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Synthesis of glycolipid analogs via highly regioselective macrolactonization catalyzed by lipase

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Abstract—Highly regioselective lipase catalyzed macrolactonization has been used in synthesizing first feedstock based glycolipid analogs. These macrolides containing common disaccharides maltose (4-*O*-α-D-glucopyranosyl-β-D-glucose) and melibiose (6-*O*-α-D-galactopyranosyl-β-D-glucose) were synthesized by employing chemoenzymatic methodologies. Maltose and Melibiose were coupled with methyl 15-hydroxy pentadecanoate and then subjected to a highly regioselective macrolactonization at the C-6″ position using *Candida antarctica* lipase-B to yield the desired products.

1. Introduction

Glycolipids containing macrolidic subunit are interesting group of natural products that have complex structures and interesting biological activities.[1] Woodrosin I, Simonin I, Sophorolipid lactone (SL), etc. belong to such a class of glycolipids which contain a ω-hydroxy acid as an agylcon that is tied back to form a macrolactone ring spanning two or more units of their saccharide backbone (Fig. 1). Though most of these compounds have been isolated from plants, sophorolipids produced by the yeast *Candida bombicola* are of growing commercial interest as biodegradable emulsifiers.[2]

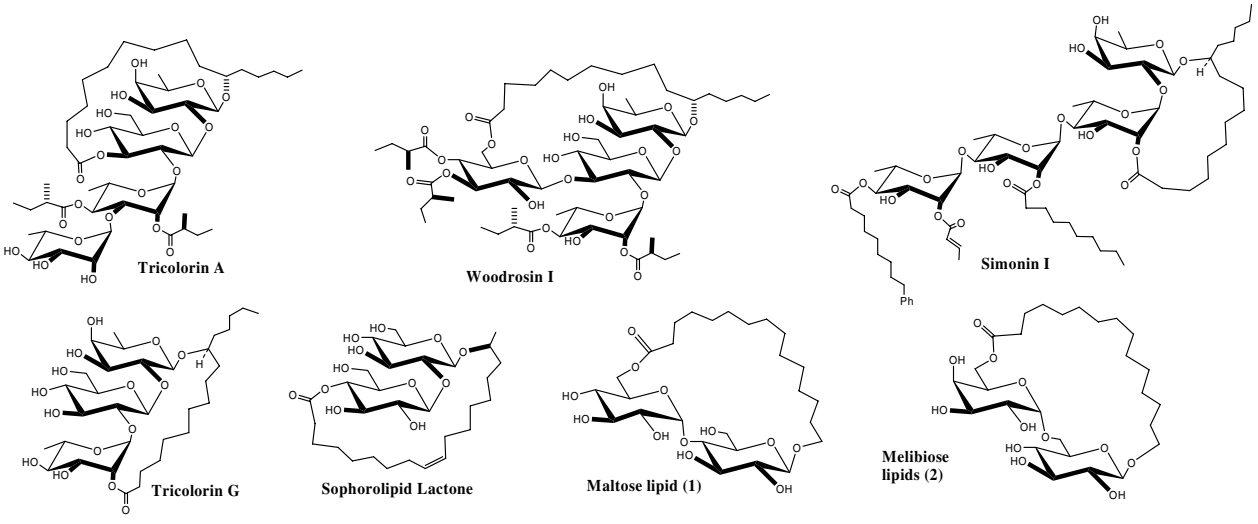

Figure 1. Glycolipids containing a macrolactone ring.

Keywords: Maltose; Melibiose; Macrolactonization; Lipase.
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Although the biological properties of glycolipids have not been fully assessed, a closer look at this class of natural products seems highly promising in view of the existing data on their potential as immunomodulators for Parkinson's disease, Alzheimer's disease, psoriasis, AIDS treatment, as well as for antiviral immunostimulation.[3] For example, sophorolipds have been reported to cause differentiation and protein kinase C inhibition in the HL60 leukemia cell line.[4] Tricolorin-A, isolated from a Mexican plant *Ipomoea tricolor*, has been reported to have significant cytotoxicity against cultured P-388 and human breast cancer cell lines.[5] Although very little is known regarding structure–activity relationship, it has been established that the intact macrolactone ring is essential for bioactivity.[6] There is clearly a need for generating analogs of these compounds to probe their promising and diverse biological effects.

Whole cell biocatalytic approaches have been investigated for the synthesis of microbial sophorolipids and their derivatives by the selective feeding of lipophilic substrates.[7] For example, changing sunflower to canola oil resulted in a large increase in the yield of the lactonic portion of sophorolipids.[8] Unsaturated C-18 fatty acids such as oleic acid may be incorporated unchanged into sophorolipids and result in dramatic change in their compositions.[9] However, it is clear that this approach is largely limited to compositional change or incorporation of select aglycons. Clearly, a different synthetic approach is needed but the intricate structure of these glycolipids poses many synthetic difficulties and consequently very little effort has been devoted to this area. The major challenge resides in the regioselective formation of the macrolactone ring which so far has been accomplished by Yamaguchi,[10] Corey,[11] or Mitsunobu macrolactonization conditions.[12] More recently the use of ring closing metathesis reaction has been explored for the formation of macrolides.[1,13] Although very useful, these approaches to the formation of the macrolides require strategically placed reactive groups necessitating a number of protection/deprotection steps resulting in a long synthetic sequence.[14]

2. Present study

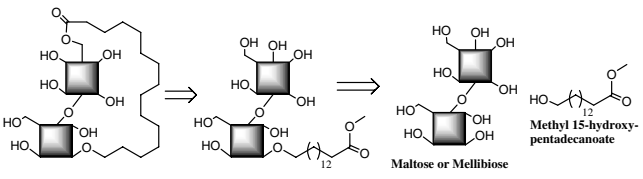
In this letter, we explore lipase catalyzed macrolactonization for the formation of glycolipid analogs maltose lipid (**1**), and melibiose lipid (**2**) (Fig. 1). These compound would otherwise be difficult to synthesize or unavailable for subsequent evaluation of their properties and bioactivities. The main advantages of using this strategy is (i) low cost starting materials, (ii) the ability to incorporate a variety of carbohydrate residue in the head group and different aliphatic hydrophobic aglycon that would allow a handle to manipulate physical properties, and (iii) biocompatibility of the lipase catalysts is in accordance with the principles of green chemistry.

Lipase catalyzed acylations and transesterification reactions have been evaluated for the formation of macrolactones.[15] Stemming from our interest in biocatalysis,[16] we outlined an efficient short synthetic route to compounds **1** and **2**, starting from readily available disaccharides maltose and melibiose (Scheme 1).

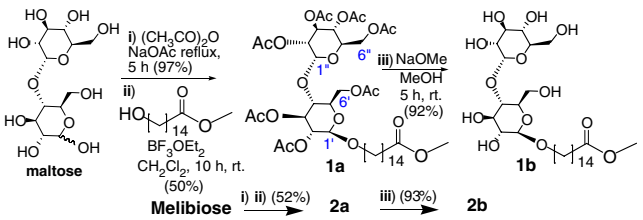
The formation of the macrolactone was investigated using a number of lipases to demonstrate the regioselectivity of the lipase catalyst. In a previous study by Bisht et al.[17] it was demonstrated that sophorose, the glycon portion of sophorolipids, is able to fit in the active site of lipase CAL (from *Candida antarctica*) and that CAL catalyzed macrolactone formation between 17-hydroxyoctadec-9-enoic acid subunit and the sophorose backbone. The utilization of maltose and melibiose is highly desired because of their origin in agriculture-based feedstock. The aglycon, 15-hydroxypentadecanoic acid is obtained from ω-pentadecalactone a known substrate for the *C. antarctica* lipase.[18]

The ring opening reaction of pentadecalactone using sodium methoxide in methanol provided methyl 15-hydroxy pentadecanoate in a 93% yield. Maltose and melibiose were peracetylated using sodium acetate and acetic anhydride to form respective octaacetates in nearly quantitative yields (Scheme 2). Taking advantage of the increased anomeric reactivity, the peracetates were directly coupled with methyl 15- hydroxypentadecanoate in the presence of boron trifluoride etherate in freshly distilled anhydrous dichloromethane to afford **1a** (46%) and **2a** (50%), respectively (Scheme 2).[19]

The stereochemical assignments of the anomeric carbon in **1a** and **2a** were achieved from ^{1}H NMR, where the J_{12} value (8.3 Hz in **1a** and 8.1 Hz in **2a**) for H-1′ indicated the β-D-configuration.[20] The global deprotection of **1a** and **2a** to **1b** and **2b**, respectively, was achieved in nearly a quantitative yield upon stirring with sodium methoxide in anhydrous methanol for 5 h at room temperature. The structural characterization of **1b** [MALDI-TOF MS $m/z = 619.5$ $(M+Na)^+$] and **2b** [MALDI-TOF MS $m/z = 619.4$ $(M+Na)^+$] was accomplished using extensive spectrometric analysis. Partial DEPT NMR spectra of **1b** and **2b** are included in Figures 2 and 3, respectively. The absence of the acetate carbonyl and methyl



Scheme 1. Synthetic approach to the maltose and melibiose lipids.



Scheme 2. Synthesis of **1b** and **2b**.

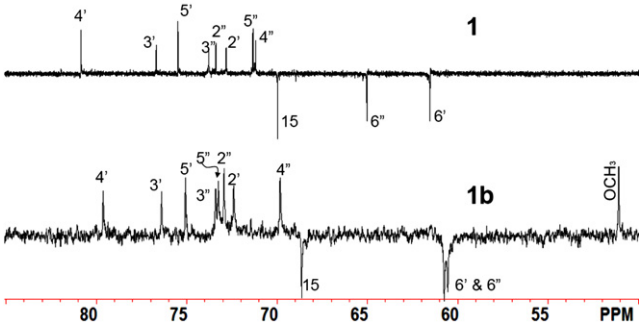


Figure 2. Portion of DEPT-135 ^{13}C NMR of **1b** and **1**, showing downfield shift of C-6″.

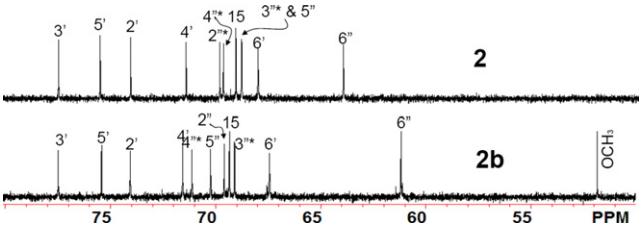


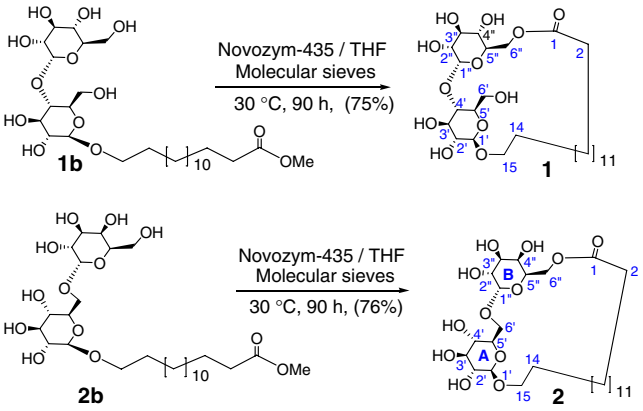
Figure 3. Portion of DEPT-45 ^{13}C NMR of **2b** and **2**, showing downfield shift of C-6″. * interchangeable.

resonances confirmed complete deacetylation. The spectra, however, retained the resonances for the methyl ester (51.2 ppm in **1b**; 51.9 ppm in **2b**) and the carbonyl group (174.2 ppm in **1b**; 174.9 ppm in **2b**) from the side chain confirming the structures as **1b** and **2b**.

With **1b** and **2b** in hand, the lipase catalyzed macrolactonization was attempted. Lipases are excellent biocatalysts, since they have the remarkable ability of assuming a variety of conformations to accommodate substrates of varying sizes and complexities.[21] A number of lipases, namely PPL (from porcine pancreas), CCL (from *Candida rugosa*), AK (from *Pseudomonas cepacia*), and CAL (from *C. antarctica*; Novozym 435®) were investigated. The reactions were performed in anhydrous THF at 30 °C for 96 h. Control reactions were set up similarly but without added lipase. Of all the lipases tested only the lipase CAL showed any activity and led to compounds **1** and **2** (Scheme 3).

Compounds **1** [White solid, MALDI-TOF MS $m/z =$ 587.1 $(M+Na)^+$] and **2** [White solid, MALDI-TOF MS $m/z = 587.3$ $(M+Na)^+$] were isolated after column chromatography using silica gel as the stationary phase and 20% methanol in dichloromethane as the eluent. All fractions collected were monitored using TLC in the same solvent system and compared alongside with the respective starting material. Product being less polar than starting material moved up on the TLC plate. Fractions containing the product were combined and concentrated in vacuo.

The extensive structural analysis of products **1** and **2**, purified by column chromatography, was undertaken using ^{1}H, ^{13}C and 2D NMR spectral techniques. Previ-



Scheme 3. Synthesis of glycolipids **1** and **2**.

ously, Bisht et al.[17] carried out a detailed NMR analysis on a sophoroselipid lactone and noted, because of complex overlapping of resonances, limited utility of the ^{1}H NMR spectra in establishing the structure of the glycolipid and assignments of various signals. The authors noted that ^{13}C NMR spectrum because of its wider range (0–200 ppm) is better suited for assignment of different carbons in these complex glycolipid analogs. In this letter ^{13}C NMR, DEPT, COSY, HMQC, and HMBC (carbon–proton long range correlation) were carefully analyzed to obtain assignments of different carbon resonances shown in Figures 2 and 3.

The spectra of compounds **1** and **2** were compared to their corresponding starting materials, **1b** and **2b**. In the ^{1}H NMR spectra, the resonance for methyl ester protons (~3.5 ppm) was not present in products **1** and **2**. Hydrolysis of the methyl ester was ruled out as a resonance for the free carboxylic acid (~12 ppm) was not observed. The absence of resonances corresponding to the methyl ester or the free carboxylic acid thus suggested the formation of a macrolactone. Although the proton resonances of the sugar skeleton appeared perturbed, it was not possible to decipher any useful information due to overlapping of the various resonances in the ^{1}H NMR. The carbon-13 NMR spectra was edited using a DEPT pulse sequence and compared with those of the corresponding starting materials. The wide distribution of the resonance frequencies in the DEPT spectra allowed for the assignment of each carbon resonance using COSY, HMQC, and HMBC correlations. A comparison of the DEPT spectra of the product and the respective starting compound allowed for an interesting observation to be made (Figs. 2 and 3).

In DEPT ^{13}C NMR for compound **1**, the resonance for the methyl ester group was not observed (~50 ppm), a carbonyl resonance was observed at 174.9 ppm, and a downfield shift of 4 ppm was observed for the C-6″ carbon when compared to the starting compound **1b**. Importantly, the HMBC spectra also showed a cross-peak ($^3J_{CH}$) between the carbonyl carbon (C1, in the side chain) and C-6″ hydrogens, confirming the formation of macrolactone between the carbonyl carbon of the side chain and C-6″ of the maltose head group. An

upfield shift in the resonance position of C-5″ is consistent with the γ-effect that is caused by the attachment of the acyl groups at the C-6″ hydroxyl. No significant shift in the resonance position of the other carbons in **1**, in comparison to those in **1b**, were found. These observations unequivocally established the formation of lactone ring at C-6″ position and the structure of the compound was established as **1**. The regioselectivity of the lipase is remarkable as only the C-6′ hydroxyl, which is in competition with six other hydroxyl groups (one primary (C-6′) and five secondary), was involved in the macrolactone formation exclusively.

In compound **2**, the DEPT ^{13}C NMR did not show the methyl ester resonance which was observed in **2b** at 51.9 ppm which together with the observed ester carbonyl resonance at 176 ppm suggested a macrolactone formation. Additionally, the C-6″ carbon resonance was observed at 64 ppm, a 3 ppm downfield shift in its position compared to in **1b** (observed at 61 ppm; Fig. 3), further suggesting the macrolactone formation at C-6″. Interestingly, resonances corresponding to carbons in the α-D-glucopyranose ring (ring-A) did not show any significant shifts in their resonance positions. However, significant shifts in the resonance position of the carbons in the α-D-galactopyranose ring (ring-B) of **2** were observed, which may be attributed to the conformational changes associated with the formation of the macrolactone ring. An observed cross-peak ($^3J_{CH}$) between carbonyl carbon (C-1; 176 ppm) and H-6″ (4.10 ppm) in the HMBC spectrum of **2** confirmed the formation of the macrolactone at the C-6″ carbon of the melibiose head group. The reaction was highly regioselective and is suggestive of the remarkable selectivity offered by the lipase CAL.

3. Conclusion

In summary we have demonstrated the highly regioselective formation of glycosidic macrolactones. This, to the best of our knowledge, is the first report of well-defined glycolipid analogs synthesis from maltose and melibiose, two readily available disaccharides. Presently we are engaged in making different analogs by changing the hydrophobic side chain and studying physical and biological properties of these analogs. These macrolidic glycolipid analogs have potential applications in the cosmetic, formulation, and food industry. Their utility in technical purposes such as oil pollution abatement can be envisaged as these biosurfactants can enhance the emulsification of hydrocarbons increasing their bioavailability for microbial degradation.

Acknowledgments

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Supplementary data

The detailed experimental procedures and NMR spectra for compounds **1b**, **2b**, **1**, and **2** are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.10.041.

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