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Large scale synthesis of amphiphiles for biological use and analytical profile of polar extracts from mastic gum

Duane Joseph Mancini
University of Toledo

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A Thesis

entitled

Large Scale Synthesis of Amphiphiles for Biological Use and Analytical Profile of

Polar Extracts from Mastic Gum

by

Duane Mancini

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Medicinal Chemistry

_____
Paul W. Erhardt, Ph.D., Committee Chair

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Jeffery G. Sarver, Ph.D., Committee Member

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Ritesh Mittal, Ph.D., Committee Member

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Patricia Komuniecki, Ph.D., Dean
College of Graduate Studies

The University of Toledo

August 2014

An Abstract of

**Large Scale Synthesis of Amphiphiles for Biological Use and Analytical Profile of
Polar Extracts from Mastic Gum**

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This thesis includes many different production and research projects that can help make many positive advances in the pharmaceutical and biomedical area. The first part of my thesis describes my practical experience at Anatrace Products, LLC. Included in this chapter is the large scale synthesis of amphiphiles for biological uses. The second part of the thesis describes my attempt to build an analytical profile of polar extracts from mastic gum for the potential treatment of chronic obstructive pulmonary disease.

Acknowledgements

First off, I would like to thank my committee chair, Paul Erhardt, Ph.D. Dr. Erhardt is an unbelievable professor and was constantly there for me whenever I had any questions or concerns. He is always looking out for what is best for me and I couldn't ask anymore from him.

I would also like to thank my committee members, Jeff Sarver, Ph.D., and Ritesh Mittal, Ph.D. Jeff spent countless hours teaching me everything I know about HPLC, and considering I knew nothing about HPLC work going in must have been a frustrating task. Ritesh who answered every single one of my questions about synthesis and mechanisms no matter how busy he was at work.

Lastly, but certainly not least, I would like to thank my fiancé Emily, my mother and father, and my sister Gina for all the love and support over my entire college career.

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List of Schemes

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List of Abbreviations

ACN ....Acetonitrile
AgOTf……………….Silver trifilate

BzCl…………………Benzoyl Chloride

CMC………………...Critical micelle concentration
COPD……………….Chronic obstructive pulmonary disease

DCM………………...Dichloromethane
DMF………………...Dimethylformamide

ETA………………....Ethanolamine

HBr………………….Hydrogen bromide
HCl…………………..Hydrogen chloride
HPLC……………….High performance liquid chromatography

LAH………………...Lithium aluminum hydride

MeOH………………Methanol

NaH…………………Sodium hydride
NG…………………..Neopentyl glycol

PDA………………...Photodiode Array
PPE…………………Personal protective equipment

QC…………………..Quality control

TEA………………...Triethylamine
TLC………………...Thin layer chromatography

UT……………….….University of Toledo

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434 W. Dussel Drive, Maumee, OH, 43537

Chapter 1

Large Scale Synthesis of Amphiphiles for Biological Use

1.1 Introduction

This portion of my thesis was a practicum experience at a company named Anatrace Products, LLC. (434 W. Dussel Dr. Maumee, OH 43537). Anatrace specializes in the synthesis of high-purity biological detergents and lipids. The always-advancing field of membrane protein science is due in large part to advances in tools and reagents used to manipulate the proteins. The detergents at Anatrace are used for protein extraction, purification and manipulation. Due to the complexity of dealing with proteins, a detergent that is used for the extraction may not be compatible for the purification or manipulation step. Therefore, Anatrace offers many different classes of detergents and many different types of detergents within those classes. I had the opportunity to synthesize several different detergents during this practical training. The large scale synthesis of these chemical reactions with an emphasis upon safety and quality control aspects proved to be a unique educational enhancement to my academic program. These are further discussed below.

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Complementing my studies at UT, I was able to gain experience in both synthetic and analytical chemistry from Anatrace. I had the opportunity to deploy techniques I learned previously on a small scale, within a large-scale synthetic environment. I also learned many new synthetic procedures and performed them on a large scale as well. Considering how the large scale reactions were run, it was very important to practice the absolute best safety and lab techniques possible. Normally minor mishaps can easily become magnified under these large scale conditions. During this time I also looked for ways to improve the final yields and when possible scale some reactions to even larger levels. The analytical portion of my experience at Anatrace also proved to be very useful. I was able to use techniques such as thin-layer plate chromatography (TLC) and column chromatography on a large scale. The opportunity to run high performance liquid chromatography (HPLC) in the analytical lab proved to be very practical because of my work with HPLC on mastic gum undertaken as part of my UT studies. For example, one benefit was that the Anatrace HPLC operating system was different, giving me the opportunity to learn and master two different HPLC operating systems during my thesis work. Finally, it was very convenient to learn the strict documentation for working in the industry and developing the habit of good documentation for all of my future technical work.

During my tenure at Anatrace, I was put in charge of synthesizing three different classes of amphiphiles. The first being the "Neopentyl Glycol (NG)" class of amphiphiles. This set of compounds is a very popular and new class that included three different detergents. The second class was "Fos-Choline", which included five different

compounds, all varying in chain length. The last class, which only has one compound, was named, "Cholesterol Hemisuccinate Tris Salt".

1.2 Amphiphiles in Membrane Protein Science

Membrane protein science is an increasingly advancing field and this is partly due to the advances in different amphiphiles. These amphiphiles are key players in membrane protein extraction, purification and manipulation. The chemical properties of the amphiphiles (Figure 1.1) allow them to interact and extract hydrophobic membrane proteins, while preserving the water-solubility of the protein outside their native bilayer environment. Unfortunately, solubility does not always translate to native structure and stability. Therefore, a detergent that is useful for extraction may not be compatible with purification or biochemical studies.
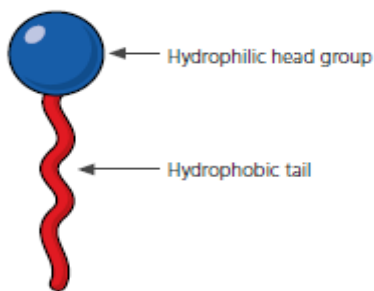
Figure 1.1. Amphiphile.

Amphiphiles interact with membrane proteins in a manner called micelles (Figure 1.2 and Figure 1.3). The process of surface active compounds forming non-covalent clusters in solution is known as micellization. The process of micellization is driven by the hydrophobic effect.[1] Micellization is a critical phenomenon when choosing the right detergent for an application. The critical micelle concentration, or CMC, is a key way to

characterize a detergent. CMC is defined as the concentration of detergent above which monomers self-assemble into non-covalent aggregates (micelles).[1,2,3] The CMC does not actually occur at a single concentration of detergent, but over a small range of concentration. It is important to have the total detergent concentration greater than the CMC to ensure all detergent monomers will go into micelles. If the total detergent concentration is below the CMC the detergent monomers are free in the bulk solution. Although the molecular details of how detergent micelles extract proteins from a membrane are still not completely understood, it is generally accepted that once a protein has been solubilized, the detergent molecules form a sphere around the hydrophobic transmembrane domains.[4]

Detergents are an essential component when working with membrane proteins. These amphiphiles offer unique characteristics that allow these compounds to partition into biological membranes, extract proteins, and maintain protein solubility in solution. These amphiphiles are also used in other applications including PAGE, inclusion body solubilization, and lipid raft preparation.
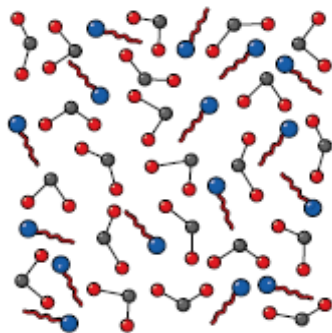
Figure 1.2. Proteins and detergents in solution.
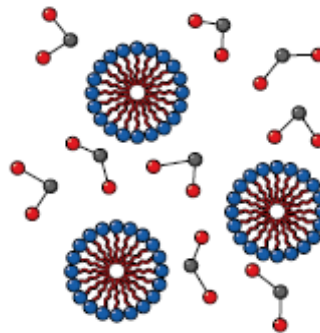
Figure 1.3 Micelles forming around proteins.

When a nonpolar group is introduced into an aqueous solution, the hydrogen bonding network formed by the existing water molecules is disrupted and the water molecules order themselves around the nonpolar entity to satisfy hydrogen bonds (Figure 2A). This

results in an unfavorable decrease in entropy in the bulk water phase. As additional nonpolar groups are added to the solution, they self-associate thus reducing the total water-accessible surface of the complex relative to the monodisperse state. (Figure 2B) Now, fewer water molecules are required to re-arrange around the collection of nonpolar groups. Therefore, the entropy associated with the complex is less unfavorable than for the monodisperse detergents.[5]

1.3 Neopentyl Gycol (NG)

The Neopentyl Glycol (NG) class of amphiphiles is a novel class of surfactants that began making a significant impact in the field of membrane protein research in late 2010.   This class of molecules has been utilized in the crystallization of several biologically important proteins such as the β_2 adrenergic receptor-GPCR complex,[6] the rhodopsin-transducin complex,[7] and the membrane component Alkβ.[8] The NG class of surfactants encompasses, but is not limited to, lauryl maltose neopentyl glycol (LMNG), octyl glucose neopentyl glycol (OGNG) and decyl maltose neopentyl glycol (DMNG). These molecules are beneficial in membrane protein studies due to unique properties conferred by their revolutionary architecture. The general structure consists of a central quaternary carbon with two hydrophilic heads and two lipophilic tails, generating subtle constraints on overall conformational flexibility. This allows the molecule to pack densely when forming a micelle and significantly enhance membrane protein extraction efficiencies and overall stabilization profile. Additionally, the NG class has been shown to produce extremely low critical micelle concentrations (CMC) while retaining significant water solubility.

The NG class of amphiphiles is the second generation of products for three of the most popular detergents: lauryl maltoside, octyl glucoside and decyl maltoside. An advantage of the new NG class is the decrease in amount of surfactant needed to achieve the same CMC. To achieve the same CMC, approximately 17-fold less of the NG class surfactant is needed when compared to the relevant monomeric derivatives of maltose and glucose. Moreover, membrane proteins surrounded by NG micelles can often be purified away from excess free surfactant using size-exclusion chromatography techniques. The unique property suggests that NG micelles do not rapidly interchange like traditional micelles do and that in fact these compounds may be more aptly defined as water soluble lipids.

1.3.1 Synthesis of LMNG and DMNG

The syntheses of 2,2-didecylpropane-1,3-bis-β-D-maltopyranoside (LMNG, **10**) and 2,2-dioctylpropane-1,3-bis-β-D-maltopyranoside (DMNG, **11**) make use of many popular synthetic strategies at a kilogram scale (scheme 1.1). Both of these amphiphiles

a. NaH, DMF, 0°C; **b.** LAH, THF, 0°C; **c.** 33% HBr in AcOH, DCM, -10°C;
d. AgOTf, 4A MS, ACN, DCM, -20°C > RT; **e.** NaOMe, MeOH
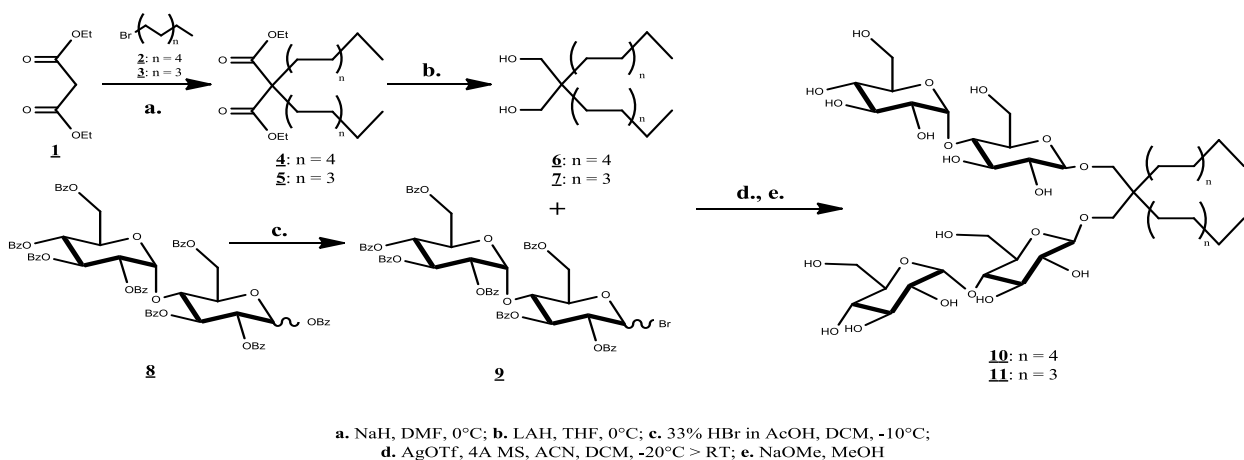
Scheme 1.1. Synthesis of LMNG and DMNG.

have a similar structure, containing two maltose sugars and two long carbon chains. The only difference between LMNG and DMNG is the length of the carbon chains, twelve and ten respectively. The synthetic scheme for these products begins with a deprotonation of the acidic carbon on diethyl malonate (**1**) using sodium hydride. Once this is accomplished, the carbon is mono-alkylated with its appropriate bromoalkane (**2**, LMNG; **3**, DMNG) to produce a transient intermediate which is again deprotonated and, in turn, alkylated for a second time. Dialkylated diesters (**4**, LMNG; **5**, DMNG) are then no longer reactive under these conditions. This is a dangerous reaction to perform because of the amount of sodium hydride (400 g) being used to effect two deprotonations on large scale. Sodium hydride can ignite in air, especially when reacting with water causing the release of hydrogen gas. Thus, it is very important to always handle this reagent with extreme care. Reduction of the diesters in **4** and **5** using lithium aluminum hydride (LAH) produces the diols (**6**, LMNG; **7**, DMNG). Like the previous step, this step also poses many safety precautions because of the reactivity of LAH (400 g). The reactivity in the presence of water is extremely violent and like sodium hydride, also has the possibility of igniting in air. Considering the solvent is tetrahydrofuran (THF), which can contain a small percentage of water, this is a dangerous reaction that requires proper personal protective equipment along with the slow addition of LAH to the THF. The initial additions of LAH react with residual traces of water in the THF. Subsequent additions are then less reactive but still must be exercised with care. Commercially available octabenzoyl maltose (MOB, **8**) is then bromonated at the anomeric carbon position to yield α-bromo-perbenzoyl maltose (BBM, **9**). This reaction carries many hazards associated with the volatility of the reagents and must be done with a half-face

respirator in a walk-in fume hood. The bromonation of MOB requires the addition of 1.2 L of 33% hydrogen bromide (HBr) in acetic acid and must be added extremely carefully because of the extreme toxicity of HBr. The product is stereoselective to alpha (80% - 90%) leaving only 10% - 20% beta formation. **6** (LMNG) or **7** (DMNG) is then combined with **9** to yield the final desired product of LMNG (**10**) or DMNG (**11**). During the first part of this process, it is essential to make sure that moisture does not interfere with the reaction because of the use of silver triflate (AgOTf) which can be quenched if it comes in contact with water. AgOTf plays many roles in this reaction, the first being the binding of the bromine atoms to the silver and precipitating out as a salt. This is important because removal of the bromine ions can help to drive the reaction to completion. It is useful to have the trifilate because it is an excellent leaving group. This is important because the addition of the diol to the BBM needs to happen quickly to enhance *beta*-stereochemistry. AgOTf is used in this reaction as a Lewis acid and is required for the coupling of the respective diol (**6**, **7**) and BBM (**9**). The acetonitrile also plays an important role, not just as a solvent for the AgOTf, but also to diminish formation *alpha*-stereochemistry. To ensure complete dryness, 4Å molecular sieves are added, and the reaction is done under argon. The stepwise addition of BBM to the reaction mixture helps to prevent the formation of monomer. The low temperatures (-20 °C) also help to aid the stereoselective *beta* formation over *alpha*. After the coupling has been completed, the benzyl protecting groups are removed from the sugar moieties by using sodium methoxide and the compound is then ready for final purification. The purification of these compounds requires the use of a twenty kilogram, C18 column (6" x

8

96"). Using a MeOH and water gradient, the products are collected with a 20 L round-bottom flask so that MeOH can then be removed on an industrial-sized rotovap. Once
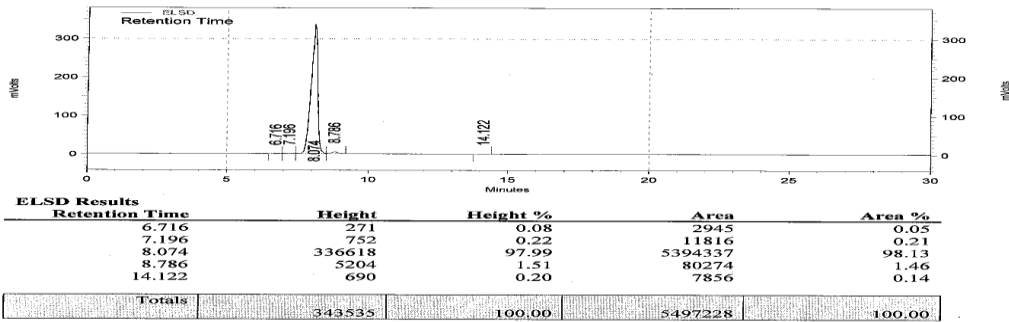
Figure 1.3. HPLC Chromatogram of LMNG.
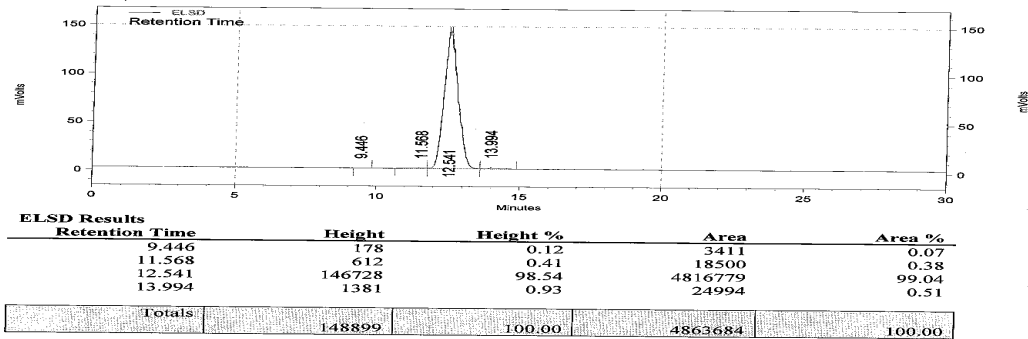
Figure 1.4. HPLC Chromatogram of DMNG.

concentrated in just water, the final compounds (**10**, **11**) are ready for lyophilization. After lyophilization analytical characterizations are performed by high performance liquid chromatography (HPLC) (Figures 1.3 and 1.4). In order for each product to pass quality assurance and be dispensed, it must be greater than 98% pure by HPLC.

1.3.2 Synthesis of OGNG

The synthesis of 2,2-dihexylpropane-1,3-bis-β-D-glucopyranoside (OGNG, **18**) is

a. NaH, DMF, 0°C; b. LAH, THF, 0°C; c. BzCl, Pyridine, -20°C > RT; d. 33% HBr in AcOH, DCM, -10°C;
e. AgOTf, 4A MS, ACN, DCM, -20°C > RT; f. NaOMe, MeOH
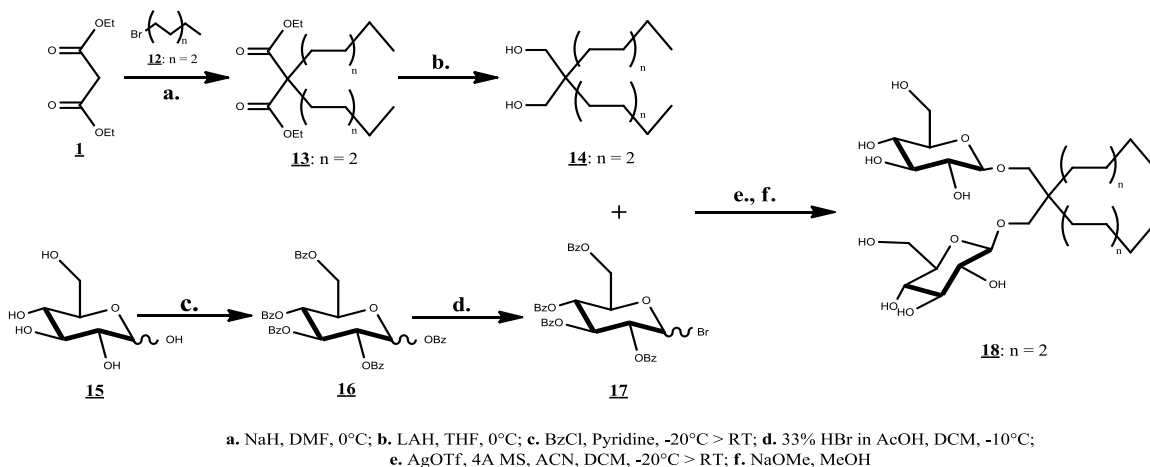
Scheme 1.2. Synthesis of OGNG.

very similar to the synthetic scheme used for LMNG and DMNG. The synthesis of

OGNG is shown in scheme 1.2 and differs in only a few areas when compared to LMNG

and DMNG. The first difference that is most evident is the use of a glucose molecule

instead of a maltose as the polar portion of the amphiphile. The second, is the difference

in the carbon length when compared to LMNG and DMNG. Another difference, is that

instead of using ACN as the solvent in the last step, the OGNG utilizes diethyl ether as

solvent. The final difference in synthetic schemes is the addition of another step. In

LMNG and DMNG, it is cheaper to purchase the already protected maltose molecule.

Alternatively for OGNG it is cheaper to perform the protection of glucose. The

protection of α-D(+)-glucose (**15**) to form penta-benzoyl glucose (GPB, **16**) makes use of

some very innovative chemistry procedures at a kilogram scale, some of which pose

safety hazards. One of the most concerning safety issues when performing this reaction

10

is the dangerous and toxic inhalation of benzoyl chloride (BzCl, 4 L) and pyridine (12 L). Due to the large amounts of these chemicals being used, it is important to make sure there is proper ventilation and that a half-face respirator is being worn while adding chemicals to the
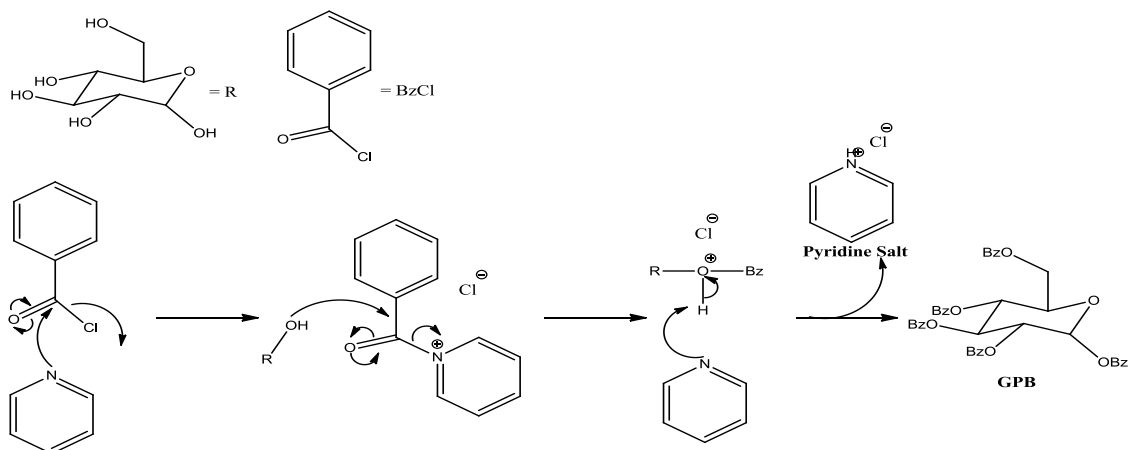
Scheme 1.3. Mechanism of GPB.

reaction. Despite pyridine's toxicity upon chronic exposers, it is ideal in this reaction because it has three different purposes. The pyridine acts as a solvent to solubilize the glucose, an activator of the benzoyl chloride so that the protecting group can be added to the free hydroxyls, and it serves as a base to form a salt with the free chlorines in the reaction mixture. The last two roles played by pyridine are depicted in scheme 1.3. Once the glucose has been protected, the rest of the synthetic scheme is the same as that for LMNG and DMNG. In order

Sample ID: OGNG26
Method Name: C:\CLASS-VP\Enterprise\Projects\Default\Method\70%MeOH_Ch.met
Data: C:\QC\2014\January 2014\OGNG26
Acquired: 1/4/2014 10:54:22 AM   Instrument: Chewbacca (Offline)   Printed: 5/19/2014 1:46:20 PM

ELSD Results

Retention Time	Height	Height %	Area	Area %
9.569	546	0.34	13014	0.28
12.714	546	0.34	12183	0.26
14.131	160394	98.62	4621747	99.38
16.324	1147	0.71	3647	0.08
Totals	162633	100.00	4650591	100.00

Figure 1.5. HPLC Chromatogram of OGNG.

for OGNG to pass quality assurance it must be greater than 98% pure by HPLC peak area (Figure 1.5), the same as LMNG and DMNG.

1.4 Fos – Choline Detergents

The fos – choline line of detergents is another type of detergent that is primarily used for extraction of the protein from the membrane. This detergent, when compared to others offered at Anatrace Products, LLC., is typically a much harsher amphiphile making it good for the extraction of membrane proteins but not good for maintaining their structures. All of the fos – choline products carry the same base structure, only differing in carbon chain length. The base structure is a modified phosphorus oxychloride, that in the end yields a zwitter ion. This phospholipid-like detergent has been used in multiple studies including the study of direct binding of cholesterol.[9] While

I was at Anatrace, the synthesis of fos – choline 8 (Fos8), fos - choline 10 (Fos10), fos –

choline 12 (Fos12), fos – choline 13 (Fos13), and fos – choline 16 (Fos16) was

completed.

1.4.1 Synthesis of Fos – Choline Detergents

The synthesis of the fos – choline class of detergents posed a new set of safety

concerns while adding their related chemicals into the kilogram scale synthetic repertoire

which Anatrace encompasses. The procedure (Scheme 1.4) for n-octylphosphocholine

a. TEA, THF, -20°C; b. ETA, TEA, THF; c. AcOH/H₂O, Acetone; d. (CH₃)₂SO₄, K₂CO₃, DCM, H₂O/iPr
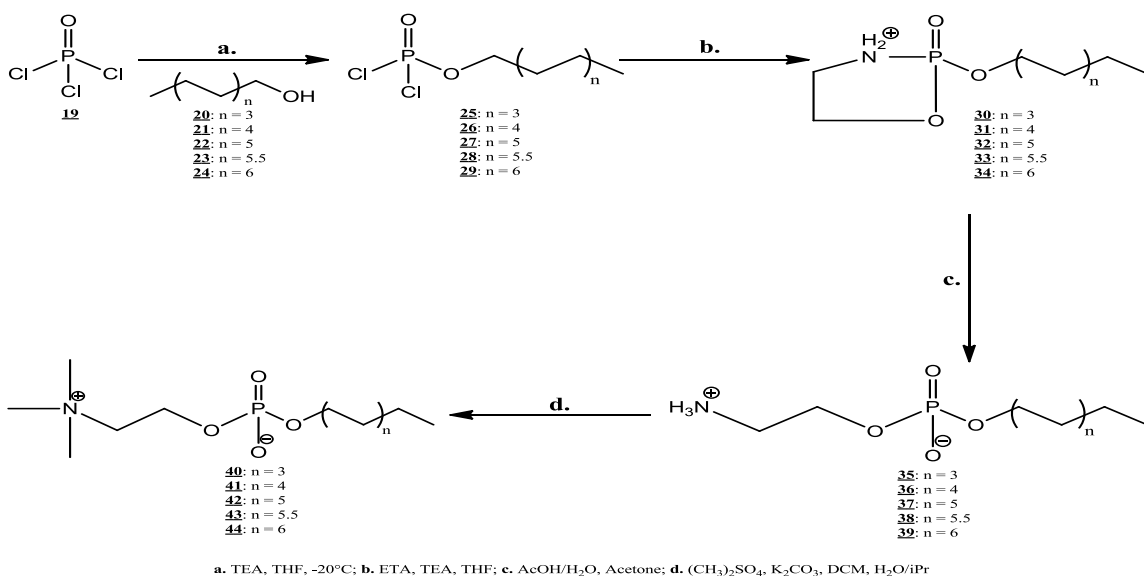
Scheme 1.4. Synthesis of Fos – Choline Detergents.

(Fos8, **40**), n-decylphosphocholine (Fos10, **41**), n-dodecylphosphocholine (Fos12, **42**),

n-tridecylphosphocholine (Fos13, **43**), and n-hexadecylphosphocholine (Fos16, **44**) are

all the same with the exception of the specific alcohols (**20 - 24)** added in the first step.

The synthesis begins with commercially available phosphorus oxychloride (**19**) in THF

and the addition of the desired alcohol (**20 - 24)** for the corresponding fos – choline

product. The addition of triethylamine (TEA) is important to quench the HCl formed during the reaction. The result of this reaction provides attachment of the proper length carbon chain to the phosphorus oxychloride (**25 - 29**). In the next step, ethanolamine is added to the reaction and forms the cyclic intermediate **30 - 34**. Ethanolamine is a very unique molecule because both of its ends can be nucleophilic. In this reaction, both of the nucleophilic ends attack the same phosphorus and displace the two remaining chlorine atoms. The nitrogen end of the ethanolamine is more nucleophilic than the oxygen, so one might think that a nitrogen from another ethanolamine might displace the third chlorine.   However, because intramolecular reactions are favored over intermolecular, the oxygen more quickly displaces the other chlorine atom. The chlorine atoms form HCl salts with TEA that is added to the reaction, similar to the previous step. The addition of water and acetic acid starts off the next step to break the phosphorus – nitrogen bond, not the oxygen – phosphorus bond, resulting in the structures **35 - 39**. The reason that the phosphorus – nitrogen bond is broken and not the phosphorus – oxygen bond is because the phosphorus – oxygen bond is stronger than the phosphorus – nitrogen bond.[10] These intermediates are taken to the next step where the use of dimethyl sulfate adds three methyl groups to produce the quaternary ammonium products. The addition of potassium carbonate neutralizes the reaction so that the dimethyl sulfate can continue to donate a methyl group to the nitrogen atom. Once three methyl groups are donated to the nitrogen the reaction is complete and Fos8 (**40**), Fos10 (**41**), Fos12 (**42**), Fos13 (**43**), and Fos16 (**44**) are produced. These final compound mixtures are filtered, concentrated to just water, and subjected to lyophilization. The products are moved to quality assurance

where they must be greater than 99% purity via HPLC testing to pass (Figure 1.6). This

synthetic

Sample ID: FOS8-17
Method Name: C:\CLASS-VP\Enterprise\Projects\Default\Method\55%MeOH_Ch.met
Data: C:\QC\2014\January 2014\FOS8-17.dat
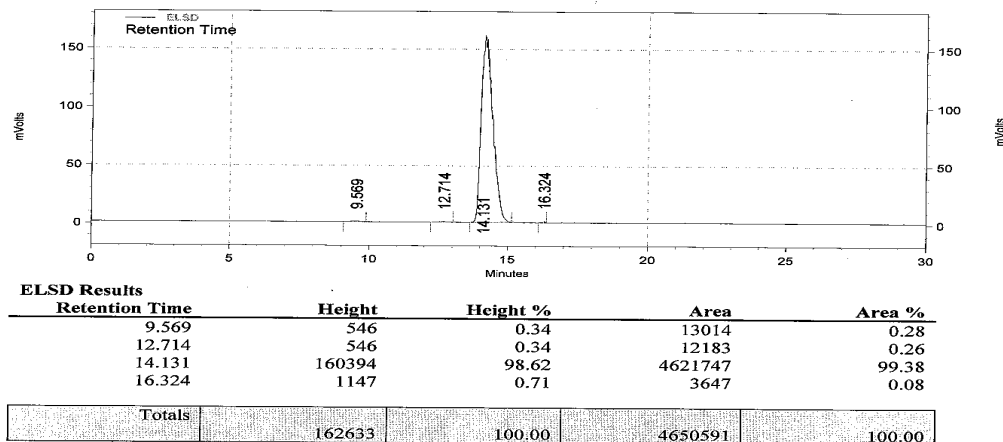Acquired: 1/4/2014 8:25:36 AM   Instrument: Chewbacca (Offline)   Printed: 5/19/2014 1:46:58 PM

ELSD Results

Retention Time	Height	Height %	Area	Area %
5.388	548	0.34	10965	0.22
7.485	160709	98.30	4880667	99.64
9.371	919	0.56	2667	0.05
9.728	594	0.36	1309	0.03
10.528	725	0.44	2815	0.06
Totals	163495	100.00	4898423	100.00
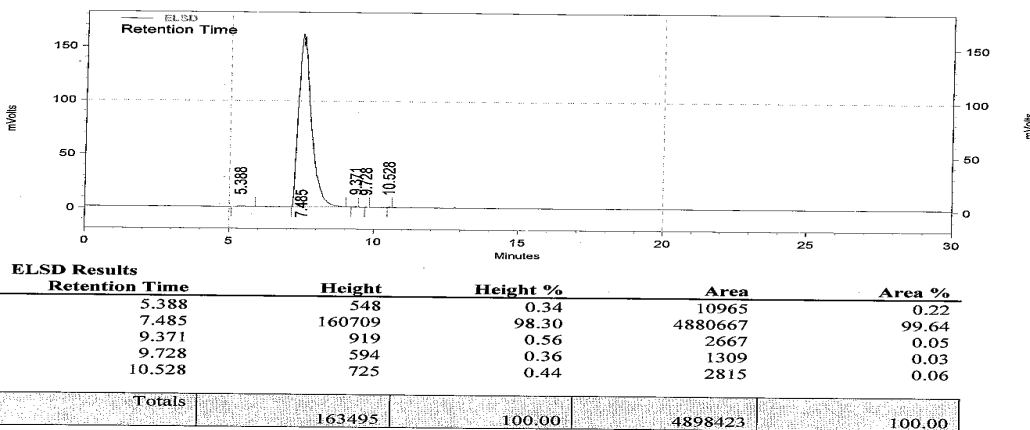
Figure 1.6. HPLC Chromatogram of Fos 8.

scheme poses two very hazardous situations, the use of phosphorus oxychloride at the

onset and dimethyl sulfate during the final stage. Phosphorus oxychloride (448 mL) is

corrosive and can cause acute toxicity if the vapors are inhaled. For this reason, when

working with this chemical it is important to wear a half-face respirator and make sure

the chemical is used underneath the hood when the cap is off. Dimethyl sulfate (900 mL)

poses many hazards as well because it is carcinogenic, highly poisonous, corrosive,

environmentally hazardous and presents an inhalation problem as well. Dimethyl sulfate

is so corrosive it is considered a chemical weapon. There is no distinct odor for dimethyl

sulfate, making it all the more viciously dangerous when dealing with this chemical.

Extreme caution is required while wearing proper personal protective equipment (PPE).

Proper PPE entails a lab coat, gloves, a respirator, eye glasses and a face shield.

1.5 Cholesteryl Hemisuccinate Tris Salt (CHS)

Cholesteryl hemisuccinate tris salt (CHS) consists of succinic acid esterified to the L-hydroxyl group of cholesterol and a trizma base molecule.[11] CHS is considered a lipid-cholesterol by Anatrace Products' definition. The goal when originally making CHS was to make a cholesterol molecule more soluble. In doing so, this made it more of a lipid which is why it is classified under the lipid-cholesterol section of Anatrace's website. The reason for the use of trizma base is to help form the salt and add polarity to the molecule to make it more water soluble. CHS is another one of Anatrace's popular products and is used in a wide variety of studies.

1.5.1 Synthesis of Cholesteryl Hemisuccinate Tris Salt (CHS)

CHS is one of the more difficult Anatrace products to synthesize at a large
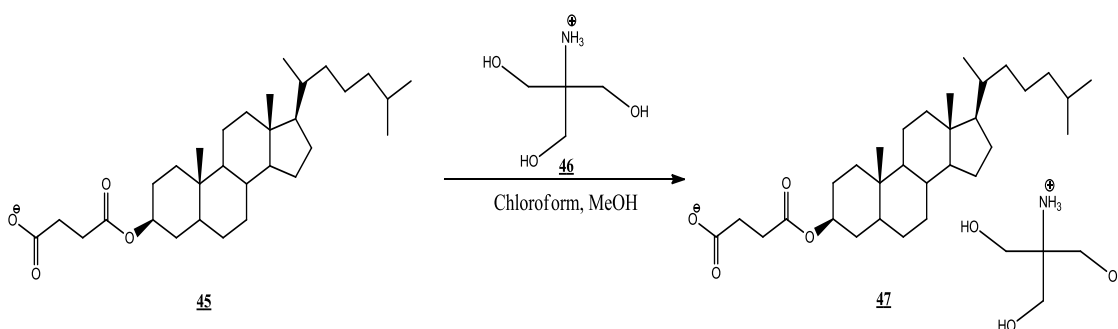
Scheme 1.5. Synthesis of CHS.

scale, not because of the number of steps (Scheme 1.5), but because of its hygrosopicity and the amount of moisture CHS absorbs through either air or solvent. The synthesis of this molecule on paper appears to be very easy, simply forming a salt. The difficult part

begins during the purification of this product. After the initial reaction, the product salts out and is filtered through a fritted funnel. During filitration, a huge problem arises because of the thickness of CHS. CHS will quickly sink to the bottom and often slow filtration down significantly. The difficult part about this is ensuring dryness because the CHS has absorbed so much methanol and chloroform. Once dry from all solvents, acetone is added to the CHS to solubilize the impurities (starting material) and leave the CHS because it is not soluble in acetone. After stirring for a day, the CHS is ready to be filtered through a fritted funnel. Once the CHS is dry, it is ready to be crushed up and stored in a vacuum oven until it is ready for QC testing. QC is performed by an outside third-party contractor.

1.6 Equipment Used

One of the more unique skills developed during my time at Anatrace, was operation and care of the kilogram scale equipment. There were many opportunities for me to use equipment at a much larger scale than that used in laboratory classes during my undergraduate and graduate academic lab time. The pictures below portray some of the equipment being used and include: 20 L rotovap (Picture 1); 20 L separatory funnel (Picture 2); and a 12" x 96" C – 18 column (Picture 3).

Picture 1. 20 L rotovap flask and industrial rotovap
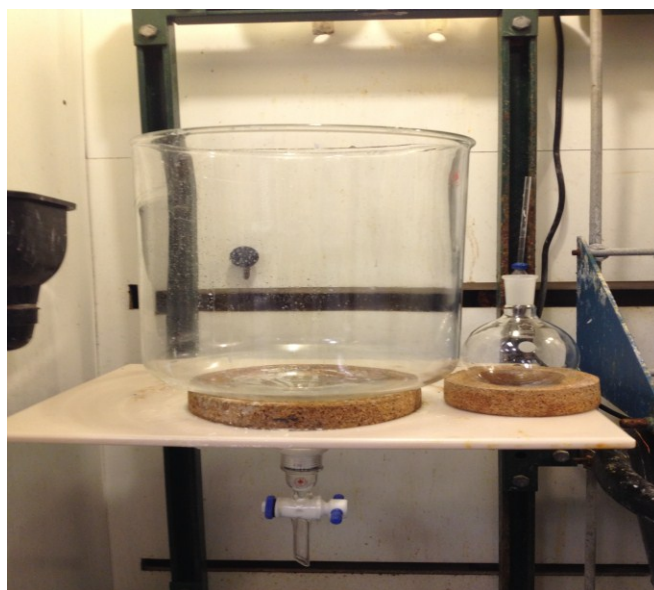
Picture 2. 20 L separation funnel vs. a 500 mL round-bottom flask

Picture 3. 8" x 96" C18 Column with a large scale fraction collector

1.7 Summary

The practicum experience at Anatrace proved to be a important and unique learning experience. I learned how to effectively contribute as a participating member on industry-style synthesis team which have common but individually assigned goals that need to be accomplished within strict timeframes due to their interdependence. I was able to master practical techniques and how to deploy specific equipment associated with conducting process chemistry reactions at a scale common to industrial settings but typically not found in academic labs. I learned how to manipulate large volumes of solvents and safely deploy extremely dangerous and toxic reagents on large scale. I reinforced my fundamental knowledge about several key organic reactions and their mechanisms by applying them in a very practical manner during multiple-step synthetic schemes. Also furthered my knowledge in the area of NMR spectroscopy as applied to

sugar molecules and their derivatives. Lastly, I learned how to efficiently merge final purification techniques with industry-standard quality control measures that insure product integrity, including exposure to an HPLC system different from that used in my academic lab experience.

For Anatrace, I was able to suggest practical modifications for the synthesis of LMNG, DMNG, and OGNG. I helped to strengthen valuable relationships between Anatrace and UT. The dollar amount of the number of products I synthesized for Anatrace was about 2 million dollars.

1.8 Future Studies

Anatrace Products, LLC. continues to explore the development and discovery of new amphiphiles to be utilized as tools that can improve membrane protein studies. With the increasingly growing field, it is important for Anatrace as a company to explore new compounds. It is also important to continue to look for ways to reduce the cost of product syntheses. Anatrace will continue to look for ways to optimize the synthesis of products to improve costs.

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Chapter 2

Analytical Profile of Polar Extracts from Mastic Gum

2.1 Introduction

This portion of my thesis took place at the Center for Drug Design and Development (CD3) at the University of Toledo (UT) in collaboration with Drs. James Willey and Michael Apostolis within UT's College of Medicine and Life Sciences. The project was based around a natural material called Chios mastic gum (*Pistacia lentiscus var. chia*) which among other properties is thought to have antimicrobial activity.[12] 'Mastic' is a white, semitransparent, natural resin that is obtained as a trunk exudate from mastic trees.[12] Mastic tree is an evergreen bush indigenous to the southern part of the Greek island called Chios. This natural product has found extensive use in the pharmaceutical and nutritional supplement industry. One of its major therapeutic applications has been as a treatment for hepatic inflammation.

The therapeutic indication that we are interested in is for the potential treatment of chronic obstructive pulmonary disease (COPD). COPD is characterized by chronically poor air flow. The biggest cause of COPD is tobacco smoking, but other factors include genetics and air pollution. COPD is a very deadly disease, ranking third in the leading

cause of death in America. Effective treatments for COPD are still to be identified and thus this area presently constitutes an unmet medical need.[13] Dr. Michael Apostolis working with Dr. James Willey suspects that there may be specific components within 'mastic' that have anti-inflammatory properties. Furthermore, he has proposed that these components may be amenable to a formulation that can potentially be applied toward a useful treatment of COPD.

2.2 Previous Characterization of Mastic Gum

Mastic gum has been analyzed previously and its chemical composition determined by researchers at the Univeristy of Warwick in the United Kingdom.[14] For those studies the composition of mastic gum was studied by gas chromatography using mass spectrometry for detection (GC-MS). Numerous components were identified that are amenable to this technique, particularly non-polar and volatile materials. These components include the well-known *alpha*-pinene and *beta*-myrcene isoprenaline-derived natural products as two of the more prominent materials (Table 2.1). As indicated, *alpha*-

Table 2.1. Chemical Composition of Mastic Gum.[12]

compound	RI^b	identification methods^c	percentage (%)^a					
			mastic gum	mastic oil	fraction 1	fraction 2	fraction 3	fraction 4
octyl formate	923	MS	—^d	tr^e	tr	tr	tr	—
tricyclene	926	MS	—	0.1	—	—	—	—
α–pinene	939	GC–MS	40.9	63.3	66.3	52.6	2.4	3.1
camphene	953	MS	1.0	0.6	1.2	1.4	tr	—
sabinene	976	MS	0.3	0.4	0.8	1.5	0.1	0.2
β-pinene	980	GC–MS	1.7	3.3	5.4	8.8	0.6	1.2
β-myrcene	991	GC–MS	9.0	25.0	13.1	21.6	7.8	17.0
methyl-o-cresol	1009	MS	0.3	0.6	1.0	1.8	0.7	1.4
p-cymene	1026	GC–MS	—	0.1	0.1	0.2	0.1	0.3
limonene	1031	GC–MS	0.8	1.5	1.7	3.2	1.8	3.8
(Z)-β-ocimene	1040	MS	—	tr	tr	0.1	—	—
(E)-β-ocimene	1050	MS	—	tr	0.1	0.1	tr	0.2
α-terpinolene	1087	MS	—	tr	—	—	—	—
α-pinene epoxide	1095	MS	—	—	1.4	1.4	4.2	7.7
linalool	1098	GC–MS	0.8	0.5	0.4	0.3	2.7	3.2
perillene	1099	MS	0.8	0.5	0.8	0.8	2.6	4.3
cis-verbenol	1100	MS	—	0.1	0.4	0.4	3.5	5.2
α-campholene aldehyde	1126	MS	0.3	0.1	0.1	0.2	0.6	0.9
trans-pinocarveol	1139	MS	—	0.1	0.2	0.2	2.3	2.3
trans-verbenol	1143	MS	0.5	0.3	0.8	0.8	9.6	10.4
β-pinene epoxide	1156	MS	—	—	tr	tr	0.5	0.5
myrtenal	1193	MS	0.5	0.1	0.2	0.2	1.7	1.9
α-terpineol	1180	GC–MS	—	tr	—	—	—	—
myrtenol	1188	MS	—	tr	—	—	—	—
verbenone	1189	GC–MS	0.8	0.1	0.4	0.4	6.5	6.1
trans-carveol	1217	MS	—	tr	tr	tr	0.3	0.1
dihydrocarveol	1226	MS	—	—	—	—	0.2	0.2
neral	1240	MS	—	0.1	—	—	—	—
linalyl acetate	1257	MS	—	tr	tr	tr	0.5	0.2
(E)-anethole	1283	GC–MS	tr	0.1	tr	tr	0.5	0.4
α-fenchyl acetate	1290	MS	—	tr	—	—	0.2	0.1
neryl acetate	1365	MS	—	—	0.2	0.2	1.3	1.7
α-copaene	1381	MS	—	tr	—	—	0.2	0.1
β-caryophyllene	1419	GC–MS	5.3	0.9	0.1	0.1	2.5	2.1
α-humulene	1454	MS	0.1	0.1	—	tr	0.4	0.2
(E)-methylisoeugenol	1500	GC–MS	0.9	tr	—	—	0.4	0.1
(Z,Z)-farnesol	1713	MS	11.9	0.1	—	—	6.3	3.2
(E,Z)-farnesol	1742	MS	0.1	tr	—	—	1.8	1.6

pinene was the most abundant of all the chemicals, (Z,Z)-farnesol and *beta*-myrcene also making up a large portion of the composition. While this represents a thorough characterization of the non-polar and reasonably volatile materials present in 'mastic', there is still a need to characterize or fingerprint materials that may be highly polar and have larger molecular weights. An ideal way to approach the latter is to deploy high performance liquid chromatography (HPLC) using a reverse phase column.[14] By using a reverse phase column, more polar materials are eluted earlier and this can provide for better resolution of their chromatographic peaks.

2.3 Source of Mastic Gum

The 'mastic gum' was obtained through Mastha Therapy in capsule form with the help of Dr. Michael Apostolis.
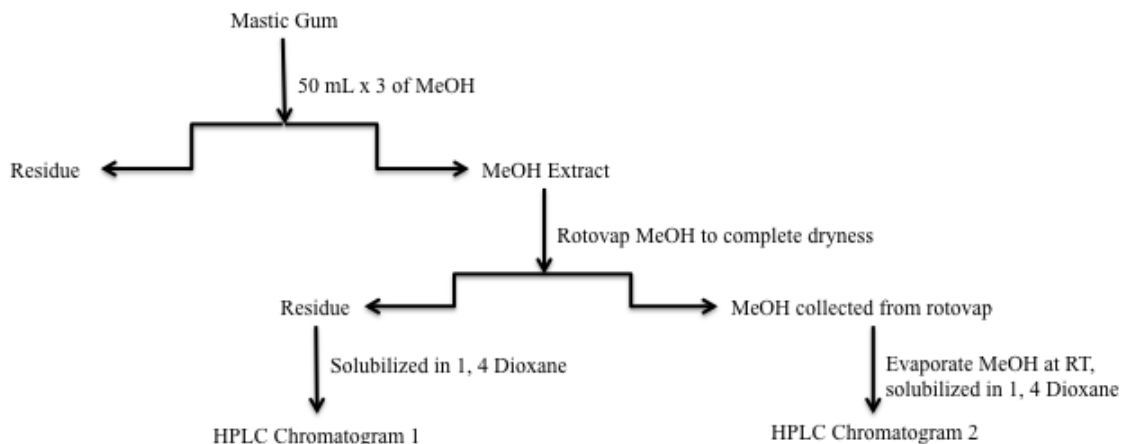
2.4 Equipment Used During Analysis

In our experiments mastic gum was analyzed on a Waters 2695 HPLC system equipped with a photodiode array (PDA) detector. The PDA detector measures absorbance from 200 nm to 700 nm, thus allowing the capability to see all compounds that will absorb light between those wavelengths. The column used was a reverse phase C18 column.

2.5 Polar Extraction of Mastic Gum and Analysis by HPLC

The immediate goal of this project was to obtain a robust extract of mastic gum and fingerprint the extraction by using reverse phase HPLC analysis. In general, polar solvents tend to extract a broader range of natural product constituents than do non-polar solvents. Our initial thought was to try using water as a solvent for the extraction to see how much of the mastic gum and certain of its constituents would be removed. However, water actually clumped the already powdered mastic gum into a solid residue that

rendered it a poor surface for extraction. Methanol (MeOH) was the next polar solvent to be examined. MeOH worked extremely well, taking up most of the mastic gum and only

Mastic Gum

50 mL x 3 of MeOH

Residue ←   → MeOH Extract

Rotovap MeOH to complete dryness

Residue ←   → MeOH collected from rotovap

Solubilized in 1, 4 Dioxane

Evaporate MeOH at RT, solubilized in 1, 4 Dioxane

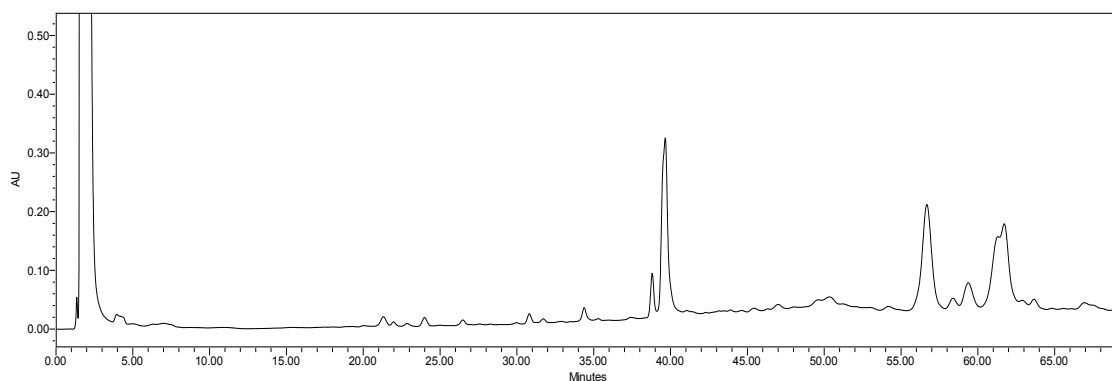HPLC Chromatogram 1   HPLC Chromatogram 2

Scheme 2.1. Mastic gum extraction procedure.

leaving behind a small amount of non-polar residue. The extraction consisted of using 50 mL of MeOH three times (Scheme 2.1). Room temperature and reflux conditons were examined for 15 minute periods. Both conditions provided the same result and so the more convenient room temperature procedure was adopted. The MeOH extracts were originally used directly for analysis by HPLC. These chromatograms turned out to be extremely difficult to fingerprint because MeOH absorbs at 200 nm detector wavelength which is where several of the mastic gum extraction materials appear to also absorb. With this being an issue, it was decided to rotovap the MeOH extracts to complete dryness and solubilize their residues in 1,4-dioxane, itself another type of reasonably polar solvent. Evaporation was achieved under water aspirator vacuum while warming the flask at no more than 25 °C so as to prevent the loss of volatile components. After taking them up in 1,4-dioxane the samples were analyzed *via* a C18 HPLC
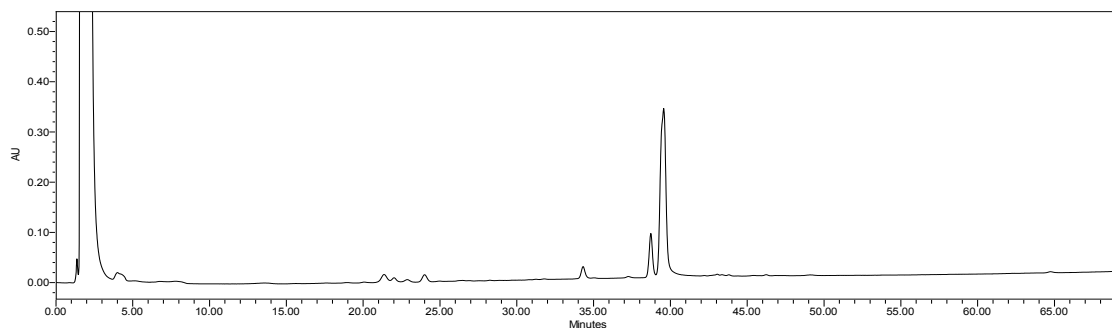
Figure 2.1 1,4-Dioxane blank.

Figure 2.2 MeOH extract (Chromatogram 1).

column. Figure 2.1 and 2.2 are representative of the results. Initially a gradient mobile phase was used (5% ACN and Water to 95 % ACN and water) but when used with MeOH injections this proved to give chromatograms with large drifts of the baseline throughout the chromatogram. This problem was not observed when 1,4-dioxane was used for injections. Even though the overall run time is rather long (70 minutes) the chromatograms have provided clear excellent baseline stability and separations of multiple peaks. Overall, this provided an excellent HPLC fingerprint for the MeOH extract of mastic gum that identified several distinct peaks (Figure 2.2). In particular, there is an interesting group of peaks that appear after the last solvent peak which has a retention time of 40 minutes (Figure 2.1). Thus, their location on the chromatogram

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suggests that they are less polar compounds than 1,4-dioxane. Surprisingly, there were only traces of materials providing small peaks prior to 1,4-dioxane on the chromatogram.

To assess if volatile components may have been present in the original extract, the solvent that was collected from the rotovap trap during evaporation of MeOH was also evaporated but at room temperature and under normal, rather than reduced pressure. It was done under these conditions to prevent loss of any volatile compounds that might come off under more rigorous conditions of evaporation. After dryness was achieved it was apparent that there was no significant residue, but just in case there were traces of material that were not visible to the naked eye, the flask was rinsed with 1,4-dioxane and the latter was also analyzed *via* HPLC on the C18 column. This experiment showed that there was nothing in the flask detectable by the PDA method as the chromatogram looked identical to the solvent blank.

2.6 Quantification of Methanol Extraction

The quantification of the methanol extraction of mastic gum was done three times starting with 1 g to ensure that our process was representative of the bulk of the material and that it was reproducible. When extracting the mastic gum, 93.1%, 91.6%, and 92.4% of the original 'mastic' weight went into the MeOH extraction. For instance, 'mastic' was weighed out to about 1 gram in a pre-weighed flask. After extraction with MeOH, the flask was dried and then re-weighed to determine the amount of 'mastic' residue remaining in the flask. From that, we calculated the amount of 'mastic' that had been taken-up by MeOH extraction sample.

2.7 Preliminary Experiments to Identify HPLC Peaks

The next set of experiments were to add internal standards to the sample to begin to determine what the peaks were in our HPLC version of the fingerprinted mastic gum. After re-examining Table 1, *alpha*-pinene was chosen because of the large amount previously shown to be present in mastic gum, and *beta*-myrcene due to the much less expensive price as compared to (Z,Z)-farnesol. First it was important to see where
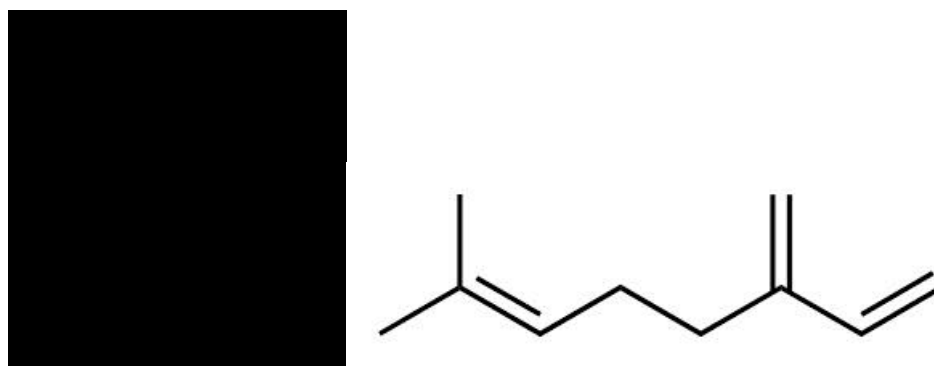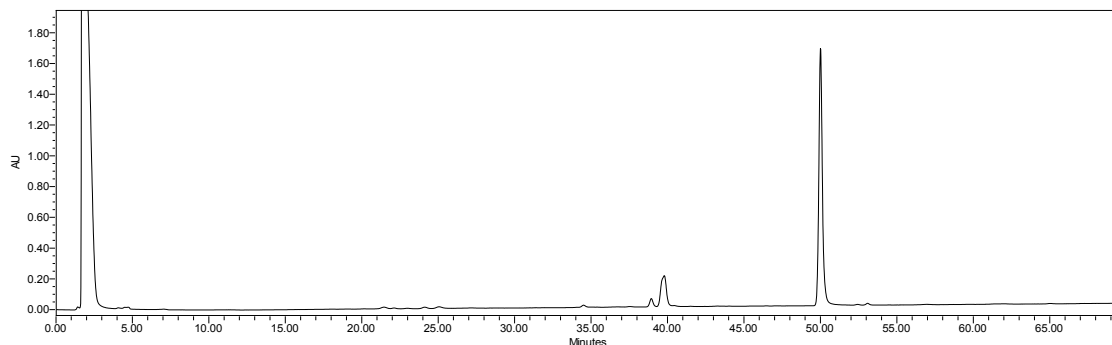

Figure 2.3. *alpha*-Pinene (left) and *beta*-Myrcene (right) are non polar, low molecular compounds that would be expected to have moderate volatility.

pure *alpha*-pinene and *beta*-myrcene themselves would appear as peaks when using our current HPLC method. These results are depicted in Figure 2.4 and 2.5. After observing
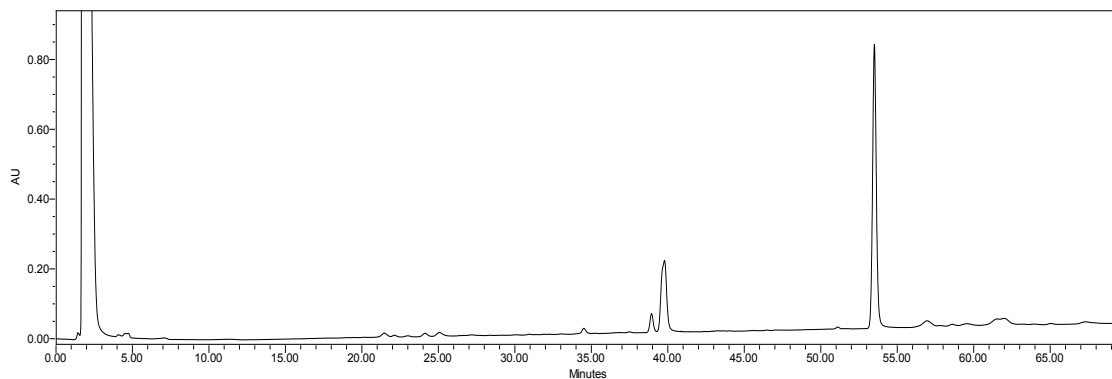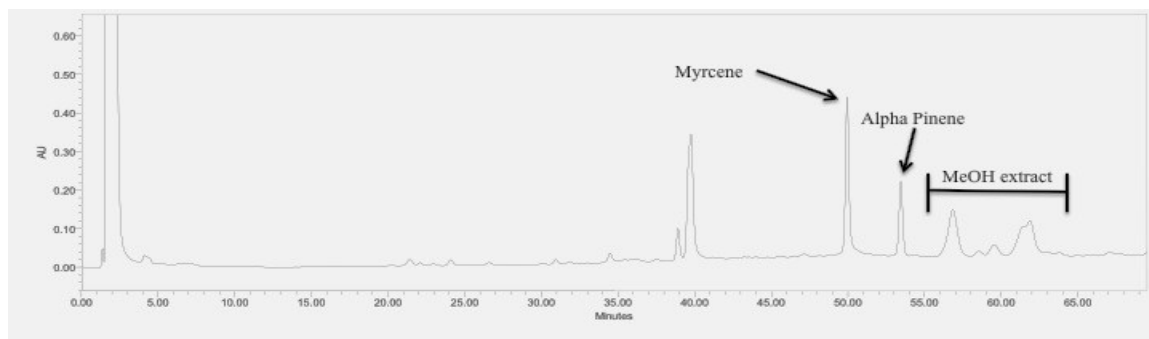
Figure 2.4. *alpha*-Pinene HPLC chromatogram.



Figure 2.5 *beta*-Myrcene HPLC chromatogram.

where these peaks appeared, it was apparent that the standards didn't correspond to any of the peaks from the MeOH extract. However, there remained the possibility that perhaps the standards were being masked or shifted on the chromatogram by other

Figure 2.6. Chromatogram of MeOH extract spiked with *alpha*-pinene and *beta*-myrcene,

compounds in the extract. To check for this possibility, a MeOH extract spiked with extra *beta*-myrcene and *alpha*-pinene was also analyzed (Figure 2.6). If any of the mastic gum peaks were *alpha*-pinene or *beta*-myrcene, one would expect to see some peaks become much larger when spiked with these standards. Instead of this happening, the two separate standard peaks re-appeared. This was unanticipated because both of the standard compounds are not volatile and should not be lost from our non-spiked samples

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during the rotovap process. Thus, the question remained why we were not seeing either *alpha*-pinene and *beta*-myrcene in our extracts. To further test this situation, an initial mastic gum sample was spiked prior to the original MeOH extraction. A dry-ice acetone cold finger was also used to collect the solvent during the evaporation step to improve subsequent chemical recovery if that was acting as the source of loss for moderately volatile materials. The same procedure was followed as in scheme 2.1, expecting to see the standard peaks show up in the chromatogram. However, when the sample was analyzed, the chromatogram looked identical to the MeOH extract of the non-spiked mastic gum with no trace of either standard. After this finding we further tested the solvent waste from the rotovap specially devised dry-ice acetone trap. Upon analysis of the waste, it was evident that there weren't any compounds in the waste. These results were unexpected because of the use of a cold finger during the rotovap process to capture any volatile compounds that may come off under heat and pressure. As another experiment we then spiked immediately before the rotovap process to see if the standards were somehow being lost during the initial extraction process. Again, we obtained the same result as when the mastic gum was spiked at
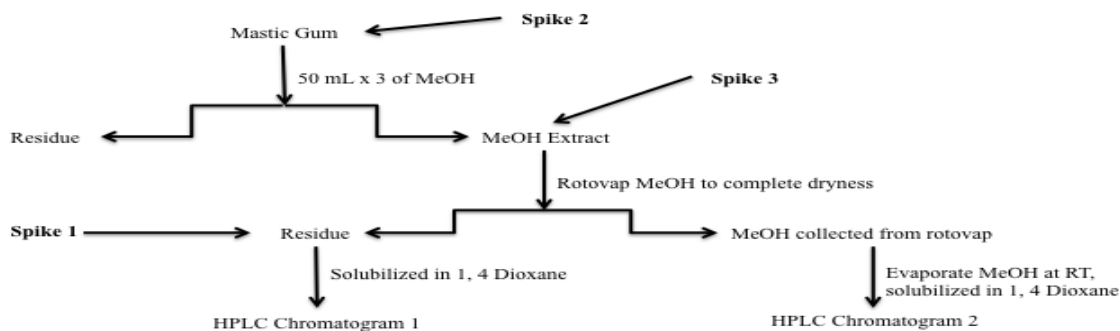
Scheme 2.2. MeOH extract and standard spiking experimental protocols.

the beginning. Both chromatograms resulted in neither one of the standards being recovered. As shown in Scheme 2.2, there were three separate places in which spikes occurred. It was very perplexing that neither spike 2 or 3 could be recovered during the process. *Alpha*-pinene and *beta*-myrcene are both soluble in MeOH, which would mean one would expect to see them in the MeOH extract. There are possible theories to why the standards were not showing up in the chromatograms. We feel we have ruled-out their loss due to volatility. Alternatively, another theory that could explain this is the possibility of the mastic gum itself forming a complex with these standards that renders them non-extractable by our process.

2.8 Summary

This project proved to be a major learning experience for me for several reasons. I learned how to effectively contribute as a participating member on an academic 'drug discovery' team composed of several highly interdisciplinary scientists and MDs. I also learned hands-on techniques and how to deploy a Waters HPLC chromatographic system equipped with a reverse-phase column and a PDA detector. It helped to reinforce my theoretical knowledge about chemical and physical properties, chromatographic separations and specific HPLC instrumentation by applying all of them in a very practical manner while engaged in this project overall. I was able to gain experience on how to devise and undertake specific natural product extraction paradigms and how to conduct HPLC assay method development studies.

For the project we were able to establish an extraction procedure that removes greater than 90% of the constituents present in a commercial grade of mastic gum material. The product from this procedure is suitable for both HPLC analysis and biological testing. We also devised an HPLC method that provides a robust fingerprint of the extract in a very consistent fashion. We helped to add to our academic knowledge about mastic gum in that it does not appear to have significant levels of polar materials and instead consists primarily of non-polar materials similar to those identified by previous investigators using assay methods especially suited for the later. Lastly, we added to our academic questions about mastic gum in that when subjected to the combination of our extraction and assay protocols, appears capable of masking the presence of certain standard compounds when spiked into samples at any step prior to the last 1,4-dioxane pick-up of the final residue that is then subjected to HPLC assay.

2.9 Future Studies

The future studies on this project remain promising and exciting. The first study that will need to be done is to test the MeOH extract of mastic gum for potential biological activity against inflammation. Another study that might address the perplexing loss of the standards would be to do TLC work to try to determine what exactly is happening to the standards. The TLC work would include spotting the MeOH extract prior to evaporation *via* rotovap against solutions of the pure *beta*-myrcene and *alpha*-pinene standards. The mastic gum should have multiple spots including a distinct *alpha*-pinene and *beta*-myrcene spot. Similarly, MeOH solutions of the pure standards shown

to have TLC spots could be treated with mastic gum to see if the latter can complex with them and thus decrease their appearance by TLC. Lastly, other studies that can be done are to devise different extraction protocols of mastic gum using varying solvents and then test those for biological activity as well.

Chapter 3

Experimental Section

3.1 Practicum Synthesis of Amphiphiles

Materials and Methods

All reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich, Fisher Scientific, or EMD) and were used without purification. Thin-layer chromatography (TLC) was done on 250 μ fluorescent TLC plates acquired from VWR and visualized by using UV light or ninhydrin. Buchi rotavapors were used for concentration using a vacuum pump. For all HPLC runs, a shimadzu unit was used for all runs. ^{1}H NMR spectra were recorded on a INOVA600 instrument and were referenced using residual non-deuterated solvent as an internal standard. Coupling constants are expressed in Hertz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplets, m = multiplet.

2,2-Didecylpropane-1,3-ethyl Ester, (4)

To a 22 L 3-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe was added dimethylformamide (8 L). Sodium hydride powder (400 g) was added to the DMF very slowly until the sodium hydride was done reacting with the trace amount of water in the DMF. Once this was finished the sodium hydride was added much more quickly through a funnel. The reaction mixture was then cooled to an internal temperature of 0 – 5 °C. Diethyl malonate (**1**) (680 mL, 4.5 mol) was added dropwise using a 1 L addition funnel over 1.5 – 2 hrs., paying close attention to the temperature and not allowing it to heat above 5 °C. Once the addition was complete the reaction was stirred for 30 minutes. Bromodecane (**2**) (10 mol, 2075 mL) was then added dropwise via 1 L addition funnel over 1.5 – 2 hrs., again paying close attention to the temperature to ensure that it does not rise above 5 °C. The resulting mixture was stirred for 24 hrs. and allowed to warm to room temperature overnight. The reaction was then poured equally into 2 open buckets containing ammonium chloride solution (6 M, 4 L) and stirred to ensure quenching of the reaction. The resulting mixture was then extracted in a 20 L separation funnel using EtOAc (5 L x 2). The organic phases from each bucket were combined and washed with brine solution (5 L x 2). The EtOAc layer was then dried over anhydrous sodium sulfate (1000 g). The dried EtOAc layer was concentrated to yield a pale yellow liquid. This crude product was used in the next step without further purification.

2,2-Dioctylpropane-1,3-ethyl Ester, (5)

To a 22 L 3-neck round-bottom flask that is equipped with an overhead stirrer and internal temperature probe was added dimethylformamide (8 L). Sodium hydride powder (400 g) was added to the DMF very slowly until the sodium hydride was done reacting with the trace amount of water in the DMF. Once this was finished the sodium hydride was added much more quickly through a funnel. The reaction mixture was then cooled to an internal temperature of 0 – 5 °C. Diethyl malonate (**1**) (680 mL, 4.5 mol) was added dropwise using a 1 L addition funnel over 1.5 – 2 hrs., paying close attention to the temperature and not allowing it to heat above 5 °C. Once the addition was complete the reaction was stirred for 30 minutes. Bromooctane (**3**) (10 mol, 1730 mL) was then added dropwise via 1 L addition funnel over 1.5 – 2 hrs., again paying close attention to the temperature to ensure that it does not rise above 5 °C. The resulting mixture was stirred for 24 hrs. and allowed to warm to room temperature overnight. The reaction was then poured equally into 2 open buckets containing ammonium chloride solution (6 M, 4 L) and stirred to ensure quenching of the reaction. The resulting mixture was then extracted in a 20 L separation funnel using EtOAc (5 L x 2). The organic phases from each bucket were combined and washed with brine solution (5 L x 2). The EtOAc layer was then dried over anhydrous sodium sulfate (1000 g). The dried EtOAc layer was concentrated to yield a pale yellow liquid. This crude product was used in the next step without further purification.

2,2-Didecylpropane-1,3-diol, (6)

To a 22 L 3-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe was added tetrahydrofuran (10 L) and the mixture cooled to 0 – 5 °C. Lithium aluminum hydride powder (400 g) was added very slowly to the reaction until the LAH was done reacting with the trace amounts of water in the THF. Once the LAH was done reacting with the trace amounts of water, the remainder was added quickly through a funnel. 2,2-Didecylpropane-1,3-ethyl ester (dissolved in 2 L of THF) was added dropwise via a 1 L addition funnel over 3 – 4 hrs. This resulting mixture was then stirred and allowed to warm to room temperature overnight. The reaction was cooled to 0 – 5 °C, and water (400 mL) was added dropwise via a 1 L addition funnel. Sodium hydroxide solution (15%, 400 mL) was added dropwise and then followed by water (1200 mL). The mixture was stirred overnight and allowed to warm to room temperature. A white precipitation was formed and removed by filtering through a 6 L Buchner funnel. This side-product was rinsed with THF (2 L) into a 20 L filter flask. The filtrate was then loaded onto a 20 L rotovap flask and concentrated to a pale yellow oil. Dichloromethane (DCM) (2 – 3 L) was added to solubilize the oil. The DCM solution was washed with ammonium chloride (2M, 2 L x 2) using a partition funnel and then dried over anhydrous sodium sulfate (1000 g), filtered and once again concentrated to a pale yellow oil which was used in the next step without any other purification.

2,2-Dioctylpropane-1,3-diol, (7)

To a 22 L 3-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe was added tetrahydrofuran (10 L) and the mixture cooled to 0 – 5 °C. Lithium aluminum hydride powder (400 g) was added very slowly to the reaction until the LAH was done reacting with the trace amounts of water in the THF. Once the LAH was done reacting with the trace amounts of water, the remainder was added quickly through a funnel. 2,2-Dioctylpropane-1,3-ethyl ester (dissolved in 2 L of THF) was added dropwise via a 1 L addition funnel over 3 – 4 hrs. This resulting mixture was then stirred and allowed to warm to room temperature overnight. The reaction was cooled to 0 – 5 °C, and water (400 mL) was added dropwise via a 1 L addition funnel. Sodium hydroxide solution (15%, 400 mL) was added dropwise and then followed by water (1200 mL). The mixture was stirred overnight and allowed to warm to room temperature. A white precipitation was formed and removed by filtering through a 6 L Buchner funnel. This side-product was rinsed with THF (2 L) into a 20 L filter flask. The filtrate was then loaded onto a 20 L rotovap flask and concentrated to a pale yellow oil. Dichloromethane (DCM) (2 – 3 L) was added to solubilize the oil. The DCM solution was washed with ammonium chloride (2M, 2 L x 2) using a partition funnel and then dried over anhydrous sodium sulfate (1000 g), filtered and once again concentrated to a pale yellow oil which was used in the next step without any other purification.

α-Bromo-perbenzoyl Maltose (BBM), (9)

A solution of maltose octabenzoyl (MOB) **(8)** (5000 g, 4.25 mol) in dichloromethane (DCM) (7 L) was cooled to 0 °C while stirring in a 22 L 3-neck round-bottom flask. A solution of 33% hydrogen bromide in acetic acid (1.2 L) at room temperature was poured into the reaction flask via graduated cylinder. The reaction mixture was allowed to attain room temperature while stirring overnight. The reaction was monitored by silica gel thin layer chromatography (TLC) using Hexanes:EtOAc:DCM (4:1:1) as the developing system and UV (254 nm) for visualization [R_f 0.44]. After completion of reaction (24 hrs.), the reaction mixture was washed with water (10 L x 2) followed by a wash with saturated sodium bicarbonate (5 L x 2). These were accomplished by the use of a 20 L separation funnel. The organic layer was then dried with anhydrous sodium sulfate (1000 g) and stored as a DCM solution to be used directly to the next step without further purification.

2,2-Didecylpropane-1,3-bis-β-D-maltopyranoside, (10)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer, internal temperature probe and argon inlet, acetonitrile (4 L), 4Å molecular sieves (500 g), and silver triflate (500 g, 1.95 mol) were added and stirred under argon for one hour at room temperature. Dichloromethane (3 L) was added to the flask followed immediately by 2,2-didecylpropane-1,3-diol (275 g, 0.77 mol). This reaction mixture was then cooled to

-15 °C via a 6 L methanol and dry ice bath. A solution of α-bromo-perbenzoyl maltose (2030 g, 1.8 mol) in dichloromethane (10 L) was prepared in a 15 L plastic carboy and stirred until all the α-bromo-perbenzoyl maltose was dissolved. Once the reaction mixture reached an internal temperature of -15 °C, 2 L of the α-bromo-perbenzoyl maltose solution was added to the reaction via a 2 L graduated cylinder. The reaction was allowed to stir for an hour before the next 2 L addition of α-bromo-perbenzoyl maltose solution. This step was repeated until a total of 5 additions were made. The reaction mixture was then allowed to attain room temperature and stirred for 48 hrs. The reaction mixture was monitored by silica gel thin layer chromatography (TLC) using Hexanes:EtOAc:DCM (4:1:1) as the developing system and UV (254 nm) for visualization [R_f 0.28].   After competition of the reaction (48 hrs.), the reaction mixture was quenched using triethylamine (405 mL) and then filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap flask. To the oil was added sodium methoxide (250 g, 4.63 mol) and methanol (8 L) to facilitate deprotection of the product. The solution was heated to 65 °C for 2 hours on the rotovap with stirring and no vacuum, and then cooled to 35 °C and allowed to rotate for another 24 hrs. The reaction mixture was transferred to a 15 L carboy and stirred with an overhead stirrer. The mixture was neutralized to a pH of 6 – 7 by IR120 (200 g). Once neutralized the reaction mixture was filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was then concentrated to a thick oil and solubilized by adding deionized water (5 L). The solution was then extracted with EtOAc (3 L x 2) via a 20 L separation funnel. Once completed, the product was purified by a C18 (20 kg of C18 resin) column (6" x 96")

using a stepwise gradient system having methanol and deionized water. ^{1}H NMR (600 MHz, CD3OD) δ5.153-5.147 (d, 4H, *J* = 3.6 Hz), 4.365-4.351 (d, 6H, *J* = 8.4 Hz), 3.879-3.877 (d, 5H, *J* = 1.2 Hz), 3.831-3.815 (m, 10H, *J* = 9.6 Hz), 3.722-3.592 (m, 26H, *J* = 96 Hz), 3.536-3.516 (t, 6H, *J* = 12 Hz), 3.471-3.425 (m, 11H, *J* = 27.6 Hz), 3.279-3.230 (m, 14H, *J* = 29.4 Hz).

2,2-Dioctylpropane-1,3-bis-β-D-maltopyranoside, (11)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer, internal temperature probe and argon inlet, acetonitrile (4 L), 4Å molecular sieves (500 g), and silver triflate (500 g, 1.95 mol) were added and stirred under argon for one hour at room temperature. Dichloromethane (3 L) was added to the flask followed immediately by 2,2-dioctylpropane-1,3-diol (275 g, 0.77 mol). This reaction mixture was then cooled to -15 °C via a 6 L methanol and dry ice bath. A solution of α-bromo-perbenzoyl maltose (2030 g, 1.8 mol) and dichloromethane (10 L) was prepared in a 15 L plastic carboy and stirred until all the α-bromo-perbenzoyl maltose was dissolved. Once the reaction mixture reached an internal temperature of -15 °C, 2 L of the α-bromo-perbenzoyl maltose solution was added to the reaction via a 2 L graduated cylinder. The reaction was allowed to stir for an hour before the next 2 L addition of α-bromo-perbenzoyl maltose solution. This step was repeated until a total of 5 additions were made. The reaction mixture was then allowed to attain room temperature and stirred for 48 hrs. The reaction mixture was monitored by silica gel thin layer chromatography (TLC) using Hexanes:EtOAc:DCM (4:1:1) as the developing system and UV (254 nm) for

visualization [R_f 0.25].   After competition of the reaction (48 hrs.), the reaction mixture was quenched using triethylamine (405 mL) and then filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap flask. To the oil was added sodium methoxide (250 g, 4.63 mol) and methanol (8 L) to facilitate deprotection of the product. The solution was heated to 65 °C for 2 hours on the rotovap with stirring and no vacuum, and then cooled to 35 °C and allowed to rotate for another 24 hrs. The reaction mixture was transferred to a 15 L carboy and stirred with an overhead stirrer. The mixture was neutralized to a pH of 6 – 7 by IR120 (200 g). Once neutralized the reaction mixture was filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was then concentrated to a thick oil and solubilized by adding deionized water (5 L). The solution was then extracted with EtOAc (3 L x 2) via a 20 L separation funnel. Once completed, the product was purified by a C18 (20 kg of C18 resin) column (6" x 96") using a stepwise gradient system having methanol and deionized water.

2,2-Dihexylpropane-1,3-ethyl Ester, (13)

To a 22 L 3-neck round-bottom flask that is equipped with an overhead stirrer and internal temperature probe was added dimethylformamide (8 L). Sodium hydride powder (400 g) was added to the DMF very slowly until the sodium hydride was done reacting with the trace amount of water in the DMF. Once this was finished the sodium hydride was added much more quickly through a funnel. The reaction mixture was then cooled to an internal temperature of 0 – 5 °C. Diethyl malonate (**1**) (680 mL, 4.5 mol) was added

dropwise using a 1 L addition funnel over 1.5 – 2 hrs., paying close attention to the temperature and not allowing it to heat above 5 °C. Once the addition was complete the reaction was stirred for 30 minutes. Bromohexane (**12**) (10 mol, 1400 mL) was then added dropwise via 1 L addition funnel over 1.5 – 2 hrs., again paying close attention to the temperature to ensure that it does not rise above 5 °C. The resulting mixture was stirred for 24 hrs. and allowed to warm to room temperature overnight. The reaction was then poured equally into 2 open buckets containing ammonium chloride solution (6 M, 4 L) and stirred to ensure quenching of the reaction. The resulting mixture was then extracted in a 20 L separation funnel using EtOAc (5 L x 2). The organic phases from each bucket were combined and washed with brine solution (5 L x 2). The EtOAc layer was then dried over anhydrous sodium sulfate (1000 g). The dried EtOAc layer was concentrated to yield a pale yellow liquid. This crude product was used in the next step without further purification.

2,2-Dihexylpropane-1,3-diol, (14)

To a 22 L 3-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe was added tetrahydrofuran (10 L) and the mixture cooled to 0 – 5 °C. Lithium aluminum hydride powder (400 g) was added very slowly to the reaction until the LAH was done reacting with the trace amounts of water in the THF. Once the LAH was done reacting with the trace amounts of water, the remainder was added quickly through a funnel. 2,2-Dihexylpropane-1,3-ethyl ester (dissolved in 2 L of THF) was added dropwise via a 1 L addition funnel over 3 – 4 hrs. This resulting mixture was then

stirred and allowed to warm to room temperature overnight. The reaction was cooled to 0 – 5 °C, and water (400 mL) was added dropwise via a 1 L addition funnel. Sodium hydroxide solution (15%, 400 mL) was added dropwise and then followed by water (1200 mL). The mixture was stirred overnight and allowed to warm to room temperature. A white precipitation was formed and removed by filtering through a 6 L Buchner funnel. This side-product was rinsed with THF (2 L) into a 20 L filter flask. The filtrate was then loaded onto a 20 L rotovap flask and concentrated to a pale yellow oil. Dichloromethane (DCM) (2 – 3 L) was added to solubilize the oil. The DCM solution was washed with ammonium chloride (2M, 2 L x 2) using a partition funnel and then dried over anhydrous sodium sulfate (1000 g), filtered and once again concentrated to a pale yellow oil which was used in the next step without any other purification.

Glucose Penta-benzoyl (GPB), (16)

To a 22 L round-bottom flask, α-D(+)-glucose (**15**) (1000 g, 5.55 mol) was solubilized in pyridine (12 L, 149.16 mol). The mixture was cooled to -20 °C prior to dropwise addition of benzoyl chloride using a 1 L addition funnel (1 L x 4, 34.43 mol) without allowing the temperature to rise above -10 °C. After addition, the reaction was allowed to warm to room temperature while continuing to stir overnight/weekend. TLC [R_f 0.44, 0.5, Hexanes:EtOAc:DCM (4:1:1)] was used to confirm completion of the reaction (24 hrs.) after which the reaction mixture was filtered through a fritted funnel. The crude product mixture was then extracted with DCM leaving the pyridinium hydrochloride salt behind. The filtrate was concentrated to a solid and then solubilized in DCM (4-6 L).

The DCM solution was then washed with 0.5 M HCl solution (10 L x 2) to remove the remaining pyridine. This was accomplished using a 20 L separation funnel. The DCM layer was dried over anhydrous sodium sulfate (1000 g). The dried solution was then refrigerated until the next step.

Benzoylbromoglucose, (17)

A solution of glucose penta-benzoyl (GPB) **16** (5000 g, 7.1 mol) in dichloromethane (DCM) (10 L) was cooled to 0 °C while stirring in a 22 L 3-neck round-bottom flask. A solution of 33% hydrogen bromide in acetic acid (4.2 L) at room temperature was poured into the reaction flask via graduated cylinder. The reaction mixture was then allowed to attain room temperature while stirring overnight. The reaction was monitored by silica gel thin layer chromatography (TLC) using Hexanes:EtOAc:DCM (4:1:1) as the developing system and UV (254 nm) for visualization [R_f 0.57]. After completion of reaction (24 hrs.), the reaction mixture was washed with water (10 L x 2) followed by a wash with saturated sodium bicarbonate (5 L x 2). These were accomplished by the use of a 20 L separation funnel. The organic layer was then dried with anhydrous sodium sulfate (1000 g) and stored as a DCM solution to be used directly to the next step without further purification.

2,2-dihexylpropane-1,3-bis-β-D-glucopyranoside, (18)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer, internal temperature probe and argon inlet, ethyl ether (4 L), 4Å molecular sieves (500 g), and silver triflate (500 g, 1.95 mol) were added and stirred under argon for one hour at room temperature. Dichloromethane (3 L) was added to the flask followed immediately by 2,2-dihexylpropane-1,3-diol (275 g, 0.77 mol). This reaction mixture was then cooled to -15 °C via a 6 L methanol and dry ice bath. A solution of benzoylbromoglucose (2030 g, 1.8 mol) and dichloromethane (10 L) was prepared in a 15 L plastic carboy and stirred until all the benzoylbromoglucose was dissolved. Once the reaction mixture reached an internal temperature of -15 °C, 2 L of the benzoylbromoglucose solution was added to the reaction via a 2 L graduated cylinder. The reaction was allowed to stir for an hour before the next 2 L addition of benzoylbromoglucose solution. This step was repeated until a total of 5 additions were made. The reaction mixture was then allowed to attain room temperature and stirred for 48 hrs. The reaction mixture was monitored by silica gel thin layer chromatography (TLC) using Hexanes:EtOAc:DCM (4:1:1) as the developing system and UV (254 nm) for visualization [R_f 0.23]. After competition of the reaction (48 hrs.), the reaction mixture was quenched using triethylamine (405 mL) and then filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap flask. To the oil was added sodium methoxide (250 g, 4.63 mol) and methanol (8 L) to facilitate deprotection of the product. The solution was heated to 65 °C for 2 hours on the rotovap with stirring and no vacuum, and then cooled to 35 °C and allowed to rotate for another 24 hrs. The reaction mixture was transferred to a 15 L carboy and stirred with an overhead stirrer. The mixture was neutralized to a pH of 6 – 7 by IR120 (200 g). Once neutralized the

reaction mixture was filtered through a 6 L fritted funnel equipped with a celite pad (500 g) into a 20 L filter flask. The filtrate was then concentrated to a thick oil and solubilized by adding deionized water (5 L). The solution was then extracted with EtOAc (3 L x 2) via a 20 L separation funnel. Once completed, the product was purified by a C18 (20 kg of C18 resin) column (6" x 96") using a stepwise gradient system having methanol and deionized water. ^{1}H NMR (600 MHz, CD3OD) δ4.638 (s, 1H) 4.329-4.316 (d, 4H, 7.8 Hz), 3.876-3.855 (t, 5H, 12.6 Hz), 3.745-3.729 (d, 4H, 9.6 Hz), 3.683-3.655 (m, 5H, 16.8 Hz), 3.465-3.448 (d, 4H, 10.2 Hz), 3.364-3.334 (t, 6H, 18 Hz), 3.279-3.268 (d, 8H, 6.6 Hz), 3.201-3.173 (m, 5H, 16.8 Hz).

Octyl-dichlorophosphate, (25)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tetrahydrofuran (1 L) and phosphoryl chloride (**19**) (448 mL, 4.8 mol) were added and stirred for ten minutes at -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (612 mL, 4.39 mol) and octanol (**20**) (632 mL, 4.00 mol) was added dropwise using a 1 L addition funnel over 3 - 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. Once the addition was complete, the reaction mixture was then allowed to attain room temperature while stirring overnight. The entire reaction mixture was taken to the next step without further purification or filtration.

Decyl-dichlorophosphate, (26)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tetrahydrofuran (1 L) and phosphoryl chloride (**19**) (448 mL, 4.8 mol) were added and stirred for ten minutes at -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (612 mL, 4.39 mol) and decanol (**21**) (632 mL, 4.00 mol) was added dropwise using a 1 L addition funnel over 3 - 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. Once the addition was complete, the reaction mixture was then allowed to attain room temperature while stirring overnight. The entire reaction mixture was taken to the next step without further purification or filtration.

Dodecyl-dichlorophosphate, (27)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tetrahydrofuran (1 L) and phosphoryl chloride (**19**) (448 mL, 4.8 mol) were added and stirred for ten minutes at -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (612 mL, 4.39 mol) and dodecanol (**22**) (632 mL, 4.00 mol) was added dropwise using a 1 L addition funnel over 3 - 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. Once the addition was complete, the reaction mixture was then allowed to attain room temperature while stirring

overnight. The entire reaction mixture was taken to the next step without further purification or filtration.

Tridecyl-dichlorophosphate, (28)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tetrahydrofuran (1 L) and phosphoryl chloride (**19**) (448 mL, 4.8 mol) were added and stirred for ten minutes at -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (612 mL, 4.39 mol) and tridecanol (**23**) (632 mL, 4.00 mol) was added dropwise using a 1 L addition funnel over 3 - 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. Once the addition was complete, the reaction mixture was then allowed to attain room temperature while stirring overnight. The entire reaction mixture was taken to the next step without further purification or filtration.

Hexadecyl-dichlorophosphate, (29)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tetrahydrofuran (1 L) and phosphoryl chloride (**19**) (448 mL, 4.8 mol) were added and stirred for ten minutes at -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (612 mL, 4.39 mol) and hexadecanol (**24**) (632 mL, 4.00 mol) was added dropwise using a 1 L addition funnel over 3 - 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. Once the addition was complete, the reaction mixture was then allowed to attain room temperature while stirring

overnight. The entire reaction mixture was taken to the next step without further purification or filtration.

Octyl-2-oxo-1,2,3-oxazaphospholane, (30)

In a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, the reaction mixture from octyl-dichlorophosphate was cooled to -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (1340 mL, 9.61 mol), and ethanolamine (292 mL, 4.84 mol) was added dropwise using a 1 L addition funnel over 3 – 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. The reaction mixture was then allowed to attain room temperature while stirring overnight. The mixture was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap. The oil was allowed to cool to room temperature before being used in the next step without further purification.

Decyl-2-oxo-1,2,3-oxazaphospholane, (31)

In a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, the reaction mixture from decyl-dichlorophosphate was cooled to -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (1340 mL, 9.61 mol), and ethanolamine (292 mL, 4.84 mol) was added dropwise using a 1 L addition funnel over 3 – 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. The reaction mixture was then allowed to attain room temperature while stirring overnight. The mixture was filtered through a 6 L fritted funnel into a 20 L filter flask.

The filtrate was concentrated to an oil via a 20 L rotovap. The oil was allowed to cool to room temperature before being used in the next step without further purification.

Dodecyl-2-oxo-1,2,3-oxazaphospholane, (32)

In a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, the reaction mixture from dodecyl-dichlorophosphate was cooled to -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (1340 mL, 9.61 mol), and ethanolamine (292 mL, 4.84 mol) was added dropwise using a 1 L addition funnel over 3 – 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. The reaction mixture was then allowed to attain room temperature while stirring overnight. The mixture was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap. The oil was allowed to cool to room temperature before being used in the next step without further purification.

Tridecyl-2-oxo-1,2,3-oxazaphospholane, (33)

In a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, the reaction mixture from tridecyl-dichlorophosphate was cooled to -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (1340 mL, 9.61 mol), and ethanolamine (292 mL, 4.84 mol) was added dropwise using a 1 L addition funnel over 3 – 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. The reaction mixture was then allowed to attain room temperature while stirring overnight. The mixture was filtered through a 6 L fritted funnel into a 20 L filter flask.

The filtrate was concentrated to an oil via a 20 L rotovap. The oil was allowed to cool to room temperature before being used in the next step without further purification.

Hexadecyl-2-oxo-1,2,3-oxazaphospholane, (34)

In a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, the reaction mixture from hexadecyl-dichlorophosphate was cooled to -20 °C. A mixture of tetrahydrofuran (4 L), triethylamine (1340 mL, 9.61 mol), and ethanolamine (292 mL, 4.84 mol) was added dropwise using a 1 L addition funnel over 3 – 4 hrs., paying close attention to the temperature and not allowing it to rise above -20 °C. The reaction mixture was then allowed to attain room temperature while stirring overnight. The mixture was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to an oil via a 20 L rotovap. The oil was allowed to cool to room temperature before being used in the next step without further purification.

Octylphosphoethanolamine, (35)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, octyl-2-oxo-1,2,3-oxazaphospholane (4 mol. from prior reaction) and THF (1 L) was added and stirred at room temperature for 10 minutes. A mixture of glacial acetic acid (940 mL, 16.4 mol) in deionized water (400 mL) was added dropwise using a 1 L addition funnel over 30 – 40 minutes, paying close attention to the temperature and not allowing it to rise above 35 °C. The reaction was stirred for 1 hour, once completed acetone (8 L) was added to the reaction. The product was precipitated

out and filtered through a 6 L fritted funnel. The solid was allowed to sit under vacuum until completely dry. The product was then taken to the next step without further purification.

<u>Decylphosphoethanolamine, (36)</u>

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, decyl-2-oxo-1,2,3-oxazaphospholane (4 mol. from prior reaction) and THF (1 L) was added and stirred at room temperature for 10 minutes. A mixture of glacial acetic acid (940 mL, 16.4 mol) in deionized water (400 mL) was added dropwise using a 1 L addition funnel over 30 – 40 minutes, paying close attention to the temperature and not allowing it to rise above 35 °C. The reaction was stirred for 1 hour, once completed acetone (8 L) was added to the reaction. The product was precipitated out and filtered through a 6 L fritted funnel. The solid was allowed to sit under vacuum until completely dry. The product was then taken to the next step without further purification.

<u>Dodecylphosphoethanolamine, (37)</u>

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, dodecyl-2-oxo-1,2,3-oxazaphospholane (4 mol. from prior reaction) and THF (1 L) was added and stirred at room temperature for 10 minutes. A mixture of glacial acetic acid (940 mL, 16.4 mol) in deionized water (400 mL) was added dropwise

using a 1 L addition funnel over 30 – 40 minutes, paying close attention to the temperature and not allowing it to rise above 35 °C. The reaction was stirred for 1 hour, once completed acetone (8 L) was added to the reaction. The product was precipitated out and filtered through a 6 L fritted funnel. The solid was allowed to sit under vacuum until completely dry. The product was then taken to the next step without further purification.

Tridecylphosphoethanolamine, (38)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, tridecyl-2-oxo-1,2,3-oxazaphospholane (4 mol. from prior reaction) and THF (1 L) was added and stirred at room temperature for 10 minutes. A mixture of glacial acetic acid (940 mL, 16.4 mol) in deionized water (400 mL) was added dropwise using a 1 L addition funnel over 30 – 40 minutes, paying close attention to the temperature and not allowing it to rise above 35 °C. The reaction was stirred for 1 hour, once completed acetone (8 L) was added to the reaction. The product was precipitated out and filtered through a 6 L fritted funnel. The solid was allowed to sit under vacuum until completely dry. The product was then taken to the next step without further purification.

Hexadecylphosphoethanolamine, (39)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, hexadecyl-2-oxo-1,2,3-oxazaphospholane (4 mol. from prior reaction) and THF (1 L) was added and stirred at room temperature for 10 minutes. A mixture of glacial acetic acid (940 mL, 16.4 mol) in deionized water (400 mL) was added dropwise using a 1 L addition funnel over 30 – 40 minutes, paying close attention to the temperature and not allowing it to rise above 35 °C.   The reaction was stirred for 1 hour, once completed acetone (8 L) was added to the reaction.   The product was precipitated out and filtered through a 6 L fritted funnel. The solid was allowed to sit under vacuum until completely dry.   The product was then taken to the next step without further purification.

Octylphosphocholine, (40)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, 2-propanol (4.3 L) and the octylphosphoethanolamine were added and stirred for 15 minutes at 40 °C. A solution of potassium carbonate (504 g, 3.65 mol) and deionized water (720 mL) was added to the reaction followed by a solution of dimethyl sulfate (308 mL, 3.25 mol) in 2-propanol (180 mL). This addition sequence was carried out three times. The reaction mixture was allowed to attain room temperature overnight while stirring. The solution was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to a thick oil and then redissolved in deionized water. The deionized water solution was placed on the lyphilizer and once completely dry

yielded our final product. ^{1}H NMR (600 MHz, CD3OD) δ4.253-4.241 (m, 2H, 7.2 Hz), 3.887-3.854 (m, 2H, 19.8 Hz), 3.635-3.620 (m, 2H, 9 Hz), 3.222 (s, 9H).

Decylphosphocholine, (41)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, 2-propanol (4.3 L) and the decylphosphoethanolamine were added and stirred for 15 minutes at 40 °C. A solution of potassium carbonate (504 g, 3.65 mol) and deionized water (720 mL) was added to the reaction followed by a solution of dimethyl sulfate (308 mL, 3.25 mol) in 2-propanol (180 mL). This addition sequence was carried out three times. The reaction mixture was allowed to attain room temperature overnight while stirring. The solution was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to a thick oil and then redissolved in deionized water. The deionized water solution was placed on the lyphilizer and once completely dry yielded our final product.

Dodecylphosphocholine, (42)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, 2-propanol (4.3 L) and the dodecylphosphoethanolamine were added and stirred for 15 minutes at 40 °C. A solution of potassium carbonate (504 g, 3.65 mol) and deionized water (720 mL) was added to the reaction followed by a solution of dimethyl sulfate (308 mL, 3.25 mol) in 2-propanol (180 mL). This addition sequence

was carried out three times. The reaction mixture was allowed to attain room temperature overnight while stirring. The solution was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to a thick oil and then redissolved in deionized water. The deionized water solution was placed on the lyphilizer and once completely dry yielded our final product.

Tridecylphosphocholine, (43)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, 2-propanol (4.3 L) and the tridecylphosphoethanolamine were added and stirred for 15 minutes at 40 °C. A solution of potassium carbonate (504 g, 3.65 mol) and deionized water (720 mL) was added to the reaction followed by a solution of dimethyl sulfate (308 mL, 3.25 mol) in 2-propanol (180 mL). This addition sequence was carried out three times. The reaction mixture was allowed to attain room temperature overnight while stirring. The solution was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to a thick oil and then redissolved in deionized water. The deionized water solution was placed on the lyphilizer and once completely dry yielded our final product.

Hexadecylphosphocholine, (44)

To a 22 L three-neck round-bottom flask equipped with an overhead stirrer and internal temperature probe, 2-propanol (4.3 L) and the hexadecylphosphoethanolamine were

added and stirred for 15 minutes at 40 °C. A solution of potassium carbonate (504 g, 3.65 mol) and deionized water (720 mL) was added to the reaction followed by a solution of dimethyl sulfate (308 mL, 3.25 mol) in 2-propanol (180 mL). This addition sequence was carried out three times. The reaction mixture was allowed to attain room temperature overnight while stirring. The solution was filtered through a 6 L fritted funnel into a 20 L filter flask. The filtrate was concentrated to a thick oil and then redissolved in deionized water. The deionized water solution was placed on the lyphilizer and once completely dry yielded our final product.

Cholesterol Hemisuccinate Tris Salt (47)

To a 22 − L round bottom flask was added cholesteryhydrogensuccinate (**45**) (1200 g, 4 mol), trizma base (**46**) (300 g, 4 mol), methanol (6 L) and chloroform (6 L) and the mixture stirred for 5 hours. The stirring was stopped and the reaction flask was allowed to sit at room temperature overnight. The product precipitates as a white solid and is filtered through a fritted funnel. The collected product is then added to a 20 − L bucket with acetone (8 L) and stirred overnight at room temperature. The suspension was filtered through a fritted funnel and the product was dried in a vacuum oven until dry. Once complete the product is moved to quality assurance for purification testing.

3.2 Mastic Gum Experiemental

Materials and Methods

All reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich, Fisher Scientific, or EMD) and were used without purification. Thin-layer chromatography (TLC) was done on 250 μ fluorescent TLC plates acquired from VWR and visualized by using UV light or ninhydrin. Buchi rotavapors were used for concentration using a vacuum pump. For all HPLC runs, a waters unit was used for all runs. A Water Xterra C18 column was used for HPLC analysis.

Extraction of Mastic Gum

Chios Mastiha capsules, obtained from Mastha Therapy, was crushed up and filtered through 0.7 mm mesh. 'Mastic' (1 gram) was placed in a pre-weighed round-bottom flask equipped with a stir bar. MeOH (50 mL x 3) was added to the flask and stirred for 15 minutes. The MeOH was then removed from the flask, leaving behind residue, and rotovapped to complete dryness. The solid was then solubilized in 20 mL of 1,4-dioxane and HPLC analysis was taken. The waste from the rotovap was evaporated at room temperature and then solubilized minimal 1,4-dioxane and HPLC analysis was taken. The samples are stored in a 4 °C fridge until analysis.

Analysis of Mastic Gum via HPLC

All samples were ran on the same gradient of 5% ACN in water to 95% ACN in water over a 70 minute time frame.

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Appendix A

NMR Spectra and HPLC Chromatograms for Synthesized Compounds

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ELSD Results

Retention Time	Height	Height %	Area	Area %
6.716	271	0.08	2945	0.05
7.196	752	0.22	11816	0.21
8.074	336618	97.99	5394337	98.13
8.786	5204	1.51	80274	1.46
14.122	690	0.20	7856	0.14
Totals	343535	100.00	5497228	100.00

66
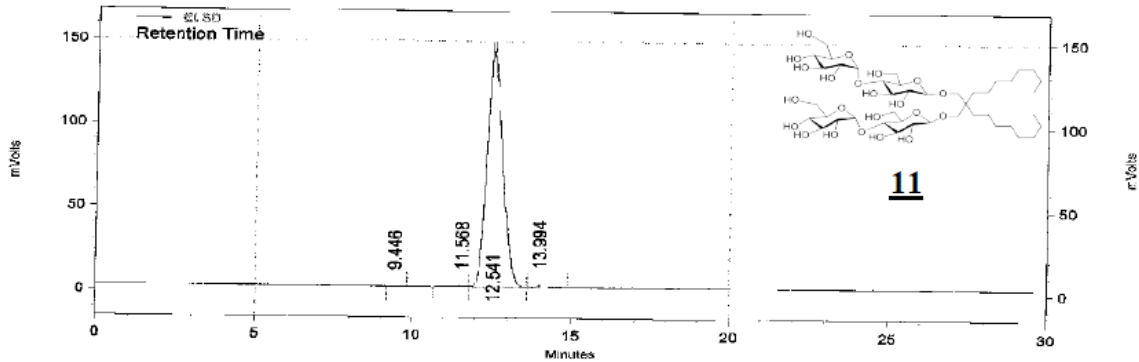
Anatrace, Inc.

ELSD Results

Retention Time	Height	Height %	Area	Area %
9.446	178	0.12	3411	0.07
11.568	612	0.41	18500	0.38
12.541	146728	98.54	4816779	99.04
13.994	1381	0.93	24994	0.51
Totals	148899	100.00	4863684	100.00

Anatrace, Inc.
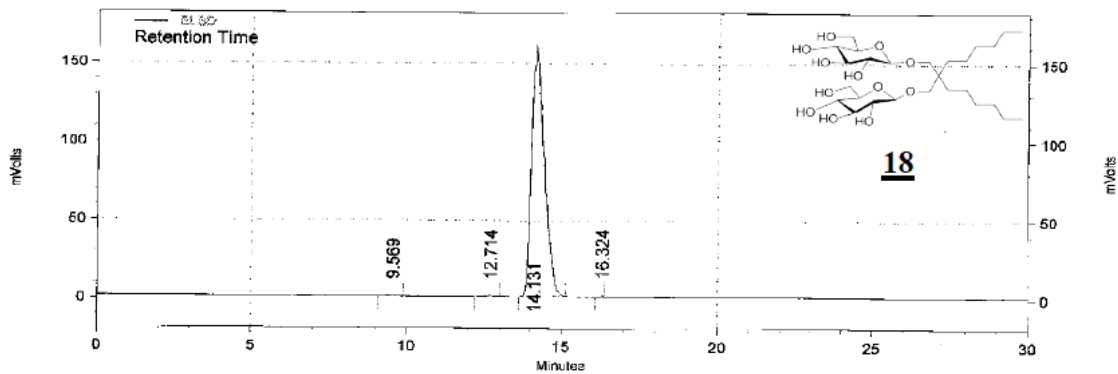
ELSD Results

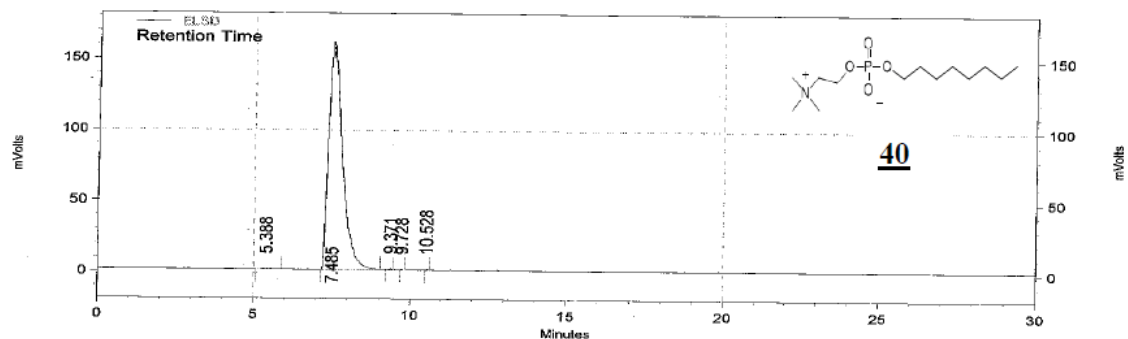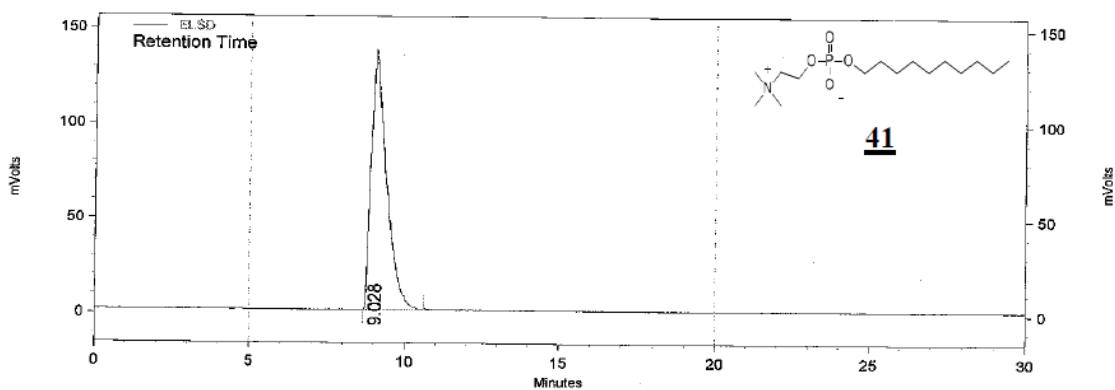| Retention Time	Height	Height %	Area	Area %
9.569	546	0.34	13014	0.28
12.714	546	0.34	12183	0.26
14.131	160394	98.62	4621747	99.38
16.324	1147	0.71	3647	0.08
Totals	162633	100.00	4650591	100.00

Anatrace, Inc.

Sample ID: FOS8-17
Method Name: C:\CLASS-VP\Enterprise\Projects\Default\Method\55%MeOH_Ch.met
Data: C:\QC\2014\January 2014\FOS8-17.dat
Acquired: 1/4/2014 8:25:36 AM   Instrument: Chewbacca (Offline)   Printed: 5/19/2014 1:46:58 PM

ELSD Results

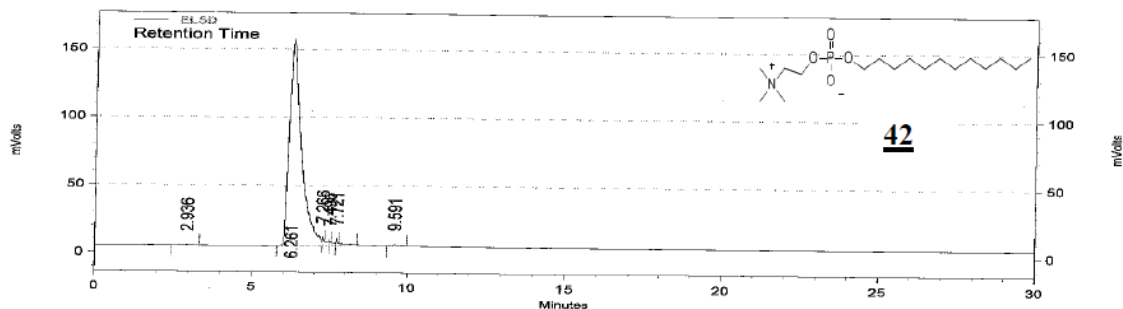| Retention Time	Height	Height %	Area	Area %
5.388	548	0.34	10965	0.22
7.485	160709	98.30	4880667	99.64
9.371	919	0.56	2667	0.05
9.728	594	0.36	1309	0.03
10.528	725	0.44	2815	0.06
Totals	163495	100.00	4898423	100.00

Anatrace, Inc.

Sample ID: FOS10-48
Method Name: C:\CLASS-VP\Enterprise\Projects\Default\Method\65%MeOH_Ch.met
Data: C:\QC\2014\January 2014\FOS10-48.dat
Acquired: 1/23/2014 1:18:01 PM   Instrument: Chewbacca (Offline)   Printed: 5/19/2014 1:47:22 PM

ELSD Results

Retention Time	Height	Height %	Area	Area %
9.028	137426	100.00	4652979	100.00
Totals	137426	100.00	4652979	100.00

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Anatrace, Inc.

ELSD Results

Retention Time	Height	Height %	Area	Area %
2.936	434	0.27	8448	0.20
6.261	152234	93.65	4204245	99.08
7.266	2961	1.82	4340	0.10
7.496	2160	1.33	3182	0.07
7.721	3604	2.22	8714	0.21
9.591	1172	0.72	14404	0.34
Totals	162565	100.00	4243333	100.00

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