Creating a C-12 Series of Glycolipids for Use as Micelles in Drug Delivery

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Abstract. Micelles are critically important molecules with a variety of uses. They have historically been used in the multibillion dollar soap industry. More recently, their efficacy in drug delivery has been demonstrated (1). Normal oxygen-linked glycosides are susceptible to hydrolysis (2). We are seeking to develop a series of carbon-linked glycolipids of varying chain lengths and functional groups in order to observe trends in their micelle properties, including Critical Micelle Concentration (CMC). Presented here are the C-12 Glycoside series.

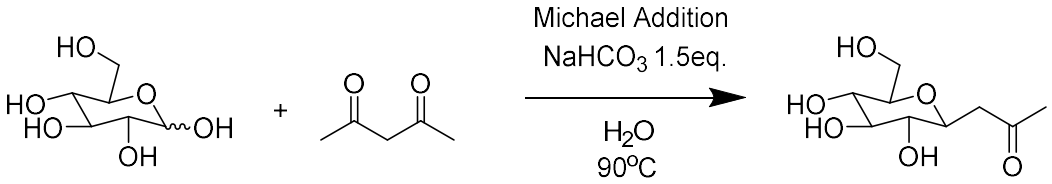
Introduction. The world surfactant market was valued at over 30 billion U$ dollars in 2015, and is expected to grow (Market Report: World Surfactant Market). The market value is split across a variety of categories including cosmetics, bioremediation, and soaps. Many surfactants are skin irritants, or may even be toxic to the environment. Thus, the development of new surfactants is a pressing matter (3).

More recently, surfactants have been examined for their use in creating micelles capable of drug delivery. Micelles are favorable candidates for drug delivery vectors for multiple reasons. Micelles are capable of encapsulating hydrophobic drugs, which would not normally be soluble in the body. The hydrophilic exterior of a micelle also is capable of forming a tight shell of water using hydrogen bonding. This shell can protect against degradation by hydrolysis or enzymatic activity (4). Furthermore, micelles can be triggered to degrade using temperature increases or either external factors which allows for quick or targeted-release of a drug.

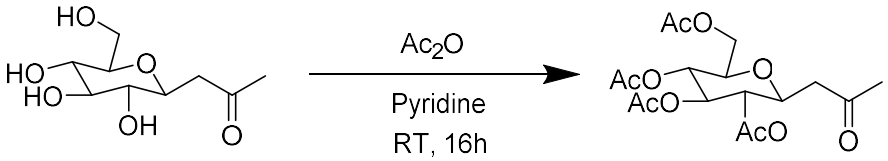
We seek to expand this field through the development of new glycolipids. We will be using glucose (blood sugar) to provide the polar head and testing different linkages including Carbon, Sulfur, and Oxygen. Glucose has the ability to selectively protect and react alcohol groups (5). The lengths of the lipid chains and the functional groups attached will also be varied. We will be measuring the surface tension of liquids in which our glycolipids are dissolved in order to determine the Critical Micelle Concentration, (CMC) the concentration at which micelles form. Presented here is the synthesis of the Carbon linked C-12 Glycolipids.

Methods. Glycolipid synthesis is done as described by Foley et. al.(6) and Lalitha et. al. (7). Our glycolipid synthesis began with a Michael Addition using D-Glucose as an electrophile reacting with Pentane 2,4-dione (1.2 eq) as a nucleophile. The reaction was done in H_2O and the presence of $NaHCO_3$ (1.5 eq). It was refluxed at 90°C overnight as shown in **Schematic 1**. The product was washed using CH_2Cl_2 and the pH of the solution was brought to ~3 using DOWEX ion exchange resin. The resulting product was protected through acetylation using Ac_2O (6 eq) in the presence of pyridine (isovolumetric to Ac_2O). The reaction was stirred at RT for 16 h as is depicted in **Schematic 2.** This product was used as a starting material for a series of reactions. To make the C-12 Glycosides, an Aldol Condensation was performed by dissolving the nonulose sugar in CH_2Cl_2 with pyrrolidine (1 eq). The reaction mixture was left to stir for 15 minutes before decanal (1 eq) was added. The solution was stirred at room temperature overnight. **Schematic 3** shows this reaction. The product of the Aldol Condensation was purified using HPLC. The product was loaded and washed with Hexanes. The column was run in 20% EtOAc in Hexanes with an

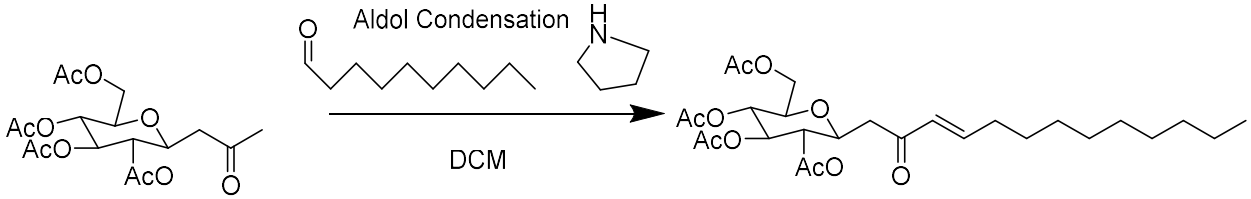
elution solvent of 30% EtOAc in Hexanes. The current product is the enone form of the C12 Glycoside. To deacetylate the enone, a Zemplén Deacetylation reaction was used. The enone was dissolved in MeOH and NaOMe was added until the pH reached ~9. The reaction was stirred overnight and quenched with DOWEX ion exchange resin as is shown in **Schematic 4**. The resulting product was collected and stored. Starting again with acetylated enone C12 Glycoside, a hydrogenation was performed by dissolving the enone in EtOAc, flushing the container with Argon, adding catalytic Pd/C, and attaching a H_2 balloon overnight. To work up the reaction, Cellite® was added, the mixture was filtered and then washed with CH_2Cl_2. The solution was diluted in EtOAc, H_2O was added, and the organic layer was collected. Evaporated solvent to yield ketone C12 Glycoside. Ketone form was deacetylated using the Zemplén Deacetylation and collected. Using acetylated ketone glycoside, a Wolff-Kishner Reduction was done by dissolving the ketone in MeOH and adding tosylhydrazone (1.1 eq). The reaction was stirred at RT overnight. The solution was diluted in CH_2Cl_2 and washed with water. The organic layer was collected and dried with Magnesium Sulfate. Solvent was evaporated. Dissolved in 9:1 CH_2Cl_2:MeOH. Sodium Borohydride (2.5 eq) was added and the reaction stirred overnight. The reaction was worked up using Ethyl Acetate and water. The organic layer was collected. A Zemplén Deacetylation was performed to collect the Alkyl C12 Glycoside. Starting from the acetylated ketone C12 glycoside, a reduction was performed by dissolving the ketone in 9:1 CH_2Cl_2:MeOH. Sodium Borohydride (2.5 eq) was added and stirred overnight. The reaction was stopped by addition of Cellite®. After filtering a Zemplén Deacetylation was performed and the alcohol form of the C12 Glycoside was collected. **Schematic 5** shows the synthesis of the alkyl and alcohol glycosides and their deacetylations.



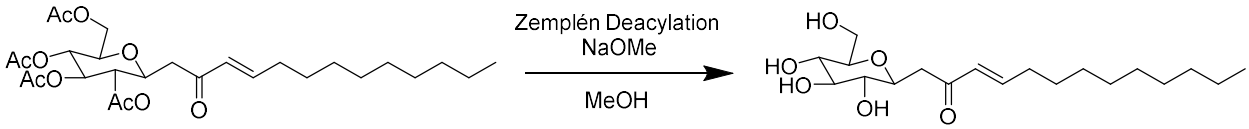
Schematic 1: Michael Addition between D-Glucose and Pentane 2,4-dione.



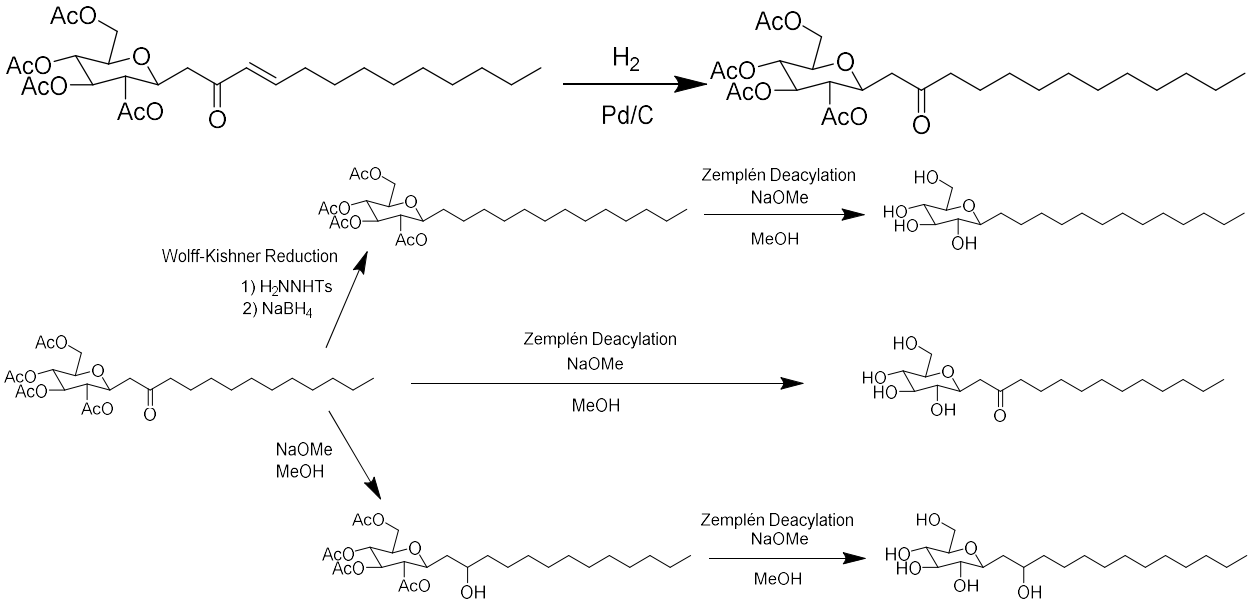
Schematic 2: Protection of the Hydroxyl groups using Acetylation.



Schematic 3: The Aldol reaction of a nonulose sugar with decanal to form the C12 Glycoside.



Schematic 4: Zemplén Deacetylation of the enone form C12 Glycoside.



Schematic 5: Synthesis of the alkyl C12 Glycolipid was achieved through a Wolff-Kishner Reduction of the ketone and synthesis of the alcohol C12 Glycolipid was achieved through a Sodium Borohydride reduction of the ketone. Also, Zemplén Deacetylations of the ketone, alkyl, and alcohol glycosides was performed to yield the final products.

Results. The C-12 Glycolipids series was successfully synthesized and confirmed through NMR analysis. **Figures 2** and **3** show the results of the hydrogenation reaction. After hydrogenation occurs, the olefin peaks around 5.5 and 7.0 ppm disappear. **Figures 4** and **5** illustrate the difference between acetylated alcohols and their deprotected form after a Zemplén reaction. The exposed alcohols result in significantly altered peaks. **Figures 3** and **4** show the transition from ketone to alcohol forms. Peaks around 4 and 5 PPM are retained, but there are significant changes in other parts of the spectrum. **Figure 6** shows the acetyl protected alkyl glycolipid. Many of the samples were more difficult to dissolve for NMR study upon deacetylation. We resolved this issue by using perdeuterated methanol (D_3COD) in place of $CDCl_3$ as the solvent. The difference in solvent choice is shown in **Figures 7** and **8** with **Figure 7** showing the sample dissolved in D_3COD and **Figure 8** shows the sample dissolved in $CDCl_3$. **Figure 7** features much sharper peaks which can be recognized as the product. This change in resolution is thought to occur due to reverse micelle formation. Reverse micelles occur when there is a small concentration of water in the nonpolar solvent. The polar heads will cluster around the water molecules with the tails pointing outwards (8) as shown in **Figure 1.**

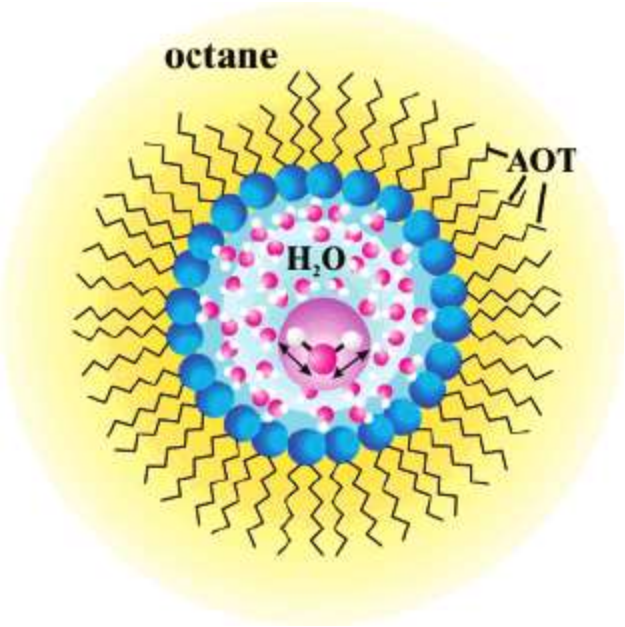


Figure 1: Cross section of a reverse micelle in which polar heads have oriented themselves around a water droplet while immersed in a nonpolar solvent (9).

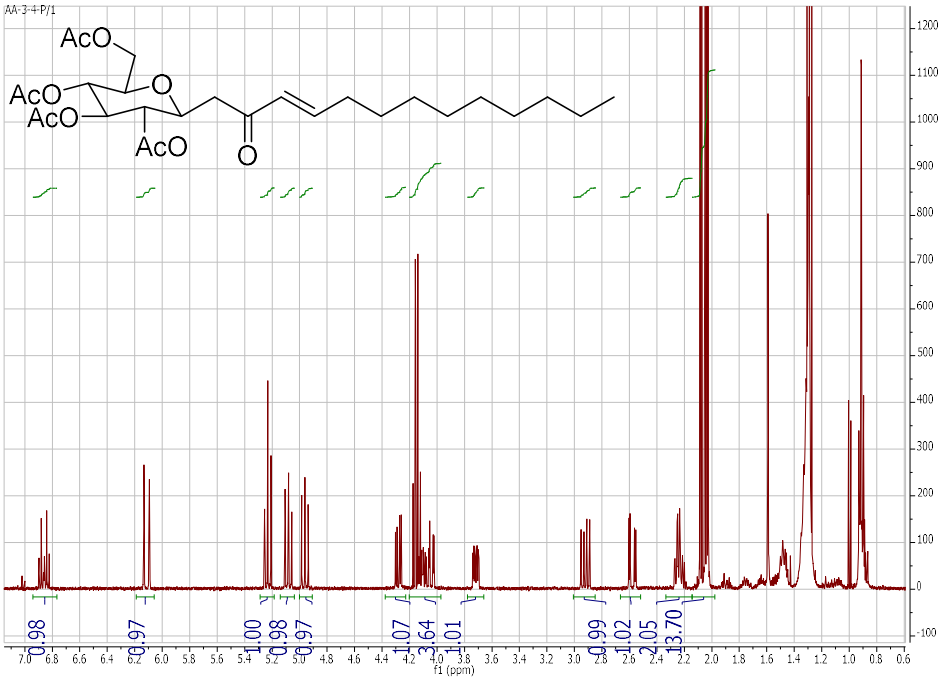


Figure 2: NMR spectrum obtained for acetyl protected enone glycolipid.

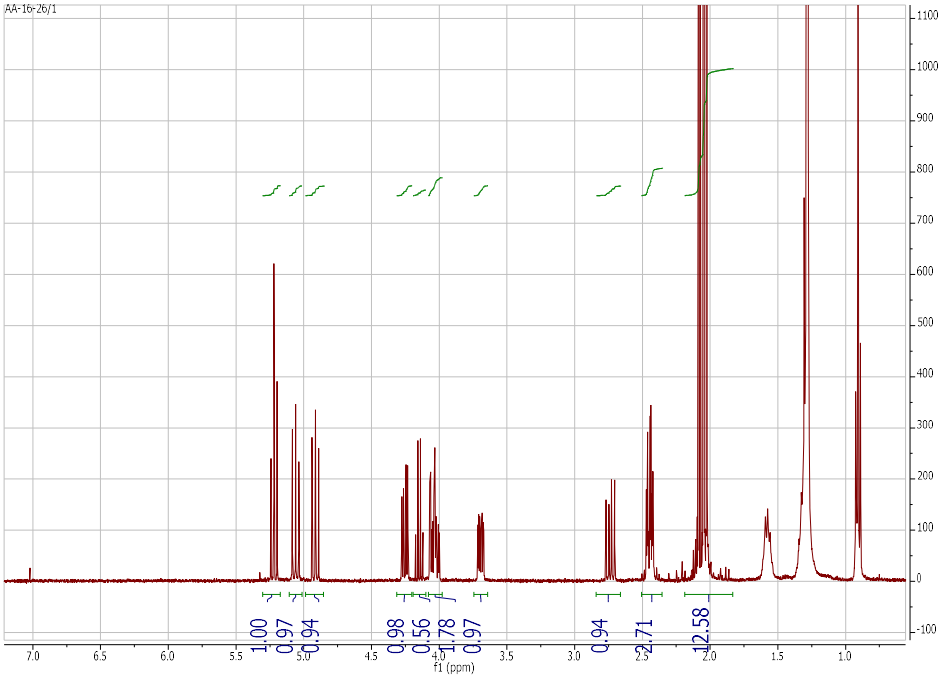


Figure 3: NMR spectrum for the acetyl protected ketone glycolipid. Note the loss of olefin peaks around 6 and 7 PPM which were present in figure 2.

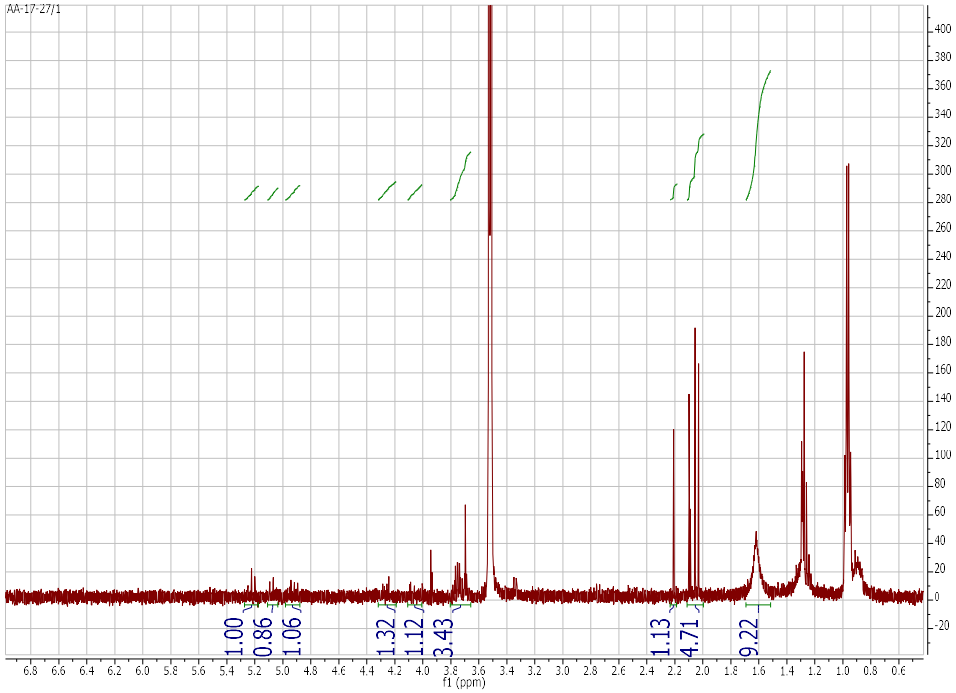


Figure 4: NMR spectrum of the acetylated alcohol glycolipid retains the presence of peaks around 5 PPM.

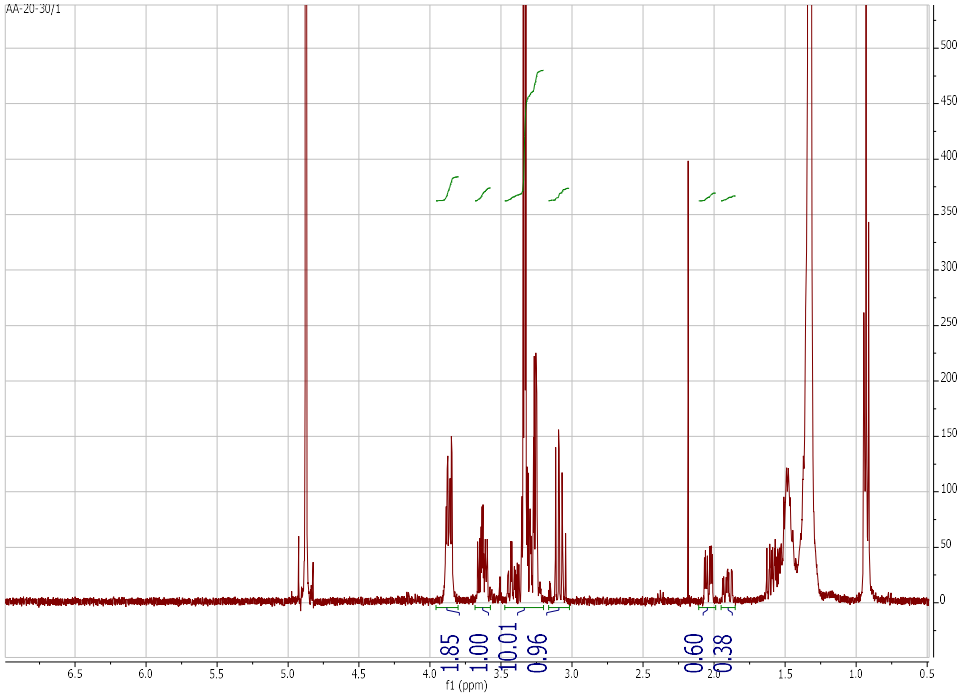


Figure 5: NMR spectrum of the deacetylated alcohol glycolipid. Notice the loss of peaks from acetyl protecting groups.

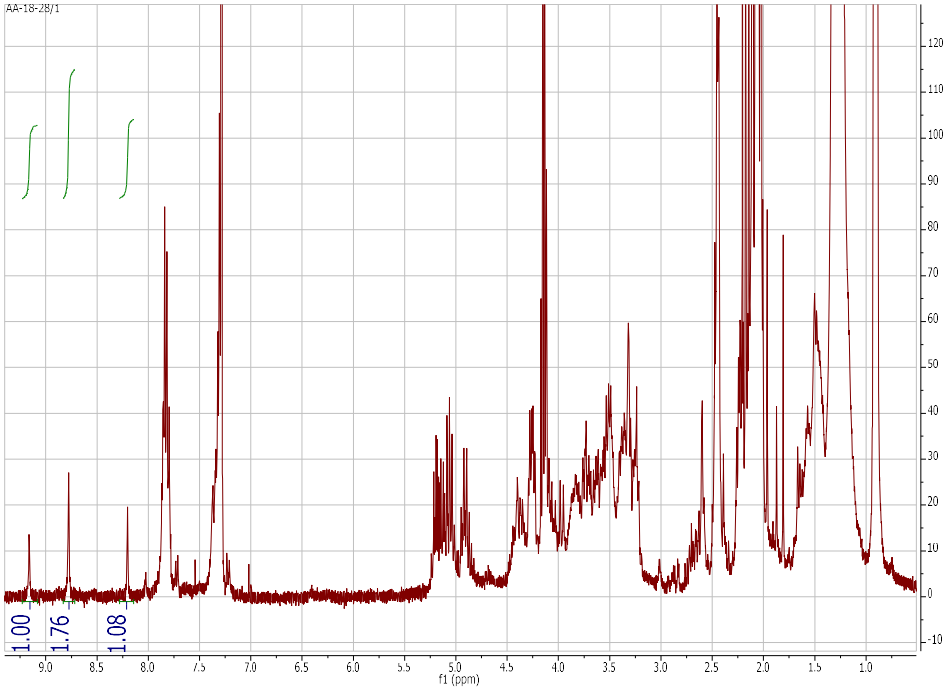


Figure 6: NMR spectrum of the acetylated alkyl glycolipid.

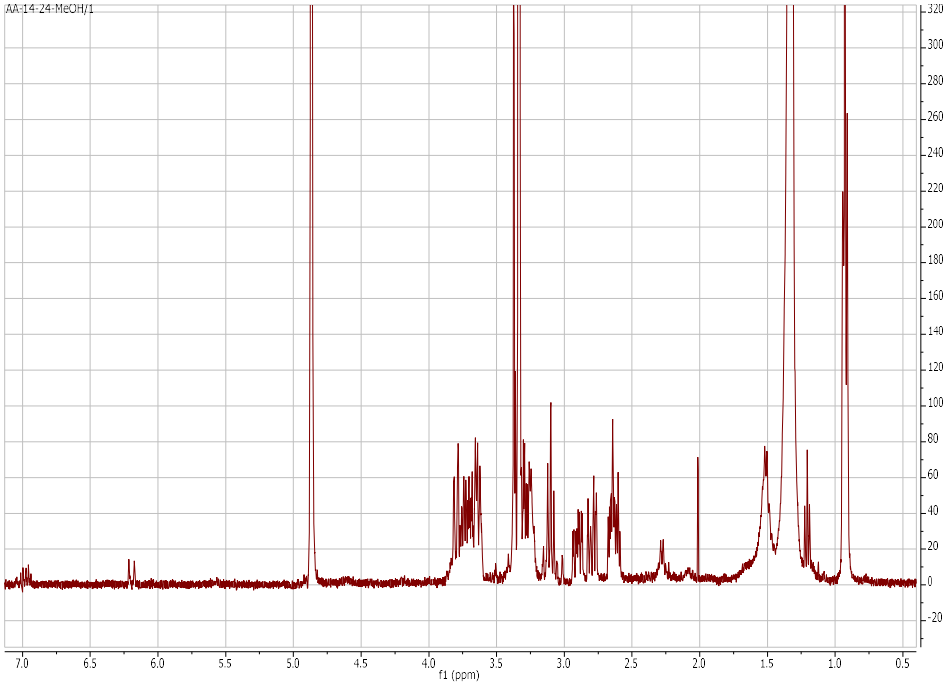


Figure 7: Spectrum for deacetylated enone glycoside using perdeuterated CD_3OD as the solvent to better dissolve sample and prevent micelle or reverse-micelle formation.

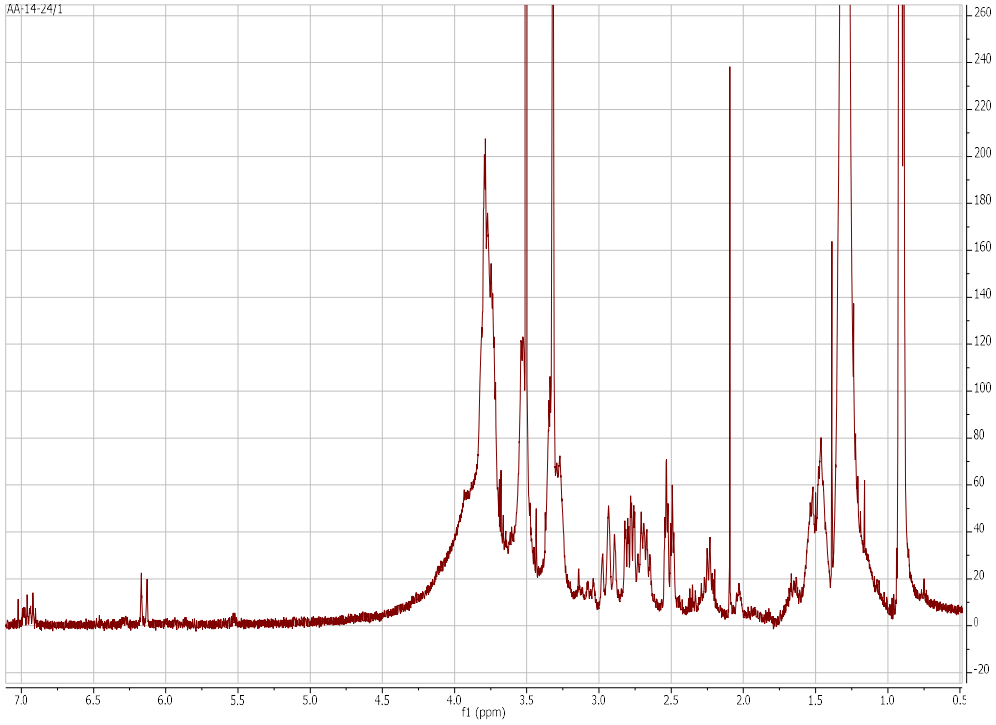


Figure 8: Spectra of the deacetylated enone glycoside taken in deuterated chloroform. Peaks show poor resolution likely due to the formation of micelles.

Discussion. With the successful synthesis of the C-12 Glycolipids, we will continue with making the C-n glycolipids of other chain lengths. We also will be investigating the S and O linked glycolipids. The next step with the C-12 Glycolipids is to determine the Critical Micelle Concentration. We will accomplish this by measuring the surface tension at different concentrations of glycolipid. By plotting this change in surface tension, we can determine when glycolipids are forming micelles rather than adhering to the surface. An example of this is shown using the C-8 glycolipid series in **Figure 9.** After these tests, we will be testing the ability of glycopeptides to incorporate into the micelles which could someday lead to drug delivery through micelles.

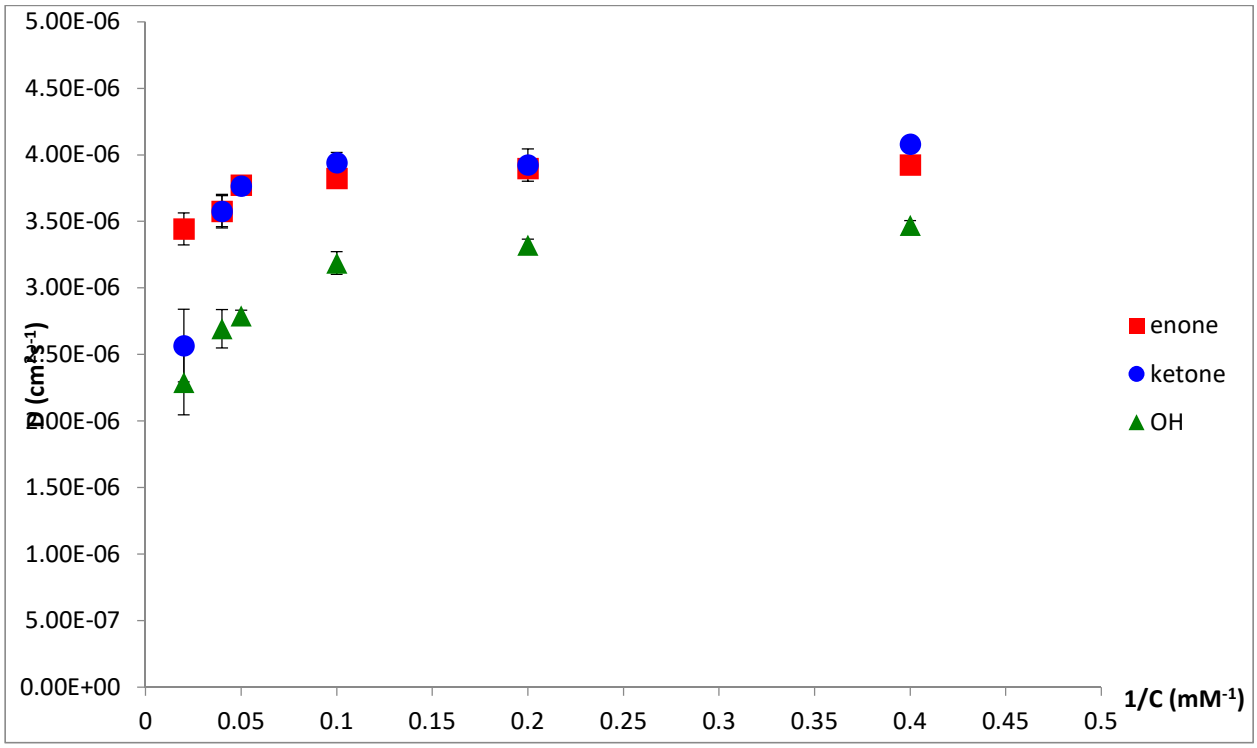


Figure 9: Measurements of the surface tension of the C-8 glycolipid series determined by Dillon Hanrahan. CMCs obtained for the compounds were as follows: Ketone: 19mM Enone: 9.2mM Alcohol: 13mM

References:

1. Crombez L, Morris MC, Deshayes S, Heitz F, Divita G. Peptide-based nanoparticle for ex vivo and in vivo drug delivery. Current pharmaceutical design. 2008;14(34):3656-65.
2. Bragnier N, Scherrmann M-C. One-Step Synthesis of β-C-Glycosidic Ketones in Aqueous Media: The Case of 2-Acetamido Sugars. Synthesis. 2005;2005(05):814-8.
3. Walters RM, Gandolfi L, Mack MC, Fevola M, Martin K, Hamilton MT, et al. In vitro assessment of skin irritation potential of surfactant-based formulations by using a 3-D skin reconstructed tissue model and cytokine response. Alternatives to laboratory animals : ATLA. 2016;44(6):523-32.
4. Rösler A, Vandermeulen GWM, Klok H-A. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Advanced Drug Delivery Reviews. 2012;64, Supplement:270-9.
5. Subbiah N, Das TM. Facile one-pot synthesis of sugar-quinoline derivatives. Carbohydrate Research. 2009;344(8):1028-31.
6. Foley PM, Phimphachanh A, Beach ES, Zimmerman JB, Anastas PT. Linear and cyclic C-glycosides as surfactants. Green Chemistry. 2011;13(2):321-5.
7. Lalitha K, Muthusamy K, Prasad YS, Vemula PK, Nagarajan S. ChemInform Abstract: Recent Developments in β-C-Glycosides: Synthesis and Applications. ChemInform. 2015;46(14):no-no.
8. Pileni MP. Structure and reactivity in reverse micelles. 1989.
9. Cringus D, Bakulin A, Lindner J, Vöhringer P, Pshenichnikov MS, Wiersma DA. Ultrafast Energy Transfer in Water−AOT Reverse Micelles. The Journal of Physical Chemistry B. 2007;111(51):14193-207.