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The development of automated solution-phase synthesis of oligosaccharides

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The development of automated solution-phase synthesis of oligosaccharides

by

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
AIBN	2,2'-Azobisisobutyronitrile
ASW	Automated Synthesis Workstation
BGH	Blood group H
BSA	Bovine serum albumin
CAN	Cerric ammonium nitrate
CSA	Camphorsulfonic acid
DCC	*N, N*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,2-Dichloro-5,6-dicyano-*p*-benzoquinone
DIPEA	*N,N*-Diisopropylethylamine
DMAP	*N,N*-Dimethylaminopyridine
DMF	*N,N*,-Dimethylformamide
DNA	Deoxyribonucleic acid
ECA	*Erythrina crystagalli*
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI-MS	Electrospray ionization mass spectrometry
FITC-Con A	Fluorescein isothiocyanate concanavalin A
FMOC	Fluorenylmethoxycarbonyl
F-tag	Fluorous tag
FSPE	Fluorous Solid-Phase Extraction
FT-IR	Fourier transform-infrared
Gal	Galactose
GAS	Group A streptococcus
Glc	Glucose
GlcNAc	*N*-Acetylglucosamine
HATU	*N,N,N',N'*-Tetramethyl- *O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate
HTF	Heat transfer oil
HOBT	Hydroxybenzotriazole
HPLC	High performanc liquid chromatography
ITC	Isothermal titration calorimetry
Lev	Levulinate
LPS	Lipopolysaccharide
MALDI-TOF-MS	Matrix assisted laser desorption time-of-flight mass spectrometry

Man	Mannose
MeCN	Acetonitrile
MeOH	Methanol
MeOTf	Methyl trifluoromethanesulfonate
m/z	Mass/charge ratio
NBS	*N*-Bromosuccinimide
NDP	Nucleotide diphosphate
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
OMP	-*O*-*para*-methoxyphenyl
PEG	Polyethylene glycol
PCR	Polymerase chain reaction
PDC	Pyridinium dichromate
PTSA	*para*-Toluenesulfonic acid
Rha	Rhamnose
RG	Rhamnogalacturonan
RCM	Ring-closing metathesis
SPE	Solid-phase extraction
TBAA	Tetrabutylammonium acetate
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TEA	Triethylamine
TFA	Trifluoroacetic acid
TESOTf	Triethylsilyl trifluoromethanesulfonate
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tol	Toluene
TMSOTf	Trimethylsilyl trifluoromethanesulfonate

ABSTRACT

This dissertation introduces the development and scope of automated solution-phase oligosaccharide synthesis based on fluorous tags. Compared to previously developed solid-phase and one-pot strategies, the new automated solution-phase methodology offers a more versatile platform for various solution-phase reaction conditions, with lower amounts of donor building blocks wasted. In addition, the fluorous-tag assisted solid-phase extraction was shown to be robust enough for automated purification steps to eliminate excess reagents. The optimization process that was required to be taken into account when designing a program to construct the complex oligosaccharide compounds is described in detail. The optimized protocols for automated oligosaccharide synthesis were then applied to the construction of oligosaccharides associated with anthrax and cholera infection. Chemistry was developed that allowed the simultaneous automated synthesis of both the anthrax- and cholera-associated oligosaccharides. Finally, a solution to solve limitations of a single fluorous tag strategy is presented. A di-tagged saccharide molecule was synthesized and tested under conventional bench-top conditions to obtain information on its robustness as applied to a glucosamine monosaccharide. This initial work was then extended to the automation platform and compared to the original mono-tagged strategy. For direct comparison, the two fluorous tags were incorporated into the initial rhamnose building block and were successfully made robotically. A second fluorous protecting group and new donor strategy were also introduced into the automated platform, thereby expanding the scope of the reactions and building block possibilities for future studies. By introducing the

development, application and alternative strategic examples, this dissertation offers a new convenient and robust synthetic method for oligosaccharide construction.

CHAPTER 1

Review: Automated Solution-Phase Oligosaccharide Synthesis

Portions of this chapter will be submitted to *Advances in Carbohydrate Chemistry and Biochemistry*

Gisun Park and Nicola L. B. Pohl*

General Introduction

Dissertation organization

This dissertation is divided into six chapters. The first chapter is a general literature review that is related to the chemistry discussed in chapters 2 through 5. Chapter 1 is a review of the development and scope of automated solution-phase oligosaccharide synthesis. The chapter compares the advantages and limitations of this new strategy with the previously developed solid-phase and one-pot strategies. Chapter 2 discusses the original process to develop the first automated solution-phase synthesis on an Automated Synthesis Workstation (ASW) 1000 robotics platform. This chapter describes the detailed optimization processes that were required to design a program to construct oligosaccharide compounds through iterative coupling/deprotection cycles using a single fluorous tag for fluorous solid-phase extractions (FSPE) between cycles. Chapter 3 demonstrates the application of the automated oligosaccharide synthesizer to the construction of biologically relevant molecules. Methods were developed to synthesize both the anthrax- and cholera-associated antigenic tetrasaccharides side by side on the platform simultaneously. Chapter 4 was published in *The*

Journal of Fluorine Chemistry in 2008. This chapter follows up the previous chapters by introducing a solution to the occasional problem of purification issues related to incomplete reactions. A di-fluorous-tagged saccharide molecule was synthesized and tested under conventional bench-top conditions to obtain information about the robustness of FSPE-based separation of the di-fluorous-tagged molecule from mono- or untagged molecules. In chapter 5, work to automate this new di-fluorous-tagged strategy is reported and compared to the original strategy discussed in chapter 2. Two fluorous tags were incorporated into a rhamnose building block for robotically-controlled synthesis. A second fluorous protecting group and a new donor strategy were also introduced into the automated platform, thereby expanding the scope of reactions and building block possibilities available for future work with this platform. Chapter 6 provides a summary and general conclusions for the entire dissertation.

1. Introduction

2. Challenges in the chemical synthesis of carbohydrates

2-1. Background

Structure/function studies of carbohydrates have clearly lagged behind related studies of peptides and nucleic acids. Isolation of pure structurally well-defined oligosaccharides from natural sources is particularly challenging given the heterogeneity and diversity of this class of biomolecules. However, the growing recognition of the importance of carbohydrates in cell-cell interactions-between different cell types in the same organism and between two

organisms, such as a pathogen and its host, has spurred greater interest in the problems related to obtaining pure oligosaccharides of known composition.

Chemical synthesis has proven to be a great solution to creating diverse structurally well-defined peptides and nucleic acids, especially as the stepwise iterative chemical synthesis of these biomolecules has been amenable to automation. Although oligosaccharides in their protected form rarely have the same aggregation problems that plague peptide synthesis, for example, oligosaccharides present their own set of special challenges. Unlike linkages between nucleic acid and amino acid building blocks, the linkages between sugars can be of two different stereochemical orientations that profoundly alter the shape and consequently also bioactivity of the resulting structures. In addition, sugars contain many identical functional groups, namely hydroxyls, which must be differentiated to control the regioselectivity of the linkages between monomers.

Given the chemical challenges of linking monomeric sugar building blocks, the protecting (or masking) group strategy of each building block is a crucial part of oligosaccharide synthesis. Protecting groups can control the stereochemistry of the anomeric linkage during a glycosylation reaction through anchimeric assistance or conformational control and can tune the relative reactivity of a glycosyl donor or acceptor. In addition, protecting groups are the most reliable way to block the many hydroxyl groups and possibly other functional groups that constitute sugar building blocks to allow only the desired linkage to form. Some functional groups can be masked with groups that are only removed at the very end of the synthesis, whereas hydroxyl groups involved in interglycosidic linkages are

masked with a group that can be selectively removed prior to coupling. Although necessary given the very slight, if any, inherent reactivity differences between hydroxyl groups, the use of various protecting groups significantly lengthens the synthesis of each individual monomer unit. A monomer can require 5-14 linear steps to make, thereby making the building blocks particularly precious.

Finally, the incredible diversity of naturally-occurring sugars further complicates their facile synthesis. Most proteins can be made from 23 canonical amino acids. Human oligosaccharides contain primarily only nine basic building blocks. However, the synthesis of bacterial, plant, and other oligosaccharides requires access to a much larger variety of monosaccharides.

3. Automated oligosaccharide synthesis

3-1. One-pot method

The first automated process for at least oligosaccharide synthesis design is called the one-pot method and was introduced by C.-H. Wong and coworkers [1, 2]. This method relies on the differential reactivity of carbohydrate building blocks provided by different protecting groups. The most reactive glycosyl acceptor building block reacts first with a glycosyl donor; then this newly formed disaccharide is activated for reaction with the next most reactive glycosyl acceptor. Data from empirical testing of dozens of protected building blocks, primarily those associated with mammalian glycosides, are now part of a computer program

that allows a user to quickly sort out the best building block combinations for a successful one-pot manual synthesis [3, 4]. Unfortunately, the challenges in providing building blocks with significant enough reactivity differences to avoid mixtures limits the method practically to the formation of two glycosidic linkages. This elegant approach does avoid the problems of requiring large excesses of donor building blocks required for biphasic processes. Future studies could possibly result in the development of an interface between this computerized oligosaccharide design program and a robotics platform to carry out the optimal synthesis.

3-2. Solid-phase oligosaccharide synthesis

The first example of the robotically controlled synthesis of oligosaccharides comparable to automated nucleic acid and peptide synthesis was reported in 2001 by P. Seeberger and coworkers [5]. This process relies on the conversion of a conventional solid-phase peptide synthesizer with the addition of a cooling unit required for some trickier glycosylation reactions. As in solid-phase peptide synthesis, a robot delivers soluble reagents and building blocks to a reactor containing the resin with the growing oligosaccharide chain and then rinses excess reagents from the resin. Unfortunately, the reported process calls for 20 equivalents of building blocks at each coupling cycle. Therefore, even very efficient coupling yields would still generate 57 equivalents of wasted building blocks after only three cycles. In addition, the success of reactions cannot easily be monitored on the resin while a program runs. Even if mistakes are caught using solid-phase NMR spectrometry, the only options are to stop the run entirely or to continue to the end with the hope of purifying away

all the undesired sequences. The growing chain cannot be removed from the synthesizer, purified, and readily returned to the automation platform.

4. Principles of automated solution-phase oligosaccharide synthesis

4-1. Automation platform

Unlike polynucleotides [6] and peptides/proteins [7], oligosaccharides still lack a commercially available platform for their synthesis. The existing platforms for automation are based on the use of solid-phases, which provide robotic rinsing of resins. For the first automated solution-phase platform, we optimized a Chemspeed Automated Synthesis Workstation (ASW) 1000. The ASW consists of three main stations, the reactor block, SPE (solid-phase extraction) block and reagent racks (Fig. 1-1, Fig. 1-2). The reactor block contains the reactor vials that are made of double-jacked glassware and varies in size from 8 mL (× 16) to 100 mL (× 4) in volume, even for gram scale syntheses. Between the double-layered glassware flows the HTF (heat-transfer) oil that circulates around the reactor vials and connects to a unit that is capable of cooling the HTF to -20 ^{o}C or heating it to 250 ^{o}C for temperature-controlled reactions. A ceramic drawer opens/closes the reactor vials to air/inert gas/vacuum depending on the task that it is programmed to perform. The SPE (solid phase extraction) block was reengineered as described below in descriptions of the purification tool.

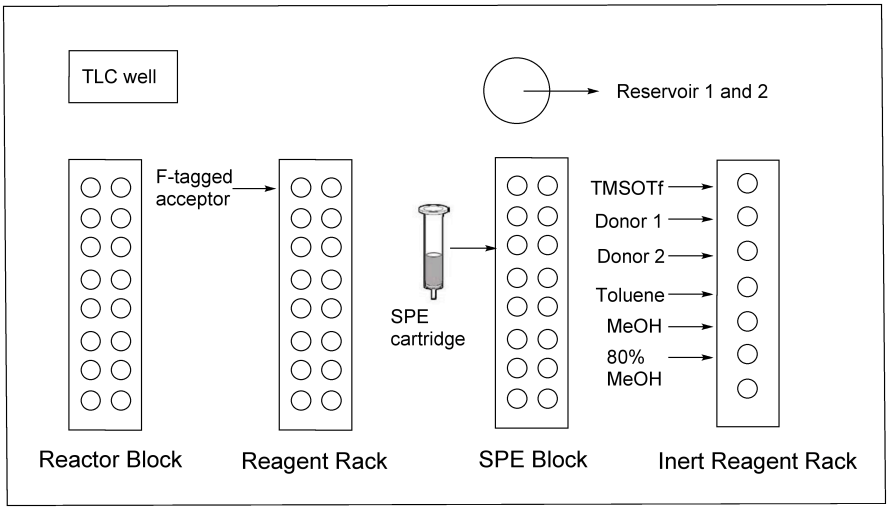

Figure 1-1. Layout of the Automated Synthesis Workstation.

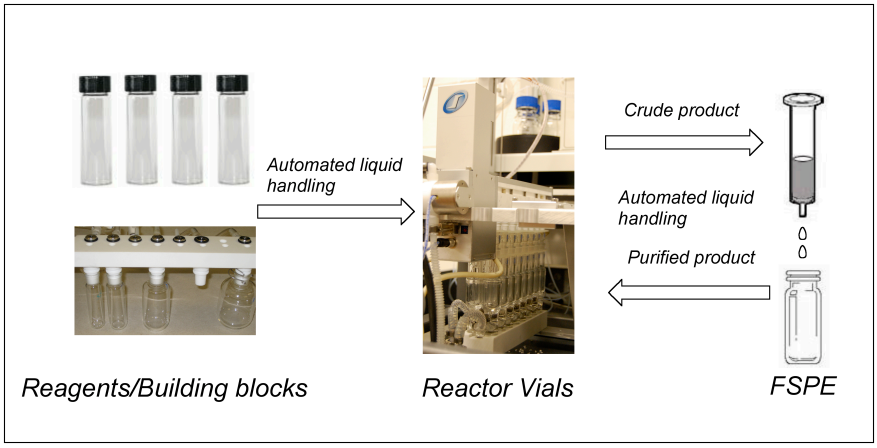

Figure 1-2. Schematic Design of the Automated Synthesis Workstation 1000

5-2. Purification tool

Although Bruce Merrifield used a solid-phase to prevent aggregation of growing peptide chains through spatial segregation, aggregation of growing oligosaccharide chains is rarely if ever a problem. The main use for a solid-phase resin then is for ease of isolation of the intermediates and removal of unreacted building blocks, decomposed building blocks, and excess reagents and their byproducts. Unfortunately, the resulting biphasic conditions then require an excess of not only reagents, but also the precious carbohydrate building blocks that often require up to ten or more steps to synthesize. In addition, the heterogeneity of reactive sites within the resins significantly complicates the translation of traditional solution-phase chemistry onto a solid-phase. For these reasons, a solution-phase approach is preferable. However, an appropriately robust alternative purification strategy is needed as well as a completely new type of automation platform.

A fluorous synthesis approach has the potential to be such an alternative purification strategy. Fluorocarbon solvents can form a 'third phase' that usually is immiscible both with aqueous and conventional organic solvents [8]. Therefore, a standard liquid-liquid extraction can be used to isolate the fluorous molecules into a fluorocarbon layer, while other reagents and materials partition into aqueous or organic layers. Unfortunately, for reliable extraction into the fluorocarbon layer, organic molecules often have to be tagged with multiple fluorous tags, so-called heavy fluorous synthesis, thereby often rendering the molecules insoluble in standard organic reaction solvents. When this simple yet effective method is applied, the reaction mixtures can be purified without standard silica gel chromatography, which is a challenging step to have as an automated process. The fluorous

properties were demonstrated in many aspects and proved to be generally applicable to a wide range of synthetic conditions.

Soluble fluorocarbon tags have been employed in the synthesis of a variety of carbohydrate molecules. A heavy fluorous tag could be incorporated into a protecting group to allow easy extraction or a light fluorous tag could be used to attract the tagged molecules by using a fluorinated silica gel in a solid-phase extraction process. [9] Although both heavy and light tags were developed for oligosaccharide synthesis, using heavy fluorous tags as protecting groups [10] was less attractive in our method, because of the necessity to include multiple fluorocarbon tails that results in a narrow solvent range for reaction conditions. On the other hand, using a single light fluorous tag was completely compatible with most of the oligosaccharide reaction conditions (Fig. 1-3). In addition, unlike other tagging methods that have been applied to carbohydrate chemistry, such as lipid or polyethylene glycol tags, [11-14] the fluorous tags are silent under standard characterization methods and orthogonal with different protecting group strategies. After simply loading the crude mixture from the reactor vials onto the FSPE cartridge, untagged molecules are eluted out and directed to the waste container, while the tagged molecules are directed to a vial placed underneath by changing the fluorophilicity of the solvent used. [15] Also a 2-carbon less C_6F_{13} tag was tested to prove that it contained enough fluorous content to serve as a purification tool under identical procedures as the C_8F_{17} [15].

As mentioned above, the SPE (solid phase extraction) block needed to be reengineered to hold the larger amount of fluorous silica gel required for fluorous solid-phase

extractions as opposed to normal silica gel-based solid-phase extractions. Commercially available 10 cc FSPE cartridges allow up to 200 - 300 mg of crude product to load for purification during the synthesis cycles. The question, however, was whether such a noncovalent fluorous interaction between tagged compounds and the fluorous silica gel would be reliably robust enough to find a single protocol for facile purification of the desired tagged compound from other reaction byproducts. Fortunately, we have now shown that even with a variety of saccharides, FSPE works successfully under automated conditions to retain fluorous-tagged compounds more strongly than the non-fluorous containing compounds. The FSPE task is important in that it offers an opportunity during the synthetic cycle to filter out any excess reagents or failed sequences to avoid accumulation of undesired byproducts in the solution mixture. This purification method then provides a convenient way to make a solution-phase approach in operating iterative cycles for oligosaccharide synthesis actually viable.

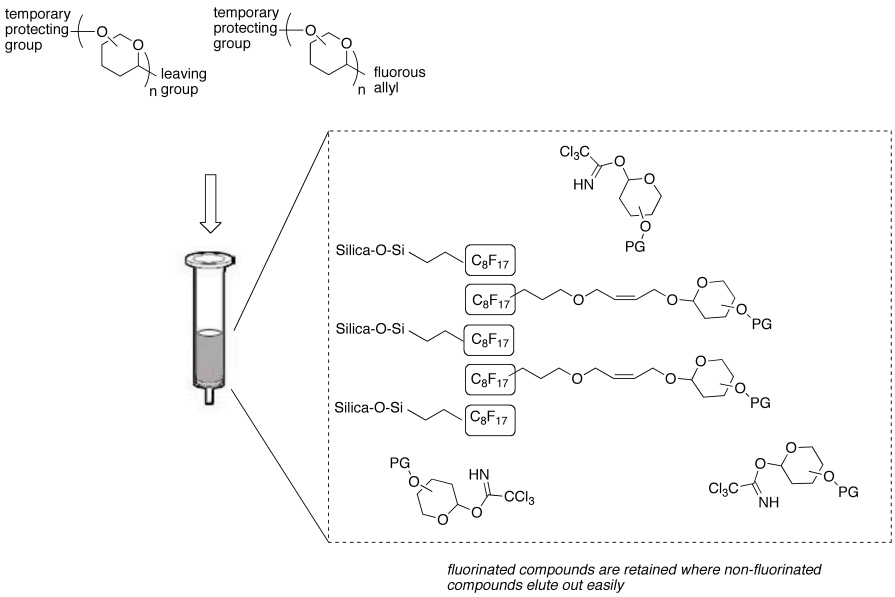

Figure 1-3. Fluorous Solid-Phase Extraction.

5-3. Automated Synthesis of Poly α-(1→2)-L-Rhamnosides

To further consider the variables for optimizing the reaction conditions on the automated platform, we started with our fluorocarbon tagged α-(1→2) rhamnoside building blocks **1** as initial substrates. After having the rhamnose building block activated as the trichloroacetimidate form **2**, [16] it is attached to the fluorous-tagged acceptor **1** under acidic conditions. Ester functionality was required to be present at the *O*-2 position for stereochemical control of the anomeric center for glycosylation reactions. Having the first fluorous-tagged acceptor in hand, the synthetic cycle was built in a way for the activated donor to add on to the acceptor to elongate the saccharide chain (Fig. 1-4). Each synthetic cycle consists of three steps. The first step is to glycosylate using trimethylsilyl

trifluoromethanesulfonate (TMSOTf) to activate a trichloroacetimidate donor. Next is a deacetylation step with sodium methoxide in the same reactor vial. The automated solution-phase approach is extremely attractive, because all the traditional manual solution phase chemistry could be directly transferred into the automated workstation without changes. Also, most importantly, the approach does not require a large excess of donor building blocks for the reactions. For example, for most of the glycosylation reactions, less than 3 equivalents of donor building blocks were used. After the deprotection step, the crude reaction mixture is carried to the fluorous solid-phase extraction (FSPE) workstation, where the desired fluorinated compound and the non-fluorinated byproducts can be easily separated due to noncovalent fluorous-fluorous interaction between the fluorous-tagged oligosaccharide and the fluorinated silica gel [17]. Removal of the excess donor/byproducts is simply accomplished by washing the FSPE cartridge with an 80% methanol/water solution several times. Finally, the robotically-controlled needle pushes the FSPE block so that the cartridge is aligned directly above a vial to collect the eluted solution when the solvent is changed to 100% methanol. The eluted solution containing the desired tagged product is carried to a clean reactor vial for solvent removal, especially of water that could interfere with the following glycosylation reaction, and the chain-elongated glycosyl acceptor is ready for the next cycle.

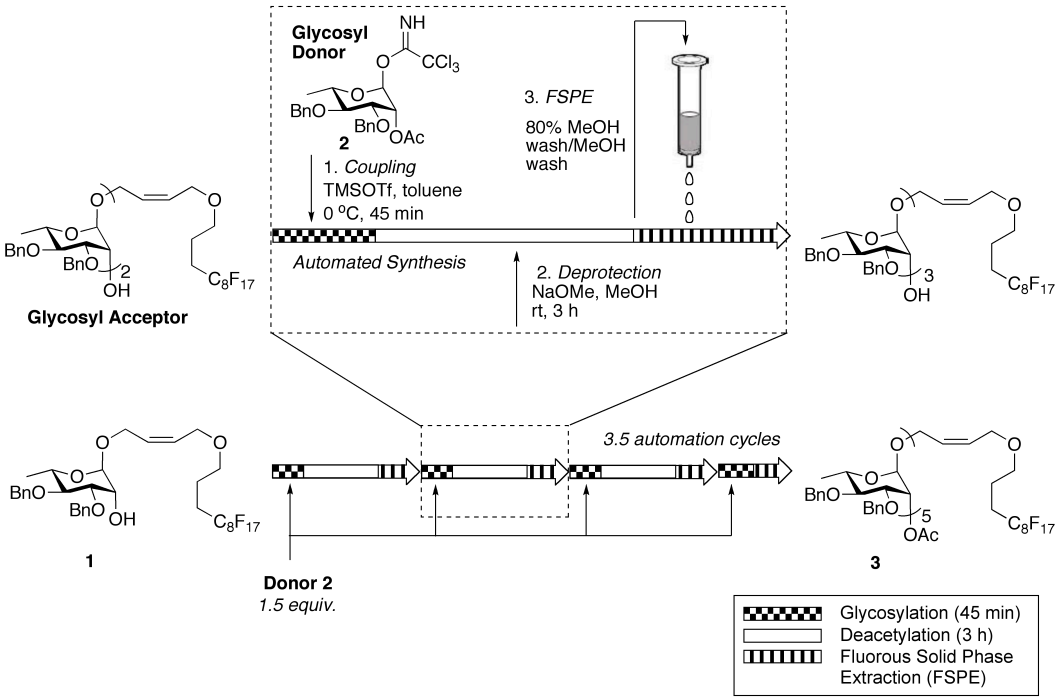

Figure 1-4. Automated solution phase synthesis of rhamnose pentasaccharide.

For the rhamnose building block, after 4 cycles, pentamer **3** was obtained in 24 hours and 16% overall yield under automated conditions. For comparison, when made under conventional conditions, the entire scheme would take more then 30 hours; even more time would be required if conventional silica gel purification schemes had to be worked out after each reaction step. To make the acquisition general for a wide range of sugar substrates, the process required further optimization. For glycosylation conditions, toluene was substituted for 1,2-dichloromethane due to the heat generated during the machine run that resulted in partial evaporation of stock solutions that altered concentrations. In addition, toluene, in which water is somewhat miscible, was used as the reservoir rinsing solvent for rinsing the needle to eliminate any small quantities of water and impurities. One major remaining

concern was the observation that most of the loss in overall yields occurred because of incomplete transfer tasks. Therefore, to decrease the amount of dead volume during transfer, flat tip needles and canonically shaped sample vials were used. With these necessary changes made (Table 1-1), the method was able to produce an average of approximately 80% yield per reaction step consistently.

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	1.5 equivalent donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.3 equivalent TMSOTf, 0 °C	45 min
2	TLC sample	30 μL of crude reaction mixture withdrawn	
3	Quenching	0.5 mL TEA	
4	Evaporation	40 °C	45 min
5	Deacetylation	3 equivalent of NaOMe solution	3 h
6	TLC sample	30 μL of crude reaction mixture withdrawn	
7	Quenching	0.3 mL 0.5 M Acetic acid solution in MeOH	
8	Evaporation	50 °C	45 min
9	FSPE preparation	0.4 mL DMF	
	Sample loading	0.7 mL crude sample transferred to cartridge	
10	Wash	4.7 mL 80% methanol wash	
11	Wash	1.5 mL methanol wash (repeated 3 times)	
12	Transfer	4.7 mL collected sample transferred to clean vial	
13	Evaporation	50 °C	45 min
14	Transfer	2 mL toluene added	
15	Evaporation	50 °C	45 min
		1-15 repeated for additional cycles	

Table 1-1. Description of Tasks Programmed in a Single Cycle.

6. Examples of automated solution-phase oligosaccharide synthesis

6-1. 1,2-*Trans* Glycosidic Linkages

a. Synthesis of Pentamannose

With an initial solution-phase-based automation platform and basic robotics protocols for the requisite glycosylation/deprotection/purification steps in hand, the next challenge was testing the robustness of these protocols for the synthesis of a range of sugars. Since automation is adopted to save time, extensive reprogramming of the machine for each new oligosaccharide desired has to be avoided. To start, the automation protocol was applied to the simplest oligosaccharide synthesis problems, those in which neighboring group assistance can be used to easily control the synthesis of 1,2-*trans*-linkages. Once the original program was created and the general problems were solved, we started to apply the method to different oligosaccharide compounds. To begin with, we decided to construct α-mannosides, which are substantially studied because of their occurrence in biological structures, such as glycolipids and *N*-linked glycoproteins. [18, 19] Having a neighboring group participant on the O-2 position will help achieve the desired 1,2-*trans* glycosidic linkage during the automated process. In addition, the initial building block should bear the fluorous tag on its reducing end for the chain extension/purification to occur. The α-mannoside **4** (Fig. 1-5) is a perfect example to test the generality of the new method for its use for more complex molecules.

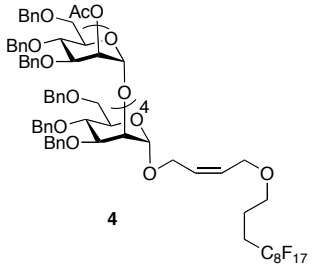

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Figure 1-5. α-Mannoside.

With the appropriate protecting groups in place, the fluorous-tagged monomer was injected into the machine along with the trichloroacetimidate donor molecule. [20] After 3.5 automation cycles, the desired pentasaccharide was synthesized within approximately 25 hours in 11% yield. Each cycle only required 2 equiv. of donor molecules per glycosylation, rather than the 20 equiv reported for the solid-phase synthesis cycles of a mannose oligomer [5], and the excess donor was washed away during the FSPE step before starting a new cycle (Fig. 1-6). The initial program for the rhamnose oligosaccharide was directly applied and successfully produced the desired oligosaccharide compound.

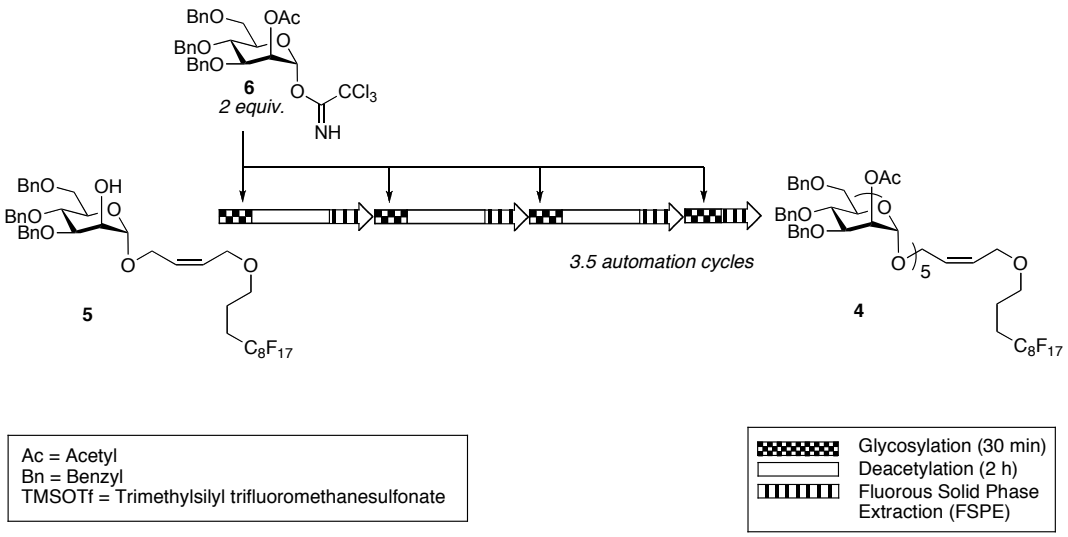


Figure 1-6. Automated synthesis of pentamannose.

a. Synthesis of Cholera tetrasaccharide

Cholera, a diarrheal disease caused by the gram-negative bacterium *Vibrio cholerae* is known to have a repeating terminal hexasaccharide of Ogawa lipopolysaccharide (LPS). [21] Interestingly, synthetic oligosaccharide antigens that were conjugated to BSA showed immunogenicity regardless of the number of saccharide monomers present. The minimum number of sugars tested were reported to be four. The tetrasaccharide version of the oligosaccharide unit was programmed to be synthesized under automated conditions. The repeating D-rhamnose building block contains a 4-amino functionality, which could be installed as a more stable azido group for the automated chain elongation process. Also, the 1,2-trans glycosidic linkage requires a neighboring group participant for the stereoselectivity of the molecule.

The initial acceptor molecule with the fluorous tag on the reducing end [22] was then injected into the automation workstation (Fig. 1-7). After 18 hours, the desired perosamine tetrasaccharide was synthesized in 2.5 cycles in 23% yield.

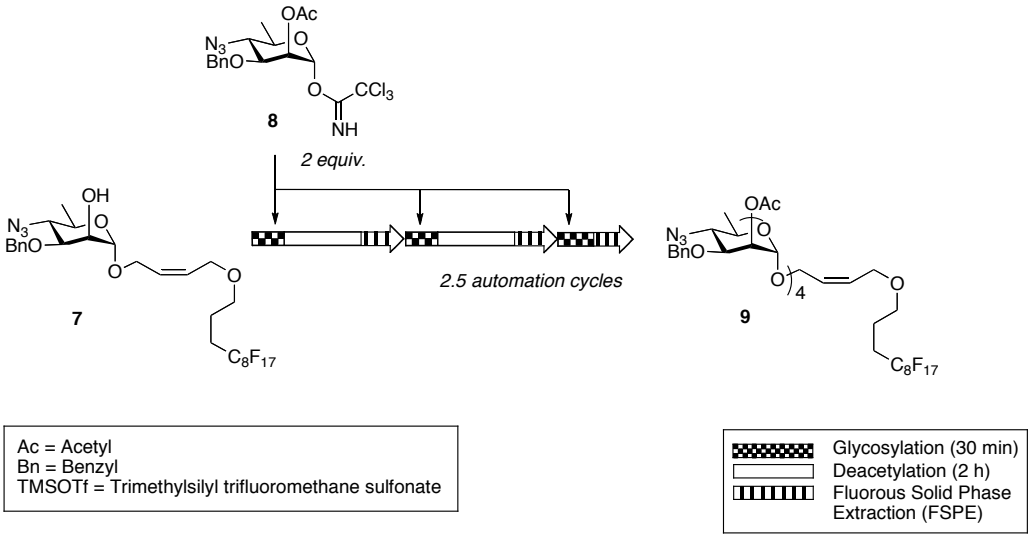


Figure 1-7. Automated synthesis of an antigenic cholera-associated tetrasaccharide.

After careful silica gel purification, the resulting azido tetrasaccharide was subjected into AIBN, Bu_3SnH conditions to reduce the azido groups to amines and was attached to the prepared side chains. After global deprotection, the target tetrasaccharide was completed. Conveniently, the mono-, di-, and tri- versions of the cholera oligosaccharide could be accomplished in one single machine run for further studies. The cholera tetrasaccharide was successfully synthesized by using the standard glycosylation protocols and conditions that were previously established requiring no additional setup time.

c. Synthesis of anthrax tetrasaccharide

Anthrax tetrasaccharide was also synthesized under automated conditions. *Bacillus anthracis*, which has spores called anthrax, is a well known cause of serious infections in

cattle and other herbivores [23]. The structure of an antigenic surface oligosaccharide of the bacteria contains an exotic sugar called anthrose. This unusual terminal sugar is not found even in closely related species, which makes it an interesting target for carbohydrate-based vaccines and diagnostics for anthrax. A silyl protecting group was first introduced as a temporary protecting group to be used under automation. The *tert*-butyldimethylsilyl group was used to mask the *O*-3 position of the first donor and was deprotected under tetrabutylammonium fluoride conditions. Also the second donor was designed to bear a levulinate protecting group on the *O*-2 position to act as both a neighboring group participant and a temporary protecting group. For the deprotection of both new protecting groups, a TBAF solution in THF and the hydrazine acetate in methanol/dichloromethane were prepared in vials and placed at the reagent rack. After 35 hours, the desired tetrasaccharide **13** was synthesized in 18% overall yield. [22] Only 2 equivalents of donor was used for each glycosylation reaction (Fig. 1-8) and after careful standard silica gel purification, the obtained molecule was ready to be methylated, coupled to the side chain, and fully deprotected to give the anthrax tetrasaccharide.

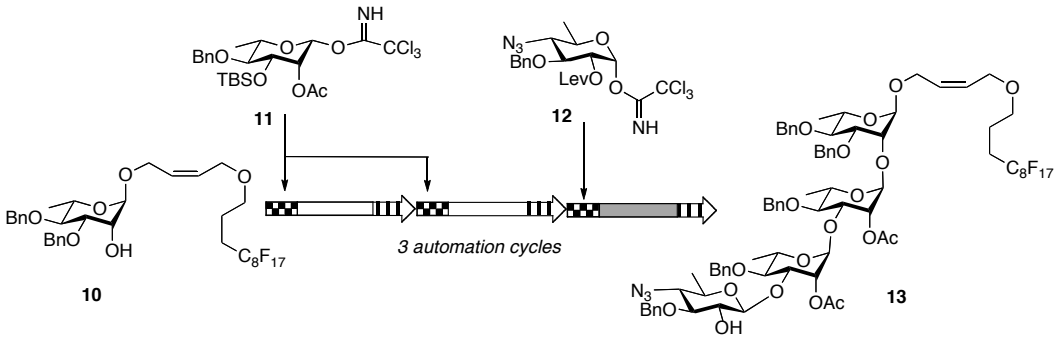


Figure 1-8. Automated synthesis of anthrax tetrasaccharide.

This was an excellent example where it shows that most of the solution-phase chemistry was easily transferrable to the automated solution-phase conditions. Silyl and levulinoyl groups proved to be good choices as temporary protecting groups to be unmasked in high yields to provide further chain elongation sites.

6-2. 1,2-*Cis* Glycosidic linkages

a. Synthesis of Blood Group H

1,2-*Cis* glycosidic linkages are well known to be challenging to synthesize in good yields, but are also often found in a range of naturally-occurring oligosaccharides [24]. To expand the utility of the automated platform, the next goal was to design a program to target 1,2-*cis* linked carbohydrates. Blood group H was chosen as an ideal target because it contains both a 1,2-*cis* and a 1,2-*trans*-linkage. For the β-(1,4)-*trans* galactosidic linkage, a neighboring group strategy could be applied. For the *cis*-linked fucose, a nonparticipating protecting group at the 2-position hydroxyl and careful solvent choice could aid formation of this alpha-linkage.

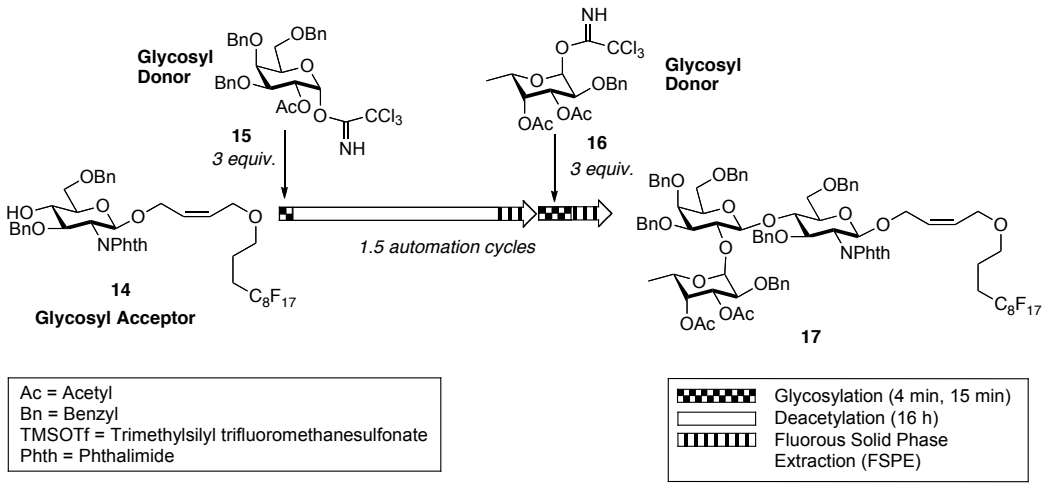


Figure 1-9. Automated Synthesis of Blood Group H.

After careful design of the protecting group strategy for the three saccharide building blocks, each was synthesized from glucosamine hydrochloride, galactose or fucose [25]. With the building blocks in hand, the automated-solution-phase synthesis protocol was applied (Fig. 1-9). The α-fucosidic linkage was programmed to use 3 equivalents of donor molecule at -15 ^{o}C for 15 minutes. After 1.5 cycles and careful purification, the desired trisaccharide was obtained in 34% overall yield. The α-(1,2-*cis*) fucosidic configuration was supported by the small $J_{1,2}$ coupling constant (J = 3.6 Hz) in the proton NMR spectra and the $J_{1,2}$ coupling constant ($J_{1,2}$ = 8.0 Hz) associated with the glucose building block demonstrated the β-(1,2-*trans*) glucosidic linkage. The anomeric proton on the galactose moiety overlaps with other protons and the anomeric configuration was determined by the one bond ^{13}C-^{1}H coupling constant ($^{1}J_{C1,H1}$ = 162 Hz). [26] The coupling constants from NMR experiments prove that the 1,2-*cis* glycosidic linkage was achieved under unattended matter by simply applying solvent effects into the reactor vial.

b. Synthesis of Rhamnogalacturonan I

Rhamnogalacturonans (RGs) are a group of closely related cell wall pectic polysaccharides that contain a backbone of a repeating disaccharide with alternating 1,2-*cis* and 1,2-*trans* glycosidic linkages throughout the structure of the oligosaccharide. Pectins have roles in plant growth, development and diseases resistance and are used as gelling agents in the food industry and coatings in medical devices. [27] Studies have revealed that the altering RG structure may have significant affects on plant growth and development. [28] However, little is known about the biosynthesis of RG1 and its specific molecular interactions as well-defined structures are difficult to isolate from heterogeneous natural sources and the RG compound itself has not been made by chemical synthesis. [29]

To access specific molecular fragments for such studies, we have developed a flexible strategy to access a range of RG1 structures from five different rhamnose and galactose building blocks. An α-1,4-linked galacturonic acid and β-1,2-linked rhamnose units were prepared for the synthetic method. The rhamnose residues could be substituted at *O*-4 with linear and/or branched size-heterogeneous oligoglycosyl side chains composed of α-L-Araf and β-D-Galp residues. These building blocks served as inputs for a new fluorous-tag based automated protocol (Fig. 1-10) for the iterative solution-phase synthesis of a discrete library of rhamnogalacturonan 1 fragments.

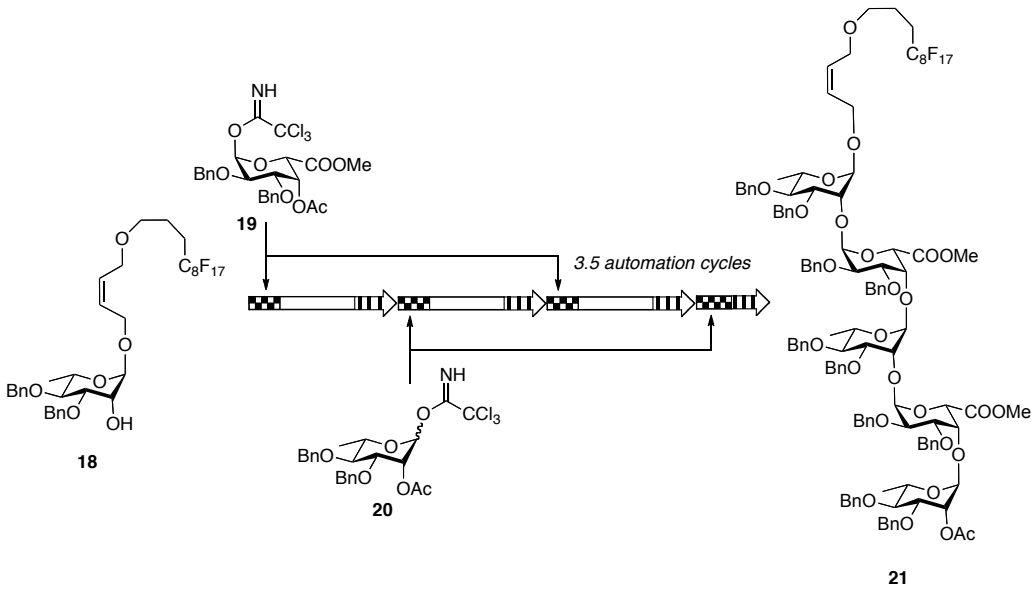


Figure 1-10. Automated Synthesis of RG1 Backbone.

c. Synthesis of β-Mannosides

The prevalence of β-mannoside residues in glycoconjugates and other naturally occurring compounds such as *N*-linked glycoproteins [30] and plant polysaccharides, [31] *O*-specific polysaccharides of Gram negative bacteria [32] has resulted in the development of a number of methodologies toward their synthesis. However, the synthesis of a β-manoside linkage has been a challenge for a long time. This linkage is disfavored by the stereoelectronic effects of the n→σ* interaction between the nonbonding electrons of the ring oxygen and the antibonding orbitals of the anomeric substituent when it is in the α-position. As a result, any generation of the carbonium ion tends to favor the α-mannoside. [33]

For the synthesis of this target, a new automated strategy had to be pursued (Fig. 1-11). A glucose donor **22** was prepared instead of the mannose trichloroacetimidate donor. And after obtaining the β-anomer by using a neighboring group participant, the *O*-2 position went through an oxidation/reduction step to invert the stereochemistry for achieving an axial hydroxyl group, thereby resulting in a β-mannoside linkage. [34]

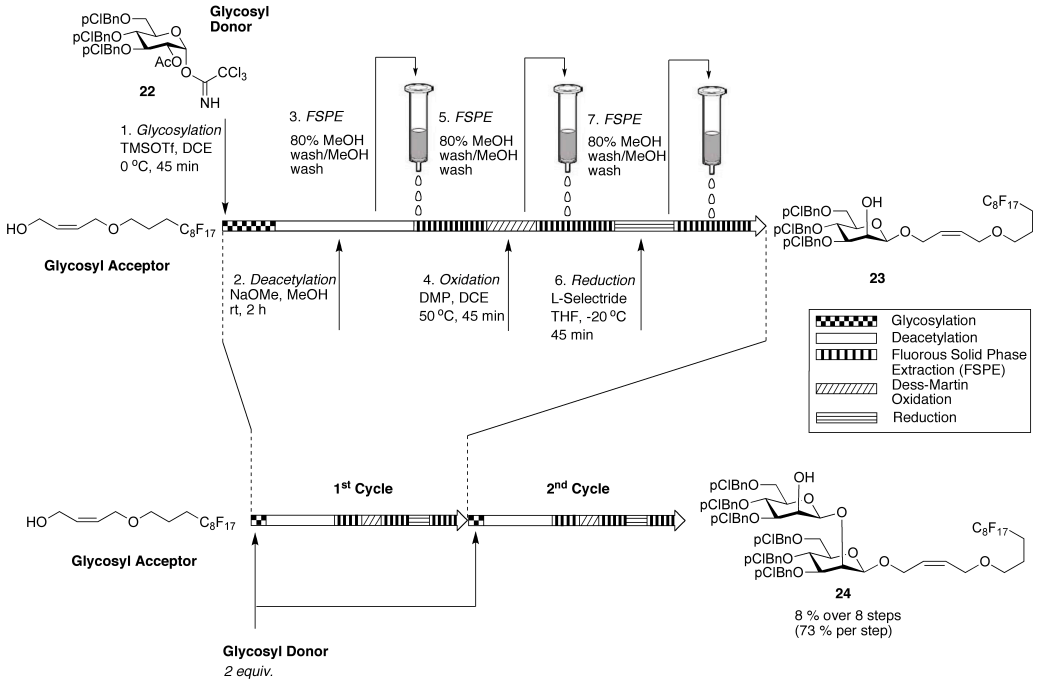


Figure 1-11. Automated Synthesis of β-Mannosides: Oxidation/Reduction Strategy

However, unlike other cases in which only a single FSPE is required at the end of each cycle, a single cycle here required two additional purification tasks. These additional transfer steps resulted in a substantial amount of material loss even when programmed to go through only two cycles. The automated oxidation/reduction protocol proved to be successful under similar automated conditions as previous molecules, but would not be as efficient when building saccharide chains containing multiple β-mannosidic-linkages.

To improve the product yields while achieving the difficult 1,2-*cis* linkage, an alternative attempt was pursued. It was shown that glycosylations of mannuronic ester donors resulted in high α stereoselectivity producing the 1,2-*cis* linkages [35] by a remote stereodirecting effect of the C-5 carboxylate group. Therefore, after synthesis of the requisite mannuronic acid building block, similar reaction conditions were applied to the mannuronic ester donor and successfully produced the desired β-di-mannuronic ester compound in moderate yields (Fig. 1-12). The resulting disaccharide **27** was then reduced at the C-6 position to give the β-di-mannoside compound. The design and implementation of these different strategies to make the beta-mannosides shows the range of reactions that can be readily accessible on a solution-phase automation platform.

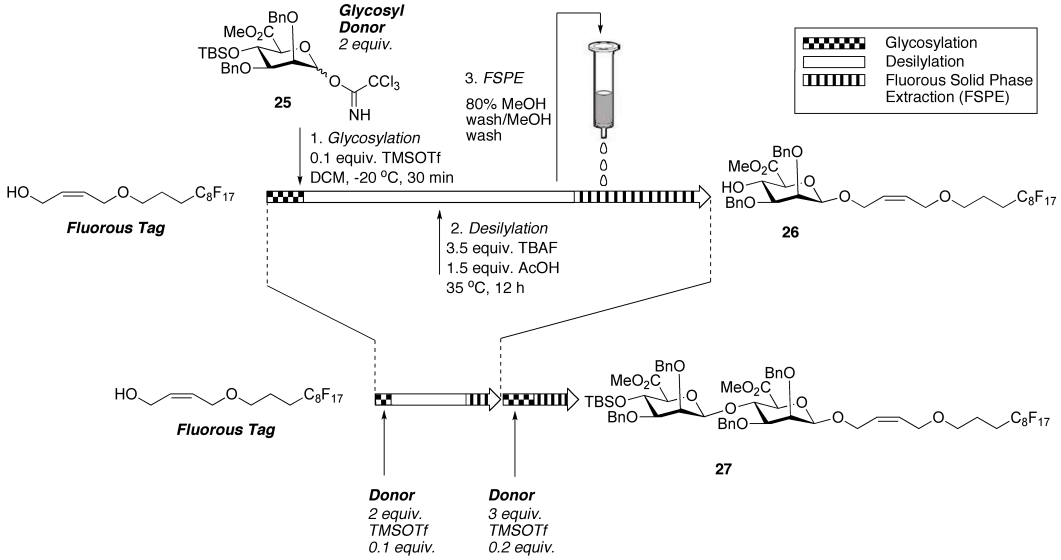


Figure 1-12. Alternative Scheme for Automated Synthesis of β-Mannosides

6-3. Branched Sugars

a. Synthesis of β-Glucans

An orthogonal protecting group strategy is especially important if there are specific positions that need to be unmasked for further chain elongations. In addition to linear oligosaccharide targets, a branched β-glucan fragment was selected as a target molecule. By synthesizing a tetrasaccharide version of the β-glucan fragment, we envisioned that this would allow us to demonstrate the ability to extend two sugar chains independently from a single branch point sugar unit. The β-glucan tetrasaccharide consists of 1,2-*trans* glycosidic linkages among the structure requiring a neighboring group participant as *O*-2 position protecting groups, as well as temporary protecting groups that are orthogonal to the rest of the masked positions on the branch point sugar molecule. The levulinate protecting group was introduced for use as a temporary protecting group for the synthesizer. The required deprotection conditions are mild and orthogonal to the rest of the molecule and indeed proved to be amenable to automation.

With the requisite building blocks in hand the automated synthesis of **32** was undertaken (Fig. 1-13). The target molecule **32** was obtained in 9.4% overall yield [36] over five chemical steps in seventeen hours.

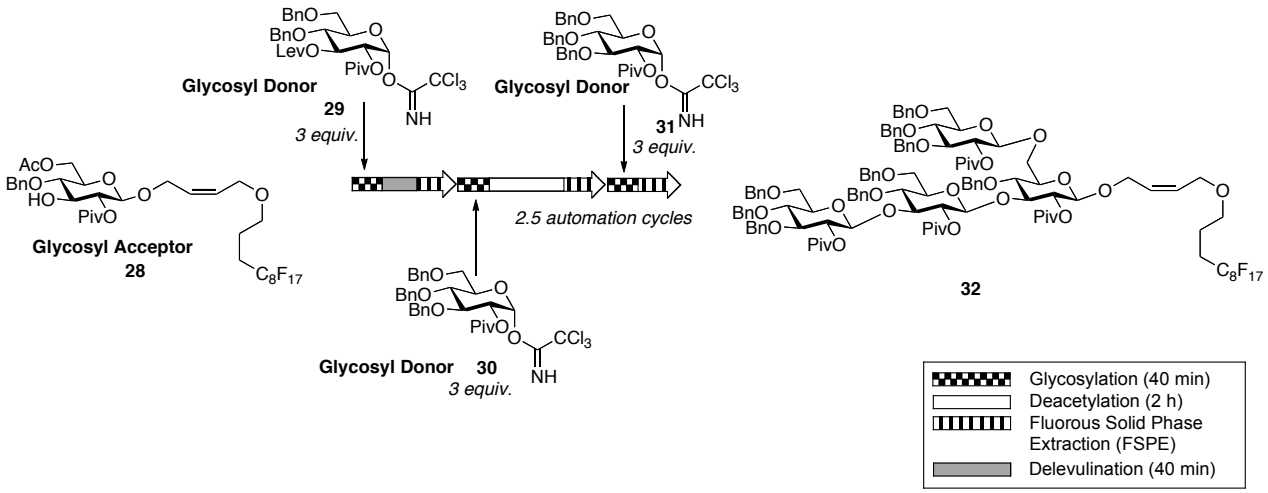


Figure 1-13. Automated Synthesis of β-Glucans.

b. Synthesis of streptococcus

Group A streptococcus (GAS) and *Staphylococcus aureus* are known causes of cellulitis, a poorly treatable skin disease. There is no known vaccine for the treatment of the disease, and in order to develop any against these bacterial infections, the key cell wall oligosaccharide **33** [37] was synthesized under automated conditions (Fig. 1-15).

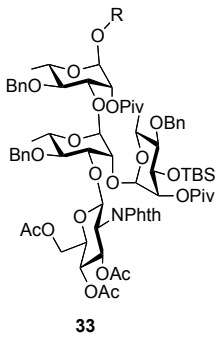


Figure 1-14. Automated synthesis of a streptococcus-associated oligosaccharide.

The polysaccharide contains a dirhamnose/glucosamine repeating unit. The core trisaccharide unit (tetrasaccharide unit shown in Fig. 1-14) was designed to be synthesized with the initial fluorous tagged-rhamnose building block. The second rhamnose and the glucosamine building blocks were then incorporated to build up the growing saccharide chain. The second rhamnose unit also acts as the potential branch point for further glycosylations to occur to access other naturally-occurring polysaccharide fragments.

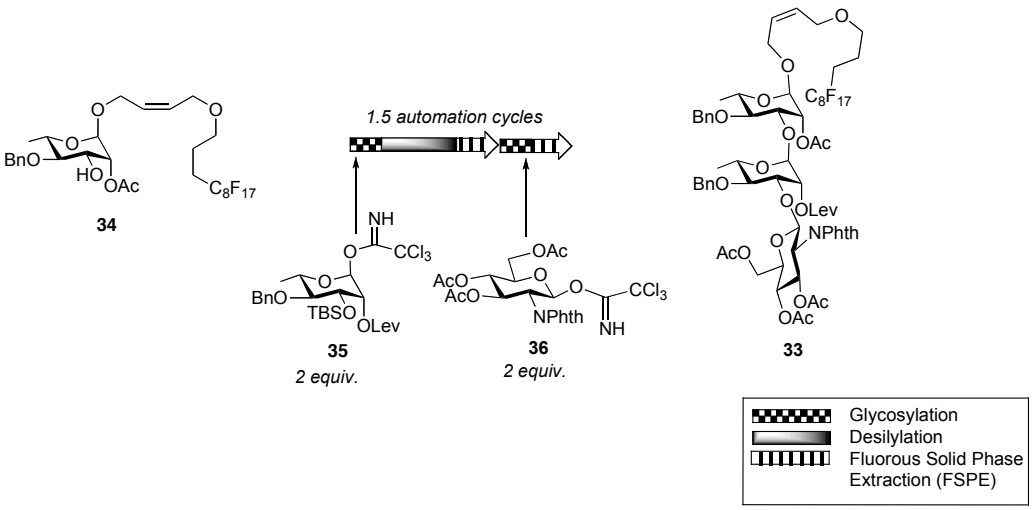


Figure 1-15. Automated synthesis of a streptococcus-associated oligosaccharide.

6-4. Purification Strategies and Limitations

a. Synthesis of Glucosamine oligomers: a ditagging strategy

The fluorous tag is a crucial tool in this whole process of constructing various oligosaccharides. However, one drawback is that there are failed acceptor molecules that are carried along with the desired molecule that results in by-product build up during the process. Therefore, at the end of the programmed acquisition, the failed sequences are collected along with the desired product. Although often these failed sequences can be readily removed at the end of the synthesizer run, sometimes the purification is not trivial.

To solve this problem, we tested how the property of the sugar molecule would change if there were a second fluorous tag present. [38] A glucosamine molecule was prepared with an ester derived fluorous protecting group on the *O*-4 position. The newly prepared glucosamine donor was activated and attached to the allyl-fluorous tag. The crude mixture was then carried to the 2 cc FSPE cartridge to test its activity (Fig. 1-16). After several trials it was shown that the additional tag indeed provided a strong enough interaction with the fluorinated silica gel compared to the singly tagged molecule. When 90% methanol/water solvent was used, the singly-tagged excess donor and failed acceptor eluted out.

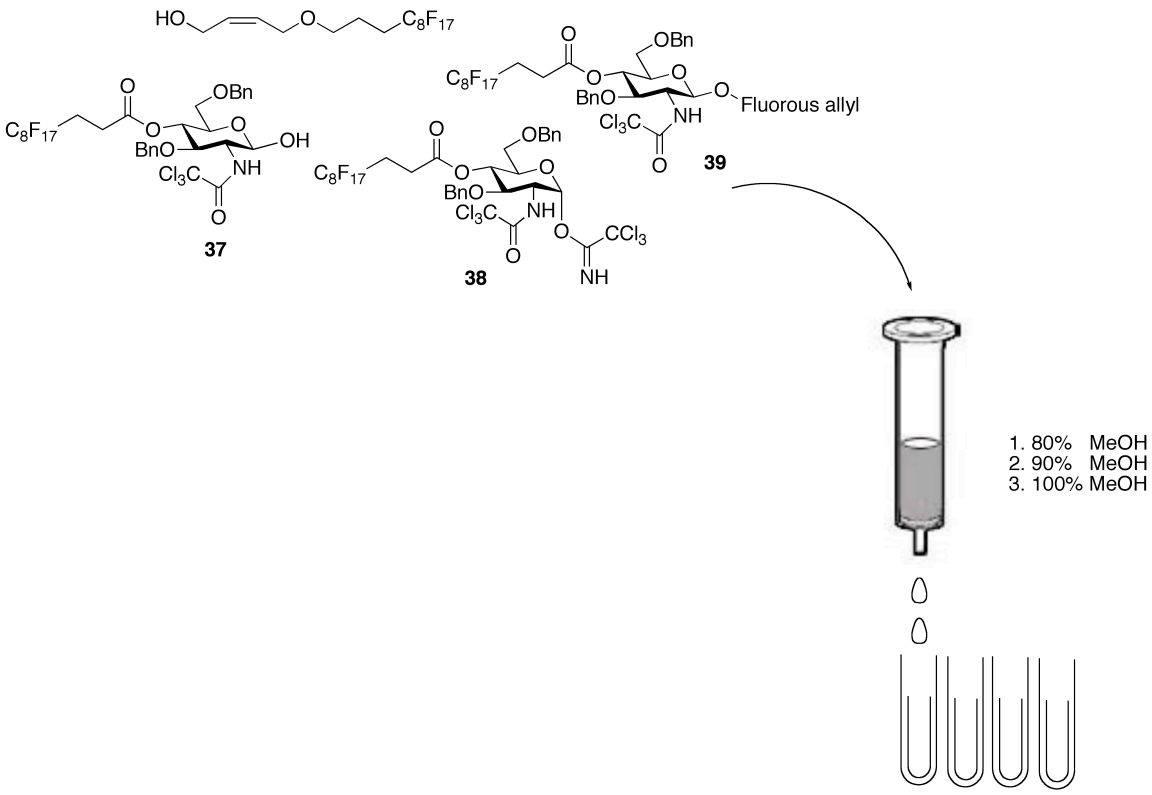


Figure 1-16. Separation of a di-fluorous tagged saccharide.

b. Automated synthesis of a di-tagged rhamnose pentasaccharide

With the preliminary results in hand with the di-tagged glucosamine molecules, we next decided to transfer the conditions for automation (Fig. 1-17). The rhamnose donor **1** that was initially used for optimizing the automated conditions was used again for comparison. Instead of the acetate protecting group that was placed on the *O*-2 position, the ester derived C_8F_{17} group was installed. Unlike the initial reaction programs that were applied to the other molecules, the FSPE step was programmed to occur right after the glycosylation step to filter out the acceptor that has failed to react as well as the excess

donor. The HPLC traces for the crude final compound from the synthesizer appeared to be pure compared to the singly-tagged saccharide compounds.

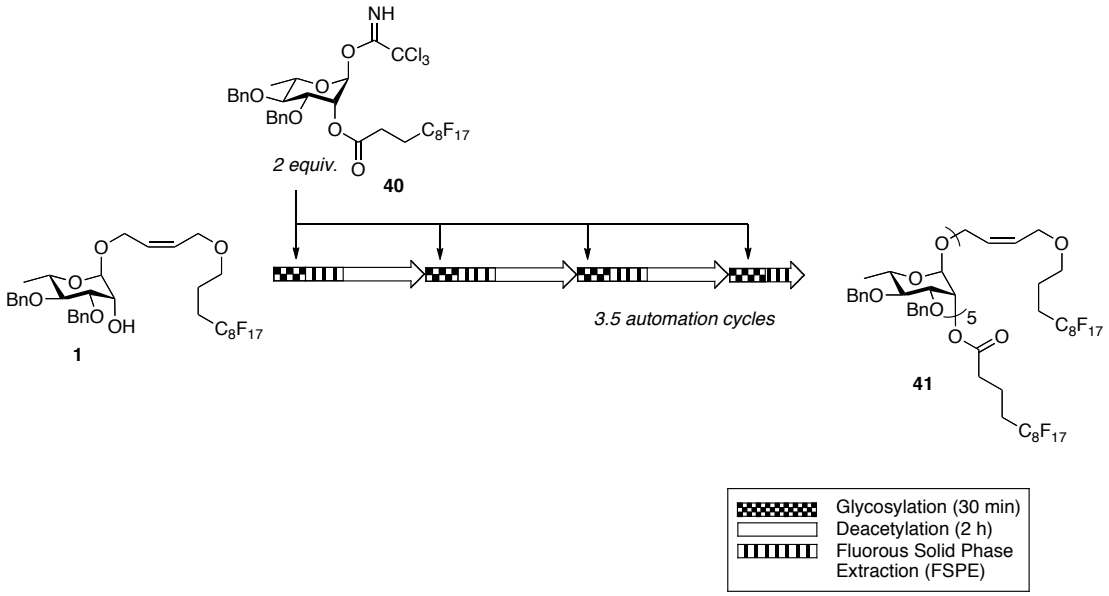


Figure 1-17. Automated synthesis of di-fluorous tagged rhamnose pentasaccharide.

c. Synthesis of 16mer oligosaccharide

To date, the fluorous tail has been compatible with most of the conditions including building block synthesis and glycosylation/deprotection conditions. The FSPE step acted as a convenient tool to purify the desired oligosaccharides from the non-fluorinated reagents and by-products. However, a question remained as to the size of the sugar chain that could be made without problems with purification and transfer steps. Once the oligosaccharide molecule exceeds the pentasaccharide scale, the solubility and separation within the FSPE cartridge is unclear.

To test the feasibility and possibility of pursuing the fluorous tail strategy to make larger oligosaccharide compounds, a known tetrasaccharide donor was used. [39] The repeating tetrasaccharide is found in the *O*-specific polysaccharide of *Shigella dysenteriae* type 1. Under automated conditions, the molecule is programmed to go through the identical coupling/deprotection steps to produce a hexadecasaccharide compound. Also, the introduction of the deprotection of the temporary protecting chloroacetyl group via thioacetate conditions expands the scope of the protecting groups that could be used.

By increasing the reaction time and equivalents of donors used, the desired hexadecasaccharide **44** was synthesized successfully in an overall 14% yield (Fig. 1-18). The higher-membered oligosaccharide fluorous tagged intermediates behaved as consistently as the small saccharide compounds and good separations resulted during the FSPE steps-an important finding for the future synthesis of larger molecules.

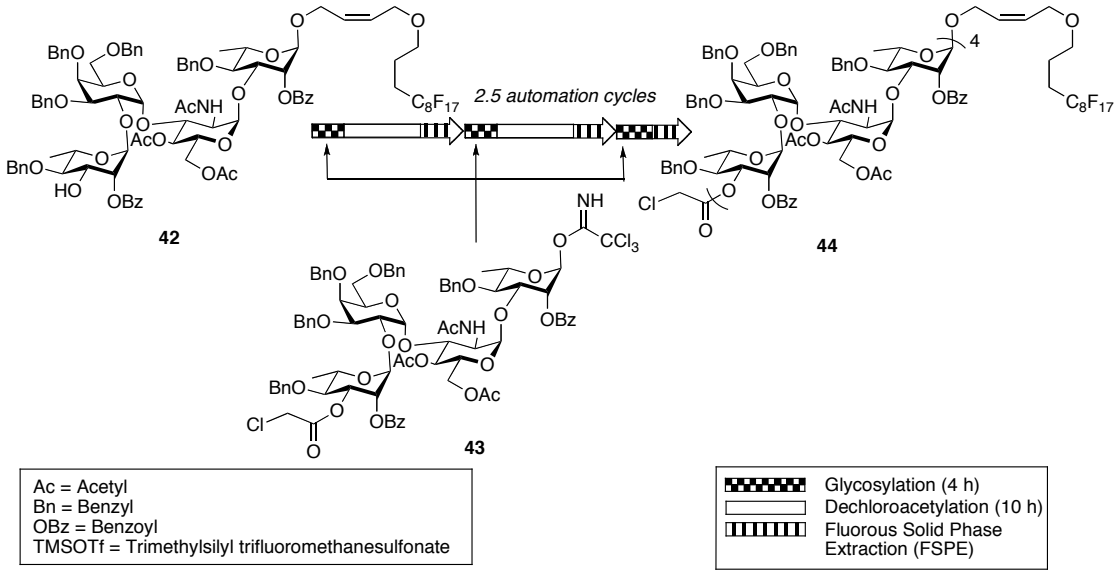
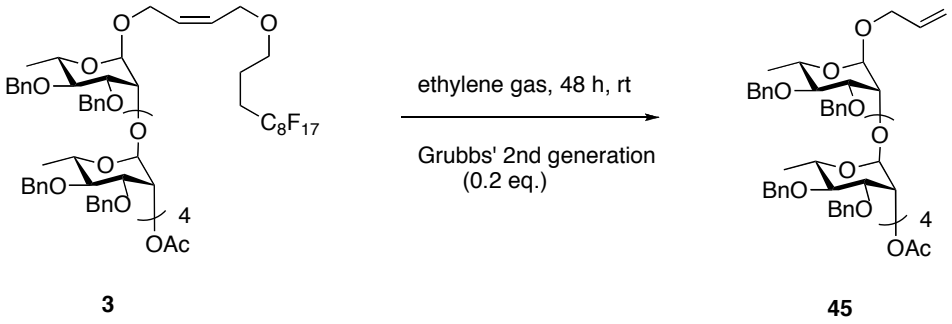


Figure 1-18. Automated synthesis of 16mer oligosaccharide.

6-5. Tag modification/removal

The allyl-C_8F_{17} fluorous tag has proved to be a convenient tool for synthesizing larger oligosaccharide compounds not only by conventional means [40], but also under unattended automated conditions by taking advantage of the fluorous-fluorous interactions. The tag was able to survive a wide range of synthetic conditions, making it useful for the glycosylation/deprotection cycles. However, after developing the general procedure for synthesizing various oligosaccharides with protecting groups, demonstrating the removal and modification of the fluorous tag was necessary. For the automated process to be an attractive method, the removal of the fluorous tag should be a mild conditioned and highly converted process. The fluorous allyl tag is designed to be a flawless group to be used

because its versatility allows it to be converted to different functional groups for various future applications. The alkene functionality offers a versatile route for the final oligosaccharide to be functionalized for any future applications. The automatically synthesized rhamnose pentasaccharide **3** was carried out under olefin cross-metathesis conditions [41] to give the resulting allyl pentasaccharide **45** in 72% yield as shown in Figure 1-19. The anomeric allyl group is a common protecting group that is used in oligosaccharide synthesis. The further removal of the anomeric allyl group could be accomplished by using palladium reagents [42] or an iridium catalyst [43], followed by either iodine or mercury conditions. [44]



3 **45**

Figure 1-19. Deprotection of the fluorous tag of an automatically synthesized rhamnose pentasaccharide under cross metathesis conditions.

The allyl fluorous-tagged molecules can also directly be introduced to ozonolysis conditions [45] for cleavage (Fig. 1-20). The resulting aldehyde, compound **47**, could then be easily purified using an FSPE cartridge to be separated from the fluorous aldehyde by-

product. The aldehyde could then be further oxidized to a carboxylic acid to be coupled to a carrier of interest, which makes it convenient in many applications.

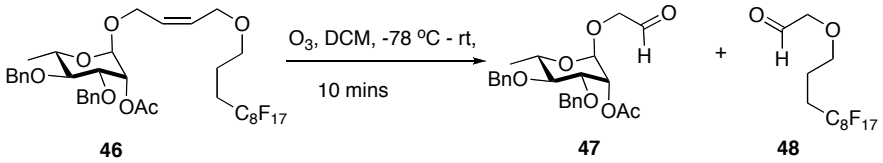


Figure 1-20. Modification of the fluorous tag of rhamnose under ozonolysis conditions.

For microarraying purposes, the fluorous tail is crucial for immobilizing the sugar molecules on the fluorinated glass slide. The final product from the automated synthesizer will be purified and introduced to a series of global deprotection conditions to result in a fully deprotected oligosaccharide **50** with a fluorous tail as shown in Figure 1-21.

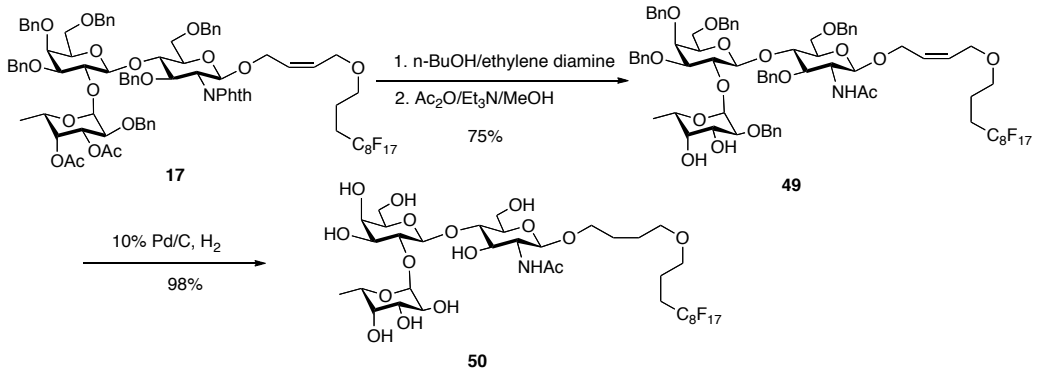


Figure 1-21. Deprotection of Automatically Synthesized BGH

7. From automation synthesis to microarray fabrication

7-1. Background

Microarrays are known to be one of the most highly efficient tools for unmasking biomolecular interactions. With the minimum amount of sample, the screening process is fast and reliable. For instance, DNA chips have been particularly successful and produced several practical assays using glass slides for immobilization. [46] However, for cases such as small molecules, like carbohydrates, the immobilization process generally requires certain functionalities of the small molecule for covalent bond formation, creating multiple chemical manipulation processes. It has been reported previously from our group utilizing the fluorous tag as an affinity tag for immobilizing the target saccharide molecules on the fluorinated glass slide. [47] A single C_8F_{17} tag proved to be efficient enough to have a stable binding with the fluorinated glass slide to tolerate repetitive incubation and rinsing steps for testing specific interactions with a certain protein. In addition, larger saccharide molecules and charged sugars were also compatible with the designed strategy. [48] Not only does this approach simplify the arraying techniques for highly functionalized hydrophilic carbohydrate molecules, but it also was proved to be equally sufficient for hydrophobic druglike molecules. [49]

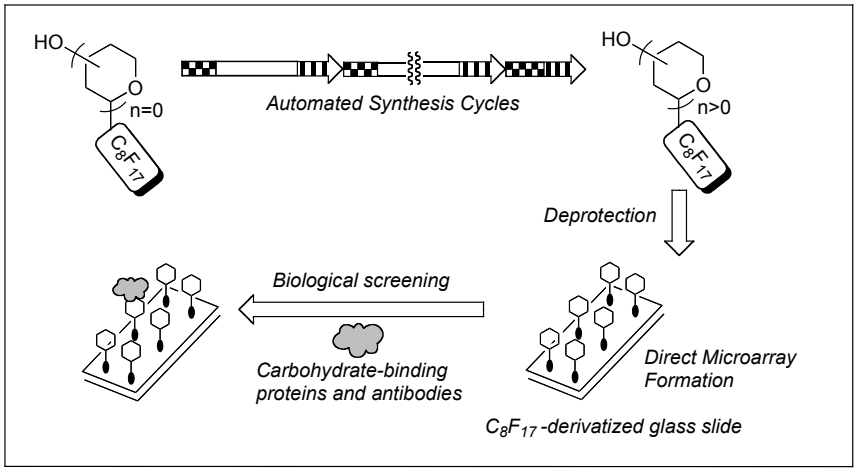


Figure 1-22. Strategy for the synthesis of carbohydrates and direct formation of fluorous-based carbohydrate microarrays.

To first test the feasibility of the surface patterning, several sugars were prepared with the fluorous-tagged allyl linker. After multiple chemical manipulation, the requisite fluorous-tagged monosaccharides were spotted on a C_8F_{17}-derivatized glass slide using a standard robot that is also used for DNA arraying. The spotted slide was then washed to eliminate any unbound excess molecules and was incubated with a solution of fluorescein isothiocyanate-labeled concanavalin A (FITC-ConA) for 20 minutes. As was hypothesized, the fluorous tag offered as a sturdy handle even when exposed to continuous rinsing steps with buffer and distilled water. And the detection of the incubated slide showed specific binding to only mannose-containing spots.

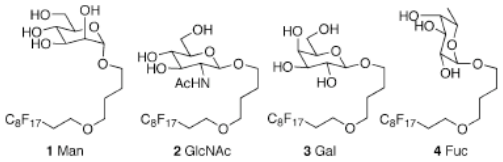


Figure 1-23. Fluorous-tagged carbohydrates synthesized for microarray formation

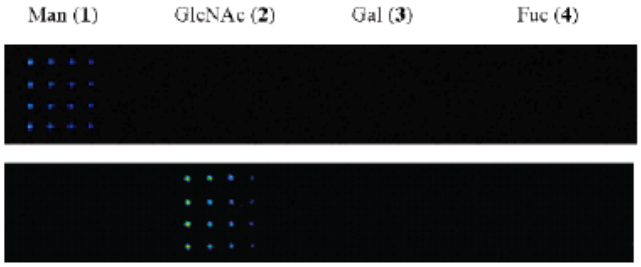


Figure 1-24. Fluorescence images of arrayed carbohydrates probed with FITC-labeled lectins. Columns of 4 spots each of 2, 1, 0.5, 0.1 mM carbohydrates were incubated for 20 min with FITC-ConA (top) or FITC-ECA with 1% TWEEN-20 detergent (bottom) with BSA. (reproduced with permission from reference 51. Copyright 2005 American Chemical Society.)

The feasibility of fluorous tags capped with an aminooxy functionality was also tested under similar conditions. The advantage of this approach is to introduce a robust tool that allows the incorporation of the reducing sugars directly onto a fluorous-based microarray. [50] With the new fluorous glycosylation method, the reducing oligosaccharides from natural sources can be tagged without activation of the anomeric center in a few number of steps. Due to its accessibility and high yield, the process is envisioned to be an attractive

assay for the area of glycobiology. Although it requires more study to fully understand its strength and limitation, the noncovalent forces that the fluorous-tags offer are reliable and generally applicable to many small molecule assays.

7-2. Examples

Synthesis of a Brucella-associated pentasaccharide

Brucella, which is known to be the cause of brucellosis, is transmitted by ingesting infected food, direct contact with an infected animal, or inhalation of aerosols but not by human-to-human transmission. The economical impacts are mostly in North America and developing countries and the protection and detection of the disease is highly desirable. [52] Although structurally distinct, the *O*-antigenic polysaccharides of *Brucella abortus* and *Brucella melitenis* both contain 2-*O*-linked 4,6-dideoxy-4-formamido-α-D-mannopyranosyl residues. For microarraying purposes, we have attempted to synthesize multiple fragments of the brucella oligosaccharide (Fig. 1-25) for diagnosis and its future possibility as carbohydrate-based vaccines.

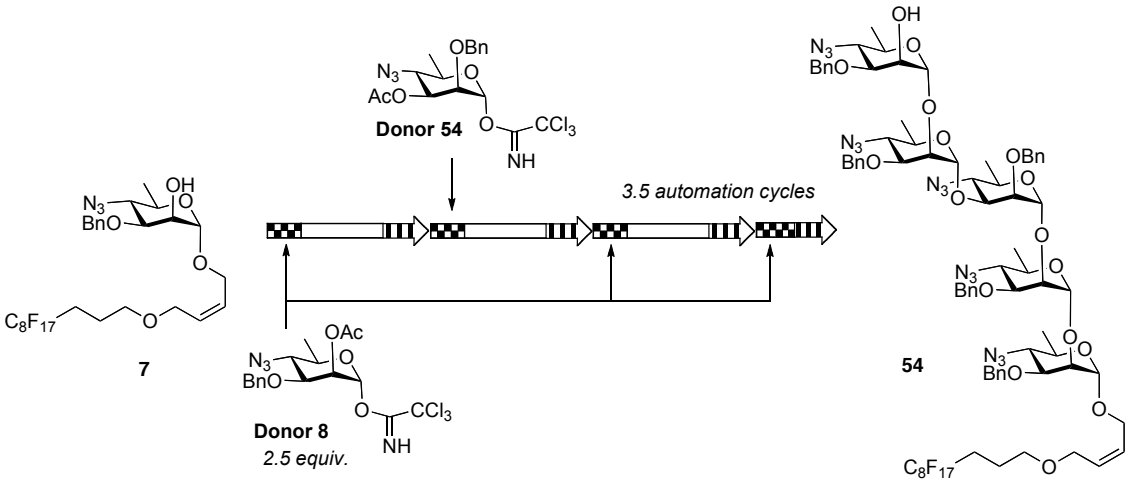


Figure 1-25. Automated synthesis of Brucella pentasaccharide for use in fluorous microarrays.

The initial acceptor monosaccharide was prepared from mannose with an azido group in the *O*-4 position. Two donors were prepared to be attached onto the molecule. After 3.5 cycles, the *Brucella* pentasaccharide was synthesized in moderate yields. The resulting compound was deprotected and arrayed on a fluorous surface for screening with infected animal sera. Antibodies in infected, but not in uninfected unvaccinated cattle, readily bound to the synthetic sugars to allow diagnosis of exposure to *Brucella*. These results open the possibility of diagnostics based on sugars made by automated synthesis.

8. Summary and future directions

Although known to be one of the three most important biomolecules, it is surprising how far behind studies of carbohydrates are compared to those of peptides and oligonucletides. The development of the customized automated solution-phase synthesizer has opened up the possibility of providing structurally well-defined complex oligosaccharide compounds conveniently. Since the automated solution-phase synthesizer using fluorous solid-phase extraction steps for purification of intermediates has proven to possess the ability to construct a range of saccharide compounds, the possibility of combinatorial carbohydrate libraries is now open.

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CHAPTER 2

Automated Solution-Phase Synthesis of Oligosaccharides

Manuscript in preparation

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Abstract

Automated solid-phase synthesis has allowed the commercial production of nucleic acid and peptide strands that drive genomics and proteomics studies. However, the synthesis of building blocks for linear and branched oligosaccharides is more complex than what is required for nucleic acid and peptide strands and therefore the solid-phase approach to oligosaccharide synthesis is more difficult and expensive. We here present an alternative automated iterative solution-phase approach to oligosaccharide synthesis and demonstrate the reliability of automated separations based on fluorous solid-phase extraction (FSPE).

Introduction

Automated chemical synthesis has allowed the commercial production of nucleic acid and peptide strands that help drive genomics and proteomics studies. These automated processes rely on a solid-phase matrix for covalent attachment of the growing biopolymer chain for simple purification of intermediates [1]. This solid-phase can be robotically rinsed with reagents and solvents as needed to iteratively attach building blocks and remove temporary protecting groups from the attached blocks to unmask the next reactive functional group. Bruce Merrifield outlined the advantages of this approach in his 1984 speech for the Nobel Prize in Chemistry [2, 3]: excesses of reagents could be used to drive reactions to completion and then could be rinsed from the solid-phase. Since his initial development of solid-phase chemistry, Merrifield's idea has been applied in a range of settings including nucleic acid synthesis [4, 5], oligosaccharide synthesis [6, 7], and the combinatorial synthesis of drug-like molecules [8]. Unfortunately, solid-phase reactions cannot be directly translated from traditional solution-phase reactions, thereby lengthening development times and effectively limiting the range of reactions that can be easily carried out in this manner [9]. The requirement for excesses of reagents also limits the effective range of this process despite the relative ease of automating solid-phase-based reaction sequences.

Results and discussion

Solution-phase-based Automated Synthesis

Given the limitations of solid-phase chemistry, soluble tags [10] to simplify the purification of soluble intermediates in iterative synthesis cycles have been explored. The unique physical properties of these tags can be used for precipitation, extraction into a liquid phase, or affinity purification/solid-phase extraction. We found that precipitation and liquid-phase extractions are too variable for reliable automation of the process. However, solid-phase extractions—a form of affinity chromatography—held promise. The required tags had to be relatively chemically inert and therefore hydrocarbon and fluorocarbon tags were investigated [11].

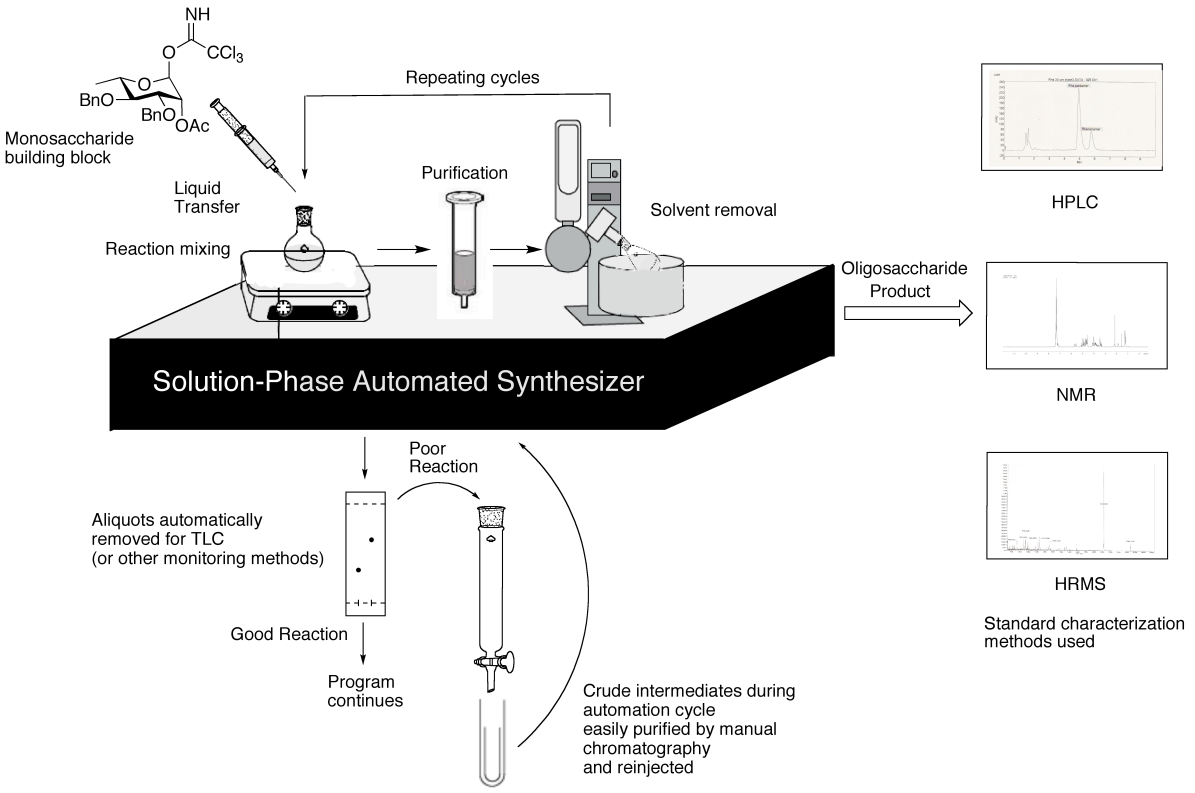


Figure 2-1. Automated Synthesis Workstation.

A key question for the success of an automated process based on fluorous tags is the robustness of these fluorous-based interactions [12, 13] between the soluble fluorous tag and the solid support during the purification step. Covalent attachment of a growing chain on a solid-support has the distinct advantage of dependable partition of the product regardless of its chemical nature from solvents even if reagents can be trapped by the supports themselves. In contrast, compounds containing a lipophilic tag [14, 15] usually require two 12 to 18-carbon hydrocarbon chains for reliable separation on C18-modified supports and separation times will vary based on the lipophilicity of the other portion of the molecule and its protecting groups. Given that traditional protecting groups are lipophilic but nonfluorous, we reasoned that a fluorous-based strategy would present less variability in the separation steps

such that an automated process would be feasible without reprogramming of the purification protocols for each new molecule. Carbohydrates, even in their protected form, contain so many atoms that can form hydrogen bonds with the water necessary for fluorous-based solid-phase extractions that they present a particularly good target for an alternative to automated solid-phase synthesis. In addition, an appreciation of the importance of carbohydrates in cancer, development, and immune recognition processes has been steadily growing, although work is still limited in part by the lack of ready availability of a range of oligosaccharides for structure/function studies [16].

To test the feasibility of an automated approach to oligosaccharide synthesis based on fluorous-solid-phase-extraction (FSPE), we needed to create an appropriate robotics platform. In addition to the liquid handling and temperature control functions of a solid-phase synthesis platform, our platform would have to carry out reaction mixing, fluorous-solid-phase extractions, and solvent evaporation functions reliably. Although many automation platforms for solid-phase iterative synthesis are now commercially available, commercial automation platforms for solution-phase chemistry tend to break apart functions and thereby require, for example, a machine to carry out the reaction, another one for the purification step, and a third for removal of solvents and volatile reagents. In the end, we machined a commercial Chemspeed ASW (Automated Synthesis Workstation) 1000 (figure 2-1) to carry out all the necessary individual steps. A crucial issue was the reengineering of the solid-phase extraction block to carry cartridges large enough to handle the larger quantities of fluorous-modified silica gel needed for separation of 200-300 milligrams of fluorous-tagged intermediates.

Once the physical platform was in place, programming of the automation cycles commenced. Building block **2** (Fig. 2-2) [17], useful for the synthesis of polyα-(1→2) rhamnosides, was chosen as an initial test substrate. Polyrhamnose is part of the coating of streptococcal group A and C bacteria [18]. This basic building block can now be made in 6 steps and 2 days and contains an acetyl group at the C2-position for neighboring group assistance in setting the stereochemistry of the glycosidic linkage. The block is activated as a trichloroacetimidate for ready activation by acids amenable to liquid handling such as trimethylsilyl trifluoromethanesulfonate (TMSOTf) [19]. A fluorous tag was chosen to contain an alkene handle for either later removal, for functionalization to attach the oligosaccharide to, for instance, fluorescent reporter groups, or for reduction of the tag for fluorous-based microarray formation [20-24]. This tag was added by acid-catalyzed glycosylation to form the initial rhamnoside **1** to test various reaction and purification conditions.

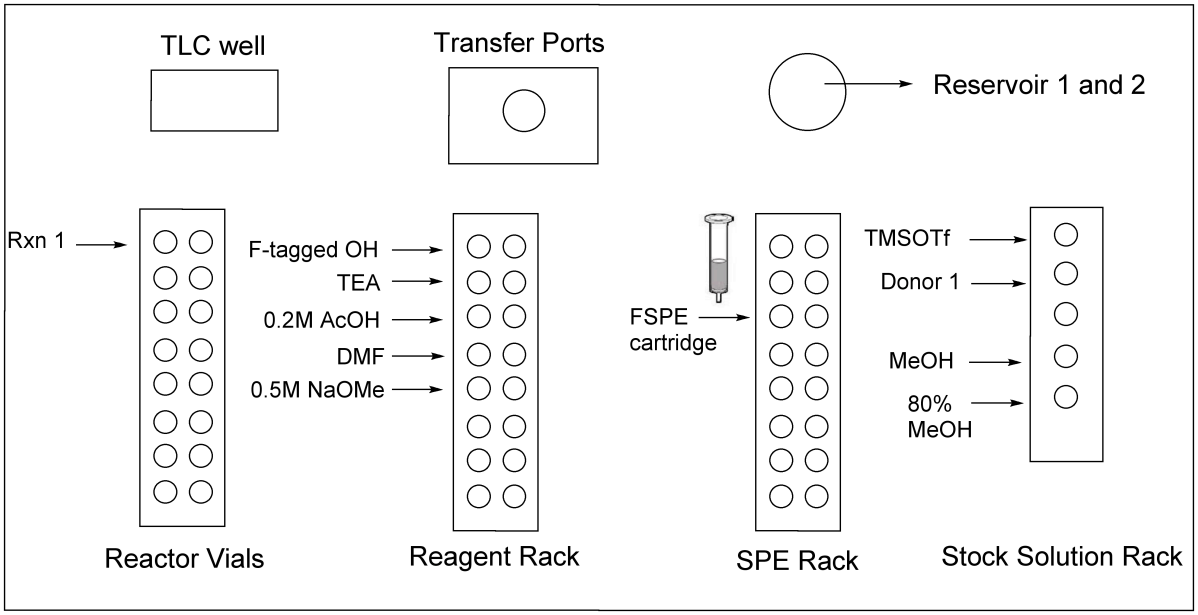
Each synthetic cycle to add a new building block consists of three steps: glycosylation for addition of the next unit to the growing chain, protecting group removal to unmask the next reactive hydroxyl for chain extension, and purification by FSPE. The automated solution-phase approach is extremely attractive because we found that general solution-phase reaction protocols could be directly transferred onto the automated workstation. In addition, the platform could be programmed to remove an aliquot at various times in the automation cycle in order to readily troubleshoot steps at the end of a programmed run by thin layer chromatography (tlc). The ability to quickly pinpoint problem

steps made development of this process much faster than a solid-phase-based process. Most importantly, the automated solution-phase chemistry did not require the 5- to 20-fold excesses [7] of activated donor building blocks required for solid-phase oligosaccharide synthesis. For most of the glycosylation reactions, less than 2 equivalents of donor building blocks were used for each nucleophilic acceptor attached to a fluorous tag.

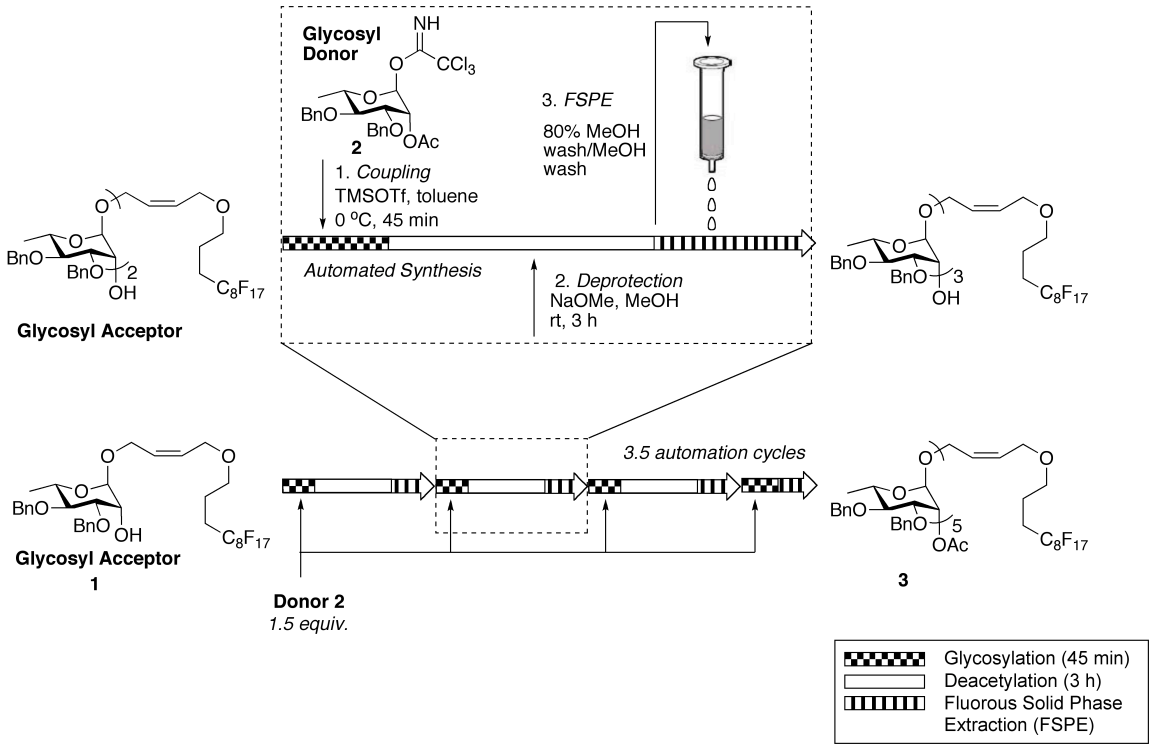
After glycosylation, the crude reaction mixture was quenched with triethylamine as a base and then carried to the fluorous solid phase extraction (FSPE) workstation for column loading. Removal of the excess donor from the mixture generates the second acceptor ready for the next cycle after deprotection. After initial failures evident by tlc in retention of the fluorous-tagged material on the fluorous silica gel, we found automation conditions that readily retained fluorous-tagged compounds on the column while only non-fluorous compounds eluted. Switching to a nonaqueous solvent allowed subsequent complete removal of the fluorous-tagged material. Because the same FSPE column is conveniently used throughout the iterative cycles, complete removal of the tagged compounds is necessary. Further optimization showed that deprotection with sodium methoxide of the acetyl group for chain extension could be carried out directly after the glycosylation cycle, thereby saving the time of a purification cycle. Solvent removal steps also required careful programming to avoid splashing of material to the top of the reaction vial and thereby loss of that material to the next reaction step. In a final iteration of the new protocols, after 4 cycles with rhamnose building block **2**, pentasaccharide **3** was obtained in 24 unattended hours and 16% overall isolated yield under automated conditions (Fig. 2-2). For comparison, conventional manual synthesis with normal silica gel chromatography between steps would require at least 3 days

for generation of this pentamer. Proton and carbon nuclear magnetic resonance (NMR) spectra showed that only alpha-anomers were produced.

A



B



C

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	1.5 equivalent donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.3 equivalent TMSOTf, 0 °C	45 min
2	TLC sample	30 μL of crude reaction mixture withdrawn	
3	Quenching	0.5 mL TEA	
4	Evaporation	40 °C	45 min
5	Deacetylation	3 equivalent of NaOMe solution	3 h
6	TLC sample	30 μL of crude reaction mixture withdrawn	
7	Quenching	0.3 mL 0.5 M acetic acid solution in MeOH	
8	Evaporation	50 °C	45 min
9	FSPE preparation	0.4 mL DMF	
10	Sample loading	0.7 mL crude sample transferred to cartridge	
11	Wash	4.7 mL 80% methanol wash	
12	Wash	1.5 mL methanol wash (repeated 3 times)	
13	Transfer	4.7 mL collected sample transferred to clean vial	45 min
14	Evaporation	50 °C	
	Transfer	2 mL toluene added	45 min
15	Evaporation	50 °C	
		1-15 repeated	

D

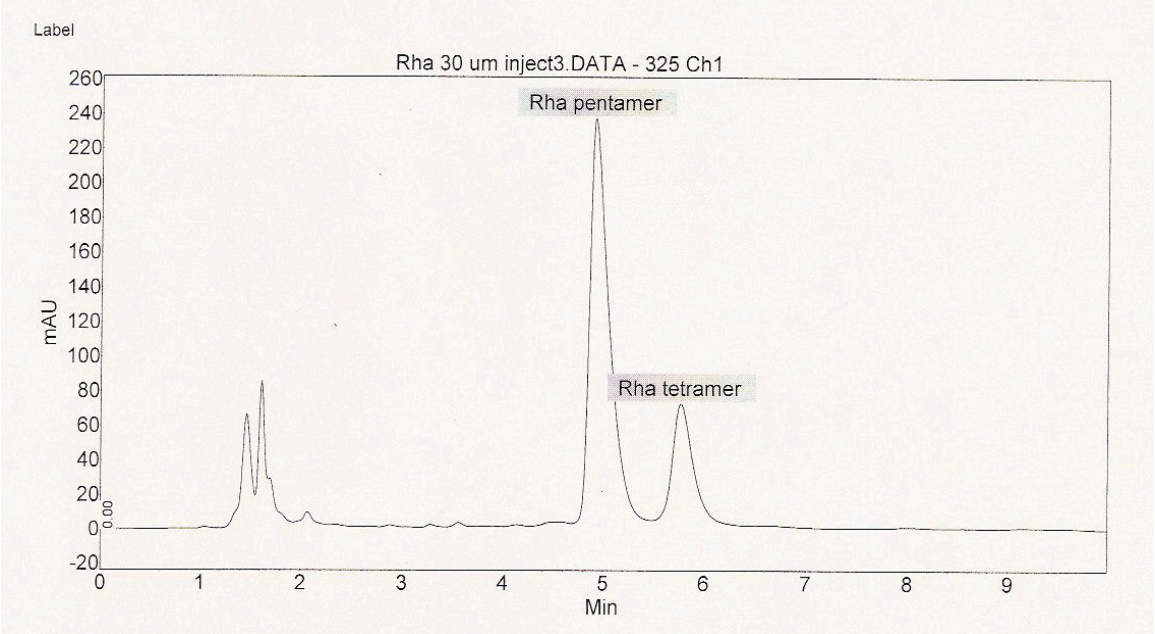


Figure 2-2. (A) ASW Automated Synthesizer layout (B) Schematic of the Automated Solution-Phase Synthesis of Rhamnose Pentasaccharide (C) Description of the tasks performed (D) Analytical HPLC chromatogram of Rhamnose pentamer synthesis (1 mL/min flowrate, 20% Ethyl acetate/Hexane, 10 minute run).

Of course, the utility of the process depends on its dependability using a range of monomers without reprogramming. For this, further optimization of the protocols and their application to a variety of monomers was required. For glycosylation reactions, toluene was substituted for 1,2-dichloroethane or dichloromethane to prevent evaporation of stock solutions from heat generated during the machine run. Toluene, which forms an azeotrope with water, was also used to rinse the needle used for transfers to eliminate any small quantities of water that might interfere with the glycosylation yields. At this point, most of

the loss in yields occurred during the transfer tasks. Therefore, a flat tip needle and conically-shaped sample vial was used to decrease the amount of dead volume during transfer.

Depending on the complexity of the structure of the desired oligosaccharide, various compounds were made under similar or slightly modified automated conditions. The automated synthesis of oligomannose, branched β-glucan fragment, blood group H (BGH) trisaccharide are discussed elsewhere [25].

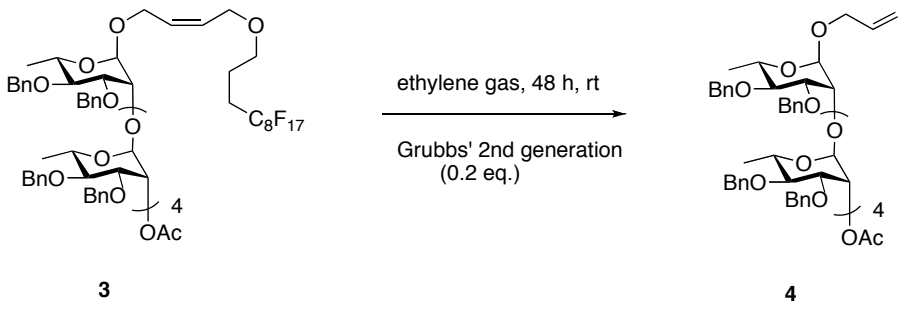


Figure 2-6. Example of further modifications of the fluorous tag

The alkene functionality of the fluorous tag also offers a versatile route for the final oligosaccharide to be functionalized for any future applications. For example, rhamnose pentasaccharide **3** was subjected to olefin cross-metathesis conditions [26] to give the resulting allyl pentasaccharide in 72% yield (Fig. 2-6). Also any fluorous-tagged compound obtained from the new automation platform can be subjected to ozonolysis conditions to obtain the aldehyde functionality for further conjugation or other reactions. Also the fully deprotected fluorous-tagged oligosaccharides could be used directly for arraying purposes [20-24].

Conclusion

In conclusion, this work [27] illustrates that noncovalent fluorous interactions are strong enough to support automated iterative synthetic protocols that reliably separate a growing carbohydrate chain from undesired by-products in a process that mimics solid-phase protocols that rely on covalent interactions. Such a solution-phase automation process requires 2 to 10-fold fewer equivalents of building blocks than equivalent processes based on solid-phase chemistry and allows greater flexibility to interrupt the synthetic process. Traditional solution-phase methods can be directly translated into this automation strategy without the significant optimization times required for the development of solid-phase synthetic strategies. In addition, the fluorous tag itself can be used to not only aid compound purification, but also be used to directly attach the compounds to microarrays for screening. These significant differences should greatly accelerate the synthesis of well-defined oligosaccharide structures to drive the field of glycobiology and particularly immunology. Finally, this new approach to automated iterative synthesis is not only an important leap to accelerate oligosaccharide synthesis, but should also aid in the automated iterative synthesis of other molecules that have been difficult to obtain by solid-phase automation strategies.

Experimental section

General methods

All commercial reagents and solvents were used as received without further purification unless indicated. Reaction solvents were distilled from calcium hydride for dichloromethane and from sodium metal and benzophenone for diethyl ether. 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 162 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 μL 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.

General procedure for automated synthetic cycles using ASW 1000 with polyrhamnose

1. Sample Preparation

Donor molecule (265.4 mg, 500 μmol) was dissolved with dried toluene (2.5 mL) in the 13 mL vial and placed at the stock solution rack (Donor 1) under argon. 0.27 M trimethylsilyltrifluoromethanesulfonate (TMSOTf, 1.05 mL) solution in toluene was prepared in the 8 mL vial and placed as indicated on the stock solution rack under argon. Dried toluene (1 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Methanol (100 mL), 80% methanol/water (100 mL), triethylamine (8 mL) were prepared in stock solution bottles and connected to the transfer ports. Acceptor molecule (43.7 mg, 50 μmol) was dissolved with dried toluene (0.5 mL) in a Wheaton 8 mL E-Z extraction vial (conically- bottomed) capped with pre-punctured septa and placed at the reagent rack where indicated (F-tagged OH).

0.5 M Sodium methoxide solution in methanol (5 mL) was prepared in 8 mL vial capped with pre-punctured septa and placed at the reagent rack. 0.2 M acetic acid solution in methanol (5 mL) was prepared in 8 mL vial capped with pre-punctured septa and placed at the reagent rack where indicated. N, N-dimethylformamide (DMF, 5 mL) directly from Fluka was transferred in 8 mL vial capped with pre-punctured septa and placed at the reagent rack

where indicated. Fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol(/water) and placed at machined SPE block. An empty Wheaton 8 mL E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle each of the 16 reactor vials (13 mL) were rinsed with 8 mL toluene and 8 mL methanol 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solutions were prepared by azeotroping the building blocks individually with toluene and dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Method Run

3.1. Glycosylation

Reactor vials were cooled to 0 ^{o}C during the 5 minutes wait time by the heat transfer oil. Then the flat-tipped open vial needle transferred the acceptor molecule (F-tagged OH) solution (0.6 mL) to the reaction vial 1, followed by the transfer of the acceptor molecule solution (0.5 mL) and the TMSOTf solution (56 μL). After each individual transfer, the needle was rinsed by 2 mL of toluene inside and out before operating the next task. The

reaction mixture was vortexed at 800 rpm for 30 minutes at 0 ^{o}C under inert gas. After the reaction time the needle withdrew 30 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. Triethylamine (0.5 mL) was added to the solution for quenching and solvent evaporated under reduced pressure.

3.2. Deacetylation

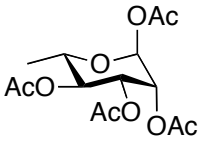
To the dried residue, methanol (0.5 mL) was added to the reactor vial followed by sodium methoxide solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 3 hours at room temperature. After the reaction time the needle withdrew 30 μL from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. Acetic acid (0.75 mL) solution was added to the reactor vial for quenching, followed by the addition of toluene (1 mL) and the solvent was evaporated under reduced pressure.

3.3. FSPE

DMF (0.4 mL) was added to dissolve the crude mixture and the vials were vortexed at 100 rpm for 5 minutes. The reaction mixture (0.7 mL) was carried to the FSPE cartridge at the SPE rack dispensed at a speed of 20 mL/s via the 10 mL syringe. Then 80% methanol (4.5 mL) was used to rinse the empty reactor vial. 80% Methanol solvent was removed from the reactor vial each time (1.7 mL, 1.5 mL, 1.5 mL) and delivered to the FSPE cartridge. An

additional 80 % methanol solution (1 mL) 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 (1.5 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the vial and deliver it to the clean reactor vial for the next reaction. Toluene (1 mL) was added to the solution and the solvent was evaporated under reduced pressure. After the evaporation cycle, once again toluene (1 mL) was added and removed under reduced pressure for complete dryness.

Synthetic Procedures



1, 2, 3, 4-Tetra-O-acetyl-α-L-rhamnopyranosde.

α/β-L-(+)-Rhamnose monohydrate (2.00 g, 10.98 mmol) was dissolved in 10 mL of pyridine/acetic anhydride (1:1). The reaction mixture was stirred at room temperature overnight. TLC confirmed the complete conversion of the starting material. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (3 × 50 mL), and brine (3 × 50 mL). The organic layer was collected and dried over $MgSO_4$. The solvent was

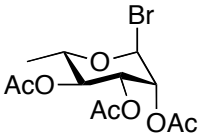
removed under reduced pressure to obtain the title compound (3.57 g, 10.76 mmol, 98%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.57 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.99 (d, J = 1.6 Hz, 1H, H-1), 5.29 (dd, J = 10.0 Hz, 3.6 Hz, 1H, H-3), 5.23 (dd, J = 3.6 Hz, 1.6 Hz, 1H, H-2), 5.12 (t, J = 10.0 Hz, 1H, H-4), 3.91 (m, 1H, H-5), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.22 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 170.0, 169.9, 168.5, 90.7, 70.6, 68.9, 68.7, 68.6, 21.1, 21.0, 20.9, 20.8, 17.6.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 355.1005; Found: 355.1566.



1-Bromo-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside.

To a solution of 1, 2, 3, 4-tetra-*O*-acetyl-α-L-rhamnopyranoside (3.56 g, 10.7 mmol) in anhydrous dichloromethane (40 mL) was added 30% hydrobromic acid in acetic acid (25 mL) dropwise in a 0 ^{o}C ice bath. The reaction mixture was stirred at room for 5 hours. TLC confirmed the complete conversion of the starting material. The reaction mixture was diluted with dichloromethane (30 mL) and washed with ice water (3 × 50 mL), 2 N hydrochloric acid (3 × 50 mL) and brine (3 × 50 mL). The organic layer was collected and dried over

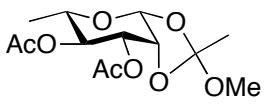
$MgSO_4$. The solvent was removed under reduced pressure to obtain the title compound (3.35 g, 9.50 mmol, 89%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.83 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.25 (d, J = 0.8 Hz, 1H, H-1), 5.67 (dd, J = 10.4 Hz, 3.6 Hz, 1H, H-3), 5.43 (dd, J = 3.6 Hz, 1.6 Hz, 1H, H-2), 5.17 (t, J = 10.0 Hz, 1H, H-4), 4.08 (m, 1H, H-5), 2.15 (s, 3H, OAc), 2.07 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.28 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.9, 169.8, 169.7, 83.7, 72.4, 71.1, 70.3, 68.4, 20.8, 20.7, 20.6, 17.0.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 325.0055; Found: 325.0921.



3, 4-O-Di-acetyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose. [28]

To a solution of 1-bromo-2, 3, 4-tri-*O*-acetyl-α-L-rhamnopyranoside (2.80 g, 7.95 mmol) in anhydrous dichloromethane (20 mL) was added tetrabutylammonium bromide (3.8 g, 11.9 mmol) and 94% *N,N*-dimethylformamide dimethyl acetal (1.96 mL, 11.9 mmol). The reaction mixture was refluxed for 5 hours. TLC confirmed the complete conversion of the starting material. The reaction mixture was diluted with dichloromethane (30 mL) and washed with water (3 × 50 mL) and brine (3 × 50 mL). The organic layer was collected and

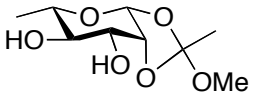
dried over $MgSO_4$. The crude product was purified by silica column chromatography (ethyl acetate/hexane, 1/4, v/v) to afford the title compound title compound (2.10 g, 6.92 mmol, 87%) as a white solid.

R_f (ethyl acetate/hexane): 0.57 (2/3).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.67 (d, J = 2.0 Hz, 1H, H-1), 5.35 (dd, J = 10.0 Hz, 4.0 Hz, 1H, H-3), 5.12 (t, J = 9.6 Hz, 1H, H-4), 4.78 (dd, J = 4.4 Hz, 2.4 Hz, 1H, H-2), 3.82 (m, 1H, H-5), 3.39 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 1.80 (s, 3H, CH_3), 1.34 (d, J = 6.4 Hz, 3H, H-6).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 170.4, 170.4, 124.1, 97.4, 77.4, 70.8, 70.6, 68.4, 50.0, 25.7, 20.9, 20.8, 17.0.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 327.1056; Found: 327.2201.



1,2-O-Methoxyethylidene-β-L-rhamnopyranose.

To a solution of 3,4-*O*-di-acetyl-1,2-*O*-methoxyethylidene-β-L-rhamnopyranose (2.00 g, 6.58 mmol) in methanol (20 mL) was added small pieces of sodium. The reaction mixture was stirred at room temperature for 5 hours. TLC confirmed the complete conversion of the starting material. The reaction mixture was concentrated under reduced pressure and loaded onto silica gel for column chromatography (ethyl acetate/hexane, 9/1, v/v) purification to

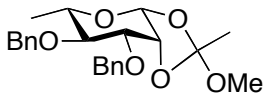
afford the title compound. The solvent was removed under reduced pressure to obtain the title compound (1.39 g, 6.32 mmol, 96%) as a white solid.

R_f (chloroform/methanol): 0.67 (4/1).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.48 (d, J = 2.4 Hz, 1H, H-1), 4.52 (dd, J = 4.0 Hz, 2.0 Hz, 1H, H-2), 3.72 (dd, J = 8.8 Hz, 4.4 Hz, 1H, H-3), 3.34-3.29 (m, 5H), 1.67 (s, 3H, CH_3), 1.34 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 125.0, 98.9, 81.6, 73.5, 73.1, 72.0, 49.8, 25.8, 18.1.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 243.0845; Found: 243.3398.



3,4-Di-O-benzyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose.

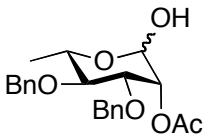
To a solution of 1,2-*O*-methoxyethylidene-β-L-rhamnopyranose (1.20 g, 5.45 mmol) in anhydrous *N, N* - dimetylformamide (30 mL) was added 60% NaH (458 mg, 11.5 mmol) and BnBr (1.36 mL, 11.5 mmol). The reaction mixture was stirred at room temperature for 2 hours. TLC confirmed the complete conversion of the starting material. The reaction mixture was diluted with dichloromethane (50 mL) and washed with water (3 × 50 mL), and brine (3 × 50 mL). The organic layer was collected and dried over $MgSO_4$. The crude product was purified by silica column chromatography (ethyl acetate/hexane, 1/4, v/v) to afford the title compound (1.98 g, 4.96 mmol, 91%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.74 (2/3).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.53-7.27 (m, 10H), 5.37 (d, J = 2.0 Hz, 1H), 4.90 (d, J = 10.4 Hz, 1H), 4.79 (d, J = 11.6 Hz, 1H), 4.70 (d, J = 10.0 Hz, 1H), 4.68 (d, J = 9.2 Hz, 1H), 4.57 (dd, J = 4.0 Hz, 2.4 Hz, 1H), 3.85 (dd, J = 8.8 Hz, 4.0 Hz, 1H), 3.38 (m, 1H), 3.27 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.29 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 140.0, 139.7, 129.6, 129.5, 129.3, 129.3, 129.0, 128.9, 125.2, 99.1, 81.0, 80.1, 78.9, 76.3, 73.0, 71.3, 50.0, 25.7, 18.4.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 423.1784; Found: 423.1708.



2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranose.

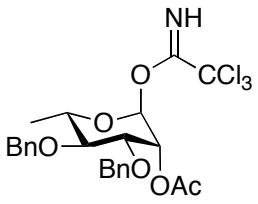
To a solution of 3,4-di-*O*-benzyl-1,2-*O*-methoxyethylidene-β-L-rhamnopyranose (1.0 g, 2.50 mmol) 80% AcOH/water solution (30 mL) was added and then stirred at room temperature for 1 hour. TLC confirmed the complete conversion of the starting material. The reaction mixture was diluted with dichloromethane (50 mL) and washed with water (3 × 50 mL), and brine (3 × 50 mL). The organic layer was collected and dried over $MgSO_4$. The crude product was purified by silica column chromatography (ethyl acetate/hexane, 1/4, v/v) to afford the title compound (0.87 g, 2.25 mmol, 90%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.74 (2/3).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.34-7.22 (m, 10 H), 5.43 (dd, J = 3.2 Hz, 1.6 Hz, 1H, H-2), 4.86 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 11.2 Hz, 1H), 4.63 (d, J = 10.8 Hz, 1H), 4.60 (d, J = 2.0 Hz, 1H), 4.50 (d, J = 11.2 Hz, 1H), 3.87 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.66 (m, 1H), 3.42 (t, J = 9.2 Hz, 1H), 3.34 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 1.20 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 172.0, 140.0, 139.5, 129.5, 129.5, 129.4, 129.2, 129.0, 128.9, 100.1, 81.3, 79.4, 72.9, 70.3, 68.9, 55.4, 21.0, 18.4.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 409.1627; Found: 409.8701.



2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranoside trichloroacetimidate (2)

To a solution of 2-*O*-acetyl-3,4-di-*O*-benzyl-α/β-L-rhamnopyranose (260 mg, 0.702 mmol) in anhydrous dichloromethane (10 mL) was added anhydrous trichloroacetonitrile (0.35 mL, 3.51 mmol) and cesium carbonate (595 mg, 1.76 mmol). The reaction mixture was stirred at room temperature for 1 hour. TLC confirmed the complete conversion of the starting material. The reaction mixture was filtered over Celite and loaded onto silica gel for column

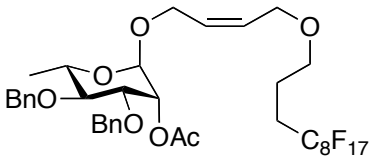
chromatography (ethyl acetate/hexane, 1/9, v/v) purification to afford the title compound (335 g, 0.632 mmol, 90%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.67 (3/7).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.68 (s, 1H, NH), 7.39-7.20 (m, 10H), 6.21 (d, J = 2.0 Hz, 1H, H-1), 5.58 (dd, J = 3.2 Hz, 2.4 Hz, 1H, H-2), 4.98 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 10.8 Hz, 1H), 4.62 (d, J = 11.2 Hz, 1H), 4.04 (dd, J = 9.6 Hz, 3.6 Hz, 1H, H-3), 3.98 (m, 1H, H-5), 3.58 (t, J = 9.6 Hz, 1H, H-3), 2.22 (s, 3H), 1.40 (d, J = 6.4 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 160.3, 138.3, 137.7, 129.2, 128.6, 128.6, 128.5, 128.4, 128.3, 95.4, 91.0, 79.5, 77.3, 75.8, 72.2, 70.9, 67.7, 21.6, 21.2, 18.2.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 552.0723; Found: 552.6624.



cis-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl-α-L-rhamnopyranoside.

To a solution of 2-*O*-acetyl-3, 4-di-*O*-benzyl-α-L-rhamnopyranosyl tricholoroacetimidate (**2**) (0.20 g, 0.46 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (0.13 g, 0.23 mmol) in dry toluene (5 mL) was added TMSOTf (0.02 mL, 0.11 mmol) at 0 ^{o}C. The reaction

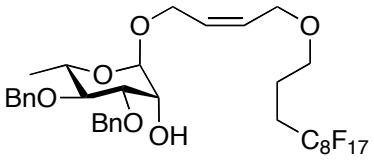
mixture was stirred at room temperature for 30 min. The reaction was quenched with triethylamine (0.5 mL) and concentrated. The crude product was purified by solid phase extraction using a fluoro flash column. Nonfluorous compounds were eluted with 5 mL 80% MeOH/water and the desired product was eluted by 5 mL 100% MeOH. The solvent was removed under reduced pressure to obtain the title compound (0.15 g, 0.175 mmol, 76%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.83 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.24 (m, 10H, Ph*H*), 5.80-5.15 (m, 2H, C*H*=), 5.37 (dd, J = 3.2 Hz, 1.6 Hz, 1H, H-2), 4.93 (d, J = 8.1 Hz, 1H, CH_2Ph), 4.76 (s, 1H, H-1), 4.71 (d, J = 8.4 Hz, 1H, CH_2Ph), 4.62 (d, J = 7.8 Hz, 1H, CH_2Ph), 4.5 (d, J = 8.4 Hz, 1H, CH_2Ph), 4.19-4.15 (dd, J = 9.3 Hz, 4.5 Hz, 1H, =CHCH_2O), 4.10-4.06 (dd, J = 9.6 Hz, 5.1 Hz, 1H, =CHCH_2O), 4.04 (d, J = 3.9 Hz, 2H, =CHCH_2O), 3.95-3.92 (dd, J = 6.9 Hz, 2.4 Hz, 1H, H-3), 3,79-3,72 (m, 1H, H-5), 3.5 (t, J = 5.1 Hz, 2H, CH_2O), 3.44 (t, J = 9 Hz, 1H, H-4), 2.30-2.14 (m, 5H, $C_8F_{17}CH_2CH_2CH_2O$, OAc), 1.95-1.80 (m, 2H, $C_8F_{17}CH_2CH_2CH_2O$), 1.45 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.6 (OAc), 139.1, 138.2 (2 × *C*H=), 131.0, 129.1, 128.9, 128.8, 128.2 (8 × C_{Ar}), 98.2 (C-1), 80.2 (C-3), 78.34 (C-2), 75.5 (C-4), 72.01 (C-5), 69.3, 69.0 (2 × *C*H_2Ph), 68.4 ($C_8F_{17}CH_2CH_2CH_2O$), 66.8 (*C*H_2O), 62.98 (*C*H_2O), 21.2 ($C_8F_{17}CH_2$), 20.8 ($C_8F_{17}CH_2CH_2$), 18.0 (C-6).

HRMS-ESI (m/z): $[M+Na]^+$ Calcd.: 939.2166; Found: 939.2122.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3,4-O-dibenzyl- α-L-rhamnopyranoside (1)

To a solution of cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-*O*-acetyl-3,4-*O*-dibenzyl-α-L-rhamnopyranoside (315 mg, 0.343 mmol) in methanol (2 mL) was added 0.5 M NaOMe (0.67 mL, 0.34 mmol) and the reaction mixture was stirred at ambient temperature for 20 min. The solution was neutralized with Amberlyst acidic resin. The mixture was filtered and washed with MeOH (10 mL) and then concentrated *in vacuo*. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/H_2O and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain deacetylated rhamnose **1** (273 mg, 0.312 mmol, 91%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.7 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.24 (m, 10H, Ph*H*), 5.73-5.66 (m, 2H, C*H*=), 4.92 (d, J = 11.2 Hz, 1H, CH_2Ph), 4.85 (s, 1H, H-1), 4.71 (s, 2H, CH_2Ph), 4.67-4.65 (d, J = 10.8 Hz, 1H, CH_2Ph), 4.22-4.17 (dd, J = 12.4 Hz, 5.2 Hz, 1H, =CHCH_2O), 4.12-4.05 (m, 4H, =CHCH_2O, H-2), 3.88-3.85 (dd, J = 9.2 Hz, 3.2 Hz, 1H, H-3), 3.79-3.72 (m, 1H, H-5), 3.51-

3.46 (m, 3H, CH_2O, H-4), 3.46 (s, 1H, O*H*), 2.57-2.16 (m, 2H, $C_8F_{17}CH_2CH_2CH_2O$), 1.95-1.80 (m, 2H, $C_8F_{17}CH_2CH_2CH_2O$), 1.45 (d, J = 6 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 138.5, 138.2 (2 × *C*H=), 130.1, 128.9, 128.7, 128.5, 128.3, 128.3, 128.0, 127.9 (8 × C_{Ar}), 98.2 (C-1), 80.2 (C-3), 80.2 (C-2), 75.5 (C-4), 72.3 (C-5), 68.9, 68.7 (2 × *C*H_2Ph), 67.5 ($C_8F_{17}CH_2CH_2CH_2O$), 66.4 (*C*H_2O), 62.6 (*C*H_2O), 28.0 ($C_8F_{17}CH_2$), 20.8 ($C_8F_{17}CH_2CH_2$), 18.0 (C-6).

HRMS-ESI (m/z): [*M*+Na]$^+$Calcd.: 897.2060; Found: 897.1884.

cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (3)

Synthesis of rhamnose pentamer-ASW rhamnose pentamer method run (3.5 cycles)

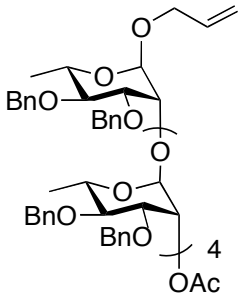
After the 4th FSPE, the methanol elution collected in the 8 mL vial was removed from the instrument and concentrated. The solvent was removed under reduced pressure to obtain the crude title compound (13 mg) as a colorless oil. After purification, 9 mg of the titled compound was obtained (16% overall yield).

R_f (ethyl acetate/haxane): 0.51 (20/80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40-7.15 (m, 50H), 5.80-5.60 (m, 2H), 5.67 (d, J = 3.2 Hz, 1.6 Hz, 1H), 5.28-4.54 (m, 25H), 4.13 (m, 18H), 3.49-3.31 (m, 7H), 2.30-2.14 (m, 5H), 1.95-1.80 (m, 2H), 1.45 (m, 15H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 138.7, 138.6, 138.6, 138.6, 138.5, 138.5, 138.5, 138.4, 138.4, 138.3, 129.9, 129.9, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 127.9, 127.9, 127.9, 127.7, 100.8, 100.5, 99.3, 98.2, 80.6, 80.5, 80.4, 80.4, 80.3, 80.3, 80.3, 79.6, 79.1, 78.0, 78.0, 77.9, 77.4, 75.7, 75.6, 74.9, 74.8, 74.77, 72.4, 72.3, 72.2, 72.1, 72.0, 69.1, 69.0, 68.7, 68.5, 68.2, 66.7, 62.7, 28.2, 21.4, 21.0, 18.2, 18.2.

HRMS-ESI (m/z): [*M*+Na]$^+$Calcd.: 2243.8238; Found: 2243.8886.



Allyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)- 3,4-di-O-benzyl-α-L-rhamnopyranoside (4)

To a solution of fluorous-tagged rhamnose pentasaccharide (25 mg, 0.011 mmol) in dry CH_2Cl_2 (2 mL), 2nd generation Grubbs' catalyst (1.8 mg, 2.10 μmol) was added. The

suspension was degassed by bubbling in ethylene gas for 30 minutes. Then after evacuating air under reduced pressure, an ethylene balloon was attached. The reaction flask was stirred at room temperature for 48 hours under an ethylene atmosphere. The suspension was filtered over a short pad of celite and silica, then concentrated. Purification by flash silica chromatography yielded the allyl saccharide (14 mg, 8.08 mmol, 71.8%).

R_f (ethyl acetate/haxane): 0.51 (20/80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.41-7.16 (m, 50 H), 5.92-5.82 (m, 1H), 5.62 (d, J = 3.2 Hz, 1.6 Hz, 1H), 5.30-4.59 (m, 27H), 4.18-3.33 (m, 22H), 2.17 (s, 3H), 1.30-1.22 (m, 15H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 138.7, 138.6, 138.6, 138.5, 138.4, 138.4, 138.2, 138.0, 137.9, 133.9, 129.2, 128.9, 128.8, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.3, 125.5, 117.3, 100.8, 100.7, 100.4, 99.3, 98.1, 80.4, 80.4, 80.3, 80.0, 79.8, 79.5, 79.4, 79.2, 78.9, 78.6, 78.3, 78.2, 78.0, 77.4, 75.7, 75.5, 74.8, 74.2, 72.4, 72.3, 72.0, 71.7, 69.6, 69.1, 68.8, 68.7, 68.6, 68.5, 68.3, 68.1, 67.8, 67.3, 21.3, 18.2, 18.1.

HRMS-MALDI (m/z): $[M+H]^+$ Calcd.: 1731.8193; Found: 1731.8204.

Additional Information: Experimental details, including copies of the ^{1}H NMR spectra, for the synthesis are found in the Appendix at the end of this dissertation.

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CHAPTER 3

Automated Solution-Phase Synthesis of Anthrax- and Cholera-Associated Antigenic Tetrasaccharides

Portions of this chapter will be submitted to *The Journal of the American Chemical Society*

Gisun Park and Nicola L. B. Pohl*

Abstract

The first automated solution-phase syntheses of two key antigenic tetrasaccharides associated with anthrax and cholera is reported. Two protecting group strategies were newly introduced into an automated strategy that relies on robotic fluorous solid-phase extractions (FSPE) for purification of intermediates and both groups proved to be good masking entities under automated conditions. After careful design of the automated synthetic protocol, both tetrasaccharides were successfully synthesized side by side simultaneously. In addition to providing two important antigenic saccharides for possible antibody screening, these results lend credence to the prospect of using this platform for the synthesis of carbohydrate libraries.

Introduction

To extend the scope of the automated solution-phase oligosaccharide synthesis platform [1, 2], two new oligosaccharides were tested to investigate the general applicability of the methods. *Bacillus anthracis*, which has spores called anthrax, is a well known cause of serious infections in cattle and other herbivores [3]. The structure of an antigenic surface oligosaccharide of the bacteria contains an exotic sugar called anthrose. This unusual terminal sugar is not found even in closely related species, which makes it an interesting target for carbohydrate-based vaccines and diagnostics for anthrax. During its synthesis [4], an intermediate from the anthrose building block resembles the repeating building block of perosamine that causes cholera [5]. Herein, we report the linear automated synthesis of anthrax (**1**) and cholera (**2**) tetrasaccharide by a divergent synthetic strategy targeting both building blocks. Key steps in our synthetic strategy for making the oligosaccharide include the selective inversion of the 6-deoxygenated D-galactose, microwave-accelerated reactions, and fluorous-tail assisted separation via fluorous column.

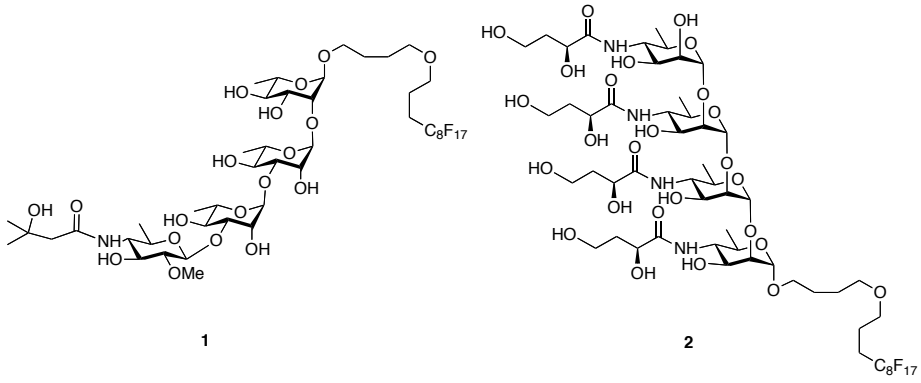


Figure 3-1. Structures of the anthrax- and cholera-associated antigenic tetrasaccharide targets.

Results and discussion

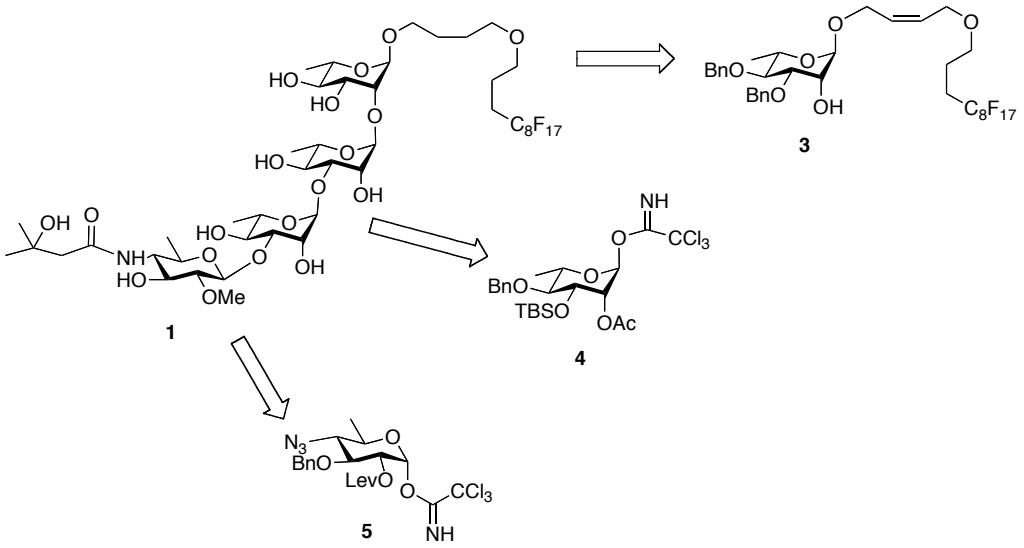


Figure 3-2. Retrosynthetic analysis of the anthrax-associated tetrasaccharide target.

Our synthetic scheme was designed to have a linear approach for both the anthrax- and cholera-antigenic tetrasaccharides and thereby mimic the biosynthetic pathway for these oligosaccharide compounds (Figure 3-2). The anthrax tetrasaccharide was started by synthesizing the first three building blocks from L-rhamnose (Figure 3-3). The 1,2- and 1,3-glycosidic linked building blocks were both made starting from the known 1,2-ortho ester-rhamnose pyranoside [6]. Treating compound **6** with 1.3 equiv**.** benzyl bromide and sodium hydride provided not only the dibenzylated compound **7** but also the desired mono-benzylated compound **8** in a 1:1.4 ratio. For the synthesis of the anthrax teterasaccharide, donors **9** and **4** are required in a 1:2 ratio. Therefore, these reaction conditions conveniently provided both donors [7]. The mono-benzylated compound was then protected at the 3-position with a silyl group and further activated, after in situ opening of the orthoester to reveal a free anomeric hydroxyl, to give the desired trichloroacetimidate **4**. Compound **7** was

directly activated in a similar manner to give the imidate compound **9**, which was activated and then glycosylated to a fluorous tail [8] acceptor to provide (**3**). The fluorous tail at the anomeric position of the reducing end of the initial rhamnose building block will serve as a purification handle during the automation process.

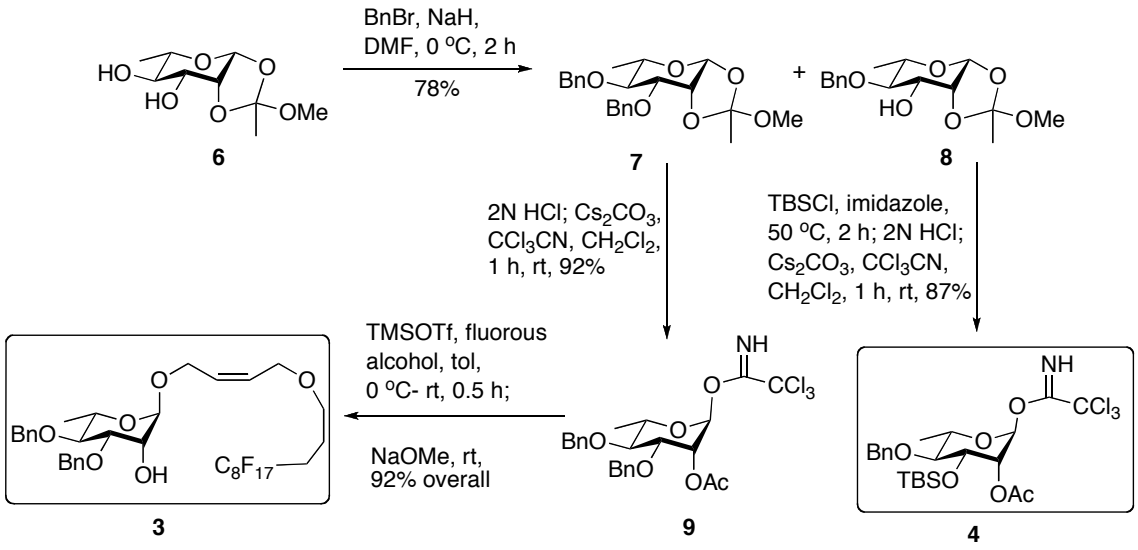


Figure 3-3. Synthetic strategy for the rhamnose building block.

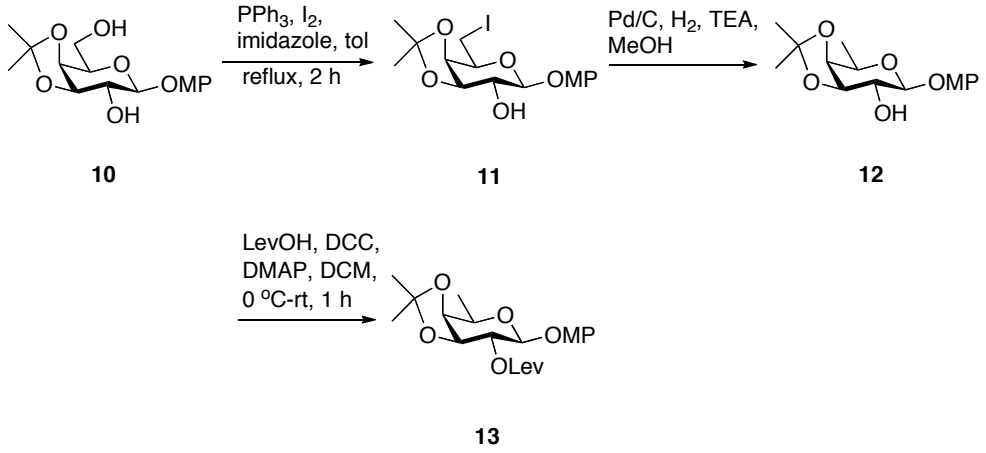


Figure 3-4. Synthetic strategy for the anthrose/perosamine building block from D-galactose

The anthrose/perosamine building block was started from D-galactose to give compound **10** in 4 steps. The D-fucose molecule was achieved by iodination followed by hydrogenation over Pd/C conditions. And after the 2 position was selectively protected and acetonide group removed, compound **14** was synthesized. To introduce the equatorial azide functionality **14** was introduced to a series of reaction conditions. After the triflate addition on the 4-position of the fucose derivative, the inversion step was conducted under microwave conditions with sodium azide to give compound **15** in 1 h. Under well-known procedures [9], the *p*-methoxyphenol(OMP) group was removed, and the building block was completed after activation to the trichloroacetimidate to give compound **5** for the anthrose building block. From the common intermediate **15**, the perosamine building block was made by unmasking the levulinic ester group and performing a second inversion under microwave conditions. Triflate addition followed by inversion using tetra butyl ammonium acetate under microwave conditions ultimately provided compound **16**. Finally, removal of the OMP group provided the anomeric alcohol and after activation to the trichloroacetimidate, the perosamine building block **17** was in hand. In order to provide the handle for automated purification in our solition-phase synthesis platform, a fluorous allyl alcohol was attached at the anomeric center of the reducing end of the molecule. Deacetylation of the fluorous-tagged monomer gave **18** as the initial molecule to extend the chain under automation.

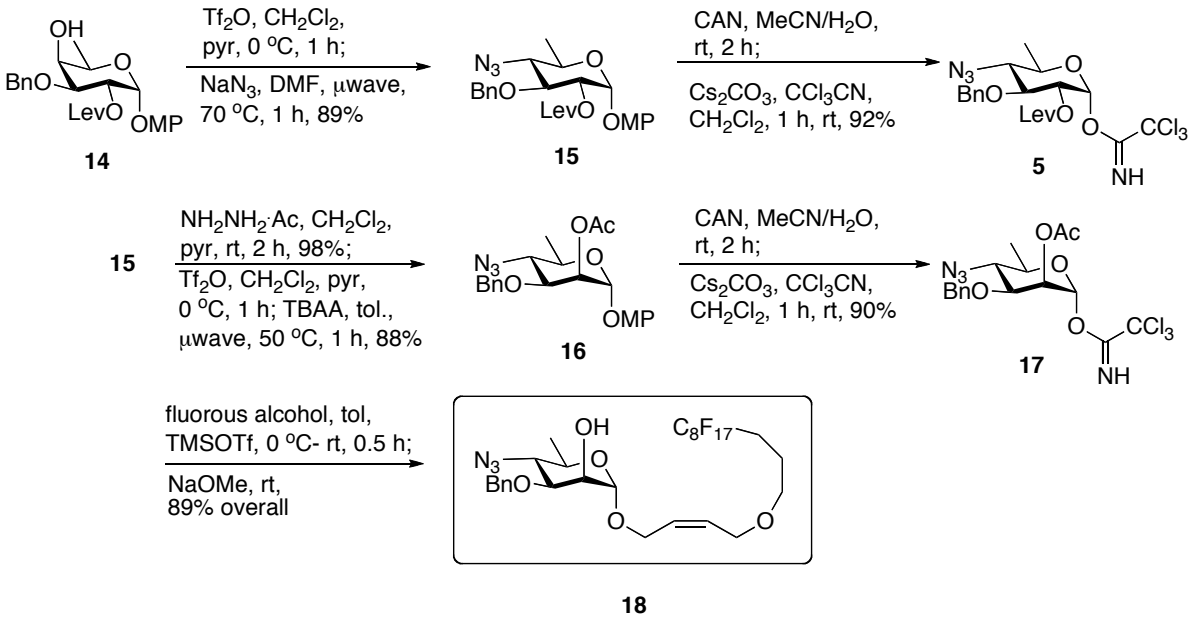


Figure 3-5. Divergent synthetic strategy for the anthrose and perosamine building block.

With the initial acceptor monomers and donors in hand, programming of the Chemspeed Automated Synthesis workstation (ASW1000) was initiated to build the target molecules robotically. As a prelude to the construction of carbohydrate libraries, we were particularly interested in developing conditions to synthesis these different oligosaccharides simultaneously. However, the variations in reaction times coupled with the unknown stability of intermediates posed challenges at the onset. First, the synthesis of each oligosaccharide was attempted singly. The anthrax tetrasaccharide was programmed to go through 3 cycles. Each cycle generally consists of three main tasks: the coupling, deprotection and fluorous solid phase extraction (FSPE). The fluorous tag is incorporated on every initial acceptor building block for ease of purification of the growing chain by washing away any excess donor molecules and reagents that are not tagged. The synthesis was started with the previously discussed acceptor **3**. After 3 cycles, tetrasaccharide **19** was constructed in 35

hours in 22% overall isolated yield. The anthrax tetrassaccharide automation protocol was necessarily different from the initial rhamnose pentasaccharide study because silyl and levulinate protecting groups were introduced. Due to the position of the protecting group (the *O*-3 position), longer than expected reaction times were required using tetrabutylammoniumfluoride (TBAF)/tetrahydrofuran (THF) for the desilylation conditions. However, the both deprotection conditions ultimately proved to be amenable to automation and thereby provided options in designing an orthogonal protecting group strategy in the future. The newly programmed deprotection conditions also resulted in high yields of the deprotected intermediate. The HPLC traces of the crude product from the robotics platform showed only one major product that was later characterized to be the desired tetrasaccahride compound. The robotics platform succesfully synthesized the anthrax-associated tetrasaccharide from two different donors and with two different deprotection protocols.

After careful purification of the product collected from the synthesizer, tetrasaccharide **19** was further elaborated manually to provide the final compound ready for incorporation into fluorous-based microarrays [10] (Figure 3-6). First, the methylation was accomplished under silver oxide conditions with refluxing methyl iodide as solvent itself. Other conditions using various solvents instead of neat condition were reported to be inefficient to provide moderate yield [4]. The 4-azido group was then reduced to an amide for side chain attachment. The side chain synthesis was completed in 2 steps starting with ethyl bromoacetate (**20**) (Figure 3-7). Surprisingly, under microwave conditions, the Reformatsky reaction [11] was completed within 10 minutes even with unactivated zinc to give the desired compound **21**. Following the completion of these studies on the side chain synthesis, another

group published experiments that show similar microwave-acceleration of the Reformatsky reaction [11]. Following purification of the Reformatsky product, the compound was oxidized to give the corresponding acid.

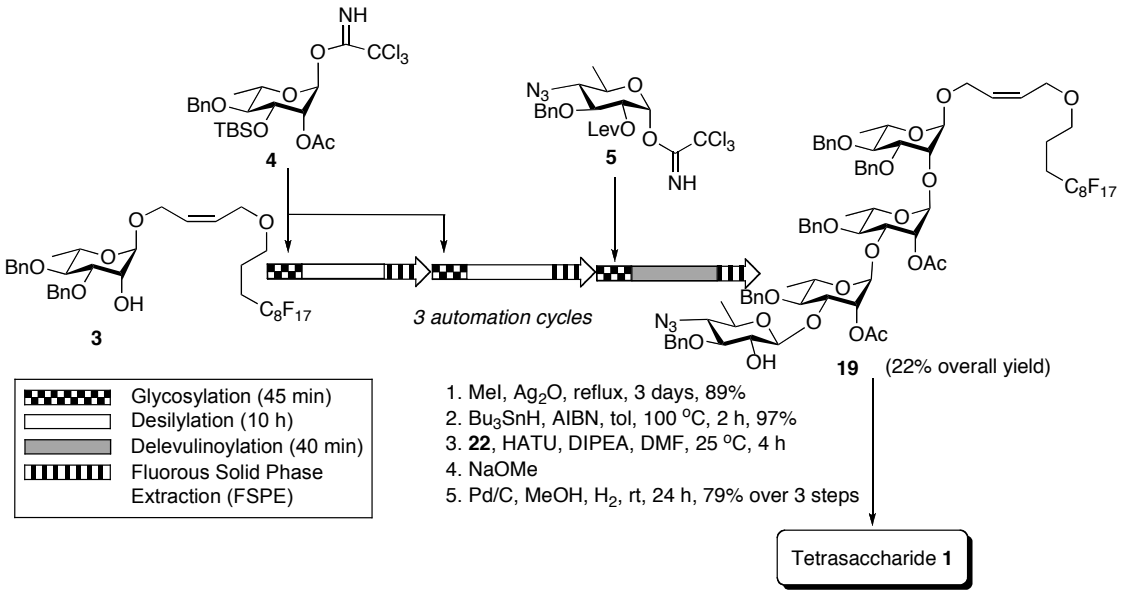


Figure 3-6. Automated synthesis of the anthrax-associated tetrasaccharide and post-automation modifications.

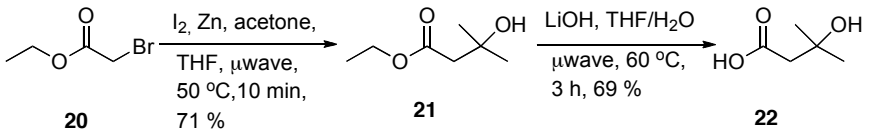


Figure 3-7. Side chain synthesis of the anthrax tetrasaccharide

After carboxylic acid **22** was synthesized, it was attached to the tetrasaccharide compound under 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl

uroniumhexafluorophosphate (HATU), diisopropylethylamine (DIPEA) conditions to give the side chain attached compound. Global deprotection under standard basic and then hydrogenation conditions provided the final desired fluorous-tagged anthrax tetrasaccharide **1**.

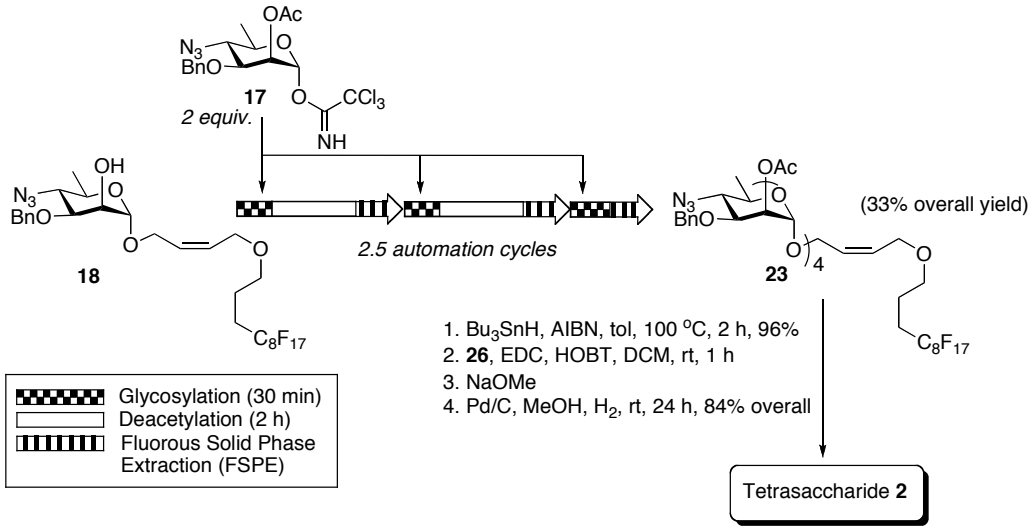


Figure 3-8. Automated synthesis of the cholera-associated tetrasaccharide and post-automation modifications.

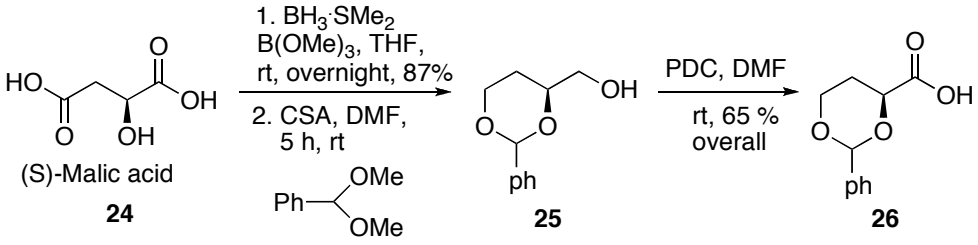


Figure 3-9. Side chain synthesis of cholera tetrasaccharide.

Next, the cholera tetrasaccharide automation program was approached (Figure 3-8). The prepared fluorous-tagged acceptor **18** was used as the initial building block, and 2.5

cycles were executed to build the tetrasaccharide. In approximately 16 hours the ASW provided the desired compound **23** in high yields. For the perosamine tetrasaccharide, the identical program used for the optimization study with the rhamnose pentasaccharide molecule was used. After purification, the tetrasaccharide was subjected to radical reaction conditions to reduce the azido groups. The necessary amide-linked side chain was synthesized by a known method in 3 steps using (S)-malic acid as starting material [12] (Figure 3-9). The requisite acid compound was then attached to the amino groups of the molecule and, after global deprotection, the repeating unit cholera tetrasaccharide **2** was accomplished (Figure 3-8).

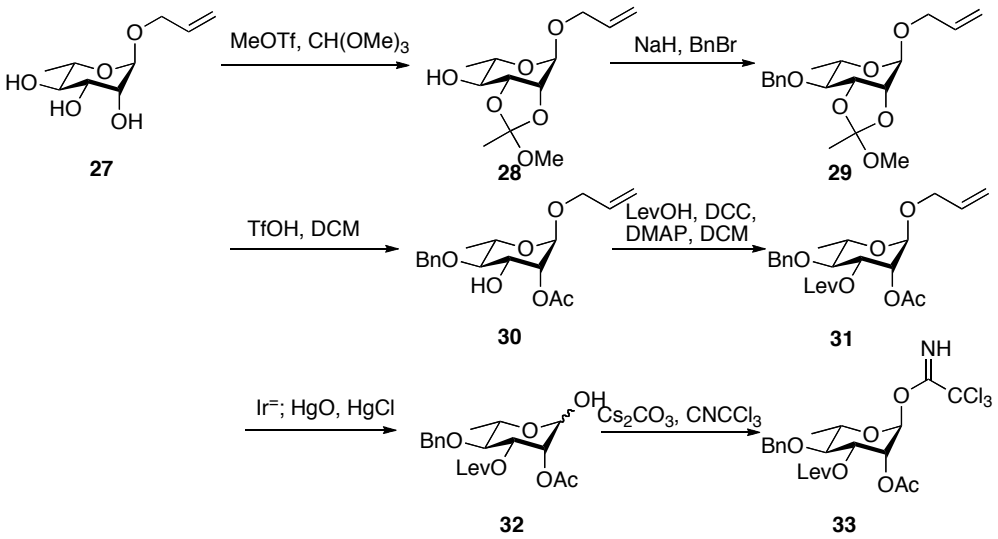


Figure 3-10. Alternative synthetic route for the modified rhamnose donor **33**.

After establishing initial programs for the synthesis of each of the two antigenic oligosaccharides, we next attempted to construct both compounds simultaneously on the automation platform. To this end, the protecting group on the first rhamnose building block of the anthrax tetrasaccharide was modified from a silyl group to a levulinate group in order

to have the same deprotection time during the cycles (Figure 3-10). Then, using the program originally designed for the cholera oligosaccharide, it was possible to have a single program to make both compounds in parallel. The differing reaction times were not problematic; there was no evidence by thin layer chromatography (TLC) of significant decomposition of products between cycles. Both antigenic tetrasaccharides were successfully synthesized in high overall yields: 28% and 33% for the anthrax- and cholera- associated tetrasaccharides respectively (Figure 3-11). These overall yields translate to yields of 81% and 80% respectively per step for each of the two tetrasaccharides.

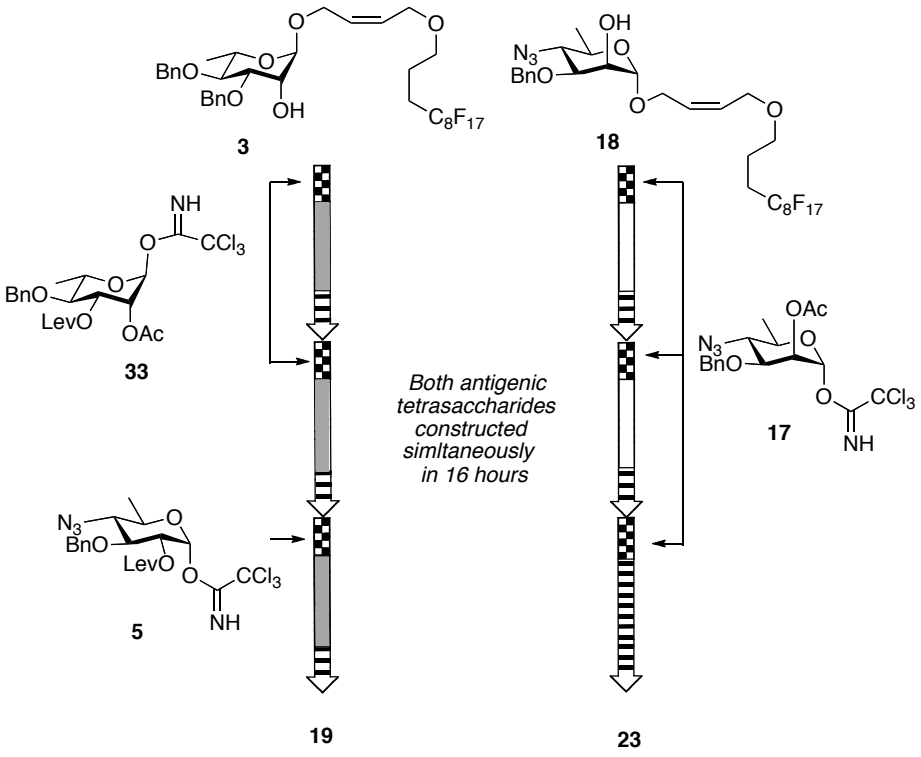


Figure 3-11. Simultaneous synthesis of the two tetrasaccharides under automated conditions.

Conclusion

The successful synthesis of both anthrax- and cholera-associated tetrasaccharides in parallel shows the power of the solution-based automation platform for the synthesis of pure structurally well-defined carbohydrate compounds. Automation protocols were developed that were capable of adding various building blocks for standard glycosylation conditions and able to carry out different solution-phase deprotection conditions to completion. The fluorous tag offers a convenient and apparently robust purification tool for automated oligosaccharide synthesis to eliminate byproducts throughout the synthesis. In addition to providing two important antigenic saccharides for possible antibody screening, these results lend credence to the prospect of using this platform for the synthesis of carbohydrate libraries.

Experimental section

General methods

All commercial reagents and solvents were used as received without further purification unless indicated. Reaction solvents were distilled from calcium hydride for dichloromethane and from sodium metal and benzophenone for diethyl ether. 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 162 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 μL 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.

General procedure for automated synthesis cycles using ASW 1000

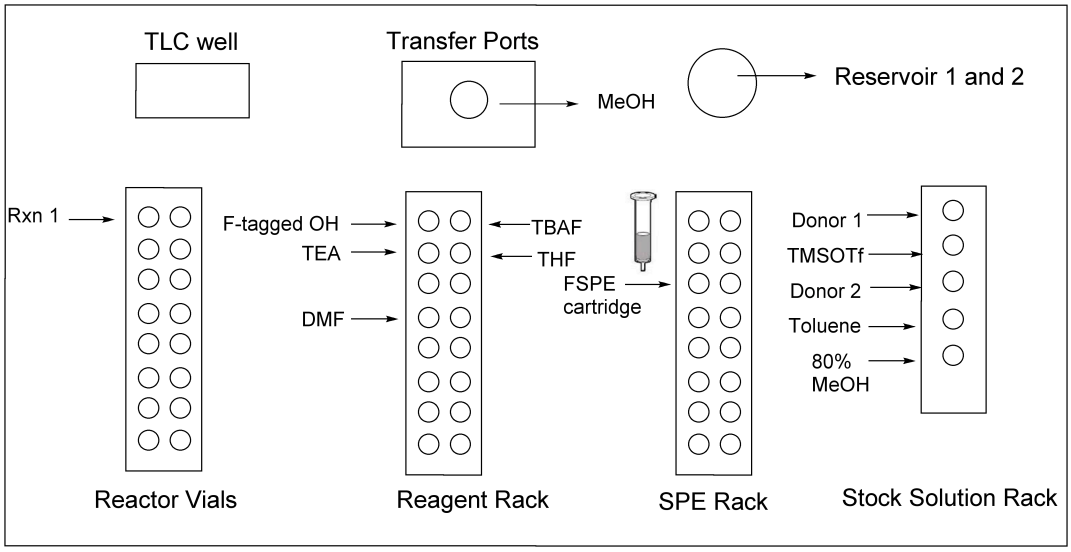


Figure 3-12. ASW Automated Synthesizer layout for anthrax associated tetrasaccharide.

1. Sample Preparation

Donor molecule (265.4 mg, 500 μmol) was dissolved with dried toluene (2.5 mL) in the 13 mL vial and placed at the stock solution rack (Donor 1) under argon. 0.27 M trimethylsilyltrifluoromethanesulfonate (TMSOTf, 1.05 mL) solution in toluene prepared in the 8 mL vial and placed as indicated on the stock solution rack under argon. Dried toluene (1 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Methanol (100 mL), 80% methanol/water (100 mL), triethylamine (8 mL) were prepared in stock solution bottles and connected to the transfer ports. Acceptor molecule (43.7 mg, 50 μmol) was dissolved with dried toluene (0.5 mL) in a Wheaton 8 mL E-Z extraction vial (conically- bottomed) capped with pre-punctured septa and placed at the reagent rack where indicated (F-tagged OH).

0.5 M sodium methoxide solution in methanol (5 mL) was prepared in 8 mL vial capped with pre-punctured septa and placed at the reagent rack. 0.2 M acetic acid solution in methanol (5 mL) (or TBAF solution in THF, Hydrazine acetate in MeOH/DCM) was prepared in 8 mL vial capped with pre-punctured septa and placed at the reagent rack where indicated. N, N-dimethylformamide (DMF, 5 mL) directly from Fluka was transferred in 8 mL vial capped with pre-punctured septa and placed at the reagent rack where indicated. Fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol(/water) and placed at machined SPE block. An empty Wheaton 8 mL E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle each of the 16 reactor vials (13 mL) were rinsed with 8 mL toluene and 8 mL methanol 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solutions were prepared by azeotroping the building blocks individually with toluene and dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Method Run

3.1. Glycosylation

Reactor vials were cooled to 0 ^{o}C during the 5 minutes wait time by the heat transfer oil. Then flat-tipped open vial needle transferred the acceptor molecule (F-tagged OH) solution (0.6 mL) to the reaction vial 1, followed by the transfer of the acceptor molecule solution (0.5 mL) and the TMSOTf solution (56 μL). After each individual transfer, the needle was rinsed by 2 mL toluene inside and out before operating the next task. The reaction mixture was vortexed at 800 rpm for 30 minutes at 0 ^{o}C under inert gas. After the reaction time the needle withdrew 30 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. Triethylamine (0.5 mL) was added to the solution for quenching and solvent evaporated under reduced pressure.

3.2. Deacetylation

To the dried residue, methanol (0.5 mL) was added to the reactor vial followed by sodium methoxide solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 3 hours at room temperature. After the reaction time the needle withdrew 30 μL from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. Acetic acid (0.75 mL) solution was added to reactor vial for quenching followed by addition of toluene (1 mL) and solvent was evaporated under reduced pressure.

3.3. Desilylation

To the dried residue, THF (0.5 mL) was added to the reactor vial followed by TBAF solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 10 hours at room temperature. After the reaction time the needle withdrew 30 μL from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. Toluene (1 mL) was added tor eactor vial and solvent was evaporated under reduced pressure.

3.4. Delevulination

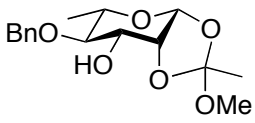
To the dried residue, DCM (0.5 mL) was added to the reactor vial followed by Hydrazine Acetate/methanol solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 3 hours at room temperature. After the reaction time the needle withdrew 30 μL from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. Acetone (0.75 mL) solution was added to reactor vial for quenching followed by addition of toluene (1 mL) and solvent was evaporated under reduced pressure.

3.5. FSPE

DMF (0.7 mL) was added to dissolve the crude mixture and the vials were vortexed at 100 rpm for 5 minutes. Reaction mixture (0.8 mL) was carried to the FSPE cartridge at the SPE rack dispensed at a speed of 20 mL/s via the 10 mL syringe. Then 80% methanol (4.5

mL) was used to rinse the empty reactor vial. 80% methanol solvent was removed from the reactor vial each time (1.7 mL, 1.5 mL, 1.5 mL) and delivered to the FSPE cartridge. Additional 80 % methanol solution (1 mL) 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 (1.5 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the vial and deliver it to the clean reactor vial for the next reaction. Toluene (1 mL) was added to the solution and solvent was evaporated under reduced pressure. After evaporation cycle, once again toluene (1 mL) was added and removed under reduced pressure for complete dryness.

Synthetic Procedures



4-O-Benzyl-1,2-O-methylethylidene-β-L-rhamnopyranose (8)

To a solution of **6** (3.34 g, 15.2 mmol) in dry DMF (25 mL) were added sodium hydride (0.72 g 13.3 mmol) and benzylbromide (2.67 mL, 22.5 mmol) at 0 ^{o}C. The reaction mixture was stirred for 2 hours at 0 ^{o}C. Then reaction mixture was diluted with ethyl acetate (100 mL) and the organic layer was washed with 2N HCl solution (2 × 100 mL), brine (2 × 100

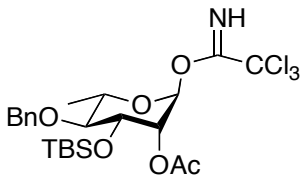
mL) and dried (Mg_2SO_4), concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the dibenzylated (1.94 g, 4.85 mmol, 32%, white solid) and monobenzylated product (2.16 g, 6.98 mmol, 46%, colorless oil).

R_f (ethyl acetate/hexane): 0.56 (2/3).

^{1}H NMR (CD_3OD, 400MHz): δ (ppm) 7.46-7.29 (m, 5H, Ar-H), 5.46 (d, J = 2.1 Hz, 1H, H-1), 4.73 (s, 2H, CH_2), 4.84 (dd, J = 4.2 Hz, 2.4 Hz, 1H, H-2), 3.58 (dd, J = 9.3 Hz, 4.2 Hz, 1H, H-3), 3.69 (t, J = 9.3 Hz, 1H, H-4), 3.30 (m, 1H, H-5), 3.20 (s, 3H, OCH_3), 1.59 (s, 3H, CH_3), 1.26 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 139.9, 129.5, 129.3, 128.9, 125.1, 99.1, 80.4, 79.1, 73.4, 72.5, 72.0, 49.8, 25.5, 18.1.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 333.1314; Found: 333.1470.



2-O-Acetyl-4-O-benzyl-3-O-tertbutyldimethylsilyl-β-L-rhamnopyranosyl Trichloroacetimidate (4)

To a solution of the monobenzylated orthoester (**8**) (650 mg, 2.09 mmol) in DMF (20 mL) were added imidazole (428 mg, 6.28 mmol) and TBSCl (632 mg, 4.18 mmol). The reaction

mixture was stirred for 3 hr at 50 ^{o}C. After TLC starting material was completely converted, reaction mixture was diluted with dichloromethane (50 mL). 2N HCl solution (100 mL) was added to the diluted organic mixture and was stirred for 30 min. The organic layer was washed with additional 2N HCl solution (2 × 100 mL), brine (2 × 100 mL) and dried (Mg_2SO_4), concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide 2-*O*-acetyl-4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-α/β-L-rhamnopyranose (760 g, 1.79 mmol, 86%, colorless oil).

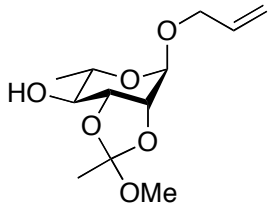
To a solution of 2-*O*-acetyl-4-*O*-benzyl-3-*O*-tertbutyldimethylsilyl-α/β-L-rhamnopyranose (760 mg, 1.85 mmol) in dry CH_2Cl_2 (10 mL) were added distilled trichloroacetonitrile (0.927 mL, 9.25 mmol) and Cs_2CO_3 (1.50 mg, 4.65 mmol). The reaction mixture was stirred for 1 h at room temperature. Then after reaction mixture was filtered over Celite, solvent was concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as a colorless oil (890 mg, 1.65 mmol, 87%).

R_f (ethyl acetate/hexane): 0.60(1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.65 (s, 1H), 7.39-7.29 (m, 5H), 6.14 (s, 1H), 5.24 (t, J = 2.4 Hz, 1H), 4.94 (d, J = 10.8 Hz, 1H), 4.64 (d, J = 10.8 Hz, 1H), 4.22 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.93 (m, 1H), 3.48 (t, J = 9.6 Hz, 1H), 2.19 (s, 3H), 1.31 (d, J = 6.0 Hz, 1H), 0.92 (s, 9H), 0.11 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 160.3, 138.3, 128.6, 128.2, 128.0, 95.2, 91.2, 80.8, 75.9, 71.5, 71.1, 71.0, 26.0, 21.2, 18.1, 18.1, -0.5, -0.5.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 576.1119; Found: 576.2440.



Allyl 4-O-benzyl-2,3-O-methoxyethylidene-α-L-rhamnopyranose (28)

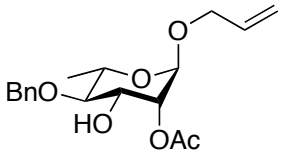
To a solution of **27** (4.46 g, 21.3 mmol) in DCM (50 mL) was added $CH_3C(OMe)_3$ (6.8 mL, 53.4 mmol) and CSA (247 mg, 1.07 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After TLC showed completed conversion of the starting material the reaction was quenched with a few drops of TEA. Solvent was removed under reduced pressue and crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the titled product as colorless oil (4.91g, 18.9 mmol, 88%).

R_f (ethyl acetate/hexane): 0.57 (2/3).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 6.03 (m, 1H), 5.47 (dd, J = 17.2 Hz, 1.6 Hz, 1H), 5.25 (dd, J = 10.4 Hz, 1.2 Hz, 1H), 5.00 (s, 1H), 4.33 (d, J = 6.0 Hz, 1H), 4.22 (m, 2H), 4.08 (dd, J = 11.6 Hz, 4.8 Hz, 1H), 3.65 (m 1H), 3.26 (m, 1H), 1.63 (s, 3H), 1.29 (d, J = 6.0 Hz, 3H).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 135.7, 122.7, 117.8, 97.3, 77.9, 75.3, 69.1, 66.6, 50.1, 23.6, 17.8.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 283.1158; Found: 283.1760.



Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (30)

To a solution of **28** (458 mg, 1.76 mmol) in DMF (10 mL) was added BnBr (0.313 mL, 2.64 mmol), and NaH (60%, 140 mg, 3.52 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 hr. After TLC confirmed complete starting material consumption the reaction mixture was diluted with dichloromethane (20 mL), washed with water (20 mL), $NaHCO_3$ solution (20 mL), and brine (20 mL), and dried over $MgSO_4$. Solvent was removed under reduced pressure and the crude product allyl 4-*O*-benzyl-2,3-*O*-methoxyethylidene-α-L-rhamnopyranose (589 mg, 1.68 mmol, 96 %) was used for the next step without purification.

To a solution of allyl 4-*O*-benzyl-2,3-*O*-methoxyethylidene-α-L-rhamnopyranose (538 mg, 1.538 mmol) in dry dichloromethane (10 mL), was added TfOH/water (v:v=3:2, 0.95 mL) solution at room temperature. The reaction mixture was stirred at room temperature for 1 hr. After TLC confirmed complete starting material consumption, the reaction mixture was diluted with dichloromethane (20 mL), washed with water (20 mL), $NaHCO_3$ solution (20

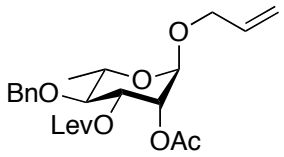
mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 25% EtOAc/hexane as eluent to obtain the title compound (501 mg, 1.49 mmol, 97%) as colorless oil.

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

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.35-3.26 (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 ($CDCl_3$, 100 MHz): 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-MALDI(m/z): $[M+Na]^+$ Calcd: 359.1471; found: 359.2668.



Allyl 2-O-acetyl-4-O-benzyl-3-O-levulinyl-α-L-rhamnopyranoside (31)

To a solution of **30** (457.5 g, 1.36 mmol) in anhydrous dichloromethane (5 mL), was added DMAP (165 mg, 1.36 mmol), levulinic acid (0.167 mL, 1.63 mmol), DCC (335.8 g, 1.63 mmol) at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 1 hr under nitrogen. After TLC

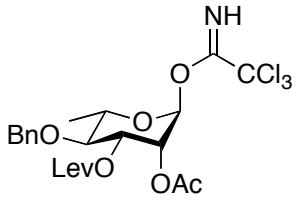
showed complete conversion, reaction mixture was filtered and concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 30% EtOAc/hexane as eluent to provide the product as colorless oil (507 mg, 1.17 mmol, 86%).

R_f (ethyl acetate/hexane): 0.40 (3/7).

^{1}H NMR ($CDCl_3$, 400 MHz): 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 ($CDCl_3$, 100 MHz): 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-MALDI(m/z): $[M+Na]^+$ Calcd: 457.4693; Found: 457.3035.



2-O-Acetyl-4-O-benzyl-3-O-levulinyl-α/β-L-rhamnopyranosyl Trichloroacetimidate (33)

To a solution of **31** (527 mg, 1.21 mmol) in dry THF (6 mL), was added $Ir(COD)(PMePh_2)_2 \cdot HPF_6$ (10 mg, 12.1 μmol). After the reaction flask was evacuated, H_2 balloon was added until the reaction mixture turned to a pale yellow. The hydrogen balloon was removed and the reaction mixture was stirred under nitrogen at room temperature for an additional 3 h. The reaction was diluted with dichloromethane (20 mL), washed with water (20 mL), HCl (2N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. The solvent was concentrated under reduced pressure to give crude product to be used directly for the next step.

To a solution of the crude isomerized compound (520 mg, 1.19 mmol) in acetone/water (5/1, 6 mL) was added $HgCl_2$ (328.5 mg), and HgO (262 mg) at room temperature. The reaction mixture was stirred under nitrogen for 5 hours then was diluted with dichloromethane (20 mL), wased with aqueous KI solution (20 mL), water (20 mL), HCl (2 N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 15% EtOAc/hexane as eluent to obtain the free anomeric intermediate **32** (458 mg, 0.16 mmol, 97%) as colorless oil.

To a solution of 32 (456.9 mg, 1.159 mmol) in dry CH_2Cl_2 (10 mL) were added distilled trichloroacetonitrile (0.581 mL, 5.79 mmol) and Cs_2CO_3 (944 mg, 2.90 mmol). The reaction mixture was stirred for 1 h at room temperature. Then after reaction mixture was filtered over Celite, solvent was concentrated under reduced pressure. Crude product was purified by flash

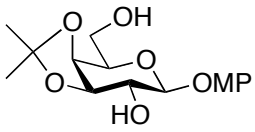
column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as colorless oil (604.65 mg, 1.19 mmol, 97.4%).

R_f (ethyl acetate/hexane): 0.6 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): 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 ($CDCl_3$, 100 MHz): 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-ESI (m/z): $[M+Na]^+$ Calcd: 560.0622; Found: 560.0199.



4-Methoxyphenyl 3,4-O-isopropylidene-β-D-galactopyranoside (10)

To a solution of 4-methoxyphenyl β-D-galactopyranoside (452 mg, 1.57 mmol) in dry DMF (5 mL) were added 2,2-dimethoxy propane (0.40 mL, 3.23 mmol) and CSA (74 mg, 0.31 mmol). The reaction mixture was stirred for 12 h at room temperature. After reaction mixture was quenched with triethylamine (1 mL), solvent was evaporated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 30%

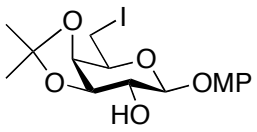
EtOAc/hexane as eluent to provide the titled product as colorless oil (453 mg, 1.38 mmol, 88%).

R_f (ethyl acetate/hexane): 0.15 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.99 (d, J = 9.2 Hz, 2H, Ar-H), 6.84 (d, J = 8.8 Hz, 2H, Ar-H), 4.72 (d, J = 8.4 Hz, 1H, H-1), 4.20-4.16 (m, 2H), 4.09-3.97 (d, 2H), 3.87-3.80 (d, 2H), 3.76 (s, 3H, $-OCH_3$), 2.69 (d, J = 2.4 Hz, 1H, OH), 2.20 (dd, J = 9.2 Hz, 3.2 Hz, 1H, OH), 1.53 (s, 3H, CH_3), 1.38 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.4, 151.1, 118.8, 114.6, 110.5, 101.5, 79.1, 73.8, 73.7, 73.2, 62.1, 55.7, 26.4, 25.6.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 349.1263; Found: 349.1263.



4-Methoxyphenyl 6-iodo-3,4-O-isopropylidene-β-D-galactopyranoside (11)

To a solution of **10** (616 mg, 1.89 mmol) in toluene (10 mL), was added iodine (718.8 mg 2.83 mmol), imidazole (257 mg, 3.78 mmol) and triphenylphosphine (743 mg, 2.83 mmol). The reaction mixture was refluxed for 2 h. After TLC showed complete conversion, reaction mixture was cooled to room temperature and diluted with ethyl acetate. The mixture was washed with Na_2CO_3 solution (3 × 30 mL), water (3 × 30 mL) and brine (3 × 30 mL). The

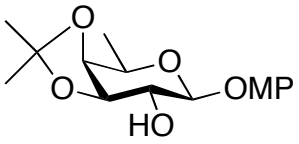
organic layer was dried (Mg_2SO_4) and concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as colorless oil (669.65 mg, 1.60 mmol, 85%).

R_f (ethyl acetate/hexane): 0.4 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.05 (d, J = 9.2 Hz, 2H, Ar-H), 6.83 (d, J = 8.8 Hz, 2H, Ar-H), 4.64 (d, J = 8.4 Hz, 1H, H-1), 4.35 (dd, J = 5.6 Hz, 2 Hz, 1H), 4.19 (dd, J = 7.2 Hz, 5.2 Hz, 1H), 4.02 (dt, J = 6.4 Hz, 2.4 Hz, 1H), 3.83 (dd, J = 7.6 Hz, 2.4 Hz, 1H), 3.78 (s, 3H, -OCH_3), 3.42 (m, 2H, H-6), 2.81 (d, J = 2.8 Hz, 1H, OH), 1.58 (s, 3H, CH_3), 1.39 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.8, 151.9, 118.8, 114.6, 110.5, 102.0, 78.9, 74.3, 74.0, 73.2, 62.1, 55.8, 28.1, 26.5, 2.0.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 459.0281; Found: 459.0212



4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-β-D-galactopyranoside (12)

To a solution of **11** (143 mg, 0.33 mmol) in methanol (1 mL) and triethylamine (0.15 mL), was added 10% Pd/C (50 mg). After the air was evacuated from the reaction flask, a hydrogen balloon was attached. The reaction mixture was stirred at room temperature for 10 h. After TLC showed complete conversion, reaction mixture was filtered over Celite and concentrated under reduced pressure. Crude product was purified by flash column

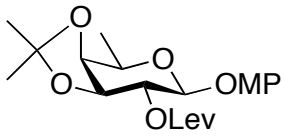
chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the titled product as colorless oil (98 mg, 0.32 mmol, 96%).

R_f (ethyl acetate/hexane): 0.43 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.01-6.98 (d, J = 11.6 Hz, 2H, Ar-H), 6.84-6.81 (d, J = 11.6 Hz, 2H, Ar-H), 4.65 (d, J = 8.4 Hz, 1H, H-1), 4.14 (dd, J = 5.6 Hz, 7.2 Hz, 1H, H-3), 4.06 (dd, J = 2.4, Hz, 5.2 Hz, 1H, H-4), 3.97 (m, 1H, H-5), 3.80 (t, J = 7.6 Hz, 1H, H-2), 3.77 (s, 3H, -OCH_3), 1.57 (s, 3H, CH_3), 1.45 (d, J = 6.4 Hz, 3H, CH_3), 1.38 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.8, 151.4, 119.0, 114.6, 110.2, 101.2, 79.0, 76.8, 73.3, 69.3, 55.8, 28.4, 26.5, 16.5.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 333.1314; Found: 333.1417.



4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-2-levulinoyl-β-D-galactopyranoside (13)

To a solution of **12** (1.64 g, 5.27 mmol) in anhydrous dichloromethane (50 mL), was added DMAP (640 mg, 5.27 mmol), levulinic acid (1 mL, 10.54 mmol), DCC (2.1 g, 10.54 mmol) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 1 h under nitrogen. After TLC showed complete conversion, reaction mixture was filtered and concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 30%

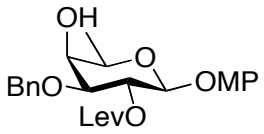
EtOAc/hexane as eluent to provide the titled product as colorless oil (2.0 g, 4.90 mmol, 93%).

R_f (ethyl acetate/hexane): 0.33 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.02-6.99 (d, J = 11.6 Hz, 2H, Ar-H), 6.85-6.81 (d, J = 11.6 Hz, 2H, Ar-H), 5.20 (t, J = 8.0 Hz, 1H, H-2), 4.77 (d, J = 8.4 Hz, 1H, H-1), 4.21 (dd, J = 7.2 Hz, 5.2 Hz, 1H, H-3), 4.16 (dd, J = 5.6 Hz, 2.0 Hz, 1H, H-4), 3.97 (m, 1H, H-5), 3.76 (s, 3H, -OCH_3), 2.95-2.59 (m, 4H, CH_2), 2.15 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.45 (d, J=6.4 Hz, 3H, CH_3), 1.38 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.8, 155.6, 151.6, 118.9, 114.6, 110.5, 100.2, 77.2, 76.4, 73.2, 69.2, 55.8, 38.1, 30.1, 28.1, 27.9, 26.6, 16.8.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 431.1682; Found: 431.1441.



4-Methoxyphenyl 3-O-benzyl-6-desoxy-2-O-levulinoyl-β-D-galacotopyranoside (14)

To a solution of **13** (200 mg, 0.49 mmol), 80% acetic acid/water (10 mL) was added. The reaction mixture was brought to 80 ^oC and stirred under nitrogen for 1 h. After TLC showed complete comversion, the reaction flask was cooled to room temperature and solvent was removed under reduced pressure. Crude product was directly used for next reaction without further purification.

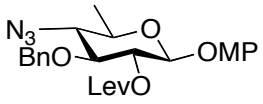
To a solution of 4-methoxyphenyl 6-desoxy-2-*O*-levulinoyl-β-D-galacotopyranoside (63 mg, 0.17 mmol) in toluene (3 mL), was added dibutyltin oxide (85 mg, 0.34 mmol) and brought to reflux. The reaction was stirred for 1 h until the reaction mixture turned clear, and then cooled to room temperature. To the cooled reaction mixture, tetrabutyl ammonium iodide (69 mg, 0.19 mmol), benzyl bromide (0.02 mL, 0.21 mmol) was added and the flask was brought to reflux again for 5 hours. After TLC showed no starting material left, the reaction solvent was removed under reduced pressure to obtain crude product. After purification using silica gel flash chromatography eluting with 20% EtOAc/Hexane to give titled compound (62 mg, 0.14 mmol, 80%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.27(β) (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40-7.32 (m, 5H, Ar-H), 6.98 (d, J = 8.8 Hz, 2H, Ar-H), 6.79 (d, J = 8.8Hz, 2H, Ar-H), 5.39 (dd, J = 9.6 Hz, 8.4 Hz, 1H, H-2), 4.78 (d, J = 8.0 Hz, 1H, H-1), 4.74 (d, J = 12.4 Hz, 1H, CH_2), 4.61 (d, J = 12.4 Hz, 1H, CH_2), 3.84 (d, J = 2.8 Hz, 1H, H-4), 3.77 (s, 3H, CH_3), 3.66 (m, 1H, H-5), 3.58 (dd, J = 9.6 Hz, 3.2 Hz, 1H, H-3), 2.86-2.51 (m, 2H, CH_2), 2.74-2.61 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 1.4 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.8, 151.7, 137.6, 128.8, 128.3, 128.1, 118.9, 114.6, 100.9, 78.9, 71.9, 71.0, 70.0, 68.9, 55.8, 38.1, 30.1, 28.2, 16.6.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 481.1838; Found: 481.2004.



4-Methoxyphenyl 4-azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α/β-D-glucopyranoside (15)

To a solution of **14** (736 mg, 1.61 mmol), in CH_2Cl_2 (10 mL) and pyridine (1.3 mL), was added triflic anhydride (0.41 mL, 2.4 mmol) at 0 ^oC. The reaction was stirred for 1 h, then quenched with few drops of water, and extracted with ethyl ether (3 × 30 mL). The organic mixture was washed with saturated Na_2CO_3 solution (3 × 30 mL), dried (Mg_2SO_4), concentrated under reduced pressure and used directly for the next step.

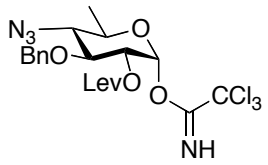
To a solution of triflate (138 mg, 0.24 mmol) in DMF (4 mL), was added NaN_3 (155.37 mg, 2.39 mmol). The mixture was placed in a CEM Voyager microwave and set at 70 ^oC for 1 h, then quench with 1 mL of water. Mixture was extracted with ethyl ether (3 × 20 mL), and organic mixture was washed with saturated Na_2CO_3 solution (3 × 20 mL), dried (Mg_2SO_4), concentrated under reduced pressure and then purified using silica gel flash chromatography eluting with 15% EtOAc/Hexane to give titled compound (94 mg, 0.20 mmol, 82%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.57(β) (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.38-7.32 (m, 5H, Ar-H), 7.02 (d, J = 8.8 Hz, 2H, Ar-H), 6.83 (d, J = 8.8Hz, 2H, Ar-H), 5.25 (t, J = 8.4 Hz, 1H, H-2), 4.88-4.68 (dd, d, J = 11.2 Hz, 8.4 Hz, 3H, H-1, CH_2), 3.77 (s, 3H, CH_3), 3.63 (t, J = 9.2 Hz, 1H, H-4), 3.41-3.24 (m, t, J = 9.6 Hz, 2H, H-3, H-5), 2.74-2.61 (m, 2H, CH_2), 2.57-2.47 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 1.4 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 206.23, 170.87, 157.33, 150.91, 137.66, 128.70, 128.56, 128.22, 118.43, 114, 89, 100.50, 81.22, 75.02, 73.69, 71.17, 67.84, 55.97, 38.78, 30.01, 27.99, 19.13.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 506.1903; Found: 506. 2989.



4-Azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α-D-glucopyranosyl Trichloroacetimidate (5)

To a solution of **15** (166 mg, 0.39 mmol) in acetonitrile (10 mL)/water (2.5 mL) mixture. Cerium ammonium nitrate (2.13 g, 3.89 mmol) was added to the solution and stirred for 2 h at room temperature. After TLC confirmed consumption of starting material, the mixture was diluted with ethyl acetate and was washed with water (3 × 30 mL), dried (Mg_2SO_4), concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 hexane/ethyl acetate) to afford 147 mg of 4-azido-3-*O*-benzyl-6-desoxy-2-*O*-levulinoyl-α-D-glucopyranoside as pale yellow oil.

To a solution of 4-azido-3-*O*-benzyl-6-desoxy-2-*O*-levulinoyl-α-D-glucopyranoside (147 mg, 0.390 mmol) in dry CH_2Cl_2 (4 mL) were added distilled trichloroacetonitrile (0.20 mL, 1.95 mmol) and Cs_2CO_3 (317 mg, 0.97 mmol). The reaction mixture was stirred for 1 h at room temperature. Then after reaction mixture was filtered over Celite, solvent was concentrated

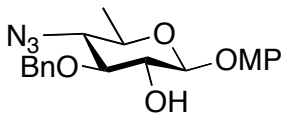
under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as a colorless oil (184 mg, 0.35 mmol, 91%).

R_f (ethyl acetate/hexane): 0.67 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.60 (s, 1H, NH), 7.40-7.27 (m, 5H, Ar-H), 6.40 (d, J = 3.3 Hz, 1H, H-1), 5.15 (dd, J = 9.9 Hz, 3.6 Hz, 1H, H-2), 4.89-4.78 (m, 2H, CH_2), 3.92 (t, J = 9.6 Hz, 1H, H-3), 3.81 (m, 1H, H-5), 3.33 (t, J = 9.6 Hz, 1H, H-4), 2.68 (t, J = 6.8 Hz, 2H, CH_2), 2.51 (t, J = 6.8 Hz, 2H, CH_2), 2.12 (s, 3H, CH_3), 1.39 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100MHz): δ (ppm) 205.5, 171.7, 160.8, 137.1, 128.7, 128.4, 128.2, 93.7, 75.0, 72.6, 69.9, 67.3, 37.7, 29.8, 27.6, 18.2.

HRMS-ESI (m/z): $[M+Na]^+$ Calcd: 543.0581; Found: 543.0608.

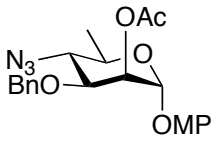


4-Methoxyphenyl 4-azido-3-O-benzyl-4,6-dideoxy-β-D-glucopyranoside

To a solution of **15** (73 mg, 0.15 mmol) in CH_2Cl_2 (3.7 mL)/methanol (0.37 mL) mixture were added hydrazine acetate (16 mg, 0.3 mmol). The reaction mixture was stirred for 3 h at room temperature. Then after reaction TLC showed the completion of the reaction solvent was evaporated and crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as pale yellow oil (55 mg, 0.14 mmol, 96%).

R_f (ethyl acetate/hexane): 0.6 (2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.49-7.00 (m, 5H, Ar-H), 6.94 (d, J = 8.8 Hz, 2H, Ar-H), 6.78 (d, J = 8.8 Hz, 2H, Ar-H), 4.91 (d, J = 10.8 Hz, 1H, CH_2), 4.88 (d, J=10.8 Hz, 1H, CH_2), 4.72 (d, J = 8.0 Hz, 1H, H-1), 3.82-3.69 (m, 4H, H-2, OCH_3), 3.52 (t, J = 9.2 Hz, 1H, H-3), 3.36 (m, 1H, H-5), 3.23 (t, J = 9.6 Hz, 1H, H-5), 2.57 (d, J = 2.8 Hz, 1H, OH), 1.40 (d, J = 6.0 Hz, 3H, CH_3).



4-Methoxyphenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α/β-D-mannopyranoside (16)

To a solution of 4-methoxyphenyl 3-*O*-benzyl-6-desoxy-α/β-D-galactopyranoside (117 mg, 0.30 mmol), in CH_2Cl_2 (4 mL) and pyridine (0.23 mL), was added triflic anhydride (0.10 mL, 0.60 mmol) at 0 ^oC. The reaction was stirred for 1 h, then quenched with few drops of water, and extracted with ethyl ether (3 × 10 mL). The organic mixture was washed with saturated Na_2CO_3 solution (3 × 10 mL), dried (Mg_2SO_4), concentrated under reduced pressure and used directly for the next step.

To a solution of triflate (145 mg, 0.28 mmol) in toluene (3 mL), was added tetrabutyl ammonium acetate (843 mg, 2.8 mmol). The mixture was placed in a CEM Voyager

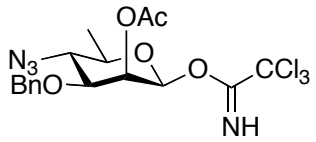
microwave and set at 70 ^{o}C for 1 h, then quenched with water (1 mL). Mixture was extracted with ethyl ether (3 × 10 mL), and organic mixture was washed with saturated Na_2CO_3 solution (3 × 10 mL), dried (Mg_2SO_4), concentrated under reduced pressure and then purified using silica gel flash chromatography eluting with 15% EtOAc/Hexane to give titled compound (110 mg, 0.26 mmol, 92%) as colorless oil.

R_f (ethyl acetate/hexane): 0.5(α) (1/1).

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.42-7.32 (m, 5H, Ar-H), 6.96 (d, J = 9.2 Hz, 2H, CH_2), 6.82 (d, J = 8.8 Hz, 2H, CH_2), 5.78 (d, J = 2.4 Hz, 1H, H-2), 4.94 (d, J = 0.8 Hz, 1H, H-1), 4.81 (d, J = 10.8 Hz, 1H, CH_2), 4.55 (d, J = 11.2 Hz, 1H, CH_2), 3.77 (s, 3H, CH_3), 3.57 (dd, J = 9.6 Hz, 3.2 Hz, 1H, H-3), 3.48 (t, J = 9.6 Hz, 1H, H-4), 2.23 (s, 3H, CH_3), 1.4 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100MHz): δ (ppm) 170.6, 155.5, 150.9, 136.8, 128.6, 128.5, 128.2, 118.2, 114.5, 97.1, 78.4, 71.5, 71.3, 67.1, 63.6, 55.7, 21.1, 18.6.

HRMS-MALDI (m/z): [*M*+Na]$^+$ Calcd: 450.1641; Found: 450.1639.



2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-β-D-mannpyranosyl Tricloroacetimidate (17)

To a solution of **16** (141 mg, 0.33 mmol) in acetonitrile (1 mL)/water (1 mL) mixture, cerium ammonium nitrate (904 mg, 1.7 mmol) was added to the solution and stirred for 2 h at room

temperature. After TLC confirmed complete consumption of starting material, the mixture was diluted with ethyl acetate and was washed with water (3 × 30 mL), dried (Mg_2SO_4), concentrated under reduced pressure. The crude product was purified by column chromatography (1:2 hexane/ethylacetate) to afford 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α/β-D-mannopyranoside (82 mg, 0.25 mmol) as pale yellow oil.

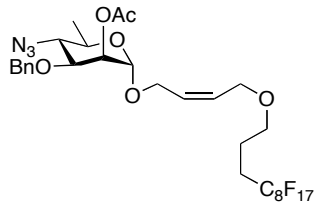
To a solution 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α/β-D-mannopyranoside (82 mg, 0.25 mmol) in dry CH_2Cl_2 (5 mL) were added distilled trichloroacetonitrile (0.125 mL, 1.25 mmol) and Cs_2CO_3 (203 mg, 0.63 mmol). The reaction mixture was stirred for 1 h at room temperature. Then after reaction mixture was filtered over Celite, solvent was concentrated under reduced pressure. Crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the product as colorless oil (95 mg, 0.20 mmol, 80.5%).

R_f (ethyl acetate/hexane): 0.5 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.52 (s, 1H, NH), 7.40-7.31 (m, 5 H, Ar-H), 6.19 (d, 1H, J = 1.6 Hz, H-1) 5.48 (t, J = 2.4 Hz, 1H, H-2), 4.78-4.53 (dd, J = 11.2 Hz, 8.4 Hz, 2H, CH_2), 3.88 (dd, J = 10.0 Hz, 3.2 Hz, 1H, H-3), 3.73 (m, 1H, H-5), 3.55 (t, J = 10.0 Hz, 1H, H-4), 2.19 (s, 3H, CH_3), 1.40 (d, J = 6.1 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.1, 159.8, 136.7, 128.6, 1288.5, 128.4, 128.2, 94.9, 75.2, 71.8, 69.9, 65.9, 63.4, 20.9, 18.6.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 487.0319; Found: 487.0596.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside

To a solution of fluorous alcohol (67 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (0.5 mL) at 0 ^oC was added the donor (37 mg, 0.08 mmol) in anhydrous CH_2Cl_2 (0.5 mL) followed by trimethylsilyl trifluoromethanesulfonate (1.45 μL, 8.0 μmol). The reaction mixture was stirred for 0.5 h and then quenched with a few drops of triethylamine. The reaction mixture was concentrated under reduced pressure and dissolved with DMF (0.4 mL) to load onto a 2 g FSPE cartridge. The loaded cartridge was first washed with 3 mL (1 column volume) 80% methanol/20% water mixture 5 times. After TLC confirmed that no compounds were eluting from the cartridge, the solvent was changes into 100% methanol (10 mL) to elute out the desired compound. The eluent was concentrated under reduced pressure to provide the titled compound (61 mg, 0.07 mmol, 91%) as colorless oil.

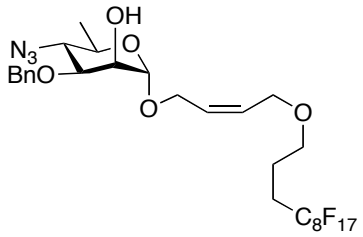
R_f (ethyl acetate/hexane): 0.53 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40-7.25 (m, 5H, Ar-H), 5.79-5.73 (m, 1H, CH), 5.71-5.65 (m, 1H, CH), 5.34 (s, 1H, H-2), 4.78 (s, 1H, H-1), 4.73 (d, J = 12.8 Hz, 1H, CH_2), 4.54 (d, J = 10.8 Hz, 1H, CH_2), 4.20 (dd, J = 12.8 Hz, 5.6 Hz, 1H, CH_2), 4.11 (dd, J = 12.8

Hz, 7.2 Hz, 1H, CH_2), 4.05 (d, J = 6.0 Hz, 2H, CH_2), 3.81 (dd, J = 10.0 Hz, 3.2 Hz, 1H, H-3), 3.59-3.54 (m, 1H, H-5), 3.51 (t, J = 6.0 Hz, 2H, CH_2), 3.45 (t, J = 9.6 Hz, 1H, H-4), 2.37-2.15 (m, 2H, CH_2), 2.14 (s, 3H, CH_3), 1.94-1.84 (m, 2H, CH_2), 1.36 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.5, 137.5, 130.5, 128.6, 128.5, 128.2, 128.1, 97.1, 76.4, 71.9, 69.1, 67.6, 67.3, 66.6, 64.2, 63.1, 28.2, 21.2, 21.0, 18.7.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 874.1761; Found: 874.0923.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (18)

To a solution of cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (27 mg, 0.03 mmol) in methanol (1 mL) at rt was added 0.5 M NaOMe solution (0.01 mL). The reaction mixture was stirred for 0.5 h and when TLC showed complete conversion of starting material, acidic resin was added for neutralization. The reaction mixture was concentrated under reduced pressure and dissolved with DMF (0.4 mL) to load onto a 2 g FSPE cartridge. The loaded cartridge was first washed with 3 mL (1 column volume) 80% methanol/20% water mixture 5 times. After TLC confirmed that no compounds were eluting from the cartridge, the solvent was changes into 100% methanol (10 mL) to elute out the desired compound. The eluent was concentrated

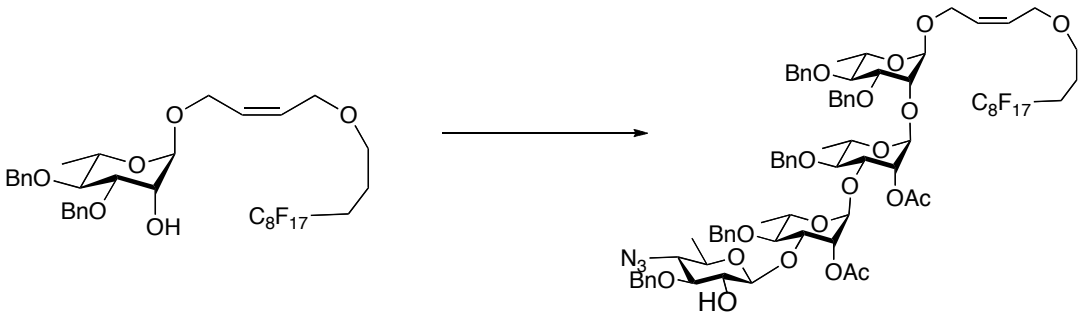
under reduced pressure to provide the titled compound (25 mg, 0.03 mmol, 97.7%) as colorless oil.

R_f (ethyl acetate/hexane): 0.23 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.70-7.31 (m, 5H, Ar-H), 5.79-5.74 (m, 1H, CH), 5.73-5.65 (m, 1H, CH), 4.86 (d, J = 1.2 Hz, 1H, H-1), 4.76 (d, J = 11.6 Hz, 1H, CH_2), 4.66 (d, J = 11.2 Hz, 1H, CH_2), 4.16 (dd, J = 12.8 Hz, 5.6 Hz, 1H, CH_2), 4.10 (dd, J = 12.8 Hz, 7.2 Hz, 1H, CH_2), 4.05 (d, J = 6.0 Hz, 2H, CH_2), 3.99 (t, J = 1.6 Hz, 1H, H-2), 3.75 (dd, J = 9.6 Hz, 3.2 Hz, 1H, H-3), 3.55 (m, 1H, H-6), 3.49 (t, J = 6.0 Hz, 2H, CH_2), 3.43 (t, J = 10.0 Hz, 1H, H-4), 2.44 (d, J = 1.6 Hz, 1H, OH), 2.27-2.13 (m, 2H, CH_2), 1.96-1.86 (m, 2H, CH_2), 1.33 (d, J = 6.4 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 137.2, 130.3, 128.7, 128.3, 128.1, 128.1, 98.1, 78.4, 77.3, 72.1, 68.9, 67.3, 66.8, 66.5, 64.9, 62.7, 28.0, 20.8, 18.5.

HRMS-ESI (m/z): [*M*+Na]$^+$ Calcd: 832.1665; Found: 832.1965.

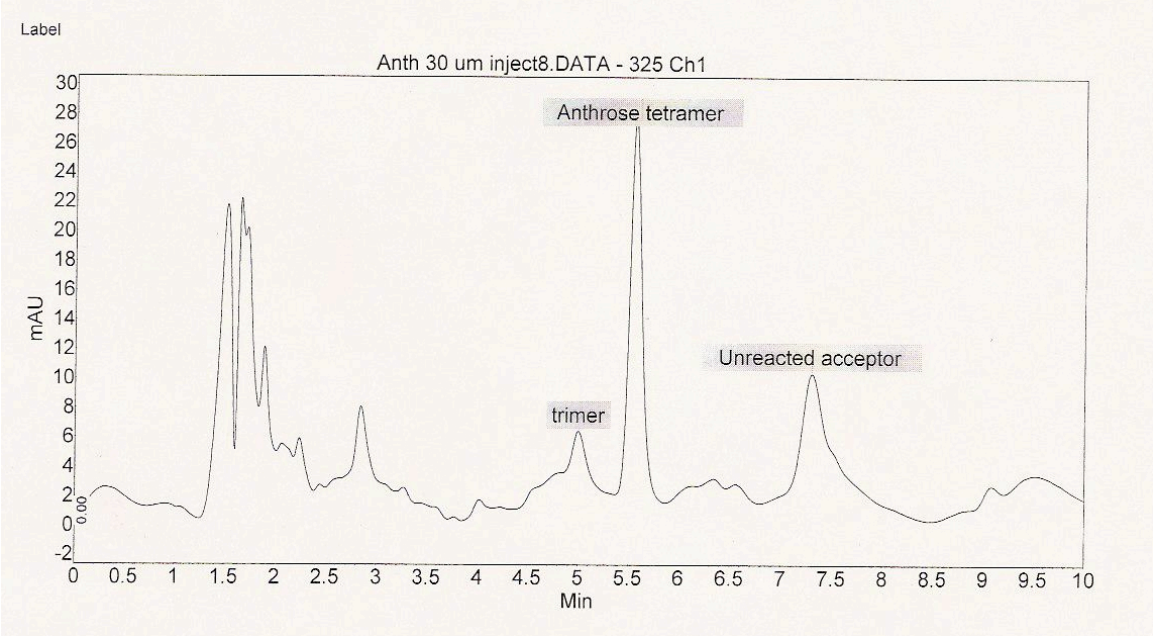


cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-azido-3-O-benzyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside (19)

- 3 cycles completed for the synthesis of anthrax tetrasaccharide

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	1.5 equivalent first donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.1 equivalent TMSOTf, rt	45 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.5 mL TEA	
4	Evaporation	40 °C	45 min
5	Desilylation	0.4 mL 1.0 M TBAF/THF solution in 1 mL THF, rt	10 hrs
6	TLC sample	20 μL of crude reaction mixture withdrawn	
7	Evaporation	40 °C	45 min
8	FSPE	0.4 mL DMF used to dissolve crude mixture	
9	Sample loading	0.7 mL crude sample transferred to cartridge	
10	Wash	5.5 ml 80% methanol wash	
11	Wash	2.0 mL methanol wash (repeated 3 times)	
12	Transfer	5.3 mL collected sample transferred to clean vial	
13	Evaporation	50 °C	45 min
14	Transfer	2 mL toluene added	
15	Evaporation	50 °C	45 min
		Repeat 1-15 for trisaccharide	
31	Glycosylation	2 equivalent second donor (100 μmol) in 1 mL toluene, 0.1 equivalent TMSOTf, 0 °C	45 min
32			
33	TLC sample	20 μL of crude reaction mixture withdrawn	
34	Quenching	0.5 mL TEA	
35	Evaporation	40 °C	45 min
36	Delevulinoylation	Hydrazine acetate solution in CH_2Cl_2/MeOH	1 hr
37	TLC sample	20 μL of crude reaction mixture withdrawn	
38	Evaporation	50 °C	45 min
39	FSPE	0.4 mL DMF used to dissolve crude mixture	
40	Sample loading	0.7 mL crude sample transferred to cartridge	
41	Wash	5.5 mL 80% methanol wash	
42	Wash	2.0 mL methanol wash (repeated 3 times)	

HPLC traces of crude anthrax tetrasaccharide run



15% Ethyl acetate/Hexane run for 10 minutes.

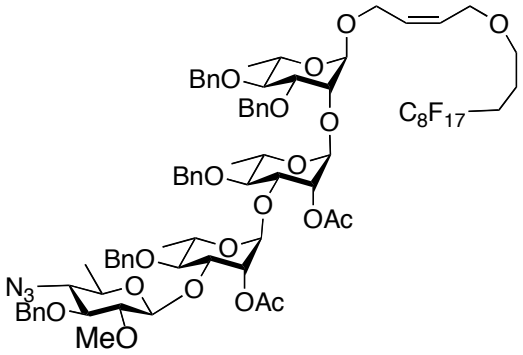
The 100% methanol elution from the last FSPE wash was removed from the ASW and concentrated under reduced pressure. Crude product was purified by preparative TLC using 30% EtOAc/hexane as eluent to provide the desired compound (16.9 mg, 0.010 mmol, 20% overall) as colorless oil.

R_f (ethyl acetate/hexane): 0.27 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.39-7. 27 (m, 25H, Ar-H), 5.73 (m, 2H, CH), 5.31 (s, 1H), 5.25 (s, 1H), 5.06 (s, 1H), 5.01 (s, 1H), 4.92-4.78 (m, 5H), 4.72-4.56 (m, 6H), 4.32 (t, J = 6.0 Hz, 1H), 4.26 (d, J = 7.6 Hz, 1H), 4.19-4.10 (m 3H), 4.02-3.98 (m, 2H), 3.92 (s, 1H), 3.83 (m, 3H), 3.63 (m, 2H), 3.55-3.40 (m, 6H), 3.28 (t, J = 9.2 Hz, 1H), 3.07-2.96 (m, 3H), 2.27-2.12 (m, 8H), 1.89 (m, 2H), 1.32-1.27 (m, 9H), 1.16 (d, J = 6.0 Hz, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 170.2, 139.8, 138.3, 139.2, 138.1, 137.2, 136.5, 133.0, 130.0, 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.6, 127.5, 127.4, 105.1, 99.3, 99.2, 98.2, 82.2, 81.0, 79.8, 79.8, 77.6, 77.3, 76.5, 75.4, 74.8, 72.0, 71.0, 71.0, 68.9, 68.6, 68.1, 67.9, 67.8, 67.4, 66.8, 66.6, 62.8, 28.0, 21.1, 20.8, 18.6, 18.4, 18.1, 17.3.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 1714.5482; Found: 1714.5214.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside

To a solution of the fluorous-tagged tetrasaccharide **19** (54 mg, 0.032 mmol) in anhydrous methyl iodide (1 mL) was added freshly prepared silver oxide (146 mg, 0.63 mmol). The reaction mixture was then brought to reflux and stirred under argon for 3 days. After TLC confirmed complete conversion of starting material, the reaction mixture was filtered over Celite and concentrated under reduced pressure. Crude product was purified by flash column

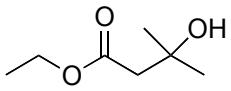
chromatography on silica gel using 20% EtOAc/hexane as eluent to provide the titled product as colorless oil (44 mg, 0.25 mmol, 81%).

R_f (ethyl acetate/hexane): 0.67 (3/7).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.51-7.26 (m, 25H, Ar-H), 5.74-5.67 (m, 2H, CH), 5.56 (dd, J = 2.8 Hz, 2.1 Hz, 1H, CH), 5.51 (dd, J = 2.8 Hz, 2.1 Hz, 1H, CH), 5.23 (d, J = 1.4 Hz, 1H, CH), 4.96-4.93 (m, 3H), 4.82 (d, J = 10.5 Hz, 1H, CH_2), 4.74-4.64 (m, 6H), 4.50-4.49 (m, 2H), 4.20 (dd, J = 13.3 Hz, 5.6 Hz, 1H, CH_2), 4.01-4.03 (m, 3H), 3.98 (t, J = 2.8 Hz, 1H, CH), 3.90-3.87 (m, 2H), 3.81-3.77 (m, 3H), 3.72-3.46 (m, 10H), 3.33 (m, 1H), 3.12-3.03 (m, 3H), 2.23-2.15 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.90-1.86 (m, 2H, CH_2), 1.34-1.25 (m, 12H, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 170.2, 138.6, 138.6, 138.4, 138.1, 137.6, 130.0, 129.4, 129.0, 128.7, 128.7, 128.5, 128.2, 128.2, 128.1, 128.1, 127.9, 127.7, 103.1, 99.8, 99.7, 98.7, 85.0, 82.9, 80.1, 80.0, 78.7, 78.0, 75.7, 75.4, 72.5, 72.5, 71.2, 70.2, 69.1, 68.7, 68.4, 68.3, 68.2, 67.9, 67.8, 66.8, 63.1, 60.8, 28.3, 28.2, 21.4, 21.3, 21.1, 18.7, 18.7, 18.3, 18.0.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 1728.5638; Found: 1728.5759.

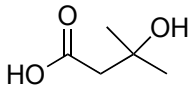


Ethyl 3-hydroxy-3-methylbutanoate (21)

To a solution of ethyl bromoacetate **20** (500 mg, 2.99 mmol) in dry THF was added distilled acetone (2.19 mL, 29.9 mmol), unactivated ⫧inc dust (391 mg, 6.0 mmol) and I_2 in room temperature. The reaction flask was placed in the CEM microwave and was stirred at 50 ^oC for 15 min. Reaction mixture was filtered over Celite and diluted with THF (20 mL). The organic layer was washed with water (3 × 10 mL), saturated Na_2CO_3 solution (3 × 10 mL), dried (Mg_2SO_4), concentrated under reduced pressure and then purified using silica gel flash chromatography eluting with 15% EtOAc/Hexane to give titled compound (340 mg, 2.33 mmol, 78%) as colorless oil.

R_f (ethyl acetate/hexane): 0.7 (4/6).

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 4.24 (q), 2.44 (s, 2H), 1.32 (m, 9H).

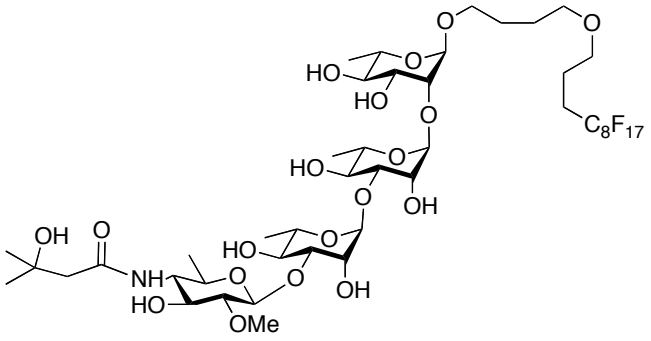


3-Hydroxy-3-methylbutanoic acid (22)

To a solution of **21** (300 mg, 2.05 mmol) in THF/water (2 mL, v:v/1:1) was added $LiOH{\cdot}H_2O$ (2.58 g) at room temperature. Reaction mixture was stirred under microwave conditions at 50 ^oC for 1 h. Mixture was diluted with THF (20 mL) and the organic layer was washed with water (3 × 10 mL), 2N HCl (3 × 10 mL), saturated Na_2CO_3 solution (3 × 10 mL), dried (Mg_2SO_4), concentrated under reduced pressure and then purified using silica gel flash chromatography eluting with 80% EtOAc/Hexane to give titled compound (166 mg, 1.41 mmol, 69%) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.48 (br, 1H), 2.52 (s, 2H), 1.28 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 177.1, 69.8, 46.2, 29.1, 29.1.



4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-butyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-β-D-glucopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→2)- α-L-rhamnopyranoside (1)

To a solution of the fluorous-tagged tetrsaccharide (40.7 mg, 0.023 mmol) in anhydrous toluene (1 mL) was added tributyl tinhydride (0.184 mL, 0.69 mmol) and 2,2'-azobisisobutyronitrile, 98% (0.33 mg, 0.002 mmol). The reaction mixture was degassed with argon bubbling in for 30 minutes. The mixture was then brought to reflux and stirred under argon for 2 hours. The mixture was cooled to room temperature and filtered through a short plug of silica gel. The crude product was then concentrated and purified by a FSPE cartridge to give the product as colorless oil (34.8 mg, 0.020 mmol, 90%).

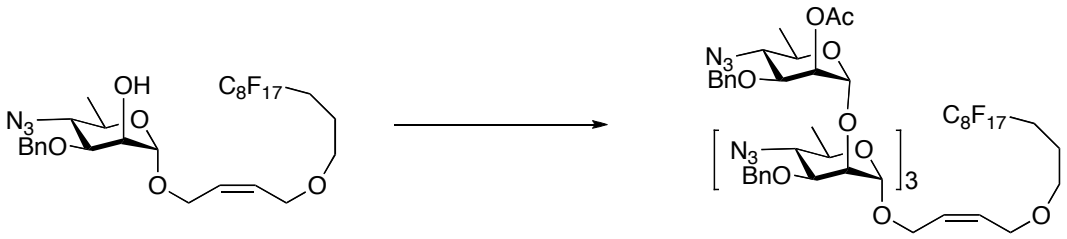
To a solution of the animo tetrasaccharide (34.8 mg 0.02 mmol), in DCM (2 mL), 3-hydroxy-3-methylbutanoic acid (5 mg, 0.03 mmol), HATU (11.4 mg, 0.03 mmol) and DIPEA (5 μL, 0.03 mmol) was added. Reaction mixture was stirred at room temperature for 2 h. After the reaction time, crude product was then concentrated and purified by a FSPE cartridge to give the product as colorless oil (31.7 mg, 0.0178 mmol, 89%).

To a solution of the side chain attached tetrasaccharide (31.7 mg, 0.0178 mmol), in MeOH (2 mL), 10% Pd/C (5 mg) was added. The reaction flask was evacuated and a hydrogen balloon was attached. Reaction mixture was stirred at room temperature overnight. After the reaction time, crude product was then concentrated and filtered over Celite and then again purified by a FSPE cartridge to give the product as colorless oil (16.8 mg, 0.0135 mmol, 76%).

^{1}H NMR (CD_3OD, 400MHz): δ (ppm) 5.03 (m, 1H), 4.96 (m, 1H), 4.91 (d, J = 1.8 Hz, 1H), 4.77 (d, J = 1.6 Hz, 1H), 4.62 (d, J = 7.8 Hz, 1H), 4.18 (dd, J = 3.3 Hz, J = 1.7 Hz, 1H), 4.05 (dd, J = 3.2 Hz, J = 1.9 Hz, 1H), 3.90 (dd, J = 9.4 Hz, J = 3.3 Hz, 1H), 3.83 (m, 1H), 3.80 (m, 2H), 3.70-3.78 (m, 3H), 3.67 (m, 2H), 3.66 (s, 3H), 3.55-3.60, (m, 3H), 3.51 (t, J = 9.6 Hz, 1H), 3.40-3.44 (m, 3H), 3.38 (m, 1H), 3.02 (dd, J = 9.1 Hz, J = 7.9 Hz, 1H), 2.36 (m, 2H), 2.15 (m, 2H), 1.68 (m, 2H), 1.29 (m, 9H), 1.25 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 3H).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 174.7, 105.5, 104.0, 103.6, 100.4, 85.7, 82.0, 80.1, 79.2, 75.1, 74.4, 73.4, 73.2, 72.3, 72.2, 71.9, 71.8, 70.8, 70.6, 70.2, 70.0, 68.0, 61.2, 58.2, 49.8, 31.5, 30.0, 29.7, 29.6, 18.5, 18.2, 18.1, 18.0.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 1270.3855; Found: 1270.8002.



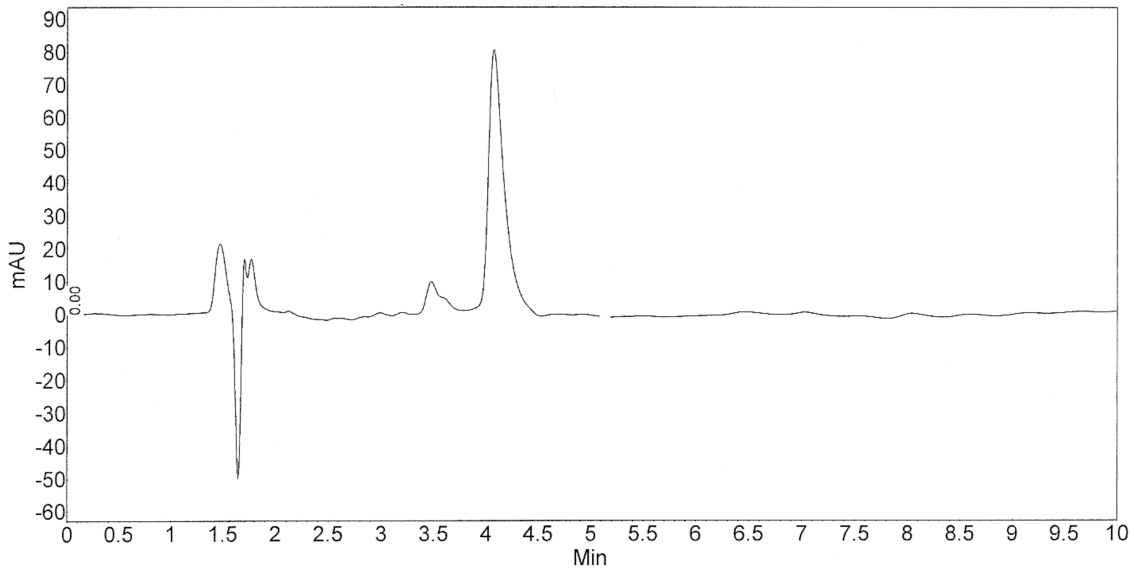
cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (23)

- 2.5 cycles completed for the synthesis of cholera tetrasaccharide

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	1.5 equivalent donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.1 equivalent TMSOTf, rt	45 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.5 mL TEA	
4	Evaporation	40 °C	45 min
5	Deacetylation	3 equivalent of NaOMe solution, rt	3 h
6	TLC sample	30 μL of crude reaction mixture withdrawn	
7	Quenching	0.3 mL 0.5 M Acetic acid solution in MeOH	
8	Evaporation	50 °C	45 min
9	FSPE	0.4 mL DMF used to dissolve crude mixture	
10	Sample loading	0.7 mL crude sample transferred to cartridge	
11	Wash	5.5 mL 80% methanol wash	

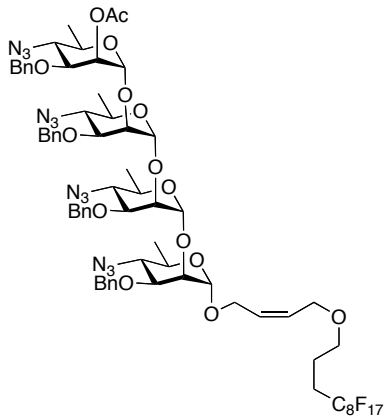
12	Wash	2.0 mL methanol wash (repeated 3 times)	
13	Transfer	5.3 mL collected sample transferred to clean vial	
14	Evaporation	50 ^{o}C	45 min
15	Transfer	2 mL toluene added	
16	Evaporation	50 ^{o}C	45 min
		Repeat 1-16	
33	Glycosylation	1.5 equivalent donor (75 μmol) in 0.5 mL toluene,	45 min
34		0.1 equivalent TMSOTf, rt	
35	TLC sample	20 μL of crude reaction mixture withdrawn	
36	Quenching	0.5 mL TEA	
37	Evaporation	40 ^{o}C	45 min
38	Deacetylation	3 equivalent of NaOMe solution, rt	3 hr
39	TLC sample	30 μL of crude reaction mixture withdrawn	
40	Quenching	0.3 mL 0.5 M Acetic acid solution in MeOH	
41	Evaporation	50 ^{o}C	45 min
42	FSPE	0.4 mL DMF used to dissolve crude mixture	
43	Sample loading	0.7 mL crude sample transferred to cartridge	
44	Wash	5.5 mL 80% methanol wash	
45	Wash	2.0 mL methanol wash (repeated 3 times)	

HPLC traces of crude cholera tetrasaccharide run



15% Ethyl acetate/Hexane run for 10 minutes. (Desired tetrasaccharide eluted in 4.07 minutes)

The 100% methanol elution from the last FSPE wash was removed from the ASW and concentrated under reduced pressure. Crude product was purified by preparative TLC using 30% EtOAc/hexane as eluent to provide the titled compound (35.12 mg, 0.021 mmol, 28.9% overall) as colorless oil.



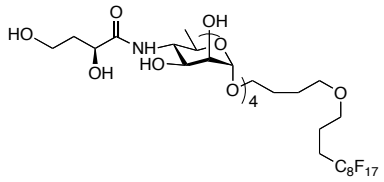
cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (23)

R_f (ethyl acetate/hexane): 0.5 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.38-7.31 (m, 20H, Ar-H), 5.78-5.61 (m, 2H, 2×CH), 5.42 (dd, J = 2.8 Hz, 2.0 Hz, 1H, CH), 4.95 (s, 1H, CH), 4.87 (s, 1H, CH), 4.86 (s, 1H, CH), 4.75-4.53 (m, 9H, CH, 4×CH_2), 4.20-3.19 (m, 21H, 15×CH, 3×CH_2), 2.27-2.20 (m, 2H, CH_2), 2.12 (s, 3H, CH_3), 1.93-1.86 (m, 2H, CH_2), 1.29-1.16 (m, 12H, 4×CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.0, 137.6, 137.5, 137.3, 130.3, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.5, 128.3, 128.3, 129.2, 128.2, 100.7, 100.3, 99.3, 98.0, 77.4, 75.7, 74.2, 73.6, 73.6, 72.4, 72.3, 72.2, 71.8, 69.1, 68.0, 67.9, 67.5, 67.3, 66.6, 64.5, 64.4, 64.2, 64.0, 63.0, 29.9, 28.2, 21.2, 18.8, 18.3, 18.2, 18.1

HRMS-MALDI (m/z): [*M*+Na]$^+$ Calcd: 1657.5101; Found: 1657.5433.



4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-butyl 4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (2)

To a solution of the fluorous-tagged tetrsaccharide **23** (87.7 mg, 0.054 mmol) in anhydrous toluene (5 mL) was added tributyl tinhydride (0.866 mL, 3.22 mmol) and 2,2'-azobisisobutyronitrile, 98% (0.88 mg, 0.005 mmol). The reaction mixture was degassed with argon bubbling in for 30 minutes. The mixture was then brought to reflux and stirred under argon for 2 hours. The mixture was cooled to room temperature and filtered through a short plug of silica gel. The crude product was then concentrated and purified by a FSPE cartridge to give the product cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl 2-*O*-

acetyl-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-amino-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside as colorless oil (81 mg, 0.053 mmol, 98%).

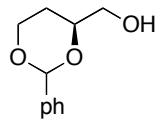
To a solution of the tetramino compound (40 mg, 0.026 mmol) in DCM (3 mL) was added 2,4-*O*-benzylidene-3-deoxy-L-glycero-tetronic acid (20 mg, 0.159 mmol), EDC (30 mg, 0.159 mmol) HOBT (21 mg, 0.159 mmol) Reaction mixture was stirred at room temperature for 2 h. After the reaction time, crude product was then concentrated and purified by a FSPE cartridge to give cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl 2-*O*-acetyl-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside as colorless oil (45.8 mg, 0.020 mmol, 77%).

To a solution of the side chain attached tetrasaccharide (45.8 mg, 0.020 mmol), in MeOH (2 mL), 10% Pd/C (5 mg) was added. The reaction flask was evacuated and a hydrogen balloon was attached. Reaction mixture was stirred at room temperature overnight. After the reaction time, crude product was then concentrated and filtered over Celite and then again purified by a FSPE cartridge to give the product as colorless oil (21.2 mg, 0.0138 mmol, 69%).

^{1}H NMR (CD_3OD, 400MHz): δ (ppm) 5.38-5.29 (m, 3H), 5.01 (s 1H), 4.99 (s 1H), 4.87 (t, J = 9.6 Hz, 1H), 4.76-4.55 (m, 8H), 4.22–3.76 (m, 8H), 3.66 (m, 2H), 3.54-3.42 (m, 8H), 2.74–2.44 (m, 8H), 2.22–2.05 (m, 10H), 1.97–1.79 (m, 2 H), 1.38–1.25 (m, 12H).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 174.7, 175.5, 175.4, 175.4, 105.5, 104.0, 103.6, 100.4, 85.7, 82.0, 80.1, 79.2, 75.1, 74.4, 73.4, 73.2, 72.3, 72.2, 71.9, 71.8, 70.8, 70.6, 70.2, 70.0, 69.5, 69.3, 68.0, 61.2, 58.2, 49.8, 31.5, 30.0, 29.7, 29.6, 26.7, 25.6, 18.5, 18.2, 18.1, 18.0.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 1561.4992; Found: 1562.8831.



2,4-Benzylidene-(S)-1,2,4-butanetriol (25)

To a solution of BH_3·SMe, (2M solution, 3.915 mL, 7.83mmol) and $B(OMe)_3$ (98%, 0.84 mL, 7.83 mmol) was added (S)-malic acid **24** (350 mg, 2.61mmol) in anhydrous THF (1.7 mL) solution dropwise in a 0 ^{o}C ice bath. After 10 min, ice bath was removed and reaction flask was stirred under argon at room temperature overnight. Reaction mixture was quenched with MeOH (6 mL) and solvent was removed under reduced pressure. The crude product was then concentrated and purified by silica gel flash column chromatography to give the product as colorless oil (187 mg, 1.76 mmol, 68%) and was used directly for the next step.

To a solution of the triol (187 mg, 1.76 mmol), $PhCH(OMe)_2$ (2.65 mL, 17.6 mmol) and PTSA (76 mg, 0.35 mmol) was added. Reaction mixture was stirred at room temperature

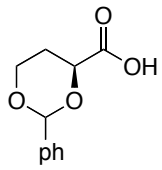
under argon for 6 h. TEA (1 mL) was added to quench the reaction and solvent was removed under reduced pressure. The crude product was purified by silica gel flash column chromatography to give the product as colorless oil (235 mg, 1.21 mmol, 69%).

R_f (methanol/chloroform, 20/80): 0.87 (1/4).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.52-7.51 (m, 2H), 7.40-7.36 (m, 3H), 5.55 (s, 1H), 4.33-4.29 (ddd, J = 11.2 Hz, 4.8 Hz, 0.8 Hz, 1H), 4.02-3.96 (m, 2H), 3.67 (m, 1H), 2.26 (m, 1H), 1.92 (dq, J = 12.0 Hz, 5.2 Hz, 1H), 1.46 (qd, J = 13.6 Hz, 1.6 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 138.4, 129.0, 128.4, 126.2, 101.3, 77.6, 66.7, 65.7, 26.9.

HRMS-MALDI (m/z): $[M+Na]^+$ Calcd: 217.0841; Found: 217.2892.



2,4-O-Benzylidene-3-deoxy-L-glycero-tetronic acid (26)

Pyridinium dichromate (2.39 g, 6.36 mmol) was added at room temperature to a solution of **25** (308.9 mg, 1.59 mmol) in DMF (3 mL), and the mixture was stirred overnight. When TLC showed absence of starting material, the reaction was quenched with water (5 mL) and was stirred for an additional few minutes. The reaction mixture was dilluted with ethyl acetate (50 mL) and was washed with water (3 × 50 mL). The organic layer was dried

(Mg_2SO_4) and then concentrated under reduced pressure. Spectral data matched the previous publication. [10]

Additional Information: Experimental details, including copies of ^1H NMR spectra, for the synthesis and production of the carbohydrate microarrays and the complete ref 2h, and competition experiment plots are found in the Appendix at the end of this chapter.

References

1. G. Park, N. L. Pohl, *Abstracts of Papers*. 233rd National Meeting of the American Chemical Society, Chicago, IL, March 28 2007; American Chemical Society: Washington, DC, 2007, ORGN 629.

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CHAPTER 4

Mono- vs. Di-fluorous-tagged Glucosamines for Iterative Oligosaccharide Synthesis

A paper published in *Journal of Fluorine Chemistry*[1]

Gisun Park, Kwang-Seuk Ko, Aleksandra Zakharova and Nicola L. Pohl*

Abstract

Fluorous-tagged protecting groups are attractive tools for elongating carbohydrate chains in oligosaccharide synthesis. To eliminate the accumulation of failed sequences during automated oligosaccharide synthesis conditions, an additional C_8F_{17} ester derived protecting group was attached to the glycosyl donor to better retain the desired doubly-tagged glycosylation product on fluorous solid phase extraction (FSPE) cartridges. Initial studies show that the double-fluorous-tagging strategy offers a robust enough separation using a commercial FSPE cartridge using simple gravity filtration to separate the desired product from the singly-fluorous-tagged starting materials and their decomposition products. In addition, removal of the fluorous acetate and its byproducts after sodium methoxide treatment and neutralization required only dissolution of the desired sugar in toluene and subsequent removal of the toluene layer from the denser fluorous byproducts.

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Introduction

The isolation of pure, structurally well-defined oligosaccharides from nature is generally a tedious and inefficient process, because these biomolecules are often present in low concentrations as microheterogeneous mixtures. A better understanding of the growing numbers of biological processes that carbohydrates mediate [1] demands efficient ways to access these structures. Studies of proteins and nucleic acids have thrived since automated solid-phase chemistry has been discovered [2]. The same principle was applied to automated oligosaccharide synthesis as well to produce various oligosaccharide compounds [3]. However the production of these compounds required a large excess of donor molecules to achieve reasonable coupling yields on a solid support and reaction monitoring is more difficult than in solution. To circumvent some of the inherent limitations of automated solid-phase synthesis, we have pursued a solution-phase approach. A lipid introduced to a monosaccharide building block [4] while maintaining solubility in organic solvents offers a handle to easily purify the desired compound; however, this tag complicates reaction monitoring by proton NMR. Due to its facile purification from non-fluorous compounds and silence in proton NMR spectra, fluorous tags have become an attractive tool for many synthetic processes [5]. Highly fluorinated versions of silyl, ether, and ester protecting groups [6] have been applied to oligosaccharides synthesis using liquid phase extractions. Unfortunately, solubility of compounds with heavy fluorous tags in organic solvents is hard to predict. A light fluorous (C_8F_{17}) tag, on the other hand, has been shown to cap failed

sequences in solid-phase oligosaccharide synthesis [7] and more recently has been shown [8] to be stable to all the reaction conditions required for the requisite glycosylation and deprotection conditions with no problems with solubility or purification of intermediates using fluorous solid phase extraction [9] (FSPE). The fast and robust separation of the fluorinated compounds also makes the method amenable to automation.

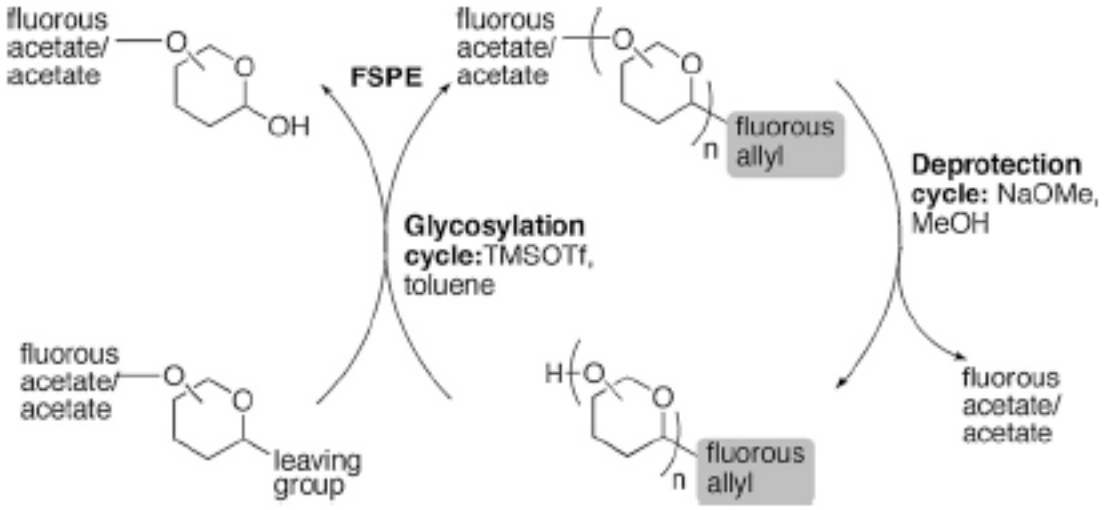


Figure 4-1. Comparison of a single- versus double-fluorous-tag strategy for iterative oligosaccharide synthesis.

A potential problem with a single-fluorous-tag strategy occurs when a reaction does not go to completion. Addition of a fluorous tag to both rather than just one of the coupling partners offers a chance to eliminate by-product build up during the synthesis without addition of a capping [7] step (Figure 4-1). The structure/retention trends in fluorous chromatography have been studied previously [10] where the larger the tag size, the stronger its retention on fluorinated silica gel. However, these comparison studies were performed on a FluoroFlash^{TM} HPLC column. Success needs to be demonstrated using a simple gravity

elution strategy for ready incorporation into an automation scheme. In addition, the benefit of removing singly-tagged starting materials and their decomposition products from the doubly-tagged desired products after a glycosylation cycle might be lost if the two single-fluorous-tagged products generated during the deprotection cycle are not easily separated by means other than FSPE. Herein we report the introduction of a light fluorous acetate protecting group for oligosaccharide synthesis and the viability of separating mono- from di-tagged saccharides using only simple FSPE cartridges to remove any excess/unreacted reagents in the context of building 1-4-linked glucosamine oligomers. We also report a simple method to remove the fluorous acetate byproduct from the desired fluorous-tagged sugar in the deprotection cycle.

Results and discussion

In order to investigate the ease of purification of di-tagged saccharide products, the synthesis of a fluorous tagged donor and fluorous-tagged acceptor was necessary. In our previous studies using fluorinated tags for oligosaccharide synthesis [11], fluorous allyl groups are attached at the anomeric position of the reducing end of the oligosaccharide for chain extension at the nonreducing end. This fluorous allyl group would again serve as a good acceptor. The synthesis of an activated glucosamine donor was then undertaken in which a fluorous acetate was installed at the chain extension position. Among the different functionalities of fluorinated-protecting groups that could be applied, an ester derived fluorous protecting group was selected for its simple and readily automated deprotection conditions. The known partially-protected glucosamine building block 1 (Figure 4-2) [12]

with an easily removable *tert*-butyl dimethyl silyl group on the anomeric position served as a good starting point. The 4-position hydroxyl **1** was treated with 2H, 2H, 3H, 3H-perfluoro undecanoic acid to provide compound **2**. This fluorous-tagged intermediate was then further elaborated to the trichloroacetimidate [13] donor 4 ready for glycosylation.

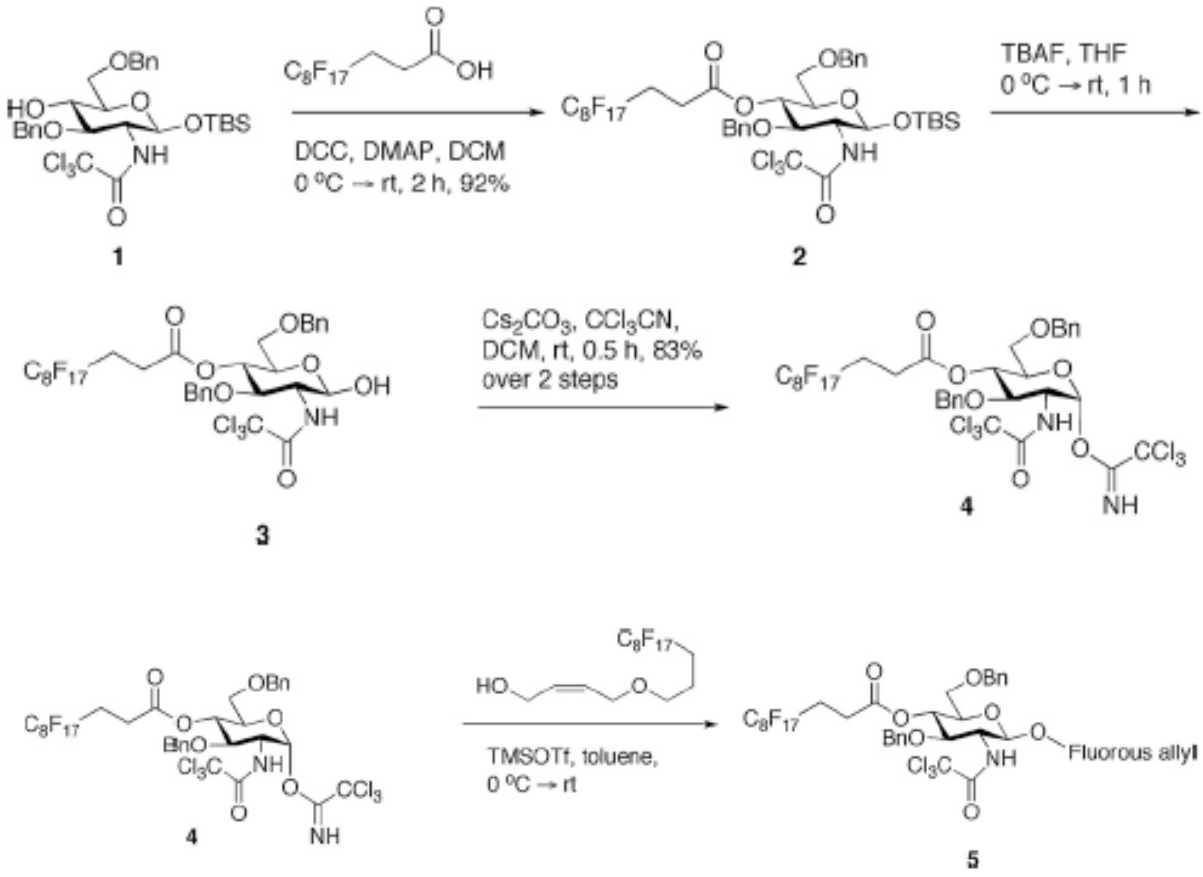


Figure 4-2. Synthesis of di-fluorous-tagged compound **5**.

With the fluorous-tagged monosaccharide donor 4 in hand, we next tested the use of the di-tagging strategy by addition of the donor to a fluorous allyl [11d] group (Figure 4-2). After the 0.5 h reaction time, the quenched crude mixture was concentrated and then loaded onto a

2 g fluorous solid phase extraction cartridge (Figure 4-3). The cartridge was first eluted with 80% methanol/20% water for 5 column volumes. All the non-fluorinated compounds were thereby eluted. The elution solvent was then changed to 90% methanol/10% water for 5 column volumes to investigate any elution of fluorinated compounds. Fortunately, the excess donor that was now decomposed to hydroxyl **3** started to elute out of the cartridge along with a trace amount of the unreacted fluorous allyl tag (Figure 4-3). The desired di-fluorous tagged compound was readily retained in the FSPE cartridge while all the unreacted/excess reagents were removed. Finally, the desired di-tagged compound **5** was eluted from the cartridge using 100% methanol as eluent. HPLC traces (Figure 4-3) of the eluents confirmed the complete separation of the desired di-fluorous-tagged compound from the crude glycosylation mixture.

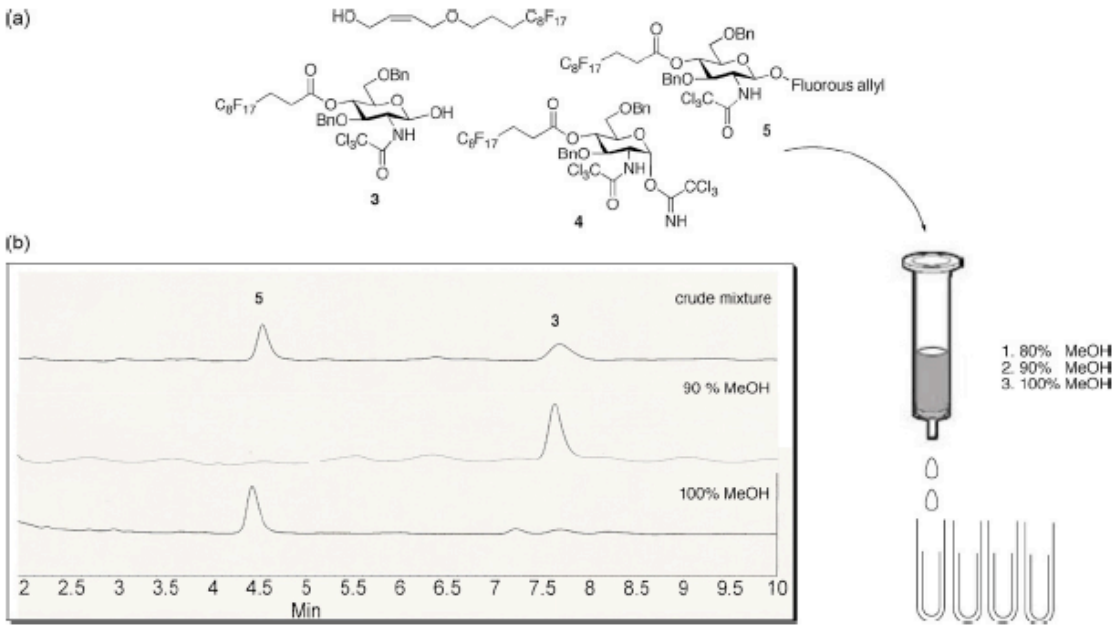


Figure 4-3. a) Selective elution of the multiple fluorous-tagged compounds using a FSPE cartridge and various elution conditions. b) HPLC traces of the compounds eluted off the FSPE cartridge with 90% or 100% methanol elution. (HPLC conditions: 15% ethyl acetate/85% hexane, 1mL/min flow rate, injection peak appeared at 1.5 min on a standard silica gel column).

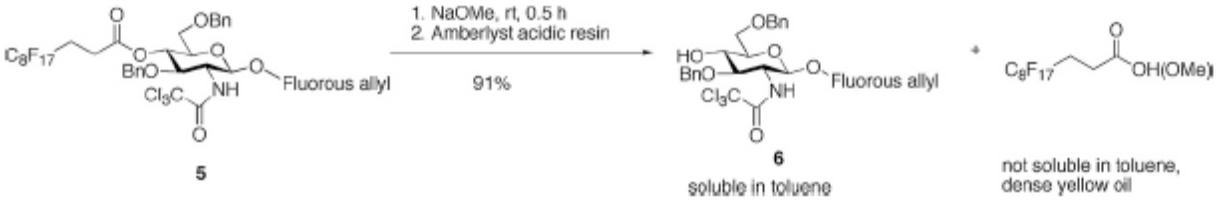


Figure 4-4. Deprotection of fluorous acetate protecting group.

The di-tagged monosaccharide **5** was then deprotected at the 4-position to allow further chain elongation (Figure 4-4). Removal of the 2H, 2H, 3H, 3H-perfluoro undecanoyl group was performed under standard sodium methoxide conditions to provide compound **6**. Now, of course, the desired product and the by-product both contain just one C_8F_{17} tag and therefore FSPE is no longer a simple strategy for separation. However, alcohol **6**, containing a large organic portion in addition to the fluorous tag, readily dissolved in toluene whereas the fluorous acetate byproducts formed a separate denser layer for easy separation. Hence, the physical property differences could be exploited for facile separation. Because growing saccharide chains with the fluorous allyl tag have good solubility in toluene as a rule, this method of fluorous acetate removal from the desired compound after the deprotection reaction should be a general strategy. In addition to fluorous acetate, other fluorous

protecting groups have the potential to be used after testing of their solubility and volatility properties.

Conclusion

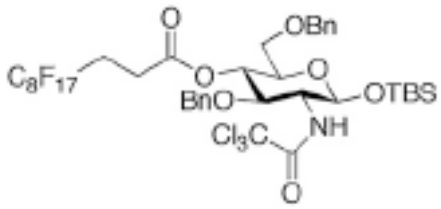
In conclusion, we have introduced a fluorous acetate group as a second protecting group to use in conjunction with our previously reported fluorous allyl group for light-fluorous-based carbohydrate synthesis. Use of a fluorous tag on not just the glycosyl acceptor, but also the glycosyl donor proved useful in the separation of the desired glycosylation product from the unreacted or decomposed starting materials with only simple gravity filtration over a commercially available FSPE cartridge. The doubly-tagged carbohydrate compound was retained in the cartridge sufficiently well to allow facile separation with a discrete increase of methanol concentrations in the aqueous eluent. The problem of thereby generating two singly-fluorous tagged components in the deprotection cycle was avoided by exploiting the difference in solubility between the tagged sugar and the fluorous acetate protecting group byproducts. Work is now in progress to test the feasibility of this double-fluorous-tagging approach in the iterative synthesis of oligosaccharides using FSPE separations on a solution-based automation platform.

Experimental section

General methods

Commercial reagents and solvents were used as received without further purification unless otherwise stated. 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. 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 162 MHz. ^{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). HPLC traces were obtained using a Varian Inc. HPLC with a Waters Nova-pak 4 μL 3.9 ×150 mm silica column. High relosution mass spectrometry was obtained with Applied Biosystems QSTAR XL Hybrid System.

Synthetic Procedures



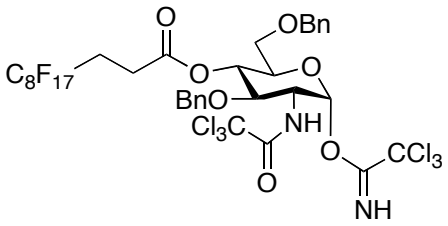
*tert-Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-4-2H, 2H, 3H, 3H, -perfluoroundecanoatyl-β-D-glucopyranoside (**2**)*

To a solution of *tert-butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside* [9] **1** (100 mg, 0.16 mmol) in dichloromethane (10 mL) cooled to 0 °C, was added DCC (66.5 mg, 0.32 mmol), DMAP (19.5 mg, 0.16 mmol) and *2H, 2H, 3H, 3H,* -perfluoroundecanoic acid (157 mg, 0.32 mmol). The reaction mixture was allowed to warm to room temperature over 2 h. The reaction was diluted with dichloromethane (20 mL), washed with water (20 mL), HCl (2N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 15% EtOAc/hexane as eluent to obtain the title compound (160 mg, 0.14 mmol, 92%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.23 (6H, d, $2CH_3$), 0.9 (9H, s, t-Bu-), 2.11-2.29 (4H, m, CH_2CH_2), 3.45 (1H, m, H-3), 3.56 (2H, m, H-6), 3.74 (1H, m, H-5), 4.35 (1H, dd, J = 9.2 Hz, 10.4 Hz, H-2), 4.46-4.74 (4H, m, $2CH_2$), 5.12 (1H, t, J = 9.2 Hz, H-4), 5.23 (1H, d, J = 7.8 Hz, H-1), 7.06 (1H, d, J = 7.6 Hz, NH), 7.22-7.40 (10H, m, $-CH_2C_6H_5$).

^{13}C NMR (162, MHz, $CDCl_3$): δ -5.2, -4.2, 17.9, 25.7, 61.1, 69.8, 72.5, 72.8, 73.6, 74.2, 76.8, 77.1, 77.3, 92.4, 94.0, 127.7, 127.7, 127.8, 128.0, 128.4, 128.5, 137.6, 137.7, 161.8, 170.0.

MS (ESI) *m/z* = calcd. for $C_{39}H_{41}Cl_3F_{17}NNaO_7Si$: 1014.1344; found : 1014.1367 $[M+Na]^+$.



*3,6-Di-O-benzyl-2-deoxy-2-trichloroacetimido-4-(2H, 2H, 3H, 3H-perfluoroundecanoyl)-α-D-glucopyranosyl trichloroacetimidate (**4**)*

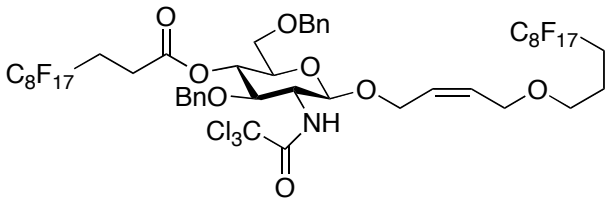
To a solution of *tert-butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside* **2** (100 mg, 0.09 mmol) in anhydrous THF, 1.0 M TBAF solution in THF (0.18 mL, 0.18 mmol) was added dropwise in a 0 ^oC ice bath. The reaction mixture was allowed to warm to room temperature over 1 h. After the solvent was removed under reduced pressure, crude reaction mixture was dissolved with DMF (0.4 mL) and loaded onto a 2 g FSPE cartridge. The cartridge was washed with 80% methanol (10 mL) followed by 100% methanol (10 mL) to elute the fluorinated desired product. The solvent was removed under reduced pressure to obtain the product (80 mg, 0.08 mmol, 89%) as colorless oil. The compound was used directly for the next step without further purification.

To a solution of the alcohol (80 mg, 0.08 mmol) in anhydrous DCM (5 mL) was added Cs_2CO_3 (66.6 mg, 0.2 mmol) and distilled trichloroacetonitrile (0.04 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 0.5 h and then filtered over a pad of Celite. The filtered solution was concentrated under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 10% EtOAc/hexane as eluent to obtain compound **4** (83 mg, 0.083 mmol, 93%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22-2.54 (4H, m, CH_2CH_2), 3.50-3.67 (2H, m, H-3, H-5), 4.01-4.09 (2H, m, H-6), 4.37 (1H, t, J = 10.0 Hz, H-2), 4.43-4.70 (4H, m), 5.40 (1H, t, J = 9.9 Hz, H-4), 6.47(1H, d, J = 3.6 Hz, H-1), 6.67 (1H, d, J = 8.8 Hz, NH), 7.25-7.39 (10H, m, C_6H_5), 8.80 (1H, s, NH).

^{13}C NMR (162, MHz, $CDCl_3$): δ 61.2, 68.3, 69.8, 69.9, 70.0, 72.1, 73.2, 73.6, 76.0, 90.7, 92.1, 94.3, 127.7, 128.2, 128.3, 128.4, 128.4, 128.7, 137.0, 137.8, 158.9, 161.8, 170.0.

MS (ESI) *m/z* = calcd. for $C_{35}H_{27}Cl_6F_{17}N_2NaO_7$: 1142.9576; found : 1142.9590 $[M+Na]^+$.



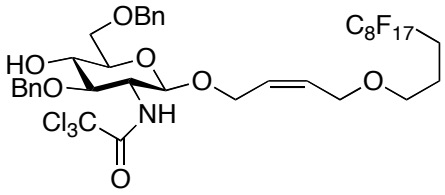
*cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl-3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-4-(2H, 2H, 3H, 3H, -perfluoroundecanoyl)-β-D-glucopyranoside (**5**)*

To a solution of the imidate (83 mg, 0.07 mmol) in anhydrous toluene (2 mL) at 0 ^{o}C was added fluorous allyl alcohol [11d] (25.5 mg, 0.05 mmol) in anhydrous toluene (2 mL) followed by trimethylsilyl trifluoromethanesulfonate (0.9 μL, 5.0 μmol). The reaction mixture was stirred for 0.5 h and then quenched with 3 drops of triethylamine. The reaction mixture was concentrated under reduced pressure and dissolved with DMF (0.4 mL) to load onto a 2 g FSPE cartridge. The loaded cartridge was first washed with 3 mL (1 column volume) 80% methanol/ 20% water mixture 5 times and then was washed with 3 ml 90% methanol/10% water mixture 5 times. After TLC confirmed that no other compounds were eluting from the cartridge, the solvent was changed into 100% methanol (10 ml) to elute out the desired compound. The eluent was concentrated under reduced pressure to provide **5** as a colorless oil (65 mg, 0.044 mmol, 87%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87-1.88 (2H, m, -$OCH_2CH_2CH_2C_8F_{17}$), 2.01-2.43 (6H, m, $C(O)CH_2CH_2C_8F_{17}$,-$OCH_2CH_2CH_2C_8F_{17}$), 3.46 (3H, m, CH_2O-, H-3), 3.56 (2H, d, J = 4.8 Hz, H-6), 3.66 (1H, m, H-5), 4.04 (2H, t, J = 5.2 Hz, CH_2CH=), 4.26-4.38 (3H, m, H-2, CH_2CH=), 4.46-4.73 (4H , m, 2 CH_2), 5.07 (1H, d, J = 8.0 Hz, H-1), 5.12 (1H, t, J = 9.6 Hz, H-4), 5.64 – 5.76 (2H, m, -CH=CH-), 7.01 (1H, d, J = 6.8 Hz, NH), 7.27-7.35 (m, 10H).

^{13}C NMR (162, MHz, $CDCl_3$): δ 25.4, 26.3, 64.9, 66.6, 69.0, 69.7, 72.5, 72.7, 73.1, 73.2, 73.8, 74.8, 77.4, 77.5, 97.6, 98.5, 127.7, 128.2, 128.3, 128.4, 128.4, 128.7, 130.8, 130.9,137.0, 137.7, 162.0, 170.2.

MS (ESI) *m/z* = calcd. for $C_{48}H_{38}Cl_3F_{34}NNaO_8$: 1530.1018; found : 1530.1198 $[M+Na]^+$.



*cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl-3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-β-D-glucopyranoside (**6**)*

To a solution of the glycosylated compound **5** (50 mg, 0.03 mmol) in methanol (1 mL) was added 0.5 M NaOMe solution (0.06 mL, 0.03 mmol). The reaction mixture was stirred for 0.5 h and then was neutralized with Amberlyst acidic resin. The reaction mixture was filtered and the solvent was removed under reduced pressure. The crude product was shaken with toluene (1 mL). The clear solution was removed with a pipette from a denser yellow oil and the solvent was removed under reduced pressure to yield **6** as a colorless oil (31 mg, 0.027 mol, 91%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72-1.80 (2H, m, $-OCH_2CH_2CH_2C_8F_{17}$), 2.04-2.17 (2H, m, $-OCH_2CH_2CH_2C_8F_{17}$), 3.01 (1H, d, J = 2.2 Hz, -OH), 3.46 (2H, m, CH_2O-), 3.60-3.65 (1H, m, H-5), 3.75-3.91 (5H, m, H-4, H-6, CH_2CH=), 4.05-4.21 (4H, m, H-2, H-3, CH_2CH=), 4.50-4.77 (4H, m, $2CH_2$), 5.12 (1H, d, J = 8.0 Hz, H-1), 5.64 – 5.76 (2H, m, -CH=CH-), 7.00 (1H, d, J = 6.9 Hz, NH), 7.27-7.35 (10H, m).

MS (ESI) *m/z* = calcd. for $C_{37}H_{35}Cl_3F_{17}NNaO_7$: 1056.1105; found : 1056.1177 $[M+Na]^+$.

Acknowledgements

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CHAPTER 5

Automated Solution Phase Synthesis: A Di-Fluorous-Tagging Strategy

Manuscript in preparation

Gisun Park and Nicola L. B. Pohl*

Abstract

Automated solution-phase oligosaccharide synthesis relying on fluorous solid-phase extraction (FSPE) for purification of intermediates is a powerful tool to construct highly functionalized oligosaccharides. A potential problem in some cases of incomplete glycosylation reactions with only tagging the reducing end of the growing chain can be failed sequences that would be carried along with the desired product during the automated sequence. In order to solve this issue, a di-fluorous tagged building block was synthesized and tested under conventional manual conditions to explore its physical properties. Initial results using FSPE had shown that the di-tagged saccharides had a stronger ability to interact with the fluorinated silica gel, therefore offering a robust separation from the mono-tagged saccharide molecules. However, it was unclear whether the differences in retention time would be great enough for robotically-controlled separations. Fortunately, careful observations of retention times of compounds on the FSPE cartridge allowed the programming of a robust protocol that can be introduced when needed for particularly challenging glycosylation cycles In this process of this work, automated protocols were also

developed to introduce a new per-fluorinated silyl group as a temporary masking group and the pentenyl activating group as a alternative glycosyl donor.

Introduction

Automated solution-phase oligosaccharide synthesis relying on fluorous solid-phase extraction (FSPE) for purification of intermediates is a powerful tool to construct highly functionalized oligosaccharides. [1]. Major benefits of this methodology are that the entire synthetic process can be easily monitored, most of the conventional solution-phase chemistry is readily applicable, and significantly less building block waste is generated. In addition, the use of the fluorous allyl tag at the reducing end of the first building block (Figure 5-1) allows a FSPE step during the iterative synthetic cycle that eliminates the by-products and excess donors that do not have the fluorous group.

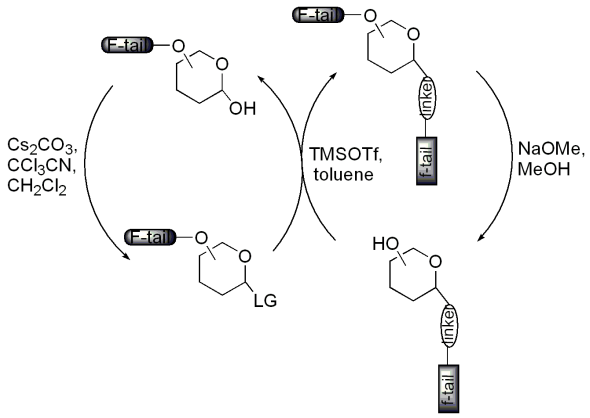


Figure 5-1. Schematic of the fluorous allyl tag used for the iterative automated synthesis of oligosaccharides.

A potential problem with only tagging the reducing end of the growing chain can be failed sequences in instances of incomplete glycosylation reactions; these failed sequences would be carried along with the desired product during the automated protocol. Although often these failed sequences are easily removed at the end of an entire synthesizer run, in some cases the final separation is quite challenging. A potential solution to this problem would be the addition of a single fluorous tag to not only the glycosyl acceptor, but also to the glycosyl donor. The resulting coupling product would then have two fluorous tags whereas the starting materials and excess reagents would have lower or no fluorous content. However, although the differences between fluorous-tagged and untagged compounds proved great enough for reliable automated separation using a standard protocol, it was unclear whether the differences in retention time between the doubly-tagged and singly-tagged fluorous compounds would be great enough for robotically-controlled separations. To test this strategy, an additional C_8F_{17} ester or a perfluoro silyl-derived protecting group was attached to the *O*-2 position of the donor molecule as a second fluorous handle. In the course of this work, another donor activation strategy, in addition to the trichloroacetimidate donor, was also tested to expand the possible kinds of activated donors used in an automated protocol.

Results and discussion

To investigate the possibility of forming di-fluorous-tagged compounds under automated conditions, an activated rhamnosyl donor was prepared. Rhamnose was chosen to allow comparisons with the initial studies of the single tagged oligosaccharides using the

automation protocols with this sugar. To this end, the synthesis of an activated rhamnosyl donor was started from the known orthoester derivative **1** [2]. Compound **2** was provided after selective opening of the orthoester with allyl alcohol under acidic conditions followed by deprotection of the resulting acetate to reveal a free hydroxyl group. This 2-position hydroxyl was then treated with 2H, 2H, 3H, 3H-perfluoro undecanoic acid to provide singly-fluorous-tagged compound **3**. After deprotection of the anomeric allyl group under well-established catalytic iridium conditions [3], the fluorous acetylated intermediate was then further elaborated to the desired trichloroacetimidate donor **5**.

The separation of a di-fluorous-tagged compound from a singly-tagged monosaccharide was previously studied using a glucosamine building block. Results show that the separation between the two was possible with a commercially available FSPE cartridge by simply changing the solvent used to elute out the two compounds. Also, deprotection of the fluorous acetate group demonstrated that the solubility difference of the sugar chain and the detached fluorous tag was big enough in toluene to have good separation based on such physical properties.

With this information in hand, the synthesis of a 1,2-linked pentarhamnose was attempted under automated conditions. The initial acceptor **5** was prepared in 7 steps using known conditions [4]. The synthetic protocol and sample preparation mostly followed the previously reported procedure [1]. The major change came in the FSPE task, which was modified by using an additional 90% methanol wash before the usual 100% methanol wash.

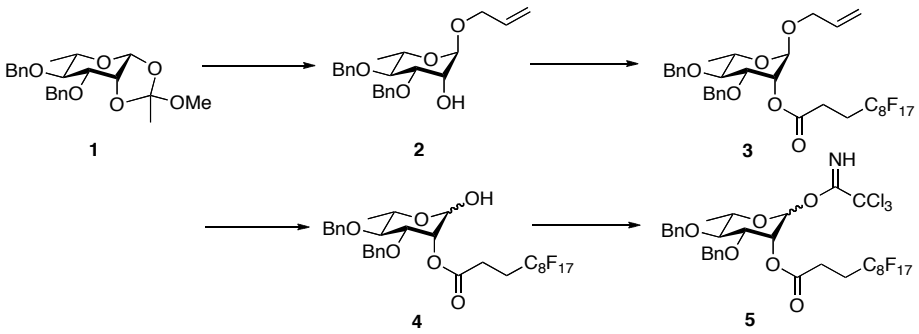


Figure 5-2. Synthesis of the fluorous acetate protected donor **5**.

Unfortunately, the previous protocol failed to produce the desired pentamer; the initial protocol would require modifications. Unlike the conventional bench-top conditions, the FSPE cartridge can easily dry out and become unusable when performing the solid-phase extraction step. The robotic needle is programmed to transfer a certain amount of solvent to elute out compounds from the FSPE cartridge, but occasionally the cartridge becomes dried out resulting a poor separation between the mono-tagged and di-tagged compounds. Surprisingly, it was observed that the separation was especially difficult between the imidate form of the donor and the di-tagged compound, as opposed to the decomposed donor and other by-products that are singly-tagged. Clearly, separations using fluorous silica gel are not always completely predicated on fluorous content alone. In order to get around this unexpected problem, we decided to add a water addition step to the glycosylation pot before quenching to ensure complete decomposition of any excess donor. With this modification to the glycosylation step, the modified strategy now successfully delivered only the di-tagged compound to the new pot for the deprotection reaction.

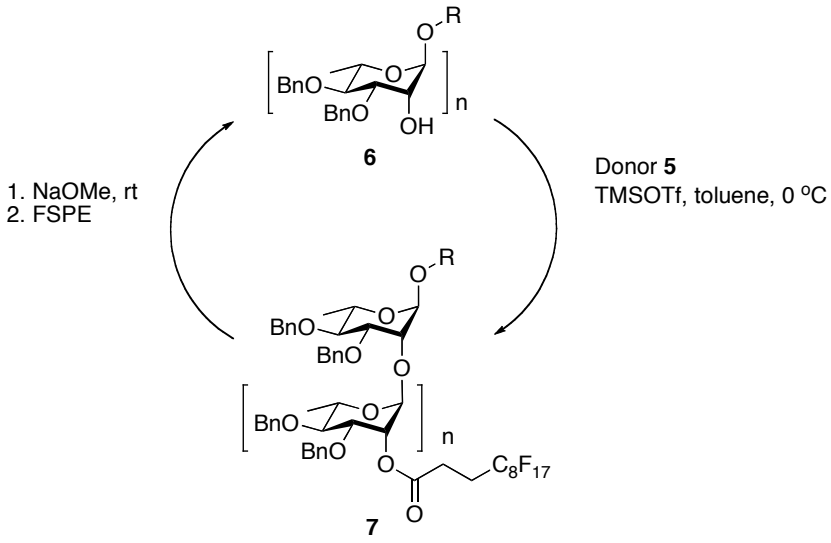


Figure 5-3. Automated iterative synthesis of a linear rhamnose pentamer.

After conditions were discovered to circumvent purification difficulties after the glycosylation step, a second problem was encountered in purifying the product resulting from the deprotection step. This challenge was more expected, however, as now a mono-fluorous-tagged product would need to be separated from the removed fluorous acetate protecting group. Prior experience with the manual synthesis of linked-glucosamines with a di-tagging plan [5] fortunately hinted at a potential resolution. Because toluene is a strongly fluorophobic solvent, the crude reaction mixture could be separated after the deprotection step by simply adding toluene. The desired saccharide compound was dissolved in toluene whereas the fluorous-containing material formed an oil on the wall of the reactor vial. Unfortunately, the acetic acid solution used to quench the sodium methoxide-containing reaction generates a good amount of salt that leads to the failure of the separation. Simply changing the acid to a solid resin instead of a solution form solved the problem of generating salts in the reaction pot. In the revised protocol, the robot was programmed to transfer the

reaction mixture to a reactor vial that was pre-packed with an acidic solid resin and then again transferred to a clean vial for solvent removal and separation via toluene addition.

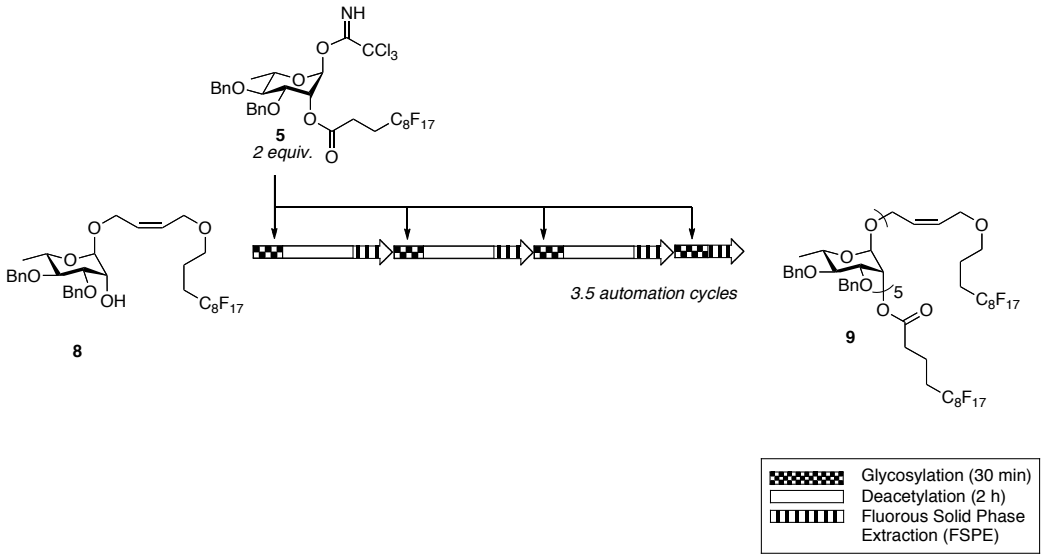
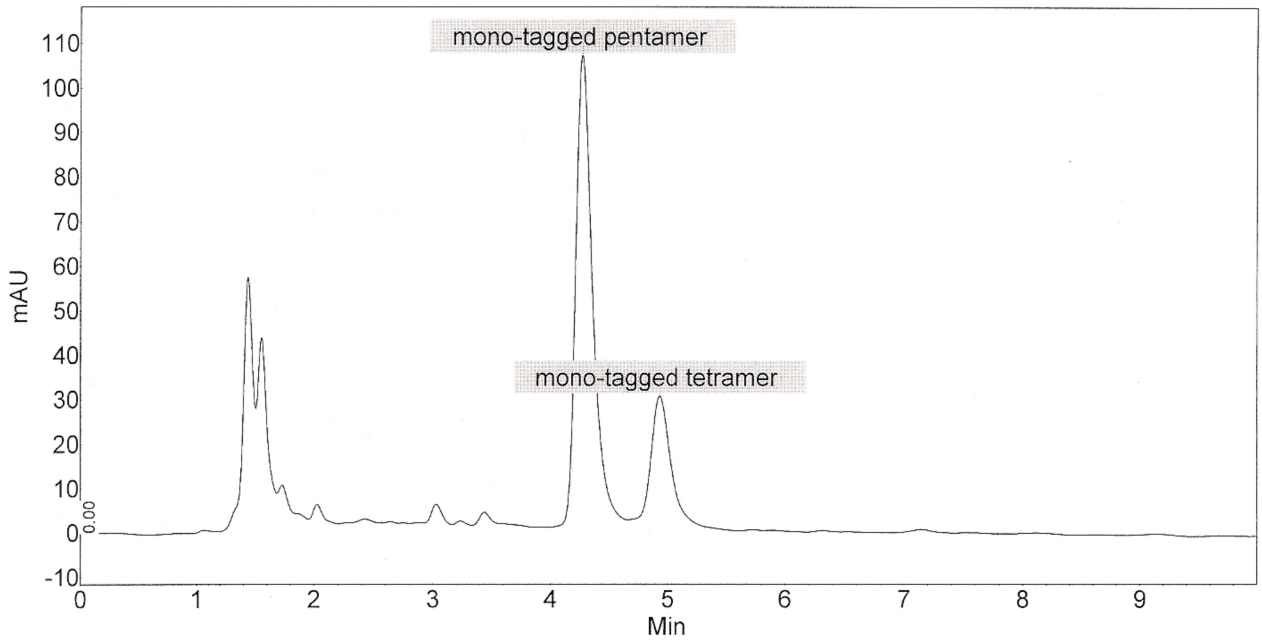


Figure 5-4. Overall schematic of the automated synthesis of a ditagged-rhamnose pentasaccharide.

In order to examine the success of this modified method in the synthesis of the original pentarhamnose target, the fluorous tailed acceptor **8** was again injected into the machine. After 22 hours, the di-tagged rhamnose pentamer was successfully retrieved from the robotics platform. For comparison, an HPLC trace of the crude product taken directly after the run is shown below along with the HPLC trace of the crude mono-tagged rhamnose pentamer, which was previously synthesized by the initial protocol [1].

Step	Task	Reagents/Operation	Time
1	Glycosylation	Donor **5**, TMSOTf, toluene, 0 °C	45
2	TLC		
3	Water	water	
4	Quench	triethylamine	
5	Evaporation	40 °C	45
6	FSPE	80% methanol (6 mL) 90% methanol (6 mL) 100% methanol (6 mL)	
7	Evaporation	40 °C	45
8	Deprotection	NaOMe, rt	180
9	TLC		
10	Quench	Acidic Amberlyst resin	
11	Evaporation	40 °C	45
12	Separation	toluene	
13	Evaporation	40 °C	45

Table 5-1. Modified tasks for the automated synthesis platform.



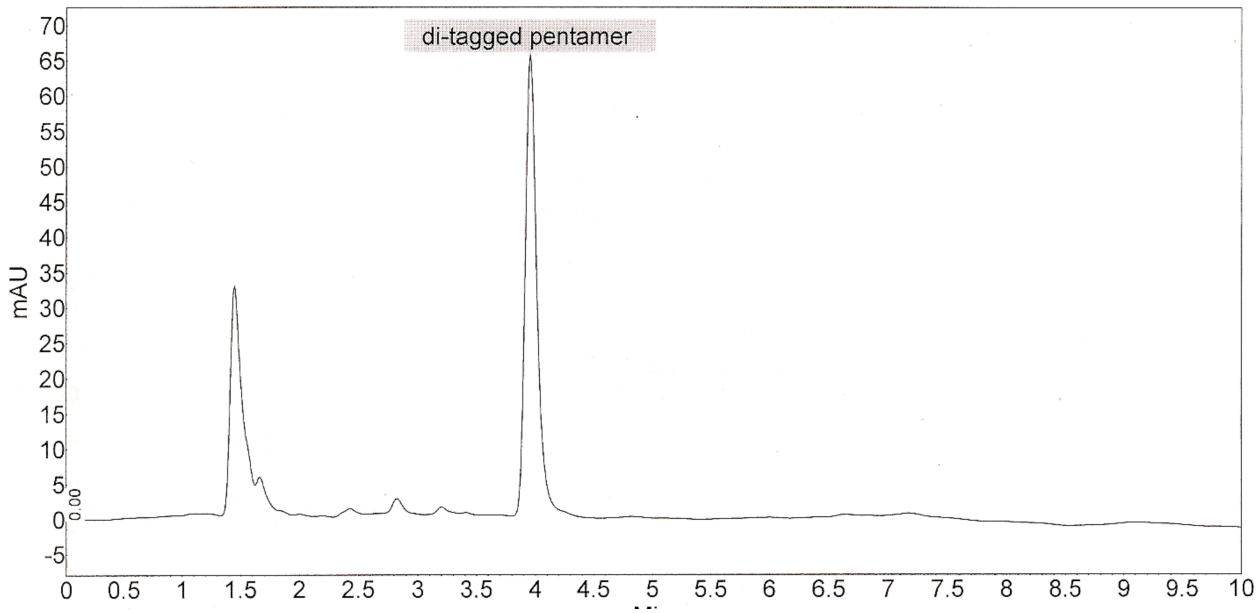


Figure 5-5. HPLC traces for the two compounds obtained from the automated synthesis platform (1 mL/min flow rate, 20% ethyl acetate/hexane, 10 minute run a standard silica column.)

With these initial results in hand, the next goal was to synthesize a silyl-derivatized fluorous protecting group for application to a similar robotically-controlled synthesis run. Because ester derived protecting groups are used frequently throughout the construction of oligosaccharide molecules, an orthogonal protecting group with different functionality and that lacked the ability to aid in neighboring group participation in the glycosylation steps would be useful. A second fluorous protecting group would also test the generality of the solutions devised for a successful di-tagging approach to automated oligosaccharide synthesis. Numerous types of silyl ether protecting groups have been used in oligosaccharide synthesis mainly due to their stability throughout the various synthetic procedures [6]. Among the many known silyl derivatives, *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl

groups are the most frequently used groups. However fluorous versions of the *tert*-butyldimethyl and *tert*-butyldiphenyl groups were not readily available. From a commercially available diisopropyl-(1H,1H,2H,2H,-perfluorodecyl)silane, the triflated intermediate was synthesized [7] and used directly to protect the free hydroxyl group of **13** to obtain the silyl derived fluorous-tail protecting group.

In parallel with testing the generality of the di-tagging protocol with another protecting group, we also probed the use of a different glycosyl donor activation strategy. To date, only the trichloroacetimidate group [8] has been used in the automated synthesis of various oligosaccharides. Activation of a free anomeric hydroxyl group to the trichloroacetimidate group is a convenient process that is high yielding. In addition, the trichloroacetimidate donors are reliably reactive species for high conversion to the glycosylated products. These attributes make the group one of the most commonly used methods for donor building block activation [9]. However depending on the reactivity of the resulting glycosyl donor, the activated building block can sometimes be unstable such that long term storage is challenging. The rearrangement of the acetimidate to the amide moiety is also a concern and was observed in a number of cases. As a potential solution for cases in which the trichloroacetimidate proves too unstable for use, a more stable pentenyl donor [10] was synthesized and investigated under automated conditions. Pentenyl glycosides, first reported by Fraser-Reid, have been extensively studied and have proven to be a promising activation/protecting group at the anomeric position for constructing complex oligosaccharides [10].

A study of the utility if these *n*-pentenyl glycosides (NPGs) as a component for automated oligosaccharide synthesis is attractive for a number of reasons. The synthesis of individual monosaccharide building blocks that bear the correct arrangements of orthogonal protecting groups is often a tedious process. The synthetic process to obtain a final donor monosaccharide can take as few as 3 steps or as many as 16 synthetic steps. The majority of these building block synthetic schemes involve the protection/deprotection and activation of the anomeric position. Use of the NPG group as the anomeric protecting group for the building block synthesis has the advantage of not usually requiring repetitive protection/deprotection/activation steps on the C-1 position throughout the synthesis. The NPG group can be introduced at the C-1 position at the beginning of the synthesis and carried through the synthesis as the other protecting groups are incorporated at the designated position of the sugar molecule. In the end, a protection and deprotection step is saved to thereby shorten the overall building block scheme. The pentenyl groups are then generally activated by halogenation of the pentenyl double bond, which results in cyclization and the release of the agylcone in the form of a halogenated furanose moiety as well as of the active glycosylation species [11]. The activation of these NPGs is achieved by using various promoters such as iodonium dicollidine perchlorate (IDC), or the corresponding triflate (IDCT), iodine [12]. Alternatively, the use of NBS, NIS in combination with either TfOH or TESOTf has been reported as well [10].

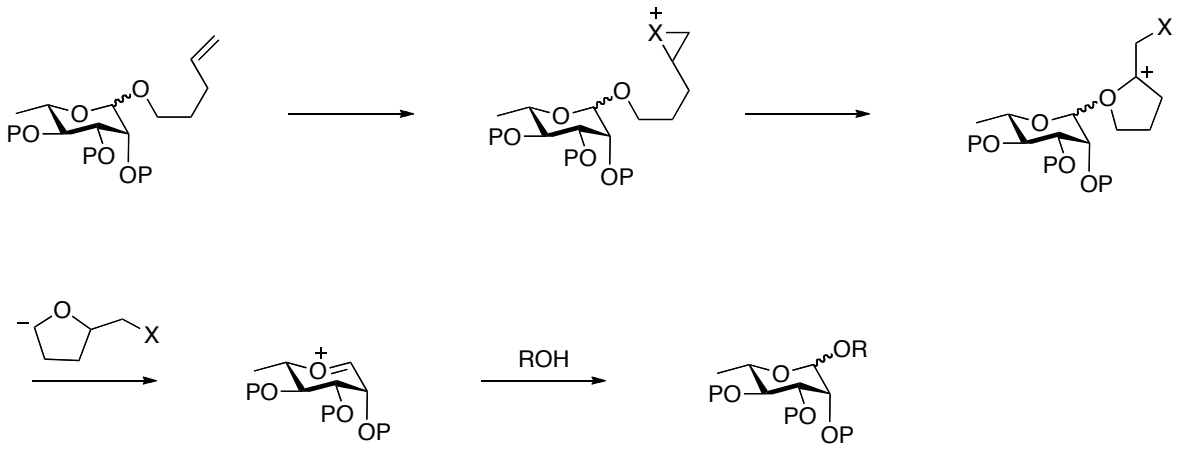


Figure 5-6. *n*-Pentenyl glycoside activation process. [6]

Finally and most importantly, the NPGs provide excellent stability under a variety of protecting group manipulations. In addition, unlike the trichloroacetimidate species, long term storage of these donors should be possible both in solution on the robotics platform when running long programs and also neat under low temperature conditions for future use without fear of rearrangement by-products.

To test the feasibility of incorporation of pentenyl glycosides into automated oligosaccharide synthesis runs, a new rhamnose hexasaccharide **10** was selected as a target. This target molecule has been found in streptococcal group A bacteria as a polyrhamnose (polyRha) core [13] in alternating 1,2 and 1,3 linkages with *N*-acetylglucosamine (GlcNAc) residues β-1,2-linked to available 3-positions of the rhamnose core [14]. The synthesis of the target hexasaccharide was designed to have a disaccharide repeating unit **11** that contains the alternating 1,2 and 1,3-linkages (Figure 5-7). The initial disaccharide contains the fluorous

tag at the anomeric position for ease of purification during automated cycles, and the second fluorous tag is attached to the *O*-3 position of the second rhamnose to test the new silyl derived fluorous protecting group for purification. Also, instead of a trichloroacetimidate donor, the *n*-pentyl group was introduced into the synthetic scheme.

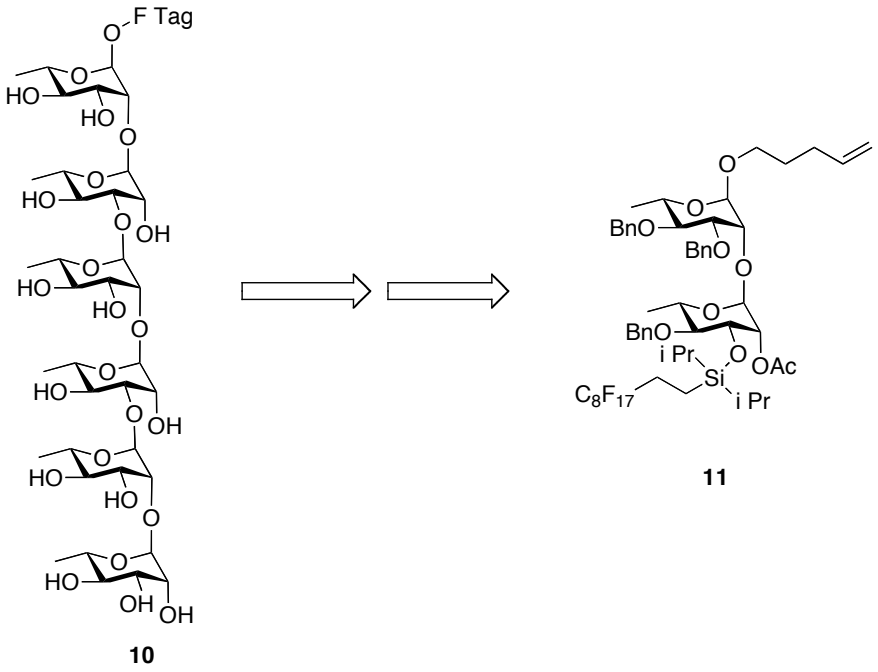


Figure 5-7. Retrosynthesis of the rhamnose hexasaccharide repeating building block.

The synthesis of the disaccharide building block was started by a previously discussed allyl-rhamnose intermediate with a free *O*-3 position (**12**). The commercially available diisopropyl-(1H,1H,2H,2H,-perfluorodecyl)silane was converted into its triflated intermediate [7] and was used directly in the same pot to protect the *O*-3 position of the rhamnose monosaccharide. The diisopropyl perfluoro silyl group will serve as the second fluorous tag to test its robustness as a second purification handle under automation. This

silyl-protected building block was carried through a series of reactions to remove the allyl group and further activate the group into the trichloroacetimidate donor. The pentenyl rhamnose acceptor **15** was prepared from a known rhamnose orthoester intermediate [2] and was deprotected at the *O*-2 position to attach the second rhamnose building block and complete the disaccharide repeating unit. With the disaccharide repeating unit containing both the perfluoro silyl protecting group and the *n*-pentenyl anomeric group, the next step was to subject this repeating unit to glycosylation reaction conditions in the automated platform and build up the target hexasaccharide.

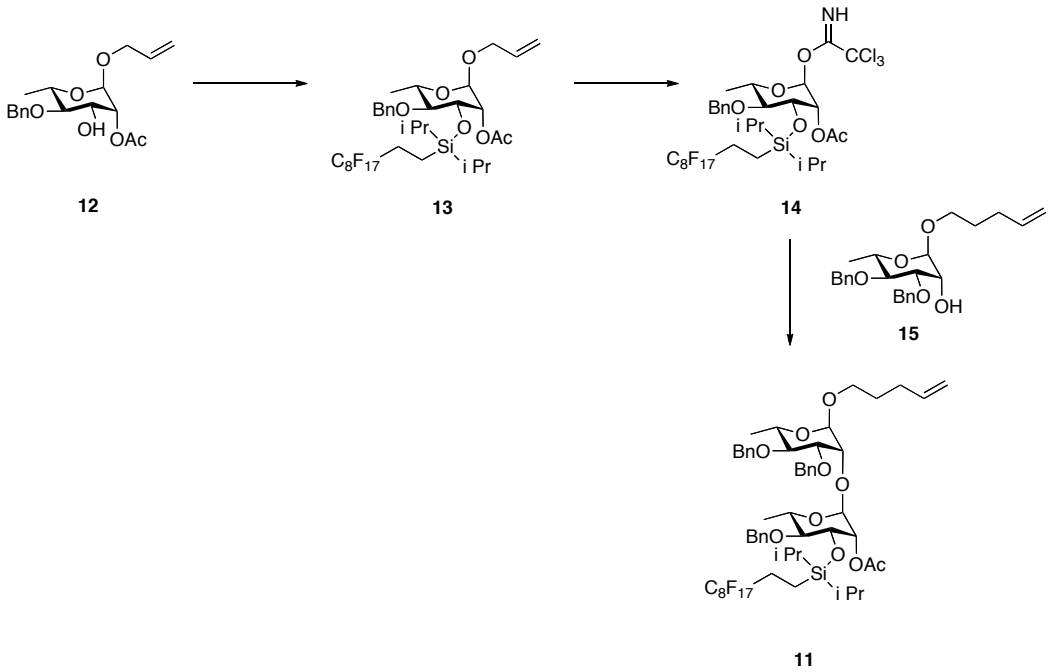


Figure 5-8. Synthetic scheme for the disaccharide repeating unit building block for the automated synthesizer.

The initial idea for the modified glycosylation conditions was to use NIS as the promotor along with TESOTf in dichloromethane conditions. However, these conditions turned out to be problematic under the automation platform because the solubility of NIS in dichloromethane was poor. Preparing a homogenous solution of NIS is important for the needle to robotically transfer an accurate quantity of NIS into the reactor vial and thereby fully convert the donor into its active species. To make this condition amenable for automation, a dual solvent reaction condition was attempted by making a NIS/acetonitrile stock solution vial to transfer the exact equimolar amount of NIS solution to the programmed reactor vial where the donor disaccharide/dichloromethane solution is located. After 20 minutes of vortexing, the TLC aliquot collected showed a high conversion of donor molecule to the anomeric fluorous tail product. The crude sample was then transferred to the FSPE station for purification. Fortunately, use of the same solvent conditions described earlier allowed the di-fluorous tagged molecule to be easily separated from the mono-fluorous tagged compounds. The purified di-tagged intermediate was then carried back to a clean reactor vial for deprotection of the fluorous silyl protecting group. In this case, too, the desired mono-fluorous product could be removed from the fluorous-containing silyl material using differential solubility in toluene. The reaction conditions and times are described in the table below.

Step	Task	Reagents/Operation	Time
1	Glycosylation	NIS/MeCN, TESOTf, Donor, DCM,	30
2	TLC	25 °C	
3	Water		
4	Quench	sodium thiosulfate solution	
5	Evaporation	40 °C	45
6	FSPE	80% methanol (6 mL) 90% methanol (6 mL) 100% methanol (6 mL)	
7	Evaporation	40 °C	45
8	Deprotection	TBAF/THF, rt	600
9	TLC		
10	Quench		
11	Evaporation	40 °C	45
12	Separation	toluene	
13	Evaporation	40 °C	45

Table 5-2. Description of the rhamnose hexasaccharide synthesis protocol.

After 56 hours, the di-tagged rhamnose hexamer was successfully synthesized under automated means in a pure form in 11% overall yield, which corresponds to approximately 70% per step yields. The separation conditions proved robust enough for application to this new activation and protecting group strategy.

Conclusion

In summary, this report presents a new protecting group strategy for complex oligosaccharide syntheses under automated conditions. We have demonstrated the convenient synthesis of the fluorous acetate protecting group and how it could be used to easily purify the elongated oligosaccharide chains away from failed sequences with a simple gravity filtration. Also, conditions to deprotect the fluorous acetate group and remove these fluorous by-products from the desired singly-tagged saccharide chain were demonstrated. In addition,

the fluorous silyl protecting group was introduced into automated oligosaccharide synthesis and tested to serve as an orthogonal protecting group that delivers some of the same advantages of the fluorous acetate group. Finally, the development of conditions for the inclusion of *n*-pentenyl glycosyl donor into automated oligosaccharide protocols offers more choices in glycosyl donor and a more stable and convenient option compared to some trichloroacetimidate donors. With protocols now in hand that expand the range of protecting group, purification, and donor activation options in automated oligosaccharide synthesis, an even greater range of complex oligosaccharides should succumb to synthesis and the possibilities for carbohydrate library synthesis are significantly expanded.

Experimental section

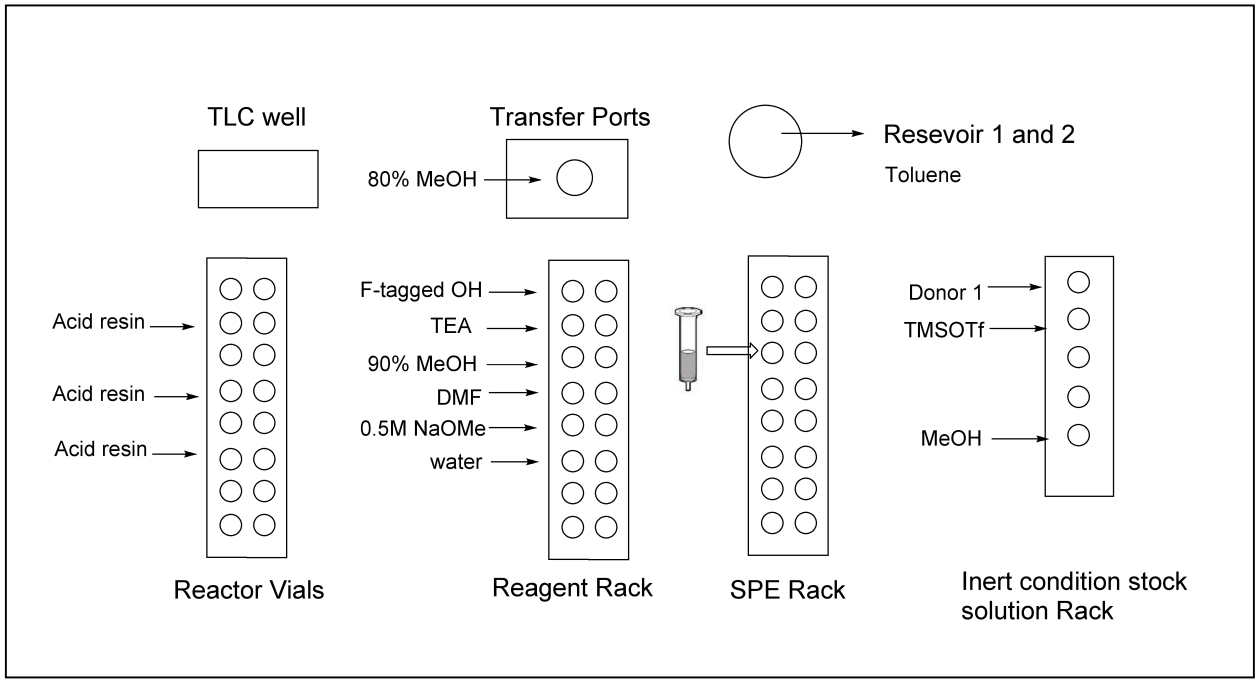
General methods

Commercial reagents and solvents were used as received without further purification unless otherwise stated. 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. 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 162 MHz. ^{1}H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (7.27ppm) as an internal reference. ^{13}C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (77.23 ppm). HPLC traces were obtained using a Varian Inc. HPLC with a Waters Nova-pak 4 μL 3.9 ×150 mm silica column. High-resolution mass spectrometry was obtained with Applied Biosystems QSTAR XL Hybrid System.

General procedures of Automated Synthetic Cycles using ASW 1000

<ASW 1000 platform>



1. Sample Preparation

Donor molecule (361.1 mg, 375 μmol) was dissolved with dried toluene (2.5 mL) in the 8 mL vial and placed at the stock solution rack (Donor 1) under nitrogen. 0.27 M trimethylsilyltrifluoromethanesulfonate (TMSOTf, 1.05 mL) solution in toluene prepared in the 13 mL vial and placed as indicated on the stock solution rack under nitrogen. Dried toluene (800 mL) was prepared in the 1 L reservoir bottle and placed with the syringe tubing connected for reaction conditions and reservoir solution for rinsing. Methanol (100 mL) was prepared in the 100 mL vial and placed under nitrogen at the inert condition rack. 80% methanol/water (100 mL), triethylamine (10 mL) were prepared in stock solution bottles and connected to the transfer ports. Acceptor molecule (43.7 mg, 50 μmol) was dissolved with dried toluene (0.5 mL) in a Wheaton 8 mL E-Z extraction vial (conical bottomed) capped with pre-punctured septa and placed at the reagent rack where indicated (F-tagged OH).

0.5 M sodium methoxide solution in methanol (5 mL) was prepared in 8 mL vial capped with pre-punctured septa and placed at the reagent rack where indicated. *N,N*-dimethylformamide (DMF, 5 mL) directly from Fluka was transferred to an 8-mL vial capped with pre-punctured septa and placed at the reagent rack where indicated. Fluorous solid phase extraction (FSPE) cartridges (2 g, 10 cc) were preconditioned with 80% methanol and placed on the machined SPE block. An empty Wheaton 8 mL E-Z extraction vial (conical bottomed) was placed under the FSPE cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle each of the 16 reactor vials (13 mL) were rinsed with 8 mL toluene and 8 mL methanol 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solvents were prepared by azeotroping the building blocks individually with toluene and dried under high vacuum. After the reactor vials were dry, the reagents were transferred into the vials to be placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Method Run

3.1. Glycosylation (Trichloroacetimidate donor method)

Reactor vials were cooled to 0 ^{o}C during the 5 minutes wait time by the heat transfer oil. The needle transferred the acceptor molecule (F-tagged monomer) solution (0.6 mL) to the reaction vial 1, followed by the transfer of the acceptor molecule solution (0.5 mL) and the TMSOTf solution (56 μL). After each individual transfer, the needle was rinsed by 2 mL toluene inside and out before operating the next task. The reaction mixture was vortexed at 800 rpm for 45 minutes at 0 ^{o}C under inert gas. After the reaction time, the needle withdrew 20 μL from the reaction mixture and placed it onto the first well of the microtiterplate for

thin layer chromatography monitoring. Water (0.5 mL), triethylamine (0.5 mL) was added to the solution for quenching and solvent evaporated under reduced pressure.

3.2. Glycosylation (Pentenyl donor method)

In a clean reactor vial the needle transferred the donor molecule (disaccharide repeating unit) solution (0.6 mL) to the reaction vial 1, followed by the transfer of the NIS/MeCN solution and the TESOTf solution (56 μL). After vortexing the reactor vials at 300 rpm for 3 min, accpetor monosaccharide (0.5 mL) was added to the same vial. Between each individual transfer, the needle was rinsed by 2 mL toluene inside and out before operating the next task. The reaction mixture was vortexed at 800 rpm for 30 minutes at 25 ^{o}C under inert gas. After the reaction time, the needle withdrew 20 μL from the reaction mixture and placed it onto the first well of the microtiterplate for thin layer chromatography monitoring. Triethylamine (0.5 mL) was added to the solution for quenching and solvent evaporated under reduced pressure.

3.3. FSPE

DMF (0.4 mL) was added to dissolve the crude mixture and the vials were vortexed at 100 rpm for 5 minutes. Reaction mixture (0.7 mL) was carried to the FSPE cartridge at the SPE rack dispensed at a speed of 20 mL/s via the 1 mL syringe. Then 80% methanol (4.5 mL) was used to rinse the empty reactor vial. 80% methanol solvent was removed from the reactor vial each time (1.7 mL, 1.5 mL, 1.5 mL) and delivered to the FSPE cartridge. Additional 80% methanol solution (1.5 mL) 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. 90% methanol solution (2 mL) was used to rinse the FSPE cartridge 3 times. After all the undesired compounds were eluted out, methanol (2 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as ‘SPE collect’ to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into ‘SPE direct’ for the needle to withdraw the collected sample from the vial and deliver it to the clean reactor vial for the next reaction. Toluene (1 mL) was added to the solution and solvent was evaporated under reduced pressure. After evaporation cycle, once again toluene (1 mL) was added and removed under reduced pressure for complete dryness.

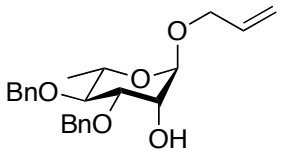
3.4. Deacetylation

To the dried residue, methanol (0.5 mL) was added to the reactor vial followed by sodium methoxide solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 3 hours at room temperature. After the reaction time, the needle transferred the reaction mixture to a clean reactor vial that contains acidic Amberlyst resin to quench the reaction. The reaction mixture was vortexed at 800 rpm for 15 minutes and then again transferred to a clean vial. Solvent was evaporated under reduced pressure to give crude product. Then 1 mL of toluene was added, and vortexed for an additional 15 minutes. The resulting solution was transferred to a clean vial and the solvent was evaporated under reduced pressure.

3.5. Desilylation

To the dried residue, THF (0.5 mL) was added to the reactor vial followed by TBAF solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 10 hours at room temperature. After the reaction time, solvent was evaporated under reduced pressure to give crude product. Then 1 mL of toluene was added, and vortexed for an additional 15 minutes. The resulting solution was transferred to a clean vial and the solvent was evaporated under reduced pressure.

Synthetic Procedure



Allyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (2)

To a solution of rhamnose orthester [15] (591 mg, 1.48 mmol), allyl alcohol (0.6 ml, 8.86 mmol) in dry CH_2Cl_2 (10 mL), was added TfOH (3 μL) at -40 ^oC bath. After stirring at -40 ^oC under nitrogen for 30 minutes, the reaction mixture was stirred for another 30 minutes to be brought up to room temperature. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution, water and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in a solution of NaOMe in MeOH (10 mL). The mixture was stirred at room temperature for 1 h, then was quenched with acidic Amberlyst resin. After the resin was filtered, the solvent was concentrated under

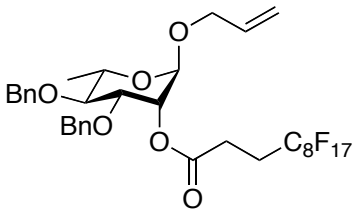
vacuum. The crude product was purified by silica gel chromatography to give the titled compound [16] as a colorless syrup (449 mg, 1.17 mmol, 79%).

R_f (ethyl acetate/hexane): 0.67 (40/60)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.49-7.32 (m, 10H, OBn), 5.99-5.89 (m, 1H, CH=), 5.36 (dd, J = 17.1 Hz, 1.5 Hz, 1H, CH_2=), 5.26 (dd, J = 10.5 Hz, 1.5 Hz, 1H, CH_2=), 5.00 (d, J = 10.8 Hz, 1H, CH_2), 4.92 (s, 1H, H-1), 4.75 (s, 2H, CH_2), 4.72 (d, J = 11.1 Hz, 1H, CH_2), 4.24 (dd, J = 13.2 Hz, 6.4 Hz 1H, CH_2), 4.12 (dd, J = 3.2 Hz, 1.6 Hz, 1H, H-2), 4.00 (dd, J = 12.9 Hz, 5.1 Hz, 1H, CH_2), 3.95 (dd, J = 9.2 Hz, 3.2 Hz, 1H, H-3), 3.83 (m, 1H, H-5), 3.73 (t, J = 9.6 Hz, 1H, H-4), 2.85 (s, 1H, OH), 1.40 (d, J = 6 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 162 MHz): δ (ppm) 139.0, 138.2, 134.0, 128.8, 128.7, 128.3, 128.2, 128.1, 128.0 118.0, 98.5, 80.4, 80.3, 75.8, 72.5, 68.8, 68.1, 67.7, 18.4.

MS (ESI) *m/z* = calcd: 407.1834; found: 407.1941 $[M+Na]^+$.



Allyl 3,4-di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranoside (3)

To a solution of **2** (306 mg, 0.79 mmol) in dry dichloromethane (10 mL) cooled to 0 °C, was added DCC (327 mg, 1.7 mmol), DMAP (97 mg, 0.79 mmol) and *2H, 2H, 3H, 3H,* -perfluoroundecanoic acid (624 mg, 1.6 mmol). The reaction mixture was allowed to warm to

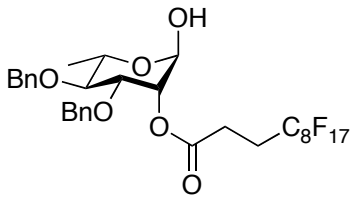
room temperature over 2 h. The reaction was diluted with dichloromethane (20 mL), washed with water (20 mL), HCl (2 N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 15% EtOAc/hexane as eluent to obtain the title compound (622 mg, 0.72 mmol, 91%) as a colorless oil.

R_f (ethyl acetate/hexane): 0.73 (40/60)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.49-7.32 (m, 10H, OBn), 5.97-5.86 (m, 1H, CH=), 5.49 (dd, J = 3.2 Hz, 1.6 Hz, 1H, H-2), 5.35 (dd, J = 10.5 Hz, 1.5 Hz, 1H, CH_2=), 5.24 (d, J = 10.8 Hz, 1H, CH_2), 4.98 (d, J = 11.1 Hz, 1H, CH_2), 4.84 (d, J = 1.6 Hz, 1H, H-1), 4.74 (d, J = 11.1 Hz, 1H, CH_2), 4.66 (d, J = 11.1 Hz, 1H, CH_2), 4.62 (d, J = 11.1 Hz, 1H, CH_2), 4.21 (dd, J = 12.8 Hz, 5.2 Hz, 1H, H-3), 4.03 (m, 2H, CH_2), 3.85 (m, 1H, H-5), 3.47 (t, J = 9.2 Hz, 1H, H-4), 2.78 (m, 2H, CH_2), 2.56-2.40 (m, 2H, CH_2), 1.39 (d, J=6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.0, 138.4, 138.1, 133.9, 128.5, 128.44, 128.2, 127.9, 117.9, 96.5, 80.0, 78.1, 75.6, 72.0, 70.0, 68.1, 67.9, 26.8, 25.8, 18.9.

HRMS-MALDI *m/z* = calcd: 859.1927; found: 859.1351 [*M*+Na]$^+$.



3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranoside (4)

To a solution of **3** (380 mg, 0.44 mmol) in dry THF (5 mL), was added $Ir(COD)(PMePh_2)_2 \cdot HPF_6$ (3.7 mg, 4.4 μmol). After the reaction flask was evacuated, a H_2 balloon was added until the reaction mixture turned to a pale yellow. The hydrogen balloon was removed and the reaction mixture was stirred under nitrogen at room temperature for an additional 3 hours. The reaction was diluted with dichloromethane (20 mL), washed with water (20 mL), HCl (2 N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. The solvent was concentrated under reduced pressure to give crude product to be used directly for the next step.

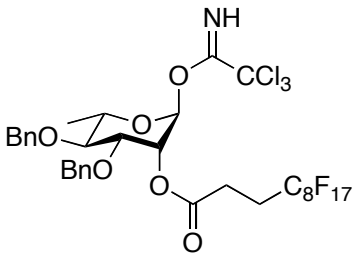
To a solution of the crude isomerized compound (337 mg, 0.39 mmol) in acetone/water (5/1, 6 mL) was added $HgCl_2$ (106 mg, 0.39 mmol), and HgO (85 mg, 0.39 mmol) at room temperature. The reaction mixture was stirred under nitrogen for 5 hours then was diluted with dichloromethane (20 mL), washed with water (20 mL), HCl (2 N, 20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 15% EtOAc/hexane as eluent to obtain the title compound (252 mg, 0.31 mmol, 79%) as colorless oil.

R_f (β): (ethyl acetate/haxane) 0.60 (60/40)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.49-7.32 (m, 10H, OBn), 5.45-5.43 (dd, J = 3.2 Hz, 2 Hz, 1H, H-2), 5.15 (d, J = 1.6 Hz, 1H, H-1), 4.94 (d, J = 11.1 Hz, 1H, CH_2), 4.70 (d, J = 11.1 Hz, 1H, CH_2), 4.65 (d, J = 11.1 Hz, 1H, CH_2), 4.58 (d, J = 11.1 Hz, 1H, CH_2), 4.03-3.97 (m, 2H), 3.42 (t, J = 9.6 Hz, 1H, H-4), 2.75 (t J = 8.0 Hz, 2H, CH_2), 2.45 (m, 2H, CH_2), 1.33 (d, J = 6 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 162MHz): δ (ppm) 171.1, 138.4 138.1, 128.6, 128.4, 128.6, 128.4, 128.1, 128.0, 92.6, 81.7, 77.8, 75.6, 72.1, 70.4, 68.1, 26.8, 25.8, 18.2.

HRMS-MALDI *m/z* = calcd: 841.1434; found: 841.1511 $[M+Na]^+$.



3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranosyl trichloroacetimidate (5)

To a solution of the alcohol **4** (80 mg, 0.08 mmol) in anhydrous DCM (5 mL) was added Cs_2CO_3 (66.6 mg, 0.20 mmol) and distilled trichloroacetonitrile (0.04 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 0.5 h and then filtered over a pad of Celite. The filtered solution was concentrated under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 10% EtOAc/hexane as eluent to obtain titled compound (83 mg, 0.08 mmol, 93%) as a colorless oil.

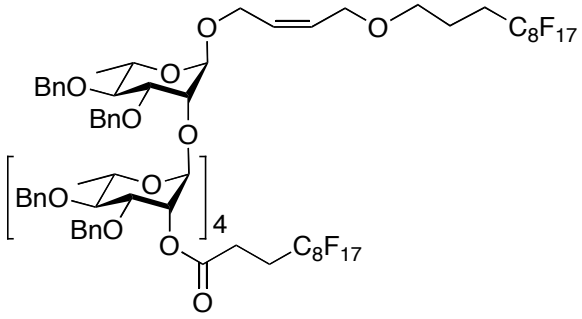
R_f: (ethyl acetate/haxane) 0.93 (60/40)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.60 (s, 1H, NH), 7.36-7.15 (m, 10H, OBn), 6.19 (dd, J = 1.6 Hz, 1H, H-1), 5.51 (dd, J = 3.2 Hz, 2.0 Hz, 1H, H-2), 4.92 (d, J = 10.4 Hz, 1H, CH_2), 4.71 (d, J = 10.4 Hz, 1H, CH_2), 4.64 (d, J = 10.4 Hz, 1H, CH_2), 4.59 (d, J = 10.4 Hz, 1H, CH_2), 4.02 (dd, J = 9.6 Hz, 3.2 Hz, 1H, H-3), 3.97-3.92 (m, 1H, H-5), 3.52 (t, J = 9.6 Hz, 1H,

H-4), 2.82-2.72 (m, 2H, CH_2), 2.54-2.41 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.36 (d, J = 6.0 Hz, 3H, H-6).

^{13}C NMR ($CDCl_3$, 162MHz): δ (ppm) 170.8, 160.7, 138.1, 137.9, 128.8, 128.7, 128.5, 128.4, 128.2, 128.1, 95.1, 79.8, 77.3, 75.9, 72.4, 71.0, 68.5, 26.8, 25.8, 18.2.

HRMS-MALDI *m/z* = calcd: 984.0530; found: 984. 0599 $[M+Na]^+$.



- 3.5 cycles completed for the synthesis of the di-tagged pentasaccharide

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	1.5 equivalent first donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.1 equivalent TMSOTf, 0 °C	45 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.5 mL water, then 0.5 mL TEA	
4	Evaporation	40 °C	45 min
5	FSPE	0.4 mL DMF used to dissolve crude mixture 80% methanol (6 mL), 90% methanol (6 mL), 100% methanol (6 mL)	
6	Transfer	6 mL collected sample transferred to clean vial	
7	Evaporation	40 °C	45 min
8	Deacetylation	NaOMe, rt	3 hrs
9	TLC sample	20 μL of crude reaction mixture withdrawn	
10	Quenching	Transfer mixture to prepacked acidic resin reactor vial	
11	Transfer	Transfer solution to clean reactor vial	
12	Evaporation	40 °C	45 min
13	Separation	3 mL toluene used to dissolve out desired compound	
14	Evaporation	40 °C	45 min
		Repeat 1-14 (x 2)	
43	Glycosylation	1.5 equivalent first donor (75 μmol) in 0.5 mL toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL toluene; 0.1 equivalent TMSOTf, 0 °C	45 min
44	TLC sample	20 μL of crude reaction mixture withdrawn	
45	Quenching	0.5 mL water, then 0.5 mL TEA	
46	Evaporation	40 °C	45 min
47	FSPE	0.4 mL DMF used to dissolve crude mixture 80% methanol (6 mL), 90% methanol (6 mL), 100% methanol (6 mL)	
48	Transfer	6 mL collected sample transferred to clean vial	

Table 5-3. List of synthetic procedures programmed

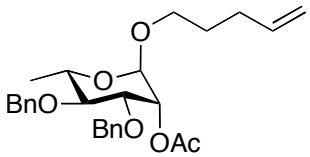
cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (9)

R_f: (ethyl acetate/haxane) 0.77 (60/40)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.36-7.15 (m, 50H), 5.80-5.60 (m, 2H), 5.68 (d, J = 3.2 Hz, 1.6 Hz, 1H), 5.28-4.54 (m, 25H), 4.13 (m, 18H), 3.49-3.31 (m, 7H), 2.30-2.14 (m, 7H), 1.95-1.80 (m, 4H), 1.45 (m, 15H).

^{13}C NMR ($CDCl_3$, 162 MHz): δ (ppm) 171.3, 138.7, 138.6, 138.6, 138.6, 138.5, 138.5, 138.5, 138.4, 138.4, 138.3, 129.9, 129.9, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 127.9, 127.9, 127.9, 127.7, 100.8, 100.5, 99.3, 98.2, 80.6, 80.5, 80.4, 80.4, 80.3, 80.3, 80.3, 79.6, 79.1, 78.0, 78.0, 77.9, 77.4, 75.7, 75.6, 74.9, 74.8, 74.77, 72.4, 72.3, 72.2, 72.1, 72.0, 69.1, 69.0, 68.7, 68.5, 68.2, 66.7, 62.7, 28.9, 21.6, 21.2, 18.9, 18.3.

HRMS-MALDI *m/z* = calcd: 2675.8045; found: 2675.8102 $[M+Na]^+$.



n-Pentenyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranoside.

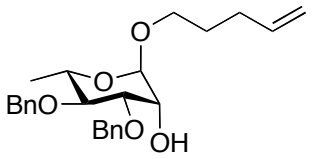
To a solution of rhamnose orthester [15] (705 mg, 1.76 mmol), *n*-pentenyl alcohol (1.07 mL, 10.6 mmol) in dry CH_2Cl_2 (5 mL), was added TfOH (5 μL) at -40 ^oC bath. After stirring at -40 ^oC under nitrogen for 30 minutes, the reaction mixture was stirred for another 30 minutes to be brought up to room temperature. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution, water and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in a solution of NaOMe in MeOH (10 mL). The mixture was stirred at room temperature for 1 h, then was quenched with acidic Amberlyst resin. After the resin was filtered, the solvent was concentrated under vacuum. Crude product was purified by silica gel chromatography to give titled compound as a colorless syrup (632 mg, 1.39mmol, 79%).

R_f: (ethyl acetate/hexane) 0.90 (40/60)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34-7.27 (m, 10H, OBn), 5.84-5.77 (m, 1H, CH=), 5.37 (s, 1H), 5.06-4.91 (m, 3H), 4.73 (m, 2H), 4.63 (d, J = 10.8 Hz, 1H), 4.56 (d, J = 11.2 Hz, 1H), 3.96 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.78 (m, 1H), 3.67 (m, 1H), 3.44-3.35 (m, 2H), 2.16 (s, 3H), 2.10 (m, 2H), 1.67 (m, 2H), 1.35 (d, J = 6.0 Hz, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.0, 138.4, 138.1, 133.9, 128.5, 128.44, 128.2, 127.9, 117.9, 96.5, 80.0, 78.1, 75.6, 72.0, 70.0, 68.1, 67.9, 26.8, 25.8, 18.9.

HRMS-MALDI $[M+Na]^+$ *m/z* = calcd: 477.2253; found: 477.8130.



n-Pentenyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (15)

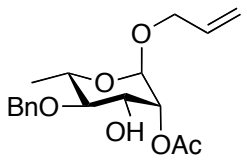
To a solution of n-pentenyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (500 mg, 1.10 mmol) in methanol (5 mL), was added a small piece of sodium (~ 10 mg) at room temperature. After stirring at rt under nitrogen for 30 minutes, the reaction mixture was quenched with acidic Amberlyst resin and filtered. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography to give the titled compound as a colorless syrup (449 mg, 1.09 mmol, 99%).

R_f: (ethyl acetate/hexane) 0.83 (40/60)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40-7.31 (m, 10H, OBn), 5.84-5.81 (m, 1H, CH=), 5.07 (dd, J = 17.2 Hz, 1.6 Hz, 1H), 5.09 (dd, J = 12.4 Hz, 2.4 Hz, 1H), 4.92 (d, J = 10.8 Hz, 1H), 4.81 (d, J = 1.2 Hz, 1H), 4.72 (s, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.04 (dd, J = 2.8 Hz, 1.6 Hz, 1H), 3.88 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.77-3.66 (m, 2H), 3.50 (t, J = 9.2 Hz, 1H), 3.44 (m, 1H), 2.53 (d, J = 2.0 Hz, 1H), 2.15 (m, 1H), 1.71 (m, 1H), 1.34 (d, J = 6.4 Hz, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 138.6, 138.2, 138.1, 128.7, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 115.1, 99.1, 80.3, 80.2, 75.6, 72.2, 68.8, 67.4, 67.1, 30.5, 28.8, 18.0.

HRMS-MALDI $[M+Na]^+$ *m/z* = calcd: 435.2147; found: 435.9012.



Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (12)

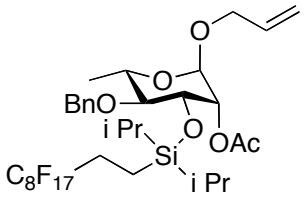
To a solution of allyl 4-*O*-benzyl-2,3-*O*-methoxyethylidene-α-L-rhamnopyranose (538 mg, 1.54 mmol) in dry dichloromethane (10 mL), was added TfOH/water (v:v=3:2, 0.95 mL) solution at room temretature. The reaction mixture was stirred at room temperature for 1 hour. After TLC confirmed complete starting material comsumption the reaction mixture was diluted with dichloromethane (20 mL), washed with water (20 mL), $NaHCO_3$ solution (20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 25% EtOAc/hexane as eluent to obtain the title compound (501 mg, 1.49 mmol, 97%) as colorless oil.

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

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.35-3.26 (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 ($CDCl_3$, 100 MHz): 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-MALDI $[M+Na]^+$ *m/z* = calcd: 359.1471; found: 349.1108.



Allyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α-L-rhamnopyranoside (13)

To a solution of diisopropyl-(1H, 1H, 2H, 2H, perfluorodecyl)silane (130 mg, 0.23 mmol) in anhydrous dichloromethane (1.5 mL) was added triflic acid (18.7 μL, 0.23 mmol) at room temperature. After the reaction mixture was stirred for 30 min, a solution of **12** (25 mg, 0.07 mmol) and 2,6-lutidine (45 μL) in dry dichloromethane (1 mL) was added at room temperature and the solution was stirred for an additional 15 min. Afer TLC confirmed complete starting material consumption, the solution was diluted with dichloromethane (20 mL), washed with water (20 mL), saturated $NaHCO_3$ solution (20 mL), and brine (20 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane as eluent to obtain the title compound (59 mg, 0.07 mmol, 86%) as colorless oil.

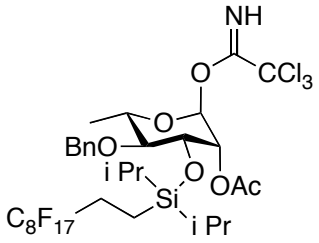
R_f: (ethyl acetate/hexane) 0.93 (40/60)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.32-7.26 (m, 5H), 5.84 (m, 1H), 5.27 (dd, J = 17.2 Hz, 1.2 Hz, 1H), 5.27 (dd, J = 17.4 Hz, 1.4 Hz, 1H), 5.10 (s, 1H), 4.87 (d, J = 11.2 Hz, 1H),

4.69 (s, 1H), 4.61 (d, J = 11.6 Hz, 1H), 4.22 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 4.11 (dd, J = 13.2 Hz, 4.8 Hz, 1H), 3.93 (dd, J = 13.2 Hz, 5.6 Hz, 1H), 3.69 (m, 1H), 3.44 (t, J = 9.2 Hz, 1H), 2.11 (m, 7H), 1.29 (d, J = 6.0 Hz, 3H), 1.05 (m, 12H), 0.84 (m, 2H).

^{13}C NMR ($CDCl_3$, 162 MHz): δ (ppm) 170.0, 138.2, 133.5, 128.2, 127.5, 127.4, 116.8, 96.7, 81.6, 71.4, 68.2, 67.9, 20.6, 18.5, 18.2, 17.8, 17.3, 17.3, 17.3, 17.0, 16.9, 12.9, 12.8.

HRMS-MALDI $[M+Na]^+$ *m/z* = calcd: 833.1203; found: 833.5600.



2-O-Acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α/β-L-rhamnopyranosyl trichloroacetimidate (14)

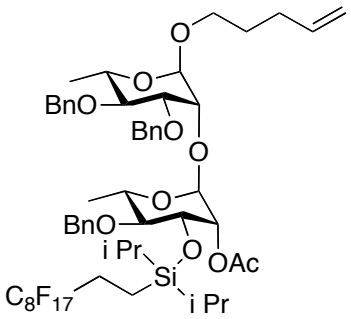
To a solution of the alcohol (653 mg, 0.76 mmol) in anhydrous DCM (5 mL) was added Cs_2CO_3 (621 mg, 1.9 mmol) and distilled trichloroacetonitrile (0.38 mL, 3.8 mmol). The reaction mixture was stirred at room temperature for 0.5 h and then filtered over a pad of Celite. The filtered solution was concentrated under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 10% EtOAc/hexane as eluent to obtain titled compound (663 mg, 0.07 mmol, 87%) as a colorless oil.

R_f: (ethyl acetate/hexane) 0.53 (20/80)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.68 (s, 1H), 7.53-7.19 (m, 5H), 6.14 (s, 1H), 5.26 (s, 1H), 4.88 (d, J = 11.2 Hz, 1H), 4.65 (d, J = 11.2 Hz, 1H), 4.35 (dd, J = 9.2 Hz, 2.8 Hz, 1H), 3.91 (m, 1H), 3.51 (t, J = 9.6 Hz, 1H), 2.16-2.02 (m, 7H), 1.26 (d, J = 6.0 Hz, 3H), 1.05 (s, 12 H), 0.93 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 160.2, 138.0, 128.6, 128.0, 127.8, 127.7, 94.9, 80.9, 75.8, 71.8, 71.3, 71.2, 21.0, 18.2, 17.8, 17.7, 17.6, 13.0, 12.9, 0.7.

HRMS-MALDI $[M+Na]^+$ *m/z* = calcd: 935.9986; found: 935.1280.



Pent-4-enyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl) diisopropylsilyl}-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (11)

To a solution of the rhamnose donor **14** (48 mg, 0.048 mmol) in dry dichloromethane (0.5 mL), was added the rhamnose pentenyl acceptor **15** (20 mg, 0.048 mmol) in dichloromethane (0.5 mL). TMSOTf (1 μL) was added to the solution and the reaction mixture was stirred at room temperature for 20 min. TLC of mixture shows complete conversion of the starting material. Reaction mixture was quenched with a few drops of triethylamine and solvent was concentrated under reduced pressure. The crude product was dissolved in DMF (0.5 mL) loaded onto a 2 g FSPE cartridge. The SPE cartridge was washed with 80% methanol/water

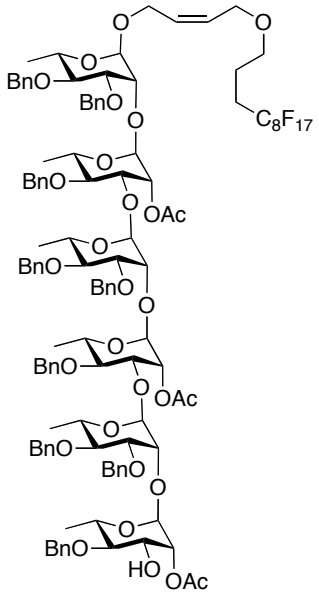
(10 mL) and 100% methanol (10 mL). The methanol solution was concentrated under reduced pressure to give the titled compound (49 mg, 0.042 mmol, 88%) as colorless oil.

R_f: (ethyl acetate/hexane) 0.53 (20/80).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34-7.24 (m, 15H, OBn), 5.82 (m, 1H), 5.25 (s, 1H), 4.96 (m, 3H), 4.85 (dd, J = 10.4 Hz, 6.0 Hz, 1H), 4.67-4.53 (m, 5H), 4.29 (dd, J = 9.2 Hz, 2.8 Hz, 1H), 3.9 (s, 1H), 3.84-3.78 (m, 2H), 3.66-3.58 (m, 2H), 3.37 (m, 3H), 2.10-2.05 (m, 9H), 1.65 (m, 2H), 1.25 (s, 3H), 1.23 (s, 3H), 1.04 (m, 12H), 0.85 (m, 2H).

^{13}C NMR ($CDCl_3$, 162 MHz): δ (ppm) 170.3, 138.7, 138.6, 138.3, 138.2, 128.6, 128.5, 128.4, 127.9, 127.8, 127.7, 127.7, 127.6, 115.1, 99.6, 98.9, 81.6, 80.7, 79.7, 75.7, 75.4, 73.2, 72.0, 71.5, 68.9, 68.0, 67.0, 30.5, 28.8, 21.2, 18.2, 18.1, 17.9, 17.8, 17.7, 17.4, 17.3, 13.0, 13.0, 13.0, 0.2.

HRMS-MALDI *m/z* = calcd: 1187.3034; found: 1187.9891. $[M+Na]^+$.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (10)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34-7.24 (m, 45H), 5.82 (m, 2H), 5.58 (s, 1H), 5.47 (s, 1H), 5.29 (s, 1H), 5.20 (s, 1H), 4.96-4.55 (m, 18H), 4.48 (m, 2H), 4.35 (m, 2H), 4.23 (m, 2H), 4.10-3.89 (m, 6H), 3.77-3.36 (m, 20H), 3.25 (m, 6H), 2.18-1.96 (m, 11H), 1.85 (m, 2H), 1.28-1.23 (m, 18H).

HRMS-MALDI *m/z* = calcd: 2675.8054; found: 2675.2209. $[M+Na]^+$.

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CHAPTER 6

Conclusions and Future Directions

This dissertation reports the first development of an automated platform for solution-phase oligosaccharide synthesis. Synthetic protocols in templated tasks were designed in order to build up continuous sequences for iterative synthetic cycles. A major advantage for this new strategy is that the majority of the bench-top solution-phase reaction conditions are directly transferrable to the automated platform. Therefore, when performing glycosylation reactions, less than 3 equivalents of donor molecules are required, which saves more than 7 fold of material waste compared to standard solid-phase oligosaccharide synthesis protocols. In addition, monitoring of the reaction progress is much easier compared to the previously described solid-phase automated synthesis. An aliquot for thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) was programmed to be withdrawn from the reaction mixture to determine whether additional purification steps might be required. Another major advantage to a solid versus solution-phase-based approach is that the instrument could be stopped and started at any stage of the programmed acquisition. Most importantly, the fluorous-tag, which is incorporated in the initial acceptor monosaccharide, offers a robust means of purification by simply taking advantage of the fluorous-fluorous interactions between the tagged molecule and fluorinated silica gel. Although the fluorous-tail strategy has been used before for convenient purification, this report is the first to demonstrate that this noncovalent interaction is robust enough to fully automated iterative

synthesis protocols with fluorous solid-phase extraction (FSPE) processes. The automated solution-phase oligosaccharide synthesis method was tested with various oligosaccharide compounds in order to probe its versatility and robustness. Not only linear, but branched oligosaccharides were successfully composed and chemically challenging 1,2-*cis* glycosidic linkages were achieved under carefully controlled conditions. Rhamnose pentasaccharide was chosen to be used as a model study for optimizing the automated conditions. Based on the successful acquisition of the linear rhamnose synthesis, both anthrax- and cholera associated antigenic tetrasaccharides were achieved simultaneously by running a slightly modified method. During the optimization process, future limitations were foreseen and strategies such as the di-tagged methodology and *n*-pentenyl glycoside formation were tested to serve as additional possibilities when needed. Results with the reported oligosaccharides offer suitable alternative methods when difficulties in intermediate purification or instability of the usual trichloroacetimidate donors is encountered.

The development of the first automated solution-phase oligosaccharide synthesis platforms now opens greater possibilities for obtaining pure, structurally well-defined oligosaccharide compounds. For future work, this strategy should be effective in constructing various combinations of sugar molecules to study structure-function relationships to contribute to the development of carbohydrate-based combinatorial libraries and ultimately carbohydrate-based vaccines and diagnostics. In addition, a labor-saving method to assemble oligosaccharides should aid in providing authentic samples to foster oligosaccharide structure determination methodologies such as mass-spectrometry-based sequencing and to carry out

carbohydrate structure and conformation work, especially with carbohydrate-binding proteins.

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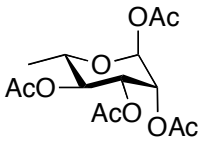
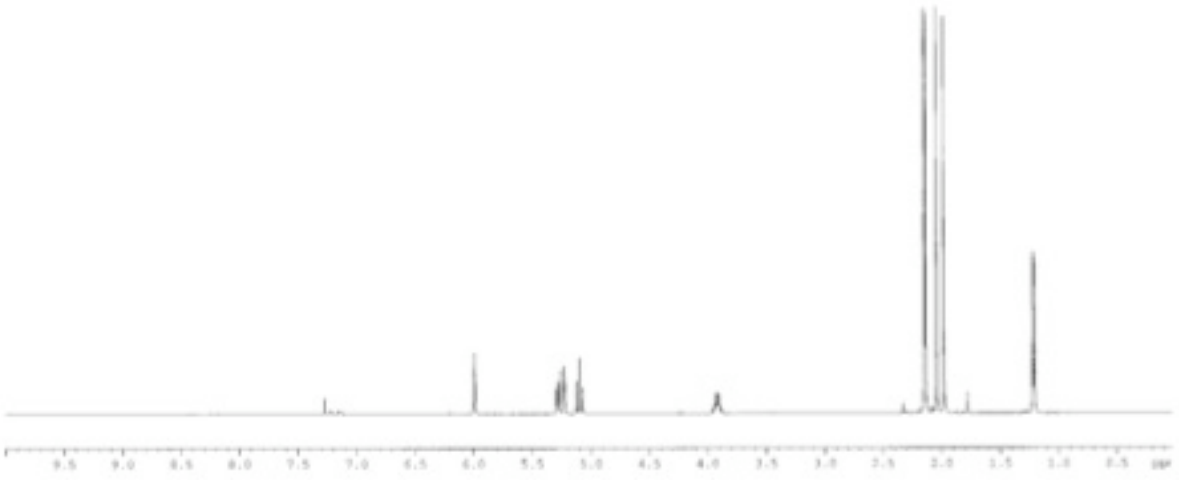
Finally, I give special thanks to my greatest supporters my parents, brother, last but not least Steven Raders for their constant love, faith and always being there for me.

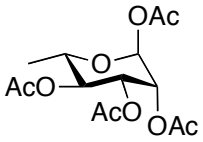
APPENDIX A. CHAPTER 2 ^{1}H AND ^{13}C NMR SPECTRA



1, 2, 3, 4-Tetra-O-acetyl-α-L-rhamnopyranosde

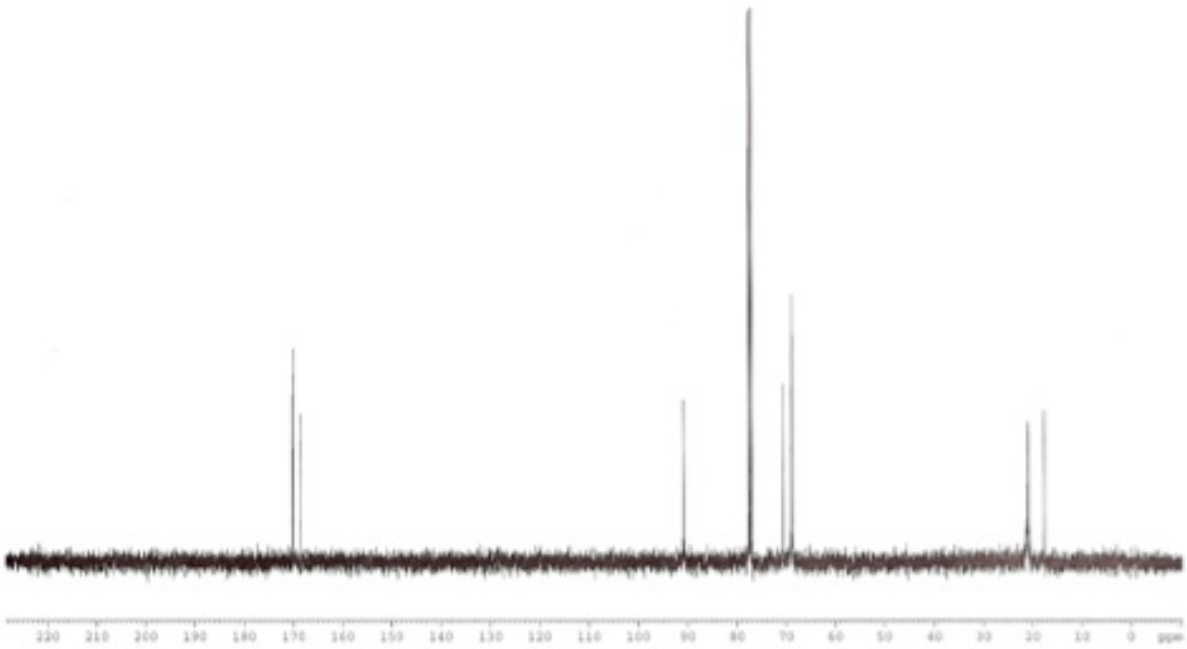
$CDCl_3$, 400 MHz

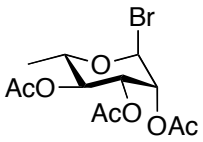




1, 2, 3, 4-Tetra-O-acetyl-α-L-rhamnopyranosde

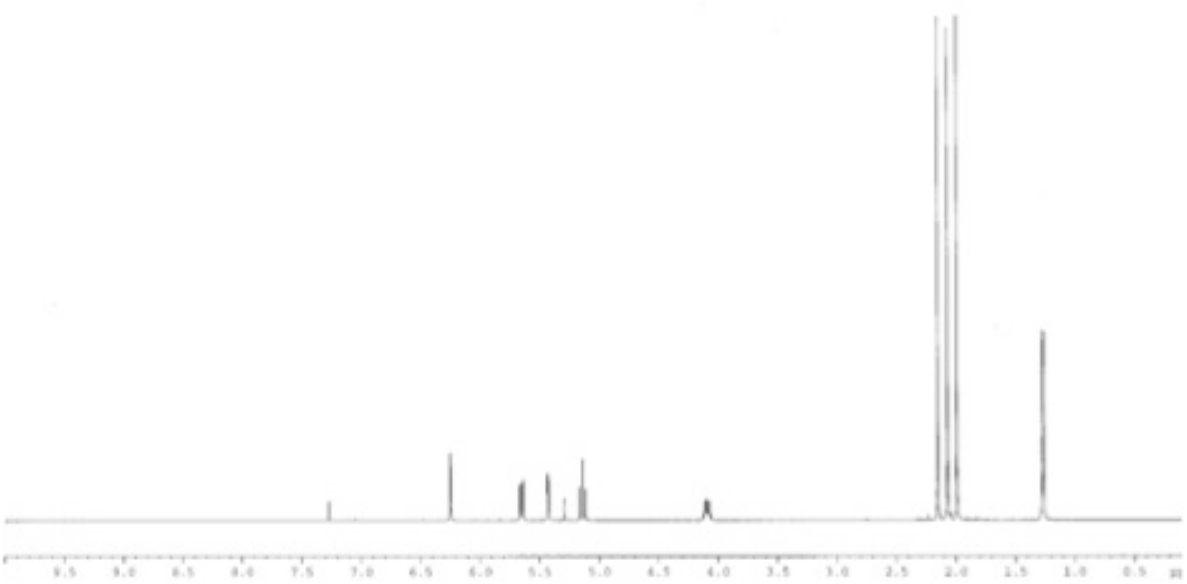
$CDCl_3$, 100 MHz

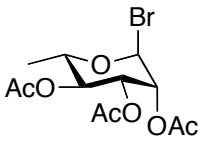




1-Bromo-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside

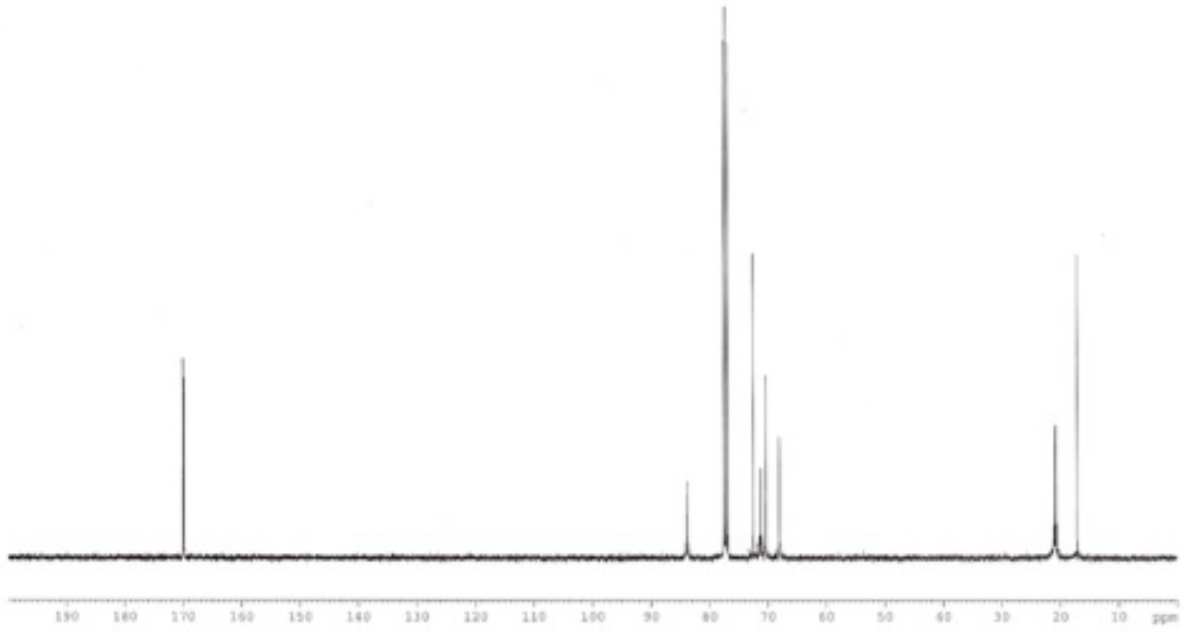
$CDCl_3$, 400 MHz

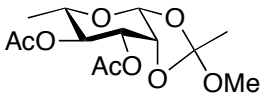




1-Bromo-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside

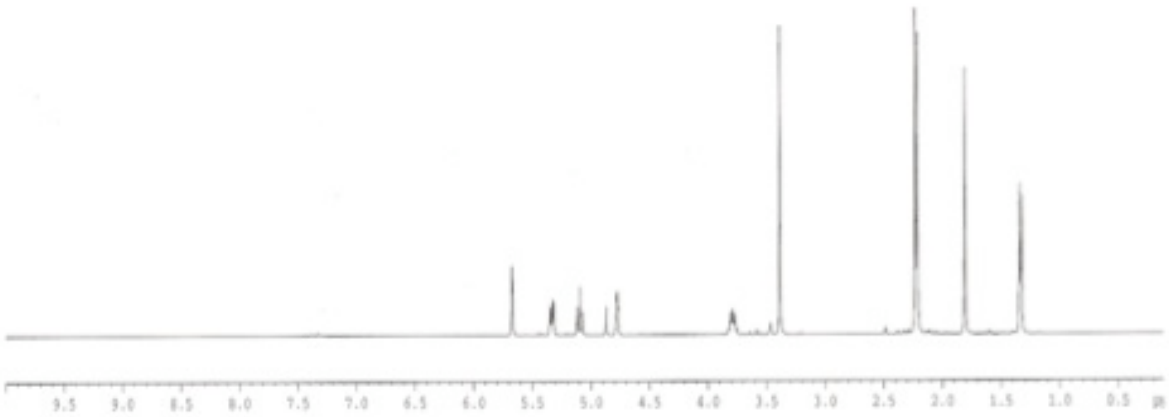
$CDCl_3$, 100 MHz

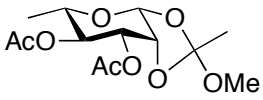




3, 4-O-Di-acetyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose

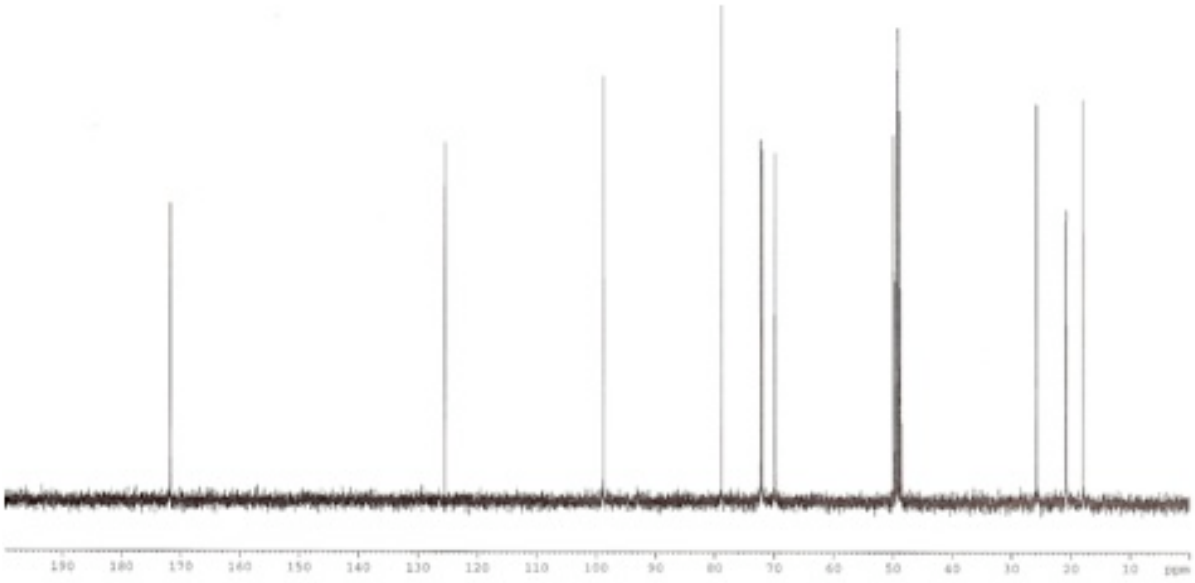
CD_3OD, 400 MHz

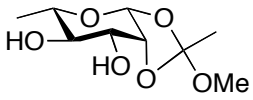




3, 4-O-Di-acetyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose

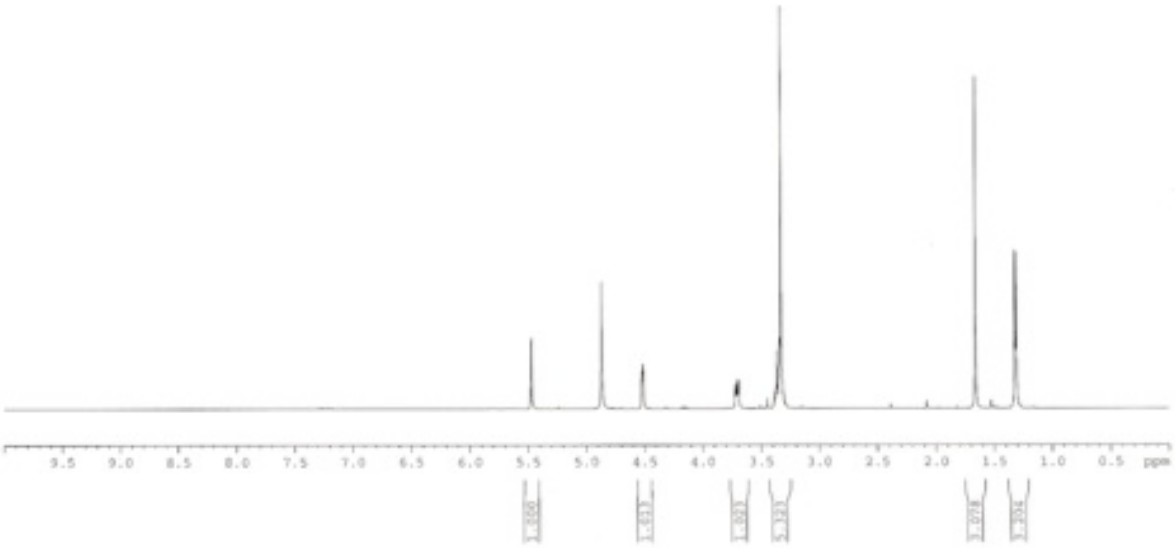
CD_3OD, 100 MHz

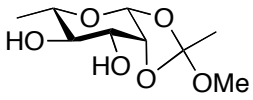




1,2-O-Methoxyethylidene-β-L-rhamnopyranose

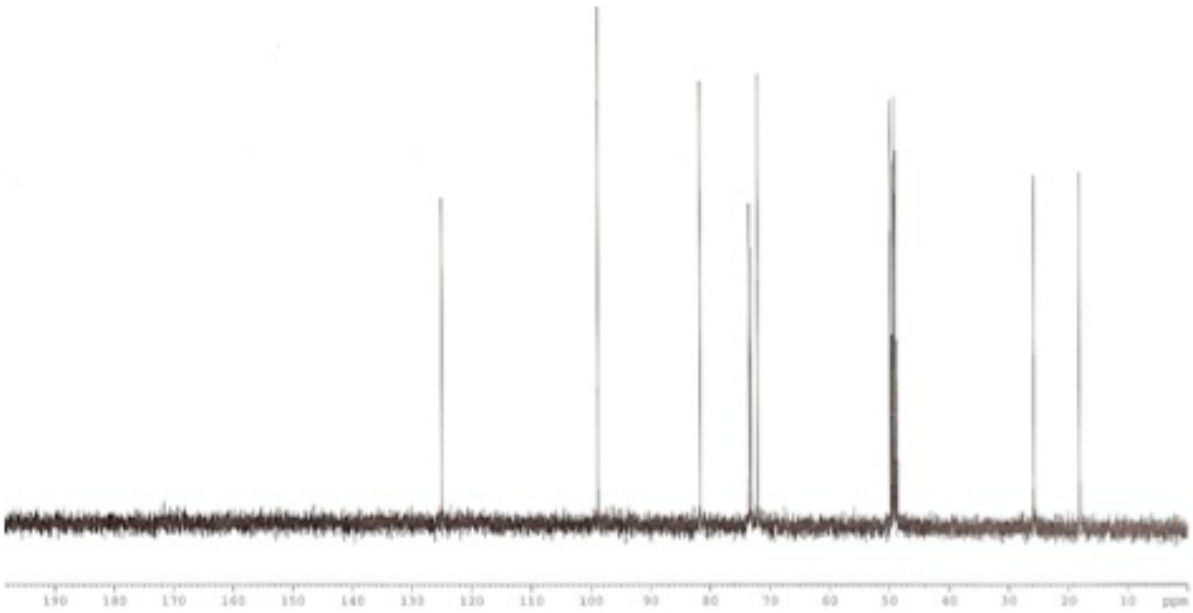
CD_3OD, 400 MHz

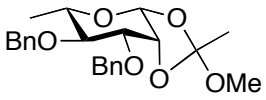




1,2-O-Methoxyethylidene-β-L-rhamnopyranose

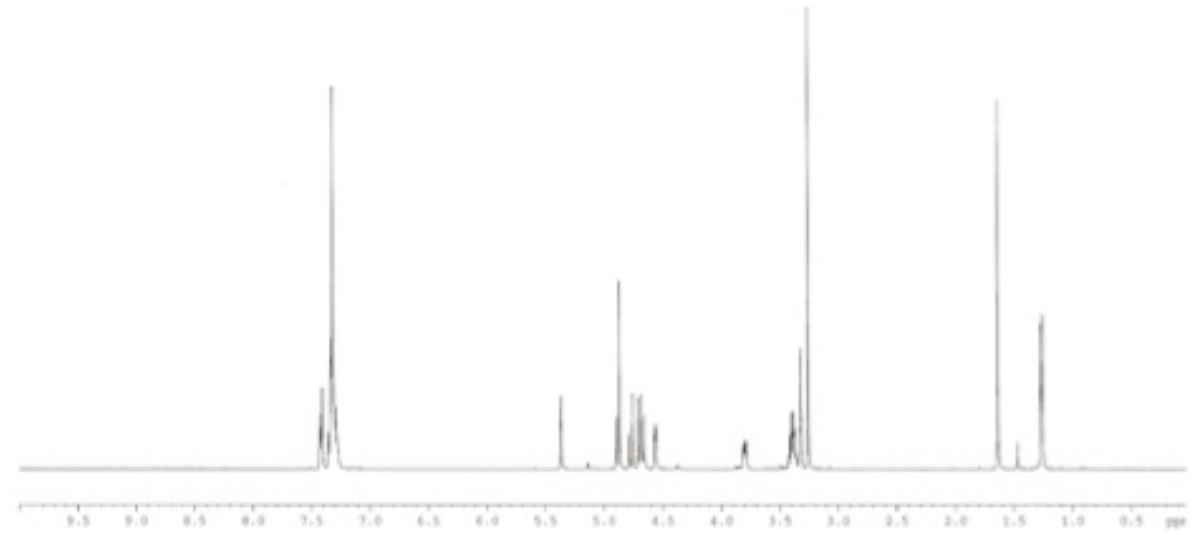
CD_3OD, 100 MHz

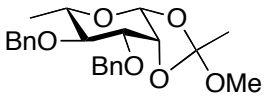




3,4-Di-O-benzyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose

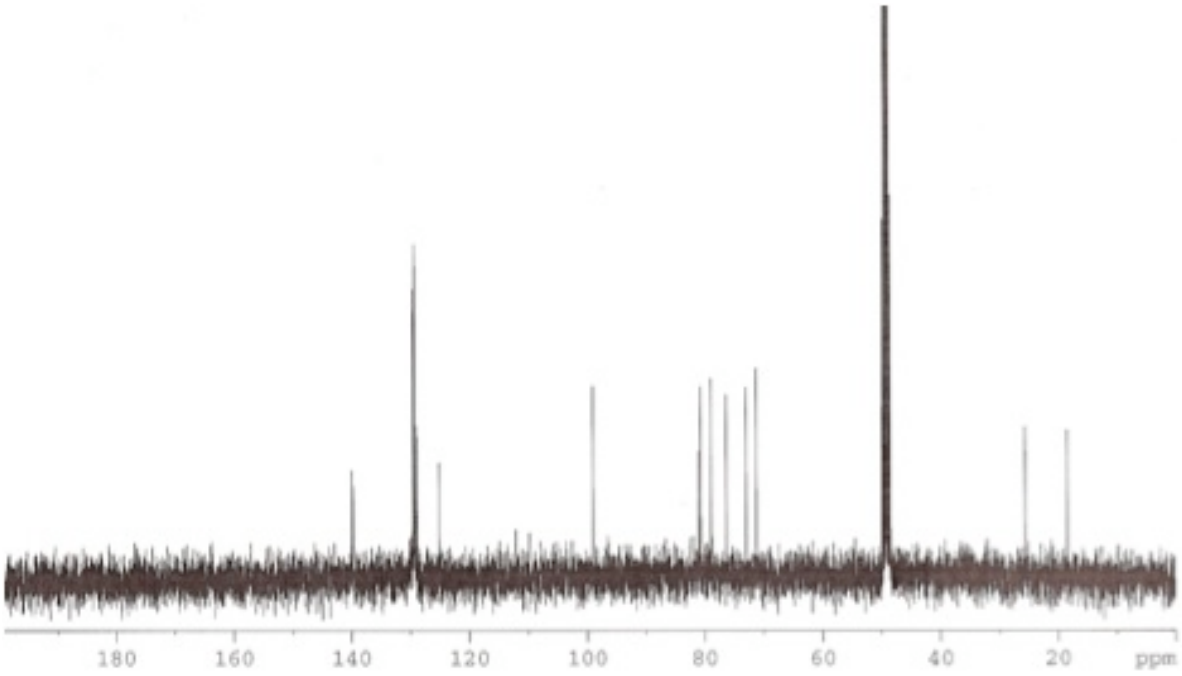
CD_3OD, 400 MHz

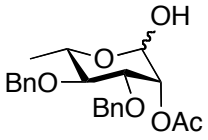




3,4-Di-O-benzyl-1,2-O-methoxyethylidene-β-L-rhamnopyranose

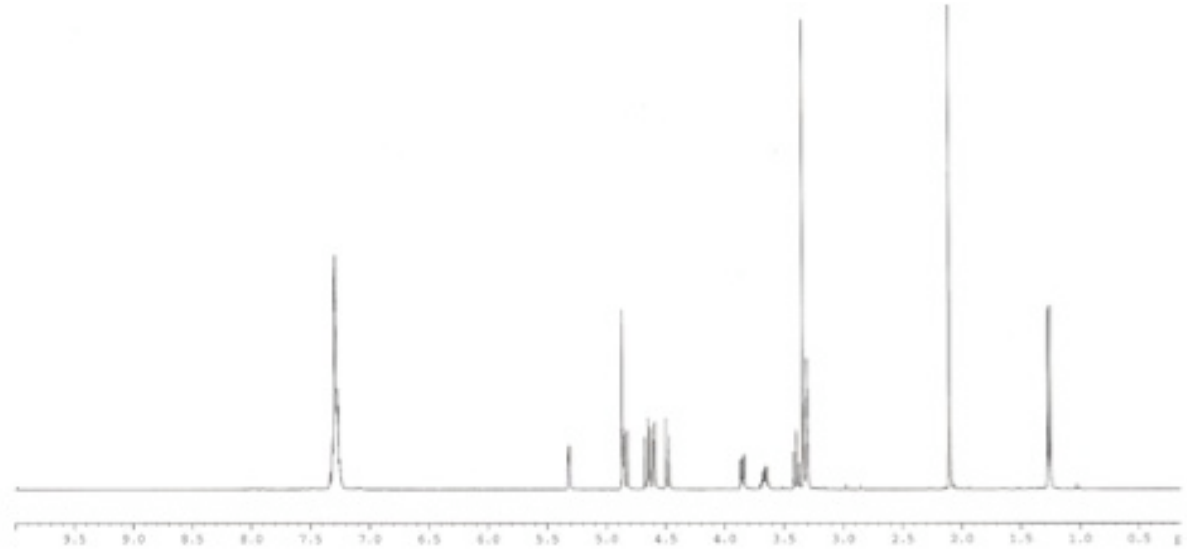
CD_3OD, 100 MHz

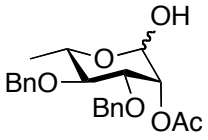




2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranose

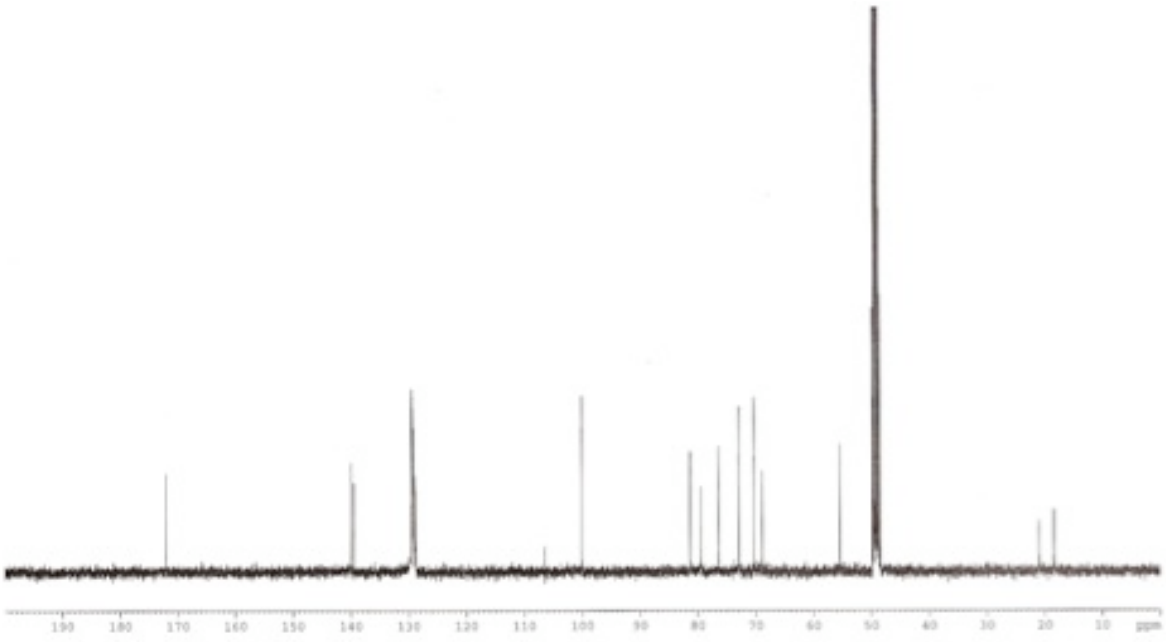
CD_3OD, 400 MHz

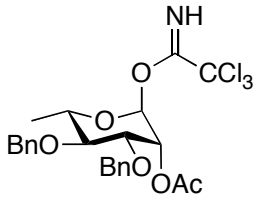




2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranose

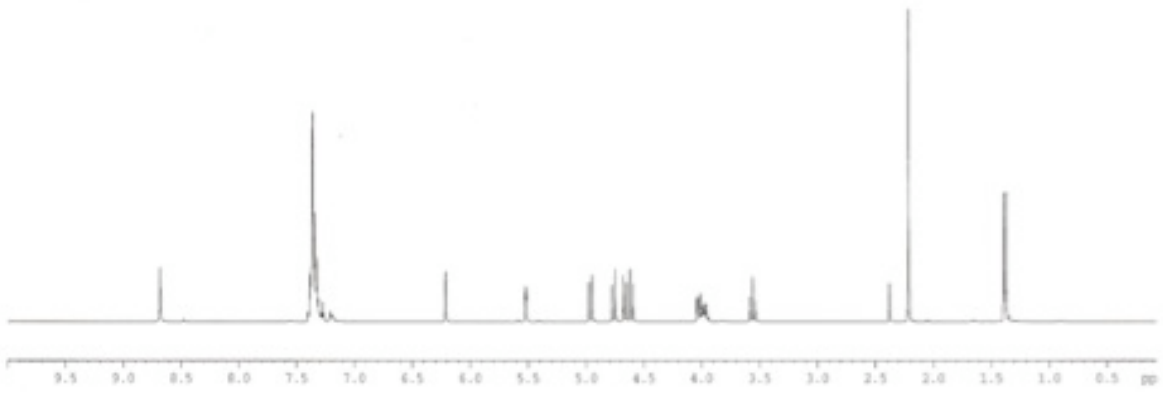
CD_3OD, 100 MHz

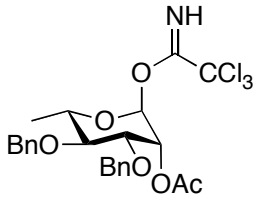




2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranoside trichloroacetimidate (2)

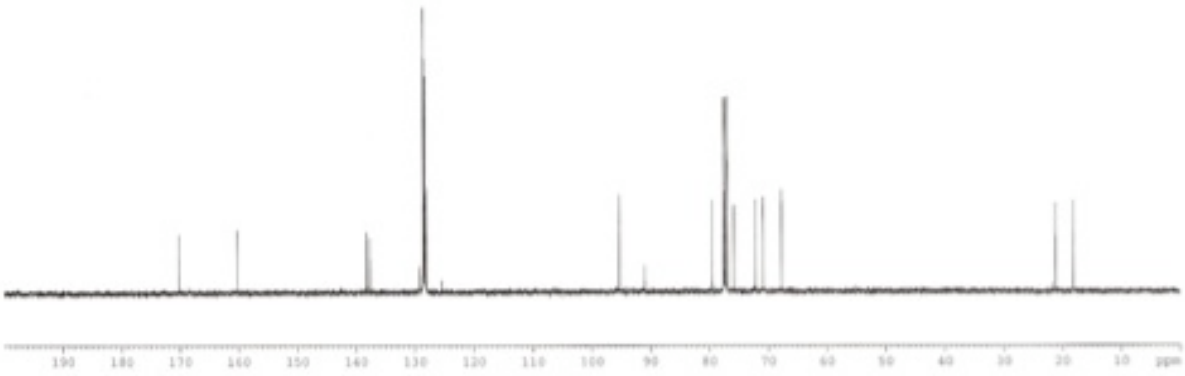
$CDCl_3$, 400 MHz

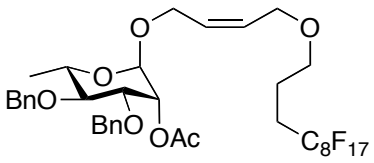




2-O-Acetyl-3,4-di-O-benzyl-α/β-L-rhamnopyranoside trichloroacetimidate (2)

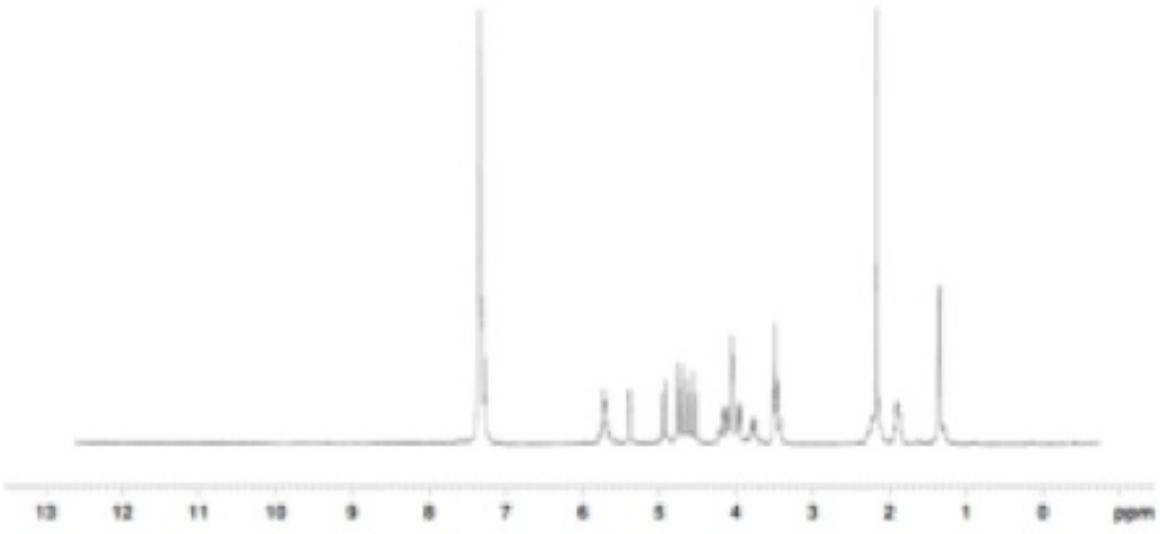
$CDCl_3$, 100 MHz

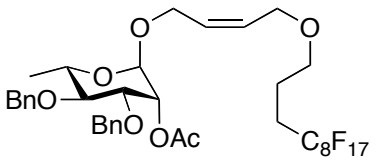




cis-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl-α-L-rhamnopyranoside

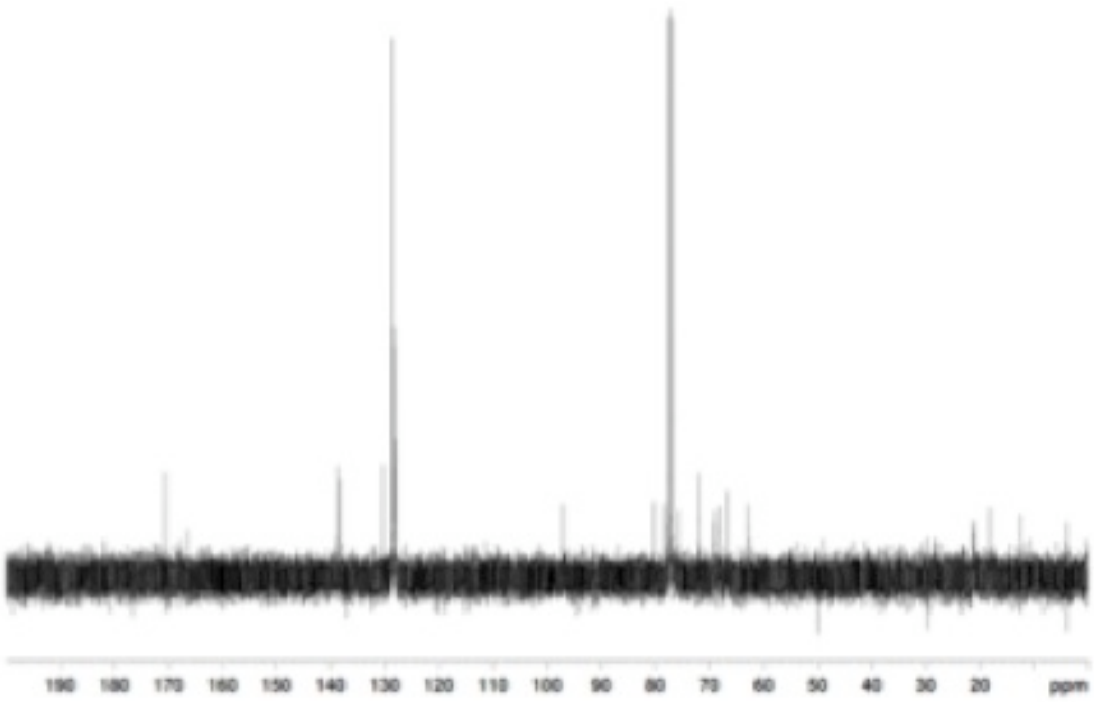
$CDCl_3$, 400 MHz

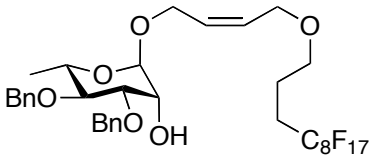




cis-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl-α-L-rhamnopyranoside

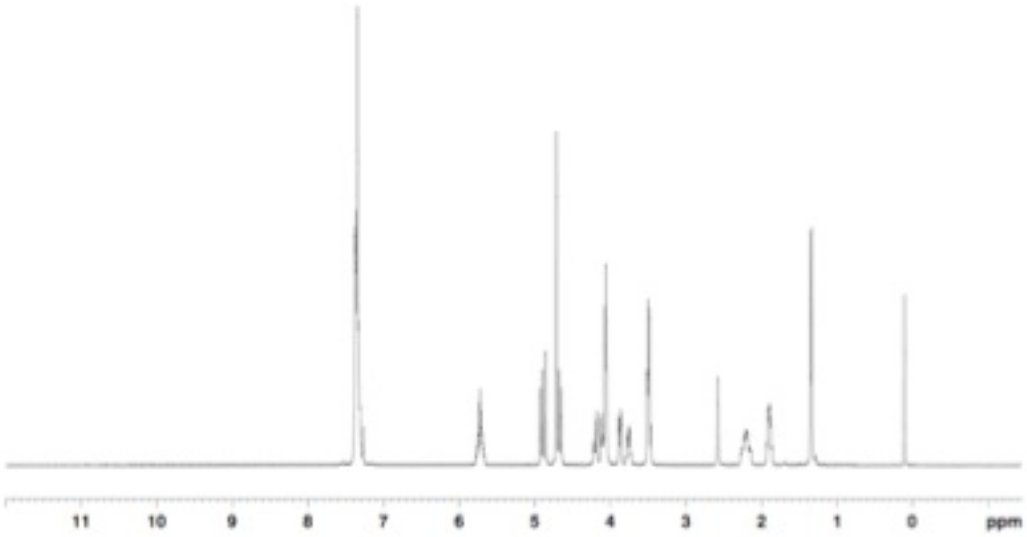
$CDCl_3$, 100 MHz

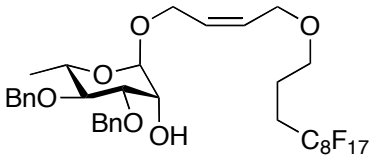




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3,4-O-dibenzyl- α-L-rhamnopyranoside (1)

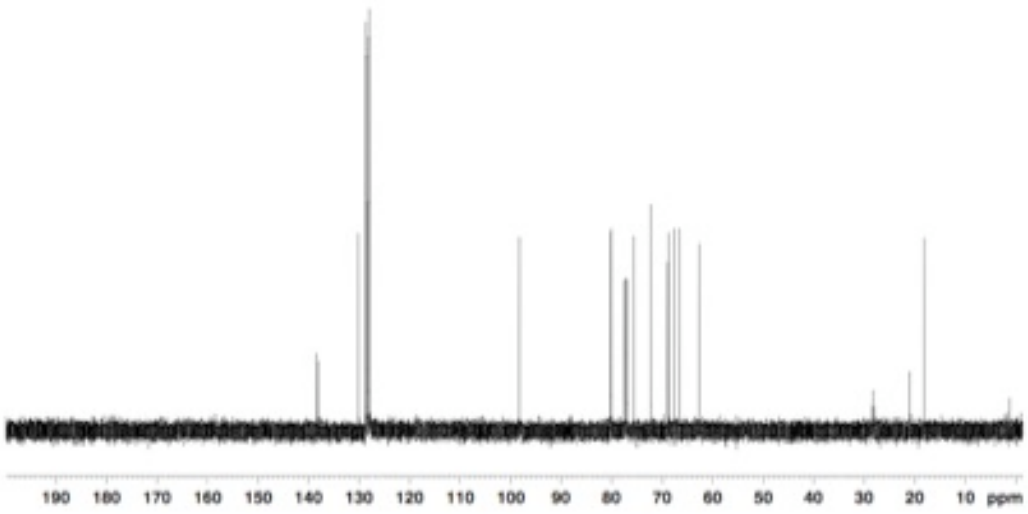
$CDCl_3$, 400 MHz

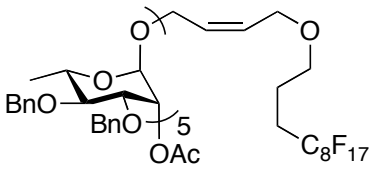




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3,4-O-dibenzyl- α-L-rhamnopyranoside (1)

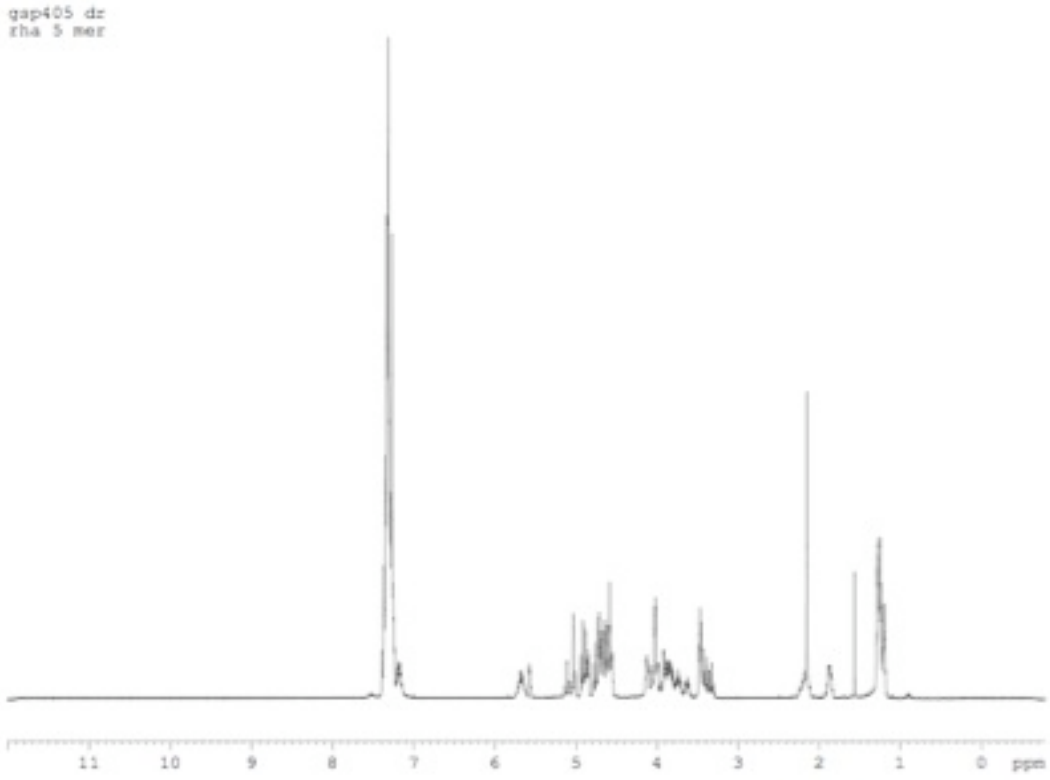
$CDCl_3$, 100 MHz

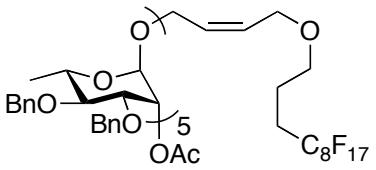




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (3)

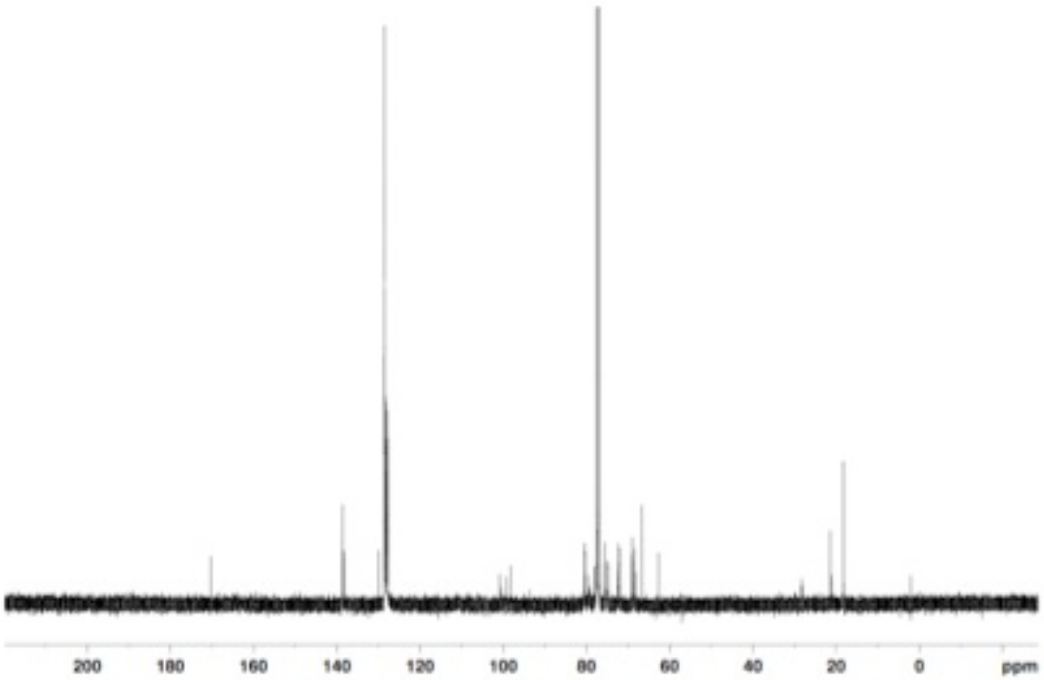
$CDCl_3$, 400 MHz

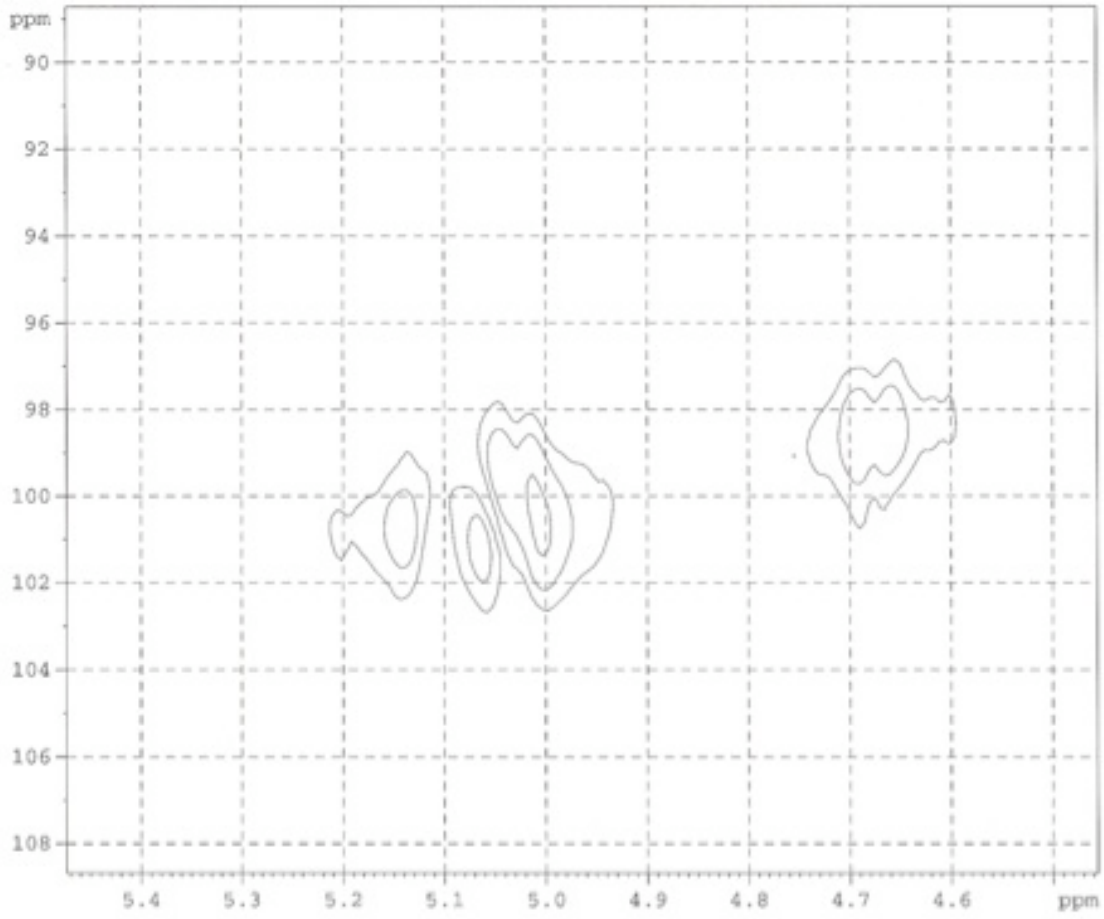




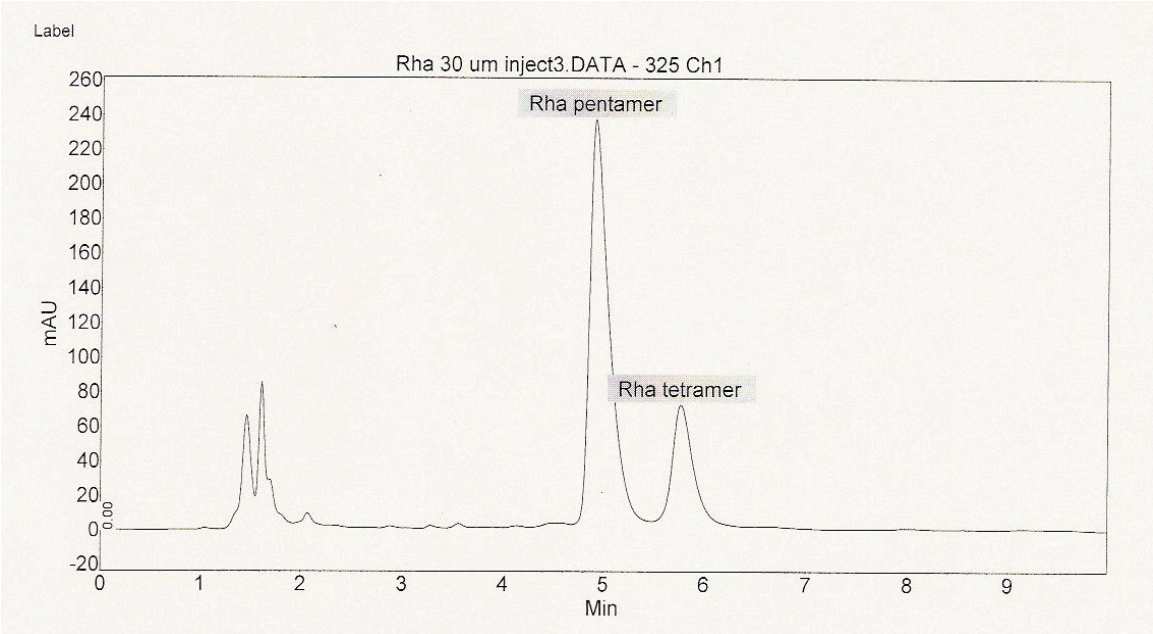
cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (3)

$CDCl_3$, 100 MHz

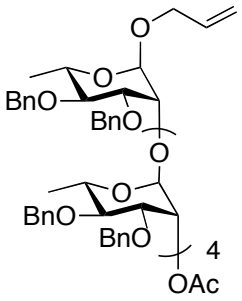




HPLC traces of rhamnose pentasaccharide run

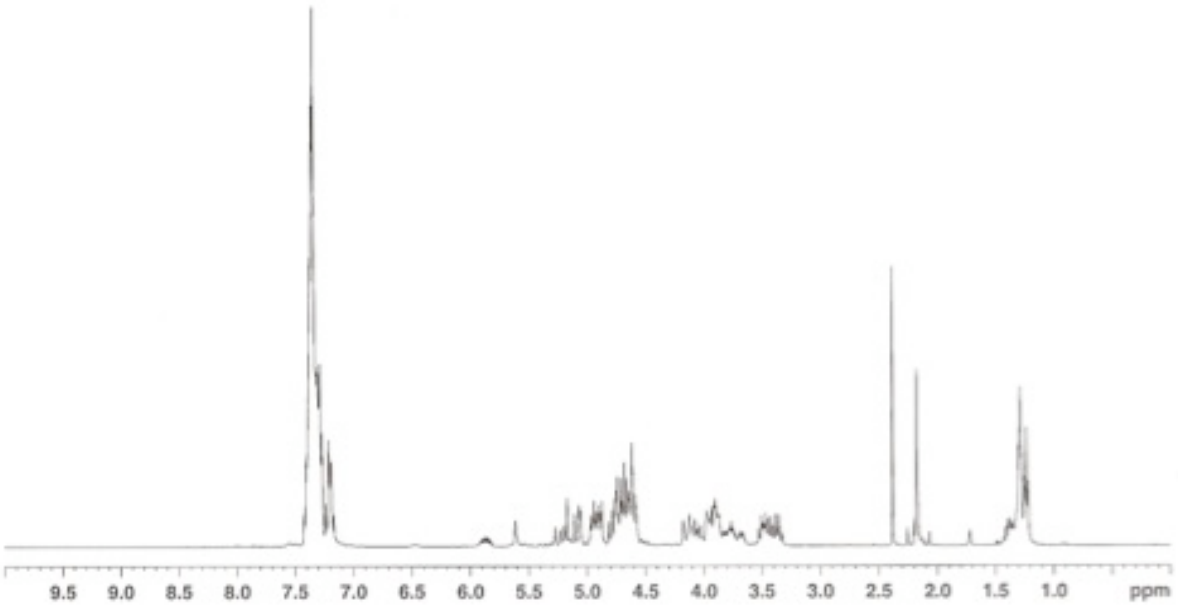


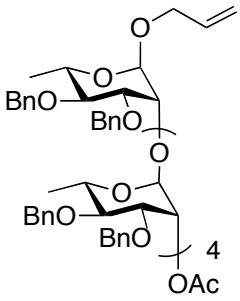
Analytical HPLC chromatogram of Rhamnose pentamer synthesis (1 mL/min flowrate, 20% Ethyl acetate/Hexane, 10 minute run).



Allyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)- 3,4-di-O-benzyl-α-L-rhamnopyranoside (4)

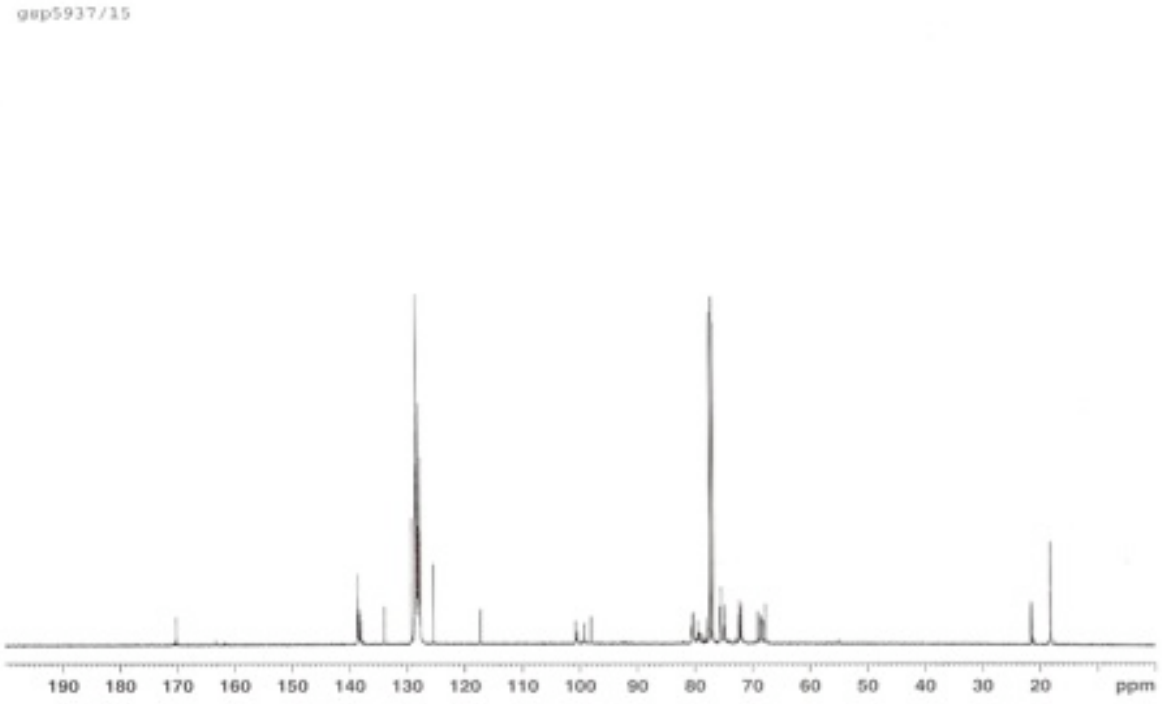
$CDCl_3$, 400 MHz



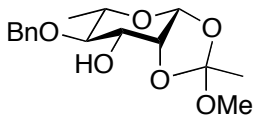


Allyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)- 3,4-di-O-benzyl-α-L-rhamnopyranoside (4)

$CDCl_3$, 100 MHz

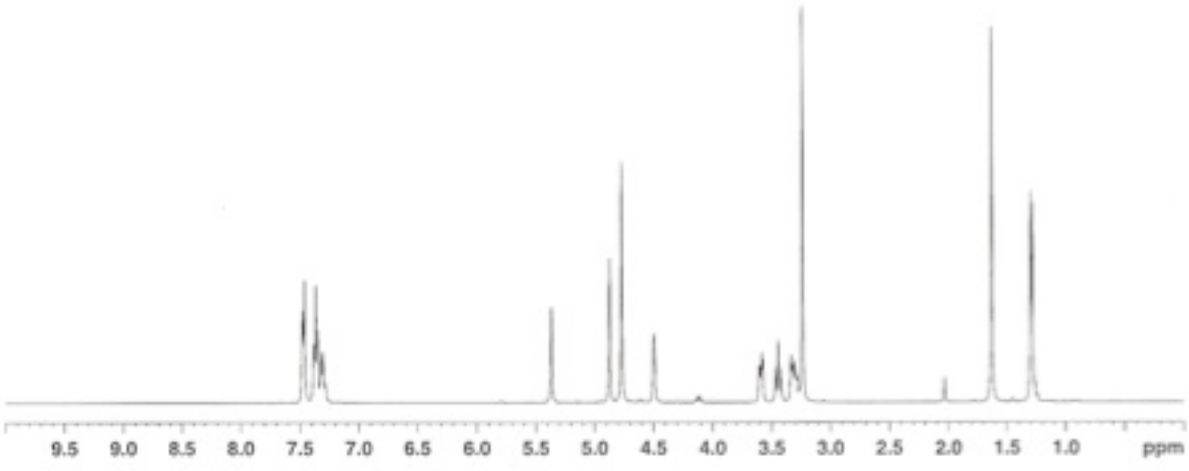


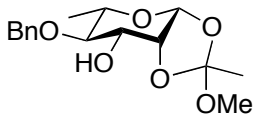
APPENDIX B. CHAPTER 3 ^{1}H AND ^{13}C NMR SPECTRA



4-O-Benzyl-1,2-O-methylethylidene-β-L-rhamnopyranose

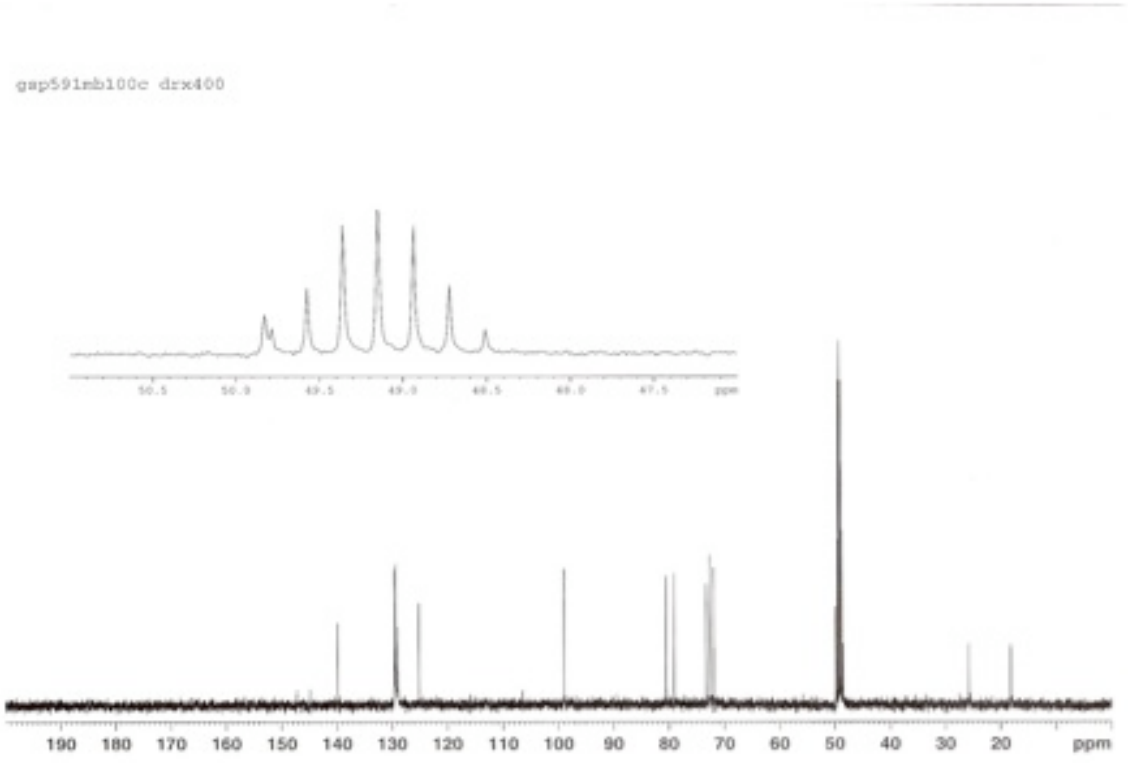
CD_3OD, 400MHz

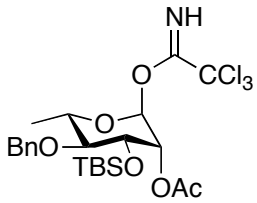




4-O-Benzyl-1,2-O-methylethylidene-β-L-rhamnopyranose

CD_3OD, 100MHz

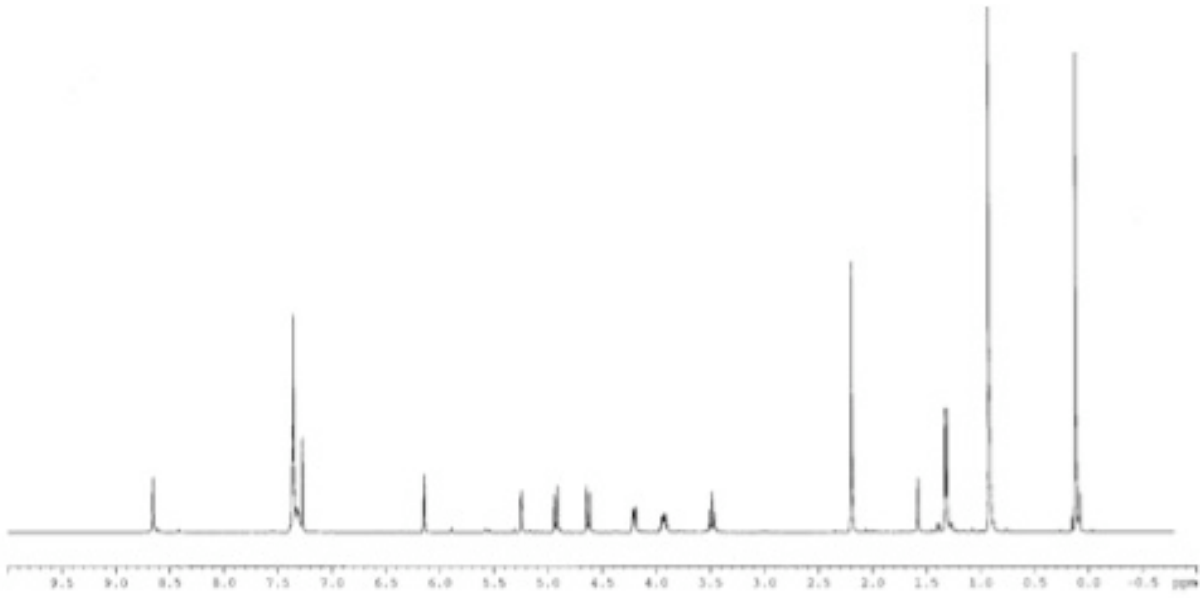


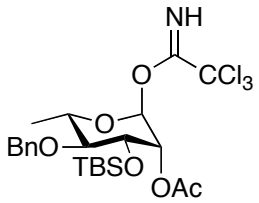


2-O-Acetyl-4-O-benzyl-3-O-tertbutyldimethylsilyl-β-L-rhamnopyranosyl

Trichloroacetimidate (4)

$CDCl_3$, 400 MHz

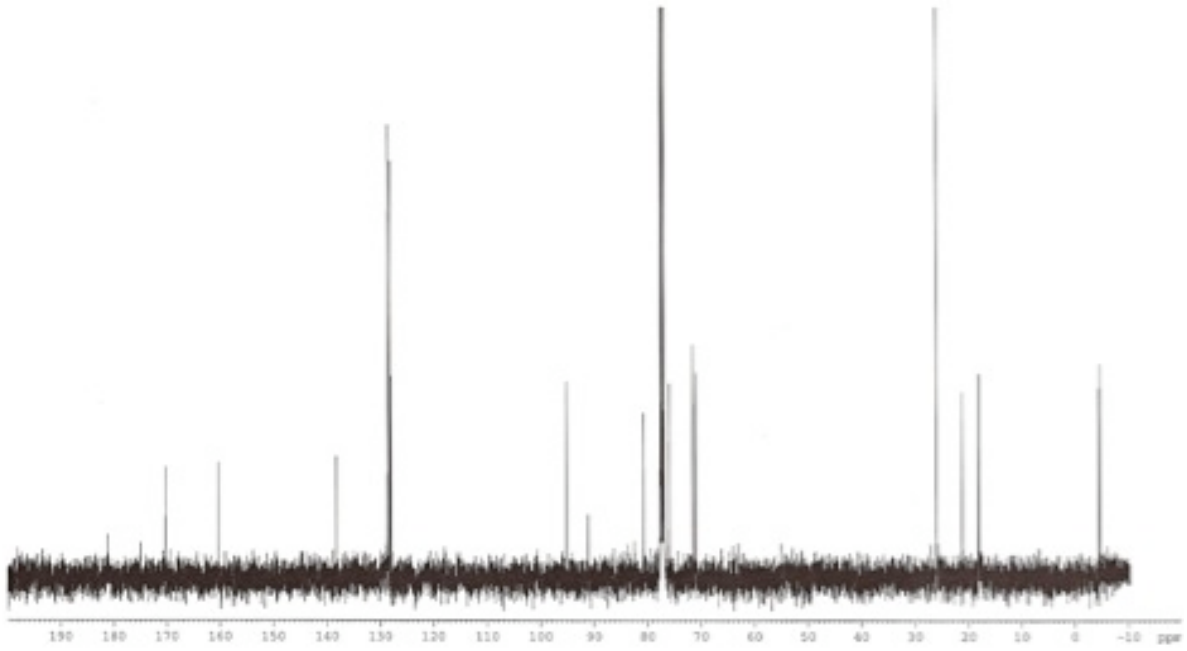


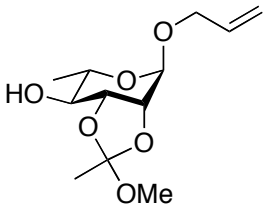


2-O-Acetyl-4-O-benzyl-3-O-tertbutyldimethylsilyl-β-L-rhamnopyranosyl

Trichloroacetimidate (4)

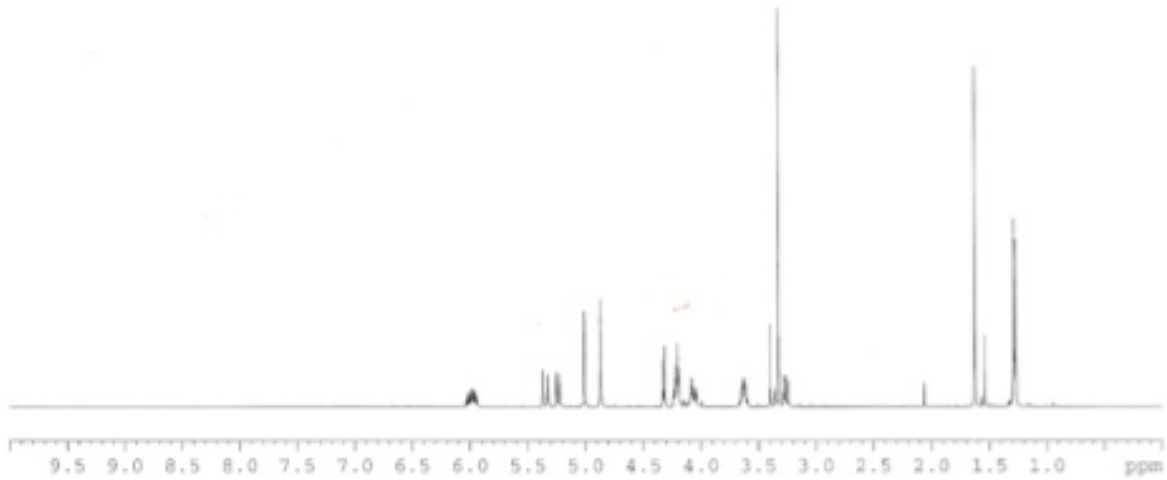
$CDCl_3$, 100 MHz

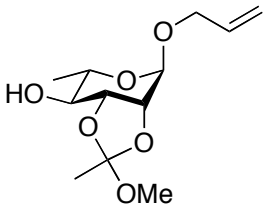




Allyl 4-O-benzyl-2,3-O-methoxyethylidene-α-L-rhamnopyranose (28)

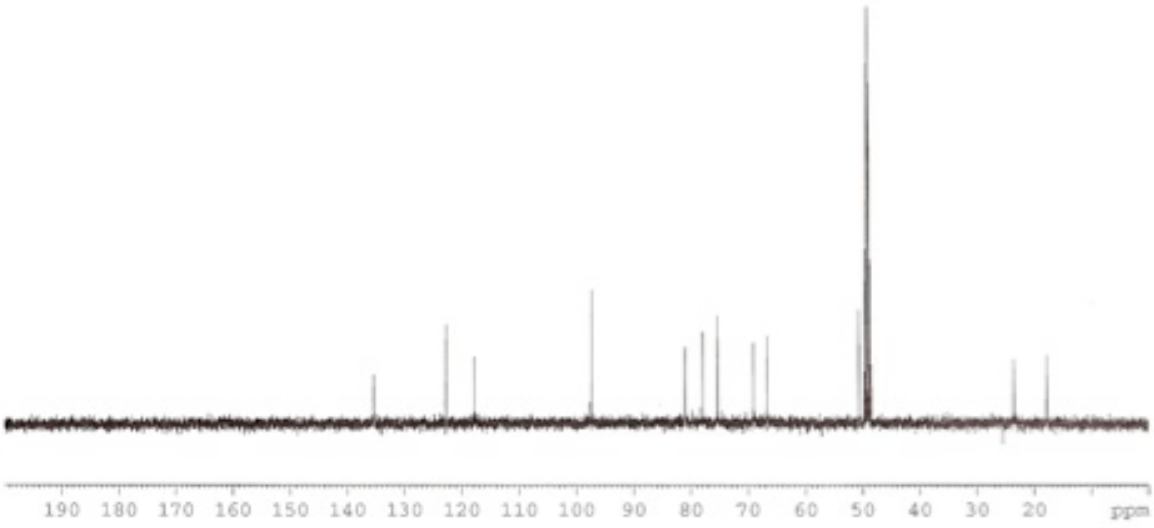
CD_3OD, 400 MHz

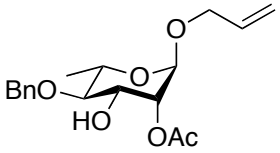




Allyl 4-O-benzyl-2,3-O-methoxyethylidene-α-L-rhamnopyranose (28)

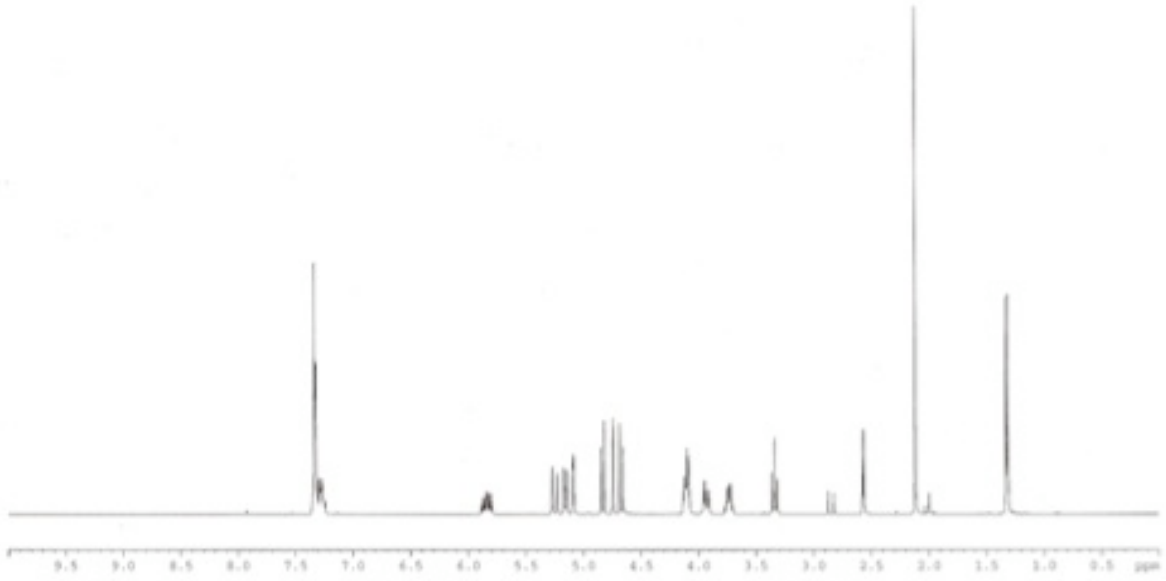
CD_3OD, 100 MHz

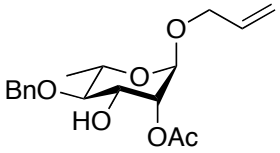




Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (30)

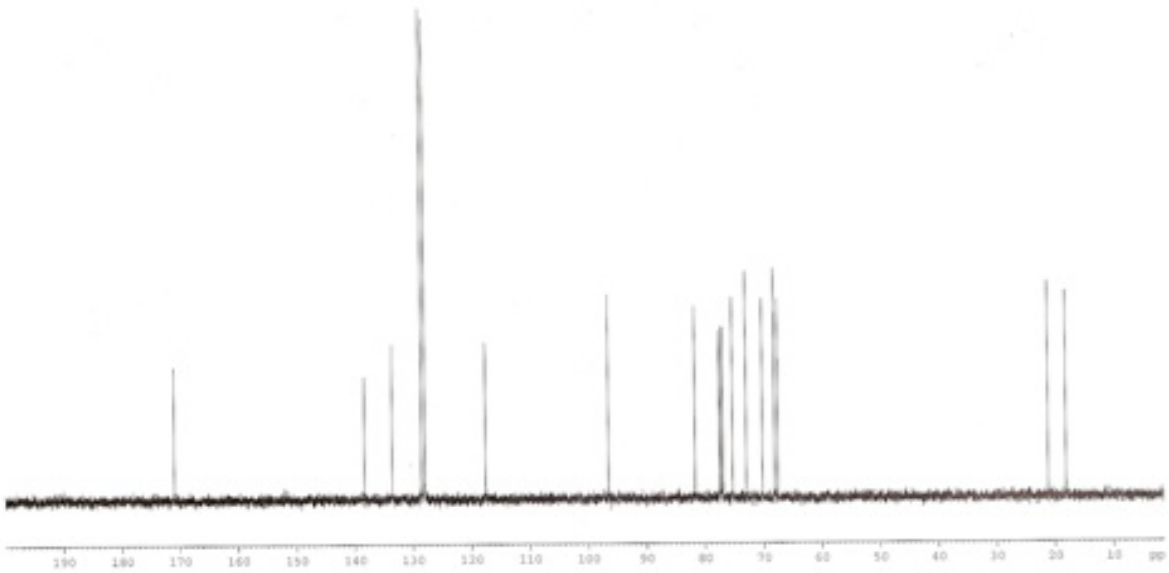
$CDCl_3$, 400 MHz

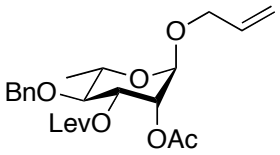




Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (30)

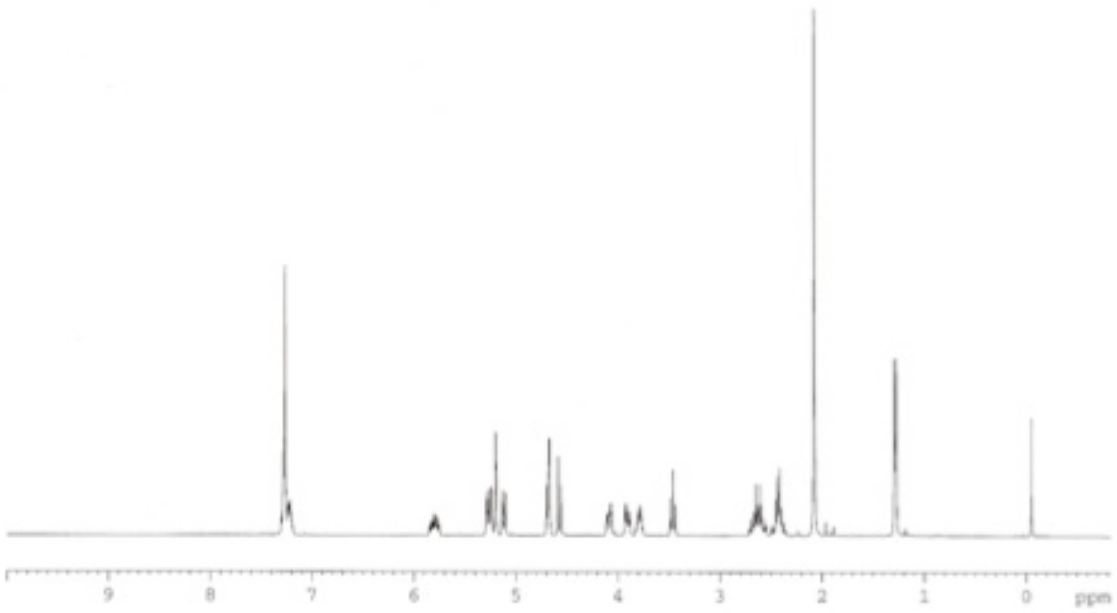
$CDCl_3$, 100 MHz

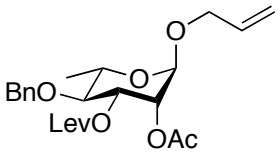




Allyl 2-O-acetyl-4-O-benzyl-3-O-levulinyl-α-L-rhamnopyranoside (31)

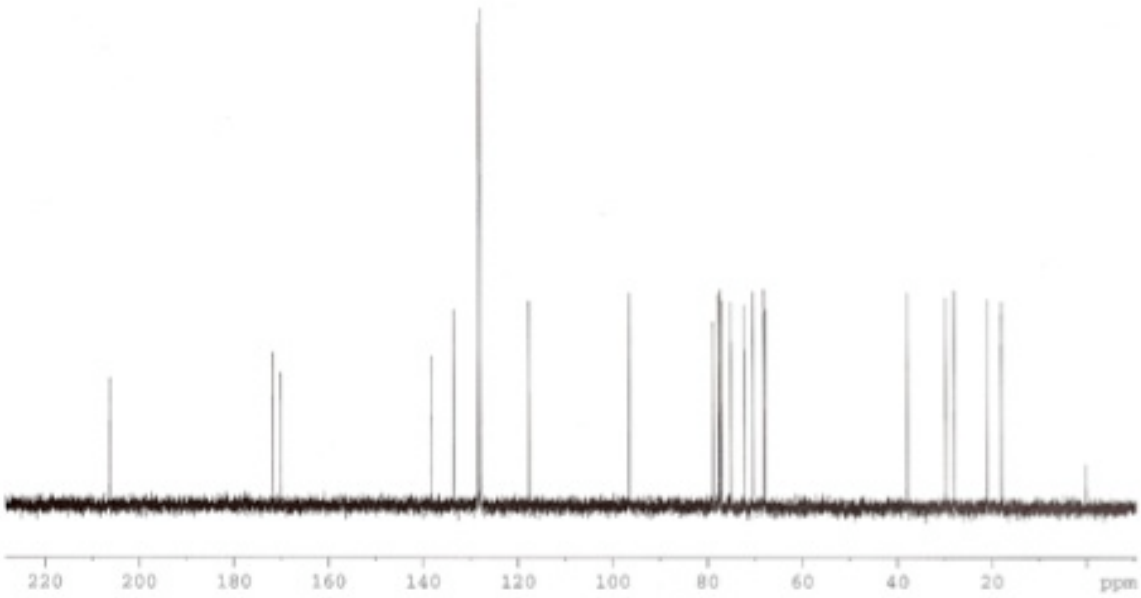
$CDCl_3$, 400 MHz

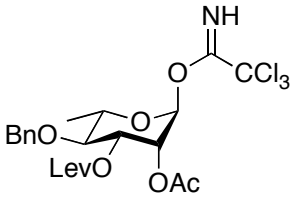




Allyl 2-O-acetyl-4-O-benzyl-3-O-levulinyl-α-L-rhamnopyranoside (31)

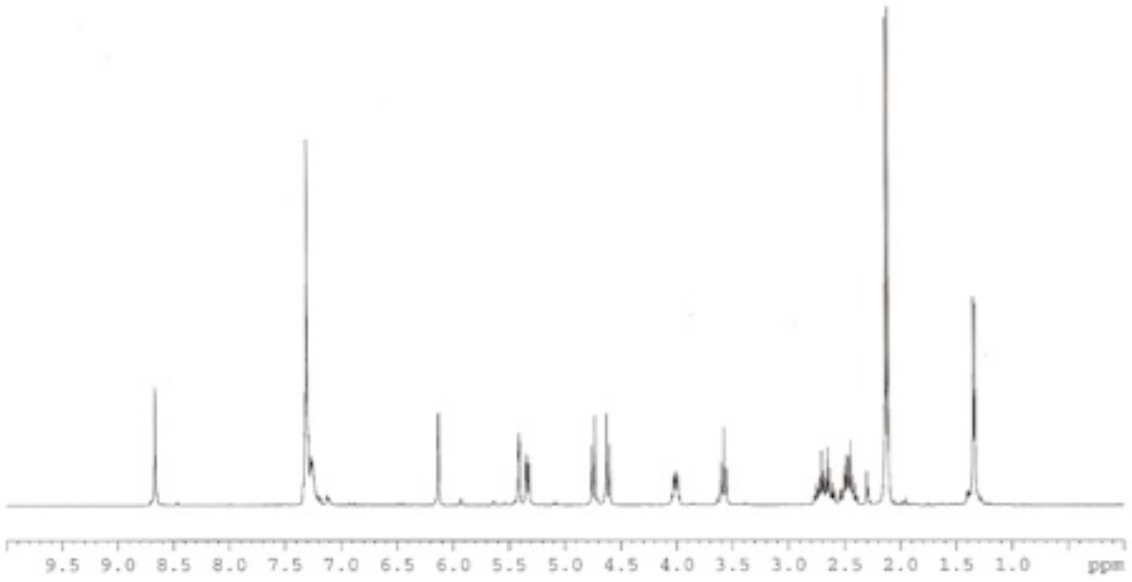
$CDCl_3$, 100 MHz

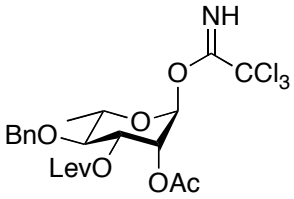




2-O-Acetyl-4-O-benzyl-3-O-levulinyl-α/β-L-rhamnopyranosyl Trichloroacetimidate (33)

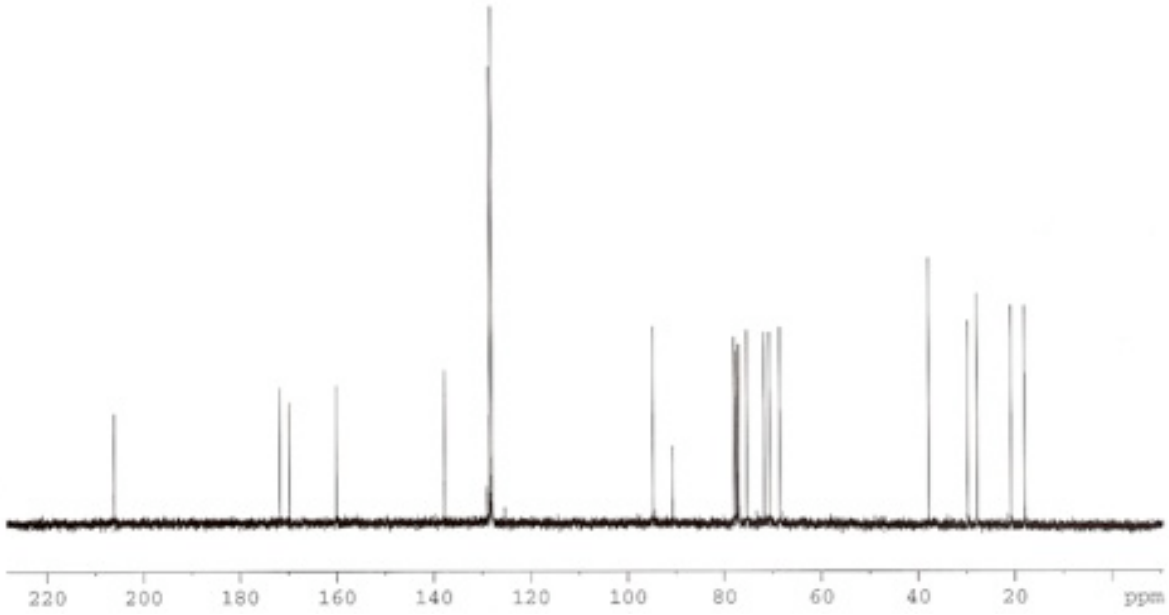
$CDCl_3$, 400 MHz

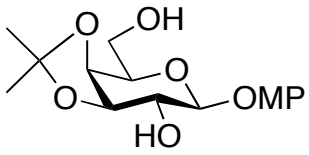




2-O-Acetyl-4-O-benzyl-3-O-levulinyl-α/β-L-rhamnopyranosyl Trichloroacetimidate (33)

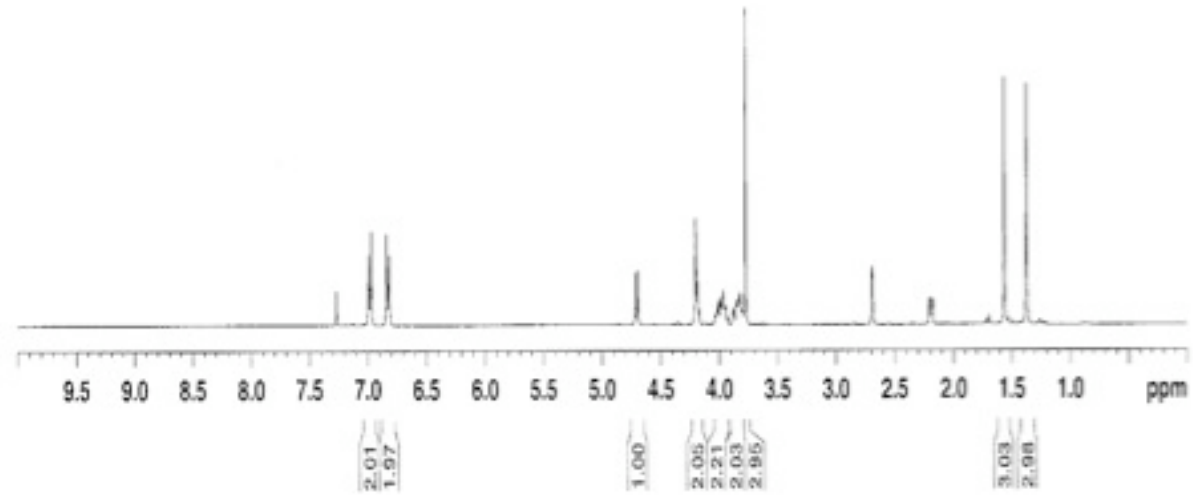
$CDCl_3$, 100 MHz

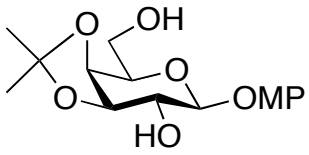




4-Methoxyphenyl 3,4-O-isopropylidene-β-D-galactopyranoside (10)

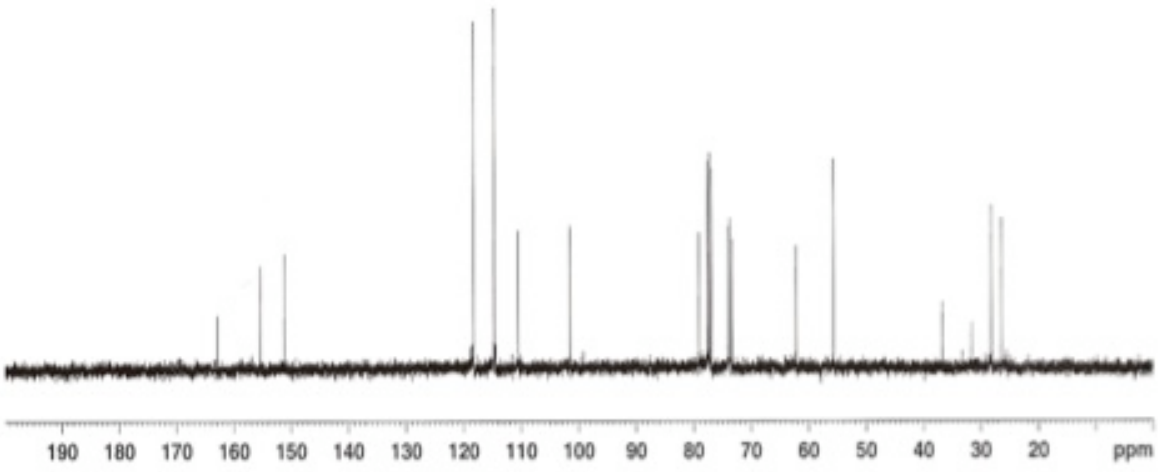
$CDCl_3$, 400 MHz

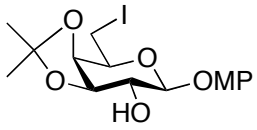




4-Methoxyphenyl 3,4-O-isopropylidene-β-D-galactopyranoside (10)

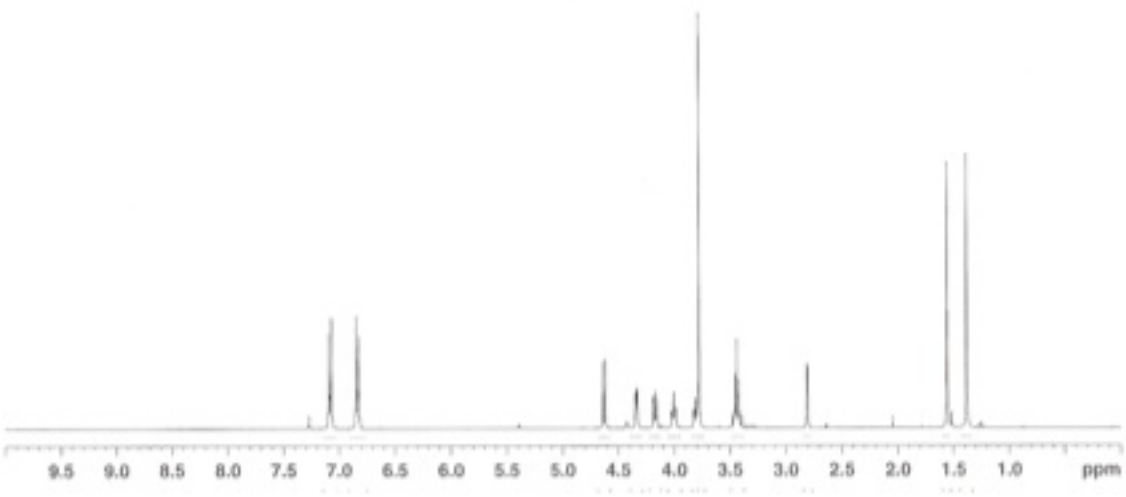
$CDCl_3$, 100 MHz

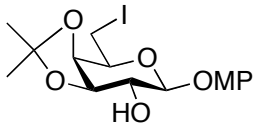




4-Methoxyphenyl 6-iodo-3,4-O-isopropylidene-β-D-galactopyranoside (11)

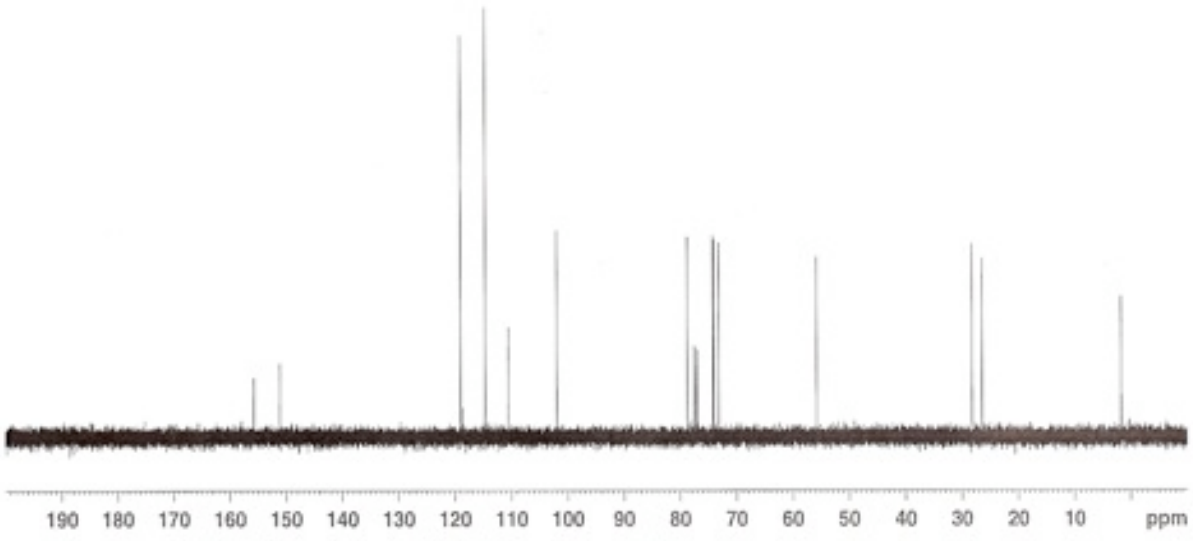
$CDCl_3$, 400 MHz

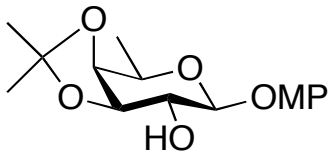




4-Methoxyphenyl 6-iodo-3,4-O-isopropylidene-β-D-galactopyranoside (11)

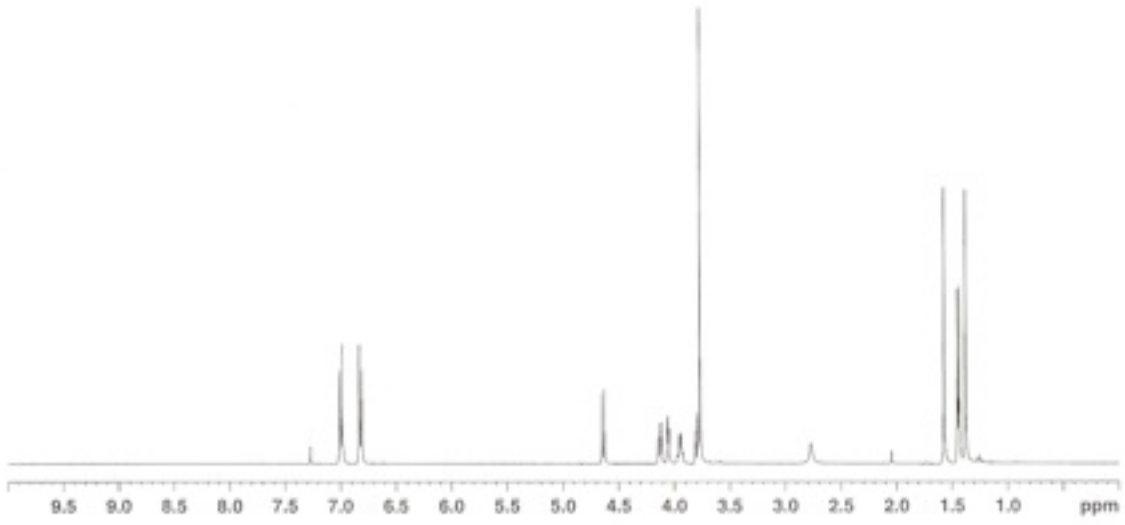
$CDCl_3$, 100 MHz

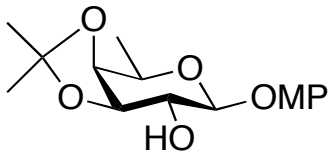




4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-β-D-galactopyranoside (12)

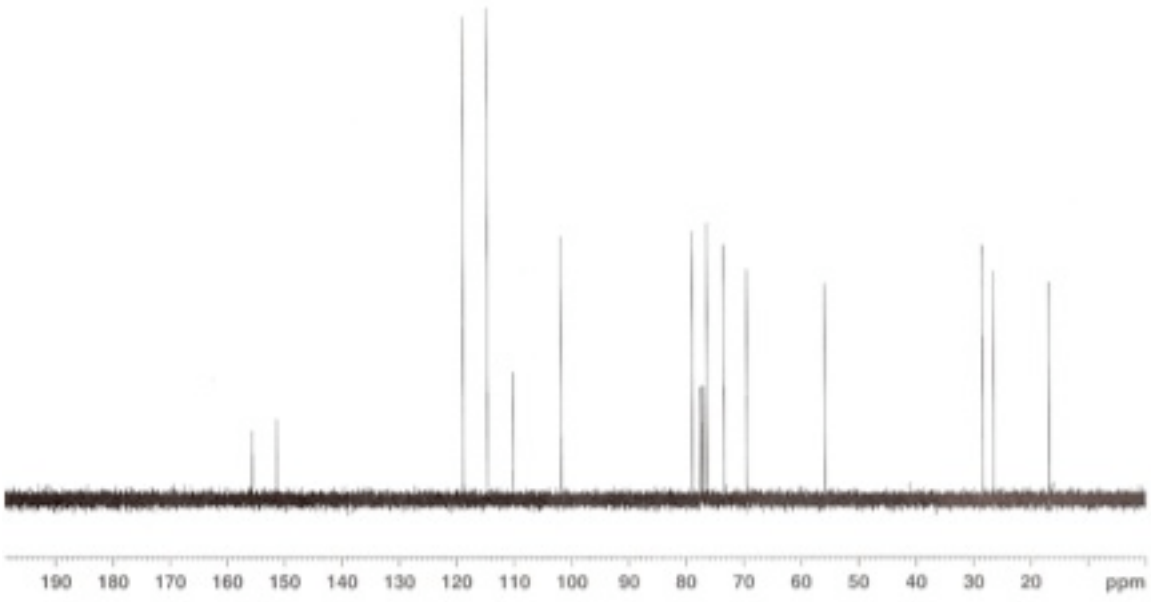
$CDCl_3$, 400 MHz

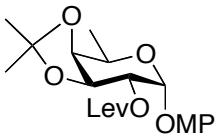




4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-β-D-galactopyranoside (12)

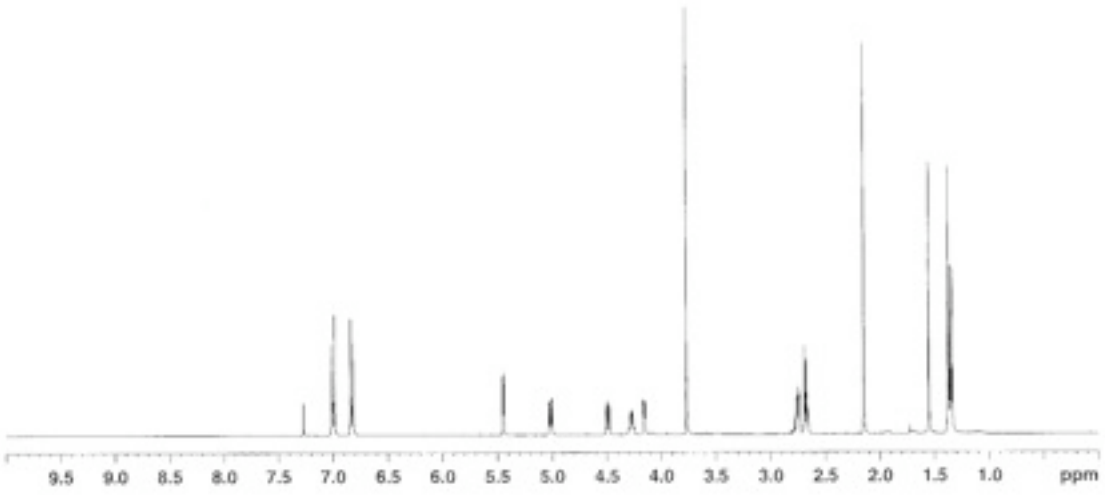
$CDCl_3$, 100 MHz

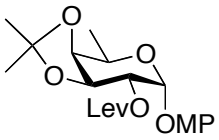




4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-2-levulinoyl-β-D-galactopyranoside (13)

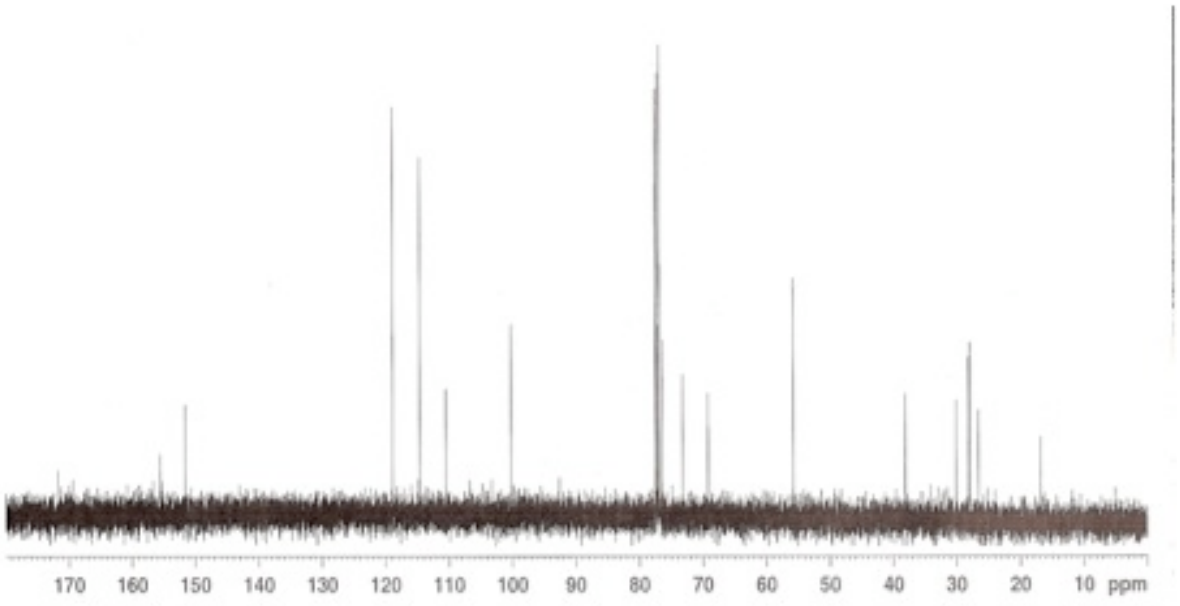
$CDCl_3$, 400 MHz

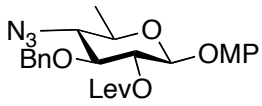




4-Methoxyphenyl 6-desoxy-3,4-O-isopropylidene-2-levulinoyl-β-D-galactopyranoside (13)

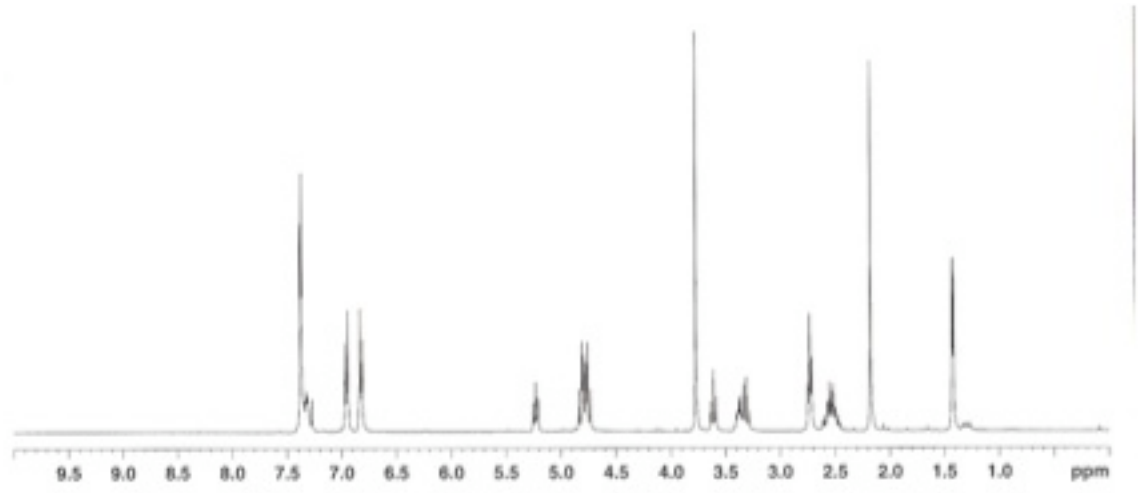
$CDCl_3$, 100 MHz

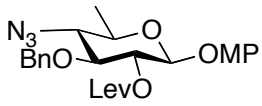




4-Methoxyphenyl 4-azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α/β-D-glucopyranoside (15)

$CDCl_3$, 400 MHz

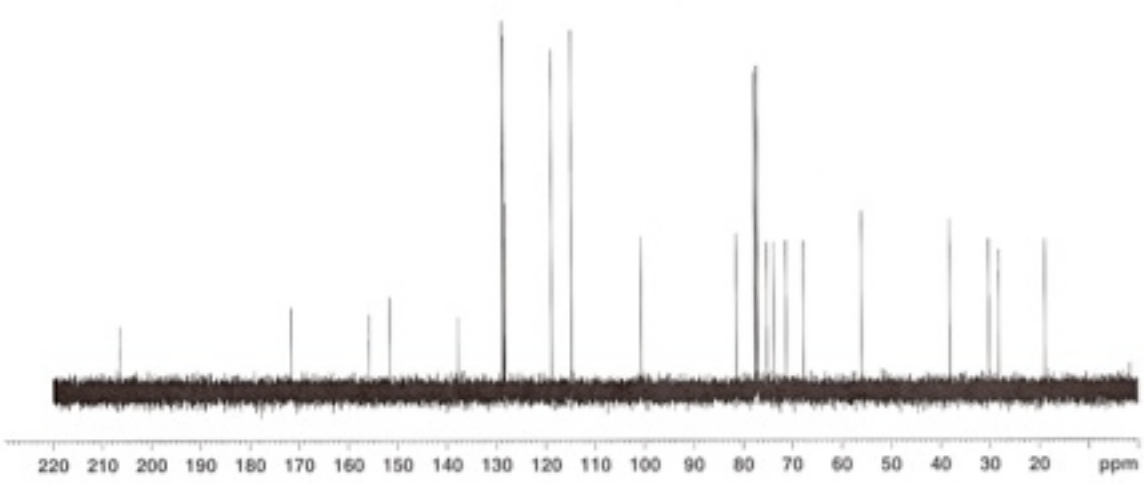


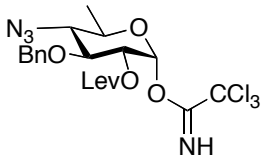


4-Methoxyphenyl 4-azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α/β-D-glucopyranoside

(15)

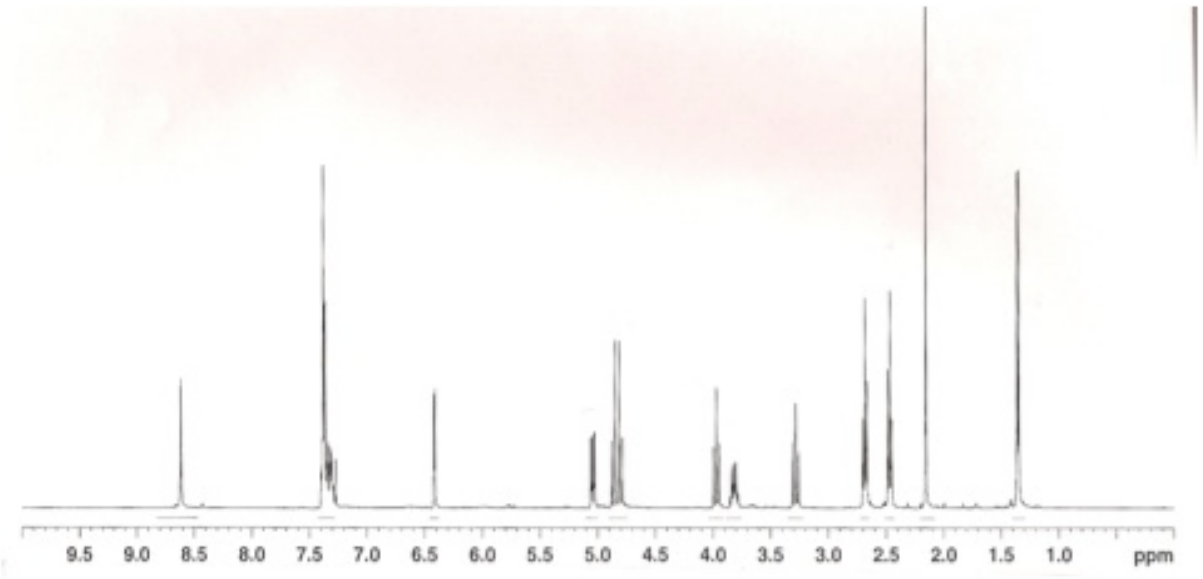
$CDCl_3$, 100 MHz

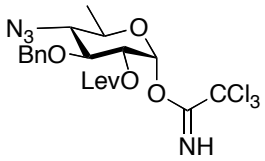




4-Azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α-D-glucopyranosyl Trichloroacetimidate (5)

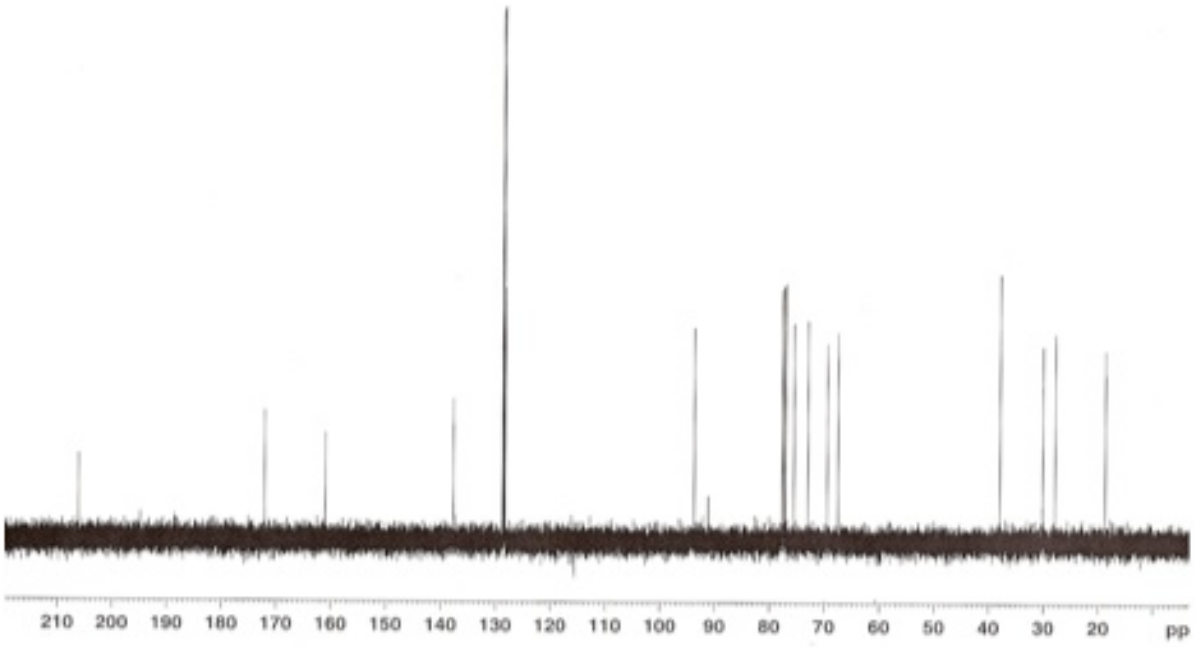
$CDCl_3$, 400 MHz

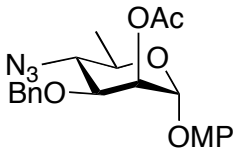




4-Azido-3-O-benzyl-4,6-dideoxy-2-O-levulinoyl-α-D-glucopyranosyl Trichloroacetimidate (5)

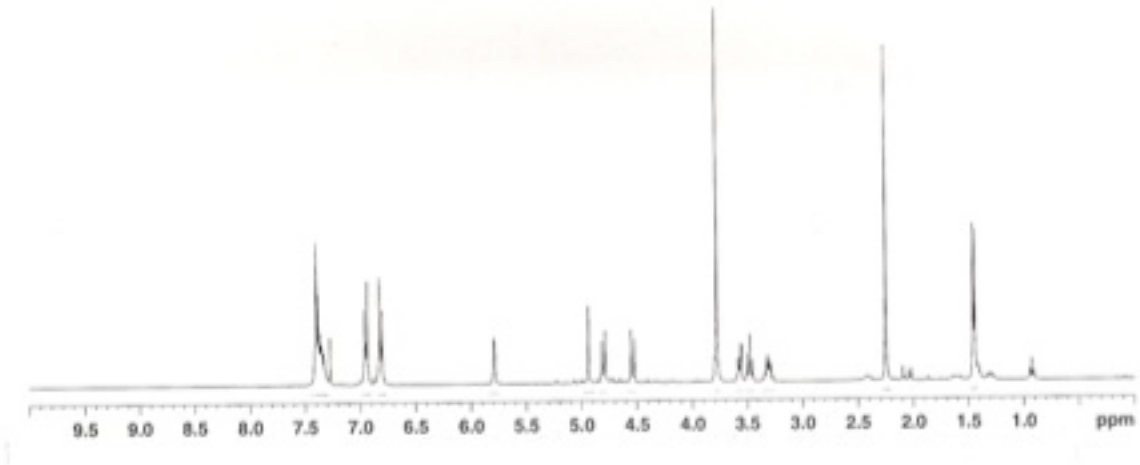
$CDCl_3$, 100 MHz

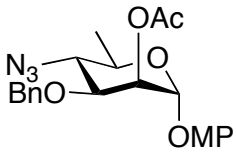




4-Methoxyphenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α/β-D-mannopyranoside (16)

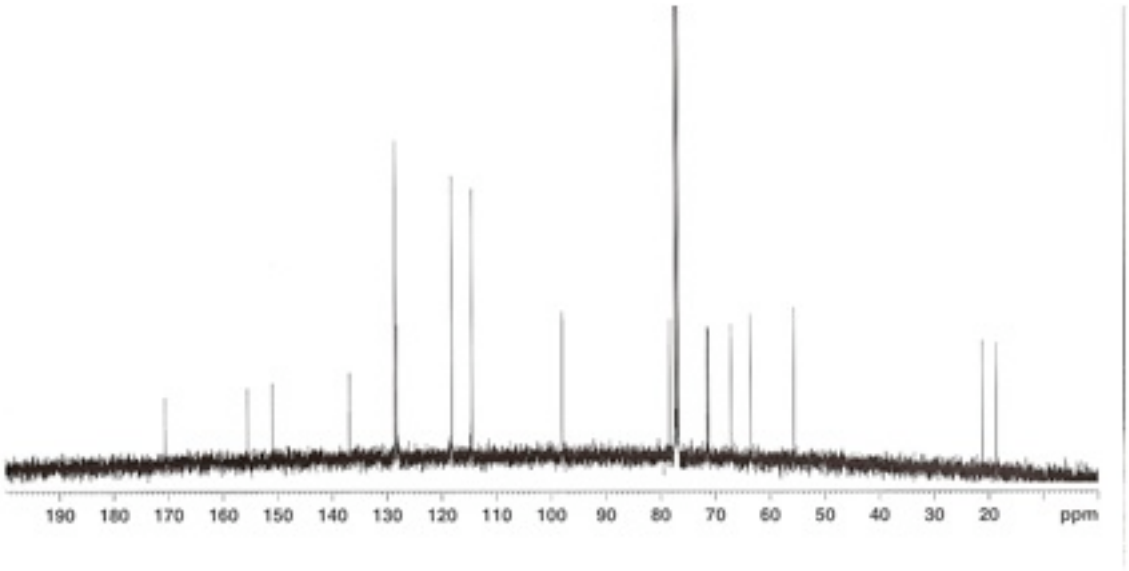
$CDCl_3$, 400MHz

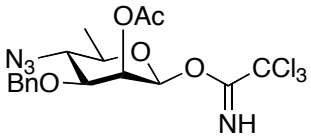




4-Methoxyphenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α/β-D-mannopyranoside (16)

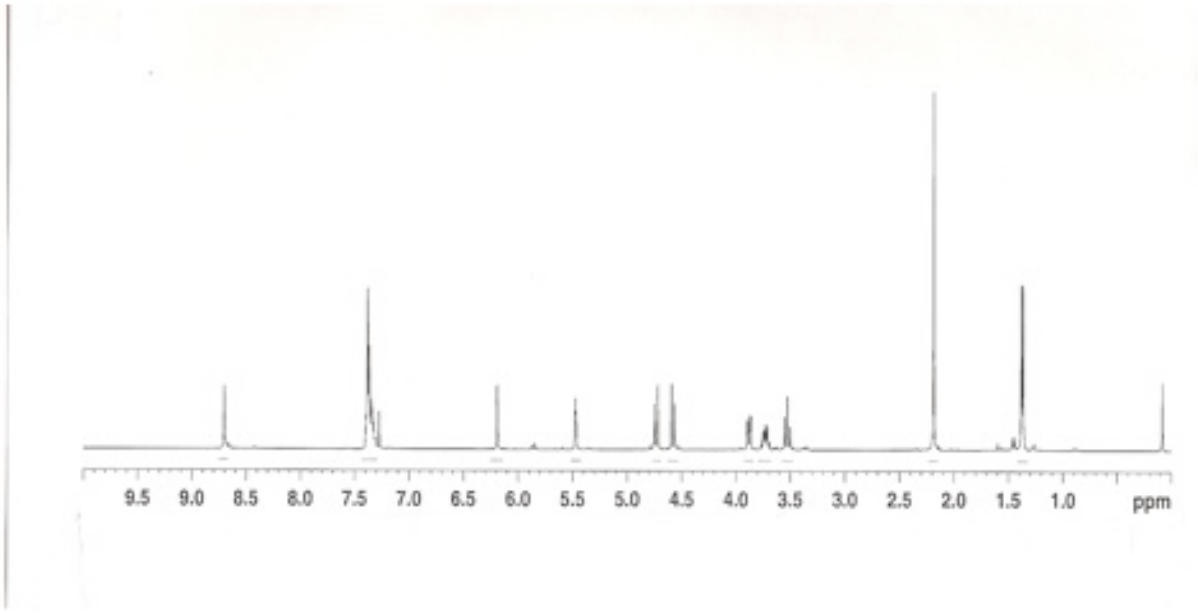
$CDCl_3$, 100MHz

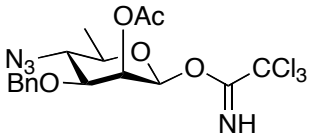




2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-β-D-mannpyranosyl Tricloroacetimidate (17)

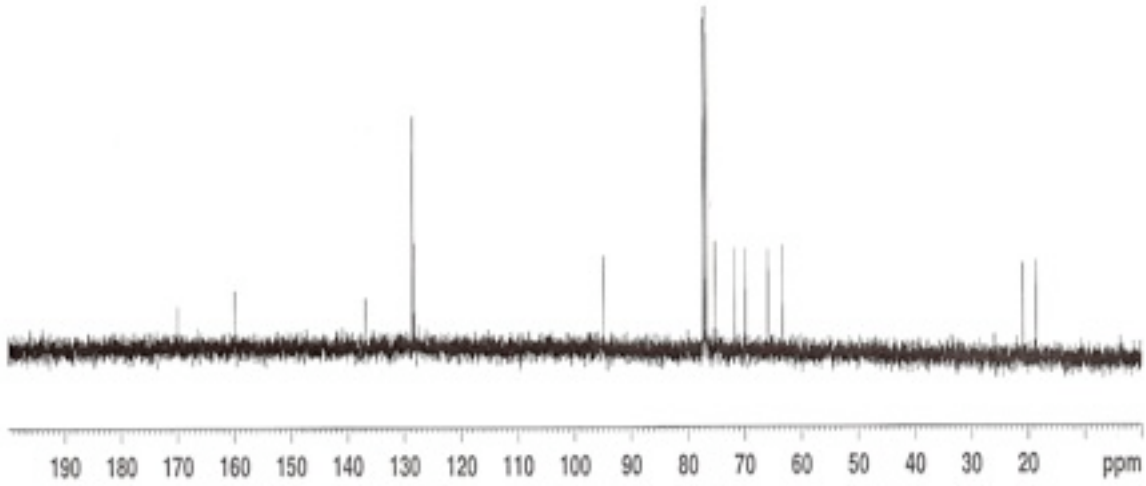
$CDCl_3$, 400 MHz

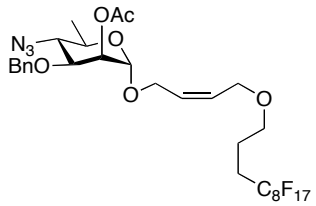




2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-β-D-mannpyranosyl Tricloroacetimidate (17)

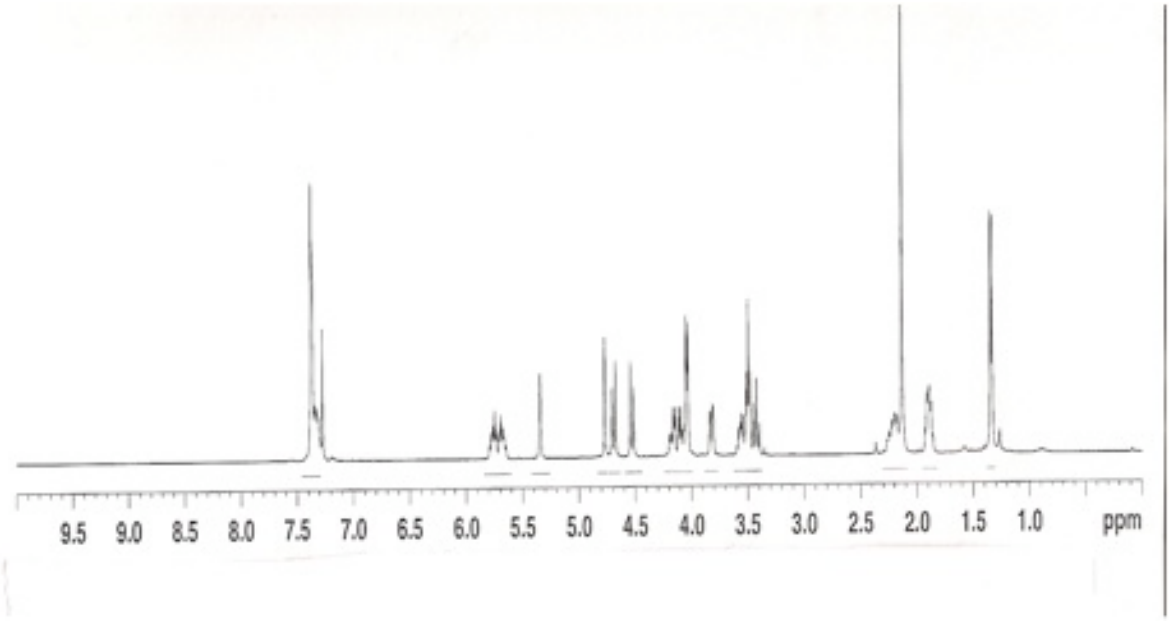
$CDCl_3$, 100 MHz

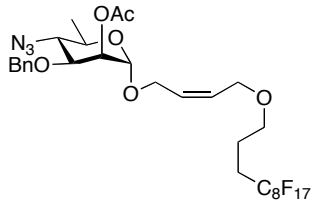




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside

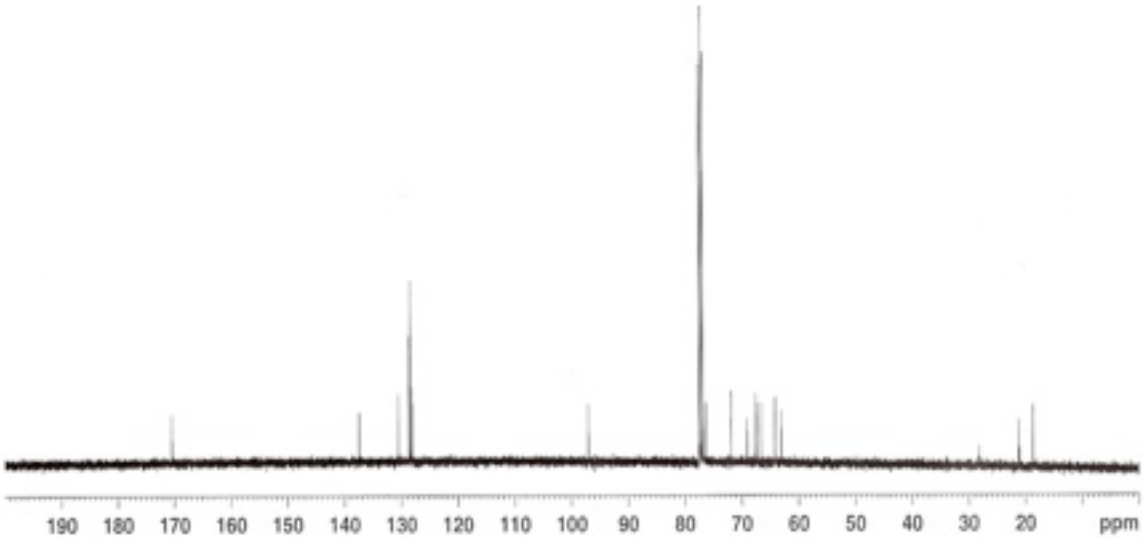
$CDCl_3$, 400 MHz

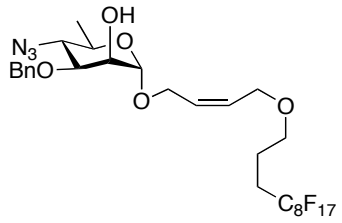




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-Acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside

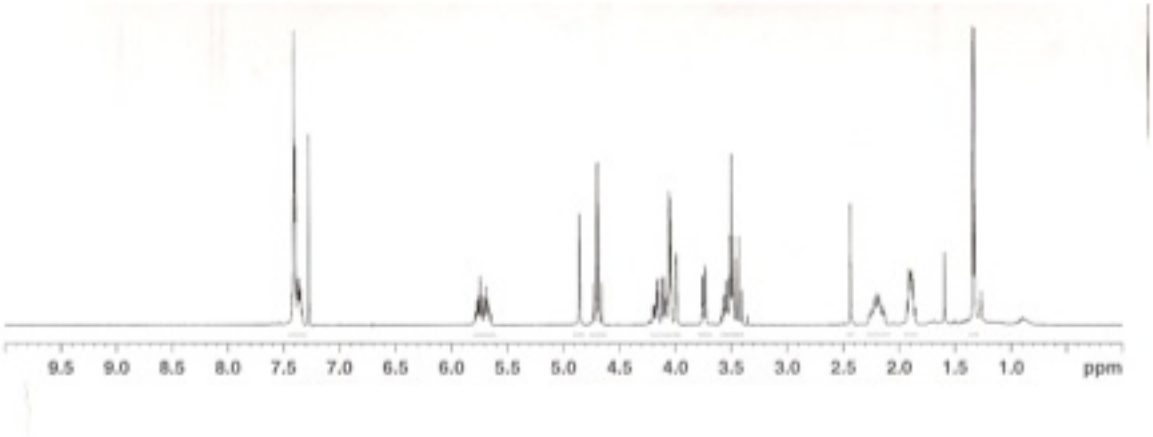
$CDCl_3$, 100 MHz

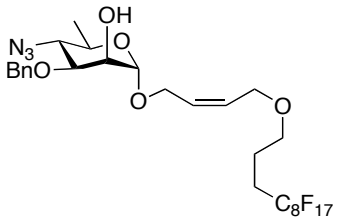




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (18)

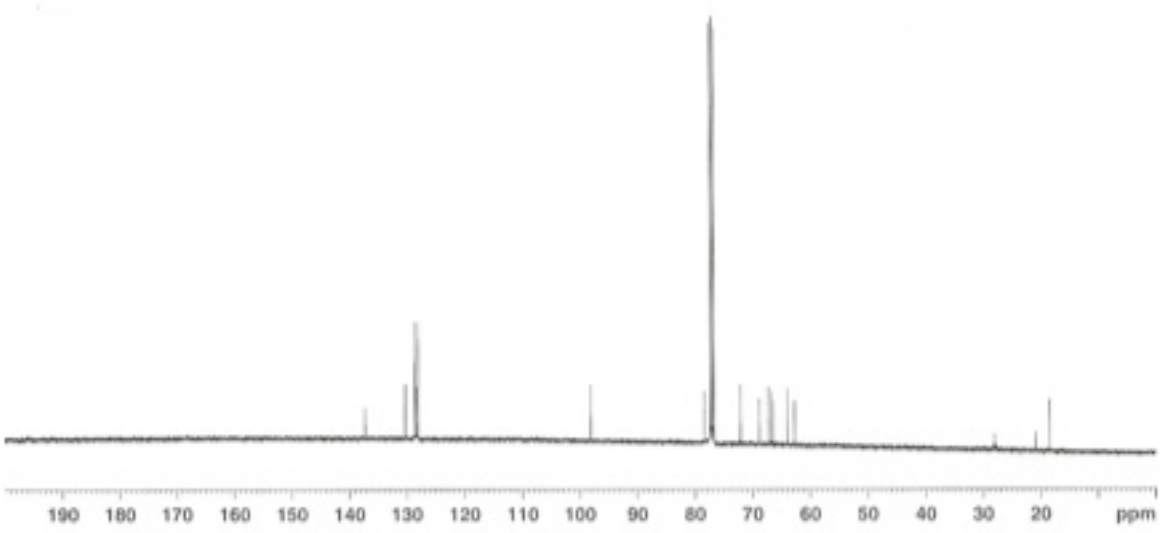
$CDCl_3$, 400 MHz

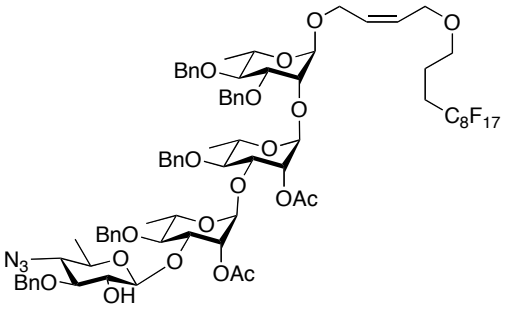




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (18)

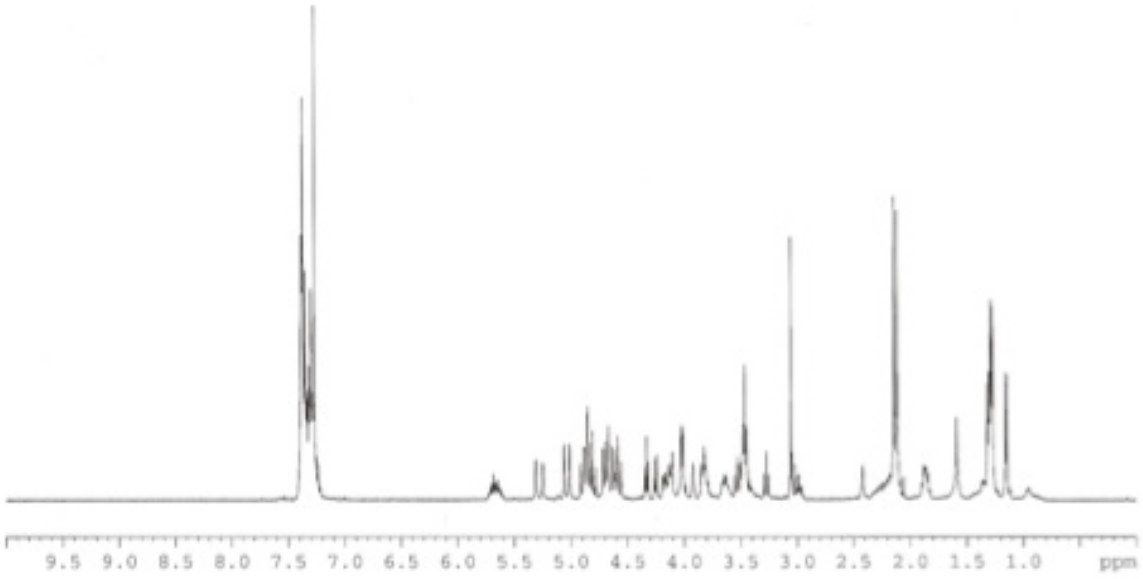
$CDCl_3$, 100 MHz

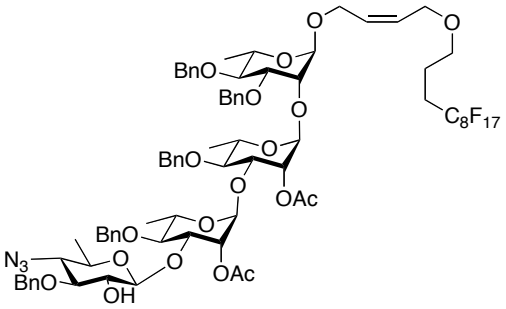




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-azido-3-O-benzyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside (19)

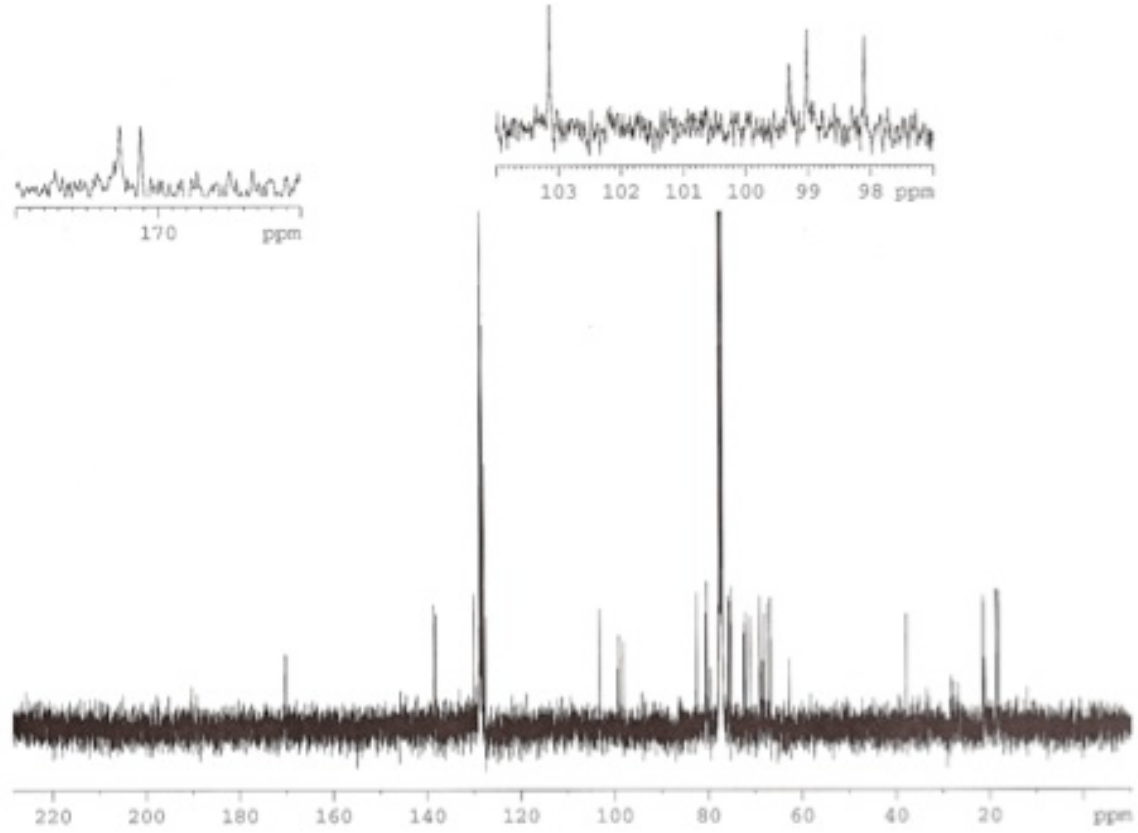
$CDCl_3$, 400 MHz

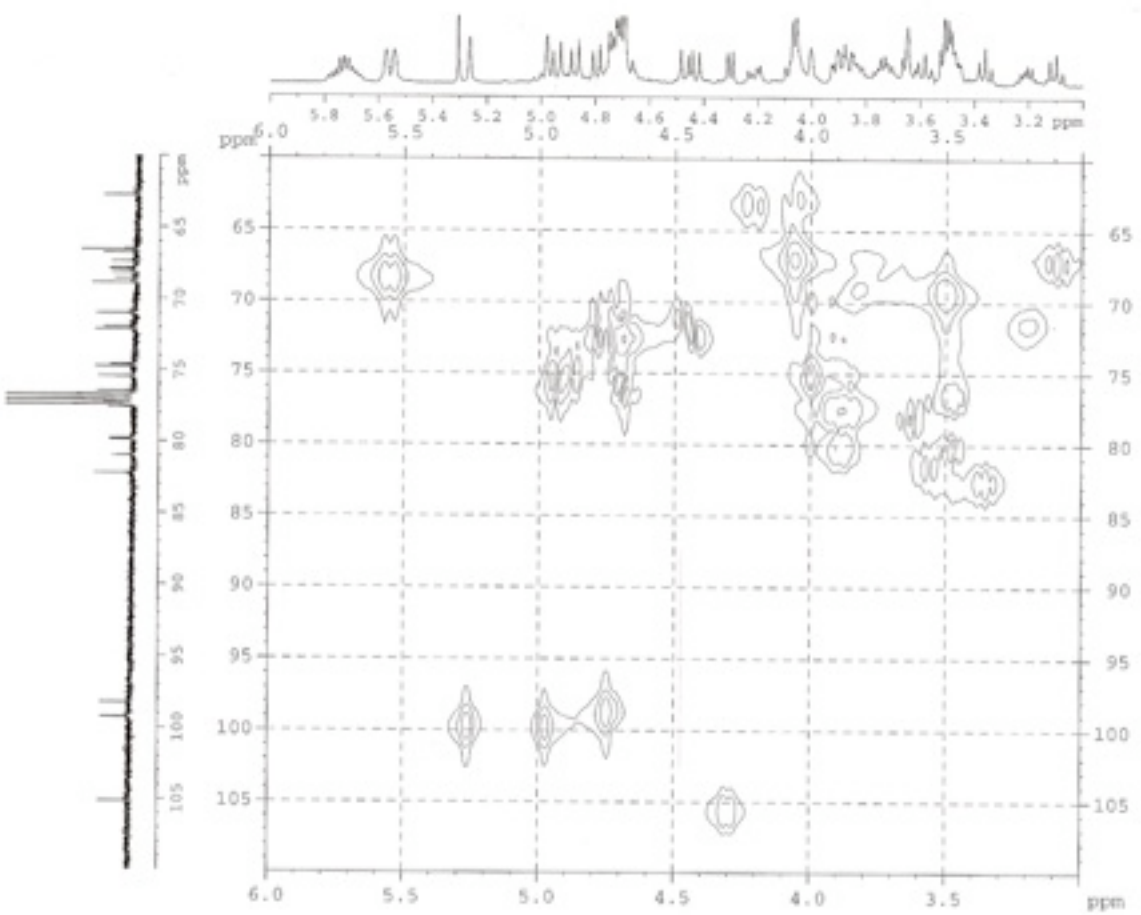


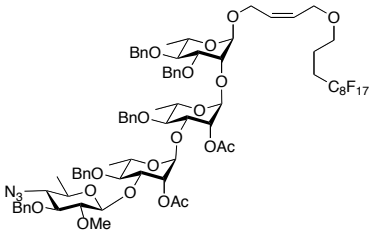


cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-azido-3-O-benzyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside (19)

$CDCl_3$, 100 MHz

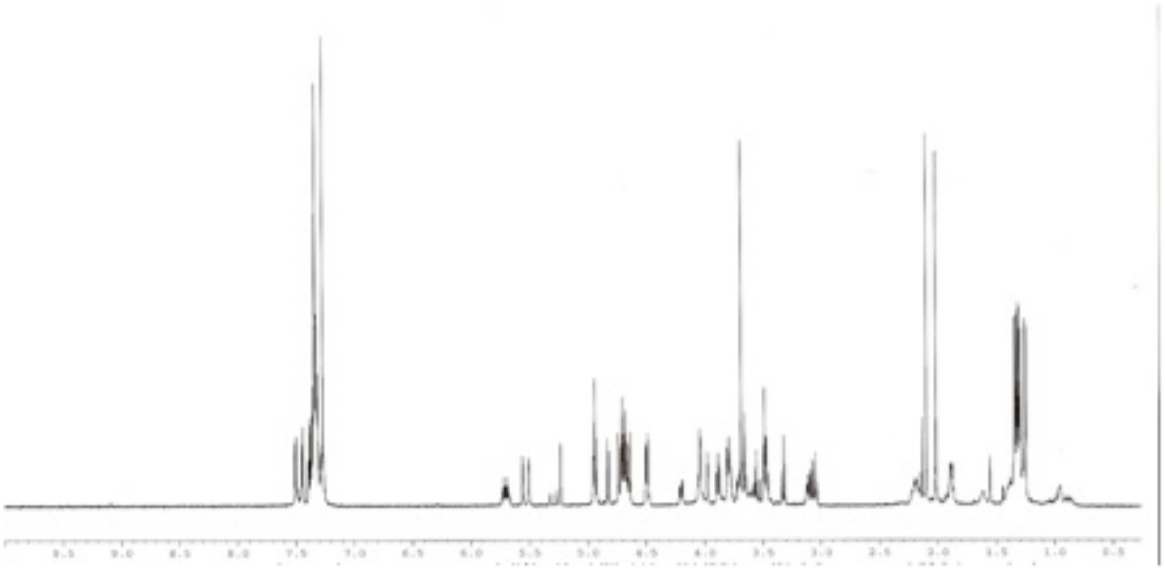


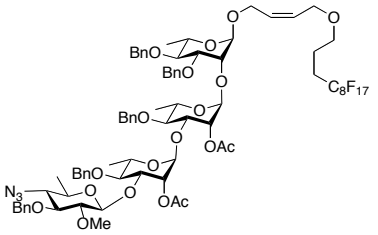




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside

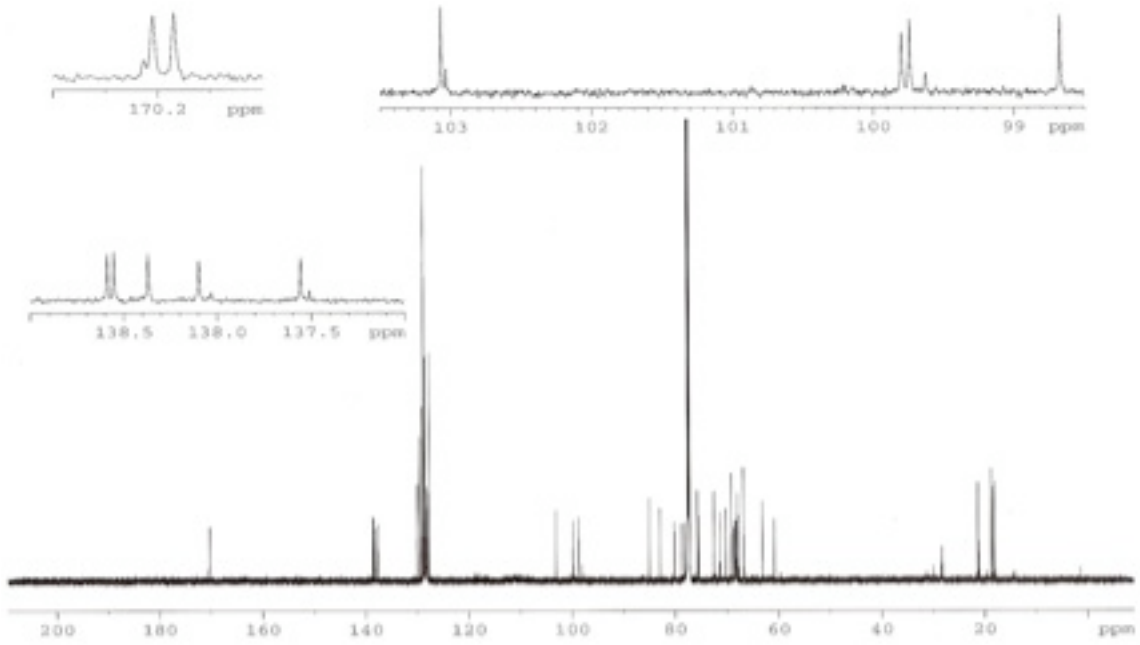
$CDCl_3$, 400 MHz

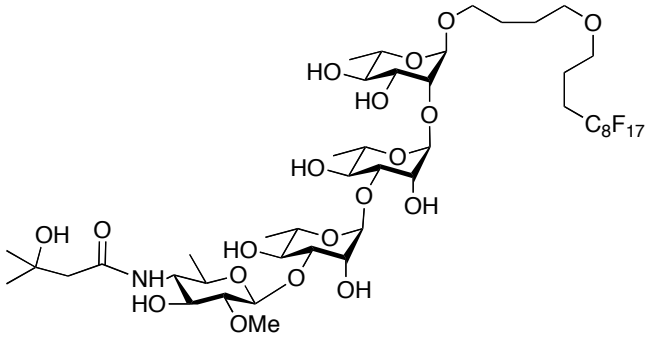




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-Azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-β-D-glucopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-benzyl-α-L-rhamnopyranoside

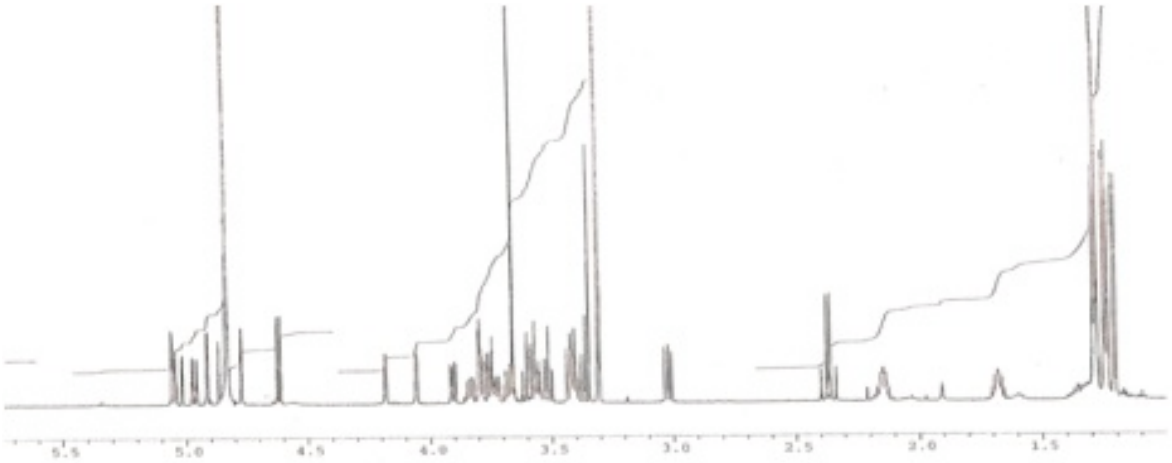
$CDCl_3$, 100 MHz

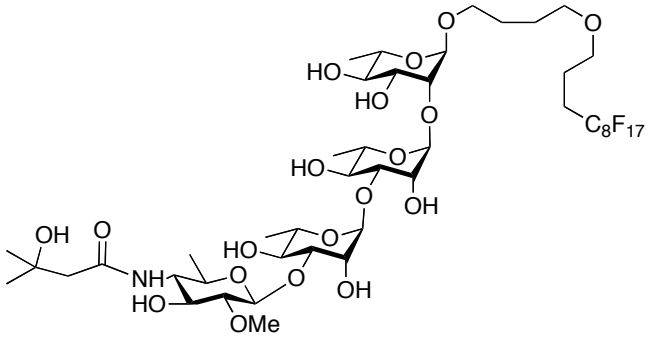




4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-butyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-β-D-glucopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→2)- α-L-rhamnopyranoside (1)

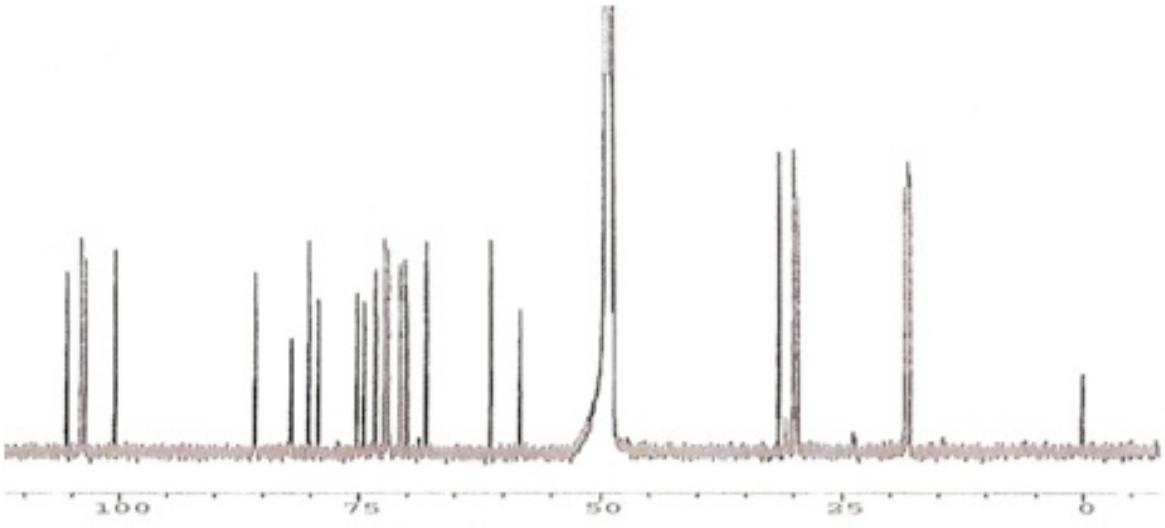
CD_3OD, 400 MHz

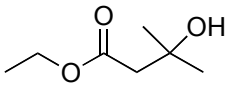




4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-butyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-β-D-glucopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→2)- α-L-rhamnopyranoside (1)

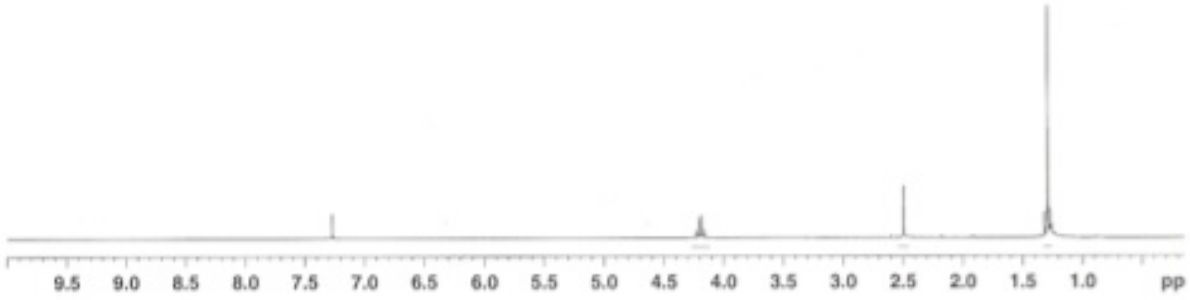
CD_3OD, 100 MHz

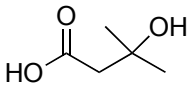




Ethyl 3-hydroxy-3-methylbutanoate (21)

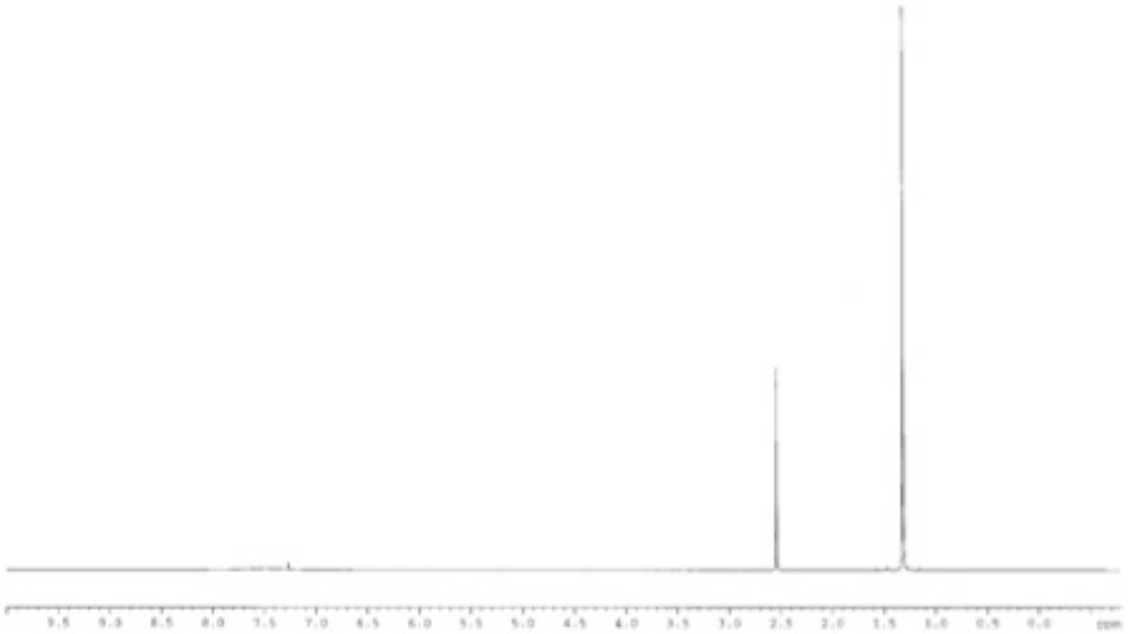
$CDCl_3$, 400 MHz

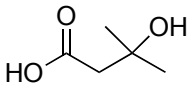




3-Hydroxy-3-methylbutanoic acid (22)

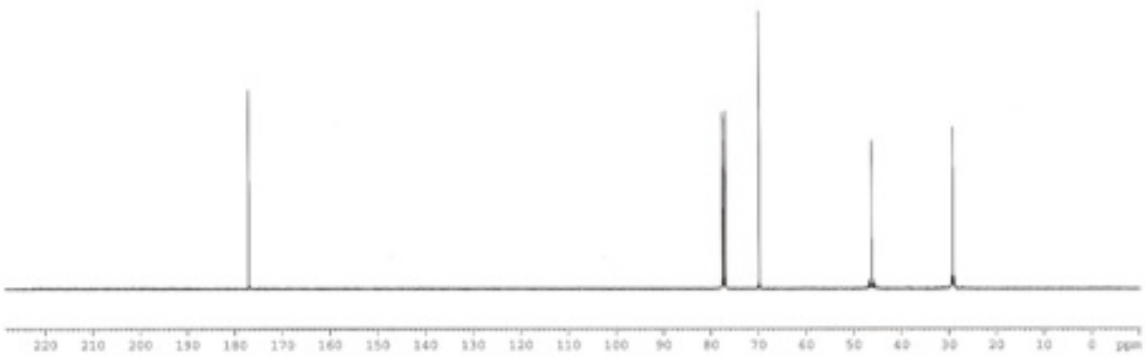
$CDCl_3$, 400 MHz

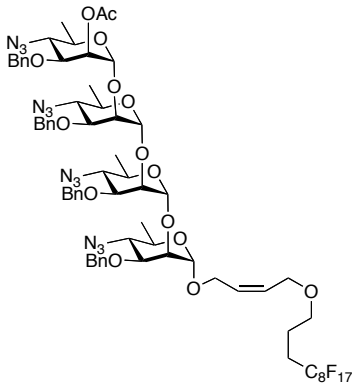




3-Hydroxy-3-methylbutanoic acid (22)

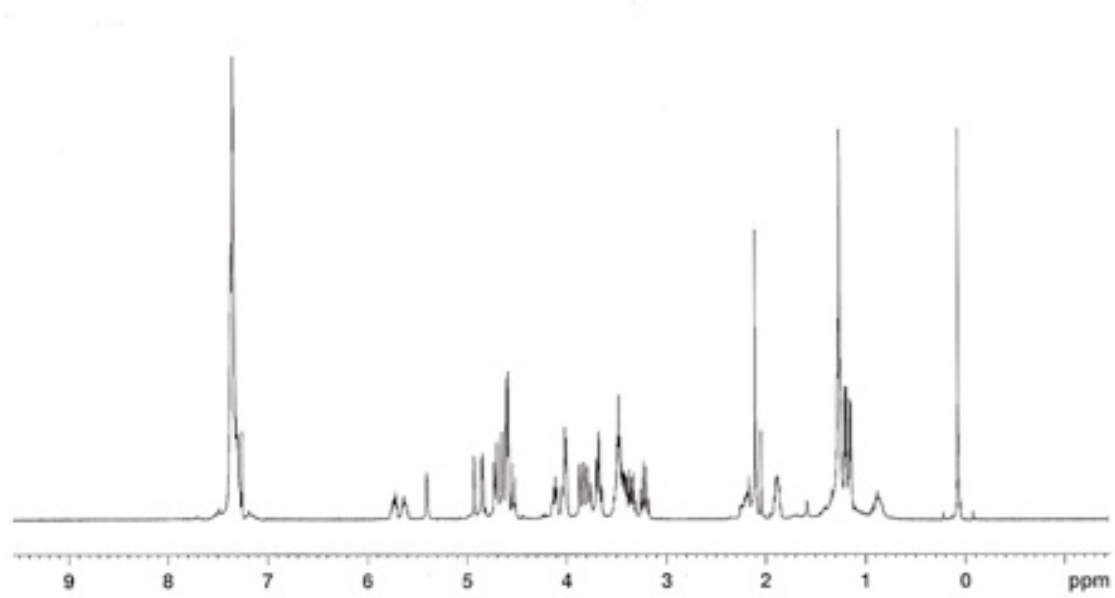
$CDCl_3$, 100 MHz

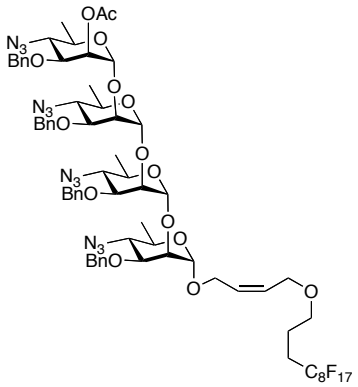




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (23)

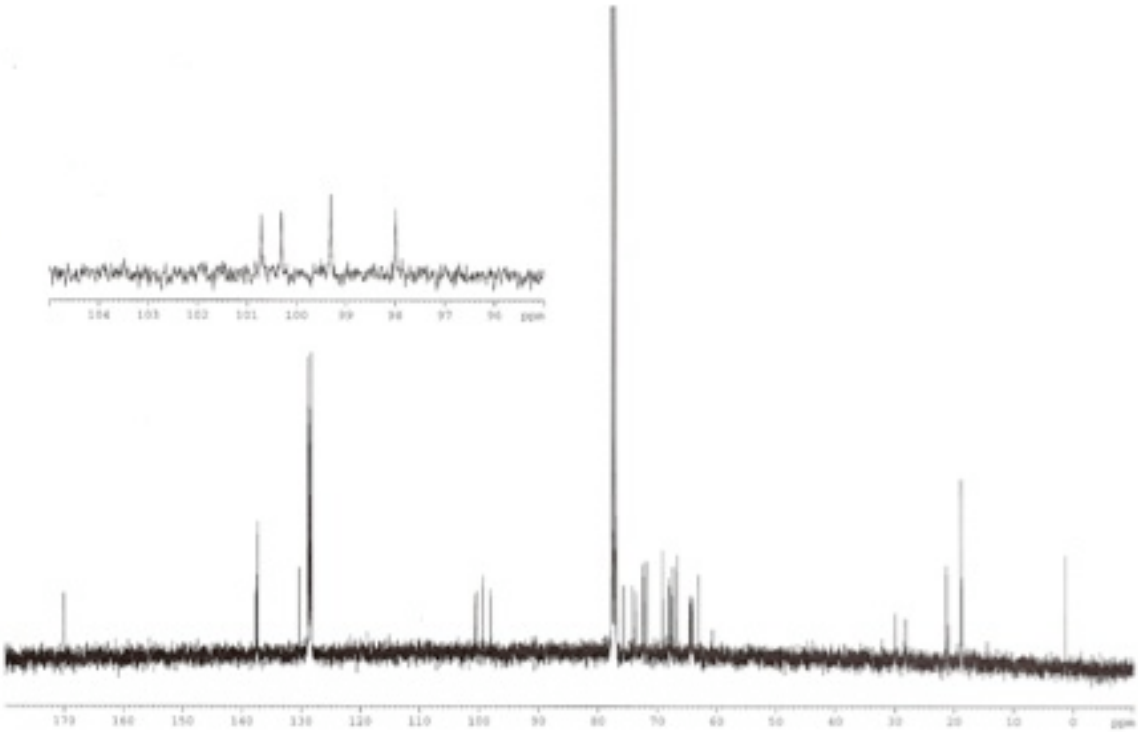
$CDCl_3$, 400 MHz

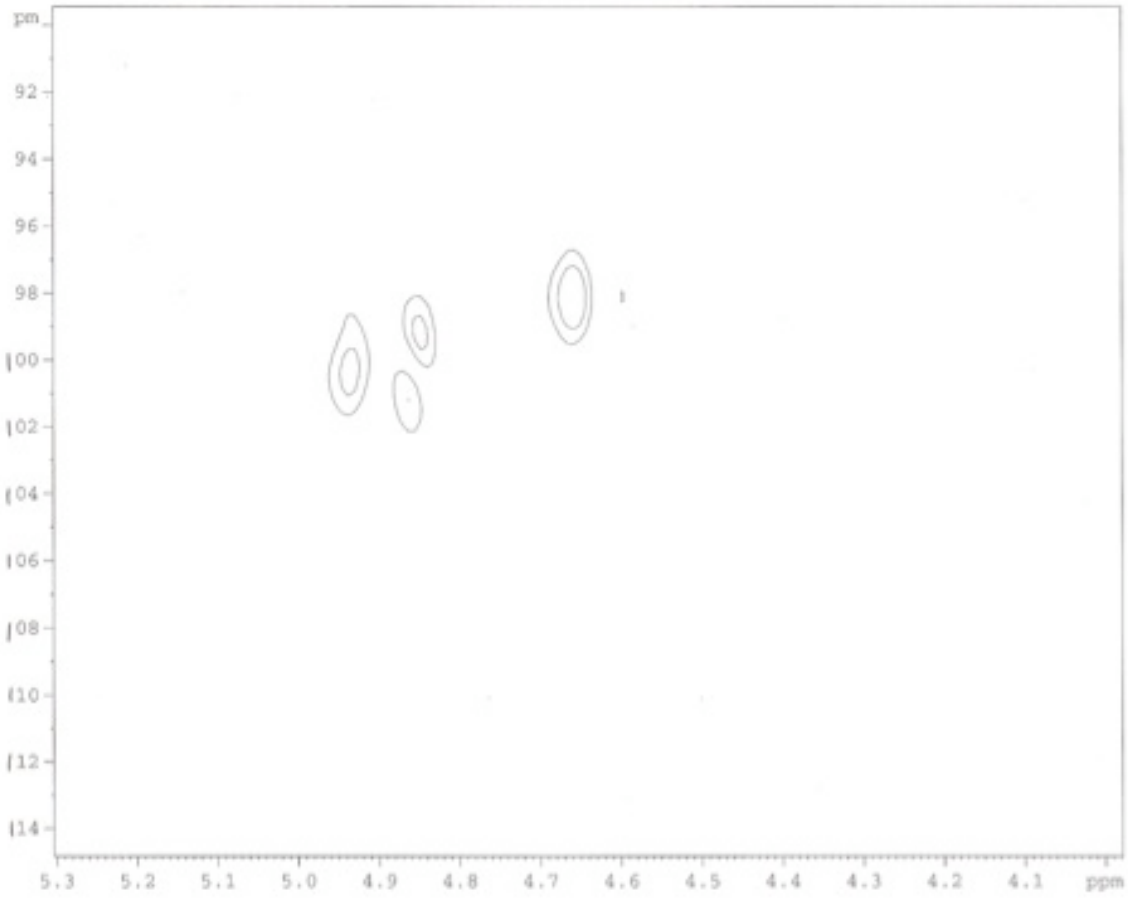


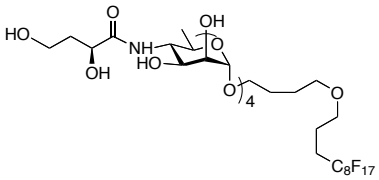


cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (23)

$CDCl_3$, 100 MHz

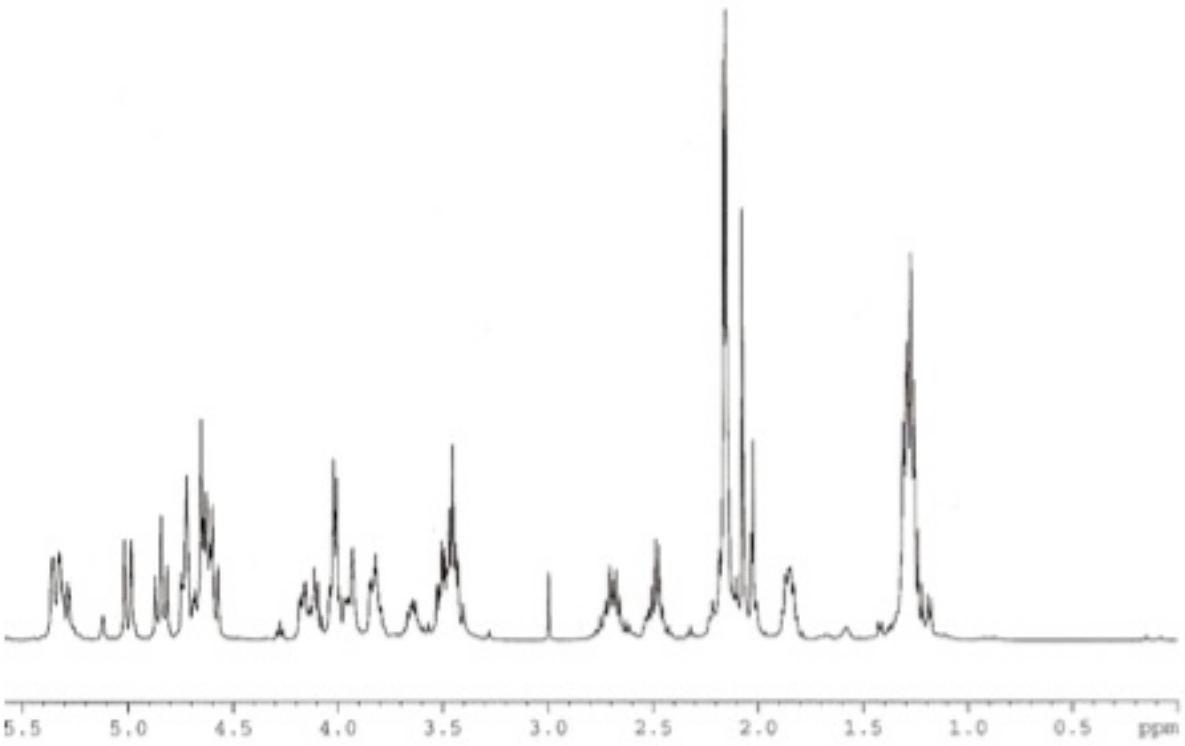


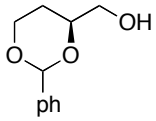




4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-butyl 4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (2)

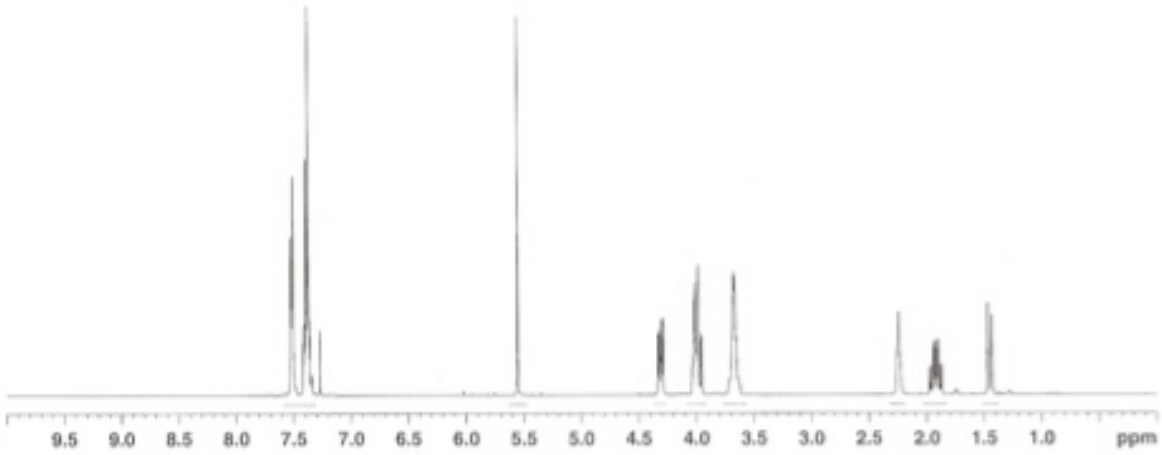
CD_3OD, 400MHz

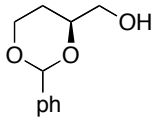




2,4-Benzylidene-(S)-1,2,4-butanetriol (25)

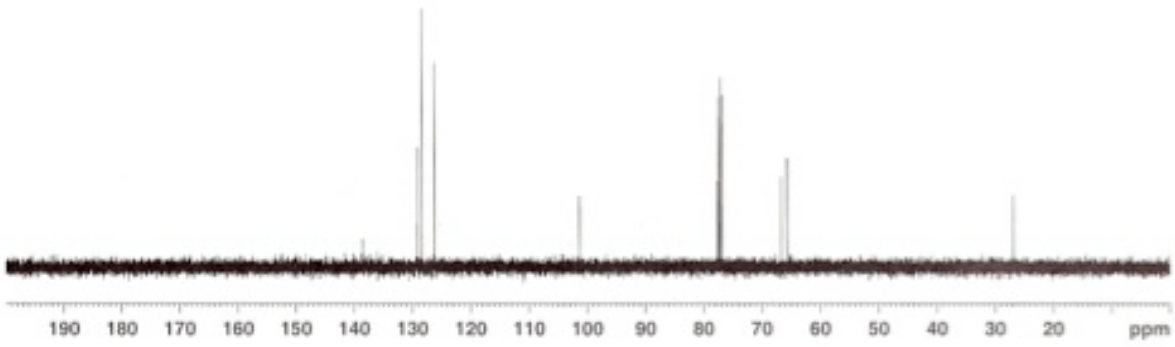
$CDCl_3$, 400 MHz



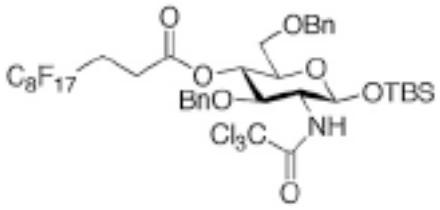


2,4-Benzylidene-(S)-1,2,4-butanetriol (25)

$CDCl_3$, 100 MHz

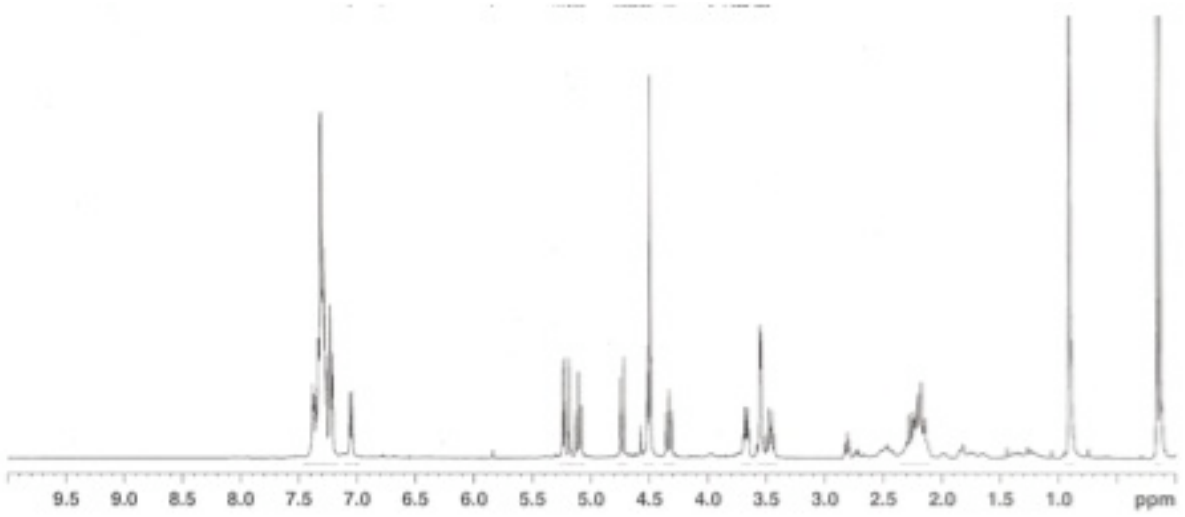


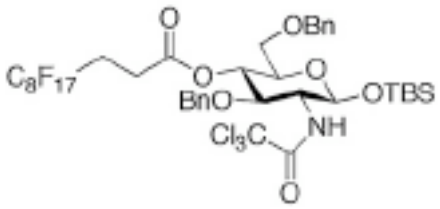
APPENDIX C. CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA



*tert-Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-4-2H, 2H, 3H, 3H, -perfluoroundecanoatyl-β-D-glucopyranoside (**2**)*

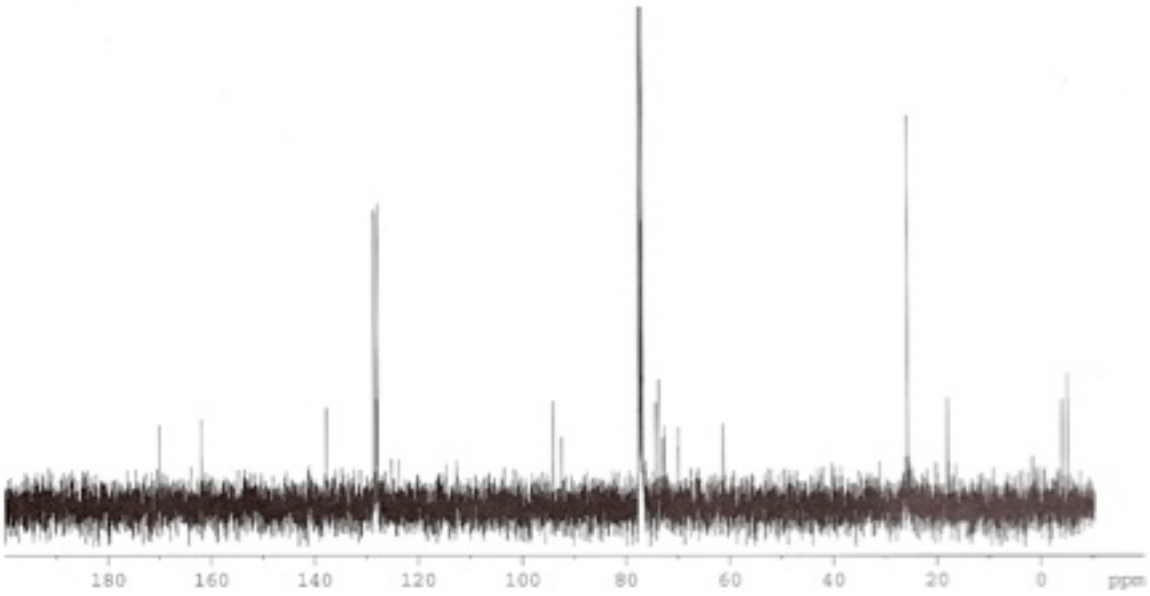
400 MHz, $CDCl_3$

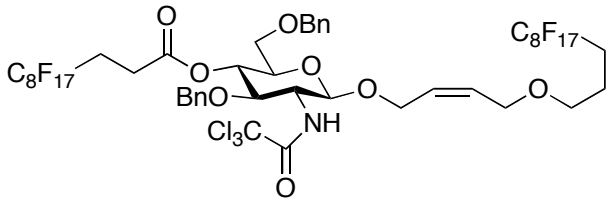




*tert-Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-trichloroacetimido-4-2H, 2H, 3H, 3H, -perfluoroundecanoatyl-β-D-glucopyranoside (**2**)*

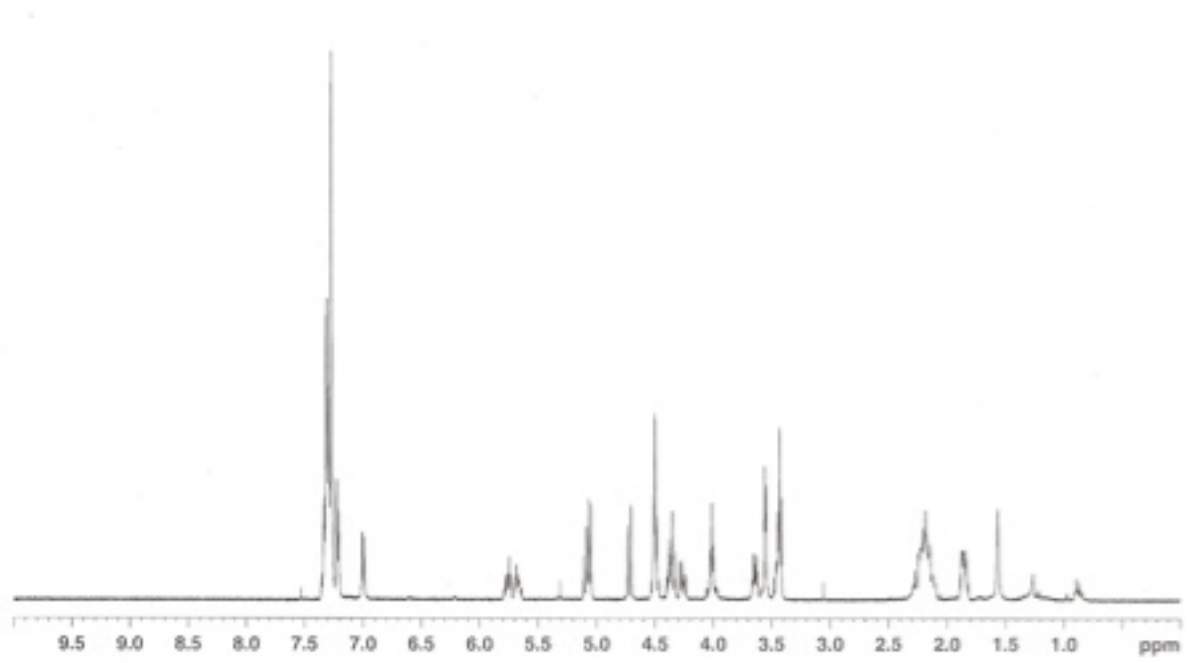
100 MHz, $CDCl_3$

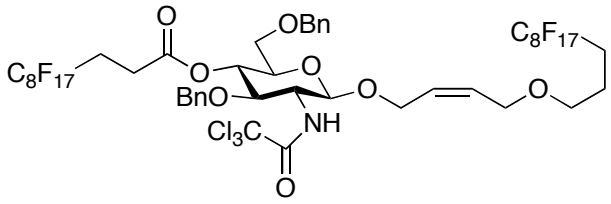




*3,6-Di-O-benzyl-2-deoxy-2-trichloroacetimido-4-(2H, 2H, 3H, 3H-perfluoroundecanoyl)-α-D-glucopyranosyl trichloroacetimidate (**4**)*

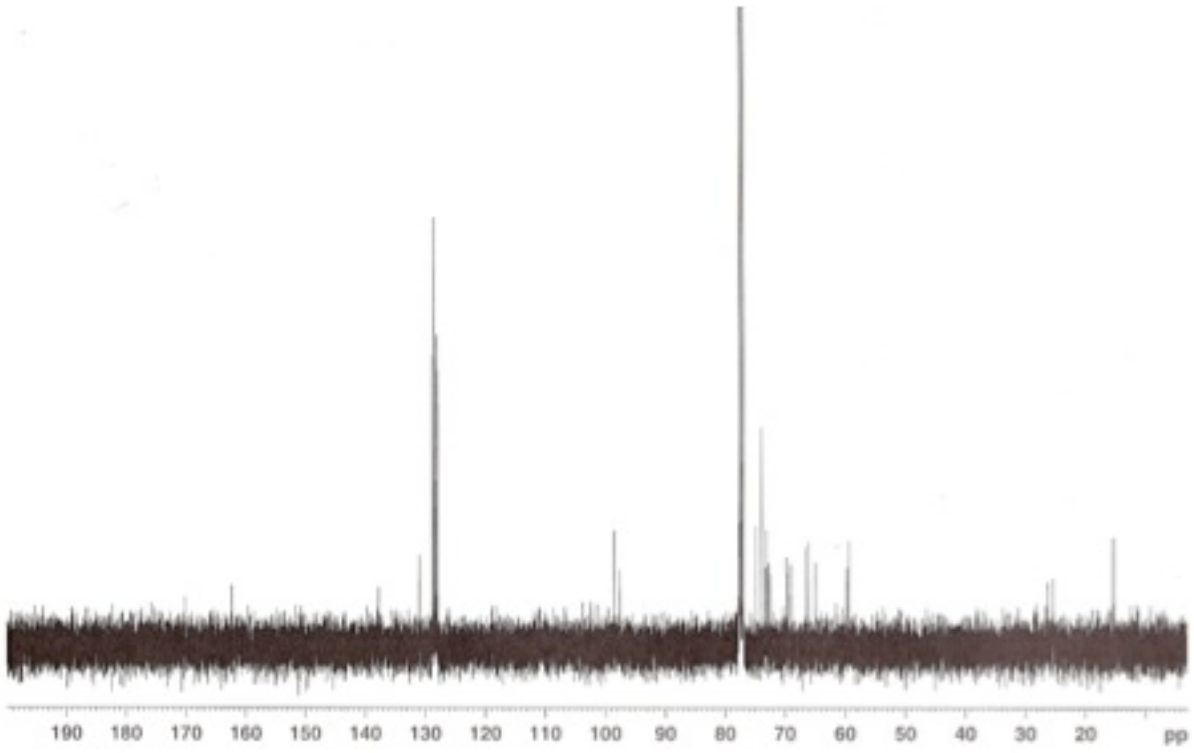
400 MHz, $CDCl_3$



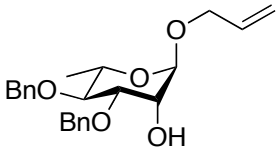


*3,6-Di-O-benzyl-2-deoxy-2-trichloroacetimido-4-(2H, 2H, 3H, 3H-perfluoroundecanoyl)-α-D-glucopyranosyl trichloroacetimidate (**4**)*

100 MHz, $CDCl_3$

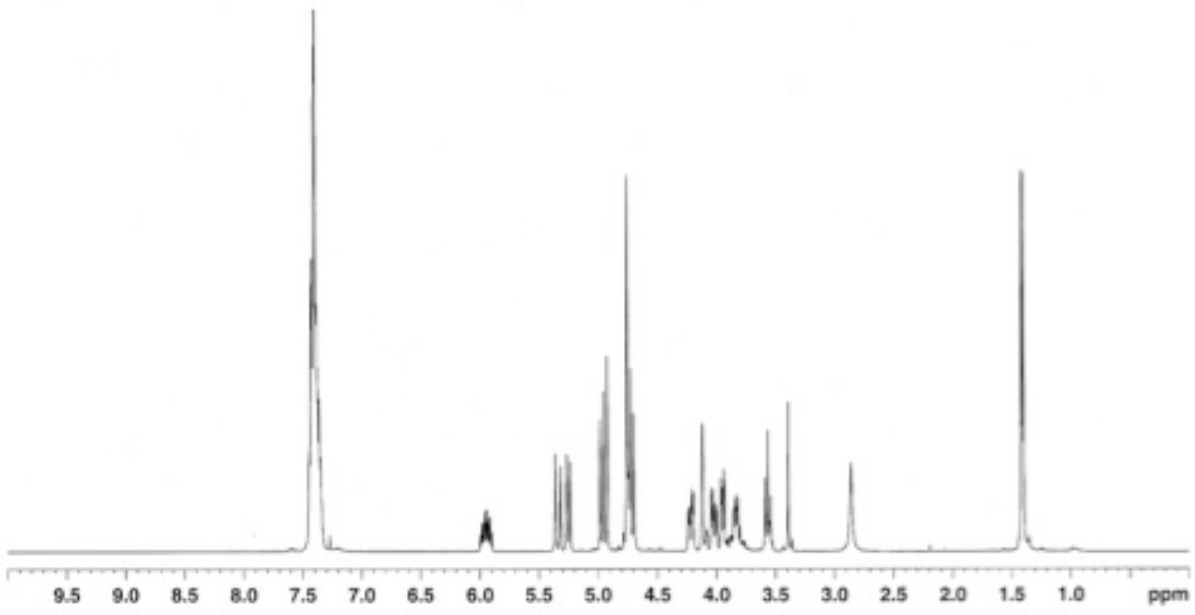


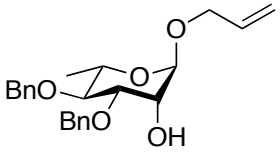
APPENDIX D. CHAPTER 5 ^{1}H AND ^{13}C NMR SPECTRA



Allyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (2)

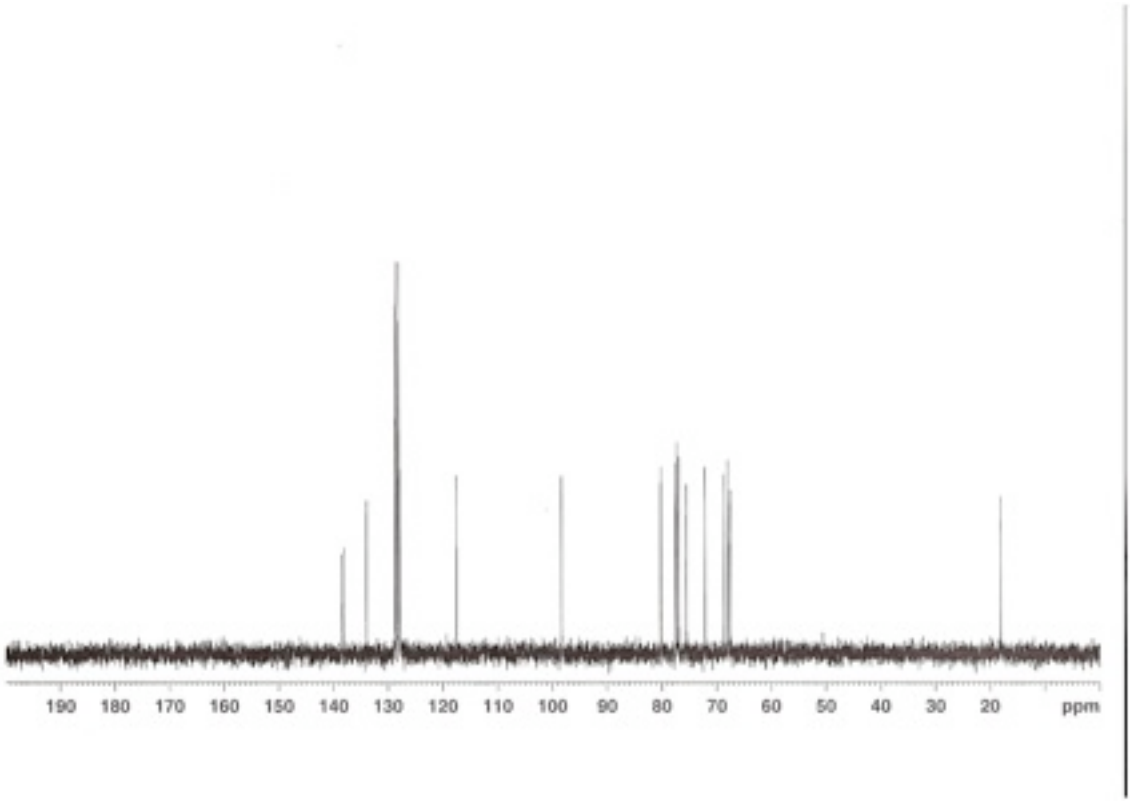
$CDCl_3$, 400 MHz

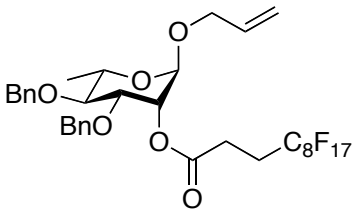




Allyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (2)

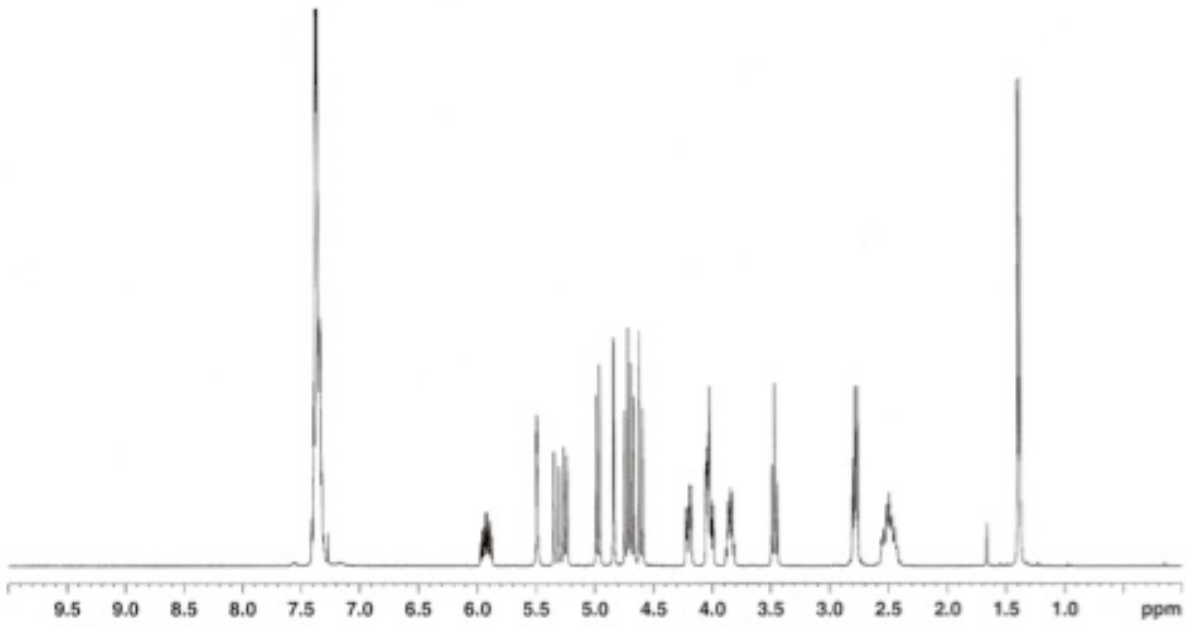
$CDCl_3$, 100 MHz

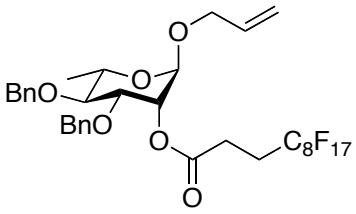




Allyl 3,4-di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranoside (3)

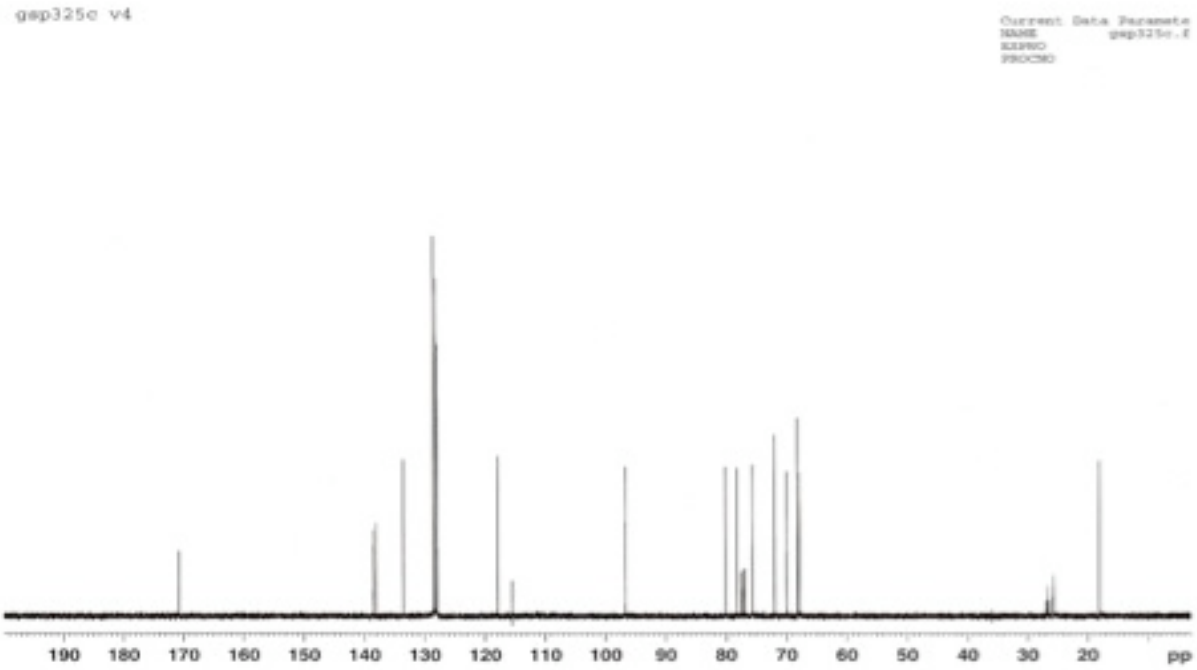
$CDCl_3$, 400 MHz

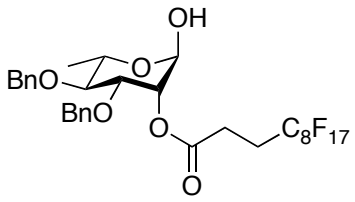




Allyl 3,4-di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranoside (3)

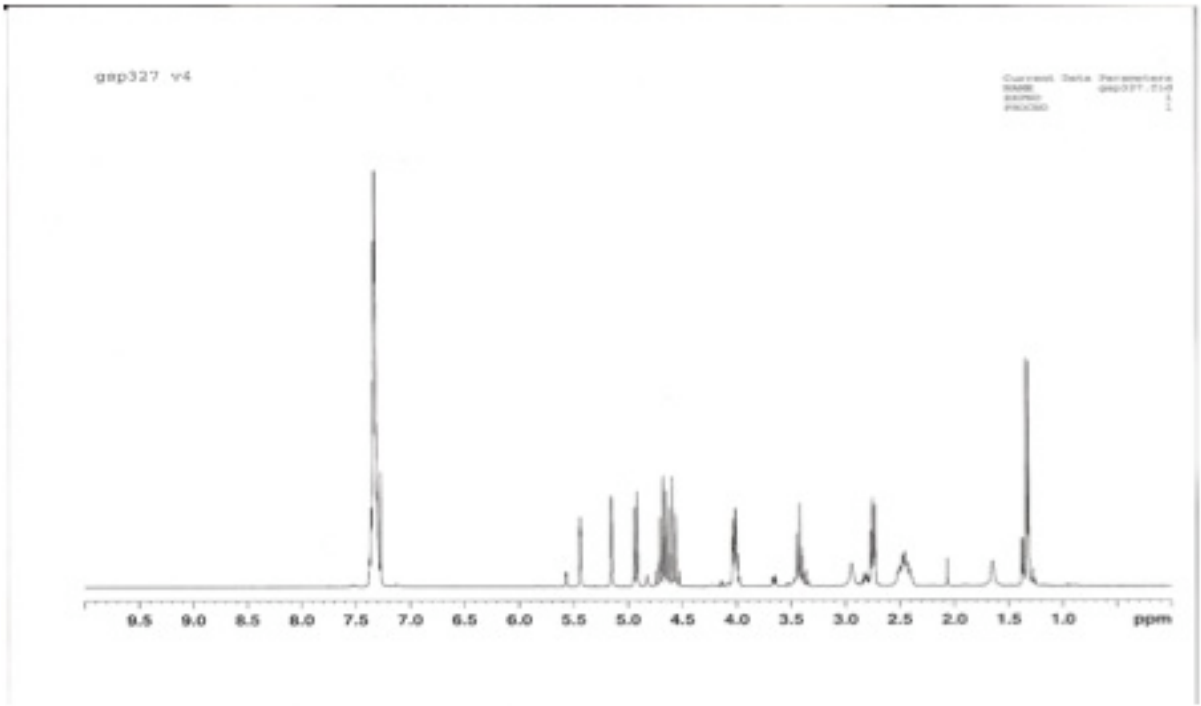
$CDCl_3$, 100 MHz

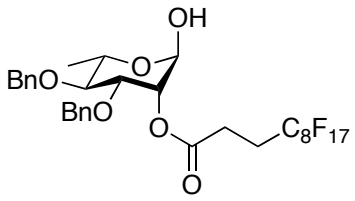




3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranoside (4)

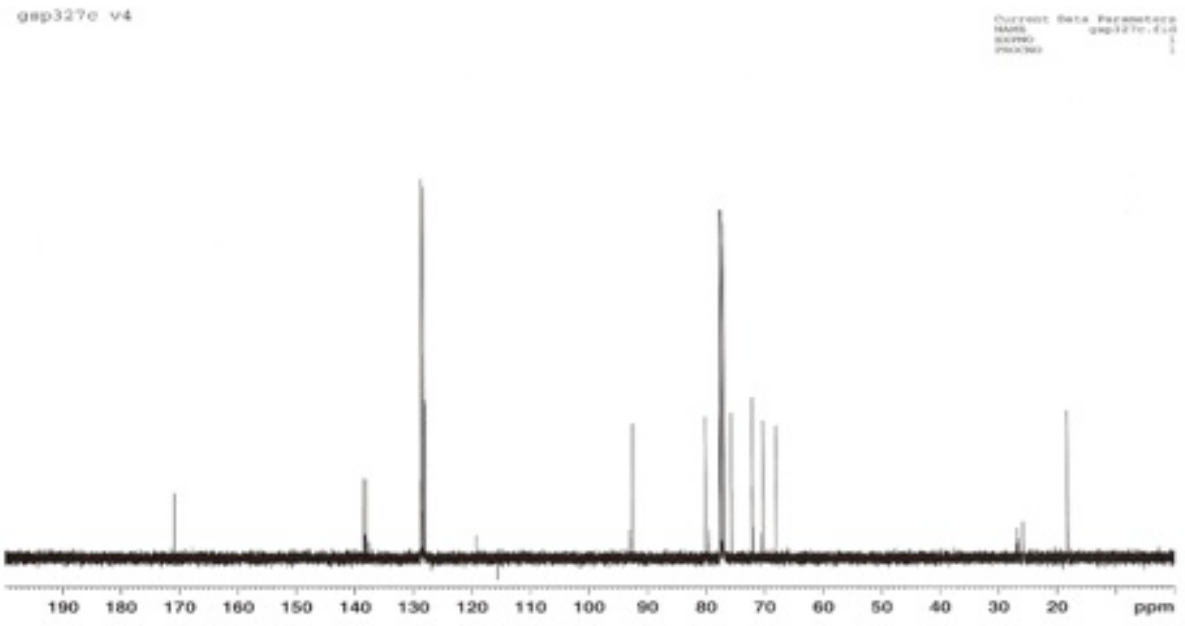
$CDCl_3$, 400 MHz

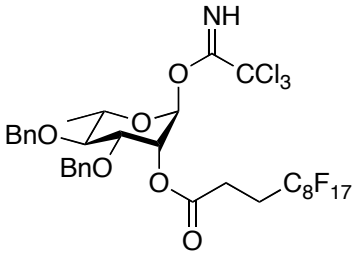




3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranoside (4)

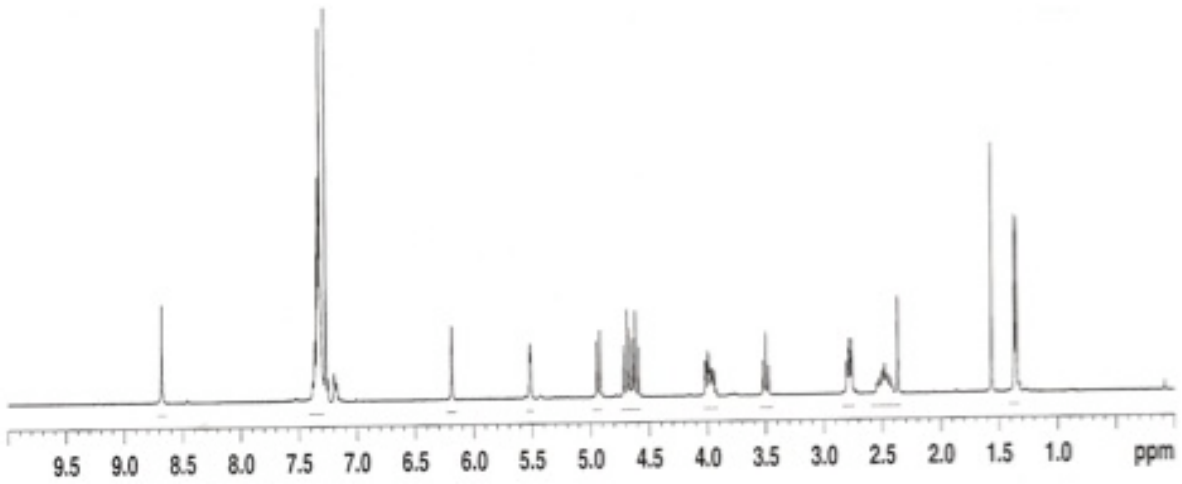
$CDCl_3$, 100 MHz

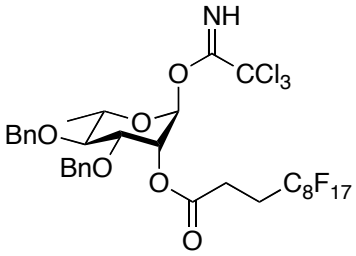




3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranosyl trichloroacetimidate (5)

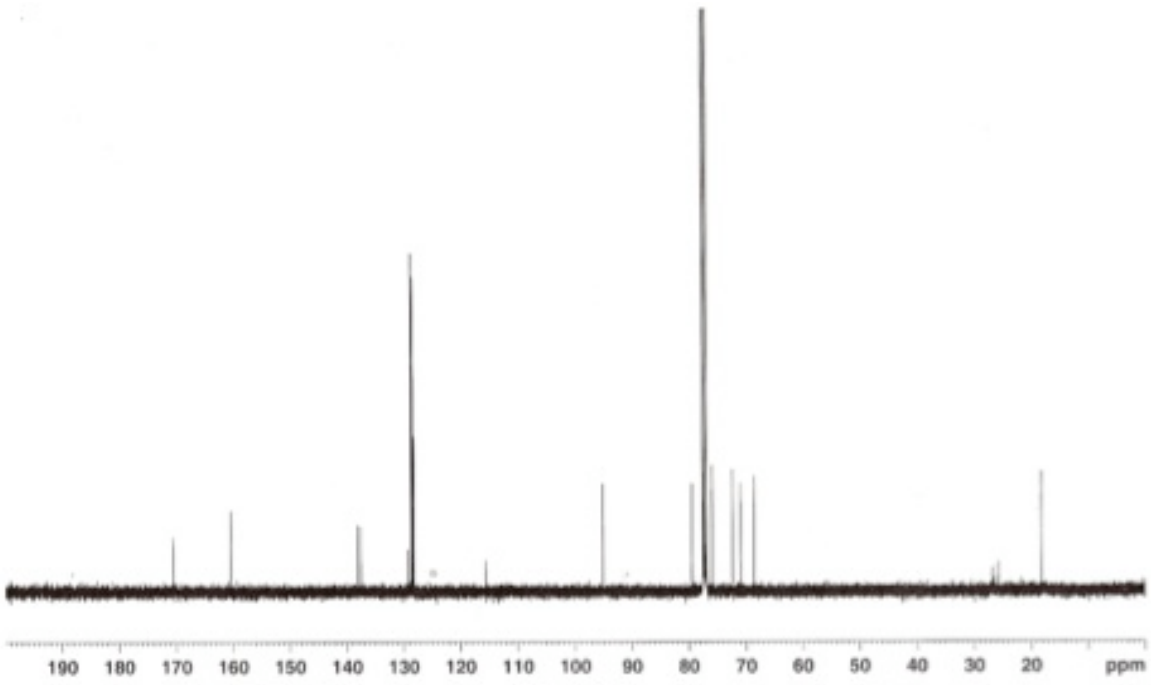
$CDCl_3$, 400 MHz

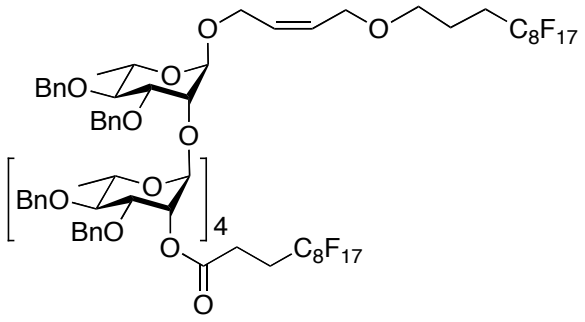




3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α/β-L-rhamnopyranosyl trichloroacetimidate (5)

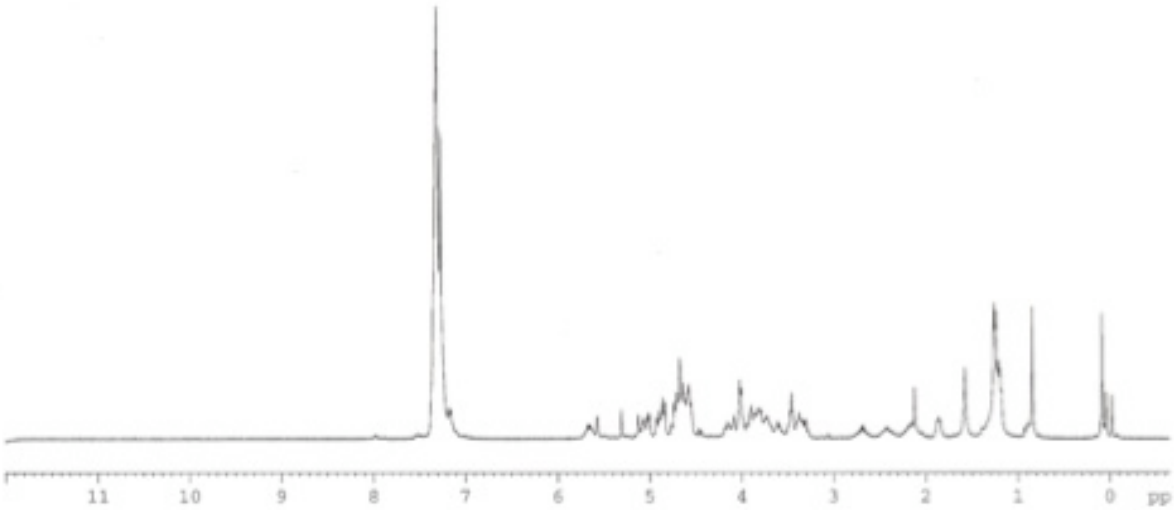
$CDCl_3$, 100 MHz

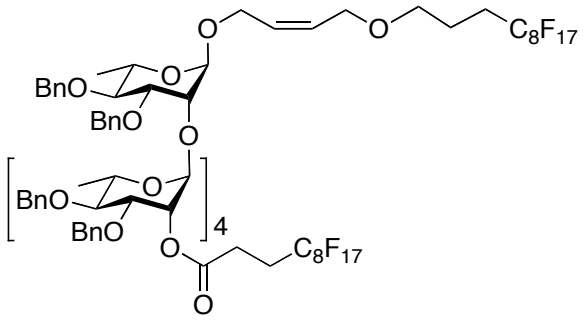




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (9)

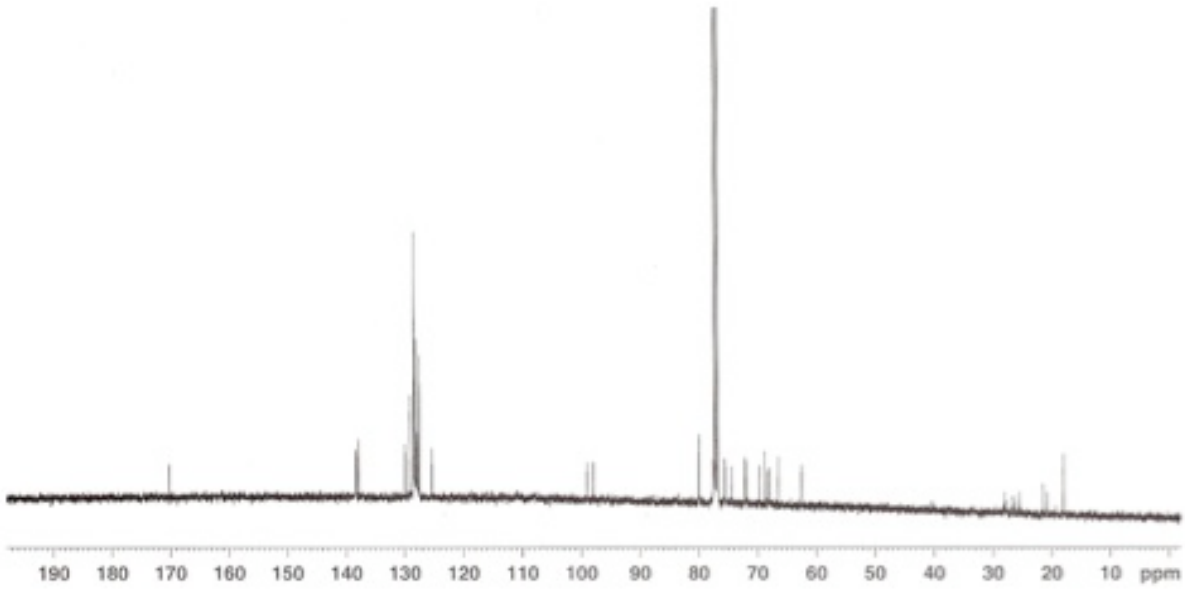
$CDCl_3$, 400 MHz

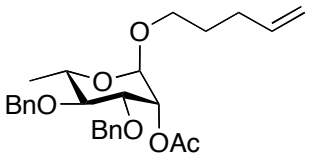




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3,4-Di-O-benzyl-2-(2H, 2H, 3H, 3H-perfluoroundecanoatyl)-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (9)

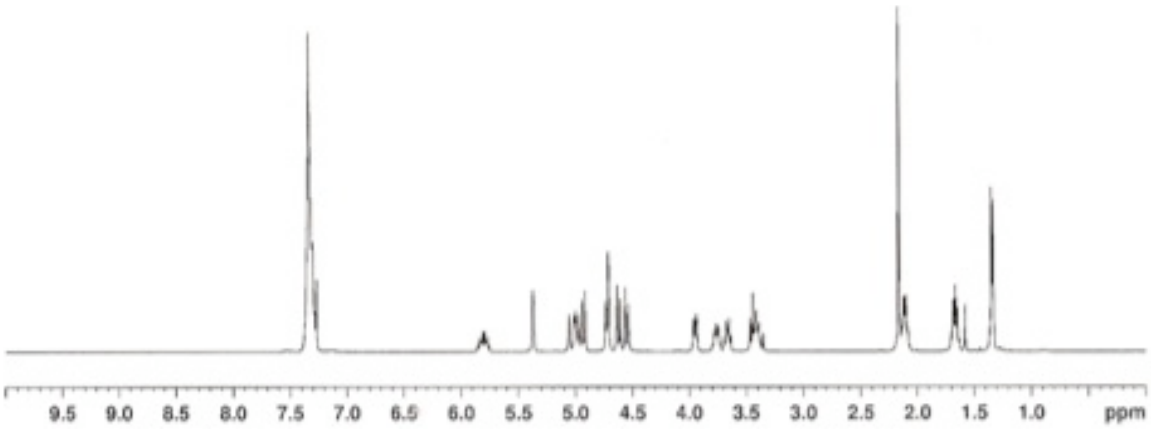
$CDCl_3$, 100 MHz

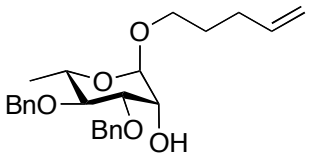




n-Pentenyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranoside.

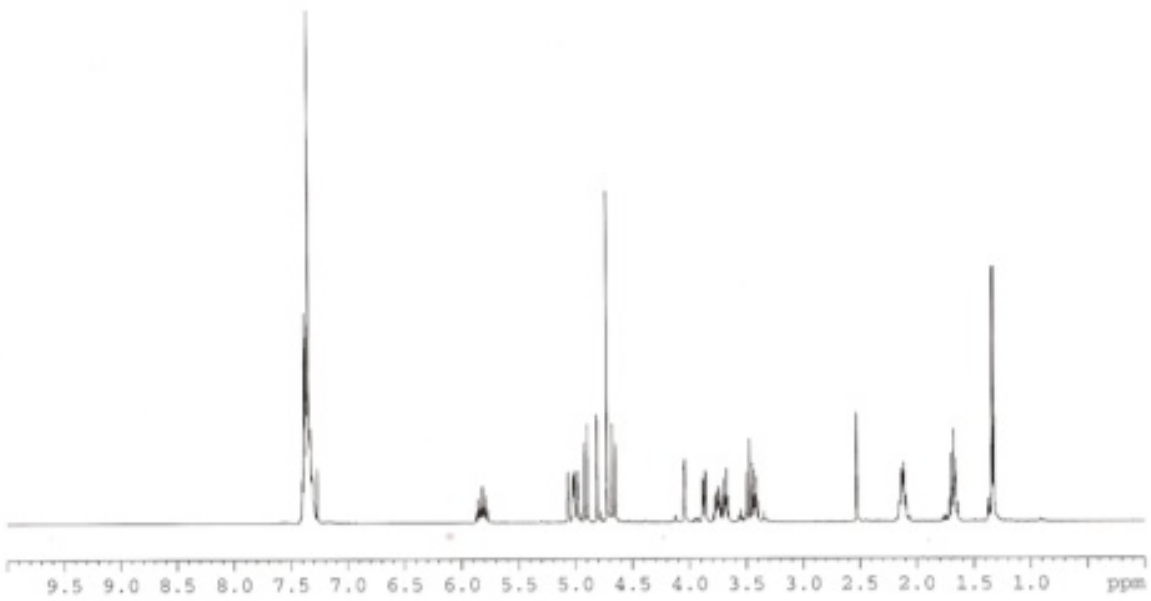
$CDCl_3$, 400 MHz

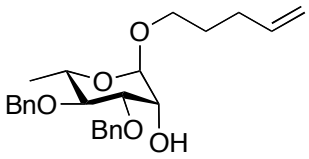




n-Pentenyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (15)

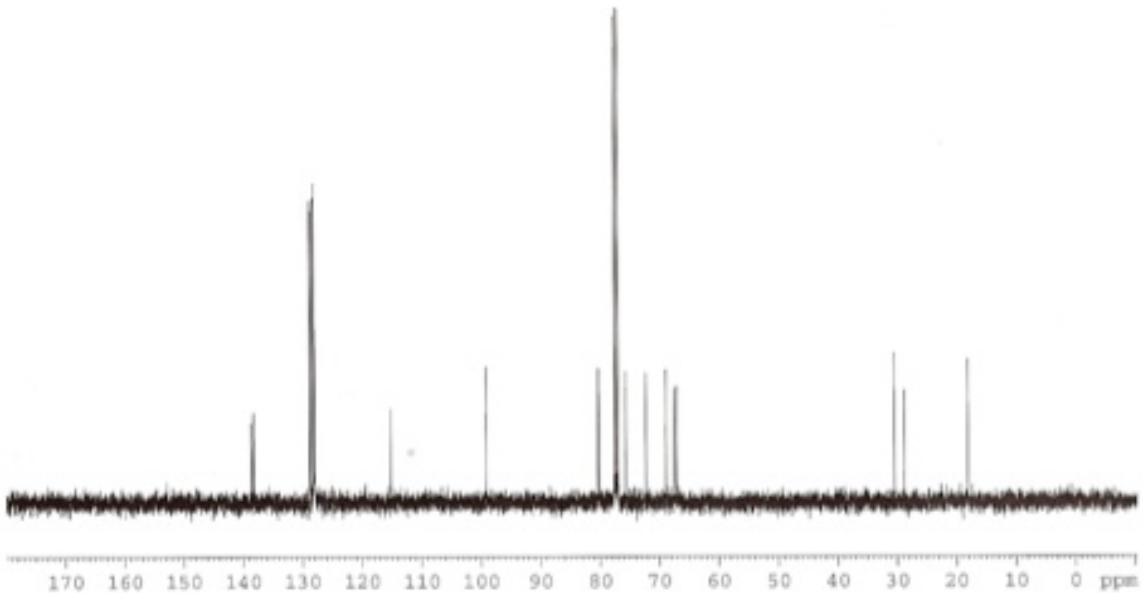
$CDCl_3$, 400 MHz

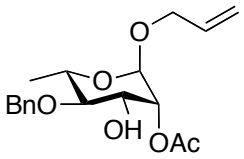




n-Pentenyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (15)

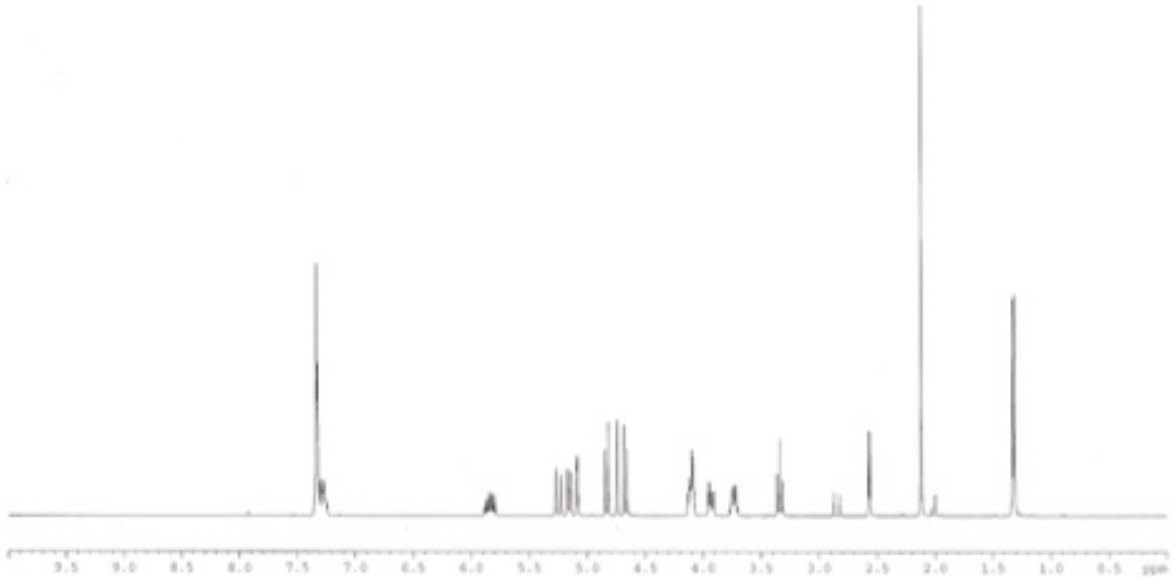
$CDCl_3$, 100 MHz

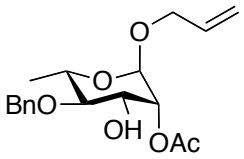




Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (12)

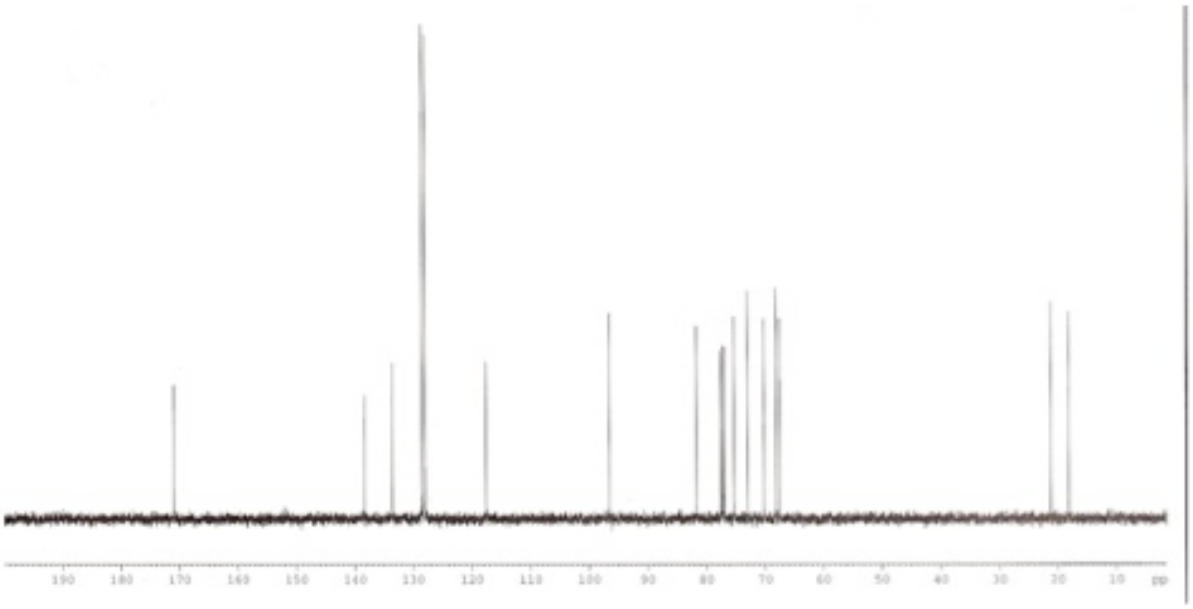
$CDCl_3$, 400 MHz

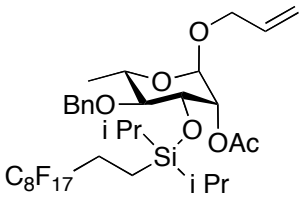




Allyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (12)

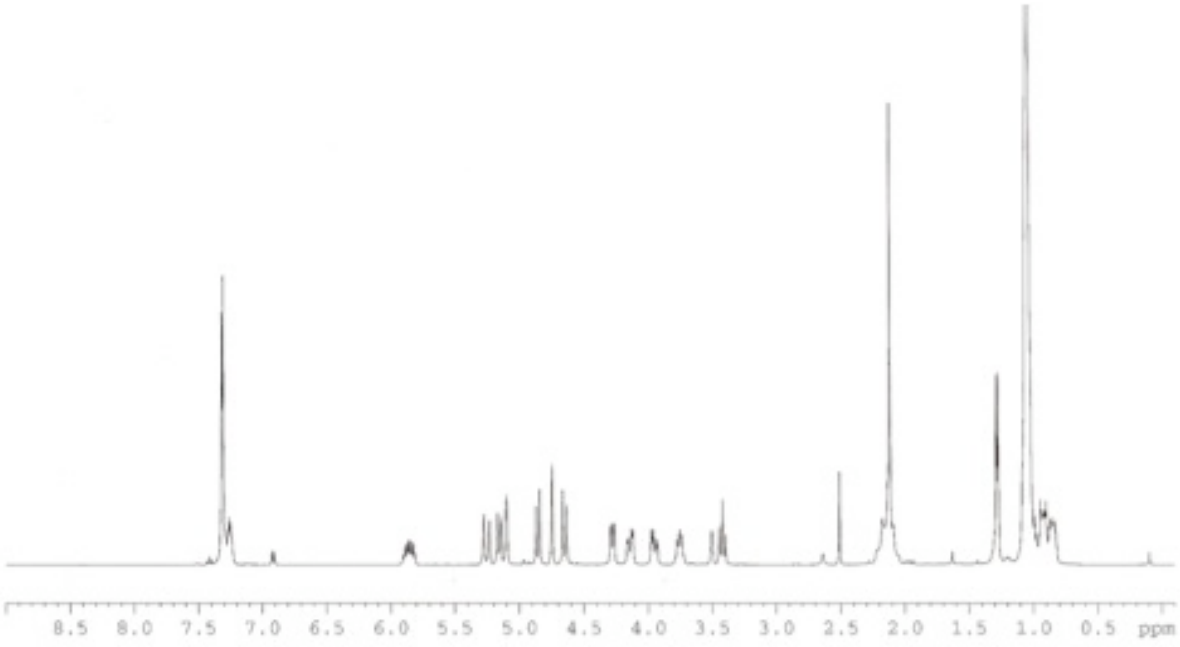
$CDCl_3$, 100 MHz

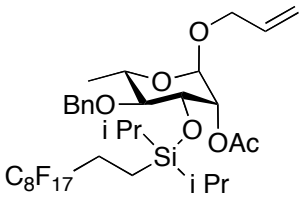




Allyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α-L-rhamnopyranoside (13)

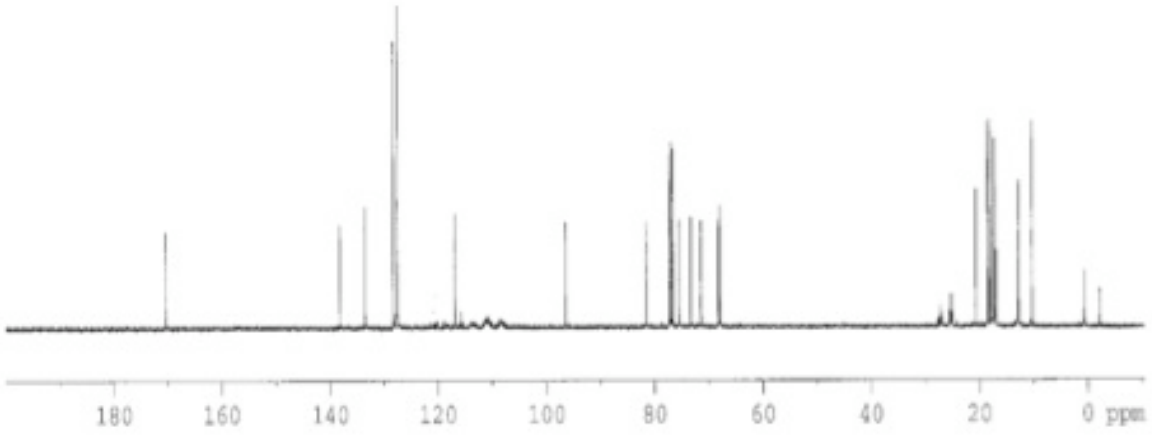
$CDCl_3$, 400 MHz

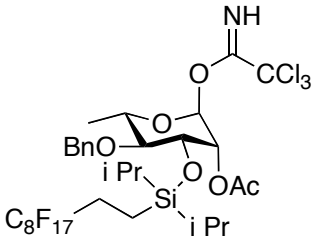




Allyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α-L-rhamnopyranoside (13)

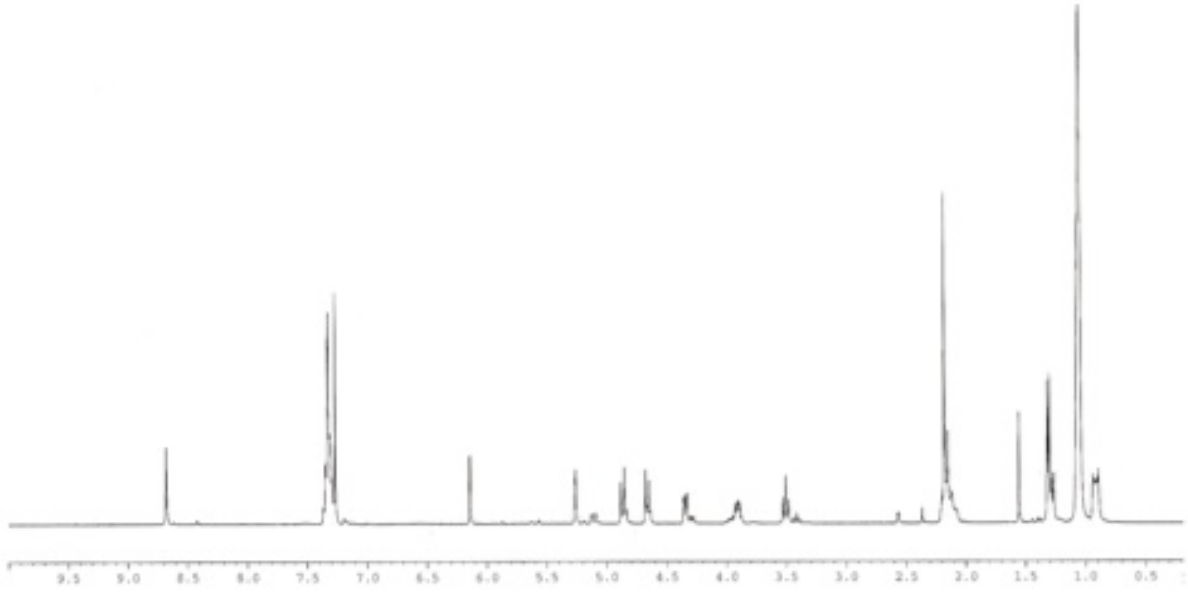
$CDCl_3$, 100 MHz

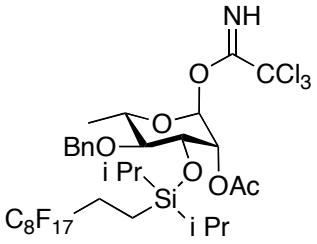




2-O-Acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α/β-L-rhamnopyranosyl trichloroacetimidate (14)

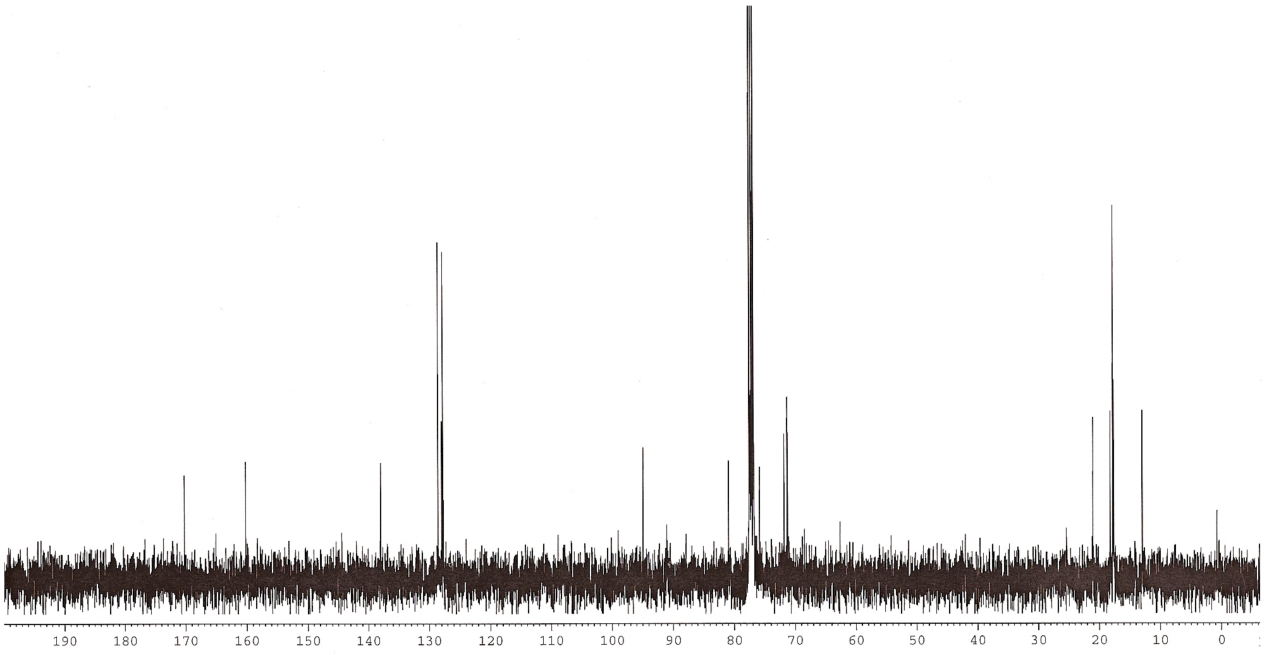
$CDCl_3$, 400 MHz

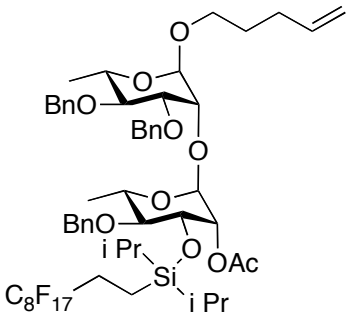




2-O-Acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl)-diisopropylsilyl}-α/β-L-rhamnopyranosyl trichloroacetimidate (14)

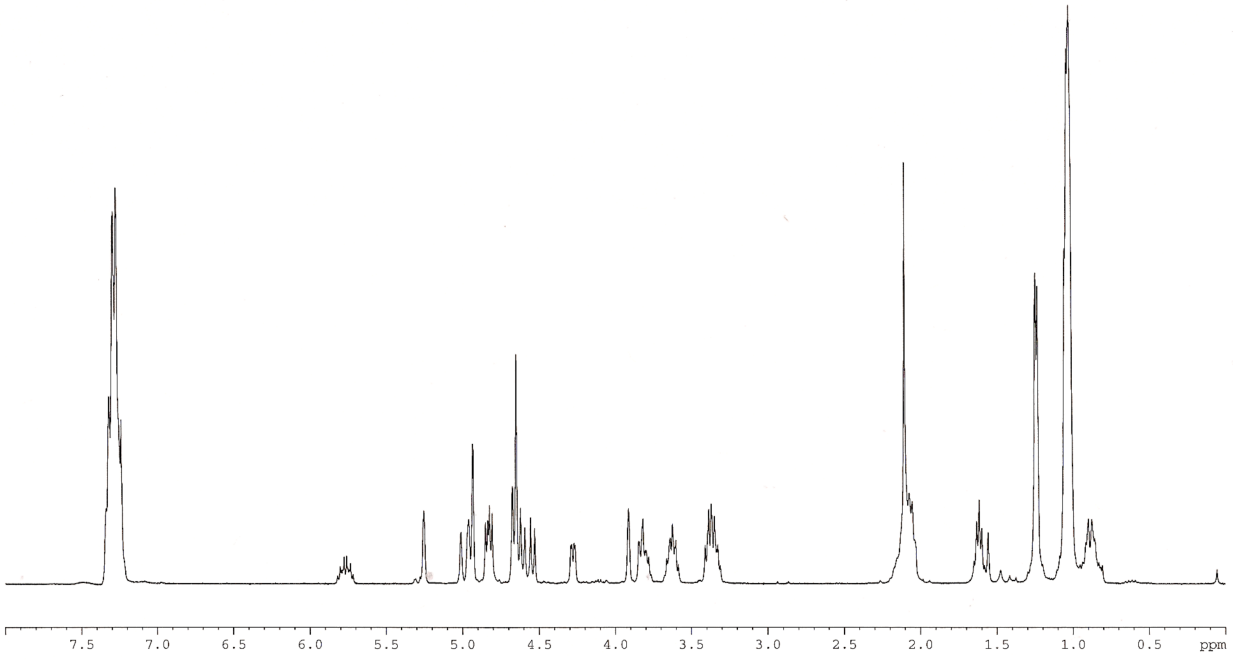
$CDCl_3$, 100 MHz

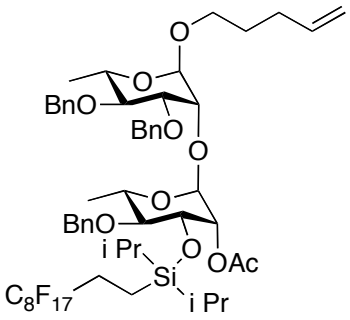




Pent-4-enyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl) diisopropylsilyl}-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (11)

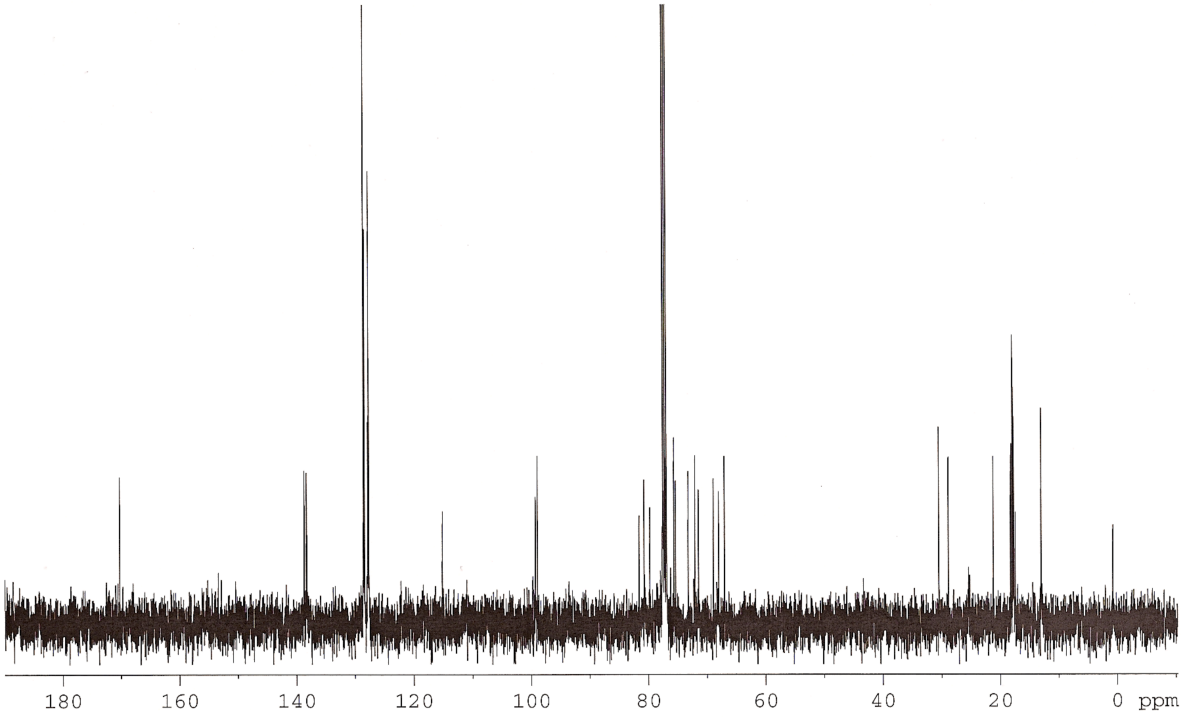
$CDCl_3$, 400 MHz

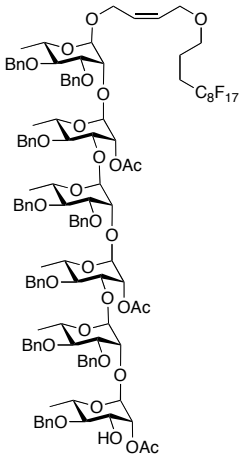




Pent-4-enyl 2-O-acetyl-4-O-benzyl-3-O-{(1H, 1H, 2H, 2H-perfluorodecyl) diisopropylsilyl}-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (11)

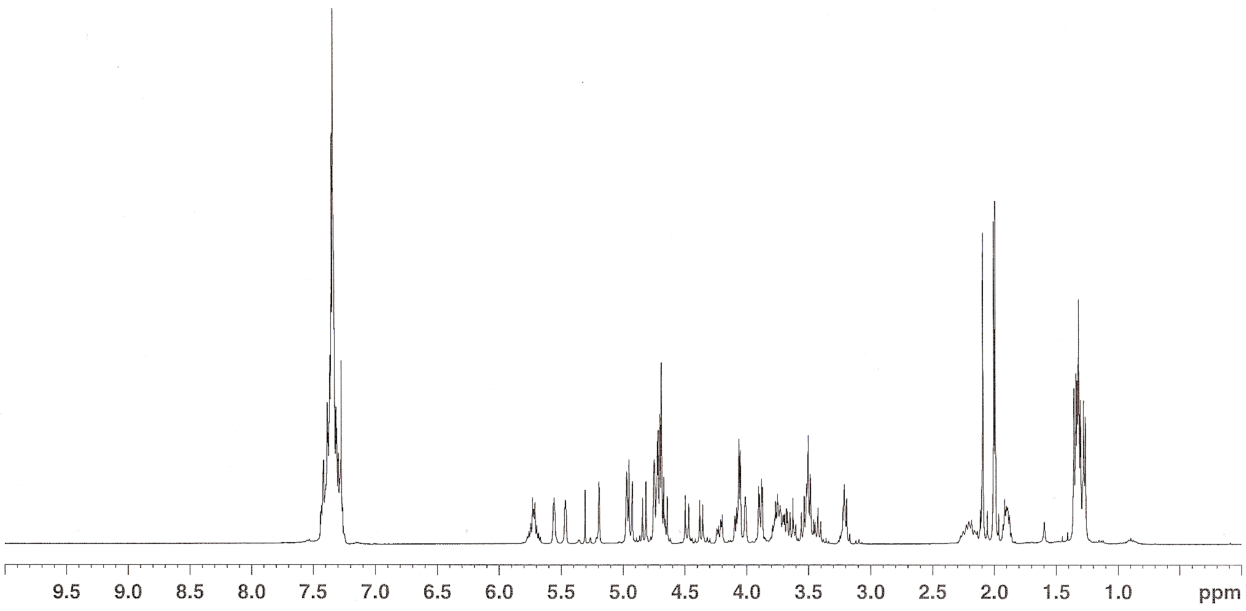
$CDCl_3$, 100 MHz

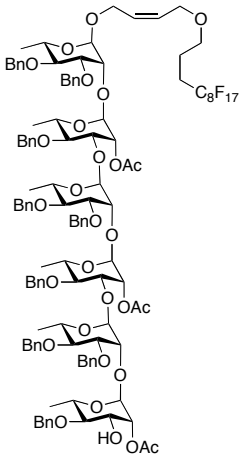




cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (10)

$CDCl_3$, 400 MHz





cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (10)

$CDCl_3$, 400 MHz