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An expeditious synthesis of an analogue of (−)-steviamine by way of the 1,3-dipolar cycloaddition of a nitrile oxide with a 1-*C*-allyl iminosugar

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ABSTRACT

In our continuing effort to develop inhibitors of the mycobacterial galactan biosynthesis, we planned to synthesize original iminosugar-based analogues of UDP-galactofuranose by way of the 1,3-dipolar cycloaddition reaction between a 1-*C*-allyl iminosugar and a nitrile oxide, followed by the reductive cleavage of the resulting isooxazoline. In initial studies, it was found that this last step led in one pot to a new polyhydroxylated indolizidine derivative closely related to the recently isolated (−)-steviamine, in good yield, by way of a sequence involving at least five individual reactions. The activity of this new compound as a glycosidase inhibitor was evaluated against a panel of glycosidases and compared to (−)-steviamine.
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In the context of our studies on iminosugars of therapeutic interest,[1] we engaged in a research program dedicated to finding new inhibitors of mycobacterial galactan biosynthesis. Indeed while D-galactose is widely distributed in higher eucaryotes in the pyranose form (Gal*p*), the furanose form (Gal*f*) is found only in prokaryotes, protozoans, and fungi[2] as well as in a few lower eukaryotes.[3] As it is specific to a number of pathogenic bacteria, the biosynthesis of galactofuranose-containing glycans is becoming an important target for the development of new antibiotics.[4] The enzymes involved in the biosynthesis of galactans are UDP-Gal mutase (UGM),[5] which is responsible for the isomerization of UDP-Gal*p* into UDP-Gal*f*, and two or more UDP-Gal*f* transferases (*GlfT*),[6] which transfer Gal*f* units to build the galactan core (Scheme 1). As part of our investigations on the synthesis of potential inhibitors of these enzymes,[7,8] we prepared the iminosugar-based analogue of UDP-Gal*f* **1**, as well as a number of disaccharide mimics such as **2** (Fig. 1) which all exhibited very weak activity on UGM.[7d] We also prepared a β-linked UDP-Gal*f* mimic **3** (Fig. 1),[7b] which was found to have significant activity as an inhibitor of UDP-Gal*f* transferase *GlfT2*.[9]

In continuation of this program, we planned to synthesize more simple analogues of UDP-Gal*f* having the general structure **A** shown in Scheme 2. The 1,4-dideoxy-1,4-imino-D-galactitol unit of **1** and **3** would be replaced by a more easily accessible L-*arabino* iminopentitol, as in **4**, and the nucleoside moiety would be mimicked by a simple substituted aromatic ring.

This type of structure could be derived from the isoxazoline moiety obtained by way of the 1,3-dipolar cycloaddition of a 1-*C*-allyl iminosugar such as **4** and a nitrile oxide **C**, generated in situ and carrying the aromatic group.

However, precursor **B** was found to be highly prone to dehydration and intramolecular reductive amination reactions during the final hydrogenolysis step, leading unexpectedly to a new indolizidine iminosugar **5**, which was found to be an analogue of the natural product (−)-steviamine (Fig. 2), recently isolated from *Stevia rebaudiana* leaves.[10] The steviamine structure was fully elucidated by X-ray crystallography of its hydrobromide salt[11] and glycosidase inhibition studies[12] showed it to be the first natural product to have a (weak) inhibition effect on an α-galactosaminidase.

Thus with the initial objective of making simple UDP-Gal*f* analogues by means of a nitrile oxide 1,3-dipolar cycloaddition, we first investigated the synthesis of the protected β-1-*C*-allyl-1,4-dideoxy-1,4-imino-L-arabinitol **4**. This iminoalditol could be readily obtained in seven steps from D-xylose (Scheme 3): kinetic glycosylation of D-xylose into its methyl furanoside, benzylation of the hydroxyl groups, and hydrolysis of the glycosidic bond led to 2,3,5-tri-*O*-benzyl-D-xylofuranose **6**[13] in excellent yield. Under conditions we reported earlier,[14] addition of benzyl carbamate directly to the free hemiacetal **6** in the presence of TMSOTf provided the protected xylofuranosylamine **7** (52% after purification, 5 g-scale). We then used the chain extension methodology we developed for the synthesis of UDP-Gal*f* mimics:[7d] addition of $AllylSiMe_3$ to the N-protected glycosylamine **7** in the presence of TMSOTf led to amino D-iditol derivative **8** in excellent yield with high *syn*-diastereoselectivity, as no trace of the other (D-*gulo*)

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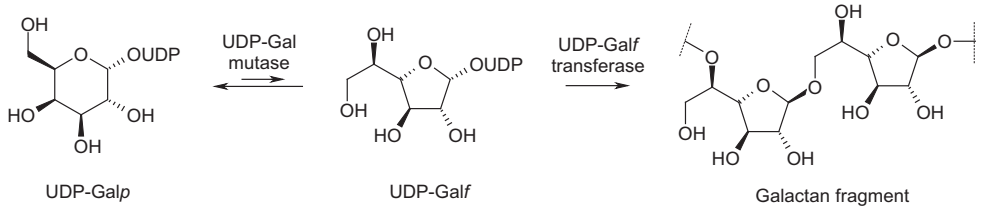


Scheme 1. UDP-galactofuranose and galactan biosynthesis.

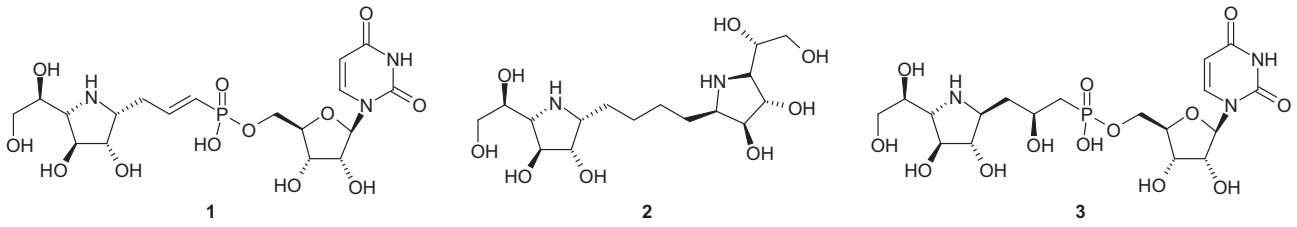
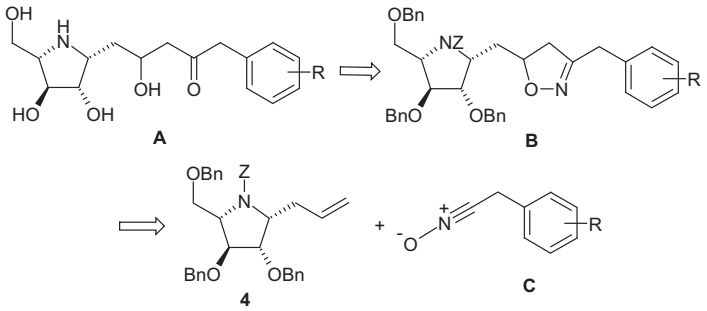


Figure 1. Previously synthesized inhibitors of UGM and *GlfT2*.



Scheme 2. Retrosynthetic analysis of compounds of structure A.

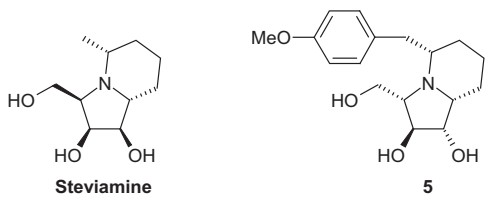
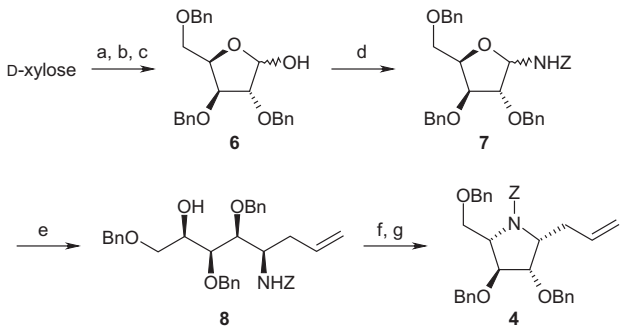


Figure 2. Chemical structures of (−)-steviamine and its synthesized analogue.

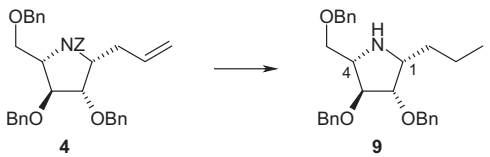


Scheme 3. Reagents and conditions: (a) HCl (0.11 M), MeOH, 30 °C, 3.5 h; (b) NaH (6 equiv), BnBr (4.5 equiv), DMF, 0 °C to rt; (c) 1 M HCl:AcOH (1:4), 80 °C, 55% (three steps); (d) H_2NCO_2Bn (2 equiv), TMSOTf (1 equiv), CH_2Cl_2, 4 Å MS, rt, 52%; (e) AllTMS (7 equiv), TMSOTf (1 equiv), CH_3CN, −20 °C, 85%; (f) MsCl (2.1 equiv), NEt_3 (2.2 equiv), CH_2Cl_2, 4 Å MS, rt; (g) *t*BuOK (2 equiv), THF, rt, 59% (two steps).

diastereoisomer could be observed by NMR. The stereochemistry of the new chiral center was determined after the cyclization step. Ring closure was achieved by a two-step sequence: mesylation of the free alcohol function of **8**, then treatment of the resulting mesylate with *t*BuOK gave the desired iminosugar **4** in the L-*arabino* series.

Determination of the configuration of the pseudo-anomeric carbon by NMR was not possible directly on compound **4**, because of the presence of *Z*-group rotamers. Deprotection of the nitrogen atom in **4** was achieved selectively by hydrogenolysis under basic conditions leading to iminosugar **9** (Scheme 4), which could be fully characterized by NMR. NOESY experiments on this compound confirmed unambiguously the 1,2-*cis* configuration with the presence of a correlation between H1 and H4.

Having the allylic iminosugar **4** in hands, we then prepared the nitrile oxide moiety (structure **C**) in order to perform the 1,3-dipolar cycloaddition reaction. Because of their facile dimerization, nitrile oxides are usually generated in situ by the dehydration of primary



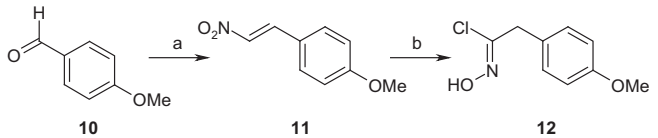
Scheme 4. Reagents and conditions: H_2, 10% Pd/C, NEt_3 (0.25 equiv), *i*PrOH, rt, 97%.

nitro compounds[15] or by base-induced dehydrohalogenation of hydroximoyl chlorides.[16] The first method was not easily applicable to the preparation of the planned nitrile oxides, as corresponding starting nitro compounds are not commercially available. Consequently we used the second methodology from hydroximoyl chlorides, which are usually prepared by chlorination or oxidation of aldoximes. We followed Kulkarni's procedure,[17] which would provide a great diversity of nitrile oxides of structure **C** possessing a methylene knuckle, from the conjugated nitrostyrene precursors. These compounds could be conveniently prepared by the addition of nitromethane on different commercially available substituted benzaldehydes.[18] As a first example, *p*-anisaldehyde **10** was efficiently converted into the conjugated nitrostyrene compound **11**,[17b] which was then submitted to the action of titanium chloride in the presence of triethylsilane to give the desired hydroximoyl chloride **12**;[17b] this precursor was used as a crude material in the cycloaddition reaction (Scheme 5).

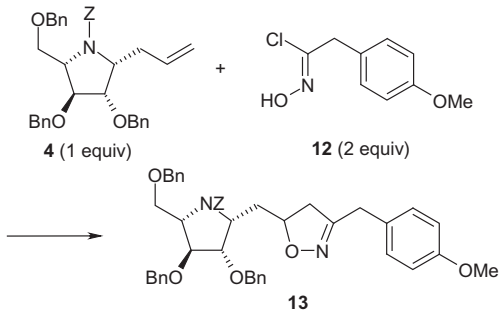
1,3-Dipolar cycloaddition was then performed at room temperature by mixing the dipolarophile **4** with an excess of the hydroximoyl chloride **12** in the presence of triethylamine, for in situ generation of the corresponding nitrile oxide (Scheme 6). The reaction proved to be highly regioselective in favor of the 5-substituted isoxazoline, which is in accordance with the predictions based on frontier orbital theory.[19] However the stereoselectivity was not as high, as two diastereoisomers were obtained in a 3:2 ratio; these isomers were quite difficult to separate by flash chromatography on silica gel. Nevertheless a sufficient amount of one diastereoisomer was obtained to perform the final hydrogenolysis step.

With the goal of cleaving the isoxazoline and deprotecting the iminosugar moiety, compound **13** was submitted as a single diastereoisomer to catalytic hydrogenolysis under aqueous acidic conditions, in order to favor the hydrolysis of the intermediate imine into a ketone. Quite surprisingly, this reaction gave directly the indolizidine derivative **5**[20] (Scheme 7), in good yield and as a single diastereoisomer.[21] Although the formation of **5** can be readily explained, this result is remarkable in that it must involve a series of at least 9 individual steps including a 5-step sequence of cleavage of the N–O bond, hydrolysis of the resulting imine, dehydration to an α,β-unsaturated ketone, hydrogenation to a saturated ketone, and stereoselective intramolecular reductive amination.

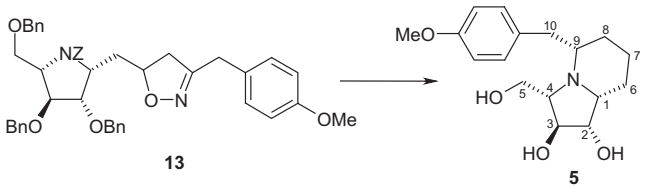
The stereochemistry of the chiral center resulting from the reductive amination was unambiguously determined by a NOESY experiment, which indicated by the correlations between H1, H4, and H9 that the three protons are all on the same side of the molecule. Compound **5** is an analogue of the recently isolated natural product (−)-steviamine,[10] bearing a *p*-methoxybenzyl group instead of the methyl group and having a 'β-L-*arabino*' configuration in the pyrrolidine instead of the 'α-D-*lyxo*' configuration. Because of its relation with the well-known glycosidase inhibitors, this new indolizidine was tested against a panel of glycosidases. With the exception of β-glucuronidase from *Escherichia coli*, for which a weak inhibition effect was observed, compound **5** did not show significant inhibitory properties toward other glycosidases (Table 1).



Scheme 5. Reagents and conditions: (a) $AcONH_4$ (1.1 equiv), CH_3NO_2, reflux, 82%; (b) Et_3SiH (2.1 equiv), $TiCl_4$ (2.2 equiv), CH_2Cl_2, rt, crude.



Scheme 6. Reagents and conditions: NEt_3 (6 equiv), CH_2Cl_2, rt, 67% (3:2 mixture of two diastereoisomers).



Scheme 7. Reagents and conditions: H_2, 10% Pd/C, H_2O (2 equiv), 1 N HCl (1 equiv), $MeOH/CH_2Cl_2$ 1:1, rt, 57%.

Table 1
Inhibition profile of **5** toward different glycosidases

Enzymes		Inhibition (%) at 1000 μM
α-Glucosidase	Yeast	4.7
	Rice	8.7
	Rat intestinal maltase	24.9
β-Glucosidase	Almond	10.7
	Bovine liver	37.8
α-Galactosidase	Coffee beans	19.2
β-Galactosidase	Bovine liver	34.7
α-Mannosidase	Jack beans	6.7
β-Mannosidase	Snail	0
α-L-Fucosidase	Bovine kidney	0.6
β-Glucuronidase	Bovine liver	6.5
	E. coli	47.8
α,α-Trehalase	Porcine kidney	4.4
Amyloglucosidase	*Aspergillus niger*	7.2
α-L-Rhamnosidase	*Penicillium decumbens*	23.0

In conclusion, we have developed an efficient synthesis of protected β-1-*C*-allyl-1,4-dideoxy-1,4-imino-L-arabinitol, an iminosugar derivative which could be used in a diversity of coupling reactions to prepare UDP-Gal*f* mimics. It was successfully engaged as a dipolarophile in a 1,3-dipolar cycloaddition reaction with a nitrile oxide. Further elaboration of the resulting isoxazoline by hydrogenolysis led, by way of a remarkable sequence of steps, to a new indolizidine, which is an analogue of the natural product (−)-steviamine. Inhibition studies showed only weak inhibitory properties for this new compound toward a panel of glycosidases. Further work is in progress in our laboratory to achieve the synthesis of iminosugar-based analogues of UDP-galactofuranose related to **A** as potential inhibitors of the mycobacterial galactan biosynthesis, using nitrile oxide 1,3-dipolar cycloaddition reaction.

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20. *Characterization data for* ***5*** [(***1S,2S,3S,5S,8aR*****)-3-hydroxymethyl-5-p-methoxybenzyl-indolizidine-1,2-diol***]: $[\alpha]_D = -28$ (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, MeOD, numbering as in Scheme 7) δ 7.08 (d, J = 8.3 Hz, 2H, H_{arom}), 6.83 (d, J = 8.3 Hz, 2H, H_{arom}), 4.02 (s, 1H, H_3), 3.75–3.73 (m, 4H, H_{5b}, OCH_3), 3.68 (d, J = 2.8 Hz, 1H, H_2), 3.57 (dd, J = 7.2, 10.8 Hz, 1H, H_{5a}), 3.11 (dd, J = 3.4, 12.1 Hz, 1H, H_{10b}), 2.88 (dd, J = 2.1, 7.2 Hz, 1H, H_4), 2.64 (dt, J = 2.8, 2.8, 10.0 Hz, 1H, H_1), 2.50 (tt, J = 3.4, 10.8 Hz, 1H, H_9), 2.37–2.28 (m, 1H, H_{10a}), 1.75–1.72 (m, 1H, H_{7b}), 1.61–1.52 (m, 2H, H_6), 1.39–1.36 (m, 1H, H_{8b}), 1.25–1.13 (m, 1H, H_{7a}), 1.11–1.01 (m, 1H, H_{8a}). ^{13}C NMR (100 MHz, MeOD, numbering as in Scheme 7) δ 159.52 (C_{arom}), 132.43 (C_{arom}), 131.34 (CH_{arom}), 114.69 (CH_{arom}), 80.54 (C_3), 79.18 (C_2), 73.11 (C_4), 69.09 (C_1), 68.15 (C_9), 65.94 (C_5), 55.64 (OCH_3), 42.33 (C_{10}), 32.08 (C_8), 26.35 (C_6), 25.67 (C_7). HRMS (ESI) calcd for $C_{17}H_{26}NO_4$ $[M+H]^+$ 308.18563, found 308.18564.
21. When the reaction was performed on the minor stereoisomer of **13**, the reaction gave a mixture (~1:1) of **5** and of the corresponding derivative having conserved the OH group (7-OH derivative of **5**).