycin **13.**



Scheme 2 Retrosynthetic route for the formation of 6-amino-1,6-didoxy-L-gulonojirimycin **13**

Synthesis of 2,6-diazabicyclo[3.2.1]octan-4,8-diol through a domino process.

Our efforts towards the synthesis of 6-amino-1,6-dideoxy-L-gulonojirimycin **13** started with benzoylation of the hydroxyl group of **9**. An intriguing observation was that the expected esterification reaction, while did not go to completion with 1.2 equiv. of BzCl in presence of triethyl amine and 20 mol% of DMAP, afforded a mixture of two compounds. Separation of them by column chromatography and through careful analysis of their spectral data, they were identified as compounds **14** and **15**, the latter arising out of an initial *O*-benzoylation followed by a selective *N*-benzoylation at C-1 nitrogen atom. On the other hand, use of 2.5 equiv. of BzCl in presence of 2.6 equiv. of Et_3N and 20 mol% of DMAP led to complete conversion of **9** resulting only in the formation of **15** in 83% yield (Scheme 3).



Scheme 3 Benzoylation reaction of diamino alcohol **9**.

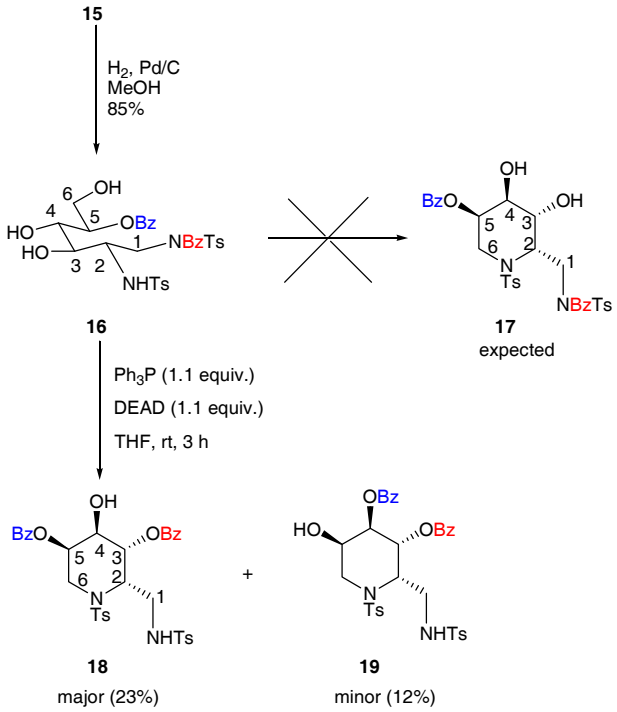
The chemoselective *N*-benzoylation at C-1 nitrogen and not at C-2 nitrogen was confirmed from the ^{1}H and ^{13}C-NMR spectral data of **15**. In its ^{1}H-NMR spectrum, signals of the two diastereotopic protons at C-1 appeared more deshielded and resonated at δ 3.92 and 3.77 as compared to the protons at C-1 of compound **9** that resonated at δ 2.88 and 2.79 respectively. Further, in the ^{13}C-NMR spectrum, there was an appreciable downfield shift in the signal of C-1 carbon from δ 44.4 in the hydroxyl compound **9** to δ 47.0 in the product **15**.

The dibenzoylation reaction observed above, though not expected, was indeed advantageous, as it ensured the participation of only C-2 nitrogen atom (and not C-1 nitrogen atom) of **15** in our planned intramolecular cyclization reaction, eventually leading to the formation of our required 6-amino-1,6-dideoxy-L-gulonojirimycin derivative **17** (Scheme 4). In order to proceed in this direction, the benzyl groups of **15** were cleaved through catalytic hydrogenation in presence of 10% Pd/C to get the triol **16** in 85% yield.

When intramolecular cyclization of compound **16** was investigated under Mitsunobu condition, the reaction was not as straight-forward as anticipated. The product formation was found to be dependent on the stoichiometry of the reagents. With 1.1 equiv. of Ph_3P and DEAD, the reaction while remained incomplete, afforded two products, a major and a minor one. Separation of them by column chromatography over silica gel followed by careful analysis of their NMR spectral data revealed that none of these two products correspond to the expected compound **17**.

From the HRMS data of the major product, its molecular composition was deduced to be $C_{34}H_{34}N_2O_9S_2$ suggesting that an intramolecular cyclization indeed had taken place with the loss of one molecule of water. Interestingly, its ^{1}H-NMR spectrum displayed a signal at δ 5.57 resonating as a *triplet* (J = 5.1 Hz, exchangeable with D_2O), indicating the presence of an acidic

proton that has two neighbouring protons to couple with. Subsequently, through a detailed analysis of the spectral data, the structure of the major compound was identified as **18** (Scheme 4). The formation of compound **18** was thus a result of a facile *N*→*O* 1,3-benzoyl migration of the initially formed piperidine derivative **17** under the reaction condition. Fortunately, compound **18** could be crystallized and the structural assignment was established through single crystal X-ray analysis as well (Fig. 2).[21] The possibility of benzoyl migration during the debenzylation of **15** to **16** was ruled out from the ^{13}CNMR spectral analysis. There was hardly any shift in the signals of the C-1 carbons of **15** and **16**, confirming the retention of the benzoyl group at the C-1 nitrogen of **16** during debenzylation.



Scheme 4 Synthesis and Mitsunobu cyclization of compound **16**

The molecular composition of the minor compound was also found to be the same as that of **18** and its ^{1}H-NMR spectrum also displayed a signal due to a proton that resonated at δ 5.64 as a *triplet* (J = 5.4 Hz) that was exchangeable with D_2O. After thorough analysis of its ^{1}H and ^{13}C-NMR spectral data, its structure was deduced as **19**. The structural assignment of **19** was subsequently substantiated through a chemical transformation. Since both compounds **18** and **19** differ only in the position of one of their benzoyl groups, upon hydrolysis with Na in MeOH, both of them afforded the same product **20** (Scheme 5) thereby clearly establishing the structure of compound **19**.

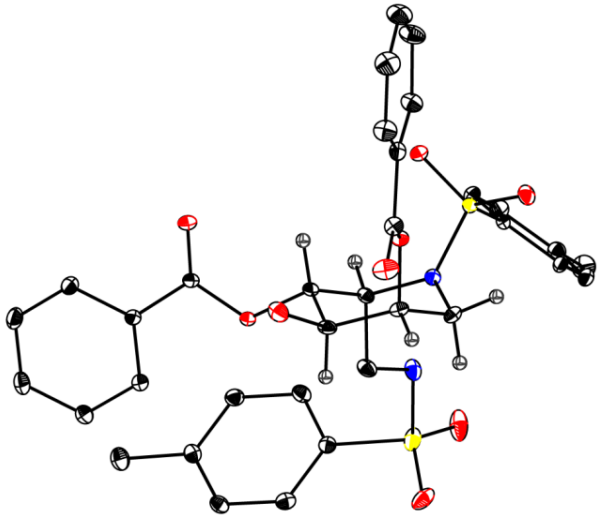
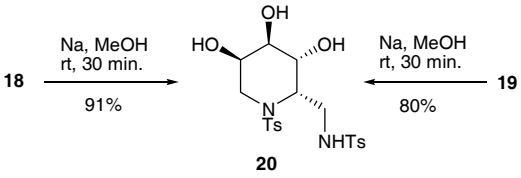


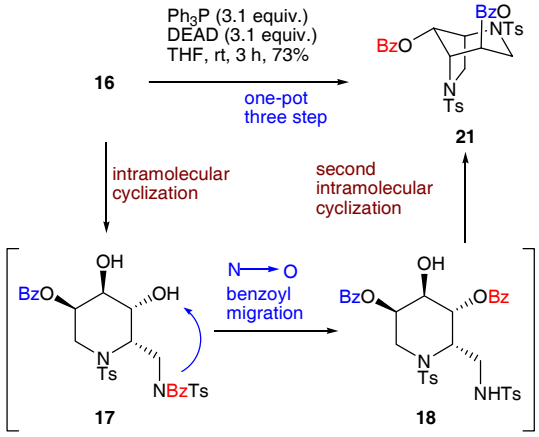
Figure 2 Single crystal X-ray structure of compound **18**. Solvent (THF) and hydrogens other than those on the piperidine ring are omitted for clarity.



Scheme 5 Basic hydrolysis of compounds **18** and **19** to give the triol **20**

Deeper investigations on the Mitsunobu reaction of triol **16** with varying proportions of Ph_3P and DEAD further led to some unusual and surprising results. When the reaction was performed with 2.2 equiv. of Ph_3P and DEAD, the reaction was still incomplete. Rather, appearance of a new non-polar spot was noticed in TLC. Interestingly, with 3.1 equiv. of Ph_3P and DEAD, not only did the reaction go to completion, but also was very clean resulting in the formation of the new non-polar compound exclusively. Under this condition, formation of compounds **18** and **19** were not at all noticed. The HRMS data of the compound revealed the molecular composition to be $C_{34}H_{32}N_2O_8S_2$, indicating the loss of two molecules of water. Moreover there were no exchangeable protons in the molecule as revealed from its ^{1}H-NMR spectra. Through detailed analyses of various 2D-NMR spectra (supporting information), the structure of the product was identified as the bridged bicyclic compound **21** (Scheme 6). Its structure was subsequently confirmed through single crystal X-ray analysis[22] of its di

debenzoylated derivative **22** (Fig. 3), which was obtained by exposing it to Na in MeOH (Scheme 7).



Scheme 6 Synthesis of bridged bicyclic compound **21** through a domino process

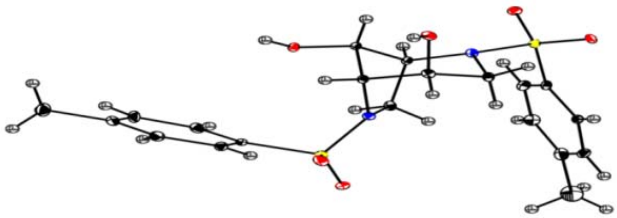
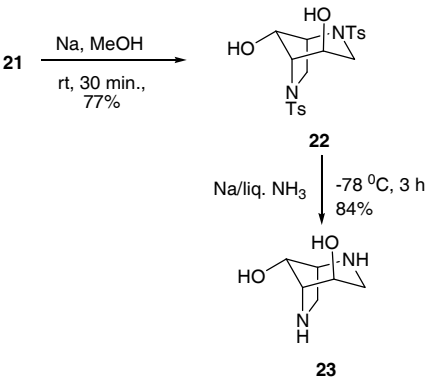


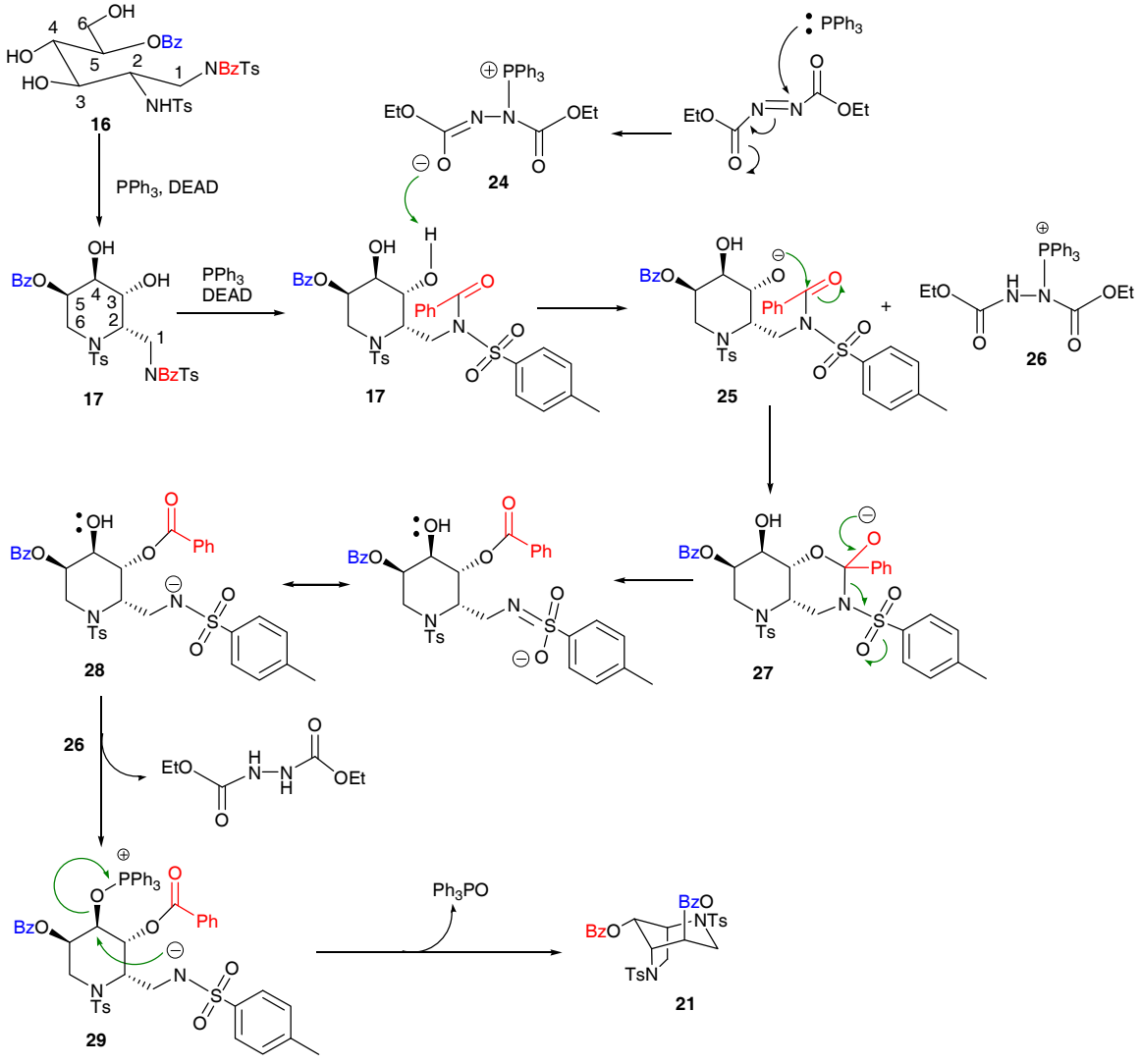
Figure 3 Single crystal X-ray structure of compound **22**



Scheme 7 Synthesis of 2,6-diazabicyclo[3.2.1]octan-4,8-diol **23**

The formation of the protected 2,6-diaza bicyclo [3.2.1]octane-4,8,diol **21** directly from **16** can be visualized as an *one-pot three-step* domino intramolecular "*cyclization-benzoyl migration-cyclization*" process (Scheme 6).

A plausible mechanism for the direct formation of the bicyclic derivative **21** from **16** is depicted in Scheme 8. First intramolcular cyclization of compound **16** in presence of Ph_3P and DEAD would give rise to the piperidine derivative **17** in which the C-3 hydroxyl group and C-1 amino functionality (numbering based on parent carbohydrate), being in *cis* geometry to each other, are properly positioned for a possible migration of the benzoyl group. Abstraction of proton from the C-3 hydroxyl group of **17** by the anion adduct **24** generated from the second equivalent of Ph_3P and DEAD would give rise to the alkoxy anion **25**. Intramolecular nucleophilic addition of the alkoxy anion **25** to the carbonyl carbon of the benzoyl group at C-1 nitrogen atom would result in the formation of a six-membered cyclic intermediate **27**, which on ring opening, with the departure of the benzoyl group to C-3 position, would then lead to the resonance stabilized anion **28**. In the adduct **28**, the nucleophilic nitrogen being *trans* to C-4



Scheme 8 Proposed mechanism for domino one-pot formation of bicyclic derivative **18**.

hydroxyl group is suitably oriented for a second cyclization. Nucleophilic attack of the C-4 hydroxyl group on the phosphonium salt **26** to give the alkoxy phosphonium salt **29**, which when displaced by the anionic nitrogen atom, in an intramolecular fashion, would deliver the novel bridged bicyclic compound **21**. In order to provide an additional support for the proposed mechanism, compound **18** was independently subjected to a second Mitsunobu cyclization with 1.2 equiv. of PPh_3 and 1.4 equiv. of DEAD. The reaction proceeded smoothly to afford the bicyclic derivative **21** 80% yield in just 30 min thereby confirming that **18** is indeed an intermediate in the formation of **20**.

Even though two equivalents of Mitsunobu reagents are sufficient for the formation of the bridged bicyclic compound **21**, experimentally, it was obtained only in about 50% yield even with 2.2 equivalents. This is probably due to the competing formation of compound **17** in a reasonable amount. However, with the use of 3.1 equivalents, the reaction was quite rapid affording the bicyclic compound **21**, as the only isolable product, in 69% yield in just 30 min. A slight, but not an appreciable, increase in the yield (73%) was noticed when the reaction time was increased to 3 h.

Migration of functional groups during Mitsunobu reactions though reported,[23] to our knowledge, only a lone example is available on the migration of a benzoyl group and too in an intermolecular fashion.[24] The result observed in this paper is quite unique as the benzoyl group migration was not only very facile, but it also provided the opportunity for a successive second intramolecular cyclization to take place *in situ* affording the bicyclic compound in a single step.

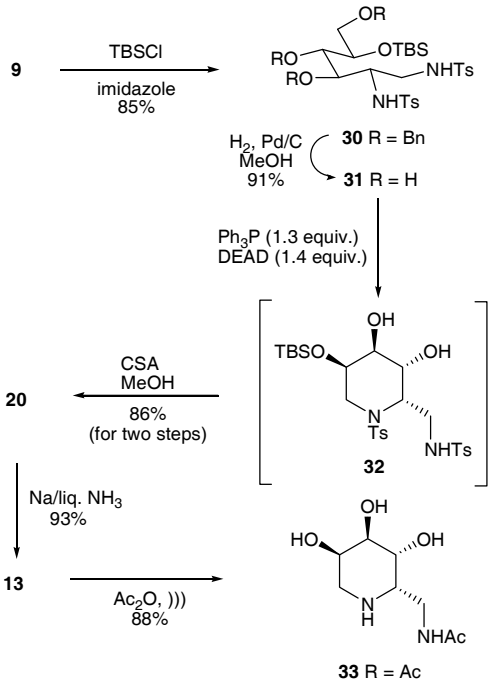
Bridged bicyclic iminosugars, calystegines and related compounds such as teloidine, baogongton, erycibelline have attracted the attention of organic chemists owing to their biological significance as glycosidase inhibitors.[1,25] There are also examples of *endo-endo* type bridged bicyclic conformationally restricted diamines (CRDA).[26] However, there are no reports on the synthesis of bridged bicyclic *di*azasugar possessing a [3.2.1] skeleton and the present work perhaps represents the first of its kind.

In order to investigate the glycosidase inhibition activities, deprotection of the tosyl groups of **22** was perfomed with Na/liq. NH_3 to get the final compound, 2,6-diaza-bicyclo[3.2.1]octan-4,8-diol, **23** in 84% yield (Scheme 7).

Synthesis of 6-amino-1,6-dideoxy-L-gulonoijirimycin 13

In light of the unexpected results observed with the benzoyl protected triol **16**, we were interested in protecting the hydroxyl group of **9** as its silyl ether and study its effect on the

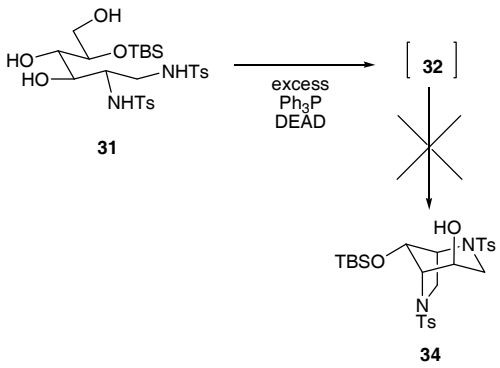
outcome of the Mitsunobu cyclization. Thus, silylation of **9** was accomplished smoothly with TBS chloride and imidazole to get the TBS ether **30** in 85% yield. Catalytic hydrogenation of **30** in presence of 10% Pd/C afforded the triol **31** (91%). Intramolecular Mitsunobu reaction of triol **31** with 1.3 equiv. Ph_3P and 1.5 equiv. of DEAD proceeded in a regioselective manner to give *exclusively* the protected 6-amino-1,6-didexoy-L-gulonojirimycin **32** (Scheme 9). However, as the separation of compound **32** from diethyl hydrazodicarboxylate, the reduction product of DEAD, was found to be difficult during purification by column chromatography, the crude product was carried over for subsequent desilylation step. Exposure of crude **32** to camphor sulfonic acid (CSA) conveniently cleaved its TBS group to provide the triol **19** in 86% yield (for two steps). Detosylation of triol **19** with Na/liq. NH_3 delivered the *hitherto unreported* 6-amino-1,6-dideoxy-L-gulonojirimycin **13** (93%) which on chemoselective acetylation at the side chain nitrogen atom with acetic anhydride under solvent free sonication condition afforded the acetyl derivative **33** in 88% yield (Scheme 9).



Scheme 9 Synthesis of 6-amino-1,6-dideoxy-L-gulonojirimycin **13** and its acetyl derivative **33**

Conformational analysis of compounds 18 and 32

An interesting and noteworthy observation is that, unlike the previous case, formation of the corresponding bicyclic compound **34** from **32**, through a second intramolecular cyclization, was not at all observed, even with an excess (3.1 equiv.) of the reagents and longer reaction



Scheme 10 Attempted Mitsunobu reaction of compound **31** with excess of the reagents

times (Scheme 10). Given the nature and bulkiness of the protecting groups, it is quite likely that compounds **18** and **32** prefer to exist in different conformations. Their feasibility toward second intramolecular cyclization, through a S_N2 reaction, may thus depend upon the attainability of requisite orientation by the nucleophile and the leaving group, in their respective conformations. Thus, in order to get an insight in to the observed difference in the reactivity of compounds **18** and **32** towards second intramolecular cyclization, conformational analysis of these compounds were carried out. In literature, unprotected as well as protected six-membered azasugars have been shown to exist in $^1C_4$ or $^4C_1$ conformation.[27] Based on these literature precedence, one may consider that the protected azasugar molecules **18** and **32** also adopt one of the two stable chair conformations i.e. $^1C_4$ or $^4C_1$ (Figures 4 and 5). In order for these molecules to participate in the second intramolecular cyclization, proper orientation of the nucleophile for back side attack at the reaction site (C-3 carbon, Fig. 4) is an essential requisite. Such an orientation demands that, in both the molecules, the side chain CH_2NHTs bearing the nucleophilic nitrogen atom should occupy the axial position. This type of arrangement is possible only when the compounds adopt $^4C_1$ conformation. Construction of a molecular model of compound **18** revealed its preferred conformation to be $^4C_1$ and not $^1C_4$. In this conformation, despite the C-2 benzoyl and CH_2NHTs groups occupying the axial positions (Fig. 4), there is least steric hindrance between various protecting groups. The existence of compound **18** in its $^4C_1$ conformation was also evidenced from the coupling constants of various protons in its ^{1}H-NMR spectrum, which displayed the appearance of the signal of H-4 at δ 5.26 ppm as a double doublet with coupling constants of 9.6 Hz ($J_{4a,3a}$) and 6.0 Hz ($J_{4a,5e}$) confirming that H-4 is in the axial position. The signal of H-3 appeared at δ 4.02 ppm as a double doublet with coupling constants of 9.6 Hz ($J_{3a,4a}$) and 2.7 Hz ($J_{3a,2e}$). There was hardly any coupling between H-2 and both H-1_{axial} as well as H-$1_{equatorial}$

indicating that H-2 is in equatorial position. ^{1}H-NMR spectral data thus provided convincing evidence for the existence of compound **18** in its $^4C_1$ conformation. Structure deduced from single crystal X-ray analysis was also supportive of this observation (Fig. 2). On the other hand, in $^1C_4$ conformation, the molecular model displayed greater steric hindrance between the axial C-4 benzoyl group and the tosyl group attached to the ring nitrogen. This would make $^1C_4$ a less preferred conformation. In order to verify this, conformational search was done using HyperChem.[28] Keeping the absolute configurations of all chiral centres fixed, conformational search was initially carried out for compound **18** using molecular mechanics, with an acceptance energy criterion of a maximum of 10 kcal/mol between various possible conformations. The optimization cycle was kept at 1000. A total of 13 structures, possessing different conformations and orientations of the substituents, were obtained during the initial geometry optimization using molecular mechanics calculations. Then, geometry optimization was done for each of these 13 structures, independently, using AM1 calculations to obtain the binding energy and heat of formation. The results from these calculations confirmed that the most stable conformation of compound **18** is $^4C_1$ (Fig. 6). The calculated binding energy and heat of formation of compound **18** are −8715.17 kcal/mol and −238.03 kcal/mol respectively. The $^4C_1$ conformation was found to be more stable than its $^1C_4$ conformation by 4.8 kcal/mol. In this conformation, the side chain CH_2NHTs group is suitably positioned for S_N2 attack at C-3 and hence compound **18** could undergo the intramolecular cyclization readily to give the bicyclic compound **21**.

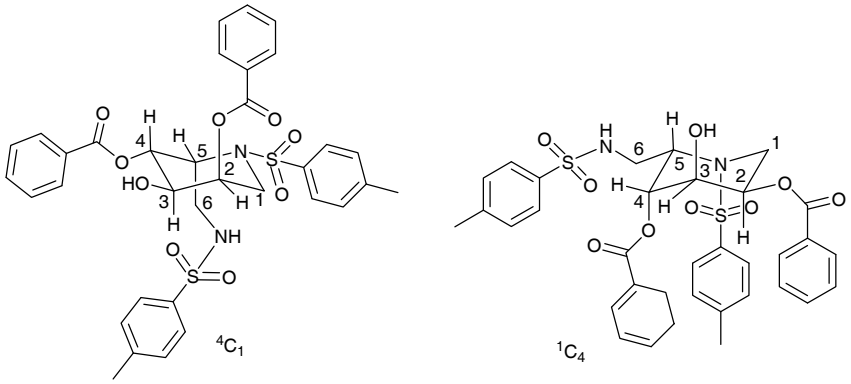


Figure 4 $^4C_1$ and $^1C_4$ conformations of compound **18** (numbering as per parent DNJ **3**)

In case of compound **32**, it was expected that the bulkiness of the TBS group might play a greater role in dictating its preferred conformation. In its $^4C_1$ conformation (Fig. 5), as revealed by the molecular model, the two bulky groups, TBS and CH_2NHTs, would occupy the axial positions making the molecule in this conformation less stable. On the other hand, in $^1C_4$

conformation, the less bulky hydroxyl groups are axially positioned leaving the bulkier TBS and CH_2NHTs in the equatorial positions, a conformation that was supposed to be energetically more preferred. The conformation of compound **32** could not be deduced from its ^{1}H-NMR H-NMRspectral data due to the difficulty in its separation from diethyl hydrazodicarboxylate during column chromatography. Hence, in order to identify the preferred conformation of compound **32**, AM1 calculations were performed as before. In this case, a total of 20 structures, with different conformations and orientations of the substituents, were obtained during the initial geometry optimization using molecular mechanics calculations. Subsequently, when geometry optimization was carried out for each of these 20 structures, independently, using AM1 calculations, the calculations revealed a skew conformation ($^1S_5$) (Fig. 6) as the preferred one for compound **32** with a binding energy and heat of formation of −7709.07 kcal/mol and −297.75 kcal/mol respectively. It was found to be more stable than its energetically nearest $^4C_1$ and $^1C_4$ conformations by 2.5 and 4.6 kcal/mol respectively. In its $^1S_5$ conformation, the CH_2NHTs bearing the nucleophilic nitrogen atom is probably in an orientation not suitable for a S_N2 attack at C-3 (Fig. 6) indicating a possible higher energy transition state for the cyclization. Thus, preliminary conformational analysis based on AM1 calculations suggests the existence of compounds **18** and **32** in different preferred conformations thereby providing support for the observed difference in their reactivity towards second intramolecular cyclization reaction. Further detailed studies in this direction are expected to throw more light on this interesting aspect.

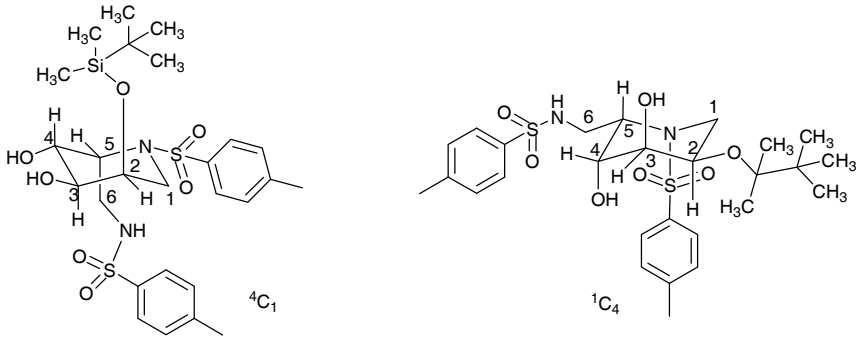


Figure 5 $^4C_1$ and $^1C_4$ conformations of compound **32** (numbering as per parent DNJ **3**)

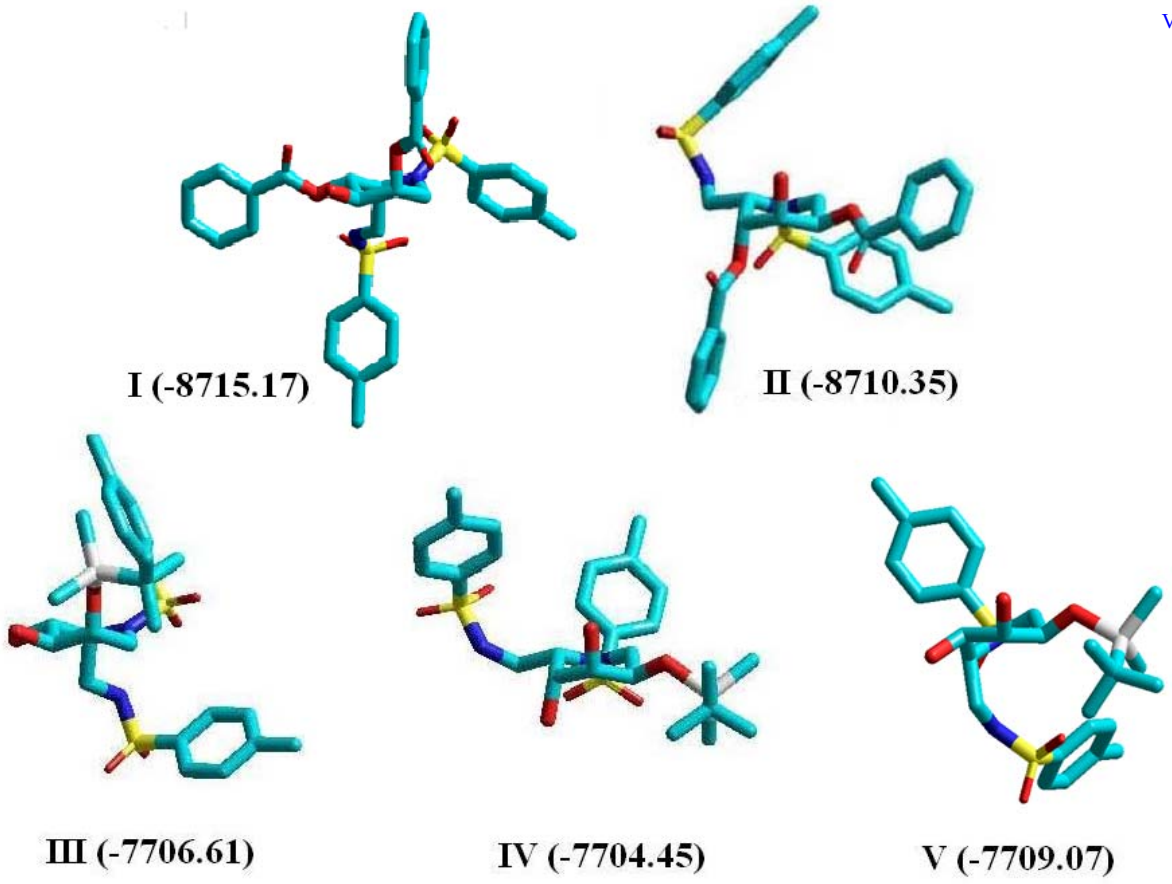


Figure 6 Different conformations and their calculated binding energies (in parenthesis) of compounds **18** and **32** based on AM1 calculations. Hydrogen atoms are omitted for clarity. **I** $^4C_1$ conformation of **18**; **II** $^1C_4$ conformation of **18**; **III** $^4C_1$ conformation of **32**; **IV** $^1C_4$ conformation of **32**; **V** $^1S_5$ conformation of **32**.

Glycosidase Inhibition Studies:

Compounds **13**, **23** and **33** were screened for their glycosidase inhibition activities against five commercially available enzymes.[19,29] These compounds did not inhibit any of the common glycosidases such as α– and β–glucosidases, and α– and β–galactosidases. However, all of them showed moderate inhibition against β–*N*–acetylhexosaminidase from jack bean (Table 1), with compound **33**, among them being a better inhibitor with an IC_{50} value of 385 μm.

Table 1 Glycosidase inhibition studies and IC_{50} values of compounds **13, 23** and **33**

Entry	Enzyme (source, conditions)	IC_{50}, mM		
		13	**23**	**33**
1.	α–glucosidase type I (bakers yeast, 37 °C & 6.8 pH	NI	NI	NI
2.	β–glucosidase (almond, 37 °C & 5 pH)	NI	NI	NI
3.	α–galactosidase (green coffee beans, 25 °C & 6.5 pH)	NI	NI	NI
4.	β–galactosidase (*Escherichia coli*, 37 °C & 7.3 pH)	NI	NI	NI
5.	β–*N*–acetylhexosaminidase (jack bean, 37 °C & 5 pH)	3.6	9.6	0.385

NI no inhibition was observed up to 10 mM inhibitor concentration

Conclusions:

In conclusion, we have disclosed the synthesis of skeletally distinct bridged bicyclic diazasugar **23** and 6-amino-1,6-dideoxy-L-gulonojirimycin **13** from a single starting material, through protecting group dictated diversity. The novel benzoyl migration and cascade reaction observed under Mitsunobu condition is quite unique and is likely to draw the attention of synthetic organic chemists. Conformational studies provide support for the observed diversity. Selective glycosidase inhibition activities of compounds **13**, **23** and **23**, though moderate, against β-*N*-acetylhexosaminidase may provide vital information regarding the structure-activity relationship of such classes of compounds. Further, we expect that compound **23** being a chiral conformationally restricted diamine (CRDA)[26] would find wide applications in the area of asymmetric catalysis, chiral base, coordination and supramolecular chemistry. Our research focus in this direction is currently underway.

Experimental Section:

All solvents were purified using standard procedures. Thin-layer chromatography (TLC) was performed on Merck silica gel pre-coated on aluminium plates. Flash column chromatography was performed on 230-400 mesh silica gel. Optical rotations were recorded on an Autopol V (Rudolph Research Flanders, New Jersey) instrument. All the rotations were measured at 589 nm (sodium D' line). Melting points of the compounds are uncorrected. IR

spectra were taken within the range 4000-400 cm^{-1} as KBr pellets on a Nicolet (Madison, USA) FT-IR spectrophotometer (Model Protege 460). All the ^{1}H and ^{13}C NMR spectra were recorded on a 300 or 500 MHz Bruker Spectrospin DPX FT-NMR. Chemical shifts are reported as δ values (ppm) relative to internal standard Me_4Si. Mass spectra were recorded using Bruker MicroTOF-QII instrument.

5-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (14) and **5-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1-*N*-benzoyl-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (15).** In a 50 mL round bottomed flask, compound **9**[19] (0.400 g, 0.527 mmol) was taken and dissolved with CH_2Cl_2 (10 mL). The solution was cooled in an ice bath. Et_3N (0.095 mL, 0.685 mmol) was then added to the reaction mixture followed by DMAP (0.013 g, 0.105 mmol). The reaction mixture was stirred at the same temperature for 10 min under N_2 atmosphere. BzCl (0.071 mL, 0.632 mmol) was then added, after which the ice bath was removed and the reaction mixture was stirred at 35 °C. The progress of the reaction was monitored by TLC which displayed two new spots in addition to the spot corresponding to the starting material. However, there was no significant change in the intensities of the different spots in TLC from 30 min. to 3 h. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layer was washed with aqueous $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Purification of the products by flash column chromatography over 230-400 mesh silica gel using 15% ethyl acetate in hexane as an eluent afforded compounds **14** (0.250 g, 55%) and **15** (0.030g, 6%) as colorless liquids along with unreacted starting material (0.060g, 15%).

Compound 14: R_f: 0.4 (hexane : ethyl acetate = 2 : 1); $[\alpha]^{29}_{D}$ +19.1 (*c* 0.722, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3283, 3031, 2921, 2866, 1719, 1597, 1452, 1334, 1160, 704, 549; δ_H (300 MHz, $CDCl_3$, Me_4Si) 7.96 (2H, d, *J* = 7.5 Hz); 7.63 (2H, d, *J* = 8.1 Hz); 7.60–7.53 (2H, m); 7. 43 (2H, t, *J* = 7.5 Hz); 7.32–7.22 (16H, m); 7.14–7.12 (2H, m), 7.07 (2H, d, *J* = 8.1 Hz), 5.24-5.23 (2H, m, including one exchangeable proton); 4.65 (1H, d, *J* = 10.5 Hz); 4.59–4.40 (6H, m); 3.94 (1H, d, *J* = 7.2 Hz); 3.87 (1H, dd, *J* = 10.5, 5.4 Hz); 3.67–3.63 (2H, m); 3.45 (1H, m); 2.92–2.79 (2H, s); 2.40 (3H, s), 2.11 (3H, s); δ_C (75 MHz, $CDCl_3$, Me_4Si): 165.4, 143.5, 143.4, 138.0, 137.7, 137.4, 137.1, 136.5, 133.2, 129.8, 129.7, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 127.1, 78.8,

76.6, 75.0, 74.5, 73.3, 72.5, 67.6, 53.5, 44.8, 21.5, 21.2; HRMS (ESI): $[M+Na]^+$ Found: 885.2849, $C_{48}H_{50}N_2O_9S_2Na$ requires 885.2850.

Compound 15: R_f: 0.5 (hexane : ethyl acetate = 2 : 1); $[\alpha]^{29}_D$ –1.8 (*c* 0.99, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3293, 3061, 3031, 2922, 2856, 1718, 1685, 1597, 1451, 1360, 1270, 1164, 1093, 704, 663; δ_H (300 MHz, $CDCl_3$, Me_4Si): 8.00 (2H, d, *J* = 7.5 Hz); 7.31 (2H, d, *J* = 7.5 Hz); 7.56 (2H, t, *J* = 7.2 Hz); 7. 42 (3H, t, *J* = 7.2 Hz); 7.27–7.09 (24H, m); 5.61 (1H, br s, exchangeable with D_2O); 5.39 (1H, s); 4.67 (2H, s), 4.60–4.48 (4H, m); 4.27–4.24 (1H, m); 4.09 (1H, s); 3.99–3.88 (4H, m); 3.78–3.73 (1H, m); 2.38 (3H, s); 2.24 (3H, s); δ_H (300 MHz, $CDCl_3$+DMSO-d_6, Me_4Si): 7.87 (2H, d, *J* = 7.2 Hz); 7.61 (2H, d, *J* = 8.1 Hz); 7.46 (2H, t, *J* = 7.2 Hz); 7. 32 (5H, t, *J* = 7.2 Hz); 7.21–7.05 (19H, m); 7.02 (3H, t, *J* = 8.4 Hz), 6.14 (1H, d, *J* = 7.2 Hz, exchangeable with D_2O); 5.41 (1H, q, *J* = 4.5 Hz); 4.61-4.34 (6H, m); 4.08 (1H, dd, *J* = 10.8, 4.2 Hz); 3.9–3.80 (5H, m); 3.62 (1H, dd, *J* = 10.8, 4.2 Hz); 2.27 (3H, s); 2.13 (3H, s); δ_C (75 MHz, $CDCl_3$, Me_4Si): 171.8, 165.5, 144.9, 143.4, 138.1, 137.9, 137.4, 137.3, 135.2, 135.1, 133.0, 131.3, 130.5, 129.8, 129.76, 129.72, 128.7, 128.47, 128.40, 128.34, 127.85, 127.81, 127.5, 127.3, 78.1, 77.5, 74.1, 73.9, 73.2, 73.1, 67.8, 53.5, 47.0, 21.6, 21.4; δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 171.3, 165.0, 144.5, 142.8, 137.7, 137.5, 137.4, 137.0, 134.8, 134.7, 132.7, 131.0, 129.6, 129.38, 129.31, 128.25, 128.22, 128.0, 127.97, 127.91, 127.8, 127.4, 127.3, 127.29, 127.21, 127.18, 126.7, 77.0, 73.51, 73.0, 72.6, 67.5, 52.9, 46.5, 21.2, 21.0; HRMS (ESI): $[M+Na]^+$ Found: 989.3151, $C_{55}H_{54}N_2O_{10}S_2Na$ requires 989.3112.

5-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1-*N*-benzoyl-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (15). In a 50 mL round bottomed flask, compound **9** (1.0 g, 1.31 mmol) was taken and dissolved with CH_2Cl_2 (15 mL). The solution was cooled in an ice bath. Et_3N (0.474 mL, 3.40 mmol) was then added to the reaction mixture followed by DMAP (0.032g, 0.26 mmol). The reaction mixture was stirred at the same temperature for 10 min under N_2 atmosphere. BzCl (0.382 mL, 3.29 mmol) was then added, after which the ice bath was removed and the reaction mixture was stirred at 35 °C for 3 h. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layer was washed with aqueous $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Purification of the product by flash column chromatography over 230-400 mesh silica gel using 15% ethyl acetate in hexane as an eluent afforded compound **15** (1.08 g, 83%) as a colorless liquid, the spectral data of which matched precisely with the product obtained in the previous experiment.

1-*N*-Benzoyl-5-*O*-benzoyl-1,2-dideoxy-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (16). 10% Pd on charcoal (0.520 g, 100% w/w) was taken in a 50 mL three necked round bottomed flask. Compound **15** (0.520 g, 0.538 mmol) dissolved in methanol (3 mL) was added to it and the reaction mixture was stirred at 42 ^{o}C. Hydrogen gas was then bubbled continuously into reaction mixture. Progress of the reaction was monitored by TLC and after completion (3 h), the reaction mixture was filtered through a celite pad and washed with methanol. The filtrate was then concentrated to get the product **16** (0.320 g) in 85% yield as a viscous liquid. R_f: 0.1 (hexane : ethyl acetate = 2 : 1); $[\alpha]^{29}_{D}$ +1.4 (*c* 0.1, MeOH); ν_{max}(KBr)/cm^{-1} 3415, 1708, 1274, 1163, 812, 709, 665; δ_H (300 MHz, $CDCl_3$, Me_4Si): 8.09 (2H, d, *J* = 6.3 Hz); 7.8 (2H, d, *J* = 6.9 Hz); 7.58-7.44 (6H, m); 7.20–7.18 (8H, m); 6.58 (1H, *br* s, exchangeable with D_2O); 4.94 (1H, *br* s); 4.38 (1H, *br* d, exchangeable with D_2O); 4.11 (3H, m, with two exchangeable protons); 3.88–3.79 (6H, m); 2.41 (3H, s); 2.33 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 171.6, 165.8, 144.8, 143.0, 137.1, 134.8, 134.6, 132.9, 131.2, 129.7, 129.5, 129.4, 129.3, 128.2, 128.1, 127.7, 127.4, 127.0, 74.5, 70.0, 66.9, 60.6, 56.9, 47.1, 21.3, 21.2; HRMS (ESI): $[M+Na]^+$ Found: 719.1711, $C_{34}H_{36}N_2O_{10}S_2Na$ requires 719.1704.

(2*S*,3*R*,4*R*,5*R*)-3,5-Di-benzoyloxy-4-hydroxy-2-(*N*-*p*-toluenesulfonyl)aminomethyl-1-*N*-(*p*-toluenesulfonyl)-piperidine (18) and **(2*S*,3*R*,4*R*,5*R*)-3,4-Di-benzoyloxy-5-hydroxy-2-(*N*-*p*-toluenesulfonyl)aminomethyl-1-*N*-(*p*-toluenesulfonyl)-piperidine (19).** A 50 mL three necked round bottomed flask was flame dried and cooled under argon atmosphere. Compound **16** (0.300 g, 0.430 mmol) was taken in it and dissolved with dry THF. PPh_3 (0.124 g, 0.47 mmol) was then added and the reaction mixture was cooled to 0 °C. DEAD (0.074 mL, 0.47 mmol) was slowly injected into the reaction mixture drop wise. The reaction mixture was then brought to room temperature (30 °C) and stirred for 3 h under argon atmosphere, after which the reaction was stopped and the solvent was evaporated. Flash chromatography of the crude residue was performed over silica gel (230-400 mesh) using a mixture of hexane and ethyl acetate (7 : 2) as an eluent to get compound **18** (0.066 g, 23%) and compound **19** (0.038 g, 12%) along with unreacted starting material **16** (0.101 g, 33%).

Compound 18. R_f: 0.4 (hexane : ethyl acetate = 3 : 2); M.p.: 94 ^{o}C; $[\alpha]^{29}_{D}$ +10.4 (*c* 0.440, THF); ν_{max}(KBr)/cm^{-1} 3328, 1723, 1269, 1157, 967, 886, 811, 711; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 8.00 (2H, d, *J* = 8.1Hz); 7.72 (2H, d, *J* = 8.1Hz); 7.65 (2H, d, *J* = 8.1Hz); 7.59–7.50 (4H, m); 7.44 (2H, t, *J* = 7.5 Hz); 7.33 (2H, t, *J* = 7.5 Hz); 7.16 (2H, d, *J* = 8.1 Hz); 6.89 (2H, d, *J* = 7.8 Hz); 5.57 (1H, t, *J* = 5.1 Hz, exchangeable with D_2O); 5.26 (1H, dd, *J* = 9.6,

6.0 Hz); 5.14 (1H, *br* m); 4.47 (1H, m); 4.14 (1H, d, J = 15.9 Hz); 4.02 (2H, dd, J = 9.6, 2.7 Hz with one exchangeable proton); 3.32–3.19 (2H, m); 3.12 (1H, d, J = 15.9 Hz); 2.30 (3H, s); 2.13 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 165.8, 165.7, 143.49, 143.41, 136.7, 136.2, 133.5, 133.0, 129.77, 129.71, 129.5, 129.2, 128.9, 128.4, 128.1, 127.0, 126.6, 71.1, 70.1, 66.4, 54.4, 42.6, 37.9, 21.4, 21.3; HRMS (ESI): $[M+Na]^+$ Found: 701.1599, $C_{34}H_{34}N_2O_9S_2Na$ requires 701.1598.

Compound 19: R_f: 0.6 (hexane : ethyl acetate = 3 : 2); M.p.: 197 °C; $[\alpha]^{29}_D$ –89.7 (c 0.274, THF); ν_{max}(KBr)/cm^{-1} 3549, 3351, 1717, 1597, 815, 712; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 7.82 (6H, dd, J = 15.3, 8.1 Hz); 7.65 (2H, d, J = 6.9 Hz); 7.55–7.41 (2H, m); 7.35–7.26 (6H, m); 7.15 (2H, d, J =7.5 Hz); 5.64 (1H, t, J = 5.4 Hz, exchangeable with D_2O); 5.28 (1H, dd, J = 11.1, 6.0 Hz); 5.17 (1H, dd, J = 11.1, 2.7 Hz); 4.29 (1H, q, J = 5.4 Hz); 4.13 (1H, br s); 4.02 (1H, d, J = 15.6 Hz); 3.55 (1H, d, J = 3.3 Hz, exchangeable with D_2O); 3.27 (2H, m); 3.05 (1H, d, J = 15.6 Hz); 2.41 (3H, s); 2.36 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 165.6, 164.8, 143.6, 143.1, 136.3, 136.2, 133.3, 133.1, 129.58, 129.53, 129.4, 129.3, 129.0, 128.5, 128.3, 128.1, 127.8, 127.0, 70.6, 66.6, 66.0, 54.2, 44.8, 37.5, 21.4, 21.3; HRMS (ESI): $[M+Na]^+$ Found: 701.1590, $C_{34}H_{34}N_2O_9S_2Na$ requires 701.1598.

(2*S*,3*R*,4*R*,5*R*)-2-(*N*-*p*-Toluenesulfonyl)aminomethyl-3,4,5-trihydroxy-1-*N*-(*p*-toluenesulfonyl)-piperidine (20)

From compound 18. Compound **18** (0.032 g, 0.040 mmol) was taken in a 50 mL round bottomed flask and dissolved with MeOH (3 mL). The reaction mixture was cooled to 0 °C. Sodium metal (0.020 g, 0.869 mmol) was added in portions. The reaction mixture was then brought to room temperature (30 °C) and stirred for 30 min., after which the reaction was stopped and solvent was evaporated. Water was added to the resulting residue and the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated to get compound **20** as a white solid in 91% (0.020 g) yield.

From compound 19. Compound **19** (0.070 g, 0.103 mmol) was taken in a 50 mL round bottomed flask and dissolved with MeOH (5 mL). The reaction mixture was cooled to 0 °C. Sodium metal (0.045 g, 1.96 mmol) was added in portions. The reaction mixture was then brought to room temperature (30 °C) and stirred for 30 min., after which the reaction was stopped and solvent was evaporated. Water was added to the resulting residue and the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated to get compound **20** as a white solid in 80% (0.039 g) yield.

Rf: 0.1 (100% ethyl acetate); M.p.: 125 °C; $[\alpha]^{29}_{D}$ +12.1 (*c* 0.387, THF); ν_{max}(KBr)/cm^{-1} 3507, 2920, 2593, 1314, 1154, 1039, 667, 555; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 7.56–7.51 (4H, m); 7.10 (2H, m); 7.03 (2H, d, *J* = 7.5 Hz); 5.55 (1H, *br* s, exchangeable with D_2O), 3.77–3.73 (2H, m); 3.61 (1H, s), 3.33–3.31 (1H, m); 3.21–3.11 (3H, m, including one exchangeable proton); 2.69–2.64 (2H, m); 2.22 (3H, s); 2.19 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 142.7, 142.6, 136.4, 136.3, 129.1, 128.7, 127.3, 126.5, 69.7, 67.2, 66.6, 55.5, 44.2, 37.4, 21.0; HRMS (ESI): $[M+Na]^+$ Found: 493.1093, $C_{20}H_{26}N_2O_7S_2Na$ requires 493.1074.

(1*S*,4*R*,5*S*,8*R*)-4,8-Di-*O*-benzoyl-2,6-(di-*N*-*p*-toluenesulphonyl)-2,6-diazabicyclo-[3.2.1]octane-4,8-diol (21). A 50 mL three necked round bottomed flask was flame dried and cooled under argon atmosphere. Compound **16** (0.300 g, 0.430 mmol) was taken in it and dissolved with dry THF. PPh_3 (0.349 g, 1.33 mmol) was then added and the reaction mixture was cooled to 0 °C. DEAD (0.209 mL, 1.33 mmol) was slowly injected into the reaction mixture drop wise. The reaction mixture was then brought to room temperature (30 °C) and stirred for 3 h under argon atmosphere, after which the reaction was stopped and the solvent was evaporated. Flash chromatography of the crude residue was performed over silica gel (230-400 mesh) using a mixture of benzene and ethyl acetate (30 : 1) as an eluent to get compound **21** (0.207 g, 73%) as a colorless liquid. *Rf*: 0.8 (hexane : ethyl acetate = 3 : 2); $[\alpha]^{29}_{D}$ –16.3 (*c* 0.927, THF); ν_{max}(KBr)/cm^{-1} 1725, 1350, 1262, 1162, 1097, 711, 668; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4-Si): 7.95 (2H, d, *J* = 7.8 Hz); 7.68–7.43 (10H, m); 7.34–7.29 (2H, m); 7.20 (2H, d, *J* = 8.1 Hz); 6.97 (2H, d, *J* = 8.1 Hz); 5.37 (1H, m); 5.32 (1H, *br* m); 4.85 (1H, *br* m); 4.55 (1H, *br* m); 3.98 (1H, d, *J* = 14.4 Hz); 3.58–3.48 (2H, m); 3.32 (1H, d, *J* = 10.8 Hz); 2.32 (3H, s); 2.03 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 171.8, 165.5, 144.9, 143.4, 138.1, 137.9, 135.1, 133.0, 131.3, 130.0, 129.8, 129.7, 128.7, 128.4, 128.3, 127.8, 127.5, 127.3, 74.1, 73.2, 73.1, 67.8, 53.5, 47.0, 21.6, 21.1; HRMS (ESI): $[M+Na]^+$ Found: 683.1476, $C_{34}H_{32}N_2O_8S_2Na$ requires 683.1492.

(1*S*,4*R*,5*S*,8*R*)-2,6-(Di-*N*-*p*-toluenesulphonyl)-2,6-diazabicyclo[3.2.1]octane-4,8-diol (22). Compound **21** (0.1 g, 0.151 mmol) was taken in a 50 mL round bottomed flask and dissolved with MeOH (3 mL). The reaction mixture was cooled to 0 °C. Sodium metal (0.080 g, 3.48 mmol) was added in portions. The reaction mixture was then brought to room temperature (30 °C) and stirred for 3 h, after which the reaction was stopped and solvent was evaporated. Water was added to the resulting residue and the reaction mixture was extracted with ethyl acetate. The

organic layer was dried over anhydrous sodium sulphate, filtered and concentrated to get compound **22** as a white solid in 77% (0.053 g) yield. R_f: 0.1 (hexane : ethyl acetate = 2 : 1); M.p.: 164 °C; $[\alpha]^{28}_D$ −54.8 (*c* 0.268, THF); ν_{max}(KBr)/cm^{-1} 3449, 1340, 1280, 1162, 1098, 966, 668; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 7.52–7.48 (4H, m); 7.25 (2H, d, *J* = 8.1 Hz); 7.12 (2H, d, *J* = 8.1 Hz); 4.19 (2H, *br* m); 4.00 (1H, *br* m); 3.87 (1H, d, *J* = 4.8 Hz); 3.45 (1H, d, *J* = 12.6 Hz); 3.08 (1H, dd, *J* = 10.2, 3.9 Hz); 2.82 (1H, dd, *J* = 12.6, 2.1 Hz); 2.58 (1H, d, *J* = 10.2 Hz); 2.34 (3H, s); 2.27 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 143.5, 142.9, 135.0, 134.6, 129.6, 128.9, 127.1, 126.7, 69.7, 68.2, 65.4, 59.7, 46.4, 45.6, 21.2, 21.1; HRMS (ESI): $[M+Na]^+$ Found: 475.0968, $C_{20}H_{24}N_2O_6S_2Na$ requires 475.0968.

(1*S*,4*R*,5*S*,8*R*)-2,6-diaza-bicyclo[3.2.1]octane-4,8-diol (23). Liquid ammonia (25 mL) was collected in a 100 mL three necked round bottomed flask at −78 °C. Sodium metal (0.088 g, 3.83 mmol) was added to it. Deep blue colour appeared. Compound **22** (0.800 g, 1.768 mmol) dissolved in THF was added to the reaction mixture and stirred at −78 °C for 3 h. Then the reaction was quenched by the addition of benzene until the blue colour disappeared followed by water. The reaction mixture was allowed to slowly warm to room temperature. The organic layer was separated and the crude aqueous reaction mixture was dried in a lyophilizer. Column chromatography of the residue over deactivated silica gel using a mixture of CH_3CN and aq. NH_4OH (8 : 2) as an eluent yielded compound **23** as a low melting solid in 84% (0.215 g) yield. R_f: 0.3 ($CHCl_3$: MeOH : NH_4OH = 1 : 3 : 1); $[\alpha]^{20}_D$ −11 (*c* 0.7, H_2O); ν_{max}(KBr)/cm^{-1} 3288, 1612, 1402, 1133, 617; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 4.46 (1H, *br* m); 4.10 (1H, *br* m); 3.79–3.73 (2H, m); 3.48–3.41(1H, m); 3.33–3.28 (1H, m); 2.90 (2H, m); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 69.0, 65.1, 62.5, 57.1, 44.2, 41.9; HRMS (ESI): $[M+H]^+$ Found: 145.0971, $C_6H_{13}N_2O_2$ requires 145.0972.

5-*O*-(*tert*-Butyldimethylsilyl)-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (30). In a 50 mL round bottomed flask, compound **9** (4.60 g, 6.06 mmol) was taken and dissolved with dry dichloromethane (25 mL). The reaction mixture was cooled to 0 °C. Imidazole (0.91 g, 13.33 mmol) was added to the reaction mixture followed by TBSCl (1.00 g, 6.67 mmol). The reaction mixture was allowed to warm to room temperature. When the reaction was over (vide TLC) (3 h), it was quenched with iced water and the reaction mixture was extracted with dichloromethane (3 x 80 mL). The combined organic layer was then washed thoroughly with saturated brine solution, dried over anhydrous sodium sulphate and filtered. The reaction mixture was then concentrated. Flash chromatography of crude reaction mixture was

performed over silica gel using a mixture of hexane and ethyl acetate (11 : 2) as an eluent to get compound **30** in 85% (4.50 g) yield as a low melting colorless solid. R_f: 0.5 (hexane : ethylacetate = 2 : 1); $[\alpha]^{29}_D$ + 8.1 (*c* 0.718, MeOH); ν_{max}(KBr)/cm^{-1} 3361, 3256, 2927, 1598, 1212, 1162, 1088, 1049, 757, 668; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 7.63 (2H, d, *J* = 7.8 Hz); 7.41–7.15 (21H, m); 5.08 (1H, d, *J* = 8.7 Hz, exchangeable with D_2O); 4.73–4.66 (2H, m); 4.59–4.50 (3H, m); 4.35 (1H, *J* = 11.1Hz); 4.24–4.17 (2H, m, including one exchangeable proton); 3.79–3.67 (3H, m); 3.57 (1H, m); 3.36 (1H, dd, *J* = 9.6, 4.8 Hz); 2.76–2.71 (1H, m); 2.57–2.50 (1H, m); 2.39 (6H, s); 0.86 (9H, s); 0.09 (6H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 143.4, 143.2, 138.7, 137.98, 137.91, 136.5, 129.7, 129.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.4, 127.05, 127.02, 82.2, 77.3, 74.82, 74.76, 73.3, 72.2, 71.3, 54.0, 44.3, 25.9, 21.53, 21.50, 18.0, –4.5, –4.8; HRMS (ESI): $[M+H]^+$ Found: 873.3666, $C_{47}H_{61}N_2O_8S_2Si$ requires 873.3633.

5-*O*-(*tert*-Butyldimethylsilyl)-1,2-dideoxy-1,2-(di-*p*-toluenesulfonamido)-D-glucitol (31). 10% Pd on charcoal (3.43 g, 100% w/w) was taken in a 50 mL three necked round bottomed flask. Compound **30** (3.43 g, 3.928 mmol) dissolved in methanol (15 mL) was added to it and the reaction mixture was stirred at 42 ^oC. Hydrogen gas was then bubbled slowly into reaction mixture. Progress of the reaction was monitored by TLC and after completion (30 min), the reaction mixture was filtered through a celite pad and washed with methanol. The filtrate was then concentrated to get the product **31** (2.16 g) in 91% yield as a colorless viscous liquid. R_f: 0.1 (hexane : ethylacetate = 2 : 1); $[\alpha]^{29}_D$ +30.8 (*c* 0.816, MeOH); ν_{max}(KBr)/cm^{-1} 3491, 3285, 2932, 1599, 1330, 1159, 1093, 665, 552; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 7.75 (2H, d, *J* = 8.1Hz); 7.62 (2H, d, *J* = 8.1Hz); 7.28 (4H, t, *J* = 8.1 Hz); 5.76 (1H, *br* s, exchangeable with D_2O); 5.53 (1H, *br* s, exchangeable with D_2O); 3.98 (1H, d, *J* = 3.9 Hz); 3.81–3.79 (1H, m); 3.66–3.60 (3H, m); 3.32 (3H, *br* m, including two exchangeable protons); 2.96 (2H, m); 2.42 (6H, s); 0.86 (9H, s); 0.09 (3H, s); 0.078 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 143.5, 143.3, 136.6, 136.15, 129.66, 129.63, 127.2, 126.9, 72.7, 71.6, 68.2, 63.1, 55.5, 43.4, 25.6, 21.45, 21.4, 17.8, –4.7,–5.0; HRMS (ESI): $[M+Na]^+$ Found: 625.2047, $C_{26}H_{42}N_2O_8S_2SiNa$ requires 625.2044.

(2*S*,3*R*,4*R*,5*R*)-2-(*N*-*p*-Toluenesulfonyl)aminomethyl-3,4,5-trihydroxy-1-*N*-(*p* toluenesulfonyl)-piperidine (20). A 50 mL three necked round bottomed flask was flame dried and cooled under argon atmosphere. Compound **31** (2.7 g, 4.479 mmol) was taken in it and dissolved with dry THF. PPh_3 (1.52 g, 5.823 mmol) was then added and the reaction mixture was

cooled to 0 °C. DEAD (1.045 mL, 6.721 mmol) was slowly injected into the reaction mixture drop wise. The reaction mixture was then brought to room temperature (30 °C) and stirred for 30 min under argon atmosphere, after which the reaction was stopped and the solvent was evaporated The crude reaction mixture was then dissolved in MeOH (30 mL) and camphor sulfoinic acid (3.618 g, 14.4 mmol) was added to it. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by TLC. After 6 h, when TLC indicated the disappearance of the starting material, the reaction was quenched with water and the reaction mixture was extracted with ethyl acetate (3x50 mL). The combined organic layer was then washed with saturated $NaHCO_3$ solution and dried over anhydrous sodium sulfate and filtered. Flash chromatography of the residue over silica gel using a mixture of hexane and ethyl acetate (1:1) as an eluent afforded 1.82 g (86%, for two steps) of compound **20** as a white solid. The specific rotation and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, HRMS) were found to be identical with those of compound **20** prepared earlier from compounds **18** and **19**.

(2S,3*R*,4*R*,5*R*)-2-Aminomethyl-3,4,5-trihydroxy-piperidine (13) [6-Amino-1,6-dideoxy-L-*gulo*nojirimycin]. Liquid ammonia (25 mL) was collected in a 100 mL three necked round bottomed flask at −78 °C. Sodium metal (0.08 g, 3.48 mmol) was added to it. Deep blue colour appeared. Compound **20** (0.600 g, 1.27 mmol) dissolved in THF was added to the reaction mixture and stirred at −78 °C for 3 h. Then the reaction was quenched by the addition of benzene until the blue colour disappeared followed by water. The reaction mixture was allowed to slowly warm to room temperature. The organic layer was separated and the crude aqueous reaction mixture was dried in a lyophilizer. Purification of the product was performed by flash column chromatography over silica gel using a mixture of CH_3CN and NH_4OH (8 : 2) as an eluent to get compound **13** (0.192 g, 93 % yield) as a low melting solid. R_f: 0.3 ($CHCl_3$: MeOH : NH_4OH = 1:3:1); $[\alpha]^{20}_D$ +7.3 (*c* 1.56, H_2O); ν_{max}(KBr)/cm^{-1} 3126, 1619, 1401, 1105, 619; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 3.75–3.65 (3H, m); 3.14 (1H, m); 2.98–2.93 (1H, m); 2.88–2.81 (1H, m); 2.77–2.71 (1H, m); 2.61–2.54 (1H, m); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 68.9, 68.3, 63.7, 57.1, 43.2, 38.7; HRMS (ESI): $[M+Na]^+$ Found: 185.0888, $C_6H_{14}N_2O_3Na$ requires 185.0897.

(2*S*,3*R*,4*R*,5*R*)-2-Acetamidomethyl-3,4,5-trihydroxy-piperidine (33) [6-Acetamido-1,6-dideoxy-L-*gulo*nojirimycin]. In a 50 mL round bottomed flask, compound **13** (0.05 g, 0.308 mmol) was taken and cooled to 0 °C. Acetic anhydride (0.032 mL, 0.339 mmol) was added to it. The reaction mixture was then brought to room temperature and sonicated under solvent free

condition for 30 min, after which the reaction was quenched with water (1 mL). Triethyl amine (approx. 2 mL) was then added to the reaction mixture until the pH of the solution rose to 9. The added triethylamine was washed out by extracting the solution with $CHCl_3$ (4x20 mL). Purification of the product from the aqueous layer by column chromatography over silica gel using a mixture of CH_2Cl_2 and MeOH (5 : 1) as an eluent afforded compound **33** in 88% (0.056 g) yield as a low melting colourless solid. R_f: 0.4 ($CHCl_3$: MeOH : NH_4OH = 1 : 3 : 1); $[\alpha]^{20}_D$ +8.6 (*c* 0.81, H_2O); ν_{max}(KBr)/cm^{-1} 3126, 1640, 1401, 1242, 1117; δ_H (300 MHz, $CDCl_3$ + DMSO-d_6, Me_4Si): 4.08–4.04 (1H, m); 3.89 (2H, s); 3.48–3.27 (3H, m); 3.12–3.06 (1H, m); 2.89–2.85 (1H, m); 1.86 (3H, s); δ_C (75 MHz, $CDCl_3$ + DMSO--d_6, Me_4Si): 175.1, 68.2, 67.3, 62.1, 53.2, 42.3, 37.8, 21.8; HRMS (ESI): $[M+H]^+$ Found: 205.1184, $C_8H_{17}N_2O_4$ requires 205.1183.

Procedure for glycosidase inhibition studies.

Glycosidase inhibition studies were carried out, spectrophotometrically, following the standard procedure,[19,29] by measuring the enzyme velocity at constant substrate concentration with varying concentrations of an inhibitor (iminosugar), utilizing the corresponding *p*–nitrophenyl glycosides as the substrates. α–Glucosidase type I from Baker's yeast, α–galactosidase from green coffee beans, β–galactosidase from *Escherichia coli*, β-*N*-acetylhexosaminidase from Jack bean, 4-nitrophenyl-*N*-acetyl-β-D-glucosmainide and 4-nitrophenyl-α-D-galactopyranoside were purchased from Sigma Chemicals Co. USA. β–glucosidase from almond, 4-nitrophenyl-α-D-glucopyranoside, 4-nitrophenyl-β-D-glucopyranoside and 4-nitrophenyl-β-D-galactopyranoside were purchased from SRL Chemicals Ltd., India.

Glycosidase was pre-incubated with various concentrations (0.5-12 mM) of inhibitor for 30 min. at its optimum pH and temperature (see Table 1). 20μL of 25mM *p*-nitrophenyl glycopyranoside (*p*-NPG) in 0.1M phosphate buffer was added to the reaction mixture to initiate the reaction. In the case of β–glucosidase acetate buffer was used. The final volume of the reaction mixture was adjusted to 1.1 mL with buffer. Control was also run in parallel without inhibitor. The reaction was then incubated at the same pH and temperature for 10 min. and quenched by adding 1 mL of 1M Na_2CO_3 solution. The glycosidase activity was determined by measuring the *p*-nitrophenol released from *p*-nitrophenyl glycopyranosides at 405 nm using a UV-visible Microplate Reader (M/s. Biotek Instrumets Inc. USA, Model Synergy 2 SAD).

Experimental data were plotted as V_i/V_0 (fractional activity) versus the inhibitor concentration [I] at a constant concentration of substrate, where V_i and V_0 represent the enzyme velocity (activity) in the presence and absence of inhibitor respectively. IC_{50} values were obtained from the inhibitor concentration [I] corresponding to fractional activity of 0.5. IC_{50} value was defined as the concentration of the inhibitor to inhibit 50% of enzyme activity under the assay conditions.

Each experiment was repeated thrice to get a range of IC_{50} values and the mean IC_{50} values were reported in the manuscript.

Acknowledgements:

We thank the Department of Science and Technology, New Delhi, India for financial support, Prof. Nalin Pant and Mr. Amit Singh for their help in AM1 calculations and the single crystal X-ray analysis respectively. MG and RVS thank IIT Delhi and CSIR, New Delhi for research fellowships. We acknowledge DST-FIST and IIT Delhi for funding of the single crystal X-ray diffractometer and ESI-HRMS facility at IIT Delhi. DST and SAIF-IIT Madras are acknowledged for recording of 2D NMR spectra.

† **Electronic supplementary information (ESI) available**: Copies of ^1H and ^{13}C NMR spectra of all new compounds are provided.

References

(1) For recent reviews on azasugars: (*a*) I. Dragutan, V. Dragutan, V and A. A. Demonceau, *RSC Advances* 2012, **2**, 719−736; (*b*) A. E. Stütz and T. M. Wrodnigg, *Adv. Carbohydr. Chem. Biochem*., 2011, **66**, 187−298; (*c*) B. L. Stocker, E. M. Dangerfield, A. L. Win-Mason, G. W. Haslett and M. S. M. Timmer, *Eur. J. Org. Chem.,* 2010, 1615−1637; (*d*) P. Compain and O. R. Martin, *Iminosugars-From Synthesis to Therapeutic Applications*; John Wiley & Sons Ltd: West Sussex England, 2007.

(2) For some very recent articles: (*a*) B. J. Ayers, B. N. Ngo, S. F. Jenkinson, R. F. Martínez, Y. Shimada, I. Adachi, A. C. Weymouth-Wilson, A. Kato, G. W. J. Fleet, *J. Org. Chem.,* 2012, **77**, 7777–7792; (*b*) S. Martinez-Montero, S. Fernandez, Y. S. Sanghvi, J. Chattopadhyaya, M. Ganesan, N. G. Ramesh, V. Gotor and M. Ferrero, *J. Org. Chem*. 2012, **77**, 4671−4678; (*c*) F. Chevallier, A. Lumbroso, I. Beaudet, E. Le Grognec, L. Toupet and J.-P Quintard, *Eur. J. Org. Chem.* 2011, 4133−4144; (*d*) I. Dragutan, V. Dragutan, C. Mitan, H. C. M. Vosloo, L. Delaude, L and A. Demonceau, *Beils. J. Org. Chem.* 2011, **7**, 699−716.

(3) G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams and R. Storer, *Drug Discov. Today* 2011, **16**, 107−118.

(4) B. G. Winchester, *Tetrahedron: Asymmetry* 2009, **20**, 645−651.

(5) (*a*) J. Liu, M. M. D. Numa, H. Liu, S.-J. Huang, P. Sears, A. R. Shikhman A and C.-H. Wong, *J. Org. Chem.* 2004, **69**, 6273−6283; (*b*) C. Saotome, C.-H. Wong and O. Kanie, *Chem. Biol.* 2001, **8**, 1061−1070; (*c*) M. Takebayashi, S. Hiranuma, Y. Kanie, T. Kajimoto, O. Kanie, and C.-H. Wong, *J. Org. Chem*. 1999, **64**, 5280−5291; (*d*) Y. Takaoka, T. Kajimoto, and C.-H. Wong, *J. Org. Chem*. 1993, **58**, 4809−4812.

(6) (*a*) G. Pototschnig, C. M. De Csáky, J. R. M. Burke, G. Schitter, A. E. Stütz, C. A. Tarling, S. G. Withers and T. M. Wrodnigg, *Bioorg. Med. Chem. Lett.,* 2010, **20**, 4077–4079; (*b*) T. M. Wrodnigg, F. Diness, C. Gruber, H. Hausler, I. Lundt, K. Rupitz, A. J. Steiner, A. E. Stütz, C. A. Tarling, S. G. Withers and H. Wolfler, *Bioorg. Med. Chem*., 2004, **12**, 3485−3495; (*c*) T. M. Wrodnigg, S. G. Withers and A. E. Stütz, *Bioorg. Med. Chem. Lett*., 2001, **11**, 1063−1064; (*d*) A. Hermetter, H. Scholze, A. E. Stütz, S. G. Withers and T. M. Wrodnigg, *Bioorg. Med. Chem. Lett*., 2001, **11**, 1339−1342; (*e*) T. M. Wrodnigg, W. Gaderbauer, P. Greimel, H. Häusler, F. K. Sprenger, A. E. Stütz, C. Virgona and S. G. Withers, *J. Carbohydr. Chem*., 2000, **19**, 975−990; (*f*) T. M. Wrodnigg, A. E. Stütz and S. G. Withers, *Tetrahedron Lett*., 1997, **38**, 5463−5466.

(7) S. H. Kang and D. H. Ryu, *Tetrahedron Lett*., 1997, **38**, 607−610.

(8) F. Popowycz, S. Gerber-Lemaire, C. Schütz and P. Vogel, *Helv. Chim. Acta* 2004, **87**, 800–810.

(9) R. C. Reynolds, N. Bansal, J. Rose, J. Friedrich, W. J. Suling and J. A. Maddry, *Carbohydr. Res.*, 1999, **317**, 164–179.

(10) I. McCort, S. Fort, A. Duréault and J.-C. Depezay, *Bioorg. Med. Chem.,* 2000, **8**, 135–143.

(11) P. Merino, I. Delso, T. Tejero, F. Cardona, M. Marradi, E. Faggi, C. Parmeggiani and A. Goti, *Eur. J. Org. Chem.,* 2008, 2929–2947.

(12) E.-L. Tsou, Y.-T. Yeh, P.-H. Liang and W.-C. Cheng, *Tetrahedron* 2009, **65**, 93–100.

(13) (*a*) A. L. Win-Mason, S. A. K. Jongkees, S. G. Withers, P. C. Tyler, M. S. M. Timmer and B. L. Stocker *J. Org. Chem.,* 2011, **76**, 9611−9621; (*b*) A. L. Win-Mason, E. M. Dangerfield, P. C. Tyler, B. L. Stocker and M. S. M. Timmer, *Eur. J. Org. Chem*., 2011,

4008–4014; (*c*) E. M. Dangerfield, S. A. Gulab, C. H. Plunkett, M. S. M. Timmer and B. L. Stocker, *Carbohydr. Res*., 2010, **345**, 1360–1365.

(14) P.-H. Liang, W.-C. Cheng, Y.-L. Lee, H.-P. Yu, Y.-T. Wu, Y.-L. Linand and C.-H. Wong, *ChemBioChem* 2006, **7**, 165–173.

(15) (*a*) A. Kilonda, F. Compernolle, K. Peeters, G. J. Joly, S. Toppet and G. J. Hoornaert, *Tetrahedron* 2000*,* **56**, 1005–1012; (*b*) A. Kilonda, F. Compernolle and G. J. Hoornaert, *J. Org. Chem.* 1995, **60**, 5820–5824; (*c*) A. Kilonda, F. Compernolle, S. Toppet and G. J. Hoornaert, *J. Chem. Soc., Chem. Commun*., 1994, 2147–2148.

(16) (*a*) T. Tite, M.-C. Lallemand, E. Poupon, N. Kunesch, F. Tillequin, C. Gravier-Pelletier, Y. Le Merrer and H.-P. Husson, *Bioorg. Med. Chem.*, 2004, **12**, 5091–5097; (*b*) E. Poupon, B.-X. Luong, A. Chiaroni, N. Kunesch and H.-P. Husson, *J. Org. Chem*. 2000, **65**, 7208–7210.

(17) M. B. Pampín, F. Fernández, J. C. Estévez and R. J. Estévez, *Tetrahedron: Asymmetry* 2009, **20**, 503–507.

(18) J. A. Tamayo, F. Franco and D. L. Re, *Synlett* 2010, 1323–1326.

(19) M. Ganesan, R. V. Madhukarrao and N. G. Ramesh, *Org. Biomol. Chem.,* 2010, **8**, 1527–1530.

(20) (*a*) V. Kumar and N. G. Ramesh, *Org. Biomol. Chem*., 2007, **5**, 3847–3858; (*b*) V. Kumar and N. G. Ramesh, *Chem. Commun*., 2006, 4952–4954.

(21) CCDC number 881845.

(22) CCDC number 881846.

(23) (*a*) A. Conejo-Garcia, M. E. Garcia-Rubino, J. A. Marchal, M. C. Nunez, A. Ramirez, S. Cimino, M. A. Garcia, A. Aranega, M. A.; Gallo and J. M. Campos, *Eur. J. Med. Chem*., 2011, **46**, 3795–3801; (*b*) R. S. Perali, S. Mandava and V. R. Chunduri, *Tetrahedron Lett*., 2011, **52**, 3045–3047; (*c*) Y. Yoshimura, K. Asami, H. Matsui, H. Tanaka and H.Takahata, *Org. Lett.* 2006, **8**, 6015–6018; (*d*) Y. Marsac, A. Nourry, S. Legoupy, M. Pipelier, D. Dubreuil and F. Huet, *Tetrahedron Lett.* 2004, **45**, 6461–6463; (*e*) A. Viso, N. Poopeiko and S. Castillon, *Tetrahedron Lett.* 2000, **41**, 407–411; (*f*) N. Poopeiko, R. Fernandez, M. I. Barrena, S. Castillon, J. Fornies-Camer and C. J. Cardin, *J. Org. Chem.* 1999, **64**, 1375–1379; (*g*) S. Takano, H. Iida and K. Ogasawara, *Heterocycles* 1993, **36**, 2203–2204.

(24) C. Tănase, M. T.; Căproiu, C. Drăghici, F. G. Cocu, C. Enache and G. Manda, *Rev. Chim*., (Bucharest) 2011, **62**, 56–59.

(25) For a review on calystegines: B. Dräger, *Nat. Prod. Rep.* 2004, **21**, 211–223.

(26) For a review on CRDA: O. O. Grygorenko, D. S. Radchenko, D. M. Volochnyuk, D. M. A. A. Tolmachev and I. V. Komarov, *Chem. Rev.* 2011, **111**, 5506–5568.

(27) (*a*) V. Pistarà, A. Rescifina, F. Punzo, G. Greco, V. Barbera and A. Corsaro, *Eur. J. Org. Chem*., 2011, 7278–7287; (*b*) V. D. Bussolo, A. Fiasella, M. R. Romano, L. Favero, M. Pineschi and P. Crotti, *Org. Lett*., 2007, **9**, 4479–4482; (*c*) S. D. Markad, N. S. Karanjule, T. Sharma, S. G. Sabharwal and D. D. Dhavale, *Bioorg. Med. Chem*., 2006, **14**, 5535–5539; (*d*) D. D. Dhavale, M. M. Matin, T. Sharma and S. G. Sabharwal, *Bioorg. Med. Chem*., 2006, **14**, 5535–5539; (*e*) D. D. Dhavale, S. D. Markad, N. S. Karanjule and J. P. Reddy, *J. Org. Chem*., 2004, **69**, 4760–4766.

(28) HyperChem 8.0.7. F. L. Gainesville, 1997, Hypercube Inc., Florida Science and Technology Park, 1115 N.W. 4th Street, Gainesville, FL, 32601, USA.

(29) (*a*) J. M. T. Hamilton-Miller, *Biochem. J.* 1966, **101**, 40c; (*b*) Y.-T. Li, Y.-T.; S.-C. Li, in *Methods in Enzymology;* Ginsberg, V., Ed.; Academic Press, 1972, *28*, part B, p. 702.