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α-Glycosphingolipids via Chelation-Induced Anomerization of *O*- and *S*-Glucuronic and Galacturonic Acid Derivatives

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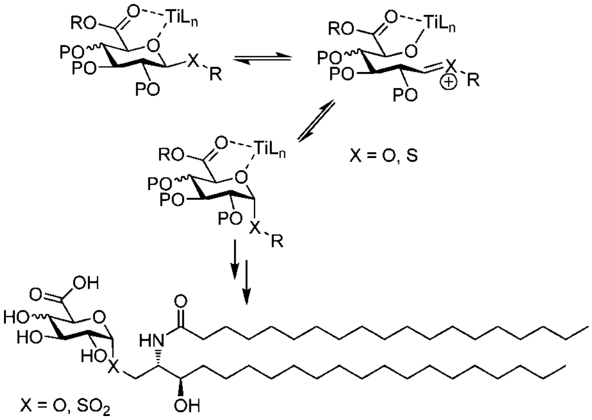
ABSTRACT



Bacterial glycolipids containing either α-glucuronic acid or α-galacturonic acid residues have an important role in the innate-type immune response to Gram-negative bacteria. Synthesis of closely related compounds, including a novel α-SO$_2$ glycolipid mimetic, is described from carbohydrate precursors where anomerization is a key step. Very high stereoselectivites (>97:3 in favor of α) were observed from *O*-glycoside precursors.

Sphingomonous cell walls that do not contain lipopolysaccharide (LPS) present glycosphingolipids such as GSL-1 **1**,[1] PBS30 **2**, and PBS59 **3**.[2] These contain glucuronic acid or galacturonic acid residues α-linked to sphingosine derivatives (Figure 1). These glycolipids are structurally related to the antitumor agent KRN7000 **4**. The glycoprotein CD1d presents **4** and stimulates natural killer T (NKT) cells to release cytokines.[3] Recently, **1**−**3** have been shown to be targets for mice and human NKT cells.[1,2] They can lead to septic shock and bacterial clearance in infected mice, demonstrating that glycolipids stimulate an innate-type immune response to gram negative bacteria not presenting LPS. Although *β*-glycosyl ceramides are more abundant in

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10.1021/ol802915h CCC: $40.75

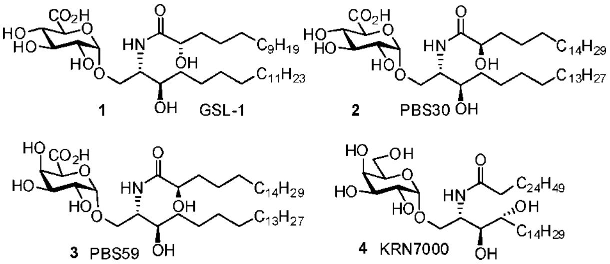
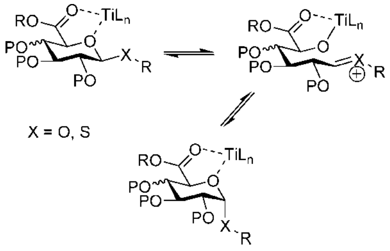


Figure 1. Structures **1**−**4**.

nature, the α-glycosidic linkage in **1**−**3** is essential for their biological properties.

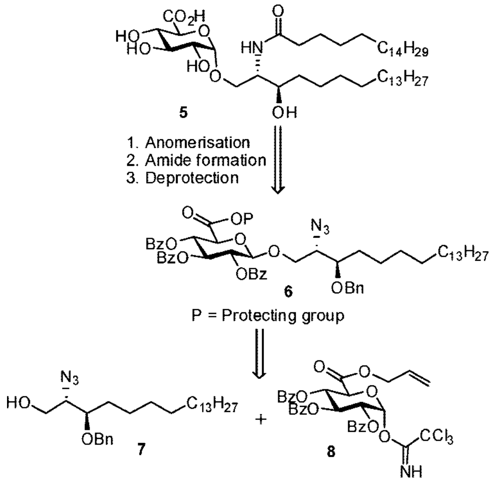
The development of novel strategies for the synthesis of α-glycolipids and their mimetics is thus important in order to generate and to evaluate the potency of new glycolipid antigens. A number of syntheses of the glucuronic and galacturonic acid based glycolipids have been reported, and these have been based on the oxidation of a preformed glycoside[4] or glycoside bond formation using a uronic acid based donor containing nonparticipating and "armed" protecting groups at C-2.[5] The latter approach gave mixtures of anomers in the glycoside bond forming reactions in some cases. Herein, we describe the synthesis of the α-linked uronic acid based glycolipids based on chelation-induced anomerization (Scheme 1), which generates the α-linkage with very high stereoselectivity (>97:3) for both glucuronic acid and galacturonic acid derivatives, even though participating and disarmed protecting groups are present. Chelation-induced anomerization of *S*-glycosides is also demonstrated.

Scheme 1. Chelation-Induced Anomerization



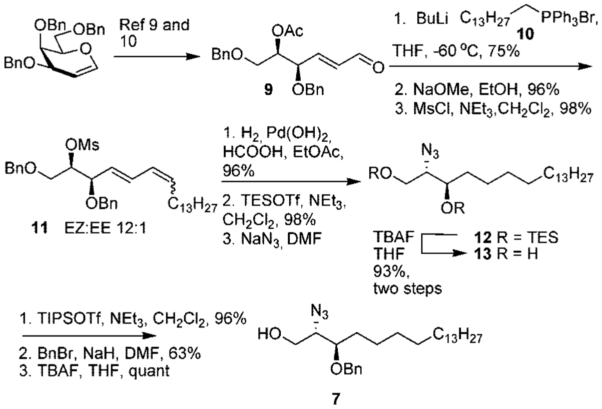
The retrosynthetic analysis of the initial target compound **5** is shown in Scheme 2. The rates of anomerization have recently been observed to be faster for some glucuronic acid derivatives than for their corresponding glucosides.[6] This rate enhancement was explained by chelation-induced anomerization (Scheme 1) wherein a $SnCl_4$ or $TiCl_4$ catalyst gives a 5-membered ring chelate after coordination to both the pyranose ring oxygen atom and to the C-6 carbonyl group. Consequently, anomerization would occur via cleavage of the C1−O5 bond[7] (endocyclic cleavage) as opposed to cleavage of the glycosidic C1−O1 bond (exocyclic cleavage). The synthesis of **6** was thus undertaken in order to determine whether anomerization could be used to generate an intermediate with the desired α-linkage that after subsequent amide formation and deprotection would give **5**. It was envisaged that **6** would be obtained via glycoside coupling of the acceptor **7** and donor **8**, which would in turn be prepared respectively from D-galactal and D-glucose.

Scheme 2. Retrosynthesis of **5**



The dihydroceramide derivative **7** was first obtained from 3,4,6-tri-*O*-benzyl-D-galactal (Scheme 3).[8] Initial reaction of the galactal under the Perlin conditions[9] and subsequent acetylation, as indicated also recently,[10] gave the aldehyde **9**.[11] Reaction of **9**with the Wittig reagent prepared by reaction

Scheme 3. Synthesis of **7**



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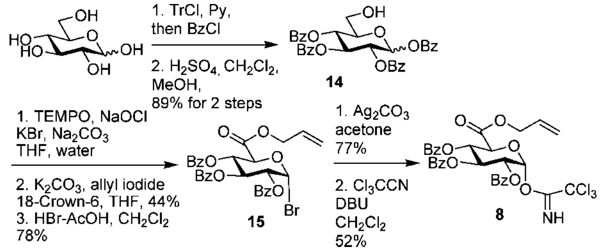
Scheme 4. Synthesis of **8**

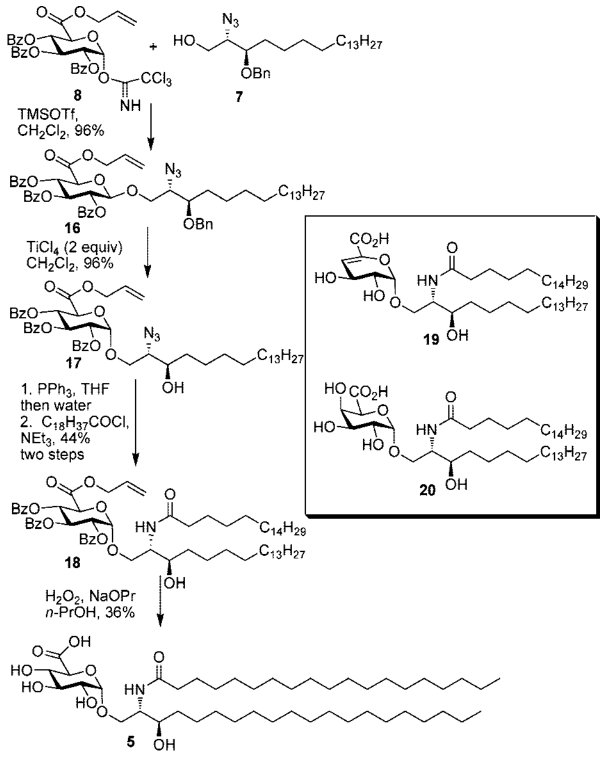


of the phosphonium salt **10**[12] with *n*-butyllithium and subsequent de-*O*-acetylation gave a diene as a mixture of stereoisomers. Subsequent mesylation of this intermediate diene gave **11**. In contrast with the previous finding,[10] we believe the *EZ* isomer (*EZ*/*EE*, 12:1) is the major product from the Wittig reaction in this sequence. Our assigment is made on the basis of signals in the ^{1}H NMR spectra (500 MHz) of the diene product mixture of **11**. For H-6, a signal for the *EZ* isomer is observed at δ 6.00 as an apparent triplet with a coupling constant (J) of 11.0 Hz; the H-6 signal of the *EE* isomer (minor product) appears at δ 6.06 (double doublet overlapping) with a J of 15.1 Hz observed. These J values are consistent with the *EZ* stereochemical assignment for the major product from the Wittig reaction. Catalytic hydrogenation of **11**, followed by introduction of TES groups and subsequent substitution of the mesyl group with azide, gave **12**. Removal of the TES groups from **12** using TBAF−THF gave the diol **13**. Efforts to carry out glycosylation reactions with a variety of acylated glucuronic acid donors and acceptors **12**[13] or **13** were unsuccessful. Thus, **13** was converted to the benzyl derivative **7** via the regioselective introduction of a TIPS group at the primary alcohol group, benzylation of the secondary hydroxyl group, and subsequent removal of the TIPS group. The use of the TIPS groups was found to be necessary in this sequence of reactions, as it was more stable than a TBS protecting group, for instance, which migrated during attempted benzylation of the secondary hydroxyl group. Glycoside bond formation from **7** was ultimately successful.

The glycosyl donor **8** was obtained in seven steps from D-glucose. Partially protected derivative **14**[14] was first prepared from D-glucose. Oxidation using TEMPO, subsequent base-promoted esterification of the acid with allyl iodide, and reaction of the corresponding ester with HBr−AcOH gave the α-glycosyl bromide **15**. This bromide was converted to the trichloroacetimidate donor **8** by hydrolysis of **15** promoted by silver carbonate and reaction of the resulting hemiacetal with DBU and trichloroacetonitrile.

The glycoside-coupling reaction of **7** and **8** was next investigated (Scheme 5). This reaction gave the β-glycoside **16** in excellent yield. The acetylated analogue of **8** was also investigated as a donor, but in glycosylation reactions an orthoester was obtained rather than the desired glycoside. With the β-glycoside **16** in hand, its anomerization reactions were investigated. Gratifyingly, anomerization with concomitant removal of the benzyl protecting group proceeded efficiently using $TiCl_4$ in dichloromethane to give **17** with high stereoselectivity (α/β, 97:3) and yield. The Staudinger reaction of azide **17** gave an amine that was converted to amide **18** after treatment with nonadecanoyl chloride in the presence of triethylamine. Removal of the protecting groups from **18** gave target compound **5**. The deprotection was effected using hydroperoxide generated in *n*-propanol from sodium propoxide and hydrogen peroxide. The use of stronger and harder bases such as hydroxide and methoxide led to the elimination of benzoic acid and formation of the undesired unsaturated compound **19**. The synthesis of the

Scheme 5. Synthesis of **5**



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(10) For a closely related strategy to sphingosines and related compounds recently published, see: Kokatla, H. P.; Sagar, R.; Vankar, Y. D. *Tetrahedron Lett.* **2008**, *49*, 4728–30.

(11) For a recent application of this reaction in synthesis of dorrigocin A analogues see Anquetin, G.; Rawe, S. L.; McMahon, K.; Murphy, E.; Murphy, P. V. *Chem. Eur. J.* **2008**, *14*, 1592–1600.

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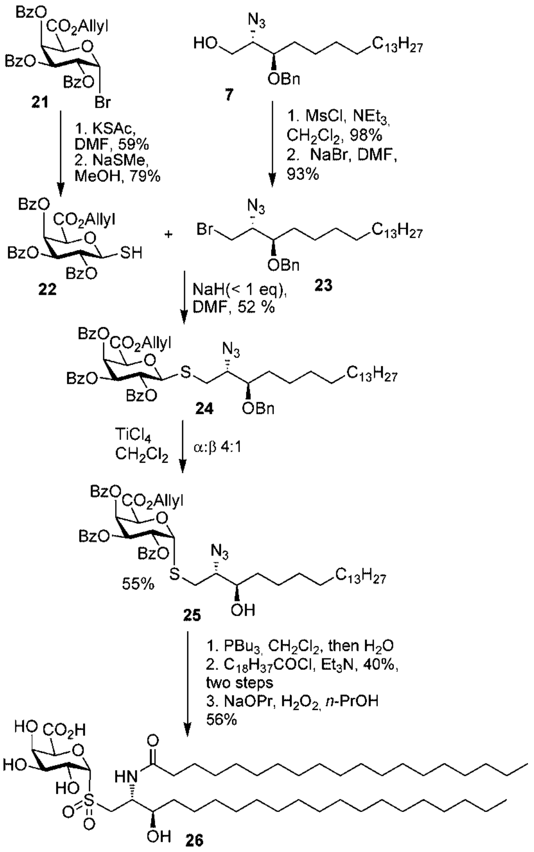
(14) Kovac, P.; Glaudemans, C. P. J. *J. Carbohydr. Chem.* **1988**, *7*, 317.

Scheme 6. Synthesis of α-SO_2 Containing Glycolipid



galacturonic acid derivative **20** was also achieved using an identical strategy from the corresponding galacturonic acid donor. Within this sequence, the anomerization reaction gave the desired α-*O*-galacturonic acid product in quantitative yield and the *β*-anomer was not detected by NMR analysis, indicating the stereoselectivity was >97:3.

Having achieved the synthesis of the target *O*-glycolipids, we next investigated *S*-glycolipid anomerization. To the best of our knowledge, the anomerization of *S*-glycosides have rarely, if at all, been applied to complex neoglycoconjugate synthesis.[15] Thus, *β*-glycosyl thiol **22** was next prepared in two steps from the galacturonosyl bromide derivative **21**, which had been obtained from a route similar to that described for preparation of **15**. Alcohol **7** was converted to bromide **23** via a mesylated intermediate. Next, coupling of thiol **22** and alkyl bromide **23** using NaH (<1 equiv) gave the *β*-*S*-thioglycoside **24**. Gratifyingly, the $TiCl_4$-catalyzed anomerization gave the α-*S*-glycoside **25** in 55% yield. A 4:1 mixture of α- and *β*-thioglycolipids were obtained in this latter reaction, and they were separated by silica gel chromatography. Conversion of the azide group to an amine and subsequent acylation followed by removal of the protecting groups and concomitant oxidation of the sulfur atom under the deprotection conditions gave **26**, which can be considered a novel glycolipid mimetic. The α-*S*-glycolipid[16] would also be an interesting compound to obtain, but conditions have not yet been established in our group that preclude the use of hydrogen peroxide, which oxidizes the thioglycoside.

In summary, we have described the synthesis of glycolipids and mimetics by a chelation-induced anomerization[17] of protected *β*-glycolipid precursors (Scheme 6). An advantage of this strategy is that both *β*- and α-glycolipids can in principle be generated. Deprotection conditions that will preclude oxidation of sulfur need to be established with a view to generating the *S*-glycolipid. The biological properties of the new analogues will be described in due course.[18]

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Supporting Information Available: Experimental procedures and ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) The results described herein were outlined in an oral communication recently: Murphy, P. V.; Pilgrim, W. Novel synthesis of glycolipids containing glucuronic acid. *Abstract of Papers*, 236th ACS National Meeting, Philadelphia, PA, United States, August 17−21, 2008; American Chemical Society: Washington, DC, 2008; CARB-092.