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The design of fluorous chemistry for a middle school classroom, for carbohydrate microarrays, and for the automated solution-phase synthesis of bacterial oligosaccharides from Group A Streptococcus and Staphylococcus aureus

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The design of fluorous chemistry for a middle school classroom, for carbohydrate microarrays, and for the automated solution-phase synthesis of bacterial oligosaccharides from Group A Streptococcus and *Staphylococcus aureus*

by

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS iv

CHAPTER 1. General Introduction 1
Dissertation organization 1
Introduction 1
Fluorous Chemistry 1
Implementation in a classroom 2
Carbohydrate Microarray fabrication 3
Challenges in the chemical synthesis of oligosaccharides 5
Automated oligosaccharide synthesis 7
One-Pot Synthesis 7
Solid-phase oligosaccharide synthesis 7
Automated solution-phase oligosaccharide synthesis 8
Purification strategies 9
Tag modification/removal 10
Summary and future directions 11

CHAPTER 2. Introduction of Surface Tension, Emulsions, and Hydrophobicity/ Fluorophilicity in Middle School Science 12
Abstract 12
Introduction 12
Brief summary of the procedure and its place in the middle school curriculum 15
Constant relevance to real world applications 18
Teacher and student perspectives of the hydrophobicity/fluorophilicity unit 19
Conclusion 23
Hazards 23

CHAPTER 3. Probing the Limitations of Fluorous Content for Tag-Mediated Microarray Formation 25
Abstract 25
Introduction 25
Results and discussion 26
Conclusion 32
Experimental section 33

CHAPTER 4. Automated Solution-Phase Synthesis of the Cell Wall Oligosaccharides of Group A Streptococcus 52
Abstract 52
Introduction 52

Results and discussion 55
Conclusion 61
Experimental section 62

CHAPTER 5. Automated Solution-Phase Synthesis of the Cell Wall Oligosaccharides of *Staphylococcus aureus* 84
Abstract 84
Introduction 84
Results and discussion 87
Conclusion 93
Experimental Section 94

CHAPTER 6. Conclusions and Future Directions 96

ACKNOWLEDGMENTS 98

APPENDIX A. CHAPTER 2 WORKSHEETS 99

APPENDIX B. CHAPTER 3 ^{1}H AND ^{13}C NMR SPECTRA 124

APPENDIX C. CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA 136

BIBLIOGRAPHY 146

LIST OF ABBREVIATIONS

AcOH Acetic acid
AIBN 2,2'-Azobisisobutyronitrile
ASW Automated Synthesis Workstation
Bn Benzyl
BSA Bovine serum albumin
CAN Ceric ammonium nitrate
CSA Camphorsulfonic acid
DCC N, N-Dicyclohexylcarbodiimide
DCM Dichloromethane
DMAP N,N-Dimethylaminopyridine
DMF N,N,-Dimethylformamide
FITC-Con A Fluorescein isothiocyanate concanavalin A
F-tag Fluorous tag
FSPE Fluorous Solid-Phase Extraction
Fuc Fucose
FucNAc N-Acetyl Fucosamine
Gal Galactose
GAS Group A streptococcus
Glc Glucose
GlcNAc N-Acetylglucosamine
HTF Heat transfer oil
HOBT Hydroxybenzotriazole
HPLC High performanc liquid chromatography
HRMS Hig Resolution Mass Spectrometry
Lev Levulinate
MALDI-TOF-MS Matrix assisted laser desorption time-of-flight mass spectrometry
ManNAcA N-Acetyl Mannosaminuronic acid
MeCN Acetonitrile
MeOH Methanol
m/z Mass/charge ratio
NMR Nuclear magnetic resonance
Piv Pivaloylate
PMB Para methoxy benzyl
PTSA para-Toluenesulfonic acid
Rha Rhamnose
SPE Solid-phase extraction
TBAB Tetrabutylammonium bromide
TBAF Tetrabutylammonium fluoride
TBS tert-Butyldimethylsilyl
TEA Triethylamine
Tf Triflate
TFA Trifluoroacetic acid
THF Tetrahydrofuran

TLC Thin layer chromatography
Tol Toluene
TMSOTf Trimethylsilyl trifluoromethanesulfonate

CHAPTER 1

GENERAL INTRODUCTION

Dissertation Organization

This dissertation is comprised of six different chapters. Chapter 1 is a review of the basic concepts and principles that are involved with chapters 2 thru 5. Chapter 2 reports a hydrophobicity/fluorophilicity unit introduced into the middle school classroom. Chapter 3 discusses the synthetic route of a di-fluorous tag. Comparative studies of the di-fluorous tag versus the mono-fluorous tags were carried out by microarray experiments. Chapter 4 demonstrates the automated solution-phase synthesizer in the synthesis of Group A Streptococcus (GAS) branched oligosaccharide. In chapter 5, *Staphylococcus aureus* monosaccharides were developed in order to be synthesized by the automated solution-phase synthesizer. This chapter will discuss the difficulties in building the *S. aureus* monomers and the methodology of the certain protecting groups. Finally, chapter 6 will conclude the dissertation with a summary of the projects and the future directions of each project.

1. Introduction

2. Fluorous Chemistry

Fluorous Chemistry is the study of highly fluorinated molecules. The studies include the structure, composition and reactions of the fluorinated compounds. (Ubeda, and Dembinski, 2006; Gladysz, and Curran, 2002) The perfluoro alkyl is a carbon attached to the fluorine. The

C-F has a short bond length, is electron withdrawing, and is chemically inert. (Biffinger, et al. 2004) It is known that the perfluoro alkyl has hydrophobic properties, is highly dipolar, and is fairly nonpolarizable. (Biffinger, et al. 2004, Dalvi, and Rossky, 2010) The most common perfluoro alkyl is Teflon®, due to the non-stick properties. Teflon® can repel both organic and inorganic solvents. (Curran, 2008) The property of perfluoro alkyls has become popular in the applications of pharmaceuticals, separation, microarrays, and etc... The importance of fluorous chemistry has grown in the past decade and been developed to provide innovative methods.

3. Implementation in the classroom

Fluorous chemistry has become very popular in the past decade in pharmaceuticals, non-stick products, small molecule libraries, and purifications. The one concern is that many students are not aware of fluorous chemistry and how it affects them in everyday life. In organic labs, professors have come up with using fluorous chemistry in the purification of desired products. The idea is for the students attach a fluorous linker to the starting material. Once the reaction occurs the students can use a fluorous solution-phase extraction (FSPE) to purify the desired product. The advantage is that the FSPE resin can be used more than once. The disadvantages are that some fluorous molecules are very expensive and that some of the reactions to attach a fluorous linker could take more time than what is in the class. There is still work that needs to be done to create ways to implement fluorous chemistry into the laboratory.

It seems that incorporating fluorous chemistry into the classroom has been more of a challenge. College classroom introductions to fluorous chemistry allow students to broaden their knowledge in environmental and experimental applications. (Ubeda and Dembinski, 2006)

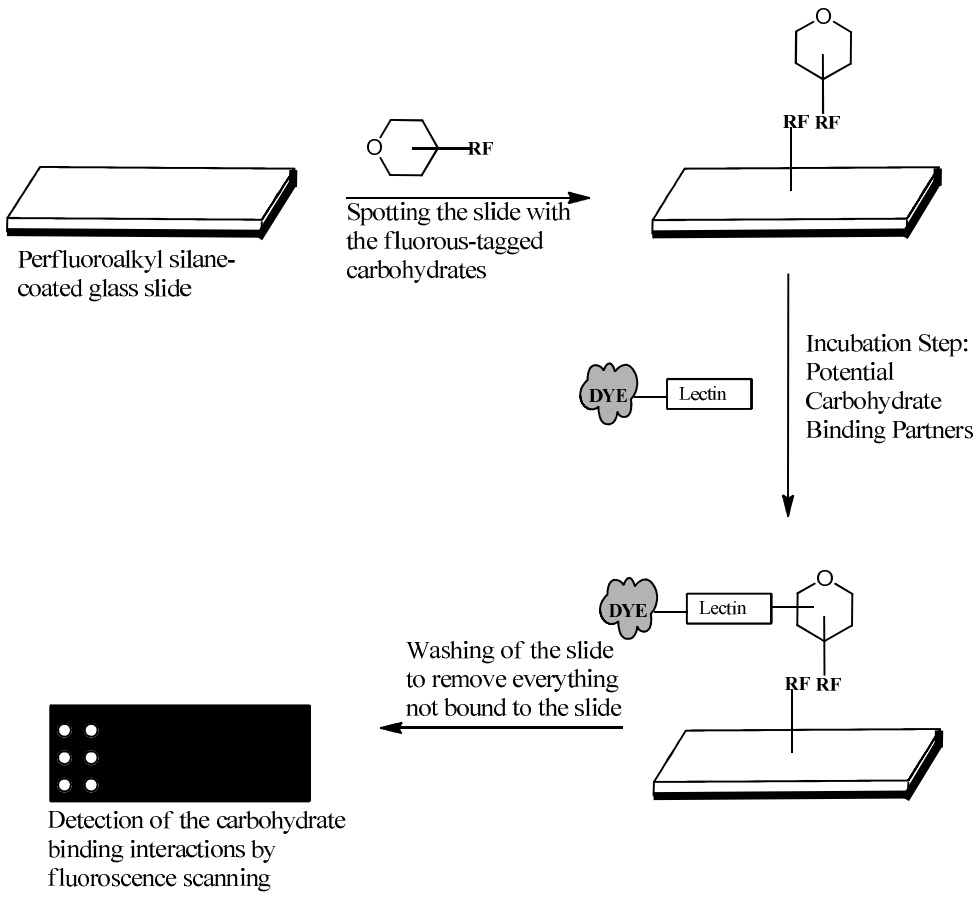
Applications and processes that combine the easy isolation procedures, and advantages of solution-phase reactions, and bring environmental awareness are of great interest. (Ubeda and Dembinski, 2006) The knowledge of the importance of fluorous products at a younger age such as middle school or high school could help the awareness of the science that is correlated to the products (such as Teflon®) that they use every day.

4. Carbohydrate Microarray fabrication

Carbohydrate microarrays are used to study the carbohydrate binding with a variety of protiens/lectins and antibodies. (Nagappayya, and Pohl, 2011; Song, and Pohl, 2009) The basic idea of the carbohydrate microarray relies on the covalent or non-covalent attachment of the sugar to the surface of the microarray slide. Once bond to the surface the qualitative and quantitative binding studies can occur (**Figure 1**). The advantage of using the microarray method is that is does not require a large amount of material. A lot of times the amount of material is in the nano to micro grams. For many of the large oligosaccharides, the amount of pure material is normally very low from both synthesis and isolation from a natural resource. (Nagappayya, and Pohl, 2011) The non-covalent method to carbohydrate microarray has been found to allow more flexibility to allow more binding to occur. The Pohl lab has taken the carbohydrate array and used non-covalent binding with fluorous-fluorous interactions. (Pohl, 2006; Pohl, 2008; Mamidyala, et al. 2006) The monosaccharide or oligosaccharide is attached to a fluorous linker, while the glass slide is coated with a Teflon® surface. The carbohydrate with the fluorous linker is spotted onto the fluorous-coated slide by a microarray printer. The slide is then placed in a humidity chamber where the fluorous linker can align itself on the glass. The next step is to incubate with the protein or antibody that is tagged with a fluorescent molecule, such as jack

bean IV concanavalin A (ConA) which is labeled with fluorescein isothiocyanate (FITC). (Jaipuri, et al. 2008; Ko, et al. 2005; Chen, and Pohl, 2006) The slide is then washed, removing the all of the non-bound protein from the slide. The slide is dried and taken to a scanner, where the protein that bound will show up. To get the quantitative data, there are special programs that will take the information found from the scanner and give the quantitative data of the fluorescence. The fluorous-fluorous interactions have been a crucial tool in carbohydrate microarrays.

Figure 1. Schematic of the work process for the production and screening of fluorous-based carbohydrate microarrays (Nagappayya, and Pohl, 2011).



5. The difficulties in the chemical synthesis of carbohydrate monomers and oligosaccharides

Carbohydrates are found to be the most abundant group of natural products. Due to the diverse roles of carbohydrates in nature, they have become attractive subjects to chemical and biological research. (Nicolaou, and Mitchell, 2001) The primary forms of carbohydrates found are monomers, oligomers, and polymers. In nature carbohydrates play an important role in many biological processes such as cell-cell recognition/communication, immune systems, development, and pathogenesis, and transport of energy. (Nicolaou, and Mitchell, 2001) Even though the roles of carbohydrates are known, scientists still do not understand many of the processes, mechanisms, and binding that occurs at the molecular level.

To understand some of the processes, mechanism, and binding of the oligosaccharides at the molecular level, the oligosaccharides need to be synthesized. Many of the oligosaccharides are complex and are challenging to synthesize. To build an oligosaccharide one must start with the monomer building block. In carbohydrate synthesis, protecting group chemistry is used due to all the hydroxyl groups that are part of the sugar. Amines and carboxylic acids groups are sometime present on the monosaccharides, which have to be protected. The protecting groups need to be orthogonal in order to have the linkages required with the desired oligosaccharides.

Another challenge is the reactivity of the monosaccharide that was developed to build the oligosaccharide. The reactivity of a monosaccharide may not be known. An acceptor/donor might be less reactive, so that the reaction will not occur. The other probability of a low reactive

monomer would create a low yielding reaction. Either outcome of the low reactivity is a setback, where the protecting groups may need to be changed. The opposite problem is having a very reactive donor. The reactive donor might react with itself to create another product in the mixture. This leads to more complicated purification steps. The reactivity is crucial when it comes to synthesis of the oligosaccharides.

Solvent effects can also create issues with the glycosylation reaction when building the oligosaccharides. For some monomers the anomeric position of the donor could have a stable group in a certain solvent, but once the donor is placed in a different solvent it could become unstable. The donor might react with the solvent, or the anomeric position promoting group might do a rearrangement, or the donor could just decompose at a faster rate. There are many unknowns to carbohydrate chemistry. Libraries of carbohydrates are critical to making the synthesis of monosaccharides and oligosaccharides in the future and will help with the challenges of carbohydrate synthesis.

6. Automated oligosaccharide synthesis

6-1. One-pot synthesis

The one-pot method for oligosaccharide synthesis and designed was the first automated process of oligosaccharide synthesis, which was introduced by C.-H Wong and coworkers. (Koeller, et al. 2000, Ye, et al. 2000) The one pot-synthesis method relies on many different challenges that include reactivity of the acceptors/donors, the orthogonal protecting groups, and the effects of the solvents. One-pot synthesis is based on the reactivity of the glycosyl donor and

acceptor which can be singled out by manipulation of the protecting groups. (Hsu, et al. 2011) Due to the manipulation of the protecting groups this can lead to chemoselective glycosylation. This occurs when the most reactive donor is activated and a less reactive donor is present with the identical leaving group to give the product desired. (Hsu, et al. 2011) Data from empirical testing of dozens of protected building blocks are now part of a computer program called OptiMer database. This database allows a user to quickly pick out the best building block combinations for a successful one-pot method synthesis, which can create a three monomer oligosaccharide in a matter of minutes to a couple of hours. (Hsu, et al. 2011; Sears, et al. 2001; Zhang, et al. 1999) Even though this seems to be an elegant approach to automated synthesis, there are still many challenges with each building block. Sometimes the reactivity of the building blocks may not have a significant difference, which leads to mixtures of the glycosidic linkages. To avoid this issue, there would need to be a large excess of donor building blocks. This computerized one-pot synthesis of oligosaccharide design program and robotic platform to carry out the optimal synthesis is becoming more advanced even with some of the flaws.

6-2. Solid-phase oligosaccharide synthesis

The solid-phase oligosaccharide synthesis was reported in 2001 by Seeberger and coworkers. (Plante, et al. 2001) Solid-phase oligosaccharide synthesis was the first example of a robotically controlled synthesis of oligosaccharides. This automation is comparable to the nucleic acid and peptide synthesis. A conventional solid-phase peptide synthesizer was converted with the addition of a cooling unit to be used for glycosylation process in order to obtain the desired oligosaccharides. (Plante, et al. 2001) The procedure of the synthesizer starts with the

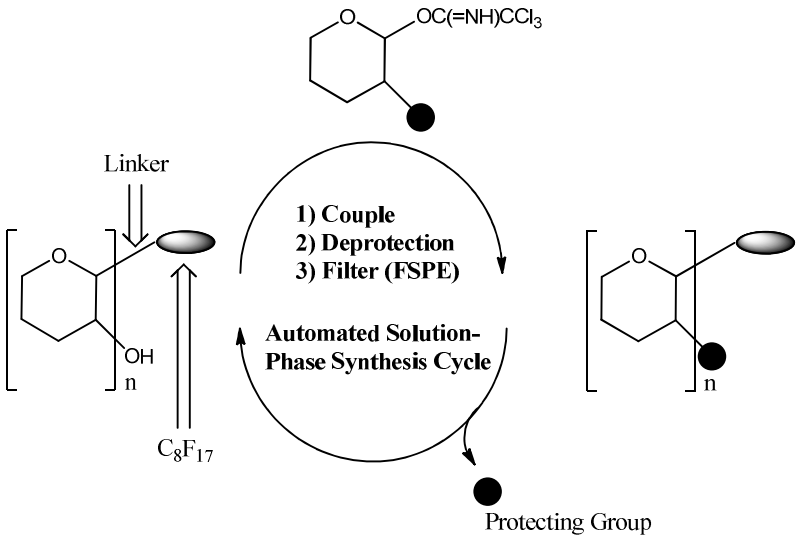
robot delivering the desired soluble reagents and building blocks to the reactor containing the resin. The resin will hold the growing oligosaccharide chain while the excess reagents are washed away. (Plante, et al. 2001; Seeberger, 2008) One of the disadvantages to the solid-phase synthesizer is that it requires 20 equivalents of donors for each glycosylation, which means that many of the building blocks are generated as waste. (Seeberger, 2008) Other complications of this process include not being able to monitor each reaction on the resin during the run. A user might be able to see the mistake with solid-phase NMR spectrometry. The user would have only two options: let the program run to the end and hope to be able to purify the mixture, or stop the run. Even with the current issues, solid-phase synthesis of oligosaccharides has been an efficient automated process.

6-3. Automated solution-phase oligosaccharide synthesis

Automated solution-phase synthesis is a fairly new method developed by Pohl and coworkers. (Jaipuri and Pohl, 2008; Pohl, 2008) The concept of the automated solution-phase synthesizer is based on bench-top chemistry, but here it is a robotic arm that delivers all the reagents and solvents to the reactor vials. The process is to take a fluorous tag acceptor and a monosaccharide donor as the first cycle as shown in **Figure 2**. The cycle starts with the glycosylation. The glycosylation can be monitored, once finished the reaction is quenched and evaporated. The solvents and reagents are delivered to the reactor vial to start the deprotection step. When the deprotection step is completed the solvents are evaporated off. The last step of the cycle is purified by FSPE and transferred to a new clean reactor vial to start the next cycle. This process continues until the desired oligosaccharide is synthesized. The advantages to the automated solution-phase synthesis of oligosaccharides are easy purification, less equivalents of donor are

used, each step can be monitored, and the reactions can be stopped in the middle of the cycle if a mistake occurs.

Figure 2. The basic schematic of the automated solution-phase synthesizer.

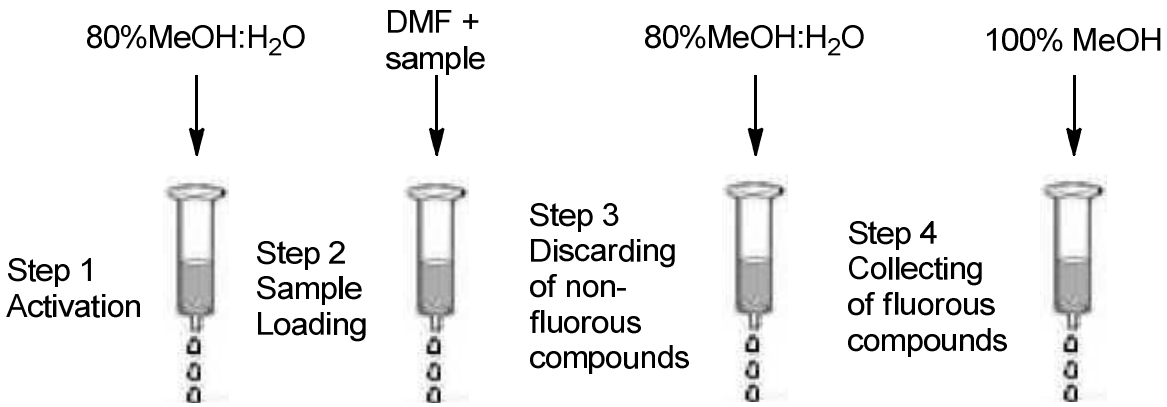


6-3.1. Purification strategies

In oligosaccharide synthesis it is crucial to have a purification strategy that can be efficient to the whole process of developing an oligosaccharide. The fluorous tag is a crucial tool in the purification process of automated solution-phase synthesis. In the Pohl lab, an allylated C_8F_{17}-tag (F-Tag) is used for the automation of oligosaccharides. (Jaipuri, and Pohl, 2008) The F-Tag is attached to the first donor molecule and after each cycle the oligosaccharide is purified by Flourous Solution-Phase Extraction (FSPE). The first step of FSPE is to activate the resin with 80% methanol:water. Next, the sample is loaded onto the FSPE by using 0.4 mL of DMF. Then 80% methanol:water solution is used to wash everything off the resin that does not

have the fluorous tag. Finally 100% methanol was used to extract all of the fluorous tag molecules off of the resin as shown in **Figure 3**. The drawback to this purification process is when one acceptor fails to react with the donor provided. This can cause the results to have a buildup of by-products. Many of these by-products can be readily removed to obtain the desired product.

Figure 3. Fluorous Solution-Phase Extraction process.



7. Tag modification/removal

In the synthesis of larger oligosaccharide compounds the allylated-C8F17 fluorous tag has proven to be a convenient tool. (Jaipuri, and Pohl, 2008) The fluorous tag was useful for the glycosylation/deprotectection cycles because of the survival rate of the fluorous tag in a variety of synthetic conditions. After the oligosaccharide was synthesized, it is necessary to remove the fluorous tag. Mild conditions and a high yielding process of the removal of the fluorous tag is required. The versatility of the fluorous allyl tag allows for it to be transformed to different

functional groups, which can be used for future applications. The fluorous tag can be converted to the allyl group by olefin cross-metathesis conditions (Ratnayake, and Hemscheidt, 2002), which can be removed later by palladium reagents (Mereyala, and Guntha, 1993) or iridium catalysts (Boullanger, et al. 1986) followed by mercury conditions (Gigg, and Warren, 1968). The allyl fluorous tag can be directly used for ozonolysis to create the aldehyde. (Gigg, and Gigg, 1966; Smith et al., 1991) The aldehyde can then be reduced to an alcohol or oxidized to a carboxylic acid. Versatility for further applications of the allyl fluorous tag has proven to be successful.

8. Summary and future directions

To conclude fluorous technology has grown in the past years creating new approaches to new and old methods of microarrays, automation platforms, small molecule libraries. Bringing the attention of fluorous compounds and environmental factors to the students of today is a key part in creating new potential safe fluorous products and methods for the future.

CHAPTER 2

Introduction of Surface Tension, Emulsions, and Hydrophobicity/Fluorophilicity in Middle School Science

Submitted to *The Journal of Chemical Education*

Heather D. Edwards, Luke Spencer and Nicola L. B. Pohl[1]

Abstract

Here we report a hydrophobicity/fluorophilicity unit for the middle school classroom. This unit was developed as a part of a National Science Foundation (NSF) GK-12 program to bring research into the classroom and serves to strengthen knowledge learned earlier of the basics of chemistry in the context of new material on physical forces. These activities, which include four hands-on laboratory experiments, helped the students to retain the chemistry knowledge they learned from previous years of education and allowed the teacher to relate the concepts in future units. The integrated set of activities and lectures also increased student awareness of the role of chemistry, especially hydrophobicity/fluorophilicity in nature.

INTRODUCTION

To convey current research using hydrophobic properties of fluorous molecules (Biffinger, et al. 2004; Yoder, and Kumar 2002) to middle school students, we proposed to modify a traditional surface chemistry unit to include concepts of hydrophobicity/fluorophilicity. This unit was chosen for modificaitaon because basic versions of this unit in surface chemistry

[1] Luke Spencer is a teacher at Warren G. Harding Middle School whose classroom this work was carried out in part.

have already been developed for use in the elementary/middle school science curriculum. (Dalvi, and Rossky 2010; Crick, and Parkin 2010) Unfortunately, many of the available surface tension and surface area hands-on experiments are designed for upper level classrooms and are taught with complex math (Hazlehurst 1942; Huang, et al. 1999; Dionísio, and Sotomayor 2000; Rosenthal 2001; Poce-Fatou 2006; Lee, et al. 2008; Eberhart, and Horner 2010; Brooks 2009) and require equipment and materials that are too expensivee and hazardous for use with younger students. (Arnáiz 1997; Silverstein 1993) Therefore, we developed simpler new labs related to surface area and surface tension at the middle school level with only pre-algebra math skills. Also, we needed to design labs that were safe (Zirkel, and Barnes 2011) for the middle school level to take the knowledge that the students have in basic chemistry such as atoms, molecules, and phases and modify and add new concepts to help explain surface science and hydrophobicity.

Middle school science is used to show students the basic concepts of several different sciences to prepare for the high school level sciences. However, students have a problem with visualizing, learning, and retaining information from the different sections. (Forbus, et al. 2005) High school teachers ideally would have students start to design their own labs without going into depth again for the basic concepts. (Iimoto, and Frederick 2005) Normally, middle school students start to learn about chemistry in the 6th grade with chemical properties and 7th grade with elements and molecules, but no chemistry is discussed in the 8th grade curriculum. (Iowa Core Curriculum) Unfortunately, the decay of knowledge of chemistry with students is apparent in the upper level classrooms. (Bunce, et al. 2011) Retention of this chemistry knowledge through the 8th grade is crucial for success in the upcoming high school years because the 9th

grade advanced physical sciences and 10th grade biological sciences incorporate molecular level thinking. (Iowa Core Curriculum) The standard 8th grade curriculum consists of the following units: forces and motion, sound and light, astronomy, and genetics. (Iowa Core Curriculum) Knowing that our particular students had either not understood or retained the basic concepts in molecular chemistry from the previous years, we decided to sneak in chemistry again at the beginning of the forces and motion unit by the development of a series of experiments in hydrophobicity/fluorophilicity within the first quarter. This tact should allow the teacher to continually go back and reference this unit to reinforce chemistry throughout the school year and thereby possible better prepare students to be in a high school setting, where they will do material science and surface science and start to develop a greater understanding of the nature of science. (Lorenzini, et al. 2011; Marchlewicz, and Wink 2011)

Our efforts to create and implement a basic chemistry unit were supported by a year-long (2011-2012) project under the National Science Foundation GK-12 STEM Fellows in Education. (Rios, and French 2011) The goal of the program is to incorporate innovative research into the classroom to show students that people are working on new things each day that can one day have an effect on students. This approach allows students to see that they are part of research everyday when using real world applications to problem solving such as baking, building something, or even playing a video game. To integrate current research and ideas in the classroom activities, the GK-12 fellowship pairs a middle school science teacher with a doctoral student. (Rios, and French 2011) To relate some of the research that goes on in our lab to 8th grade students and also reinforce basic chemistry concepts, we designed a week-long

hydrophobicity/fluorophilicity unit that would allow students to discover some of the basics of noncovalent interactions, physical properties, and surface science.

Brief Summary of Procedure and its Place in the Middle School Science Curriculum

The hydrophobicity/fluorophilicity unit was taught in three different 8th grade classes; one of these classes was 8th grade lessons for advanced 7th graders. Two of the classes contained 21 students each; the advanced class contained 19 students. The hydrophobicity/fluorophilicity unit included 4 labs that were focused on emulsion, surface area, and surface tension. Each lab had the students write a prediction/hypothesis, collect specific data, and develop a conclusion that would wrap up what they did in the experiment and how they could relate the lab to real world applications. The labs were set up to give the students instruction, but required that they have to figure out what is happening in the experiment with minimal teacher intervention.

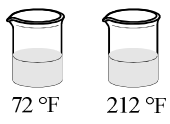
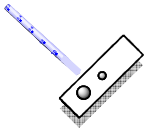
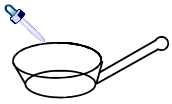
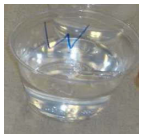
Motion Unit	Energy Unit	Friction/Fluid Unit	Friction/Fluid Unit	Energy Unit	
1. Pre-test assessment 2. Introduction to vocabulary terms 30-40 min 2. Brownian Motion Demonstration	1. Lecture 10-15 min on Emulsification 2. Emulsification Lab	1. Lecture 10-15 min on surface area 2. Qualitative analysis of surface area lab	1. A 5-10 min recap of Day 3 2. Quantitative surface area lab: Calculate the surface area of a circle	1. Lecture for 10-15 min on surface tension 2. Surface tension lab	1. Discussion and questions 2. The quiz show game 3. Post-test assessment
Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
					

Figure 1: Timeline of the hydrophobicity/fluorophilicity unit. Above in the color bars are the units that that experiment correlates to do just that lab.

An overview of the week's activities for the newly designed hydrophobicity/fluorophilicity unit is shown in Figure 1. The first day of the hydrophobicity/fluorophilicity unit is an introduction to the entire week. The teacher gives a pre-assessment test to find out what the students know at the moment. This brief test is followed by a lecture that starts out by asking the students if their chairs and tables are moving. This opening allows the teacher to start to explain motion at a molecular level and includes a demonstration of Brownian motion demonstration to aid student understanding that everything is in continuous movement whether as a solid, liquid, or a gas. The students could see that the water may look still, but that it is composed of smaller units (molecules) that are moving more rapidly. Fluorocarbons verses hydrocarbons are also introduced and served as a way to also talk about elements on the periodic table and how these might have similar hydrophobic properties. Here the teacher talked about Teflon™ and vegetable oil. The teacher can also introduce the concept of polarity and explain how the properties of two different molecules result in their ability to associate with one another or not. The teacher-led demonstration leads to an explanation of new science vocabulary and an understanding of basic concepts needed for the week's planned experiments. (A vocabulary list is included in the supporting information.)

An emulsification lab was the first lab of the unit and served as a way to show hydrophobicity in action without yet introducing concepts of surface area or surface tension. We wanted the students to be able to reflect back to this lab when running the other experiments to

relate all the labs together in the end. Emulsification is another way to use motion—to create the emulsion—and then forces are acting on the globules to make them slowly go back to the original liquid. Emulsification puts an emphasis on the phrase "like dissolves like." Many research groups have found ways to create emulsification labs for the college level by either their focus on emulsion (lotions (Mabrouk 2004)) or emulsifiers (chocolates (Rowat, et al. 2011)). Here we have the students create an emulsion (salad dressing) using vinegar and vegetable oil with egg yolk as the emulsifier. We chose to do this experiment because every student was familiar with salad dressing and thereby could relate the new concept to a real world application. In addition, the inexpensive supplies readily fit into the required budget with limited safety concerns.

The third and fourth days introduce surface area in the context of two different labs. These labs were designed to help students understand how surface area relates to the emulsification process they just carried out. At the end, the students could explain that the surface area of the droplets from the emulsification experiments determines the time it will take for the two liquids to separate from one another with no remaining globules. The new surface area labs allowed the students to visualize how different surfaces react with water and other liquids and to start to see that the same concept could apply to a variety of liquids. In addition, surface area was related to friction, a topic that was required for 8th grade. The first day is quantitative and the students use slides that are covered with different materials. They place a spot of different liquids on slides and observe the different dimensions (height and width) of the spots. The second day is more quantitative and students measure the surface area of a circle by

using different liquids and different surfaces. The students measure the diameter of the spot made by a liquid to then find the surface area of the circle produced.

Surface tension has always been a hard concept to explain, something the American Chemical Society (ACS) has tackled with activities that help to explain surface tension. (American Chemical Society) Using this ACS activity as a starting point, we modified the activity for incorporation into the 8th grade classroom. We modified the lab by adding different liquids to test, so the students could physically see what was happening. The surface tension labs are to show that surface tension can be explained as forces that are acting upon the surface. The students were directed to come to the conclusion that the liquid in the middle has forces acting on it from every direction but the liquid at the surface only has the forces from the bottom and the sides acting upon it. The final day is allotted for discussion time, for answering questions, and for playing a game show quiz that serves to refresh students on what they have learned for the past week. A post-assessment test closes the unit. If time does not allow a full week, the hydrophobicity/fluorophilicity unit can be used as smaller units. In **Figure 1,** the top bar shows the units that a particular lab will fit into if needed. The whole unit was designed to be versatile for teachers to pick whether to do the whole lab or just certain labs that pertain to what is being taught.

RELEVANCE TO REAL WORLD APPLICATIONS

We found that it was very important to maintain student interest and spark their critical thinking skills throughout by relating the abstract concepts to real world applications. The

students were explicitly asked to think about how each lab related to things that they saw everyday. In the emulsification lab, many students wrote that the lab itself applies to everyday situations because salad dressing—an emulsion—can be used on many types of food. Students found it more difficult to find everyday applications of the surface area lab. A few were able to think about how leaves have a waxy surface on top and so when it rains the water will bead up and slide off the leaves. Others thought about rainy days and how we use umbrellas. The umbrella will have the same effects as the leaves. Many other students could think of shoes and coats. Surface tension was a trickier concept for the students. The students had a hard time grasping real world applications. One that was presented to them was water striders. A water strider is a bug that moves along the surface of the water without breaking the surface. Another example that the students came up with was a lizard that could run across water. Even though the lizard does break the surface tension a little with the feet, the tail seems to never break the surface.

Another part to this weeklong unit was to convey information about fluorous molecules—their hydrophobicity and their known utility. In the beginning, most students had never even heard of Teflon™. The ones that had heard of Teflon™ said that the material was in pots and pans. The students were introduced to Teflon™ on the first day when talking about fluorocarbons. The students were able to see how Teflon™ is used in other materials for non-stick applications and lubrications (pans, tape, bicycle chain lubrication, different type of polishes, etc.) in the surface area labs.

TEACHER AND STUDENT PERSPECTIVES ON THE WEEK-LONG UNIT

Sixty-one students were asked this question at the end of the week: What made learning easier for you this week? Not surprisingly, most students answered that the hands-on learning/manipulatives helped them to understand the concepts better. The hands-on experiments were important to help them understand how the scientific concepts relate to things that they do everyday. The discussion at the end of the week was to have students critically think about what they had done throughout the whole unit. Questions that they still had could be answered. The discussion time could also be used to allow students to brainstorm how to set up other experiments that would apply to the concepts that were taught.

Additional assessment revealed that in the initial emulsification experiment the students had a difficult time writing and explaining what they were observing and relating those observations to real world applications when answering the conclusion questions. As the students progressed through the other labs during the course of the week, their observations became notably more detailed and the conclusions became more thoughtful, thereby showing that they were starting to understand how everything related to one another. By the last lab, on surface tension, the students were able to write in their conclusions more about how the lab could apply to the real world. It was important that in the conclusion questions the students started to think more critically about what they had done and how it related to other things that they had done previously. For example, the surface tension lab asked them to critically think about how emulsification and surface tension can relate to one another. The students were able to talk about the surface tension of the globules and how they make larger masses in the emulsion. This was

good because they would try to critically think about how the process worked and how it related to the real world.

The pre-assessment test and the post-assessment test asked essentially the same questions. The pre-assessment had a few vocabulary terms that the students had learned from previous weeks, but the rest of the vocabulary terms and questions were left blank. The post-assessment test had five specific questions based on vocabulary terms, fluorinated compounds, "like dissolves like", Teflon™, and hydrophobicity in nature. For the vocabulary term section 83% of the students had a better understanding of the terms from pre- to post-assessment. By the end, 80% of the students could comprehend the phrase "like dissolves like"—a nice finding given how hard the concept had been for them to understand at the beginning of the unit. Most of them had answers that substances with the same properties will mix together while substances with uncommon properties will not mix. Only 4% of the students could not answer what Teflon™ is and where it can be found. By the end, 51% of the student understood what was meant by flourinated compounds. This result was good as the teacher did not particularly emphasize this idea throughout the week. About 47% of the students did not answer the fluorinated question, so we could not determine whether they did or did not understand the concept, but presume the latter. The hydrophobicity question asked where in nature do you find things that repel (keep away) water? This question stumped 22% of the students. The remaining 78% had answers that ranged from waxy leaves, to the oils found in the coats of otters and in the feathers of ducks. We had asked the students to think why hydrophobicity is useful in the context of what they had answered. The students had answered that for the waxy leaves it is to keep the plants from drowning when it rains, while for otters and ducks they felt that the oil helps to repel

the water, so that the animals would not freeze to death when in cold water. Another answered that the duck feathers need to be kept dry for when they need to fly off when a predator approaches. Some of the students would try really hard and if they were not sure at the end when handing it in they would ask and try to discuss it with the teacher. Motivation and critical thinking at this level is very important because they can harness these skills for the future classes. As the week started the students seemed to just do the bare minimum and not answer the questions or try to think of why certain things happened, but as the week went on the students started to discuss more and wanted to explore concepts in more depth.

For the teacher, there is a presentation, lab write ups, and vocabulary for this unit. Each day was designed to be a 40-50 minute class period. For longer class times and in cases where few students actually read the procedures, the students could write the procedure in their own words as a way of helping them clarify any misunderstandings prior to the lab activities. Each day, the teacher was able to get through the labs in the time that was allotted, except for the surface area of a circle lab. This lab seemed to to take the most time and would be easier to conduct in two 50-minute class periods versus one class period. Otherwise, the activities could be done within the time allotted for each lesson and lab.

Interestingly, the structure of the experiments led some students to start generating their own hypotheses, which followed nicely from their initial introduction to the nature of science at the beginning of 8th grade. For example, some asked what the difference in surface area would be of a drop of water on a white paper towel versus a brown paper towel that the school uses. Time allowed these students to design and carry out their proposed experiment; they found that the

surface area of the water droplets were larger on the brown paper towels than the white paper towels. This set of experiments led to an interesting discussion that led students to question the reason for the difference and to debate which type of towel they would rather use given this data.

CONCLUSION

The concepts of hydrophobicity/fluorophilicity could successfully be incorporated into the 8th grade classroom in the usual discussion of forces and used to reinforce basic chemistry concepts through this integrated set of activities. The hands-on learning experiments proved particularly valuable in relating the material to students. The hydrophobicity/fluorophilicity unit utilizes the labs to engage the students to develop a familiarity with the basic chemistry principles of motion. The labs serve as a source for introducing and connecting the relevance of chemistry to the everday experiences, while connecting to the research and representations used by scientists. Although this lab was designed for the 8th grade classroom to fit in with forces and motion, it could also fit readily into other curriculums/learning objectives in the 6th and 7th grades. We hope that this hydrophobicity/fluorophilicity unit will serve other teachers and also encourage them to introduce more chemistry into the middle school science classroom.

HAZARDS

The isopropyl alcohol is flammable. Isopropyl alcohol and soap can be irritants. Depending on the source of the eggs, a very small risk of infectious agents could be present. Do

not touch the face with your hands during the experiment and make sure to wash hands after the experiment when handling the eggs. All glassware should also be handled with care.

CHAPTER 3

Probing the Limitations of Fluorous Content for Tag-Mediated Microarray Formation

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Heather D. Edwards, Sahana K. Nagappayya and Nicola L. B. Pohl, *Chem. Commun*., **2012**, *48*, 510-512, DOI: 10.1039/C1CC16022B

Abstract

The synthesis of a di-perfluorohexyl tag is reported for use in a fluorous-based carbohydrate microarray. A comparative microarray study with this di-perfluorohexyl tag and a mono-perfluorooctyl and mono-perfluorohexyl tag found the increased fluorous content conducive to better spot morphology and easier washing protocols without precluding reuse of the fluorous slide.

Introduction

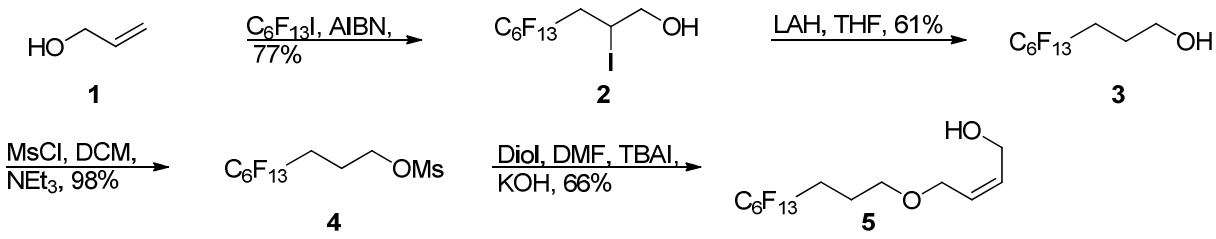
Perfluorinated compounds (PFCs) have been extensively used in the past decade in commercial, industrial, and research studies, but now PFCs are becoming a major environmental concern. (Cheng, et al. 2008; Pistocchi, and Loos 2009; Renner 2007; Renner 2009, Kato, et al. 2011; Lopez-Espinosa, et al. 2011; Loi, et al. 2011) The most environmentally persistent PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctonoate (PFOA). Recent studies have found that the growing amounts of PFOS and PFOA found in water correlate with the bioaccumulation of PFCs in human and animals globally. (Cheng, et al. 2008; Pistocchi, and Loos 2009; Renner 2007; Renner 2009; Kato, et al. 2011; Lopez-Espinosa, et al. 2011; Loi, et al.

2011) However, unlike the longer PFCs, C6 and shorter perfluorous chains are not environmentally persistent. (Lee, and Mabury 2011) Growing safety and health issues with these 8-carbon-length fluorocarbons threaten their continued bulk manufacturing and therefore also the inexpensive use of this fluorous chain length in a variety of applications in which environmental escape of the PFCs is not a concern. However, such octylfluorous tags have already proven useful not only for efficient separations using fluorous solid phase extraction (FSPE)(Curran 1998) but also as a convenient fluorous handle for microarray formation.(Curran 2008) Fluorous microarrays rely on non-covalent fluorous-fluorous interactions between the fluorous tail linked to the molecule used for screening and the fluorous coated glass slide.(Pohl 2008; Zhang, and Cai 2008) In addition to its use for carbohydrates, such octylfluorous-based microarrays have also been successful for screening other small molecule-protein interactions.(Vegas, et al. 2007; Liang, et al. 2008) More recently, biotin was adhered to a fluorous-coated glass slide and it was demonstrated that C_8F_{17}-tagged molecules were better for fluorous biotin-avidin microarrays than the C_6F_{13}-tagged molecules in terms of spot intensity, size and spot morphology.(Nicholson, et al. 2007) Given that a shorter fluorous tag was likely not an option, we set out to discover a fluorous tag that could form strong enough non-covalent interactions for robust fluorous microarray studies without reliance on the C_8F_{17} motif.

Results and Discussion

First a route to the desired mono-C_6F_{13}-tag was designed based on the synthesis of the known mono-C_8F_{17}-allyl tag for direct comparison.(Mamidyala, et al. 2006) We started the synthetic route with perfluorohexyl iodide as an inexpensive precursor to obtain our desired

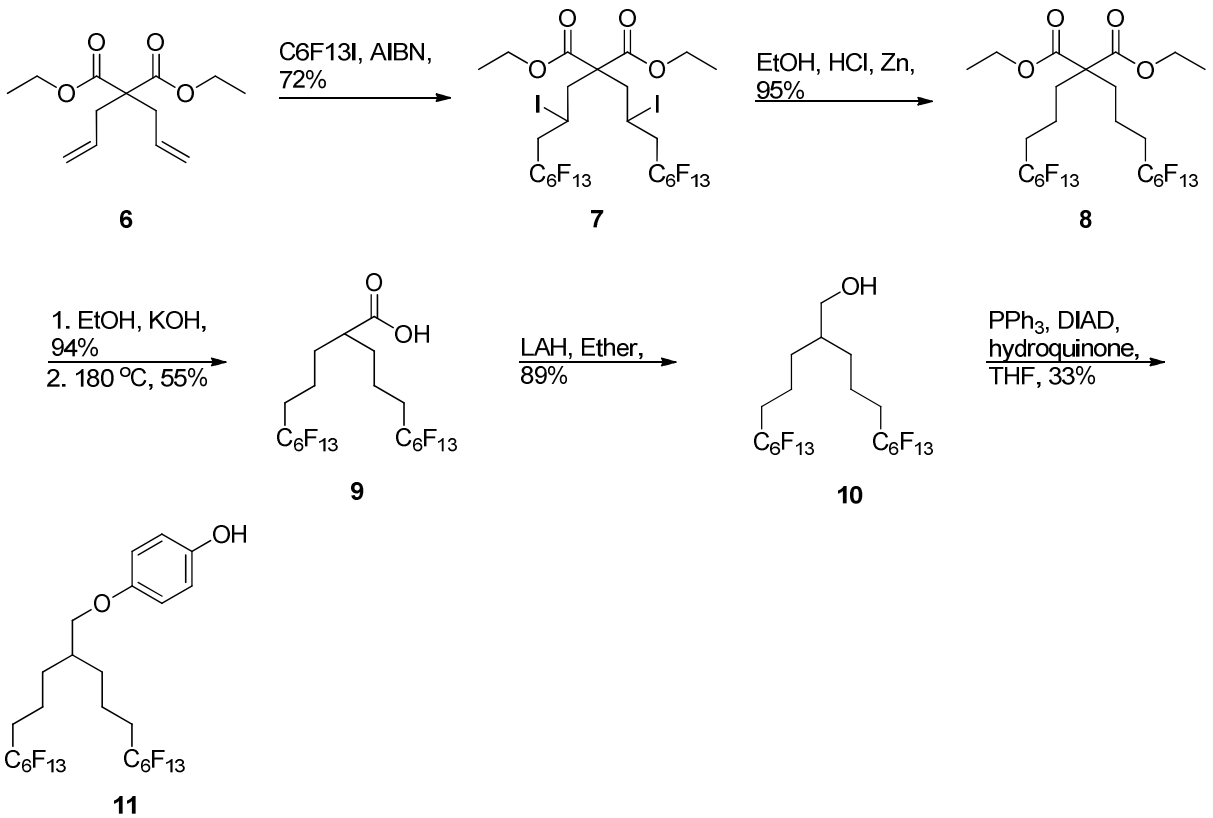
product **5** (**Scheme 1**). Radical addition of perfluorohexyl iodide to allyl alcohol followed by reduction of the iodide provided perfluorohexyl alcohol **3**. Alcohol **3** was then mesylated for displacement by (Z)-1,4-but-2-endiol to provide the desired allyl tag **5** in 28% overall yield from allyl alcohol.



Scheme 1: Synthetic route to the mono-C_6F_{13}-allyl fluorous tag.

As one hexylflourous tag was insufficient for good spot formation on a fluorous slide, we next designed a new tag containing two C_6F_{13}-moieties for direct comparison with the related mono-C_8F_{17}- and mono-C_6F_{13}- tagged carbohydrates described above. Such a di-C_6F_{13}-tag would still need to allow the attached sugar to orient away from the slide surface in such a way that protein-binding could take place. As many sugars come in the form of glycolipids, these structures were seen as inspiration for the design of compound **11** with two fluorous tails attached to a group that served as a UV-active and removable linker (**Scheme 2**). Using ceric ammonium nitrate (Tojino, and Mizuno 2008) the fluorous linker could be readily removed from the di-C_6F_{13}-tagged peracetylated glucose. The desired di-C6F13-tag synthesis then started with the known diester **6** made by addition of two allyl groups to diethylmalonate followed by radical addition of perfluorohexyl iodide to the resulting alkenes.(Brace 1964; Brace 1972; Brace 1973; Brace 1975; Brace, and Van Elswyk 1976; Brace 1979; Brace 1999a; Brace 1999b; Brace 2001)

The presence of the diesters precluded use of lithium aluminum hydride to remove the iodides; therefore, microwave-assisted conditions using zinc in acidic medium were developed for iodide removal to produce, after base-mediated saponification, diacid. The diacid was immediately subjected to heat-mediated decarboxylation to produce monoacid **9**.(Loiseau, et al. 2001; Kaplanek, et al. 2006) This monoacid was reduced to the alcohol for a Mitsunobu reaction analogous to those previously carried out with other fluorous alcohols(Mizuno, et al. 2008) to produce the desired di-fluorous tag **11** in a 14% overall yield from diester **6**. The di-fluorous tag was found to be soluble to at least 1 M concentrations in the common solvents dichloromethane and toluene at room temperature.



Scheme 2: Synthetic route to the di-C_6F_{13} fluorous tag.

With the necessary fluorous tags **5** and **11** in hand, the next step was their glycosylation with peracetylated trichloroacetimidate-activated (Schmidt, and Michel 1980) mannose, glucose, and rhamnose glycosyl donors. (All α-L-rhamnose glycosides were provided by Sahana Nagappayya) After Zemplen deacylation and reduction of the alkene, nine compounds (**Figure 1**) were obtained to perform microarray experiments with fluorescein isothiocyanate-labeled concanavalin A (FITC-ConA). ConA is known to bind to terminally α-linked D-mannose, whereas β-D-glucose and α-L-rhamnose are not ligands for this plant lectin. (Ko, et al. 2005) Previously reported FSPE protocols for the octylfluorous tagged monosaccharides were used for the purification of our new fluorous-tagged monosaccharides.(Mizuno, et al. 2008) Interestingly, the same solvents could be used for eluting the compounds with a single as for a double fluorous hexyl moiety. The aromatic ring also does not override the fluorous content in the FSPE protocol.

In order to compare the performance of the three fluorous-tags on the fluorous-coated glass slide, the carbohydrate microarray study was set up as shown (**Figure 1**). To make a 250 μM concentration of the carbohydrates, the fluorous-tagged monosaccharides were dissolved in methanol/DMSO/water (2:6:2). (Chen, and Pohl 2008) These monosaccharides were then spotted multiply in groups of nine onto a commercially available fluoroalkylsilane-derivatized glass slide using a standard microarray spotting robot. (Chen, and Pohl 2008; Jaipuri, et al. 2008) The slides were then incubated with a 200 μM solution of FITC-ConA in phosphate-buffered saline (PBS) for one hour. After incubation, the slide was washed with a 1X PBS containing 1% BSA solution twice and then washed once with distilled water.(Liao, et al. 2010) Next, the slide was scanned using a General Scanning ProScan Array HT at 488 nm to visualize the carbohydrate–ConA binding (**Figure 1**). Finally, the data collected from the scan was processed through ImaGene®

8.0 software to obtain the intensities of each fluorous-tagged monnosaccharides. (For details of the spotting and scanning, see Experimental Section.)

Multiple spotting and scanning experiments revealed several key experimental details. We found that spotting on a new fluorous glass slide resulted in uneven spots; washing the slides with a 1:1 dichloromethane:methanol solution before printing solved this problem. The morphology of the spots was found to also be affected by several physical factors like temperature, humidity and drying time. We printed the slides at three different humidity conditions (60%, 65% and 70% humidity) while maintaining the temperature at 22 °C and observed that the slides printed at 70 % humidity showed the best spots with no donut effect.

We speculate that the higher water content in the air helps the hydrophilic carbohydrates to better orient on the glass slide to create the non-covalent fluorous-fluorous interactions with the fluorous molecules on the slide. Drying of the slide after printing also had an affect on the spot morphology. Less donut effect was observed when the slides were dried for a longer period of time. After testing various drying times, we concluded that the slides should be kept in the humidity chamber for 18 hours and then outside of it for 2 hours before incubating.

After optimal spotting and drying conditions were found, comparisons among the three different fluorous tags were made. Fluorescence scans after various washing protocols show that the di-C6F13-tag-containing sugars were robust and could withstand more than two washings with 1XPBS containing 1% BSA. In many cases, the mono-C_6F_{13}-tag containing sugars were being washed away when washed more than once with the PBS solution containing BSA,

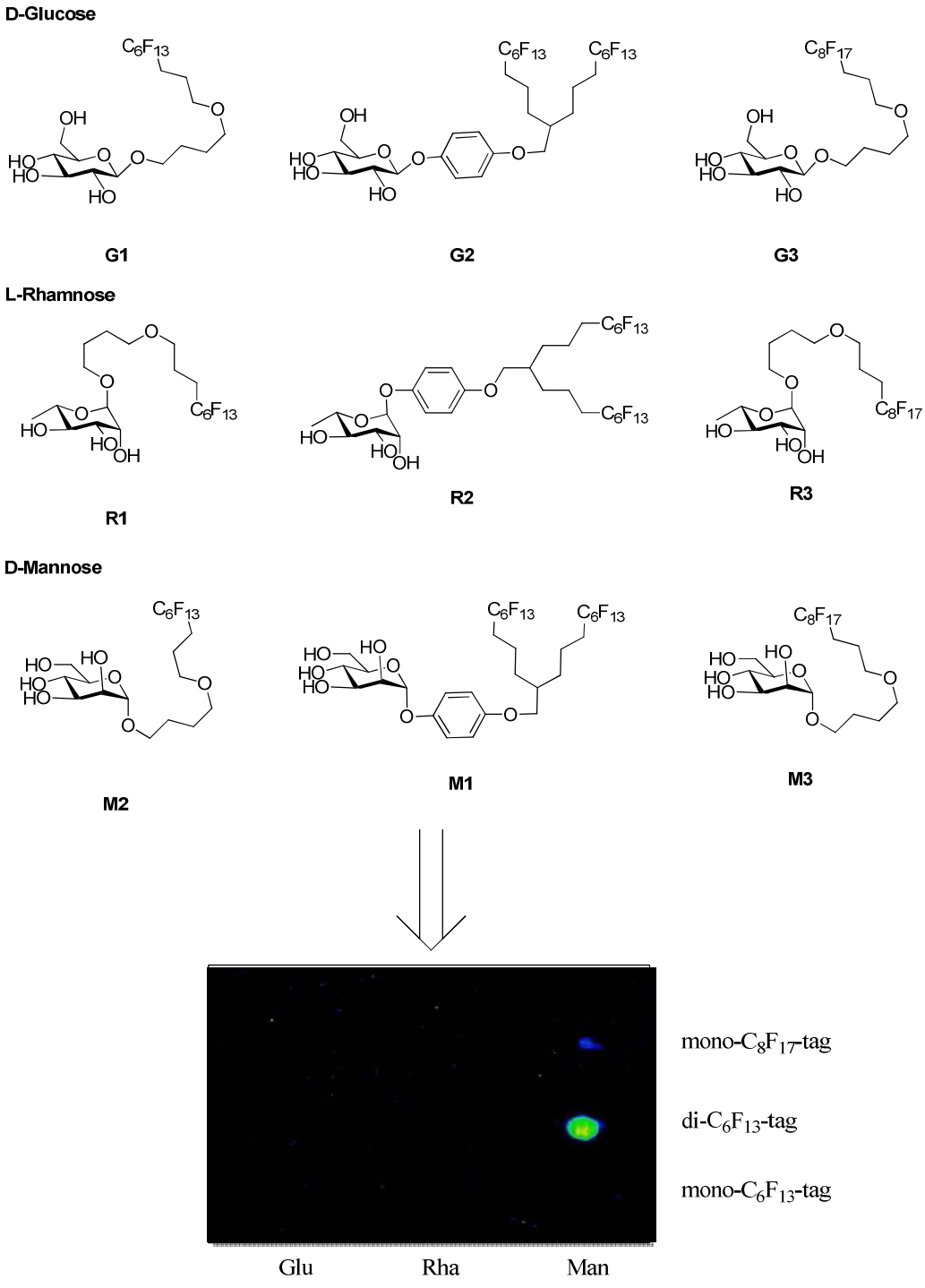


Figure 1: The nine fluorous linked saccharides that were spotted at 250 μM concentration on the

microarray slide and then screened for binding to ConA-FITC. The slide was visualized using a fluorescent scanner at 488 nm. The rhamnose compounds were synthesized by S. K. Nagappayya.

whereas the intensity of the di-C_6F_{13}-tag containing sugars was the same even after washing multiple times. There was only a slight decrease in the intensity of the $-C_8F_{17}$ tag containing sugars with multiple washings with PBS as shown by the scans. **Figure 1** shows all nine of the fluorous-linked monosaccharides spotted on the same fluorous slide. As shown, only two of these fluorous-linked monosaccharides are seen bound to the ConA-FITC. The relatively weak non-covalent fluorous interaction of the mono-C_6F_{13}-tag with the slide due to less fluorous content precluded its visualization. Note also that the β-D-glucose and α-L-rhamnose compounds did not bind at all, as expected. From the slide we can see also that the di-C_6F_{13}-tag has a larger spot size and is brighter than the mono-C_8F_{17}-tag. Despite the apparent greater adhesion of the sugars attached to the di-C_6F_{13}-tag to the slides, the fluorous slides could still be washed and reused at least five times without significantly increasing background noise.

Conclusion

By software analysis of spot intensities, we can conclude that the spot intensity of the sugars attached to the di-C_6F_{13}-tag are two to four times more intense than that of the mono-C_8F_{17}-tag and its binding ability is superior to both the mono-C_8F_{17}- and -C_6F_{13}-tag. The synthesis of the di-C_6F_{13}-tag is relatively simple and has high yielding steps which could be carried out on a larger scale. Clearly, the standard mono-C_8F_{17} tag can be effectively and

efficiently replaced by the di-C_6F_{13}-tag. Given its comparable behaviour on fluorous silica gel, this new tag could also possibly replace the fluorousoctyl tag in the purification of compounds using FSPE in both manual and automated syntheses.

Experimental Section

General materials and methods.

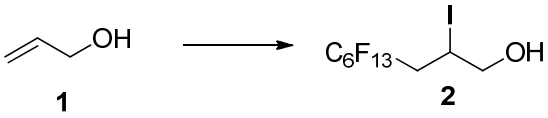
Reaction solvents were used directly from solvent tower (Swagelok). Amberlyst® 15 ion-exchange resin was washed repeatedly with methanol before use. All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (tlc) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 µm). The developed tlc plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Flash chromatography was performed with ZeoPrep 60 Eco 40-63 µm silica gel unless otherwise specified. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Microwave heating was carried out with a CEM-Discover continuous wave microwave. ^{1}H NMR and ^{13}C NMR spectra were obtained with a

Varian VXR at 400 MHz and101 MHz respectively. 1H NMR spectra were reported in parts per million relative to $CDCl_3$ as an internal reference. ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$.

Synthetic procedures.

• Synthesis of mono-C_6F_{13}-tag.

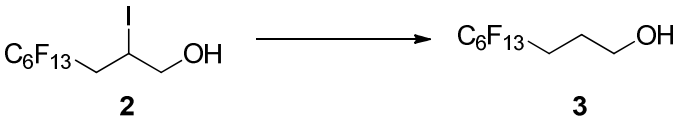
4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononan-1-ol (2).



1 2

To a mixture of allyl alcohol (1.0 g, 17 mmol) and perfluorohexyl iodide (7.7 g, 17 mmol) was added AIBN (0.30 g, 1.7 mmol) at 20 °C. The mixture was cooled to -78 °C to freeze the contents and then degassed, warmed to 20 °C, and then blanketed with argon. The reaction mixture was heated to 70 °C and stirred for 20 h. The reaction was then dissolved in hexane and poured through a fritted glass funnel and immediately purified by flash column chromatography (hexane) (82%, 7.1 g, white solid). The ^1H NMR data of compound **2** matched previously reported data. (Rábai, et al. 2007)

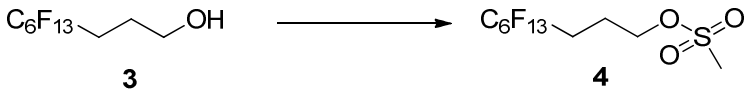
4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononan-1-ol (3).



2 3

Compound **2** (6.5 g, 13 mmol) was dissolved in tetrahydrofuran (150 mL) and cooled to 0 °C under argon. Once cooled, lithium aluminum hydride (0.98 g, 26 mmol) was slowly added to the mixture. The reaction vessel was warmed to 22 °C and stirred for 12 h. The reaction was quenched very slowly with water (30 mL) by adding it dropwise. The solution was then filtered and washed with $NaHCO_3$ (50 mL) and extracted with ethyl acetate (2 x 80 mL). The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) (83%, 4.1 g, clear oil). The ^{1}H NMR data of compound **3** matched previously reported data. (Go, et al. 2007)

4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl methanesulfonate (4).



3 4

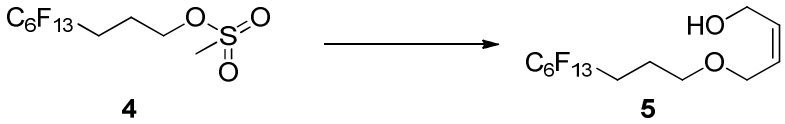
To a solution of 3-(perfluorohexyl)propanol **3** (1.7 g, 4.6 mmol) in dichloromethane (20 mL) was added triethylamine (1.3 mL, 9.1 mmol) and the mixture was cooled to 0 °C. Mesyl chloride (0.7 mL, 9.1 mmol) was added dropwise over 10 min and the reaction mixture was allowed to warm to ambient temperature over 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water (20 mL) and brine (20 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **4** (2.0 g, 98%, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 4.34-4.30 (t, *J* =12, 2H), 3.05 (s, 3H), 2.32-2.19 (m, 2H), 2.13-2.06 (m, 2H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 117.6, 68.1, 37.7, 27.9, 27.6, 27.4, 23.6, 21.0, 20.9

HRMS calcd. for $C_{10}H_9F_{13}O_3SNa$: 479.00, found$[M+Na]^+$: 478.9959

(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)but-2-en-1-ol (5).



4 **5**

A solution was made of *cis*-1,4-butenediol (3.6 mL, 44 mmol) and 3-(perfluorohexyl)propyl methyl sulfonate (2.0 g, 4.4 mmol) in DMF (20 mL). A second solution was made of tetrabutylammonium bromide (0.16 g, 0.5 mmol) in DMF (20 mL), to which powdered KOH (0.25 g, 4.4 mmol) was added. The first solution was added dropwise over 5 min to the second solution and the reaction mixture was stirred for 12 h at 22 °C then poured into water (20 mL). The aqueous layer was extracted with ethyl acetate (40 mL) and the organic layer was washed with water (30 mL) and brine (30 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **3** (1.2 g, 60%, pale yellow oil).

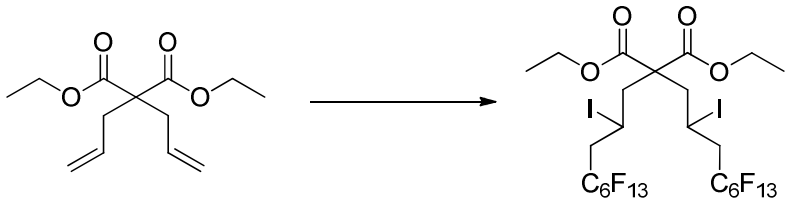
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 5.85-5.79 (m, 1H), 5.72-5.66 (m, 1H), 4.22 (s, 2H), 4.07-4.06 (d, *J* = 6, 2H), 3.53-3.50 (t, *J* = 11.6, 2H), 2.25-2.12 (m, 2H), 1.92-1.85 (m, 2H), 1.67-1.66 (m, 0.5H), 1.58 (s, 0.5H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 132.4, 128.5, 123.9, 117.6, 69.1, 66.72, 59.0, 28.2, 23.6, 21.0,

HRMS calcd. for $C_{13}H_{13}F_{13}O_2Na$: 471.06, found $[M+Na]^+$: 471.06

• **Synthesis of di-C_6F_{13}-fluorous tag.**

Diethyl 2,2-bis(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononyl)malonate (7).



6 **7**

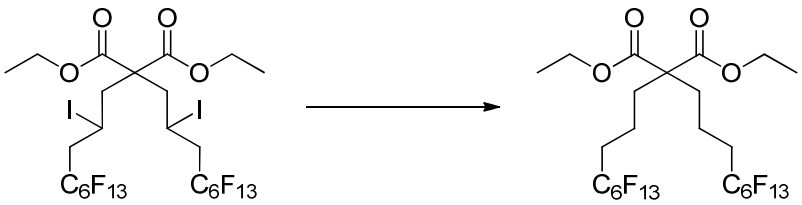
To a mixture of 2,2-diallyl-diethylmalonate (0.10 g, 0.41 mmol) and perfluorohexyl iodide (0.37 g, 0.83 mmol) was added AIBN (13 mg, 82 µmol) at 20 °C. The mixture was cooled to -78 °C to freeze the contents and then degassed, warmed to 20 °C, and then blanketed with argon. The reaction mixture was heated to 70 °C and stirred for 20 h. The reaction was then dissolved in hexane and poured through a fritted glass funnel and immediately purified by flash column chromatography (hexane) to yield compound **7** (70%, 0.33 g, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 4.23-4.18 (q, *J* = 7.2, 4H), 3.17-3.14 (m, 1H), 3.06-3.02 (t, *J* = 9.2, 1H), 2.58-2.51 (m, 3.5H), 2.33-1.99 (m, 4.5H), 1.28-1.23 (m, 6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 172.5, 172.1, 62.1, 62.0, 58.5, 45.6, 39.9, 38.6, 35.6, 29.9, 14.2, 5.8

HRMS calcd. for $C_{25}H_{20}F_{26}I_2O_4K$: 1170.90, found $[M+K]^+$-I: 1048.89

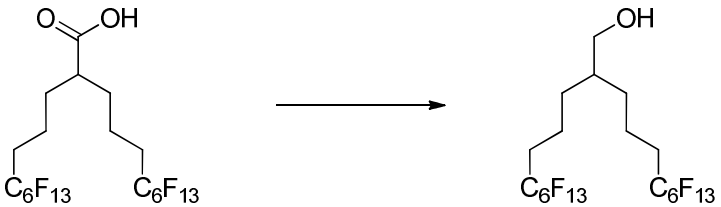
Diethyl 2,2-bis(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)malonate (8):



7 8

To compound **7** (1.4 g, 1.3 mmol), a mixture of ethanol (20 mL) and hydrochloric acid (20 mL) was added to form a pale yellow-colored solution. Next the solution was heated in the microwave at 60 °C for five min. Powdered zinc (2.0 g) was slowly added to the solution and reaction flask was placed back in the microwave at 60 °C for 15 min to form a clear solution. Water (40 mL) was added to the reaction. The aqueous layer was extracted with ethyl acetate (2 x 60 mL) and the organic layer was washed with $NaHCO_3$ (100 mL) and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to yield **8** (94%, 1.0 g, pale yellow oil). The ^1H NMR data of compound **8** matched previously reported data. (Loiseau, et al. 2001; Kaplanek, et al. 2006)

6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecan-1-ol (10).

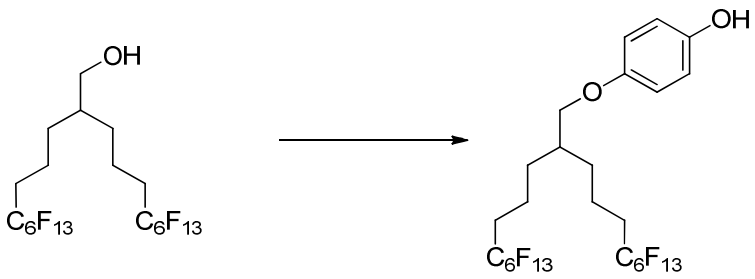


9 10

Ether (20 mL) was added to compound **9** (0.19 g, 0.25 mmol). The mixture was cooled to 0 °C. Lithium aluminum hydride (20 mg, 0.53 mmol) was added to the reaction flask. The reaction

vessel was warmed to 22 ºC and stirred for 12 h. The reaction was quenched very slowly with water (3 mL) by adding it dropwise. The solution was then filtered and washed with $NaHCO_3$ (40 mL) and extracted with ethyl acetate (2 x 40 mL). The organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane:ethyl acetate) (78%, 0.15 g, pale yellow oil). The ^{1}H NMR data of compound **10** matched previously reported data. (Loiseau, et al. 2001; Kaplanek, et al. 2006)

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenol (11).



10 **11**

To a mixture of compound **10** (0.60 g, 0.79 mmol) and hydroquinone (1.7 g, 16 mmol) in THF (20 mL) was added PPh_3 (0.41 g, 1.6 mmol) and DIAD (0.3 ml, 1.6 mmol) at 20 °C. After stirring for 6 h at ref ux condition, the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (7:3 hexane: ethylacetate) to provide compound **11** (37%, 0.25 g, white solid).

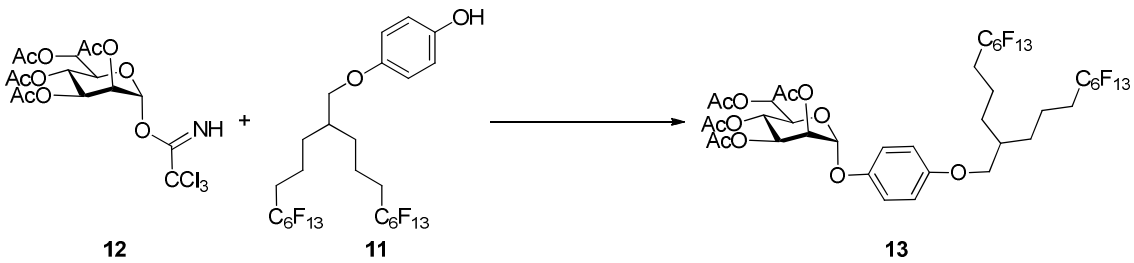
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 6.77-6.76 (d, *J* = 3.2, 4H), 4.63 (s, 1H), 3.82-3.80 (d, *J* = 8.8, 1H), 3.77-3.75 (d, *J* = 9.2, 1H), 2.58-1.01 (m, 10H), 0.91-0.81 (m, 3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 153.5, 149.7, 116.3, 116.2, 115.9, 115.8, 73.4, 38.0, 37.3, 37.1, 36.8, 35.6, 35.1, 35.0, 33.4, 31.5, 31.2, 16.8, 14.7

HRMS calcd. for $C_{26}H_{20}F_{26}O_2$: 858.10, found 857.32

• Synthesis of the mannose-linked tags.

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (13).



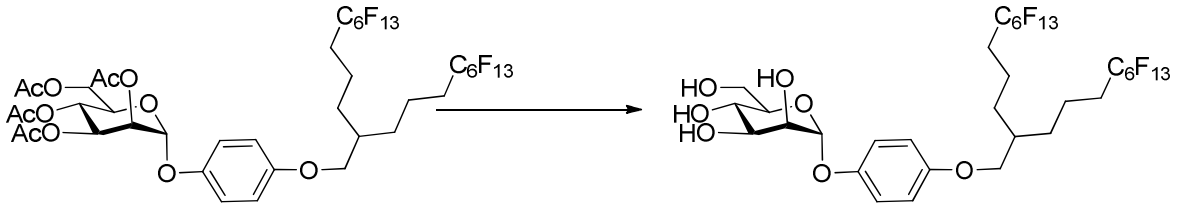
12 11 13

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (27 mg, 55 µmol) and compound **11** (24 mg, 28 µmol) in toluene (5 mL) was added TMSOTf (5.0 µL, 1.4 µmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction mixture was quenched with triethylamine (0.20 mL) and concentrated under reduced pressure. The crude product was purif ed by fluorous solid-phase extraction (FSPE) using a f uorous solid-phase extraction cartridge. Non-f uorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide **13** (27 mg, 83%, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.01-6.99 (m, 1.5H), 6.82-6.76 (m, 2.5), 5.55-5.53 (m, 1H), 5.43-5.35 (2H), 4.30-4.20 (1H), 4.15-4.06 (2H), 3.81-3.76 (2H), 2.38-2.03 (16H), 1.80-1.55 (3.5H), 1.35-1.15 (4.5H) 0.87-0.85 (d, J = 6.4, 2H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 170.8, 170.2, 170.1, 170.0, 155.2, 149.9, 149.8, 118.0, 116.2, 115.8, 115.6, 115.6, 115.5, 96.6, 73.4, 73.1, 69.7, 69.2, 69.1, 66.3, 62.4, 37.9, 37.3, 37.1, 37.0, 36.8, 35.8, 35.6, 35.1, 35.0, 33.4, 31.5, 31.4, 29.9, 21.1, 21.0, 20.9, 20.8, 16.9, 14.8

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2 (4,4,5,5,6,6,7,7,8,8,9,9,9tridecafluorononyl)undecyl)oxy)phenoxy-α-D-mannopyranoside (14).



13 **14**

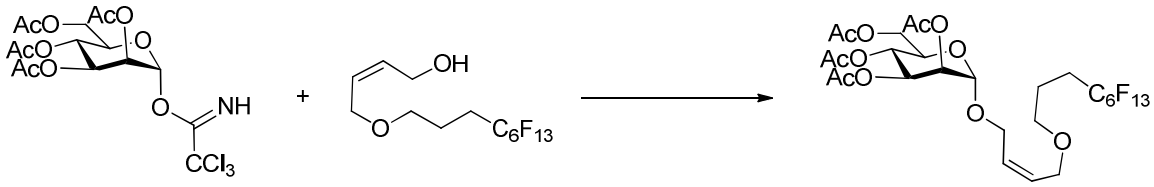
To a solution of compound **13** (27 mg, 23 µmol) in methanol (5.0 mL) was added K_2CO_3 (13 mg, 91 µmol). The reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **14** (20 mg, 88%, white solid).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.72-7.69 (m, 2H), 7.54-7.52 (m, 2H), 6.70 (s, 1H), 5.28-5.25 (1H), 4.31-4.12 (4H), 2.33-2.30 (5H), 1.89-1.83 (1H), 1.65 (2H), 1.62 (2H), 1.42-1.37 (6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 131.09, 129.01, 69.03, 68.38, 62.39, 62.33, 57.30, 57.24, 57.03, 56.90, 54.57, 54.43, 38.95, 34.22, 31.92, 30.57, 29.95, 29.90, 29.61, 29.57, 29.44, 29.21, 29.16, 29.12, 29.09, 29.06, 28.87, 28.14, 28.12, 28.08, 28.06, 27.45, 27.42, 27.17, 26.82, 26.80, 26.72, 26.39, 25.02, 23.97, 23.24, 22.93, 22.82, 14.32, 14.25, 14.217, 11.21

HRMS calcd. for $C_{32}H_{30}F_{26}O_7Na$: 1043.15, found $[M+Na]^+$: 1043.15

3-(perfluorohexyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (15).



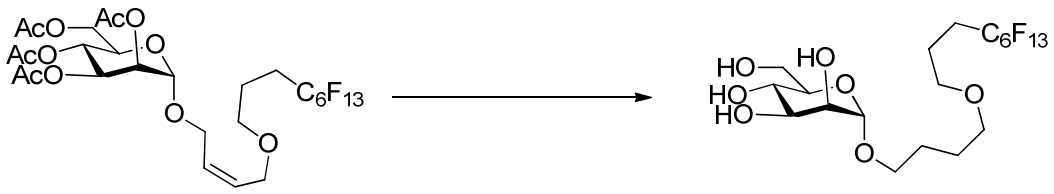
12 **5** **15**

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (21 mg, 47 µmol) and compound **5** (46 mg, 93 µmol) in toluene (5.0 mL) was added TMSOTf (0.80 µL, 4.6 µmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched with triethylamine (0.2 mL) and concentrated under reduced pressure. The crude product was purif ed by fluorous solid-phase extraction (FSPE) using a f uorous solid-phase extraction cartridge. Non-f uorous compounds were eluted with 80%MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide **15** (28 mg, 77%, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 5.78-5.67 (m, 2H) 5.36-5.31 (0.5H), 5.203-5.17 (0.5H), 4.69-4.63 (1H), 4.33-4.01 (6H), 3.51-3.49 (t, *J* = 10.8, 2H), 2.25-2.02 (15H), 1.91-1.84 (3H), 1.25 (s, 1H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 171.98, 170.88, 170.39, 16.92, 169.18, 157.35, 130.92, 130.90, 130.53, 128.03, 126.82, 99.83, 95.68, 71.60, 69.98, 69.22, 69.07, 66.68, 66.63, 63.22, 63.023, 60.36, 31.15, 31.11, 30.06, 29.94, 28.43, 28.20, 28.12, 27.97, 27.33, 21.05, 21.02, 20.95

3-(perfluorohexyl)propanyloxybutanyl-α-D-mannopyranoside (16).



15 **16**

To a solution of compound **15** (25 mg, 33 µmol) in methanol (5 mL) was added 10% Pd/C (0.1 g). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was then f ltered over Celite® and partially concentrated under reduced pressure. Then K_2CO_3 (18.0 mg, 0.33 mmol) was added to the solution and stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and f ltered over Celite®. The solvent was removed under reduced pressure to provide compound **16** (16.1 mg, 82%, white solid).

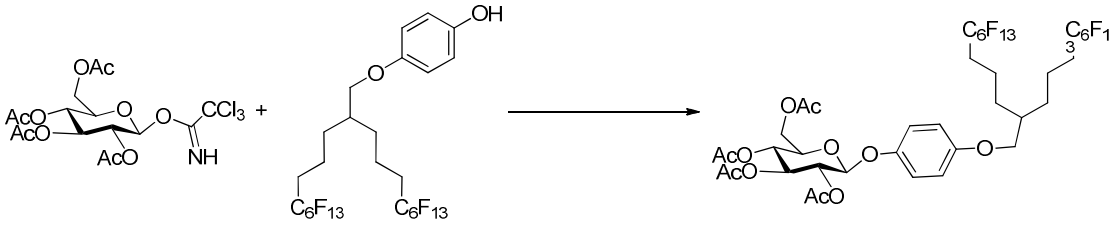
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 3.66 (s, 2H), 3.52-3.46 (4 H), 2.28-2.16 (4H), 1.98-1.86 (3H), 1.70-1.64 (5H), 1.25 (s, 3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 115.55, 71.16, 69.52, 62.94, 30.28, 30.26, 30.22, 28.38, 28.21, 28.16, 27.94, 26.80, 26.76, 20.97, 20.94

HRMS calcd. for $C_{19}H_{25}F_{13}O_7Na$: 635.13, found $[M+Na]^+$: 635.13

• Synthesis of the glucose-linked tags.

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (23).



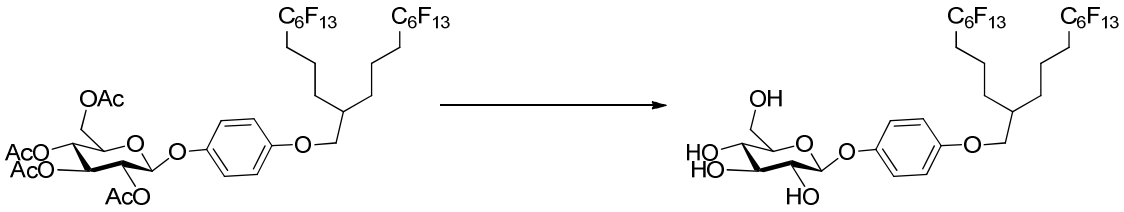
22 **11** **23**

To a solution of 2,3,4,6-tetra-O-acetyl- β-D-glucopyranosyl trichloroacetimidate (**22**) (55 mg, 0.13 mmol) and compound **11** (19 mg, 22 µmol) in toluene (5 mL) was added TMSOTf (0.20 µL, 1.1 µmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction mixture was quenched with triethylamine (0.20 mL) and concentrated under reduced pressure. The crude product was purif ed by fluorous solid-phase extraction (FSPE) using a f uorous solid-phase extraction cartridge. Non-f uorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide **23** (26 mg, 99%, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.00-6.87 (2H), 6.81-6.74 (2H), 5.29-5.13 (2H), 4.95-4.93 (d, *J* = 8,1H), 4.30-4.04 (2H), 3.82-3.76 (3 H), 3.48 (12H) 2.07-2.03 (dd, *J* = 21.2, 12H), 1.66 (2H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 170.87, 170.53, 169.65, 169.57, 155.55, 151.07, 118.901, 115.51, 115.46, 100.54, 73.12, 72.98, 72.93, 72.15, 71.46, 68.61, 68.53, 68.06, 62.17, 57.01, 51.09, 40.58, 37.25, 37.12, 36.82, 35.81, 35.61, 35.56, 35.10, 34.94, 33.40, 31.53, 29.94, 29.91, 20.95, 20.93, 20.88, 20.85, 20.71, 16.86, 14.77, 14.38, 14.35

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9 tridecafluorononyl)undecyl)oxy)phenoxy-β-D-glucopyranoside (24).



23 **24**

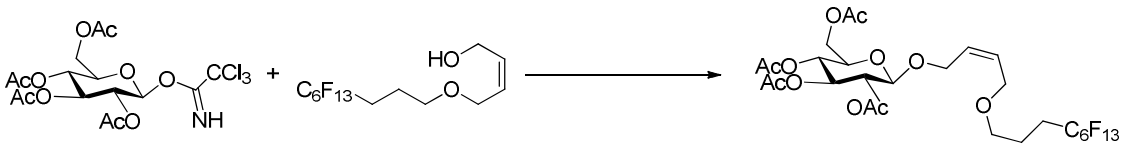
To a solution of compound **23** (22 mg, 18 µmol) in methanol (5 mL) was added K_2CO_3 (13 mg, 92 µmol). The reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **24** (14 mg, 75%, white solid).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.01-6.97 (1.5H), 6.88-6.76 (2.5H), 3.83-3.76 (4H), 3.44 (2H), 3.38-3.37 (1H), 3.33 (1H), 2.34-2.17 (10H), 1.65 (2H), 1.56 (3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 128.54, 128.30, 128.03, 127.94, 116.25, 116.24, 115.88, 115.85, 96.08, 96.04, 95.95, 80.80, 79.75, 73.38, 72.85, 68.11, 64.18, 51.81, 50.60, 37.31, 37.11, 37.08, 36.07, 35.66, 35.10, 33.46, 31.21, 29.95, 22.95, 18.10, 18.08

HRMS calcd. for $C_{32}H_{30}F_{26}O_7Na$: 1043.15, found $[M+Na]^+$2D: 1047.27

3-(perfluorohexyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (25).



22 **5** **25**

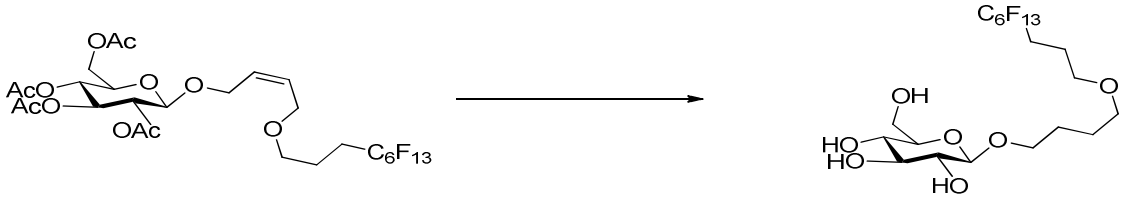
To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (**22**) (39 mg, 78 µmol) and compound **5** (18 mg, 40 µmol) in toluene (5 mL) was added TMSOTf (0.40 µL, 2.0 µmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min. The reaction mixture was quenched with triethylamine (0.40 mL) and concentrated under reduced pressure. The crude

product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80%MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide **25** (26 mg, 83%, pale yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 5.76-5.64 (m, 2H), 5.23-4.98 (3H), 4.55-4.52 (1H), 4.39-4.33 (1H), 4.25-4.23 (2H), 4.16-4.12 (1H), 4.05-4.01 (2H), 3.70-3.66 (1H), 3.50-3.47 (2H), 2.18 (2H), 2.09 (3H), 2.04-2.01 (9H), 1.90-1.86 (2H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 132.32, 123.90, 123.87, 117.64, 111.37, 99.76, 95.68, 72.063, 71.42, 69.03, 66.72, 62.12, 31.85, 29.95, 20.93, 20.83

3-(perfluorohexyl)propanyloxybutanyl-β-D-glucopyranoside (26).



25 **26**

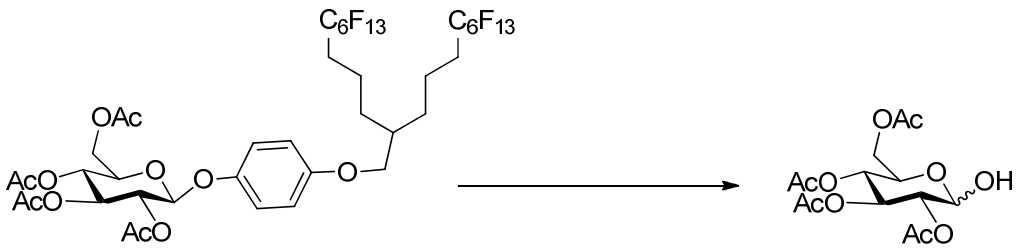
To a solution of compound **25** (21 mg, 27 μmol) in methanol (5 mL) was added 10% Pd/C (0.1 g). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was then filtered over Celite® and partially concentrated under reduced pressure. Then K_2CO_3 (15 mg) was added to the solution and stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **26** (13 mg, 77%, white solid).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 4.34-4.30 (2H), 3.66-3.65 (4H), 3.04 (3H), 2.20-2.15 (3H), 1.99-1.97 (2H), 1.88-1.85 (4H), 1.67-1.63 (4H), 1.25 (2H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 71.16, 71.12, 69.52, 69.47, 62.94, 62.90, 57.01, 37.88, 37.84, 37.80, 37.76, 37.74, 37.72, 30.26, 30.21, 30.16, 26.80, 26.75, 26.71, 21.037, 21.02, 20.93, 20.91

HRMS calcd. for $C_{19}H_{25}F_{13}O_7Na$: 635.13, found $[M+Na]^+$: 635.11

2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside (27):



23 **27**

To a solution of compound **23** (17 mg, 14 µL) in acetonitrile:water (4:1) (2 mL) was added ceric ammonium nitrate (16 mg, 27 µL). The reaction mixture was stirred for 2.5 h at 21 °C. $NaHCO_3$ (10 mL) was added to the reaction. The aqueous layer was extracted with dichloromethane (2 x 10 mL) and the organic layer was dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to yield **27** (70%, 3.5 mg, pale yellow sticky solid). The 1H NMR data of compound **27** matched previously reported data. (Schmidt, and Michel 1980)

The solubility of the di-fluorous-tag was tested in a 1M solution with dichloromethane and toluene to show that everything was dissolved.

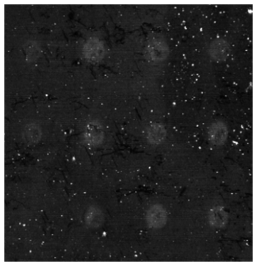
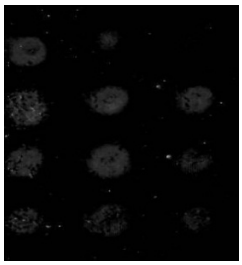
Microarray preparation, screening and notes. (All microarray experiments were conducted with Sahana Nagappayya)

Microarrays were printed by the Microarrayer**XactII™ (**LabNEXT Inc. 733 Beaver Road, Glenview, IL 60025**)** robotic pin (Xtend MP Microarray Pins 0.35 mm) deposition of the three different fluorous-tagged sugars in methanol/DMSO/water (v:v:v = 2:6:2) (250 µM solution) from a 384-well plate onto commercially available fluorinated glass slides (Fluorous Technologies, Inc.; Pittsburgh, PA) at 70% humidity and a temperature of 22 °C. (Note that the slide will work better if it is washed once with a 1:1 solution of dichloromethane:methanol.) The glass slide was allowed to dry for 15 h to 24 h inside a humidity chamber and for another 2 h outside of it. This drying procedure helped in avoiding the donut effect and allowed the molecules to orient themselves on the slides to obtain good spots. The ConA-FITC 200 µM solution was composed of the FITC-labeled ConA (Sigma, 1 mg/1 mL, 200 µL) in a 1X PBS (780 µL) 1 mM $CaCl_2$ (10 µL) and 1 mM $MnCl_2$ (10 µL) were used for the detecting protein-carbohydrate interactions. The arrays were incubated with the protein solution (150 µL) by using a PC500 CoverWell incubation chamber (Grace Biolabs, Bend, OR) for 1 h, and then washed twice with 1X PBS containing 1% BSA followed by washing once with distilled water. They were then dried and scanned at Iowa State University DNA facility. The slides were scanned using a General Scanning ProScanArray 5000 set at 488 nm. The fluorescent intensities were determined using the ImaGene 8.0 software.

During the microarray study we found that the spots had many inconsistencies depending on the environmental conditions. One of the major factors affecting the spot morphology was humidity. So, we did a comparative study by changing humidity and keeping the temperature constant at 22 ºC by using the already known mannose-C_8F_{17} tags. We started with 60% humidity as most microarray studies were previously performed at 60% humidity condition.

Then we tested 65% and 70% humidity conditions. From the slides shown below, we can conclude that the slide at 60% humidity has donut effect whereas the slide at 65% humidity has a slightly less donut effect, and the slide at 70% humidity has no donut effect at all. Also, consistently good spots were observed with the slides printed at 70% humidity. Based on these results, for all the studies in this paper, we performed the microarray at 70% humidity.

Humidity 60% **Humidity 65%** **Humidity 70%**

Also, we found that the use of new slides caused inconsistencies in the spots in terms of binding ability of the carbohydrate to the slide. The new slide always showed prominent donut effect and most of time the spot would be completely washed away. So, we washed the slides by adopting the method used by Spring and co-workers using 1:1 dichloromethane:methanol.(Nicholson, et al. 2007) We decided to wash the new slide once with the 1:1 solution first before printing. Also, we were able to wash and reuse the slides at least five times.

While printing we observed contamination of the spots from the pins. The washing protocol of the array was not very well set up for carbohydrate microarray studies. So, we had to

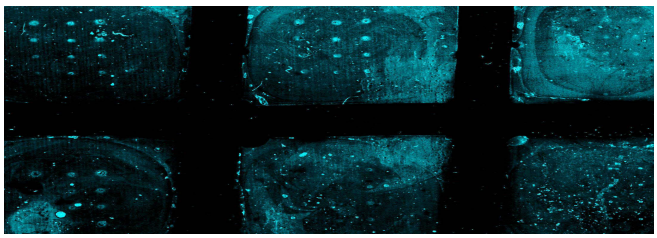
wash the pins out by hand after each spot with acetone. We used the same pin for each spot to get reliable results.

To develop a good washing protocol for the fluorous tagged carbohydrates, we scanned the slide after each washing with 1XPBS containing 1% BSA. We found that the first wash with 1XPBS containing 1% BSA was most effective in clearing the protein from the slide whereas the second wash had little effect. Also, we found that washing with distilled water had no effect on the slide. Each slide was washed twice with 1XPBS containing 1% BSA and once with distilled water to remove any remaining salts that may have deposited on the slide.

The slides below are the results that we obtained from the microarray studies. The β-D-glucose and the α-L-rhamnose (provided by Sahana Nagappayya) slide showed nothing, as expected. The α-D-mannose slide showed that the mono-C_6F_{13}-tag was washed away, while the di-C_6F_{13}-tag and mono-C_8F_{17}-tag were clearly noticeable. The last slide has all nine fluorous-tagged monosaccharides placed in one well. From this slide there are only two spots in each well indicating what the first three slides data shows.

Mannose Slide:

Mono-C_8F_{17}-Man Di-C_6F_{13}-Man Mono-C_6F_{13}-Man

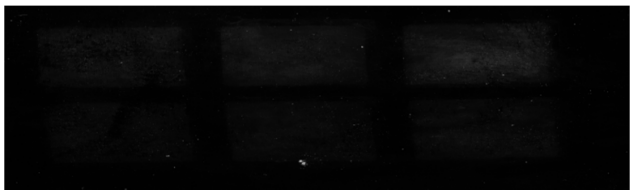


Rhamnose Slide:

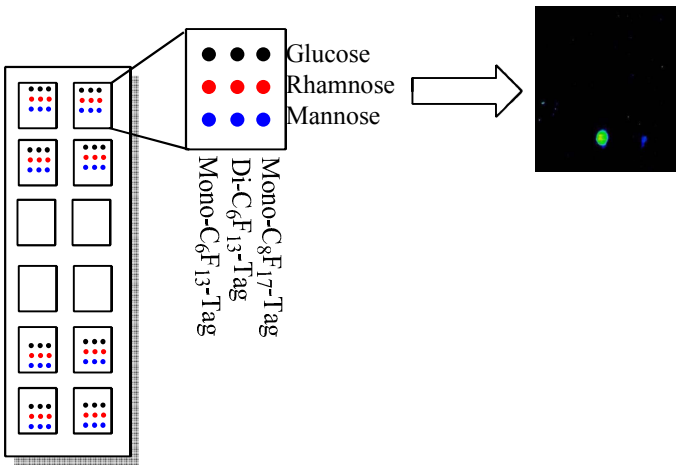
Mono-C_8F_{17}-Rha Di-C_6F_{13}-Rha Mono-C_6F_{13}-Rha

Glucose Slide:

Mono-C_8F_{17}-Glu Di-C_6F_{13}-Glu Mono-C_6F_{13}-Glu



The pattern of the slide with all nine fluorous-linked monosaccharides and the actual slide:



CHAPTER 4

Automated Solution-Phase Synthesis of the Cell Wall Oligosaccharides of Group A Streptococcus

Manuscript in Preparation

Heather D. Edwards and Nicola L. B. Pohl

Abstract

Cellulitis is a poorly treatable skin disease caused by Group A streptococcus (GAS) and *Staphylococcus aureus*. In order to develop a vaccine against these bacterial infections, the first automated syntheses have been developed of a key cell wall polysaccharide from GAS. For the synthesis of the cell wall fragment containing a repeating dirhamnose/glucosamine unit of GAS, two different rhamnose building blocks and one glucosamine building block have been synthesized with protecting groups that allow their incorporation into a growing saccharide chain. The first unit was added to a fluorous tag that allowed automated purification between iterative automated glycosylation/deprotection steps to successfully build the hexasaccharide unit.

Introduction

Carbohydrates play an important role in many biological processes such as immune response, hormone regulation, cell-cell recognition, and cell development. To better understand the biological roles of the carbohydrates, they need to be synthesized. (Hytonen, et al. 2000; Liang, et al. 2008; Mamidyala, et al. 2006; Pitner, et al. 2000) Many of these carbohydrates are

complex systems that have not been synthesized due to their complexity. Synthesizing these complex chains can help in understanding the roles they play in binding to other cells.

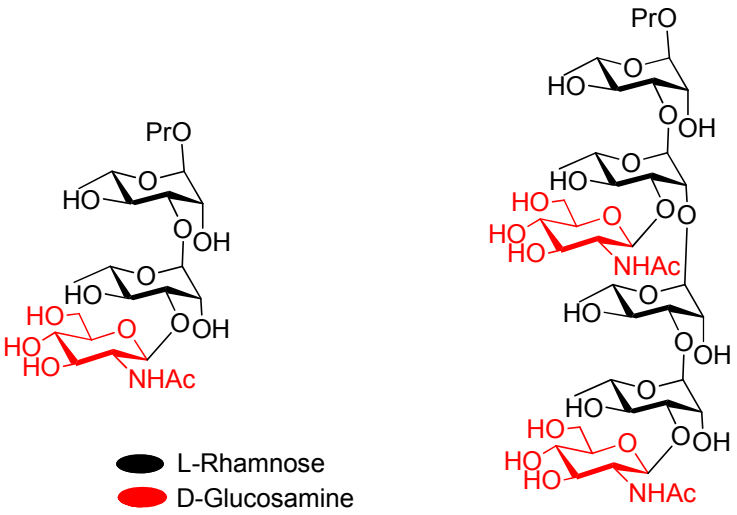
Oligosaccharides have become very important in the development of vaccines, enabling us to have a better understanding of the cells, and the interactions between each cell. (Hytonen, et al. 2000; Liang, et al. 2008; Mamidyala, et al. 2006; Pitner, et al. 2000) Many bacteria oligosaccharides have been identified. These chains that have been identified can be synthesized in order to figure out the reactivity and the binding of these oligosaccharides to different cells. Once a library of these oligosaccharides is formed, it would is possible to deduce what other bacteria might have a similar sequence and what cells in animals that have a similar sequence to that specific oligosaccharide. (Liang, et al. 2008)

Bacterial cell walls contain complex chains with many of the same oligosaccharides that human cells contain on the cell wall. The similarity creates a good interaction between the two cells, which enables bacteria or virus to take over human cells because of this interaction. Then the complex carbohydrate chains in bacteria that are not found in humans are preferred in vaccine development. The main difficulty is that many of these sugars are not well understood and are no libraries exist to help to find how and where they bind, and their reactivity. (Liang et al. 2008) The reactivity in the glycosylation and intracellular binding of each monosaccharide needs to be tested.

Group A Streptococcus is a gram-positive bacteria that is part of the *Streptococcus pyogenes* family. (Hanski and Caparont 1992) It is the cause of many different diseases. Mild

diseases caused by Group A Streptococcus include pharygitis and impetigo, and the life threatening diseases include rheumatic fever, glomerulonephritis, septicemia, myositis, cellulitis, streptococcal toxic shock syndrome, and necrotizing fasciitis. (Cartwright 1997; Kotloff, et al. 2005; Graham, et al. 2002; Scott, et al. 1987; Scott, et al. 1985; Wessels 1997) The cell wall oligosaccharide of Group A streptococcus (GAS) was first found in the 1960s by Dr. Rebecca Lancefield. (Mora, et al. 2005) Dr. Lancefield and Dr. Mario Pinto since extensively studied the oligosaccharide chain. Dr. Pinto has synthesized the GAS cell wall oligosaccharide before (shown in **Figure 1**), but some of the glycosylations used were too harsh for an automated solution-phase synthesizer. (Reimer, et al. 1992; Pinto, et al. 1991; Auzanneau, et al. 1996) It

Figure 1: The trisaccharide and hexasaccharide of Group A Streptococcus that Dr. Mario Pinto built by bench-top.



was thus necessary for new building blocks to be designed. The glucosamine building block is already known. The L-rhamnose has been found in many other bacteria such as *Klebsiella*

pneumoniae and *Shigella flexneri*. (Zhang, et al. 2001; Boutet and Mulard 2008) The rhamnose building blocks are known up to the point of tertbutyldimethylsilyl addition. Once that group was added to the rhamnose building blocks, they became new building blocks.

The initial goal was to design the building blocks of the sugars found in the cell wall polysaccharide of GAS. After synthesis of the building blocks, conditions need to be found to connect together these building blocks and take the repeating trisaccharide unit and be able to build it up into a hexasaccharide unit. To do this we would synthesize the oligosaccharide on the automated solution-phase synthesizer based on the purification of fluorous-tagged intermediates by fluorous solid phase extraction (FSPE).

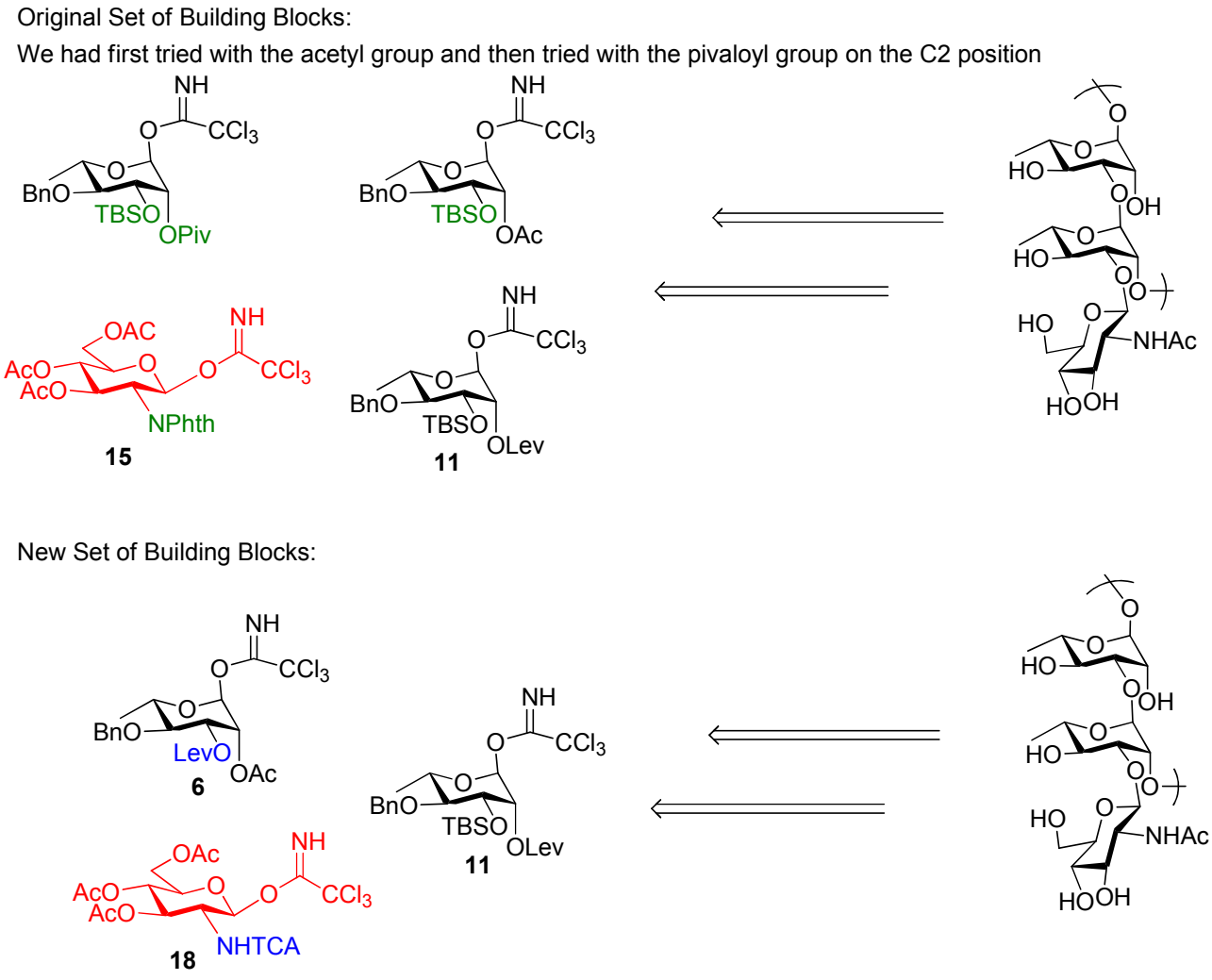
Results and Discussion

The polysaccharide contains one glucosamine and two different rhamnoses. One rhamnose has a linkage on the anomeric, C2, and C3 positions, while the other rhamnose only has a linkage at the anomeric and C3 positions. The glucosamine building block only has one linkage at the anomeric position. When designing the building blocks protecting groups selection for each position to allow selective unmasking of hydroxyls to build the chain from the reducing to nonreducing end was critical.

Routes to the two rhamnose building blocks start out the same shown in **Schemes 1**. Each rhamnose building block (compound **1**) first undergoes allylation at the anomeric position (compound **2**). (Hirooka, et al. 2001) Then trimethyl orthoacetate is used to place an orthoester

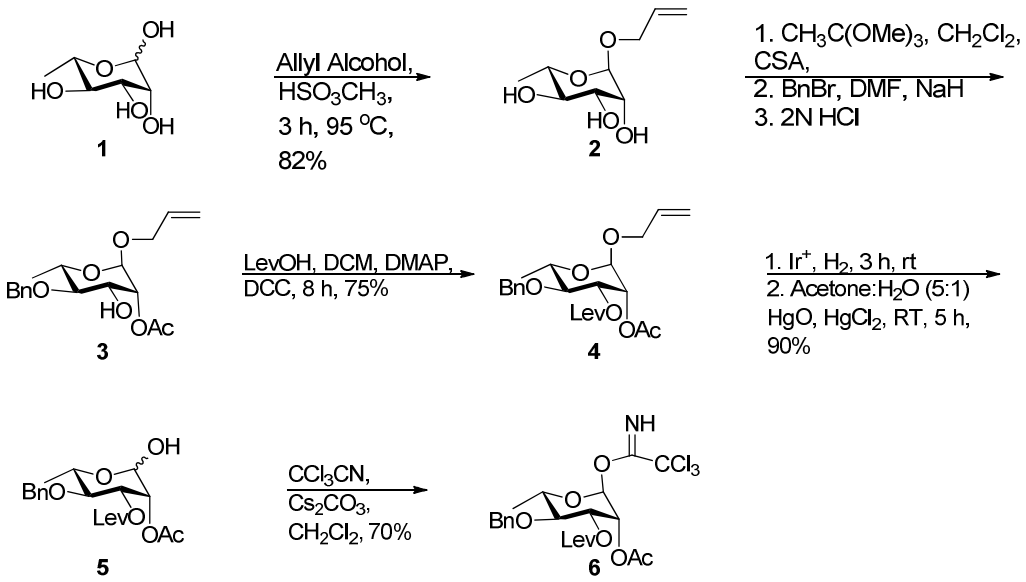
between the C2 and C3 positions as shown in **Scheme 1**. Next, benzylation occurs at the C4 position and the orthoester is opened up by an acid workup to get compound **3**. When the orthoester opens the acetate will end up on the C2 position. (Boutet and Mulard 2008) From here

Figure 2: The original set of building blocks that were first used to create the GAS oligosaccharides. Due to many complications we made a new set of building blocks to try to overcome the original challenges.



the building block strategies diverge. For the 1st rhamnose building block, initially a tertbutyldimethylsilyl (TBS) group was attached to protect the C3 position. Due to complications with steric hindrance and deprotection of the ester groups on the C2 position when deprotecting; we decided to attach a levulinate group instead at the C3 position, which gave compound **4** (**Figure 2**). The 1st rhamnose building block will keep the acetate group on the C2 position and went through deallylation (compound **5**). Finally, a trichloroacetimidate is placed on the anomeric position (compound **6**) to successfully complete the synthesis of the desired 1st rhamnose building block ready for glycosylation.

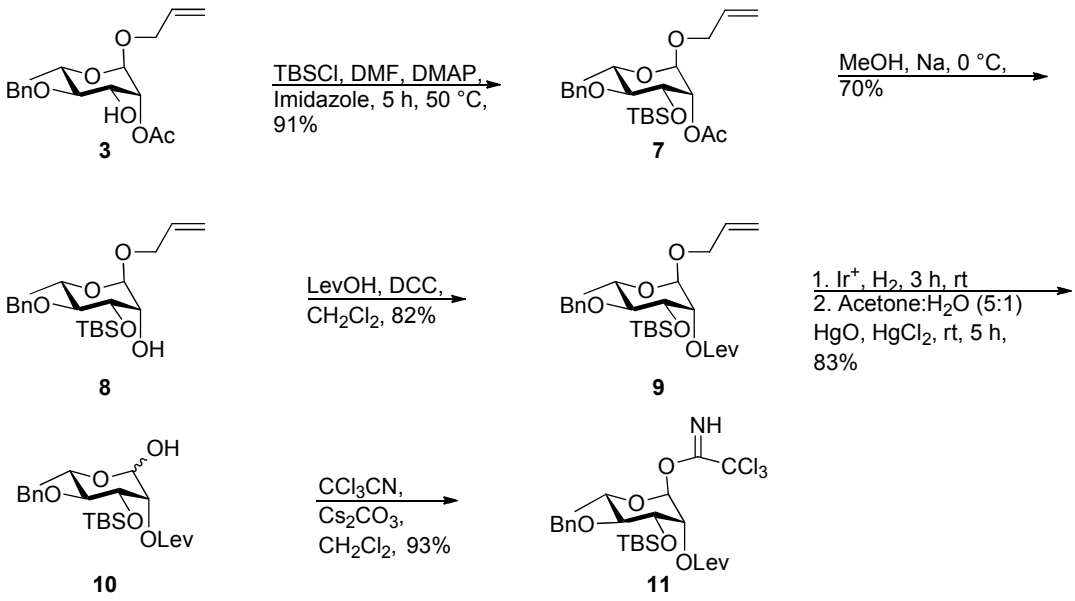
Scheme 1: The synthesis of the first rhamnose building block.



For the 2nd rhamnose building block, a tertbutyldimethylsilyl group is then used to protect the C3 position to get compound **7** (**Scheme 2**). The next step was to remove the acetate group

by very basic conditions. Unfortunately, the silyl group migrated to the C2 position under these conditions. However, cooling the reaction to 0 °C yielded more of the desired product compound **8**. The 2nd rhamnose building block had a levulinate group attached to the C2 position (compound **9**), followed by deallylation to give compound **10**. (Boullanger, et al. 1986; Barbier, et al. 2007; Warren and Jeanloz 1977) Finally, a trichloroacetimidate is placed on the anomeric position (compound **11**) to successfully complete the synthesis of the desired 2nd rhamnose building block and was ready for glycosylation.

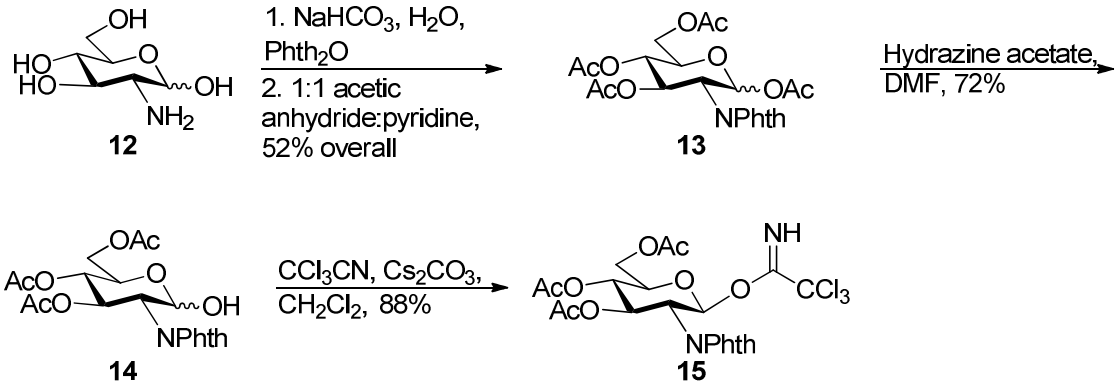
Scheme 2: The synthesis of the 2nd rhamnose building block.



The original glucosamine building block that we started with was synthesized from the commercially available glucosamine (compound **12**) that underwent peracetylation and installation of a phthalamide protecting group on the nitrogen to give compound **13** (**Scheme 3**).

(Hernandez-Torres, et al. 2002) Selective deactylation of the anomeric position (compound **14**) provided a free hydroxyl for addition of a trichloroacetimidate (compound **15**). As this final activated building block turned out to be very reactive, this final activation step needs to be carried out just before the glycosylation step that attaches this sugar to the growing rhamnose chain sugars.

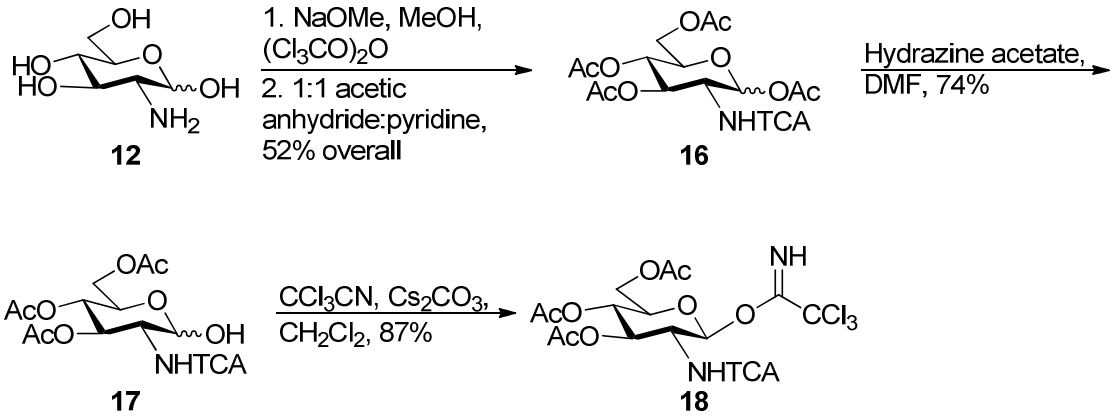
Scheme 3: The synthesis of the known glucosamine building block with phthalamide. (Hernandez-Torres, et al. 2002)



Due to the having issues of extending our oligosaccharide from a trisaccharide to a hexasaccharide we decided to change the design of our glucosamine building block. We thought that the phthalamide was just too bulky and creating steric hindrance. Therefore, the phthalamide was replaced with trichloroacetylamide group to give compound **16** (**Scheme 4**). This glucosamine building block is known and the steps were the same as the original building block after the attachment of the TCA group. (Blatter, et al. 1994; Boutet, et al. 2008) This

glucosamine building block was a white powder that was found to be more stable than the original glucosamine building block. Also, it was found that the tricholoroimidate (compound **18**) would rearrange in toluene versus the dichloromethane.

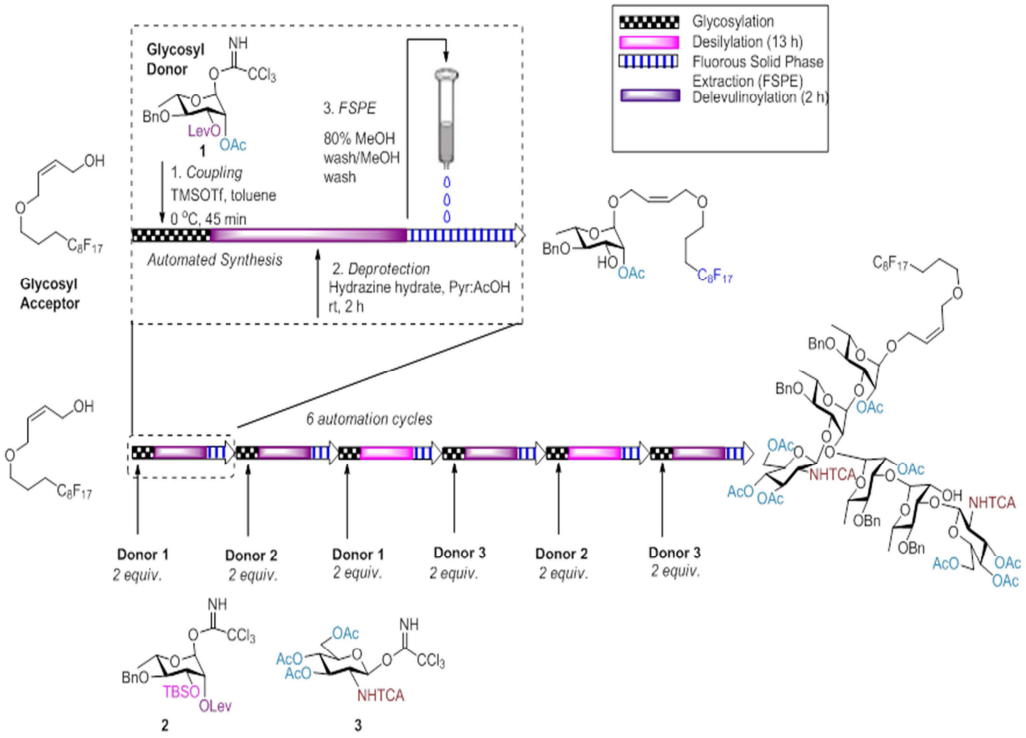
Scheme 4: The synthesis of the known glucosamine building block with TCA group. (Blatter, et al. 1994; Boutet, et al. 2008)



Once the synthesis of the building blocks was finished, the glycosylation reactions were done manually to check for possible problems that could appear in an automated procedure (**Scheme 5**). The first glycosylation was to attach a fluorous tag alcohol acceptor with two equivalents of the 1st rhamnose building block as the donor. Removal of the levulinate group with 1 M hydrazine hydrate solution unmasked the next hydroxyl group for chain extension. The resulting acceptor and two equivalents of the 2nd rhamnose building block was reacted with tetrabutylammonium fluoride to remove the silyl group. Finally, the glucosamine building block was reacted with the chain. Each glycosylation reaction was quenched with triethylamine, concentrated, and then taken directly to the deprotection step to mimic the automated synthesis

protocol. After each deprotection step, a fluorous silica gel column was used to separate the fluorous product from the non-fluorous compounds.

Scheme 4: Automated solution-phase synthesis of the hexasaccharide unit of GAS.



Conclusion

With conditions established, manual protocol was adapted to an automated synthesizer to produce one unit of the repeating trisaccharide. The run took a total of 60 hours to get to the putative final product mixture of tetrasaccharide and hexasaccharide, which was verified by mass spectrometry and NMR. After some future optimization of the final

glycosylation automated protocol, the plan is to extend the chain and to attempt synthesis of different chain lengths of the repeating units to ultimately discover the minimal structure needed for a vaccine construct against cellulitis.

Experimental Section

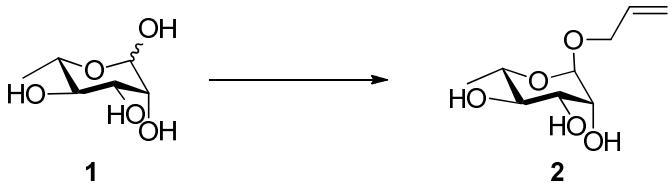
- **General materials and methods**

Reaction solvents were used directly from solvent tower (Swagelok). All commercial reagents and solvents were used as received without further purification unless indicated. Amberlyst 15 ion-exchange resin was washed repeatedly with methanol before use. The reactions were monitored and the R_f values determined using analytical thin layer chromatography (tlc) with 0.25 mm EM Science silica gel plates (60F - 254). The developed tlc plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Silica gel flash chromatography was performed with Selecto Scientific silica gel, 32-63 mm particle size. Fluorous phase chromatography was performed using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture sensitive reactions were performed in flame- or oven- dried glassware under nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker DRX400 at 400 MHz and 101 MHz, Varian400 at 400 MHz and 100 MHz or Varian300

at 300 MHz and 75 MHz respectively. ^{1}H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (7.27 ppm) as an internal reference. ^{13}C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (77.23 ppm) or CD_3OD (49.15 ppm). HPLC traces were obtained from a Varian Inc. HPLC system using a Waters Nova-pak 4 μm 3.9 ×150 mm silica column. High-resolution mass spectrometry was obtained with Applied Biosystems QSTAR XL Hybrid System from the W. M. Keck Metabolomics Research Laboratory or Applied Biosystems DE-Pro MALDI mass analyzer from the protein facility at Iowa State University. A Chemspeed ASW1000 (Chemspeed, Augst, Switzerland) synthesis platform with hood, 16 reactor vials (13-mL capacity each) and cooling unit (to –20 °C) was machined to hold the FSPE cartridges at the ISU machine shop.

- **Synthetic Procedures of monomer building blocks for Group A Streptococcus (GAS)**

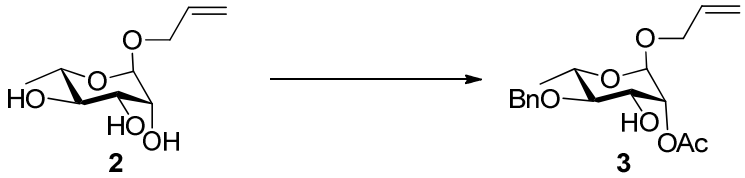
1-*O*-allyl-α-L-rhamnopyranoside (2):



A solution of L-rhamnose (2 g, 12 mmol) in allyl alcohol (10 mL, 147 mmol) was stirred at 95 °C under nitrogen, and added was methyl sulfonic acid (33 uL, 0.51 mmol). The mixture was stirred for 3 h. Then $NaHCO_3$ (12 mmol) was added to neutralize the solution. The mixture is then concentrated to a syrup. Column Chromatography (10:1 CH_2Cl_2:methanol), another column

chromatography (20:1 EtOAc:Methanol) gave compound **2** (2g, 82.4%, clear syrup). The ^{1}H NMR data of compound **2** matched previously reported data. (Hirooka, et al. 2001)

4-*O*-benzyl-2-*O*-acetyl-1-*O*-allyl-α-L-rhamnopyranoside (3):



To a solution of compound **2** (2.0 g, 10 mmol) in dichloromethane (20 mL) at room temperature, under nitrogen, 10-camphorsulfonic acid (120 mg, 0.50 mmol) and trimethyl orthoacetate (3.3 mL, 27 mmol) was added. The reaction went for 5 h and then was concentrated down to yellow syrup. Then *N,N'*-dimethyl-formamide (10 mL) was added to the syrup and stirred. Sodium hydride (800 mg, 20 mmol) and benzyl bromide (2.4 mL, 20 mmol) were added to the solution and stirred at room temperature for 7 h. Once completed the reaction was diluted with CH_2Cl_2, washed with 2NHCl solution, water, saturated $NaHCO_3$ solution and water in succession and dried ($MgSO_4$). The organic layer was concentrated to syrup. Column Chromatography (2:1 Hexane:EtOAc) gave compound **3** (2.0 g, 60%, yellow syrup).

Rf: (ethyl acetate/hexane) 0.5 (40/60)

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.35-7.26 (m, 5H), 5.87 (m, 1H), 5.27-5.23 (dd, J =17.2 Hz, 1.6 Hz, 1H), 5.17 (dd, J = 17.4 Hz, 1.2 Hz, 1H), 5.09 (dd, J = 3.6 Hz, 1.6 Hz, 1H), 4.85 (d, J = 11.2 Hz, 1H), 4.74 (d, J = 1.2 Hz, 1H), 4.68 (d, J = 11.2 Hz, 1H), 4.12-4.07 (m, 2H), 3.93 (dd,

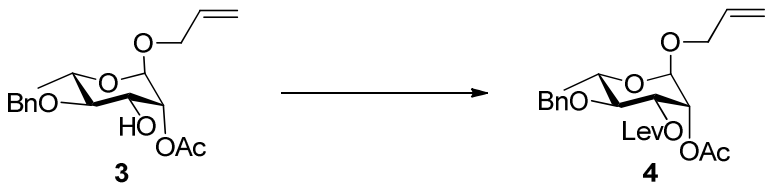
J = 12.8 Hz, 6.0 Hz, 1H), 3.75 (m, 1H), 3.36 (t, J = 9.2 Hz, 1H), 2.56 (d, J =5.2 Hz, 1H), 2.0 (s, 3H), 1.32 (d, J = 6.4 Hz, 3H).

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 171.0, 138.3, 133.6, 128.5, 128.0, 127.9, 117.6, 96.6, 81.7, 75.3, 72.9, 70.2, 68.1, 67.5, 21.1, 18.0

HRMS calcd. for $[M+Na]^+$: 359.1465; found: 359.1460

- **First Rhamnose Building Block**

4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-acetyl-1-*O*-allyl-α-L-rhamnopyranoside (4):



To a solution of compound **3** (2.5 g, 7.4 mmol) in dichloromethane at 21 °C, under nitrogen, *N,N'*-dicyclohexylcarbodiimide (3.1 g, 14 mmol),4-dimethylaminopyridine (1.8 g, 7.4 mmol), and levulinic acid (1.7 g, 14 mmol).The mixture was stirred for 8 h. Once completed the reaction was filtered and concentrated down. The crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **4** (2.4 g, 75%, clear syrup).

Rf (ethyl acetate/hexane): 0.40 (3/7)

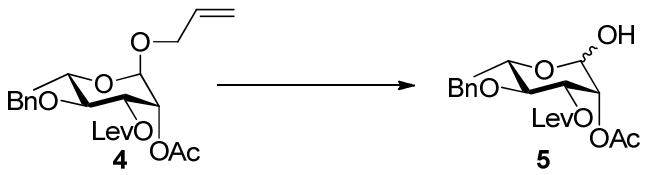
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.28-7.23 (m, 5H), 5.82 (m, 1H), 5.29 (dd, J = 10 hA, 3.6 Hz, 1H), 5.24-5.20 (m 2H), 5.13 (dd, J = 10.4 Hz, 1.6 Hz, 1H), 4.67 (m, 2H), 4.58 (d, J = 11.2

Hz, 1H), 4.06 (dd, J = 11.8 Hz, 4.0 Hz, 1H), 3.92 (dd, J = 12.8 Hz, 4.8 Hz, 1H), 3.78 (m, 1H), 3.46 (t, J = 9.6 Hz, 1H), 2.68 (m, 2H), 2.43 (m, 2H), 2.07 (s, 3H), 2.07 (s, 3H), 1.27 (d, J = 6.0 Hz, 3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 206.2, 171.7, 170.1, 138.1, 138.4, 128.4, 127.8, 96.4, 78.7, 75.0, 70.3, 68.0, 67.7, 37.8, 29.7, 27.9, 20.9, 17.9.

HRMS calcd. for $[M+Na]^+$: 457.18, found 457.1831

4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-acetyl-α-L-rhamnopyranoside (5):



To a solution of tetrahydrofuran (15 mL) and compound **9** (2.4 g, 5.5 mmol) was added (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (54 µmol, 46 mg). The reaction flask was put under vacuum and a hydrogen balloon was added. The reaction was stirred for 2 min with the hydrogen balloon. Then the hydrogen balloon was taken off and the reaction vessel was vacuumed and then flushed with nitrogen. The reaction stirred for 3 h at 21 °C. An NMR was taken of the solution to see if the reaction was done. The solvent was removed under pressure. To the reaction vessel was added mercuric oxide (0.50 g), mercuric chloride (0.50 g), and acetone:water (5:1) (30 mL). The reaction was stirred for 6 h at 21 °C. The solution was filtered through Celite®. Washed with potassium iodide (30 mL) and extracted with dichloromethane (40 mL x 2). The organic layer was washed with $NaHCO_3$(aq) (60 mL) and dried over $MgSO_4$. The solvent was reduced under pressure. The crude product was purified by

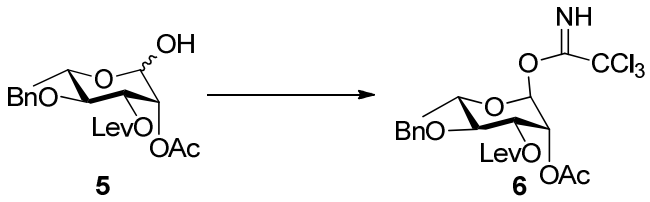
flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **5** (1.9 g, 91%, clear syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.37-7.29 (m, 5H), 5.35-5.27 (m, 2H) 5.11 (s, 1H), 4.73-4.63 (q, 2H), 4.15-4.09 (q, 4H), 3.5-3.47 (1H), 3.11-3.10 (1 H), 2.9-2.85 (1H), 2.75-2.49 (6H), 2.44-2.40 (1H), 2.25-2.19 (3H), 2.04 (s, 3H), 1.78 (s, 1H), 1.67 (s, 3H), 1.29-1.24 (3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm)

HRMS calcd. for $[M+Na]^{+}$: 417.15 found 417.1459

4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-acetyl-1-*O*-trichloroimidate-α-L-rhamnopyranoside (6):



To compound **5** (0.70 mg, 1.8 mmol) in dichloromethane (10 mL) was added cesium carbonate (0.58 g, 1.8 mmol) and trichloroacetonitrile (0.90 mL, 8.9 mmol). The reaction was stirred under nitrogen at 21 °C for 2 h. The reaction was filtered through Celite®. The solvent was reduced under pressure. The crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **11** (0.69 mg, 72%, clear syrup).

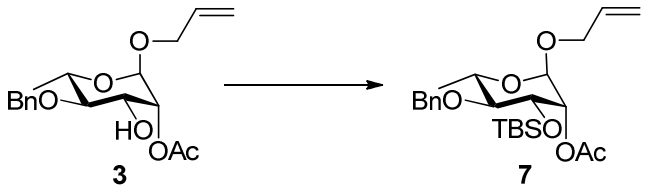
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 8.66 (s, 1H), 7.31-3.24 (m, 5H), 6.13 (s, 1H), 5.42 (dd, J = 3.2 Hz, 2.0 Hz, 1H), 5.33 (dd, J = 9.6 Hz, 3.2 Hz, 1H), 4.76 (d, J = 10.8 Hz, 1H), 6.63 (d, J = 10.8 Hz, 1H), 4.01 (m, 1H), 3.60 (t, J = 9.6 Hz, 1H), 2.72 (m, 1H), 2.52 (m, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 1.33 (d, J = 6.0 Hz, 3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 206.1, 171.8, 169.8, 137.7, 128.4, 128.0, 127.9, 94.8, 90.7, 77.9, 75.24, 71.7, 70.6, 68.5, 37.8, 29.8, 27.8, 20.8, 18.0

HRMS calcd. for $[M+Na]^+$: 560.06; Found: 560.0621

- **Second Rhamnose Building Block**

4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-2-*O*-acetyl-1-*O*-allyl-α-L-rhamnopyranoside (7):



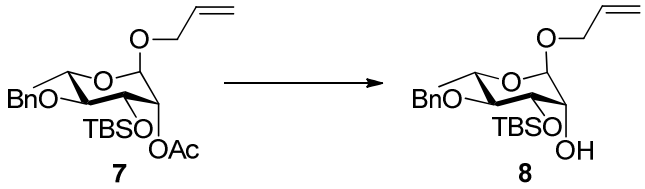
A solution of compound **3** (1.9, 5.6 mmol) in *N,N'*-dimethyl-formamide at 50 °C tertbutyldimethylsilylchloride (1.7 g, 11 mmol), imidazole (1.5 g, 22 mmol), and dimethylaminopyridine (140 mg, 1.1 mmol) were added. The mixture was stirred for 5 h. Once completed the reaction was diluted with CH_2Cl_2, washed with water, saturated $NaHCO_3$ solution and water in succession and dried ($MgSO_4$). Column Chromatography (2:1 Hexane:EtOAc) gave compound **7** (2.3 g, 91%, yellow syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.32 (5H), 5.93-5.83 (1H), 5.29-5.17 (2H), 5.08-5.07 (1H), 4.92-4.86 (1H), 4.72-4.66 (1H), 4.62-4.57 (2H), 4.14-4.10 (1H), 3.98-3.93 (1H), 3.76-3.72 (1H), 3.39-3.35 (1H), 2.14 (s, 3H), 1.33-1.28 (3H), 0.93-0.84 (9H), 0.12-0.08 (3H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 170.38, 138.05, 134.05, 128.48, 127.90, 117.48, 96.68, 81.50, 75.78, 72.84, 72.35, 71.09, 68.08, 26.02, 18.07, 4.48

HRMS calcd. for $[M+Na]^+$: 473.23, found 473.2328

4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-1-*O*-allyl-α-L-rhamnopyranoside (8):



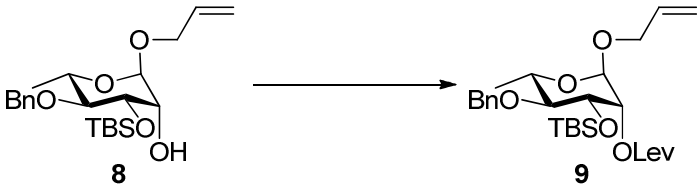
A solution of 0.50 M sodium methoxide at -10 °C was added to compound **7** (1.4 g). The reaction was stirred at -10 °C while being monitored by TLC every 10 min. The reaction was completed in 2.5 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and f ltered over Celite®. The solvent was removed under reduced pressure. Column Chromatography (2:1 Hexane:EtOAc) gave compound **8** (0.92 g, 74%, clear syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.36-7.30 (5H), 5.92-5.84 (m, 1H), 5.29-5.16 (2H), 4.85-4.82 (2H), 4.60-4.57 (1H), 4.17-4.13 (1H) 4.03-3.95 (2H), 3.81 (s, 1H), 3.74-3.69 (1H), 3.37-3.32 (1H), 2.64 (s, 1H), 1.28-1.26 (3H), 0.94-0.92 (9H), 0.15-0.08 (6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 138.52, 134.16, 134.11, 128.58, 127.92, 127.87, 127.78, 117.31, 98.11, 81.49, 75.57, 73.25, 72.16, 67.58, 26.15, 18.20, 4.36

HRMS calcd. for $[M+Na]^+$: 431.22, found 431.2225

4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-2-*O*-levulinoyl-1-*O*-allyl-α-L-rhamnopyranoside (9):



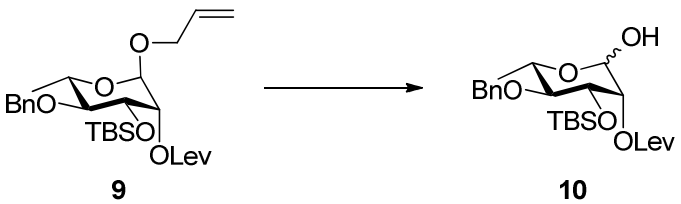
To a solution of compound **8** (0.93 g, 2.3 mmol) in dichloromethane (10 mL), under nitrogen, *N,N'*-dicyclohexylcarbodiimide (0.95 g, 4.6 mmol), 4-dimethylaminopyridine (0.28 g, 2.3 mmol), and levulinic acid (0.53 g, 4.6 mmol).The mixture was stirred for 8 h. Once completed the reaction was filtered and concentrated down. The crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **9** (1.1 g, 92%, clear oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.36-7.30 (5H), 5.92-5.82 (m, 1H), 5.28-5.16 (2H), 5.06 (1H), 4.90-4.87 (1H), 4.68 (s, 1H), 4.61-4.58 (1H), 4.15-4.09 (2H), 3.96-3.92 (1H), 3.76-3.69 (1H), 3.36-3.31 (1H), 2.79-2.68 (4H), 2.20 (s, 3H), 1.30-1.26 (3H), 0.89 (s, 9H), 0.10-0.08 (6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm)

HRMS calcd. for $[M+Na]^{+}$: 529.26, found 529.2591

4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-2-*O*-levulinoyl-L-rhamnopyranoside (10):



To a solution of tetrahydrofuran (15 mL) and compound **9** (1.1 g, 2.1 mmol) was added (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (18 mg, 0.02

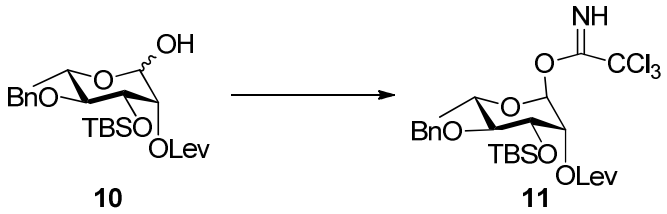
mmol). The reaction flask was put under vacuum and a hydrogen balloon was added. The reaction was stirred for 2 min with the hydrogen balloon. Then the hydrogen balloon was taken off and the reaction vessel was vacuumed and then flushed with nitrogen. The reaction stirred for 3 h at 21 °C. An NMR was taken of the solution to see if the reaction was done. The solvent was removed under pressure. To the reaction vessel was added mercuric oxide (0.13 g), mercuric chloride (0.13 g), and acetone:water (5:1) (18 mL). The reaction was stirred for 6 h at 21 °C. The solution was filtered through Celite®. Washed with potassium iodide (20 mL) and extracted with dichloromethane (30 mL x 2). The organic layer was washed with $NaHCO_3$(aq) (30 mL) and dried over $MgSO_4$. The solvent was reduced under pressure. The crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **10** (0.80 mg, 82%, clear syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.37-7.30 (m, 5H), 5.08-5.07 (1H), 4.89-4.85 (1H), 4.61-4.59 (1H), 4.15-4.11 (1H), 3.99-3.96 (1H), 3.37-3.32 (1H), 2.89-2.60 (6H), 2.23-2.17 (3H), 1.29-1.26 (3H), 0.92-0.88 (9H), 0.11-0.05 (6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 211.13, 176.97, 147.11, 143.23, 133.38, 133.14, 132.58, 109.63, 102.03, 101.62, 97.08, 85.87, 74.10, 72.99, 72.59, 72.28, 42.73, 39.68, 38.96, 35.12, 34.68, 32.30, 30.69, 30.12, 29.45, 27.4, 22.65, 17.11, 15.48, 0.11

HRMS calcd. for $[M+Na]^+$: 489.23, found 489.2276

4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-2-*O*-levulinoyl-1-*O*-trichloroimidate-α-L-rhamnopyranoside (11):



10 **11**

To compound **10** (0.20 mg, 0.43 mmol) in dichloromethane (10 mL) was added cesium carbonate (0.14 g, 0.43 mmol) and trichloroacetonitrile (0.22 mL, 2.15 mmol). The reaction was stirred under nitrogen at 21 °C for 2 h. The reaction was filtered through Celite®. The solvent was reduced under pressure. The crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product **11** (0.25 mg, 93%, clear syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 8.64 (s, 1H), 7.37-7.30 (m, 5H), 6.12-6.17 (1H), 5.23-5.22 (1H), 4.92-4.87 (1H), 4.63-4.58 (1H), 4.20-4.15 (1H), 3.93-3.89 (1H), 3.47-3.46 (1H), 2.80-2.59 (4H), 2.21-2.16 (3H), 1.33-1.21 (3H), 0.94-0.88 (9H), 0.12-0.08 (6H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 206.25, 171.86, 160.31, 138.27, 128.55, 128.03, 127.69, 95.68, 91.17, 80.52, 75.77, 72.04, 71.82, 70.92, 70.81, 38.11, 28.21, 26.50, 26.10, 25.99, 18.11, 4.51

HRMS calcd. for $[M+Na]^{+}$: 634.14, found 634.1412

General procedure for automated synthetic cycles to produce Group A Streptococcus

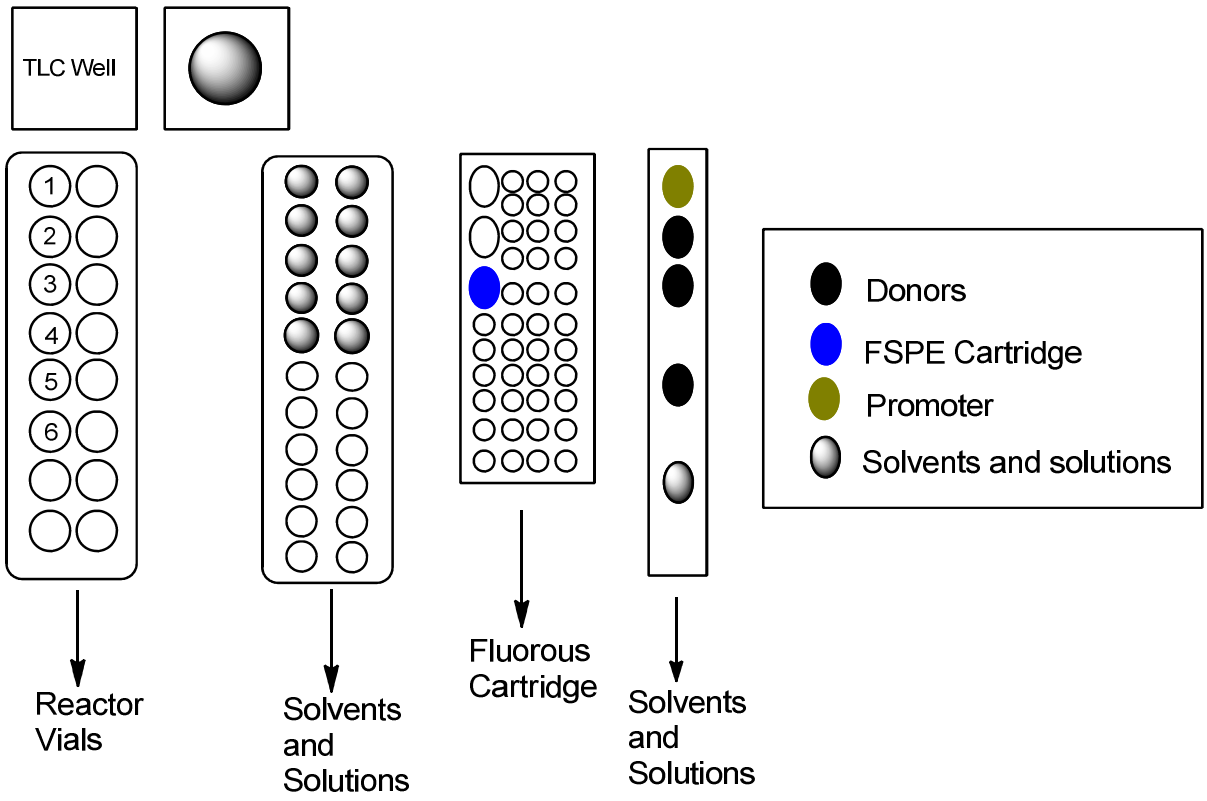


Figure S1: Basic layout of automated solution-phase oligosaccharide for the GAS hexasaccharide.

1. Sample Preparation

Donor molecules (50 µmol of each donor) was dissolved in anhydrous toluene (0.7 mL) or dichloromethane (0.7 mL) in the vials and the donors were placed at the inert reagent rack under argon. A 0.27 M trimethylsilyltrifluoromethanesulfonate (TMSOTf, 1.05 mL) solution in toluene was prepared in a vial and placed as indicated on the inert reagent rack under argon. Toluene (1.0 L) was placed in the stock solution bottle and placed at the reservoir bottle rack, so it couls be used for rinsing the needle. Acetonitrile (100 mL), 80% methanol/water (100 mL), triethylamine (8 mL), Acetone (8 mL), DMF (8 mL), Tetrahydrofuran (8 mL) with a small

amount of acetic acid (0.3 mL); these stock solutions were prepared in stock solution bottles or vials capped with a pre-punctured septa. Acceptor molecule (21.3 mg, 25 μmol) was dissolved with anhydrous toluene (0.7 mL) in an 8 mL vial that has a conically-bottomed and capped with a pre-punctured septa. Then a 1 M Hydrazine Hydrate (0.4) solution in 2:3 acetic acid:pyridine (8 mL) was placed in an 8 mL vial and capped with a pre-punctured septa. A fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol/water and placed on the FSPE block. An empty 8 mL extraction vial (conically-bottomed) was placed under the FSPE cartridge.

2. The Automated Solution-Phase Synthesizer-Cleaning Cycle

Before placing any of the reagents into the ASW1000, the reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. The cleaning cycle consist of cleaning each of the 16 reactor vials (13 mL capacity each) with toluene (8 mL) and methanol (8 mL) 3 times each. The solvents were then removed from the reactor vials, which were dried under vacuum and argon for 45 minutes.

3. Automated Solution-Phase Synthesizer-Method Run

Glycosylation

For Each glycosylation, the reactor vials were cooled to 22 ^{o}C during the 5 minutes wait time by the heat transfer oil. Then the flat-tipped open vial needle transferred the acceptor

molecule solution (1.0 mL) to the desired reaction vial, followed by the transfer of the donor molecule solution (1.0 mL) and the TMSOTf solution (100 µL). After each individual transfer, the needle (inside and outside) was rinsed by toluene (2 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 45 minutes at 22 ^{o}C under argon gas. After the reaction time the needle withdrew 20 µL of the solution from the reaction mixture and placed it into the desired well of the microtiterplate for thin layer chromatography (TLC) monitoring. There was a 10 minute wait for the TLC monitoring before the Triethylamine was added. Once the Triethylamine (1.0 mL) was added to the solution for quenching, the reaction mixture was vortexed at 800 rpm for 10 minutes. Finally, toluene (1.0 mL) was added and the solvent was evaporated under reduced pressure.

Delevulination

Here is the procedure of each delevulination that occurred during the synthesis of the GAS oligosaccharides. To the dried residue, 1 M solution of hydrazine hydrate in 2:3 acetic acid : pyridine (1.0 mL) was added to the desired reactor vial. The reaction mixture was then vortexed at 800 rpm for 2 hours at room temperature. After the 2 hours, the needle withdrew 20 µL from the reaction mixture and placed it into the desired well of the microtiterplate for TLC monitoring. There was a 10 minute wait for the TLC monitoring before Acetone was added. Acetone (1.0 mL) was added to the solution for quenching followed by addition of toluene (1.0 mL) and solvent was evaporated under reduced pressure.

Desilylation

Desilyation was carried out by adding tetrahydrofuran (1.0 mL) to the dried residue in the desired reactor vial followed by tetrabutyl ammonium fluoride 1.0M in THF solution (0.6 mL). The reaction mixture was vortexed at 800 rpm for 15 hours at room temperature. After the 15 hours, the needle withdrew 20 µL from the reaction mixture and placed it into the desired well of the microtiterplate for TLC monitoring, which was 10 minutes. Toluene (1.0 mL) was added and solvent was evaporated under reduced pressure.

Fluorous solid-phase extraction (FSPE)

For each FSPE, DMF (0.4 mL) was added to the reactor vial to dissolve the crude mixture and the vials were vortexed at 800 rpm for 2 minutes. The reaction mixture (0.7 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 20 mL/s via the 10 mL syringe for 1 minute. Next, 80% methanol (4.5 mL) was used to rinse the reactor vial and vortexed at 800 rpm for 5 minutes. Then the 80% methanol solution was removed from the reactor vial two times (2.5 mL X2) and delivered to the FSPE cartridge. Additional 80% methanol solution (2 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at 'SPE waste' for the eluted mixture to be disposed. Methanol (2 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound while the FSPE cartridge was positioned at the 'SPE collect'. The collect position has a 8 mL vial right below for collection of the sample. The next step was the position of the 'SPE direct' for the needle to withdraw the collected sample from the 8 mL conically-bottomed vial and deliver it to the next clean reactor vial for the cycle of reactions to take place.

Toluene (1.0 mL) was added to the solution and the solution was evaporated under reduced pressure. After the evaporation cycle, once again toluene (1.0 mL) was added and removed under reduced pressure to remove any residual water.

Synthesis of the Group A Streptococcus hexasaccharide.

For the synthesis of the GAS hexasaccharide, the general automation protocol was applied. The specific operation conditions are described in the table below for the 5.5 cycles completed for the synthesis of GAS hexasaccharide.

Step	Task	Reagents / Operation	Operation time
1	Glycosylation	2 equivalent donor **Rha 1** (100 μmol) in 2 mL toluene, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
2	TLC sample	30 μL of crude reaction mixture withdrawn	
3	Quenching	1 mL TEA	
4	Evaporation	50 °C	35 min
5	Delevulination	1mL of 1M solution of hydrazine hydrate in 2:3 Acetic acid:Pyridine	2 h
6	TLC sample	30 μL of crude reaction mixture withdrawn	
7	Quenching	1 mL equiv. acetone	
8	Evaporation	50 ^{o}C	35 min

9	FSPE preparation	0.4 mL DMF	
10	Sample loading	0.7 mL crude sample transferred to cartridge	
11	Wash	8 mL 80% methanol wash (repeated 4 times)	
12	Wash	6 mL methanol wash (repeated 3 times)	
13	Transfer	6.5 mL collected sample transferred to clean vial	
14	Evaporation	50 ^{o}C	45 min
15	Transfer	1 mL toluene added	
16	Evaporation	50 ^{o}C	45 min
17	Glycosylation	2 equivalent donor **Rha 2** (100 µmol) in 2 mL toluene, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
18	TLC sample	30 µL of crude reaction mixture withdrawn	
19	Quenching	1 mL TEA	
20	Evaporation	50 °C	35 min
21	Delevulination	1mL of 1M solution of hydrazine hydrate in 2:3 acetic acid : pyridine	2 h
22	TLC sample	30 µL of crude reaction mixture withdrawn	
23	Quenching	1 mL equiv. acetone	
24	Evaporation	50 ^{o}C	35 min
25	FSPE	0.4 mL DMF	

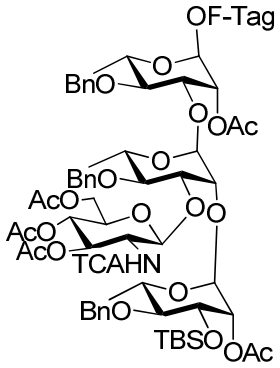
	preparation		
26	Sample loading	0.7 mL crude sample transferred to cartridge	
27	Wash	8 mL 80% methanol wash (repeated 4 times)	
28	Wash	6 mL methanol wash (repeated 3 times)	
29	Transfer	6.5 mL collected sample transferred to clean vial	
30	Evaporation	50 ^{o}C	35 min
31	Transfer	1 mL toluene added	
32	Evaporation	50 ^{o}C	35 min
33	Glycosylation	2 equivalent donor **Rha 1** (100 µmol) in 2 mL toluene, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
34	TLC sample	30 µL of crude reaction mixture withdrawn	
35	Quenching	1 mL TEA	
36	Evaporation	50 °C	35 min
37	Desilylation	1mL of tetrahydrofuran 0.6 mL of tetrabutylammonium fluoride	13 h
38	TLC sample	30 µL of crude reaction mixture withdrawn	
39	Evaporation	50 ^{o}C	35 min
40	FSPE preparation	0.4 mL DMF	

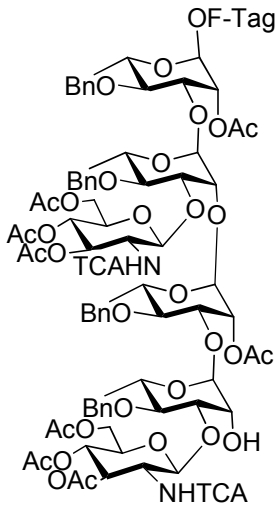
41	Sample loading	0.7 mL crude sample transferred to cartridge	
42	Wash	8 mL 80% methanol wash (repeated 4 times)	
43	Wash	6 mL methanol wash (repeated 3 times)	
44	Transfer	6.5 mL collected sample transferred to clean vial	
45	Evaporation	50 ^{o}C	45 min
46	Transfer	1 mL toluene added	
47	Evaporation	50 ^{o}C	45 min
48	Glycosylation	2 equivalent donor **GluNAc** (100 µmol) in 2 mL dichloromethane, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
49	TLC sample	30 µL of crude reaction mixture withdrawn	
50	Quenching	1 mL TEA	
51	Evaporation	50 °C	35 min
52	Delevulination	1mL of 1M solution of hydrazine hydrate in 2:3 Acetic acid:Pyridine	2 h
53	TLC sample	30 µL of crude reaction mixture withdrawn	
54	Quenching	1 mL equiv. acetone	
55	Evaporation	50 ^{o}C	35 min
56	FSPE preparation	0.4 mL DMF	
57	Sample	0.7 mL crude sample transferred to cartridge	

	loading		
58	Wash	8 mL 80% methanol wash (repeated 4 times)	
59	Wash	6 mL methanol wash (repeated 3 times)	
60	Transfer	6.5 mL collected sample transferred to clean vial	
61	Evaporation	50 ^{o}C	45 min
62	Transfer	1 mL toluene added	
63	Evaporation	50 ^{o}C	45 min
64	Glycosylation	2 equivalent donor **Rha 2** (100 µmol) in 2 mL toluene, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
65	TLC sample	30 µL of crude reaction mixture withdrawn	
66	Quenching	1 mL TEA	
67	Evaporation	50 °C	35 min
68	Delevulination	1mL of 1M solution of hydrazine hydrate in 2:3 Acetic acid:Pyridine	13 h
69	TLC sample	30 µL of crude reaction mixture withdrawn	
70	Quenching	1 mL equiv. acetone	
71	Evaporation	50 ^{o}C	35 min
72	FSPE preparation	0.4 mL DMF	
73	Sample loading	0.7 mL crude sample transferred to cartridge	
74	Wash	8 mL 80% methanol wash (repeated 4 times)	

75	Wash	6 mL methanol wash (repeated 3 times)	
76	Transfer	6.5 mL collected sample transferred to clean vial	
77	Evaporation	50 ^{o}C	45 min
78	Transfer	1 mL toluene added	
79	Evaporation	50 ^{o}C	45 min
80	Glycosylation	2 equivalent donor **GluNAc** (100 µmol) in 2 mL dichloromethane, 0.05 equivalent TMSOTf, 22 ^{o}C	45 min
81	TLC sample	30 µL of crude reaction mixture withdrawn	
82	Quenching	1 mL TEA	
83	Evaporation	50 °C	35 min
84	FSPE preparation	0.4 mL DMF	
85	Sample loading	0.7 mL crude sample transferred to cartridge	
86	Wash	8 mL 80% methanol wash (repeated 4 times)	
87	Wash	6 mL methanol wash (repeated 3 times)	

Tetrasaccharide + Hexasaccharide:





Automated Solution-Phase Synthsizer Procedure Above

Tetramer HRMS calcd. for $[M+K+H_2O+D_2]^+$: 1817.24, found 1817.2357

Hexamer HRMS calcd. for $[M+Na_2+H_2O+D_2]^+$: 2410.30, found 2410.2978

CHAPTER 5

Progress Toward the Automated Solution-Phase Synthesis of the Cell Wall Oligosaccharides of *Staphylococcus aureus*

Manuscript in Preparation

Heather D. Edwards and Nicola L. B. Pohl

Abstract

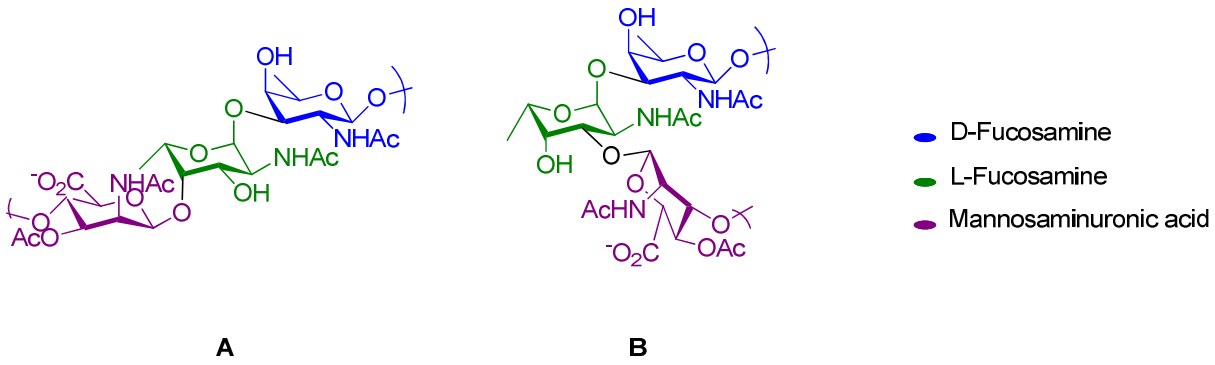
Cellulitis is a poorly treatable skin disease caused by Group A streptococcus (GAS) and *Staphylococcus aureus*. In order to develop a vaccine against these bacterial infections, we would like to develop an automated synthesis of the key cell wall polysaccharides from *S. aureus*. The synthesis of type 5 and type 8 cell wall fragments contain repeating units with d-fucosamine, l-fucosamine, and mannosaminuronic acid. With one d-fucosamine building block and 2 building blocks of each of the l-fucosamine and mannosaminuronic acid, the growing chain will depend on where the protecting groups are positioned. The difficulties of these building blocks are due to the rarity of the saccharides and the complications of the syntheses.

Introduction

The *Staphylococcus aureus* has two oligosaccharide chains found in type 5 and type 8 (**Figure 1**). Both type 5 and type 8 contain L-fucosamine, D-fucosamine, D-mannosaminuronic acid. (Jones 2005) Very little is known of these sugars. They are difficult to synthesize, and there are not many papers out on the synthesis of some of the linkages, which creates a challenge. The D-mannosaminuronic acid contains an acetate group on the end product of the oligosaccharide;

thus the protecting groups on the other sugars have to be orthogonal to the acetate. Esters are a prominent way of protecting the hydroxyl groups on sugars. Once the monosaccharides are synthesized, confirmation and binding studies can begin.

Figure 1: (A) Type 5 S. aureus oligosaccharide and (B) Type 8 S. aureus oligosaccharide. (Jones 2005)

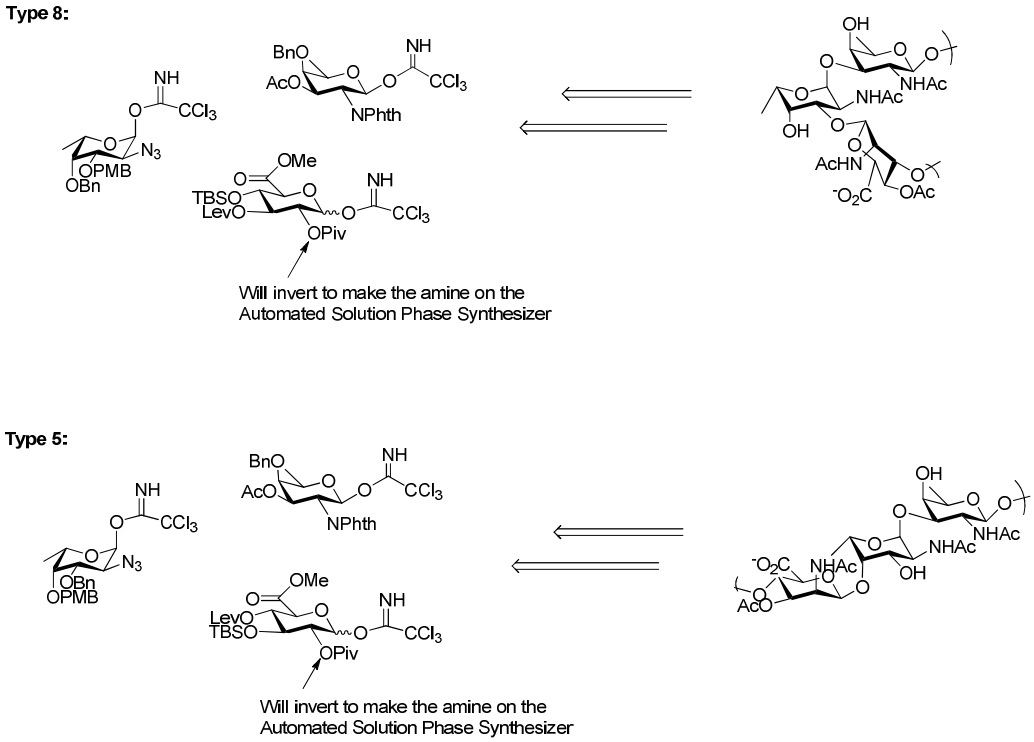


Staphylococcus aureus is a gram positive bacterium. (Eiff, et al. 2001; Lee 2001; Von Eiff, et al. 2007; Liau and Hash 1977) The bacterium has many virulence factors such as extracellular toxins, secreted enzymes, and cell associated antigens that are responsible for the pathogenicity. The bacterium can cause metastatic abscesses, septic arthritis, endocarditis, cellulitis, osteomyelitis, and wound infections. *Staphylococcus aureus* is of great concern to many scientist and doctors, due to the methicillin-resistant strains. (Fattom, et al. 2004) Vancomycin is one of the known antibiotics that is not resistant to this type of *Staphylococcus aureus*.

The capsular polysaccharides of type 5 and type 8 are considered to be a virulent factor. (Fattom, et al. 2004; Jones 2005) Therefore, development of the syntheses of the oligosaccharide

chains of type 5 and type 8 enable a better understanding of their biological roles. Dr. Peter Norris has been looking at these strains over the last couple of years. (Alhassan, et al. 2006) The sugars found in these chains are also found in many other bacteria such as *Pseudomonas aeruginosa, Escherichia freundii, Pneumococcu, Proteus mirabilis* and *Bacillus*. (Barker, et al. 1961; Barker, et al. 1966; Barrow and Wheat 1972; Barry and Roark 1964; Benzing, et al. 1981; Beynon et al. 1992; Cifonelli, et al. 1966; Dabrowski, et al. 1996; Daniel, et al. 1972; Daoust, et al. 1981; Dmitriev 1980) Building these oligosaccharides will help to discover what other bacteria have these similar monosacharides or oligosaccharide chains.

Figure 2: *Staphylococcus aureus*: Type 5 and Type 8 building block strategies



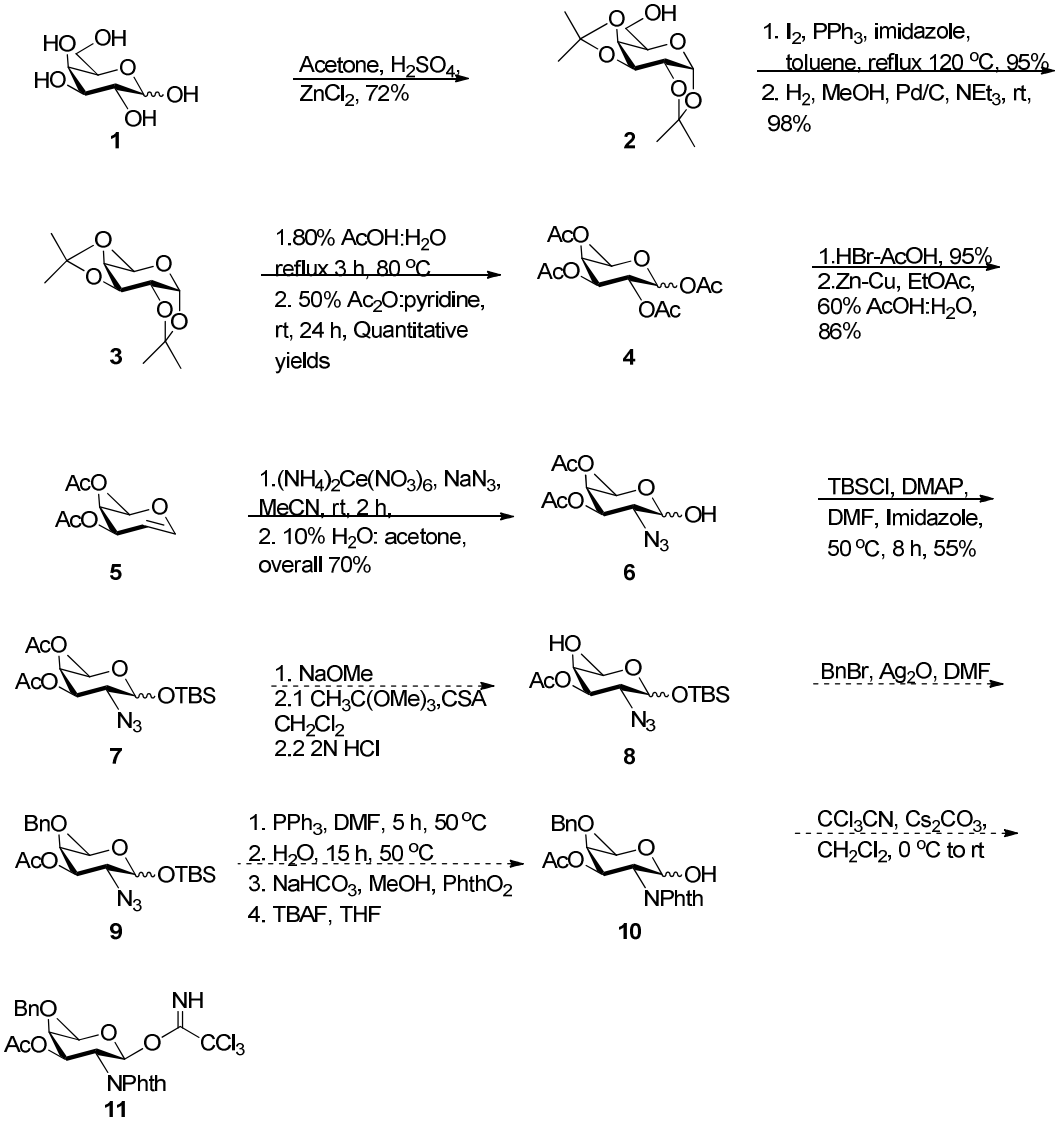
The initial goal was to design the building blocks of the sugars found in the cell wall polysaccharides of *S. aureus* type 5 and type 8. After synthesis of the building blocks, conditions will need to be found to connect together these building blocks and take the repeating trisaccharide units of type 5 and type 8. The ideal way to synthesize these trisaccharide units would be the automated solution-phase synthesizer, which is based on the purification of fluorous-tagged intermediates by fluorous solid phase extraction (FSPE). Figure 2 shows the monosaccharides that we are trying to achieve. We have gone through many different synthesis approaches to try and find the best routes possible. Some routes just have to many challenges and now we have come to the new building blocks described in the results and discussion section.

Results and Discussion

D-fucosamine is not commercially available to buy and D-fucose is very expensive. D-galactose (compound **1**) is commercially available and was used to start the D-fucosamine building block (**Scheme 1**). (De Almeida, et al. 2007; Horton, et al. 1977; Hou and Kovac 2008; Lerner 1993; Liu, et al. 2000; Szabo and Charon 1994) The first step is to take galactose and do a double acetal protection (compound **2**). Next, an iodide was placed on the C6 position followed by the palladium catalyst and hydrogen was used to transform the C6 position to a methyl group (compound **3**). The acetal groups are then removed and replaced with acetate groups (compound **4**) followed by transformation into a glycal (compound **5**). (Shull, et al. 1996) Cerium ammonium nitrate (CAN) is used with sodium azide to place the azide group on the C2 position (compound **6**). (Robbins and Trahanovsky 1971) The synthesis of this building block is known through these steps and the following are new moieties. From there the anomeric oxygen was protected with tertbutyldimethylsilyl (TBS) group (compound **7**) will be followed by acetate

removal. The next step will be to attach an orthoester and open it up to get the acetate either on the C3 or C4 position (compound **8**). Once we have the acetate in the C3 position we can precede the next step. The next step would be to protect the free hydroxyl group with a benzyl group

Scheme 1: The synthesis of the D-fucosamine building block.

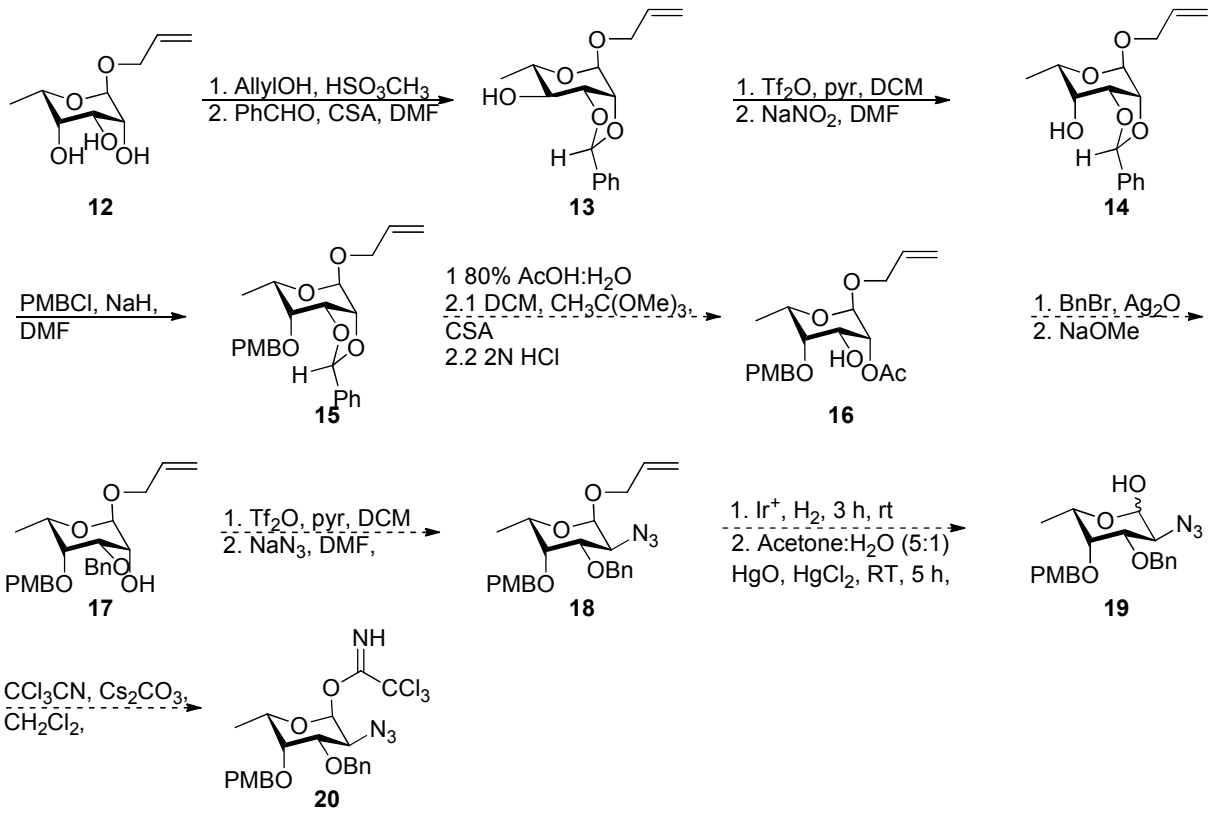


(compound **9**). The azide group would then need to be transformed into an amine and protected in order to get the neighboring group participation for the beta selectivity (compound **10**). The TBS group would then be removed and a trichloroimidate would be added to the anomeric position to give compound **11**. This would conclude the building block of the D-fucosamine. The issue with going this route was that once compound **6** was in hand, the addition of tertbutyldimethylsilyl to the anomeric center would not occur. It seemed that the reactivity of the compound had become very low, essentially meaning that a new method was needed to get the desired product of compound **7** from compound **6**.

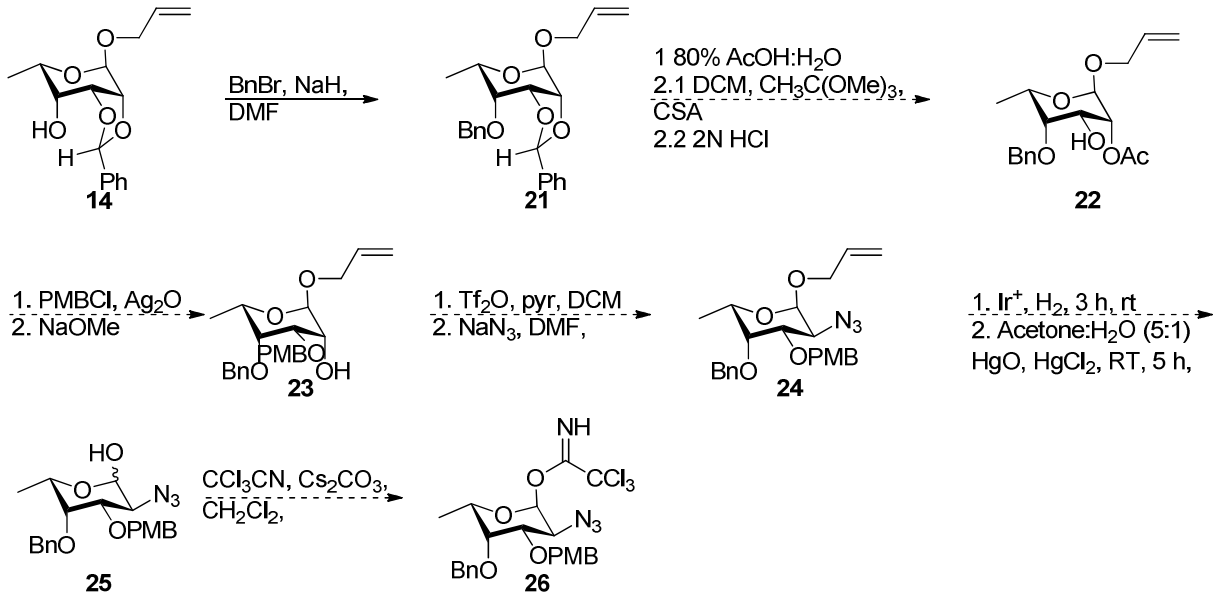
L-fucosamine is not commercially available, so the synthesis will start from L-rhamnose shown in **Schemes 2** and **3**. The first step is to allylate followed by the synthesis of the benzylidene to give compound **13** (Clode, et al. 1976). These building blocks are known up to these steps the rest are new moieties. The next step is to protect the C4 position with triflate and then invert the C4 position by using sodium nitrite to give compound **14**. Here the synthesis deviates between the two L-fucosamine building blocks. The first building block places a para-methoxy benzyl (PMB) group on the inverted C4 position (compound **15**). Then the benzylidene group is removed and replaced with the orthoester, which is opened up to have the acetate on the C2 position to provide compound **16**. Next, the C3 position can be benzylated by using silver (II) oxide and then the acetate will be removed (compound **17**). Once the acetate group is removed, the C2 position will be protected with a triflate group. This will allow for sodium azide to place an inverted azide attached to the C2 position (compound **18**). Iridium and mercury will be used to remove the allyl group (compound **19**) and a trichloroimidate would be added to the anomeric position (compound **20**) to finish the first L-fucosamine building block.

The second L-fucosamine follows the same synthetic routes except for a few minor changes in order for the linkages of the polysaccharides to be correct. For the second L-fucosamine building block, a benzyl group was used to protect the C4 position (compound **21**). The other step that differs would be the step where the C3 position would be protected with a PMB group (compound **23**). These are the two L-fucosamine building blocks that will then be used to make the repeating units of type 5 and type 8.

Scheme 2: The synthesis of the 1st L-fucosamine building block.



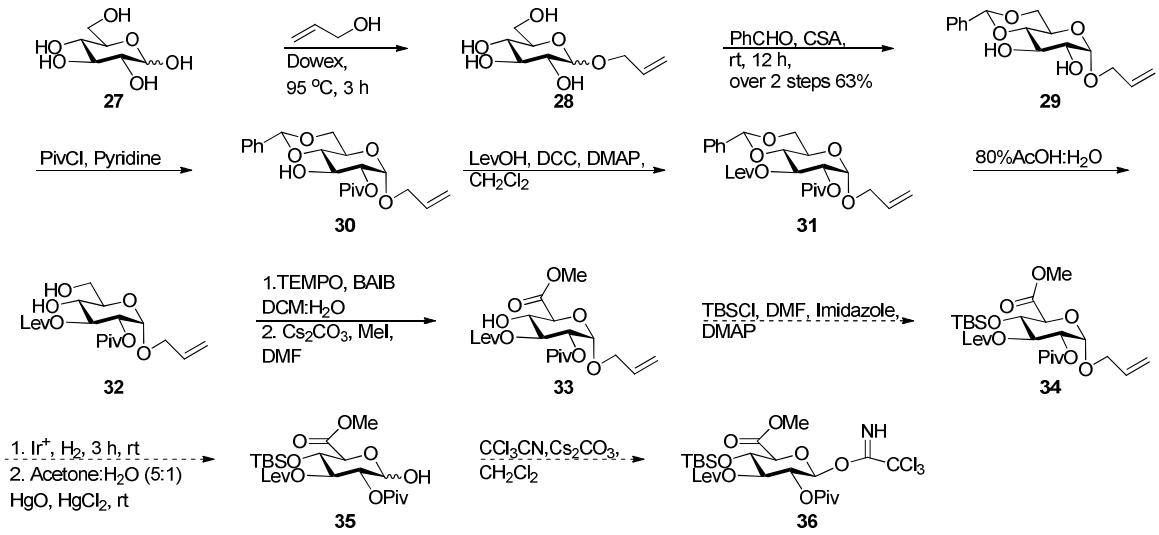
Scheme 3: The synthesis of the 2nd L-fucosamine building block.



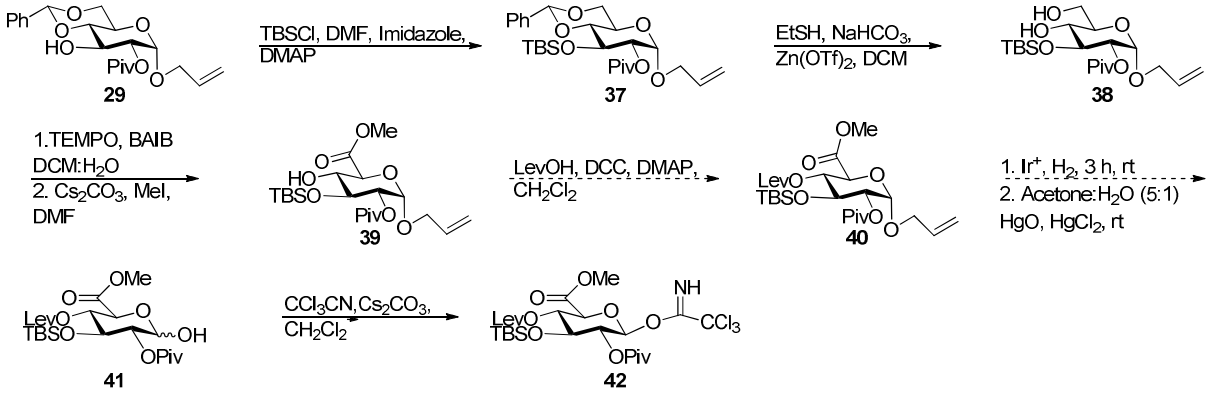
The final building block is mannosaminuronic acid. Not much is known on this building block and has only been synthesized a couple of times. (Drew et al. 2001; Kaji, et al. 1994) The ones that have been synthesized before have different linkages. The challenge of this building block is the linkages and the acetate that will need to be placed onto the building block in the end. In the end the protecting groups could prove to be difficult in making the building block be less reactive. Since mannosaminuronic acid was not available, the synthesis began with glucose (compound **27**). First, both building blocks had undergone allylation at the anomeric position followed by benzaldehyde to protect O6 and O5 (compound **29**). Pivaloylate is used to protect the C2 position (compound **30**). Then the synthesis deviates with the first building block having a levulinate group to protect the hydroxyl group on the C3 position to provide compound **31**.

Next, deprotection of the O6 and O5 positions was done with acidic solution (compound **32**) and transformation of the C6 into an ester group was attempted to afford compound **33**. A TBS group would then be placed on the C4 position to provide compound **34**. Once this is done the allyl group (compound **35**) could be removed and a trichloroimidate group could be placed at the anomeric center (compound **36**). The sugar could then be attached to another building block and then transformed into the mannosaminuronic acid in order to get the beta selectivity.

Scheme 4: The synthesis of the 1st mannosaminuronic acid building block



Scheme 5: The synthesis of the 2nd mannosaminuronic acid building block



For the synthesis for the second D-mannosaminuronic acid building block, a TBS group was placed to protect the hydroxyl group on the C3 position (compound **37**). Next, deprotection of the O6 and O5 positions was achieved by mild conditions in order to keep the TBS group to afford compound **38**. Then the transformation of the C6 into an ester group was attempted (compound **39**). A levulinate group would then be placed on the C4 position to give compound **40**. Once this was done the allyl group (compound **41**) could be removed and a trichloroimidate group could be placed at the anomeric center (compound **42**). Again, the sugar could then be attached to another building block and then transformed into the mannosaminuronic acid in order to get the beta selectivity.

After all the building blocks are synthesized, the goal will be to glycosylate in an automated solution-phase synthesizer. The repeating unit chain length will then be varied in order to study how many repeating units are needed to help in binding a cellulitis vaccine. The ultimate goal is to combine the GAS cell wall oligosaccharide and the type 5 and type 8 oligosaccharides into one vaccine.

Conclusion

To conclude, we are synthesizing D-fucosamine, L-fucosamine, and D-mannosaminuronic acid to reconstruct the oligosaccharides of *S. aureus* type 5 and type 8. The synthetic routes to each of the building blocks is challenging due to the end products needed. Once all the building blocks are at hand, we will be able to build the oligosaccharides in the

automated solution-phase synthesizer and use the FSPE for purification. Then we would be able to optimize the conditions and build the trisaccharides to longer repeating units to study the binding for a vaccine of cellulitis.

Experimental Section

General materials and methods.

The known synthetic procedures used reaction solvents directly from the solvent tower (Swagelok). Amberlyst® 15 ion-exchange resin was washed repeatedly with methanol before use. All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (tlc) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 µm). The developed tlc plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Flash chromatography was performed with ZeoPrep 60 Eco 40-63 µm silica gel unless otherwise specified. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Microwave heating was carried out with a CEM-Discover continuous wave microwave. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Varian VXR at 400 MHz and101 MHz respectively. 1H NMR spectra were

reported in parts per million relative to $CDCl_3$ as an internal reference. ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$.

Chapter 6

Conclusions and Future Directions

This dissertation reports different applications of fluorous chemistry in the classroom and the laboratory. First, a fluorous chemistry curriculum for a week was introduced in the middle school classroom. Next, the capabilities of different fluorous linkers to bind to fluorous-coated glass slides were probed to reveal that a less environmentally-persistent fluorous chain could also be used successfully to form carbohydrate microarrays. Finally, simplified purification steps using fluorous linkers when synthesizing the bacterial oligosaccharides of Group A Streptococcus and *Staphylococcus aureus* were demonstrated. Clearly, the unique properties of fluorous alkyl groups can be exploited in a variety of areas.

A new hydrophobicity/fluorophilicity unit was developed for the middle school classroom. The desire to bring research into the classroom and to strengthen the knowledge that students have learned in previous years of the basic concepts of chemistry were the motivation for this new unit. The unit was based on surface chemistry and four different laboratory experiments were developed to help the students understand the concepts. All the activities were compared with the fluorous chemistry and how the materials that they were working with had similar and different properities than fluorous compounds. This integrated set of laboratory experiments and lectures increased student awareness of the role of chemistry, especially hydrophobicity/fluorophilicity in nature. For the future, teachers will be able to use these activities and easily tie additional research from the active fluorous area into the classroom. The surface chemistry unit can be broken down into smaller useable units; different activities can fit into other parts of the curriculum in the middle school. Fluorous chemistry was applied to the

classroom atmosphere and helped in making students more aware of the chemistry that takes place around them.

Next, fluorous-fluorous interactions have shown their utility in both carbohydrate microarrays and small-molecule microarrays. The C_8F_{17}-mono linker was originally developed for carbohydrate microarrays, but because this fluoroalkyl compound has been found to bioaccumulate, alternatives are needed. A new di-C_6F_{13}-containing linker was found to form compounds spots on microarrays that appear four times more intense than spots made from the original tag when doing binding studies. The strength of this noncovalent interaction with new di-tag on fluorous surfaces provides promising evidence for the increased use of this new fluorous tag in not only carbohydrate microarrays, but also automated solution-phase oligosaccharide synthesis.

Finally, the feasibility of using fluorous solution-phase extraction to aid the synthesis of bacterial oligosaccharide fragments of Group A Streptococcus (GAS) was demonstrated and should serve well for the the synthesis of *Staphhylococcus aureus* oligosaccharides. Methods to make both tetrameric and hexameric oligosaccharide fragments of GAS using automated solution-phase synthesis were developed. The complex monosaccharide building blocks were built for construction of *S. aureus* oligosaccharides of type 5 and type 8. Future studies are needed to optimize glycosylation steps to extend the chainlength of the GAS even farther and to apply these methods to develop robust protocols for the automated solution-phase synthesis of *S. aureus* oligosaccharides. Ultimately, the successful syntheses of these classes of carbohydrate polymer fragments will allow studies to define the minimal structures needed to make a vaccine for cellulitis.

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Finally, I want to give a special thanks to my greatest supporters my parents, my little brother, and my husband for their constant love, faith, and always being there for me.

APPENDIX A. CHAPTER 2 WORKSHEETS

Lesson Plans:

Lesson Plans for the Week of Hydrophobicity Discussion

Day 1: Lesson Overview

Introduction to the basics of molecules and motion using the particle theory with a Brownian motion demonstration. Students will learn the vocabulary terms for the beginning of the week. These terms are needed to understand the rest of the week.

Vocabulary

Atoms, Molecules, Fluorine, Carbon, Hydrogen, Motion, Brownian Motion, Polarity, Fluorocarbons, Hydrocarbons, Hydrophobicity, Viscosity, Friction

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces.
2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.
2. Understand that different kinds of questions suggest different kinds of scientific investigations.
3. Select and use appropriate tools and techniques to gather, analyze and interpret data.
4. Incorporate mathematics in scientific inquiry.
5. Communicate and defend procedures and explanations.
6. Identify and explain the properties and changes of matter.
7. Describe and explain concepts related to mechanics, forces, and motion.

8. Understand the concept of energy and its various forms.

Materials

Two clear plastic cups, water, hot plate, dye,

Lesson Procedures

1. Start by giving a 10 min pretest.
2. Start to explain how molecules are constantly moving even if you can't see that they are moving. Talk about the different phases (solid, liquids, and gases). Solid molecules are packed so close together that there is only minimal amount of movement. Liquids are loosely packed allowing more movement between the molecules. Molecules of gas are freely moving all the time.
3. Do a demonstration by taking two clear cups and filling one with hot water and the other with room temperature water. Add a drop of dye into each of the cups of water and watch what happens. Explain why the dye will flow faster in the hot water because the molecules are moving faster. Heat is a form of energy. When we say that something is hot, we mean that the molecules in the sample have more energy than those of an identical sample at a cooler measured temperature. Therefore, the dye molecules also move more slowly in the room temperature water.
4. Then discuss hydrophobicity. The term hydrophobicity comes from the Greek words for water and fear; we use the term to mean that the molecule is water fearing/hating. The molecule will want to repel the water. Think of examples in nature of where something will repel water, such as the wax on leaves. Introduce hydrocarbons and fluorocarbons. Hydrocarbons are chains of carbon with hydrogen atoms attached, where fluorocarbons are chains of carbon with fluorine atoms attached.
5. Next discuss polarity and what the phase "like dissolves like" means. Talk about where you can see this in real life.
6. Have the students start to work on the vocabulary terms. They will have the week to work on the terms because each day they will learn new terms.

Evaluation/Assessment

The vocabulary terms for the day; a discussion and questions on the demonstration and what they have learned for that day.

References:

Chemistry: The Study of Matter and Its Changes; Brady, J.; Holum, R., Ed.; John Wiley and Sons, Inc.: Canada, 1993; pp. 444.

Day 2: Lesson Overview

Today the students will learn about emulsification. Emulsification can be related to hydrophobicity and the phrase “like dissolve like.” The students will do a lab where they will create their own emulsion and have to use an emulsifier. The students should constantly draw and think about what they are seeing. They should be able to relate it to real world applications.

Vocabulary

Emulsification, Emulsifier, Hydrophobicity

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces
2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.
2. Understand that different kinds of questions suggest different kinds of scientific investigations.
3. Select and use appropriate tools and techniques to gather, analyze and interpret data.
4. Incorporate mathematics in scientific inquiry.
5. Communicate and defend procedures and explanations.
6. Identify and explain the properties and changes of matter.
7. Describe and explain concepts related to mechanics, forces, and motion.
8. Understand the concept of energy and its various forms.

Materials

Per student or group of students: centrifuge tubes (50 mL), vegetable oil (15 mL), vinegar (15 mL), 1 egg yolk, 1 beaker .

Lesson Procedures

1. Do a 10 min introduction on emulsification. Introducing the vocabulary terms of emulsion and emulsifier. Ask the students if they can think of real world applications where they have emulsions and use emulsifiers.
2. Then hand out the lab and the materials for the lab.
3. Have the students do the lab. The students can do the lab individually or in groups of 2 to 3.

Evaluation/Assessment

The lab is the assessment for the day. The students need to have the hypothesis, the observations and data, and the conclusion finished. The students can work on the vocabulary terms that they learned for the day.

References

Bravo-Díaz, C.; González-Romero, E. *J. Chem. Educ.* **1996**, *73*, 844-846.
Mabrouk, S. *J. Chem. Educ.* **2004**, *81*, 83-86.
Rowat A.; Hollar, K.; Stone, H.; Rosenberg, D. *J. Chem. Educ.* **2011**, *88*, 29-33.

Day 3: Lesson Overview

Today the students will learn about surface area. The day is more about qualitative analysis. The students will be using glass slides and placing a drop of different types of liquids and observing what happens with each drop. How will each liquid react to the surface of the glass slide?

Vocabulary

Surface Area, Area, Solids, Liquids

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces

2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.

2. Understand that different kinds of questions suggest different kinds of scientific investigations.

3. Select and use appropriate tools and techniques to gather, analyze and interpret data.

4. Incorporate mathematics in scientific inquiry.

5. Communicate and defend procedures and explanations.

6. Identify and explain the properties and changes of matter.

7. Describe and explain concepts related to mechanics, forces, and motion.

8. Understand the concept of energy and its various forms.

Materials

Per student or group of students: 1 Regular glass microscope slide, 1 wax-covered glass microscope slide, 4 pipettes, water (1 mL), vegetable oil (1 mL), canola oil (1 mL) , isopropyl alcohol (1 mL), 4 small plastic cups

Lesson Procedures

1. The teacher should prepare the wax covered glass slides the day before by taking a regular glass microscope slide and spray it with a wax spray.
2. First 5 to 10 min the teacher will introduce surface area. Relate the surface area to the past few days of what they have learned.
3. Show the video of the Teflon® coated slide to have them see what they are supposed to do for today's lab.
4. Hand out the lab and the materials needed for the students to start and finish the lab. The students can do the lab individually or in groups of 2 to 3.

Evaluation/Assessment

The lab is the assessment for the day. The students need to have the hypothesis, the observations and data, and the conclusion finished. The students can work on the vocabulary terms that they learned for the day.

Day 4: Lesson Overview

This day will be a continuation of Day 3 on surface area. The students will have to do quantitative data analysis on the surface area of a circle. The students will have 4 different surfaces that will have 3 different liquids placed on each surface. The students will measure the diameter of the circles that are made by the liquids on the surface.

Vocabulary

Surface Area, Area, Solids, Liquids

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces
2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.
2. Understand that different kinds of questions suggest different kinds of scientific investigations.
3. Select and use appropriate tools and techniques to gather, analyze and interpret data.
4. Incorporate mathematics in scientific inquiry.
5. Communicate and defend procedures and explanations.
6. Identify and explain the properties and changes of matter.
7. Describe and explain concepts related to mechanics, forces, and motion.
8. Understand the concept of energy and its various forms.

Materials

Vegetable oil (5 mL), water (5 mL), isopropyl alcohol (5 mL), 3 eye droppers, 1 paper towel, 1 Teflon® pan,1 sheet of wax paper, 1 plastic sheet, 1 compass, and/or 1 ruler.

Lesson Procedures

1. For the first few minutes discuss what they learned from Day 3 and how it pertains to real world applications.
2. Talk about the surface area of a circle. Introduce the equation and the radius and diameter of the circle. The surface area of a circle is $A=Pi(r^2)$. Pi or π is 3.141593—an empirically derived number.
3. Hand out the materials needed for the lab and let the students get started. The students can do the lab individually or in groups of 2 to 3.

Evaluation/Assessment

The lab is the assessment for the day. The students need to have the hypothesis, the observations and data, and the conclusion finished. The students can work on the vocabulary terms that they learned for the day.

Day 5: Lesson Overview

This lesson will be focused on surface tension. Surface tension is a property of the surface of a liquid that allows it to resist an external force. Liquids have a tendency to assume formations with the lowest surface area. When the kinetic energy is increased to increase the surface area is when one will get surface tension. In this lab, the students will determine which liquids have strong surface tension and which liquids will have weak surface tension. They explain emulsions with surface tension and can relate polarity to surface tension.

Vocabulary

Liquids, Surface Tension, Friction, Polarity, Emulsion, Emulsification, Kinetic Energy, Force

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces

2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.
2. Understand that different kinds of questions suggest different kinds of scientific investigations.
3. Select and use appropriate tools and techniques to gather, analyze and interpret data.
4. Incorporate mathematics in scientific inquiry.
5. Communicate and defend procedures and explanations.
6. Identify and explain the properties and changes of matter.
7. Describe and explain concepts related to mechanics, forces, and motion.
8. Understand the concept of energy and its various forms.

Materials

5 Regular paper clips, 1 large paper clips, water (15 mL), soapy water (15 mL), vegetable oil (15 mL), 1 eye dropper, isopropyl alcohol (15 mL), 5 clear plastic cups

Lesson Procedures

1. For the first 10 minutes, start to introduce surface tension. Here you can relate surface tension to surface area, to emulsion, and polarity.
2. Hand out the materials needed for the lab and let the students get started. The students can do the lab individually or in groups of 2 to 3.

Evaluation/Assessment

The lab is the assessment for the da. The students need to have the hypothesis, the observations and data, and the conclusion finished. The students can work on the vocabulary terms that they learned for the day.

References

American Chemical Society Lesson 5.2: Surface Tension. http://www.middleschoolchemistry.com/lessonplans/chapter5/lesson2 (Accessed April 5, 2012)

Day 6: Lesson Overview

The last day of this short unit and to wrap it up there is a round 1 and final round ready made up game of Jeopardy. This will test what the students have learned for the week. This week was to help the students to understand the basics of chemistry that relates to the forces and motion unit in their school.

Vocabulary

Atoms, Molecules, Fluorine, Carbon, Hydrogen, Motion, Brownian Motion, Emulsification, Emulsifier, Hydrophobicity, Surface Area, Area, Solids, Liquids, Surface Tension, Friction, Polarity, Hydrocarbon, Fluorocarbon, Kinetic Energy, Viscosity, Force

Length of Lesson

50 minutes

Iowa Core Statement

1. Understand and apply knowledge of motions and forces
2. Identify and generate questions that can be answered through scientific investigations.

Content Objectives

1. Design and conduct different kinds of scientific investigations.
2. Understand that different kinds of questions suggest different kinds of scientific investigations.
3. Select and use appropriate tools and techniques to gather, analyze and interpret data.
4. Incorporate mathematics in scientific inquiry.
5. Communicate and defend procedures and explanations.
6. Identify and explain the properties and changes of matter.
7. Describe and explain concepts related to mechanics, forces, and motion.
8. Understand the concept of energy and its various forms.

Materials

The Quiz Show Presentation, Post-Test.

Lesson Procedures

1. The students will get into three different groups to play Jeopardy.
2. All the students in each group will need to know the answer before raising their hands. Once the first 2 teams raise their hands, call on the first team, but call on the person who is not paying attention. Remember it is a team game. If the first team gets it wrong, call on the next team.
3. Play the game through the first round and the final jeopardy round. Play for 35 to 40 min.
4. The last 10 minutes will be for the post-test of the unit.

Evaluation/Assessment

Jeopardy will help to have students discuss what they have learned for the week. It will also help students to form questions that they still have from the week. The post test will help to understand what the students have learned over the week.

Pre-Assessment Test (next page)

Hydrophobicity of Fluorinated Compounds

From the list below, do you know the meaning of any of the following vocabulary terms? Pick 3 vocabulary terms below.

Hydrophobicity:

_____
_____

Fluorine:

_____
_____

Motion:

_____
_____

Atom:

_____
_____

Molecule:

_____
_____

Separation:

_____
_____

Phase:

_____
_____

Emulsification:

_____
_____

Can you explain what the phrase “like dissolves like” means?

_____
_____

_____
_____

Have you ever heard of fluorinated compounds before time in this class? Yes ___
No ___

If yes, please explain:

_____
_____
_____
_____

Have you ever heard of Teflon? Yes ___ No___

If yes, name one example of where Teflon is used.

_____
_____

Where in nature do you find things that repel (keep away) water? Why do you think such a property is useful in that context?

_____
_____
_____

Vocabulary Worksheet:

Vocabulary Terms

For each of the vocabulary terms below, please follow these steps:

1. **List the definition;**
2. **Put that formal definition in your own words;**
3. **Use the word in a sentence; and**
4. **Draw a neat color picture that helps you understand the term/word.**

Vocabulary List:

1. Fluorocarbon
2. Emulsification
3. Motion
4. Emulsifier
5. Hydrocarbon
6. Hydrophobicity
7. Force
8. Surface Area
9. Surface Tension
10. Atoms
11. Molecules
12. Friction
13. Viscosity
14. Kinetic Energy
15. Polarity

Emulsification Worksheet:

Emulsification Worksheet

Background

Have you ever wondered how to describe in one word when two liquids are mixed together and form these bubbles that take seemingly forever to disappear? This phenomenon is an example of *emulsification*. The two liquids are not compatible with one another; therefore, when mixed, they will form spheres that are really repelling the other liquid and vice versa (Latin for "the other way around"). This can be related to the phrase "like dissolves like" because the two liquids do not have the same type of molecules and therefore want nothing to do with each other. Emulsifiers help the two liquids to exist together by increasing the kinetic energy in the system. Emulsifiers are incredibly useful to make some foods such as vinaigrette salad dressings into stable treats!

Hypothesis

What will happen when you add an oil to water? Will the oil sink, float, or mix with the water? Can you explain why the oil will do what you predict? State your hypothesis—your proposed explanation for this observable phenomenon—below.

_____
_____
_____
_____

Safety

After touching the eggs, **DO NOT** touch your face or mouth. Wash your hands immediately after this experiment.

Materials

Centrifuge tubes (50 mL), vegetable oil (15 mL), vinegar (15 mL), 1 egg yolk, and 1 beaker.

Procedure to Test Hypothesis

1. Pour vinegar (15 mL) into the 50 mL centrifuge tube.
2. Gently pour vegetable oil (15 mL) into the vinegar in the centrifuge tube.
3. Tightly cap the centrifuge tube.
4. Draw what you see (**Do Not Shake**).

5. Once you have drawn what you see, vigorously shake the centrifuge tube for 30 seconds.
6. Now set the centrifuge tube in the beaker and draw what you see. Can you explain what is happening?
7. Let it sit for 2 minutes and again observe and describe what is happening.
8. Now take the egg yolk and place it in the centrifuge tube. What happens when you add the egg yolk? Please draw a picture.
9. Tightly cap the centrifuge tube and shake it for 30 seconds. Draw what you see happening.

Data Collection

On a separate piece of paper please draw each step of your experiment; *be specific in the details.* (Careful observation is an important part of science and enjoying the world around you!)

Conclusion

What did you observe in this lab experiment? What did you learn about emulsification and emulsifiers? What real world applications for egg yolks could you imagine based on your observations?

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Surface Area Worksheet:

Qualitative Analysis of Surface Area with Use of Microarray Slides

Background

A microarray slide is a microscope slide (usually just a specially cut piece of glass) that is covered with specific materials for the binding of the same type of materials. Scientists will use microarray slides for the binding of very small amounts of biological or synthetic materials to find partners for these materials. Finding a good partner that binds to a certain protein, for example, is the first step in designing a very helpful drug for a disease involving that protein. One example of using the surface of a slide to bind to Teflon® coated slide (slide covered with fluorocarbons) is used to bind with the fluorocarbons that were attached to a sugar. Then the slide is put in a solution with a protein and then once the slide is washed, it will be taken to be scanned to tell whether the protein will bind to that sugar. First, we need to know what liquids we can use on the surface of a slide to make the fluorous-fluorous interaction. We do not want the liquid to start to run all over the place, we need a nice clean circular spot. Here we will test different slides with different liquids to see what liquid would work best with each slide.

Hypothesis

What do you think will happen with each of the following liquids (water, vegetable oil, isopropyl alcohol, canola oil) when you spot these liquids on the 2 different slides: one that is regular glass and the other that is waxy?

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Materials

1 Regular glass microscope slide, 1 glass slide cover with a wax, 4 pipettes, water (1 mL), vegetable oil (1 mL), canola oil (1 mL) , isopropyl alcohol (1 mL), 4 small plastic cups

Procedure

1. Take the pipette and dip its tip into the water. Then gently place a spot of water on each of the two slides.
2. Record in pictures and words what you see on the slide. (**Be Very Detailed!!**)
3. Take the pipette and dip its tip into the next liquid and place a spot of each liquid on each of the slides. Do this for each liquid. Make sure to record what you observe after each spot.

4. Observe a video of spotting of a third slide that is Teflon® coated. (One Teflon®-coated slide will be there for you to observe directly, too.)
5. Once all the liquids are on each slide, start to compare how each liquid behaved on each slide. Make sure to write your observations in your qualitative data section.

Qualitative Data

On a separate piece of paper, draw each slide with the different spots and properly label each drawing. BE SPECIFIC!

Conclusions

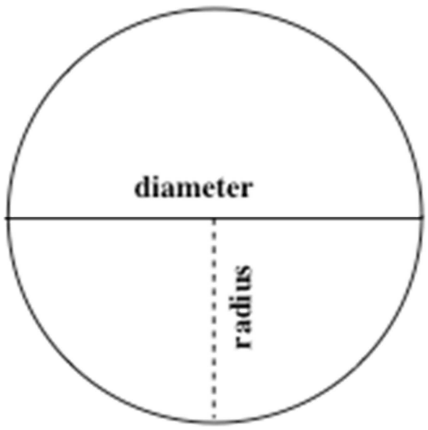
What did you observe? Does your observed data match your original hypothesis? Were any of the results surprising? If not, how would you explain your observations now? What questions do you still have?

Surface Area of a Circle Worksheet:

Surface Area of a Circle Worksheet

Background

Surface area is the measure of how much exposed area an object has in 2 dimensions and is expressed in square units. In this experiment you will be calculating the area of a circle. The circle will be the drop of liquid that will be placed on different surfaces. This experiment can serve as a method to measure relative surface tensions of liquids with surfaces by calculation of how large the circular area of coverage is. The surface area of a circle is $A=Pi(r^2)$. Pi or π is approximately 3.141593 and is defined as the number that will relate the area to the radius of *any* circle. The r is the radius of the circle. The drawing below identifies the radius and the diameter of a circle. By definition, the diameter is equal to two times the radius.



Hypothesis

What do you predict will happen with each surface (a paper towel, Teflon® pan, wax paper, plastic sheet) when you add a drop of water? Or a drop of vegetable oil? Or a drop of isopropyl (rubbing) alcohol?

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Materials

Vegetable oil (5 mL), water (5 mL), isopropyl alcohol (5 mL), 3 eye droppers, 1 paper towel, 1 Teflon® pan,1 sheet of wax paper, 1 plastic sheet, 1 compass, and/or 1 ruler.

Procedure

1. In groups, first take the paper towel and place a drop of water onto the paper towel.
2. Wait a minute and write down what you observe.
3. Now take the compass or the ruler and measure the diameter in centimeters (cm), with the diameter you should be able to calculate the radius and with the radius you can then calculate the area of the circle.
4. Now repeat the first 3 steps for the remaining 2 liquids (isopropyl alcohol and vegetable oil) for the paper towel.
5. Repeat the first 4 steps for the remaining 3 surfaces (Teflon® pan, wax paper, and plastic sheet.

Observations and Data

Please use a separate piece of paper and record (write and draw) what you observed. *Please be specific.* Write out all of your calculations.

Conclusions

What did you observe? What differences and what similarities did you see? Relate this to real world applications.

Surface Tension Worksheet:

Surface Tension Worksheet

Background

Surface tension is a property of the surface of a liquid that allows it to resist an external force. Liquids have a tendency to assume formations with the lowest surface area. An example of surface tension would be a drop of water that makes a spherical shape or beads up when on a waxy surface. Energy has to be supplied to increase the surface area of a liquid and this is known as surface tension. Remember that there are many water molecules that make up the liquid and the surface is like our skin. The molecules on the surface are constantly being pulled towards the molecules that are in the deeper layers. This force creates the surface tension. In this experiment you will figure out which liquids have strong surface tension and which have weak surface tensions. The strong surface tensions will allow the paper clip to float, whereas liquids with weak surface tension will allow the paper clip to sink.

Hypotheses

What do you think will happen if you drop a paper clip into the solutions of pure water, soapy water, vegetable oil, or isopropyl (rubbing) alcohol? What do you think will happen if you lightly place the paper clip on top of the surface of each of the liquids? You previously saw what would happen if you had water and oil mixed together, but what do you think will happen if you add a drop of oil to soapy water and why? **Please be detailed.**

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Materials

5 Regular paper clips, 1 large paper clips, water (15 mL), soapy water (15 mL), vegetable oil (15 mL), 1 eye dropper, isopropyl alcohol (15 mL), 5 clear plastic cups

Procedures

1. Each group will have to take the large paper clip and straighten it all the way out. Then bend the large paper clip into a “U” shape and bend the bottom of each end

out a little bit as shown below. You will use this shape like tweezers to place the smaller paper clip on the surface of the water.

2. Each group will have four cups each with a different liquid ¾ full. (Water, vegetable oil, soapy water and isopropyl alcohol are the four liquids to test.)
3. Each group will take a regular paper clip and drop it into a liquid. Do this for all of the liquids and record observations for each event by a picture or a chart.
4. Use the U-shaped paper clip from step 1 to pick up a paper clip. Do this by squeezing the ends of the device together a bit and placing them inside the paper clip. Then carefully lower the paper clip so that it is lying flat on the surface of the liquid. Gently and slowly squeeze the U shape paper clip to release the paper clip.
5. Do step 4 for all of the liquids and record observations for each event by a picture or a chart.
6. In a clean cup, pour a small amount of the soapy water. Then take a dropper and fill it with the vegetable oil.
7. Take the dropper and add a couple of drops of oil to the soapy water and write down your observations.

Data Collection

Please draw a picture on a piece of paper or make a chart of what you observe. **Please be specific in the details.**

Conclusion

Explain what you observed. Were your original hypotheses correct based on your experiments? Based on discussions of the last week, why does water have more surface tension then the rest of the liquids? Why did the vegetable oil and soapy water mix this time when the water and vegetable oil did not in the emulsification experiment? How does emulsification relate to surface tension? What are some real world applications of surface tension**?**

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Post Assessment Test:

Hydrophobicity of Fluorinated Compounds

From the list below, describe all the vocabulary terms listed:

Hydrophobicity:

_____

_____

Fluorine:

_____

_____

Motion:

_____

_____

Atom:

_____

_____

Molecule:

_____

_____

Separation:

_____

_____

Phase:

_____

_____

Emulsification:

_____

_____

Can you explain what the phrase “like dissolves like” means?

_____

_____

_____
_____

Have you ever heard of fluorinated compounds? Yes ___ No ___

If yes, please explain what fluorinated compounds are and where you can find them?

_____
_____
_____

Have you ever heard of Teflon? Yes ___ No___

If yes, name one example of where Teflon is used.

_____
_____

Where in nature do you find things that repel (keep away) water? Why do you think such a property is useful in that context?

_____
_____
_____

Suggested Speech for the Class Videos:

Qualitative Surface Area Experiment with Fluorous Microarray Slide

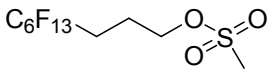
Today we will be doing a qualitative analysis lab on surface area on a microarray slide. Here is a slide that is covered with fluorocarbons (Teflon®). The first thing that we will do is we will take the oil and stick our pipette into the oil. Then we are going to put our thumb on the end of the pipette to get some of the oil into the pipette. Then we will put a droplet of oil onto the microarray slide. Next, we will do the exact same thing for propanol. Then we will do this for water. What I want you to do is look at the microarray slide carefully and I want you to see the difference of the spots/droplets. From the side you can see that the alcohol, when tilted will slide side to side. The other two spots will stay at their designated spot hardly moving. Also, you can see how the spots/droplets are: how the oil actually stays closer to the surface, while the water droplet is higher. Today you will do the same with your microarray slides. You will have a plain glass microarray slide and a wax covered microarray slide. Then you will compare it to this slide on the video.

Surface Tension Experiment with Fluorous Liquid

Today we are going to do a surface tension lab using paperclips and different liquids. In the video, the scientist is going to take methoxyperfluorobutane (fluorocarbon) and pour some of it into the beaker. The person is then going to try to take the paper clip and gently try to set it on the top of the surface of the liquid. What happened? Why did the paper clip just drop to the bottom of the liquid? The person is going to try it again. Remember the person wants to get the paper clip flat on top of the surface. After

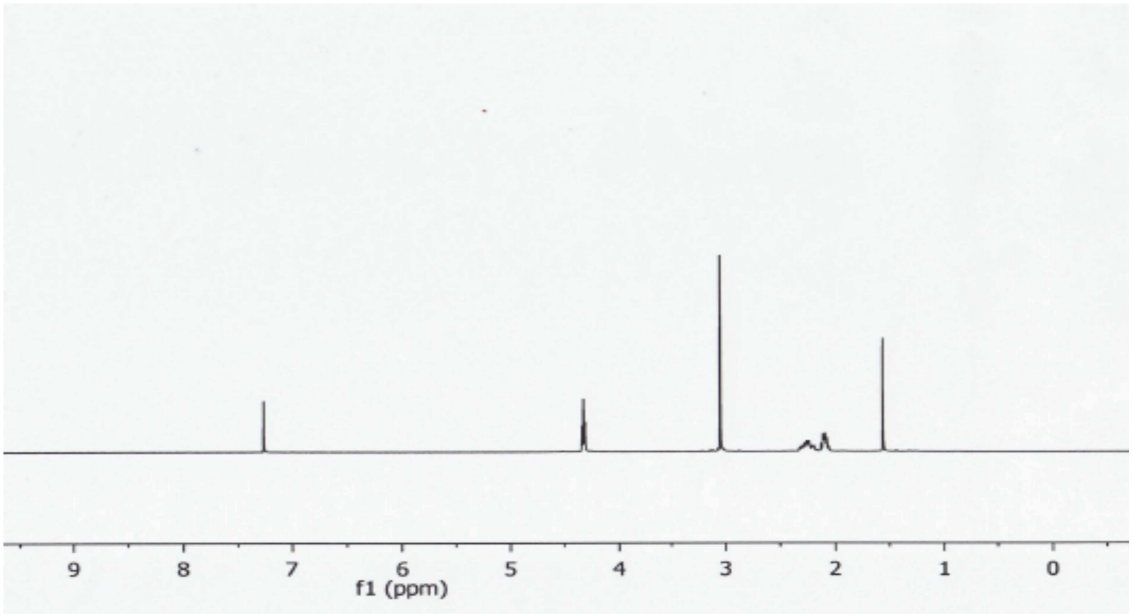
additional attempts, was the person able to get the paper clip to stay on the surface of the liquid? What do you think is occurring? Today you will test the surface tension of the water with oil. You will need to observe what is happening with the surface area/surface tension and compare it to the surface area/surface tension in the fluorous system in this video.

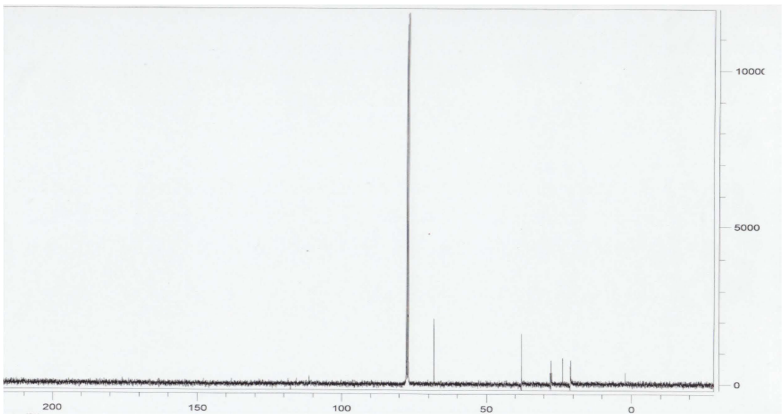
APPENDIX B. CHAPTER 3 ^{1}H AND ^{13}C NMR SPECTRA

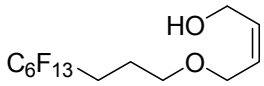


4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl methanesulfonate (4)

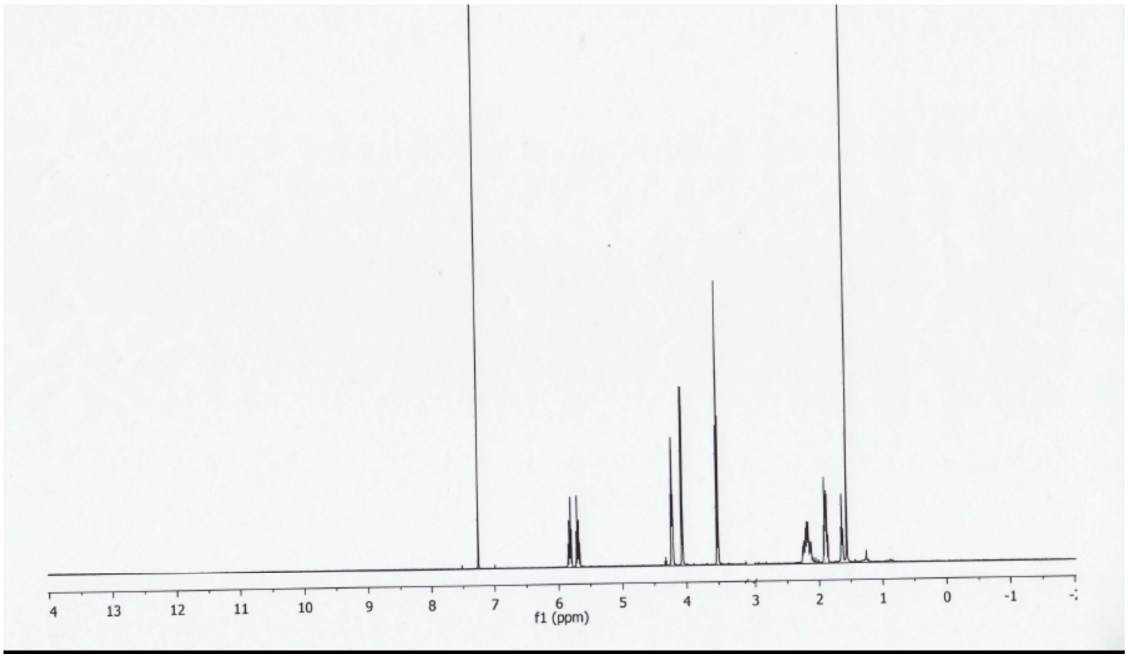
$CDCl_3$, 400MHz

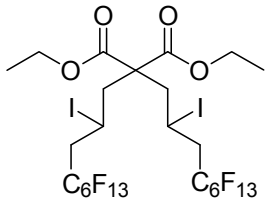




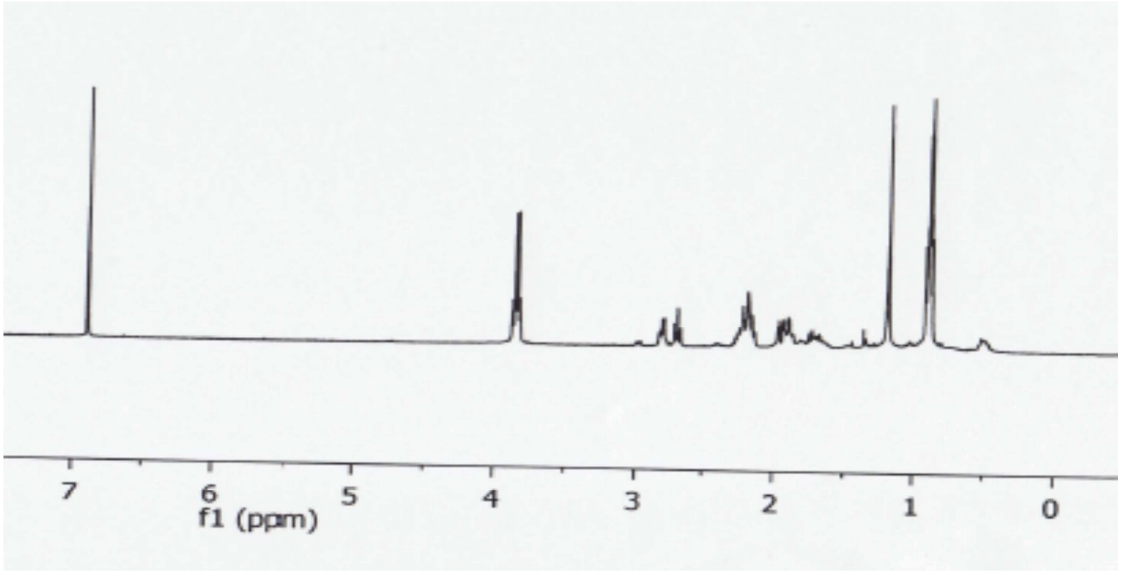


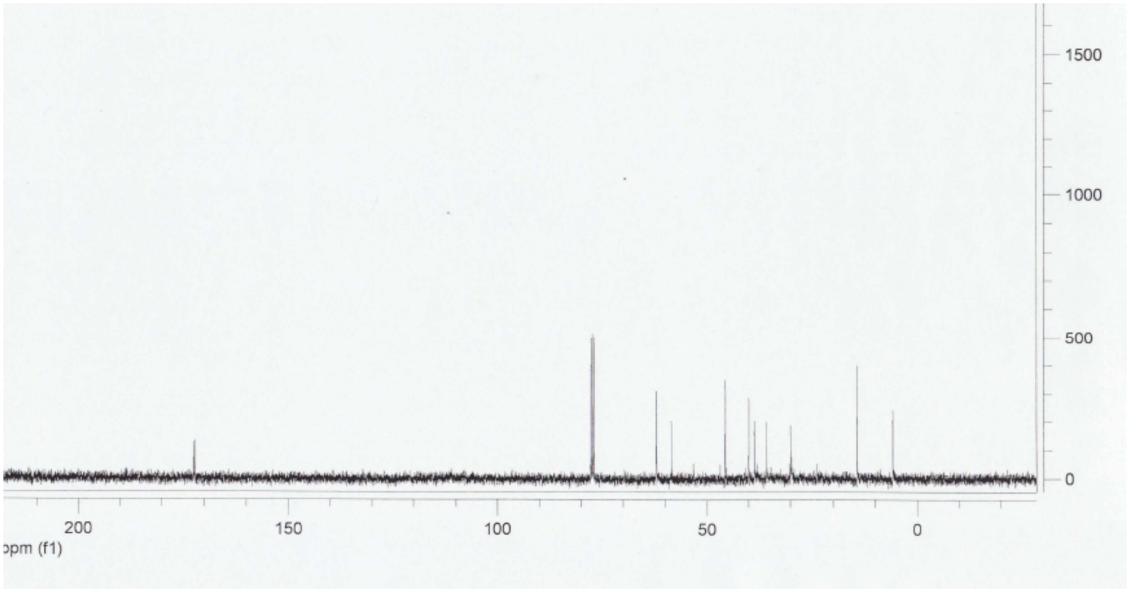
(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)but-2-en-1-ol (5)

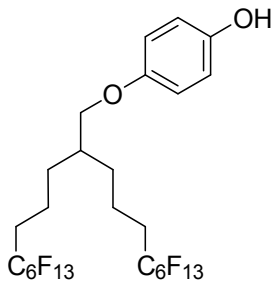




Diethyl 2,2-bis(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononyl)malonate (7)







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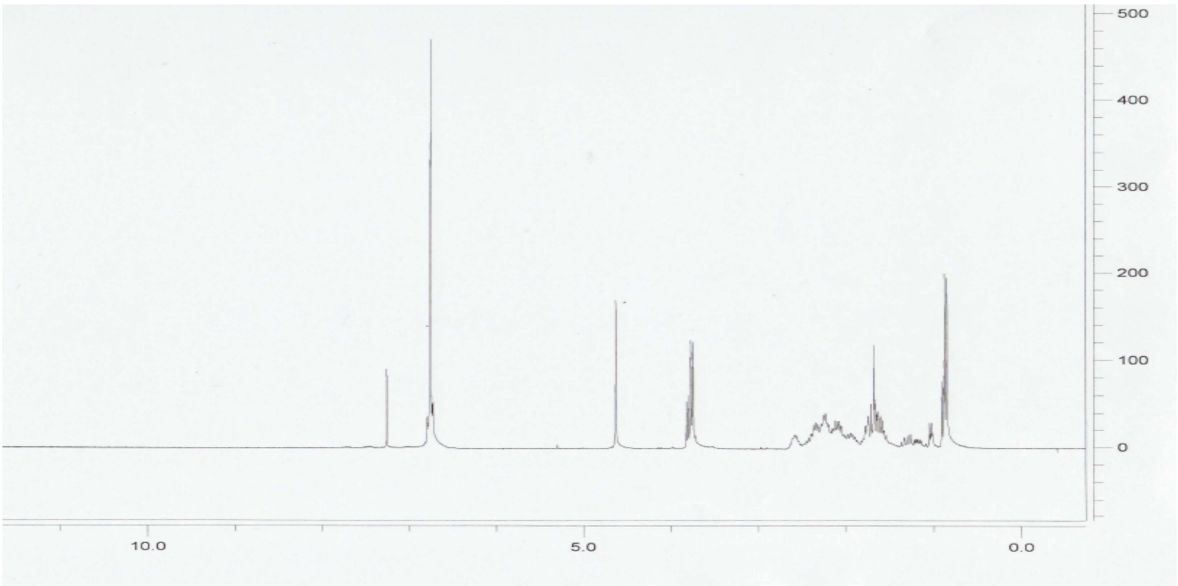

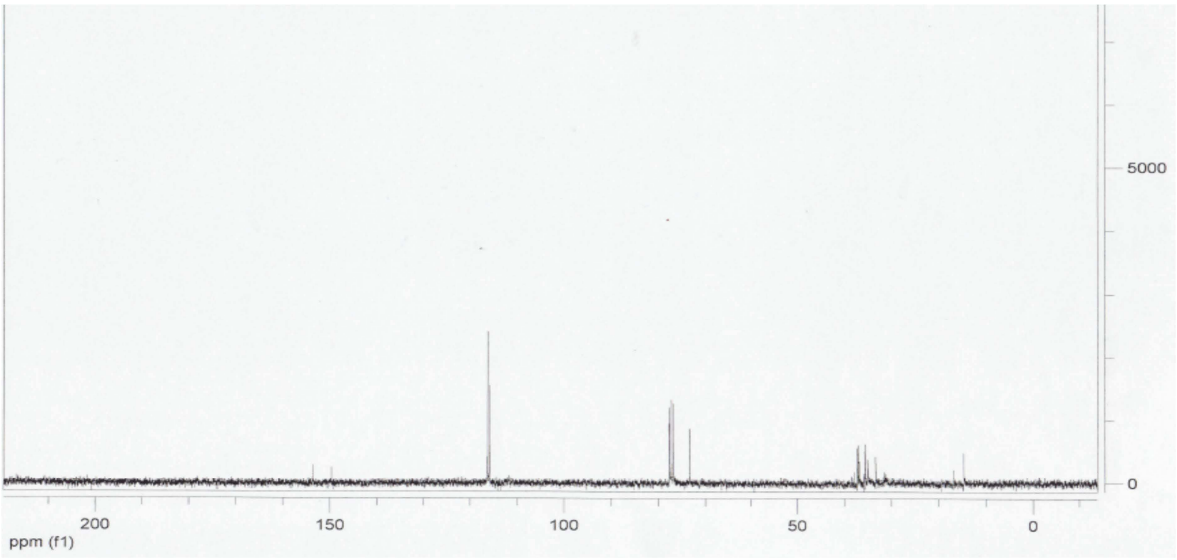

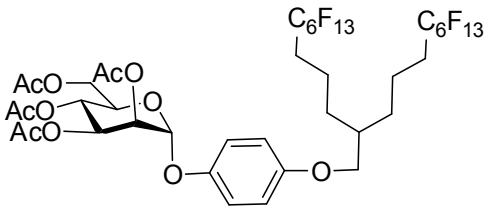

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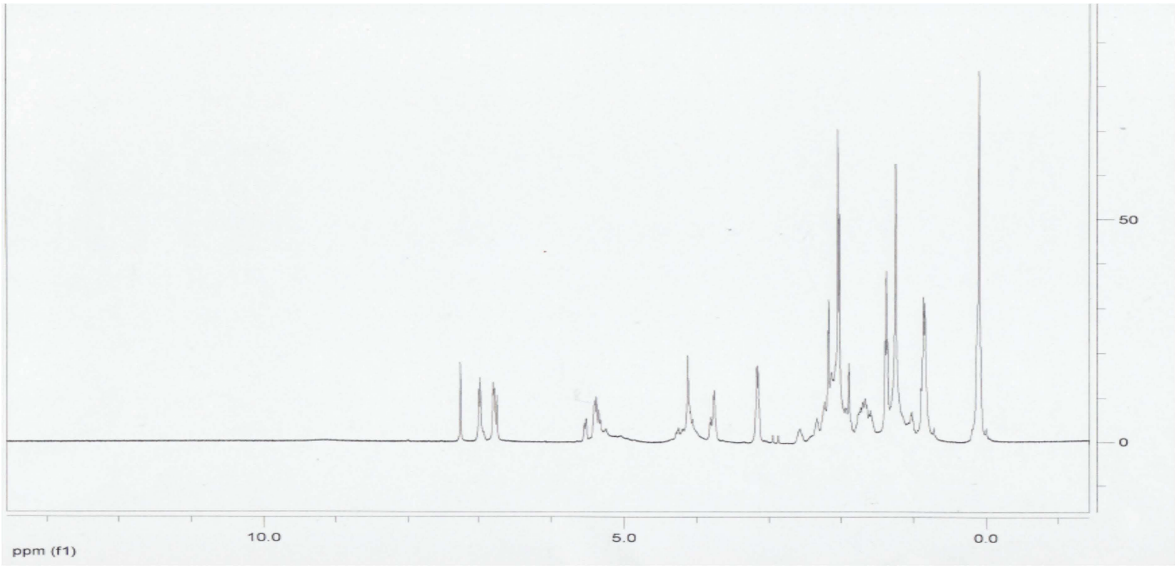

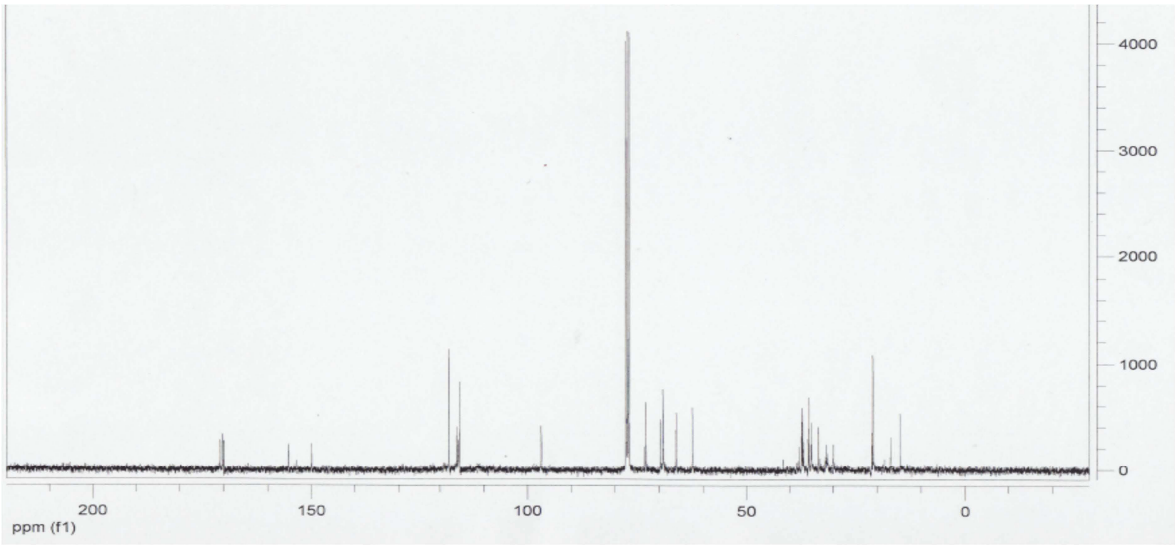

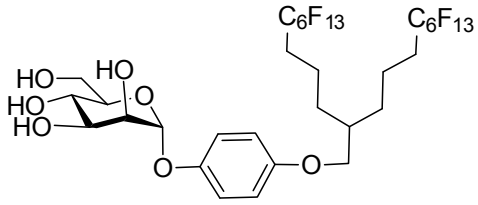

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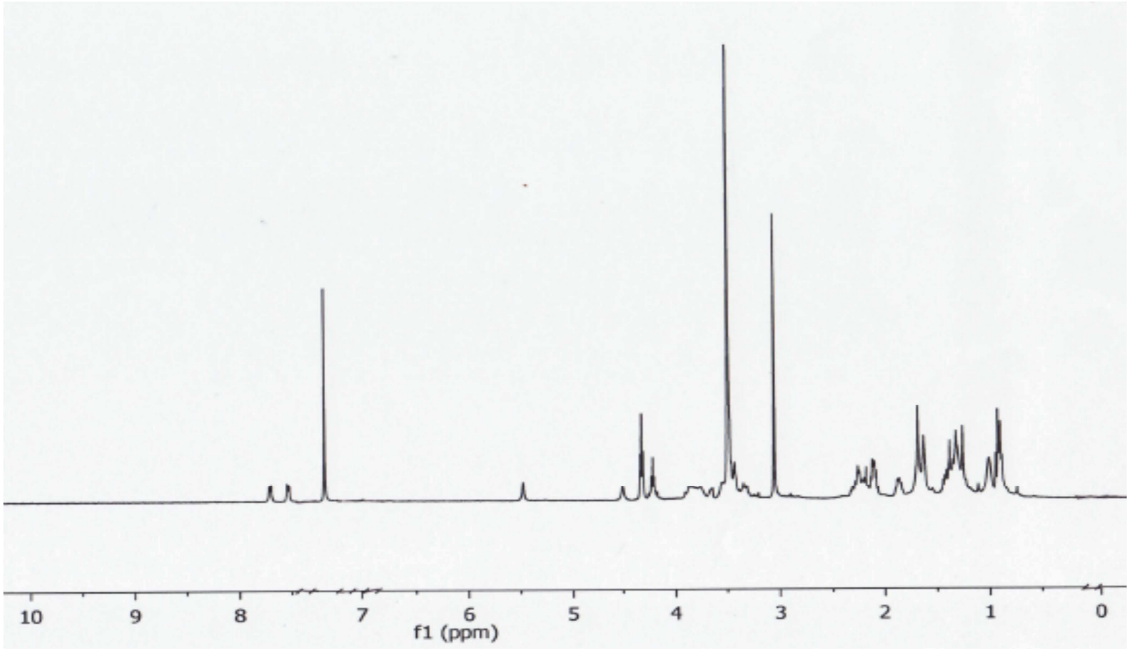
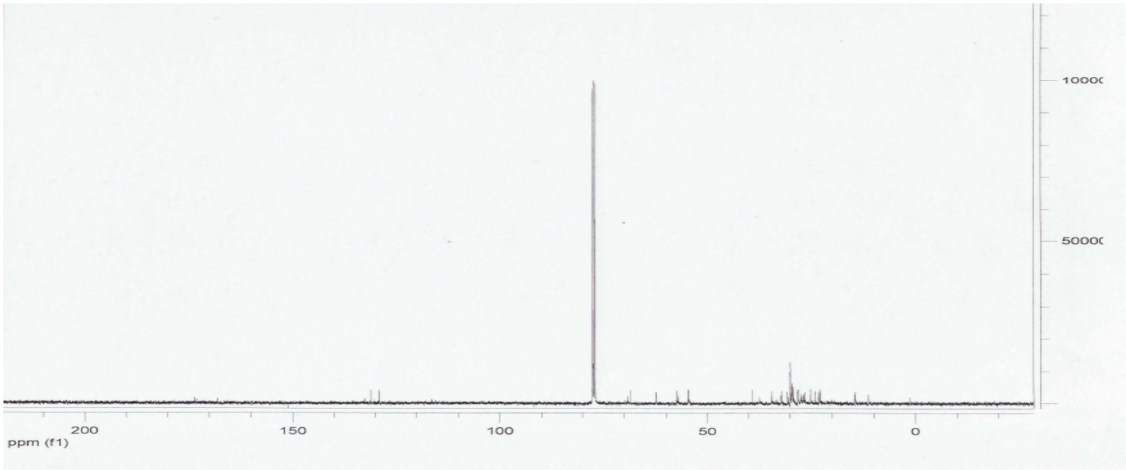
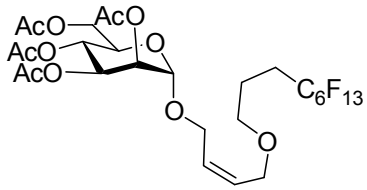
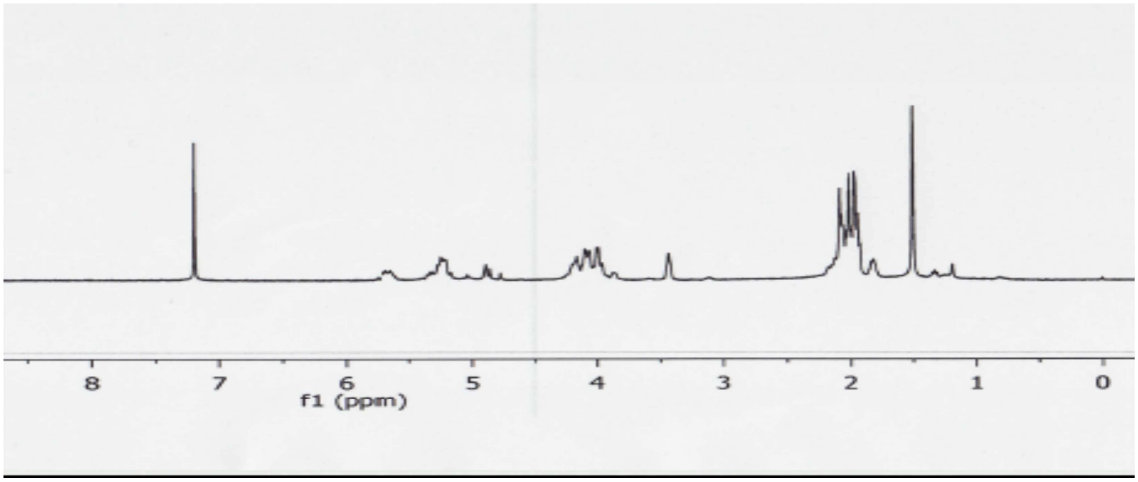
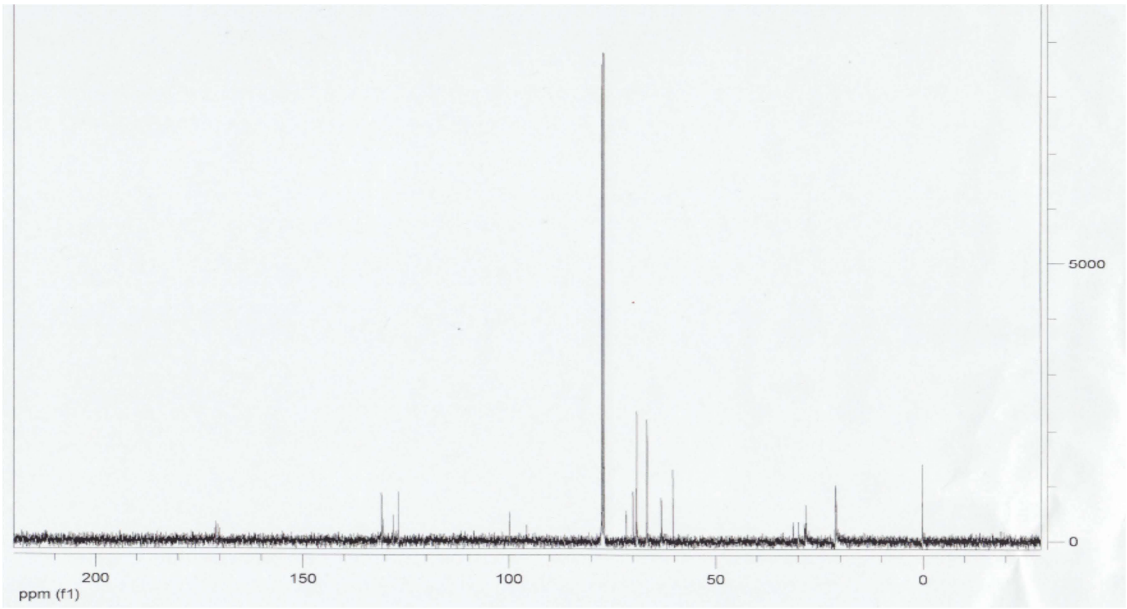


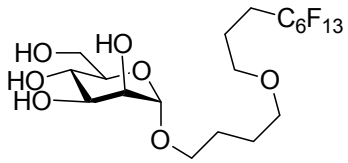




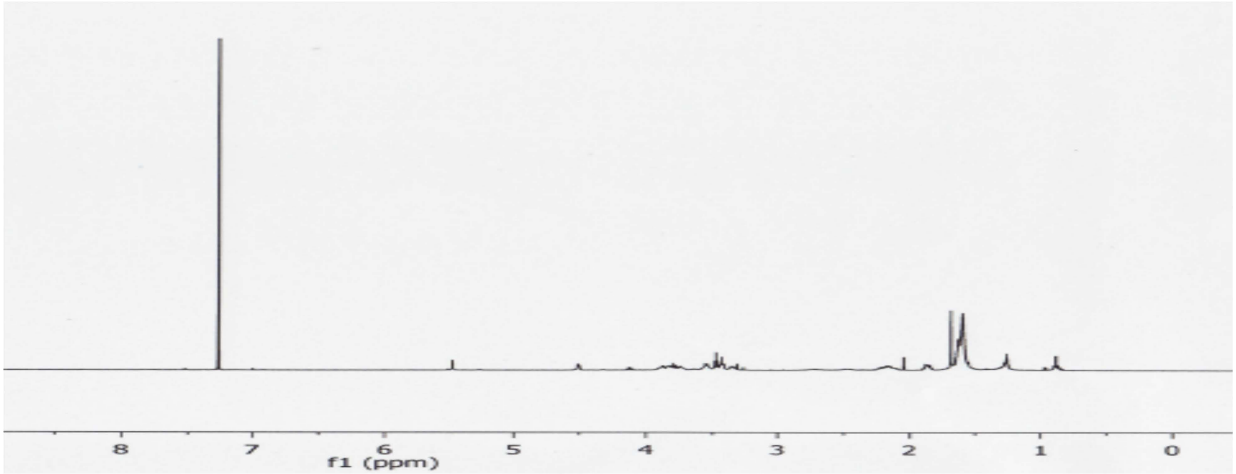
3-(perfluorohexyl)propanyloxybutenyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (15)

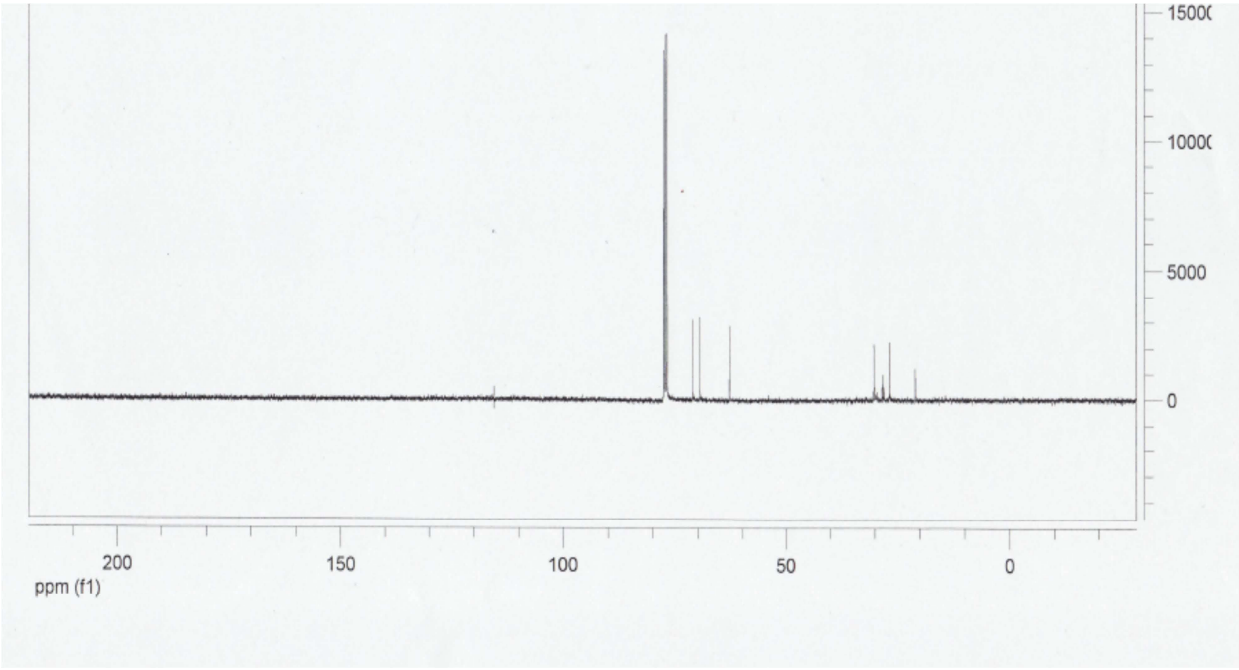


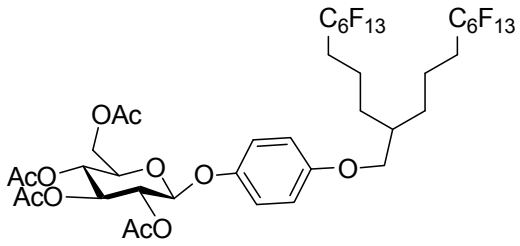




3-(perfluorohexyl)propanyloxybutanyl-α-D-mannopyranoside (16)







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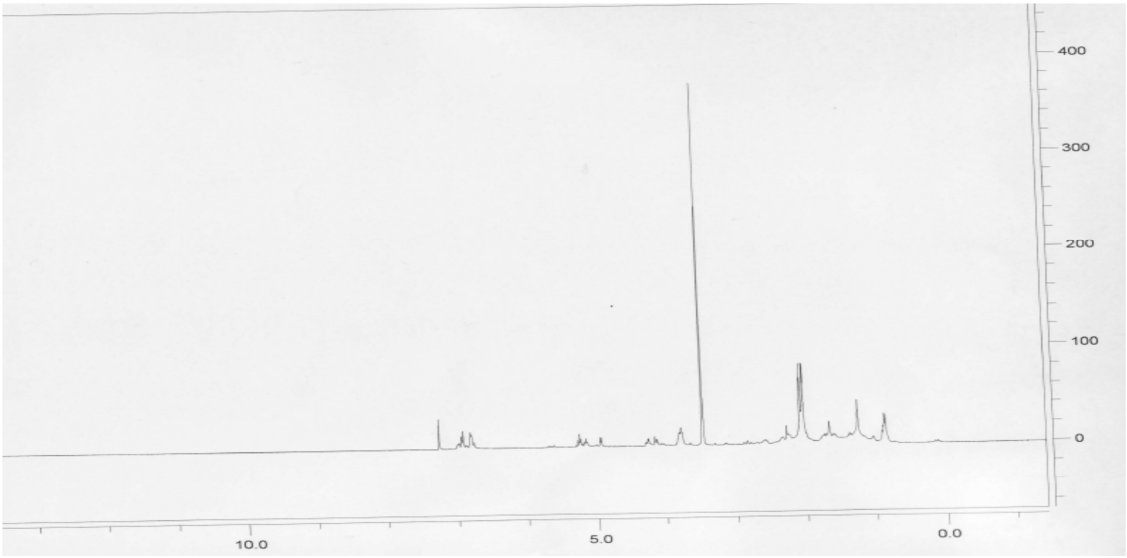

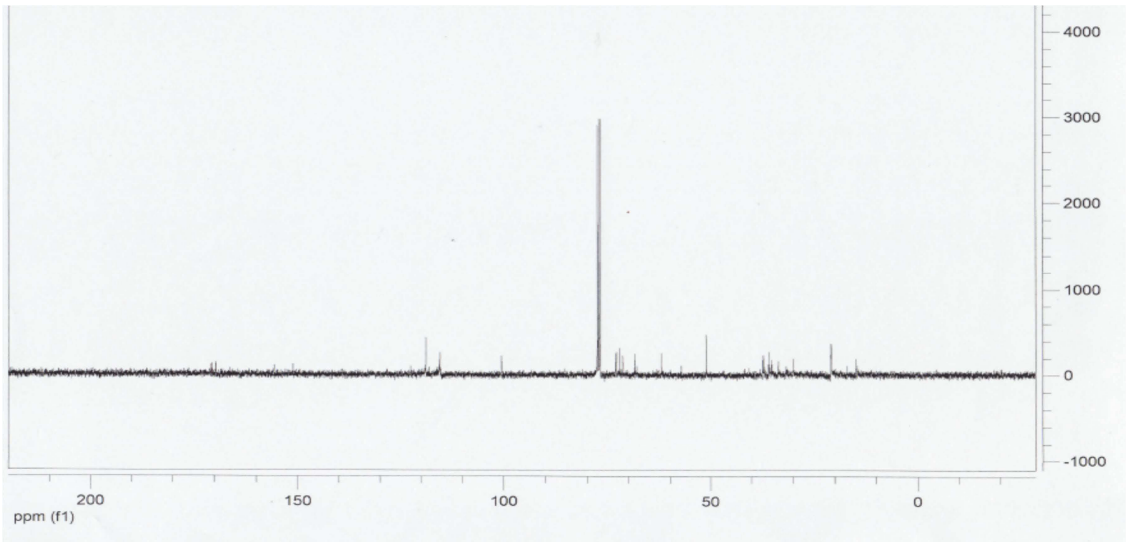

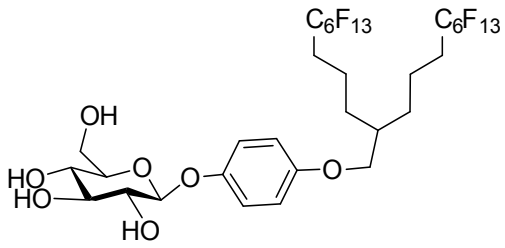

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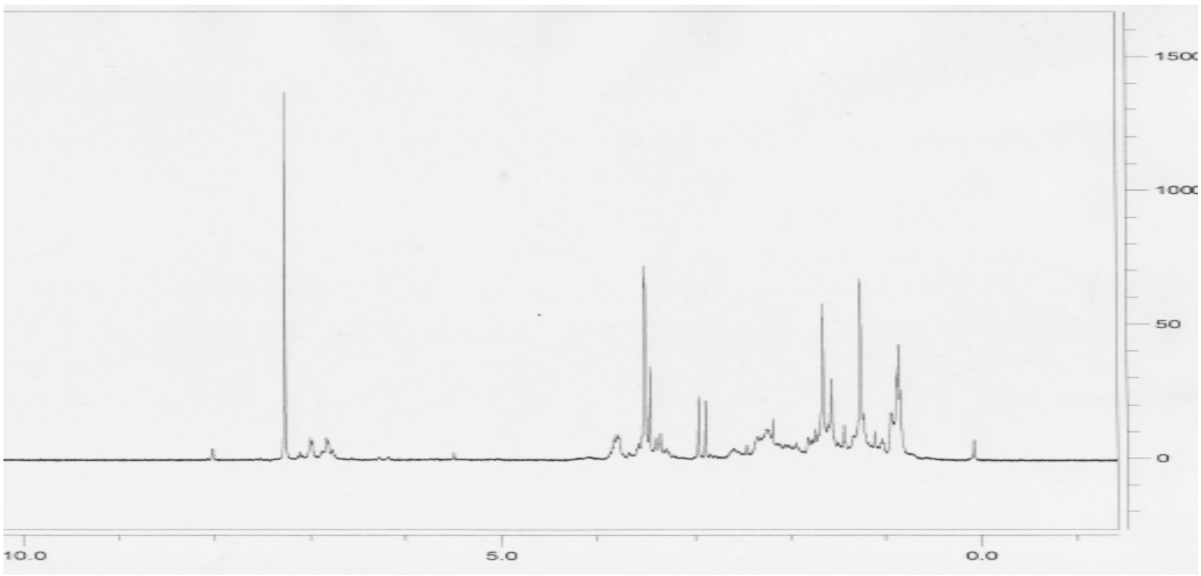

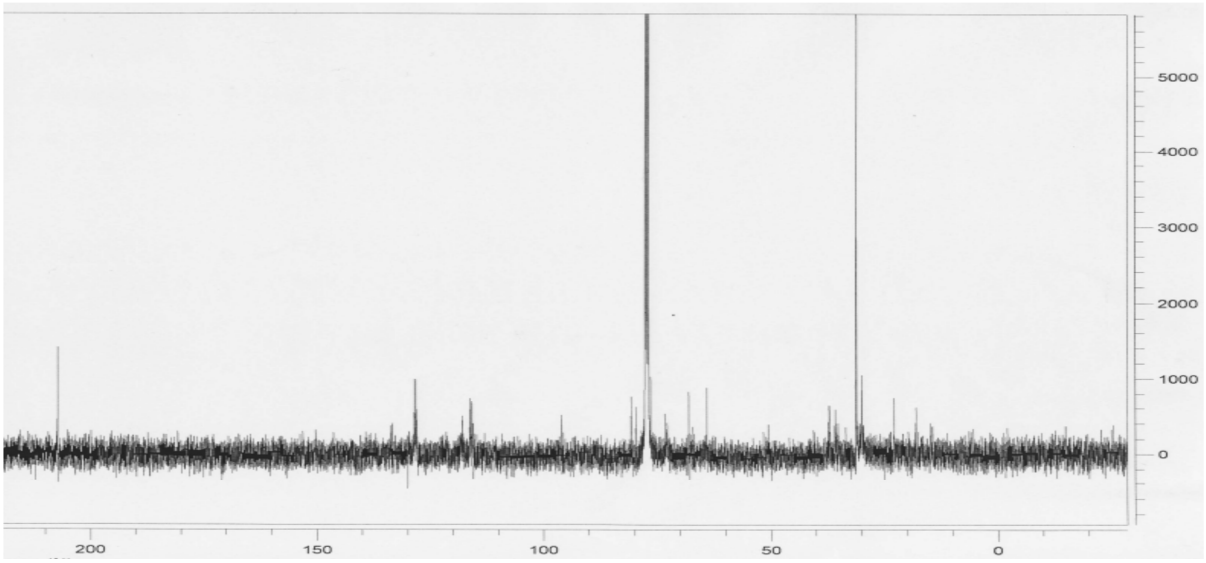

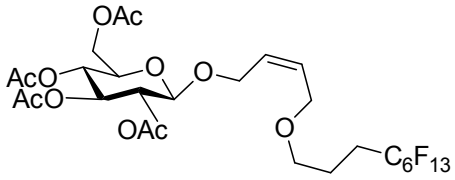

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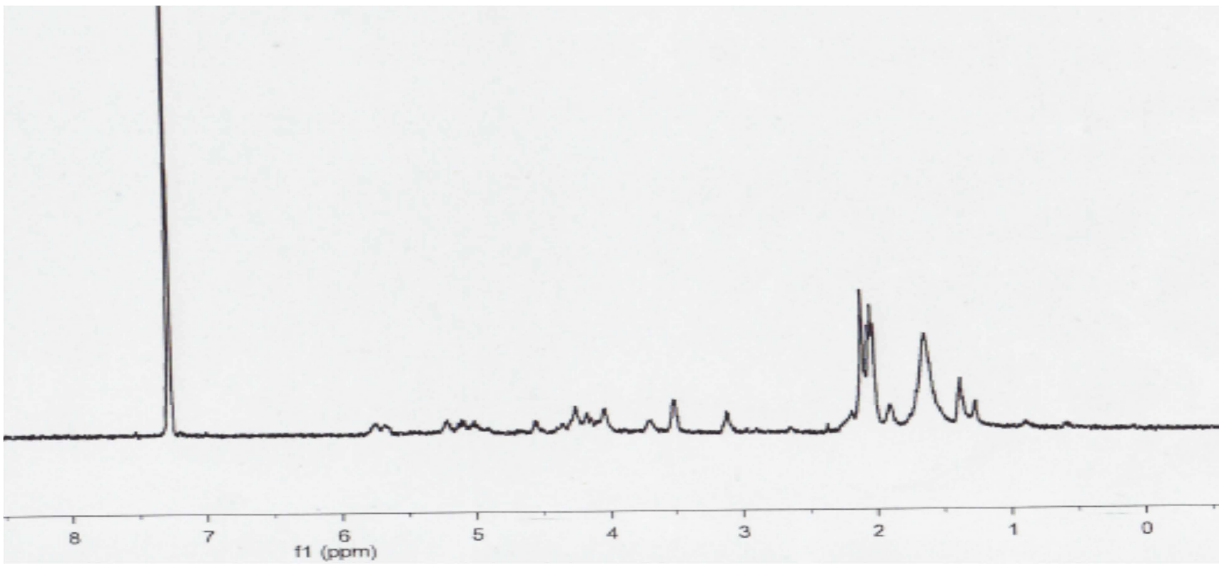

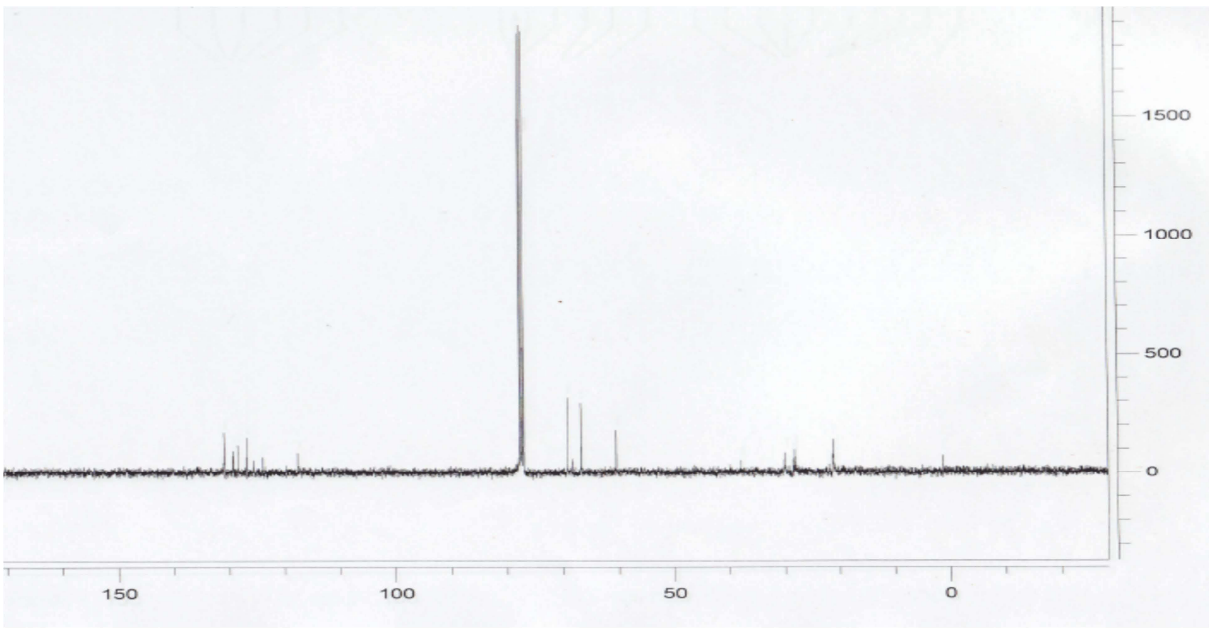

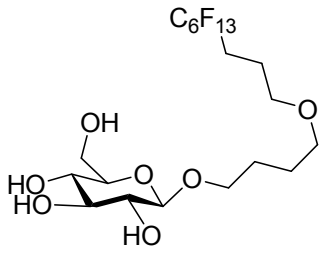

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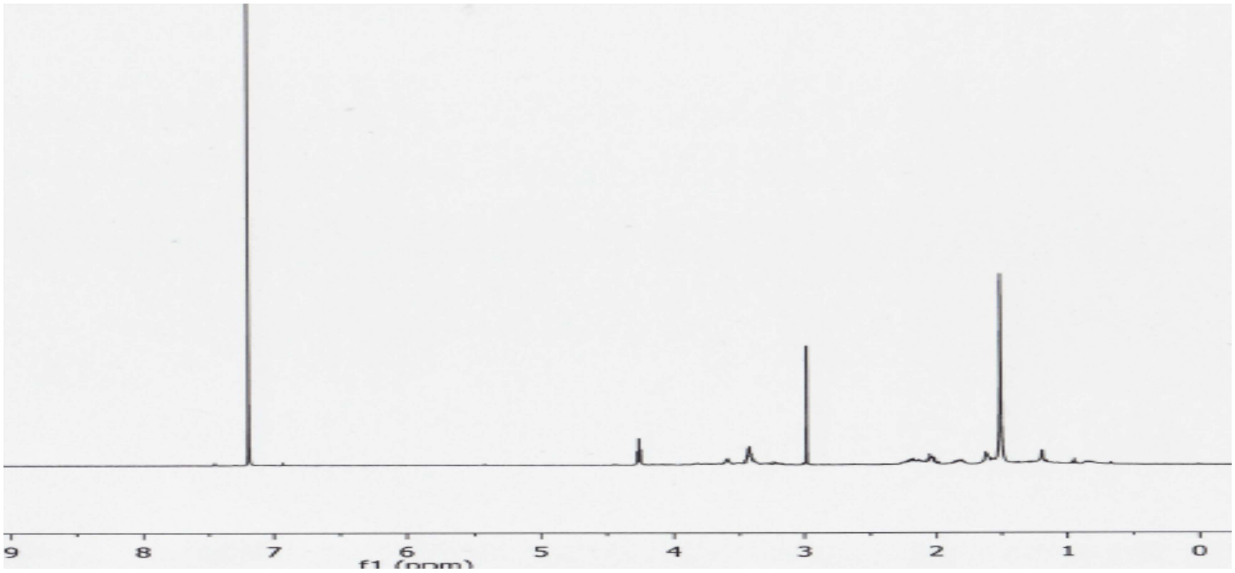
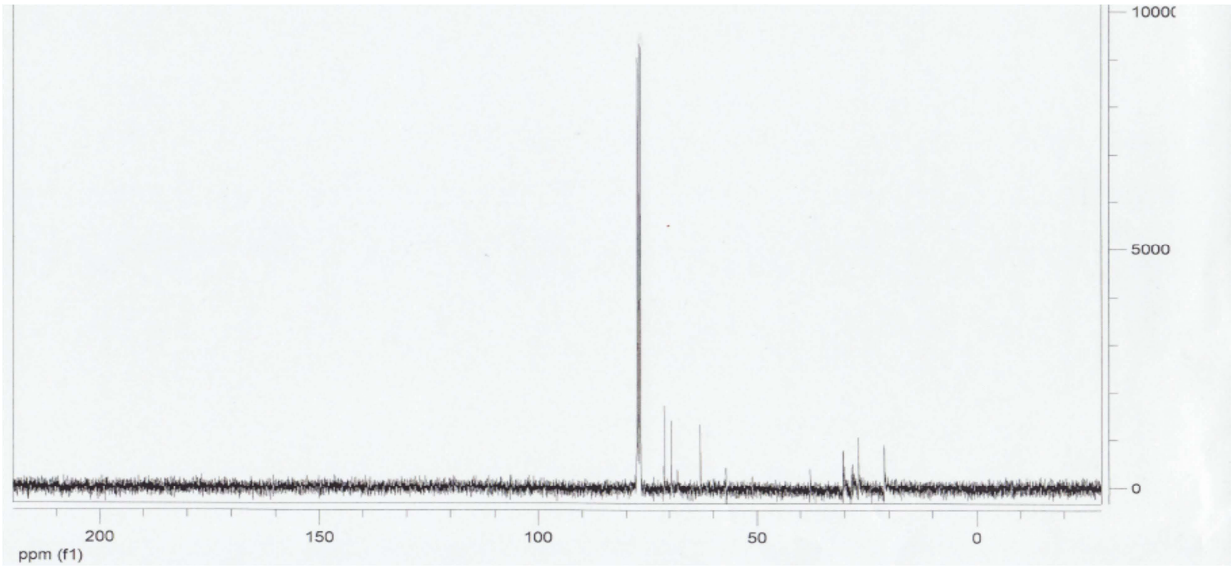
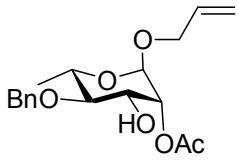




APPENDIX C. CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA



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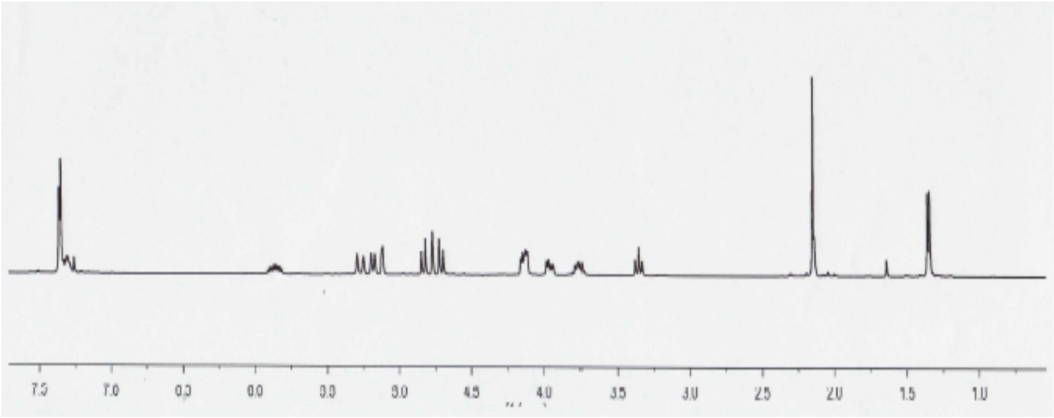

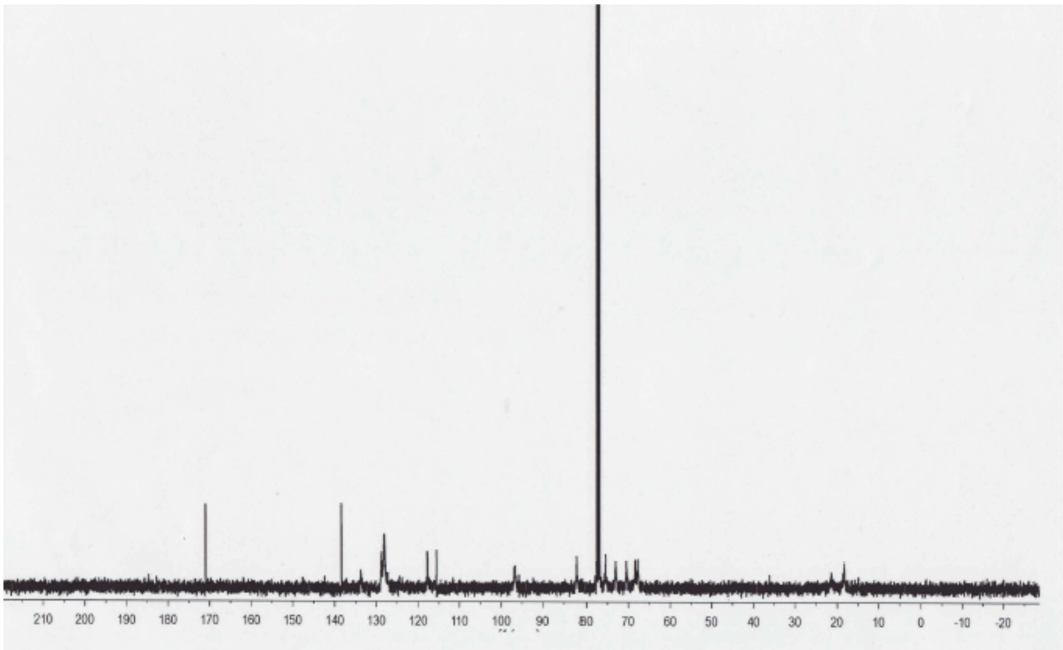

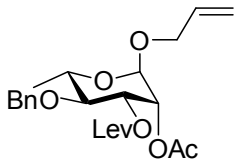

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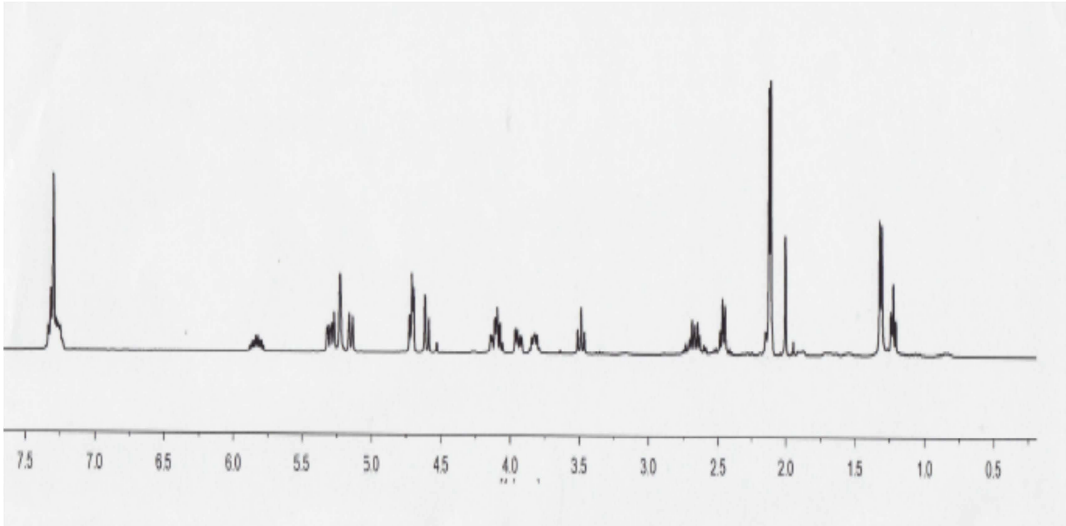

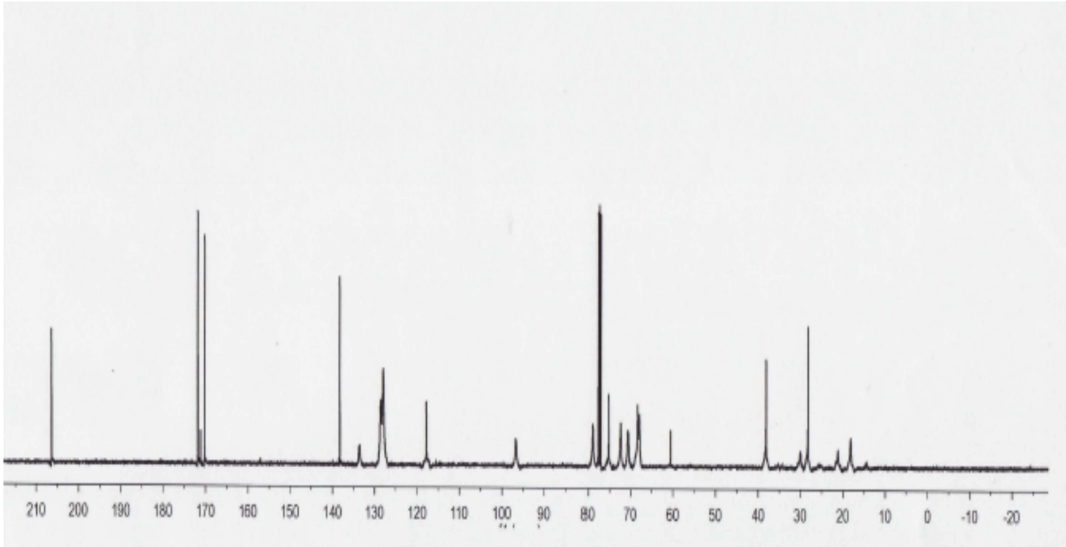

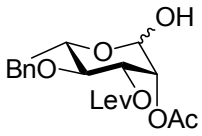

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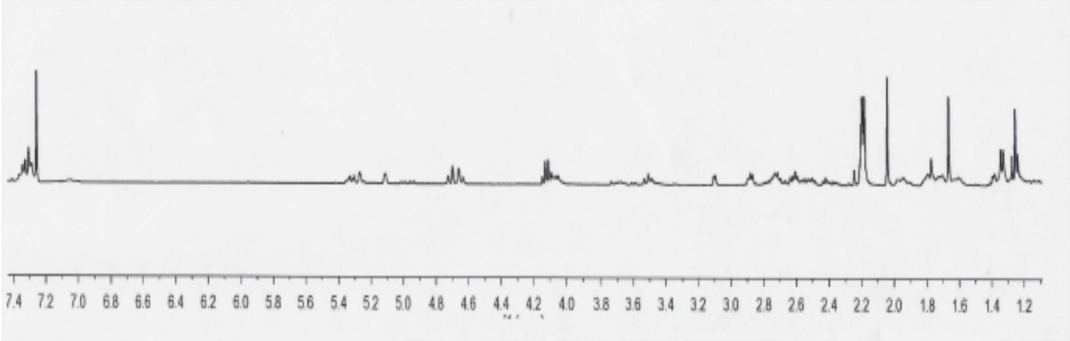

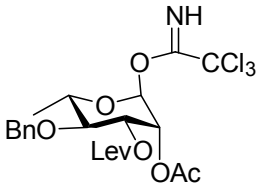

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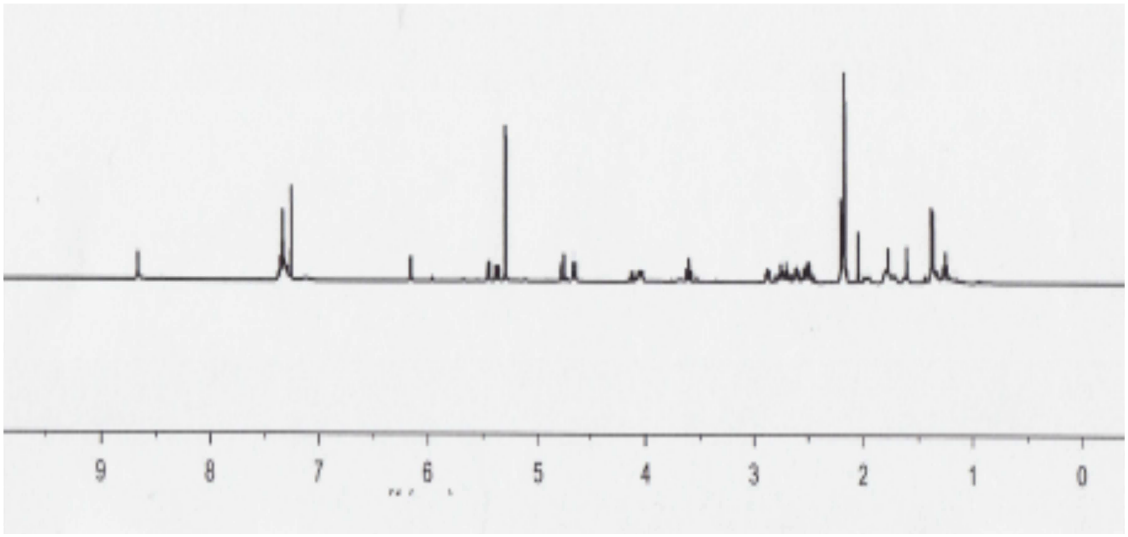

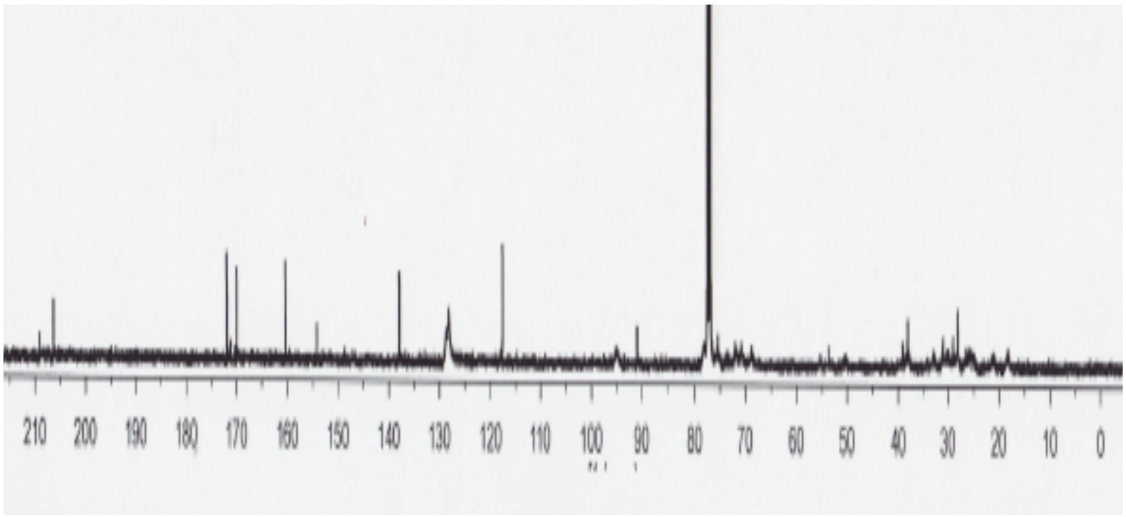

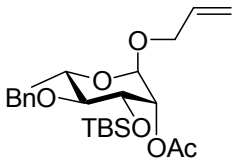

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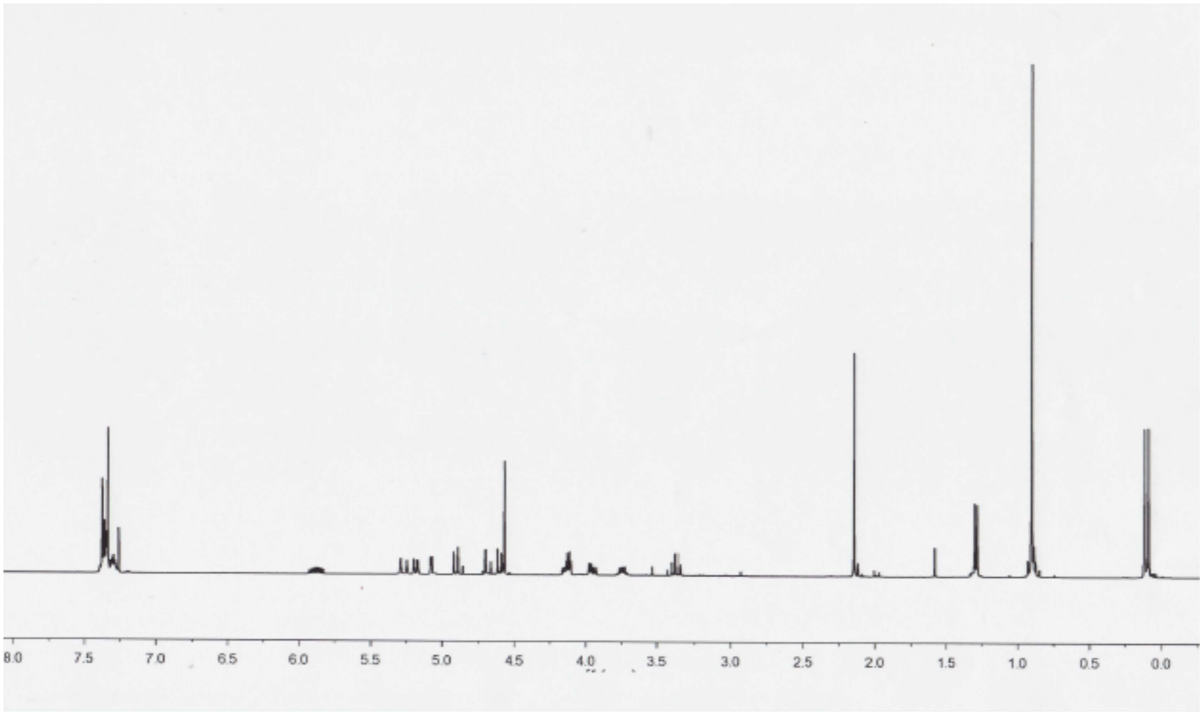

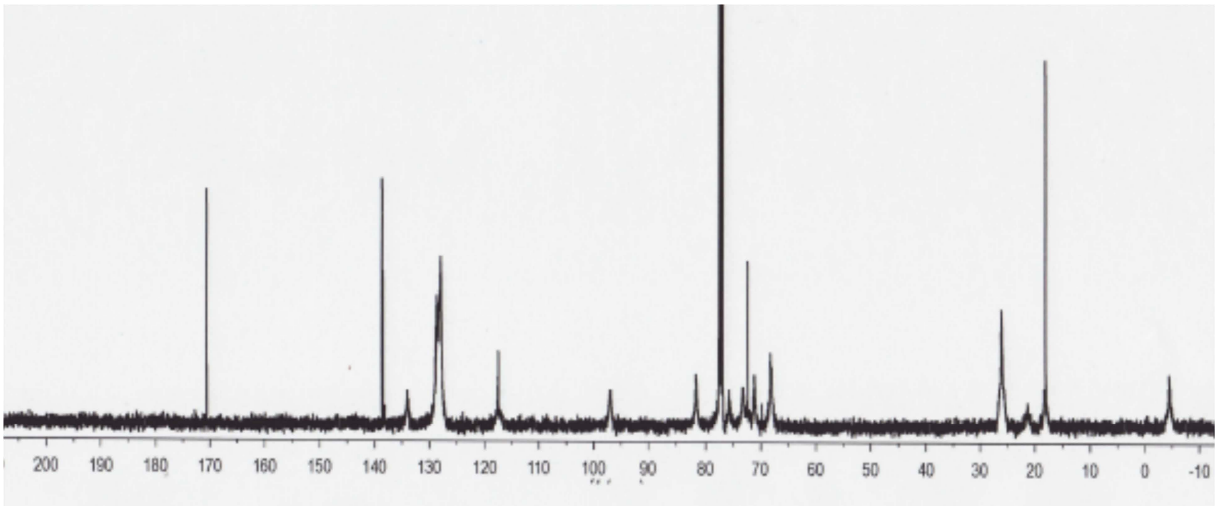

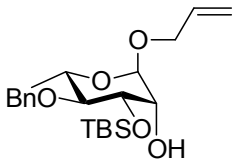

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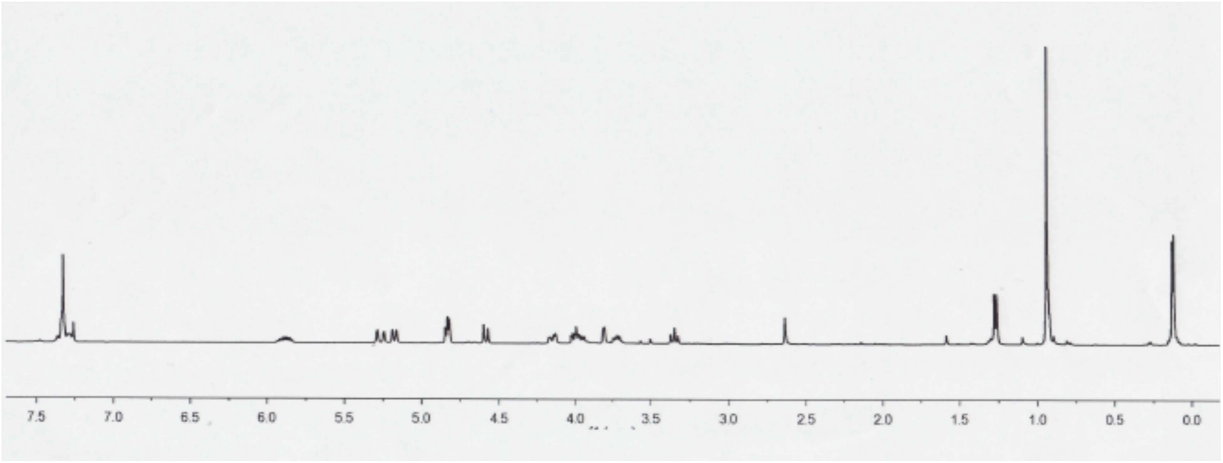

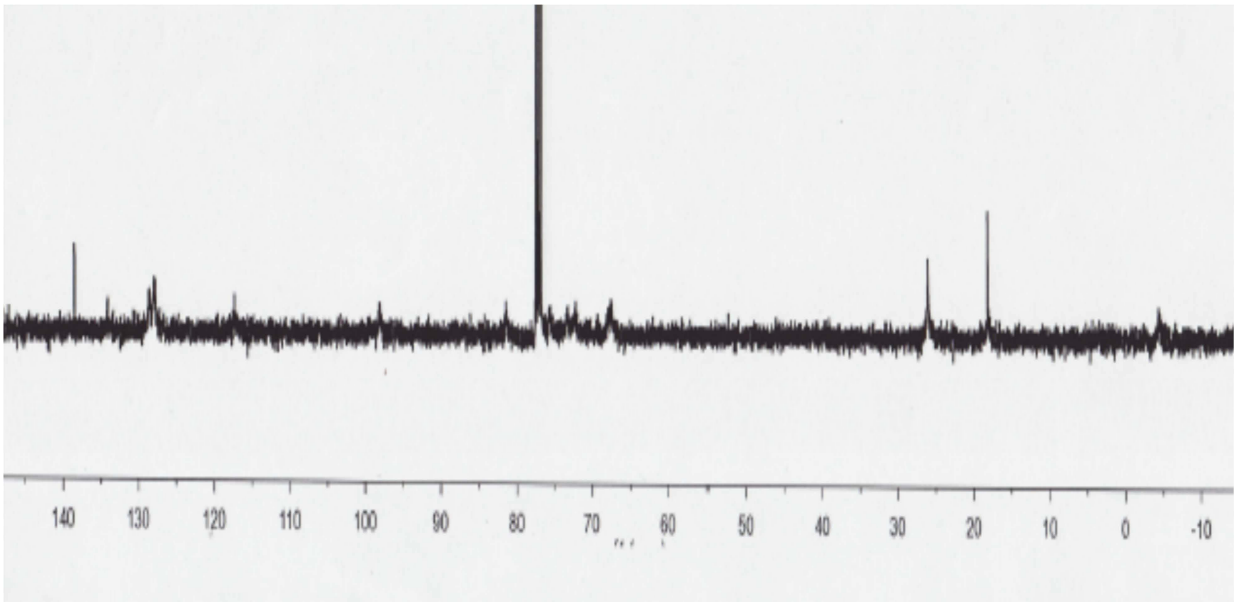

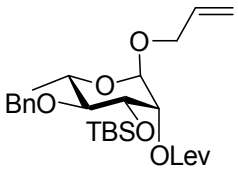

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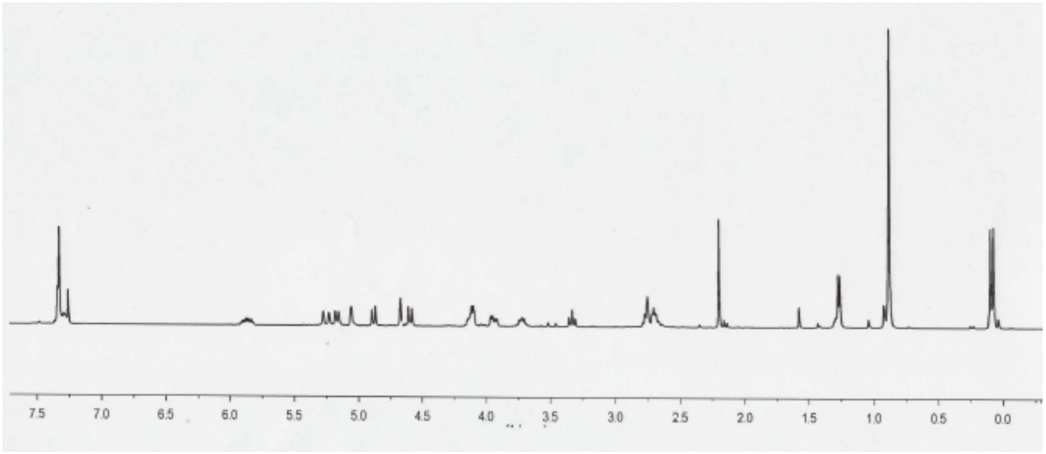

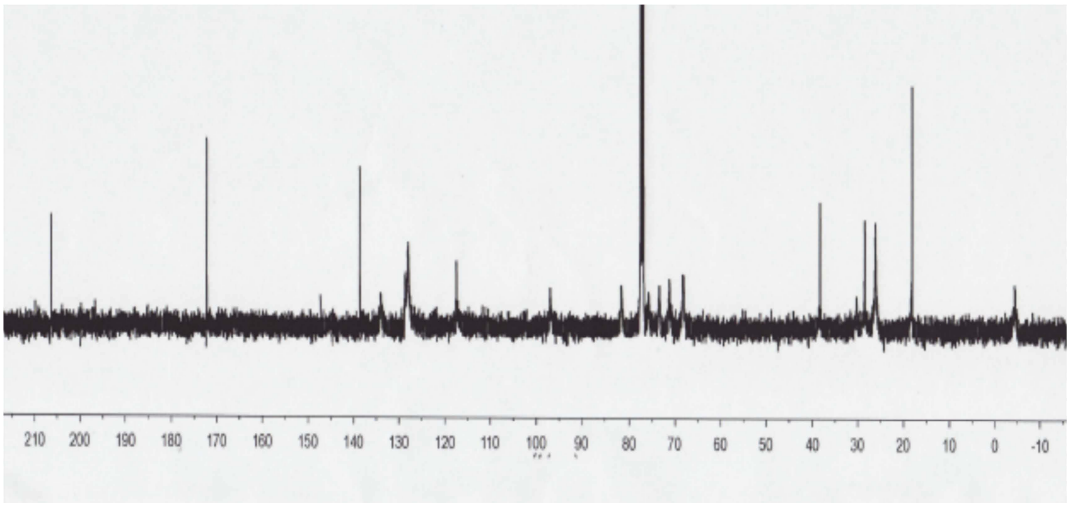

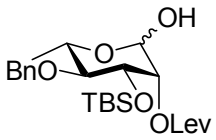

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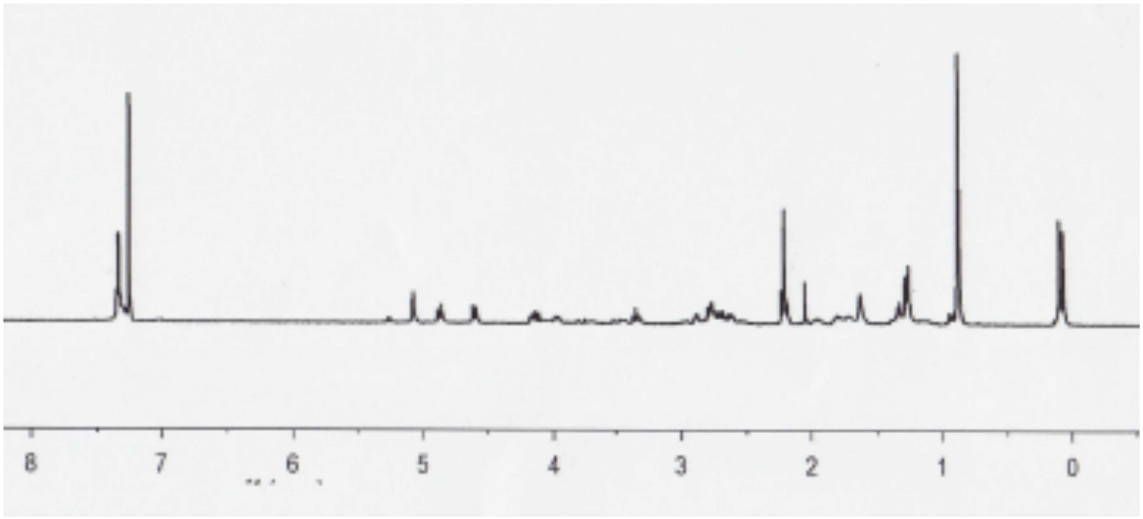

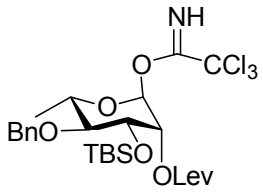

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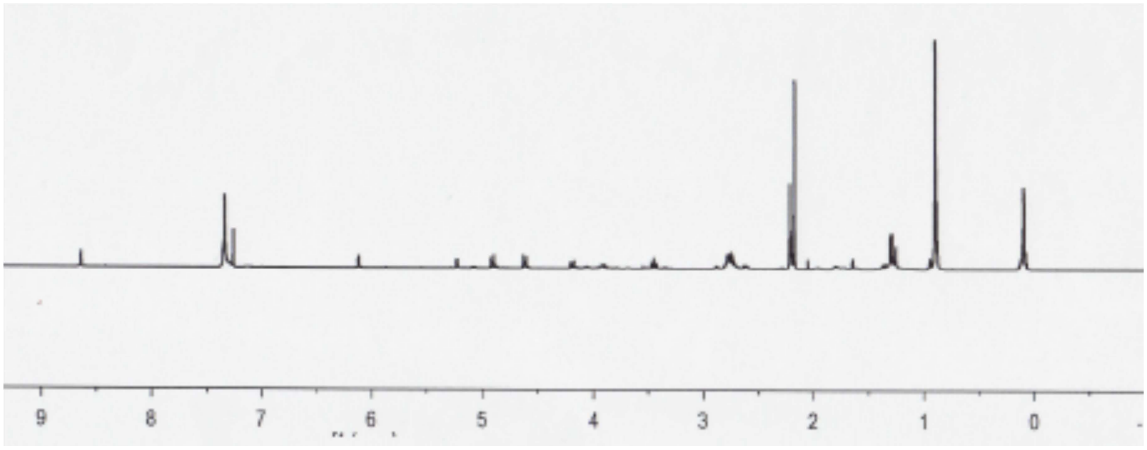
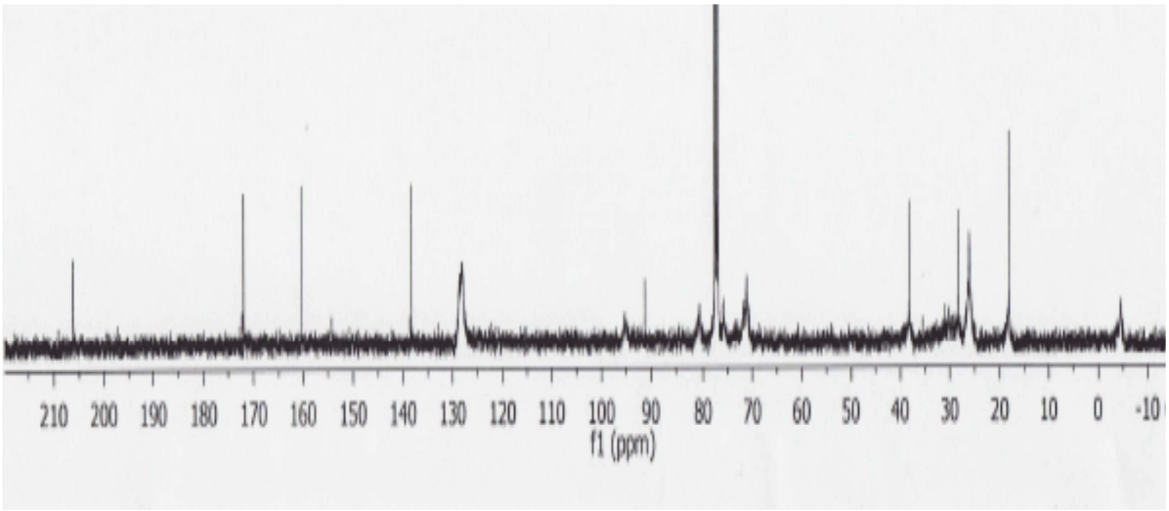
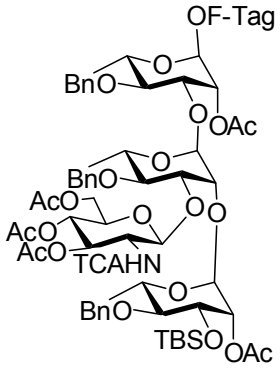
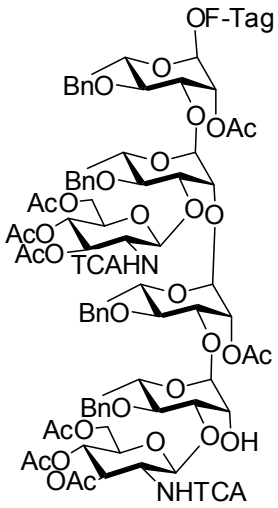
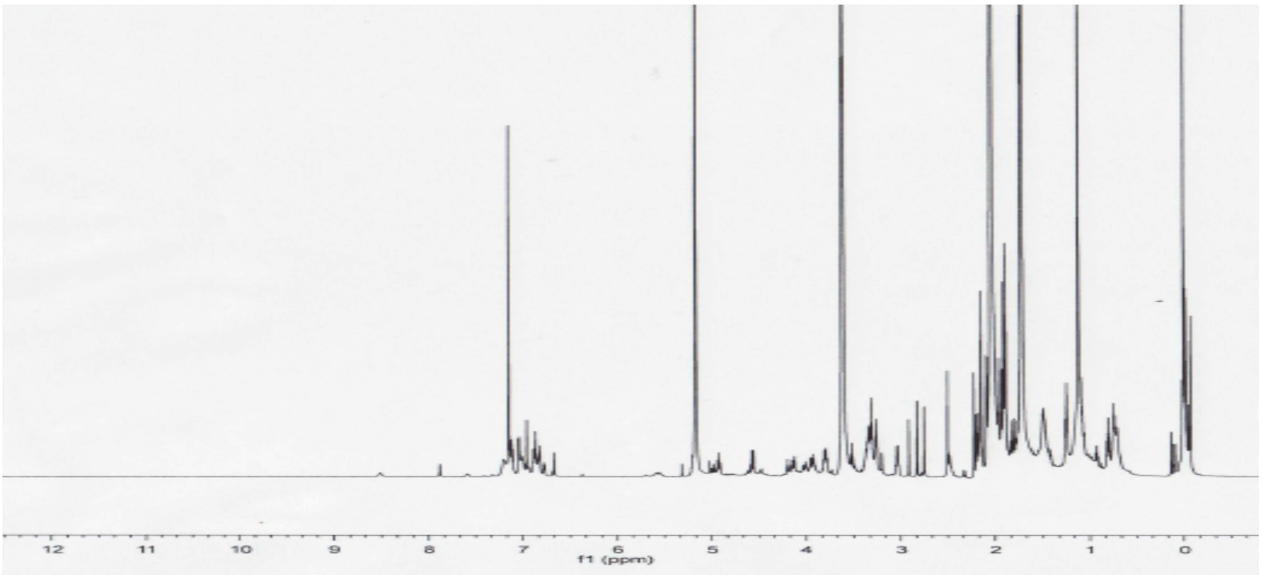




Tetrasaccharide + Hexasaccharide:







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