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Efficient synthesis of galactosylceramide analogues for iNKT cell stimulation

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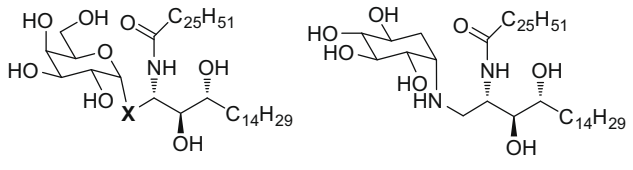
ABSTRACT

Glycolipids are potential antigens for iNKT cells recognition and demonstrate important roles in both innate and adaptive immunity. However, the difficulties in the preparation of pure configuration defined glycolipids limit the exploration of their different profiles in activating iNKT cells. We report here a concise and stereospecific preparation of novel galactosylceramide analogues by oxime ligation. This strategy would provide an efficient way to generate varied glycolipid analogues with either synthetic or natural carbohydrates for biological evaluations.

Invariant natural killer T (iNKT) cells, a subfamily of unique lymphocytes defined as natural kill T (NKT) cells, are specifically stimulated by CD1d presenting glycolipid antigens. Like other NKT cells, iNKT cells are able to rapidly secrete large quantities of interferons and interleukins upon activation by CD1d/glycolipid complex. To date, the marine sponge derived glycolipid, α-galactosylceramide (α-GalCer), is identified as the most potent ligand to stimulate iNKT cells when presented by CD1d.[1] The extraordinary potency of α-GalCer makes it the gold standard for the study of the iNKT cell stimulation. However, the controversial effects resulted from both high level inductions of Th1 and Th2 cytokines caused the failure of its therapeutic applications.[2] Therefore, better candidates with selectivity toward either Th1 or Th2 cytokines release were extensively explored. In order to achieve this goal, a lot of modifications on the prototype α-GalCer have been done and stimulation profiles of various analogues were studied. Some modifications on the lipid part[3] and galactosyl moiety[4] were found to induce either Th1 or Th2 biased responses. Surprisingly, modifications on the glycosidic linkage yielded contrasting effect on the iNKT cells stimulation (Fig. 1): The analogue with the carbon glycosidic bond (α-C-GalCer) remained the potent activity; While the replacement of anomeric oxygen by sulfur (α-S-GalCer) made it inert to iNKT cells.[5] More interestingly, the analogue (α-1C-GalCer) with missing oxygen between galactosyl moiety and ceramide not only remained the stimulatory activity, but induced Th1 biased response.[6] The most recent study demonstrated that one of the aminocyclitol ceramide analogues (HS44), which has a charged nitrogen glycosidic linkage, exhibited weak stimulatory activity against iNKT cells.[7]

Traditional glycosylation method has successfully produced a variety of α-GalCer analogues with controlled α-configuration (Scheme 1).[8] Nevertheless, this strategy required tedious protection and deprotection steps in order to generate the desired α or β glycosidic linkage. Even though the dominant stereo selectivity was achieved, the trace amount of undesired isomer challenged the separation after reactions and the biological evaluations.

In order to overcome these drawbacks, we have made a lot of efforts to explore an advanced methodology to efficiently conjugate different carbohydrates to ceramide lipid, which could quickly produce a library of glycolipids with natural or synthetic carbohydrates. It has been revealed that both α-GalCer and α-glucosylceramide (α-GlcCer) possess the ability to stimulate iNKT cells, while the α-mannosylceramide (α-ManCer) does not.[1] In addition, disaccharide (α-LacCer)[9] or trisaccharide (iGb3)[10] derived glycoceramides can also be recognized by iNKT cells. These results suggested that it is worthy to exploit all the available saccharides and thus evaluate the effects of carbohydrate part on activities of



α-GalCer: X = OCH_2
α-C-GalCer: X = CH_2CH_2
α-S-GalCer: X = SCH_2
α-1C-GalCer: X = CH_2

aminocyclitol ceramide (HS44)

Figure 1. Structures of α-GalCer analogues with varied glycosidic linkage.

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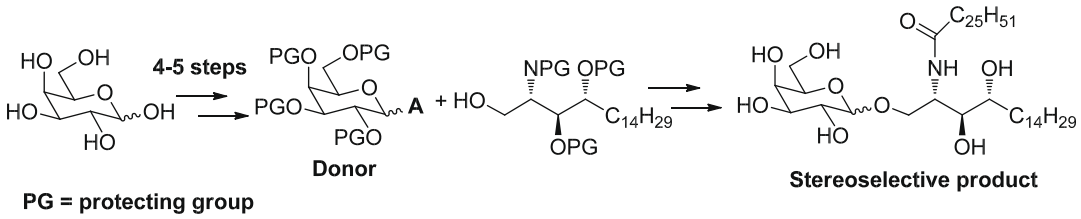


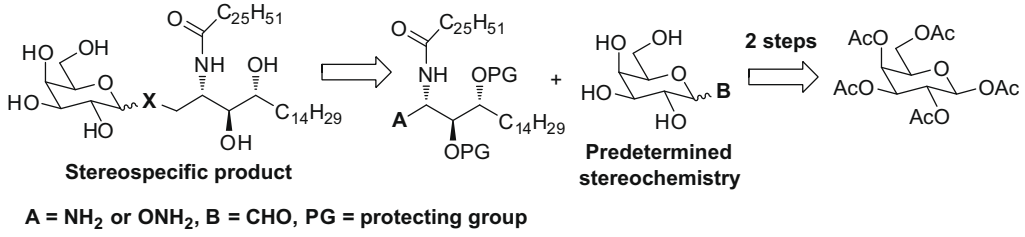
Scheme 1. Synthesis of α-GalCer and its analogues from traditional glycosylation.

glycolipids in stimulating iNKT cells. However, generation of a big library of glycoceramides seems to be impractical by using traditional glycosylation method. Recent discoveries of glycolipid antigens with modifications on the glycosidic linkage suggested the possibility to tolerate novel glycosidic linkage after applying efficient coupling method. More encouragingly, discovery of the aminocyclitol analogue (HS44) indicated that insertion of nitrogen atom will not completely diminish the biological activity. In this regard, reductive amination would be one of most perfect reactions to conjugate the saccharide to lipid part with minimal modification on the linkage part via stereospecific reaction (Scheme 2). However, our experimental trials using amino sugar and aldehyde ceramide pair failed. This was presumably due to the weak reactivity of the aminosugar. Herein, we report a concise synthesis of galactosylceramide analogues employing predetermined stereochemistry through oxime ligation (Scheme 2). The coupling reactions successfully generated novel glycolipid analogues with an oxime bond between sugar and lipid. Indeed, oxime ligations are often employed to conjugate bioorganic molecules and precious macromolecules, since oxime bond is readily formed and is stable under physiological conditions.[11]

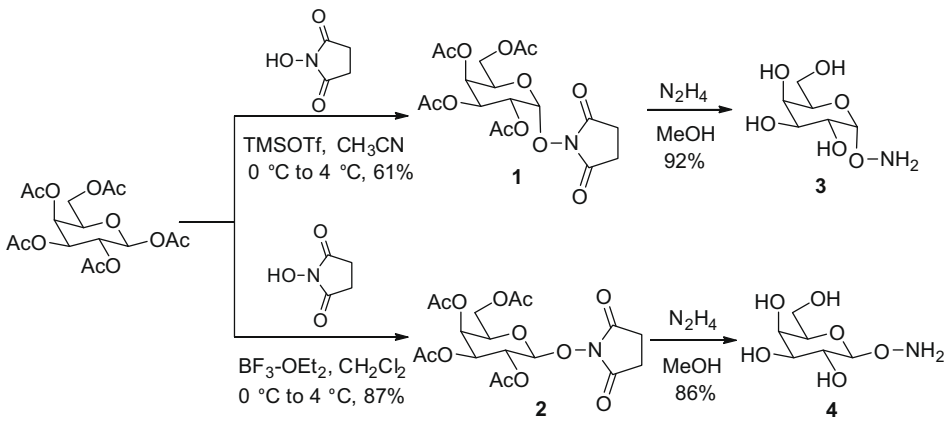
As shown in Scheme 3, both of the α and β galactosyl oxyamine intermediates were prepared from the same precursor, β-D-galactose pentaacetate. Previously, α-glycoside was produced using ether (e.g., benzyl) protected precursor in order to eliminate the neighboring group participation effect. Surprisingly, α-NHS galactoside **1** was selectively produced by treating the peracetate precursor with TMSOTf in acetonitrile at low temperature. This similar strategy was reported earlier for successful preparation of both α-selective glucosyl and lactosyl NHS analogous.[12] On the other hand, treatment of the same starting material in dichloromethane using BF_3–OEt_2 efficiently resulted in expected β-NHS galactose intermediate **2**. Then all the protecting groups were easily removed by using hydrazine in methanol to give galactosyl oxyamine **3** and **4**, respectively.

With these oxyamines ready, the coupling reactions were firstly performed by using the reported ceramide aldehyde with benzyl protecting group.[7] It turned out that this benzyl protected aldehyde was highly unstable and the final hydrogenolysis step jeopardized the oxime bond. In contrast, *tert*-butyldimethylsilyl (TBS) group was considered as a better protecting group for the ceramide since it is lipophilic and can be easily removed under mild condition. Thus, TBS protected ceramide intermediate **5** was prepared according to published procedure in three steps (Scheme 4).[8c] Oxidation of alcohol **5** using Dess–Martin periodinane (DMP) successfully yielded stable aldehyde ceramide **6**.[13]

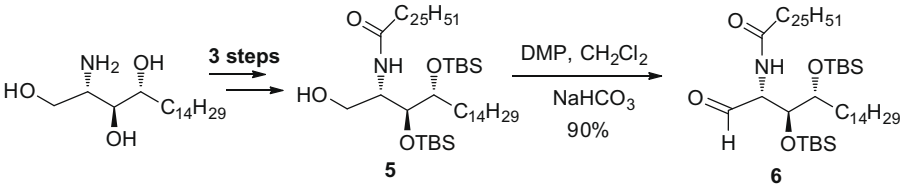
The conjugations between galactosyl oxyamines (**3** and **4**) and aldehyde **6** produced stereospecific α and β oxime intermediates



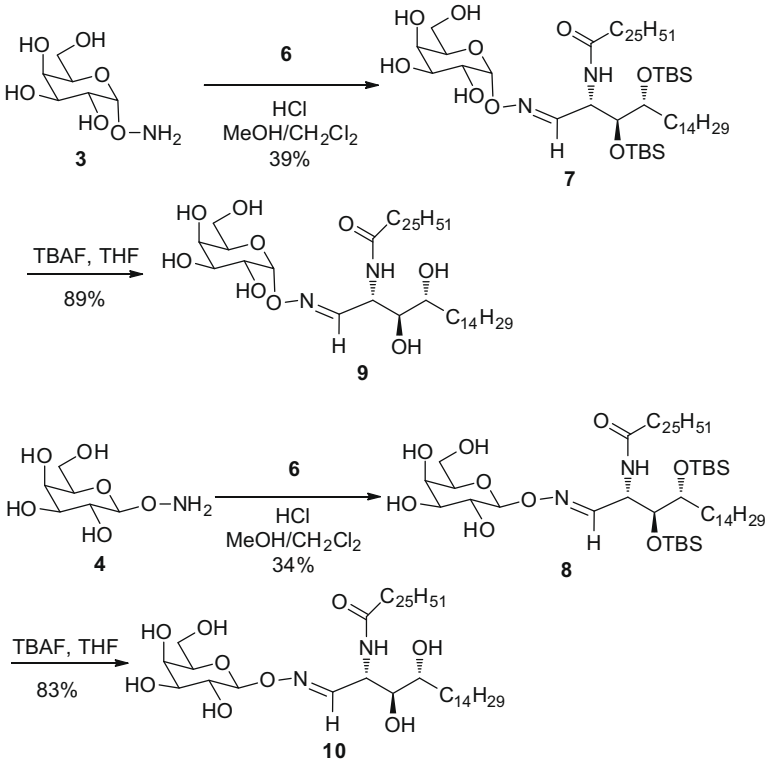
Scheme 2. Retrosynthetic strategy of glycolipid analogues by efficient coupling.



Scheme 3. Synthesis of galactosyl oxyamines.



Scheme 4. Synthesis of ceramide aldehyde.



Scheme 5. Synthesis of oxime galactosylceramide analogues.

7 and **8** in the presence of HCl (Scheme 5). Final products **9** and **10** were accomplished by the treatment of compounds **7** and **8** with TBAF in THF, respectively. This strategy efficiently and exclusively generated both α and β isomers of galactosylceramide without undergoing traditional glycosylations.

In summary, both α and β oxime galactosylceramide analogues were successfully synthesized in four steps from peracetylated galactose. These novel α-GalCer analogues are currently under biological evaluations. In our synthesis, the pre-installation of both α and β linkages, followed by oxime ligation simplified the synthesis of glycolipids as to traditional glycosylation method. Therefore, this method represents an efficient way to couple varied carbohydrates and the ceramide lipids. By extending the protocol to other available saccharides, it will lead to a facile generation of glycolipid library for the discovery of novel glycolipid ligands for iNKT cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.045.

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