Tetrahedron: *Asymmetry* 19 (2008) 1999–2002

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

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

Novel Gal*f*-disaccharide mimics: synthesis by way of 1,3-dipolar cycloaddition reactions in water

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ARTICLE INFO

Article history:
Received 23 July 2008
Accepted 6 August 2008

ABSTRACT

Galactofuranose-disaccharide analogues incorporating a 1,4-dideoxy-1,4-imino-D-galactitol moiety were obtained by 1,3-dipolar cycloadditions of a *galacto*-configured cyclic nitrone with arabino- or galacto-furanosides carrying a *C*-vinyl or *O*-allyl substituent. Remarkably, the cycloadditions could be performed highly efficiently and stereoselectively in water using unprotected nitrone and sugar-derived dipolarophile as reaction partners. The resulting pseudo-disaccharides are analogues of the Gal*f*-Gal*f* motifs found in mycobacterial cell-wall glycans and therefore constitute mimics of the acceptor substrates of mycobacterial Gal*f*-transferases.

1. Introduction

The cell wall of *Mycobacteria* is an impenetrable barrier, which provides the organism with great protection towards its environment. It consists of the so-called mAGP complex, a three-layer assembly of biomolecules (mycolic acids, arabinogalactans and peptidoglycans) and of the lipopolysaccharide LAM (lipoarabinomannan).[1] The central glycan section of the mAGP complex comprises a unique galactofuran core, which contains about 30 D-galactofuranosyl (Gal*f*) residues linked by alternating β-(1→5) and β-(1→6) bonds, and connected to the peptidoglycan base by an α-L-Rha-(1→3)-α-D-GlcNAc-1-P tether. Of particular interest is the fact that galactose in its furanose form is found only in prokaryotes and some lower eukaryote organisms.[2] Gal*f*-containing oligosaccharides are essential for the survival and infectivity of mycobacteria:[3] the biosynthesis of galactofuranose-containing glycans has thus become a promising target for the development of new antimycobacterial agents.[4]

The repeating Gal*f* unit is formed by an unprecedented ring-contraction of UDP-Gal*p* into UDP-Gal*f* catalysed by UDP-Gal mutase (UGM).[5] The sugar nucleotide is then substrate of Gal*f*-transferases[6] for the formation of the glycofuranoside linkages in the growing glycan chain. The recent work by Lowary[7] and Besra[8] has shed light on these elusive transferases and on the mechanism of galactan formation: remarkably, two Gal*f*-transferases appear to be sufficient enough to account for the assembly of the entire cell-wall Gal*f*-containing glycan of mycobacteria, both of which are behaving as bifunctional transferases.

Within a research program dedicated to the search of new compounds as potential inhibitors of mycobacterial galactan biosynthesis, we have already reported the preparation and evaluation of several novel UDP-Gal*f*-like compounds, incorporating a 1,4-iminogalactitol moiety as UGM inhibitors.[9] We have now extended our investigations to the analogues of galactan fragments, mimicking the acceptor substrate of the mycobacterial Gal*f*-transferases. Very few examples of iminosugar-containing oligo-arabino- or galacto-furanosides have been reported so far.[10] Herein, we report the first results of our synthetic studies towards novel Gal*f*-disaccharide analogues incorporating a 1,4-dideoxy-1,4-iminogalactitol moiety linked to O-5, C-5 or O-6 of the Gal*f* unit, having the general formula shown in Figure 1.

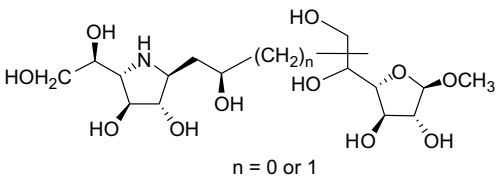

Figure 1. Target disaccharide analogues.

2. Results and discussion

Our synthetic approach is based on the coupling of the sugar entities by a 1,3-dipolar cycloaddition process[11] between the iminogalactitol-derived nitrone **1** and L-arabino- or D-galacto-furanosides, carrying a vinyl group as a dipolarophile. A major advantage of this methodology is the very high β-stereoselectivity of the cycloadditions onto nitrone **1**, as shown in our previous

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doi:10.1016/j.tetasy.2008.08.006

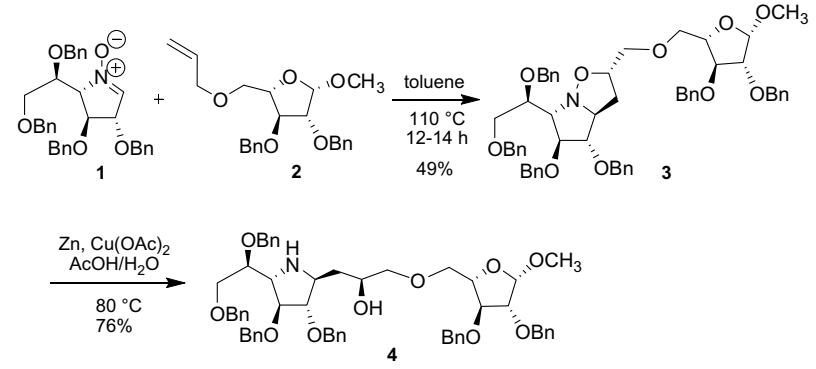
studies,[9b] which ensures the proper β-configuration of the pseudo-galactofuranose component of the disaccharide analogues. In the first experiment, we reacted 5-*O*-allyl-L-arabinofuranoside derivative **2**[12] with nitrone **1** (Scheme 1). The reaction gave the desired cycloaddition product **3**,[13] resulting from an *anti-exo* cycloaddition stereochemistry, as the major product, in 49% (isolated) yield (dr ⩾ 92%, ^{1}H NMR of crude product). The isoxazolidine ring was reductively opened with Zn in the presence of catalytic copper acetate[14] to afford protected pseudo-disaccharide **4** in good yield.

In order to reach disaccharide mimics, which more closely resemble the structure of the parent Gal*f* disaccharides, we also investigated the cycloaddition of **1** with the 5-*C*-vinyl-L-arabinofuranoside derivative **5**[15]; the cycloaddition proceeded here highly stereoselectively and in a quite satisfactory yield to give the bicyclic product **6** (Scheme 2). The N–O bond in **6** was cleaved under the same conditions to give the protected β-C-linked Gal*f*-disaccharide mimics **7**. While a diversity of imino-*C*-disaccharides has already been reported, in particular by Vogel et al.,[16] this compound is one of the rare examples of C-linked imino-disaccharides incorporating two five-membered ring units;[17] in its deprotected form, compound **7** has a structure that closely mimics a galactofuranobioside.

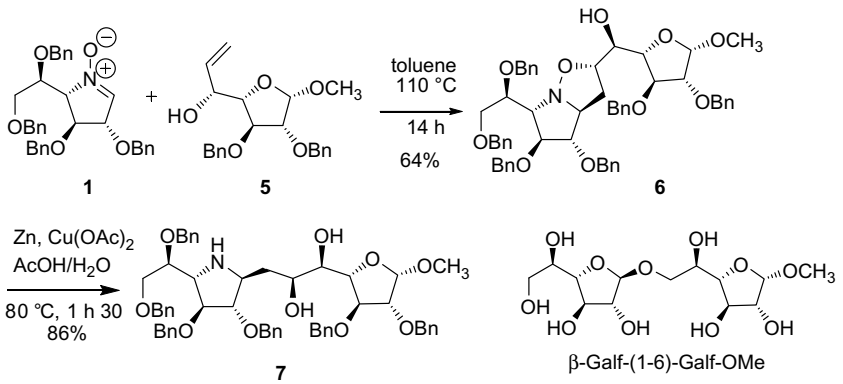
At this point, debenzylation of compounds **4** and **7** was attempted in order to obtain the corresponding pseudo-disaccharides as free glycosides for biological assays. This step met, however, with unexpected difficulties: catalytic hydrogenation under a wide variety of conditions (Pd/C, $Pd(OH)_2$, etc.), cleavage under dissolving metal conditions (Na/NH_3) and other methods (BCl_3/CH_2Cl_2)[18] either did not provide the final product or promoted degradation reactions; the BCl_3-technique was clearly unsatisfactory here because of the sensitivity of the glycosidic linkage under these conditions.

We therefore decided to investigate the cycloaddition process using deprotected reaction partners; cycloaddition reactions of this type are indeed compatible with free OH groups,[19] and can be performed in water.[20] We first prepared the unprotected 6-*O*-allyl-β-D-galactofuranoside **9** from the free galactoside[21] and examined its reaction with nitrone **1** (Scheme 3). The reaction could still be run in toluene at reflux temperature, and afforded the cycloaddition product **10** in 35% yield. We then proceeded to deprotect nitrone **1**. In spite of the sensitivity of this compound, we were able to cleave all the benzyl groups from **1** to give **8** in good yield (66%) using a large excess of BCl_3,[22] a reagent that already proved to be useful in the deprotection of other benzylated iminosugar derivatives in our work including our UDP-Gal*f* mimics.[9] The reaction of **8** with **9** was then investigated in water at 60 °C;[23] although the reaction was slow, it afforded the desired, unprotected cycloaddition product **11** in a yield of 51%, with excellent stereoselectivity. The N-O bond in **11** could be cleaved using Raney Ni[24] to give pseudo-1,6-linked galactofuranobioside **12**, thus avoiding the separation of the final product from metal salts. The cycloaddition of **8** with **9** is noteworthy, in that it provides a means of coupling two free sugar units by a carbon-carbon linkage in water, and thus opens a concise approach to a diversity of disaccharide mimics. While 1,3-dipolar cycloadditions of sugar-derived nitrones[11] have been used to prepare disaccharide mimics including an imino-*C*-disaccharide,[25] this is the first example of coupling of two free sugar derivatives by such a reaction in water.

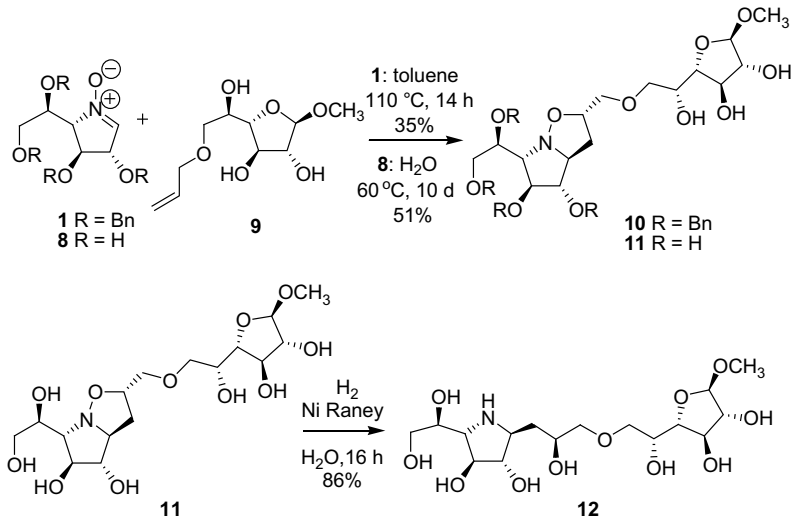
We also explored the synthesis of structures that mimic 1,5-linked galactofuranose disaccharides. For this purpose, the 5-*O*-allyl-β-D-galactofuranoside **17** was prepared from methyl β-D-galactofuranoside **13**,[26] by way of derivative **14**, as shown in Scheme 4. Acetylation of **14**, then cleavage of the isopropylidene group followed by tritylation[27] gave compound **15**, which carries a free



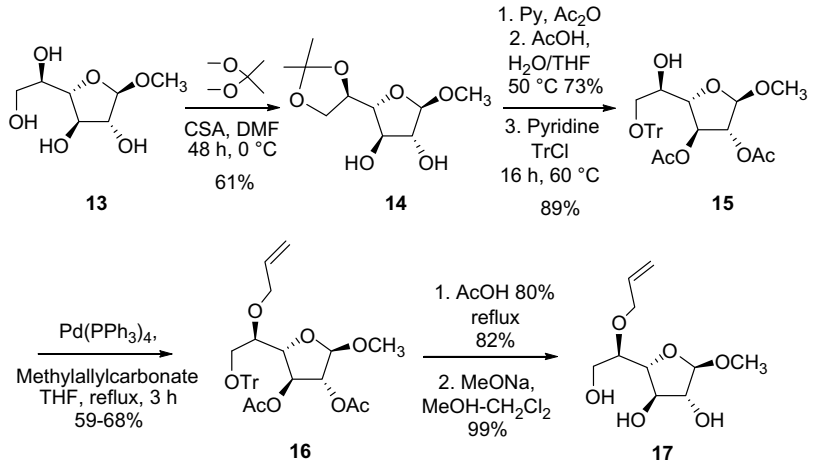
Scheme 1.



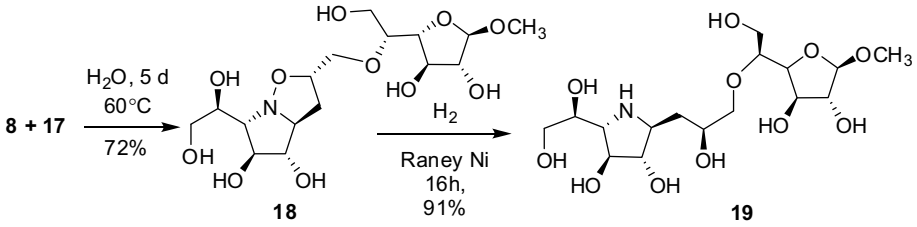
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

OH group at C-5. Allylation of this OH group proved to be a challenge: the alkylation was successful by Pd-catalysed allyl transfer onto this position, to give **16**, a process[28] that is particularly convenient for the allylation of OH groups in sensitive environments such as sugar derivatives carrying esters or aminosugar derivatives carrying carbamates. Removal of the trityl and acetyl groups in **16** afforded the selectively 5-O-allylated galactofuranose derivative **17**, in 22% overall yield from **13**. The cycloaddition of **17** with free nitrone **8** afforded bicyclic product **18** in 72% yield after 5 d at 60 °C, with excellent stereoselectivity (Scheme 5).

The N–O bond in **18** was cleaved as in compound **11** using Raney Ni. This remarkable sequence of reactions afforded pseudo-disaccharide **19** in two steps only from free monosaccharidic partners and in 66% overall yield. Compound **19** is a mimic of the β-(1→5)-linked galactobioside motif found in mycobacterial galactans. Pseudo-disaccharides **12** and **19** are also analogues of homo-DMDP-7-*O*-apioside, a natural product isolated from the leaves of bluebells (*Hyacinthoides non-scripta*).[29]

3. Conclusion

In conclusion, we have demonstrated in this study that complex structures of significant biological interest could be readily reached

by the 1,3-dipolar cycloaddition of two free sugar derivatives, carrying appropriate functional groups in water; in addition, by using this method, we have achieved the synthesis of new types of pseudo-disaccharides incorporating both an imino-galactofuranose and a normal galactofuranose unit, and thus mimicking fragments of mycobacterial galactans. Such compounds are potential inhibitors of the enzymes involved in the biosynthesis of mycobacterial cell-wall glycans. Biological investigations on these compounds are currently in progress, and the results will be reported in due course.

Acknowledgement

Dr. J. C. Jacquinet is warmly acknowledged for discussions and advice.

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23. General procedure for cycloadditions in water: Nitrone **8** and galactofuranoside **9** or **17** (1.6 equiv) were dissolved in water (0.09 M). After the appropriate stirring time at 60 °C, the reaction mixture was concentrated under reduced pressure. Purification on silica gel (CH_3Cl_3/MeOH/H_2O 7/4/0.1) afforded the desired cycloadduct.
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