Design and synthesis of new amino-modified iminocyclitols: selective inhibitors of α-galactosidase†‡§

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A new and short synthesis of hitherto unreported stereo analogue of amino-modified five-membered iminocyclitols from readily available tri-*O*-benzyl-D-glucal is described. Significantly, glycosidase inhibition studies of alkylamino substituted iminocyclitols display a high degree of selectivity towards α-galactosidase.

Over the years, the chemistry and biology of naturally occurring iminocyclitols (azasugars) and their synthetic analogues have received immense research attention due to their significant and selective inhibition of various glycosidases,[1] a feature attributable to their structural resemblance as well as mimicry of the glycosidase oxocarbenium-ion transition state.[2] The successful launch of synthetically modified six-membered azasugar based clinical drugs such as Glyset® **2** (for non-insulin dependant diabetes) and Zavesca® **3** (for the control of Gaucher's disease) (Fig. 1) has provided an added impetus for research in this area.[3] As a consequence, functional group modifications at the aglycon moieties of iminocyclitols, in order to fine tune their inhibitory activities against glycosidases, is becoming an area of increasing importance. Pioneering work on such functional group modifications on five-membered iminocyclitols was carried out by Wong *et al.* who had observed that a synthetic azasugar **6** obtained by replacement of one of the side chain hydroxyl groups of naturally occurring 2,5-dihydroxymethyl-3,4-dihydroxy pyrrolidine (DMDP) **4** by an acetamido group had pronounced inhibitory activity against *N*-acetylglucosaminidase.[4] Subsequently, a high throughput screening of a library of such 1-aminodeoxy-DMDP (ADMDP) analogues, synthesized by them, resulted in the identification of novel structures for antivirals and osteoarthritis.[5] Stütz and coworkers have carried out extensive research on the synthesis of *C*-1 amino-modified five-memebered iminocyclitols such as **5** by meticulously exploiting the Amadori rearrangement of 5-azido-5-deoxy-D-glucofuranose and similar compounds with dibenzylamine as a key step and investigated, in detail, their structure–activity relationship against various glycosidases.[6] Since the significant findings that such synthetically designed azasugars are promising structures for various disorders, there has been an upsurge in research related to their synthesis and biological studies. A recent report on the first structural basis of glycosidase inhibition by five-membered azasugars has further spurred the research activities in this area.[2a] Besides the glycosidase inhibition activities, transition metal complexes of amino-substituted polyhydroxypyrrolidines have been used as catalysts for asymmetric transformations and find applications in medicines as well.[7]

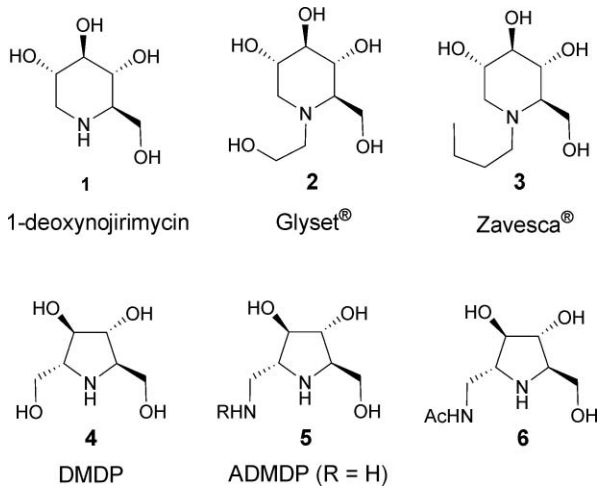

Fig. 1 Representative examples of azasugars.

A few other strategies so far reported for the synthesis of ADMDP **5** and its various stereoisomers include (i) synthetic manipulations of naturally occurring DMDP **4** and its stereo analogues by way of selective conversion of one of the side chain hydroxyl groups into an amino functionality;[5b,8] (ii) intramolecular cyclization of a C_2-symmetric amino alcohol;[9] (iii) nucleophilic ring opening of sugar based bis-aziridines;[10] (iv) transformation of an optically active γ-lactam in to ADMDP analogues;[11] and (v) nucleophilic addition of cyanide to sugar based cyclic nitrones followed by reduction.[7a,12] Postel and co-workers very recently disclosed the synthesis of 2-aminocyclopropyl polyhydroxylated pyrrolidine and its isomers through cyclopropanation of glycoaminonitriles.[13] To the best of our knowledge, there is no glycal based approach available for the synthesis of such amino-modified azasugars, despite their ready availability. Earlier we had reported a novel one-pot diamination of glycals with chloramine-T and utilized them for efficient syntheses of *N*-linked glycoamino acids and peptides.[14] We expected that the pluripotent diamine **8** could as well serve as a versatile precursor for the synthesis of amino-modified azasugar **10**, through a cleavage-recombination

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† Dedicated to Prof. Yasuyuki Kita on the occasion of his 65th birthday.

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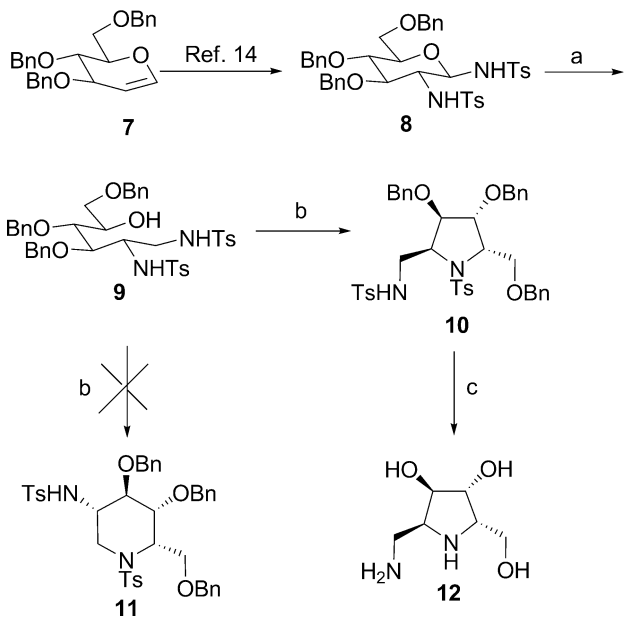
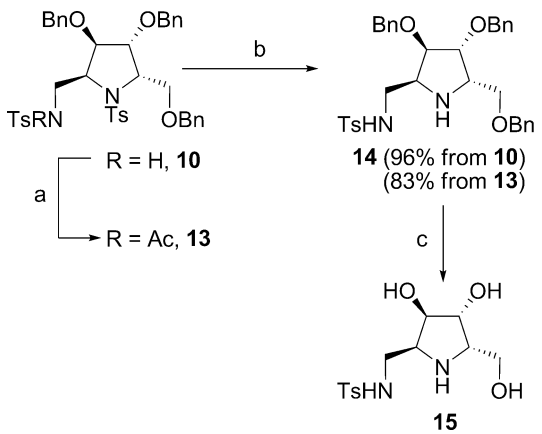
pathway. In this communication, we report a new and a very short synthesis of a *hitherto unreported* stereo analogue of ADMDP from readily available tri-*O*-benzyl-D-glucal **7** *via* cleavage of the O–C_1 bond of diamine **8** followed by an intramolecular cyclization of the diamino alcohol intermediate **9**. The glycosidase inhibition studies of these molecules reveal that azasugars with an alkylamino-substituted side chain display a high degree of selectivity towards α-galactosidase (from green coffee bean).

Our synthesis of amino-modified iminocyclitols started with the reduction of the readily available diamine **8**[14] to the diamino alcohol **9**, which was easily accomplished in 95% yield by refluxing **8** with 1.5 equiv. of $LiAlH_4$ for 20 min (Scheme 1). It was expected that an intramolecular cyclization of compound **9** could readily provide the pyrrolidine derivative **10**. Thus, when compound **9** was initially subjected to an intramolecular cyclization under Mitsunobu conditions, complete consumption of starting material was noticed (TLC) in 30 min and formation of a new product was observed. After purification and thorough characterization, the product was confirmed to be the polyhydroxypyrrolidine **10** and not the piperidine derivative **11** (Scheme 1). In the ^{1}H-NMR spectrum of the product, the signal of the NH proton (that disappeared upon D_2O exchange) resonated as a *triplet* with a coupling constant of 7.2 Hz, thereby indicating that there were two neighbouring protons for the NH to couple with. This clearly established the structure of the product to be the pyrrolidine **10**. Both the neighbouring diastereotopic methylene protons were also found to couple with the NH proton as revealed by the ^{1}H–^{1}H COSY spectrum of the above compound. On the other hand, in the case of piperidine derivative **11**, the signal of the NH proton was expected to resonate only as a *doublet* due to its coupling with the lone neighbouring CH proton. Irrespective of the reaction conditions (change in temperature, solvent, time *etc.*) and other reagents {(PPh_3, I_2, imidazole); (NaH, MsCl, THF)} employed, compound **10** was always obtained as the exclusive product in the intramolecular reaction of **9**. Among them, the best yield (90%) was obtained when the reaction was carried out under Mitsunobu conditions at 0 °C to room temperature with 1.3 equiv. of PPh_3 and 1.4 equiv. of DEAD in THF as a solvent. In order to study the inhibition properties it was imperative to deprotect the tosyl and benzyl groups of **10** to obtain the parent polyhydroxypyrrolidine **12**. This was conveniently achieved in a single step by treating compound **10** with Na/liq.NH_3 to obtain (2*S*,3*R*,4*R*,5*S*)-2-aminomethyl-5-hydroxymethyl-3,4-dihydroxypyrrolidine **12** in 86% yield. Thus, we have accomplished a very short synthesis of a *new* stereo-analogue of ADMDP in just three steps and with a total yield of 74% from **8** (Scheme 1).

We next focused our attention towards the synthesis of a few derivatives of **12**, possessing different substituents at the side chain amino functionality, with a view to investigating the role of substituents on their inhibition activities. An obvious choice was the synthesis of the tosyl derivative **15**, for which selective deprotection of the tertiary tosyl group of **10** in the presence of the side chain secondary one was an essential requisite. After several experiments, we were successful in chemoselectively deprotecting the tertiary tosyl group of **10** over the secondary one by treating it with Na–Hg. Compound **14** was obtained in 96% yield. Subsequent catalytic hydrogenation of **14** in presence of 10% Pd/C gave the tosylamido polyhydroxypyrrolidine **15** in 90% yield (Scheme 2).



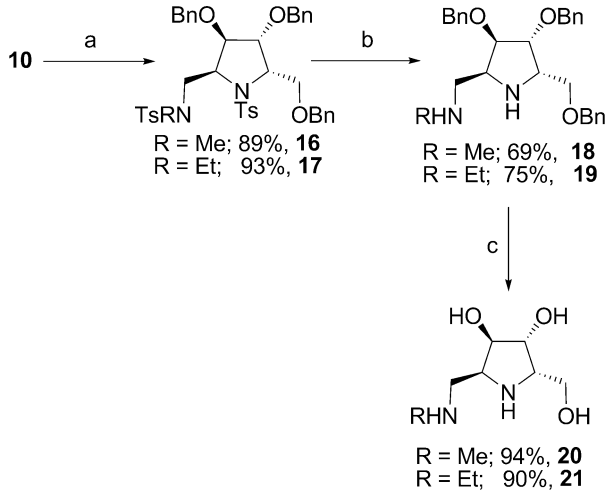
Scheme 1 *Reagents and conditions*: (a) $LiAlH_4$, THF, reflux, 20 min, 95%; (b) Ph_3P, DEAD, THF, 0 °C to rt, 30 min, 90%; (c) Na–liq.NH_3, THF, –78 °C, 3 h, 86%.



Scheme 2 *Reagents and conditions*: (a) Ac_2O, DMAP, pyridine, rt, 24 h, 95%; (b) 3% Na–Hg, Na_2HPO_4, DMF–MeOH (4 : 0.5), 60 °C, 3 h; (c) 10% Pd/C, H_2(g), MeOH, 30 °C, 5 h, 90%.

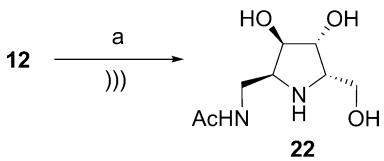
While a library of compounds bearing diverse functional groups on the side chain nitrogen of ADMDP have been reported in the literature,[4–13] those possessing simple alkyl groups were visibly missing and thus their glycosidase inhibition studies as well as structure–activity relationship largely remain uninvestigated. This prompted us to explore the possibility of synthesizing a few alkyl derivatives of **12** and undertake their inhibitory studies against glycosidases. Thus, reaction of **10** with alkyl halides in presence of sodium hydride afforded the alkyl-substituted derivatives **16** and **17** in good yields. Unexpectedly, global deprotection on compounds **16** and **17** using Na/liq. NH_3 did not give the required products **20** and **21**. Instead, cleavage of the alkyl groups also occurred along with the other protecting groups resulting in the formation of ADMDP **12**, which was rather unusual and

surprising. However, this problem was circumvented by following an alternate two-step procedure. On treatment with Na–Hg, compounds **16** and **17** underwent a smooth didetosylation reaction to give products **18** and **19** in high yields. Hydrogenation of compounds **18** and **19** in presence of 10% Pd/C and HCl delivered the required products **20** and **21** respectively (Scheme 3).



Scheme 3 *Reagents and conditions*: (a) NaH, RX, DMF, rt; (b) 3% Na–Hg, Na_2HPO_4, DMF–MeOH (4 : 0.5), 60 °C, 3 h; (c) (i) 10% Pd/C, H_2(g), dil. HCl, MeOH, 30 °C, 12 h, (ii) Et_3N.

Our next target was the synthesis of the acetyl derivative **22**. Acetylation of compound **10** using standard procedures afforded the *N*-acetyl-*N*-tosyl derivative **13** in 95% yield (Scheme 2), which however, on treatment with Na–Hg did not undergo the expected didetosylation reaction. Unlike compounds **16** and **18**, which underwent smooth didetosylation reaction with Na–Hg, in the case of **13**, it was the side chain acetyl and not the tosyl group that was cleaved along with the tosyl group attached to the ring nitrogen to afford compound **14** exclusively (Scheme 2). Such a preference of acetyl over tosyl group cleavage of *N*-acetyl-*N*-tosylamides with Na–Hg has hardly been reported in literature and thus could prove to be a useful protocol in organic synthesis. Finally, synthesis of the acetamido polyhydroxypyrrolidine **22** was carried out by direct acetylation of the free amine **12** with acetic anhydride under solvent-free conditions using a modified procedure developed in our lab (Scheme 4).



Scheme 4 *Reagents and conditions*: (a) Ac_2O, 0 °C to rt, sonication, 15 min, 85%.

All new compounds were screened for their inhibition activities against four different glycosidases (Table 1). Compound **12** and its acetyl derivative **22** did not show any inhibition against any of these glycosidases. On the other hand, very surprisingly, the tosyl derivative **15** and alkylamino derivatives **20**, **21** gave complementary results. While the tosyl derivative **15** has a broad range of inhibition against α-glucosidase, β-glucosidase and β-galactosidase, it did not inhibit α-galactosidase. Quite contrastingly, both the alkyl derivatives **20** and **21** displayed a very high degree of selectivity by inhibiting *only* α-galactosidase among the four glycosidases tested. Thus, the complementary behaviour of **15** *versus* **20** and **21** toward various enzymes is not only interesting but also could provide vital information for further understanding the structural basis of glycosidase inhibition by these amino-modified five-membered iminocyclitols.

Table 1 Glycosidase inhibition studies

	IC_{50}, mM				
Enzyme (source)	**12**	**15**	**20**	**21**	**22**
α-glucosidase type I (baker's yeast)	n.i.	3.5	n.i.	n.i.	n.i.
β-glucosidase (almond)	n.i.	6.3	n.i.	n.i.	n.i.
α-galactosidase (green coffee beans)	n.i.	n.i.	6.9	8.1	n.i.
β-galactosidase (*Escherichia coli*)	n.i.	5.4	n.i.	n.i.	n.i.

n.i., no inhibition was observed up to 12 mM inhibitor concentration

In conclusion, we have developed a glycal based route towards the synthesis of a new stereo analogue of amino-modified polyhydroxypyrrolidines. This methodology is one of the shortest (only 3 to 5 steps from the diamine **8**) routes available for the synthesis of ADMDP analogues. The glycosidase inhibitory activities of these compounds are quite interesting. Notably, the specific inhibitory nature of alkyl-ADMDP compounds **20** and **21** towards α-galactosidase is expected to spur further research on the synthesis and biological studies of alkylamino derivatives, which has largely remained elusive so far. Detailed investigation on the nature of the interaction between these inhibitors and the enzymes is currently underway. Further, since inhibitors of α-galactosidase have been found to be promising chemical chaperones for Fabry's disease,[15] compounds **20** and **21** reported here could find potential applications in this direction as well.

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