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Synthesis of α-S-Glycosphingolipids Based on Uronic Acids

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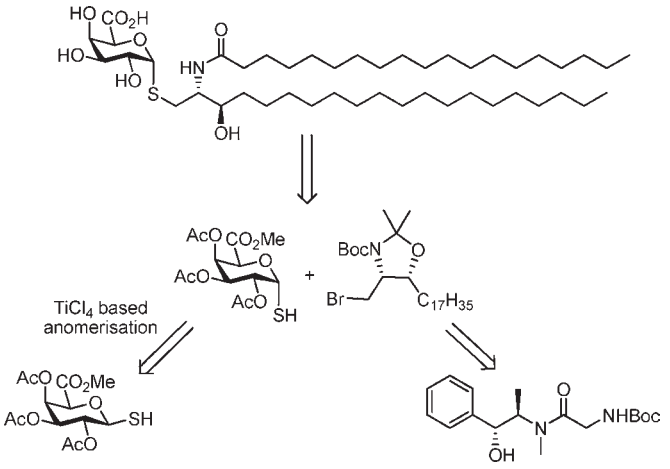
ABSTRACT



The synthesis of *S*-glycosphingolipids based on uronic acids is described. These compounds are analogous to the highly immunostimulatory antigens isolated from the cell walls of bacteria of the *Sphingomonas* family. Key to the synthetic route is a stereoselective anomerization to give α-glycosyl thiol precursors. A route to a sphinganine precursor from pseudoephedrine glycinamide is also described.

Sphingomonas cell walls that do not contain lipopolysaccharide (LPS) present glycosphingolipids based on glucuronic acid and galacturonic acid, such as GSL-1 **1**,[1] PBS30 **2**, and PBS59 **3**.[2] The uronic acids in these structures are α-linked to sphingosine derivatives (Figure 1). These glycolipids are structurally related to the highly bioactive glycolipid KRN7000 **4** and other potent analogues which have potential as anti-infective agents[3] and as vaccine adjuvants.[4] The glycoprotein CD1d presents **4** and stimulates natural killer T (NKT) cells to release cytokines.[5] The structurally related glycolipids **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 they stimulate an innate-type immune response to gram negative bacteria not presenting LPS. Although β-glycosyl ceramides are more abundant in Nature, the α-glycosidic linkage in **1**–**3** is regarded as essential for their activity. The development of the synthesis of mimetics of α-glycolipids facilitates the generation and biological evaluation of new glycolipid antigens. In this paper, we describe a strategy for the synthesis of *S*-glycolipids based on uronic acids. The route includes an approach to a sphinganine precursor from the Myers chiral glycinamide precursor and a highly

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stereoselective anomerization of β-glycosyl thiols to give α-glycosyl thiols.

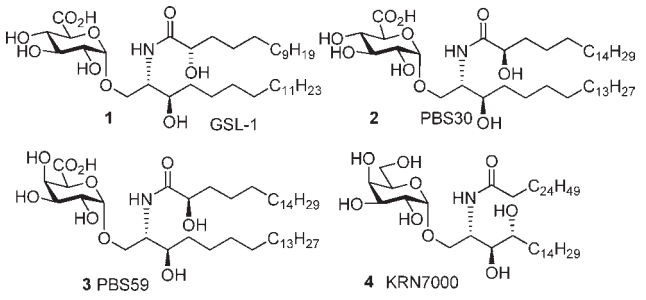
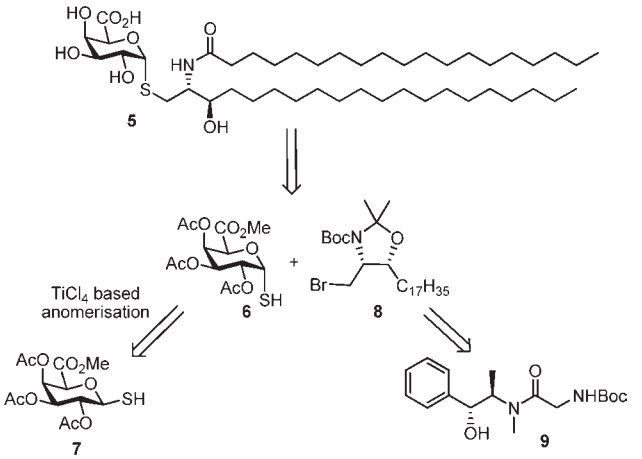


Figure 1. Immunostimulatory glycosphingolipids.

The synthesis of *S*-glycoside analogues of natural bioactive *O*-glycosides is of great interest and there has been progress on the synthesis of *S*-oligosaccharides, *S*-glycopeptides and the thioglycoside analogue of KRN7000.[6] These synthetic studies are important because *S*-glycosides are more stable *in vivo* than the corresponding *O*-glycosides.[7] Despite these synthetic achievements the *S*-glycoside analogue of **1**–**3** has not been prepared previously. Efforts from our own laboratory have, until now, failed to establish conditions for removing the ester protecting groups from the uronic acid residue. Also a previous approach from our laboratory to *O*-glycolipids, which are closely related to **1,** resulted in a 14 step route to a sphinganine precursor from D-galactal.[8] A shorter route to a sphinganine precursor was therefore desired. In our revised retrosynthesis of the *S*-glycolipid **5** (Scheme 1), the α-glycosyl thiol **6** and the bromide **8** were identified as the key precursors. It was envisaged that **6** could be generated by anomerization of the β-glycosyl thiol **7** and that the sphinangine **8** could be prepared from the glycinamide **9** which contains the pseudoephedrine chiral auxiliary. Both enantiomers of **9** are readily available, providing the potential to generate diastereoisomers or other analogues of the glycolipids for SAR studies.

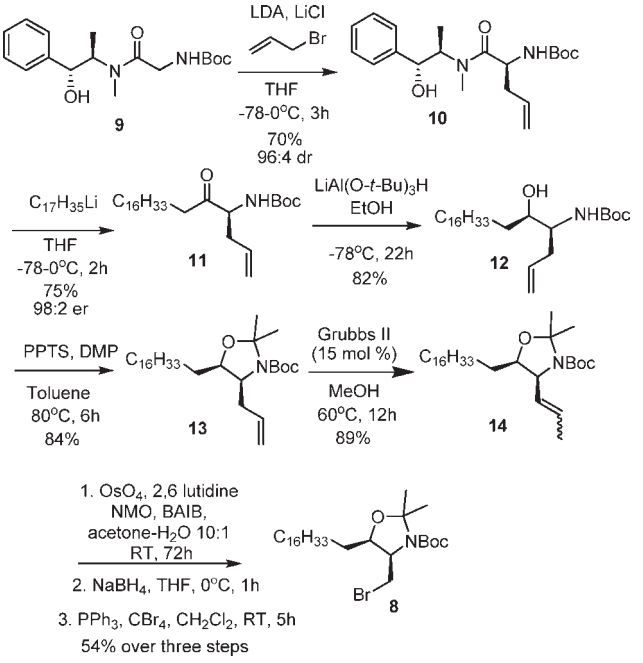
The synthesis (Schemes 2–4) began with preparation of **8**. The glycinamide **9**, shown in Schemes 1 and 2, was first allylated to give **10** in a highly stereoselective fashion according to the conditions described by Myers et al.[9] The Myers group have also shown that the pseudoephedrine derivatives of *N*-Boc protected amino acids can thereafter be converted into *N*-Boc protected aminoketone derivatives by reaction with alkyllithium reagents.[10] Therefore treatment

Scheme 1. Retrosynthetic Analysis



of **10** with heptadecyl lithium, which had been preprepared by the reaction of iodoheptadecane with *t*-BuLi,[11] gave the corresponding α-aminoketone **11** in high yield and with excellent enantioselectivity. Hoffman and co-workers have shown that access to *anti*-1,2-aminoalcohols can be achieved through treatment of carbamate protected α-aminoketones using LiAl(O-*t*-Bu)$_3$H in EtOH at −78 °C.[12]

Scheme 2. Synthesis of **8**



The aminoketone **11** was reduced under the Hoffman conditions to give the aminoalcohol **12** in high selectivity

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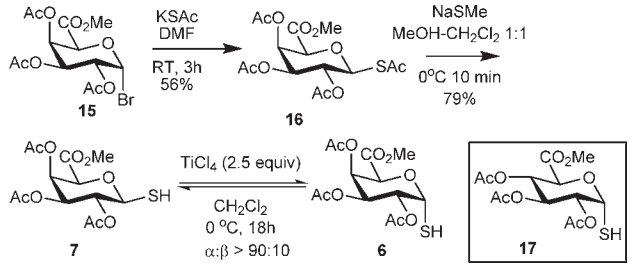
and yield.[13] Conversion of **12** to the oxazolidine **13** was then achieved[14] where **12** was reacted with catalytic pyridinium *p*-toluenesulfonate and dimethoxypropane in toluene at 85 °C. Next an isomerization of the allyl group to the propenyl derivative **14** was brought about using the Grubbs-II catalyst,[15] as described by Hanessian and coworkers;[16] the isomerization proceeded in 89% yield and gave a 1:1 ratio of both the *E* and *Z* isomers. Oxidative cleavage[17] of the propenyl derivative gave an aldehyde which was subsequently reduced using $NaBH_4$ in THF to give a primary alcohol intermediate. The reduction reaction was slow and took 3 days to complete. The desired bromide **8** was subsequently generated from the alcohol using the Appel reaction (54%, three steps). Overall the sphinganine precursor **8** was obtained in 8 steps from readily available **9**; the overall yield was ~17%.

Scheme 3. Synthesis of β-Galacturonosyl Thiol and Anomerization Reaction

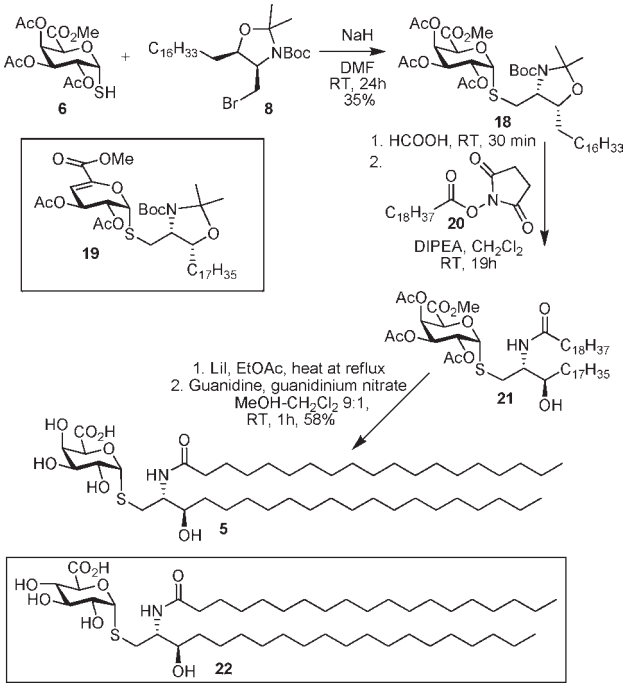


The route to α-galactosyl thiol **6** was next explored (Scheme 3). First the protected galacturonosyl bromide **15** was prepared[18] and then reacted with potassium thioacetate to give **16**. Subsequent thiolysis gave the galacturonosyl thiol **7** with the β-configuration.[19] Gratifyingly, the $TiCl_4$ induced anomerization of the glycosyl thiol proceeded successfully to give the desired α-anomer (68%) with high selectivity in the anomerization reaction. A 2.5 fold excess of the $TiCl_4$ was used to ensure the optimum proportion of the α-anomer **6**, as previously described for thioglycosides.[18a] The α:β ratio was not just dependent on $TiCl_4$ concentration but was also higher at 0 °C than at room temperature. The α:β ratio was also found to be scale dependent (α:β > 97:3 on a 100 mg scale; α:$\beta \approx$ 90:10 on a multigram scale). A similar anomerization reaction with a glycosyl thiol derived from glucuronic acid gave **17** (α:β = 7:1, 100 mg scale). The presence of the C-5 carbonyl is critical in order for the anomerization of the thiol to efficiently proceed; the anomerization reaction of the corresponding tetra-*O*-acetylated galactopyranosyl thiol, for example, with $TiCl_4$ did not proceed under identical conditions. This is believed to be due to chelation by both the C-5 carbonyl and ring oxygen to the Lewis acid, which increases the anomerization rate in uronic acid derivatives.[18a]

With **6** and **8** in hand the completion of the synthesis of the *S*-glycolipid was undertaken (Scheme 4). The coupling of **6** and **8** was brought about using NaH (< 1 equiv) to give the protected lipid derivative **18** in 35% yield. The use of other bases for this reaction led to lower yields or only trace amounts of **18** whereas the use of an excess of NaH led to the formation of the unsaturated compound **19**. The protected thioglycolipid **18** was treated with formic acid[20] for 30 min to remove the oxazolidine and Boc groups and gave the aminoalcohol intermediate in 85% yield. Reaction of this aminoalcohol with the succinate **20**[21] in dichloromethane gave the amide **21** (60%).

Attempts to remove the protecting groups from uronic acid derivatives similar to **21** were unsuccessful; the use of methoxide or hydroxide cleaved the ester protecting groups but also caused the formation of an unsaturated compound as a result of elimination of acetic acid from C-4

Scheme 4. Coupling of Fragments and Completion of Synthesis



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and C-5. The acyl protecting groups could also be removed successfully using sodium hydroperoxide, without the competing elimination reaction taking place, but oxidation to a sulfone could not be prevented.[8] Successful deprotection was finally achieved in two steps. First selective cleavage of the methyl ester was achieved by heating **21** with LiI in EtOAc at reflux for 4 h;[22] this gave the carboxylic acid. The formation of an enolate and subsequent elimination did not occur from the acid intermediate and the removal of the acetate groups from the C-2, C-3 and C-4 oxygen atoms was effected successfully using guanidine and guanidinium nitrate[23] in CH_2Cl_2-MeOH for 30 min; this gave the desired *S*-glycolipid **5**, which was purified successfully using lipophillic Sephadex LH-20. The thiol **17** (Scheme 3) gave **22** by a similar sequence (not shown).

In summary, the synthesis of *S*-glycolipids based on uronic acids which are analogous to antigenic components of bacterial cell walls has been achieved.[24] The route included anomerization[25] of β-glycosyl thiols to generate the α-anomers. There are relatively few syntheses of such 1,2 *cis* or α-glycosyl thiols reported to date.[26] Such thiols are envisaged to have potential in *S*-oligosaccharide and *S*-neoglycoconjugate synthesis. Also a route to sphinganine precursors has been described. The biological evaluation of the *S*-glycolipids and their analogues is underway and will be reported in due course.

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Supporting Information Available. Experimental procedures, NMR spectra and analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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