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

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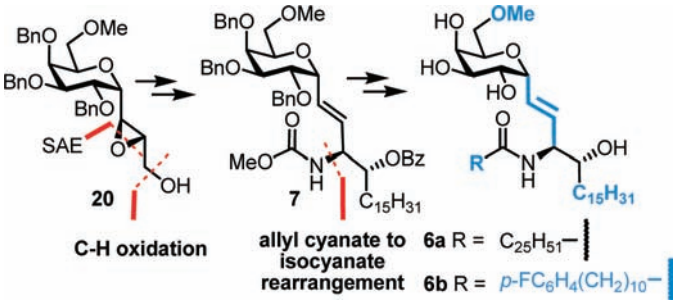
ABSTRACT



***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]

(1) For recent reviews of KRN7000 (compound **1**) and its analogues, see: (a) Banchet-Cadeddu, A.; Hénon, E.; Dauchez, M.; Renault, J.-H.; Monneaux, F.; Haudrechy, A. *Org. Biomol. Chem.* **2011**, *9*, 3080. (b) Mori, K.; Tashiro, T. *Heterocycles* **2011**, *83*, 951. (c) Murphy, N.; Zhu, X.; Schmidt, R. R. *Carbohydr. Chem.* **2010**, *36*, 64.

(2) Godfrey, D. I.; MacDonald, H. R.; Kronenberg, M.; Smyth, M. J.; Van Kaer, L. *Nat. Rev. Immunol.* **2004**, *4*, 231.

(3) (a) Kronenberg, M. *Annu. Rev. Immunol.* **2005**, *23*, 877. (b) Van Kaer, L.; Parekh, V. V.; Wu, L. *Immunotherapy* **2011**, *3*, 59.

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(5) Tashiro, T.; Nakagawa, R.; Inoue, S.; Shiozaki, M.; Watarai, H.; Taniguchi, M.; Mori, K. *Tetrahedron Lett.* **2008**, *49*, 6827.

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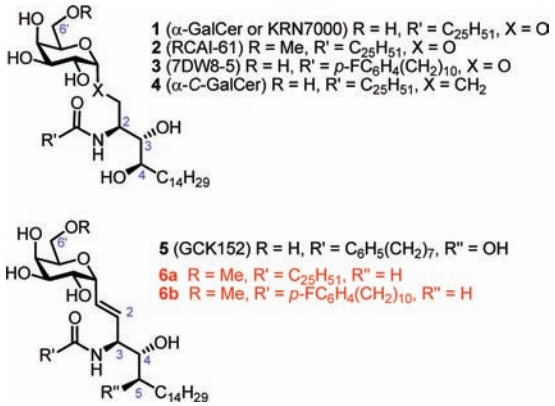
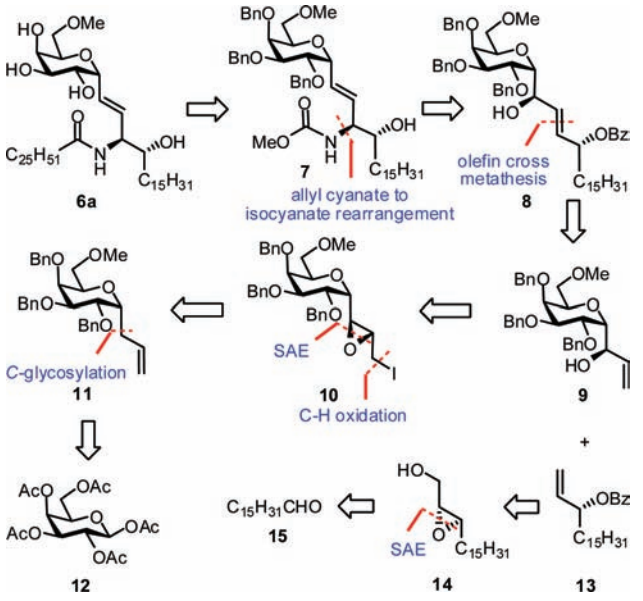


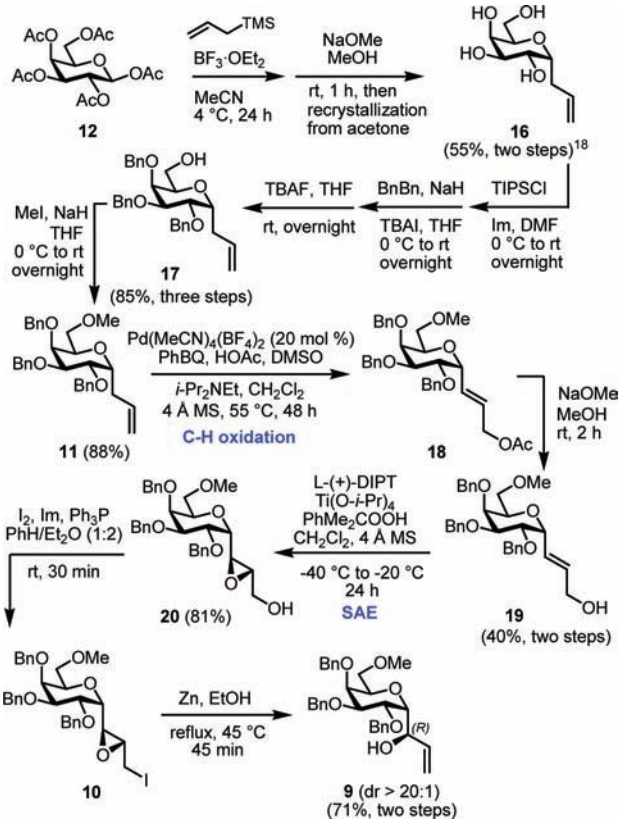
Figure 1. Structures of glycolipids **1**–**6**.

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 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-metathesis[12] (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

(7) For a review of α-*C*-GalCer (compound **4**), see: (a) Franck, R. W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692. (b) Franck, R. W. *C. R. Chimie* **2011**, in press, doi:10.1016/j.crci.2011.05.006.

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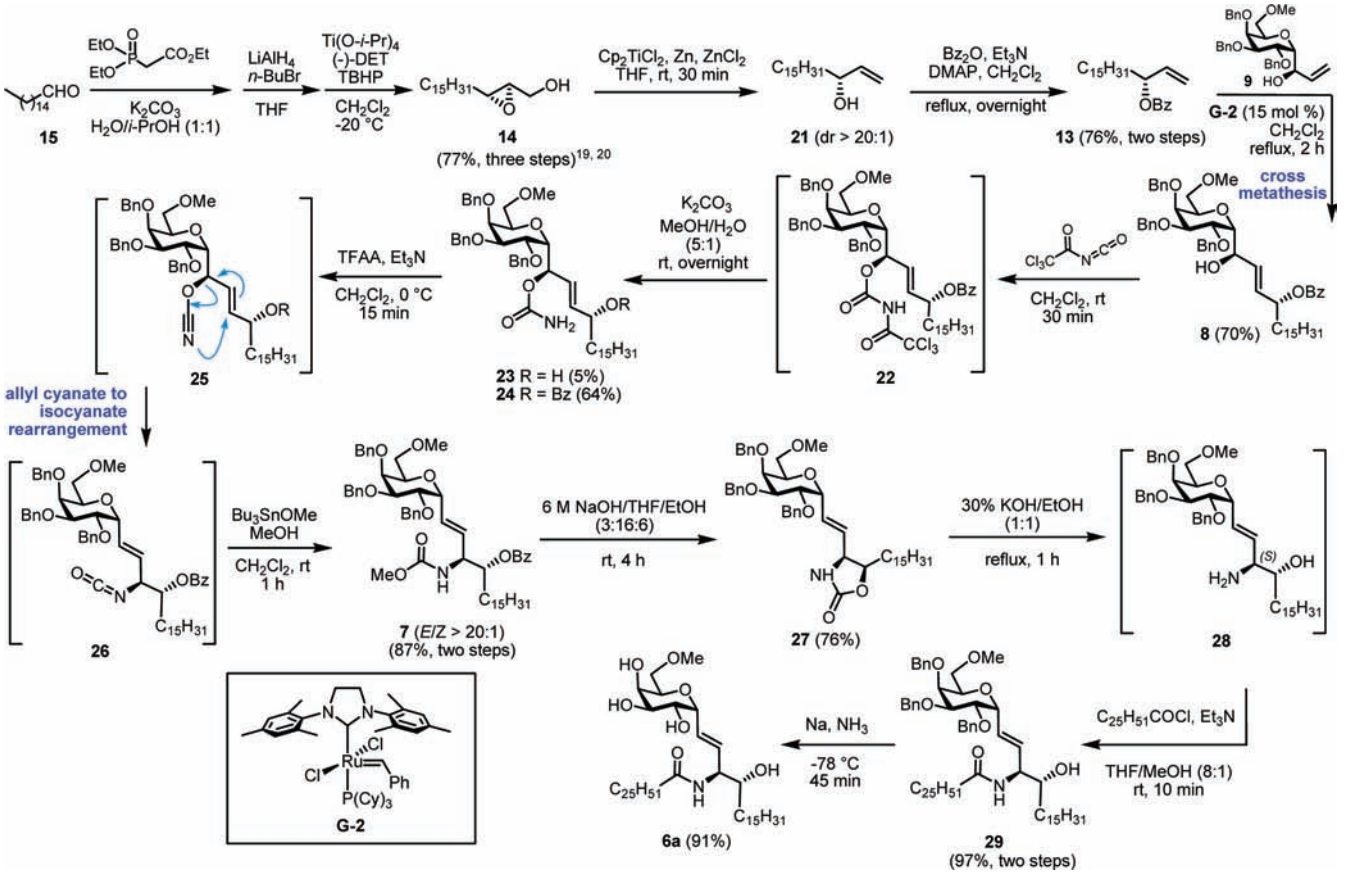
Scheme 3. Synthesis of **6a**



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 rearrangement[15] (**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 oxidation[16] 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 for 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 ^{1}H NMR spectra of the corresponding Mosher esters.

(15) For a review of the allyl cyanate to isocyanate rearrangement, see: Ichikawa, Y. *Synlett* **2007**, 2927.

(16) (a) Chen, M. S.; White, M. C. *J. Am. Chem. Soc.* **2004**, *126*, 1346. (b) Covell, D. J.; Vermeulen, N. A.; Labenz, N. A.; White, M. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 8217.

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(21) (a) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939. (b) For a review of the advanced Mosher method, see: Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17. The ^{1}H NMR spectra of all MTPA-esters/amides are included in the Supporting Information.

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 isocyanate[15] 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 rearrangement[15] 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 methoxide[24] 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 chloride[25] to afford amide **29** in 97% yield over two steps. Finally, global debenzylation using Birch reduction furnished the final analogue **6a** in 91% yield.

(22) (a) Yadav, J. S.; Shekharam, T.; Gadgil, V. R. *J. Chem. Soc., Chem. Commun.* **1990**, 843. (b) Fu, R.; Ye, J.-L.; Dai, X.-J.; Ruan, Y.-P.; Huang, P.-Q. *J. Org. Chem.* **2010**, *75*, 4230. (c) RajanBabu, T. V.; Nugent, W. A. *J. Am. Chem. Soc.* **1994**, *116*, 986. (d) Cuerva, J. M.; Justicia, J.; Oller-López, J. L.; Oltra, J. E. *Top. Curr. Chem.* **2006**, *264*, 63.

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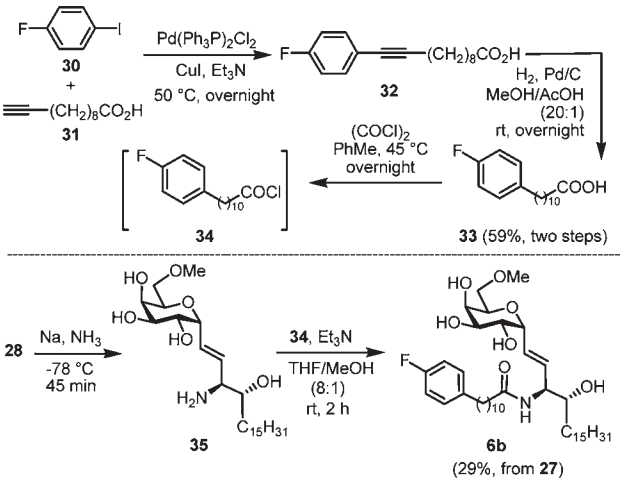
Scheme 4. Synthesis of **6b**



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, debenzylation 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 ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.