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Synthesis of α-*S*-galactosylceramides with a truncated sphingoid chain

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ABSTRACT

We present here a convenient synthesis of the truncated sphingoid iodide **15** from D-galactose, in which Mitsunobu reaction was utilized to retrieve successfully an unwanted intermediate, thereby increasing greatly the synthetic efficiency. Subsequent reaction of **15** with the pre-prepared α-galactosyl thiol **16** led smoothly to the desired thioglycoside **17** in good yield, from which the catabolically stable thioglycoside analogs of OCH **4** and **22** were synthesized.

The CD1 family of antigen-presenting proteins is monomorphic major histocompatibility complex (MHC) class I like glycoproteins that present lipid-based antigens recognized by T cells from different individuals and even across species.[1] Of all CD1 family members, only CD1d seems to have specialized in the presentation of a limited set of glycolipids[2] for recognition by natural killer T (NKT) cells,[3] the unique lymphocytes defined by their co-expression of the surface marker associated with NK cells along with a T cell antigen receptor (TCR). Extensive studies have shown that NKT cells are implicated in a broad range of diseases and are potent regulatory T cells that have the capacity to either initiate or shut down a wide variety of immune responses.[3] Hence there has been intense interest in understanding how NKT cells are stimulated by a CD1d-glycolipid complex and the extent to which NKT cell responses can be controlled.

To date, the best known class of agonist ligands for NKT cells is agelasphins **1** (Fig. 1),[4] first isolated from a marine sponge for their antitumor attributes.[5] An unusual feature of agelasphins is their α-anomeric configuration, unlike the ubiquitous β-glycosidic linkages in nearly all known natural glycolipids of normal mammalian cells. Later, a slightly simplified analog KRN7000 (**2**) was synthesized in a structure–activity study on these marine glycolipids and chosen as the lead compound for further development.[6] KRN7000 in the context of CD1d can bind the TCR with high affinity, stimulating NKT cells to release a variety of cytokines in vitro and in vivo. These cytokines are recognized subsequently by other cells of the immune system and may have a widespread influence on immune responses. One group of cytokines, including interferon-γ (IFN-γ) and interleukin-2 (IL-2), causes an inflammatory response, termed as T helper 1 (T_H1) response. In contrast, other cytokines that can be released by NKT cells include IL-4 and IL-10, and these results in an immunomodulatory or T_H2 response. Release of T_H1 cytokines is believed to be responsible for the antitumor, antiviral, and antibacterial effects of KRN7000. However, autoimmune diseases, such as multiple sclerosis, lupus, rheumatoid arthritis and type 1 diabetes, are T_H1-mediated. T_H2 cytokines can antagonize the immunostimulatory properties of T_H1 cytokines, consequently, production of T_H2 cytokines can ameliorate autoimmune diseases.

KRN7000 has thus been utilized in studies for the treatment of many diseases including cancer, diabetes, malaria, and hepatitis B, and in most cases, impressive activities have been observed.[7] Nevertheless, the efficacy of KRN7000 has been limited because it stimulates production of a mixture of T_H1 and T_H2 cytokines,[8] and these two types of cytokines are antagonistic to each other as described above. In addition, it has been shown that repeated administration of KRN7000 induces long-term NKT unresponsiveness in mice,[9] suggesting that there are also limitations to the use of KRN7000 as a therapeutic option.

Hence, many efforts have been devoted to synthesize KRN7000 analogs with the hope of developing novel lead compounds with better cytokine-inducing selectivities and appropriate potency as immunostimulatory agents.[10,11] In this context, a remarkable case is the truncated analog of KRN7000, termed OCH[12] which is the first described T_H2-biased agonist of NKT cells and has been the most studied agonist after KRN7000.[13] Stimulation of NKT cells with OCH (**3**) results in release of primarily T_H2 cytokines, as such, OCH was more efficacious than KRN7000 in animal models of the above-mentioned T_H1-mediated autoimmune diseases,[14] such as

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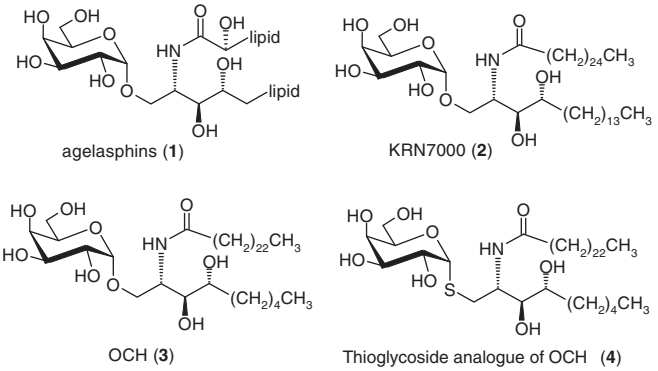


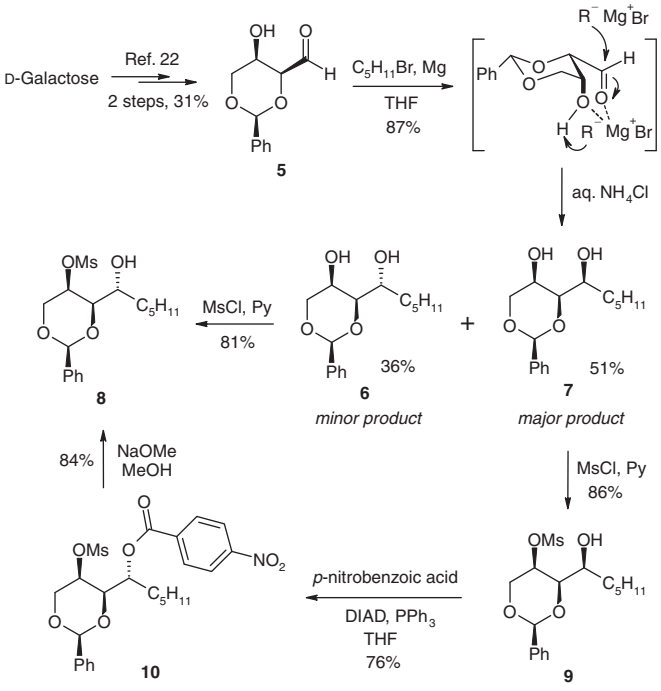
Figure 1. Structures of α-galactosylceramides **1**–**4**.

experimental autoimmune encephalomyelitis (EAE). Another interesting case is the *C*-glycoside analog of KRN7000,[15] which induces a very T_H1-biased response in vivo. It was thus significantly more potent than KRN7000 in disease models where a T_H1 response would be expected to be beneficial, such as mouse models of malaria and malignant tumors. Many other structural modifications on both the galactose residue and the lipid chains of the galactosylceramide have also been conducted,[10] and substantially altered NKT responses were also reported in some cases.

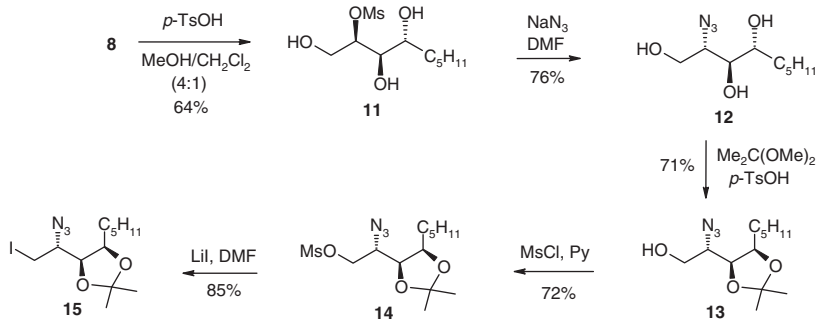
Yet, it remains unclear how these α-galactosylceramide (α-GalCer) analogs elicit qualitatively different responses. Several related X-ray crystal structures were recently elucidated,[16] revealing that the lipid chains of α-GalCer fit tightly into the CD1d binding groove and the galactose ring is positioned above the groove for recognition by NKT TCR. A series of hydrogen bonds were identified and are assumed to hold the complex together. However, from these structures, it is still not immediately apparent how the lipid chain length and the sugar moiety influence NKT cell responses although the hydrogen bonds did answer theoretically some of the structure–activity data derived from α-GalCer analogs.[16a] It has been postulated that the origin of cytokine polarization could relate to the stability of the glycolipid-CD1d complex.[8] Thus, the stability is currently a focus point in the design of potent α-GalCer analogs.

Recently, we achieved the first total synthesis of a thioglycoside analog of KRN7000 by using a nonconventional approach.[17,18] Thioglycosides are very attractive substitutes for *O*-glycosides as it is well known that they are much less susceptible to enzymatic cleavage as well as chemical degradation.[19] Moreover, unlike *C*-glycosides, thioglycosides may possibly retain all hydrogen bonding interactions with protein receptors. The subsequent bioassay demonstrated that this thioglycoside possessed similar potency to KRN7000 in human NKT cell activation, and stimulated cytokine release in a CD1d-dependent manner.[20] As such, the thioglycoside analog may be superior to KRN7000 as a parent compound for developing immune stimulants for humans in view of its bioactivity, stability, ease of synthesis, and flexibility for making other analogs. Thioglycosides usually have more flexibility around the anomeric linkage compared with the corresponding *O*-glycosides owing to the longer C–S bond and weaker stereoelectronic effects. We speculate that this increased flexibility may cause α-*S*-GalCer to sit differently in the CD1d binding groove, resulting in an altered structure of the glycolipid/CD1d complex and a change in its affinity with the TCR of NKT cells. Also, after binding with CD1d, the sugar head of α-*S*-GalCer may orientate at a different angle from that of α-GalCer as the C–S–C bond angle is significantly smaller than the C–O–C angle, which could result in differential recognition by the TCR of NKT cells.

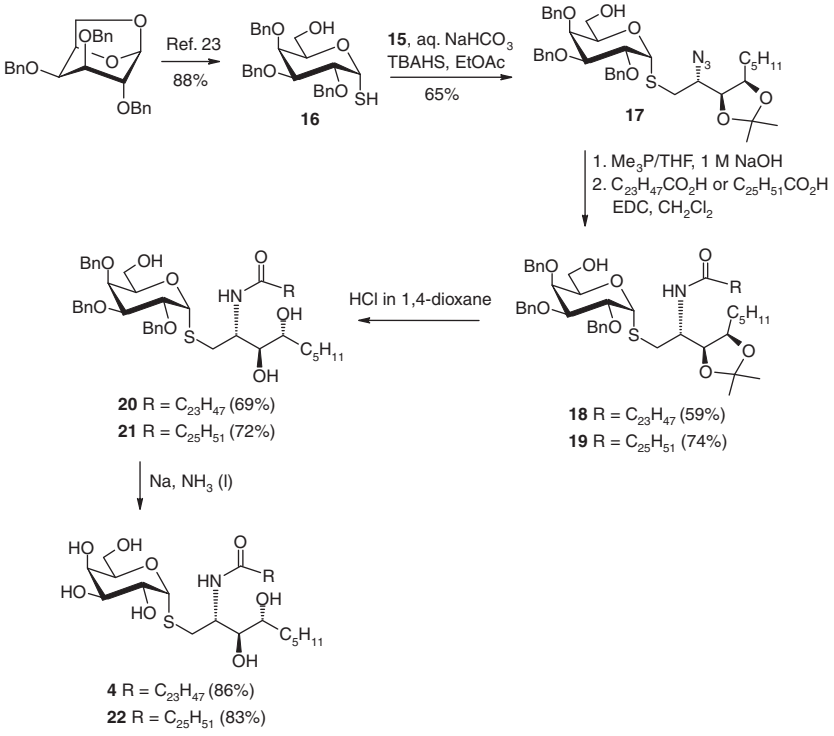
Encouraged by the bioactivity of α-*S*-GalCer,[20] we report here the total synthesis of two other thioglycoside analogs of α-GalCer, **4** and **22** containing the truncated sphingoid chain of OCH with a view to developing therapeutic analogs with shorter lipid chains. Based on our previous work,[17] a truncated sphingoid derivative needs to be prepared in order to construct the target molecules **4** and **22**. For this purpose, Schmidt's strategy[21] for the synthesis of phytosphingosine derivatives was again employed to synthesize the desired truncated sphingoid chain. As shown in Scheme 1, D-galactose was first converted into the aldehyde **5** in two steps and 31% overall yield.[22] Grignard reaction of **5** with pentylmagnesium bromide generated from 1-bromopentane and Mg was then performed, affording the adducts **6** and **7** in 87% yield. Unfortunately, as observed previously,[17] the desired isomer **6** was produced as the minor product with a ratio of **6**:**7** = 1:1.4. We presume that the observed selectivity, that is, the preferential formation of **7**, arises from substrate chelation to a magnesium cation (through the aldehyde and β-hydroxy group) and pentyl addition across the least hindered carbonyl *si* face, as depicted in Scheme 1. Attempts to reverse the selectivity to favor the formation of **6** were not made as previous studies indicated that lowering the reaction temperature or adding chelating agents only increased the formation of **7**.[21a] Subsequent treatment of **6** with 1 equivalent of methanesulfonyl chloride in the presence of pyridine led to the selectively 2-mesylated compound **8** in 81% yield. As Schmidt pointed out,[21a] the higher reactivity of the 2-OH group could be attributed to its greater nucleophilicity caused by intramolecular hydrogen bonding with the oxygen atoms of the dioxane ring. Meanwhile, in order to make use of the major product **7** for the synthesis of the target molecules, we inverted the configuration of the 4-OH group via Mitsunobu reaction (Scheme 1). As with **6**, selective mesylation of **7** gave smoothly the 2-mesylated intermediate **9** in very high yield, which underwent Mitsunobu reaction with *p*-nitrobenzoic acid to generate the 4-OH-inverted *p*-nitrobenzoic ester **10** in 76% yield. Removal of the *p*-nitrobenzoyl group was then effected by NaOMe in MeOH to give the desired 2-mesylated intermediate **8** in high yields. Thus, by using Mitsunobu reaction, both stereoisomers generated in the Grignard reaction could be capitalized for the synthesis of the target structures. With



Scheme 1. Synthesis of intermediate **8**.



Scheme 2. Synthesis of sphingoid building block **15**.



Scheme 3. Synthesis of α-*S*-galactosylceramides **4** and **22**.

sufficient amounts of **8** in hand, its transformation into the sphingoid building block was investigated. As expected, treatment of **8** with *p*-TsOH in a mixture of MeOH and CH_2Cl_2 gave rise to the desired intermediate **11** in 64% yield (Scheme 2). Next, **11** was treated with NaN_3 under heating, leading smoothly to the azide **12** in 76% yield, which was subjected to standard isopropylidenation conditions (2,2-dimethoxypropane/*p*-TsOH) to give the intermediate **13** in 71% yield. Azide **13** was subsequently mesylated with methanesulfonyl chloride in pyridine to generate compound **14** in 72% yield, which was converted into the iodide **15** in very high yield by treatment with LiI in DMF.

To access the target molecules **4** and **22**, the iodide **15** was glycosylated with the pre-prepared α-galactosyl thiol **16** as shown in Scheme 3. Thiol **16** could be readily prepared from the benzylated 1,6-anhydrogalactose following the stereospecific procedure for the synthesis of α-glycosyl thiols developed in our laboratory.[23] It is worth mentioning that this procedure is a significant advance in glycosyl thiol chemistry because there have not been any reports on direct stereoselective preparation of α-glycosyl thiols prior to our work. The desired galactosyl thiol was isolated exclusively as the α-anomer, which made purification very simple and straightforward. Thioglycosylation of **15** with **16** proceeded smoothly under the action of $NaHCO_3$ in biphasic conditions,[24] providing thioglycoside **17** in good yield (65%). It is noteworthy that the reaction worked well regardless of the free hydroxy group present in the sugar **16** due to extremely mild conditions.

Subsequently, **17** was exposed to Staudinger reduction conditions to give smoothly the required free amine, which was used directly in the subsequent acylation reaction. Coupling between the amine and tetracosanoic acid or hexacosanoic acid was then conducted in the presence of 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) in CH_2Cl_2, and expectedly, the corresponding amides **18** and **19** were produced in satisfactory yields of 59% and 74%, respectively. Subsequent exposure of **18** and **19** to a solution of HCl in 1,4-dioxane led to the corresponding partially protected glycolipids **20** and **21** in 69% and 72% yields. Finally, these intermediates were debenzylated by Birch reduction to give the desired target molecules **4** and **22** in 86% and 83% yields, respectively.

In summary, as a continuation of our previous project,[17] in this Letter, two thioglycoside analogs, **4** and **22** carrying the truncated sphingoid chain of OCH were synthesized as potential immunomodulating agents. The synthetic efficiency was greatly increased by employing Mitsunobu reaction to retrieve the unwanted

diastereoisomer. In view of the distinctive bioactivities of OCH and the significant potency of the thioglycoside analog of KRN7000, these two compounds may also exhibit interesting cytokine release profiles. Bioassays on these compounds are currently underway and the results will be reported elsewhere in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.07.103.

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