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Concise synthesis of clarhamnoside, a novel glycosphingolipid isolated from the marine sponge *Agela clathrodes*

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Abstract—The first total synthesis of a novel α-galactoglycosphingolipid clarhamnoside has been achieved through a straightforward strategy. A thiogalactosyl donor with a benzylidene group at C-4 and C-6 and nonparticipating *p*-methoxybenzyl group at C-2 was successfully employed in the stereocontrolled syntheses of α-GalGSLs. The *N*-Phth-protected trifluoroacetimidate donor for terminal disaccharide was successfully applied in constructing the [GalNAcβ-(1→6)-Gal] glycosidic linkage.

Keywords: KRN7000; Clarhamnoside; Glycosphingolipids; Immunostimulatory; Sponge; Synthesis

1. Introduction

It is well established that sponges of the genus *Agelas* and *Axinella* produce α-galactoglycosphingolipids (α-GalGSLs), unique glycosphingolipids with an α-galactose as the first sugar of the carbohydrate chain, unlike the ubiquitous β-glycosidic bond from nearly all known higher animals and plants.[1] The scientific interests in α-GalGSLs have recently increased on account of the role they could play as therapeutic agents. α-GalGSLs are potent ligands of the MHC class I-like CD1d protein, which is present on the surface of the antigen-presenting cells (APCs) and is capable of activating in vitro and in vivo a specialized population of T cells, named natural killer T cells (NKT cells), which play an important role in regulating innate and adaptive immunity during infection, tumor growth, and autoimmune diseases.[2] α-Galactosyl ceramide (KRN7000), a potent analogue of the natural agelasphins isolated from the marine sponge *Agelas mauritianus*, is an important cerebroside exhibiting immunostimulatory activity and antitumor properties.[3] A truncated analogue of KRN7000, OCH, was found to selectively induce IL-4, as opposed to IFNγ, and to offer protection in mice against experimental autoimmune encephalomyelitis (EAE)[4] and more recently has been shown to offer protection against diabetes in NOD mice[5] and against collagen-induced arthritis.[6] Agelagalastatin, isolated from the Western Pacific marine sponge *Agelas* sp., displays significant in vitro inhibitory activities against human cancer cell growth.[7]

Besides its biological activities, spongal α-GalGSLs appear to be a quite peculiar class of molecules in terms of the structure of their carbohydrate moieties.[8] Clarhamnoside, a novel α-GalGSL, which was recently identified by the Mangoni group from new specimens of *A. clathrodes*,[9] bears quite a unique structure containing α-L-Rha*p*-(1→3)-β-D-Gal*p*NAc-(1→6)-α-D-Gal*p*-(1→2)-α-D-Gal*p*. It is one of the few natural α-GalGSLs glycosylated at the inner galactose 2-OH and the only α-GalGSL with an L-rhamnose unit in the sugar head.[9] In addition, the sequential two 1,2-*cis*-α-D-galactopyranosidic linkages [Galα-(1″→2′)-Galα-(1′→1)-Cer] are also an extraordinary and rare feature in nature (Fig. 1).

As part of our effort to understand the mechanism of CD1-mediated T-cell activation, we are interested in developing a facile approach to series of α-GalGSLs with a glycosylated 2′-OH or other substituting groups,

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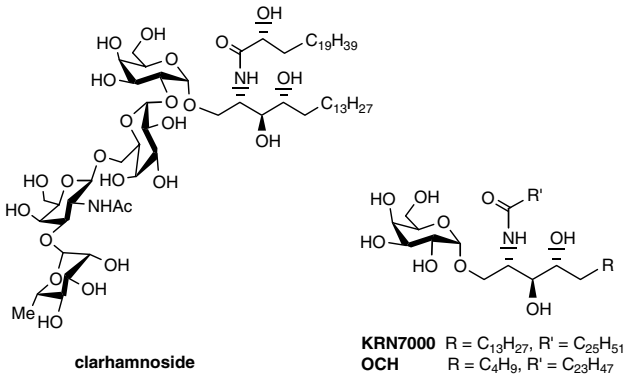


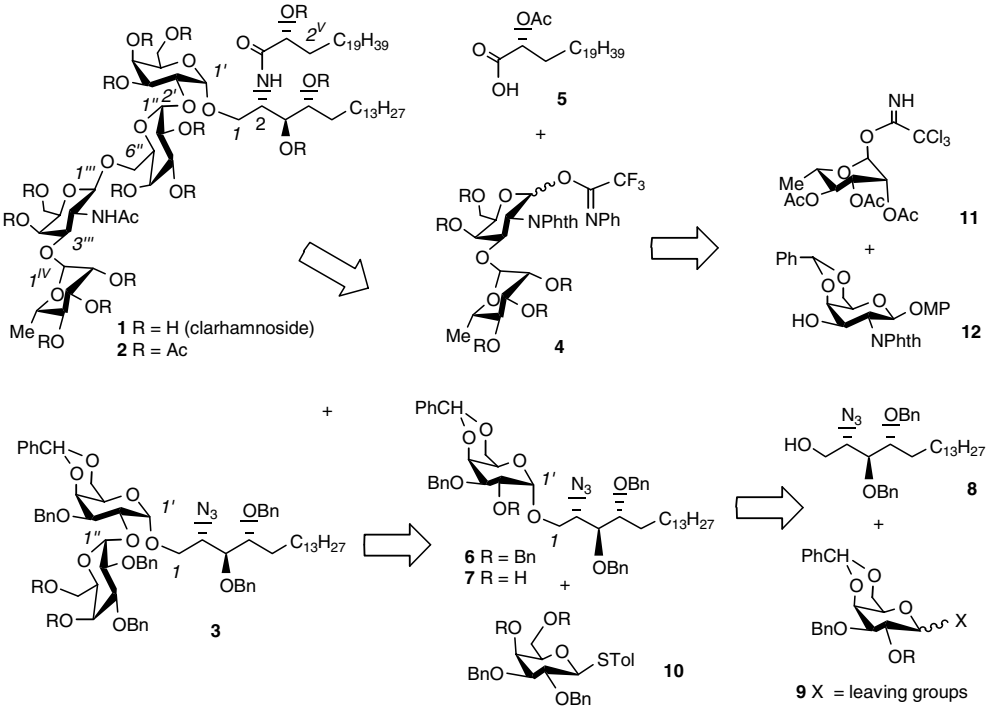
Figure 1. The structures of KRN7000, OCH, and clarhamnoside.

since structure–activity relationships showed that substitution on 2′-OH strongly affected the bioactivity of these compounds.[10] Although extensive syntheses of KRN7000 and its analogues[11] have been described, there are only a few works reported on the syntheses of C-2′ modified α-GalGSLs[10b,c,12] and spongal α-GalGSLs with a longer carbohydrate chain than KRN7000.[13] The unique structure and potent bioactivities make clarhamnoside a suitable goal for synthesis. Herein, we describe the concise synthesis of clarhamnoside using α-stereoselective glycosylations.

2. Results and discussion

Clarhamnoside consists of two synthetically distinct parts, the ceramide and the tetrasaccharide. A convergent synthesis thus calls for coupling of a tetrasaccharide donor with the aglycon. However, α-selective glycosylation of the ceramide with galactosyl donors has long been recognized as a difficult task,[13] especially for the inner galactose, which is (1→2) branched. The potent steric effect between the bulky ceramide residue and the tetrasaccharide donor requires attention in the convergent synthesis.[14,15] In addition, a general route for preparation of α-GalGSLs with a free 2′-OH group were needed in our later work. For this purpose, a straightforward strategy was described to complete the synthetic work. The retrosynthetic analysis of clarhamnoside is depicted in Scheme 1. α-Galactosyl lipid **7** with a free 2-OH group was anticipated as a key intermediate for elaboration of the tetrasaccharide lipid via sequential coupling with monosaccharide donor **10** and disaccharide donor **4**. The stereocontrolled construction of α-linked monosaccharide lipid **7** and disaccharide lipid **3** turned out to be the crucial steps in our synthesis.

For preparation of α-galactosyl ceramide (**6** or **7**), a major effort was to find a suitable galactosyl donor, which should be designed to obtain a high α:β ratio as well as a satisfactory yield. Recently, galactosyl trichloroacetimidate **15** with a benzylidene group at C-4 and C-6 and nonparticipating benzyl groups at C-2 and C-3 has been successfully used in the stereocontrolled syntheses of α-GalGSLs[16] (Scheme 2). This remarkable stereoselectivity could be attributed to the *cis*-decalin ring system with the equatorial phenyl group in **15** that hinders the attack from the β-face.[16] The result prompted



Scheme 1. Retrosynthetic analysis of clarhamnoside.