

Synthesis of C-Glycoside Analogues of α -Galactosylceramide via Linear Allylic C–H Oxidation and Allyl Cyanate to Isocyanate Rearrangement

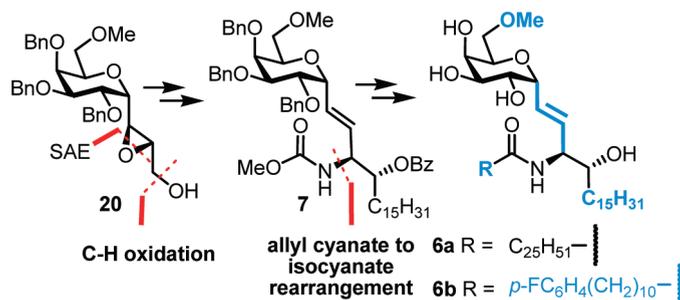
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ABSTRACT



C-Glycoside analogues of α -galactosylceramide were synthesized in which several significant modifications known to promote Th-1 cytokine production were included. The key transformations include C–H oxidation, Sharpless asymmetric epoxidation, olefin cross metathesis, and an allyl cyanate to isocyanate rearrangement.

α -Galactosylceramide (**1**, also known as α -GalCer or KRN7000), an optimized synthetic material originating from a marine sponge, is the most widely studied glycolipid antigen for activating invariant natural killer T (iNKT) cells (Figure 1).¹ These cells are a subset of T lymphocytes that interact with glycolipid antigens presented by the major histocompatibility complex class I-related glycoprotein CD1d.² Immunoregulatory cytokines, such as IFN- γ (Th-1 type) and IL-4 (Th-2 type), produced by stimulated iNKT cells hold substantial promise in immunotherapy and for development of vaccine adjuvants.³ However, phase I clinical trials of **1** in the treatment of solid

tumors have been ineffective, perhaps as a consequence of counteraction of the Th-1 and Th-2 cytokines induced by **1**.⁴

A variety of glycolipid antigens that can differentially elicit distinct effector functions in iNKT cells have been identified. For example, installing an OMe group at the 6'-position of the galactosyl moiety gave rise to the strong Th-1 biasing ligand RCAI-61 (**2**).⁵ Introduction of a *p*-fluorophenyl group at the terminus of the fatty amide chain led to 7DW8-5 (**3**), which induced selective production of IFN- γ .⁶

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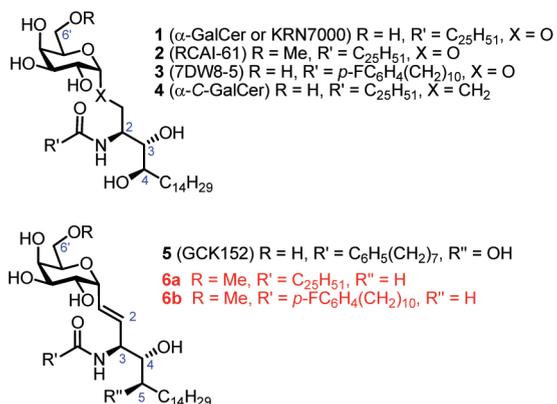
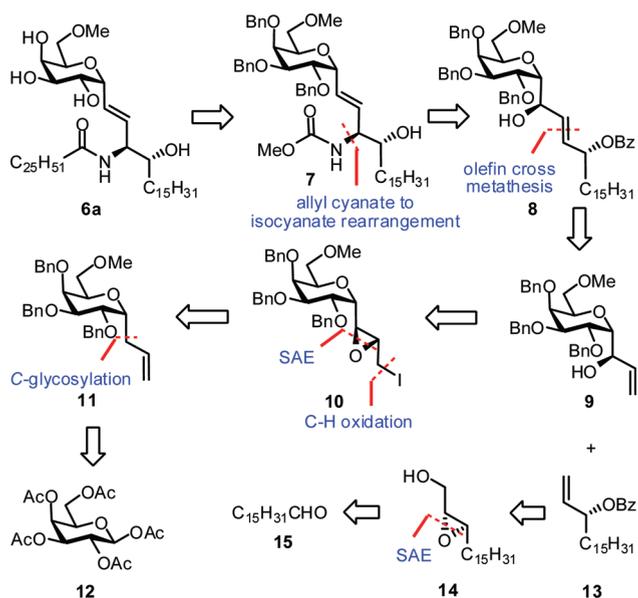


Figure 1. Structures of glycolipids 1–6.

Scheme 1. Retrosynthetic Plan For 6a



α -C-GalCer (**4**), a *C*-glycoside analogue of KRN7000,⁷ binds more stably than **1** to dendritic cells and acts as a more effective link between innate and adaptive immunity in vivo.⁸ In fact, comparisons of **1** and **4** in mouse models of disease revealed that the *C*-glycoside **4** displayed higher activity.^{7b} Interestingly, **4** was found to be a weak agonist of human iNKT cells in vitro, but *C*-glycoside analogues that feature an *E*-alkene as a spacer between the galactose moiety and the ceramide, such as GCK152 (**5**), activate

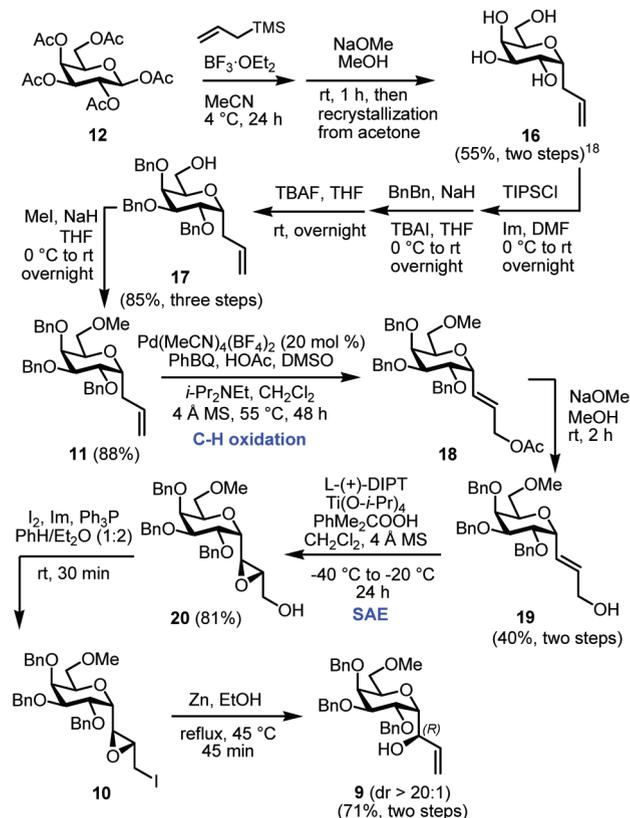
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human iNKT cells and induce the maturation and activation of human dendritic cells through iNKT-cell activation.⁹

Scheme 2. Synthesis of 9



As part of our ongoing investigations of *C*-glycoside analogues of KRN7000,¹⁰ we have designed analogues **6a,b**, which combine several significant structural modifications for selective Th-1 cytokine production. In addition to the installation of the 6'-OMe and *p*-fluorophenyl substitutions stated above, the report that 4-deoxy-KRN7000 initiates cytokine production similarly to that induced by **1** in human iNKT cells in vitro, and in its murine counterpart in vivo,¹¹ prompted the synthesis of the corresponding 4-deoxyphytosphingosine moiety in *C*-glycoside **6**. Our retrosynthetic plan is illustrated in Scheme 1. It was reported that cross-metathesis¹² (CM) of vinyl-*C*-galactosides requires a high loading of catalysts and that the yields of the coupling product are low and sensitive to the

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The synthesis of lipid olefin **21** was accomplished in a similar way, using SAE to introduce the chirality (Scheme 3). Unlike the strategy involving zinc-mediated reductive elimination of an epoxide halide, the transformation of 2,3-epoxy alcohol **14** to allylic alcohol **21** was achieved in one step by using the titanocene-induced regioselective deoxygenation protocol developed by Yadav and co-workers.²² CM with 15 mol % Grubbs second generation catalyst (**G-2**) using 3.2 molar equiv of lipid olefin **13** with **9** provided **8** in 70% yield with high *E*-selectivity (*E/Z* ratio > 20:1). This reaction required reflux for only 2 h, and 48% of **13** was recovered, along with a small amount of the easily separable homodimers of **13** (11%, based on **13**).

Treatment of **8** with trichloroacetyl isocyanate¹⁵ afforded intermediate **22**, and hydrolysis with potassium carbonate in aqueous methanol gave carbamate **24**, along with 5% of **23**. Dehydration of **24** with trifluoroacetic anhydride (TFAA) and triethylamine at 0 °C gave allyl cyanate **25**, which immediately underwent the allyl cyanate to isocyanate rearrangement¹⁵ to afford allyl isocyanate **26**. It is noteworthy that this rearrangement can occur below room temperature and, thus, is milder than the related Overman rearrangement.²³ Isocyanate **26** was further reacted with methanol in the presence of a catalytic amount of tributyltin methoxide²⁴ in situ, providing carbamate **7**. The *E* configuration of the alkene was confirmed by the coupling constants of the vinylic protons (16.0 Hz). Basic hydrolysis of **7** afforded cyclic carbamate **27**, which was further treated with 30% aqueous KOH solution at reflux in ethanol to afford amine **28**. The absolute configuration at the C-3 position was confirmed by the advanced Mosher method,²¹ which revealed the *S* configuration at C-3 in **28**. The fatty amide chain was then introduced into amine **28** by using cerotyl chloride²⁵ to afford amide **29** in 97% yield over two steps. Finally, global debenzoylation using Birch reduction furnished the final analogue **6a** in 91% yield.

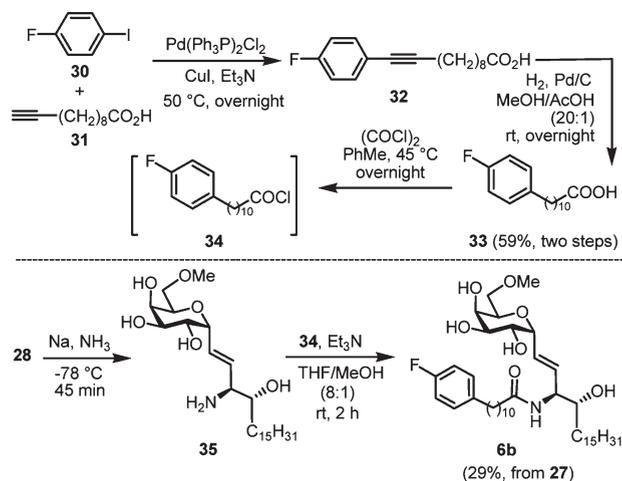
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Scheme 4. Synthesis of **6b**



As shown in Scheme 4, the corresponding carboxylic acid **33** of the amide moiety in **3**⁶ was prepared in 59% yield (two steps) from commercially available iodide **30** and alkyne **31** via Sonogashira coupling followed by catalytic hydrogenation of alkyne **32**. For in situ N-acylation, **33** was converted to acyl chloride **34**. In order to avoid defluorination during Birch reduction, debenzoylation must be carried out prior to N-acylation. As a result, target **6b** was obtained in 29% yield over three steps from **27**.

In conclusion, we have developed a highly stereocontrolled total synthesis of *C*-glycoside analogues of KRN-7000 containing an *E*-alkene linker in 20 steps starting from penta-*O*-acetyl- β -D-galactose (**12**) in 2.5% (**6a**) and 0.8% (**6b**) overall yield. The synthesis showcases the utility of a linear allylic C–H oxidation in synthetic carbohydrate chemistry and an allyl cyanate to isocyanate rearrangement for stereoselective construction of the stereogenic center in the presence of a sugar moiety. These novel α -GalCer analogues are currently undergoing biological evaluation.

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Supporting Information Available. Experimental procedures as well as ¹H and ¹³C NMR spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.