Synthesis of a Versatile Building Block for the Preparation of 6-*N*-Derivatized α-Galactosyl Ceramides: Rapid Access to Biologically Active Glycolipids

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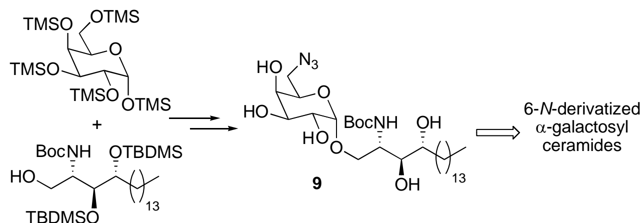


A concise route to the 6-azido-6-deoxy-α-galactosyl-phytosphingosine derivative **9** is reported. Orthogonal protection of the two amino groups allows elaboration of **9** into a range of 6-*N*-derivatized α-galactosyl ceramides by late-stage introduction of the acyl chain of the ceramide and the 6-*N*-group in the sugar head-group. Biologically active glycolipids **6** and **8** have been synthesized to illustrate the applicability of the approach.

The synthetic glycolipid α-galactosyl ceramide (α-GalCer),[1] also known as KRN7000 (**1**) (Figure 1), has been shown to bind to the protein CD1d. Recognition of the resulting glycolipid-protein complex by T cell receptors (TCRs) located on the surface of invariant natural killer T (*i*NKT) cells, leads to activation of the immune response through the release of a diverse range of cytokines, including both Th1 (IFNγ) and Th2 (IL-4) cytokines.[2-4] The release of Th1 cytokines may contribute to antitumour and antimicrobial functions, while the release of Th2 cytokines is believed to play a role in alleviating autoimmune diseases[5-7] such as multiple sclerosis[8] and arthritis.[9] When both Th1 and Th2 cytokines are released together, their effects oppose one another, which may induce mixed and unpredictable biological effects.[10] This is the case upon α-GalCer/CD1d activation of the immune response, which has complicated efforts to develop KRN7000 as a therapeutic agent. The search for analogues of this glycolipid, which induce a more biased Th1/Th2 response, is therefore a current focus of many immunological studies.

A number of α-GalCer analogues have been reported that exhibit more skewed Th1/Th2 cytokine profiles compared with that elicited by α-GalCer **1** (Figure 1).[11] Truncation of the acyl[12] (**2**) and sphingosine[8,13] (OCH (**3**)) chains, and the incorporation of unsaturation in the acyl chain (α-GalCer C20:2 (**4**))[14] result in CD1d agonists that generate a more Th2-biased response. Examples of more Th1-biasing molecules are much rarer;[15,16a] the *C*-glycosyl analogue of KRN7000, α-*C*-GalCer (**5**) is one such molecule that has been shown to induce a useful Th1-biased response.[15]

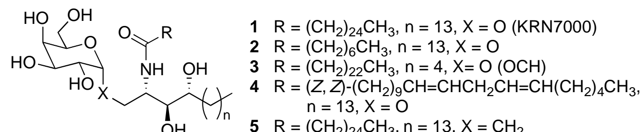


Figure 1. Prototypical KRN7000 (**1**) and biologically active analogues **2**, **3**, **4** and **5**.

There has been recent interest in galactosyl ceramides in which the hydroxyl group at the 6-position of the sugar head group has been modified (Figure 2).[16] Crystal structures of the CD1d-KRN7000 complex[17] and a TCR-KRN7000-CD1d complex[18] show that the 6-hydroxyl group is not involved directly in hydrogen bonding with either the CD1d protein or the TCR. Moreover, it has been shown that the TCR-glycolipid-CD1d interaction can tolerate derivatization at C6.[16d,19] Savage has reported that GalCer **6** (PBS-57) offers improved solubility over KRN7000, effectively stains both mouse and human NKT cells, and stimulates cytokine release at low concentrations.[16b] Wang has synthesized the biotinylated α-GalCer analogue **7** to facilitate detection of this antigen in flow cytometry assays.[16c] In this study, the problematic low solubility of these α-GalCer analogues was remedied by incorporating a truncated acyl chain. Of particular interest, Van Calenbergh recently found that substituting the 6-hydroxyl group of **1** for a range of aryl ureas, such as **8**, induced a more Th1-biased cytokine response than is observed with α-GalCer.[16a]

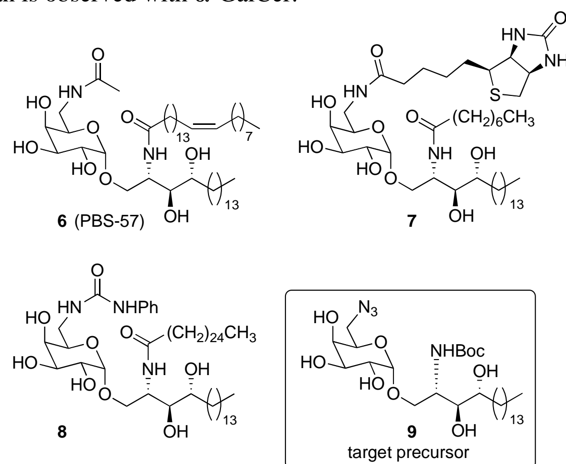


Figure 2. Biologically active 6-derivatized α-galactosyl ceramides **6**-**8** and our target precursor **9**.

Previously reported routes to 6-derivatized α-GalCer analogues are somewhat lengthy, involve extensive protecting group manipulation, and often suffer from poor overall yields. In addition, they do not allow the late-stage variation of the acyl chain; a change of acyl chain therefore requires a complete repeat of the synthetic sequence. Here we present a concise synthesis of the orthogonally protected diamino α-GalCer analogue **9** (Figure 2), which is primed for further elaboration to 6-*N*-derivatized galactosyl ceramides. The described route allows both the acyl chain of the ceramide unit and the 6-substituent of the sugar head group to be varied at a late stage of the synthesis, thus providing access to a potentially large and varied library of this class of compound.

The retrosynthesis of our target **9** is shown in Figure 3. With careful choice of protecting groups, it was envisaged that **9** (or the potentially more useful Boc-deprotected derivative **10**) could be accessed in a single step from fully-protected azide **11**, which in turn could be accessed from alcohol **12**, using a Mitsunobu reaction to install the azide. At this point we would require a selective mono-deprotection of the primary 6-OH for which there was precedent from Fernández who showed that 1,2,3,4-tetra-*O*-trimethylsilylgalactose could be obtained by treating 1,2,3,4,6-penta-*O*-trimethylsilyl-galactose **13** with acetic acid.[20] We therefore reasoned that alcohol **12** could be accessed from **14** in a similar manner. Glycoside **14** was further disconnected to glycosyl donor **15** and sphingosine acceptor **16**.

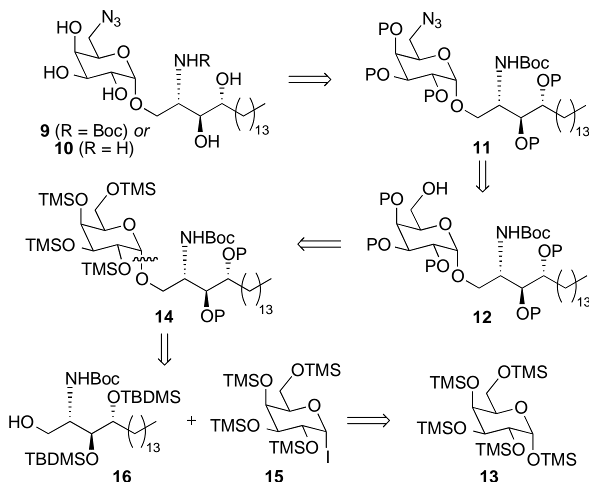
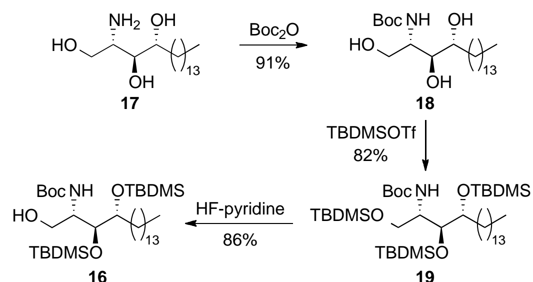


Figure 3. Retrosynthetic analysis of targets **9** and **10**.

Trimethylsilyl groups were chosen as the protecting groups for the sugar unit owing to 1) their ease of attachment to the galactose starting material;[20,23] 2) the ability to mono-deprotect the primary silyl ether selectively;[20] 3) the ease with which the remaining silyl groups can be removed when required; and 4) the 'arming' effect that silyl groups impart on the sugar donor in glycosylation reactions.[21] *tert*-Butyldimethylsilyl (TBDMS) groups were chosen as the protecting groups for the sphingosine acceptor. While conferring an increased level of stability during the synthesis, as well as providing an extra handle for ^{1}H- and ^{13}C NMR spectroscopic analysis, we envisaged these bulkier silyl groups could also be removed along with the trimethylsilyl groups of the sugar head unit in a single step.

Per-TMS-protected galactose **13** was synthesized in quantitative yield by treating D-galactose with chlorotrimethylsilane and hexamethyldisilazane in the presence of pyridine.[22] Acceptor **16** was accessed in three steps from commercially available phytosphingosine **17** (Scheme 1): Boc protection afforded carbamate **18**, which underwent threefold silylation of the triol functionality to provide silyl ether **19**. Selective mono-deprotection of the primary silyl ether in **19** with HF-pyridine[23] concluded the synthesis of glycosyl acceptor **16**.

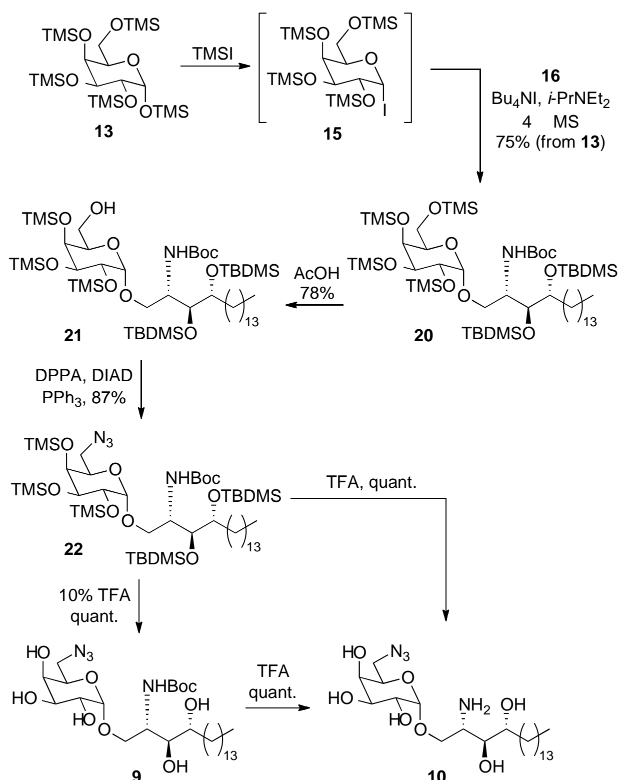


Scheme 1. Synthesis of glycosyl acceptor **16**.

The key step in the synthesis of **9** employed Gervay-Hague's elegant glycosylation methodology in which the glycosyl iodide donor **15** was generated in situ by treating per-TMS-protected galactose **13** with iodotrimethylsilane.[24] Glycosyl iodide **15** reacted with acceptor **16** in the presence of Bu_4NI and Hünig's base, to provide glycoside **20** exclusively as the α-anomer in 80% yield, thus providing a short and scalable route to galactosyl ceramide precursors (Scheme 2). With this method of glycosylation, the protecting groups are usually removed immediately via an acidic work-up.[24] However, it was desirable in our case to leave the silyl protecting groups in the product intact and effect a selective mono-deprotection of the primary trimethylsilyl group instead. To this end, following Fernández's method for 6-trimethylsilyl ether deprotection,[20] treatment of **20** with acetic acid in acetone/methanol gave mono-deprotected **21** in a satisfactory 78% yield, and provided the valuable handle for modifying the 6-position. Alcohol **21** was converted in 92% yield to azide **22** by a Mitsunobu reaction employing diphenylphosphoryl azide (DPPA), diisopropyl azodicarboxylate (DIAD) and triphenylphosphine.[25] Treating azide **22** with tetrabutylammonium fluoride (TBAF) successfully removed the five silyl protecting groups; however the residual tetrabutylammonium by-products could not be separated from the polar product by flash column chromatography. While the use of silica-supported TBAF ameliorated this problem, a better deprotection method involved stirring **22** with 10% trifluoroacetic acid (TFA) in dichloromethane overnight. Interestingly, while this method produced pentaol **9** exclusively, treatment of azide **22** with neat TFA for 10 minutes resulted in clean conversion to Boc-deprotected amine **10** (Scheme 2).

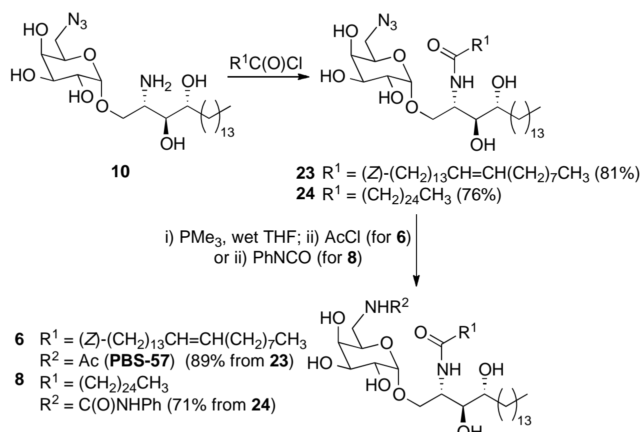
To illustrate the versatility of amine **10** we completed the synthesis of two biologically active 6-derivatized ⟨-galactosyl

ceramides **6** (PBS-57) and **8**, which differ in both the acyl chain of the ceramide base and the nitrogen-containing



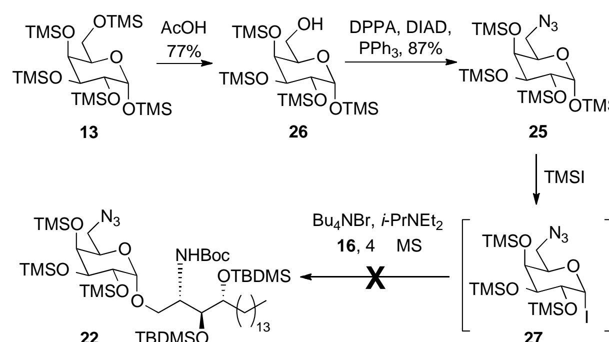
Scheme 2. Synthesis of α-galactosides **9** and **10**.

functionality at the 6-position of the sugar head group (Scheme 3). Treating a biphasic mixture of amine **10** in THF / 8 M aqueous NaOAc with nervonoyl chloride afforded 6-azido GalCer **23** in 81% yield. Subsequent Staudinger[24d,26] reaction followed by treatment with acetyl chloride afforded PBS-57 (**6**).[16b] Alternatively, acylation of **10** using hexacosanoyl chloride, to provide amide **24**, followed by Staudinger reduction[24d,26] and then treatment with phenylisocyanate, afforded Th1-biasing α-GalCer derivative **8**.[16a]



Scheme 3. Synthesis of α-galactosyl ceramides **6** and **8**.

An even more convergent route to our target molecules would employ 6-azido galactose **25** in the key glycosylation step (Scheme 4). Azide **25** was synthesized by deprotecting the primary silyl ether in per-TMS-protected galactose **13** to afford alcohol **26**, followed by Mitsunobu reaction with DPPA, DIAD and PPh_3. Following the same procedure as with per-TMS-protected galactose **13**, 6-azido galactose **25** was treated with TMSI and the resulting glycosyl iodide **27** added to a solution of acceptor **16**, Bu_4NI and Hünig's base in dichloromethane. Unfortunately, this reaction was unsuccessful, only providing various TMS-deprotection products of 6-azido galactose **25**. The failure of this sugar donor to undergo glycosylation can be understood by the electron-withdrawing nature of the azide group imparting a strongly deactivating effect.[27] Acceptors such as alcohol **16** already display attenuated nucleophilicity owing to the presence of a hydrogen bond between the alcohol oxygen atom and the carbamate hydrogen atom,[28] and while the reaction of **16** proceeds readily with 'armed' per-TMS donor **15**, the corresponding less-armed per-benzylated glycosyl iodide is unreactive to this class of acceptor.[24a,b] These observations serve to highlight how sensitive is the reactivity of these glycosyl iodide donors to small changes in the sugar substitution pattern.



Scheme 4. Attempted more convergent synthesis of α-galactoside **22**.

In summary, a short and convenient route to 6-*N*-derivatized galactosyl ceramides has been developed, which allows late-stage variation of both the *N*-acyl chain of the sphingosine unit and the substituent introduced at the 6-position of the sugar. Azides **23** and **24** are also primed for further functionalization via Click Chemistry. Future work will focus on using such derivatization strategies in the synthesis and biological evaluation of novel 6-*N*-derivatized glycolipids.

Experimental Section

General Procedure for Selective 6-Desilylation (Alcohols 21 and 26). AcOH (3.00 mmol) was added to a solution of galactoside **20** or **13** (1.57 mmol) in acetone (3.5 mL) and MeOH (4.7 mL) at 0 °C. The reaction mixture was allowed to warm to rt. After 8 h, the reaction was quenched by the addition of $NaHCO_3$ (5.95 mmol) and then filtered. After concentration of the filtrate under reduced pressure, the resulting oil was purified by flash column chromatography (EtOAc in hexane) to afford alcohol **21** (from **20**) or **26** (from **13**) as a colorless oil.

General Procedure for Mitsunobu Reaction (Azides 22 and 25). PPh_3 (1.86 mmol), DIAD (1.86 mmol) and DPPA (1.86 mmol) were added sequentially to a cooled solution of alcohol **21** or **26** (0.90 mmol) in THF (20 mL). The mixture was allowed to warm to rt. and then stirred overnight. Concentration under reduced pressure followed by purification of the residue by flash column chromatography (EtOAc in hexane) afforded azide **22** (from **21**) or **25** (from **26**) as a colorless oil.

Pentaol 9. TFA (0.50 mL, 6.6 mmol) was added dropwise over 5 min to a solution of azide **22** (200 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure to afford pentaol **9** as a colorless oil (115 mg, quant.): $[\alpha]^{20}_{D}$ +12.4 (*c* 0.5, $CDCl_3$:CD_3OD, 2:1); ν_{max}(film)/cm^{-1} 3282s br (O–H), 2114s (N_3), 1696m (C=O); δ_H(300 MHz, $CDCl_3$:CD_3OD, 2:1) 0.85 (t, *J* 6.0, 3H), 1.18-1.39 (stack, 22H), 1.40-1.71 (stack, 4H), 1.44 (s, 9H), 3.26 (A of ABX, $J_{A\text{-}B}$ 12.6, $J_{A\text{-}X}$ 4.9, 1H), 3.51-3.64 (stack, 3H), 3.65-3.98 (stack, 7H), 4.89 (d, *J* 3.3, 1H); δ_C(100 MHz, $CDCl_3$:CD_3OD, 2:1) 14.3 (CH_3), 23.2 (CH_2), 26.4 (CH_2), 28.6 (CH_3), [29.9, 30.2, 32.4, 32.8 (CH_2, resonance overlap)], 51.7 (CH), 51.8 (CH_2), 68.3 (CH_2), 69.3 (CH), 70.4 (CH), 70.6 (2 × CH, resonance overlap), 72.5 (CH), 75.3 (CH), 80.1 (C), 156.8 (C); MS (TOF ES+) *m/z* 627.3 ($[M + Na]^+$, 100%); HRMS (TOF ES+) calcd for $C_{29}H_{56}N_4O_9Na$ $[M + Na]^+$ 627.3945, found 627.3956.

Amine 10 from azide 22: TFA (1.0 mL, 13.2 mmol) was added dropwise over 5 min to azide **22** (400 mg, 0.38 mmol) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure. The resulting colorless oil was used in the next step without further purification (192 mg, quant.). **From azide 9:** TFA (0.50 mL, 6.6 mmol) was added dropwise over 5 min to azide **9** (114 mg, 0.19 mmol) at rt. After 30 min, the reaction mixture was concentrated under reduced pressure. The resulting colorless oil was used in the next step without further purification (96 mg, quant.).

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Supporting Information Available: Experimental procedures, characterization, ^1H NMR and ^{13}C NMR spectra for products **6**, **8-10**, **13**, **16**, **18** and **20-25**. This material is available free of charge via the internet at http://pubs.acs.org.

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