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Automated solution-phase synthesis of HIV- and Leishmania-associated oligosaccharides to probe structure-dependent immune responses

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Automated solution-phase synthesis of HIV- and *Leishmania*-associated oligosaccharides to probe structure-dependent immune responses

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LIST OF ABBREVIATIONS

ACN	acetonitrile
AcOH	acetic acid
ESI-MS	electrospray ionization mass spectrometry
FMOC	fluorenylmethoxycarbonyl
FT-IR	Fourier transform-infrared
Gal	galactose
Glc	glucose
FITC	fluorescein isothiocyanate
GlcNAc	*N*-acetylglucosamine
Hepes	*N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid)
HPLC	high pressure liquid chromatography
$(i\text{Pr})_2\text{NP(OBn)}_2$	dibenzyl *N*,*N*-diisopropyl phosphoramidite
ITC	isothermal titration calorimetry
IPP	inorganic pyrophosphatase
IPTG	isopropyl thiogalactoside
MALDI-TOF-MS	matrix assisted laser desorption time-of-flight mass spectrometry
Man	mannose
*m*CPBA	3-chloroperbenzoic acid
m/z	mass/charge ratio
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced form
NBS	*N*-bromosuccinimide
NDP	nucleotide diphosphate
NIS	*N*-iodosuccinimide
PEG	polyethylene glycol
P_i	phosphate
PP_i	pyrophosphate
SIM	selected ion monitoring
TBDPS	*tert*-butyldiphenylsilyl
TEA	triethylamine
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
TMSOTf	(trimethylsilyl)methanesulfonic acid

ABSTRACT

Infectious disease associated-oligosaccharides are important target molecules for synthesis to enable studies of their functions in infection mechanisms both in humans and animals. Unlike solid-phase automated chemical syntheses of oligonucleotides and oligopeptides that serve to provide these molecules for systematic structure-function relationships, automated chemical synthesis of oligosaccharides has been restricted due to the need to manage stereochemistry of each linkage and to the greater complexity of the monomeric carbohydrate building blocks. A new solution-phase automation platform that relies on fluorous solid-phase extraction (FSPE) to purify intermediates potentially offers easier access to complicated oligosaccharides with several features such as simpler monitoring of reactions, only 2-3 equivalents of building block usage per glycosylation cycle, labor savings, and easier access to previously constructed compounds. In addition, the fluorous allyl-tag used to simplify purification in the automation platform also allows not only direct incorporation into microarrays but also ready modification of the tag for facile conjugation to polymeric vehicles. Herein are reported the development of methods for this automation platform for the first construction of HIV- and *Leishmania*-associated

oligosaccharides. Automated methods to make phosphate-linked sugars as well as conventional glycosidic linkages are demonstrated. Several *Leishmania*-associated oligosaccharides—including capping structures, phosphate-linked capping structures and phosphoglycan repeats—were synthesized as probes for carbohydrate microarrays to screen sera of infect animals. The further development of efficient conjugation chemistry allowed the multivalent modification of latex beads and degradable micro-/nanoparticles with these bioactive oligosaccharides to probe carbohydrate-related structure/function relationships in the stimulation of cellular immune responses.

CHAPTER 1

General introduction and review of fluorous-based carbohydrate microarrays

Portions of this chapter have been published as a special report in *Future Medicinal Chemistry* (2009), 1, 889-896. (Copyright 2009 Future Science)

1. Dissertation organization

This dissertation consists of seven chapters. The first chapter is a review published in *Future Medicinal Chemistry* in 2009. Chapter 1 includes not only recent progress in microarray fabrication methods in order to perform fluorous-based microarrays on both covalent and non-covalent immobilized slides but also applications of fluorous-based microarrays in the screening of protein, antibody and enzyme activities. Chapter 2 describes the first automated solution-phase synthesis of HIV-associated linear α-1,2-linked pentamannose. Unlike solid phase-based automation platforms, the solution phase-based automation method allows the construction of oligosaccharides with easy reaction monitoring through conventional techniques such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), fewer equivalents of building blocks per coupling cyclce (1.5 ~ 2

equivalents), and less laborious work. Chapter 3 discusses the automated synthesis of *Leishmania*-associated carbohydrates and fluorous-based microarray for antibody screening. Six different fluorous-tagged *Leishmania*-associated carbohydrates were synthesized either through automated synthesizer or iterative synthesis for antibody screening of serum using a fluorous-based microarray. Chapter 4 discusses the synthesis of phosphate-linked *Leishmania*-associated carbohydrates by using an H-phosphonate strategy in order to investigate the role of phosphate-linkages in lipophosphoglycans (LPG). Importantly, the first automated synthesis of phosphoglycan repeats of *L. donovani* was achieved in a solution-based platform with the development of general protocols for this type of phosphate-linked carbohydrate. Chapter 5 includes the synthesis of multivalent *Leishmania*-associated capping carbohydrates for the collaborative evaluation of carbohydrate structure-dependent immune responses. Chapter 6 describes protocols for the modification of degradable polymeric particles with carbohydrates for the collaborative investigation of dendritic cell (DC) activation against carbohydrates-modified polymeric particles. Chapter 7 provides conclusions for this dissertation as well as future directions for carbohydrate research.

2. Flurous–based small-molecule microarrays for protein, antibody and enzyme screening.

Carbohydrates are one of the most abundant biomolecules on the surface of the cell membrane and thereby are crucial for the interactions of cells with one another and with pathogens such as viruses, bacteria, and fungi. Although several functions such as generating energy, mediating signal transduction between different organisms, and providing recognition makers and structural components have long been appreciated roles for carbohydrates, their regulation mechanisms are still not clear. Furthermore, the structural complexity of carbohydrates, which are derived from the incredible diversity of regiochemistries and stereochemistries possible between furanose and pyranose rings, adds to the difficulty in studying carbohydrate binding partners such as proteins, enzymes and antibodies.[1] Given the limitations in accessing large quantities of pure structurally well-defined carbohydrates, carbohydrate microarrays are increasingly used as a versatile tool for unveiling the possible binding associates for carbohydrates since the first glycan array was reported in 1985.[2]

2.1 Overview of fluorous-based microarrays.

Small molecules are an important tool for the elucidation of biological recognition processes.[3] Currently, the best way to design molecules that have biological activities for soluble proteins without structural information is to screen an assortment of possible structures. With increasing demand for useful tools for small molecule screening, small molecule microarrays have become increasingly important. Microarray techniques allow quick assessments of possible binding partners for biomolecules including nucleic acids, proteins and carbohydrates, with small amounts of sample and therefore lower costs than multiwell plate types of screening methods. Small molecule microarrays are particularly valuable for creating and probing multivalent displays of molecules such as saccharides that mimic the multivalent displays of cell-surface bound compounds.

For example, carbohydrate microarrays have clearly played a key role in facilitating access to information about carbohydrate-protein interactions.[4] After a general discussion of immobilization techniques used for small molecule microarray fabrication using carbohydrates as an example, a more detailed discussion of microarrays based on noncovalent fluorocarbon interactions will be surveyed to highlight the unique potential of fluorine in this context

2.2 Immobilization strategies for microarray fabrication

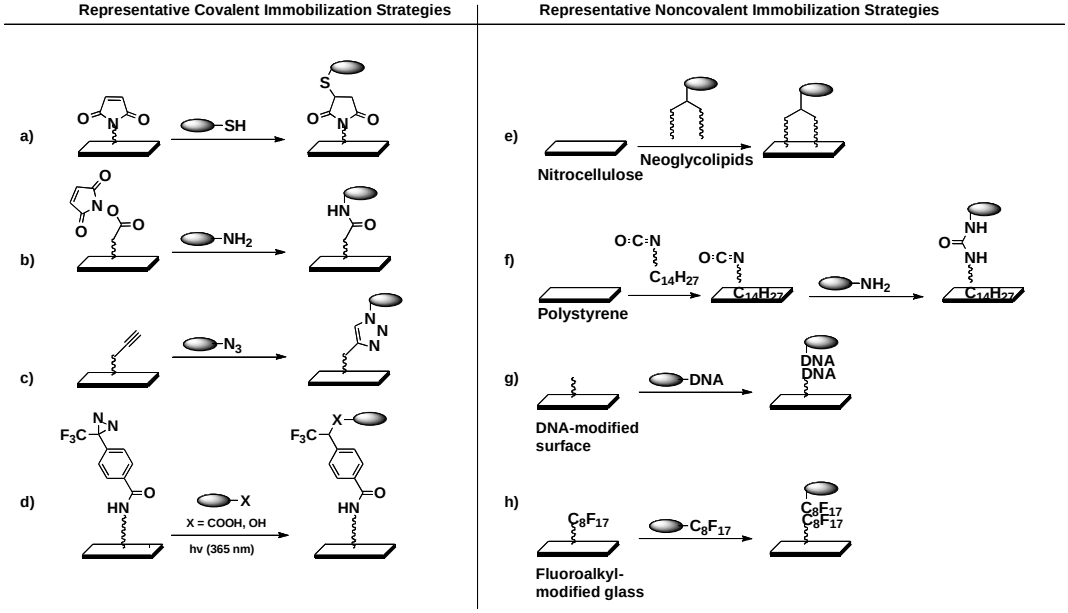

Figure 1. Representative covalent and noncovalent immobilization strategies for small molecules for microarray fabrication: a) Immobilization of thiol-functionalized molecules to maleimide-modified slides, b) Immobilization of amine-functionalized molecules to *N*-hydroxysuccinimide ester-modified slides, c) Immobilization of azide-functionalized molecules to alkyne-modified slides by Huisgen cycloaddition, d) Immobilization through photochemical activation of natural products to aryl-diazirin-coated slides, e)Immobilization of lipid-containing molecules to nitrocellulose, f) Immobilization of amine-functionalized molecules to noncovalently modified

polystyrene wells containing lipid-linked isocyanates, g) Immobilization of DNA-tagged compounds to complementary strands on surface; h) Immobilization of fluorocarbon-tagged molecules to fluoroalkyl-modified slides.

Immobilization techniques for microarray formation can be divided into two main categories that describe the method of attachment of the molecules to the slide surface: covalent immobilization and noncovalent immobilization of the molecules to the slide surface. (Figure 1) A basic overview of these methods using carbohydrate immobilization as an example follows. Among covalent immobilization techniques, forming a stable bond between maleimide-functionalized slides and thiol-containing molecules[5, 6, 7] is common, as well as reactions of amine-functionalized molecules with *N*-hydroxysuccinimde (NHS)-activated glass slides[8, 9]. Applications of Cu(I)-mediated 1,3-dipolar Huisgen cycloadditions between azide-containing molecules and alkynylated glass slides[10, 11, 12] have also been utilized to covalently attach carbohydrates or small molecules to slide surfaces for screening. When products contain hydroxyl or carboxylate moieties, photoinduced cross-linking based immobilization techniques[13] have been introduced to array small molecules on diazirin-coated glass slides.[14] Conditions (pH, time, temperature, etc.) have to be

optimized to maximize reaction yields of the covalent-bond forming step. The subsequent challenge is quantification of those yields to know the concentration of the molecules that will be screened. Each of these methods, of course, requires the introduction of the necessary functional group into both the molecules to be screened and the slides themselves. In certain cases in which the small molecules have unique reactive functional handles, at least unmodified molecules can be used with specially-modified slides. For example, in addition to the photocrosslinking strategy mentioned above, the direct immobilization of unmodified reducing sugars recently has been shown using hydrazide- and aminoxy-derivatized glass slides.[15] (Figure 2) Fifty-eight unmodified glycans including mono-, oligo- and polysaccharides were then directly arrayed on the specially derivatized slides and then tested for binding of the sugars to lectins, antibodies, and bacterial cells. Although undesired products such as acyclic adducts from hydrazide-derivatized slides and acyclic adducts with mixture of alpha/beta anomers from aminoxy-derivatized slides can be present at each microarray spot to complicate data interpretation, this one-step immobilization technique can be used to probe carbohydrate-protein binding, carbohydrate-antibody

binding and quantification of carbohydrate-protein binding without prior modification of the carbohydrates.

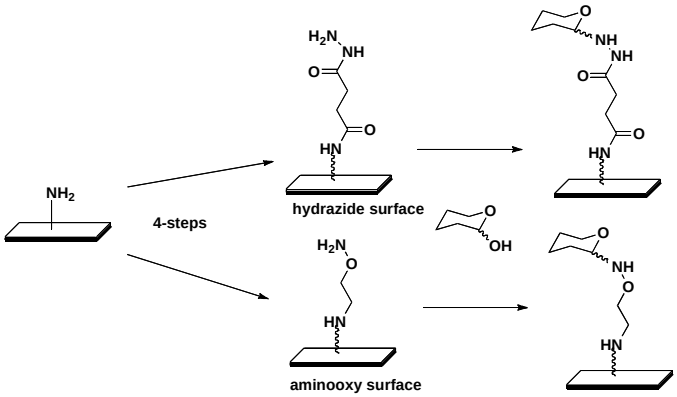

Figure 2. One-step direct immobilization of unmodified reducing sugars to hydrazide- and aminoxy-modified slide surfaces.

Although covalent immobilization techniques have provided powerful tools for investigating the binding properties of molecules in microarray formats, noncovalent immobilization techniques are also being investigated (Figure 1). In contrast to covalent immobilization techniques, non-covalent techniques should render compounds on the slide surface more flexible, thus allowing them to move for optimal binding with a protein, antibody, or enzyme target. Moreover, non-covalent

immobilization techniques have the potential advantage of allowing the recycling and reuse of slides.

Several strategies have emerged for such noncovalent schemes to create carbohydrate microarrays. An intriguing noncovalent strategy involves attachment of a nucleic acid strand to a compound and then immobilization of that compound onto a specific location of a slide where a complementary nucleic acid strand is located.[16] The authors found their DNA-directed strategy to have a lower detection limit than a noncovalent strategy for detecting immobilized glycoconjugates. Older noncovalent immobilization strategies include the attachment of neoglycolipids (NGLs) on nitrocellulose[17] and noncovalent arrays using long hydrocarbon chains[18]. The latter method requires amine-functionalized carbohydrates[16] for an isocyanate-mediated capture strategy to attach the long hydrocarbon chains.[19] Unfortunately, the use of such large hydrocarbon tags to noncovalently anchor compounds to the slide surface can be problematic, for example when detergents are required in bioassay buffers.

2.3 Noncovalent fluorous-based microarrays

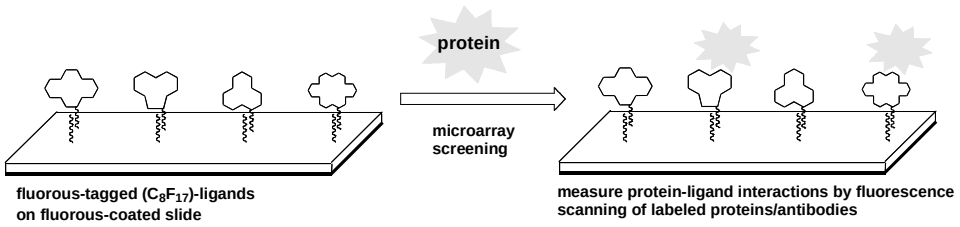

Figure 3. Application of fluorous-based microarrays for protein screening

More recently, noncovalent immobilization based on fluorophobic rather than hydrophobic effects has been tested in the context of microarray formation. Selective immobilization of a polyfluorocarbon (C8F17) chain attached to a carbohydrate to a fluorinated surface[20,21,22,23] has been shown to be surprisingly robust enough for the screening of carbohydrate-protein binding screening (Figure 3). Prior to this work, such noncovalent fluorous interactions had been used to facilitate the purification of fluorous-tagged compounds from compounds that do not contain any fluorous content using fluorous solid-phase extraction (FSPE).[24] Washing of fluorous-modified silica gel with aqueous/organic solvent mixtures allows retention of fluorous-tagged molecules, whereas non-fluorous compounds are eluted from the column. Organic solvents such as MeOH or THF then can elute fluorous-containing compounds. With this method, a compound with only a small fraction of fluorocarbon content (such as a single C6F13-containing tag) can be reliably purified. Although this tagging and

purification strategy has been used for the production of a range of bioactive molecules[25], the fluorous tag normally has to be removed from compounds in order to use traditional screening methods. The first demonstration that this fluorous tag can be directly incorporated into a microarray platform opens new avenues to think about the production and screening of compounds and other uses for fluorous tags in biological applications.[26, 27] To date, fluorous-based small molecule microarrays have shown utility for protein, antibody and enzyme screening.

2.4 Fluorous-based microarrays for protein and antibody screening

Noncovalent fluorous-based microarrays possess unique features: 1) surface blocking steps after immobilization are rendered unnecessary and 2) high signal to noise ratios and low-nonspecific binding can be achieved with fewer washing steps than required with more reactive slide surfaces. In addition, the fluorocarbon tag itself does not complicate proton NMR spectra. The initial concept was developed in the context of carbohydrate microarray fabrication. Namely, fluorous-tagged monosaccharides were noncovalently immobilized by fluorous-fluorous interactions on a glass slide surface specially modified with C8F17 chains[22]. The immobilized

monosaccharides were screened with the common fluorescein isothiocyanate(FITC)-labeled lectin concanavalin A (FITC-ConA) that is known to bind to alpha-linked mannose, glucose and *N*-acetylglucosamine. Indeed, specific binding of ConA to immobilized mannose and *N*-acetylglucosamine was observed by fluorescence imaging. More surprisingly, the use of detergents in the buffer used for screening of the sugars to a different lectin did not completely destroy the noncovalent interactions. Noncovalent fluorous-based arrays can clearly withstand detergents not tolerated by noncovalent hydrocarbon-based arrays. In further experiments, fluorous-based carbohydrate microarrays proved not only binding of ConA with two new carbohydrate ligands—both diastereomers of *glycerol*-D-*manno*-heptoses—but also facilitated the quantitative assessment of these carbohydrate-protein interactions.[21]

Spring's group[28] has reported another application of fluorous-based microarrays for screening and probed the chain length requirement of the fluorous tag. By comparison of C8F17-tagged biotin with C6F13-tagged biotin, the longer C8F17-tag was considered more reliable for the attachment of biotin to the fluorous-coated glass slide. Enhanced biotin-avidin interactions on the fluorous-coated slide were achieved using a polyethylene glycol spacer between the biotin and the fluorous tag (C8F17).

This work demonstrated that not only very hydrophilic carbohydrates could be screened successfully in this new fluorous-based format, but also more lipophilic molecules such as biotin could be reliably immobilized. In addition, efficient protocols for recycling slides by washing with organic solvents such as methanol and dichloromethane were presented.

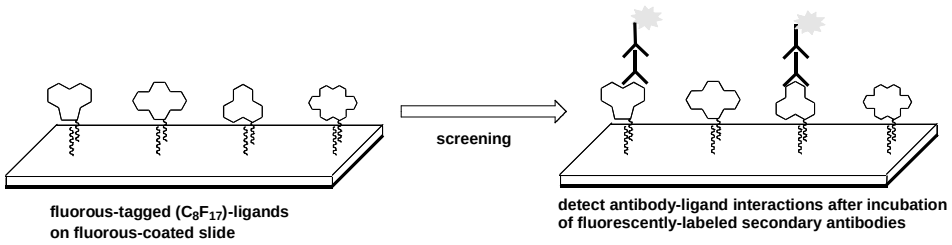

Figure 4. Application of fluorous-based microarrays for antibody screening.

Microarrays are also showing promise as possible diagnostic tools in their detection of specific antibodies, for example against Globo-H antigens in human cancer sera[11] or against *Salmonella* O-antigens in sera from *Salmonella* infected patients[29]. Recently, fluorous-tagged carbohydrate antigens associated with infectious diseases were synthesized using automated synthesis (see below). Serum samples from infected animals were incubated with the noncovalently immobilized sugars and then binding was visualized using fluorescently labeled secondary antibodies (Figure

4).[30] The fluorous-based platform proved robust for the diagnosis of animals containing antibodies against the disease agent. These initial experiments show that fluorous-based microarrays can be used to diagnose animal exposure to pathogens.

2.5 Fluorous-based microarrays for enzyme screening

Fluorus-based microarrays have also shown their use in screening for enzyme inhibitors and for enzyme activity. Small molecule microarrays were designed with compound to target a specific class of enzymes called histone deacetylases (HDACs). [31] To screen for inhibitors of this enzyme that catalyzes the hydrolysis of *N*-acetyl groups on lysine residues, fluorous-tagged compounds were printed on the fluorous-coated glass surface and screened with HDAC2, HDAC3/NCoR3 peptide complex and HDAC8. Incubation of the arrays with alexa-647-labeled anti-His antibodies subsequently permitted visualization of HDAC binding by fluorescence imaging. This approach, which takes advantage of detecting native HDACs from whole cell lysates, could serve as the basis for the discovery of a range of new HDAC inhibitors.

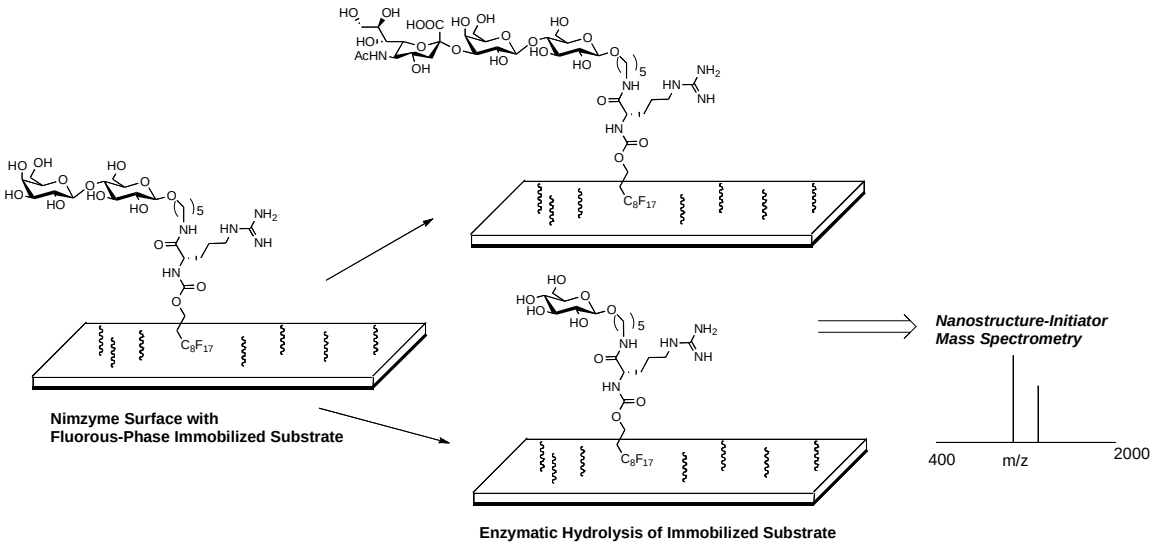

Figure 5. Illustration of the Nimzyme assay. [32]

More recently, fluorous-tagged metabolites were applied to Nanostructure-Initiator Mass Spectrometry (NIMS) for "on-chip" enzymatic assays.[32] (**Figure 5**) The use of fluorous-phase immobilization for the attachment of enzyme substrates to fluorous-coated surfaces not only allowed high signal-to-noise ratios (S/N) in the analysis of the enzymatic reactions, but also allowed conformational flexibility to the substrates that likely enhance enzymatic activities. Specifically, immobilized fluorous–tagged lactose on the NIMS surface was used for direct characterization of beta-1,4-galactosidase activity from a thermophilic microbial community lysate. With the advances being made in mass spectrometry-based enzymatic assays based on

fhuorous-phase immobilization and NIMS, the construction of printed microarrays can also be imagined for the direct screening of enzymatic activity and inhibitors of that activity.

2.6 Linking fluorous-based arrays to automated synthesis

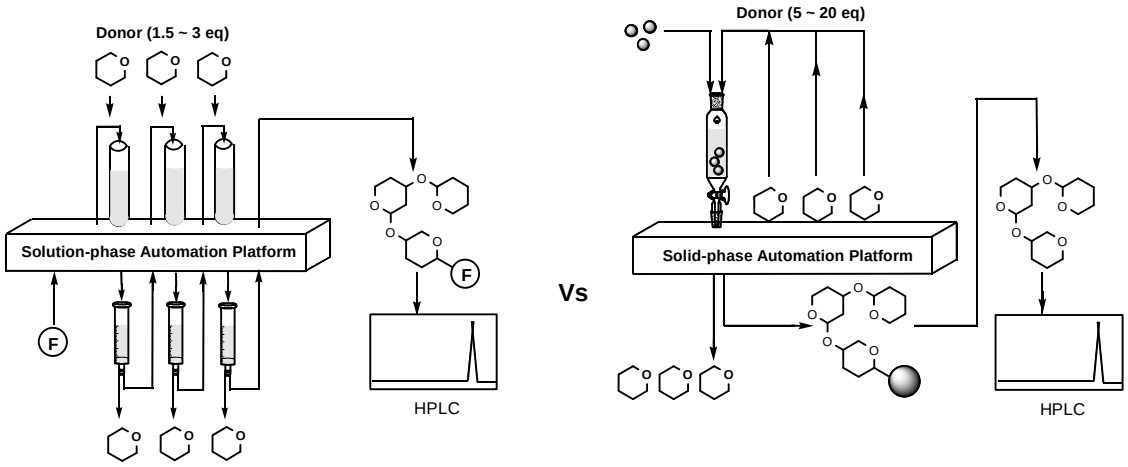

Figure 6. Solution-phase automation platform vs Solid-phase automation platform.

All microarray formats are limited by the number of compounds that can be readily produced for inclusion in the particular array format. To date, no fluorous-based microarray has incorporated hundreds or thousands of compounds. However, recent developments in the automation of multistep synthetic routes using fluorous-

phase rather than solid-phase protocols promises to rapidly expand the availability of compounds already containing fluorous-tags required for their incorporation into fluorous-based microarrays (Figure 6).[33,34] Although noncovalent fluorous interactions have been shown to be clearly robust enough to create a range of microarrays, the robustness of these interactions for repetitive robotics-based separations of a wide range of compounds is not obvious. However, these noncovalent interactions have proven reliable enough to separate intermediates in oligosaccharide synthesis on an automation platform with the same programmed protocol after investigations of parameter ranges. This new automation platform based on fluorous solid-phase extractions has been used to synthesize not only linear oligosaccharides, but also branched oligosaccharides. More recently, mono- and di-fluorous-tagged glucosamines have been examined for sequential separation using FSPE.[35] Elution conditions could be found to obtain the desired di-tagged compound separate from the mono-tagged starting materials, thereby opening more possibilities for the automation of synthetic schemes that include less than optimal coupling results. Further applications of FSPE-based automation are now in progress for the construction of a

range of oligosaccharides to probe the scope and range of this new method to provide compounds already tagged for incorporation into fluorous-based screening protocols.

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CHAPTER 2

Automated synthesis of HIV-associated linear α-1,2-linked pentamannose

Eun-Ho Song and Nicola L. Pohl

Introduction

Facile synthesis of oligosaccharide has been an issue both in organic synthesis and biomedical research due to difficulties in the construction of complicated oligosaccharides. With this demand, carbohydrate chemistry and automation method have quickly consolidated their position as practical and versatile method for the construction of oligosaccharide.

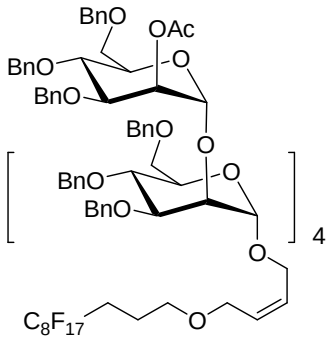

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Figure 1. α–(1-2) linked pentamannose

Oligomannose has been considered as an important target molecule due to its unique structure and function as a GPI anchors in nature.[1] In addition, poly α-mannose has been well known as ligand for concanavalin A (Con A)[2], one of important cell wall components [3,4] and HIV-associated carbohydrates on gp120[5].

Although the synthesis of oligomannose in an automation platform using solid phase has previously been reported by Seeberger's group[6], the efficiency of automation on the solid phase was not reliable due to several reasons such as consuming too much reagents (5 ~ 10 equiv of glycosyl donor per cycle) and difficulties of monitoring reaction. Recently, iterative synthesis of α–(1-2) linked tetramannose has been reported to probe the utilization of fluorous solid-phase extraction (FSPE) protocol in oligosaccharides synthesis.[7] To increase the efficiency of automated platform, FSPE technique has been employed in automation platform and newly developed solution-phase automation platform[8] enables using only 1.5 ~ 2 equivalents of donor building blocks, monitoring the completion of reaction by TLC as well as easy purification through FSPE for facile synthesis of α–(1-2) linked pentamannose (Figure 1).

Herein, we present the utilization of solution-phase automation platform as a useful tool for the construction of HIV-associated linear oligomannose.

Results and discussion

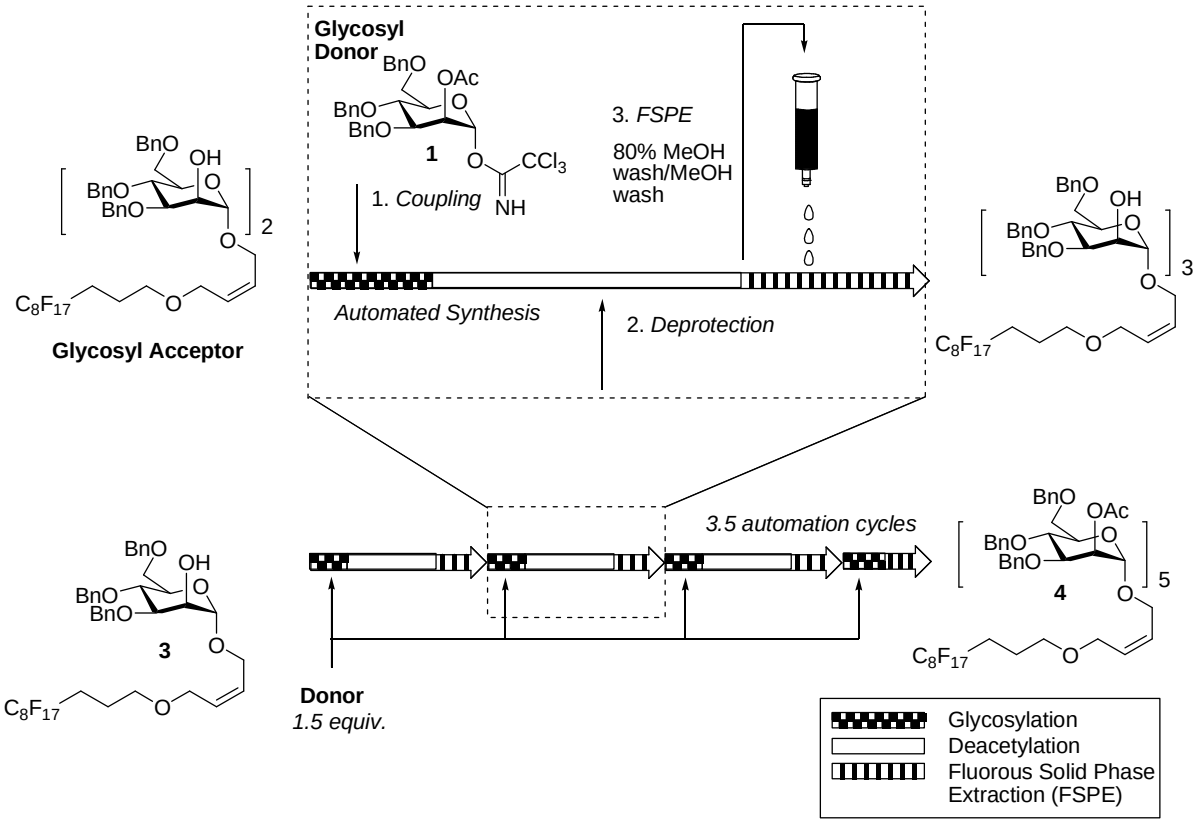


Figure 2. Automated synthesis of α–(1-2) linked pentamannose

Fluorous-tagged mannose acceptor **4**[9] has been prepared to improve the efficiency of

purification through FSPE[10]. Only 1.5 equivalent of trichloroacetimidate[11] **1** which can be activated under acidic condition (TMSOTf) has also been used as a glycosyl donor. The reaction has been done 3.5 cycles (4 x glycosylation, 3 x deacetylation and 4 x FSPE) for 24 h 56 min 39 sec without any labor since automated platform was ready to run the synthesizer for making liner pentamannose. (Figure 2) Strikingly, not only 2 equiv of glycosyl donor per one cycle was consumed for the completion of glycosylation, but the purity of crude product **5** (18 mg) shown in HPLC traces (supporting information) after 7 steps (24 h 56 min 39 sec) was also remarkable even without further purification. Simple prep TLC gave us highly pure product (15 mg, 73 % per step) to have reasonable ^{1}H-, ^{13}C-NMR and mass spectroscopy data. We evaluated the efficacy of solution-phase approach with a direct comparison of yield based on the glycosyl donor. While 27 % yield per glycosylation/deprotection cycle was produced in the solution-phase automation platform, only a 5 % per cycle yield was produced in the traditional solid-phase approach. Over many cycles, huge differences in building block loss will be produced.

Conclusion

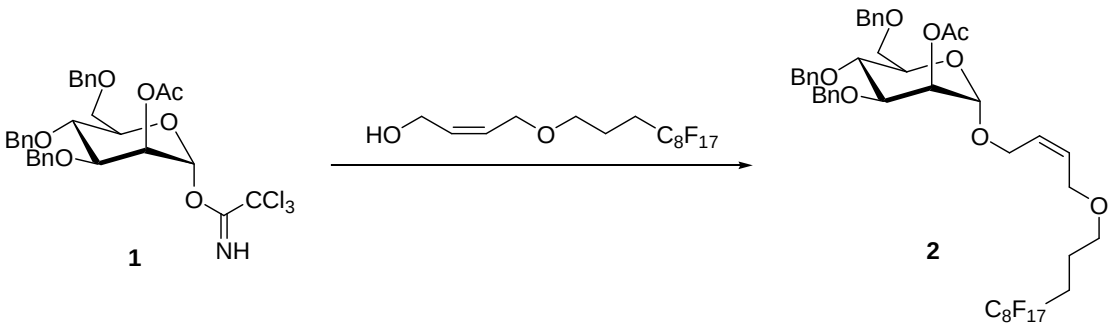
First solution-phase automated synthesis of HIV-associated linear pentamannose was achieved in the new solution-phase automation platform. In particular, fluorous-tagged oligosacchrides from solution-phase automation platform can be incorporated into microarrays for screening antibody and protein in terms of diagnostic tool. These successful results suggest that the automation of oligosaccharide can be performed with small amount of reagents (2 equiv of glycosyl donor per cycle), less laborious work, and high purity of products. From these achievements, other complicated oligosaccharide can be introduced to the same automated platform.

Experimental section

General methods

Solvents were reagent grade and in most cases dried prior to use. All other commercially available reagents were used as received unless otherwise noted. The organic extracts were dried over anhydrous $MgSO_4$. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$) prior to use. Methylene chloride (CH_2Cl_2), and triethylamine (Et_3N) were distilled from calcium hydride. Diethyl ether (Et_2O) was distilled from sodium-benzophenone ketyl.

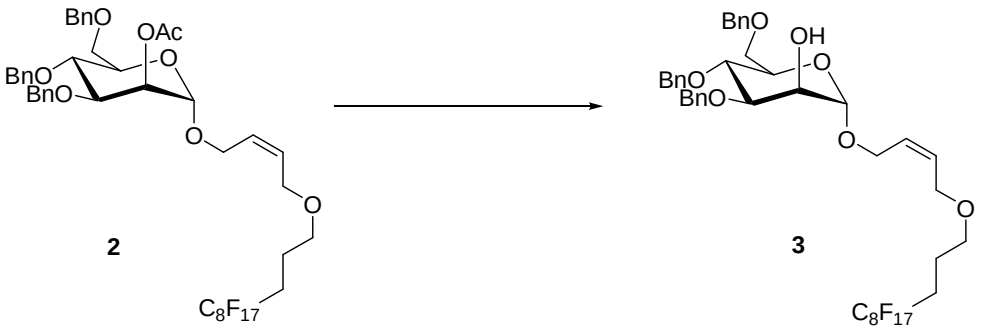
^{1}H and ^{13}C spectra NMR were obtained at 400 MHz and 100 MHz on Varian VXR-400 NMR or on Bruker DRX-400 NMR. Mass spectra (MS) were recorded on an Applied Biosytems DE-Pro MALDI mass analyzer or an Applied Biosytems QSTAR® XL Hybrid LC/MS/MS System. Chemical shifts are reported in parts per million downfield relative to tetramethylsilane (δ 0.00) and coupling constants are reported in Hertz (Hz). The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; and br = broad.



Synthesis of 3-(Perfluorooctyl)propanyloxybutenyl-2-O-acetyl-3,4,6-O-benzyl-α-D-mannopyranoside (**2**)[9]

To a solution of 2-O-acetyl-3,4,6-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate **1**[11] (0.3 g, 0.47 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol[9] (0.13 g, 0.24 mmol) in dry

dichloroethane (5 mL) was added TMSOTf (8.5 µL, 0.05 mmol) at 25 ^{o}C. The reaction mixture was stirred at room temperature for 30 min. The reaction was quenched with triethylamine (0.5 mL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using 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 desired product **2** (0.20 g, 84 %).



Synthesis of 3-(Perfluorooctyl)propanyloxybutenyl-3,4,6-O-benzyl-2-hydroxy-α-D-mannopyranoside (**3**)[7]

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-2-O-acetyl-3,4,6-O-benzyl-α-D-mannopyranoside **2** (0.2 g, 0.2 mmol) in methanol was added Na (45 mg). The reaction mixture was stirred at 25 ^{o}C for 1 h and concentrated under reduced pressure. The crude product **3** was purified to obtain the desired product (0.19 g,

100 %) by silica column chromatography.

ASW Pentamannose method run

After FSPE, the methanol elution collected in the vial was removed from the instrument and concentrated. Solvent was removed under reduced pressure to obtain the crude product (18 mg, 13 %) as colorless oil. In order to obtain pure product **5** (15 mg, 11 %) for ^{1}H NMR, ^{13} C NMR and mass spectrum, further purification was performed using prep TLC.

R_f (ethyl acetate/haxane): 0.75 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.35-7.11 (m, 75 H), 5.61-5.58 (m, 2H), 5.55-5.54 (m, 1H), 5.24 (d, *J* = 1.6, 1H), 5.23 (d, *J* = 1.2, 1H), 5.15 (d, *J* = 0.8, 1H), 5.03 (d, *J* = 0.8, 1H), 4.98 (d, *J* = 1.6 , 1H), 4.88-4.71 (m, 5H), 4.68-4.33 (m, 23 H), 4.22-4.06 (m, 6H), 3.98-3.42 (m, 29 H), 3.33 (t, *J* = 6.4, 2H), 2.13-2.04 (m, 2H), 2.12 (s, 3H), 1.82-1.74 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3 (C=O), 138.8-138.2 (m, C_q-Aryl), 128.6-127.5 (m, CH-Aryl), [101.6, 101.0, 99.7, 99.5, 98.7 ($CH_{anomeric}$)], 79.9, 78.8, 78.5, 77.5, 76.5,75.8, 75.5, 75.3, 75.2, 75.1, 74.9, 74.5, 73.5, 73.4, 73.0, 72.8, 72.4,

72.2, 72.1, 72.0, 71.9, 71.8, 69.9, 69.7, 68.9, 68.8, 66.7, 66.6, 62.9, 28.5, 28.2, 28.0, 21.4, 21.1.

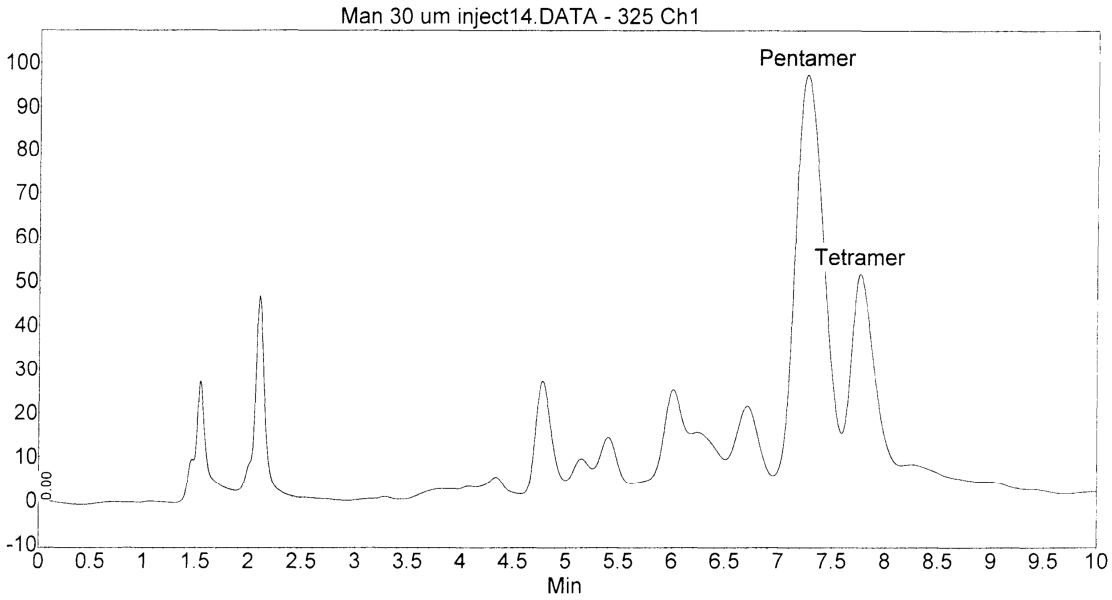
HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{152}H_{155}F_{17}O_{28}Na$, 2775.8423; Found, 2775.1026.

- 3.5 cycles (24 h 56 min) completed for the synthesis of pentamannose.

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	2 equivalent donor (100 μmol) in 0.5 mL Toluene, 1 equivalent F-tagged acceptor (50 μmol) in 1 mL Toluene; 0.1 equivalent TMSOTf, rt	30 min
2	TLC sample		
3	Quenching	30 μl of crude reaction mixture withdrawn	
4	Evaporation	0.5 ml TEA	45 min
5		40 ^oC	
	Deacetylation		2 h
6	TLC sample	3 equivalent of NaOMe solution	
	Quenching	30 μl of crude reaction mixture withdrawn	
7	Evaporation	0.3 ml 0.5 M Acetic acid solution in MeOH	45 min
8	FSPE	50 ^oC	

	preparation		
9	Sample	0.4 ml DMF	
10	loading	0.7 ml crude sample transferred to cartridge	
11		4.7 ml 80% methanol wash	
12	Wash	1.5 ml methanol wash (repeated 3 times)	
13	Wash	4.7 ml collected sample transferred to clean vial	45 min
14	Transfer	50 ^{o}C	
15	Evaporation	2 ml toluene added	45 min
	Transfer	50 ^{o}C	
	Evaporation		

- HPLC traces of pentamannose run



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CHAPTER 3

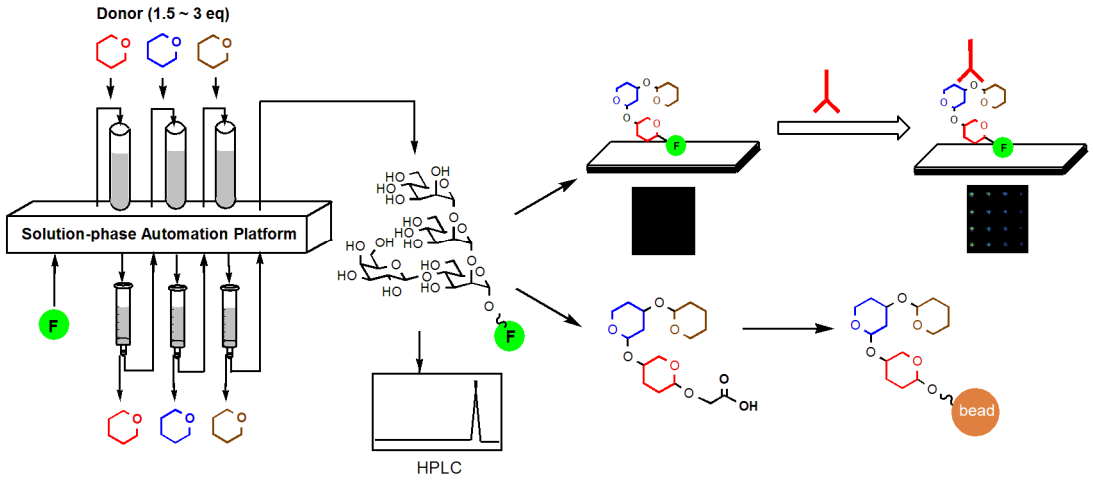
Automated synthesis of *Leishmania*-associated carbohydrates and fluorous-based carbohydrate microarrays

A paper to be submitted to *Chemical Communications*

Eun-Ho Song and Nicola L. Pohl

Introduction

The increasing demand for oligosaccharides in the field of biomedical research led to the development of synthetic methods for the complicated oligosaccharide synthesis in the field of organic chemistry. Although several approaches, such as one-pot solution-phase synthesis and solid-phase based automated synthesis, have been developed for facile synthesis of oligosaccharides,[1,2] structurally complicated oligosaccharides are still not readily available in the market due to difficulties in the formation of a stereocenter upon connection with other sugars while customized DNA sequences and peptides are commercialized in the market.



Scheme 1. Fluorous phase-based automation platform and its applications.

We have recently developed the first automation platform to carry out iterative synthesis based on fluorous-solid-phase extraction (FSPE) protocol[3], rather than solid-phase-based purification (Scheme 1). This new automation platform allows the use of 5 to 10-fold lower building block amounts than the solid-phase approaches, but also enables easy monitoring of reaction by TLC. More importantly, fluorous-tagged products from fluorous phase-based automation platform are not only directly applicable in microarrays, but also capable of conjugation to ligands or other carriers.

Leishmaniasis has been known as a parasitic disease caused by infection with over 15 species of *Leishmania*. Leishmaniasis is mostly endemic throughout Africa, India, southern Europe, and Central and South America with an estimated 12 – 15 million

individuals and 2 million new cases each year. In order to prevent leishmaniasis, WHO have designated it as a category I (emerging and uncontrolled) disease.[4] In spite of the significant effort made in the development of leishmaniasis vaccine, no vaccine against leishmaniasis is being made to support the prevention of disease.

Cell-surface glycoconjugates are not only significant in the survival of the *Leishmania* parasite[5], it also can be used as target in order to unveil the role of carbohydrates on the mechanism of infection. Lipophosphoglycan (LPG) is known as one of the major glycoconjygates in *Leishmania*.[6] As shown in Figure 1, structure analysis of LPG has shown that it has three domains, a glycosylphosphatidylinositol (GPI) anchor, a repeating phosphorylated saccharide region, and an oligosaccharide cap structure.[7,8]

But, the identification of structure-function relationship of the extracellular domain in LPG is still a challenge for both chemists and biologists. Although earlier reports for the synthesis of *Leishmania* capping structures have been featured in the construction of desired capping structures with different synthetic routs,[9-13] synthesized capping structures have never been prepared for microarrays. Early successes in microarrays for antibody binding were dedicated to detect specific

antibodies against Globo-H antigens in cancer patients' sera[14] or *Salmonella O*-antigen in *Salmonella* infected patients' sera[15]. However, because of limitations in availability of defined carbohydrate antigens and reliable tools, identifying interactions between carbohydrate antigens associated infectious diseases and antibody in serum from infected animals or human has been difficult.

In this context, facile synthesis of oligosaccharides should be accomplished as prerequisite in order to economically achieve the rapid development of fluorous-based microarray technique which enables detection of various *Leishmania* species in a short time,

Herein, we report the first solution-phase automated synthesis of *Leishmania* tetrasaccharide and iterative synthesis of *Leishmania* capping structures for the fluorous-based microarray of *Leishmania* capping structures as a possible diagnostic tool for the detection of antibodies in serum.

Results and discussion

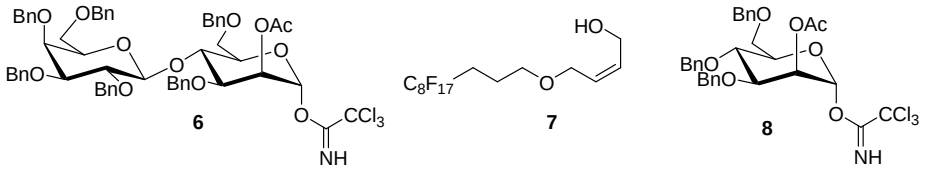
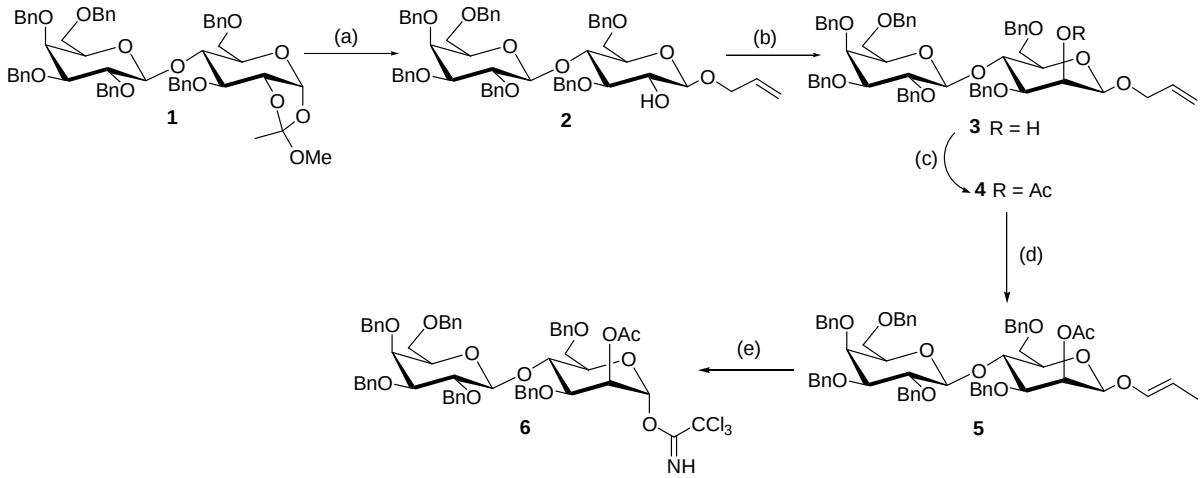


Figure 1. Fluorous alcohol and building blocks for the automated fluorous-phase synthesis of *Leishmania* tetrasaccharide.

The automated synthesis of *Leishmania* tetrasaccharide was designed to use just two building blocks: a known activated mannose building block[16] and a building block obtained from lactose (Figure 1).



Scheme 2. Synthesis of key disaccharide building block. (a) (i) CH_2=$CHCH_2OH$, TfOH, (ii) Na, MeOH (87 %); (b) (i) DMP DCM, (ii) $NaBH_4$, CH_2Cl_2/MeOH (92 %);

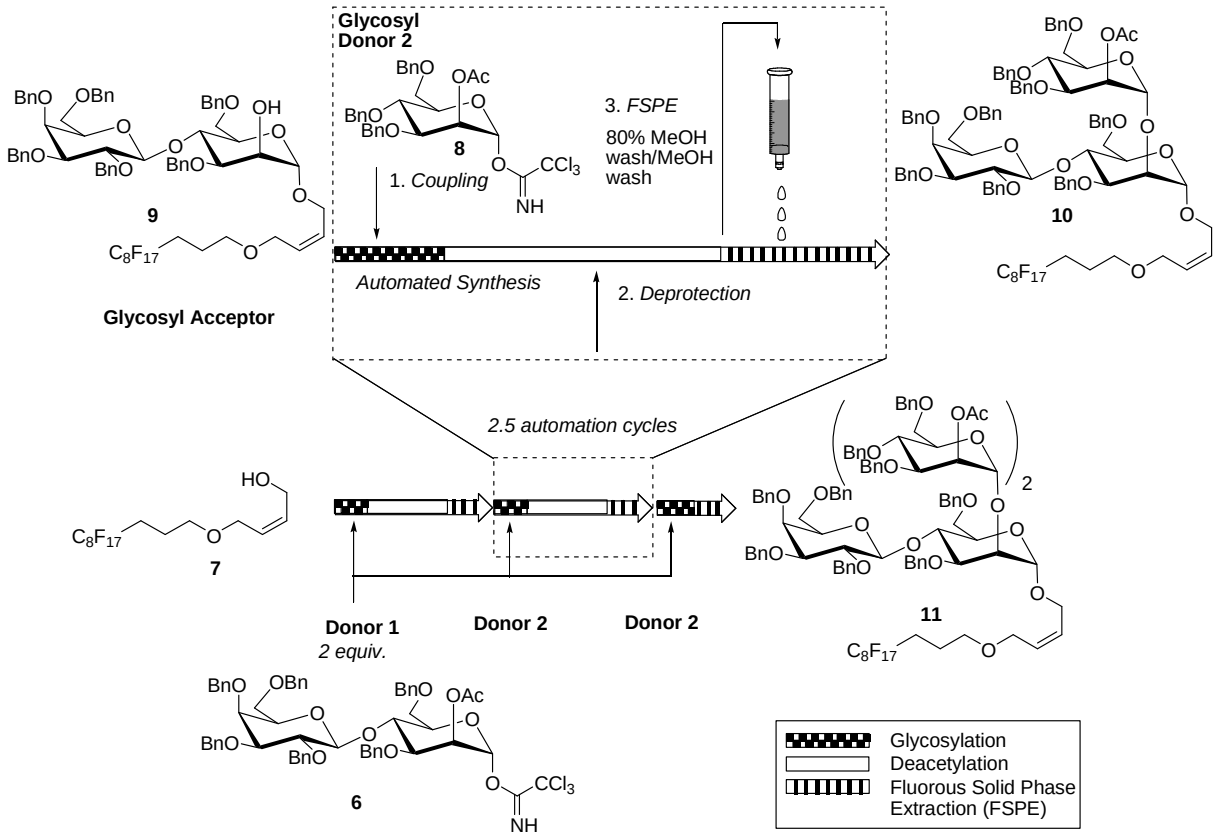
(c) Ac_2O, DMAP, TEA, CH_2Cl_2 (91 %); (d) [Ir] cat., H_2, THF (94 %); (e) (i) HgO, $HgCl_2$, $O=(CH_3)_2/H_2O$ (10/1), (ii) TCA, DBU, CH_2Cl_2 (89 %).

The key intermediate hexa-*O*-benzyl orthoester **1**[17] was prepared by orthester formation and follwed by benzylation from lactose in in good yield. One-step allylation under mild conditions using allyl alcohol and TfOH produced mainly 2-*O*-unprotected saccharide **2** along with 2-*O*-acetylated saccharide. Further deprotection by NaOMe gave the desired 2-*O*-unprotected saccharide **2** in high yield (87% over 2 steps). Conversion of β-glucoside **2** to α-mannoside **3** was achieved by a two-step oxidation-reduction process. Initial attempts to obtain 2-ulose **3** via Swern oxidation were unsatisfactory. Known oxidation condition[18] using Ac_2O/DMSO gave desired product in ~ 70 % yield, but it required long reaction time (48 h) and produced acetylated side product (5 ~ 10 %). However upon changing the oxidation conditions to Dess-Martin periodate, the reaction went smoothly at 35 ^oC for 3 h in quantitative yield. Further reduction with $NaBH_4$ to invert the stereochemistry of the C2 position gave the desired disaccharide **3** in high yield (92 % over 2 steps). Acetylation of **3** was performed under DMAP condition in 91 % yield. The allyl group **4** was removed

by isomerization to propenyl ether **5** with an iridium catalyst[19] and subsequent hydrolysis[20] under non-acidic conditions using $HgO/HgCl_2$. The desired activated glycosyl donor **6** was then prepared as needed with trichloroacetonitrile/DBU.

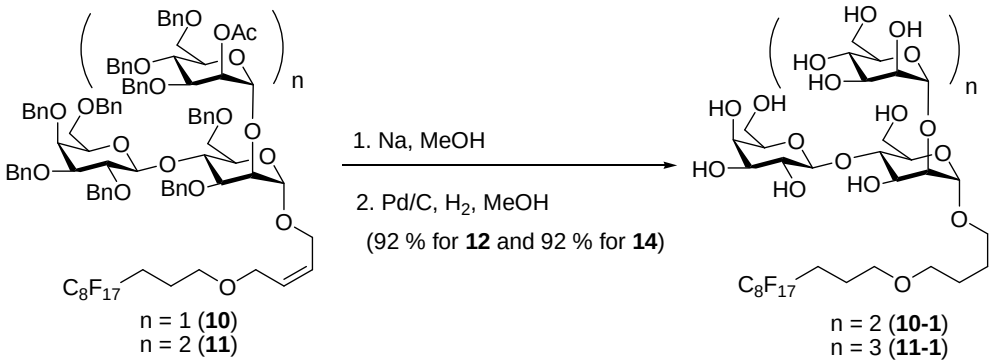
The important features of this approach include a suitable protecting group strategy, the transformation of glucose (Glc) to mannose (Man), and a fluorous solid-phase extraction (FSPE) technique for automation platform as an efficient purification method.

Fluorous-tag **7** has been prepared to improve the efficiency of purification through FSPE. Trichloroacetimidates **6** and **8** that can be activated under acidic condition (TMSOTf) have also been used as a glycosyl donor. The reaction has been done 2.5 cycles for 17 h 31 min without any labor since automated platform was ready to run the synthesizer for making *Leshimania* tetrasaccharide. (Scheme 3) Strikingly, not only 2 eq of glycosyl donor per one cycle was consumed for the completion of glycosylation, but the purity of crude product **11** (30 mg) shown in HPLC traces (supporting information) after 5 steps (17 h 31 min) was also remarkable even without further purification. Simple prep TLC gave us highly pure product (21 mg, 71 % per step) to have reasonable ^{1}H-, ^{13}C-NMR and mass spectroscopy data.



Scheme 3. Automated fluorous-phase synthesis of *Leishmania* tetrasaccharide.

These successful results demonstrated that the automation of oligosaccharide can be performed with small amount of reagents, less labor, and high purity of product. From these achievements, other oligosaccharide can be introduced to the same automated platform.



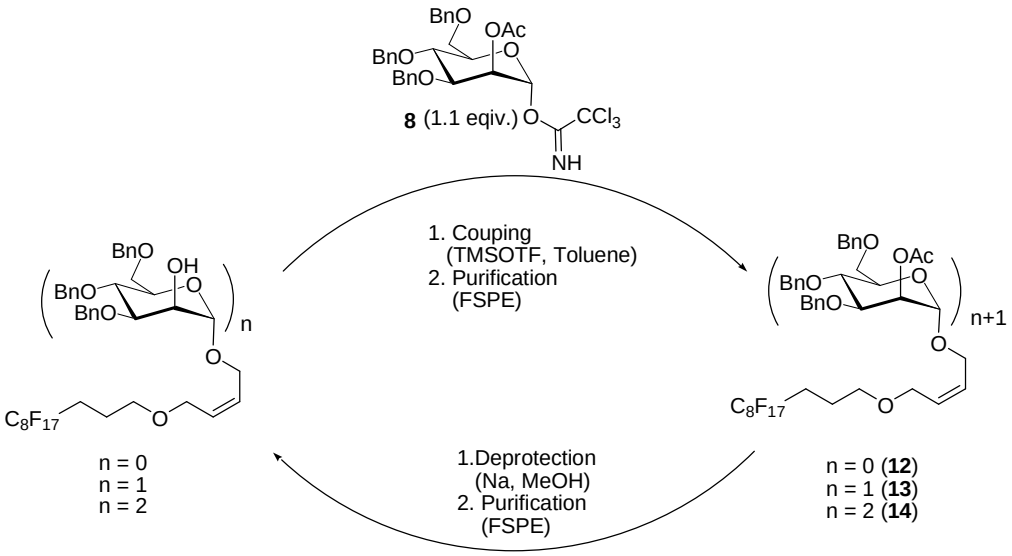
Scheme 4. Fluorous-tagged branched Tri- and Tetrasaccharide for microarray.

Fully deprotected Fluorous-tagged branched trisaccharide **10-1** and tetrasaccharide **11-1** were prepared after deacetylation, followed by simultaneous debenzylation and reduction of alkene with Pd/C/H_2.

Iterative synthesis of *Leishmania* capping structures

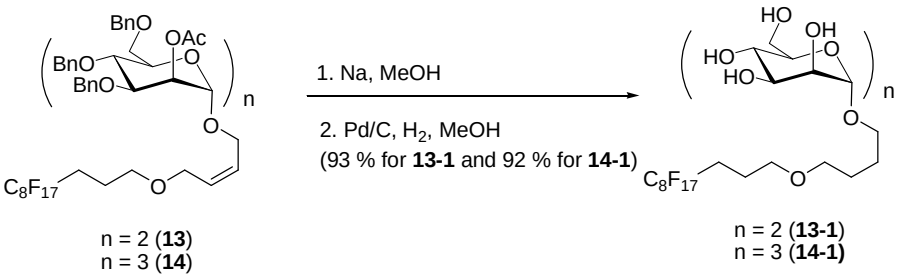
Leishmania species such as *L. major*, *L. donovani* and *L. mexicana* have linear α-1,2-linked mannose oligomer and lactose capping structures.[7,8] Iterative synthesis of linear α-1,2-linked mannose oligomers has been done in order to complete libraries for *Leishmania* capping structures. Each glycosylation was performed with 1.1 equivalent of donor rather than 1.5 equivalent of donor[21] in toluene at 25 ^{o}C for 5 min. Facile purification of crude product by FSPE enabled easy preparation of desired

linear α-1,2-linked dimannose **13** and trimannose **14** in high yield.



Scheme 5. Iterative synthesis of linear mannose oligomer.

Deacetylation, followed by simultaneous debenzylation and reduction of alkene with Pd/C/H_2, gave desired fully deprotected fluorous–tagged α-1,2-linked di- and tri-mannose in high yield (Scheme 6).



Scheme 6. Fluorous–tagged α-1,2-linked di- and tri-mannose.

Although iterative synthesis of linear α-1,2-linked dimannose **13** and trimannose **14** has previously been reported in good yield,[21] these oligomers have never been produced for the application in fluorous-based microarrays.

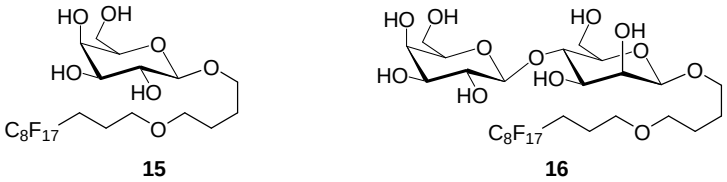


Figure 3. Fluorous-tagged galactose and lactose for microarray.

Fully deprotected fluorous-tagged galactose **15** and lactose **16** as *L. donovani* capping structures were also prepared as reported in the literature.[22]

Conclusion

In conclusion, we have shown that fully deprotected *Leishmania* capping structures for *L. donovani* and *L. major* were efficiently syntheisized either through fluorous-based automation platform or iterative synthesis using FSPE. Conversion of β-glucoside to α-mannoside as a key step for the synthesis of disaccharide building block was achieved by Dess-Martin oxidation, followed by reduction with $NaBH_4$ in high yield. Most importantly, automated fluorous-phase synthesis of *Leishmania* tetrasaccharide enabled facile synthesis of target oligosaccharides in order to be incorporated into fluorous-based microarray with high purity, less chemicals and less laborious work. This fluorous-based automated platform might be a powerful tool for the construction of carbohydrate library for the study of structure-function relationships.

Experimental section

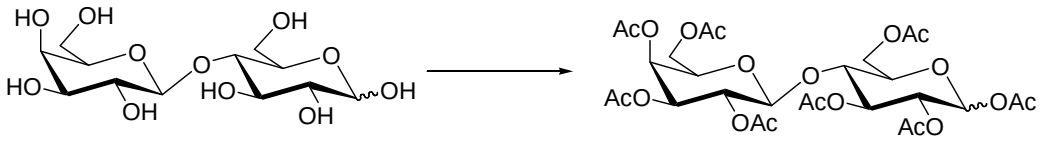
General methods

Solvents were reagent grade and in most cases dried prior to use. All other commercially available reagents were used as received unless otherwise noted. The organic extracts were dried over anhydrous $MgSO_4$. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$) prior to use. Methylene chloride

(CH_2Cl_2), and triethylamine (Et_3N) were distilled from calcium hydride. Diethyl ether (Et_2O) was distilled from sodium-benzophenone ketyl.

^1H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on Varian VXR-400 NMR or on Bruker DRX-400 NMR. Mass spectra (MS) were recorded on an Applied Biosytems DE-Pro MALDI mass analyzer or an Applied Biosytems QSTAR® XL Hybrid LC/MS/MS System. Chemical shifts are reported in parts per million downfield relative to tetramethylsilane (δ 0.00) and coupling constants are reported in Hertz (Hz). The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; and br = broad.

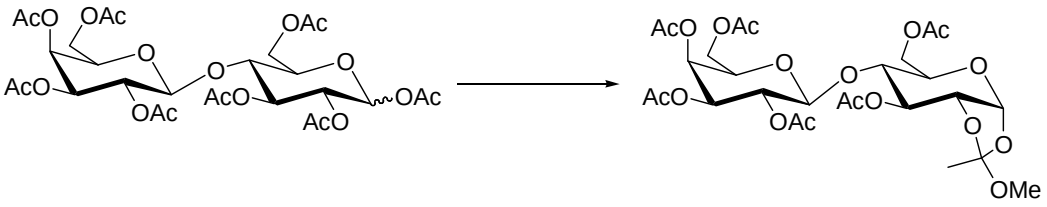
Synthesis of disaccharide building block



Synthesis of 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1-4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranoside.[19]

To a solution of lactose (1 g, 2.8 mmol) in acetic anhydride (10 mL) was added

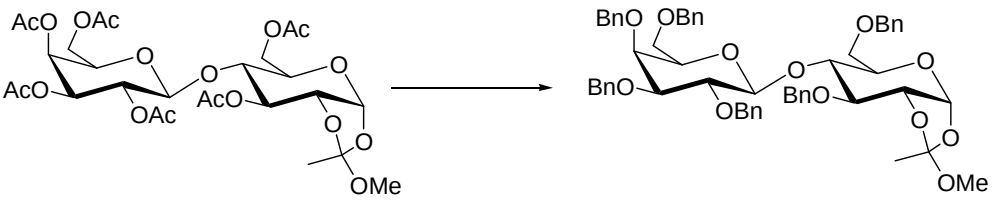
catalytic amount of iodine (14 mg, 0.1 mmol) at 25 ^{o}C. The reaction mixture was stirred at room temperature for 1 h. The dark brown reaction mixture was poured into a separatory funnel containing dichloromethane, aqueous sodium thiosulfate solution and crushed ice. The colorless solution from a separatory funnel was washed with H_2O (2 x 50 mL) and sat.$NaHCO_3$ (2 x 50 mL). It was dried with Na_2SO_4 and then concentrated under reduced vacuum to obtain the desired product with quantitative yield**.**



Synthesis of 2,3,4,6,-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-acetyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside.[19]

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1-4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranoside (1.5 g, 2.2 mmol) in dichloromethane (5 mL) was added 33 % HBr (5 mL, 28.6 mmol) in acetic acid for 10 min. The reaction mixture was stirred at 25 ^{o}C for 1 h and then checked TLC to check the completion of reaction.

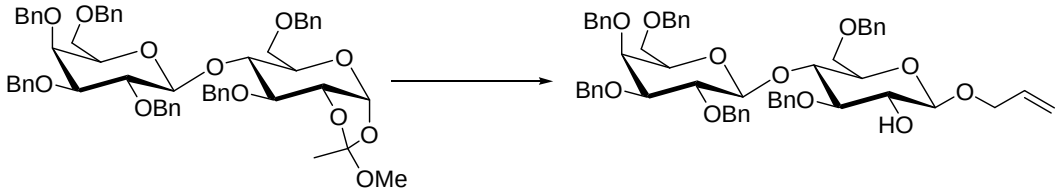
After completion of reaction, the mixture was washed with cold water three times and then followed by washed with saturated sodium bicarbonate solution three times. The neutralized solution was dried with sodium sulfate and concentrated under reduced pressure. The crude product was used for next step without further purification. To a solution of hepta-acetyl lactosyl bromide in dichloromethane was added triethylamine (0.62 mL, 4.4 mmol), Bu_4NBr (0.71 mg, 2.2 mmol) and methanol (0.077 mL, 2.64 mmol). The reaction mixture was stirred at 40 ^oC for 16 h and then washed with H_2O (1 x 50 mL). The solution was dried with Na_2SO_4 and then concentrated under reduced vacuum. The crude produce was purified to obtain the desired product (1.19 g, 83 % over 2 steps) by silica column chromatography.



Synthesis of 2,3,4,6,-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside 1.[19]

To a solution of 2,3,4,6,-tetra-*O*-acetyl-β-D-galactopyranosyl-(1-4)-3,4-di-*O*-acetyl-

1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside (1g, 1.5 mmol) in MeOH (5 mL) was added catalytic amount of Na (3.5 mg, 0.15 mmol) . The reaction mixture was stirred at 25 ^{o}C for 6 h and then concentrated under reduced vacuum to yield the crude deacetylated product as yellow foam. To a solution of deacetylated product in DMF (10 mL) was added 60 % NaH (0.47 g, 11.5 mmol), followed by addition of benzyl bromide (2.06 mL, 11.5 mmol) and tetrabuylammonium iodide (0.57 g, 1.5 mmol) at 0 ^{o}C. The reaction mixture was warmed up to 25 ^{o}C and stirred for 12 h. The reaction mixture was diluted with dichloromethane (20 mL) and then washed with H_2O (2 x 50 mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude product was purified by flash silica column chromatography to afford the desired product (**1**) (1.28 g, 89 % over 2 steps).



Synthesis of Allyl *O*-(2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-*2*-O-hydroxy-3,6-di-*O*-benzyl-β-D-glucopyranoside 2.

A solution of 2,3,4,6,-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside (**1**) (1 g, 1.1 mmol) and ally alcohol (0.45 mL, 6.6 mmol) in dichloromethane (10 mL) was cooled to – 40 ^{o}C and TfOH (6 µL, 0.07 mmol) was added dropwise over 10 min. The reaction mixture was stirred at – 40 ^{o}C for 30 min and allowed to 25 ^{o}C for 1 h followed by, addition of triethylamine to neutralize a solution. The crude product was obtained after concentration of solvent under reduced vacuum. To a crude product in MeOH was added Na (5 mg, 0.22 mmol). The reaction mixture was stirred at 25 ^{o}C for 1 h and then concentrated under reduced vacuum. The crude product was purified by flash silica column chromatography to afford the desired product (**2**) (0.86 g, 87 % over 2 steps).

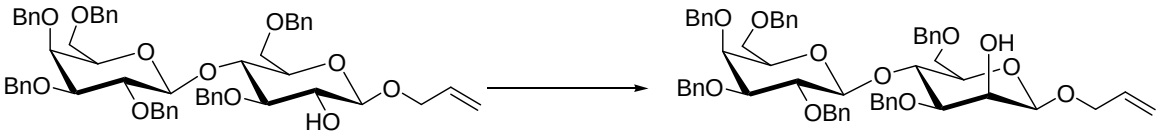
R_f (ethyl acetate/haxane): 0.50 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.36-7.18 (m, 30H, aromatics), 6.09-5.99 (m, 1H, $CH_2C\underline{H}=CH_2$), 5.33 (dd, 1H, *J* = 17.1, 1.2 Hz, $CH=C\underline{H}_2$), 5.22 (dd, 1H, *J* = 10.3, 1.2 Hz, $CH=C\underline{H}_2$), 5.08 (d, 1H, *J* = 11.1 Hz), 4.97 (d, 1H, *J* = 11.5 Hz), 4.82 (dd, 2H, *J* = 20.4, 11.1 Hz), 4.71 (dd, 2H, *J* = 22.8, 6.8 Hz), 4.70 (d, 1H, *J* = 5.3 Hz), 4.56 (dd, 2H, *J* = 11.5, 4.4 Hz), 4.43 – 4.43 (d, $1H_{anomeric}$, *J* = 7.6 Hz), 4.40 (m, 3H), 4.34 (d,

$1H_{anomeric}$, *J* = 7.2 Hz), 4.48 (d, 1H, *J* = 11.8 Hz), 4.15 (dd, 1H, *J* = 12.7, 6.4 Hz), 3.96 (d, 1H, *J* = 9 Hz), 3.92 (d, 1H, *J* = 2.7 Hz), 3.81 – 3.70 (m, 3H), 3.57 – 3.46 (m, 3H), 3.41 – 3.34 (m, 4H), 2.39 (br, 1H, O<u>H</u>).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.2, 139.1, 138.9, 138.7, 138.5, 138.2 (C_q-Aryl)], 134.1 ($CH_2\underline{C}H=CH_2$), 128.6 – 127.5 (m, CH-Aryl), 118.0 ($CH=\underline{C}H_2$), 103.0 ($CH_{anomeric}$), 101.7 ($CH_{anomeric}$), 83.0, 82.7, 80.1, 76.5, 75.6, 75.5, 74.9, 74.8, 73.7, 73.7, 73.3, 73.2, 72.8, 70.3, 68.4.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{57}H_{62}NaO_{11}$, 945.4190; Found, 945.5140.



Synthesis of Allyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-*2*-O-hydroxy-3,6-di-*O*-benzyl-β-D-mannopyranoside 3.

To a solution of allyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-β-D-glucopyranoside (**2**) (0.1 g, 0.11 mmol) in dichloromethane (5 mL) was added dess-martin periodinane (92 mg, 0.22 mmol). The reaction mixture was stirred at 35 ^oC for 4 h and then diluted with dichloromethane (10 mL). The diluted solution

was washed with sat.$NaHCO_3$ (2 x 10 mL), H_2O (1 x 10 mL), and brine (1 x 10 mL). The organic layer was dried with Na_2SO_4 and then concentrated under reduced vacuum. The crude residue was dissolved in dichloromethane/MeOH (1/1) and cooled to 0 ^oC. $NaBH_4$ (50 mg, 1.35 mmol) was added and then the reaction mixture was allowed to 25 ^oC over 1 h. The mixture was diluted with dichloromethane (10 mL) and washed with H_2O (1 x 10 mL), 1 % aqueous citric acid (1 x 10 mL) and brine (1 x 10 mL). The solvent was evaporated under reduced vacuum and dried with Na_2SO_4. The crude product was purified by flash silica column chromatography to afford the desired product (**3**) (92 m g, 92 % over 2 steps).

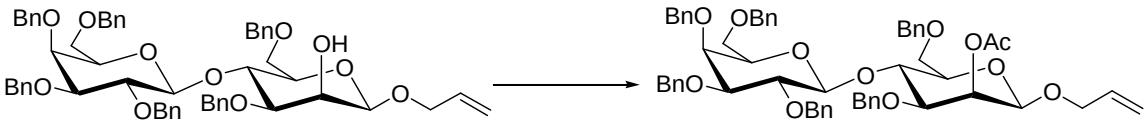
R_f (ethyl acetate/haxane): 0.45 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.36-7.21 (m, 30H), 5.97-5.87 (m, 1H, $CH_2C\underline{H}=CH_2$), 5.29 (dd, 1H, *J* = 16.5, 1.6 Hz, $CH=C\underline{H}_2$), 5.20 (dd, 1H, *J* = 10.4, 1.6 Hz, $CH=C\underline{H}_2$), 4.97 (d, 1H, *J* = 11.5 Hz), 4.81 (d, 1H, *J* = 2 Hz), 4.78 (d, 1H, *J* = 2.8 Hz), 4.75 (d, 1H, *J* = 10.8 Hz), 4.72-4.66(m, 4H), 4.60 (d, 1H, *J* = 11.5 Hz), 4.50 (d, $1H_{anomeric}$, *J* = 1.2 Hz), 4.47 (d, 1H, *J* = 10.8 Hz), 4.45 (d, $1H_{anomeric}$, *J* = 7.9 Hz), 4.41-4.37 (m, 3H), 4.30 (d, 1H, *J* = 11.6 Hz), 4.12-4.07 (m, 3H), 3.92 (d, 1H, *J* = 2.8 Hz), 3.84 (dd, 1H, *J* = 10.8, 2.8 Hz), 3.76-3.72 (m, 2H), 3.63(t, 1H, *J* = 8.68 Hz), 3.56 (dd,

1H, *J* = 8.4, 3.6 Hz), 3.51-3.39 (m, 4H), 2.39 (br, 1H, OH).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.1, 138.9, 138.7, 138.7, 138.6, 138.1 (C_q-Aryl)], 134.1 ($CH_2\underline{C}H=CH_2$), 128.6 – 127.6 (m, CH-Aryl), 118.0 ($CH=\underline{C}H_2$), 103.4 ($CH_{anomeric}$), 98.7 ($CH_{anomeric}$), 82.8, 80.1, 79.5, 75.5, 75.4, 73.7, 73.6, 73.3, 73.2, 72.8, 72.6, 70.0, 69.2, 68.9, 68.6,

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{57}H_{62}NaO_{11}$, 945.4190; Found, 945.5131.



Synthesis of Allyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside 4.

To a allyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-β-D-mannopyranoside (**3**) (80 mg, 0.09 mmol) in dichloromethane (5 mL) was added DMAP (6 mg, 0.05 mmol), triethylamine (0.02 mL, 0.18 mmol) and acetic anhydride (0.01 mL, 0.011 mmol). The reaction mixture was stirred at 25 ^oC for 1 h and then concentrated under reduced vacuum. The crude residue was dried with Na_2SO_4 and purified by flash silica column chromatography to afford the desired product (**4**) (76

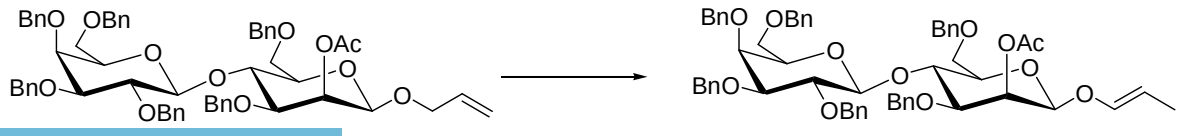
mg, 91 %)

R_f (ethyl acetate/haxane): 0.70 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.30-7.18 (m, 30H), 5.92-5.82 (m, 1H, $CH_2C\underline{H}=CH_2$), 5.58 (dd, 1H, *J* = 2.8, <1 Hz, $CHC\underline{H}(OAc)CH$), 5.27 (dd, 1H, *J* = 17.2, 1.6 Hz, $CH=C\underline{H}_2$), 5.18 (dd, 1H, *J* = 10, 1.2 Hz, $CH=C\underline{H}_2$), 4.80 (d, 1H, *J* = 10.8 Hz), 4.73-4.61 (m, 4H), 4.58 (d, 1H, *J* = 8 Hz), 4.54 (d, 1H, *J* = 3.6 Hz), 4.53 (d, $1H_{anomeric}$, *J* = <1 Hz), 4.51 (d, $1H_{anomeric}$, *J* = 12 Hz), 4.42-4.32 (m, 3H), 4.24 (d, 1H, *J* = 11.6 Hz), 4.10 (dd, 1H, *J* = 13.2, 6.4 Hz), 4.98 (t, 1H, *J* = 9.2 Hz), 3.85 (dd, 2H, *J* = 11.2, 2 Hz), 3.79-3.68 (m, 2H), 3.60-5.53 (m, 2H), 3.49-3.45 (m, 1H), 3.41-3.39 (m, 2H), 3.33 (t, 1H, *J* = 6.8 Hz), 2.03 (s, 3H, $OC(O)C\underline{H}_3$).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.8 (C=O), [139.0, 138.9, 138.7, 138.6, 138.5, 138.2 (C_q-Aryl)], 133.8 ($CH_2\underline{C}H=CH_2$), 128.6 – 127.4 (m, CH-Aryl), 117.8 ($CH=\underline{C}H_2$), 115.5, 103.0 ($CH_{anomeric}$), 97.7 ($CH_{anomeric}$), 82.9, 80.1, 78.5, 75.8, 75.3, 74.8, 74.6, 73.7, 73.6, 73.4, 72.7, 71.8, 70.0, 69.3, 68.9, 68.6, 21.2(CH_3).

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{59}H_{64}NaO_{12}$, 987.4295; Found, 987.3006.



Synthesis of Vinyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside 5.

To a solution of allyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside (**4**) (80 mg, 0.08 mmol) in THF (3 mL) was added catalytic amount of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (2 mg, 0.002 mmol). The stirred solution was degassed, placed under N_2 and degassed. The reaction mixture was placed under H_2 for 5 min and degassed once more to prevent further reduction. The mixture was stirred at 25 ^oC for 30 min under N_2 and then concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product (**5**) (75 mg, 94 %).

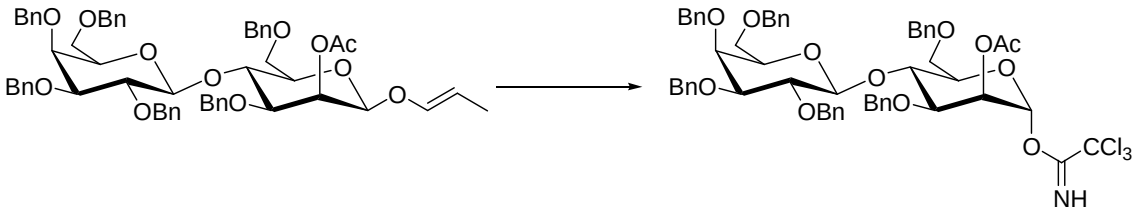
R_f (ethyl acetate/haxane): 0.72 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.19 (m, 30H), 6.23 (d, 1H, *J* = 12.3 Hz, OC<u>H</u>=CH), 5.60 (dd, 1H, *J* = 2.6, <1 Hz, CHC<u>H</u>(OAc)CH), 5.15-5.07 (m, 1H, CH=C<u>H</u>CH_3), 4.96 (d, 1H, *J* = 11.4 Hz), 4.82 (d, 1H, *J* = 10.9 Hz), 4.74-4.62 (m, 5H), 4.71 (d, $1H_{anomeric}$, *J* = <1 Hz), 4.59-4.55 (m, 2H), 4.51 (d, $1H_{anomeric}$, *J* = 17.4 Hz), 4.49-4.35 (m, 3H), 4.25 (d, 1H, *J* = 11.7 Hz), 4.00 (t, 1H, *J* = 9.1 Hz), 3.89 (d, 2H, *J* =

10.5 Hz), 3.78-3.70 (m, 2H), 3.64 (dd, 1H, *J* = 8.6, 3.2 Hz), 3.56-3.53 (m, 2H), 3.44-3.40 (m, 1H), 3.35 (t, 1H, *J* = 6.3 Hz), 2.04 (s, 3H, OC(O)C$\underline{H}_3$), 1.54 (d, 3H, *J* = 6.54 Hz, CHC$\underline{H}_3$).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.7 (C=O), 143.4(O$\underline{C}$H=CH), [139.0, 138.8, 138.7, 138.5, 138.2 (C_q-Aryl)], 128.6 – 127.4 (m, CH-Aryl), 104.9 (CH=$\underline{C}$HCH$_3$) 103.0 ($CH_{anomeric}$), 97.8 ($CH_{anomeric}$), 82.9, 80.0, 78.2, 76.1, 75.4, 74.8, 74.5, 73.7, 73.4, 72.8, 71.9, 69.2, 68.9, 68.1, , 21.2(CH_3), 12.6 (CH$\underline{C}$H$_3$).

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{59}H_{64}NaO_{12}$, 987.4295; Found, 987.3076



Synthesis of 2,3,4,6,-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate 6.

To a vinyl *O*-(2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside (**7**) (75 mg, 0.08 mmol), and mercury oxide (24 mg, 0.1 mmol) in 3 mL of acetone/H_2O (10 mL /1mL) was added a solution of

mercuric chloride (23 mg, 0.09 mmol) in 2 mL of acetone/H_2O (10 mL/1mL) for 1 min. The reaction mixture was stirred at 25 ^oC for 3 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was concentrated under reduced vacuum and dried with Na_2SO_4. To a crude residue in dichloromethane (5 mL) was added Cs_2CO_3 (25 mg, 0.08 mmol) and followed by addition of trichloroacetonitrile (0.016 mL, 0.16 mmol) at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 30 min and then concentrated under reduced vacuum and purified by flash silica column chromatography to afford the desired product (**6**) (74 mg, 89 % over 2 steps).

R_f (ethyl acetate/haxane): 0.80 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.66 (s, 1H, NH=C), 7.32-7.17 (m, 30H), 6.24 (d, $1H_{anomeric}$, *J* = 1.8 Hz), 5.43 (dd, 1H, *J* = 3.1, 2.8 Hz, CHCH(OAc)CH), 4.97 (d, 1H, *J* = 11.6 Hz), 4.81 (d, 1H, *J* = 10.8 Hz), 4.76 (d, 1H, *J* = 12 Hz), 4.69-4.65 (m, 3H), 4.57 (d, 1H, *J* = 11.2 Hz), 4.44 (d, 1H, *J* = 7.6 Hz), 4.40 (d, 1H, *J* = 11.6 Hz), 4.38 (d, 1H, *J* = 11.2 Hz), 4.28-4.22 (m, 2H), 3.95 (dd, 2H, *J* = 8.9, 3.2 Hz), 3.87 (dd, 1H, *J* = 8.9, 7.8 Hz), 3.63-3.54 (m. 2H), 3.46-3.36 (m, 3H), 1.98 (s, 3H, OAc).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.4 (C=O), 160.1 (C=NH), [139.1, 138.9, 138.7, 138.6, 138.5, 138.4 (C_q-Aryl)], 128.7 – 127.6 (m, CH-Aryl), 103.2 ($CH_{anomeric}$), 95.4 ($CH_{anomeric}$), 82.8, 80.0, 75.5, 75.4, 74.9, 74.6, 74.0, 73.9, 73.7, 73.4, 72.9, 72.8, 69.1, 68.5, 68.2, 21.1 (CH_3).

ASW *Leishmania*-tetrasaccharide method run

After FSPE, the methanol elution collected in the vial was removed from the instrument and concentrated. Solvent was removed under reduced pressure to obtain the crude product (30 mg) as colorless oil. In order to obtain pure product (**5**) (21 mg, 16 %) for ^{1}H NMR, ^{13} C NMR and mass spectrum, further purification was performed using prep TLC.

Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside 11.

R_f (ethyl acetate/haxane): 0.75 (20/80)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-7.11 (m, 30H), 5.62 (br, 2H), 5.47 (s, 1H), 5.08 (s, 1H), 4.92 (d, 2H, *J* = 15.6 Hz), 4.82-4.77 (m, 3H), 4.70-4.59 (m, 6H), 4.55-4.44 (m, 10H), 4.41-4.36 (m, 3H), 4.26 (d, 1H, *J* = 12 Hz), 4.21-4,18 (d, 1H, *J* = 12.4 Hz), 4.15 (m, 1H), 4.05-3.98 (m, 2H), 3.92-3.82 (m, 10H), 3.78-3.69 (m, 8H), 5.57 (t, 2H, *J* = 10 Hz), 3.41 (t, 2H, *J* = 9.2 Hz), 3.32-3.29 (m, 3H), 2.15-2.04 (m, 2H), 2.08 (s, 3H), 1.80-1.73 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2 (C=O), [139.4, 139.2, 139.0, 138.9, 138.8, 138.7, 138.6, 138.6, 138.6, 138.2, 138.2 (C_q-Aryl)], 128.5 – 127.2 (m, CH-Aryl), [103.1, 101.2, 99.6, 98.2 95.4 ($CH_{anomeric}$)], 83.0, 80.3, 79.7, 78.2, 75.4, 75.3, 75.2, 75.0, 74.8, 74.5, 73.5, 73.4, 73.2, 72.7, 72.4, 72.3, 72.1, 72.0, 71.9, 69.7, 69.0, 98.8, 68.5, 66.7, 63.0, 28.4, 28.3, 28.0, 21.3(CH_3).

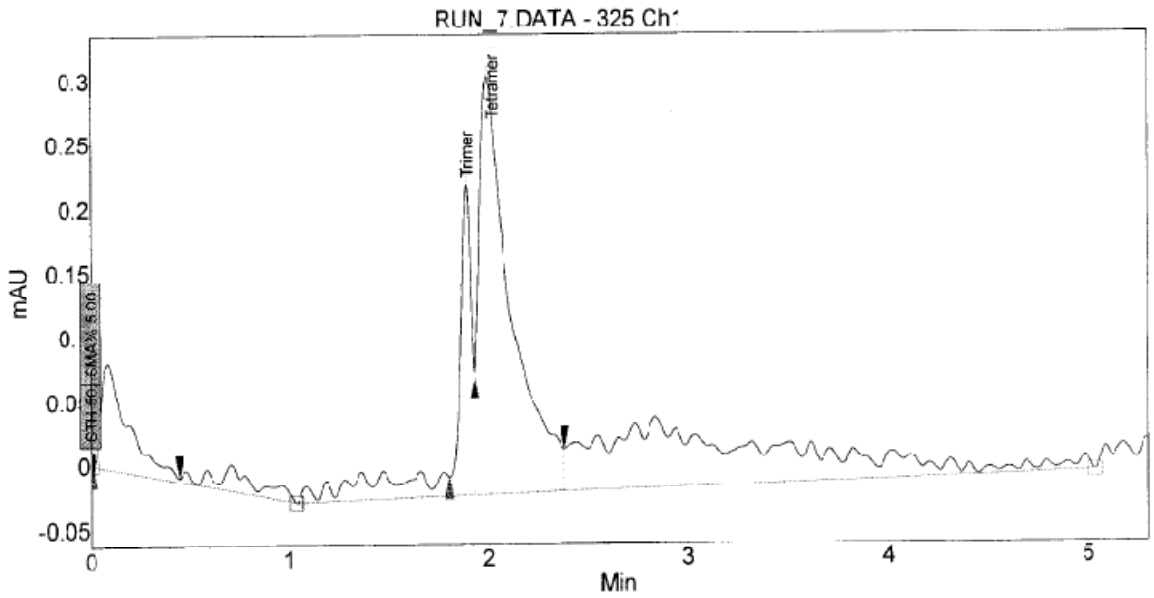
HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{125}H_{127}F_{17}NaO_{23}$ 2341.8394; Found, 2341.3591.

- 2.5 cycles (17h 31 min 39) completed for the synthesis of *Leishmania*-tetrasaccharide.

Step	Task	Reagent/ Operation	Operation Time

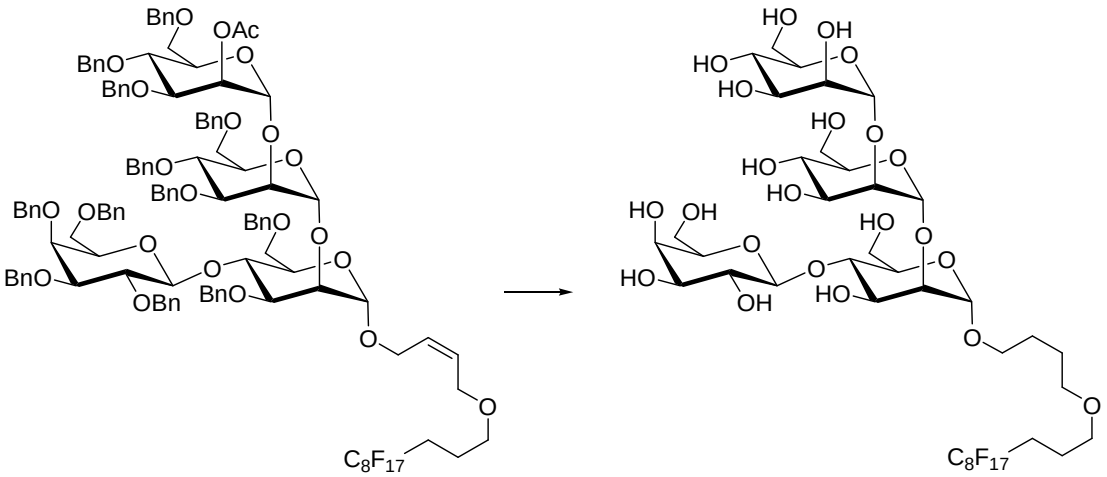
1	Glycosylation	2 equivalent donor (100 µmol) in 0.5 mL Toluene, 1 equivalent F-tagged acceptor (50 µmol) in 1 mL Toluene; 0.1 equivalent TMSOTf, rt	30 min
2	TLC sample	30 µl of crude reaction mixture withdrawn	
3	Quenching	0.5 ml TEA	45 min
4	Evaporation	40 ^{o}C	
5	Deacetylation	3 equivalent of NaOMe solution	2 h
	TLC sample	30 µl of crude reaction mixture withdrawn	
6	Quenching	0.3 ml 0.5 M Acetic acid solution in MeOH	
7	Evaporation	50 ^{o}C	45 min
8	FSPE preparation	0.4 ml DMF	
9	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 ^{o}C	45 min
14	Transfer	2 ml toluene added	
15	Evaporation	50 ^{o}C	45 min

- HPLC trace for crude product.



Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	0.09	14.38	0.1	0.0	14.381
2	Trimer	1.89	16.93	0.2	0.0	16.932
3	Tetramer	1.99	68.69	0.3	0.1	68.687
Total			100.00	0.7	0.1	100.000



3-(perfluorooctyl)propanyloxybutanyl-4-*O*-[β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-

***O*-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside 11-1.**.

To a 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-β-D-mannopyranoside (**11**) (50 mg, 0.02 mmol) in MeOH (3 mL) was added Na (1 mg, 0.07 mmol) and stirred at 25 ^{o}C for 30 min. The reaction mixture was neutralized with Dowex-ion-exchange resin and filtered through Celite pad. purified by solid phase extraction using fluoro flash column. The solvent was removed under reduced pressure to obtain deacetylated product. To a sluotion of deacetylated product in MeOH (3mL) was added 10 % Pd/C (50 mg). The reaction mixture was stirred at 25 ^{o}C under hydrogen atmosphere for 12 h. The desired (**11-1**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (25 mg, 96 %).

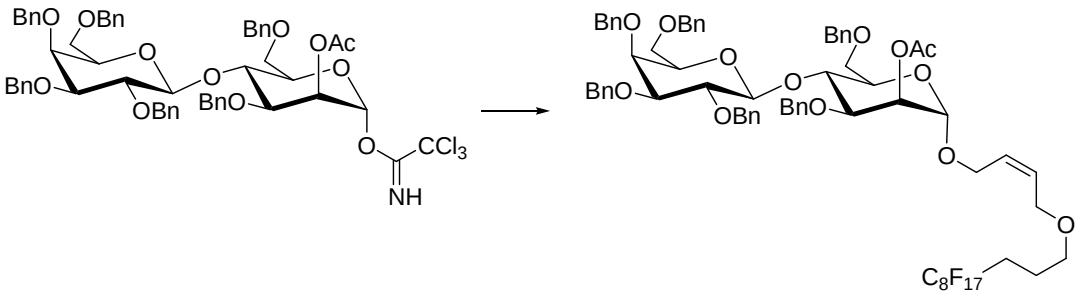
^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) [5.33 (s, 1H), 5.01 (s, 1H), 4.96 (s, 1H), 4.35 (d, 1H, *J* = 8 Hz) $CH_{anomeric}$)], 4.01-3.1 (m, 30H), 2.31-2.18 (m, 2H), 1.89-1.78 (m, 2H), 1.84-1.53 (m, 4H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) [103.9, 102.9, 101.0, 98.7 ($CH_{anomeric}$)], 79.2,

78.0, 77.6, 73.8, 73.8, 73.6, 71.8, 71.2, 70.7, 70.6, 70.5, 69.9, 69.1, 68.9, 68.0, 67.6, 67.3, 62.1, 62.0, 61.3, 61.0, 26.4, 26.1.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{39}H_{55}F_{17}NaO_{22}$ 1221.2811; Found, 1220.1206.

Iterative synthesis of *Leishmania*-capping structures



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-]-*α*-D-mannopyranoside 6-1.

To a solution of 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**6**) (0.1 g, 0.09 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (**7**) (62 mg, 0.11 mmol) in dry toluene (3 mL) was added TMSOTf (2 µL, 0.01 mmol) at 25 ^oC. The reaction mixture was stirred at room temperature for 10 min. The reaction was quenched with triethylamine

(0.01 mL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using 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 desired product (**6-1**) (0.13 g, 92 %).

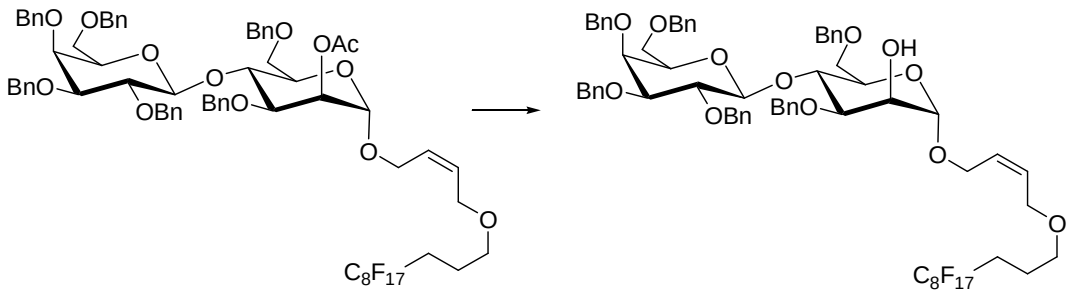
R_f (ethyl acetate/haxane): 0.75 (20/80)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.30-7.17 (m, 30H), 5.75-5.65 (m, 2H), 5.29 (s, 1H), 4.95 (d, 1H, *J* = 11.4 Hz), 4.82 (d, 1H, *J* = 11 Hz), 4.81 (d, 1H, *J* = <1 Hz), 4.70-4.62 (m, 5H), 4.57-4.47 (m, 2H), 4.39 (d, 1H, *J* = 5.6 Hz), 4.36 (d, 1H, *J* = 5.2 Hz), 4.24 (d, 1H, *J* = 11.8 Hz), 4.18 (d, 1H, *J* = 4.3 Hz), 4.10-4.06 (m, 2H), 4.01-4.00 (m, 2H), 3.90-3.85 (m, 2H), 3.80 (d, 2H, *J* = 8.7 Hz), 3,72 (t, 2H, *J* = 8.1 Hz), 3.55 (t, 1H, *J* = 9 Hz), 3.43-3.32 (m, 5H), 2.20-2.03 (m, 2H), 1.95 (s, 3H), 1.85-1.78 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.6 (C=O), [139.0, 138.9, 138.9, 138.5, 138.5, 138.2 (C_q-Aryl)], 130.6, 128.5 – 127.3 (m, CH-Aryl), [103.1, 96.8 ($CH_{anomeric}$)], 82.0, 80.1, 76.3, 75.3, 74.8, 74.7, 73.7, 73.6, 73.3, 72.7, 72.2, 71.5, 69.6, 69.1, 69.9, 68.9, 68.9, 66.6, 62.9, 28.2, 28.0, 21.1(CH_3).

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{71}H_{71}F_{17}NaO_{13}$ 1478.2783; Found,

1478.2483.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-]-(1-4)-2-*O*-hydroxy-3,6-di-*O*-benzyl-α-D-mannopyranoside 9.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-]-(1-4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (**6-1**) (0.1 g, 0.07 mmol) in MeOH (3 mL) was added Na (1 mg, 0.07 mmol) and stirred at 25 ^{o}C for 30 min. The crude product was purified by solid phase extraction using 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 desired product (**10**) (95 mg, 98 %).

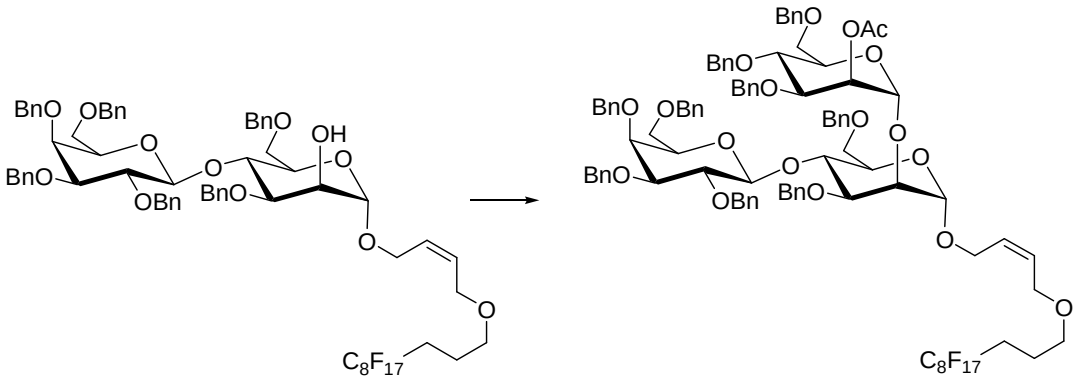
R_f (ethyl acetate/haxane): 0.45 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34-7.21 (m, 30H), 5.76-5.67 (m, 2H), 4.98 (d, 1H, *J* = 11.6 Hz), 4.91 (d, 1H, *J* = 11.6 Hz), 4.89 (d, 1H, *J* = <1 Hz), 4.82 (d, 1H, *J*

= 11.2 Hz), 4.75-4.69 (m, 3H), 4.63-4.50 (m, 3H), 4.43 (d, 1H , *J* = 10 Hz), 4.40-4.36 (m, 2H), 4.28 (d, 1H, *J* = 11.6 Hz), 4.21 (dd, 1H, *J* = 12, 3.2 Hz), 4.13-4.07 (m, 2H), 4.03-4.01 (m, 2H), 3.90 (d, 1H, *J* = 2.4 Hz), 3.79-3.69 (m, 5H), 3.58 (t, 1H, *J* = 8.8 Hz), 3.46-3.37 (m, 5H), 2.21-2.08 (m, 2H), 1.85-1.79 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.1, 138.9, 138.8, 138.6, 138.2 (C_q-Aryl)], 130.5, 128.6 – 127.6 (m, CH-Aryl), [103.3, 98.3 ($CH_{anomeric}$)], 82.8, 80.1, 78.2, 75.4, 74.5, 73.7, 73.7, 73.3, 73.0, 72.8, 71.4, 69.5, 68.9, 68.9, 68.7, 66.7, 62.7, 28.2.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{69}H_{69}F_{17}NaO_{12}$ 1435.4415; Found, 1435.3106..



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl- α -D-

mannopyranosyl]-α-D-mannopyranoside 10.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-]-(1-4)-2-*O*-hydroxy-3,6-di-*O*-benzyl-α-D-mannopyranoside (**9**) (90 mg, 0.06 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate (42 mg, 0.07 mmol) in dry toluene (3 mL) was added TMSOTf (2 µL, 0.01 mmol) at 25 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 10 min. The reaction was quenched with triethylamine (0.1 mL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using 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 desired product (**10**) (0.11 g, 89 %).

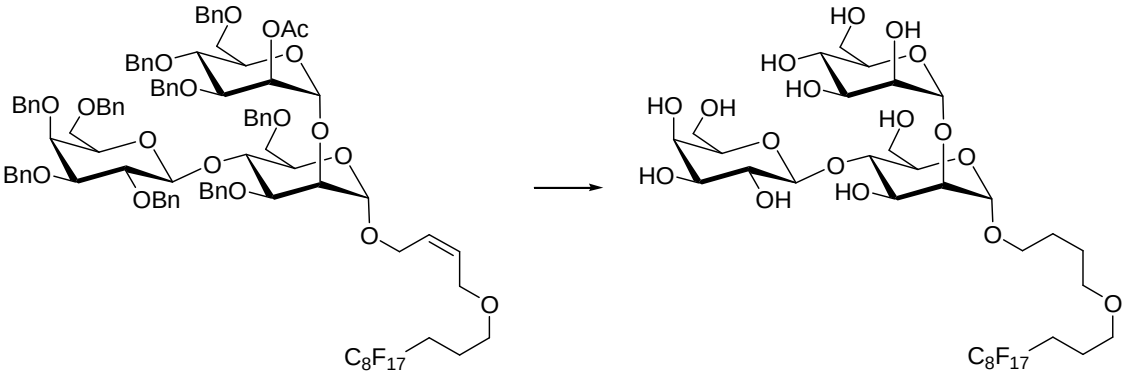
R_f (ethyl acetate/haxane): 0.75 (20/80)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.29 (m, 45H), 5.64-5.61 (m, 2H), 5.50 (dd, 1H, *J* = 2.8, 1.6 Hz), 5.05 (d, 1H, *J* = 1.2 Hz), 4.89 (d, 1H, *J* = 11.2 Hz), 4.83-4.77 (m, 4H), 4.68 (d, 1H, *J* = 11.2 Hz), 4.64-4.57 (m, 5H), 4.53 (d, 1H, *J* = 5.6 Hz), 4,51-4.45 (m, 3H), 4.42 (d, 1H, *J* = 7.6 Hz), 4.38 (d, 1H, *J* = 5.2 Hz), 4.33 (d, 1H, *J* = 10.8 Hz), 4.29 (d, 1H, *J* = 12.4 Hz), 4.21 (d, 1H, *J* = 12 Hz), 4.16-4.12 (m, 1H), 4.06

(d, 1H, *J* = 8.8 Hz), 4.02-4.00 (m, 1H), 3.97-3.91 (m, 4H), 3.85-3.82 (m, 3H), 3.80 (d, 1H, *J* = 9.6 Hz), 3.75-3.62 (m, 6H), 3.51 (t, 1H, *J* = 8.8 Hz), 3.44 (d, 1H, *J* = 2.8 Hz), 3.41-3.29 (m, 4H), 2.17-2.01 (m, 2H), 2.04 (s, 3H), 1.81-1.75 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.0 (C=O), [139.4, 139.2, 139.1, 138.9, 138.7, 138.7, 138.5, 138.3 (C_q-Aryl)], 130.1, 128.6 – 127.1 (m, CH-Aryl), [103.5, 99.7, 98.2 ($CH_{anomeric}$)], 83.0, 80.3, 78.7m 78.2, 75.5, 87.4, 75.2, 74.8, 74.6, 73.7, 73.1, 72.7, 72.7, 72.2, 72.1, 72.0, 69.5, 69.2, 68.9, 68.6, 68.4, 66.8, 62.9, 28.5, 28.3, 28.1, 21.4.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{98}H_{99}F_{17}NaO_{18}$ 1909.6458; Found, 1909.6699.



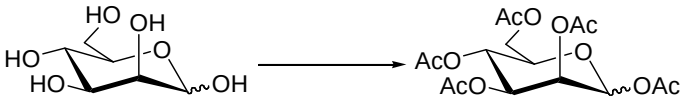
Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-4-*O*-[β-D-galactopyranosyl]-2-*O*-[2-*O*-α-D-mannopyranosyl]-α-D-mannopyranoside 10-1.

To a 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (**10**) (50 mg, 0.03 mmol) in MeOH (3 mL) was added Na (1 mg, 0.07 mmol) and stirred at 25 ^{o}C for 30 min. The reaction mixture was neutralized with Dowex-ion-exchange resin and filtered through Celite pad. purified by solid phase extraction using fluoro flash column. The solvent was removed under reduced pressure to obtain deacetylated product. To a sluotion of deacetylated product in MeOH (3mL) was added 10 % Pd/C (50 mg). The reaction mixture was stirred at 25 ^{o}C under hydrogen atmosphere for 12 h. The desired (**10-1**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (26 mg, 95 %).

^{1}H NMR (D_2O, 400 MHz): δ (ppm) [4.95 (s, 1H), 4.87 (s, 1H), 4.29 (d, 1H, *J* = 8 Hz), $CH_{anomeric}$)], 3.92-3.33 (m, 26H), 1,44-1,42 (m, 2H), 1.22-1.20 (m, 2H), 0.76-0.73 (m, 2H).

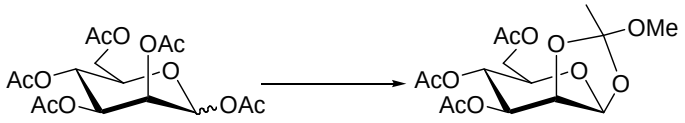
^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [103.9, 102.8, 98.7 ($CH_{anomeric}$)], 78.0, 77.7, 75.7, 73.8, 73.6, 71.8, 71.8, 71.4, 71.1, 70.6, 70.5, 69.8, 69.0, 68.9, 67.6, 67.2, 61.8, 61.2, 61.0, 60.2, 31.5, 27.6, 27.4,19.3. 13.0.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{33}H_{45}F_{17}NaO_{17}$ 1059.2283; Found, 1059.3657.



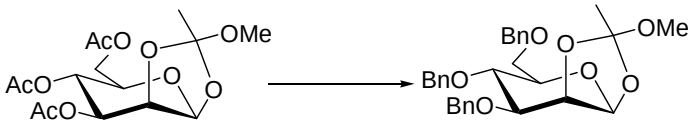
Synthesis of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranoside

To a solution of monnose (1 g, 5.6 mmol) in acetic anhydride (5 mL) was added catalytic amount of iodine (50 mg, 0.2 mmol) at 25 ^oC. The reaction mixture was stirred at room temperature for 1 h. The dark brown reaction mixture was poured into a separatory funnel containing dichloromethane, aqueous sodium thiosulfate solution and crushed ice. The colorless solution from a separatory funnel was washed with H_2O (2 x 50 mL) and sat.$NaHCO_3$ (2 x 50 mL). It was dried with Na_2SO_4 and then concentrated under reduced vacuum to obtain the desired product **1** with quantitative yield.



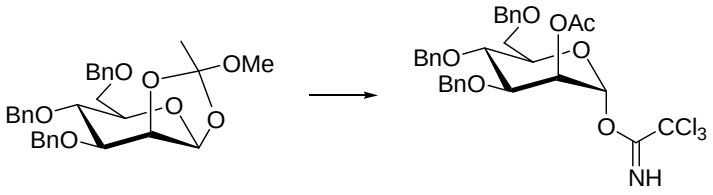
Synthesis of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidiene)-α-D-mannopyranoside

To a solution of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranoside (2.2 g, 5.6 mmol) in dichloromethane was added HBr (10 mL) for 10 min . The reaction mixture was stirred at 25 ^{o}C for 1 h and then checked TLC to check the completion of reaction. After completion of reaction, the mixture was washed with cold water three times and then followed by washed with saturated sodium bicarbonate solution three times. The neutralized solution was dried with sodium sulfate and concentrated under reduced pressure. The crude product was used for next step without further purification. To a solution of hepta-acetyl lactosyl bromide in dichloromethane was added triethylamine (1.6 mL, 11.2 mmol), Bu_4NBr (2.0 g, 6.2 mmol) and methanol (0.2 mL, 6.7 mmol). The reaction mixture was stirred at 40 ^{o}C for 16 h and then washed with H_2O (1 x 50 mL). The solution was dried with Na_2SO_4 and then concentrated under reduced vacuum. The crude produce was purified to obtain the desired product (1.56 g, 78 % over 3 steps) by silica column chromatography.



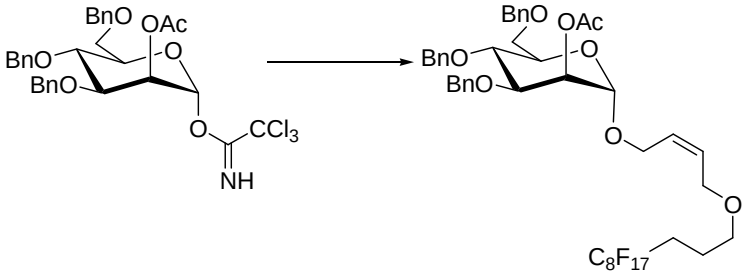
Synthesis of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-α-D-mannopyranoside

To a solution of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidiene)-α-D-mannopyranoside (1g, 2.8 mmol) in MeOH (5 mL) was added catalytic amount of Na (10 mg, 0.4 mmol) . The reaction mixture was stirred at 25 ^{o}C for 2 h and then concentrated under reduced vacuum to yield the crude deacetylated product as yellow foam. To a solution of deacetylated product in DMF (10 mL) was added 60 % NaH in mineral oil (1.33g, 11.2 mmol), followed by addition of benzyl bromide (1.31 mL, 11.2 mmol) and tetrabuyl ammonium iodide (31 mg, 0.08 mmol) at 0 ^{o}C. The reaction mixture was warmed up to 25 ^{o}C and stirred for 12 h. The reaction mixture was diluted with dichloromethane (50 mL) and then washed with H_2O (2 x 50mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude product was purified by flash silica column chromatography to afford the desired product (1.28 g, 92 %).



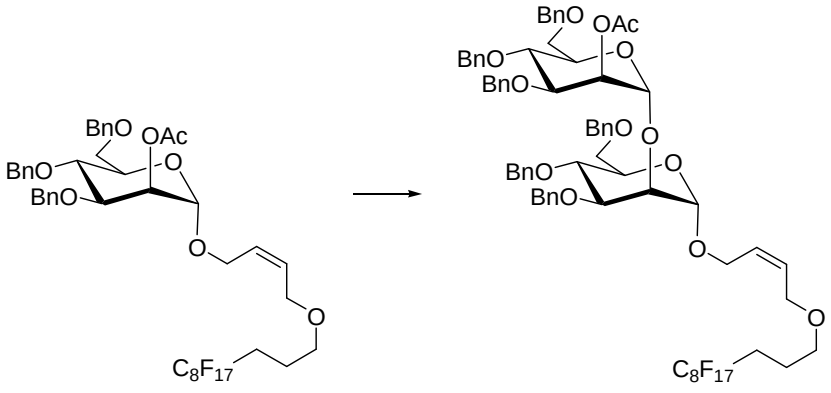
Synthesis of 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate 8 [16]

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-α-D-mannopyranoside (1g, 2 mmol) was dissolved in 80 % acetic acid (10 mL) and stirred at 25 ^{o}C for 30 min. The reaction mixture was diluted with dichloromethane (20 mL) and washed with H_2O (3 x 20 mL) followed by with sat.$NaHCO_3$ (1 x 20 mL). The solution was dried with Na_2SO_4 and concentrated under reduced pressure. To a crude residue in dichloromethane was added Cs_2CO_3 (0.32 g, 1 mmol) and followed by addition of trichloroacetonitrile (0.4 mL, 4 mmol) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 30 min and then concentrated under reduced pressure. The crude residue was dried with Na_2SO_4 and purified by flash silica column chromatography to afford the desired product (**8**) (1.15 g, 92 %).



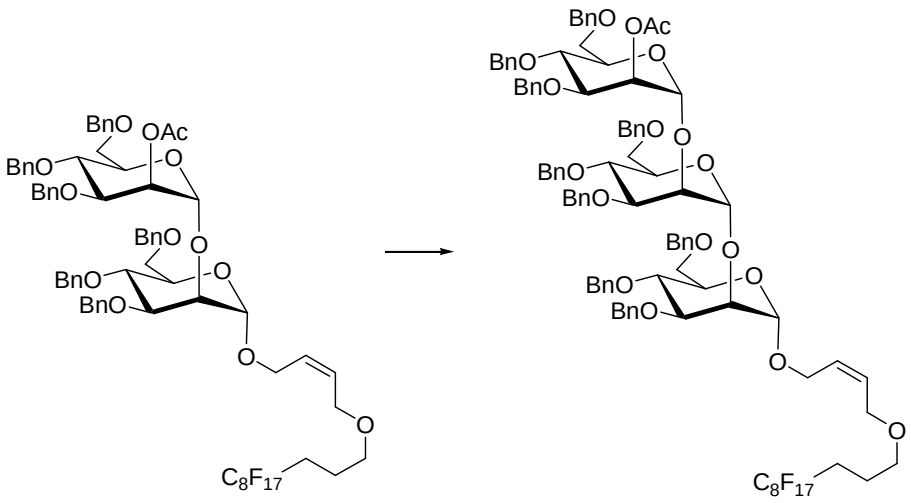
Synthesis of 3-(Perfluorooctyl)propanyloxybutenyl-2-O-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside 12.[21]

To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate (**8**) (0.3 g, 0.47 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (**2**) (0.13 g, 0.24 mmol) in dry dichloroethane (5 mL) was added TMSOTf (8.5 µL, 0.05 mmol) at 25 ^{o}C. The reaction mixture was stirred at room temperature for 30 min. The reaction was quenched with triethylamine (0.5 mL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using 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 desired product (**12**) (0.20 g, 84 %).



Synthesis of 3-(Perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)- α-D-mannopyranoside 13[21].

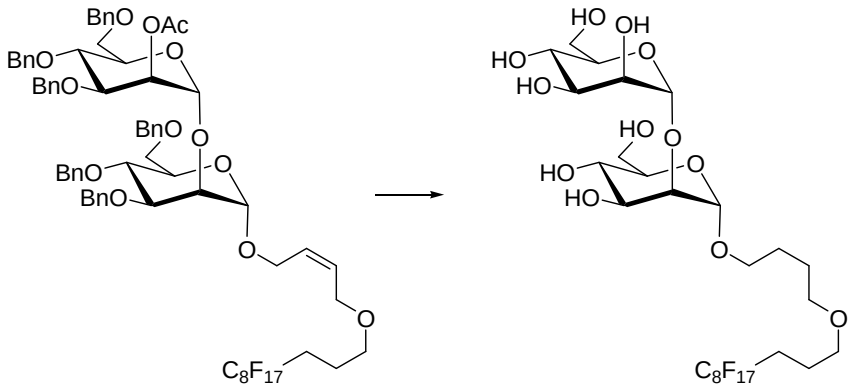
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-2-O-acetyl-3,4,6-O-benzyl-α-D-mannopyranoside (**12**) (0.2 g, 0.2 mmol) in MeOH (5 mL) was added Na (4 mg, 0.2 mmol) and stirred at 25 ^{o}C for 30 min. The crude product was purified by solid phase extraction using fluoro flash column. Nonfluorous 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 desired product (0.18 g, 94 %). To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-O-benzyl-α-D-mannopyranoside (0.18g, 0.19 mmol) and of 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate (0.13 g, 0.21 mmol) in toluene (5 mL) was added TMSOTf (2 µL, 0.11 mmol) at 25 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 10 min. The reaction was quenched with triethylamine (30 µL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using fluoro flash column. Nonfluorous compounds were eluted with 5 mL 80 % MeOH/H_2O and the desired product was eluted by 5 mL 100 % MeOH. The solvent was removed under reduced pressure to obtain the desired product (**13**) (0.23 g, 86 %).



Synthesis of 3-(Perfluorooctyl)propanyloxybutenyl-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]- α-D-mannopyranoside 14.[21]

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**13**) (0.2g, 0.14 mmol) in MeOH (5 mL) was added Na (3 mg, 0.14 mmol) and stirred at 25 ^{o}C for 30 min. The crude product was purified by solid phase extraction using 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 desired product (0.18 g, 92 %)

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-*O*-benzyl-α-D-mannopyranosyl-(1-2)-3,4,6-*O*-benzyl- α-D-mannopyranoside (0.18 g, 0.13 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate (91 mg, 0.14 mmol) in toluene (5 mL) was added TMSOTf (2 µL, 0.11 mmol) at 25 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 30 min. The reaction was quenched with triethylamine (30 µL) and concentrated under reduced pressure. The crude product was purified by solid phase extraction using fluoro flash column. Nonfluorous compounds were eluted with 5 mL 80 % MeOH/H_2O and the desired product was eluted by 5 mL 100 % MeOH. The solvent was removed under reduced pressure to obtain the desired product (**14**) (0.21 g, 86 %).



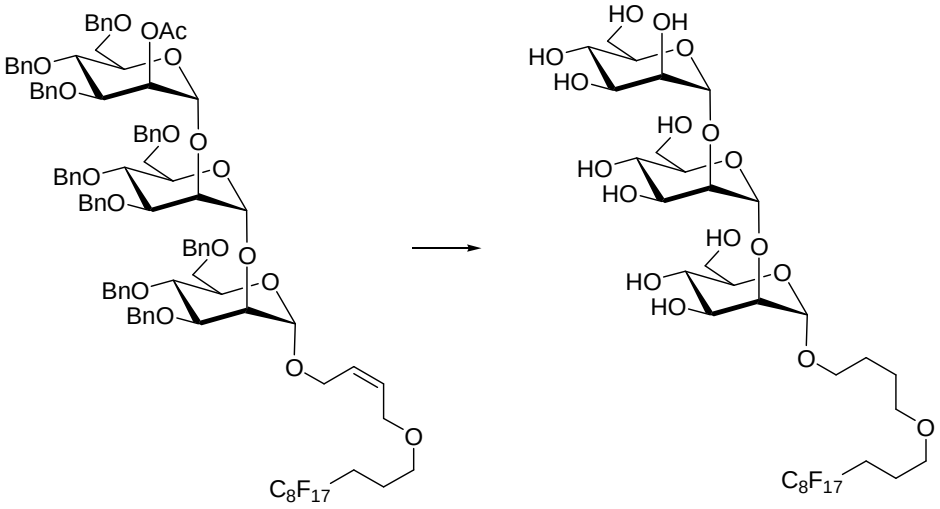
Synthesis of 3-(perfluorooctyl)propanyloxybutanyl--2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranoside 13-1.

To a 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)- α-D-mannopyranoside (**13**) (50 mg, 0.03 mmol) in MeOH (3 mL) was added Na (1 mg, 0.07 mmol) and stirred at 25 ^{o}C for 30 min. The reaction mixture was neutralized with Dowex-ion-exchange resin and filtered through Celite pad. purified by solid phase extraction using fluoro flash column. The solvent was removed under reduced pressure to obtain deacetylated product. To a sluotion of deacetylated product in MeOH (3mL) was added 10 % Pd/C (50 mg). The reaction mixture was stirred at 25 ^{o}C under hydrogen atmosphere for 12 h. The desired (**13-1**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (29 mg, 95 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) [5.06 (s, 1H), 4.96 (s, 1H) $CH_{anomeric}$)], 3.81-3.31 (m, 22H), 2.30-2.18 (m, 2H), 1.87-1.76 (m, 2H), 1.68-1.60 (m, 2H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) [103.0, 98.7 ($CH_{anomeric}$)], 79.5, 73.8, 73.4, 71.2, 71.0, 70.6, 68.9, 67.8, 67.6, 67.1, 61.9, 61.8, 60.2, 27.8, 27.6, 26.4, 26.1.

HRMS-MALDI (m/z): $[M+Na]^{+}$Calcd for $C_{27}H_{35}F_{17}NaO_{12}$ 897.1289; Found, 897.1755.



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-[2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside 14-1.

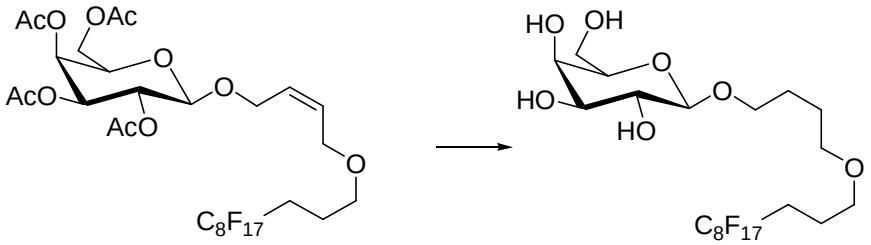
To a 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (**14**) (50 mg, 0.03 mmol) in MeOH (3 mL) was added Na (1 mg, 0.07 mmol) and stirred at 25 ^{o}C for 30 min. The reaction mixture was neutralized with Dowex-ion-exchange resin and filtered through Celite pad. purified by solid phase extraction using fluoro flash column. The solvent was removed under reduced pressure to obtain deacetylated product. To a sluotion of deacetylated product in MeOH (3mL) was added 10 % Pd/C (50 mg). The reaction mixture was stirred at 25 ^{o}C under hydrogen atmosphere for 12 h. The desired (**14-1**) was obtained through

Celite filteration followed by evaporation of solvent under reduced pressure in good yield (26 mg, 93 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) [5.27 (s, 1H), 5.06 (s, 1H), 4.97 (s, 1H) $CH_{anomeric}$)], 4.02-3.03 (m, 29H), 2.31-2.17 (m, 1H), 1.87-1.76 (m, 2H), 1.58-1.51 (m, 1H), 1.43-1.35(m, 1H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) [102.9, 101.3, 98.6 ($CH_{anomeric}$)], 79.7, 79.1, 73.8, 71.2, 71.0, 70.7, 70.5, 68.9, 68.0, 67.8, 67.6, 67.1, 62.1, 62.0, 61.8, 27.6, 27.4, 26.4, 26.1.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{33}H_{45}F_{17}NaO_{17}$ 1059.2283; Found, 1059.1600.



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-β-D-galactopyranoside 15[22.]

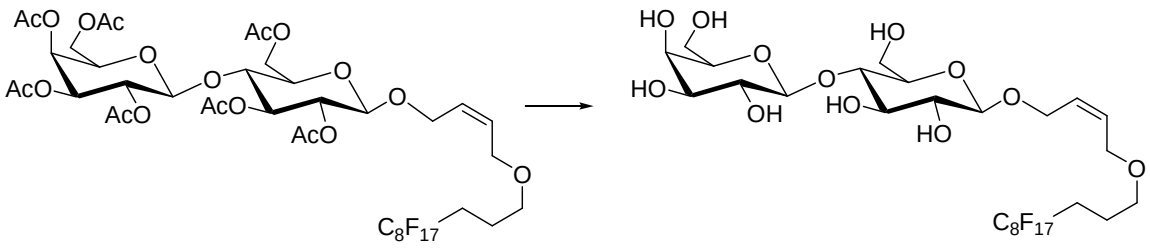
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside[22] (50 mg, 0.06 mmol) in MeOH (3 mL) was added Na (2 mg, 0.14

mmol) and stirred at 25 ^{o}C for 2 h. The reaction mixture was neutralized with Dowex-ion-exchange resin. The desired (**15**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (39 mg, 97 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.21 (s, 1H, *J* = 7.6 Hz $CH_{anomeric}$), 3.92-3.45 (m, 12H), 2.31-2.18 (m, 2H), 1.87-1.80 (m, 2H), 1.72-1.63 (m, 4H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 103.7 ($CH_{anomeric}$), 75.4, 73.8, 71.3, 70.5, 69.2, 69.0, 68.8, 61.2, 26.3, 26.1.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{21}H_{25}F_{17}NaO_7$ 735.1227; Found, 735.1095.



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-4-O-β-D-galactopyranosyl)-β-D-glucopyranoside 16.[22]

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside[22] (50 mg, 0.04 mmol) in MeOH (3 mL) was added Na (3 mg, 0.21 mmol) and stirred at 25 ^{o}C for 4 h. The

reaction mixture was neutralized with Dowex-ion-exchange resin. The desired (**16**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (36 mg, 96 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) [4.77 (d, 1H, *J* = 3.6 Hz), 4.34 (s, 1H, *J* = 7.6 Hz) $CH_{anomeric}$], 3.76-3.69 (m, 9H), 3.51-3.48 (m, 11H), 2.32-2.18 (m, 2H), 1.88-1.81 (m, 2H), 1.72-1.63 (m, 2H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) [103.8, 98.7 ($CH_{anomeric}$)], 79.7, 75.9, 73.6, 72.3, 72.3, 72.0, 71.3, 70.9, 70.5, 69.1, 68.9, 67.7, 61.3, 60.6, 26.4, 26.1.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{27}H_{35}F_{17}NaO_{12}$ 897.1755; Found, 897.1510.

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CHAPTER 4

Development of automated synthesis and screening of phosphate-linked *Leishmania*-associated carbohydrates

Eun-Ho Song and Nicola L. Pohl

Introduction

Since fluorous-based automation platform became a reliable tool for automated synthesis of oligosaccharides,[1]automated synthesis of phosphoglycans becomes a challenge due to its unique phosphate-linked structure and function. Unlike glycosylation and deacetylation for the construction of oligosaccharide in an automation platform, forming phosphate-linkage requires three steps; coupling, oxidation and deprotection.

In order to extend the versatility of fluorous-based automation platform, we examined its application for the synthesis of phophoglycan repeats in *Leishmania* surface glycolipid, lipophosphoglycan (LPG).

The role of lipophosphoglycan (LPG) in parasite virulence has been intensively reported in the field of parasite biology due to its importance during macrophage

infection.[2] Nevertheless, their role in parasite virulence is controversial issue.[3, 4] But, now there is no longer any doubt that *Leishmania* cannot survive in sand flies and macrophages without LPG.[5, 6]

As shown in figure 1, the structure of LPG has been characterized by mass spectrometry and NMR spectroscopy.[7, 8] Phsphoglycan repeats are the most abundant *Leishmania*-surface molecules in LPG due to difference in the length distribution among three domains. Moreover, each species has unique structure of phosphoglycan repeats in LPG. For example, *L. donovani* PGs consists of linear [-6Gal*p*- β 1,4-Man*p*- α 1-phosphate]$n_{avg.}$=12 repeats and *L. major* PGs consists of branched [6-(Gal- β 1,3)-Gal*p*- β 1,4- Man*p*- α 1-phosphate] $n_{avg.}$=27 repeats.

Although there have been several studies made on the identification of phosphoglycan repeats functions such as part of epitopes for recognition by macrophage receptors and playing a key role in parasite survival early in the sand fly,[9] these studies were not performed with pure phosphoglycan repeats due to the impossibility to obtain pure phosphoglycan repeats from LPG. Thus, we can only assume that PGs might play a crucial role in parasite virulence. While there has been

increasing interest in the study of PGs function, specific study has been restricted due to the limited access to pure synthesized PGs.

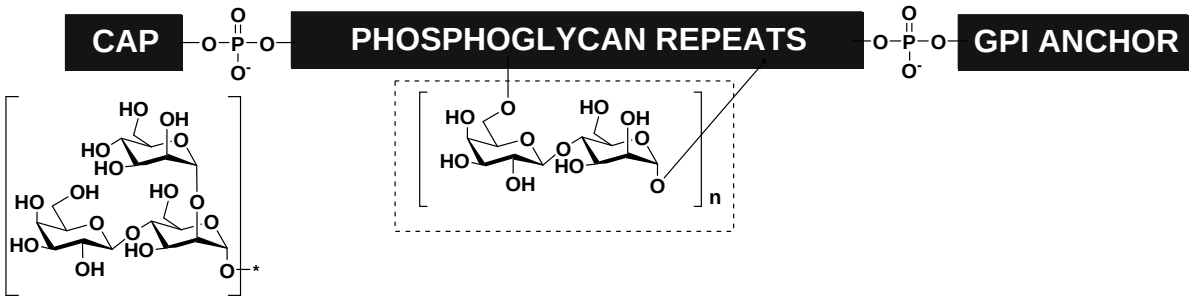


Figure 1. Structure of the lipophosphoglycan of *Leishmania donovani*.

Interestingly, capping structures are also linked to phosphoglycan repeats through phosphate. Like capping structure, phosphate groups might serve as distinct epitopes and profoundly affect antibody binding. Given this thought, it can be hypothesized that phosphate-linkages are involved in synergistic antibody bindings. Fluorous-based microarray[10] can also be used to demonstrate the effect of phosphate-linkages in antibody bindings.

In this regard, rapid and efficient chemical synthesis for the construction of *Leishmania* phosphoglycan repeats and phosphate-linked capping structures has been prerequisite due to its biological interests for unveiling PGs and phosphate specific

functions in parasite virulence and their potential role in immunomodulation for vaccine development.

Several attempts for both chemical synthesis of *L. donovani* PGs[11-13] and *L. major* PGs[14-16] have been made through either using monosaccharide building blocks (galactose and mannose) with multiple steps for protection, deprotection, and glycosylation or disaccharide building block. In the study which used disaccharide building block, iterative synthesis of PGs has been achieved by solid-phase[11], but the use of automation platform for synthesis of PGs has never been reported. Unlike oligonucleotide and peptides synthesis via a synthesizer, phosphate-linked oligosaccharides such as phosphoglycan have never been synthesized by using an automation platform due to the difficulty of managing anomeric stereochemistry and instability of the anomeric phosphodiester. In order to overcome these limitations, we focused on the development of synthetic methods under H-phosphonate chemistry[17] and it led to the discovery of an efficient method for the synthesis of phosphate-linked oligosaccharides.

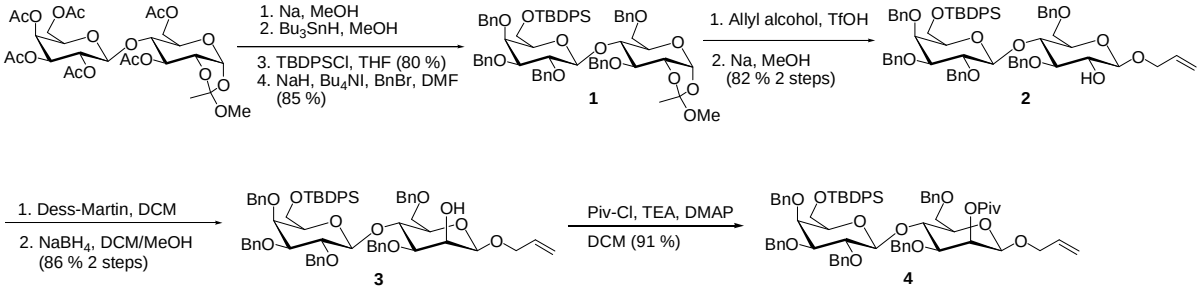
Herein, we reported the first fluorous-based automated synthesis of linear phosphoglycan repeats for *L. donovani* and phosphate-linked *Leishmania*-associated capping structures through a H-phosphonate strategy.

Results and discussion

Synthesis of *L. donovani* phosphoglycan building block

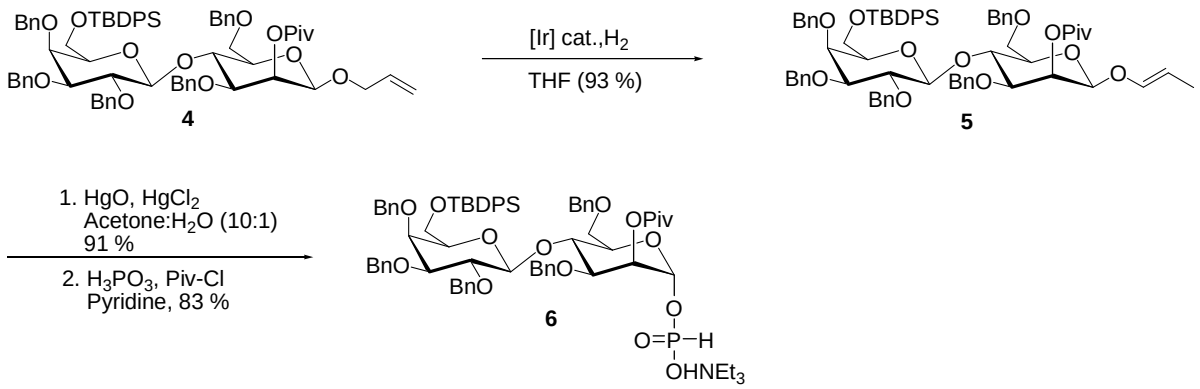
A known intermediate orthoester[18] served as starting material for the synthesis of phosphoglycan donor (Scheme 1). According to the conditions previously developed by Ruhela et al.[11], regioselective silylation of 6-position of galactose on orthester was performed by using Bu_3Sn/MeOH and TBDPSCl in reasonably high yield (80 % 3 steps). Subsequence benzylation gave the benzylated orthoester **1** in 85 % respectively. Lewis acid-mediated allylation by using allyl alcohol and TfOH was followed by reaction with NaOMe to yield 2-*O*-unprotected disaccharide **2** in high yield (82% over 2 steps)**.** With 2-*O*-unprotected disaccharide **2**, conversion of β-glucoside **2** to α-mannoside **3** was successively accomplished under a two-step oxidation-reduction process by using Dess-Martin periodate and subsequence reduction with $NaBH_4$.

Further protection of 2-*O*-unprotected disaccharide **3** with Piv-Cl gave fully protected disaccharide **5** in 91 % respectively.



Scheme 1. Synthesis of *L. donovani* phosphoglycan building block

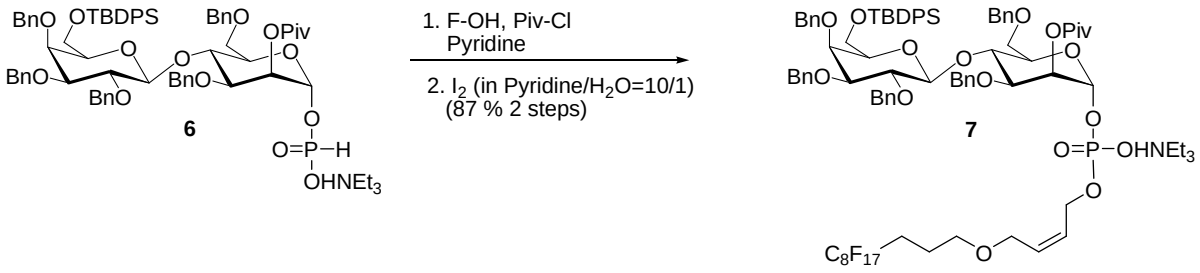
Iridium-catalyzed isomerization[19] of allyl ether **4** was carried out in order to give trans-propenyl ether **5** and then free anomeric-OH was introduced under non-acidic conditions[20] using $HgO/HgCl_2$ to furnish pure α-hemiacetal product in 91 % respectively after removal of β-hemiacetal product through silica column purification.



Scheme 2. Synthesis of *L. donovani* phosphoglycan donor

PCl_3/imidazole reagent[21] has been widely used as phosphonylating agent for formation of H-phosphonate monoester in carbohydrate due to its high reactivity. However, phsphonylation using PCl_3/imidazole requires not only laborious experimental procedure but also very careful handling of PCl_3 which is classified very toxic and corrosive by EPA.

Instead of using PCl_3/imidazole, phosphorous acid/Piv-Cl system enabled us to accomplish phsphonylation of **6** in good yield (83 %) though pivaloyl chloride-mediated coupling reaction[22]. H-phposphonate donor **6** was confirmed by unique large coupling constant of ^{31}P-^{1}H (doublet, J_{HP}= 632.4 Hz in ^{1}H NMR), ^{31}P NMR (1.31 ppm) and HRMS ($[M+Na]^+$Calcd 1177.4898; Found 1177.0978).



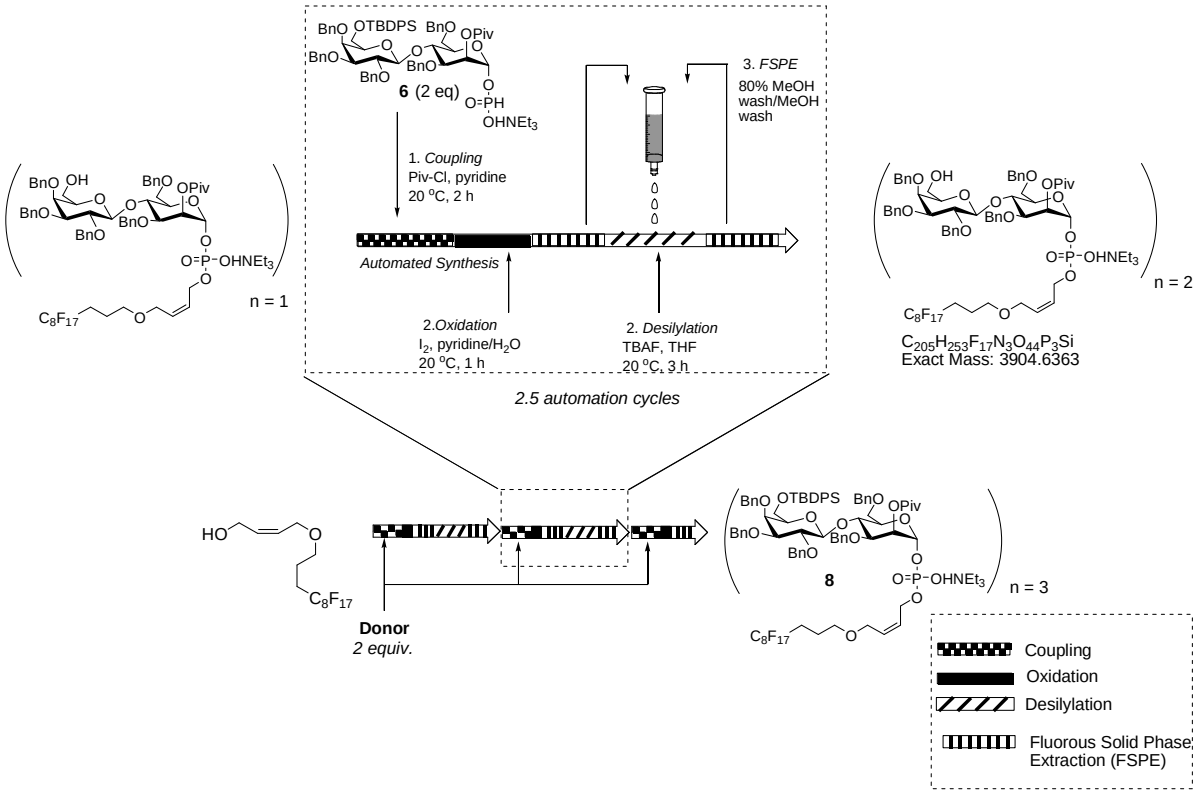
Scheme 3. Synthesis of *L. donovani* phosphoglycan

In order to optimize conditions for the synthesis of phosphodiester prior to run synthesizer, another pivaloyl chloride mediated coupling reaction for the introduction of fluorous-tag was followed by oxidation[23] with iodine in aqueous pyridine to furnish fluorous-tagged β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphodiester **7** in 87 % yield**.**

Automated synthesis of *L. donovani* phosphoglycan repeats.

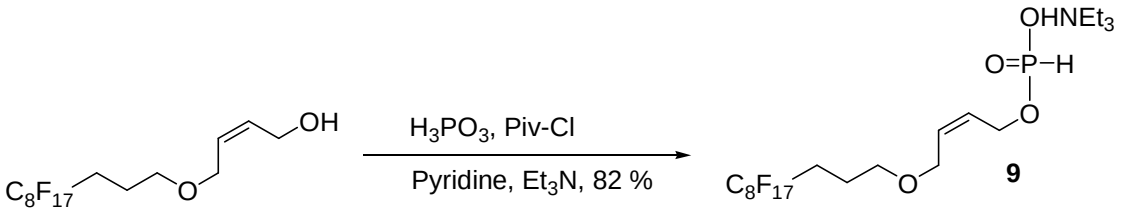
The automated synthesis of *L. donovani* phosphoglycan repeats was designed to carry out 2 and 2/3 cycles including 3 x coupling for the enlogation of disaccharide repeat, 3 x oxidation for the transformation of phosphite to phosphate, 2 x desilylation for the deprotection of TBDPS group and 5 x FSPE for the purification of crude product (Scheme 4). Each reaction cycle was programmed to use only 2 equivalent of

H-phposphonate donor **6** PIv-Cl, iodine and TBAF. As expected, the use of TLC in monitoring reactions enabled us to confirm the completion of each step of the reaction during running synthesizer. After 2.5 automation cycles, 23 mg of crude hexa-phosphoglycan repeats with high purity was obtained from automation platform without further purification. With conventional purification with prep TLC, 19 mg of pure product was provided in overall 10 % yield (75 % per step).



Scheme 4. Automated synthesis of *L. donovani* phosphoglycan repeats

Synthesis of phosphate-linked *Leishmania*-associated capping structures



Scheme 5. Synthesis of H-phosphonate fluorous-tagged alcohol.

α-Hemiacetal products of *Leishmania*-associated capping structures were prepared as reported in previous study.[24] Pivaloyl chloride mediated coupling reaction underwent efficiently to produce H-phosphonate fluorous-tagged alcohol **9** in good yields (82 %). Subsequent coupling reaction of H-phosphonate fluorous-tagged alcohol using phosphorous acid/Piv-Cl with α-hemiacetal intermediates followed by oxidation with iodine in aqueous pyridine resulted in the production of fluorous-tagged *Leishmania* capping phosphodiesters in good yields (81 % ~ 89 %)**.**

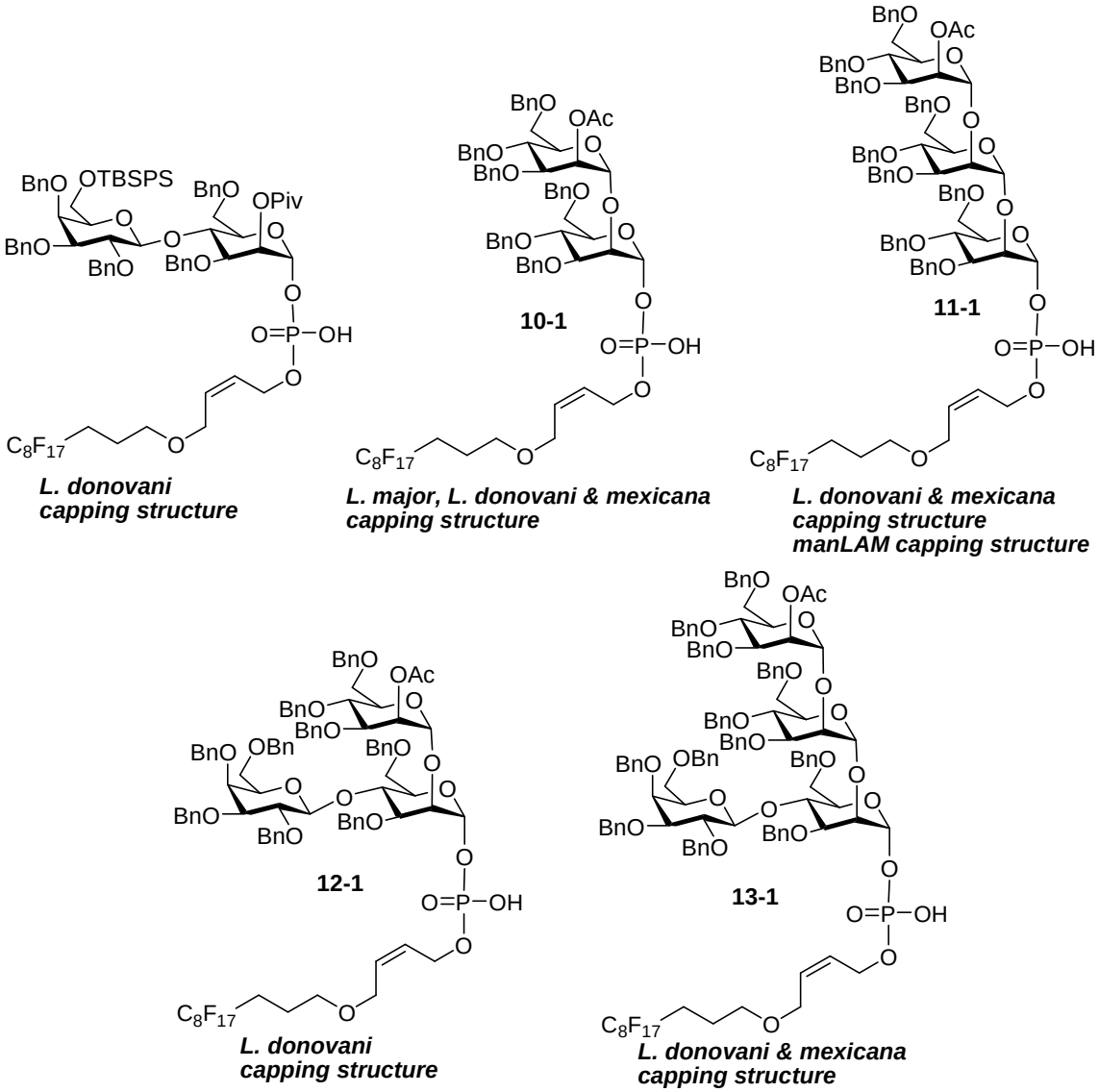


Figure 2. Phosphate-linked *Leishmania*-associated capping structures.

Conclusion

In conclusion, the first automated synthesis of *L. donovani* phosphoglycan repeats demonstrated that phosphate-linked oligosaccharide can be readily available in the

newly developed fluorous-based automation platform within a short period, with low cost and less laborious work. More importantly, the synthesis of phosphate-linked *Leishmania*-associated capping structures for *L. donovani* and *L. major* has led to advances in understanding of the role of phosphate in antibody bindings. The study for the detection of glycolipid specific antibodies in serum from *Leishmania* infected animals using phosphate-linked *Leishmania*-associated capping structures will be exploited.

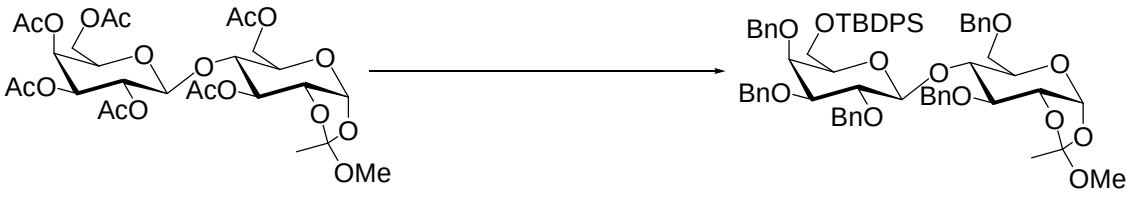
Experimental section

General methods

Solvents were reagent grade and in most cases dried prior to use. All other commercially available reagents were used as received unless otherwise noted. The organic extracts were dried over anhydrous $MgSO_4$. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$) prior to use. Methylene chloride (CH_2Cl_2), and triethylamine (Et_3N) were distilled from calcium hydride. Diethyl ether (Et_2O) was distilled from sodium-benzophenone ketyl.

^1H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on Varian VXR-

400 NMR or on Bruker DRX-400 NMR. Mass spectra (MS) were recorded on an Applied Biosytems DE-Pro MALDI mass analyzer or an Applied Biosytems QSTAR® XL Hybrid LC/MS/MS System. Chemical shifts are reported in parts per million downfield relative to tetramethylsilane (δ 0.00) and coupling constants are reported in Hertz (Hz). The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; and br = broad.



Synthesis of 2,3,4-Tri-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside 1.

To a solution of 2,3,4,6,-tetra-*O*-acetyl-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-acetyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside[18] (2 g, 3.1 mmol) in MeOH (10 mL) was added catalytic amount of Na (140 mg, 6.2 mmol). The reaction

mixture was stirred at 25 ^{o}C for 3 h and then was filtered through a Celite pad. The crude deacetylated product as yellow foam was obtained after concentration of reaction mixture under reduced vacuum. To a solution of deacetylated product in anhydrous MeOH (20 mL) was added Bu_2SnO (0.77 g, 3.1 mmol) and then the reaction mixture was heated to reflux for 4 h followed by evaporation of solvent. To a solution of a dibutyltin compound in anhydrous THF was added TBDPSCl (0.72 mL, 3.1 mL) and then the reaction mixture was stirred at 25 ^{o}C for 48 h. The solvent was concentrated under reduced vacuum. The crude product was purified by flash silica column chromatography (3 % MeOH in DCM) to afford the desired product 2,3,4-tri-*O*-hydroxy-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-hydroxy-β-D-glucopyranoside (1.88 g, 80 % over 2 steps). To a solution of TBDPS-protected product (1.88 g, 3.0 mmol) in DMF (10 mL) was added 60 % NaH (0.99 g, 30 mmol), followed by addition of benzyl bromide (3.5 mL, 30 mmol) and tetrabuylammonium iodide (1.1 g, 3.0 mmol) at 0 ^{o}C. The reaction mixture was warmed up to 25 ^{o}C and stirred for 12 h. The reaction mixture was diluted with dichloromethane (20 mL) and then washed with H_2O (2 x 20mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude product

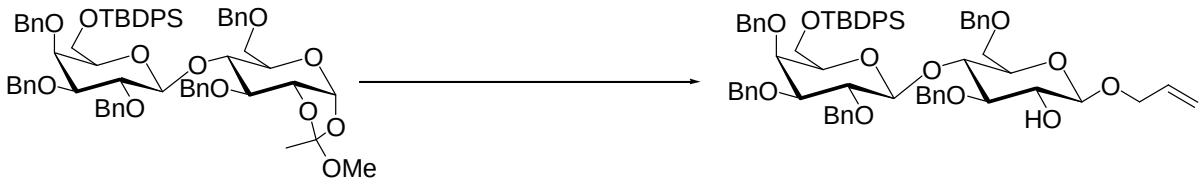
was purified by flash silica column chromatography to afford the desired product **1** (2.73 g, 85 % over 2 steps).

R_f (ethyl acetate/haxane): 0.83 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.54-7.07 (m, 35H), 5.67 (d, 1H, *J* = 5.2 Hz), 5.00 (d, 1H, *J* = 9.6 Hz), 4.85-4.77 (m, 4H), 4.63 (d, 1H, *J* = 16 Hz), 4.59 (d, 1H, *J* = 15.2 Hz), 4.55 (d, 1H, *J* = 12.8 Hz), 4.40 (t, 1H, *J* = 11.2 Hz), 4.27 (m, 1H), 4.18 (d, 1H, *J* = 8 Hz), 4.03 (br, 1H), 3.95-3.86 (m, 3H), 3.75-3.58 (m, 5H), 3.72 (dd, 1H, *J* = 8.8, 2.4 Hz), 3.28-3.25 (m, 1H), 3.22 (s, 3H), 1.54 (s, 3H), 1,02 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.3, 139.1, 138.9, 138.7, 138.3 (C_q-Aryl)], [130.2-127.8 (m, CH-Aryl)] 135.8, 135.7, [105.6, 97.8 ($CH_{anomeric}$)], 82.3, 79.7, 75.3, 74.9, 74.3, 73.4, 72.1, 70.3, 69.4, 62.0, 51.2, 27.3, 20.6, 19.5.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{66}H_{74}NaO_{12}Si$, 1109.4847; Found, 1109.6400.



Synthesis of Allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-2-*O*-hydroxy-β-D-glucopyranoside 2.

A solution of 2,3,4-Tri-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl-(1-4)-3,6-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidiene)-β-D-glucopyranoside **1** (2 g, 1.8 mmol) and ally alcohol (1.25 mL, 18 mmol) in dichloromethane (10 mL) was cooled to – 40 ^{o}C and TfOH (10 µL, 0.12 mmol) was added dropwise over 10 min. The reaction mixture was stirred at – 40 ^{o}C for 30 min and allowed to 25 ^{o}C for 1 h followed by addition of triethylamine to neutralize a solution. The crude product was obtained after concentration of solvent under reduced vacuum. To a crude product in MeOH was added Na (41 mg, 1.8 mmol). The reaction mixture was stirred at 25 ^{o}C for 1 h and then concentrated under reduced vacuum. The crude product was purified by flash silica column chromatography to afford the desired product **2** (1.62 g, 82 % over 2 steps).

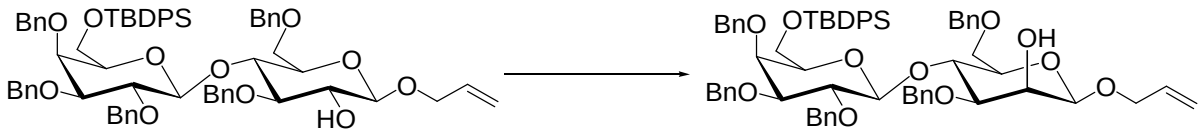
R_f (ethyl acetate/haxane): 0.50 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.56-6.98 (m, 35H), 5.98-5.88 (m, 1H), 5.32 (dd, 1H, *J* = 17.2, 1.6 Hz), 5.21 (dd, 1H, *J* = 10.4, 1.2 Hz), 5.07 (d, 1H, *J* = 11.2 Hz), 5.02 (d, 1H, *J* = 10.8 Hz), 4.81-4.73 (m, 3H), 4.60-4.54 (m, 3H), 4.39-4.30 (m, 4H),

4.13 (dd, 1H, *J* = 12.8, 6.4 Hz), 4.01 (d, 1H, *J* = 2.8 Hz), 3.88 (t, 1H, *J* = 9.2 Hz), 3.86 (d, 1H, *J* = 12 Hz), 3.81-3.64 (m, 4H), 3.446-3.44 (m, 1H), 3.41-3.33 (m, 3H), 3.24 (dd, 1H, *J* = 9.2, 5.2 Hz), 1.03 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.2, 138.9, 138.7, 138.6, 138.4 (C_q-Aryl)], 135.5, 134.0, 133.2, [129.8-127.1 (m, CH-Aryl)], 117.8, [102.7, 101.5 ($CH_{anomeric}$)], 82.8, 82.4, 80.2, 77.4, 76.8, 76.1, 75.5, 75.3, 74.9, 74.3, 73.7, 73.2, 70.0, 68.1, 61.3, 27.0, 19.2.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{66}H_{74}NaO_{11}Si$, 1093.4898; Found, 1093.4898.



Synthesis of Allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-2-*O*-hydroxy-β-D-mannopyranoside 3.

To a solution of allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-2-*O*-hydroxy-β-D-glucopyranoside **2** (1.5 g, 0.14 mmol) in dichloromethane (10 mL) was added dess-martin periodinane (1.2 g, 0.28 mmol). The reaction mixture was stirred at 35 ^oC for 4 h and then diluted with

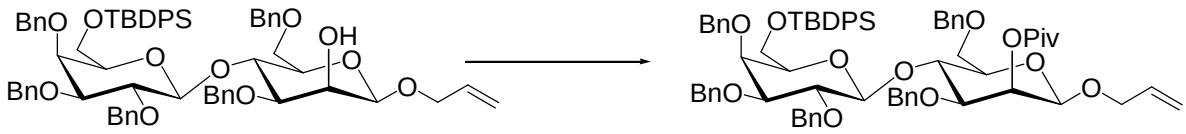
dichloromethane (10 mL). The diluted solution was washed with sat.$NaHCO_3$ (2 x 5 mL), H_2O (1 x 5 mL), and brine (1 x 5 mL). The organic layer was dried with Na_2SO_4 and then concentrated under reduced vacuum. The crude residue was dissolved in dichloromethane/MeOH (1/1) and cooled to 0 ^oC. $NaBH_4$ (0.11 mg, 0.28 mmol) was added and then the reaction mixture was allowed to 25 ^oC over 1 h. The mixture was diluted with dichloromethane (10 mL) and washed with H_2O (1 x 5 mL), 1 % aqueous citric acid (1 x 5 mL) and brine (1 x 5 mL). The solvent was evaporated under reduced vacuum and dried with Na_2SO_4. The crude product was purified by flash silica column chromatography to afford the desired product **3** (1.29 g, 86 % over 2 steps).

R_f (ethyl acetate/haxane): 0.47 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.57-7.06 (m, 35H), 5.94-5.84 (m, 1H), 5.27 (dd, 1H, *J* = 17.2, 1.6 Hz), 5.18 (dd, 1H, *J* = 10.4, 1.2 Hz), 5.05 (d, 1H, *J* = 11.2 Hz), 4.79-4.74 (m, 4H), 4.70 (d, 1H, *J* = 12 Hz), 4.63 (d, 1H, *J* = 15.6 Hz), 4.59 (d, 1H, *J* = 12 v), 4.51 (d, 1H, *J* = 12 v), 4.45 (d, $1H_{anomeric}$, *J* = 0.8 Hz), 4.40-4.35 (m, 2H), 4.39 (d, $1H_{anomeric}$, *J* = 8 Hz), 4.08-3.98 (m, 4H), 3.86 (t, 1H, *J* = 9.2 Hz), 3.79-3.71 (m, 4H), 3.48-3.40 (m, 3H), 3.29 (dd, 1H, *J* = 8.8, 5.2 Hz), 1.01 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [139.2, 138.8, 138.6, 138.5, 138.4 (C_q-Aryl)], 135.5, 133.9, 133.2, [129.8-127.2 (m, CH-Aryl)], 117.7, [103.1, 98.5 ($CH_{anomeric}$)], 82.5, 80.1, 79.1, 75.4, 75.2, 74.6, 74.3, 74.2, 73.7, 73.2, 72.8, 72.6, 69.8, 68.9, 27.0, 19.2.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{66}H_{74}NaO_{11}Si$, 1093.4898; Found, 1093.4898.



Synthesis of Allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside 4.

To a allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)-3,6-di-*O*-benzyl-2-*O*-hydroxy-β-D-mannopyranoside **3** (1 g, 0.9 mmol) in dichloromethane (5 mL) was added DMAP (57 mg, 0.5 mmol), triethylamine (0.62 mL, 0.18 mmol) and trimethyl acetylchloride (0.23 mL, 0.18 mmol). The reaction mixture was stirred at 25 ^oC for 1 h and then concentrated under

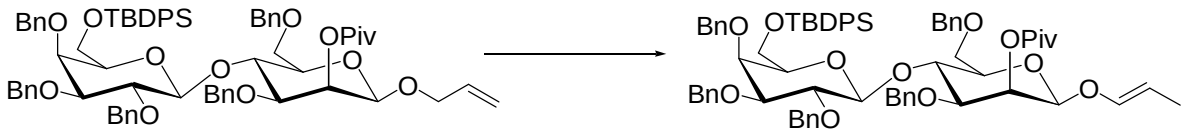
reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product **4** (1.04 mg, 96 %)

R_f (ethyl acetate/haxane): 0.65 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.54-7.00 (m, 35H), 5.89-5.79 (m, 1H), 5.46 (d, 1H, *J* = 3.2 Hz), 5.25 (dd, 1H, *J* = 17.2, 1.6 Hz), 5.15 (dd, 1H, *J* = 10.4, 1.2 Hz), 5.05 (11.6), 4.78-4.69 (m, 4H), 4.60 (d, 2H, *J* = 12 Hz), 4.54 (d, 2H, *J* = 9.6 Hz), 4.48 (d, 1H, *J* = 5.2 Hz), 4.45 (d, 1H, *J* = 12 Hz), 4.32-4.27 (m, 1H), 4.05-3.98 (m, 2H), 3.95 (d, 1H, *J* = 2.4 Hz), 3.84-3.80 (m, 3H), 3.74 (dd, 1H, *J* = 9.6, 8 Hz), 3.55-3.50 (m, 2H), 3.46-3.45 (m, 1H), 3.42 (dd, 1H, *J* = 9.6, 2.8 Hz), 3.22 (dd, 1H, *J* = 8.8, 5.2 Hz), 1.05 (s, 9H), 1.01 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 177.7 (C=O), [139.2, 138.9, 138.7, 138.6, 138.4 (C_q-Aryl)], 135.5, 133.9, 133.2, [129.8-127.0 (m, CH-Aryl)], 117.7, [102.7, 97.6 ($CH_{anomeric}$)], 82.6, 80.1, 77.7, 75.6, 75.1, 74.6, 74.2, 73.9, 73.6, 73.0, 72.9, 71.5, 69.5, 68.8, 68.5, 61.5, 38.9, 27.1, 27.0, 19.2.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{71}H_{82}NaO_{12}Si$, 1178.4788; Found, 1178.4167.



Synthesis of Vinyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside 5.

To a allyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside **4** (1 g, 0.09 mmol) in THF (10 mL) was added catalytic amount of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (10 mg, 0.01 mmol). The stirred solution was degassed, placed under N_2 and degassed. The reaction mixture was placed under H_2 for 5 min and degassed once more to prevent further reduction. The mixture was stirred at 25 ^{o}C for 30 min under N_2 and then concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product **5** (0.93 g, 93 %).

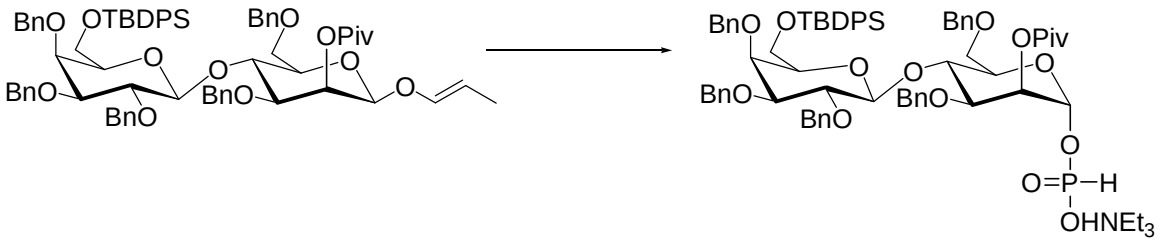
R_f (ethyl acetate/haxane): 0.68 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.54-7.00 (m, 35H), 6.18 (dd, 1H, *J* = 12.4, 1.6 Hz), 5.46 (d, 1H, *J* = 2.4 Hz), 5.04-4.99 (m ,1H), 5.02 (d, 1H, *J* = 11.6 Hz), 4.76-

4.67 (m, 6H), 4.58-4.40 (m, 7H), 3.97 (t, 1H, *J* = 9.2 Hz), 3.93 (d, 1H, *J* = 2.4), 3.81-3.77 (m, 4H), 3.73 (dd, 1H, *J* = 9.6, 8 Hz), 3.54-3.48 (m, 4H), 3.42 (dd, 1H, *J* = 9.6, 2.8 Hz), 3.21 (dd, 1H, *J* = 8.8, 4.8 Hz), 1.50 (dd, 3H, *J* = 6.8, 1.2 Hz), 1.03 (s, 9H), 0.99 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 177.8 (C=O), 143.8, [139.5, 139.2, 139.0, 138.9, 138.7 (C_q-Aryl)], 135.9, 133.5, 133.5, [128.8-127.4 (m, CH-Aryl)], 104.4, [103.1, 98.0 ($CH_{anomeric}$)], 82.6, 80.1, 77.8, 76.2, 75.5, 75.0, 74.6, 74.1, 74.0, 73.3, 73.3, 72.0, 69.0, 68.5, 61.8, 39.3, 27.5, 27.4, 19.6, 12.8..

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{71}H_{82}NaO_{12}Si$, 1178.4788; Found, 1178.8628.



Synthesis of Triethylammonium (2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl hydrogen phosphonate 6.

To a vinyl *O*-(2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranoside **5** (0.9 g, 0.8 mmol), and mercury oxide (0.24 g, 0.1 mmol) in 10 mL of acetone/H_2O (10/1) was added a solution of mercuric chloride (0.23 g, 0.9 mmol) in 5 mL of acetone/H_2O (10/1) for 1 min. The reaction mixture was stirred at 25 ^oC for 3 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the hydrolyzed product (0.79 g, 91 %). Hydrolyzed compound (0.7 g, 0.6 mmol) and phosphonic acid (57 mg, 0.7 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (5 mL) was added a solution of pivaloyl chloride (0.085 mL, 0.7 mmol) in pyridine (2 mL) and then the mixture was stirred at 25 ^oC for 30 min under nitrogen gas environment. Prydine was removed under reduced vacuum and the crude product was purified by flash silica column chromatography (with triethylamine) to afford the desired product **6** (0.86 g, 83 %).

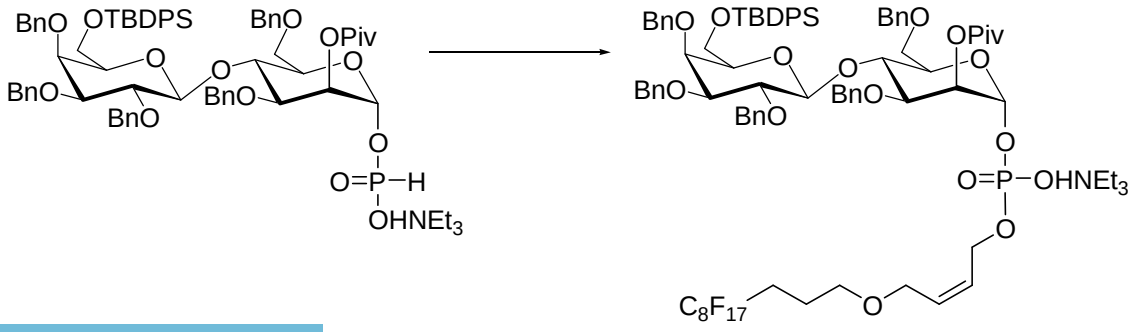
R_f (10 % MeOH in DCM): 0.5.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.51-7.15 (m, 35H), 6.98 (d, 1H, J_{HP} = 685 Hz), 5.53 (d, 1H, *J* = 8.8 Hz), 5.30 (s, 1H), 5.06 (d, 1H, *J* = 11.2 Hz), 4.80-4.70 (m, 5H), 4.66-4.56 (m, 4H), 4.46 (d, 1H, *J* = 11.2 Hz), 4.38-4.35 (m, 2H), 4.14 (t, 1H, *J* = 9.6 Hz), 4.02-3.90 (m, 5H), 3.80-3.70 (m, 3H), 3.66 (d, 1H, *J* = 9.6 Hz), 3.44 (dd, 1H, *J* = 9.2 Hz), 3.38 (dd, 1H, *J* = 9.6, 2.4 Hz), 0.99 (s, 9H), 0.93 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 177.5 (C=O), [139.4, 139.2, 139.0, 138.0, 138.9 (C_q-Aryl)], 135.6, 133.4, 133.4, [129.9-126.8 (m, CH-Aryl)], [103.3, 93.5 ($CH_{anomeric}$)], 82.7, 80.2, 75.5, 75.3, 74.8, 74.2, 74.1, 74.0, 73.1, 73.1, 73.0, 72.4, 68.6, 45.9 (NEt_3), 39.0, 27.3, 27.1, 19.4, 9.2(NEt_3).

HRMS-MALDI (m/z): $[M\text{-}NEt_3\text{-}H]^-$Calcd for $C_{68}H_{78}O_{14}PSi$, 1177.4898; Found, 1177.0978.

^{31}P NMR (162 MHz, $CDCl_3$): δ 1.31.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-*α*-D-mannopyranosyl phosphate triethylammonium salt 7.

Triethylammonium (2,3,4-tetra-*O*-benzyl-6-O-(tert-butyldiphenylsilyl)-β-D-galactopyranosyl)-(1-4)- 2-*O*-trimetylacetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl hydrogen phosphonate **6** (0.1 g, 0.08 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol[25] (43 mg, 0.08 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.02 mL, 0.16 mmol) in pyridine (0.5 mL) and then the mixture was stirred at 25 ^{o}C for 1 h under nitrogen gas environment. To the reaction mixture was added a solution of iodine (20 mg, 0.008) in pyridine/water mixture (0.2 mL, 10/1) and the reaction mixture was quenched with saturated $Na_2S_2O_3$. Excess pyridine was evaporated under reduced pressure and then the crude product was purified by flash silica column chromatography (with triethyamine) to afford the desired product **7** (0.12 g, 87 %).

R_f (10 % MeOH in DCM): 0.6.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-6.89 (m, 35H), 5.72 (m, 1H), 5.54 (m ,1H), 5.47 (d, 1H, *J* = 8.4 Hz), 5.45 (s, 1H), 5.04 (d, 1H, *J* = 11.2 Hz), 4.78-4.69

(m, 5H), 4.62 (d, 1H, *J* = 7.2 Hz), 4.57-4.54 (m, 3H), 4.49-4.41 (m, 3H), 4.37-4.34 (m, 2H), 4.10 (t, 1H, *J* = 9.6 Hz), 4.02-3.88 (m, 4H), 3.78-3.68 (m, 3H), 3.45-3.33 (m, 4H), 3.13 (dd, 1H, J = 9.2, 4.8 Hz), 2.13-2.09 (m, 2H), 1.80-1.76 (m, 2H), 0.98 (s, 9H), 0.91 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 177.3 (C=O), [139.5, 139.2, 139.1, 138.9, 138.9 (C_q-Aryl)], 135.6, 133.5, 133.4, [129.8-126.7 (m, CH-Aryl)], [103.3, 94.4 ($CH_{anomeric}$)], 82.6, 80.2, 74.2, 74.0, 73.0, 72.5, 70.7, 68.7, 68.7, 61.7, 61.2, 45.9 (NEt_3), 38.9, 28.4, 28.2, 28.0, 27.1, 19.4, 8.7 (NEt_3).

HRMS-MALDI (m/z): $[M\text{-}NEt_3\text{-}H]^-$Calcd for $C_{83}H_{89}F_{17}O_{16}PSi$, 1723.5386; Found, 1722.8274.

^{31}P NMR (162 MHz, CDCl3)δ -1.44

ASW *Leishmania*-phosphoglycan repeats method run

After FSPE, the methanol elution collected in the vial was removed from the instrument and concentrated. Solvent was removed under reduced pressure to obtain the crude product (30 mg) as colorless oil. In order to obtain pure product **8** (19 mg, 10 %) for ^{1}H NMR, ^{13} C NMR and mass spectrum, further purification was performed

using prep TLC.

- 2.5 cycles (24h 56 min 39 sec) completed for the synthesis of phosphoglycan repeats.

Step	Task	Reagent/ Operation	Operation Time
1	Coupling	2 equivalent donor (100 μmol) in 0.5 mL Pyridine, 1 equivalent F-tagged acceptor (50 μmol) in 0.5 mL Pyridine; 1 equivalent Piv-Cl	2 h
2	Oxidation	I_2 in Pyridine/Water (0.3 mL)	
3	Quenching	$Na_2S_2O_3$ (0.2 mL)	1h
4	Evaporation	Add TEA and Toluene	
		70 ^oC	2 h
5	TLC sample	30 μl of crude reaction mixture withdrawn	
6	FSPE		
7	Deprotection	5 equivalent of TBAF solution in THF	3 h
8	TLC sample	30 μl of crude reaction mixture withdrawn	
9	Evaporation	70 ^oC	
10	FSPE preparation Sample	0.4 ml DMF 0.7 ml crude sample transferred to cartridge 4.7 ml 80% methanol wash	

	loading	1.5 ml methanol wash (repeated 3 times)	
	Wash	4.7 ml collected sample transferred to clean vial	45 min
	Wash		
	Transfer	50 ^{o}C	45 min
	Evaporation	2 ml toluene added	
	Transfer	50 ^{o}C	
	Evaporation		

Synthesis of Phosphoglycan repeats 8.

R_f (10 % MeOH in DCM): 0.55.

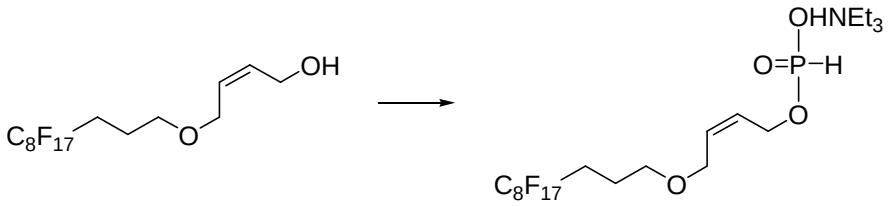
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.50-6.85 (m, 85H), 5.75 (m, 1H), 5.53-5.47 (m, 3 H), 5.41 (s, 1H), 5.34 (s, 1H), 5.04 (d, 1H, *J* = 8 Hz), 4.90 (d, 1H, *J* = 12 Hz), 4.79-4.66 (m, 11H), 4.63-4.48 (m, 12H), 4.45-4.35 (m, 8H), 4.28 (d, 2H, *J* = 12 Hz), 4.19-4.06 (m, 10H), 3.98-3.80 (m, 11H), 3.76-3.67 (m, 5H), 3.63-3.53 (m, 5H), 3.41-3.31 (m, 6H), 3.12-3.09 (m, 2H), 2.15-2.03 (m, 2H), 1.84-1.81 (m, 2H), 1.23 (s, 9H), 1.10 (s, 9H), 0.98 (s, 9H), 0.87 (s, 9H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) [178.2, 177.5, 177.1 (C=O)], 151.0, [139.7-138.7 (C_q-Aryl)], 133.4, 133.3, [129.8-126.7 (m, CH-Aryl)], [103.2,103.2, 103.0, 94.5, 94.3, 94.2 ($CH_{anomeric}$)], 82.6, 82.6, 80.2, 80.2,75.9,75.1, 74.8, 73.9, 72.9, 72.7, 72.4,

72.2, 68.9, 68.7, 68.0, 66.7, 62.7, 61.6, 61.1, 59.2, 57.0, 45.9 (NEt_3), 39.1, 38.9, 31.6, 28.7, 27.3, 27.1, 24.3, 21.0, 19.4, 19.2, 13.9, 18.9, 8.7 (NEt_3).

HRMS-MALDI (m/z): $[M-2NEt_3-3H]^{3-}$Calcd for $C_{189}H_{210}F_{17}NO_{44}P_3Si$, 3641.2936; Found, 3641.3557.

^{31}P NMR (162 MHz, CDCl3)δ -0.76, -1.24, -1.32.

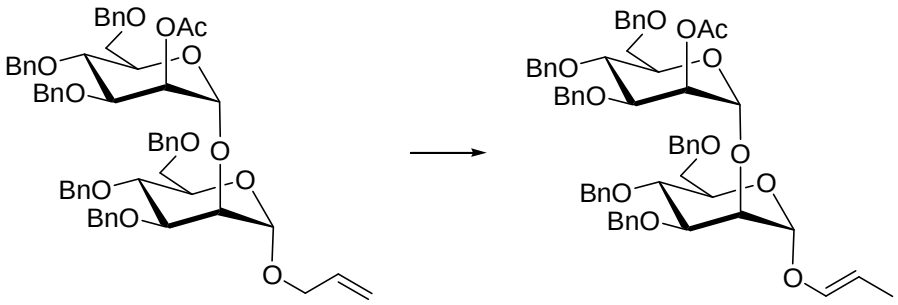


Synthesis of (perfluorooctyl)propanyloxybutenyl- hydrogen phosphonate 9.

(Perfluorooctyl)propanyloxybutenyl alcohol[25] (0.1 g, 0.18 mmol) and phosphonic acid (30 mg, 0.36 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (5 mL) was added a solution of pivaloyl chloride (0.045 mL, 0.36 mmol) in pyridine (2 mL) and then the mixture was stirred at 25 ^{o}C for 30 min under nitrogen gas environment. Prydine was removed under reduced vacuum and the crude product was purified by flash silica column chromatography (with triethylamine) to afford the desired product **9** (0.11 g, 82 %).

R_f (10 % MeOH in DCM): 0.7

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.83 (d, 1H, J_{HP} = 685 Hz), 5.77-5.71 (m, 1H), 5.65-5.59 (m, 1H), 4.46 (t ,1H, *J* = 8 Hz), 4.04 (d, 1H, *J* = 6 Hz), 3.45 (d, 2H, *J* = 6 Hz), 2.21-2.07 (m, 2H), 1.86-1.79 (m, 1H).



Synthesis of Vinyl-O-(3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-Dmannopyranosyl)-α-D-mannopyranoside 10.

To a solution of allyl-*O*-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-Dmannopyranosyl)-α-D-mannopyranoside (0.15 g, 0.08 mmol) in THF (3 mL) was added catalytic amount of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (7 mg, 0.008 mmol). The stirred solution was degassed, placed under N_2 and degassed. The reaction mixture was placed under H_2 for 1 min and degassed once more to prevent further reduction. The mixture was stirred at 25 ^oC for 1 h under N_2 and then

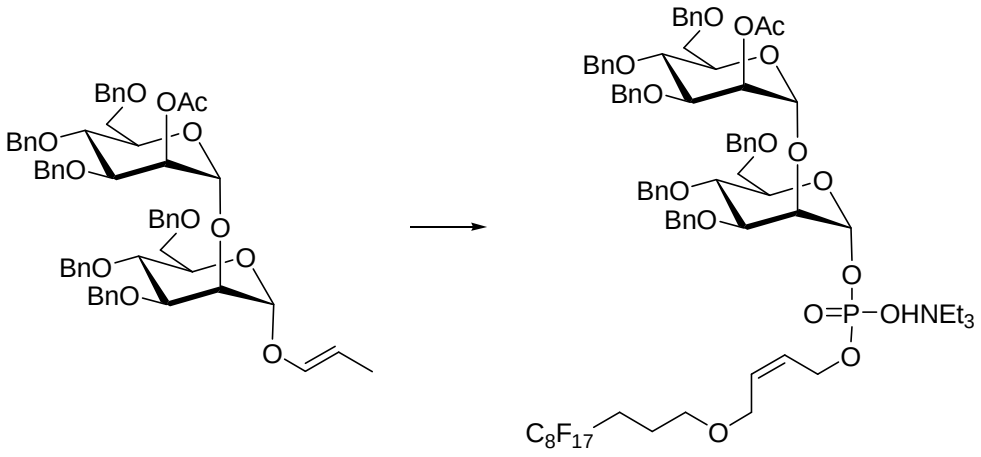
concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product **10** (0.14, 95 %).

R_f (ethyl acetate/haxane): 0.63 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-7.15 (m, 30H), 6.07 (d, 1H, *J* = 12 Hz), 5.54 (s, 1H), 5.12 (d, 1H, *J* = 20 Hz), 5.05-5.05 (m, 1H), 4.85-4.82 (m, 2H), 4.72-4.62 (m, 5H), 4.55 (d, 2H, *J* = 12 Hz), 4.48-4.43 (m, 4H), 4.39 (d, 1H, *J* = 8 Hz), 4.05 (d, 1H, *J* = 16 Hz), 3.96-3.89 (m, 3H) 3.84-3.66 (m, 6H), 2,12 (s, 3H), 1.49 (d, 3H, *J* = 8 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3 (C=O), 142.6, [138.7, 138.6, 138.5, 138.4, 138.2 (C_q-Aryl)], [128.6-127.6 (m, CH-Aryl)], 104.8, [99.9, 98.3 ($CH_{anomeric}$)], 79.7, 78.4, 75.4, 75.3, 74.6, 73.6, 73.4, 72.4, 72.2, 69.3, 69.2, 69.0, 21.4, 12.6.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{59}H_{64}NaO_{12}$, 988.1220; Found, 988.3250.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-Dmannopyranosyl)-α-D-mannopyranosyl phosphate 10-1.

To a vinyl-O-(3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-Dmannopyranosyl)-α-D-mannopyranoside **9** (0.1 g, 0.1 mmol), and mercury oxide (31 mg, 0.14 mmol) in 4 mL of acetone/H_2O (10/1) was added a solution of mercuric chloride (31 mg, 0.11 mmol) in 4 mL of acetone/H_2O (10/1) for 1 min. The reaction mixture was stirred at 25 ^oC for 3 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the hydrolyzed product (90 mg, 94 %).

The hydrolyzed product (50 mg, 0.05 mmol) and triethylammonium 3-(perfluorooctyl)propanyloxybutenyl- hydrogen phosphonate (39 mg, 0.05 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.013 mL, 0.1 mmol) in pyridine (0.5 mL) and then the mixture was stirred at 25 ^{o}C for 30 min under nitrogen gas environment. To the reaction mixture was added a solution of iodine (13 mg, 0.05) in pyridine/water mixture (0.2 mL, 10/1) and the reaction mixture was quenched with saturated $Na_2S_2O_3$. Excess pyridine was evaporated under reduced pressure and then the crude product was purified by flash silica column chromatography (with triethyamine) to afford the desired product **10-1** (67 mg, 82 %).

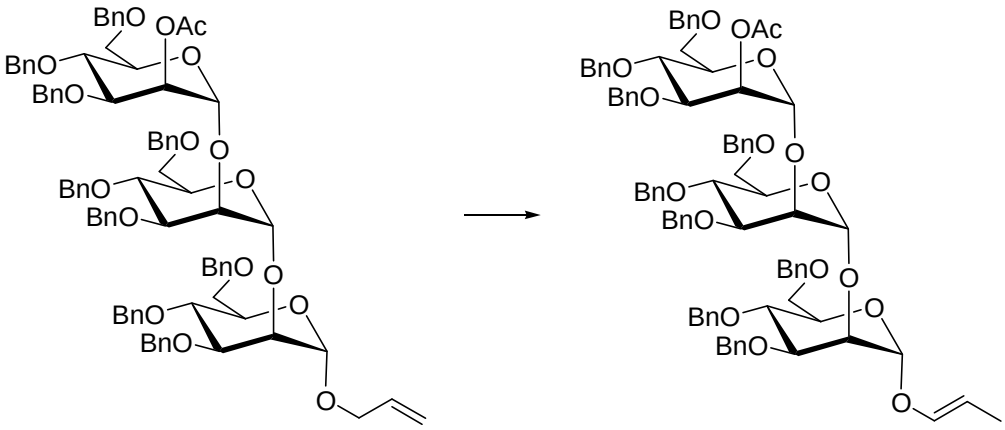
R_f (10 % MeOH in DCM): 0.65

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.28-7.06 (m, 30H), 5.66-5.61 (m, 1H), 5.57-5.54 (m, 1H), 5.50 (s, 1H), 5.05 (s, 1H), 4.78 (t, 1H, *J* = 10.4 Hz), 4.68 (d, 1H, *J* = 12 Hz), 4.63-4.61 (m, 3H), 4.54 (d, 1H, *J* = 16 Hz), 4.47-4.34 (m, 7H), 4.02 (br, 1H), 3.94-3.89 (m, 6H), 3.86 (d, 1H, *J* = 12 Hz), 3.79-3.72 (m, 2H), 3.65 (d, 1H, *J* = 12 Hz), 3.59 (br, 2H), 3.40 (br, 1H), 3.34 (t, 2H, *J* = 8 Hz), 2.14-2.02 (m, 2H), 2.08 (s, 3H), 1.79-1.72 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2 (C=O), [138.4, 138.4, 138.2, 138.0, 138.0 (C_q-Aryl)], [128.5-127.4 (m, CH-Aryl)], [99.7, 95.0 ($CH_{anomeric}$)], 79.2, 78.1, 75.2, 75.1, 74.1, 73.2, 72.8, 72.2, 72.0, 71.9, 68.9, 68.8, 68.7, 68.4, 66.5, 61.8, 28.1, 27.9, 27.5, 21.0.

^{31}P NMR (162 MHz, CDCl3):δ -3.84

HRMS-MALDI (m/z): [M-NEt_3] Calcd for $C_{71}H_{72}F_{17}O_{16}P$, 1535.2684; Found, 1535.4162.



Synthesis of Vinyl-O-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]- α-D-mannopyranoside 11.

To a solution of allyl-O-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-

D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-annopyranoside (0.11 g, 0.08 mmol) in THF (3 mL) was added catalytic amount of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (3 mg, 0.004 mmol). The stirred solution was degassed, placed under N_2 and degassed. The reaction mixture was placed under H_2 for 5 min and degassed once more to prevent further reduction. The mixture was stirred at 25 ^oC for 30 min under N_2 and then concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product **11** (0.103 g, 94 %).

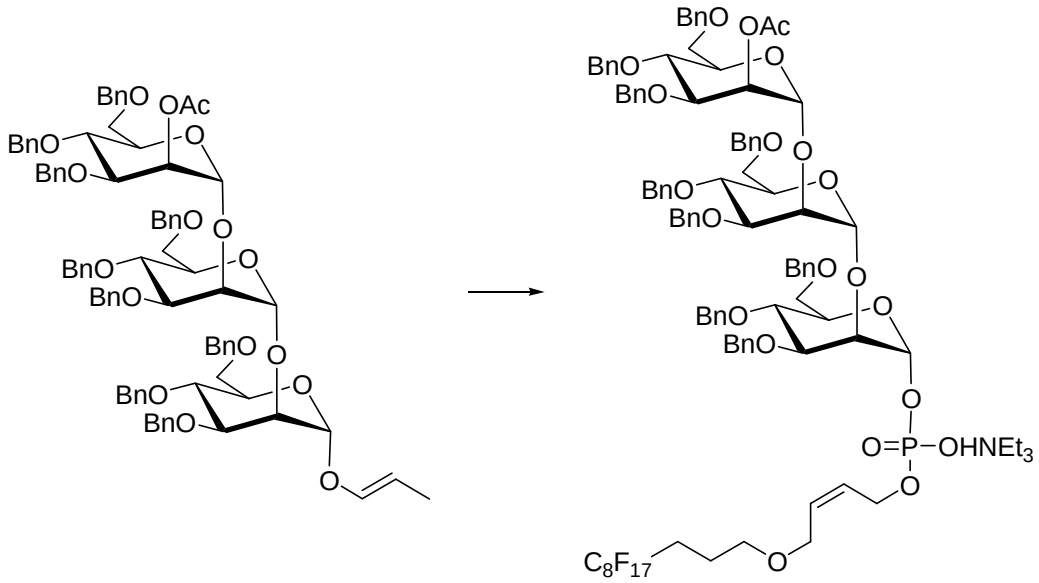
R_f (ethyl acetate/haxane): 0.70 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-7.14 (m, 45H), 6.05 (d, 1H, *J* = 12 Hz), 5.54 (s, 1H), 5.19 (d, 1H, *J* = 8 Hz), 5.06-5.05 (m, 1H), 5.03 (s, 1H), 4.85 (d, 1H, *J* = 12 Hz), 4.70-4.50 (m, 14H), 4.45-4.41 (m, 3H), 4.32 (d, 1H, *J* = 12 v), 4.09 (br, 1H), 4.01-3.89 (m, 9H), 3.83-3.81 (m, 2H), 3.73-3.64 (m, 6H), 3.54 (d, 1H, *J* = 12 Hz), 2.13 (s, 3H), 1.47 (d, 3H, *J* = 4 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3 (C=O), 142.2, [138.8, 138.7, 138.6, 138.6, 138.6, 138.5, 138.4, 138.2 (C_q-Aryl)], [128.6-127.6 (m, CH-Aryl)], 104.6, [100.9, 99.6, 98.3 ($CH_{anomeric}$)], 79.4, 78.3, 75.4, 75.3, 75.2, 74.9, 74.7, 74.5, 73.6,

73.5, 73.4, 72.5, 72.3, 72.1, 69.8, 69.3, 69.1, 69.0, 21.4, 12.6.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{86}H_{92}NaO_{17}$ 1420.6302; Found, 1420.4338.



3-(Perfluorooctyl)propanyloxybutenyl-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]- α-D-mannopyranosyl phosphate 11-1.

To a vinyl-O-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside **11** (0.1 g, 0.07 mmol), and mercury oxide (22 mg, 0.1 mmol) in 3 mL of acetone/H_2O (10/1) was added a solution of mercuric chloride (21 mg, 0.08 mmol) in 3 mL of

acetone/H_2O (10/1) for 1 min. The reaction mixture was stirred at 25 ^oC for 4 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the hydrolyzed product (92 mg, 95 %).

The hydrolyzed product (50 mg, 0.04 mmol) and triethylammonium 3-(perfluorooctyl)propanyloxybutenyl- hydrogen phosphonate (26 mg, 0.04 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.009 mL, 0.08 mmol) in pyridine (0.3 mL) and then the mixture was stirred at 25 ^oC for 30 min under nitrogen gas environment. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.013 mL, 0.1 mmol) in pyridine (0.5 mL) and then the mixture was stirred at 25 ^oC for 30 min under nitrogen gas environment. To the reaction mixture was added a solution of iodine (9 mg, 0.04) in pyridine/water mixture (0.2 mL, 10/1) and the reaction mixture was quenched with saturated $Na_2S_2O_3$. Excess pyridine was evaporated under reduced pressure and then the crude

product was purified by flash silica column chromatography (with triethyamine) to afford the desired product **11-1** (62 mg, 81 %).

R_f (10 % MeOH in DCM): 0.68

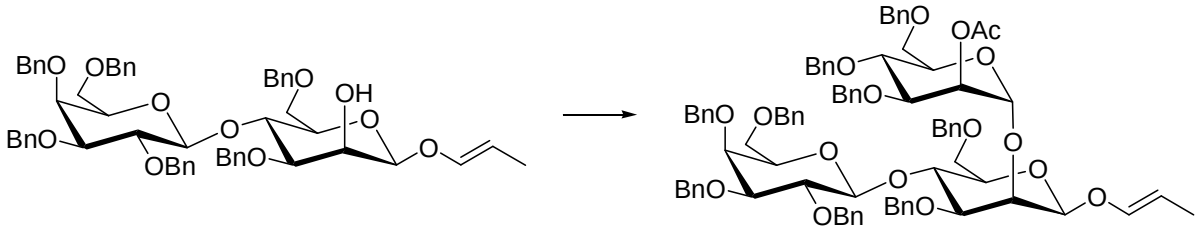
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26-7.09 (m, 45H), 5.64-5.58 (m, 1H), 5.54-5.46 (m, 3H), 5.19 (s, 1H), 5.05 (s, 1H), 4.79 (d, 1H, *J* = 8 Hz), 4.76 (d, 1H, *J* = 8 Hz), 4.67 (d, 1H, *J* = 12 Hz), 4.61 (d, 1H, *J* = 8 Hz), 4.56 (d, 1H, J = 4), 4.53-4.50 (m, 4H), 4.47-4.44 (m, 4H), 4.39-4.32 (m, 4H), 4.25 (d, 1H, *J* = 12 Hz), 4.10 (d, 1H, *J* = 28 Hz), 3.95-3.85 (m, 9H), 3.81 (d, 1H, *J* = 8 Hz), 3.74-3.69 (m, 2H), 3.65-3.56 (m, 4H), 3.44 (d, 1H, *J* = 8 Hz), 3.39 (t, 1H, *J* = 4 Hz), 3.30 (t, 2H, *J* = 8 Hz), 2.12-1.99 (m, 2H), 2.09 (s, 3H), 1.76-1.69 (2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3 (C=O), [138.4, 138.2, 138.2, 138.1, 138.1, 138.0, 137.9, 137.8 (C_q-Aryl)], [128.4-127.4 (m, CH-Aryl)], [100.2, 99.2. 95.1 ($CH_{anomeric}$)], 79.1, 78.2, 75.1, 75.1, 74.5, 74.4, 74.3, 74.2, 74.1, 73.4, 73.2, 72.7, 72.5, 72.1, 72.1, 71.9, 71.8, 69.0, 68.7, 68.5, 61.7, 61.6, 49.2, 46.3 (NEt_3),28.1, 27.8, 27.6, 21.1, 8.6 (NEt_3).

^{31}P NMR (162 MHz, CDCl3): δ -3.36

HRMS-MALDI (m/z): [M-NEt_3] Calcd for $C_{98}H_{100}F_{17}O_{21}P$ 1967.7766; Found,

1967.4717.



Synthesis of Vinyl-*O*-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-β-D-mannopyranoside 12.

To a solution of vinyl *O*-(2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-2-*O*-hydroxy-3,6-di-*O*-benzyl-β-D-mannopyranoside[24] (0.2 mg, 0.2 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzylyl-α-D-mannopyranosyl tricholoroacetimidate (0.15 g, 0.22 mmol) in dry toluene (5 mL) was added TMSOTf (3 µL, 0.02 mmol) at 25 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 10 min. The reaction was quenched with triethylamine (0.1 mL) and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography to afford the desired product **12** (0.27 g, 89 %).

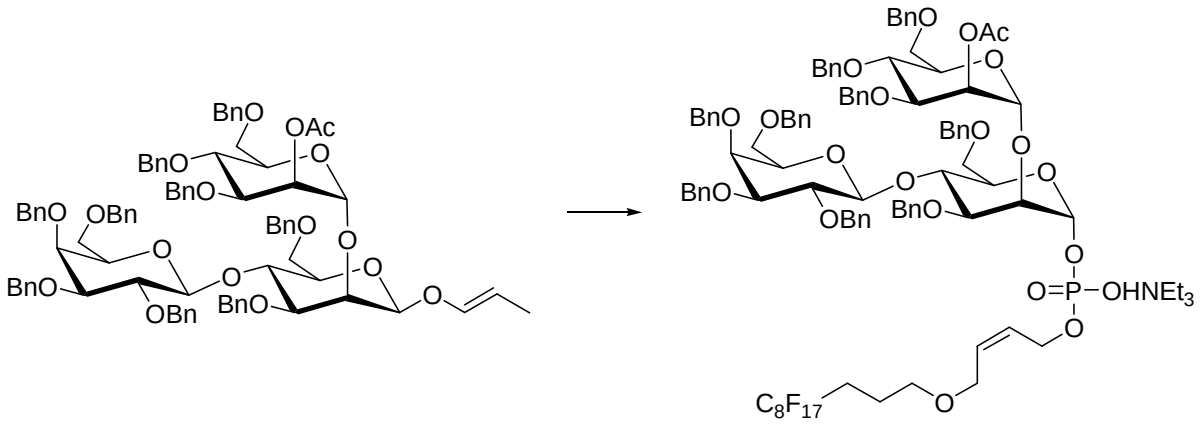
R_f (ethyl acetate/haxane): 0.63 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.14 (m, 45H), 6.22 (d, 1H, *J* = 8 Hz),

5.59 (s, 1H), 5.15 (s, 1H), 5.03-4.98 (m 1H), 4.91-4.79 (m, 4H), 4.72-4.62 (m, 8H), 4.54-4.30 (m, 11H), 4.23 (d, 1H, *J* = 12 Hz), 4.12 (t, 1H, *J* = 8 Hz), 4.05 (dd, 1H, *J* = 8, 4 Hz), 3.91-3.88 (m, 4H), 3.83 (dd, 1H, *J* = 8, 4 Hz), 3.75-3.69 (m, 3H), 3.66-3.45 (m, 6H), 3.40-3.36 (m, 2H), 2.04 (s, 3H), 1.56 (dd, 3H, *J* = 16, 8 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.0 (C=O), 144.0, [139.3, 139.2, 139.0, 139.0, 138.9, 138.7, 138.6 138.4 (C_q-Aryl)], [128.6-127.1 (m, CH-Aryl)], 104.1, [103.5, 99.9, 98.8 ($CH_{anomeric}$)], 82.9, 80.2, 79.9, 78.9, 76.2, 75.5, 75.1, 74.8, 74.6, 73.7, 73.6, 73.4, 73.1, 72.7, 72.3, 71.7, 69.4, 69.2, 68.9, 68.4, 21.4, 12.7.

HRMS-MALDI (m/z): $[M]^+$Calcd for $C_{86}H_{92}O_{17}$, 1397.6405; Found, 1397.3901.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl]-α-D-mannopyranosyl phosphate 12-1.

To a vinyl-*O*-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside **11** (0.1 g, 0.07 mmol), and mercury oxide (22 mg, 0.01 mmol) in 5 mL of acetone/H_2O (10/1) was added a solution of mercuric chloride (21 mg, 0.08 mmol) in 5 mL of acetone/H_2O (10/1) for 1 min. The reaction mixture was stirred at 25 ^oC for 3 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the hydrolyzed product (85 mg, 88 %).

The hydrolyzed product (50 mg, 0.04 mmol) and triethylammonium 3-(perfluorooctyl)propanyloxybutenyl- hydrogen phosphonate (26 mg, 0.04 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.009 mL, 0.08 mmol) in pyridine (0.3 mL) and then the mixture was stirred at 25 ^oC for 30 min under nitrogen gas environment. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.013 mL, 0.1 mmol) in pyridine (0.5 mL) and

then the mixture was stirred at 25 ^{o}C for 30 min under nitrogen gas environment. To the reaction mixture was added a solution of iodine (9 mg, 0.04) in pyridine/water mixture (0.2 mL, 10/1) and the reaction mixture was quenched with saturated $Na_2S_2O_3$. Excess pyridine was evaporated under reduced pressure and then the crude product was purified by flash silica column chromatography (with triethyamine) to afford the desired product **12-1**(63 mg, 83 %).

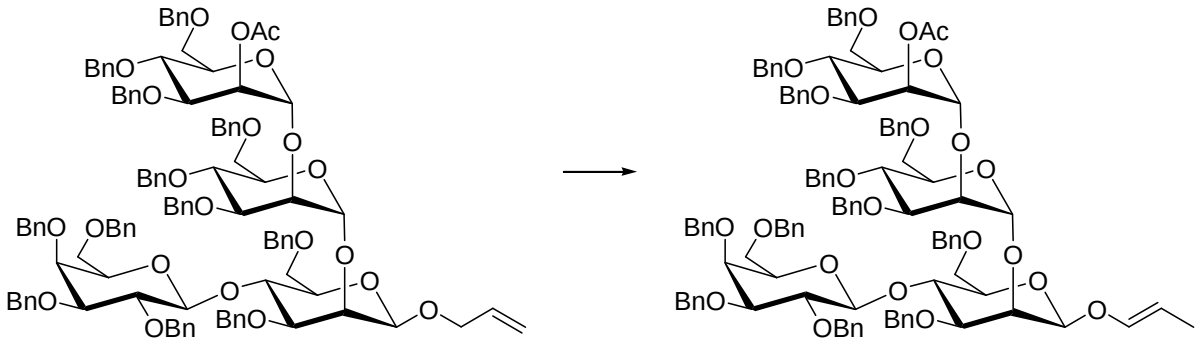
R_f (10 % MeOH in DCM): 0.68

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26-7.14 (m, 45H), 5.66-5.64 (m, 1H), 5.56-5.45 (m, 3H), 5.09 (d, $1H_{anomeric}$, *J* = < 1 Hz), 4.97 (d, 1H, *J* = 12), 4.76 (d, 1H, *J* = 12 Hz), 4.76 (d, 1H, *J* = 12 Hz), 4.67-4.56 (m, 5H), 4.52-4.35 (m, 8H), 4.31-4.24 (m, 4H), 4.17 (d, 1H, *J* = 8 Hz), 4.12 (t, 1H, *J* = 8 Hz), 4.03 (br, 1H), 3.94 (m, 8H), 3.74-3.60 (m, 5H), 3.51 (t, 1H, *J* = 8 Hz), 3.40-3.37 (m, 3H), 3.33 (t, 2H, *J* = 8 Hz), 2.15-2.06 (m, 2H), 2.01 (s, 3H), 1.79-1.72 (m, 2H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.8 (C=O), [139.1, 139.0, 138.8, 138.4, 138.3, 138.1, 138.1, 138.0, 137.9 (C_q-Aryl)], [128.3-127.0 (m, CH-Aryl)], [103.2, 99.6. 95.0 ($CH_{anomeric}$)], 82.0, 79.9, 78.4, 75.9, 75.2, 74.6, 74.0, 73.3, 73.2, 73.0, 72.7, 72.4, 71.9, 71.8,68.7, 66.5, 61.8, 61.7,46.2, 28.1, 27.7, 27.1, 21.0.

^{31}P NMR (162 MHz, CDCl3): δ -2.99

HRMS-MALDI (m/z): [M-NEt_3] Calcd for $C_{98}H_{100}F_{17}O_{21}P$ 1967.7766; Found, 1967.3716.



Synthesis of Vinyl-*O*-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside 13.

To a solution of allyl-*O*-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (0.2 g, 0.11 mmol) in THF (5 mL) was added catalytic amount of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (10 mg,

0.011 mmol). The stirred solution was degassed, placed under N_2 and degassed. The reaction mixture was placed under H_2 for 5 min and degassed once more to prevent further reduction. The mixture was stirred at 25 ^oC for 30 min under N_2 and then concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the desired product **13** (0.19 g, 93 %).

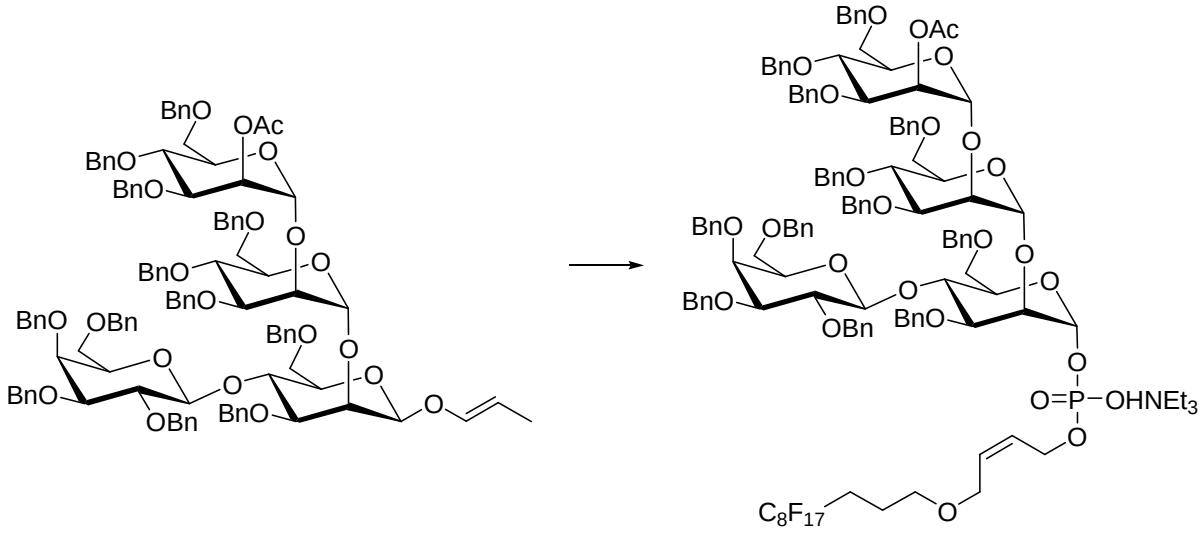
R_f (ethyl acetate/haxane): 0.70 (25/75)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.30-7.16 (m, 60H), 6.18 (d, 1H, *J* =12 Hz), 5.50 (s, 1H), 5.17 (s, 1H), 5.02-4.98 (m 1H), 4.93 (t, 2H, *J* = 6.4 Hz), 4.81 (t, 2H, *J* = 10.4 Hz), 4.74 (br, 2H), 4.66-4.65 (m, 4H), 4.63 (d, 1H, *J* = 10.4 Hz), 4.58 (d, 1H, *J* = 11.2 Hz), 4.51-4.40 (m, 7H), 4.35 (d, 1H, *J* = 10.8 Hz), 4.27-4.25 (m, 2H), 4.21-4.18 (m, 2H), 4.06 (t, 1H, *J* = 8 Hz), 4.01-3.90 (m, 7H), 3.86 (d, 2H, *J* = 9.2 Hz), 3.80-3.71 (m, 3H), 3.66 (d, 1H, *J* = 10.4 Hz), 3.61-3.48 (m, 6H), 3.45 (dd, 1H, *J* = 7.2, 2 v), 3.38 (d, 2H, *J* = 4.4 Hz), 2.07 (s, 3H), 1.51 (d, 3H, *J* = 6.8 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2 (C=O), 144.0, [139.1, 139.1, 138.9, 138.9, 138.8, 138.8, 138.6, 138.3, 138.2 (C_q-Aryl)], [128.5-127.3 (m, CH-Aryl)], 103.9, [103.0, 100.4, 99.6, 98.7 ($CH_{anomeric}$)], 80.0, 79.7, 79.2, 78.4, 75.8, 75.4, 75.1, 74.9, 74.6, 74.6, 73.5, 73.4, 73.3, 73.0, 72.9, 72.8, 72.2, 72.1, 72.1, 69.5, 69.4, 69.3,

69.1, 68.5, 21.4, 12.6.

HRMS-MALDI (m/z): $[M]^+$Calcd for $C_{113}H_{120}O_{22}$, 1828.8271; Found, 1828.4955.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranosyl phosphate 13-1.

To a vinyl-*O*-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside **12** (0.1 g, 0.06 mmol), and mercury oxide (17 mg, 0.14 mmol) in 4 mL of acetone/H_2O (10/1) was added a solution of mercuric

chloride (16 mg, 0.06 mmol) in 3 mL of acetone/H_2O (10/1) for 1 min. The reaction mixture was stirred at 25 ^oC for 3 h. The crude residue was filtered through celite and diluted with dichloromethane (5 mL). The organic layer was washed with sat. KI (1 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL). The solvent was dried with Na_2SO_4 and concentrated under reduced vacuum. The crude residue was purified by flash silica column chromatography to afford the hydrolyzed product (82 mg, 84 %).

The hydrolyzed product (50 mg, 0.03 mmol) and triethylammonium 3-(perfluorooctyl)propanyloxybutenyl- hydrogen phosphonate (20 mg, 0.03 mmol) were coevaporated with pyridine and dried under high vacuume for 30 min. To a solution of this mixture in pyridine (3 mL) was added a solution of pivaloyl chloride (0.007 mL, 0.06 mmol) in pyridine (0.3 mL) and then the mixture was stirred at 25 ^oC for 30 min under nitrogen gas environment. Prydine was removed under reduced vacuum and the crude product was purified by flash silica column chromatography (with triethyamine) to afford the desired product **13-1** (59 mg, 84 %).

R_f (10 % MeOH in DCM): 0.69

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26-7.05 (m, 60H), 5.64-5.59 (m, 1H), 5.53-5.45 (m, 3H), 5.13 (d, $1H_{anomeric}$, J = < 1 Hz), 4.91 (d, 1H, J = 12 Hz), 4.79 (d, 1H, J =

8 Hz), 4.74 (d, 1H, *J* = 8 Hz), 4.67-4.57 (m, 5H), 4.54-4.36 (m, 11H), 4.29 (t, 2H, *J* = 12 Hz), 4.23 (d, 1H, *J* = 8 Hz), 4.20 (d, 1H, *J* = 8 Hz), 4.14 (d, 1H, *J* = 12 Hz), 4.09-4.05(m, 2H), 4.00-3.94 (m, 2H), 3.90-3.78 (m, 11H), 3.73-3.52 (m, 6H), 3.41 (d, 2H, *J* = 12 Hz), 3.34-3.27 (m, 4H), 2.12 (m, 2H), 2.03 (s, 3H), 1.76-1.69 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.1 (C=O), [139.1, 139.0, 138.7, 138.5, 138.4, 138.2, 138.1, 138.0, 138.0, 138.0 (C_q-Aryl)], [128.3-127.0 (m, CH-Aryl)], [103.2, 100.8, 99.3. 95.0 ($CH_{anomeric}$)], 82.6, 79.9, 79.6, 75.2, 75.1, 74.7, 74.3, 74.1, 73.2, 73.1, 73.0, 72.6, 72.5, 72.0, 71.9, 71.8, 68.7, 68.7, 68.5, 68.1, 66.4, 61.7, 61.7, 49.5, 46.2 (NEt_3), 28.1, 27.9, 27.7, 21.1, 8.6 (NEt_3).

^{31}P NMR (162 MHz, CDCl3): δ -2.39

HRMS-MALDI (m/z): [M-NEt_3] Calcd for $C_{125}H_{128}F_{17}O_{26}P$ 2400.2848; Found, 2400.7329.

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CHAPTER 5

Synthesis of multivalent tuberculosis and *Leishmania*-associated capping carbohydrates and evaluation of structure-dependent immune responses in IL-12 production

A paper to be submitted to *Journal of the American Chemical Society*

Eun-Ho Song, Alex O. Osanya, Christine A. Petersen, and Nicola L. Pohl

Microbacterium tuberculosis is an intracellular pathogen which survives in macrophages and remains as one of the severe infectious diseases due to large number of infections with more than 2 million deaths annually worldwide.[1] Leishmaniasis is also an infectious disease caused by protozoan parasites of the genus *Leishmania*. Leishmaniasis is endemic over much of 88 countries in Africa, India, southern Europe, and Central and South America.[2] Importantly, *M.* tuberculosis[3] and leishmaniasis[4] are particularly problematic in these areas as a reactivating infection in AIDS patients. Despite considerable progress devoted to preventing and controlling tuberculosis and leishmaniasis, the disease still remains beyond efficient medical treatments such as vaccines. Therapies, including vaccines, have to deal with the parasites' ability to hide

in human macrophage cells—the white bloods cells that usually engulf and eliminate foreign materials. Herein we present the synthesis and development of new chemical tools which demonstrates that only simple changes in parasite-associated surface oligosaccharides are sufficient to change cellular immune responses and thereby let a parasite hide from immune surveillance.

The most abundant molecules found on the cell surfaces of bacteria, parasites and viruses are carbohydrates.[5] Due to structural distinctions among host cells and the distribution of carbohydrates on the outer surface of the cell, structure-function relationships involving carbohydrates have drawn attention amongst both synthetic organic chemists and immunologists. However, obtaining well-defined carbohydrate structures has been challenging due to limitations associated with current isolation protocols.[6] Simple commercially available sugars can be readily attached to beads for the identification of monosaccharide-dependent immunity[7]; however, these protocols do not lend themselves readily to larger structurally well-defined oligosaccharides.

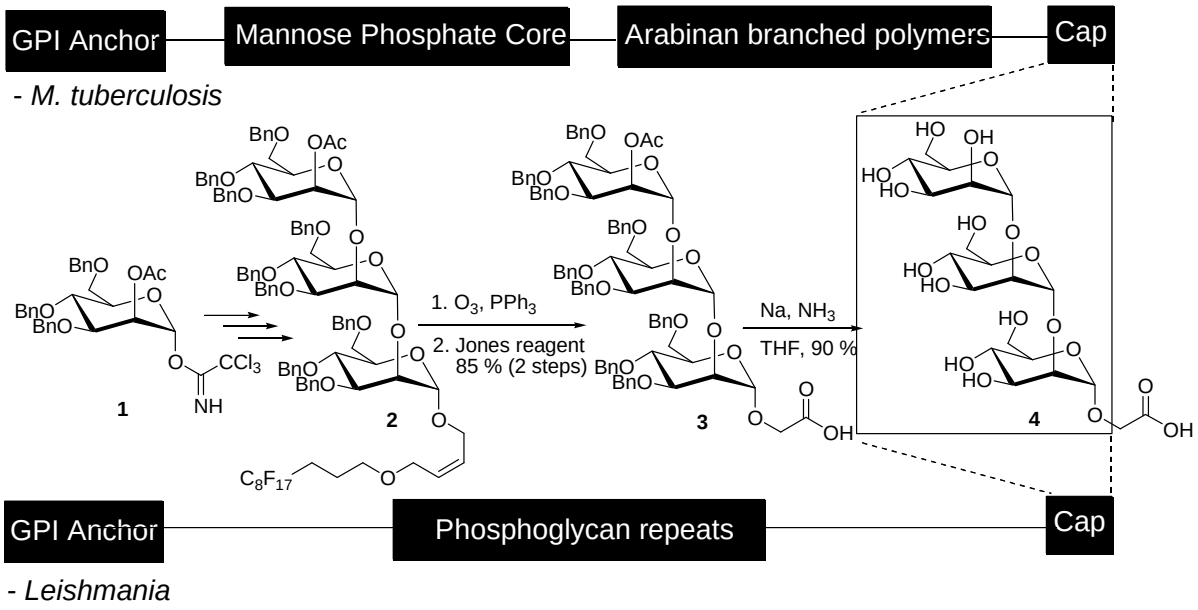


Figure 1. Capping structure in lipoarabinomannan (LAM) of *M.* tuberculosis and lipophosphoglycan (LPG) of *Leishmania*.

As shown in Figure 1, trimannose is one of the major components both in LPG of *Leishmania* parasites[8] and LAM of *M.* tuberculosis cell wall.[9] To date, several synthetic approaches have been reported to construct *Leishmania* capping structures including oligomannose[10] and branched oligosaccharide[11] for the purpose of synthetic challenge or vaccine development[12]. We wanted to develop a viable route to a range of *Leishmania* capping structures including trimannose and lactose as a control.

With this purpose, we envisioned making essentially artificial parasites of the same size (one micron) as *Leishmania* that contain only the structurally well-defined

capping polysaccharide associated with pathogens. The key challenge in the synthesis of artificial pathogens is construction of multivalent effect on latex beads in order to give more chance to induce immune responses.

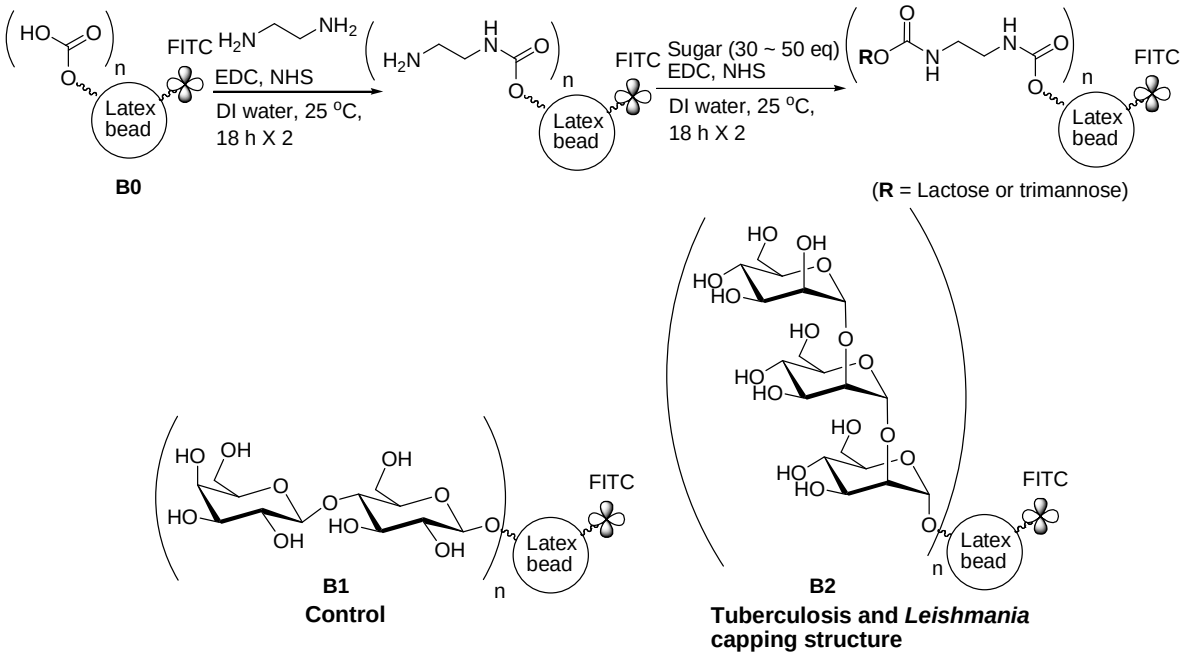
Results and discussion

Trimannose can be prepared fluorous solid-phase extraction (FSPE)-based iterative synthesis as reported in literature[10] (Scheme 1). Mannose trichloroacetimidate **1** was prepared from tri-*O*-benzyl orthoester intermediate[13]. Acid (TMSOTf)-activated glycosylation of mannose trichloroacetimidate with fluorous-tag gave fluorous-tagged disaccharide for further iterative synthesis of capping structures. This iterative synthesis using fluorous-solid-phase extraction (FSPE) takes advantage of the fact that desired oligosaccharide can be prepared without conventional slica-column purification.[14] The fluorous-tag in trimannose **2** was then cleaved with ozonolysis and subsequent oxidation with Jones reagent facilitates further coupling reaction of carboxylated-sugar with amine-functionalized beads. Global deprotection under Na/NH_3 at -78 ^oC furnished fully deprotected trimannose **4** in good yield.

Carboxylated-lactose was also efficiently prepared through conventional silica-

column purification. (see Supporting information)

With the capping sugar and the control sugar lactose in hand, a suitable one micron-sized support was required for multivalent display of these sugars. Latex beads are commonly used in immunoassays due to their inertness and commercial availability. Moreover, beads with high concentrations of imbedded fluorophores enable various fluorescent assays.[15] For example, such beads have been utilized for agglutination tests for the detection of antibodies[16] or investigation of heparin-binding properties[17], for the analysis of blood cell populations[18], for the identification of specific cell membrane markers[19] and for the measurement of sugar particle-induced immunity[7].



Scheme 1. Synthesis of multivalent tuberculosis and *Leishmania* capping structures on latex beads.

In this regard, we used commercially available Latex beads **B0** (microspheres, 1μm diameter and yellow-green fluorescent) derivatized with carboxylate groups (3.5×10^{-4} mmol carboxylate groups per mL). The chosen size of FITC-labeled latex beads mimics the size of the *Leishmania* parasite and allows use of a common immunofluorescent assay for the observation of the possible uptake of beads by macrophages. In order to avoid spatial proximity of sugars on the surface of the beads and improve the accessibility of sugars to possible macrophage binding partners, ethylenediamine as a spacer was attached to the carboxylated bead surface under standard peptide coupling conditions. Unfortunately, solvents for the coupling reaction were severely limited due to difficulties associated with the stability of the apparently noncovalent FITC linkage in organic solvents. Therefore, the choice of suitable solvent was an important consideration for both obtaining quantitative yield in coupling reaction and designing synthetic routes for sugars. Combined condition for coupling using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride

(EDC) and *N*-hydroxysuccinimide (NHS)[20] in deionized water was utilized to make peptide linkages (Scheme 2).

The subsequent coupling reaction of various capping structures containing the carboxylic acid linker with amine spacer on the beads gave *Leishmania*-associated capping carbohydrate coated-beads (Figure 1). Each coupling step was repeated twice with 30 ~ 50 equivalent of sugars to obtain a complete reaction. The density of sugar was calculated by given density of carboxylates (1.296×10^{-14} mmol / bead). As for coupling steps, Kaiser colorimetric test was used as an indicator of coupling reaction progress.[21]

To evaluate structure-dependent immune responses, lactose coated beads **B1** and trimannose-coated beads **B2** were initially incubated with murine macrophages for the analysis of cytokine production including Interleukin-12 (IL-12).[22] IL-12 plays a significant role in the link between innate and adaptive immunity

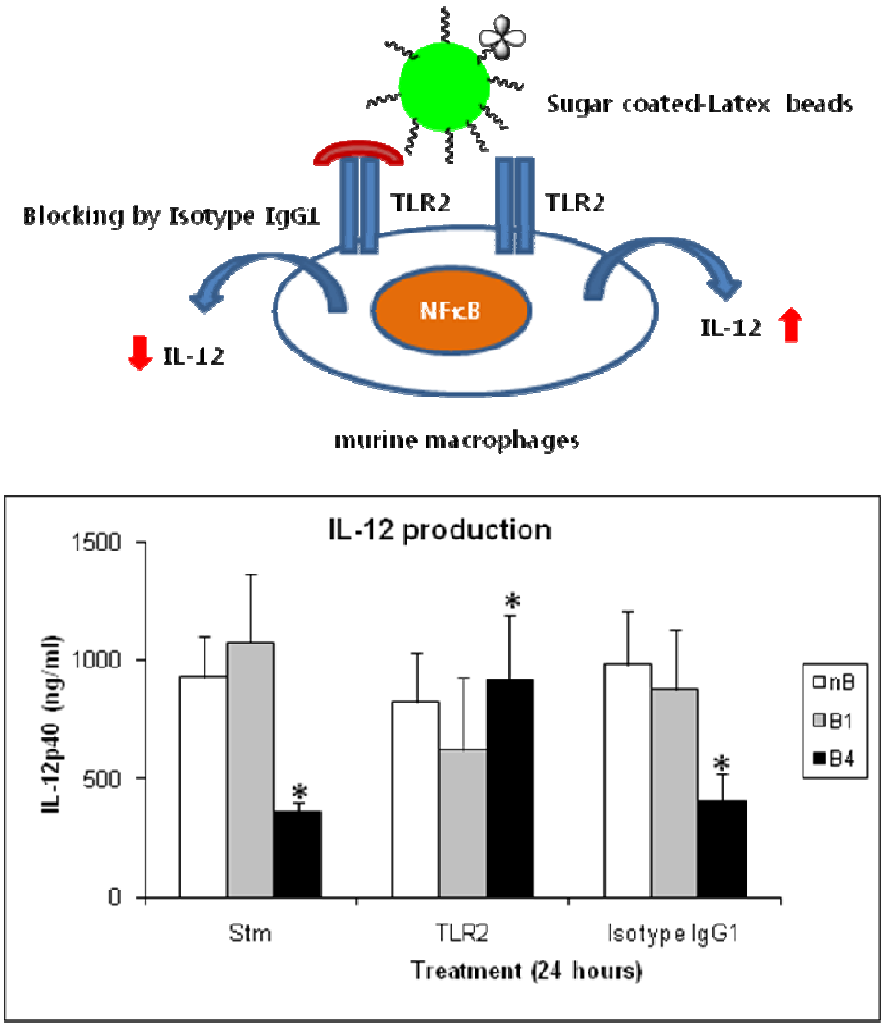
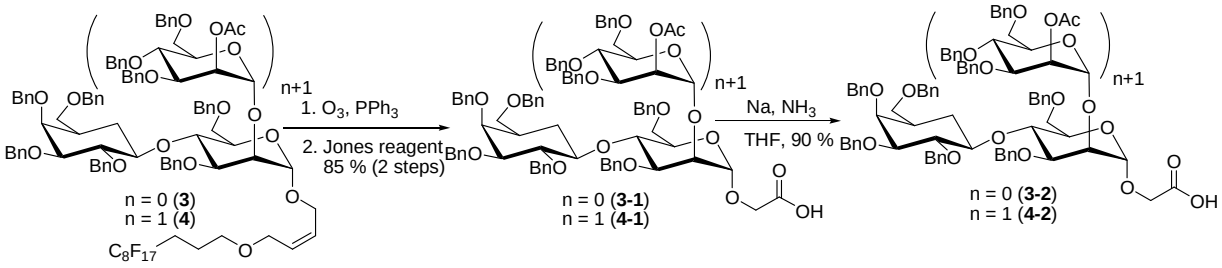


Figure 2. Effect of cap sugar coated-latex beads (B0 = latex bead, B1 = lactose coated beads, and B2 = trimannose-coated beads) on the production of IL-12p40 by murine macrophage cell line (J774) (mφ) stimulated by LPS and IFNγ. [Asterisks denote a significant change between the TLR2 blocking from control isotype and/or non-blocking-stimulation only treated cells ($p<0.05$) via Student's *t*-

test.]

As shown in Figure 2, comparable levels of IL12-p40 under two different environments including no blocking agents and isotype IgG1 antibody were produced when murine macrophages were stimulated by lactose-coated beads, whereas IL12 production stimulated by trimannose-coated beads was significantly diminished. These results indicate that structure differences in carbohydrates exhibit clear differences in the activation of innate immune responses induced by only differences in the carbohydrate structure.

In order to obtain more information about structure-dependent immune responses, other capping structures were also efficiently prepared through conventional silica-column purification for galactose[23] or FSPE-based purification for dimannose[7], branched tri-and tetrasaccharide.[24]



Scheme 2. Iterative synthesis of fluorous-tagged *Leishmania* tetrasaccaride

Branched *Leishmania* capping structures including trisaccharide **3** and tetrasaccharide **4** was also prepared either through iterative synthesis or automation platform.[22] The fluorous-tag in tetrasaccharide was then cleaved with ozonolysis and subsequent oxidation with Jones reagent facilitates further coupling reaction of carboxylated-sugar with amine-functionalized beads. Global deprotection under Na/NH_3 at -78 ^oC furnished fully deprotected saccharides (**3-2** and **4-2**) in good yield (Scheme 3).

Other capping structures were also efficiently prepared through conventional silica-column purification for galactose **5** or FSPE-based purification for dimannose **6**.

Carboxylated-*Leishmania* capping structures were then displayed on the latex beads under optimized coupling conditions (Figure 3).

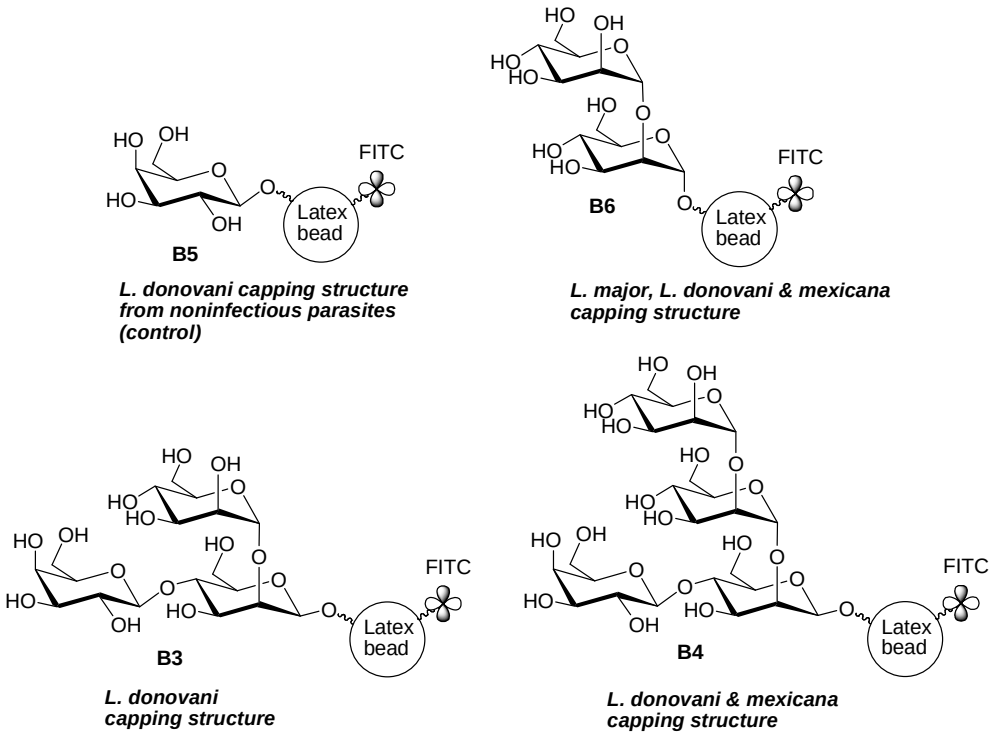


Figure 3. Multivalent *Leishmania* capping structures on latex beads

Conclusion

In conclusion, we demonstrated that the protocol of preparation of artifical parasites including well defined tuberculosis and *Leishmania*-associated capping structures such as trimannose and lactose enabled the study of structure-dependent immune reponses. In particluar, fluorous phase-based iterative synthesis provides not only a convenient purification step but also an easy transformation to carboxylic acid in order to display capping structures on the latex beads surface.

More importantly, our results with preminary IL-12 production studies showed that structure differences in carbohydrates produced a distinct differences in IL-12 p40 producton.

These results could provide a standard strategy for unveiling innate immune mechanisms induced by specific carbohydrate structure

Experimental section

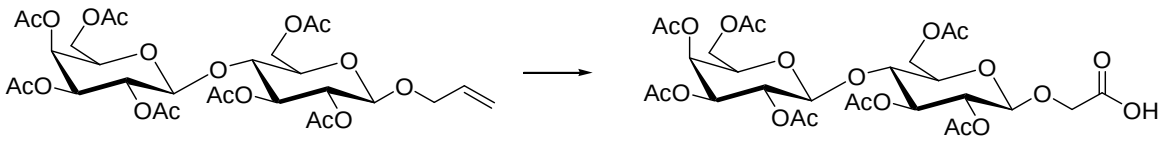
General methods

Solvents were reagent grade and in most cases dried prior to use. All other commercially available reagents were used as received unless otherwise noted. The organic extracts were dried over anhydrous $MgSO_4$. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$) prior to use. Methylene chloride (CH_2Cl_2), and triethylamine (Et_3N) were distilled from calcium hydride. Diethyl ether (Et_2O) was distilled from sodium-benzophenone ketyl.

^{1}H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on Varian VXR-400 NMR or on Bruker DRX-400 NMR. Mass spectra (MS) were recorded on an Applied Biosytems DE-Pro MALDI mass analyzer or an Applied Biosytems

QSTAR® XL Hybrid LC/MS/MS System. Chemical shifts are reported in parts per million downfield relative to tetramethylsilane (δ 0.00) and coupling constants are reported in Hertz (Hz). The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; and br = broad.

Synthesis of Fully deprotected carboxylated-*Leishmana* capping structures.



Synthesis of Carboxymethyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-2,3,6-tri-O-acetyl-β-D-galactopyranoside 1.

To a solution of 2-propynyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl] -2,3,6- tri-O-acetyl-β-D-galacto-pyranoside[25] (0.5g, 0.7 mmol) in the mixture of CCl_4 (3 mL), CH_3CN (3 mL), and H_2O (4 mL) is added $NaIO_4$ (1.27 g, 5.6 mmol). To this mixture was added $RuCl_3.H_2O$ (3.1 mg, 2.2 mol %) and the reaction mixture was stirred at 25 ^oC for 2 h. After dilution with 10 mL of DCM, the reaction mixture was filtered through a Celite pad. The aqueous layer was extracted with twice with DCM and then organic layer was washed with water and brine. The crude mixture was

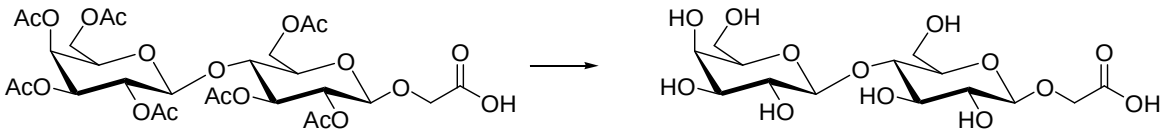
obtained after drying with Na_2SO_4 followed by concentration under reduced vacuum. The crude product was purified to obtain the desired product **1**(0.47 g, 91 %) by silica column chromatography

R_f (ethyl acetate/haxane): 0.30 (70/30)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.34 (d, 1H, *J* = 2.4 Hz), 5.22 (t, 1H, *J* = 9.2 Hz), 5.09 (t, 1H, *J* = 8 Hz), 4.97-4.93 (m, 2H), 4.62 (d, 1H, *J* = 7.6 Hz), 4.50-4.47 (m, 2H), 4.29 (s, 2H), 4.11-4.04 (m, 3H), 3.87 (t, 1H, *J* = 6.4 Hz), 3.80 (t, 1H, *J* = 9.2 Hz), 3.65-3.61 (m, 1H), 2,14 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.6, 170.6, 170.3, 170.3, 170.1, 169.9, 169.2, [101.2, 100.4 ($CH_{anomeric}$)], 76.2, 73.1, 72.5, 71.4, 71.1, 70.9, 69.2, 66.8, 65.5, 61.9, 61.0, 21.0, 21.0, 20.9, 20.9, 2.08, 20.7.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{28}H_{38}NaO_{20}$, 717.5791; Found, 717.6184.



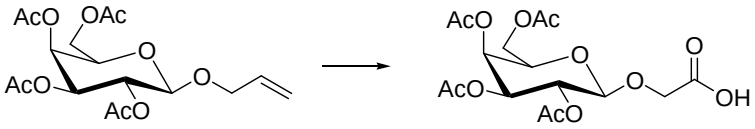
Synthesis of Carboxymethyl-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside 2.

To a carboxymethyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-2,3,6-tri-O-acetyl-β-D-galacto-pyranoside (**1**) (0.4 g, 0.57 mmol) in MeOH (5 mL) was added Na (27 mg, 1.14 mmol) and stirred at 25 ^{o}C for 2 h. The reaction mixture was neutralized with Dowex-ion-exchange resin. The desired (**2**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (0.21 g, 89 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.46 (d, 1H, *J* = 16.4 Hz), 4.30-4.23 (m, 2H), 3.81 (d, 1H, *J* = 3.2 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.0, 170.8, 170.5, 17.4, 170.2, 100.4 ($CH_{anomeric}$), 71.1, 70.7, 68.6, 67.1, 65.0, 61.4, 21.0, 20.9, 20.8, 20.8.

HRMS-ESI (m/z): $[M+Na]^{+}$Calcd for $C_{14}H_{24}NaO_{13}$, 423.1115; Found, 423.0942.



Synthesis of Carboxymethyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside 3.

To a solution of 2-propynyl-2,3,6-tri-O-acetyl-β-D-galacto-pyranoside[20] (0.5 g, 1.29 mmol) in the mixture of CCl_4 (3 mL) , CH_3CN (3 mL), and H_2O (4 mL) is added

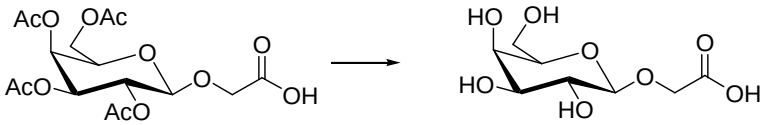
$NaIO_4$ (2.2 g, 10.3 mmol). To this mixture was added $RuCl_3$.H2O (6 mg, 2.2 mol %) and the reaction mixture was stirred at 25 ^oC for 2 h. After dilution with 10 mL of DCM, the reaction mixture was filtered through a Celite pad. The aqueous layer was extracted with twice with DCM and then organic layer was washed with water and brine. The crude mixture was obtained after drying with Na_2SO_4 followed by concentration under reduced vacuum. The crude product was purified to obtain the desired product (**3**) (0.42 g, 81 %) by silica column chromatography

R_f (ethyl acetate/haxane): 0.25 (70/30)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.41 (d, 1H, *J* = 2.8 Hz), 5.25 (dd, 1H, *J* = 7.9, 2.5 Hz), 5.06 (dd, 1H, *J* = 10.4, 3.3 Hz), 4.62 (d, 1H, *J* = 7.9), 4.36 (s, 2H), 4.20-4.09 (m, 2H), 3.94 (t, 1H, *J* = 6.7 Hz), 2.16 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.0, 170.8, 170.5, 170.4, 170.2, 100.4 ($CH_{anomeric}$), 71.1, 70.7, 68.6, 67.1, 65.0, 61.4, 21.0, 20.9, 20.8, 20.8.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{16}H_{22}NaO_{12}$,429.1009; Found,



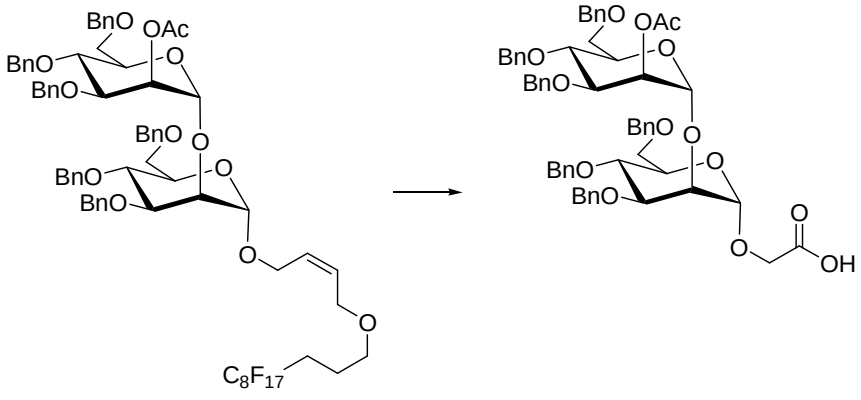
Synthesis of Carboxymethyl-β-D-galactopyranoside 4.

To a carboxymethyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**3**) (0.4 g, 0.99 mmol) in MeOH (5 mL) was added Na (45 mg, 1.98 mmol) and stirred at 25 ^{o}C for 2 h. The reaction mixture was neutralized with Dowex-ion-exchange resin. The desired product (**4**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (0.21 g, 89 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.46 (d, 1H, *J* = 16.4 Hz), 4.30-4.23 (m, 2H), 3.81 (d, 1H, *J* = 3.2 Hz), 3.75-3.68 (m, 2H), 3.60-3.53 (m, 1H), 3.51-3.47 (m, 2H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 173.1, 103.4 ($CH_{anomeric}$), 75.7, 73.4, 71.1, 69.0, 65.5, 61.3.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_8H_{14}NaO_8$, 261.0586; Found, 261.0463.



Synthesis of Carboxymethyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 5.

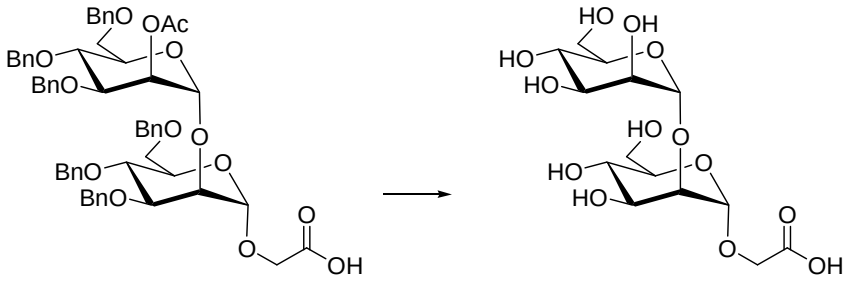
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (0.2 g, 0.15 mmol) in DCM was bubbled O_3 at -78 ^oC for 5 min. The light-blue solution was treated with PPh_3 (58 mg, 0.23 mmol) and was allowed to warm to room temperature. After 30 min, the crude aldehyde product was obtained after concentration under reduced vacuum. To a solution of aldehyde product in acetone was added Jones reagent (0.16 mL, 0.13 mmol) and the mixture was stirred for 30 min. After TLC analysis, excess reagent was quenched with 2-propanol and sold was filtered off through a Celite pad. The pure product **5** (0.12 g, 89 %) was obtained after concentration under reduced vacuum followed by purified through silica column chromatography (Hexane:Ethyl Acetate = 1:2).

R_f (ethyl acetate/haxane): 0.30 (70/30)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-7.15 (m, 30H), 5.52 (s, 1H), 5.07 (s, 1H), 4.98 (s, 1H), 4.83 (dd, 2H, *J* = 10.8, 6 Hz), 4.68 (s, 2H), 4.67-4.60 (m, 3H), 4.53-4.39 (m, 5H), 4.10-4.02 (m, 3H), 3.96-3.90 (m, 3H), 3.80-3.65 (m, 7H), 2.11 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.5 (C=O), 170.5 (C=O), [138.7, 138.6, 138.4, 138.3 (C_q-Aryl)], 128.7-127.8 (m, CH-Aryl), [99.8, 99.0 ($CH_{anomeric}$)], 79.7, 78.4, 75.4, 74.7, 73.7, 73.6, 72.7, 72.4, 72.3, 72.2, 69.4, 69.2, 69.1, 63.7, 21.4 (CH_3).

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{58}H_{62}NaO_{14}$, 1005.4037; Found, 1005.4289



Synthesis of Carboxymethyl-2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranoside 6

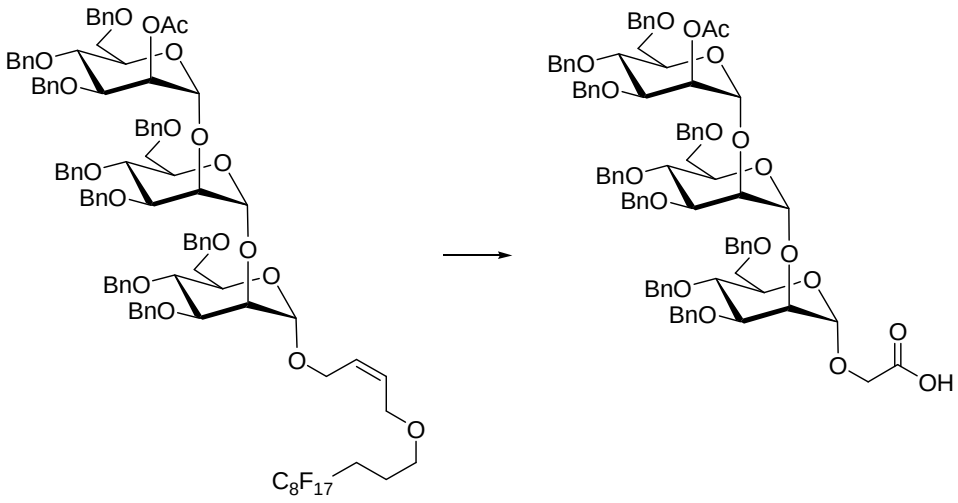
To a carboxymethyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**5**) (0.1 g, 0.11 mmol) was dissolved in THF (8 mL) and MeOH (0.5 mL) in a flask. Liquid ammonia (20 mL) was then condensed into the flask at -78 ^oC. Sodium metal was added in several portions until the solution was dark blue. The dark blue solution was stirred at -78 ^oC for 30 min. Following

disappearance of the dark blue color, EtOH (3 mL) was added and ammonia was blown off with a stream of air. The solution was then neutralized with acidic Dowex-resin to pH 7 and the resin was filtered off through a Celite pad. The desired (**6**) was obtained through evaporation of solvent under reduced pressure in good yield (34 mg, 75 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.15 (s, 1H), 4.98 (s, 1H), 4.24 (m, 2H), 3.96-3.57 (m, 12H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 172.3 (C=O), [102.9, 98.7 ($CH_{anomeric}$)], 78.8, 78.8, 74.0, 73.7, 71.2, 70.6, 67.4, 63.7, 63.4, 61.8, 61.7.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{14}H_{24}NaO_{13}$, 423.1115; Found, 423.0923.



Synthesis of Carboxymethyl-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside 7.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (0.2 g, 0.11 mmol) in DCM was bubbled O_3 at -78 ^{o}C for 5 min. The light-blue solution was treated with PPh_3 (42 mg, 0.16 mmol) and was allowed to warm to room temperature. After 30 min, the crude aldehyde product was obtained after concentration under reduced vacuum. To a solution of aldehyde product in acetone was added Jones reagent (0.11 mL, 0.08 mmol) and the mixture was stirred for 30 min. After TLC analysis, excess reagent was quenched with 2-propanol and sold was filtered off through a Celite pad. The pure product (**7**) (0.13 g, 87 %) was obtained after concentration under reduced vacuum followed by purified through silica column chromatography (Hexane:Ethyl Acetate = 1:2).

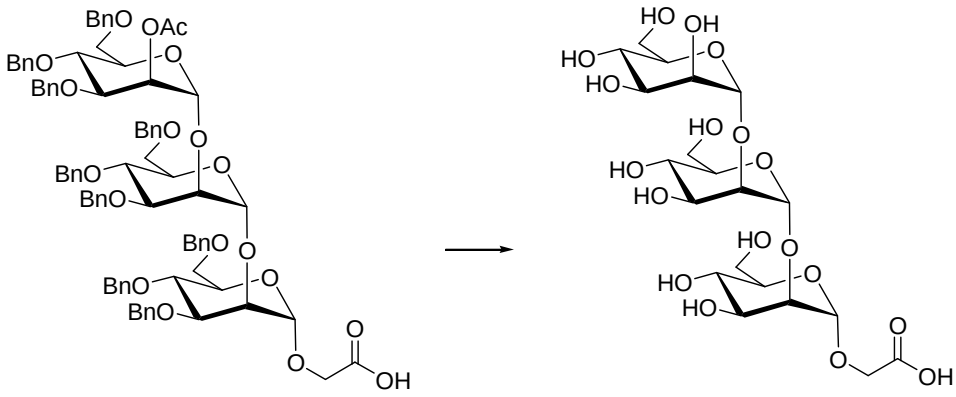
R_f (ethyl acetate/haxane): 0.13 (50/50)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26-7.12 (m, 45H, aromatics), 5.52 (t, 1H, *J* = 2.0 Hz), 5.17 (s, 1H), 5.03 (d, 1H, *J* = 8.0 Hz), 4.83 (d, 1H, *J* = 10.8 Hz), 4.80 (d, 1H,

J = 10.8 Hz), 4.62-4.41 (m, 14H), 4.33 (d, 1H, *J* = 12.0 Hz), 4.10-4.05 (m, 3H), 4.05-3.88 (m, 6H), 3.77-3.63 (m, 8H), 3.53 (d, 1H, *J* = 10.4 Hz), 2.12 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 172.4, 170.2, [138.6, 138.5, 138.5, 138.4, 138.3, 138.3, 138.1 (C_q-Aryl)], 134.1 ($CH_2\underline{C}H=CH_2$), 128.5 – 127.6 (m, CH-Aryl), [100.9, 99.5, 99.3 ($CH_{anomeric}$)], 79.1, 78.1, 75.2, 75.2, 75.0, 74.9, 74.6, 74.3, 73.4, 72.6, 72.5, 72.3, 72.2, 72.0, 71.9, 69.8, 69.2, 68.9, 68.8,

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{85}H_{90}NaO_{19}$, 1437.5974; Found, 1437.5097.



Synthesis of Carboxymethyl-2-*O*-[2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside 8.

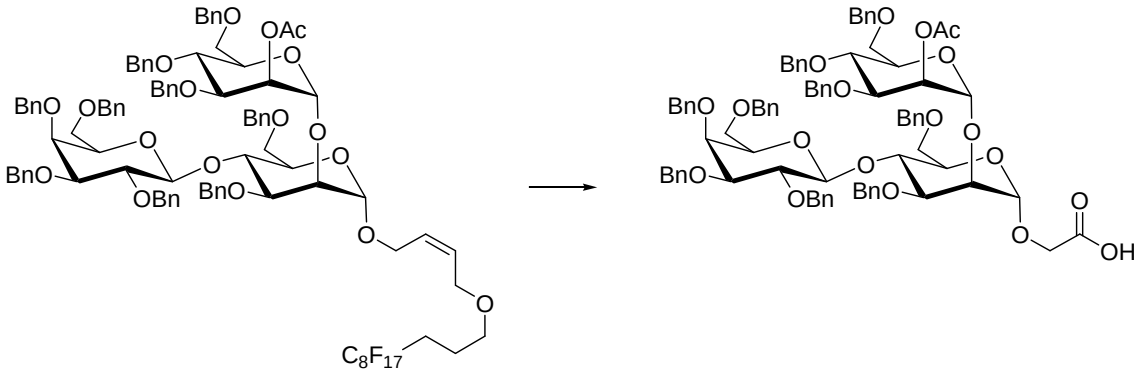
To a carboxymethyl-3,4,6-*O*-benzyl-2-O-[2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside **7**

(0.1 g, 0.07 mmol) was dissolved in THF (8 mL) and MeOH (0.5 mL) in a flask. Liquid ammonia (20 mL) was then condensed into the flask at -78 ^{o}C. Sodium metal was added in several portions until the solution was dark blue. The dark blue solution was stirred at -78 ^{o}C for 30 min. Following disappearance of the dark blue color, EtOH (3 mL) was added and ammonia was blown off with a stream of air. The solution was then neutralized with acidic Dowex-resin to pH 7 and the resin was filtered off through a Celite pad. The desired (**8**) was obtained through evaporation of solvent under reduced pressure in good yield (29 mg, 72 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.29 (s, 1H), 5.11 (s, 1H), 4.97 (s, 1H), 4.02-3.53 (m, 20H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 176.3 (C=O), [102.7, 101.1, 98.4 ($CH_{anomeric}$)], 79.1, 78.9, 73.7, 73.7, 73.5, 71.2, 70.8, 70.7,70.7, 67.9, 67.8, 67.5, 66.0, 61.8, 61.7, 61.6.

HRMS-ESI (m/z): $[M+Na]^{+}$Calcd for $C_{20}H_{34}NaO_{18}$, 585.1643; Found, 585.1528.



Synthesis of Carbpxymethyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside 9.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (0.2 g, 0.11 mmol) in DCM was bubbled O_3 at -78 ^{o}C for 5 min. The light-blue solution was treated with PPh_3 (42 mg, 0.16 mmol) and was allowed to warm to room temperature. After 30 min, the crude aldehyde product was obtained after concentration under reduced vacuum. To a solution of aldehyde product in acetone was added Jones reagent (0.11 mL, 0.08 mmol) and the mixture was stirred for 30 min. After TLC analysis, excess reagent was quenched with 2-propanol and sold was filtered off through a Celite pad. The pure product **9** (0.13 g, 85 %) was obtained after concentration under reduced vacuum

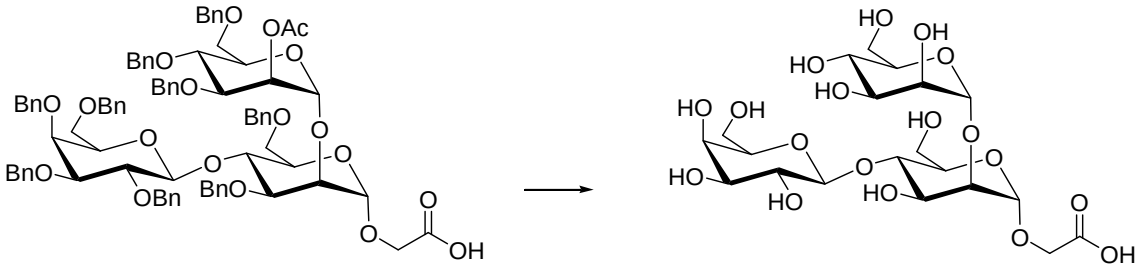
followed by purified through silica column chromatography (Hexane:Ethyl Acetate = 1:2).

R_f (ethyl acetate/haxane): 0.12 (50/50)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.29-7.13 (m, 45H, aromatics), 5.45 (s, 1H), 5.08 (s, 1H), 4.94 (d, 1H, *J* = 4 Hz), 4.90 (d, 1H, *J* = 12 Hz), 4.84-4.76 (m, 3H), 4.71-4.44 (m, 9H), 4.41-4.25 (m, 7H), 4.19-4.15 (m, 3H), 3.95-3.87 (m, 3H), 3.80-3.64 (m, 7H), 3.50 (t, 1H, *J* = 12 Hz), 3.43-3.38 (m, 2H), 2.07 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.3, 170.0, [139.2, 139.1, 139.0, 138.8, 138.6, 138.5, 138.3, 138.2, 138.1 (C_q-Aryl)], 128.5–127.2 (m, CH-Aryl), [103.7, 99.3, 99.1 ($CH_{anomeric}$)], 82.8, 80.0, 78.5, 75.4, 75.3, 75.1, 74.8, 74.5, 73.5, 73.3, 73.1, 72.9, 72.8, 72.5, 72.2, 72.1, 69.1, 69.0, 68.6, 68.4, 84.1, 21.3.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{85}H_{90}NaO_{19}$, 1437.5974; Found, 1437.7109..



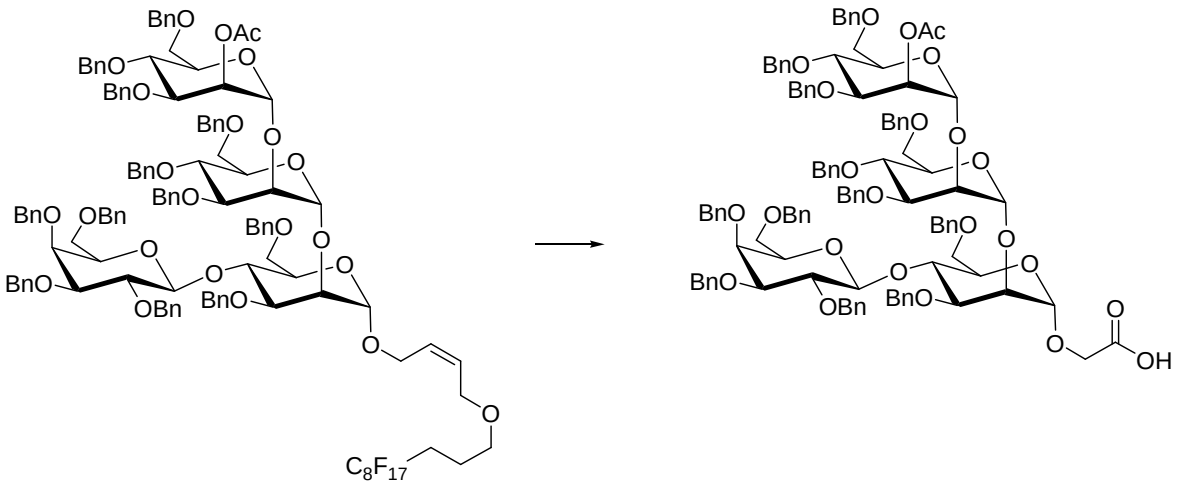
Synthesis of Carboxymethyl-4-*O*-[β-D-galactopyranosyl]-2-*O*-[2-*O*-α-D-mannopyranosyl]-α-D-mannopyranoside 10.

To a carbpxymethyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl-]-2-*O*-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (**9**) (0.1 g, 0.07 mmol) was dissolved in THF (8 mL) and MeOH (0.5 mL) in a flask. Liquid ammonia (20 mL) was then condensed into the flask at -78 ^{o}C. Sodium metal was added in several portions until the solution was dark blue. The dark blue solution was stirred at -78 ^{o}C for 30 min. Following disappearance of the dark blue color, EtOH (3 mL) was added and ammonia was blown off with a stream of air. The solution was then neutralized with acidic Dowex-resin to pH 7 and the resin was filtered off through a Celite pad. The desired (**10**) was obtained through evaporation of solvent under reduced pressure in good yield (31 mg, 78 %).

^{1}H NMR (CD_3OD, 400 MHz): 5.10 (s, 1H), 5.02 (s, 1H), 4.34 (d, 1H, *J* = 7.6 Hz), 4.19 (br, 1H), 4.04-3.48 (m, 18H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 171.0 (C=O), [103.9, 102.7, 99.0 ($CH_{anomeric}$)], 77.3, 75.7, 73.8, 73.6, 72.4, 70.6, 69.0, 67.4, 64.0, 61.7, 61.2, 60.8.

HRMS-MALDI (m/z): $[M+Na]^{+}$Calcd for $C_{20}H_{34}NaO_{18}$, 585.1643; Found, 585.6198



Synthesis of Carboxymethyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside 11.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (0.2 g, 0.09 mmol) in DCM was bubbled O_3 at -78 ^{o}C for 5 min. The light-blue solution was treated with PPh_3 (34 mg, 0.13 mmol) and was allowed to warm to room temperature. After 30 min, the crude aldehyde product was obtained after concentration under reduced vacuum. To a solution of aldehyde product in acetone was added Jones reagent (0.11 mL, 0.08 mmol) and the mixture was stirred

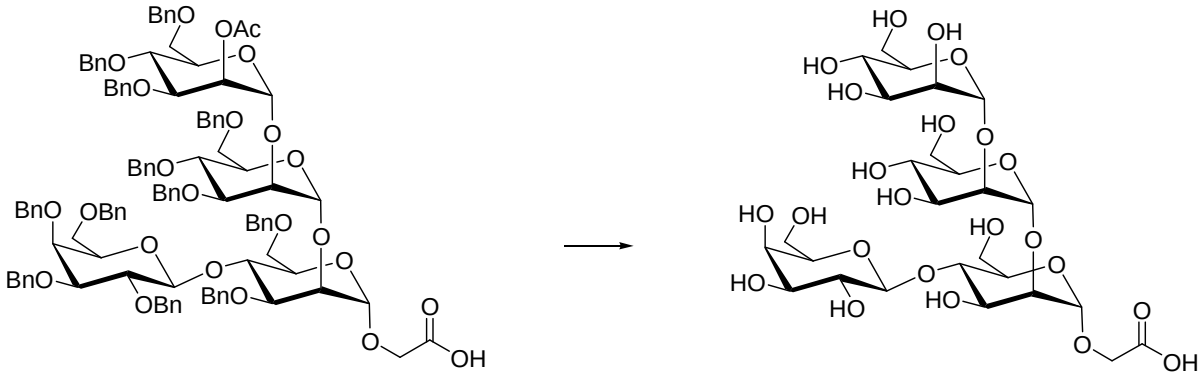
for 30 min. After TLC analysis, excess reagent was quenched with 2-propanol and sold was filtered off through a Celite pad. The pure product (**9**) (0.13 g, 85 %) was obtained after concentration under reduced vacuum followed by purified through silica column chromatography (Hexane:Ethyl Acetate = 1:2).

R_f (ethyl acetate/haxane): 0.15 (50/50)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.14 (m, 60H, aromatics), 5.46 (s, 1H), 5.15 (s, 1H), 4.99 (d, 2H, *J* = 8 Hz), 4.94 (d, 1H, *J* = 12 Hz), 4.81 (dd, 2H, *J* = 8, 4 Hz), 4.71-4.64 (m, 3H), 4.68 (s, 2H), 4.59-4.56 (m, 3H), 4.51-4.41 (m, 9H), 4.34-4.23 (m, 5H), 4.13 (d, 1H, *J* = 16 Hz), 4.00-3.83 (m, 10H), 3.73-3.61 (m, 6H), 3.59-3.50 (m, 4H), 3.42-3.37 (m, 3H), 2.11 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.1, 170.3, [139.3, 139.1, 138.9, 138.8, 138.7, 138.7, 138.6, 138.6, 138.5, 138.5, 138.3, 138.1 (C_q-Aryl)], 128.6–127.3 (m, CH-Aryl), [103.6, 100.6, 99.8, 99.5 ($CH_{anomeric}$)], 82.8, 80.1, 79.6, 75.6, 75.5, .75.3, 74.9, 74.8, 74.5, 73.5, 73.5, 73.3, 73.1, 72.9, 72.8, 72.7, 72.4, 72.2 72.1, 68.6, 69.0, 68.6, 64.5, 21.4.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{112}H_{118}NaO_{24}$, 1869.7911; Found, 1870.5026.



Synthesis of Carboxymethyl-4-*O*-[β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside 12.

To a solution of carboxymethyl-3,6-di-*O*-benzyl-4-*O*-[2,3,4,6,-tetra-*O*-benzyl-β-D-galactopyranosyl]-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (**11**) (0.1 g, 0.05 mmol) was dissolved in THF (8 mL) and MeOH (0.5 mL) in a flask. Liquid ammonia (20 mL) was then condensed into the flask at -78 ^{o}C. Sodium metal was added in several portions until the solution was dark blue. The dark blue solution was stirred at -78 ^{o}C for 30 min. Following disappearance of the dark blue color, EtOH (3 mL) was added and ammonia was blown off with a stream of air. The solution was then neutralized with acidic Dowex-resin to pH 7 and the resin was filtered off through a

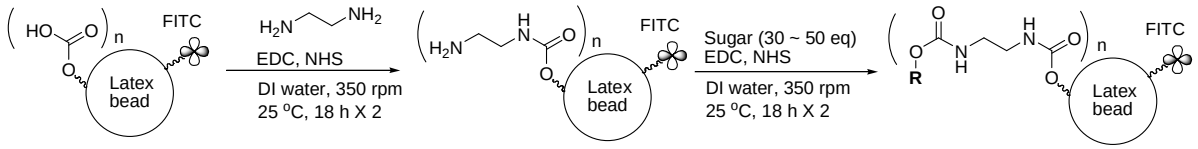
Celite pad. The desired (**12**) was obtained through evaporation of solvent under reduced pressure in good yield (36 mg, 90 %).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.33 (s, 1H), 5.08 (s, 1H), 4.97 (s, 1H), 4.35 (d, 1H, *J* = 7.2 Hz), 4.13 (s, 2H), 4.05-3.96 (4H), 3.85-3.82 (m, 8H), 3.73-3.49(13H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 172.4 (C=O), [103.9, 102.7, 100.9, 98.9 ($CH_{anomeric}$)], 79.0, 77.3, 77.3, 75.7, 73.8, 73.6, 73.5, 72.3, 71.3, 71.1, 70.6, 70.6, 69.6, 69.2, 67.9, 67.4, 63.8, 61.8, 61.3, 60.8.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{26}H_{44}NaO_{23}$, 747.6035; Found, 747.6172.

Synthesis of sugar-conjugated latex beads



mmol of COOH per 1mL of beads solution
: (2.7 x 10 ^{10} bead / mL) x (1.296 x x10 $^{-14}$ mmol/bead) = 3.5 x 10 $^{-4}$ mmol / mL

R = Lactose (**B1**), Trimannose (**B2**)
Branched-tri (**B3**), Brnched-tetra (**B4**)
Galactose (**B5**), Dimmanose (**B6**)

To a solution of latex beads (1mL, 2.7 x 10^{10} beads/ mL) was added EDC (8 mg, 0.004 mmol) solution (100 μL) in deionized water (DI water) followed by ethylenediamin in MES buffer (200 μL). The reaction mixture was stirred at 26 ^{o}C for

18 h (X 2) and then beads were washed with DI water 2 ~ 3 times for the Kaiser test. 10 μL of Beads solution was used to perform a Kaiser test (positive:deep purple color). To a solution of sugar (30 equiv of dimannose, trimannose, branched trisaccharide and tetrasaccharide; 50 equiv of lactose and galactose) in DI water (200 μL) was added EDC solution (DI water, 100 μL) followed by NHS solution (DI water, 100 μL). The reaction mixture was stirred for 10 min and then combined with amine-functionalized latex beads in MES buffer (200 μL). The mixture was stirred at 26 ^{o}C for 18 h (X 2) and then performed the Kaiser test with 10 μL of Beads solution (negative: dark brown color). Sugar-coated latex beads (2.65 x 10^{10} beads in 1mL of DI water) were stored 4 ^{o}C refrigerator.

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CHAPTER 6

Modification of degradable polymeric particles with carbohydrates for the study of in vitro activation of dendritic cells

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Introduction

Despite extensive efforts on the development of efficient delivery systems for drugs, an efficient carrier for drug to specific areas in the human body has been highlighted as a prerequisite for the optimization of drug efficiency.[1, 2] More recently, attention has been focused on the development of vaccine delivery system in order to prevent severe bacterial, viral, parasitic and respiratory infectious diseases both in human and animal.[3, 4]

Several polymers such as copolymers[5] of sebacic anhydride (SA) and 1,6-bis-(p-carboxyphenoxy)hexane (CPH), copolymers[6] of 1,6-bis-(p-carboxyphenoxy)hexane (CPH) and 1,8-bis(p-carboxyphenoxy-3,6-dioxaoctane (CPTEG) and poly(propylacrylic acid) (PPAA)[7] as vaccine delivery vehicles have been extensively studied in various biomedical researches due to their biocompatibility and degradation properties under certain pH ranges and temperatures.

Among these polymers, polyanhydride copolymers including CPH:SA and CPTEG:CPH have been well characterized in order to facilitate bulk-erodible property,

induced by hydrophilic-polymers (CPTEG), into the surface erodible polyanhydrides (CPH and SA).[8] Important features using polyanhydride copolymers are the enhancement of controlling erosion kinetics, determined by a combination of erosion mechanisms between bulk-eroding and surface-eroding, for controlled drug release kinetics and the non-acidic microenvironment produced by degradation of polymers.[5, 9] Moreover, recent study with polyanhydride nanoparticles has shown that size and chemistry of particles are important factors in cellular internalization of polymers by monocytes.[10]

To examine carbohydrate antigen-mediated activation of Dendritic cells (DCs), more research has been performed with synthetic carbohydrate ligands as antigens capable of activating DCs.[11] DCs are immune cells that are the most important antigen-presenting cells (APCs) in the early stage of immune responses. Importantly, DCs are not only capable of stimulating T cells, but also essential for connecting innate and adaptive immunities through Th1 cell or Th2 stimulation.[12] Although importance of carbohydrate antigens has been addressed in terms of immune stimulation, accessibility of carbohydrates on polymeric vehicles is still limited by lack of suitable conjugation chemistry and degradable property of polymeric particles. Previous erosion studies with polyanhydride copolymers showed that 50:50 CPTEG:CPH copolymer was significantly degraded (67 % of molecular weight loss) in water for 2 days.[5] This degradation property provides harsh conditions for the modification of polymeric particles.

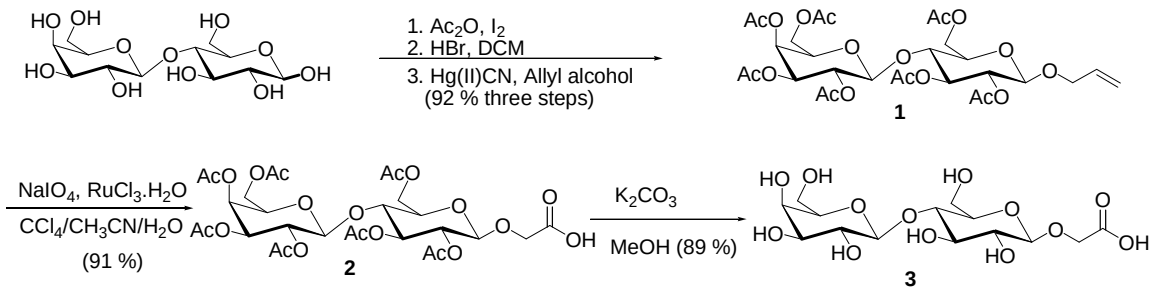
In this study, we demonstrated modification of degradable polyanhydride copolymers with lactose and dimannose in order to investigate carbohydrate antigen-dependent immune responses on uptake and activation of DCs.

Results and discussion

In order to enhance immune responses of copolymers through activation of DCs, carbohydrate antigen coated polymers were prepared.

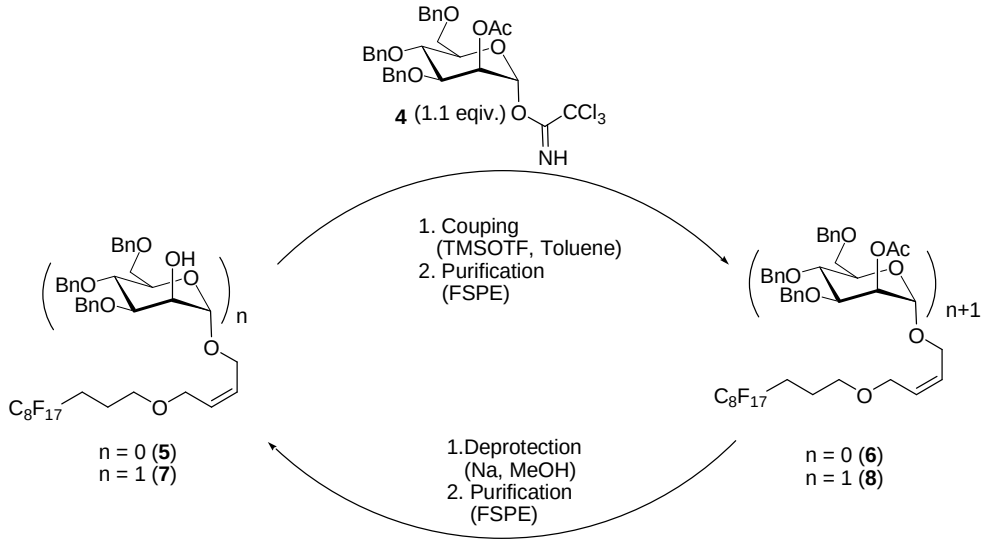
Synthesis of carboxylated-lactose and dimannose.

Mercury (II)-catalyzed allylation[13] of penta-*O*-acetyl-1-*O*-bromide lactose using Hg(II)CN and all alcohol produced β-1-*O*-allylated lactose **1** in high yield (92 % three steps) as a precursor for oxidation. One-step oxidation of olefin for the construction of carboxylic acid in 2-acetamido-2-dexoy-D-glucose has been reported by using ruthenium-catalyzed Sharpless conditions.[14, 15] As expected, using excess amounts of $NaIO_4$ (8 eq) under ruthenium-catalyzed Sharpless conditions produced the desired acid **2** in 91 % yield. But, this oxidation is not suitable in the presence of benzyl protections due to transformation of benzyl to benzoyl. Subsequent deacetlyation under mild condition using K_2CO_3 provided fully deprotected disaccharide **3**.



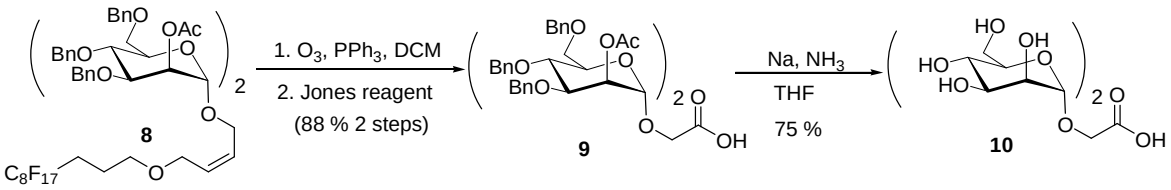
Scheme 1. Synthesis of carboxy-functionalized lactose.

Iterative synthesis of linear α-1,2-linked dimannose **8** has been reported by using fluorous-solid phase extraction (FSPE).[16] Each glycosylation was performed with 1.1 equivalent of trichloroacetimidate donor **4**[17] in toluene at 25 ^{o}C for 5 min. Facile purification of crude product by FSPE enabled easy preparation of desired linear α-1,2-linked dimannose **8** in high yield.



Scheme 2. Iterative synthesis of fluorous–tagged α-1,2-linked dimannose.

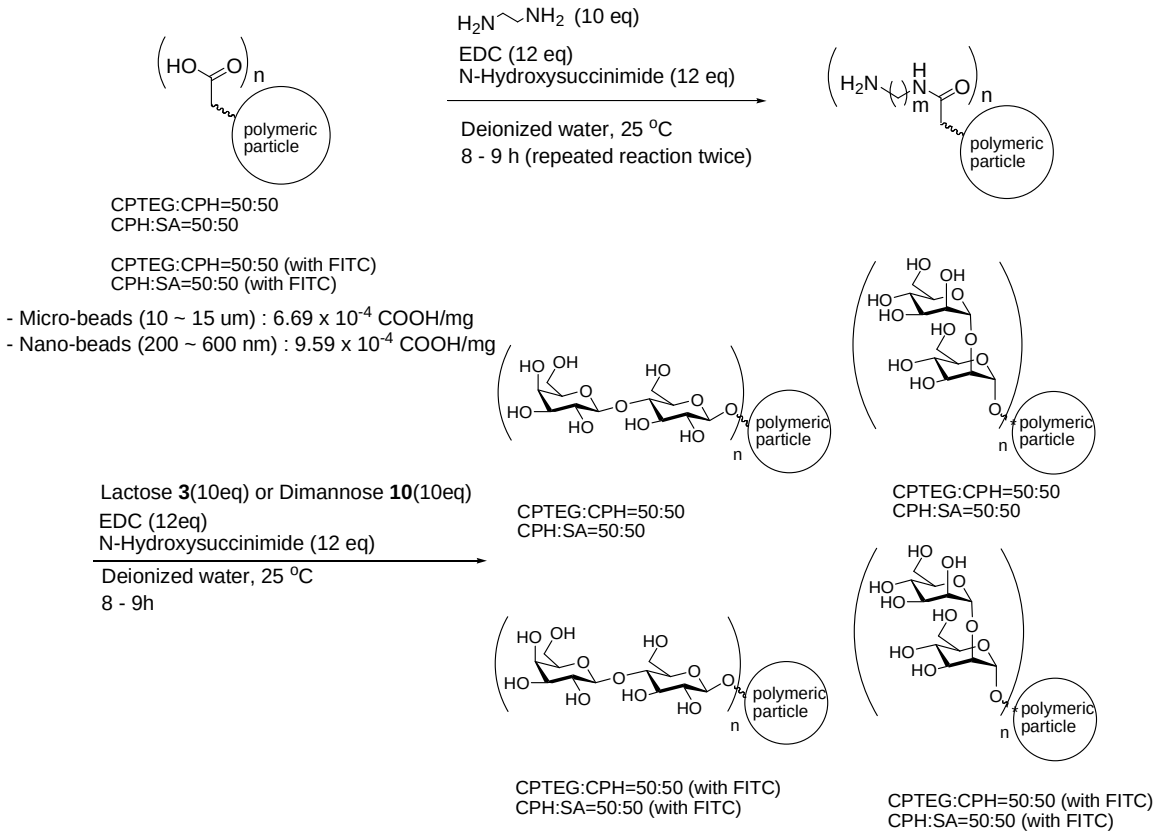
Fully deprotected α-1,2-linked dimannose **10** was obtained by ozonolysis[18] of **8**, followed by global deprotection of **9** under Birch reduction conditions[19] (Na, NH_3).



Scheme 3. Synthesis of carboxy-functionalized dimannose.

Modification of micro- and nanoparticles with lactose and dimannose.

Polyanhydride copolymers[5, 6] and FITC-dextran loaded copolymers[20] for confocal images of the internalization of polymer in DCs were prepared as reported in literatures.



Scheme 4. Modification of polymeric particles with carbohydrates.

Surface modification of polymeric particles is obviously challenging due to physical properties of polymers such as degradation and aggregation. In addition, FITC-labeling can be detached from particles during coupling steps under certain conditions such as high temperature and organic solvent because it is not chemically attached to polymeric particles.[19] To overcome these unfavorable properties of polymeric particles, optimization of coupling reaction conditions should be a prerequisite for

homogeneous density of carbohydrates on the surface.

Sugar coated polymeric particles were synthesized from carboxylic acid-functionalized lactose **3** and dimannose **10** using a procedure that has been used for the peptide-type coupling reaction. The control compound, glycolic acid, was also carried through same steps to work out a viable protocol (Scheme 1). In order to avoid fast degradation of polymeric particles, we performed coupling reactions under aqueous conditions rather than under DMF or DCM which is common solvent for solid-phase synthesis. Although 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)[21] has been used as a coupling agent for the purpose of forming amide bonds in peptide synthesis, amine reactive O-acylisourea intermediate which is activated by EDC can be hydrolyzed by water. In order to increase the efficiency of coupling reaction in aqueous condition, we added *N*-hydroxysuccinimide (NHS)[22] to form an amine reactive NHS-ester after activating carboxyl group with EDC. Ethlylenediamine as a spacer molecule was attached to carboxylated polymeric particles in order to increase accessibility of carbohydrates to the surface of particles. Peptide-type coupling reaction was repeated twice with relatively short reaction time (8 ~ 9 hours) to accomplish a complete coupling reaction. Carbohydrate-coated

polymeric particles were prepared through second coupling reaction with carboxylic acid-functionalized lactose **3** and dimannose **10** followed by dry under high-vacuum. The Kaiser test[23] (Ninhydrin color test) was employed as an indicator of coupling reaction progress. Quantitative monitoring of reaction can verify sugar loading on the beads.[24] Surface characterization by X-ray photoelectron spectroscopy (XPS) clearly showed an increase of N/C % in carbohydrate modified particles. (by Brenda R. Carrillo-Conde).

In order to evaluate the effect of surface modification with carbohydrate on immune responses, DC activation studies such as cell surface marker expression analysis, cytokine production analysis and particle uptake studies have been done by collaboration partner (Brenda R. Carrillo-Conde) in Chem. Eng. (Dr. Balaji Narasimhan). Although significant difference between unmodified particles and carbohydrate-modified particles was not observed in cytokine production studies, carbohydrate-modified particles showed higher up-regulation in the expression of MHC II, CD40, CD86, CD206, and CD209 as well as greater uptake of lactose by DCs, a marker for DC stimulation. Because cells that are associated with immune system, display a unique set of cell surface markers, identification of cell surface

markers plays a significant role in unveiling the type of immune response.

Conclusion

We demonstrated that the Surface modification of erodible polymeric particles by carbohydrates including lactose and dimannose could play an important role in activation of innate immunity. Conjugation chemistry using EDC and NHS provided a viable route of modification of erodible polymeric beads without significant loss of polymeric particles caused by degradation.

Importantly, we observed that carbohydrate-modified particles are closely related with several important cell surface markers that are capable of inducing adaptive immunity (MHC II, CD40, and CD86) or acting as a cell adhesion receptor (CD209). These preliminary results showed great promise for the study of the effect carbohydrate-modified particles on the regulation of immune responses.

In order to support to these results, using branched-/linear trisaccharide and longer spacer might be helpful to clarify carbohydrate antigen effect on immune responses

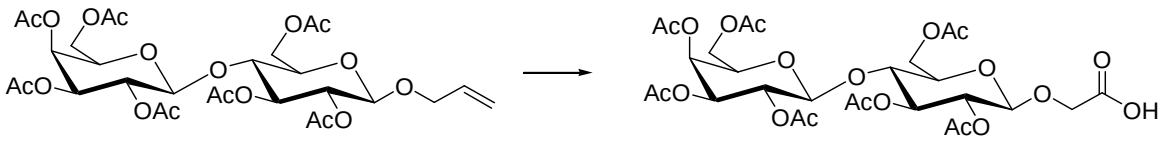
Experimental section

General methods

Solvents were reagent grade and in most cases dried prior to use. All other commercially available reagents were used as received unless otherwise noted. The organic extracts were dried over anhydrous $MgSO_4$. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride ($LiAlH_4$) prior to use. Methylene chloride (CH_2Cl_2), and triethylamine (Et_3N) were distilled from calcium hydride. Diethyl ether (Et_2O) was distilled from sodium-benzophenone ketyl.

^1H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on Varian VXR-400 NMR or on Bruker DRX-400 NMR. Mass spectra (MS) were recorded on an Applied Biosytems DE-Pro MALDI mass analyzer or an Applied Biosytems QSTAR® XL Hybrid LC/MS/MS System. Chemical shifts are reported in parts per million downfield relative to tetramethylsilane (δ 0.00) and coupling constants are reported in Hertz (Hz). The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; and br = broad.

Synthesis of carboxylated-lactose and dimannose.



Synthesis of Carboxymethyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-2,3,6-tri-O-acetyl-β-D-galactopyranoside 2.

To a solution of 2-propynyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl] -2,3,6- tri-O-acetyl-β-D-galacto-pyranoside (**1**)[25] (0.5g, 0.7 mmol) in the mixture of CCl_4 (3 mL), CH_3CN (3 mL), and H_2O (4 mL) is added $NaIO_4$ (1.27 g, 5.6 mmol). To this mixture was added $RuCl_3.H_2O$ (3.1 mg, 2.2 mol %) and the reaction mixture was stirred at 25 ^oC for 2 h. After dilution with 10 mL of DCM, the reaction mixture was filtered through a Celite pad. The aqueous layer was extracted with twice with DCM and then organic layer was washed with water and brine. The crude mixture was obtained after drying with Na_2SO_4 followed by concentration under reduced vacuum. The crude product was purified to obtain the desired product (**2**) (0.47 g, 91 %) by silica column chromatography

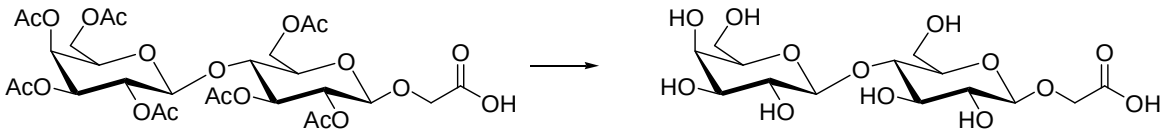
R_f (ethyl acetate/haxane): 0.30 (70/30)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.34 (d, 1H, *J* = 2.4 Hz), 5.22 (t, 1H, *J* = 9.2 Hz), 5.09 (t, 1H, *J* = 8 Hz), 4.97-4.93 (m, 2H), 4.62 (d, 1H, *J* = 7.6 Hz), 4.50-4.47 (m, 2H), 4.29 (s, 2H), 4.11-4.04 (m, 3H), 3.87 (t, 1H, *J* = 6.4 Hz), 3.80 (t, 1H, *J* = 9.2 Hz),

3.65-3.61 (m, 1H), 2,14 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.6, 170.6, 170.3, 170.3, 170.1, 169.9, 169.2, [101.2, 100.4 ($CH_{anomeric}$)], 76.2, 73.1, 72.5, 71.4, 71.1, 70.9, 69.2, 66.8, 65.5, 61.9, 61.0, 21.0, 21.0, 20.9, 20.9, 2.08, 20.7.

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{28}H_{38}NaO_{20}$, 717.5791; Found, 717.6184.



Synthesis of Carboxymethyl-4-O-(β-D-galactopyranosyl)-β-D glucopyranoside 3.

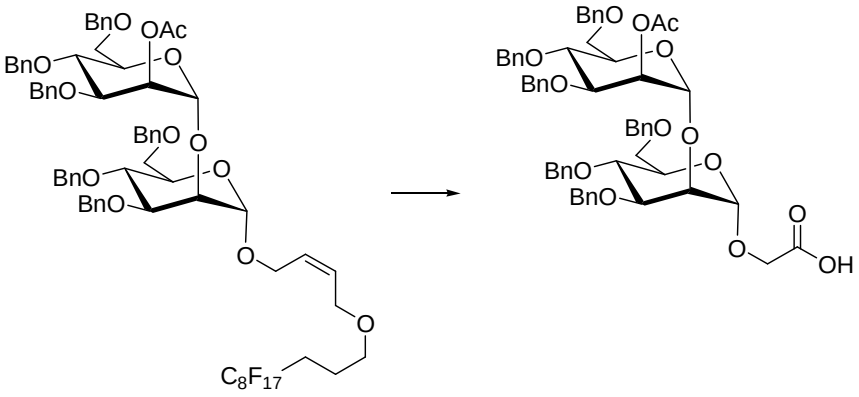
To a carboxymethyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-2,3,6-tri-O-acetyl-β-D-galacto-pyranoside (**2**) (0.4 g, 0.57 mmol) in MeOH (5 mL) was added Na (27 mg, 1.14 mmol) and stirred at 25 ^oC for 2 h. The reaction mixture was neutralized with Dowex-ion-exchange resin. The desired (**3**) was obtained through Celite filteration followed by evaporation of solvent under reduced pressure in good yield (0.21 g, 89 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.46 (d, 1H, *J* = 16.4 Hz), 4.30-4.23 (m, 2H),

3.81 (d, 1H, *J* = 3.2 Hz).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.0, 170.8, 170.5, 17.4, 170.2, 100.4 ($CH_{anomeric}$), 71.1, 70.7, 68.6, 67.1, 65.0, 61.4, 21.0, 20.9, 20.8, 20.8.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{14}H_{24}NaO_{13}$, 423.1115; Found, 423.0942.



Synthesis of Carboxymethyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 9.

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**8**) (0.2 g, 0.15 mmol) in DCM was bubbled O_3 at -78 ^oC for 5 min. The light-blue solution was treated with PPh_3 (58 mg, 0.23 mmol) and was allowed to warm to room temperature. After 30 min, the crude aldehyde product was obtained after concentration under

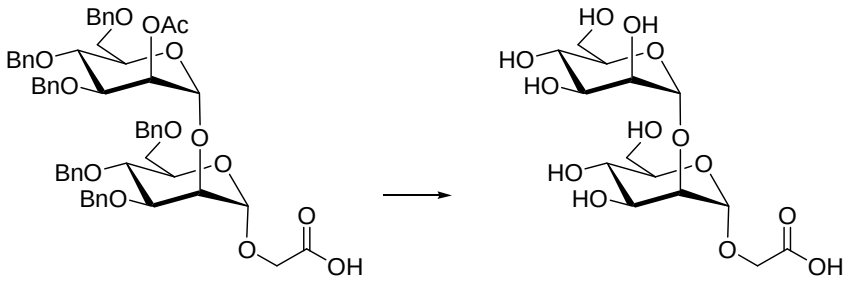
reduced vacuum. To a solution of aldehyde product in acetone was added Jones reagent (0.16 mL, 0.13 mmol) and the mixture was stirred for 30 min. After TLC analysis, excess reagent was quenched with 2-propanol and sold was filtered off through a Celite pad. The pure product (**9**) (0.12 g, 89 %) was obtained after concentration under reduced vacuum followed by purified through silica column chromatography (Hexane:Ethyl Acetate = 1:2).

R_f (ethyl acetate/haxane): 0.30 (70/30)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33-7.15 (m, 30H), 5.52 (s, 1H), 5.07 (s, 1H), 4.98 (s, 1H), 4.83 (dd, 2H, *J* = 10.8, 6 Hz), 4.68 (s, 2H), 4.67-4.60 (m, 3H), 4.53-4.39 (m, 5H), 4.10-4.02 (m, 3H), 3.96-3.90 (m, 3H), 3.80-3.65 (m, 7H), 2.11 (s, 3H).

^{13} C NMR ($CDCl_3$, 100 MHz): δ (ppm) 173.5 (C=O), 170.5 (C=O), [138.7, 138.6, 138.4, 138.3 (C_q-Aryl)], 128.7-127.8 (m, CH-Aryl), [99.8, 99.0 ($CH_{anomeric}$)], 79.7, 78.4, 75.4, 74.7, 73.7, 73.6, 72.7, 72.4, 72.3, 72.2, 69.4, 69.2, 69.1, 63.7, 21.4 (CH_3).

HRMS-MALDI (m/z): $[M+Na]^+$Calcd for $C_{58}H_{62}NaO_{14}$, 1005.4037; Found, 1005.4289



Synthesis of Carboxymethyl-2-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranoside 10.

To a carboxymethyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**9**) (0.1 g, 0.11 mmol) was dissolved in THF (8 mL) and MeOH (0.5 mL) in a flask. Liquid ammonia (20 mL) was then condensed into the flask at -78 ^{o}C. Sodium metal was added in several portions until the solution was dark blue. The dark blue solution was stirred at -78 ^{o}C for 30 min. Following disappearance of the dark blue color, EtOH (3 mL) was added and ammonia was blown off with a stream of air. The solution was then neutralized with acidic Dowex-resin to pH 7 and the resin was filtered off through a Celite pad. The desired (**10**) was obtained through evaporation of solvent under reduced pressure in good yield (34 mg, 75 %).

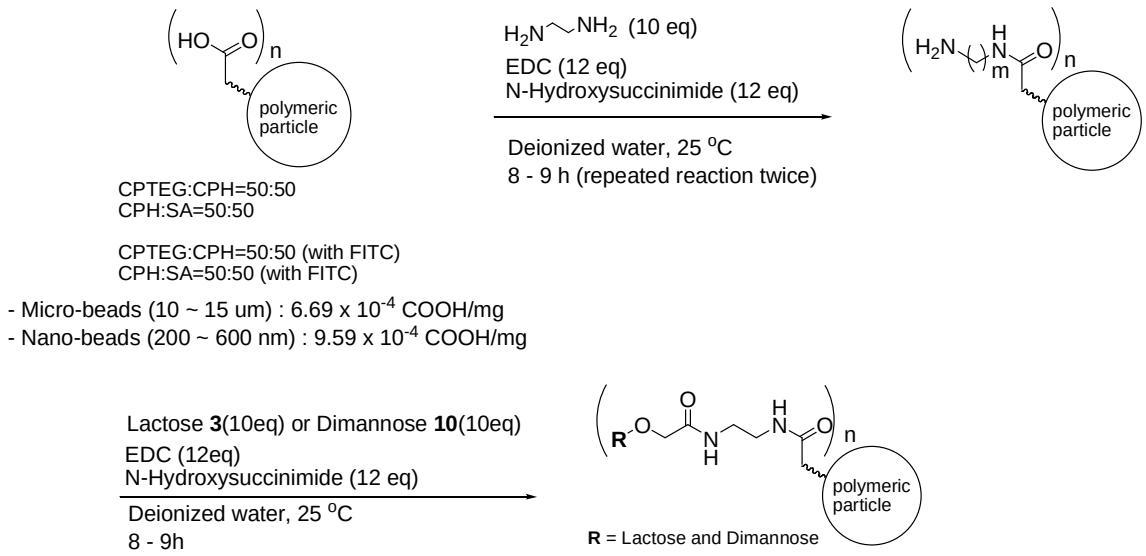
^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.15 (s, 1H), 4.98 (s, 1H), 4.24 (m, 2H),

3.96-3.57 (m, 12H).

^{13} C NMR (CD_3OD, 100 MHz): δ (ppm) 172.3 (C=O), [102.9, 98.7 ($CH_{anomeric}$)], 78.8, 78.8, 74.0, 73.7, 71.2, 70.6, 67.4, 63.7, 63.4, 61.8, 61.7.

HRMS-ESI (m/z): $[M+Na]^+$Calcd for $C_{14}H_{24}NaO_{13}$, 423.1115; Found, 423.0923.

Modification of polymeric particles with sugars.



To a solution of microshperes (20 mg, 1.34 x 10 -^{2} mmol of COOH) was added EDC (31 mg, 0.16 mmol: 12 eq) solution (100 μL) in deionized water (DI water) and NHS (19 mg, 0.16 mmol) solution (DI water, 100 μL) followed by ethylenediamin (8.9 μL, 0.13 mmol: 10 eq) in DI water (100 μL). The reaction mixture was stirred at

25 ^{o}C for 8 ~ 9 h (X 2) and then microspheres were washed with DI water 2 ~ 3 times for the Kaiser test. 1 mg of particles was used to perform a Kaiser test (positive:deep purple color). To a solution of sugar (53 mg, 0.13 mmol: 10 eq) in DI water (200 μL) was added EDC (31 mg, 0.16 mmol) solution (100 μL of DI water) followed by NHS (19 mg, 0.16 mmol) solution (DI water, 100 μL). The reaction mixture was stirred for 10 min and then combined with amine-functionalized particles in DI water (200 μL, pH = 9). The mixture was stirred at 25 ^{o}C for 8 ~ 9 h (X 2) and then performed the Kaiser test with 1 mg of particles (negative: dark brown color). Di water was removed and wet sugar-coated particles were dried under high vacuum. (Particle aggregation was prevented by using probe sonicator during coupling reaction.)

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CHAPTER 7

Conclusions and Future Directions

In this dissertation, the first automated solution-phase syntheses of oligosaccharides that are related to infectious disease such as HIV, leishmaniasis and tuberculosis are reported. The use of a new fluorous-based automation platform enabled the facile synthesis of HIV-associated linear α-(1,2)-pentamannose as well as a *Leishmania*-capping tetrasaccharide. In addition, chemistry for the construction of phosphate-linked sugars was successively programmed and applied to the synthesis of lipophosphoglycans associated with *Leishmania*. The new automated methods developed herein and featured in the construction of complicated oligosaccharides not only opens up a new era for oligosaccharide library synthesis—capable of facilitating diversity both in structure and sequence—but also easier access to carbohydrates for systematic structure-function relationship studies. The combination of the fluorous-based automation platform with fluorous-based microarrays should be a powerful tool for accelerating research progress in glycomics.

In addition to the development of automated methods for the synthesis of several

bioactive oligosaccharides, this thesis demonstrates the importance of systematic structure-function evaluations for carbohydrate structures in understanding innate immune responses to these structures. Methods to attach synthetic sugars to latex beads or erodable polymeric particles for multivalent displays are reported. Collaborative studies show that these synthetic structures exhibit clear differences in the activation of innate immune responses based solely on differences in the carbohydrate structure. Further studies on the effect of carbohydrate structures on immune responses will aid the development of carbohydrate-based antigens as vaccine adjuvants and vaccines. Future studies should also aim to evaluate the immunomodulatory effects of structurally different cap sugars in vivo. Ultimately, significant progress both in the synthetic tools available and in the study of immunity induced by carbohydrates will provide the necessary background to fully develop and evaluate the therapeutic potential of carbohydrate-based vaccines as immunomodulatory adjuvants.

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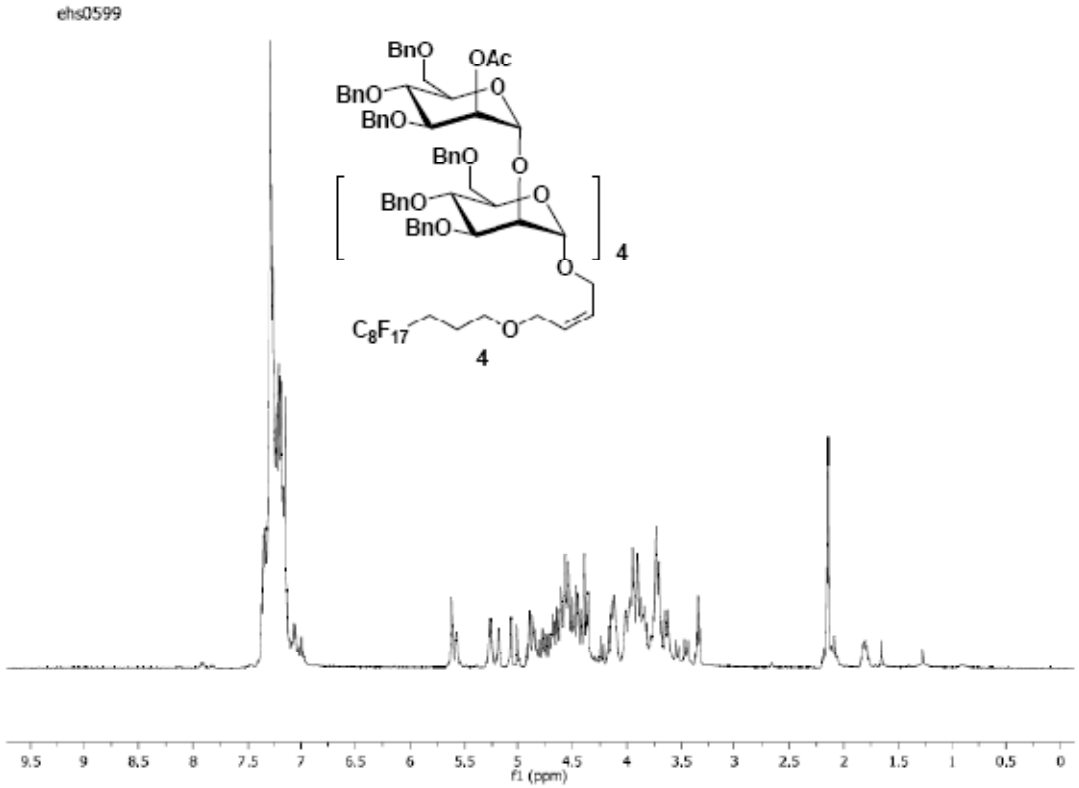
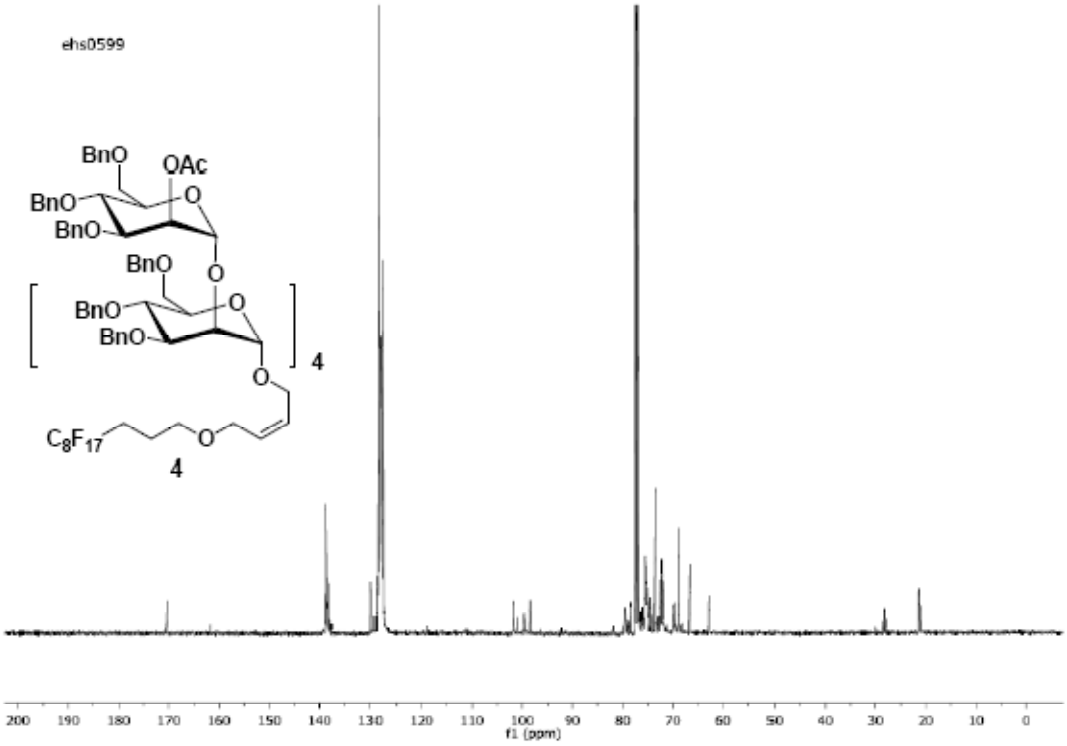
APPENDIX A.

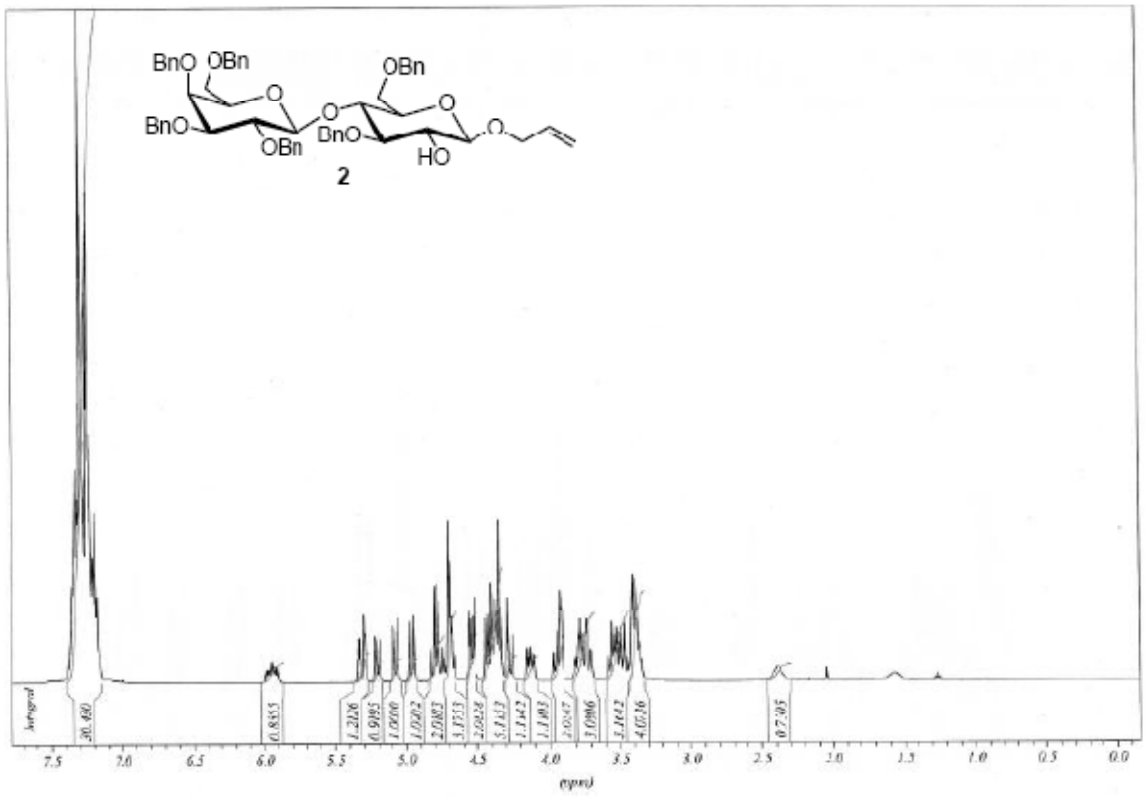
CHAPTER 2 ^{1}H AND ^{13}C NMR SPECTRA

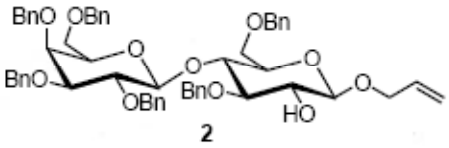




APPENDIX B.

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





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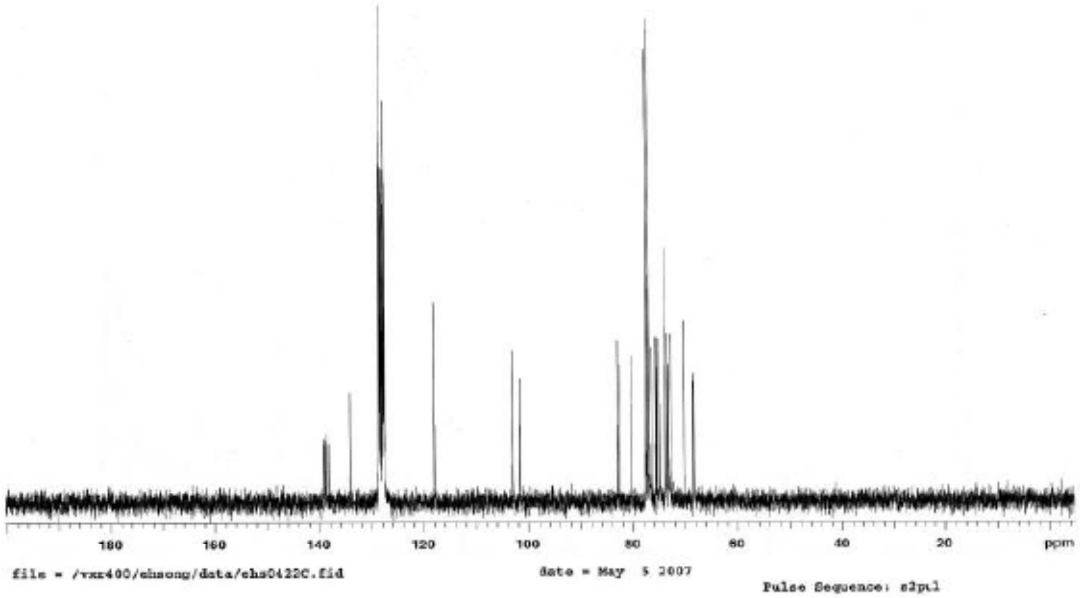

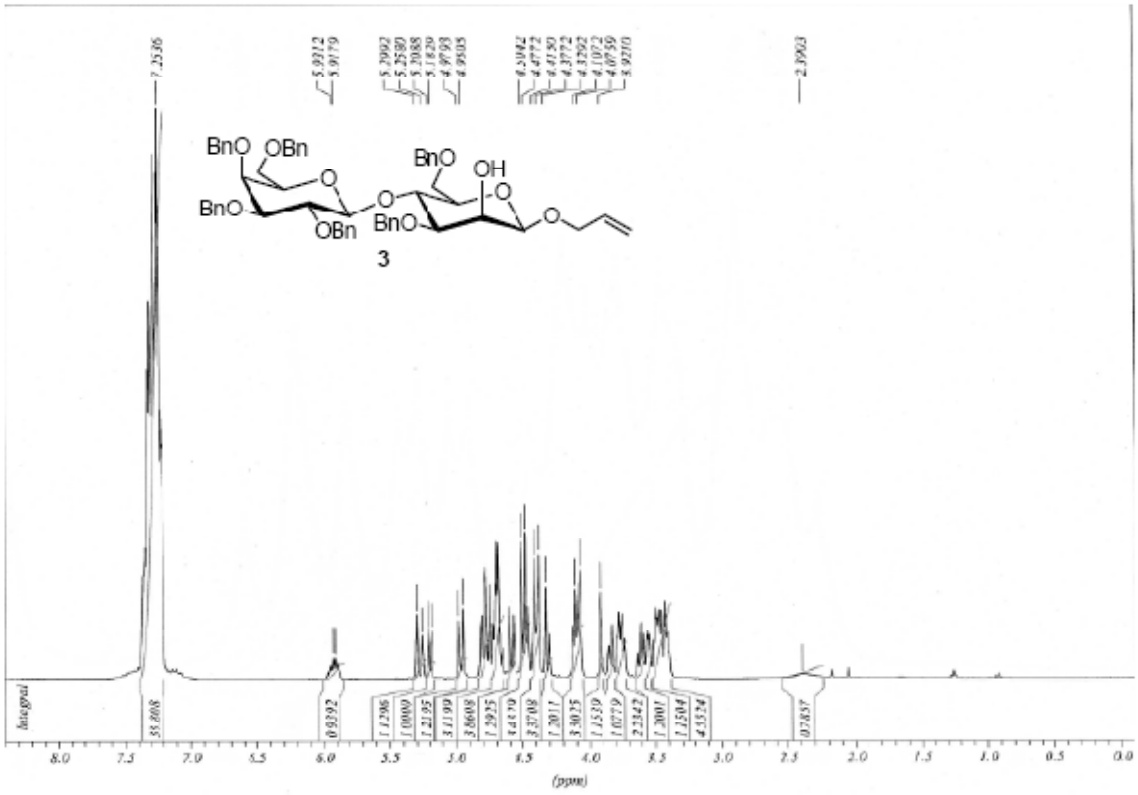

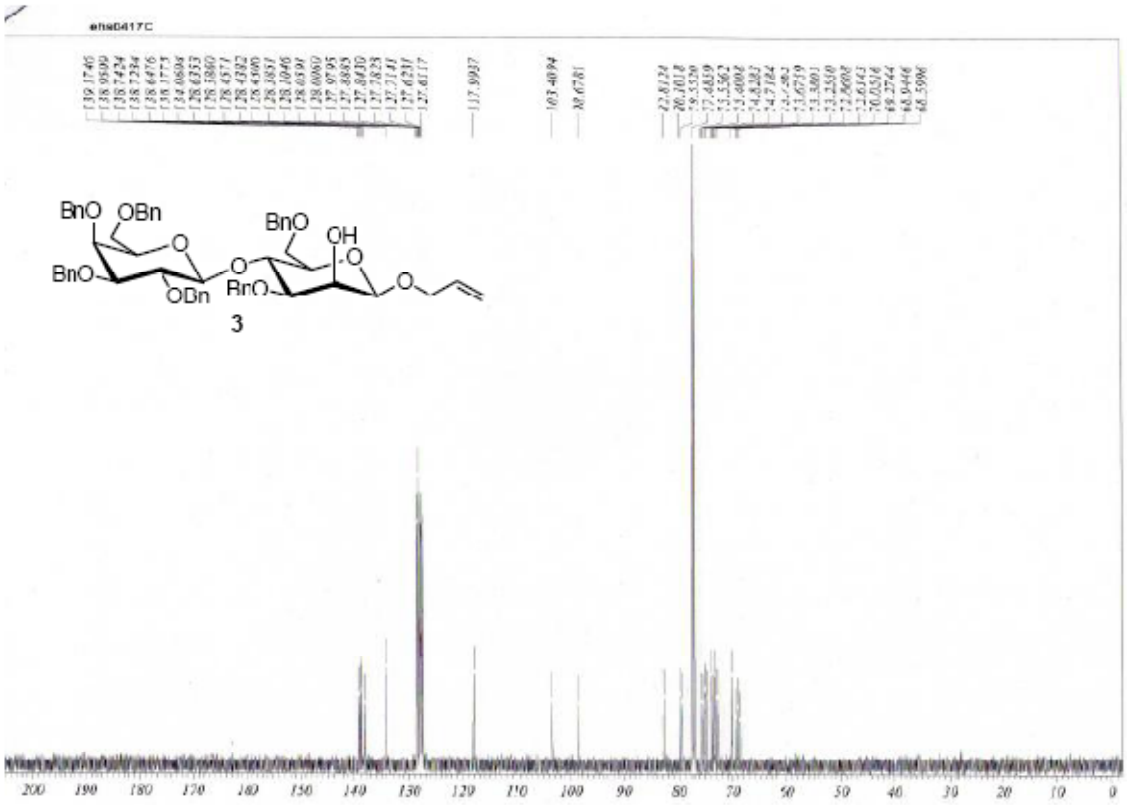

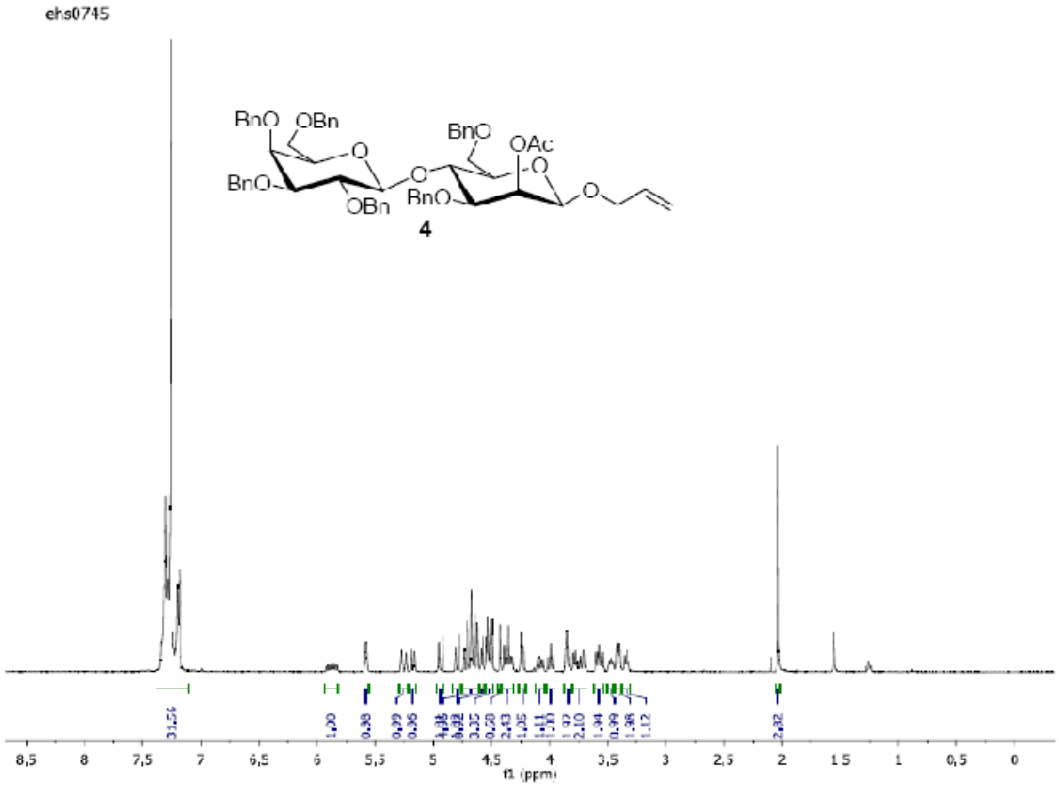

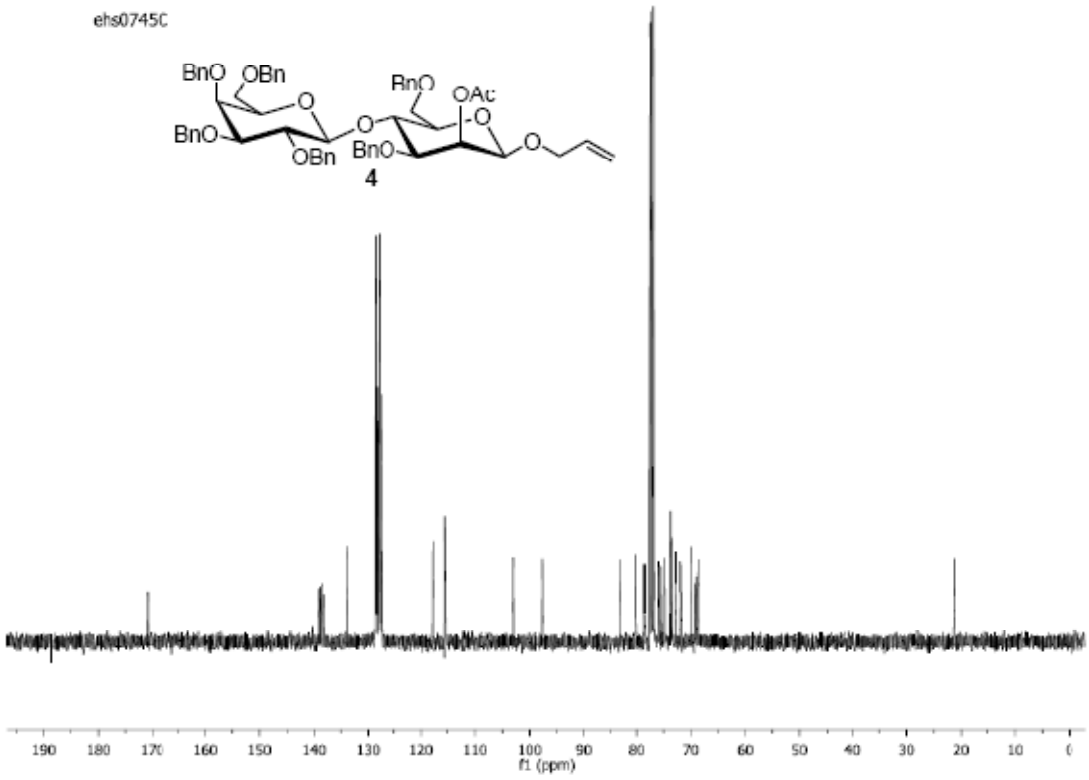

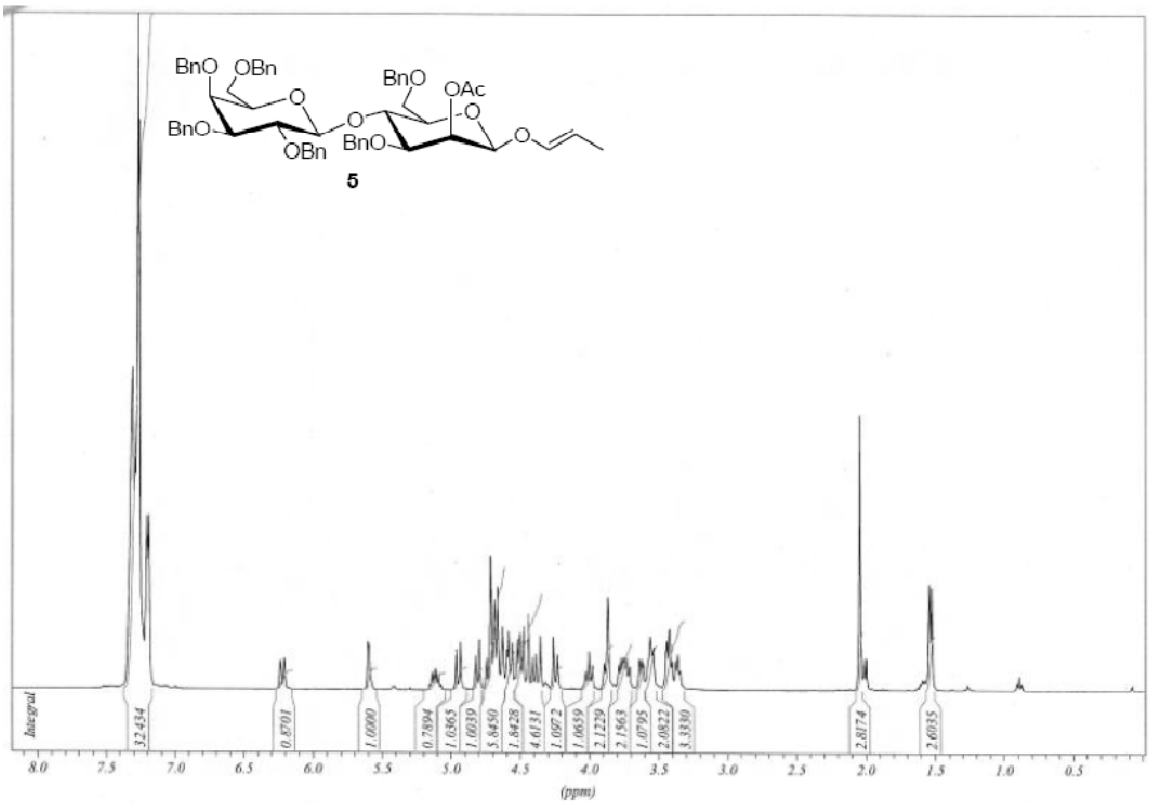

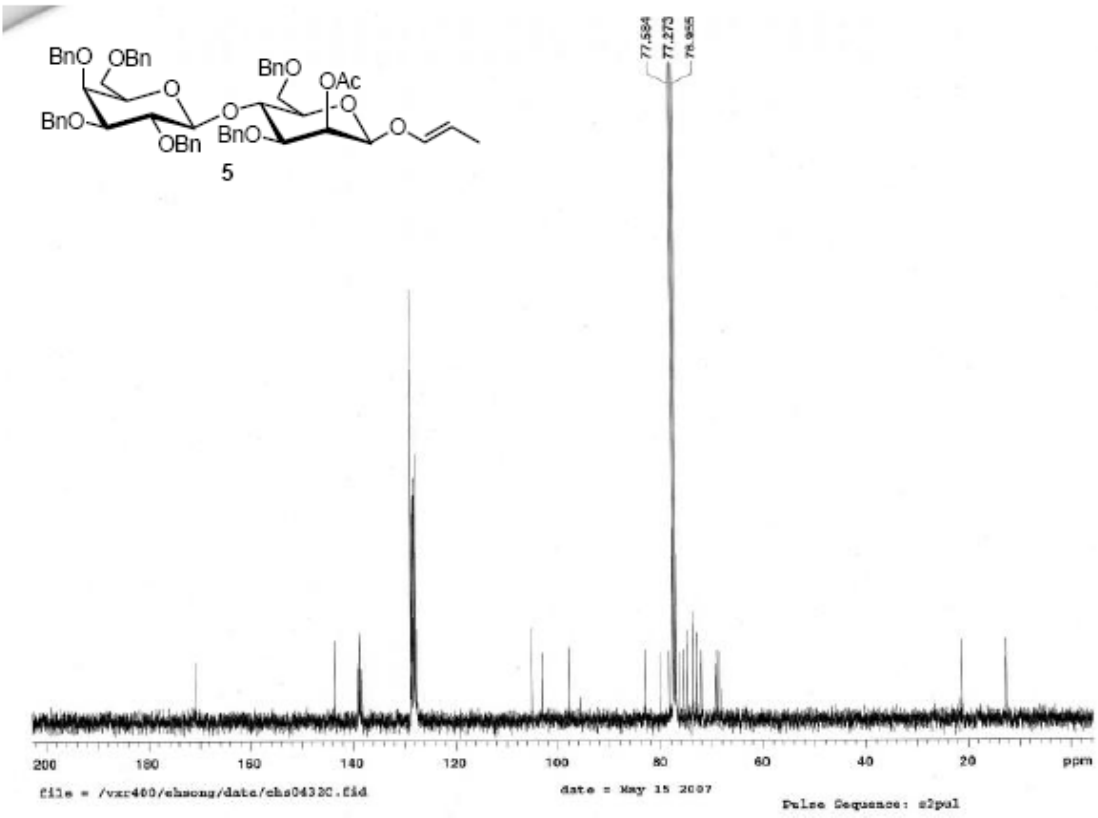

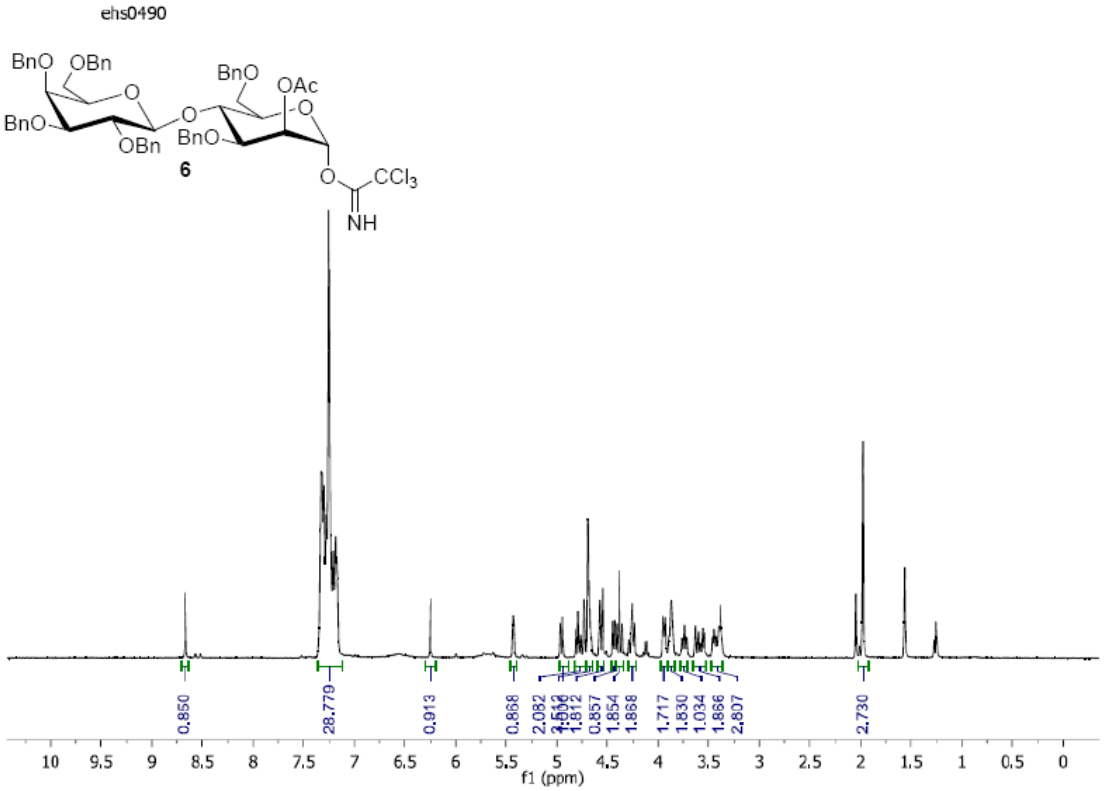

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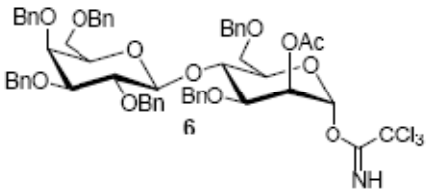

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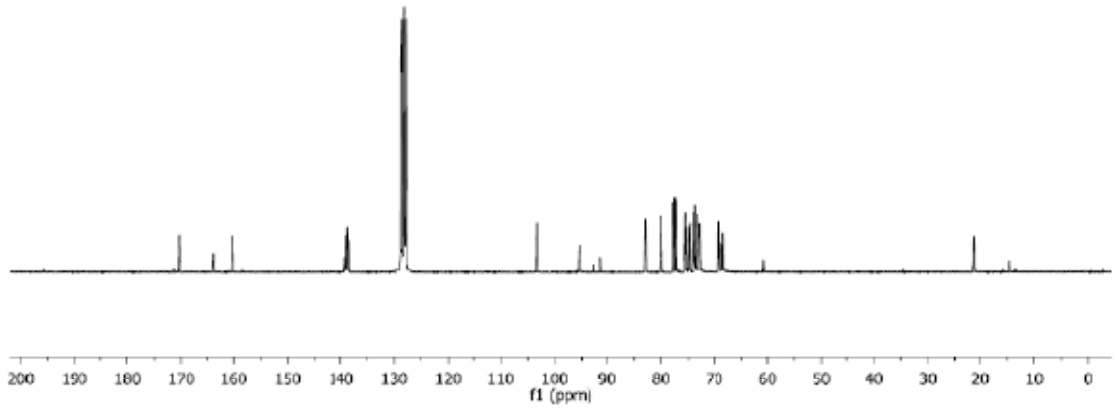

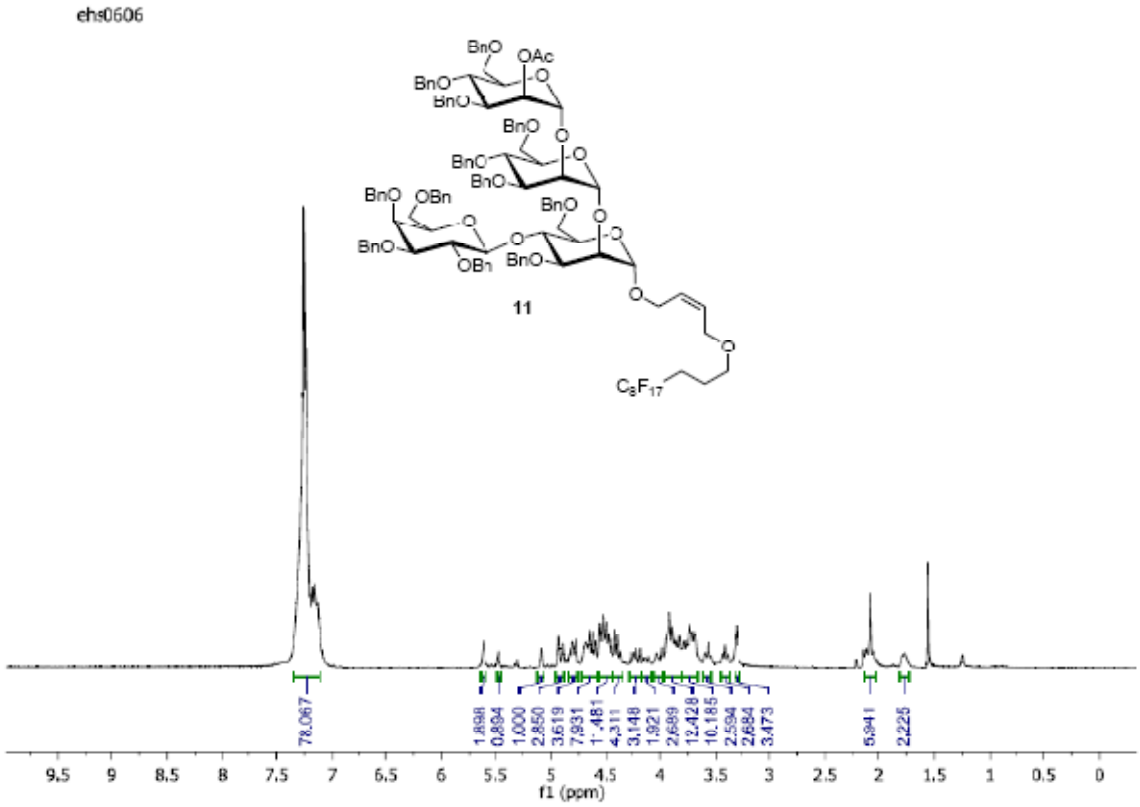

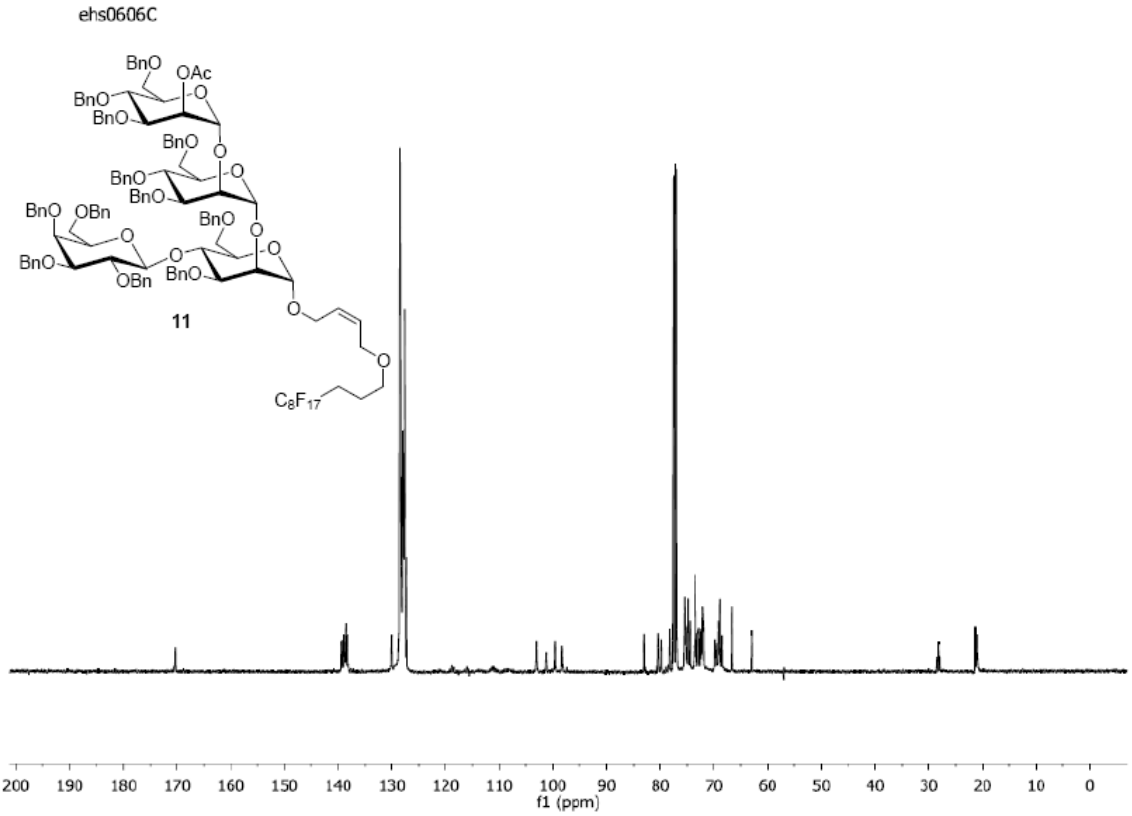

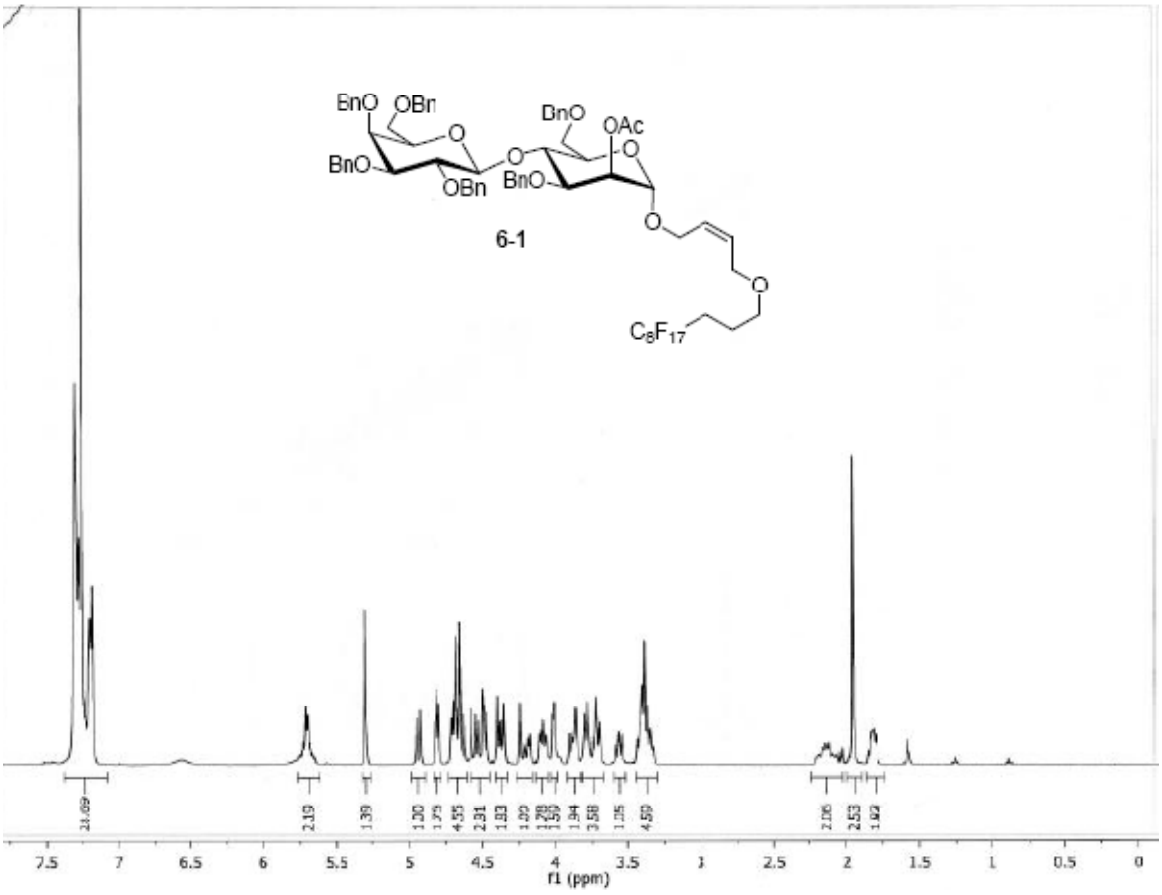

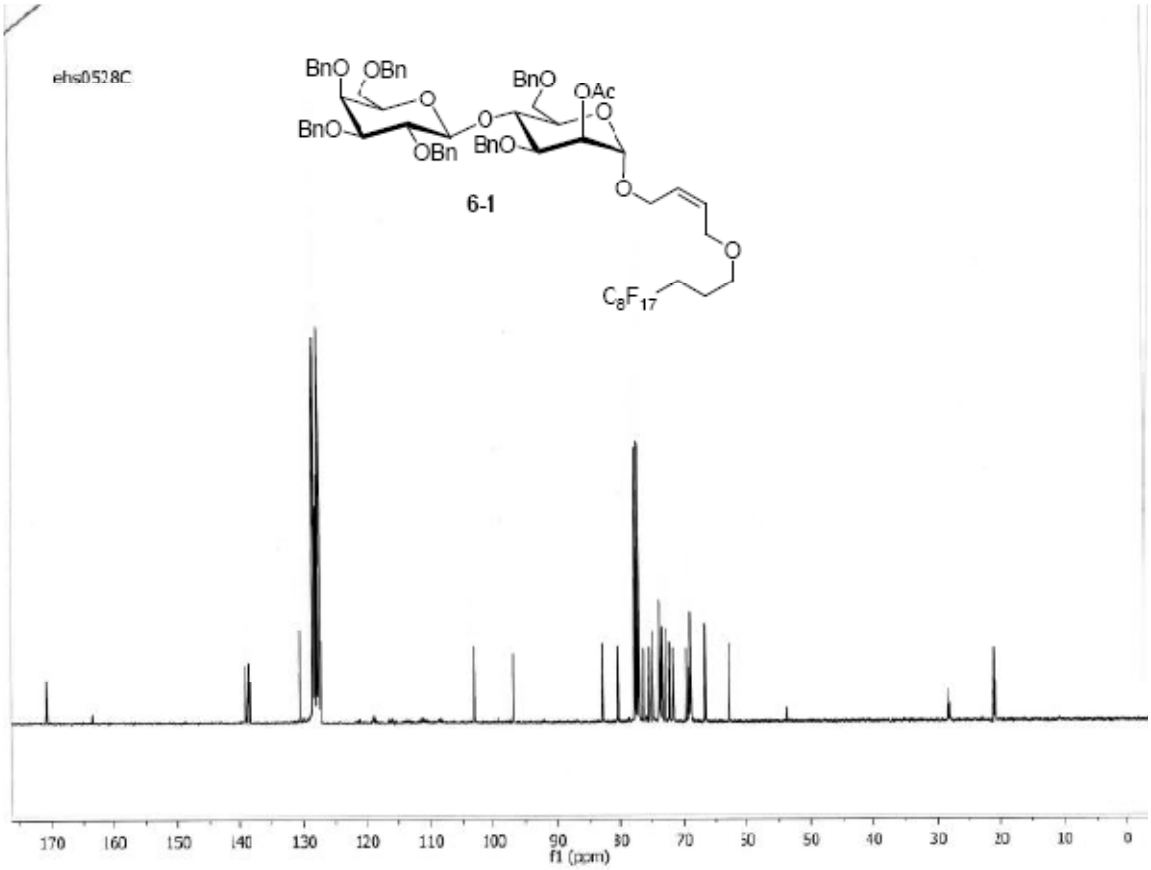

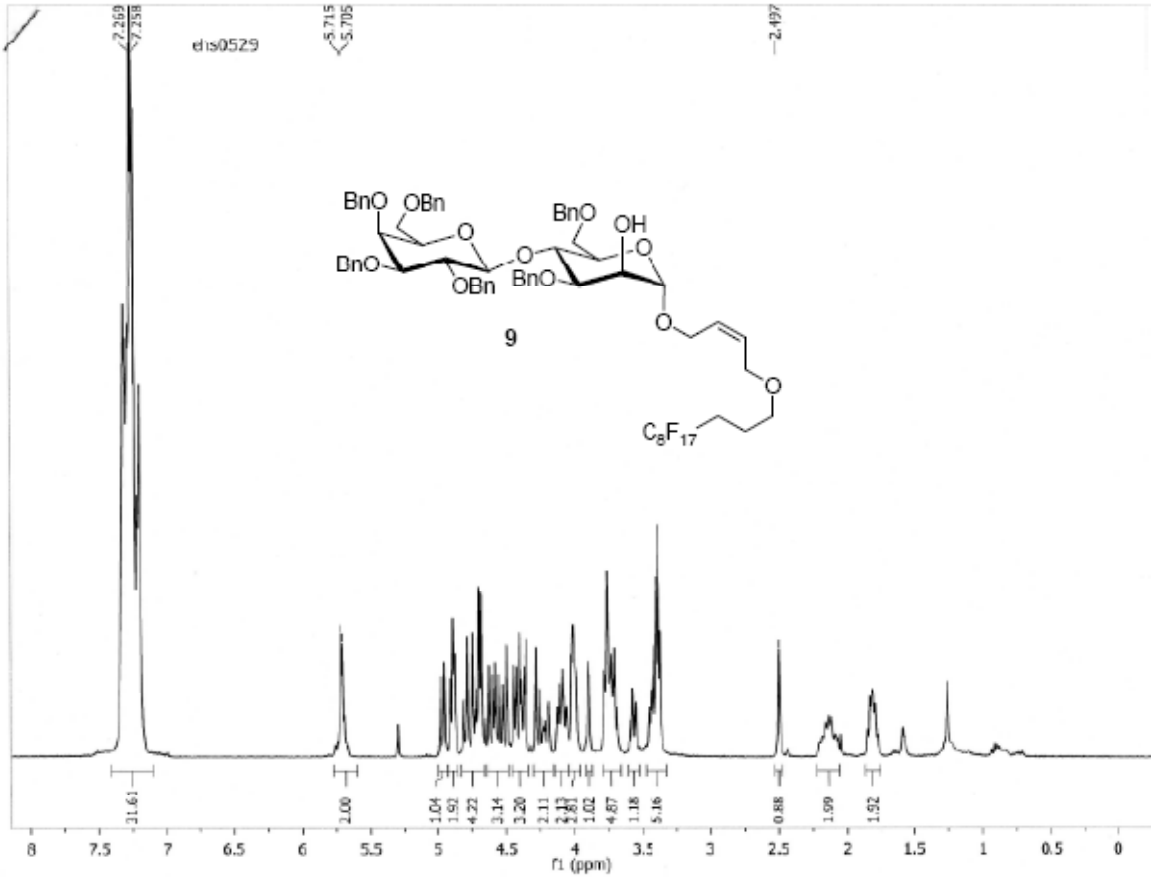

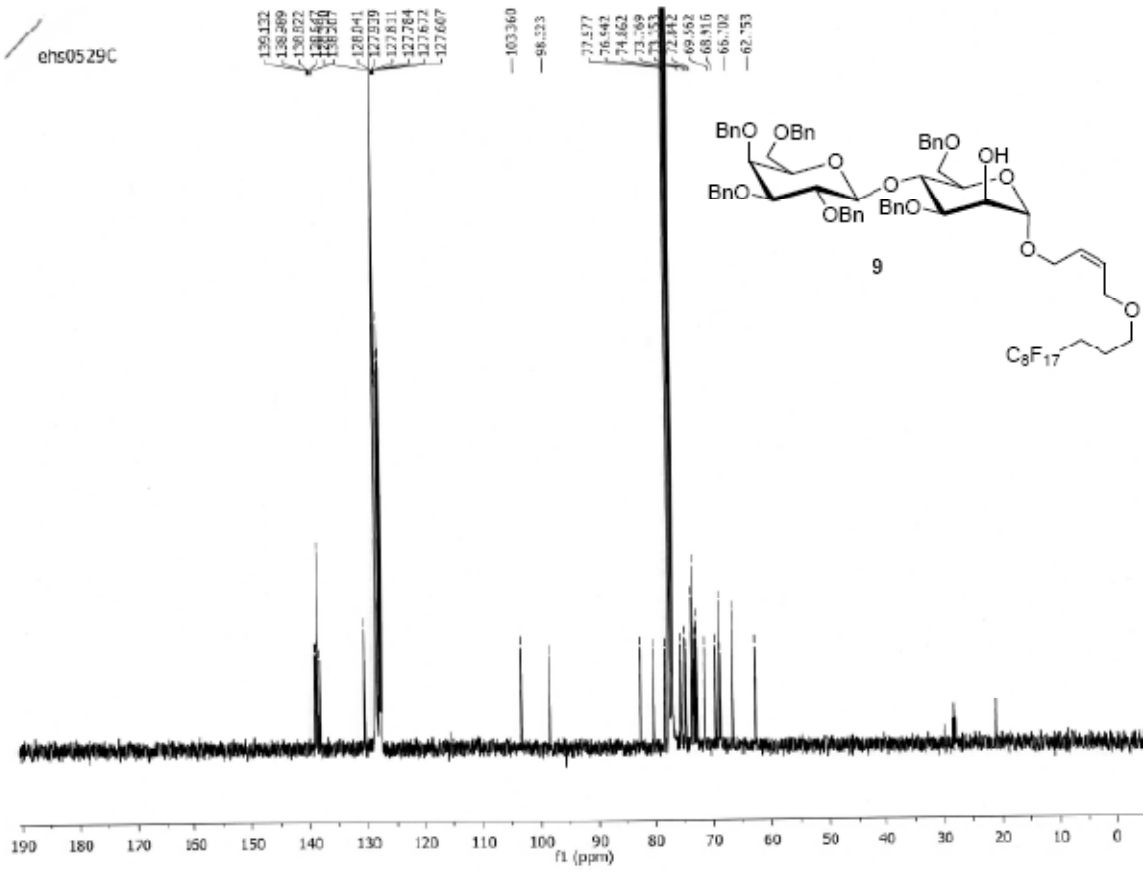

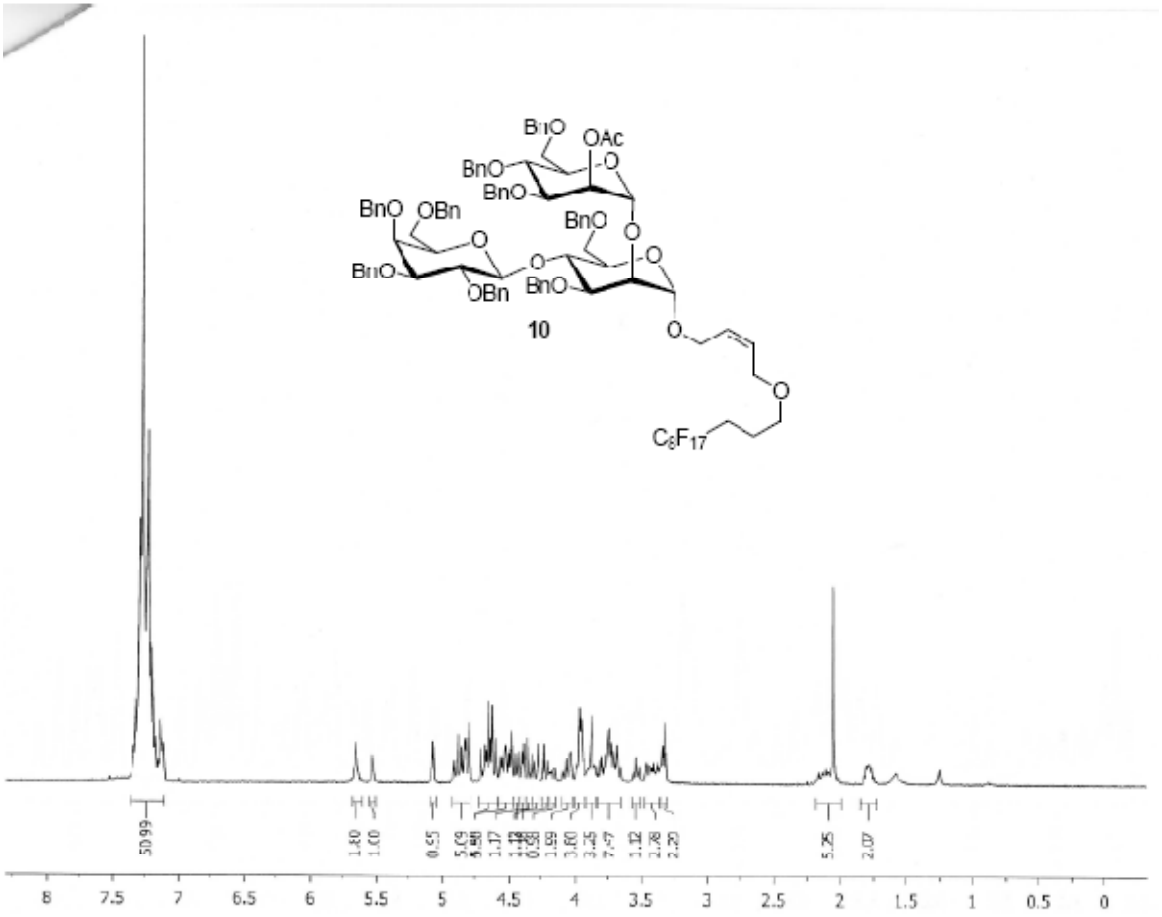

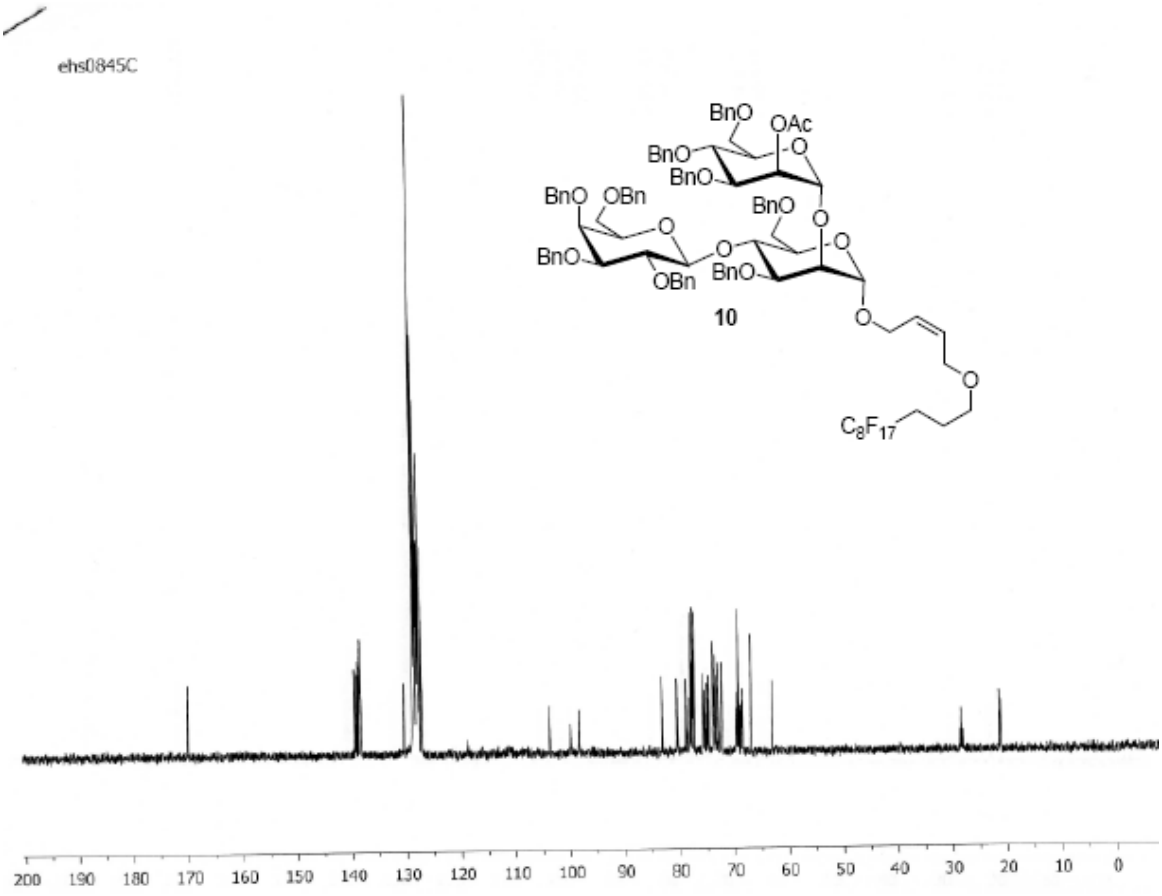

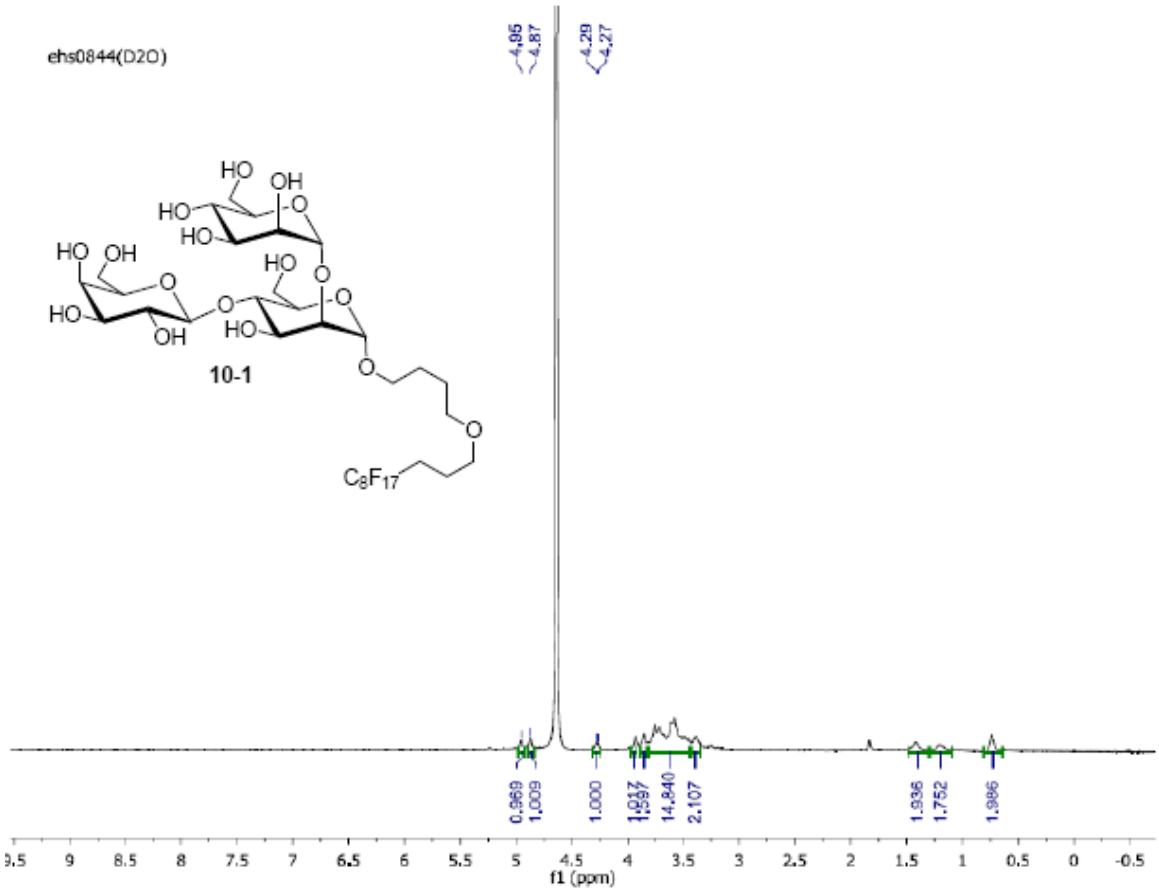

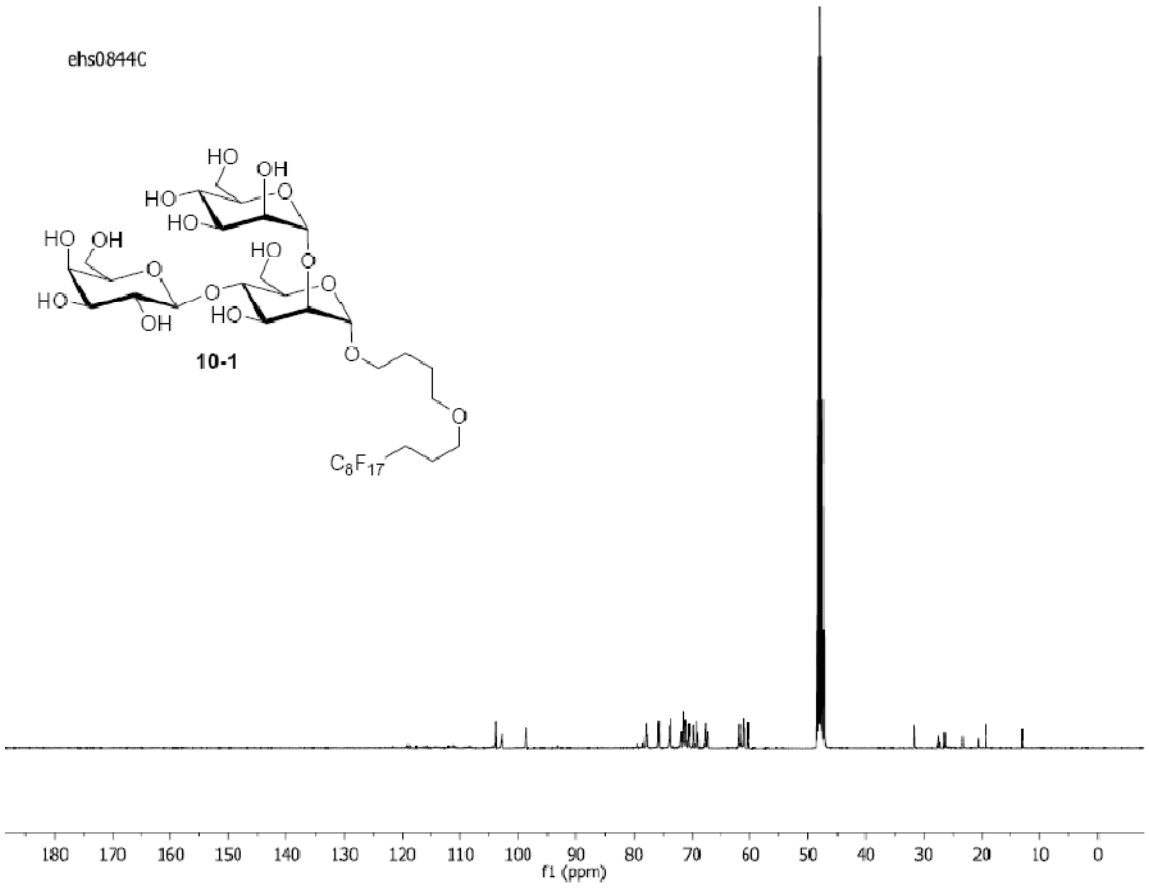

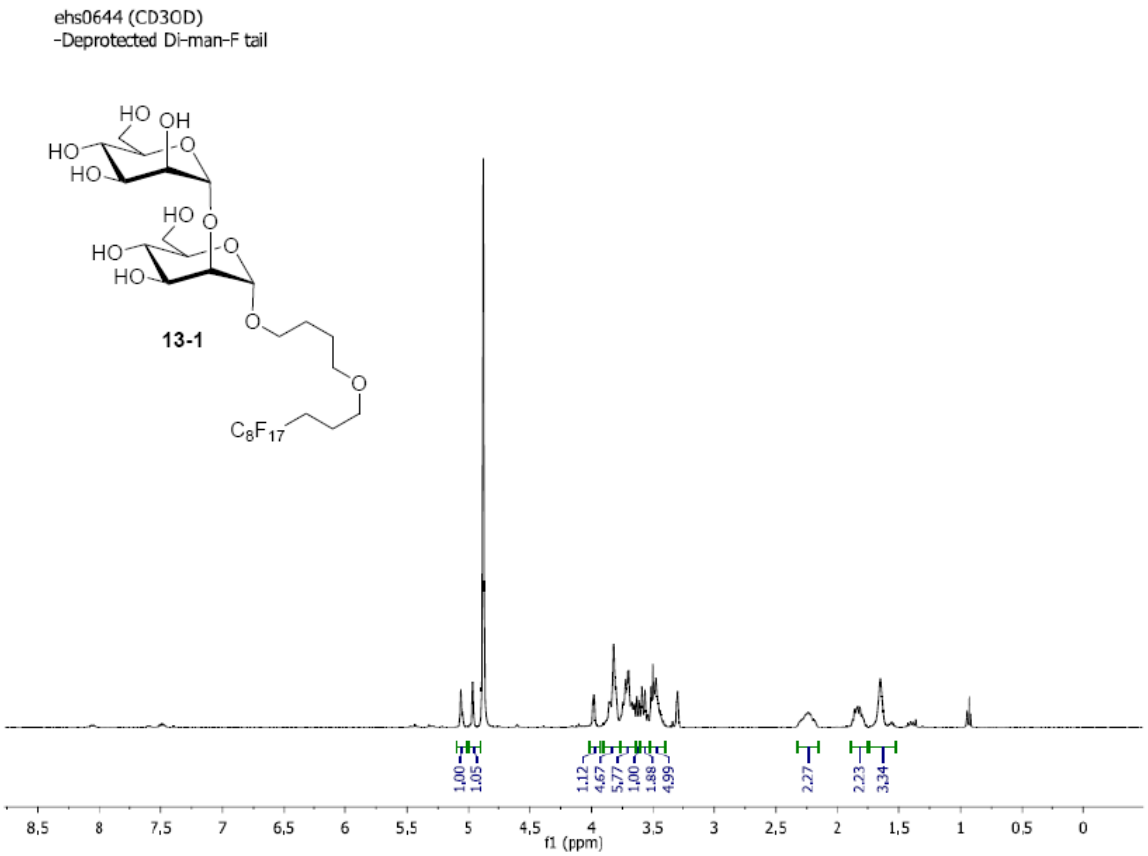

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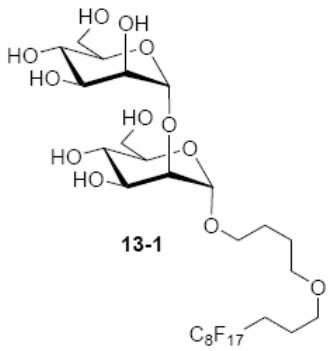

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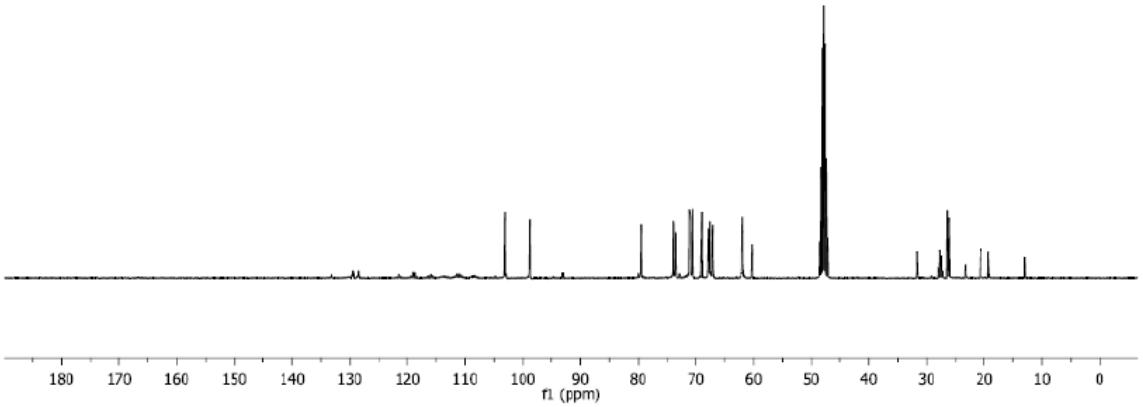

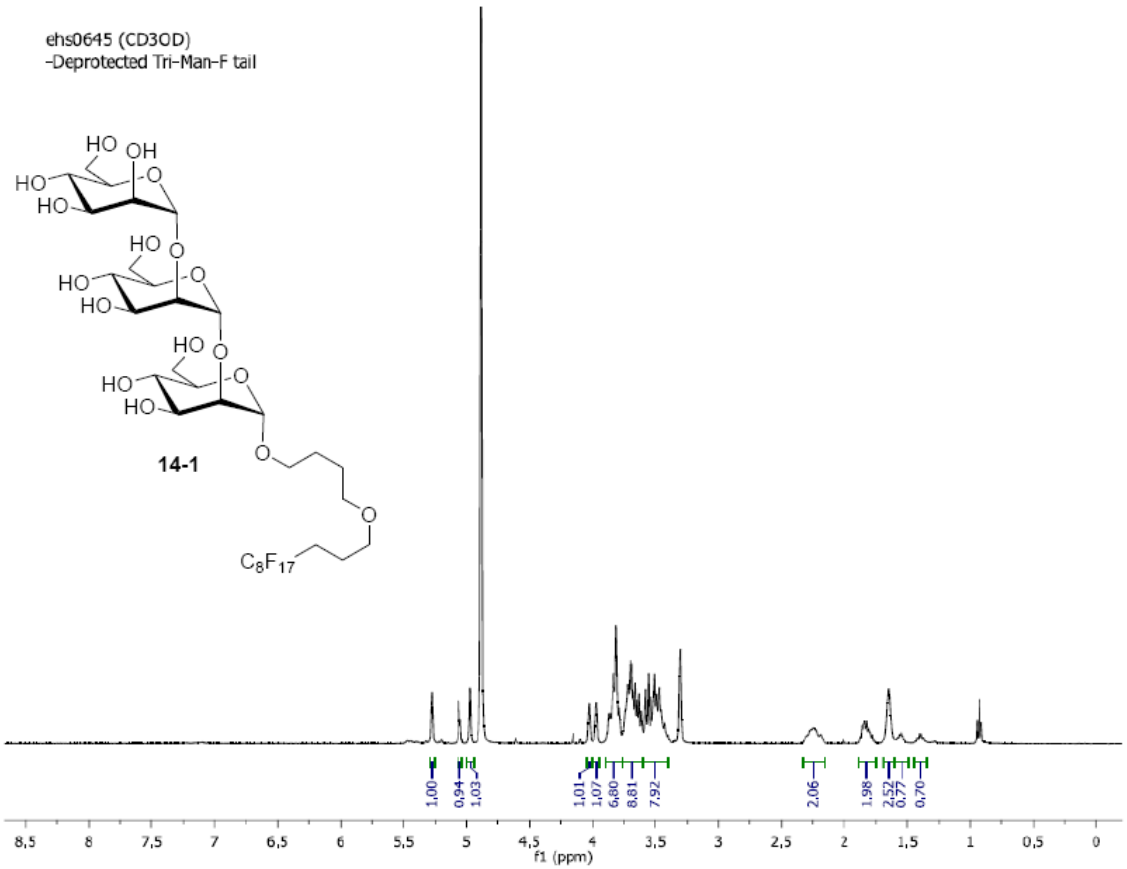

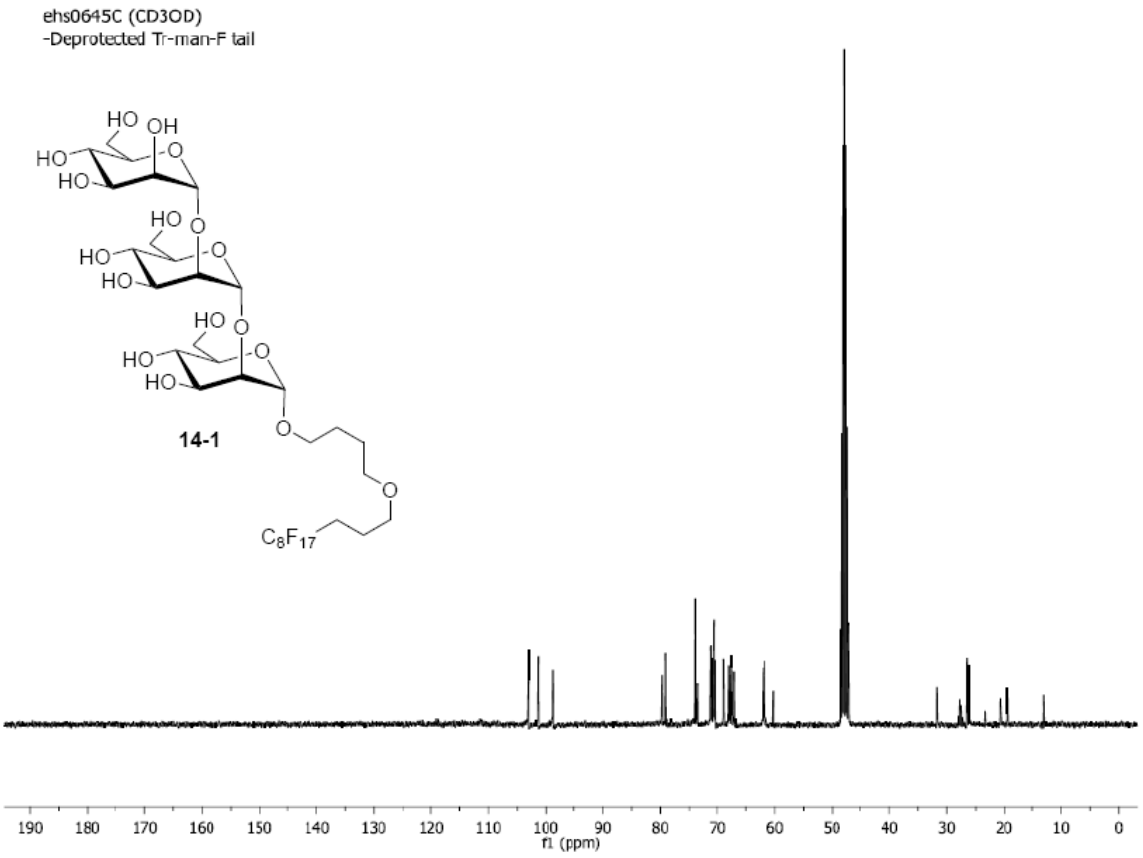

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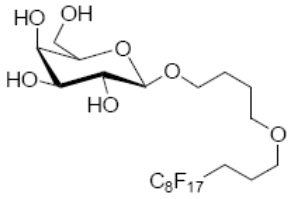

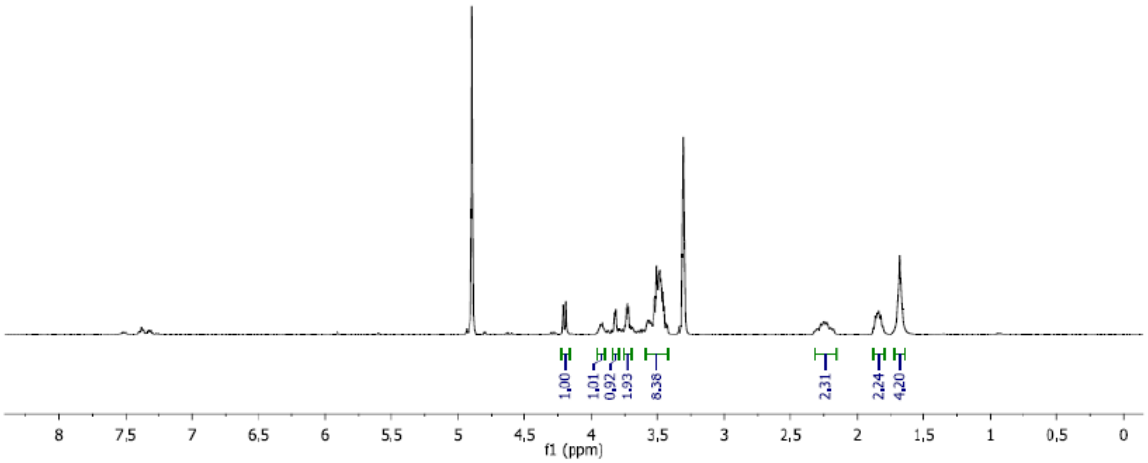

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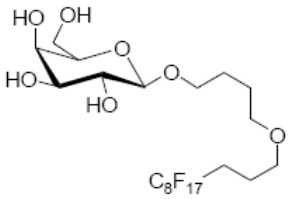
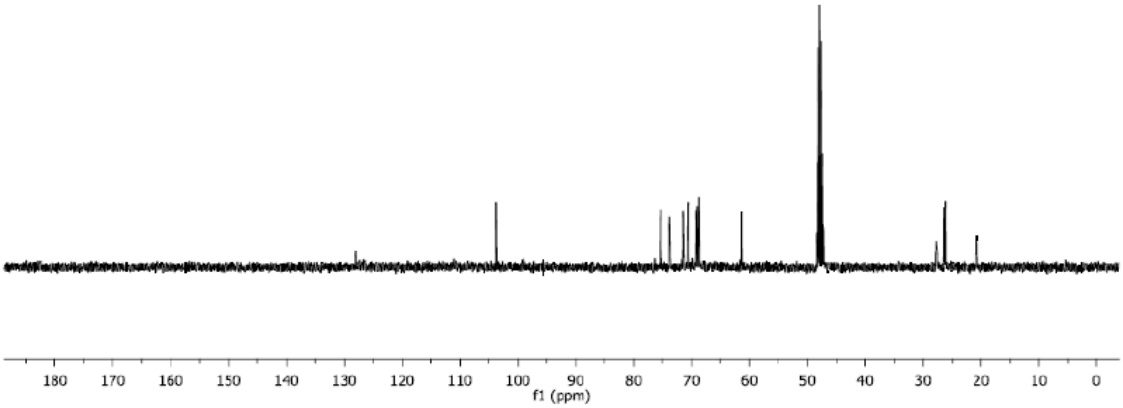
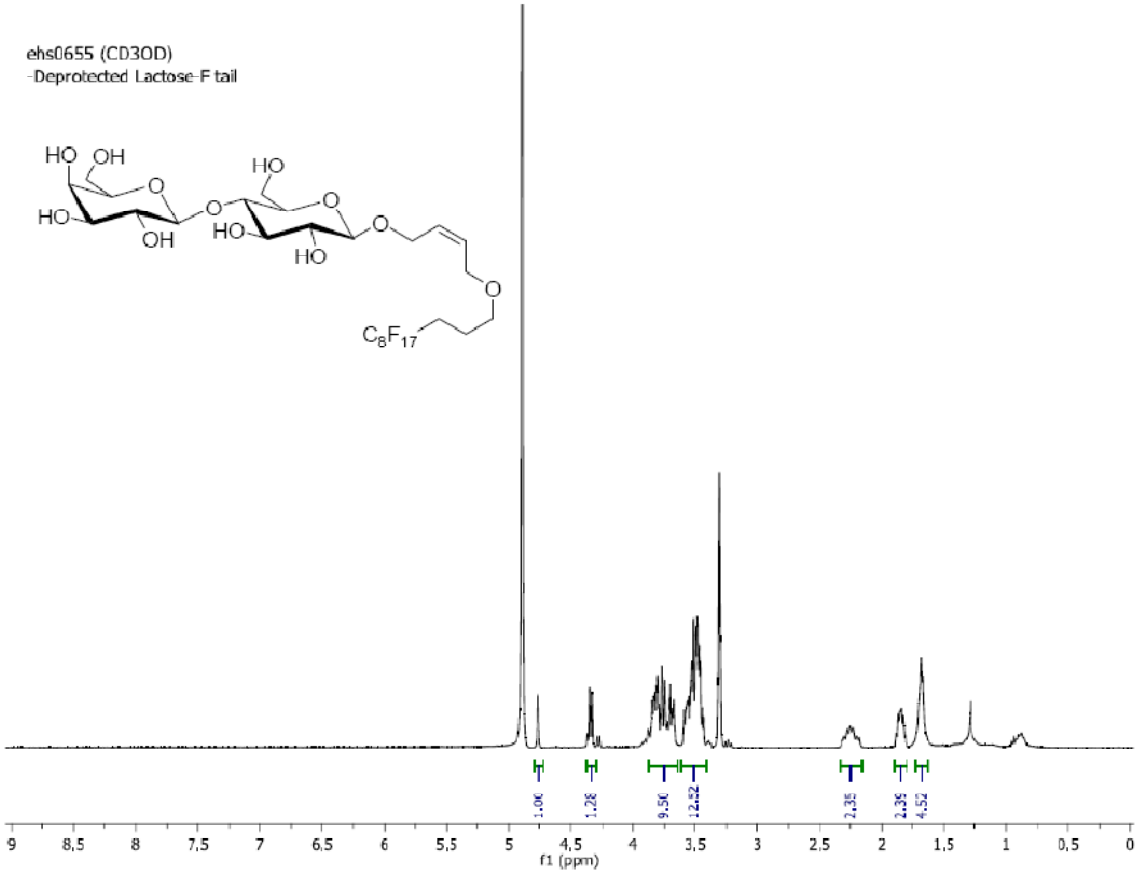
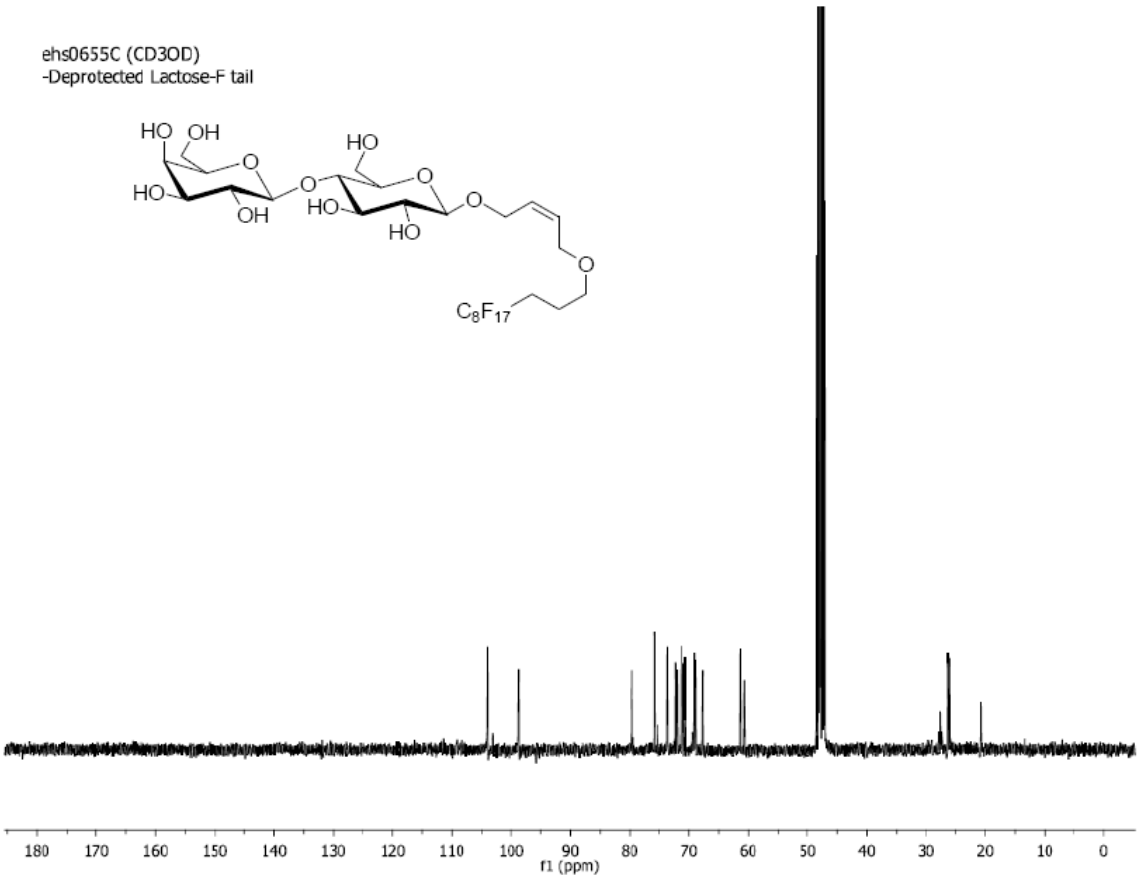


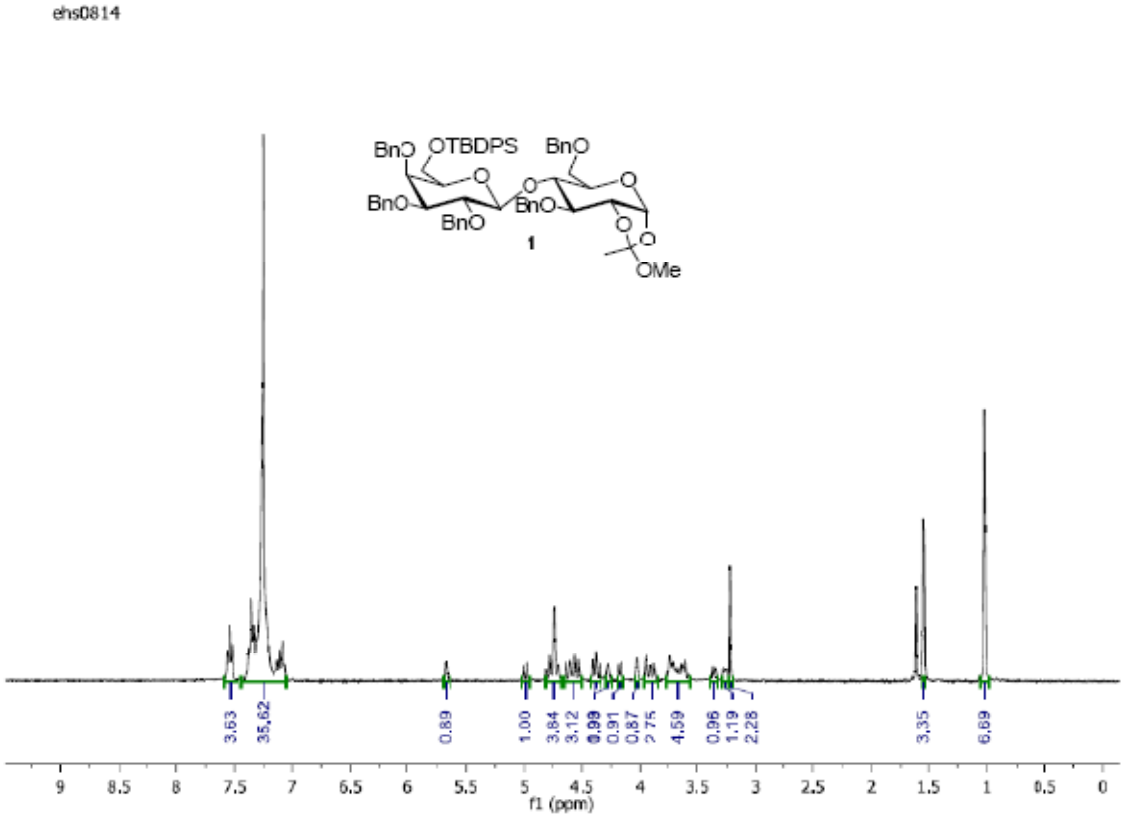


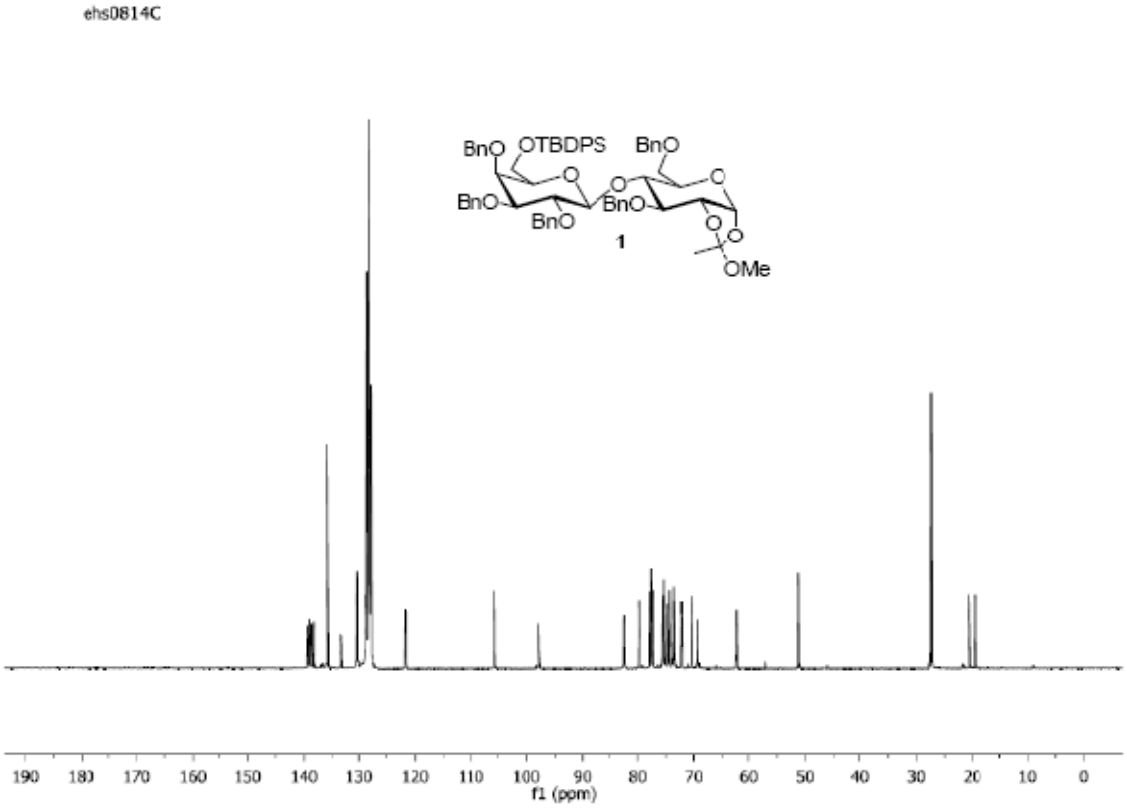


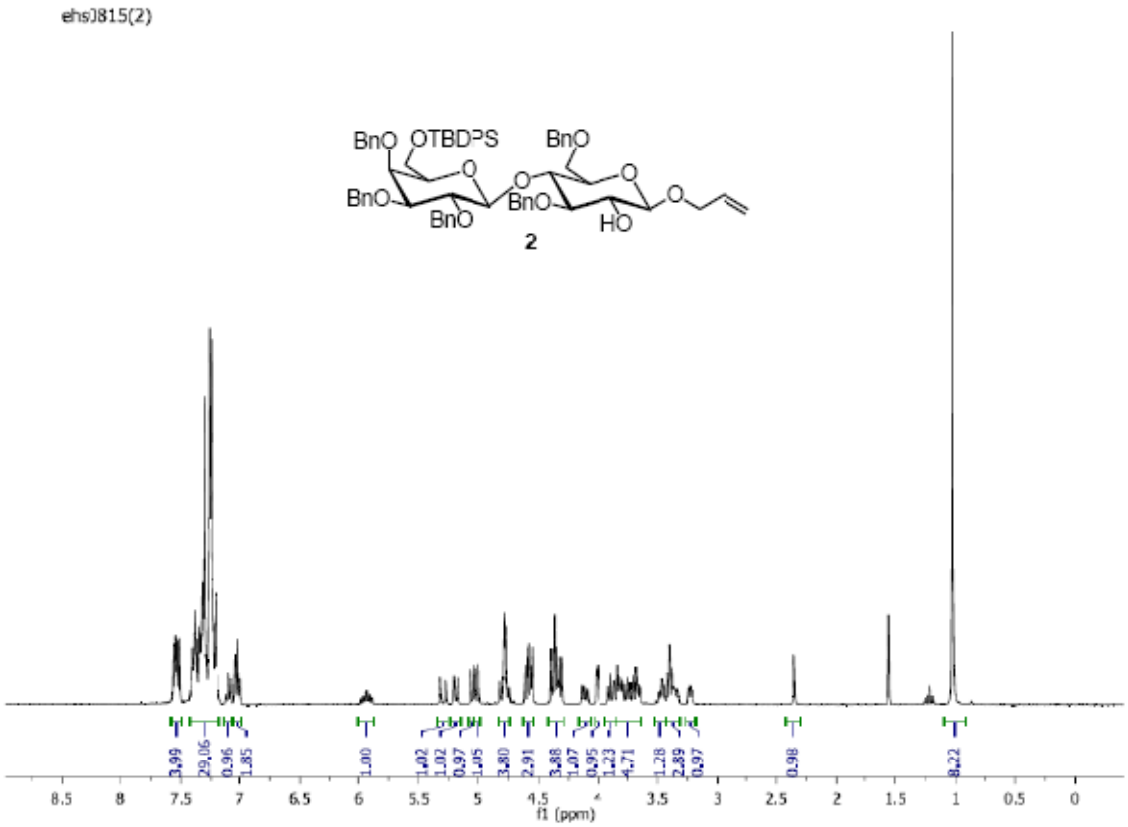


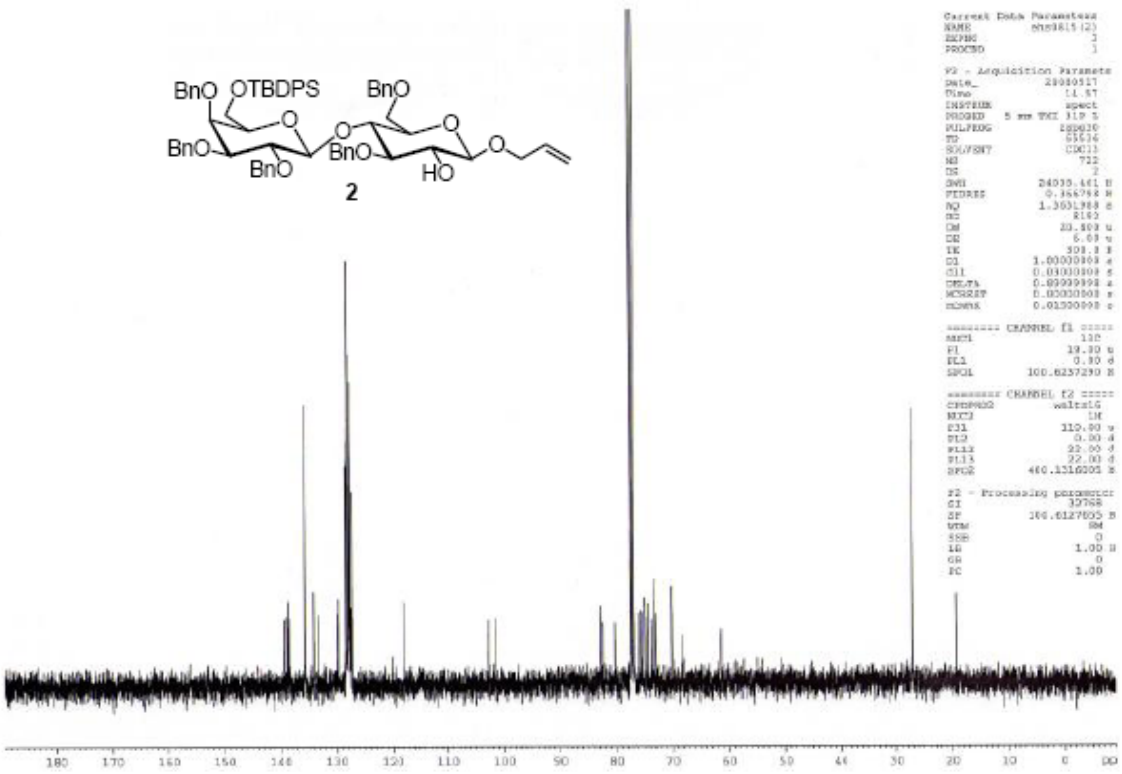
APPENDIX C.

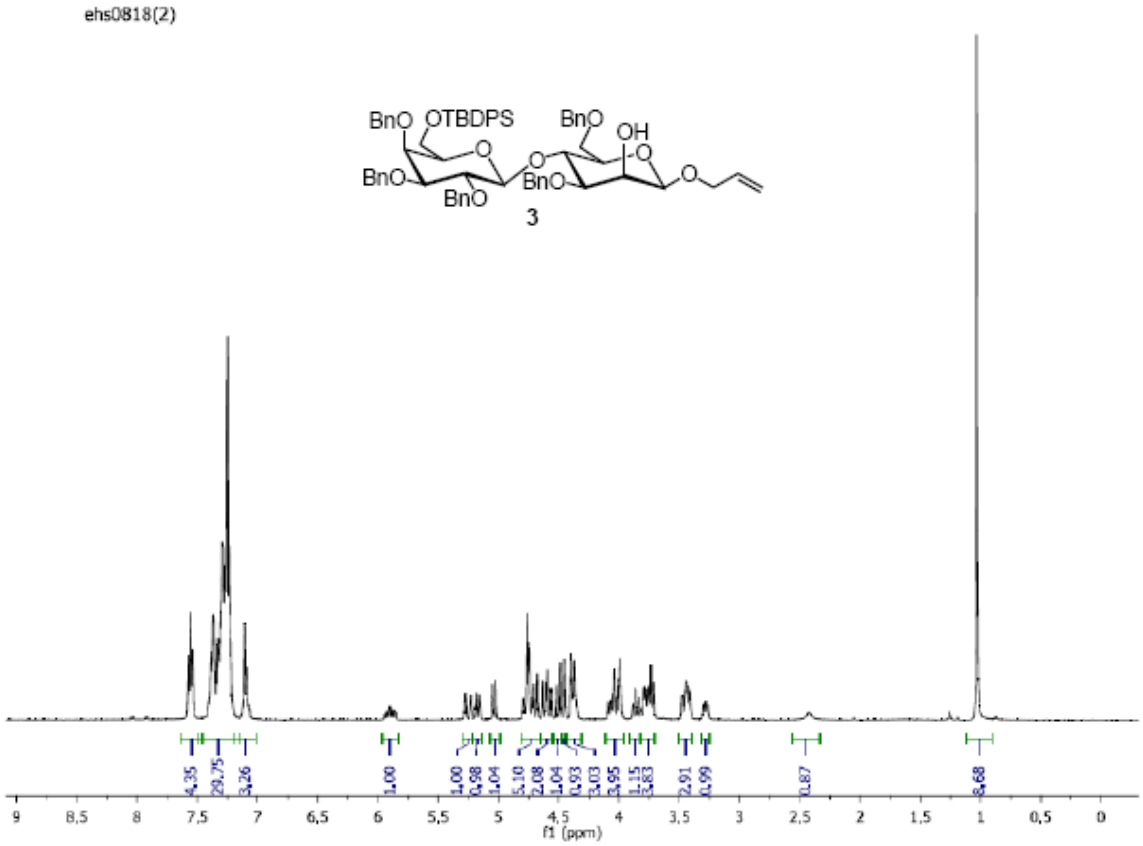
CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA

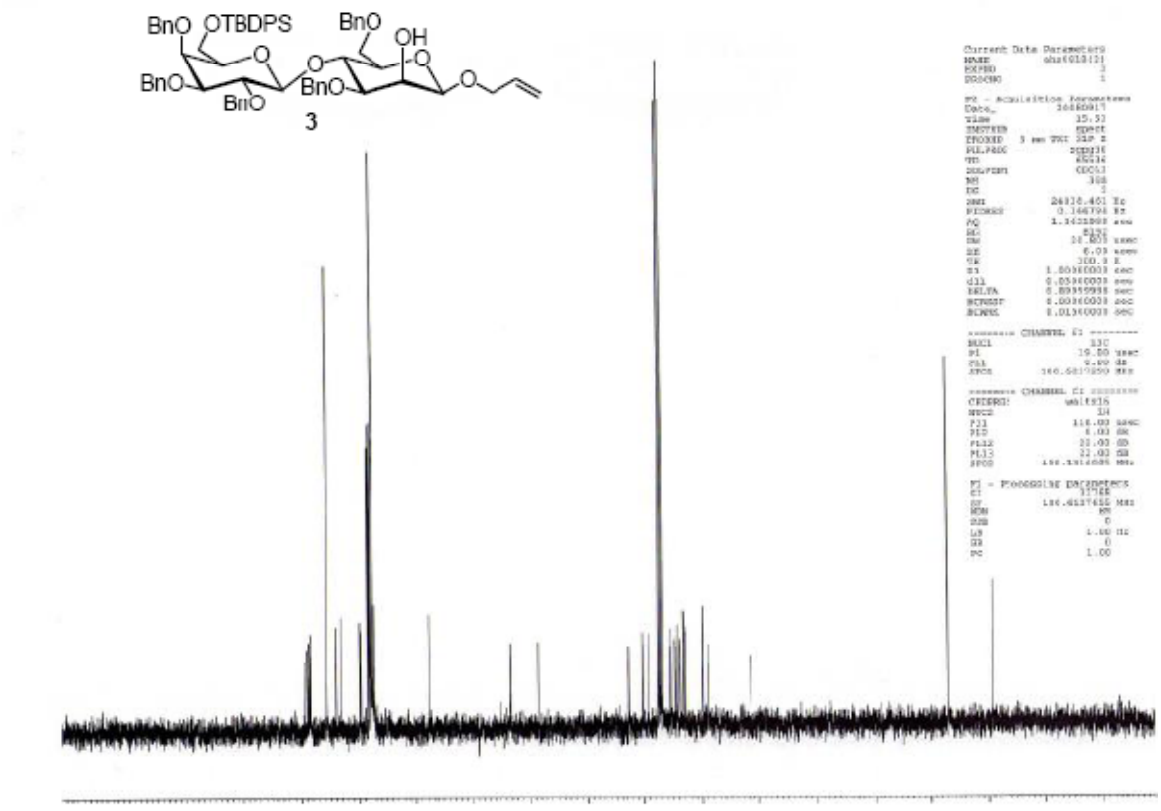


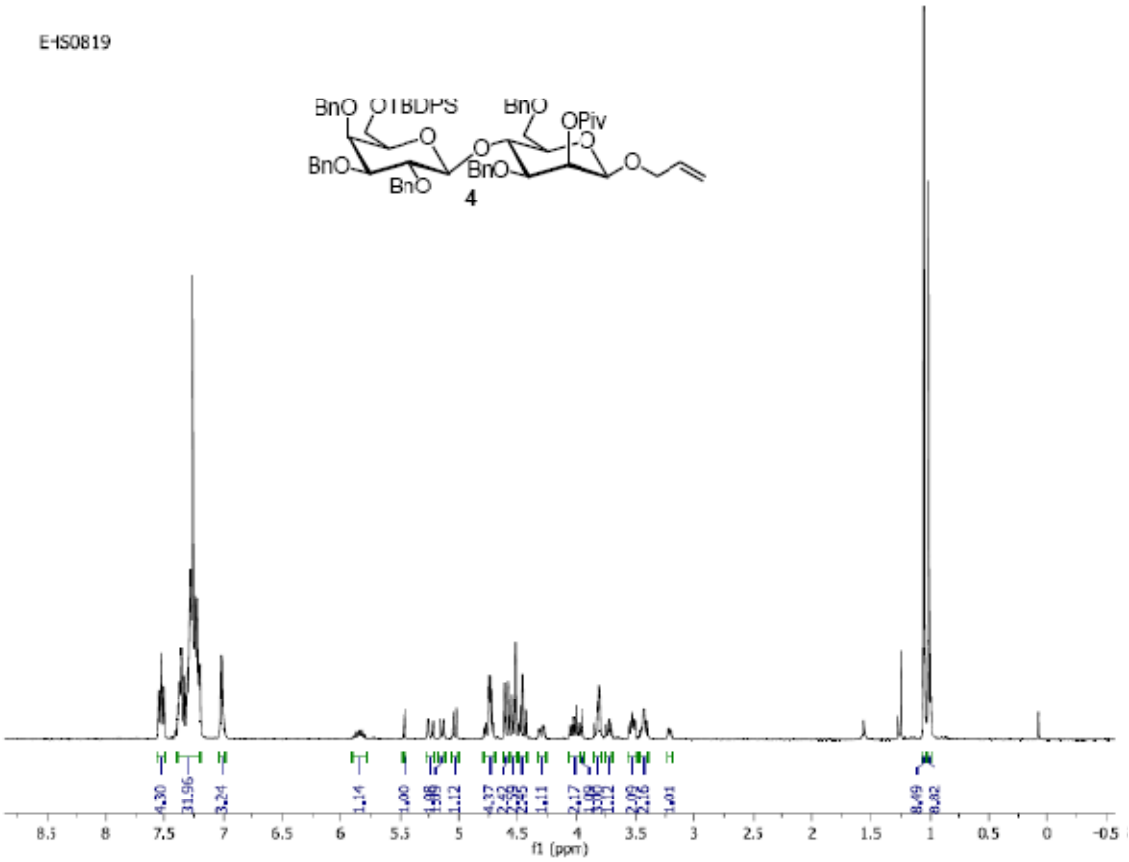


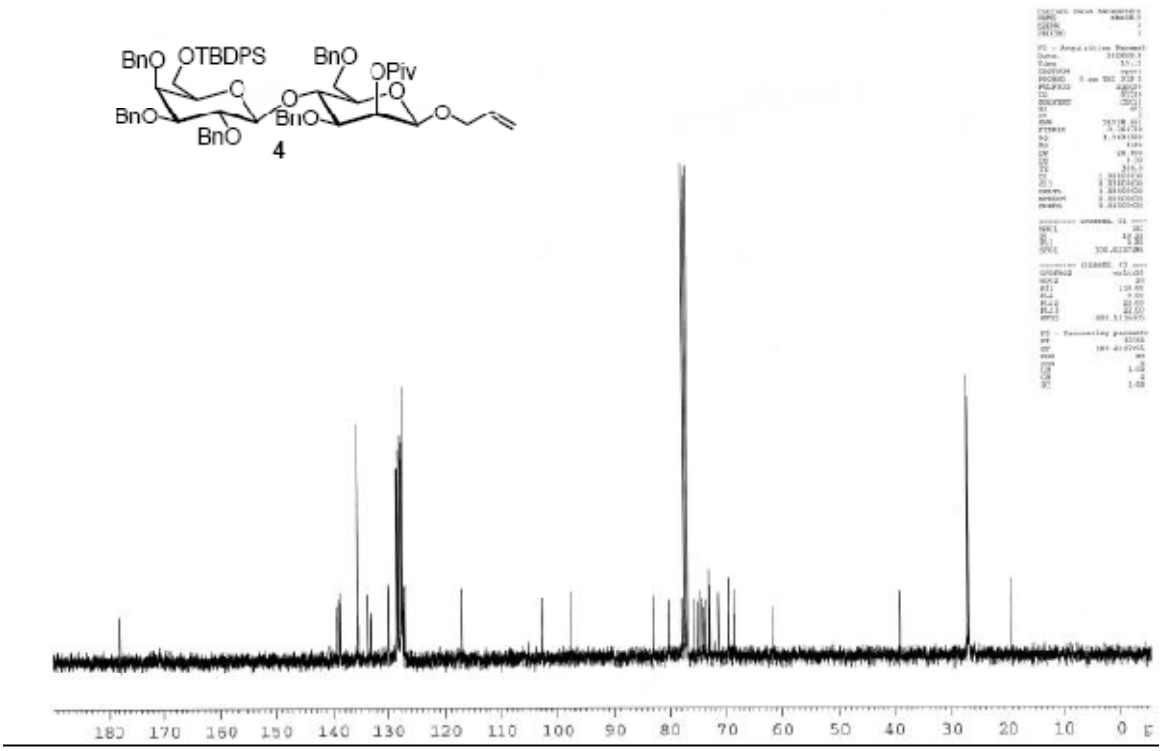


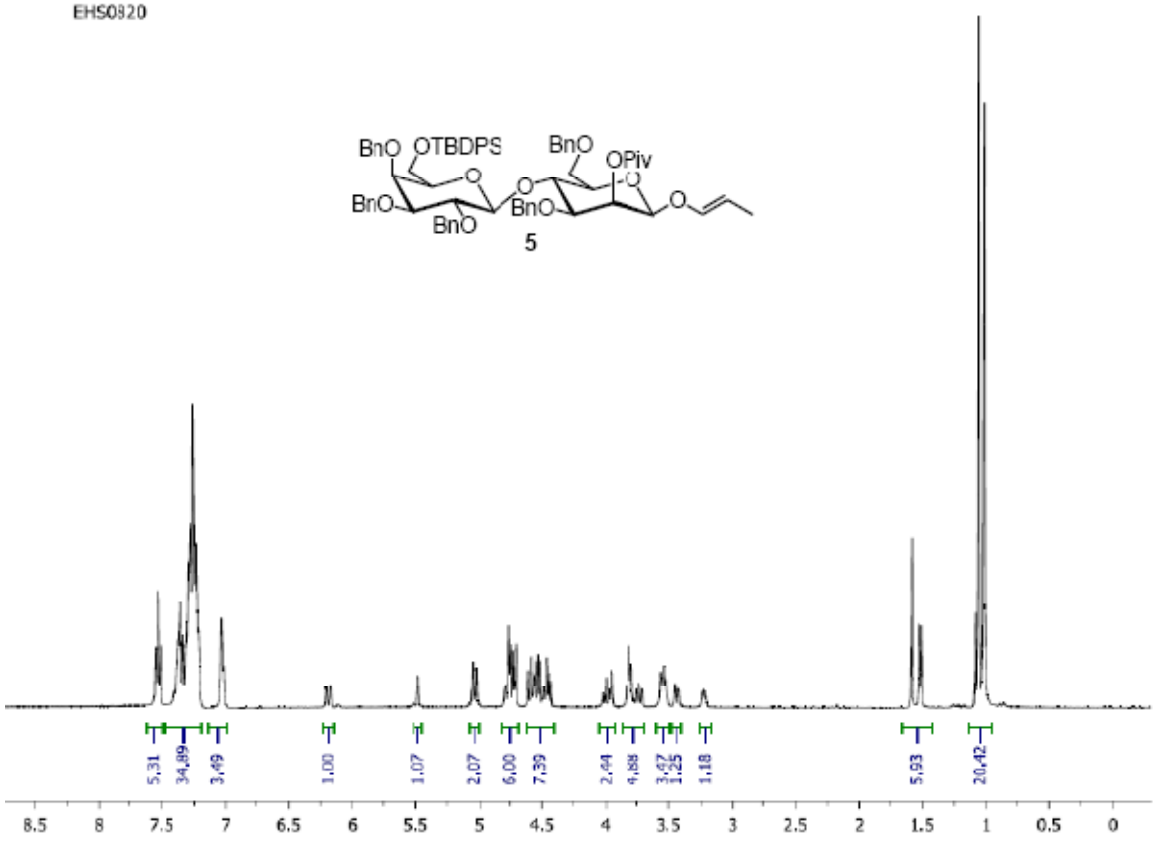


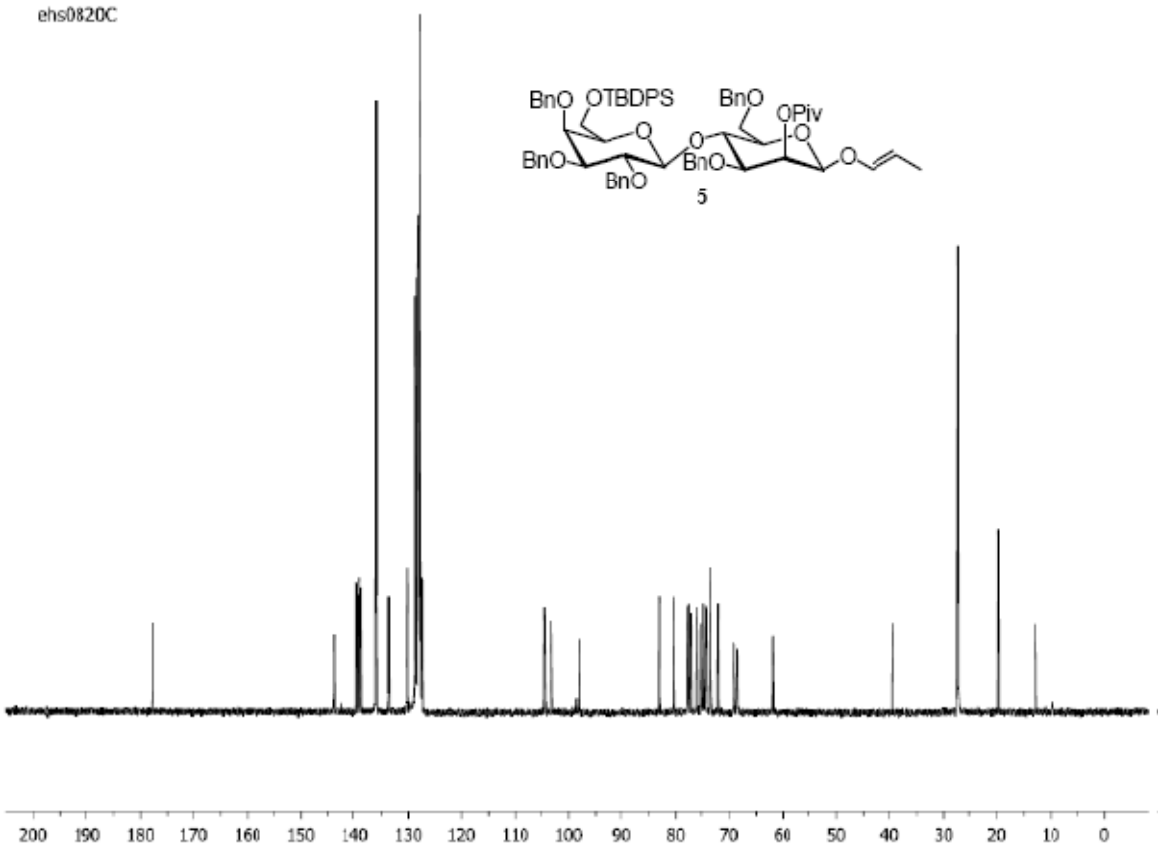


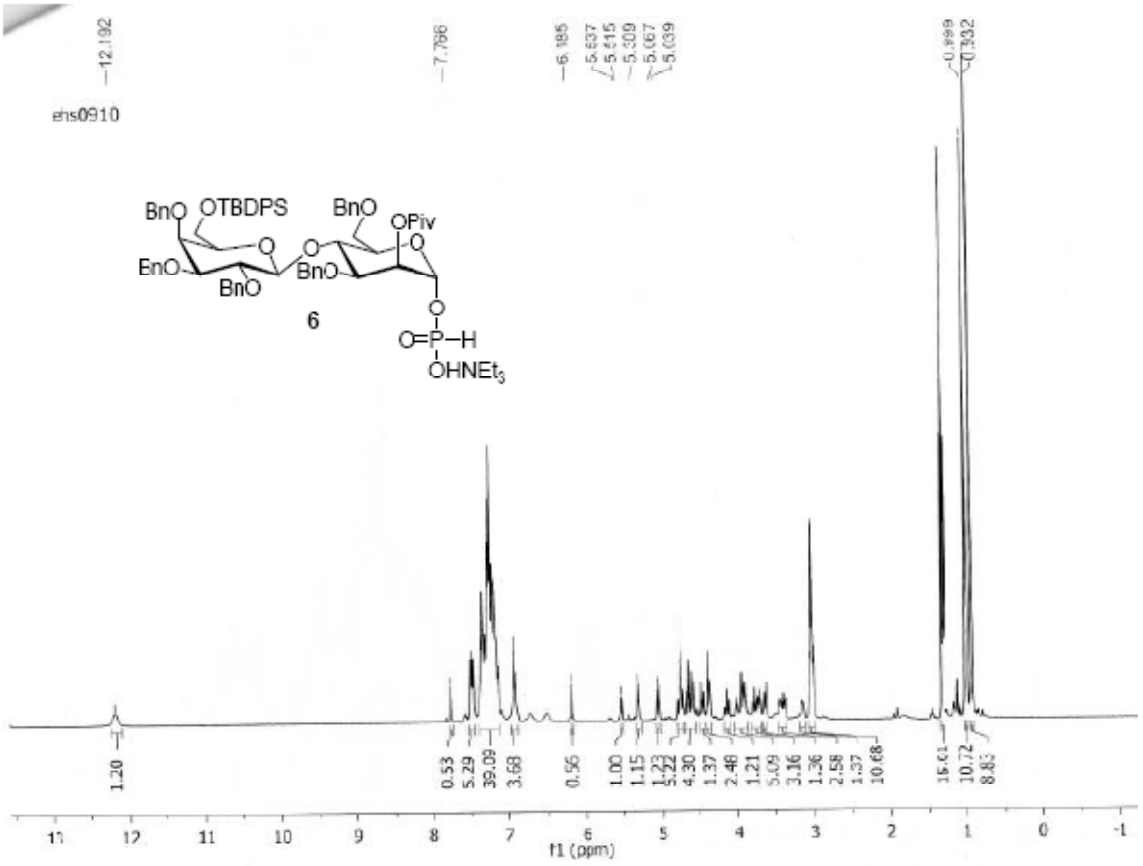












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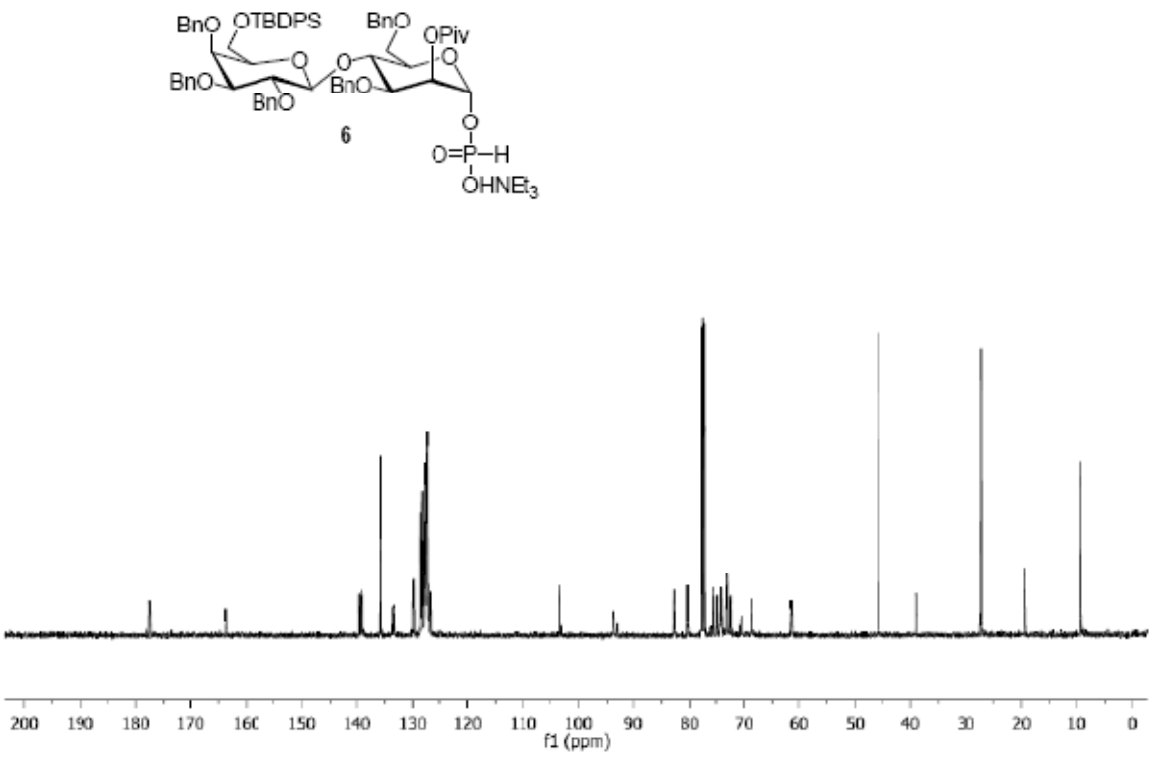

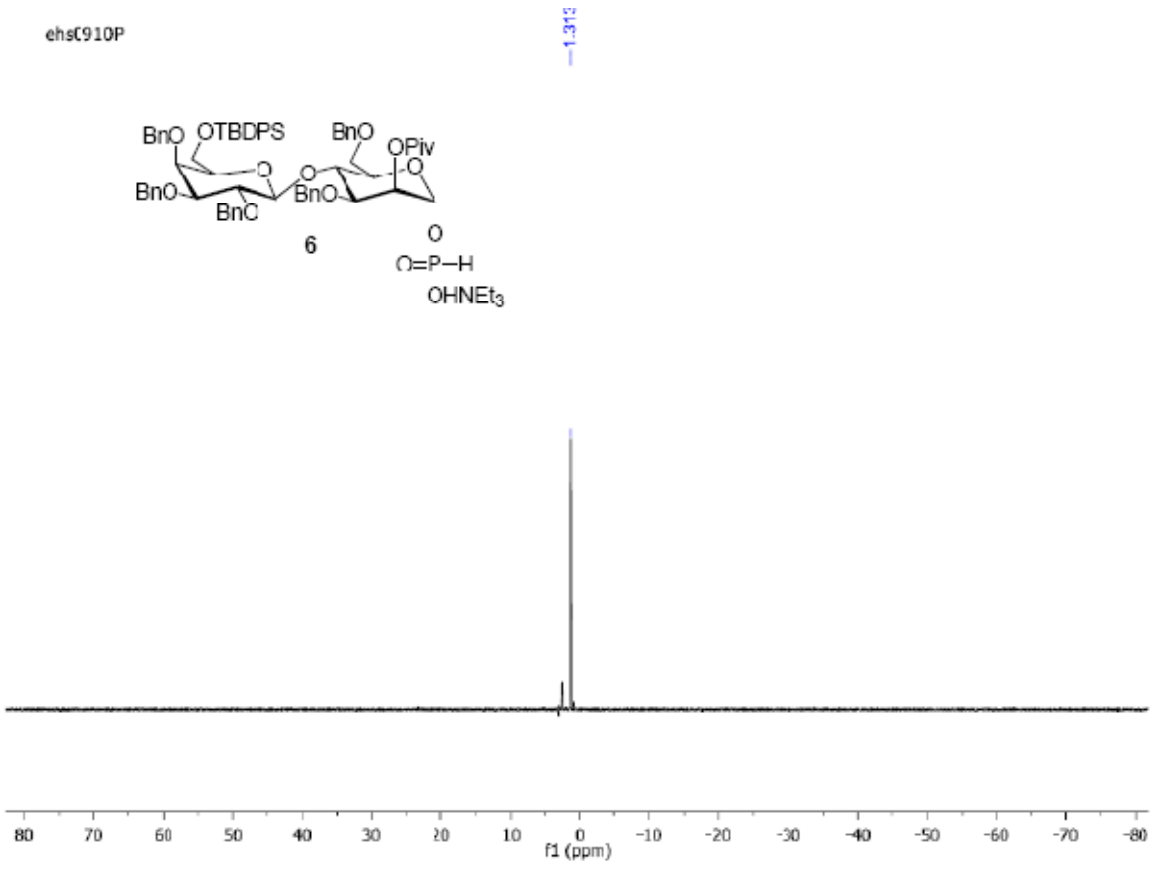

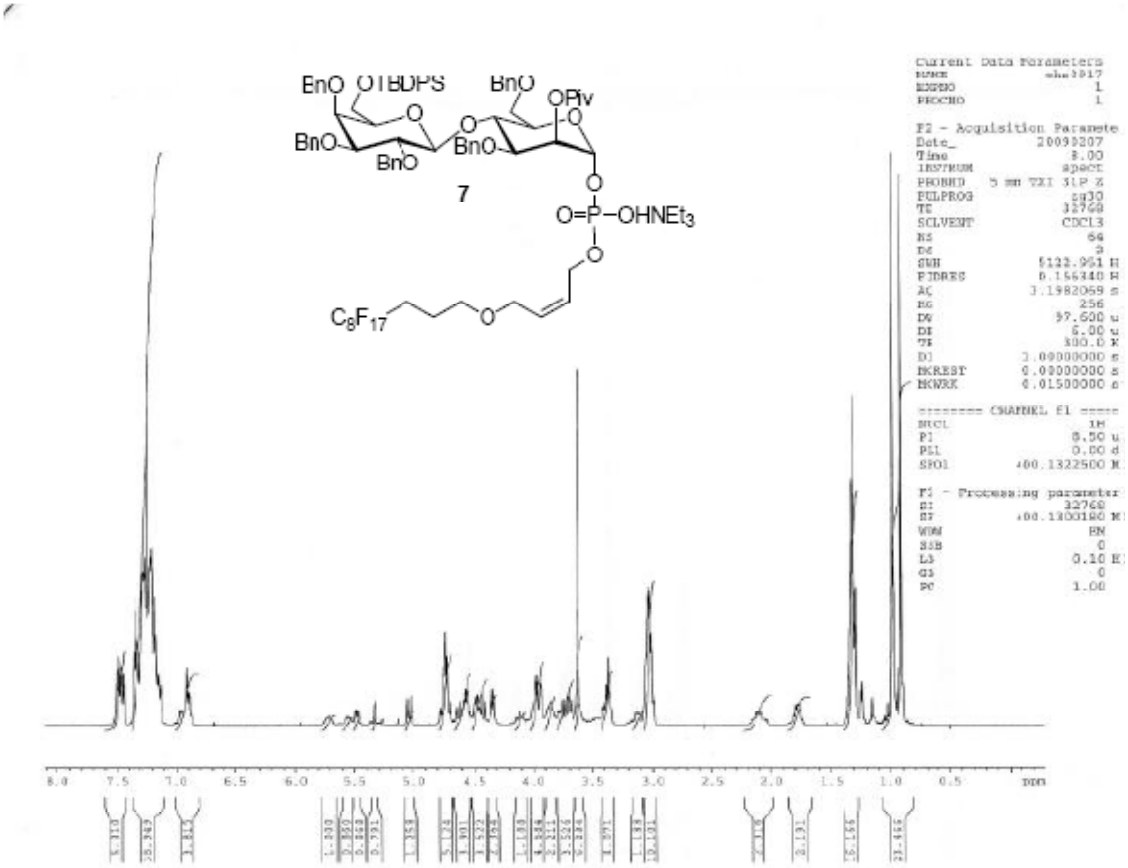

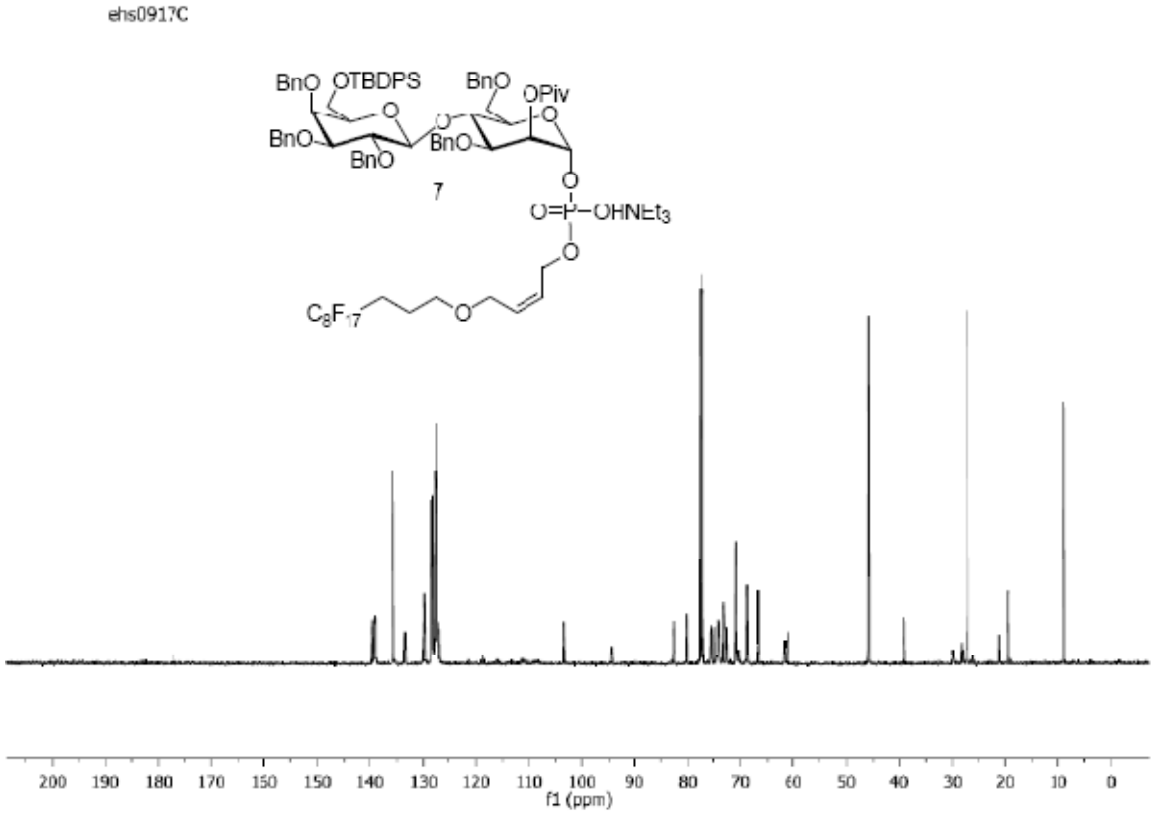

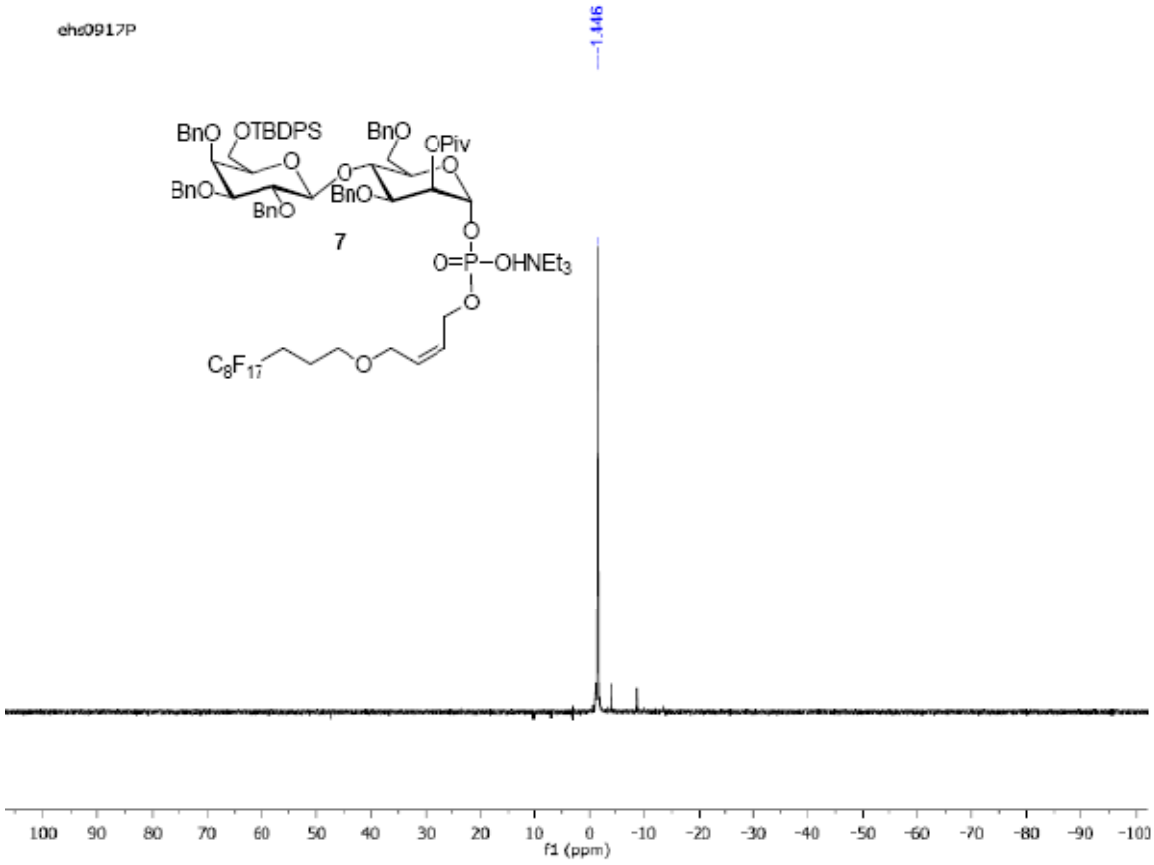

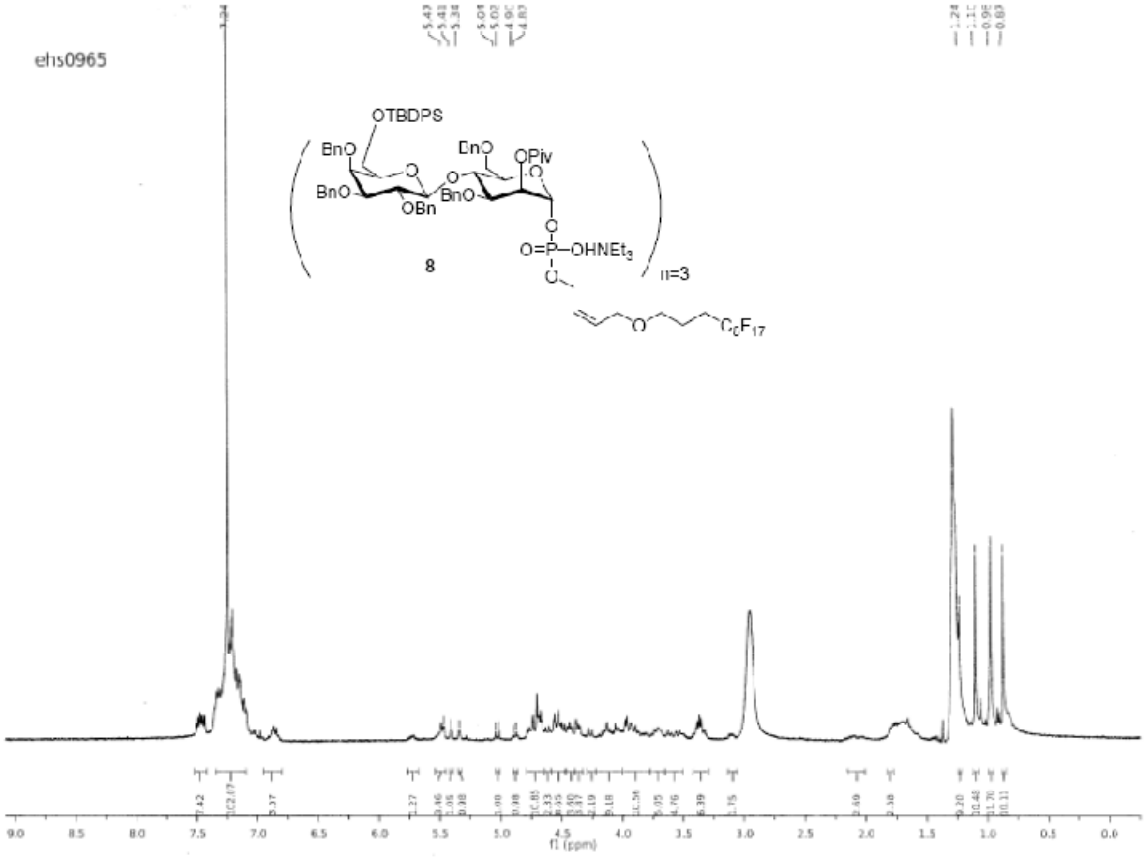

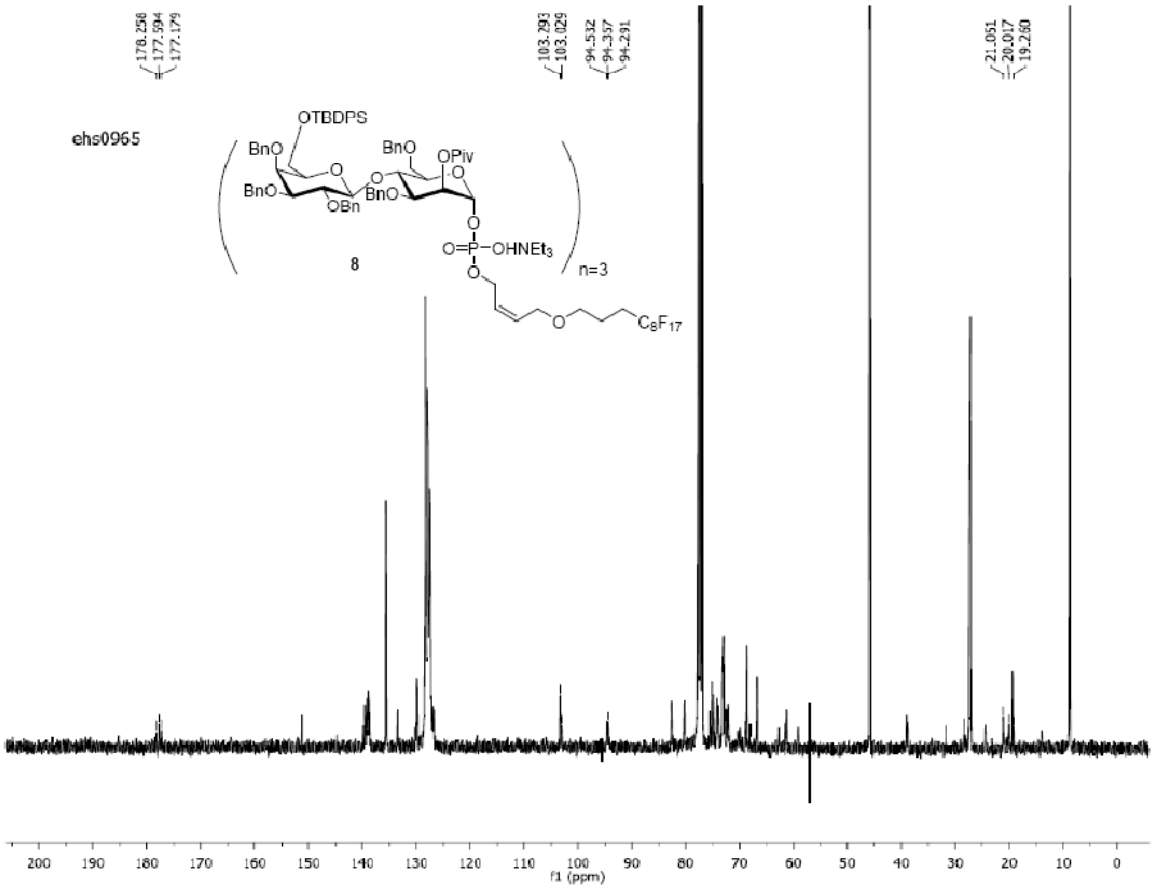

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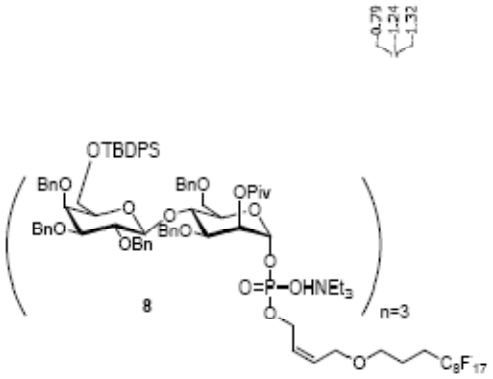
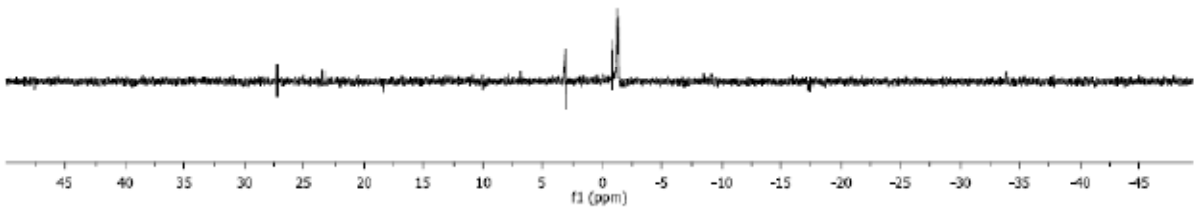
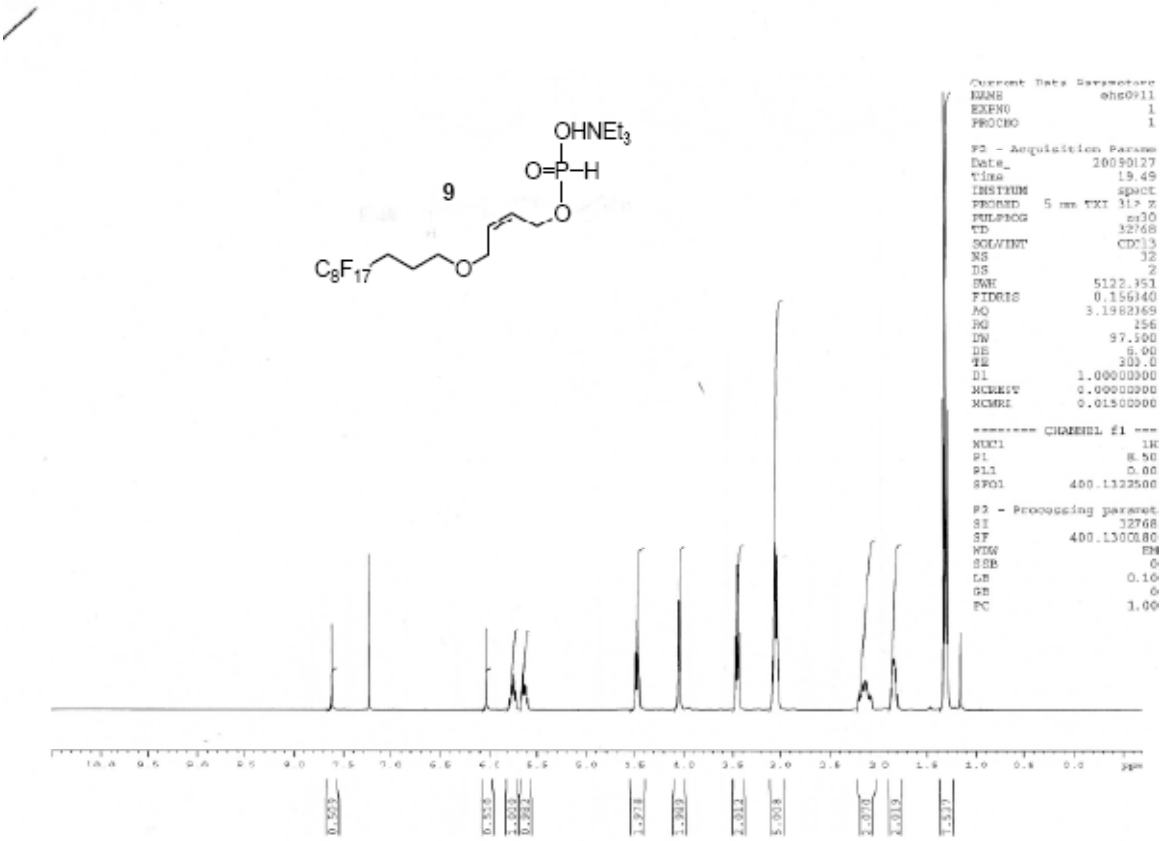
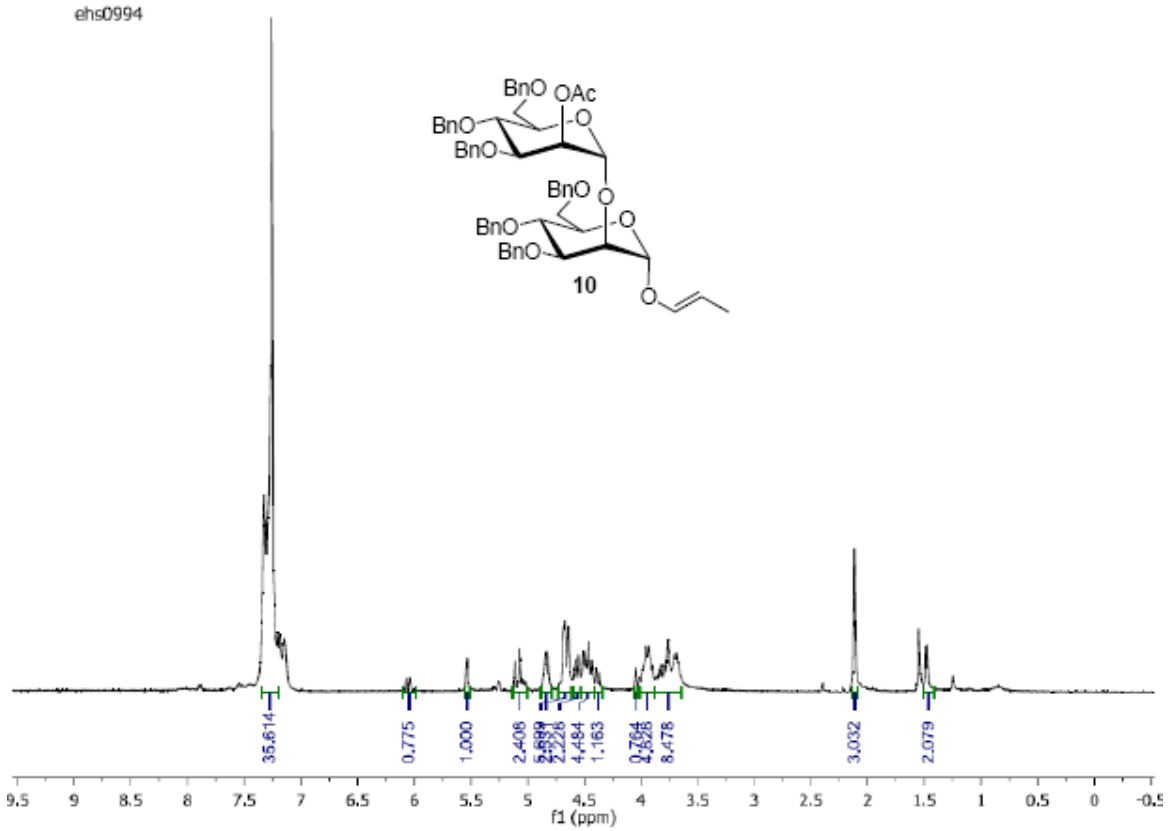
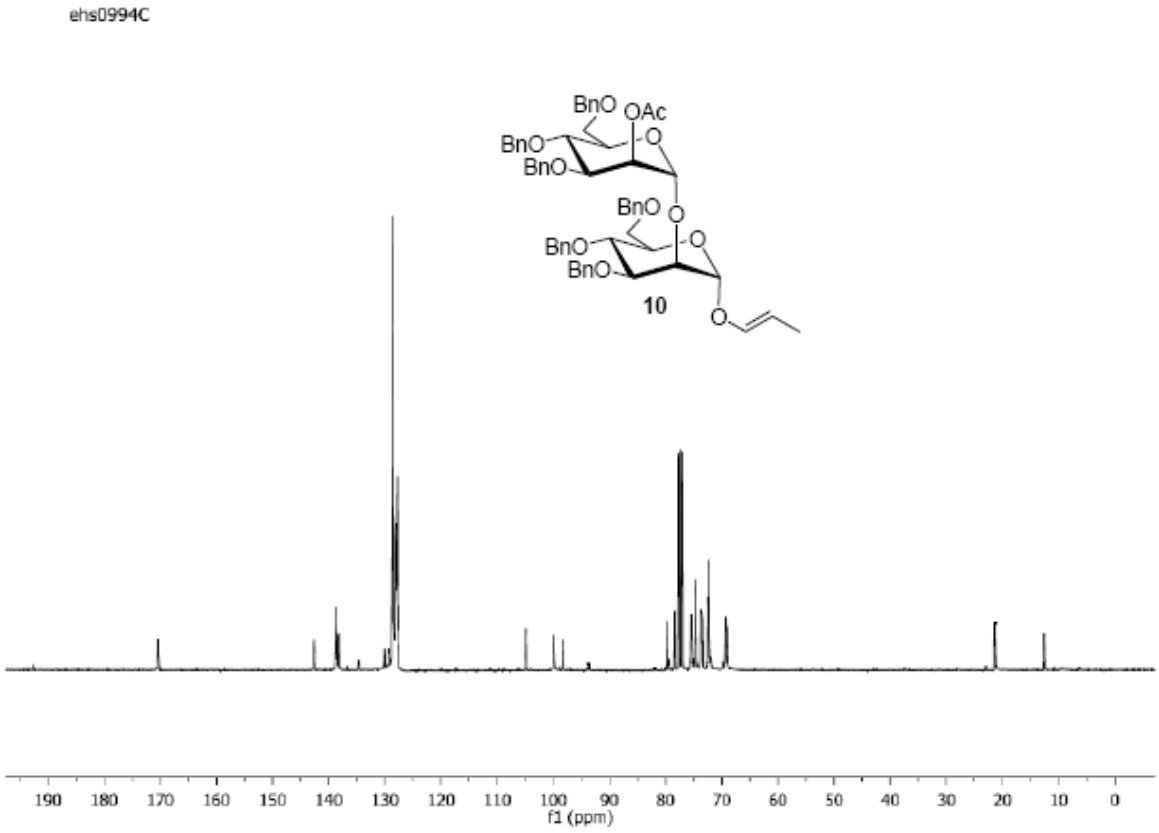
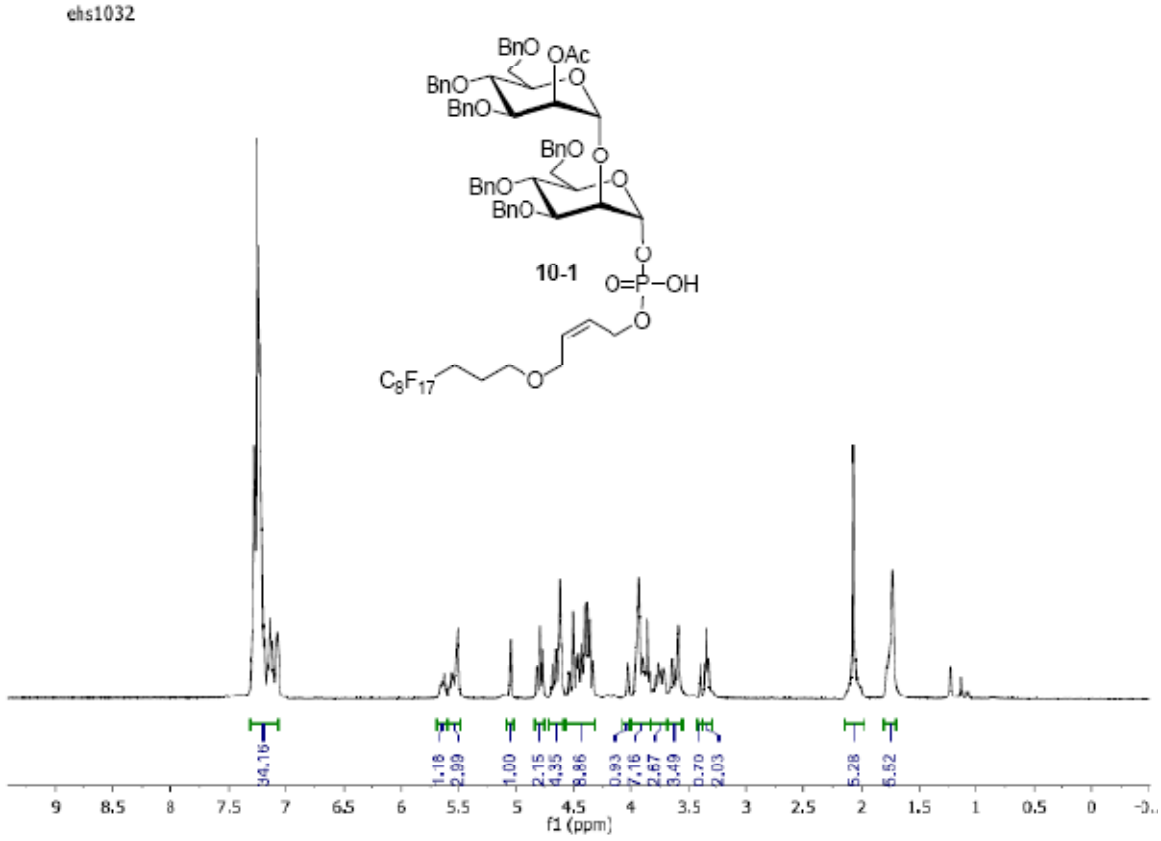


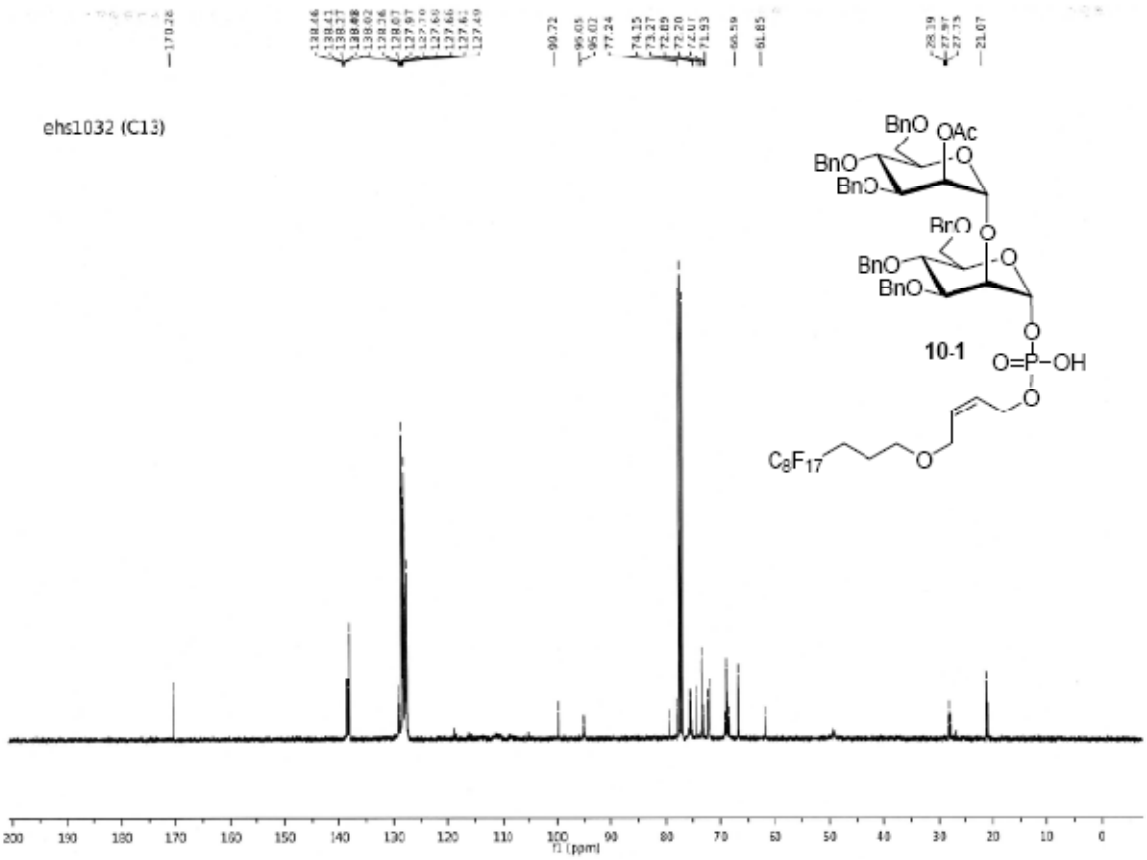


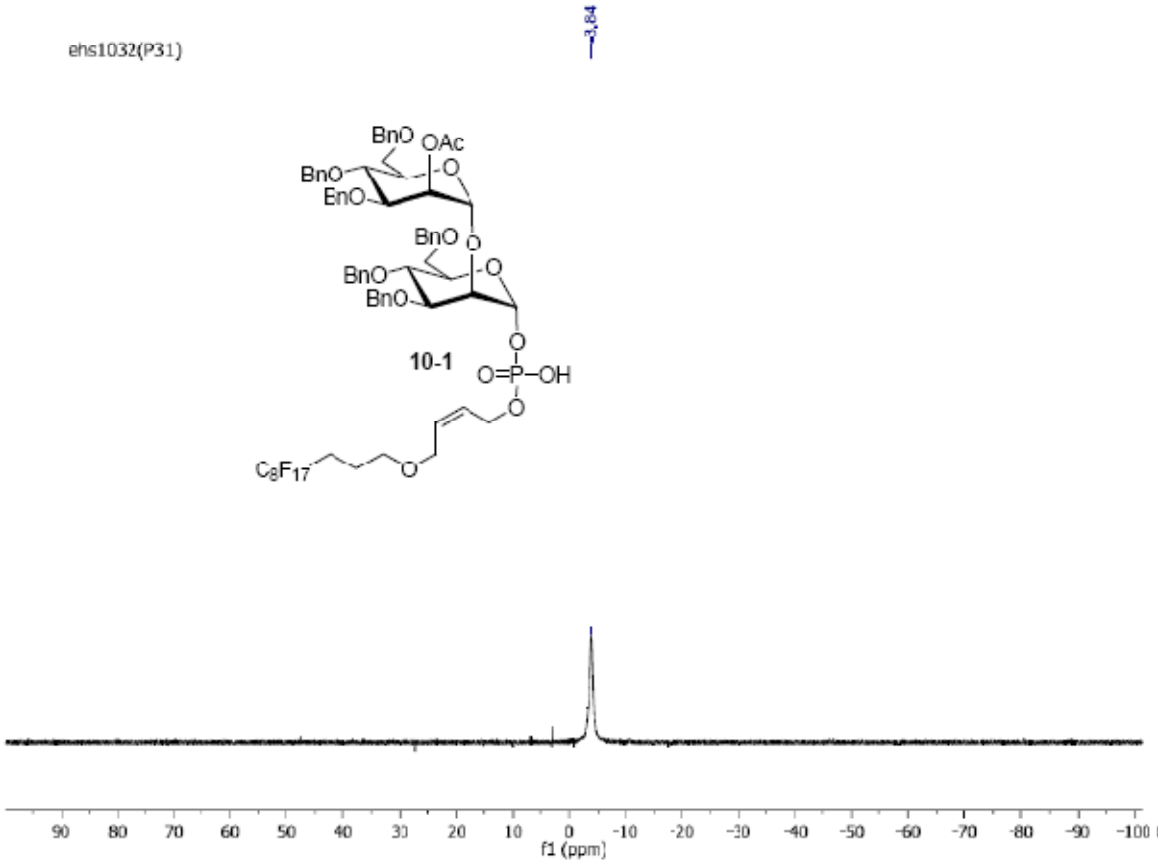


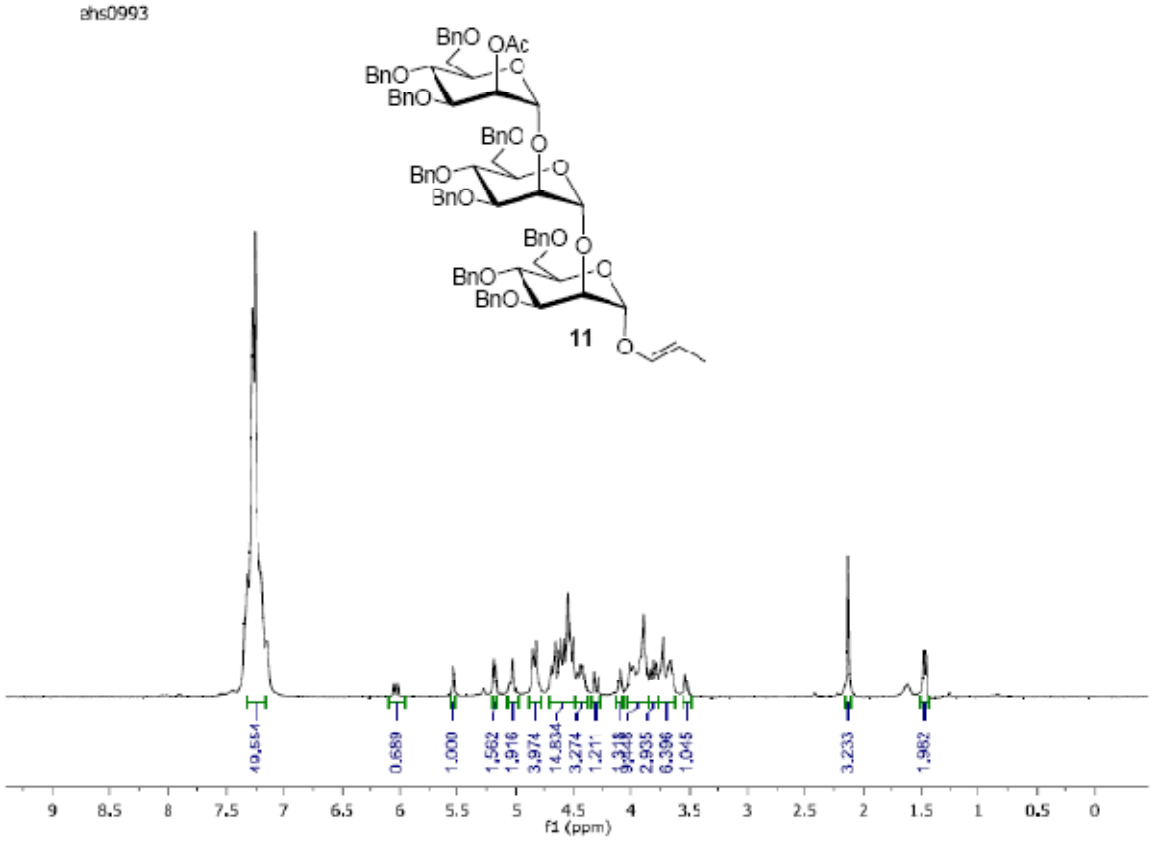


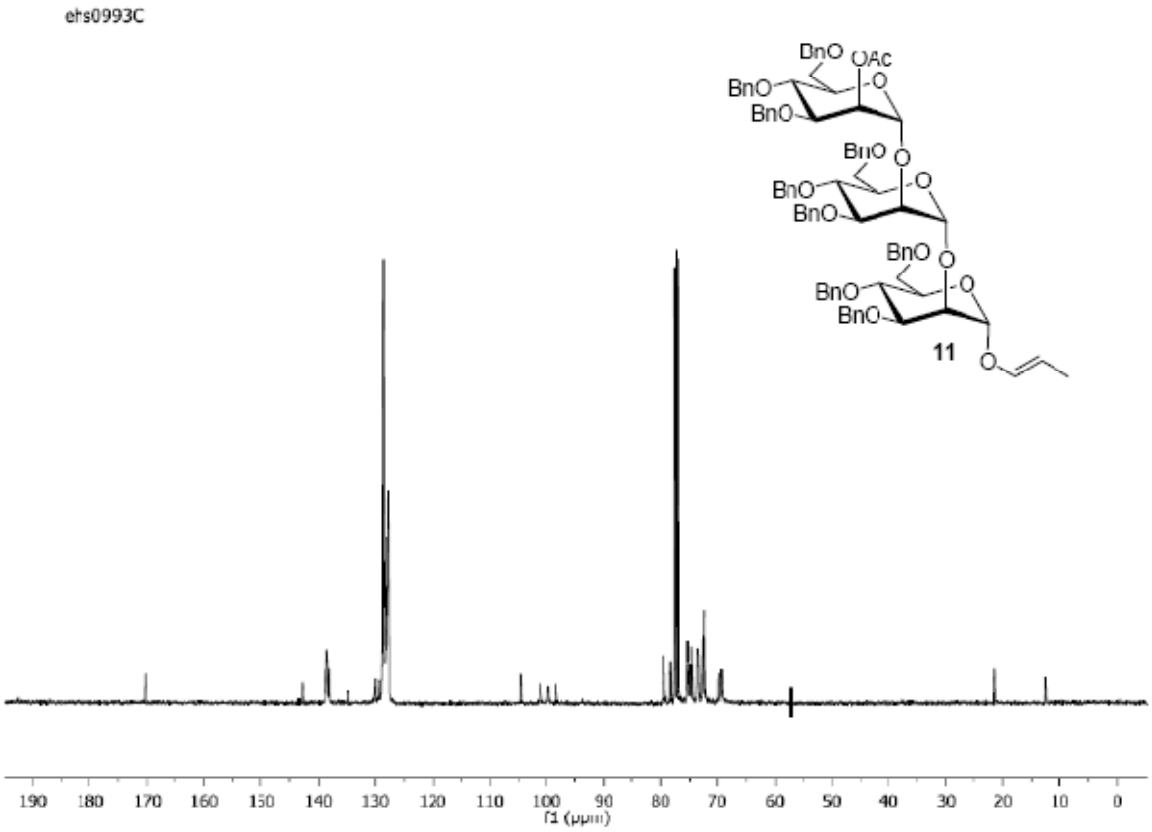


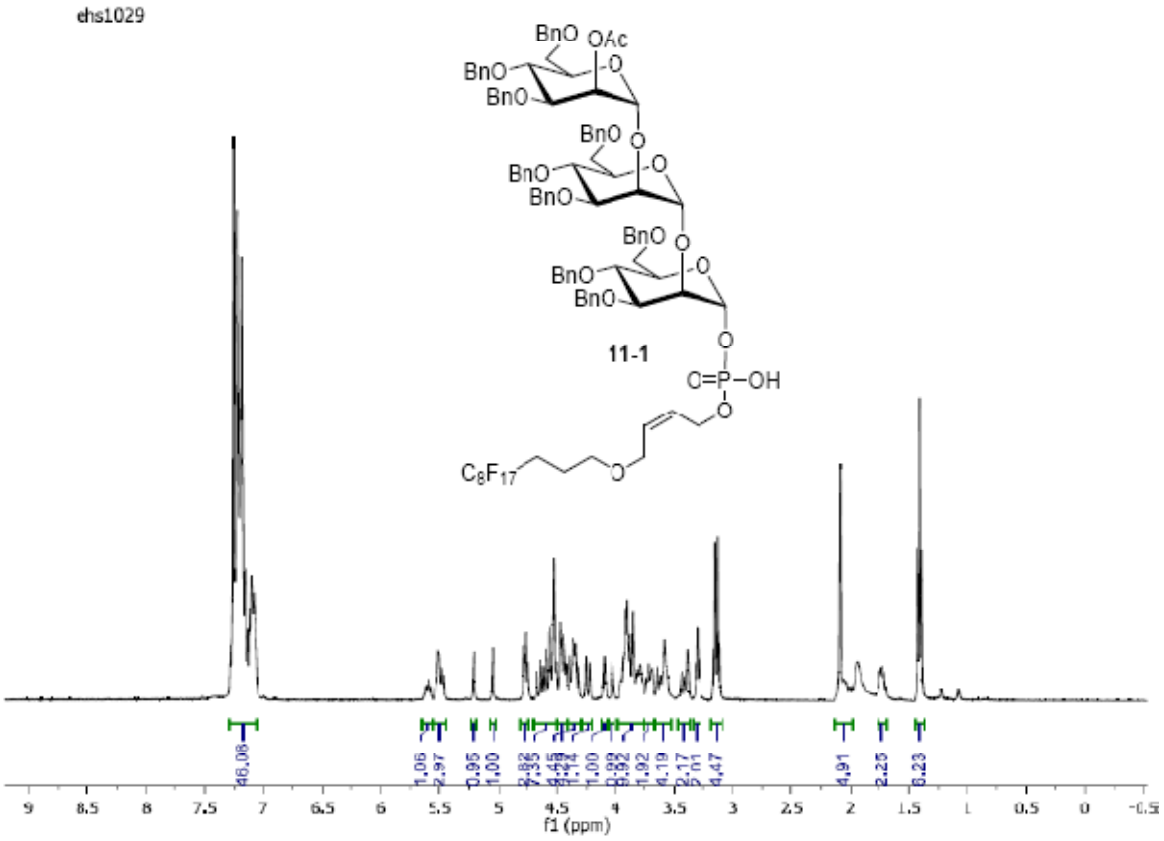


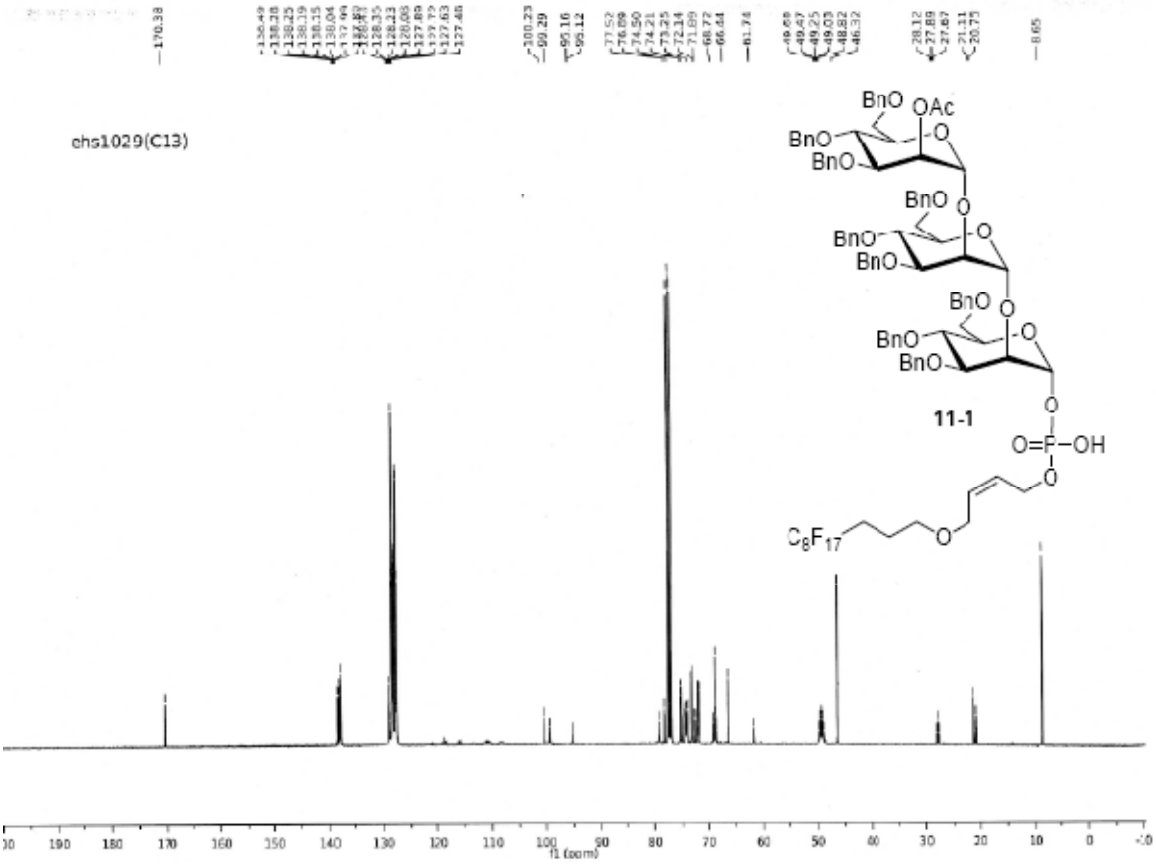


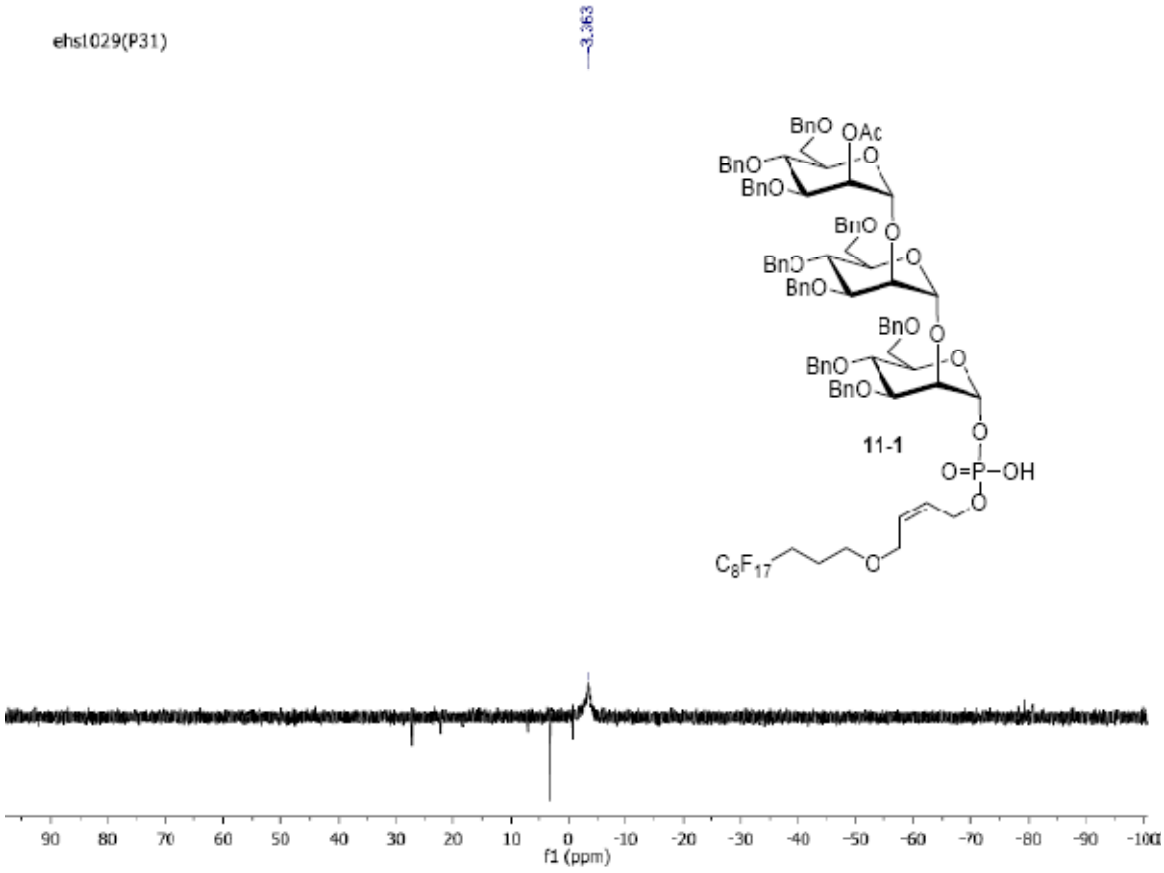


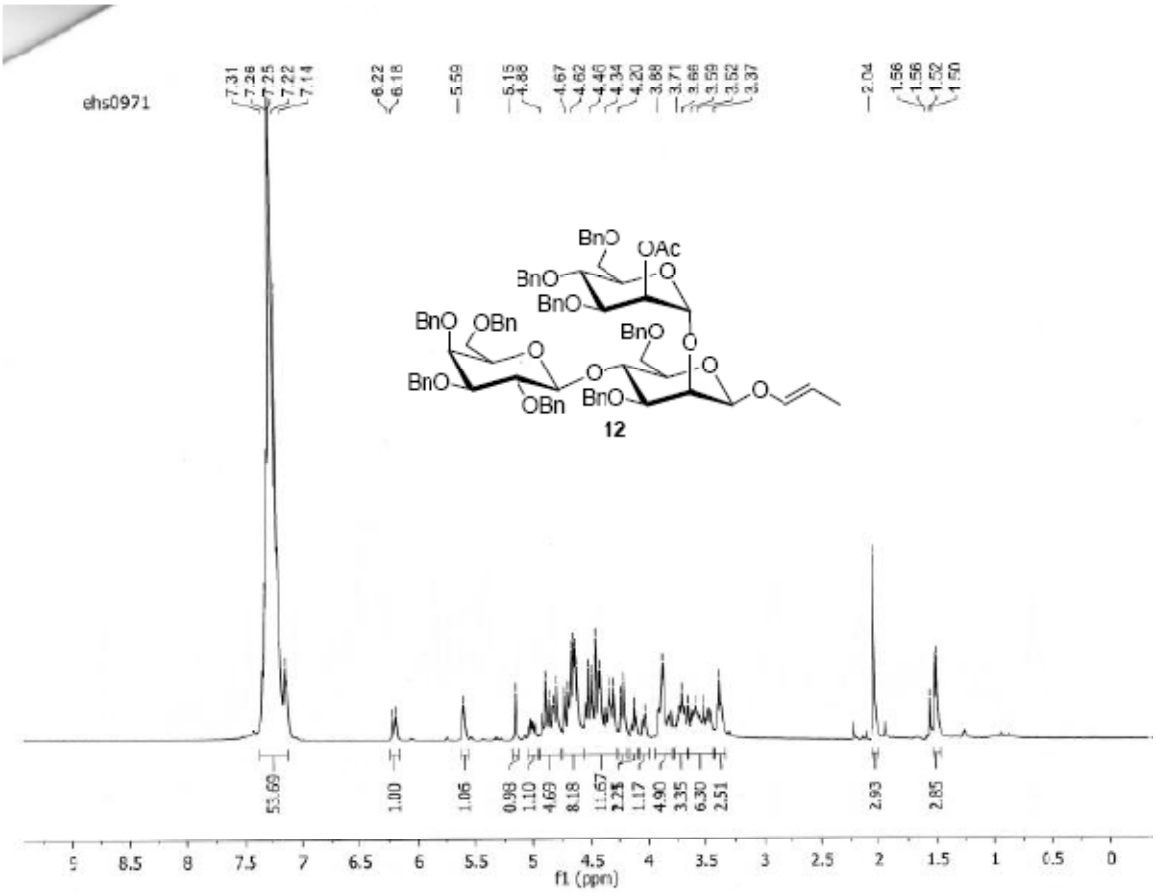


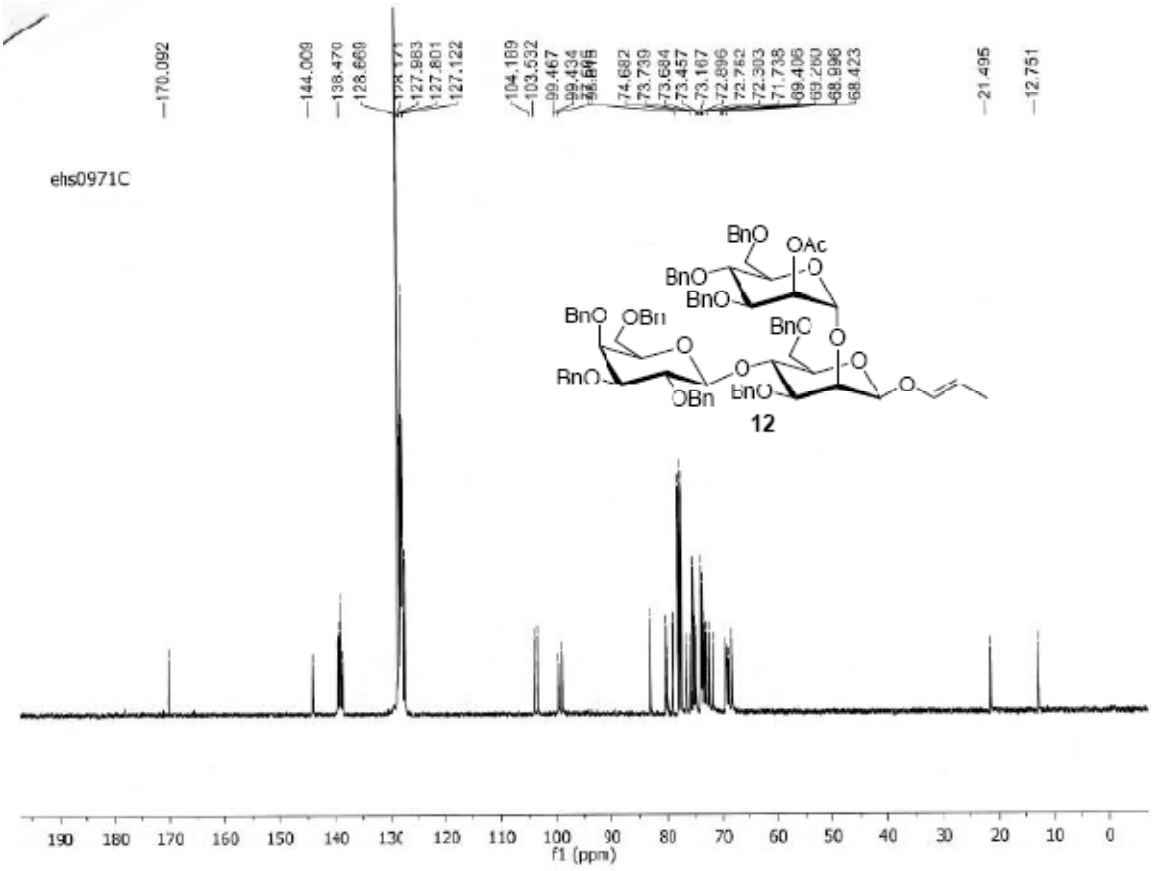


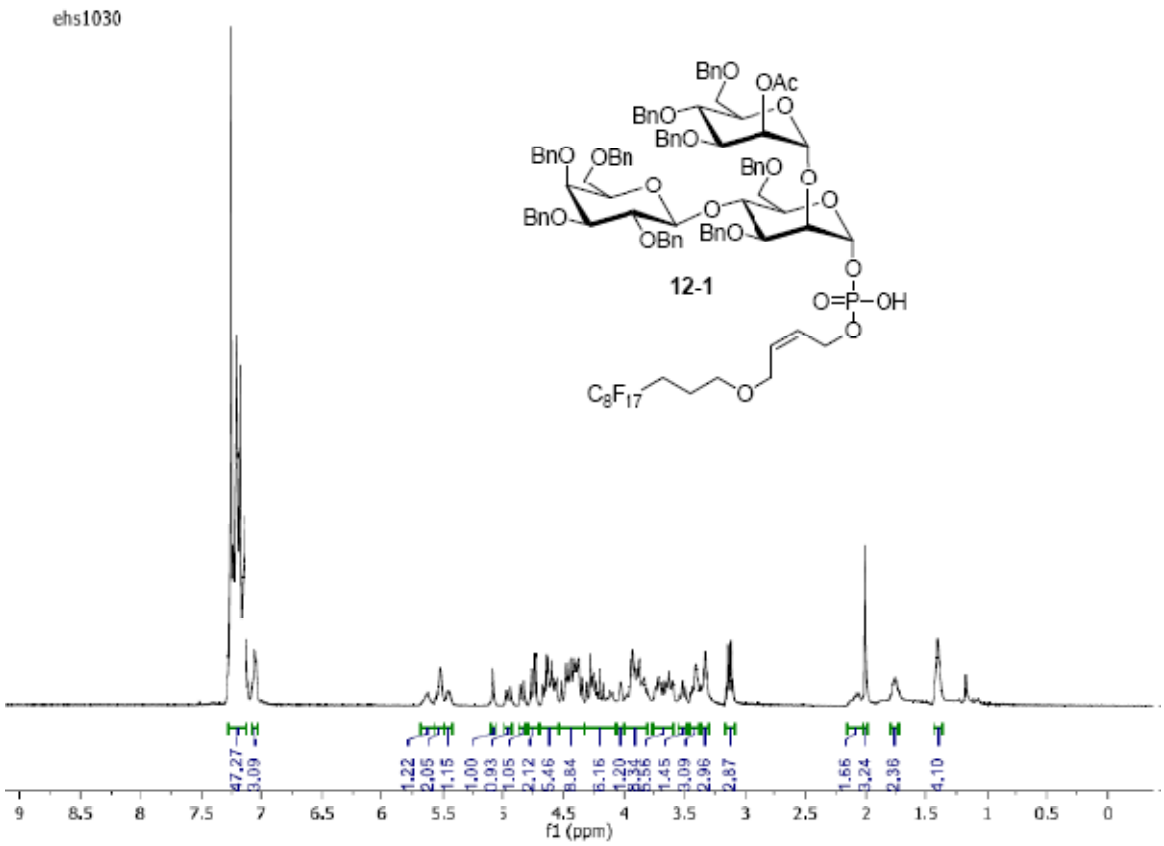


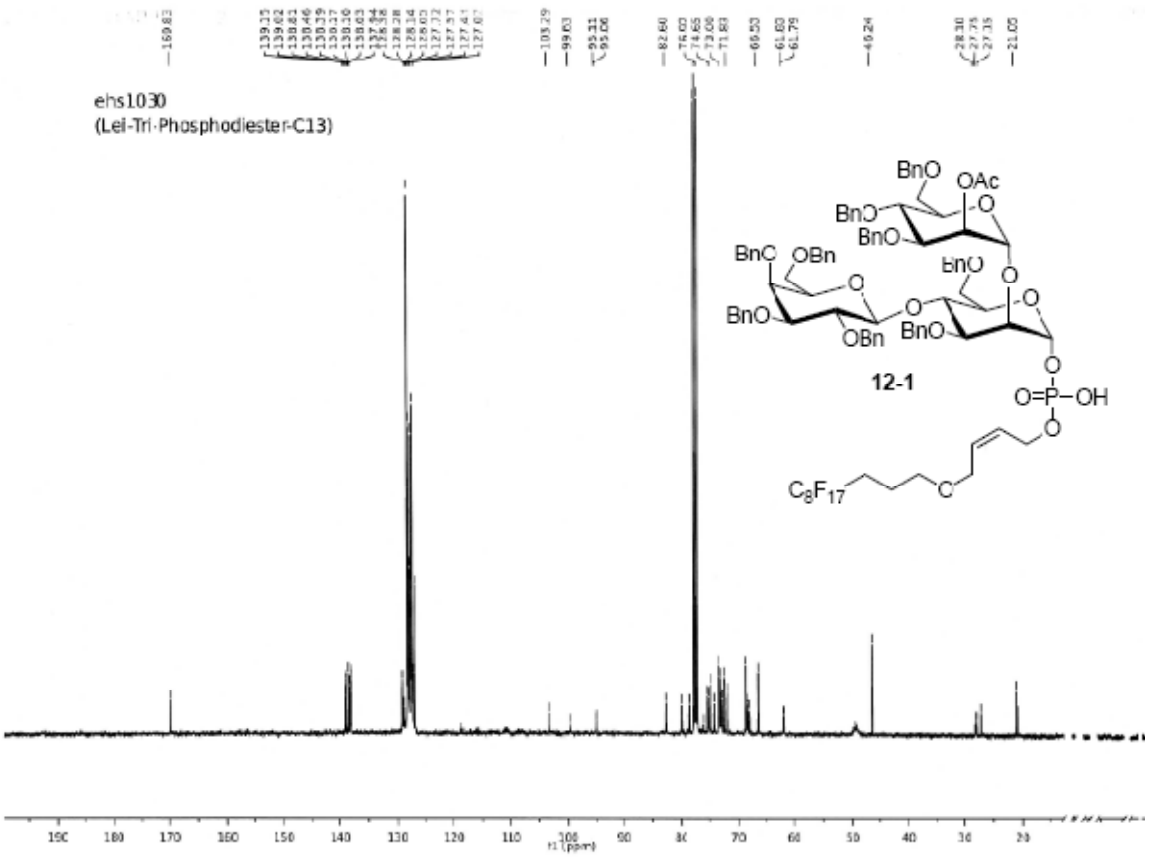


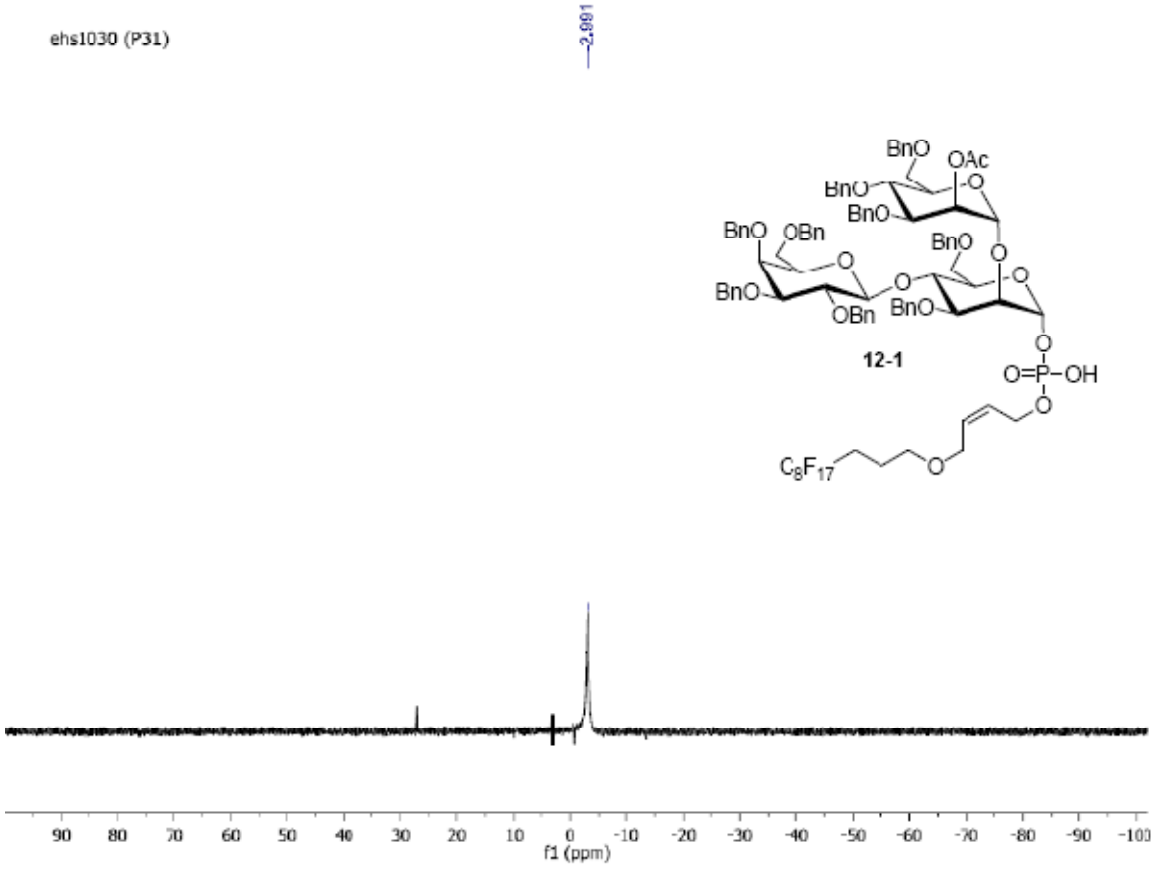


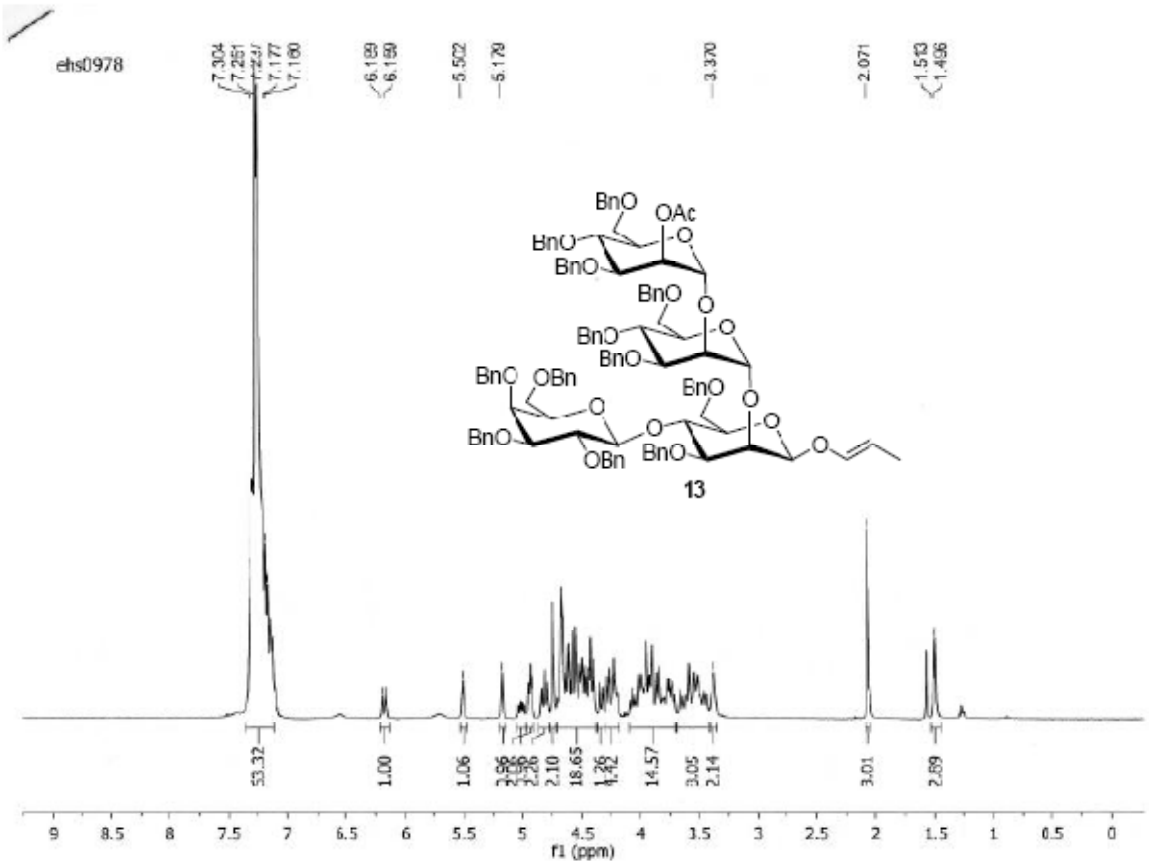


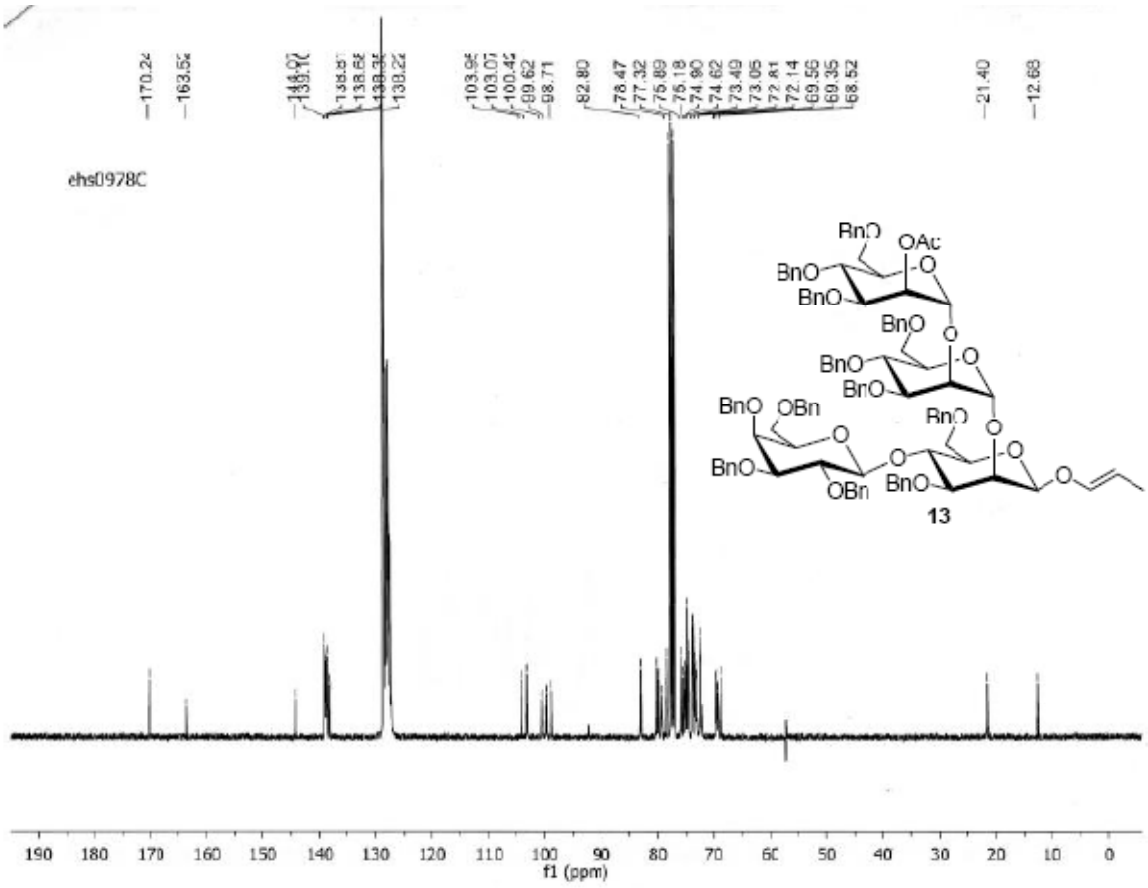


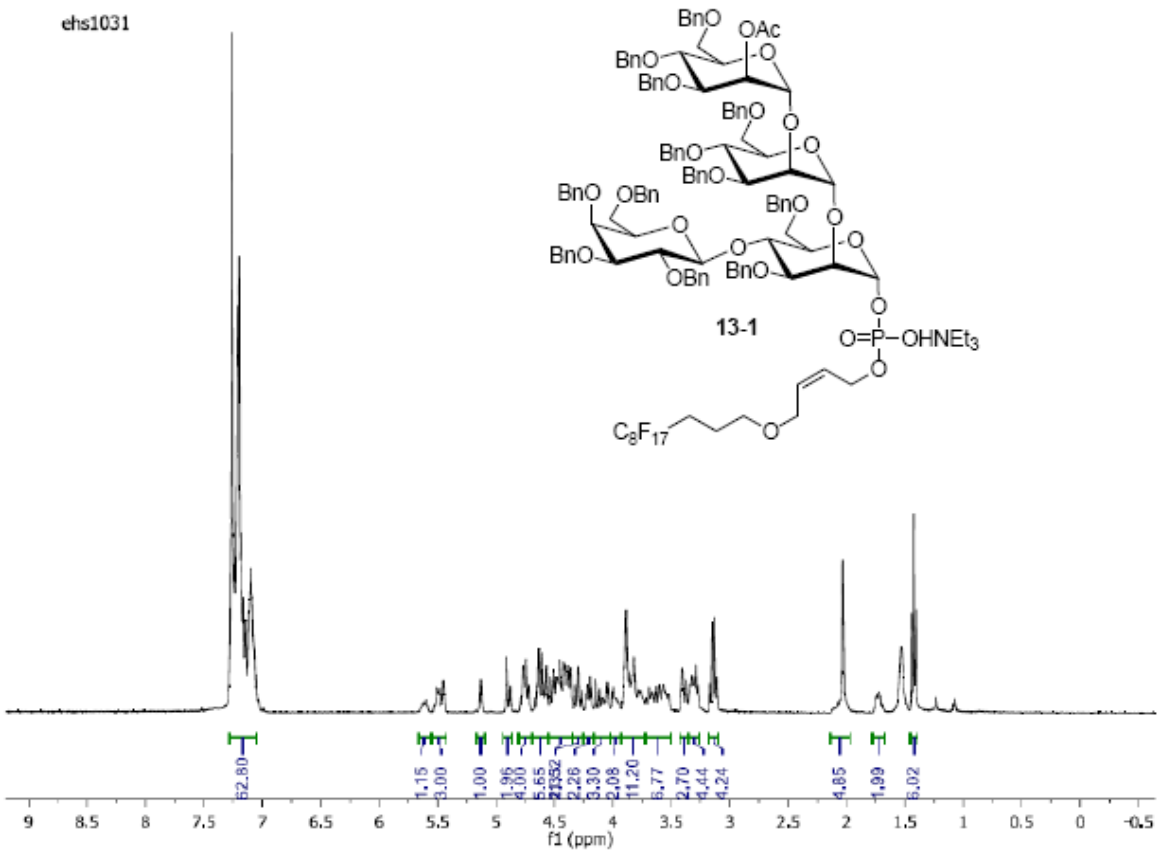


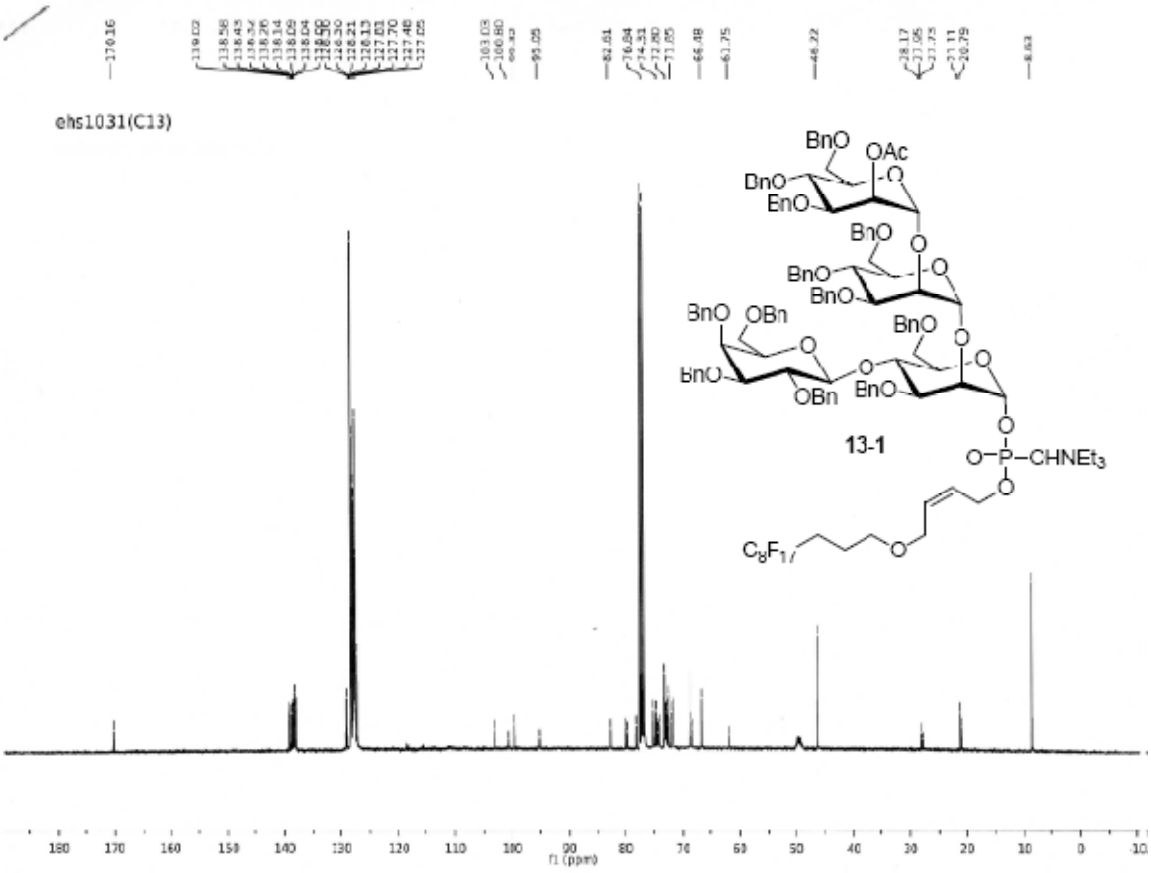


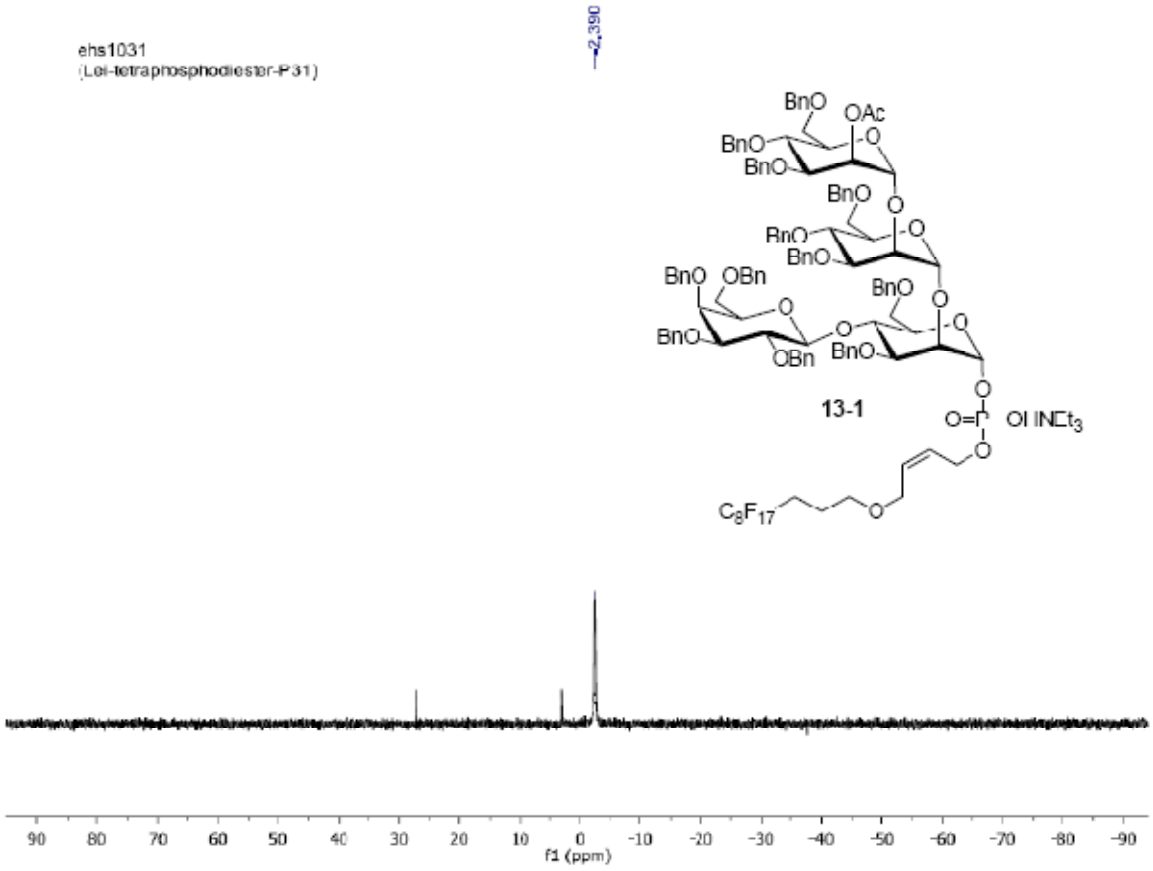






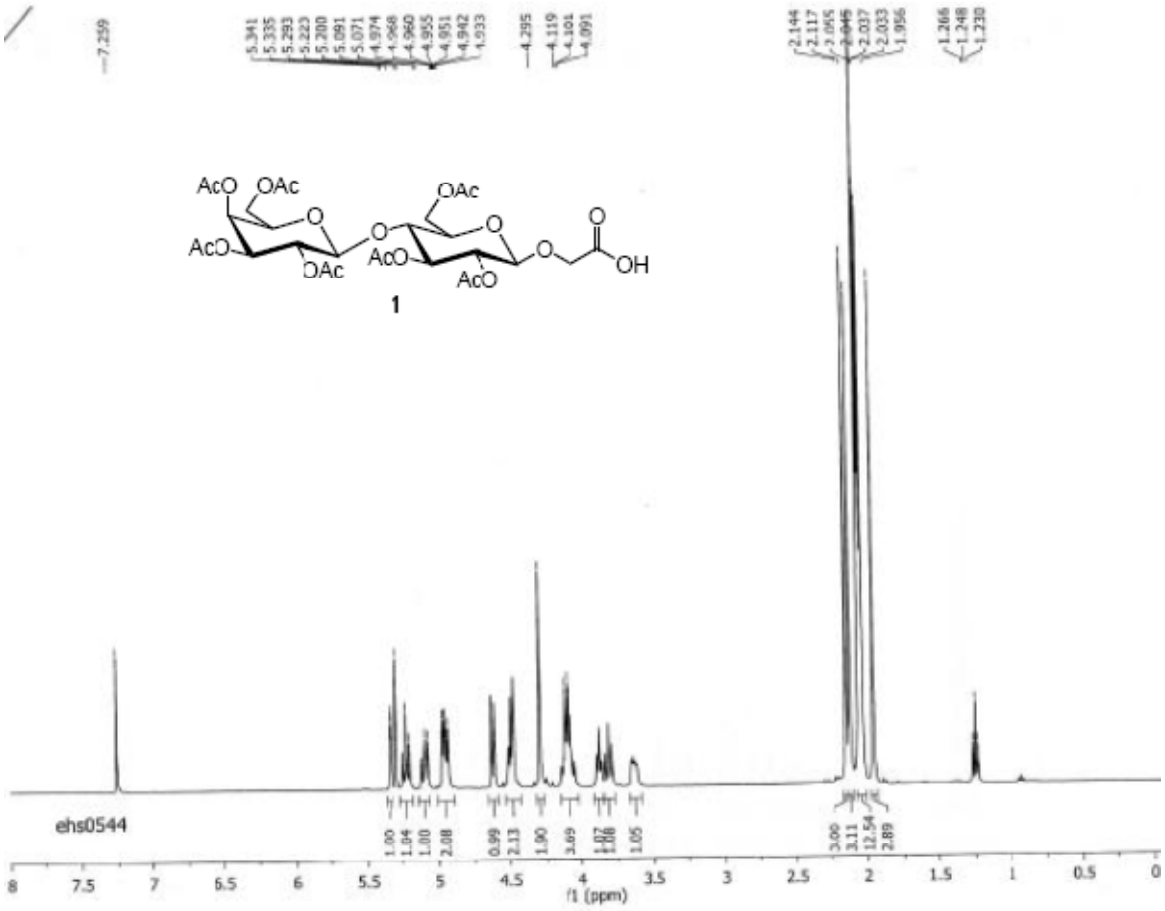


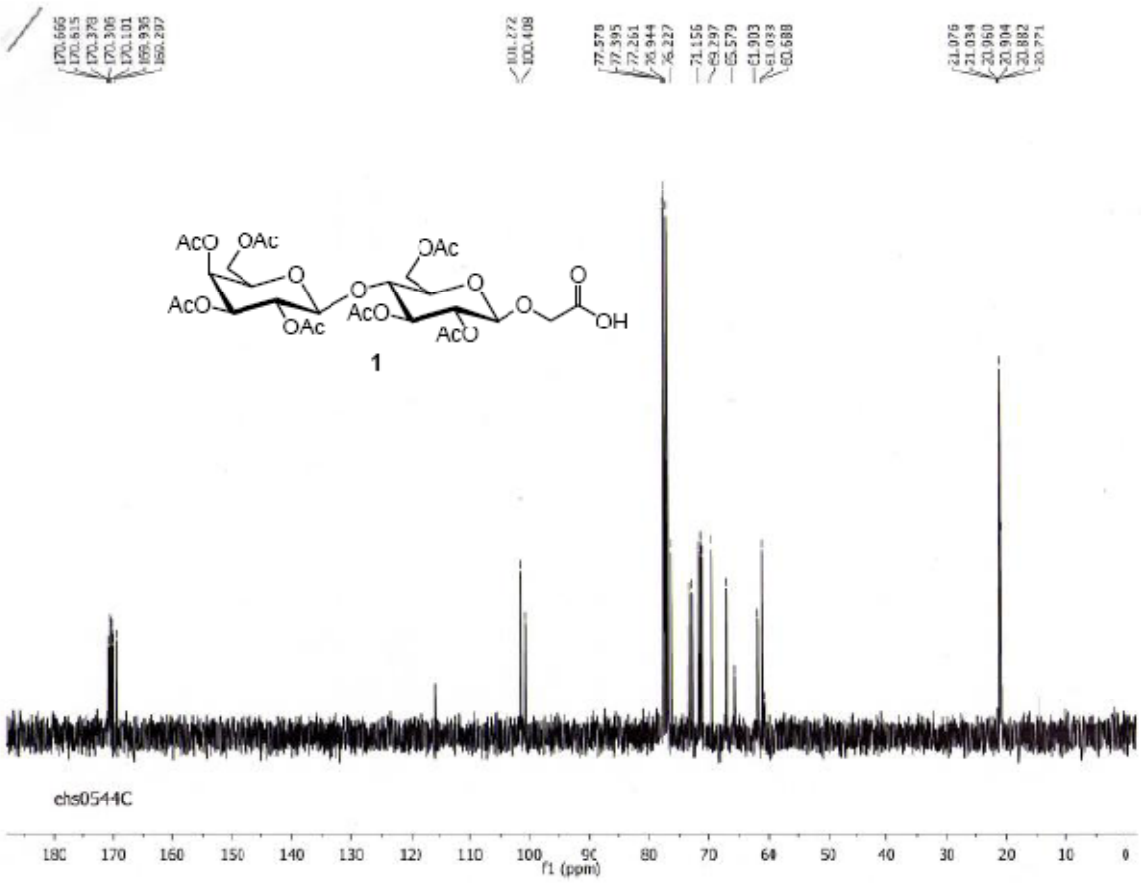




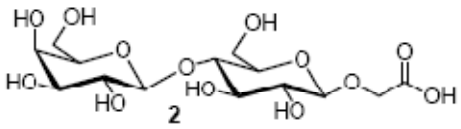
APPENDIX D.

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





ehs0661 (CD3OD)
-Deprotected Lactose-carboxylic acid



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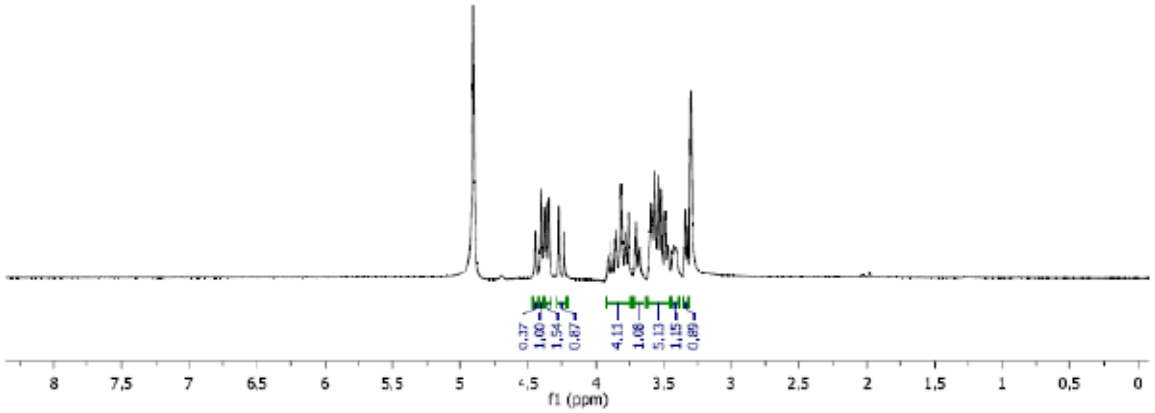

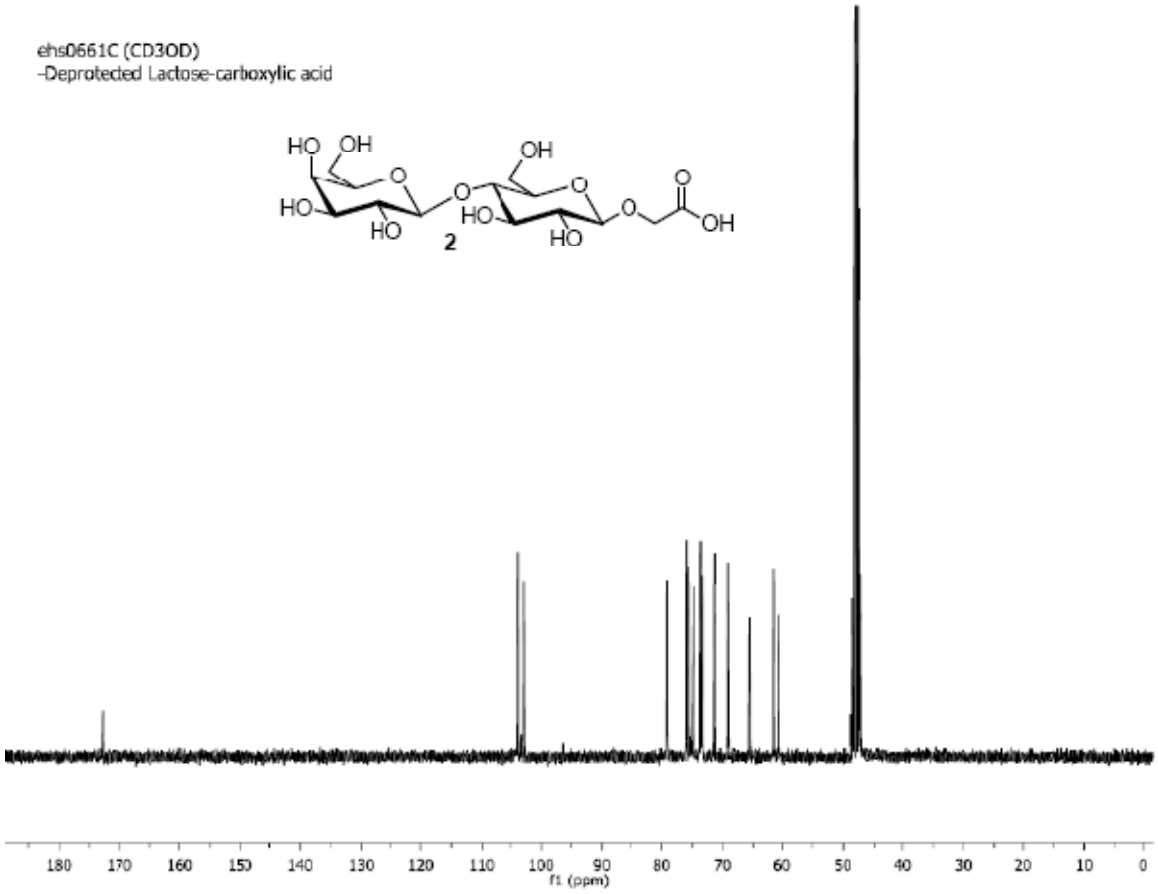

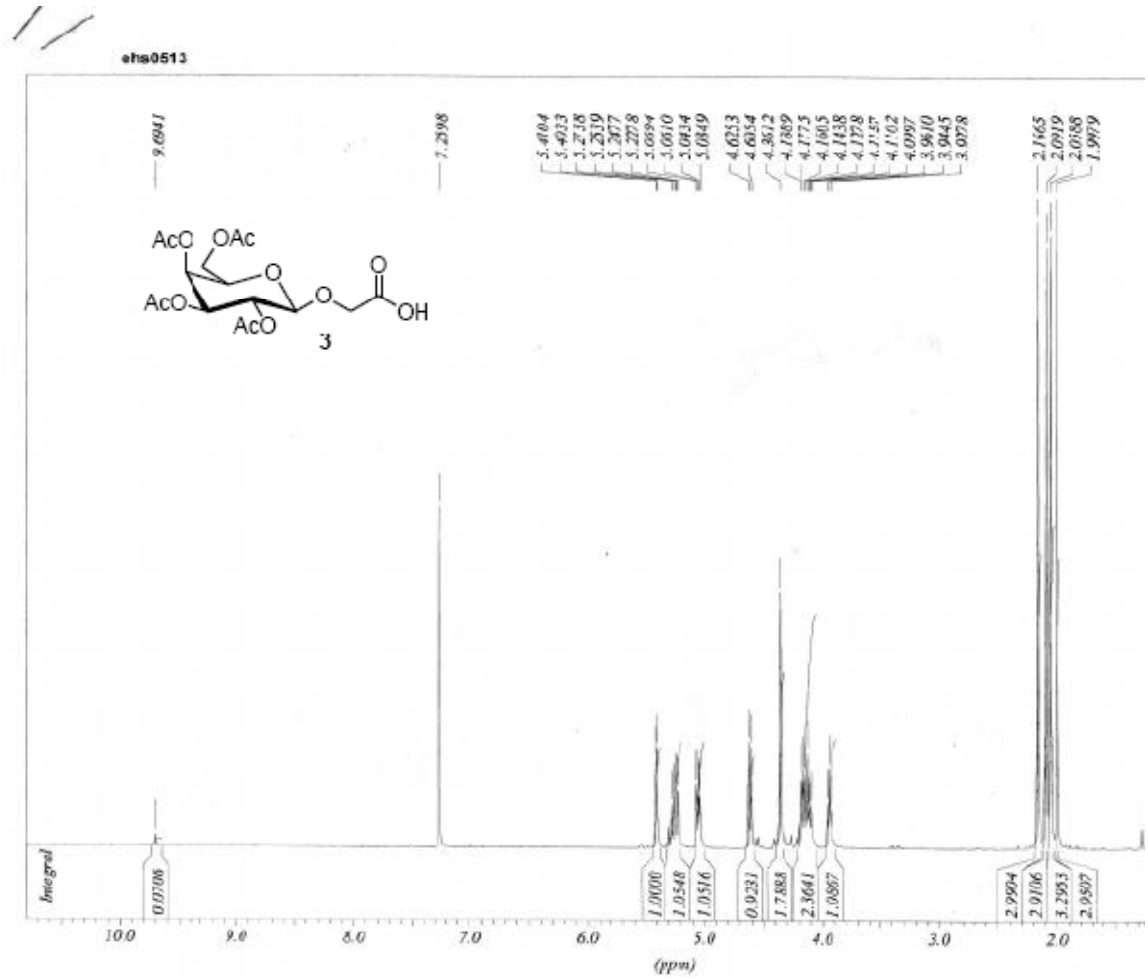

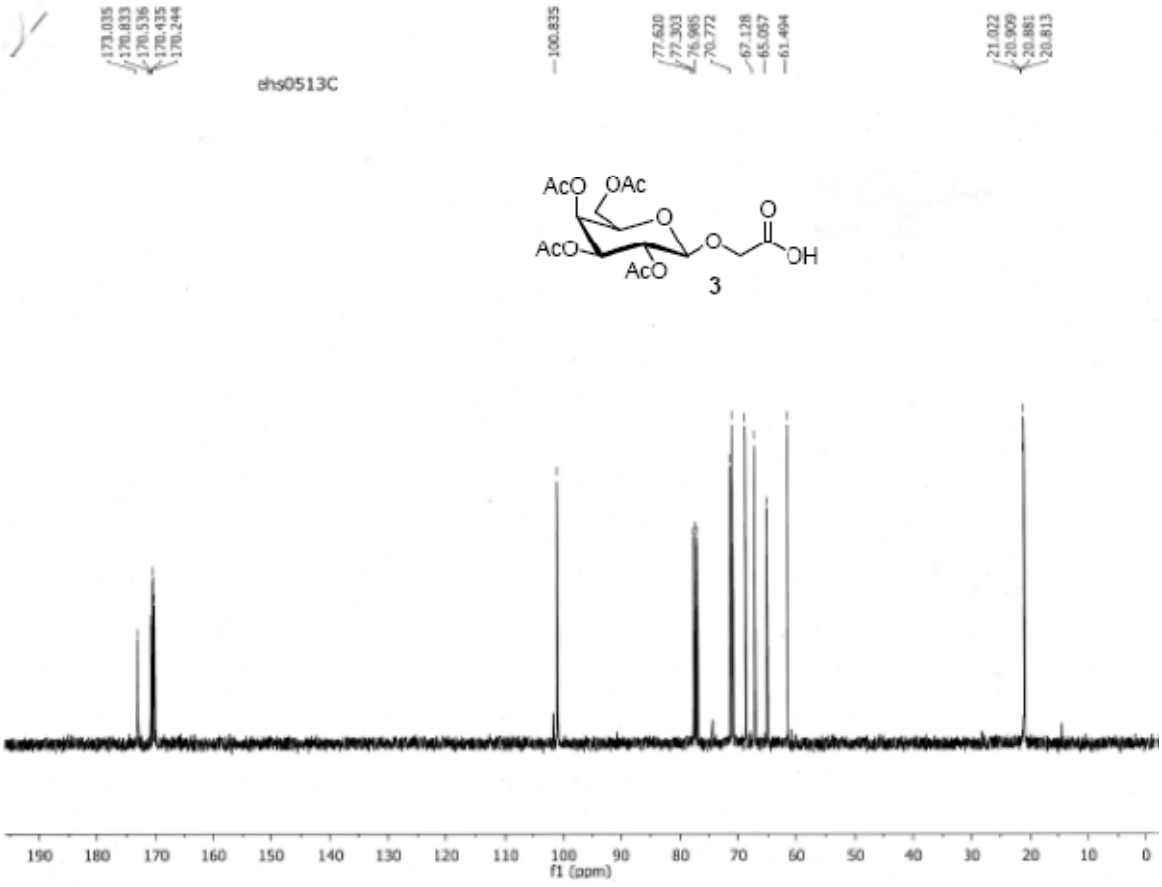

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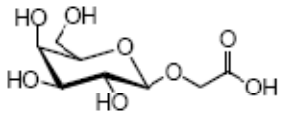
-Deprotected Galactose-carboxylic acid



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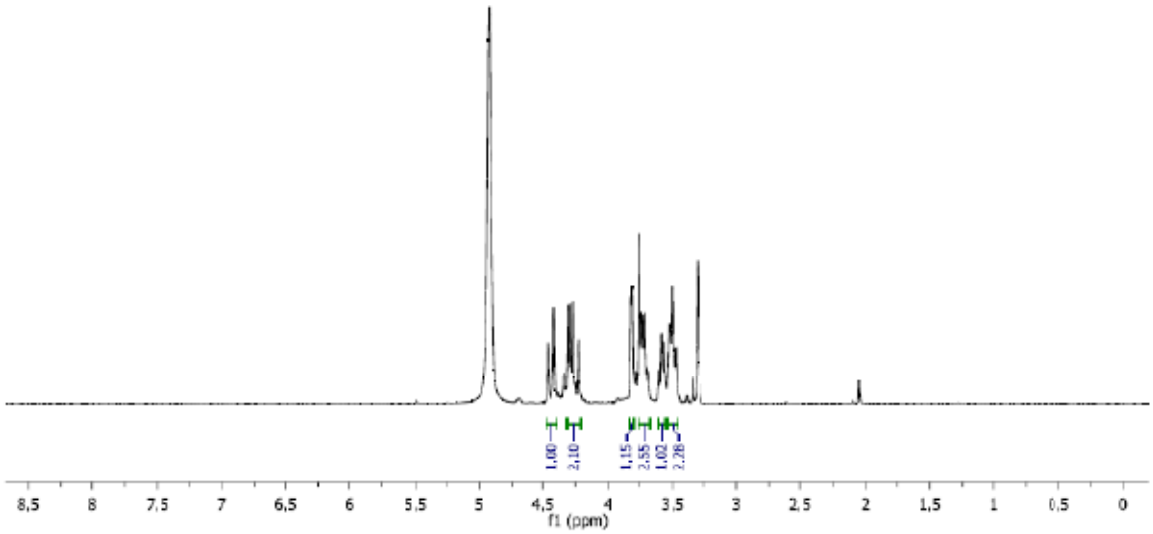

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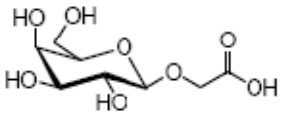

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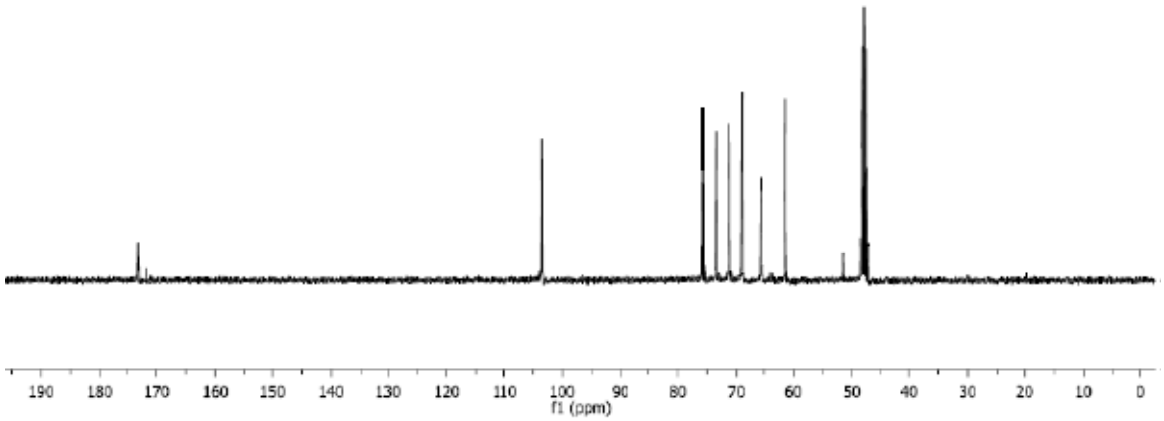
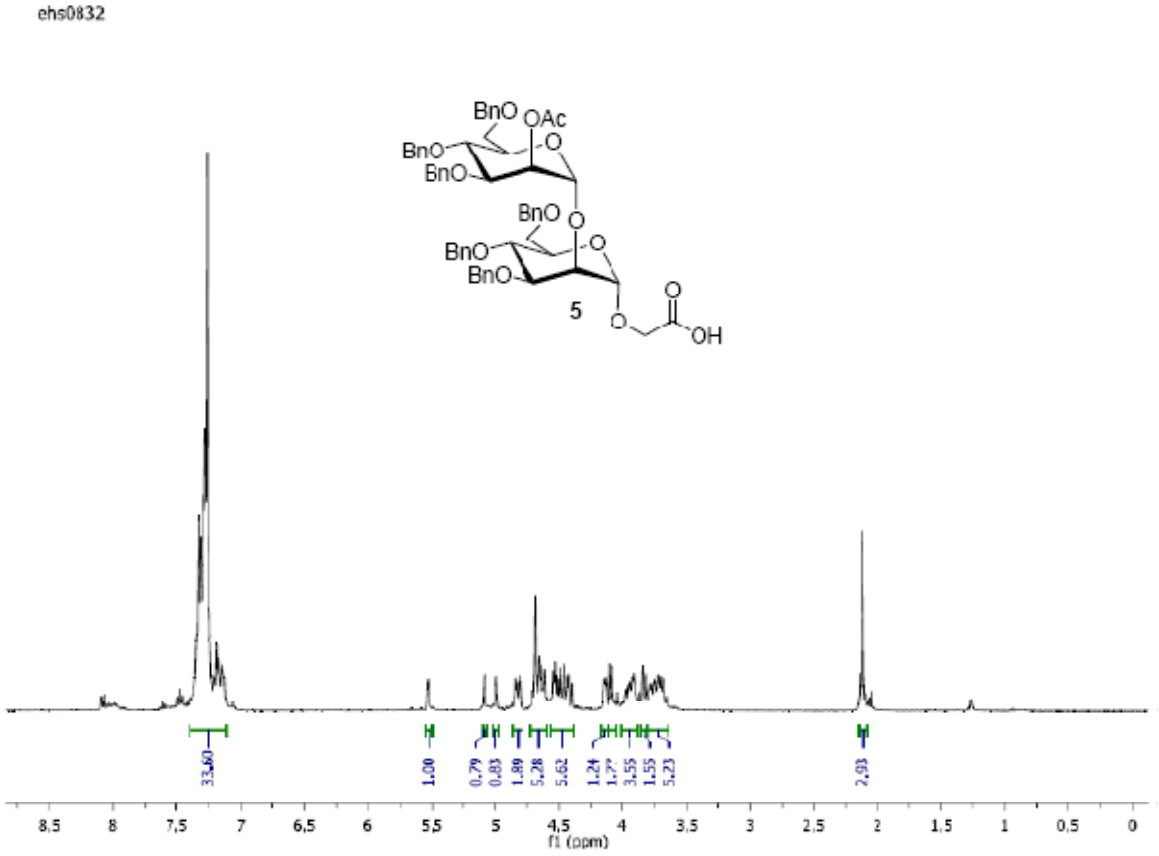
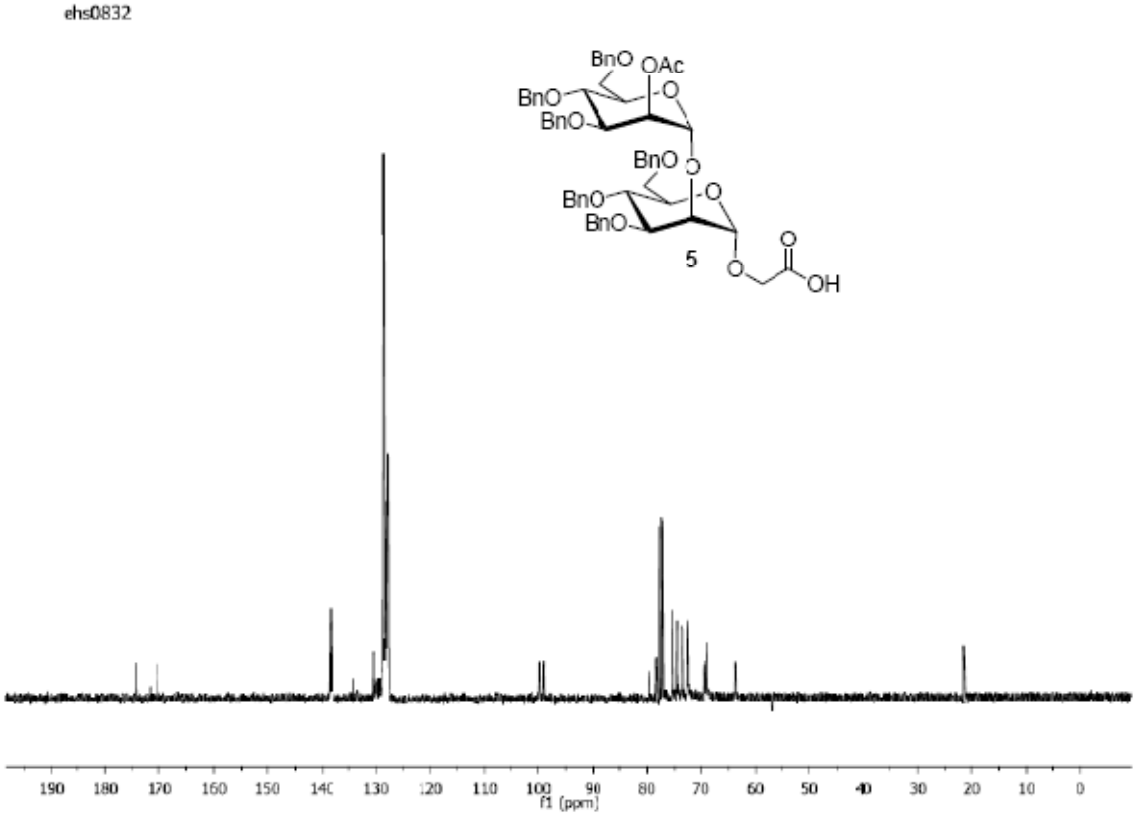
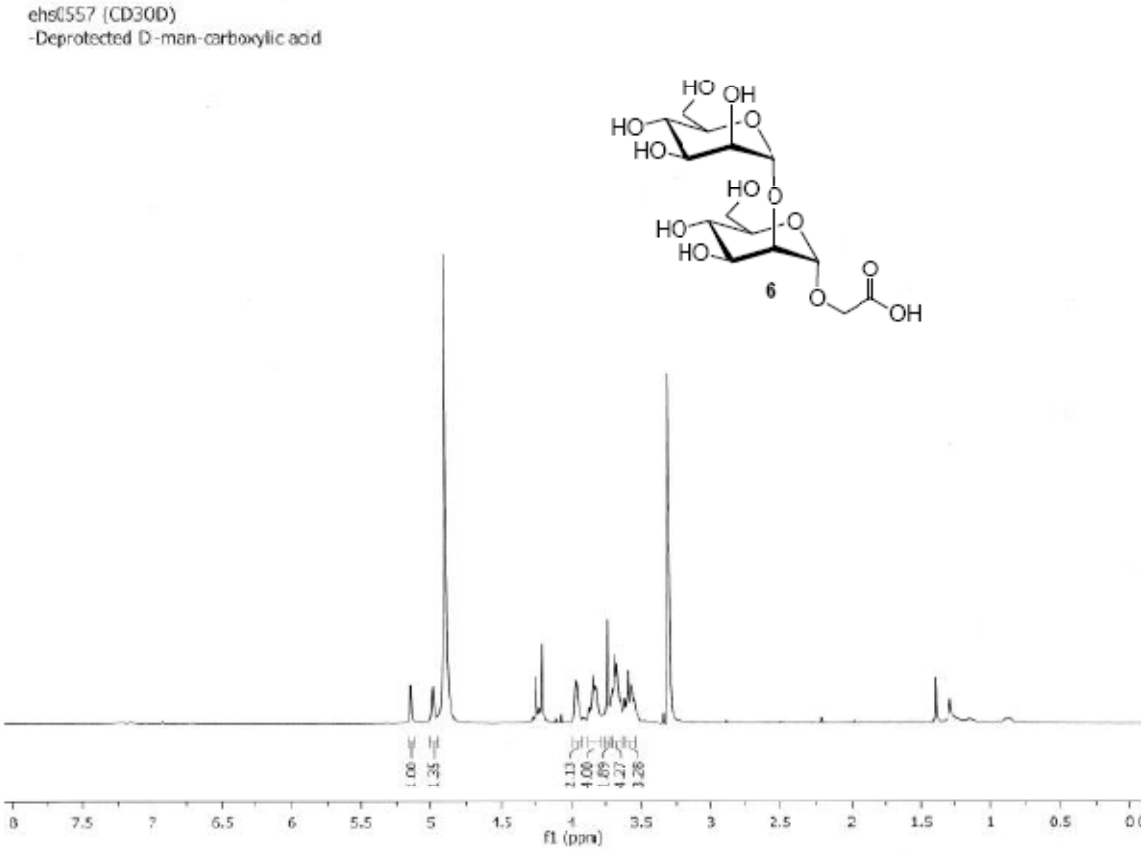
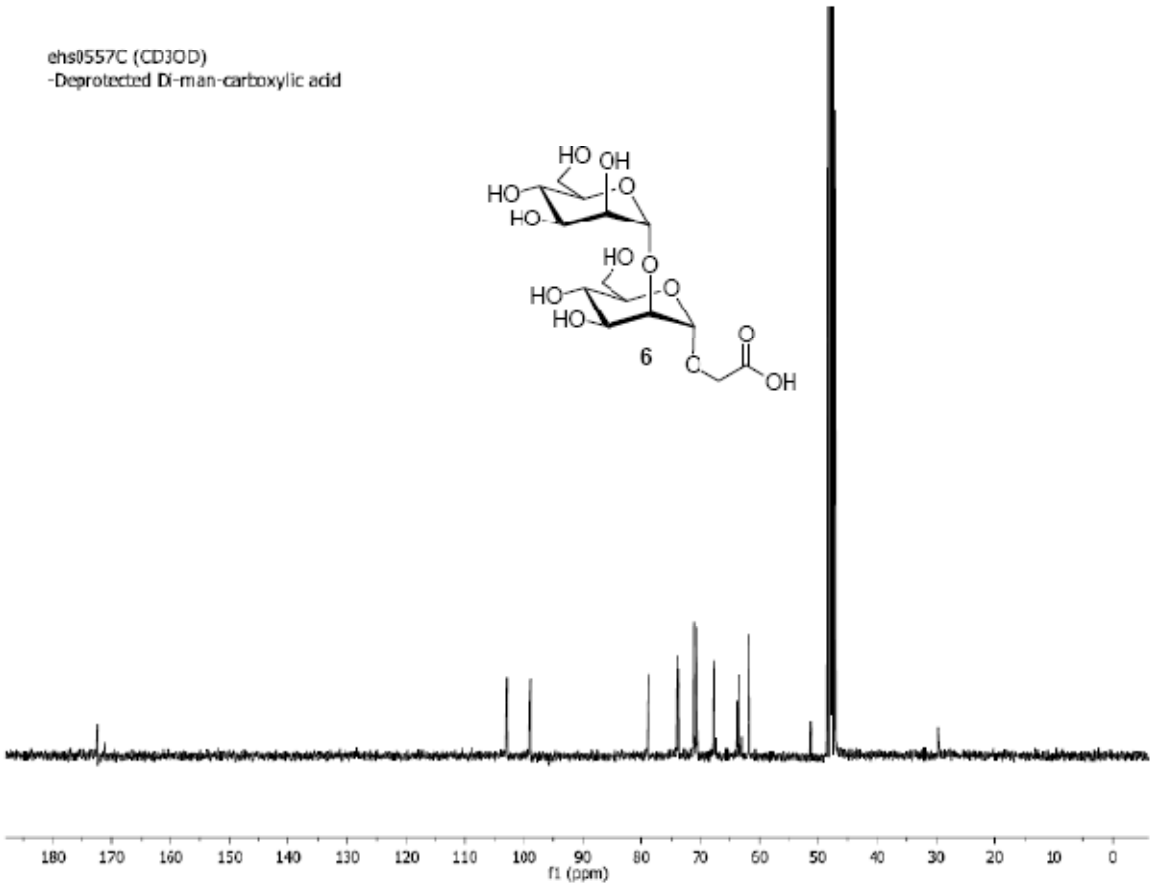
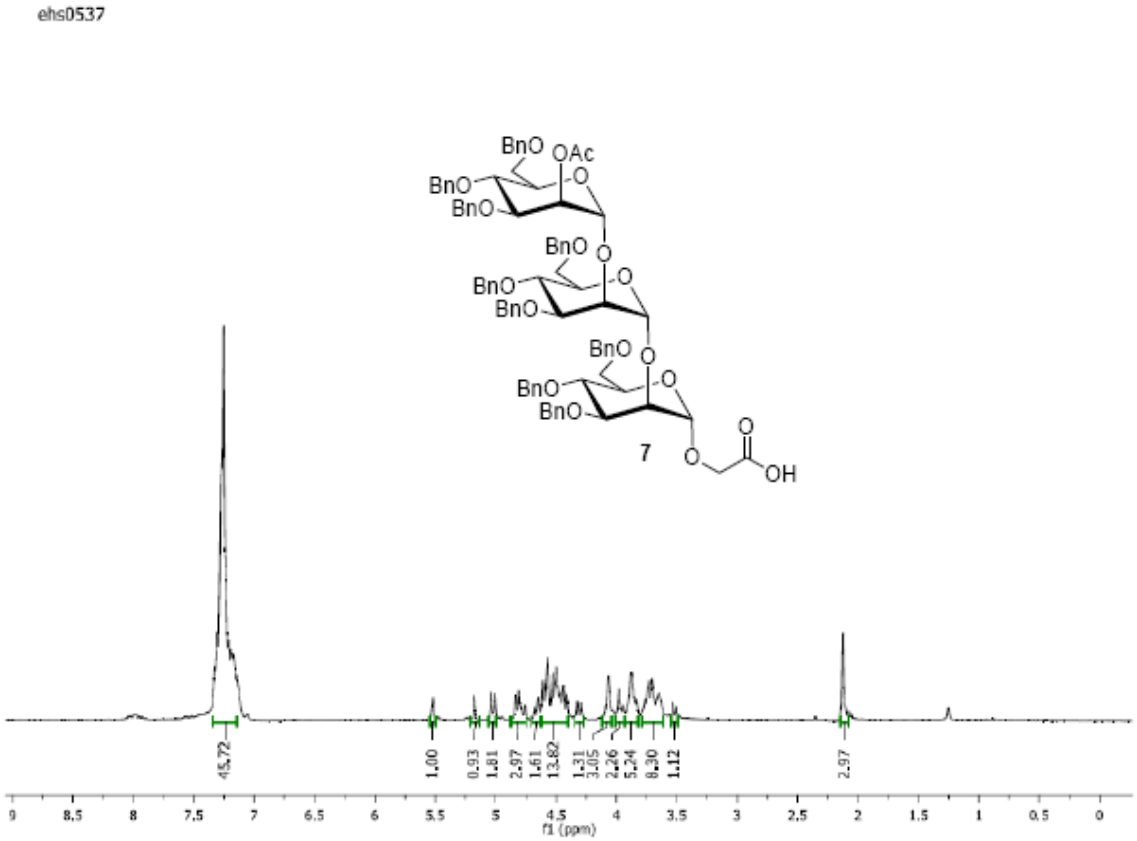


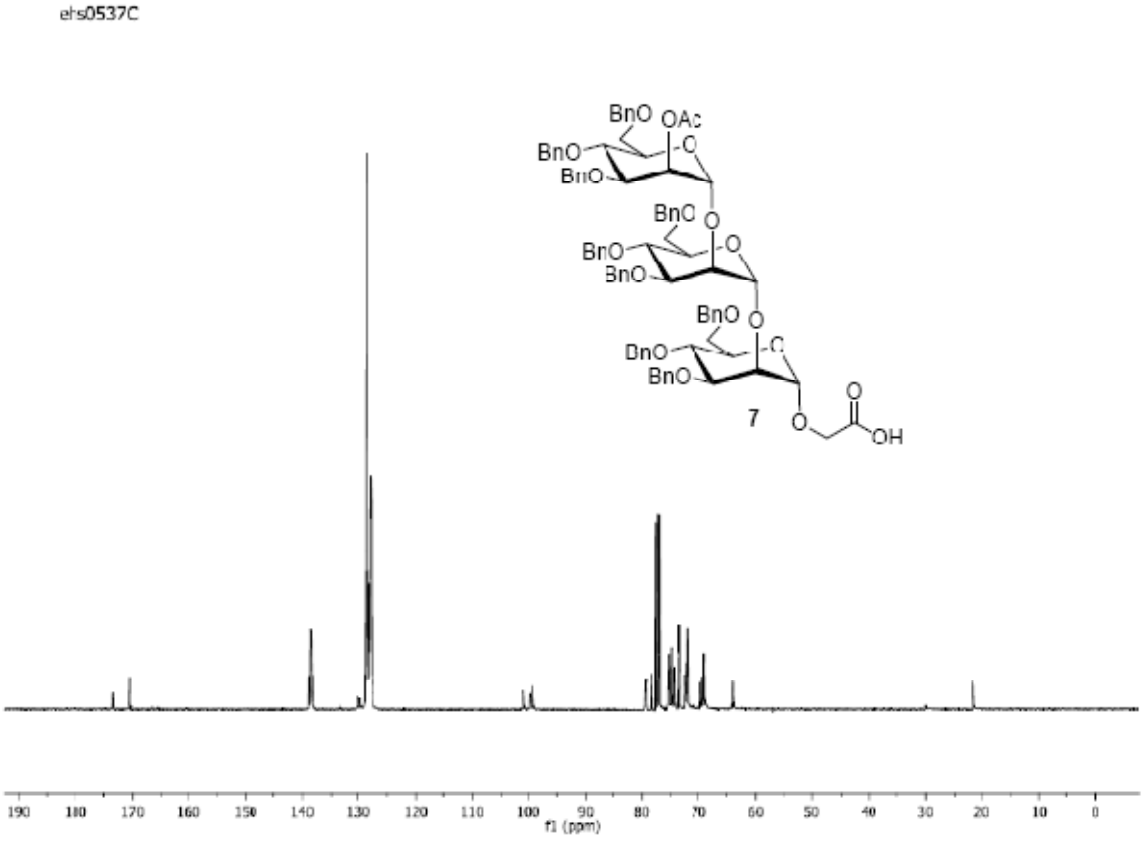


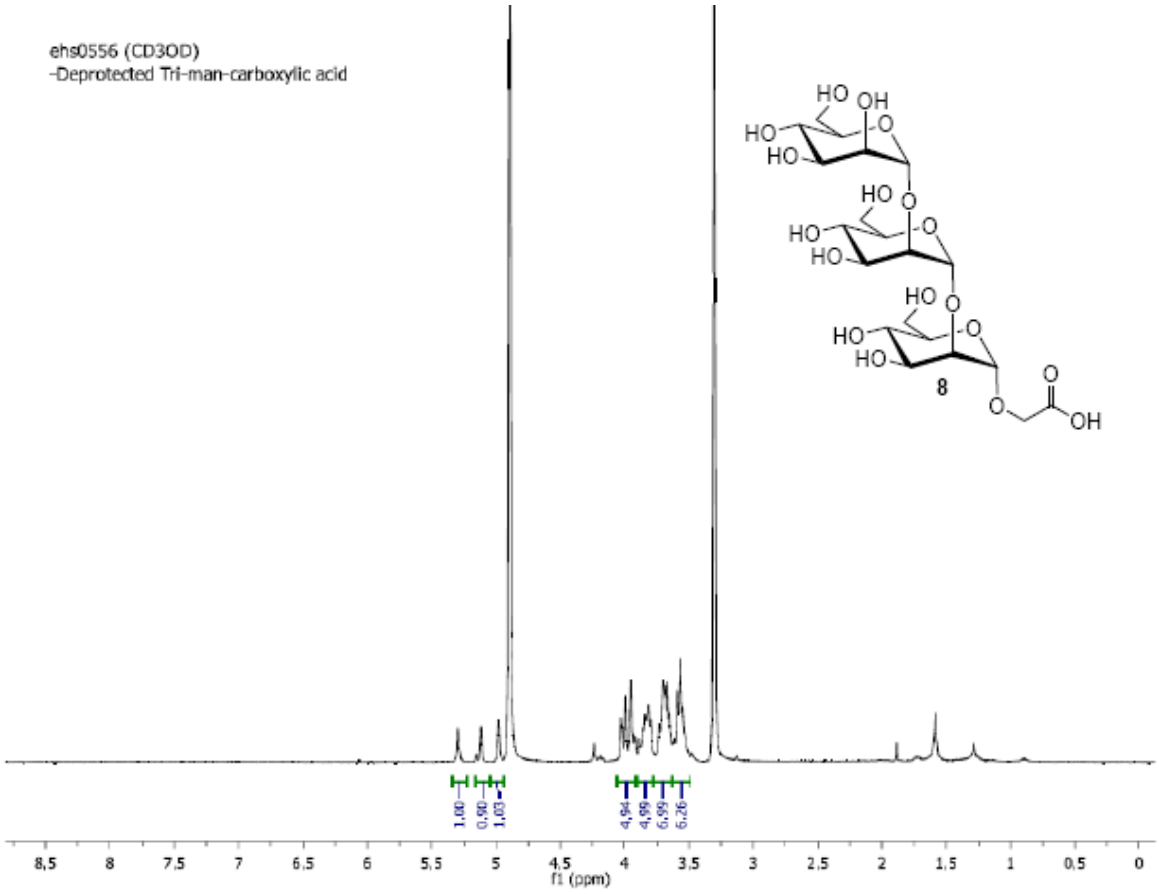


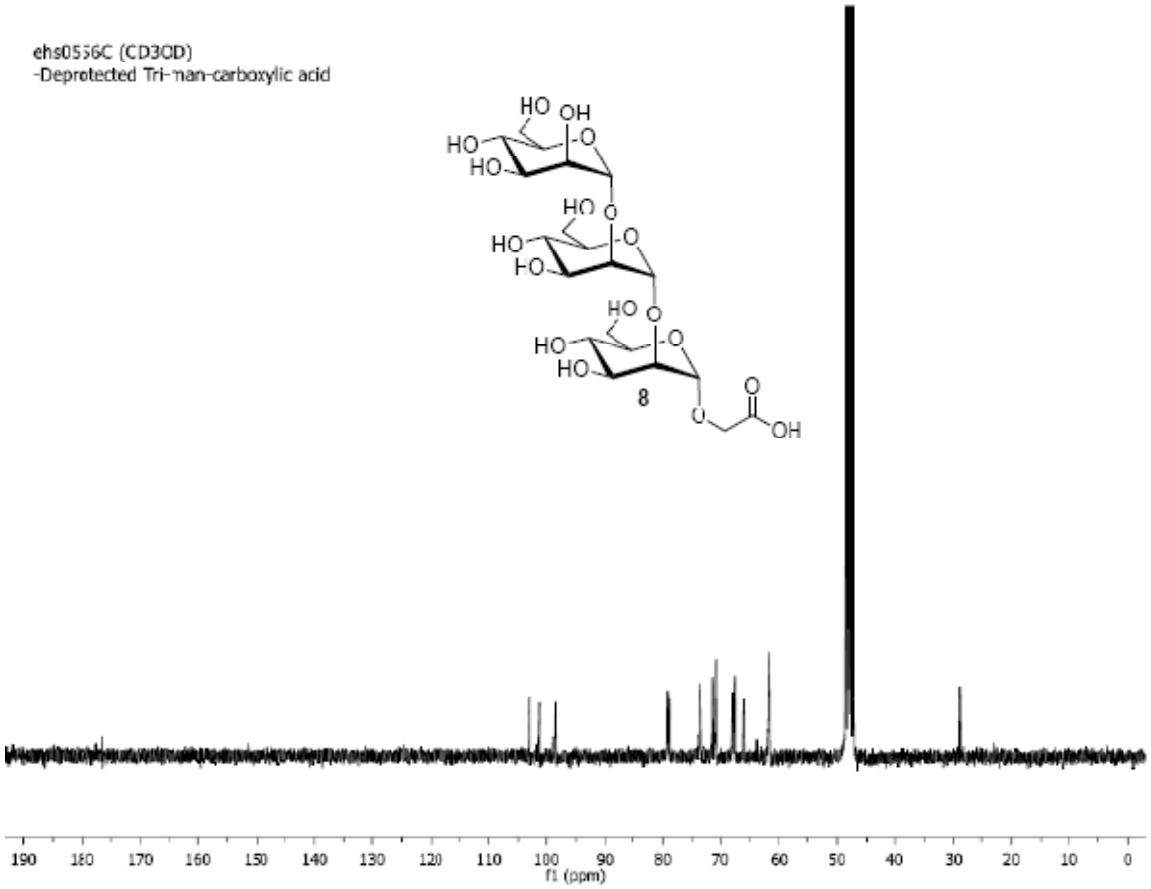


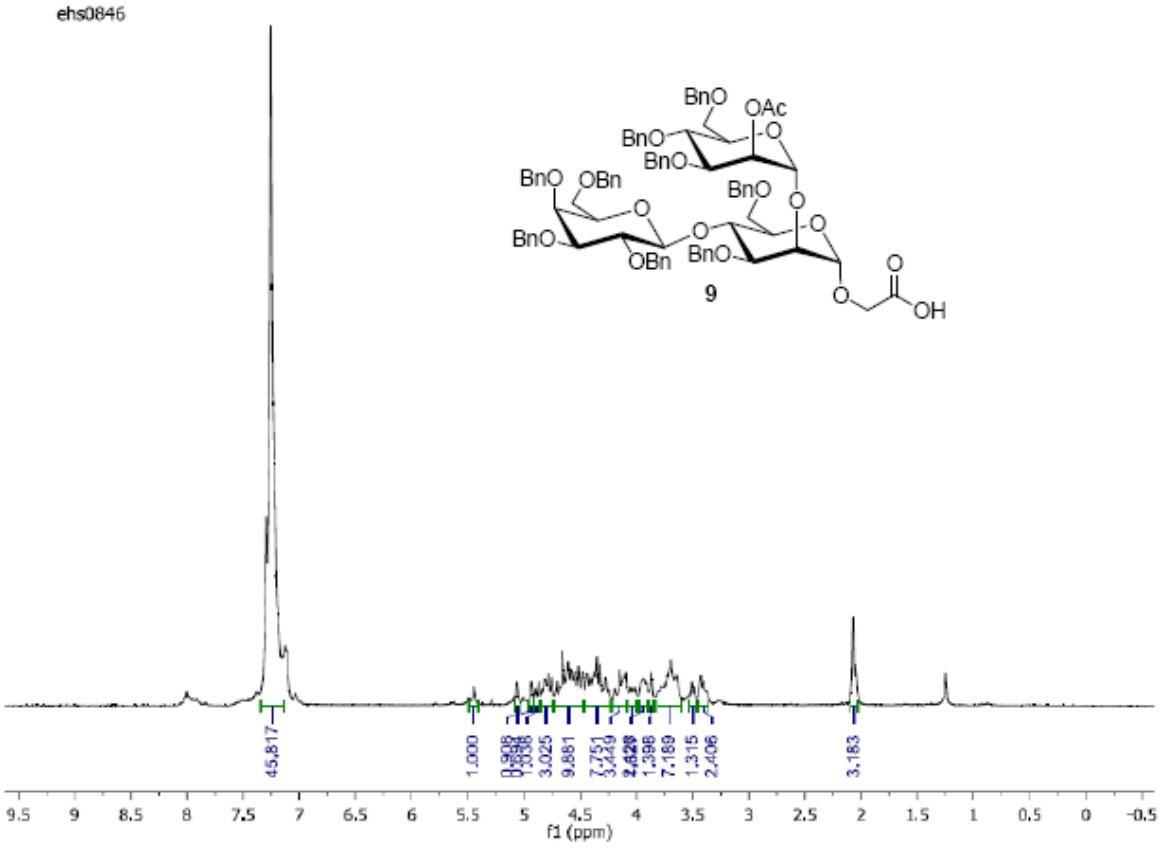


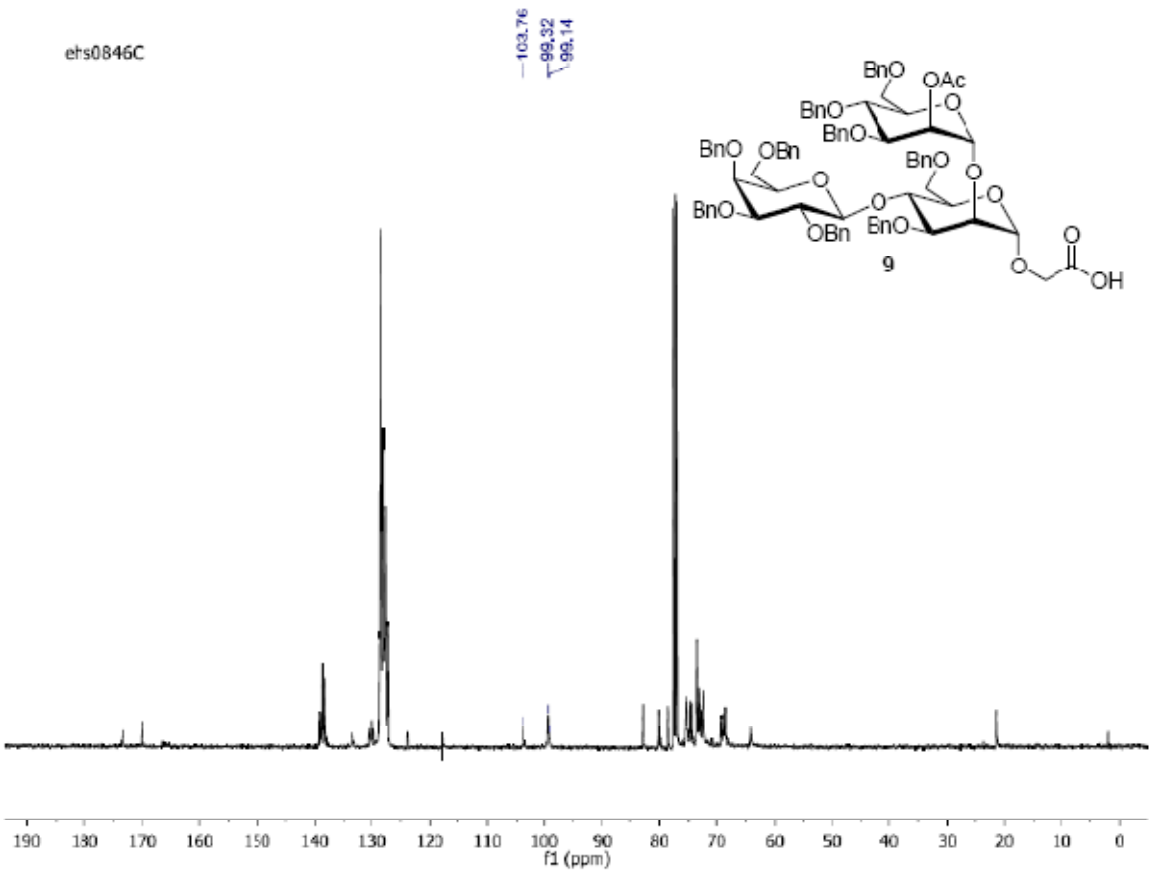


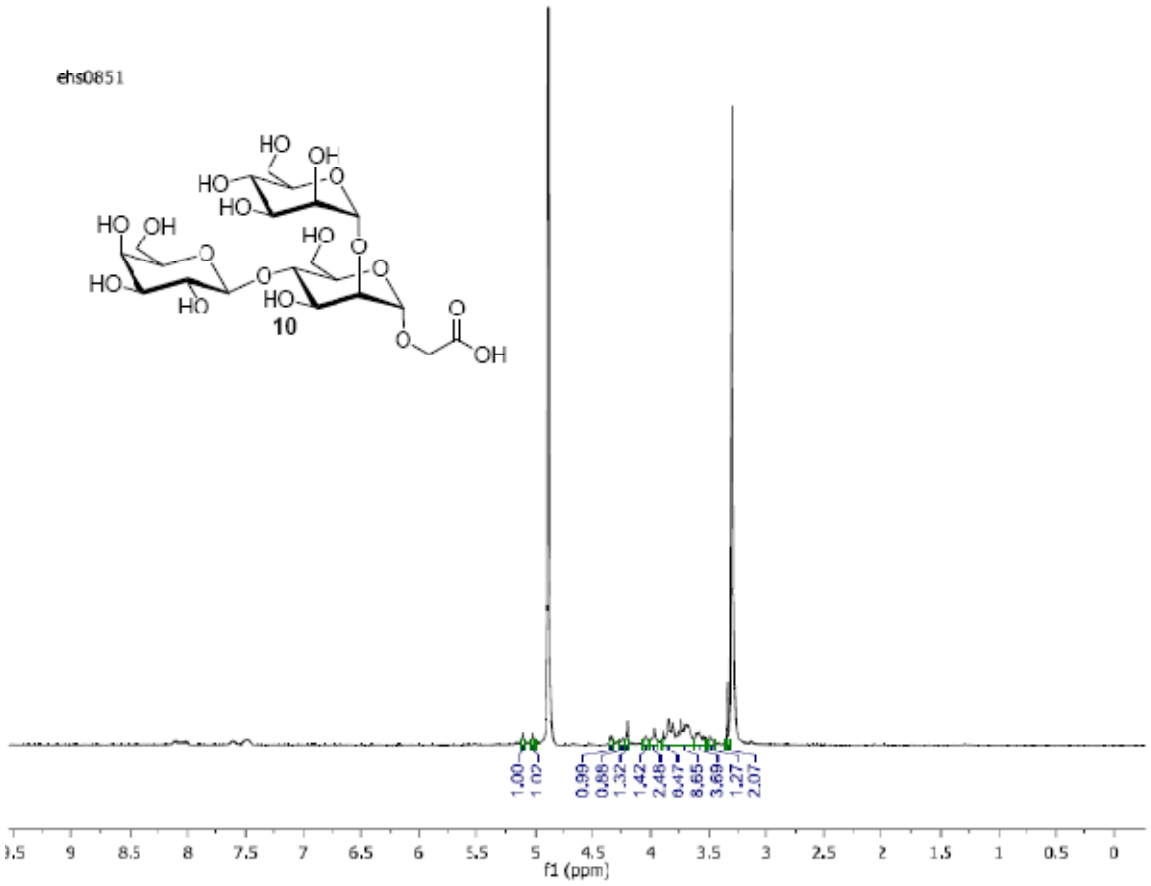


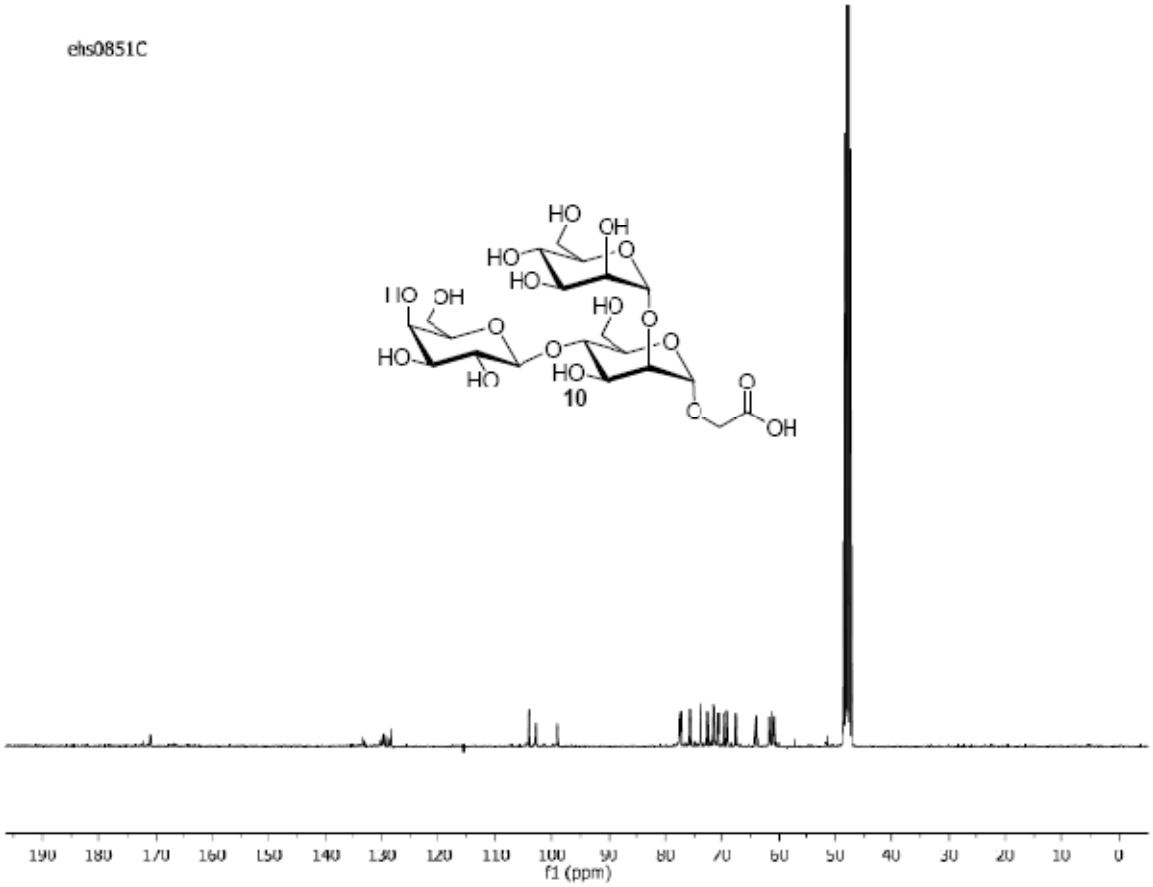


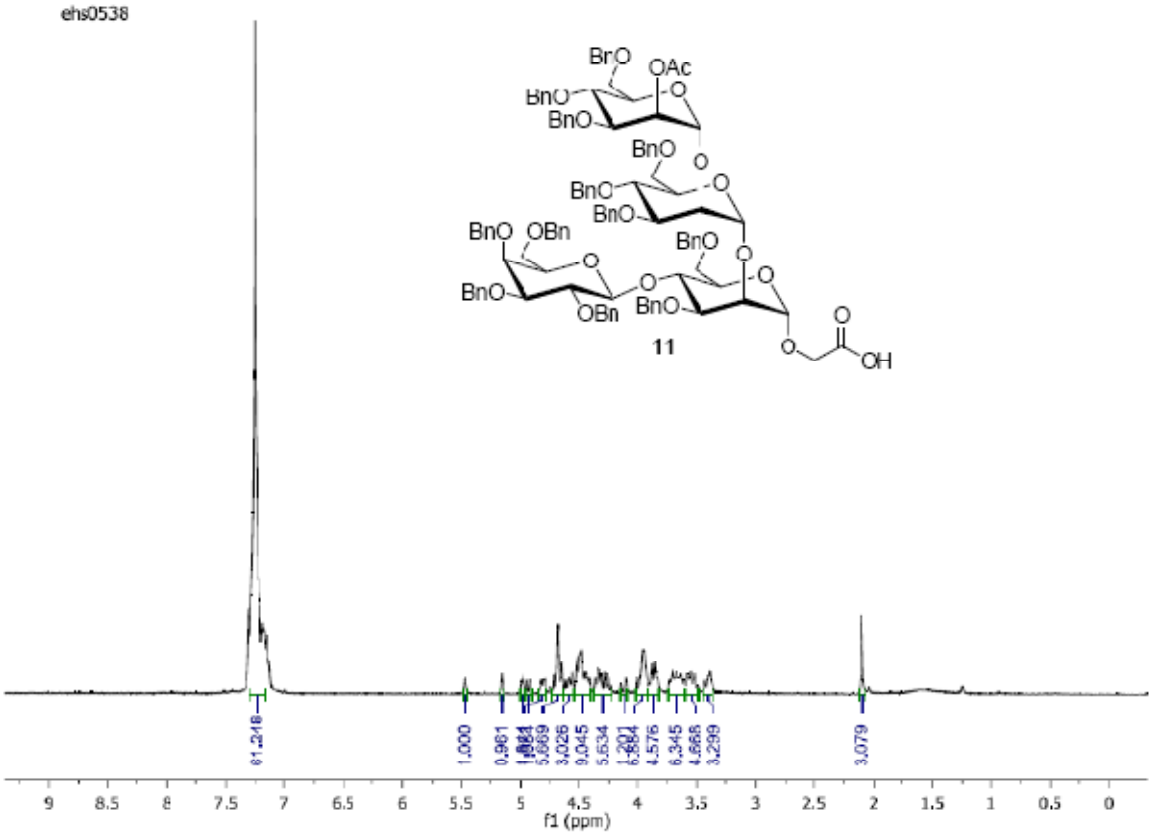


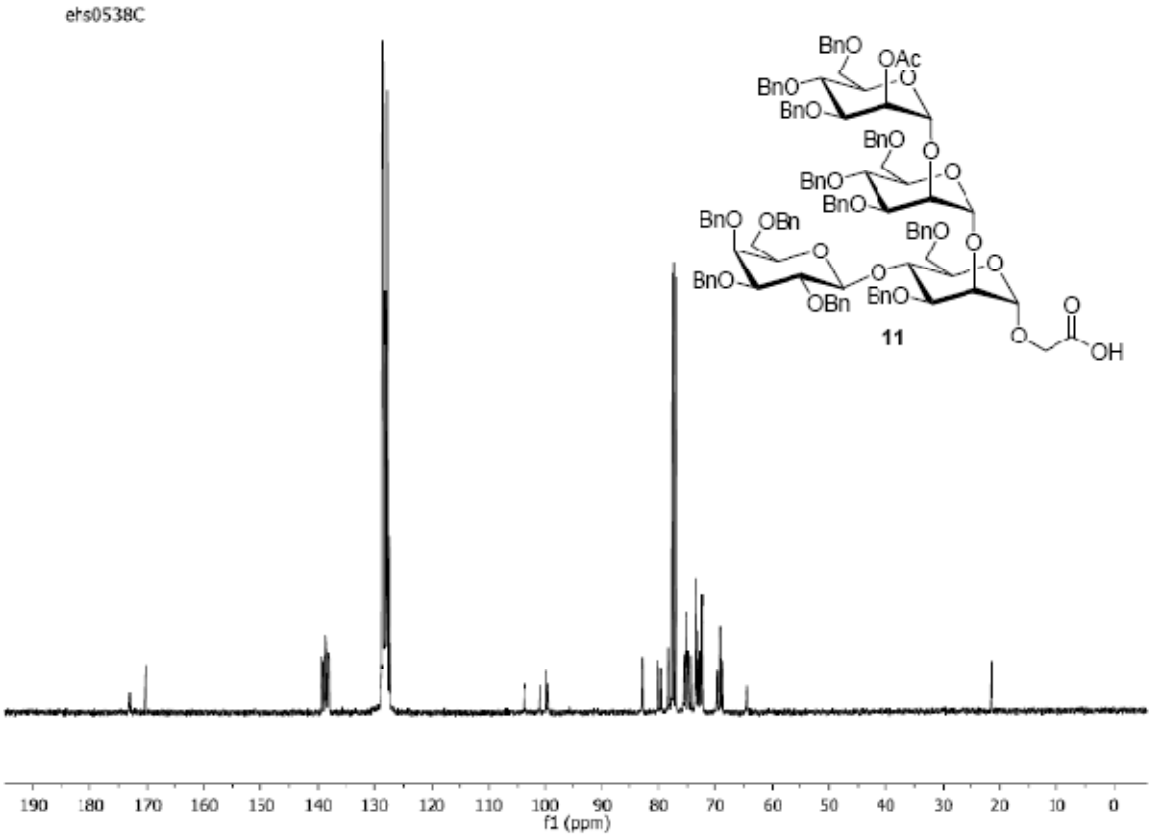


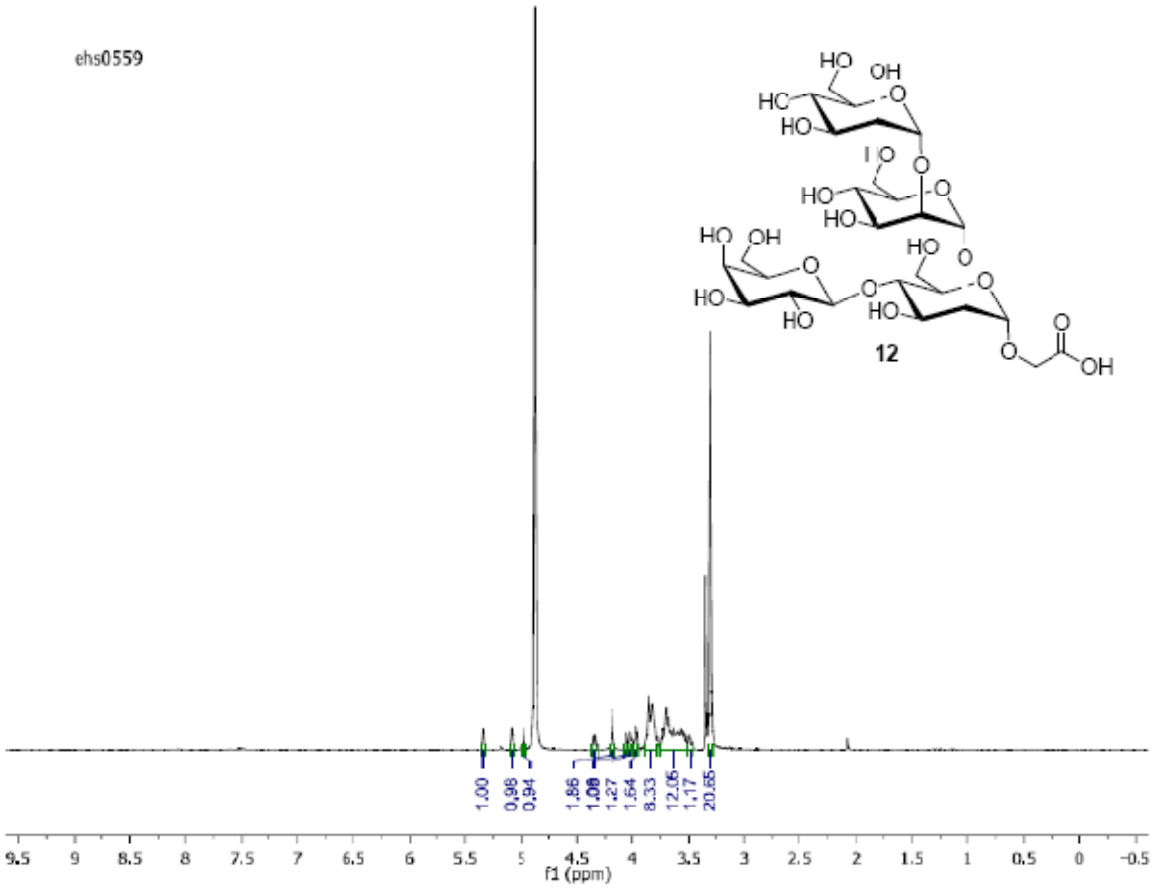


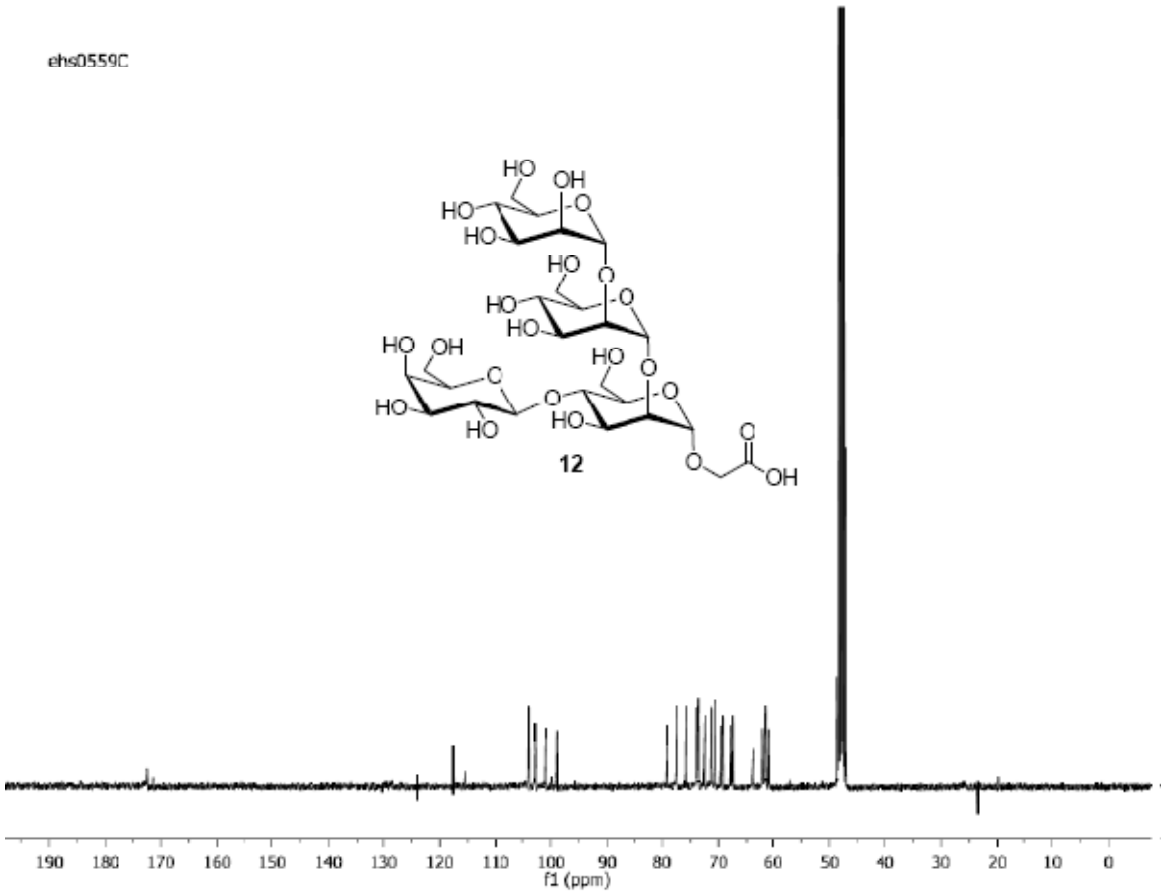






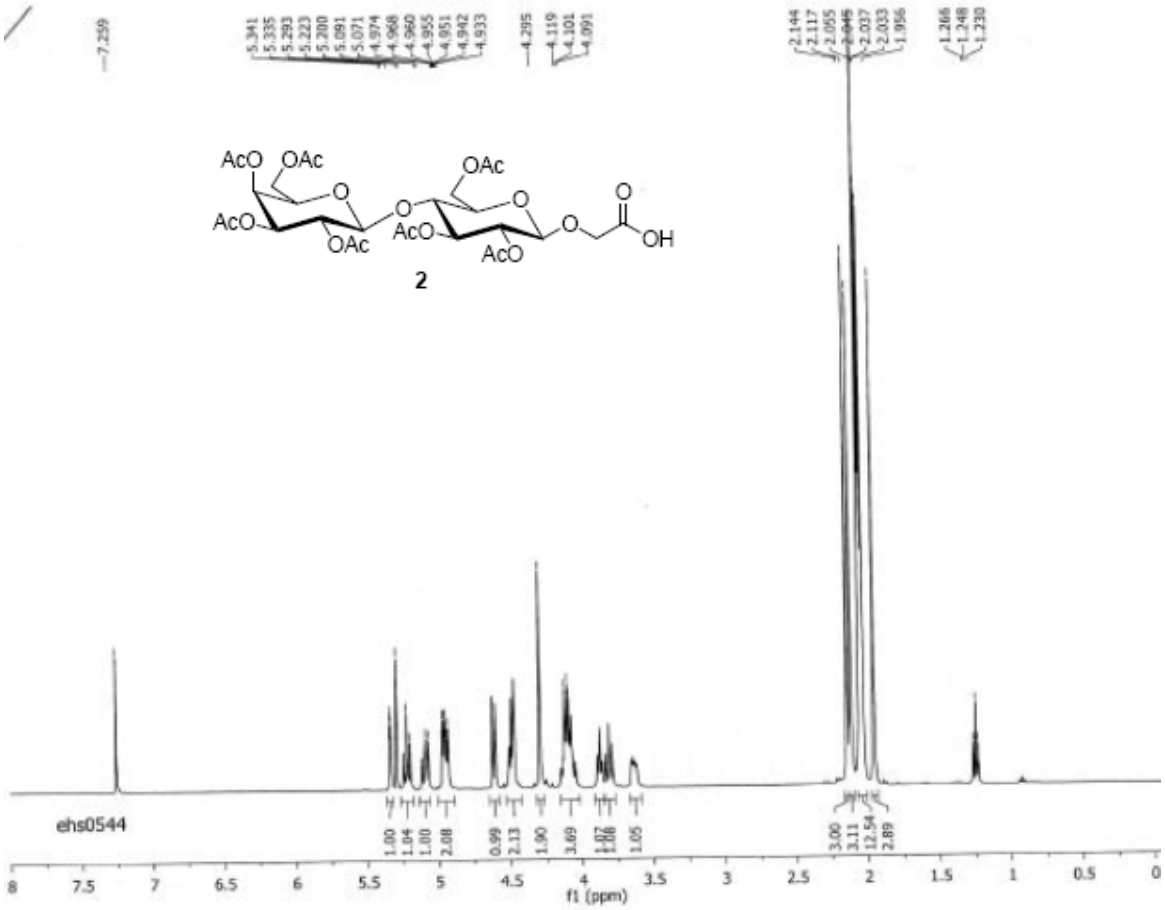


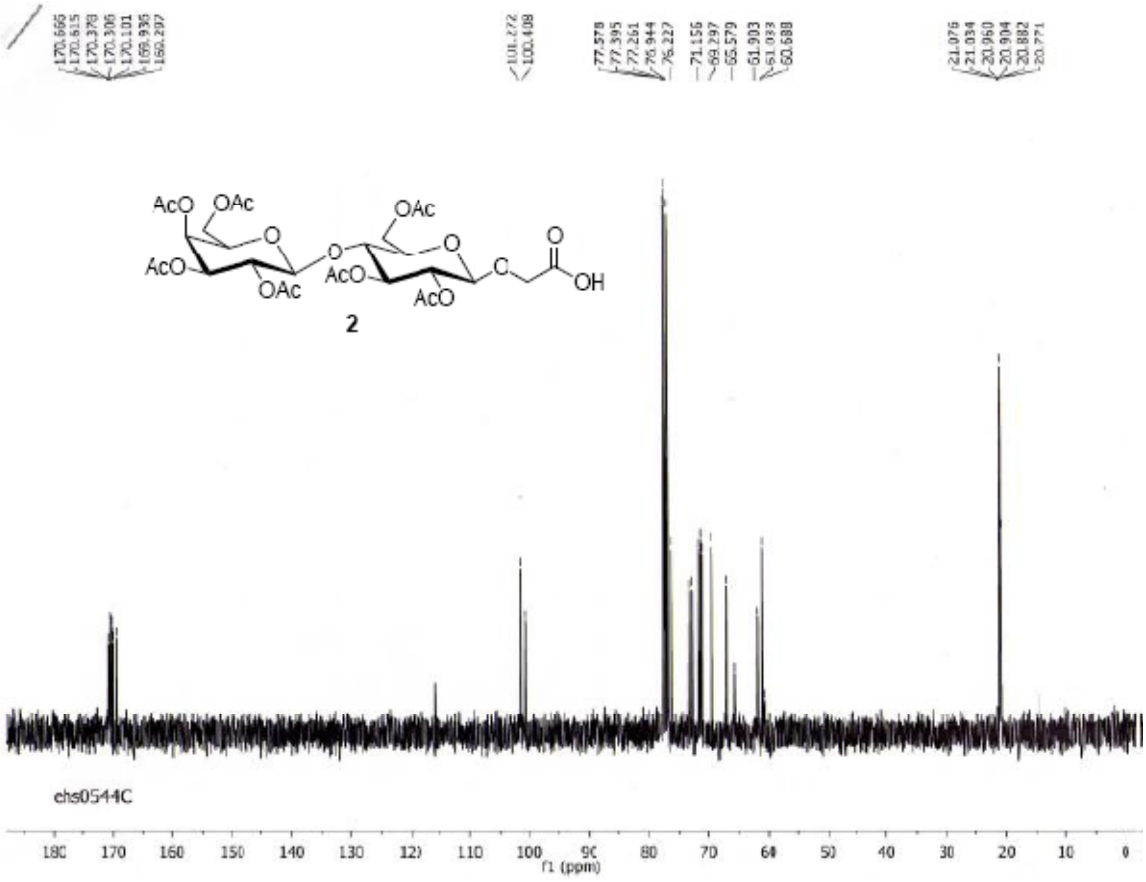




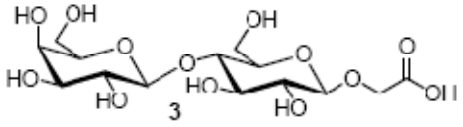
APPENDIX E.

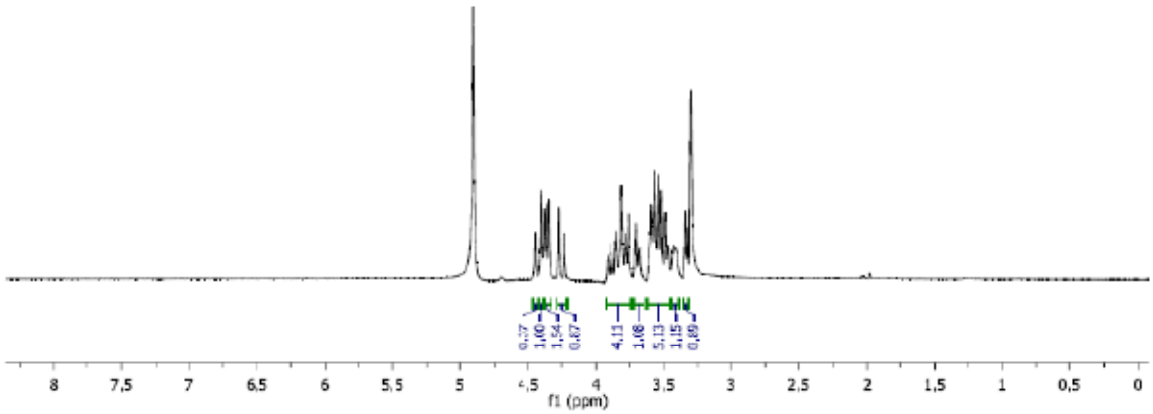
CHAPTER 6 ^{1}H AND ^{13}C NMR SPECTRA

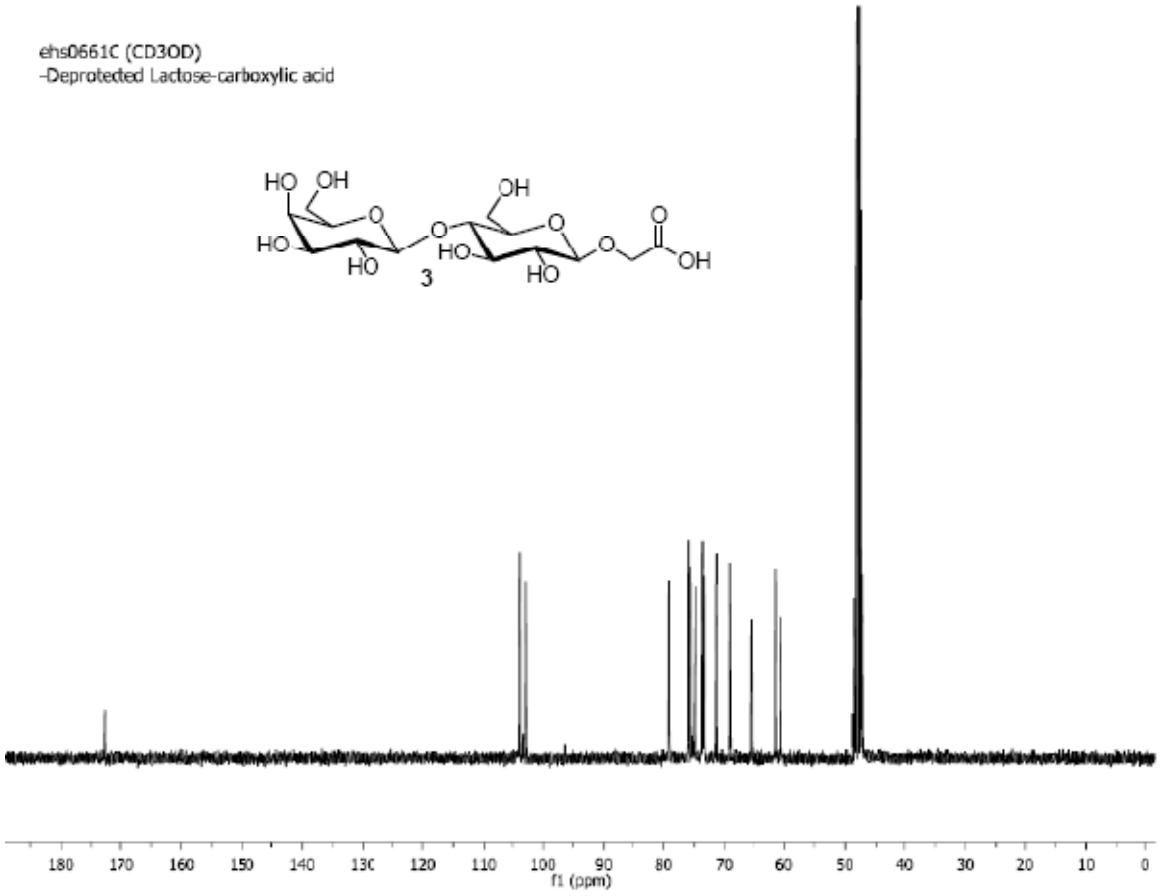


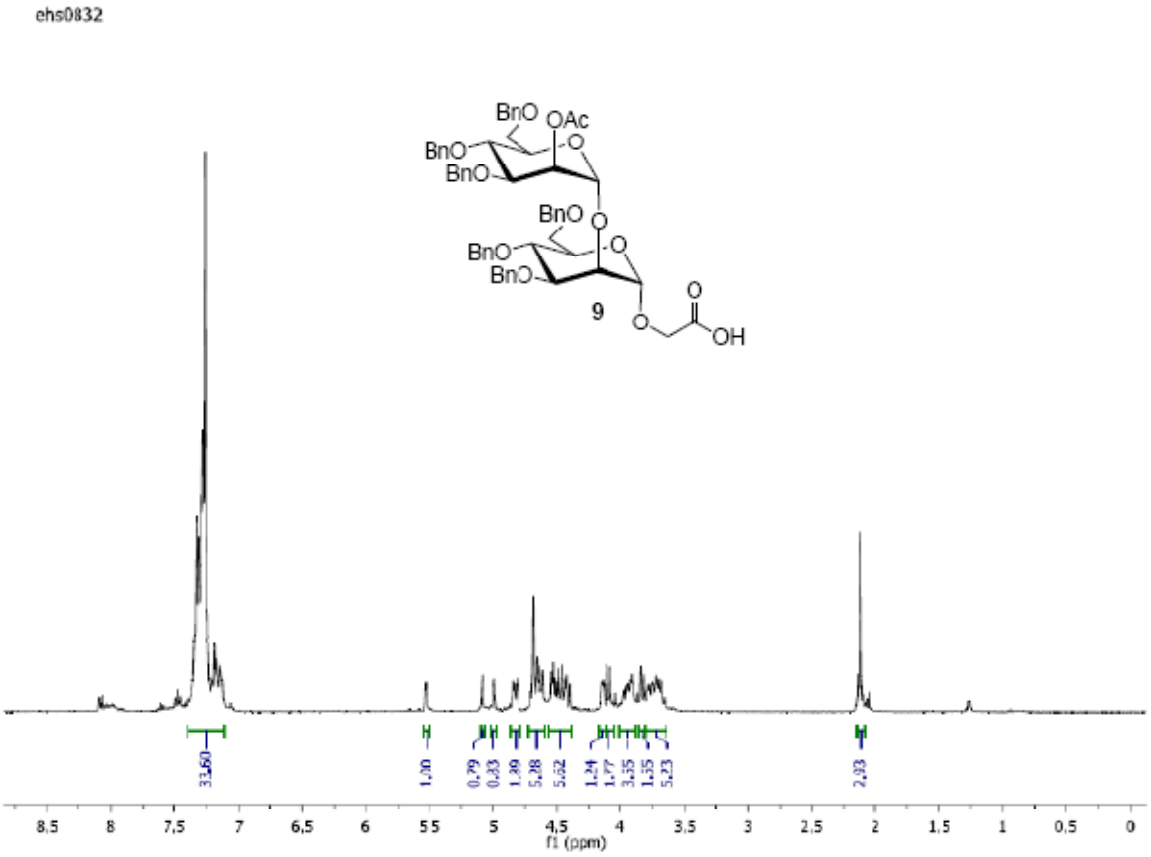


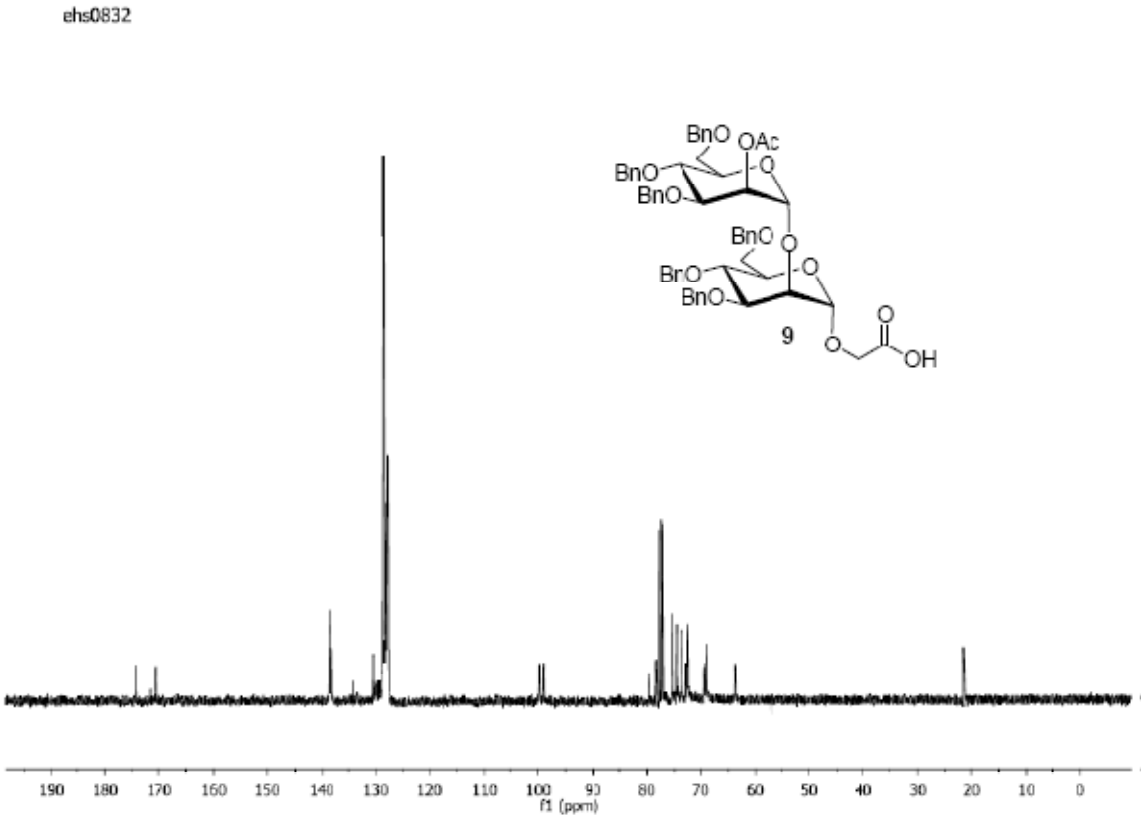
ehs0661 (CD3OD)
-Deprotected Lactose-carboxylic acid











ehs0557 (CD3OD)
-Deprotected Di-man-carboxylic acid