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Synthesis of α-C-Galactosylceramide via Diastereoselective Aziridination: The New Immunostimulant 4′-*epi*-C-Glycoside of KRN7000

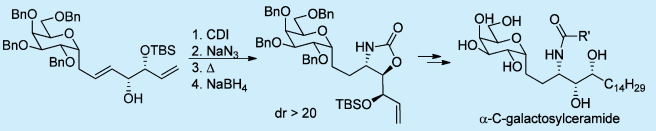
Ya-Jen Chang,*[,‡] Yi-Chen Hsuan,[†] Alan Chuan-Ying Lai,[‡] Yun-Chiann Han,[‡] and Duen-Ren Hou*[,†]

[†]Department of Chemistry, National Central University, No. 300 Jhong-Da Road, Jhong-li, Taoyuan, Taiwan 32001

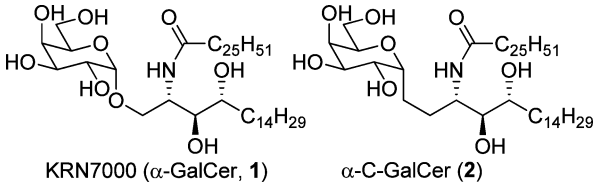
[‡]Institute of Biomedical Sciences, Academia Sinica, No. 128 Academia Road, Section 2, Nankang, Taipei, Taiwan 11529

Supporting Information

ABSTRACT: A new immunostimulant, the 4′-epimer of α-C-GalCer, was synthesized from a C_2-symmetric dienediol and α-C-allyl galactoside. The intramolecular aziridination and the following reductive ring opening provided the core of the aliphatic amino alcohol with excellent regio- and stereocontrol. The new immunostimulants **3d** and **3e** gave a better polarized Th1-type cytokine response in murine NKT cells than the benchmarked α-C-GalCer.



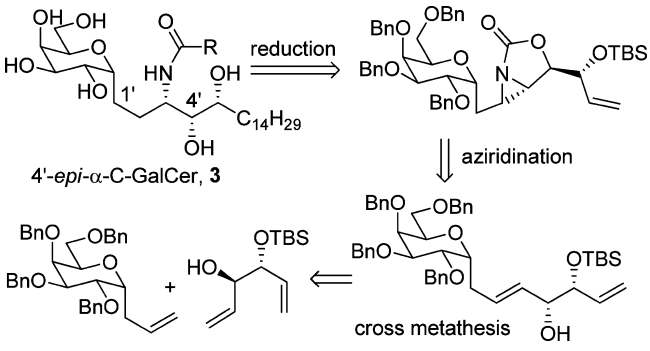
In the 1990s, agelasphins, a family of glycosphingolipids isolated from the marine sponge *Agelas mauritianus*,[1] were found to prolong the life span of mice inoculated with B16 melanoma cells.[2] These active compounds were characterized as α-galactosylceramides, which were found to be effective immunostimulants for their antitumor activity.[3] KRN7000 (α-GalCer, **1**), a synthetic analogue of the active agelasphins, was later developed by the Kirin Brewery Company to replace the scarce natural agelasphins as the prototype of a glycolipid antigen of invariant natural killer T (iNKT) cells.[3,4] The binary complex of α-GalCer and CD1d, a histocompatibility complex class I-like protein, is recognized by the T-cell antigen receptor (TCR) of iNKT, and the activated iNKT cells release cytokines.[5] The cytokines can be further classified as proinflammatory T-helper 1 (Th1) type and immunomodulatory T-helper 2 (Th2) type, such as interferon-γ (IFN-γ) and interleukin-4 (IL-4), respectively.[6] In 2003, Franck and co-workers reported that α-C-GalCer (**2**), a C-glycoside analogue of KRN7000, is more potent than **1** in mouse malaria and melanoma assays, both disease models requiring a protective Th1-type immune response.[7] These results suggest that it is possible that ligands for CD1d may lead to a selective immune response toward a Th1 or Th2 profile. Therefore, the synthesis of α-C-GalCer analogues is an attractive approach for new immunostimulants.[8−14]



A general and convenient method to access α-C-GalCer is to utilize cross-metathesis between C-alkenylgalactose and optically active allyl alcohols.[9a,11,13] However, further chemical transformations on the internal alkene to install the aminodiol stereotriad of C-glycosphingolipids were often complicated by issues of regio- and diastereoselectivity.[9,11−13]

Here we report that both issues can be resolved by the intramolecular aziridination and subsequent reductive ring opening to give C-glycosphingolipids (Scheme 1).[15] The resulting 4′-*epi*-C-glycosides of KRN7000, **3d** and **3e**, show comparable potencies and better polarized Th1-type cytokine responses than **2**.

Scheme 1. Intramolecular Aziridination To Give C-Galactosyl Ceramide



Our synthesis started with cross-metathesis between benzyl-protected α-C-allyl galactoside **4**[16] and optically active hexa-1,5-diene **5**[17] using Lipshutz's protocol[18] to give compound **6** in 75% yield (Scheme 2). The hydroxyl group was then converted to the azidoformate **7** via a two-step sequence using 1,1′-carbonyldiimidazole (CDI) and then sodium azide. Intramolecular acylnitrene addition to the olefin was induced by

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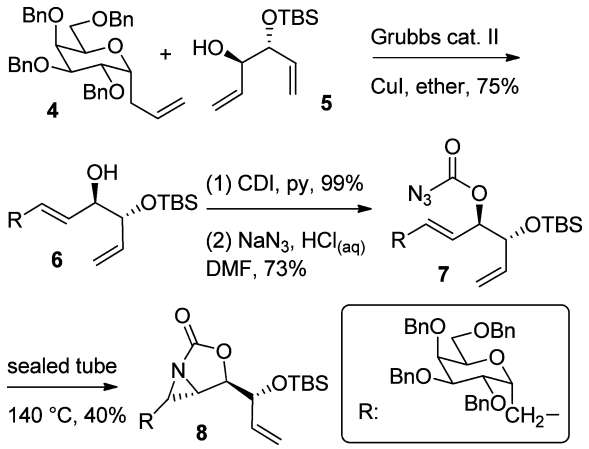
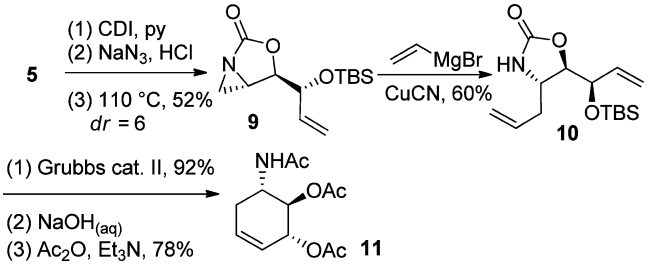
Scheme 2. Synthesis of Aziridine 9



conventional heating, microwave-assisted heating, or ultraviolet light to give aziridine **8** (see the Supporting Information).[15,19] To our delight, all of the reaction conditions screened provided diastereomerically pure *trans*-oxazolidinones **8** according to the ^{1}H NMR spectra of the crude products. Here the stereochemical assignment followed Bergmeier's studies using the azidoformates derived from 1-alkyl-substituted prop-2-en-1-ol[15b,c] and was confirmed by the parallel synthesis of the known diacetate **11**[20] from **5** (Scheme 3). The improved diastereoselectivity of the aziridination may be a consequence of the synergistic influence of the bulky C-galactosyl group and the internal (*E*)-alkene.[15b]

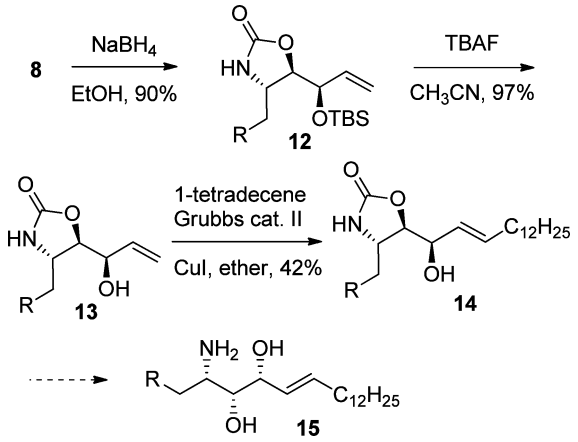
Scheme 3. Confirmation of the Stereochemistry of Aziridination



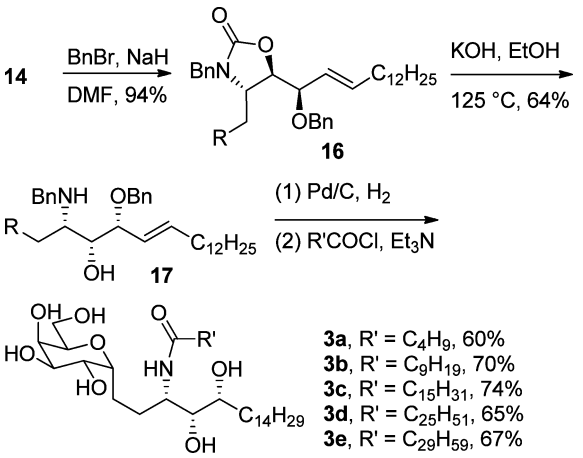
Reductive ring opening of the strained bicyclic compound **8** gave the stable oxazolidinone **12** as the sole product (Scheme 4). The TBS protecting group was then removed to facilitate the following cross-metathesis with 1-tetradecene.[21] However, the intended hydrolysis of oxazolidinone **14** to generate **15** always led to decomposed mixtures under the reported basic or acidic reaction conditions.[22,23] We suspect that the side reactions of allylic alcohol **14** were fostered by the remaining trace amounts of Grubbs catalyst, which is a common contaminant in the products of metathesis reactions.[24]

This problem was resolved by replacing the labile −OH and −NH protons of **14** with benzyl groups (compound **16**; Scheme 5). The hydrolysis of the oxazolidinone moiety then succeeded at elevated temperatures to give amino alcohol **17**. Palladium-catalyzed hydrogenation, hydrogenolysis, and subsequent N-acylation provided the desired 4′-*epi*-α-C-GalCer products **3a**−**e**, whose difference is in the chain length of the *N*-acyl lipid.

Scheme 4. Synthesis of C-Glycosphingolipid



Scheme 5. Synthesis of 4′-*epi*-C-Glycosides



The in vitro assays to determine the stimulatory efficacies of the new α-C-GalCer analogues **3a**−**e** were performed with NKT cells sorted from Vα14tg BALB/c mice. Compounds **3a**−**e** were screened at a concentration of 1 μM using α-GalCer (**1**) (0.1 μM) and α-C-GalCer (**2**) (10 μM) as the controls (Figure 1). We observed a significant reduction of the IL-4 induced by **2** and **3a**−**e** in comparison with that induced by **1** (Figure 1A). A reduced function of these C-glycosides in inducing IFN-γ production was also observed, and the differences between them were minor (Figure 1B). However, the graph of the ratios derived from the induced IFN-γ/IL-4 shows that all of the C-glycosides have a significantly higher ratio than α-GalCer. More importantly, both **3d** and **3e** show a better Th1-biased response than **2** at a lower concentration (Figure 1C; also see the Supporting Information for a comparison of the immunostimulants at 0.1 μM). However, the efficacies of compounds **3d** and **3e** require further studies using an in vivo mouse model and/or an in vitro human NKT assay.

Compound **3d** has the same *N*-acyl group as in **2**. Compound **3e**, with the extended acyl chain, gave a slightly higher IFN-γ/IL-4 ratio than **3d**. On the other hand, less polarized Th1 responses were observed for the compounds with a truncated acyl lipid chain (**3a**−**c**), which is in agreement with the previous structure−activity relationship (SAR) study of KRN7000.[8,25]

Comparing the potencies of the two stereoisomers **3d** and **2** is interesting. The corresponding (2′*S*,3′*R*,4′*R*) stereoisomer of

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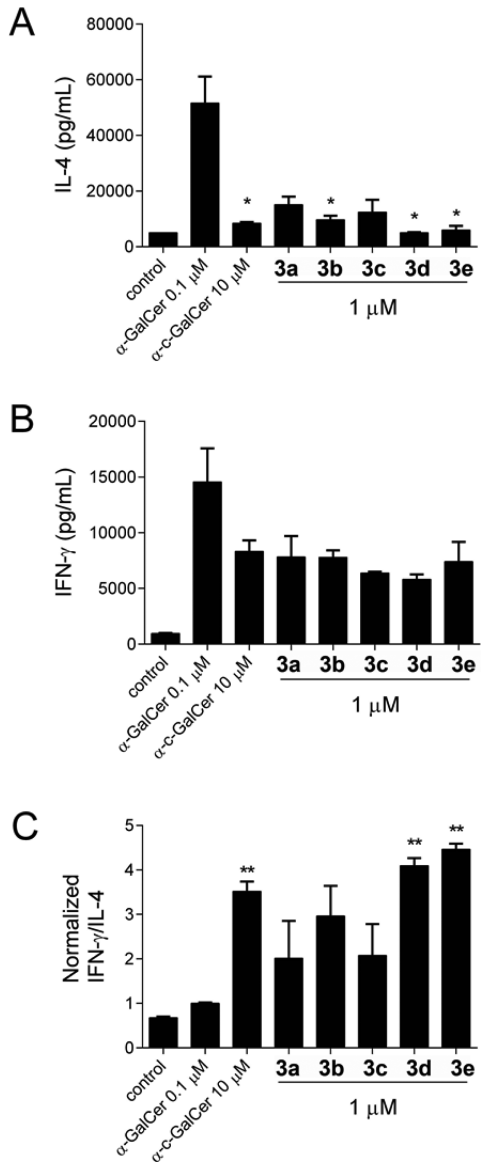

Figure 1. Potencies of α-C-GalCer analogues **3a**−**e** in stimulating *iNKT*. NKT cells sorted from Vα14tg BALB/c mice were cultured with immunostimulants for 72 h, and the supernatants were collected and analyzed with ELISA for the cytokines IL-4 and IFN-γ. (A, B) Levels of IL-4 and IFNγ, respectively. (C) IFN-γ/IL-4 ratios for the ligands normalized to the IFN-γ/IL-4 ratio for α-GalCer (**1**). Student's *t* analysis was performed by comparing all of the data to those for α-GalCer (**, $P < 0.01$; *, $P < 0.05$).

KRN7000 is known to give reduced cytokine secretion and weaker Th1 response in comparison with KRN7000.[8c,26] However, this trend does not apply to the α-C-GalCer analogues, as a slightly more Th1-polarized response and comparable secretion of cytokines induced by **3d** and **3e** relative to **2** were observed. Previous studies indicated that the lower affinity of **2** for the TCR compared with that of **1** is partially due to the loss of the hydrogen-bonding interaction between Arg95α of the TCR and the 3-OH of the phytosphingosine chain.[27] We speculate that the 4′-epimer of α-C-GalCer is beneficial to have this hydrogen-bonding interaction, leading to better potency.

In summary, a new route to prepare α-C-GalCer analogues has been developed and features intramolecular aziridination and reductive ring opening to introduce the 3′-amino group with excellent stereo- and regiocontrol. Biological assays showed that the new 4′-*epi*-α-C-GalCer analogues **3d** and **3e** can generate a Th1-biased cytokine response at a lower concentration than that of the benchmarked α-C-GalCer (**2**).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00090.

Experimental procedures and spectral data of compounds described herein (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: drhou@ncu.edu.tw.

*E-mail: yajchang@ibms.sinica.edu.tw.

Notes

The authors declare no competing financial interest.

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