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Concise syntheses of immunostimulating glycolipids, α-galactosyl ceramides

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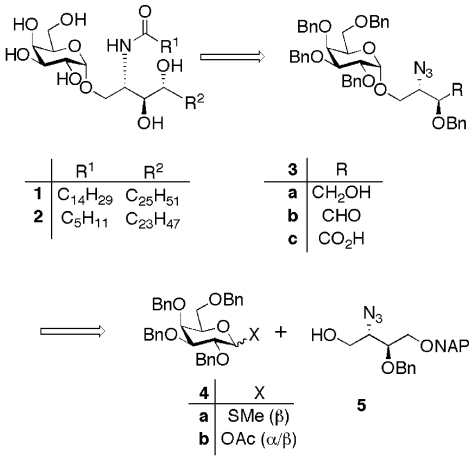
Abstract—α-Galactosylceramides (α-GalCers) are well known as immunostimulating agents having therapeutic potential. To facilitate the synthesis of α-GalCers and their derivatives, a novel and convergent strategy was designed, which is expected to be versatile for the preparation of a variety of analogues in a diversity-oriented fashion. As an initial demonstration of our strategy, KRN7000 and OCH were synthesized in eight steps from a common intermediate, which is easily obtainable in a multi-gram scale.

1. Introduction

α-Galactosyl ceramides (α-GalCers) are a class of immunostimulating agents that activate invariant natural-killer T (iNKT) cells.[1] Most representative among them are KRN7000 (**1**)[2] and OCH (**2**),[3] which are considered to be benchmark derivatives for the structure–activity relationship studies of α-GalCers (Scheme 1). The mode of their action comprises three consecutive steps. Firstly, α-GalCer bind as a ligand to the CD1d protein on antigen-presenting cells, and the resulting complex is recognized by T-cell receptor on iNKT cells. Subsequently, the ternary complex formation induces iNKT cells to produce various cytokines, such as interferon (IFN)-γ and interleukins (ILs). Production of these cytokines induces either Th1-type (e.g., IFN-γ and IL-2) or Th2-type (e.g., IL-4 and IL-13) immune response. Interestingly, subtle alternation of α-GalCer structures results in drastically different patterns of cytokine production. For instance, OCH preferentially induces Th2-type cytokines, while C-glycoside analogue of KRN7000 is a potent inducer of the Th1-type response.[4]



Scheme 1. Synthetic strategy of α-GalCers.

Due to the diversity of cytokines they can produce, iNKT cells are considered to be multi-functional cells that play important roles for the regulation of immune systems. As potent ligands of iNKT cells, α-GalCers are deemed to be promising drug candidates for various diseases, such as cancer and autoimmune diseases.[1,5] In fact, numerous efforts have been made toward synthesis and structure–activity relationship studies of α-GalCers and their analogues.[6]

In order to facilitate the synthesis of α-GalCer analogues, we set out to investigate the approach depicted in Scheme 1. It consists of (1) the synthesis of azidothreitol **5** as a 'universal' aglycon, (2) formation of common intermediate **3a**, and (3) construction of sphingosine base and attachment of fatty acid. It is our perspective

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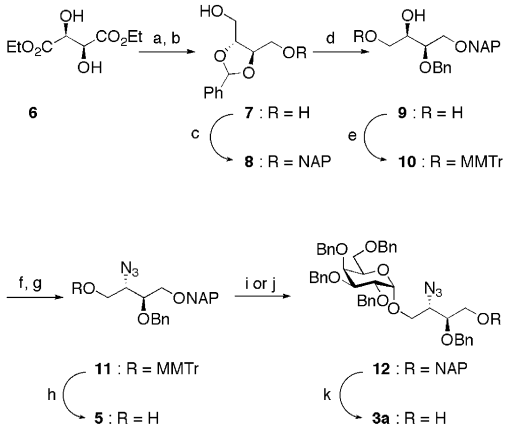
that various analogues can be synthesized from aldehyde (**3b**) or carboxylate (**3c**) readily obtainable from **3a**. We report herein the execution of this strategy to the synthesis of prototypical iNKT cell agonists α-GalCers KRN7000 (**1**) and OCH (**2**).

2. Synthesis of the common intermediate 3a

As an azidothreitol component, strategically protected triol **5** was designed and prepared as shown in Scheme 2. Namely, commercially available diethyl D-tartrate (**6**) was first converted to 2,3-*O*-benzylidene-D-threitol (**7**) according to the standard protocol.[7] Desymmetrization of diol **7** was achieved by controlled protection with 2-naphthylmethyl (NAP) group to afford mono-NAP ether **8**. Reductive ring opening of 2,3-*O*-benzylidene acetal was performed regioselectively according to Saito's protocol[8] to give 1,2-diol **9**. Its primary alcohol was then converted to monomethoxytrityl (MMTr) ether **10**, which was transformed into azide **11**, with inversion of configuration, through corresponding mesylate. Acidic detachment of the MMTr group afforded the primary alcohol **5**, which was designed as an aglycon for subsequent glycosylation.

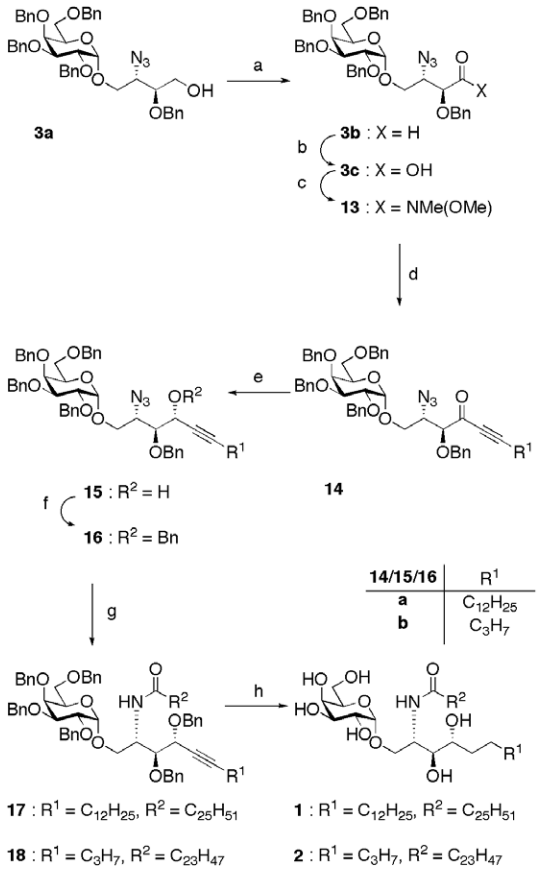
Attempted glycosylation of azidethreitol **5** with thiogalactoside **4a**[9] under standard conditions [MeOTf, 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), MS4A, CH_2Cl_2] proceeded well, but with modest selectivity (83%, α:β = 2.1:1).[10] Formation of a significant amount of β-isomer entailed careful chromatographic separation, and hampered the preparation of **12** on a larger scale. On the other hand, when MMTr ether **11** was directly used as an aglycon,[11] glycosylation with **4a** was completely stereoselective to afford **12** as the sole glycosylated product in 65% yield, which was readily isolated even in multi-gram scale preparation. Enhanced stereoselectivity observed with **4a** was not unexpected, in the light of previous reports, which employed trityl ether as an aglycon for α-selective glycosylation.[11a,d] Subsequent removal of the NAP group by DDQ afforded primary alcohol **3a**.



Scheme 2. Synthesis of a common intermediate. Reagents and conditions: (a) $PhCH(OMe)_2$, *p*-TsOH·H_2O, toluene, reflux (73%); (b) $LiAlH_4$, THF (90%); (c) NAPBr, NaH, THF (72%); (d) BH_3·SMe_2, CH_2Cl_2, then BF_3·OEt_2 (91%); (e) MMTrCl, DMAP, pyridine (92%); (f) MsCl, pyridine (88%); (g) NaN_3, DMF (94%); (h) PPTS, MeOH (86%); (i) MeOTf, DTBMP, CH_2Cl_2, MS4A (56% α, 25% β); (j) MeOTf, CH_2Cl_2 (64%, α only); (k) DDQ, CH_2Cl_2, MeOH (81%).

3. Synthesis of KRN7000 and OCH

Scheme 3 illustrates the synthesis of KRN7000 and OCH from the common intermediate **3a**. To begin with, the primary alcohol was oxidized through aldehyde **3b** to carboxylic acid **3c**, which was converted to Weinreb's amide[12] **13** by condensation with *N*,*O*-dimethylhydroxylamine. It was alkylated with lithium acetylide derived from 1-tetradecyne to afford ynone **14a**. Stereoselective reduction of the ketone was conducted with Corey's CBS system,[13] to afford propargylic alcohol **15a** in good yield, which was converted to benzyl ether **16a**. Compound **16b**, which was designed as the intermediate for OCH, was prepared in a similar manner via **14b** and **15b**.

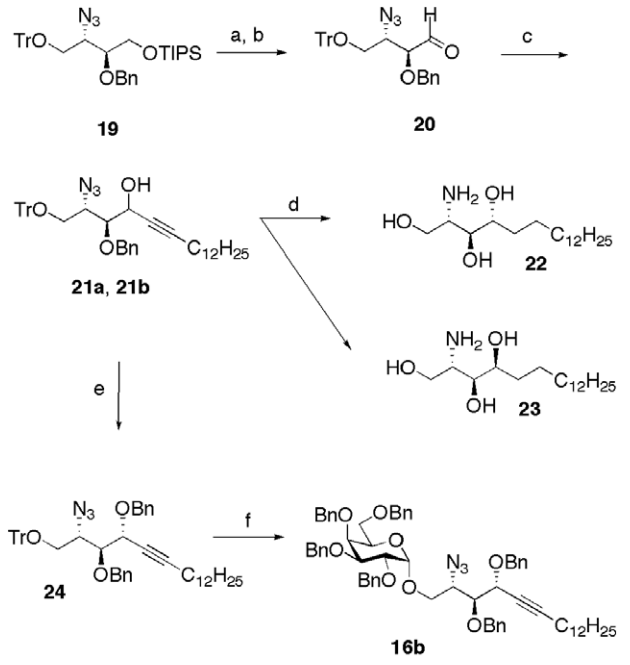


Scheme 3. Syntheses of KRN7000 and OCH. Reagents and conditions: (a) TPAP, NMO, CH_3CN, MS4A (91%); (b) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O (85%); (c) EDC·HCl, HNMe(OMe)·HCl, CH_2Cl_2 (90%); (d) 1-tetradecyne or 1-pentyne, BuLi, THF (75% for **14a**, 80% for **14b**); (e) (*R*)-B-Me-CBS, BH_3·SMe_2, toluene (91% for **15a**, 94% for **15b**); (f) NaH, BnBr, TBAI, DMF (85% for **16a**, 87% for **16b**); (g) $NaBH_4$, THF, MeOH; (h) $C_{25}H_{51}CO_2C_6F_5$ or $C_{23}H_{47}CO_2C_6F_5$, pyridine (83% for **17**, 77% for **18**); (h) H_2, $Pd(OH)_2$/C, EtOH, $CHCl_3$ (77% for **1**, 69% for **2**).

Although configuration of the newly generated stereochemistry of **15/16** seemed to be evident from well-documented selectivity of the CBS method, rigorous confirmation was made by synthesizing **16a** in an unambiguous manner (vide infra).

With compounds **16a** and **16b**, precursors for KRN7000 and OCH, in place, their azide moieties were reduced with $NaBH_4$ to give corresponding amines. Subsequent condensation with pentafluorophenyl ester of tetraicosanoic or hexaicosanoic acid furnished protected glycosphingolipids **17** (from **16a**) and **18** (from **16b**), respectively. Finally, global deprotection by hydrogenolysis accomplished the syntheses of KRN7000 (**1**) and OCH (**2**).

To eliminate any uncertainty in the configuration of C-4 hydroxyl groups of sphingosine, we prepared **16a** by an alternative approach (Scheme 4). Starting with compound **19**, which was prepared according to the reported procedure from compound **7**,[14] desilylation and oxidation of the primary alcohol by Dess–Martin periodinane[15] gave aldehyde **20**. Addition of lithium acetylide gave propargyl alcohols as a mixture of diastereomers (**21a** and **21b**). After chromatographic separation, they were converted into D-ribo (**22**)[16] and D-lyxophytosphingosine (**23**)[16] by hydrogenation of unsaturated bond and hydrogenolysis of trityl and benzyl ethers, respectively. Compound **21a**, which was correlated with **22**, was then converted to **24**. Galactosylation of **24** with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl acetate (**4b**) by using of Votéro's modified Mukaiyama procedure[11c,d] afforded α-galactoside **16b** selectively, which was identical with the one obtained in a route shown in Scheme 2.



Scheme 4. Alternative syntheses of intermediate **16** and phytosphingosines. Reagents and conditions: (a) TBAF, THF (95%); (b) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2 (94%); (c) 1-tetradecyne, BuLi, THF (97%); (d) H_2, Pd/C, EtOH (66%); (e) NaH, BnBr, DMF (90%); (f) $AgClO_4$, $SnCl_4$, Et_2O, CH_2Cl_2, MS4A (55%).

In conclusion, we established a convergent route to typical α-GalCers, KRN7000, and OCH, from the common intermediate **3a**. Further investigation to diversify **3a** to synthesize various types of α-GalCer analogues are under current investigation and will be reported in due course.

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