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

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C-Glycoside analogues of  $\alpha$ -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.

 $\alpha$ -Galactosylceramide (1, also known as  $\alpha$ -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).<sup>1</sup> These cells are a subset of T lymphocytes that interact with glycolipid antigens presented by the major histocompatibility complex class I-related glycoprotein CD1d.<sup>2</sup> Immunoregulatory cytokines, such as IFN- $\gamma$  (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.<sup>3</sup> 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).<sup>5</sup> 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- $\gamma$ .<sup>6</sup>

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Figure 1. Structures of glycolipids 1–6.





 $\alpha$ -*C*-GalCer (4), a *C*-glycoside analogue of KRN7000,<sup>7</sup> binds more stably than 1 to dendritic cells and acts as a more effective link between innate and adaptive immunity in vivo.<sup>8</sup> In fact, comparisons of 1 and 4 in mouse models of disease revealed that the *C*-glycoside 4 displayed higher activity.<sup>7b</sup> 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.<sup>9</sup>



As part of our ongoing investigations of *C*-glycoside analogues of KRN7000,<sup>10</sup> 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,<sup>11</sup> 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<sup>12</sup> (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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Scheme 3. Synthesis of 6a



protecting groups on the vinvl-C-galactoside.<sup>13,14</sup> 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<sup>15</sup> (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<sup>16</sup> followed by Sharpless asymmetric epoxidation (SAE).<sup>17</sup>  $\alpha$ -Allyl galactoside 11 could possibly be prepared from  $\beta$ -galactoside 12 through straightforward transformations according to known protocols.<sup>18</sup> 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, <sup>19,20</sup> which is accessible from palmitaldehyde (15).

As shown in Scheme 2, our synthesis commenced with the preparation of  $\alpha$ -allyl-*C*-galactoside 11 from commercially available penta-O-acetyl- $\beta$ -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<sup>16a</sup> [Pd(OAc)<sub>2</sub>, benzoquinone (BO), DMSO/AcOH (1:1), 50 °C, 100 h] afforded 18 in low yield (17%) and incomplete conversion (71%). However, treatment of 11 under conditions<sup>16b</sup> 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),<sup>21</sup> and the dr value was determined on the basis of the <sup>1</sup>H NMR spectra of the corresponding Mosher esters.

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<sup>(21) (</sup>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 <sup>1</sup>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 titanoceneinduced regioselective deoxygenation protocol developed by Yadav and co-workers.<sup>22</sup> 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<sup>15</sup> 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<sup>15</sup> 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.<sup>23</sup> Isocyanate 26 was further reacted with methanol in the presence of a catalytic amount of tributyltin methoxide<sup>24</sup> 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,<sup>21</sup> which revealed the S configuration at C-3 in 28. The fatty amide chain was then introduced into amine 28 by using cerotyl chloride<sup>25</sup> to afford amide 29 in 97% yield over two steps. Finally, global debenzylation using Birch reduction furnished the final analogue 6a in 91% yield.





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- $\beta$ -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  $\alpha$ -GalCer analogues are currently undergoing biological evaluation.

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

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