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Efficient Synthesis of α-Galactosyl Ceramide Analogues Using Glycosyl Iodide Donors

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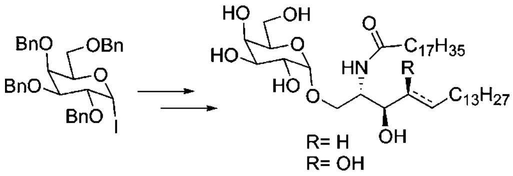
ABSTRACT



The combination of reactive galactosyl iodide donors with electron-rich acceptor lipids provides highly stereoselective and efficient routes to α GalCer analogues. Using per-*O*-silylated donors, key intermediates can be obtained in a three-step, one-pot sequence providing useful constructs for analogue development.

Since the discovery of α *O*-galactosyl ceramides from marine sponge in 1993,[1] several studies of KRN7000 (**1**) (Figure 1) and its derivatives have been reported. These investigations have revealed that tumor-associated cell-surface glycoproteins such as CD1d present exogenous lipids to natural killer T (NKT) cells causing the release of chemokines that regulate immune response to cancer.[2] The potent immunostimulatory activities of lipid antigens such as KRN7000 have led to the development of anticancer chemotherapeutics that are currently in clinical trials.[3] Moreover, α *O*-galactosyl ceramide analogues have provided important tools for elucidating signal transduction pathways involved in CD1d-mediated antigen presentation.[4]

Several syntheses of KRN7000 analogues have been reported. However, current glycosidation methods suffer from relatively low yields and sometimes poor α/β selectivities. Galactosyl fluoride and trichloroacetimidate donors have been most successfully employed in the formation of α *O*-glycosidic linkages. Yields for the fluoride typically range between 30 and 60% and are complicated by the formation of the β anomer, which is difficult to separate.[5] Stereoselectivity can be improved with the use of galactosyl trichloroacetimidates,[6] but the reaction requires portionwise addition of both the donor and acceptor, otherwise yields drop and approximately one-half of the unreacted acceptor is recovered.[7] Thioglycosides and glycosyl bromides[8] have also been utilized, and while relatively easy to employ, both yields and stereoselectivities are compromised with these donors.

Here, we report remarkably efficient syntheses of α *O*-galactosyl ceramides using galactosyl iodide donors. We recently demonstrated the utility of glycosyl iodides in the synthesis of α-glycosides.[9] Stereoselectivity is achieved from in situ anomerization of the α-iodide to a more reactive

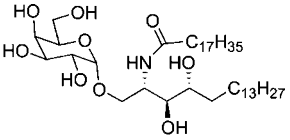


Figure 1. KRN7000 (**1**).

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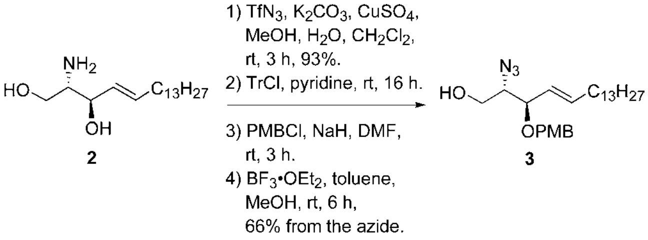
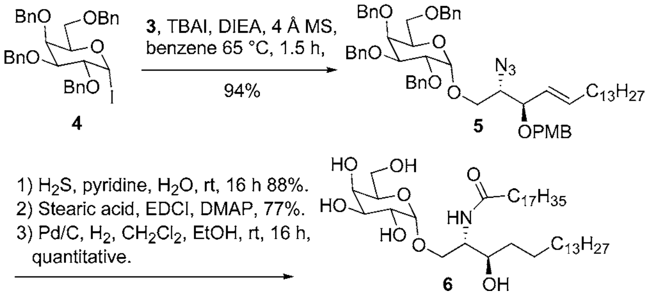
Scheme 1. Synthesis of Azido Sphingosine Acceptor



β-iodide, which subsequently undergoes S_N2-like displacement to give exclusively the α-glycoside. Lemieux first introduced the concept of in situ anomerization using glycosyl bromides.[10] However, we have found that reactions with glycosyl iodides are orders of magnitude faster, proceeding quantitatively, with exclusive delivery of the α anomer. These advantages prompted us to undertake the studies reported herein. When tetrabutylammonium iodide (TBAI) is used as a promoter, glycosidation of sphingosine and phytosphingosine proceeds in over 90% yield giving exclusively the α *O*-galactosyl linkage. The reactions are made even more efficient when per-*O*-silylated sugars are used as the iodide precursor alleviating the need for selective functionalization of the anomeric center.

Initial investigations began with commercially available sphingosine (**2**), which is also available by total synthesis.[11] The amino group was first converted to an azide (Scheme 1) in 93% yield according to the procedure reported by Wong and co-workers.[12] It is preferable to protect the amine as an azide for the glycosidation step, as amides diminish the nucleophilicity of the primary hydroxyl through hydrogen bond donation.[13] The primary alcohol was temporarily blocked with a trityl ether and the secondary alcohol was protected with an electron donating ether (*p*-methoxybenzyl (PMB)) to enhance the overall nucleophiliciy of the acceptor alcohol. Polt and co-workers have reported similar advantages when using electron-donating protecting groups in the glycosidation of serine derivatives.[13b] After deprotection of the trityl group with $BF_3{\cdot}OEt_2$, a sphingosine acceptor (**3**) was available for glycosidation.

Scheme 2. Synthesis of Desoxy-KRN7000



The iodide donor (**4**) was generated in situ from 2,3,4,6-tetra-*O*-benzylgalactosyl acetate according to a procedure previously reported by our laboratory.[9a] Using TBAI as a promoter, the reaction of **4** (3 equiv) with the sphingosine acceptor (**3**) proceeded smoothly at 65 °C in 1.5 h giving 94% yield of the α-glycoside (**5**) after column chromatography (Scheme 2). Hydrogenation of **5** to arrive at the deprotected amine was retarded, presumably due to amine deactivation of the catalyst.[14] Therefore, we decided to reduce the azide prior to hydrogenation. Staudinger reduction of the azide utilizing either triphenylphosphine or triethylphosphine did not proceed efficiently, instead we found that hydrogen sulfide in pyridine/water was preferable producing the amine in 88% yield. The synthesis of 4-desoxy KRN7000 (**6**) was completed by condensation of the amine with stearic acid followed by hydrogenation, which resulted in global deprotection and concomitant reduction of the double bond.

Encouraged by the successful synthesis of **6**, we proceeded with the synthesis of KRN7000 using the same strategy. Phytosphingosine[15] was converted into an activated acceptor (**7**) by amine to azide conversion and incorporation of PMB ethers on both of the secondary alcohols. Glycosidation with donor **4** was uneventful yielding the glycoconjugate (**8**) in 90% yield after column chromatography. Reduction of the azide, amidation, and global deprotection afforded KRN7000 in excellent overall yield (Scheme 3).

Because α galactosyl ceramides are lipid antigens, the 4,5-dehydro analogue of KRN7000 is an obvious choice for analogue synthesis. Initial attempts to achieve selective debenzylation of **5** using Lindlar catalyst resulted in relatively inefficient reactions that led to byproduct formation. In earlier work, we demonstrated that per-*O*-silylated sugars are excellent precursors to glycosyl iodides that readily undergo nucleophilic addition by cyanide.[16] These donors are particularly attractive due to their ease of synthesis and facile deprotection under methanolysis conditions. Thus reaction

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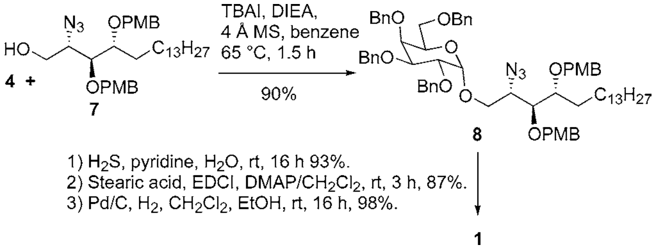
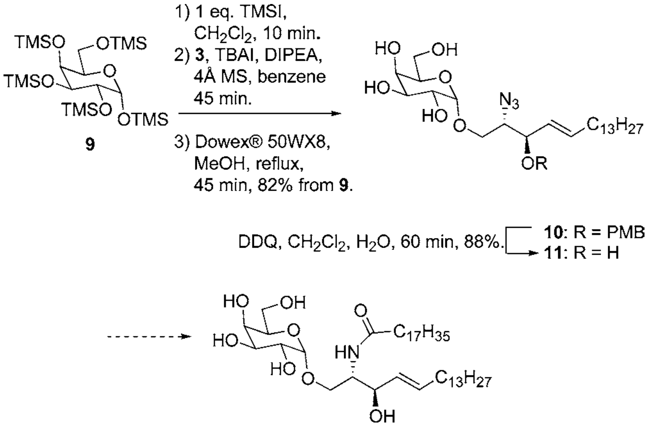
Scheme 3. Synthesis of KRN7000



of per-*O*-trimethylsilylgalactopyranose (**9**) with TMSI provided the galactosyl iodide in 10 min at 0 °C. After evaporation of the solvent, 0.33 equiv of **3**, 2 equiv of TBAI, and 1.5 equiv of DIEA in benzene were added to the iodide and stirred for 45 min, after which time TLC indicated the reaction was complete. The crude product was refluxed in methanol with Dowex H^+ resin for 45 min. The NMR spectrum of the resulting glycolipid (**10**) was remarkably clean, and only the α anomer was observed (Scheme 4). For our own purposes, the azide is a useful intermediate for analogue development so we terminated the process after removal of the PMB protecting group, but one could easily continue with reduction of the azide and subsequent condensation with a lipid of interest.

In summary, the combination of highly reactive galactosyl iodide donors with electron rich acceptor lipids provides stereoselective and efficient routes to α GalCer analogues. Key intermediates such as **10** can be obtained in a 3-step one-pot sequence providing useful intermediates for analogue development.

Scheme 4. Rapid Analogue Synthesis



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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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