Published in final edited form as:

Org Lett. 2012 January 20; 14(2): 620–623. doi:10.1021/ol2032448.

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.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 a 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

α -C-GalCer (**4**), a C-glycoside analogue of KRN7000,⁷ binds more stably 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**

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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>.

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 human iNKT cells and induce the maturation and activation of human dendritic cells through iNKT-cell activation.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 high loading of catalysts, and that the yields of coupling product are low and sensitive to the protecting groups on the vinyl-*C*-galactoside.^{13,14} Therefore, a CM disconnection of **8** was deemed more practical than for **7**, leading to the simple synthons **9** and **13**. The required amide at C-3 could potentially be delivered with stereocontrol from C-1 via an allyl cyanate to isocyanate rearrangement¹⁵ (**8** to **7**). The concerted nature of the sigmatropic rearrangement would secure a high level of [1,3]-chirality transfer during the C-O to C-N bond reorganization. The stereocontrolled construction of the hydroxyl group at the allylic position in **9** could be fulfilled via zinc-mediated reductive elimination of the asymmetric epoxide halide **10**, which might be accessible from terminal alkene **11** via linear C-H oxidation¹⁶ followed by Sharpless asymmetric epoxidation (SAE).¹⁷ α -Allyl galactoside **11** could possibly be prepared from β -galactoside **12** through straightforward transformations according to known protocols.¹⁸ Similarly, allylic ester **13**, the lipid olefin of CM, could be obtained through a sequence of SAE and reductive elimination from known epoxy alcohol **14**,^{19,20} which is accessible from palmitaldehyde **15**.

As shown in Scheme 2, our synthesis commenced with the preparation of α -allyl-*C*-galactoside **11** from commercially available penta-*O*-acetyl- β -D-galactose (**12**) and involved straightforward protecting group manipulations using **16** and **17**.¹⁸ Linear C-H oxidation of **11** under conditions developed by Chen and White^{16a} [Pd(OAc)₂, benzoquinone (BQ), DMSO/AcOH (1:1), 50 °C, 100 h] afforded **18** in low yield (17%) and incomplete conversion (71%). However, treatment of **11** under conditions^{16b} revised by White et al., followed by acetate deprotection under basic conditions, provided allylic alcohol **19** in 40% yield (two steps) with high *E*-selectivity (*E*/*Z* ratio > 20:1). SAE of **19** smoothly afforded epoxy alcohol **20** in 81% yield. Iodination of **20** afforded **10**, which upon zinc-mediated reductive elimination gave rise to **9** (71% yield, two steps). The *R* configuration of C-1 in **9** was established by the advanced Mosher method (see Supporting Information),²¹ and the *dr* value was determined on the basis of the ¹H NMR spectra of the corresponding Mosher esters.

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 coworkers.²² CM with 15 mol % Grubbs 2nd 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.

As shown in Scheme 4, the corresponding carboxylic acid **33** of 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 KRN7000 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health Grant HL-083187.

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Figure 1.
Structures of glycolipids **1-6**

Scheme 1.
Retrosynthetic Plan For 6a

Scheme 2.
Synthesis of **9**

Scheme 3.
Synthesis of 6a

Scheme 4.
Synthesis of **6b**