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Fluorous-tag assisted solution phase synthesis of mannose and heptomannose oligosaccharides and study of their binding interactions using carbohydrate microarray technology

Firoz Ali Jaipuri
Iowa State University

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by

Firoz Ali Jaipuri

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Program of Study Committee:
Nicola L. Pohl, Major Professor
George A. Kraus
L. Keith Woo
Yan Zhao
Reuben Peters

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LIST OF ABBREVIATIONS

^{13}C NMR	carbon nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
Ac	acetyl
Piv	pivaloyl
TBDMS	tert-butyldimethylsilyl
Lev	levulinyl
Bn	benzyl
Con A	Concanavalin A
Pd/C	palladium on carbon
H_2	hydrogen gas
rt	room temperature
MeOH	methanol
NaOMe	sodium methoxide
TMSOTf	trimethylsilyltrifluoromethane sulfonate
py	pyridine
HPLC	high performance liquid chromatography
TLC	thin layer chromatography
DCM	dichloromethane
EtOAc	ethyl acetate
$CDCl_3$	chloroform-d
CD_3OD	methanol-d
NaOH	sodium hydroxide
MS	mass spectrometry
ESI	electron spray ionization
Hz	hertz
MHz	megahertz
FSPE	fluorous solid-phase extraction
THF	tetrahydrofuran

ppm	parts per million
$NH_2.NH_2$	hydrazine
NMNO	*N*-methyl morpholine-*N*-oxide
DMF	*N*,*N*-dimethyl formamide
AcOH	acetic acid
CBz	benzyl carbonate
HCl	hydrochloric acid
Ms	mesylate
$MgSO_4$	magnesium sulfate
TMEDA	tetramethylethylenediamine
K_2CO_3	potassium carbonate
R_f	rate of flow
LCMS	liquid chromatography-mass spectrometry
Man	mannose
Gal	galactose
Fuc	fucose
Lac	lactose
Mal	maltose
Rha	rhamnose
GlcNAc	*N*-acetyl glucosamine
Ara	arabinose
GlcN	glucosamine
DNA	deoxynucleic acid
$PdCl_2$	palladium chloride
Cs_2CO_3	cesium carbonate
PEG	polyethylene glycol

CHAPTER 1: BACKGROUND INFORMATION

Importance of Carbohydrates

There are four major classes of macromolecules in biology, DNA, proteins, carbohydrates and lipids. Carbohydrates differ from the other classes of biological polymers in two important characteristics: they can be highly branched molecules, and many different linkage types can connect their monomeric units to one another. This complexity allows carbohydrates to present in almost unlimited structure variations. The basic building blocks of carbohydrates are monosaccharides. The carbohydrates are a major source of metabolic energy, both for plants and for animals that depend on plants for food. Apart from the sugars and starches that meet this vital nutrition role, carbohydrates play important role in carrying out various biological functions. In all living organisms, the two monosaccharides ribose and deoxyribose form part of the structure of nucleic acids DNA and RNA in which sugar rings form the backbone to which the bases encoding the genetic information are covalently linked. Carbohydrates are also linked to proteins (glycoproteins) and lipids (glycolipids) where they play important structural roles and are involved in many cell communication and signaling events.

Glycoproteins (Allen 1992, Kobata 1993, Lee 1994) are fundamental to many important biological processes including fertilization, immune defense, viral replication, degradation of blood clots, cell growth, cell-cell adhesion, and inflammation (Yaki 1986, Spohr 1988, Phillips 1990, Lasky 1992, Miller 1992, Schulze 1992, Varki 1993, Levy 1994, Tulsiani 1997,). Carbohydrates are linked to proteins either by oxygen or nitrogen and are termed as

O-linked and N-linked glycans respectively. Glycoproteins and glycolipids are major components of the outer surface of mammalian cells. Oligosaccharide structures change dramatically during development, and it has been shown that specific sets of oligosaccharides are expressed at distinct stages of differentiation. Further, alterations in cell surface oligosaccharides are associated with various pathological conditions including malignant tranformation.

The role of DNA and proteins in biological events termed as genomics and proteomics respectively, have been well studied. But study of the role of carbohydrates in biology is just beginning to expand. Glycomics examines how the addition of sugars to proteins (glycosylation) affects biological systems. In many cases it is not the protein itself that is relevant to disease status, but the modification associated with that protein. Glycosylation is one of the major posttranslational modification events affecting proteins and has been implicated in various biological events such as cancer, congenital disorders, infections, autoimmune diseases and signaling.

The interactions between saccharides and their receptors are of vital importance in many normal cellular mechanisms. These interactions are being recognized as important in a range of disease processes, including, for example, infections by bacteria, viruses and parasites, in inflammatory disorders, and possibly in cancer metastatis. In addition there are human disorders caused by disruptions in normal synthesis or catabolism of glycosylated molecules.

Glycomics has trailed the explosive growth seen in genomics and proteomics. This is mostly because of the complex nature of carbohydrates and their unavailability. The diversity of synthetic and naturally occurring carbohydrate structures means that their

automated chemical synthesis is not practical in the way it is for proteins and nucleic acids. Difficulties in chemical synthesis arise from the potential structural diversity of these compounds. Furthermore, difficulties spring because carbohydrates contain several hydroxyl groups of similar chemical reactivity: in chemically synthesizing a carbohydrate structure, each hydroxyl group must be distinguished from the rest in order to obtain the desired product with the correct stereochemistry and regiochemistry. Isolation of oligosaccharides from natural sources is difficult, time-consuming and expensive.

Two major technological breakthroughs helped genomics and proteomics to blossom. The sequencing of oligonucleotides and proteins is automated and allows for the composition of an unknown sample to be determined quickly and reliably (Hunkapiller 1991), thereby providing a starting point for structure–function studies and the design of modifications. In addition, non-experts in an automated fashion can now achieve the synthesis of defined oligonucleotides (Caruthers 1985, 1991) and peptides (Atherton 1989). Modified oligonucleotides, peptides and proteins have found use as research tools and therapeutic agents.

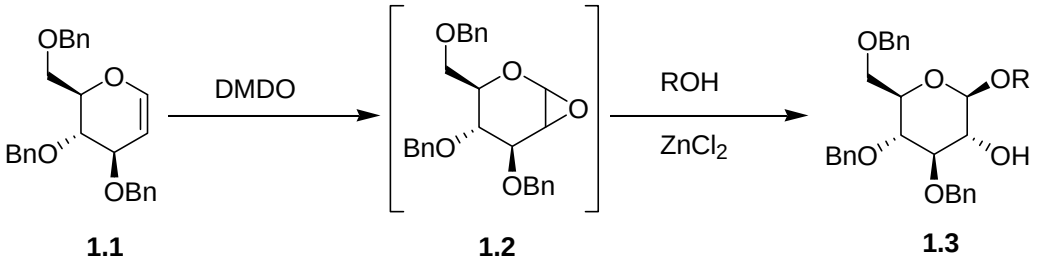
Access to pure carbohydrates for biological, biochemical and biophysical studies relies on chemical or enzymatic synthesis. Given the structural complexity of carbohydrates, regio- and stereoselectivity of glycosylation reactions is the key challenge for the assembly of oligosaccharides (ref). Synthetic chemists have developed increasingly powerful and versatile methods that have resulted in the assembly of ever more complex oligosaccharides and glycosaminoglycans. Still, the preparation of such structures is technically difficult, extremely time consuming and is carried out by a few highly specialized laboratories. Advances in enzymatic synthesis and in automated synthesis

planning have alleviated some of these challenges (ref), but still no automated synthesis method was available until recently to fuel the growing need for defined oligosaccharide structures as glycomics efforts gather steam.

Iterative Solution Phase Synthesis by Danishefsky's Glycal Method

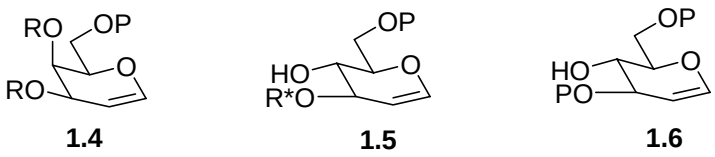
Danishefsky and coworkers has explored the use of glycals for the synthesis of oligosaccharides and glycoconjugates (Danishefsky 1996). Glycals may be converted into a number of glycosyl donors (Gordon 1990). For instance, epoxidation of glycals provides access to 1,2-anhydrosugars, which, in the presence of Lewis acid catalysts, proved to be excellent glycosyl donors in a range of glycosylation reactions (Scheme 1.1). The heart of this method involves the use of glycal-derived donors with suitably differentiated glycal acceptors, thereby providing the feature of smooth reiterability. Indeed, the adaptability of the logic of glycal assembly to solid phase synthesis has been demonstrated (Danishefsky 1996).

The Le^x-Le^y nonasaccharide is not readily available from natural sources, but could be valuable for, e.g. developing anticancer vaccines. Remarkably, this oligosaccharide can ultimately be prepared from just three glycal precursors (Scheme 1.2).



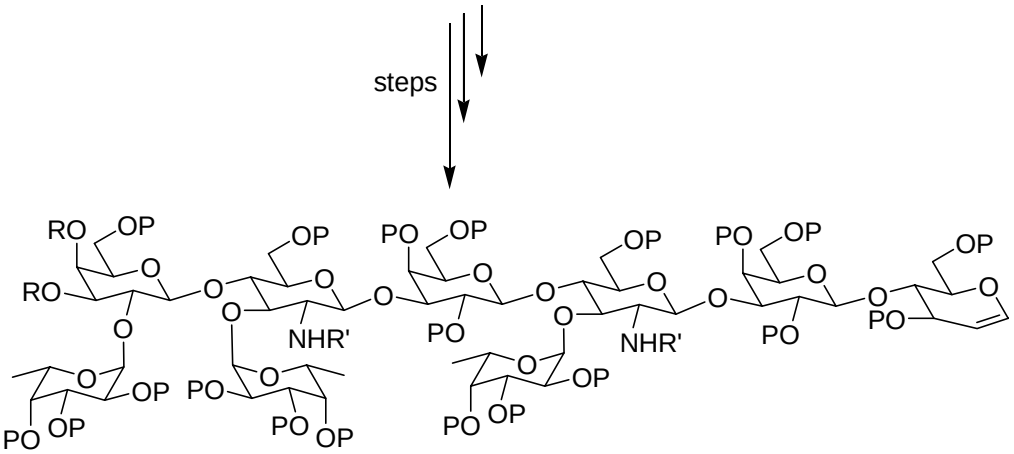
1.1 **1.2** **1.3**

Scheme 1.1 General Glycal Method.



1.4 **1.5** **1.6**

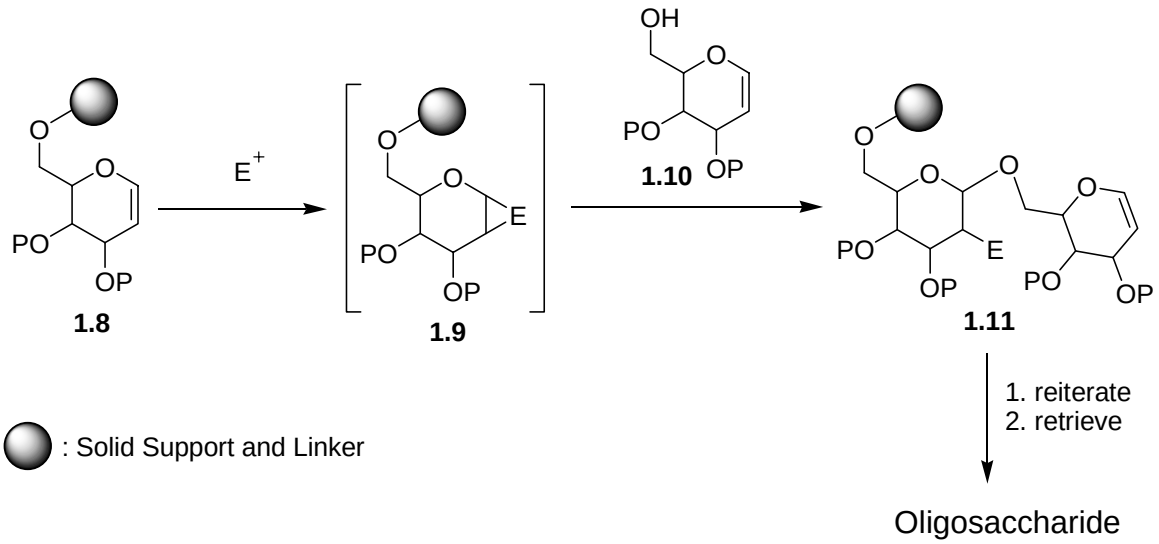
P = protecting group, R = P or H, R* = unique hydroxy protecting group and fucosylation site



1.7

Scheme 1.2 Solution phase synthesis of Le^x-Le^y nonasaccharide by glycal assembly method.

The advantages of using glycal assembly method are as follows: 1) Syntheses of important and previously inaccessible oligosaccharides were achieved. 2) Reaction conditions used in this method are mild and general. The major drawback of using solution phase glycal assembly method was that the synthesis was laborious and time consuming. Since it is using solution phase chemistry, the isolation and purification of intermediates was difficult and time consuming. Also, overall yields were less than 1%. To circumvent this problem, Danishefsky and coworkers came up with the solid phase version of the glycal assembly method (Scheme 1.3).



Scheme 1.3 General strategy for the synthesis of oligosaccharides on a solid support using the glycal assembly method.

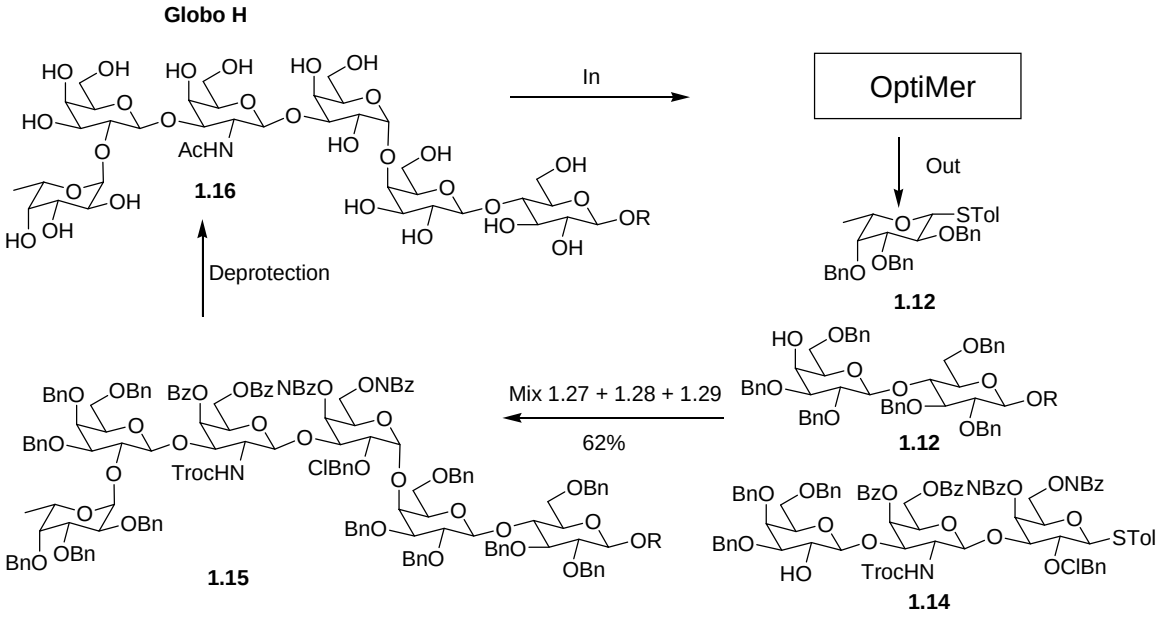
In glycal assembly method the glycal terminus of compound **1.16** could be converted to a donor function as represented in a general way with compound **1.17**. For example, compound **1.17** could be an isolable entity such as a 1,2-anhdyrosugar (Halcomb 1989), in which case E^+ corresponds to an epoxidizing agent (Scheme 1.3). Moreover, through employment of a glycal as the solution based acceptor, the scheme benefits from relative simplicity in the identification of strategic hydroxyls for glycosylation.

Wong's OptiMer Method

The Chi-Huey Wong's research group at Scripps has prepared hundreds of mono- and disaccharides with different protecting group patterns to control glycosylation rate. This means that multiple components can be combined in the same reaction: the fastest component reacts first, then the next fastest, etc. A computer program (OptiMer) has been written that knows the relative reactivities of all the potential reactants. It can carry out

retrosynthetic analyses of complex carbohydrates and suggest a set of reactants that would allow synthesis of the oligosaccharide in one single reaction. In addition to condensing multiple reactions into one, this dramatically simplifies the laborious and inefficient processes of isolation and purification.

Wong and coworkers synthesized Globo H, a carbohydrate that acts as antigen on breast cancer, using automated synthesis based on OptiMer technology (Sears 2001). The key to this approach is to have extensive quantitative data regarding the relative reactivities of different protected sugars. In Globo H, four monosaccharides are linked with one disaccharide to make Globo H hexasaccharide. This is then linked to a protein carrier to make a candidate breast cancer vaccine.



Scheme 1.4 Synthesis of the cancer antigen Globo H with OptiMer Technology.

In the synthesis of Globo H, the sequence of Globo H is entered into the computer, which predicts the best building blocks to be used. These building blocks are then mixed in

sequence, starting with the most reactive one, in the presence of activator. The product obtained is then purified and deprotected to give the target (Scheme 1.4). This technology for the one-pot synthesis of oligosaccharides is very efficient and predictable. It dramatically reduces labor associated with isolation and purification. The limitation of this method is that it requires access to a very large number of complex precursors. The synthetic method to make these complex precursors is still not available to non-chemists.

Solid Phase Oligosaccharide Synthesis

Solid phase synthesis has proven extremely efficient for the assembly of peptides and oligonucleotides as it does not require purification after each reaction step, utilizes excess reagent to drive reactions to completion and lends itself to automation (ref). A series of different approaches to solid phase oligosaccharide synthesis had been described and all critical aspects including the choice of synthetic strategy, differentially protected glycosylating agents, solid support materials, and linkers to attach the first monosaccharide to the support matrix were explored.

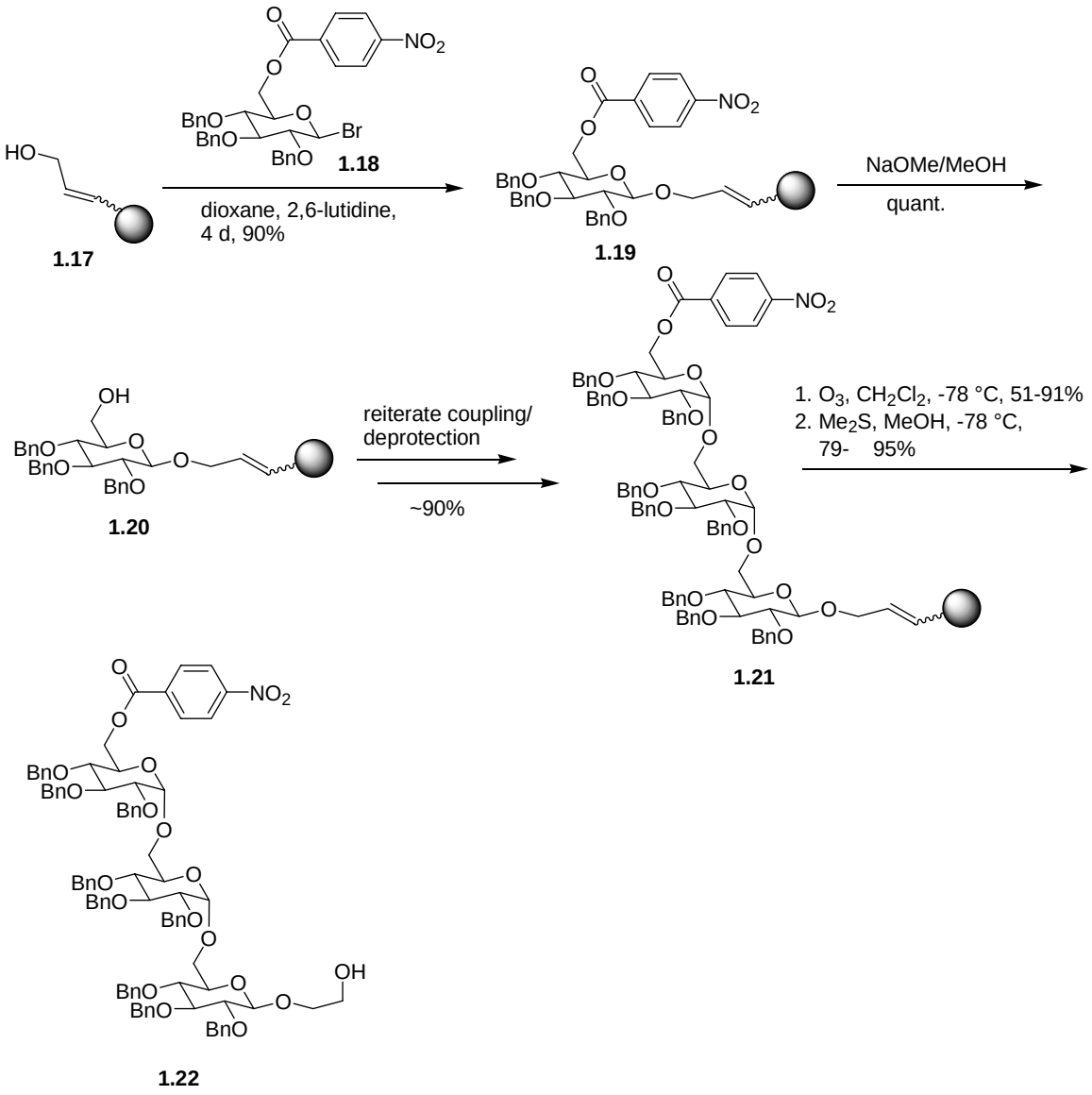
Much progress concerning different aspects of solid phase oligosaccharide assembly had been made, but no generally applicable approach had evolved towards an automated solid phase oligosaccharide synthesizer. The salient features associated with the solid-phase synthesis that attracted researchers to explore oligosaccharide synthesis on solid support included ease of purification, higher yields by use of excess reagents, and synthesis speed. The importance of solid-phase synthesis was realized in the synthesis of peptides (Merrifield 1963, 1985), which had been automated by 1970 (Merrifield 1966). The impact of automated solid-phase synthesis of oligopeptide (Atherton 1989) and later

oligonucleotide (Caruthers 1985) on the development of biochemistry and biology of these molecules was enormous. Given the importance of oligosaccharides in biological systems, the motivation for developing similar methodology for the synthesis of oligosacchides is comprehensible.

The problems associated with the synthesis of oligosaccharides on a polymer support are much greater than that of DNA and proteins. Unlike DNA and proteins that consist of linear chains, oligosaccharides have four potential positions for elongation. They are often branched, requiring flexible protecting group strategies for the effective differentiation of hydroxyl groups of similar chemical reactivity. The formation of a new stereogenic center in every coupling step further complicates oligosaccharide synthesis. Moreover, traditional acid-sensitive linker systems used in peptide synthesis are often incompatible with the Brönsted or Lewis acidic glycosylation conditions. Thus, the following problems need to be addressed in order to design a viable synthetic strategy: (1) methods of attachment of the carbohydrate to the polymeric support through the "reducing" or the "nonreducing" end, (2) choice of a polymer support, (3) selection of a linker that is stable to glycosylation and deprotection conditions, (4) a highly flexible protecting group strategy, (5) stereospecific and high-yielding coupling reactions, and (6) "on resin" methods to monitor coupling and deprotection reactions.

The first solid-phase synthesis of oligosacchrides was reported in 1971 by Fréchet and Schuerch (Fréchet 1971). Glucosyl donor **1.2** was attached to allyl alcohol functionalized Merrifield resin **1.1** by simple alcoholysis, preparing the first resin-bound monomer **1.3** (Scheme 1.5). The yield was determined by weight gain of the resin and on the basis of

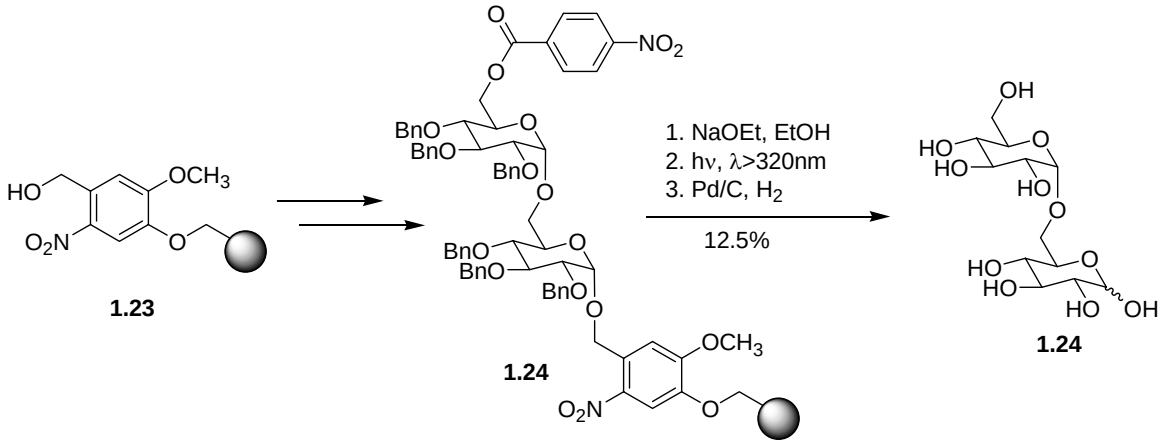
free hydroxyl groups of the latest attached sugar monomer. Cleavage from the resin was accomplished by ozonolysis followed by reduction of the ozonide with dimethyl sulfide.



Scheme 1.5 Early solid-phase approach to α-(1→6)-linked Mannose Trisaccharide **1.6**.

Zehavi and coworkers introduced the original concept of a photolabile linkage (Pillai 1980) of the first carbohydrate monomer to the polymeric phase (Zehavi 1973). They used essentially the same coupling conditions as Fréchet to synthesize disaccharide **1.8** in

approximately 90% yield per coupling cycle (Scheme 1.6). Unfortunately, photolytic release of the disaccharide from the resin did not proceed as well on a preparative scale as in previous solution-phase model studies (Zehavi 1972), and debenzylated reducing isomaltose **1.9** was obtained in only 12.5% yield.



Scheme 1.6 Use of a new photocleavable linker for the solid-phase synthesis of Isomaltose.

In addition to differently functionalized polystyrene (Merrifield's resin), controlled-pore glass, as a nonswelling inorganic polymeric support was already evaluated for solid-phase oligosaccharide synthesis in its pioneering days. Schuerch reported the attempted glycosylation of a zirconia-coated glass surface carrying unsaturated alcohol acceptor sites, but only poor glycosylation yields could be achieved (Eby 1975).

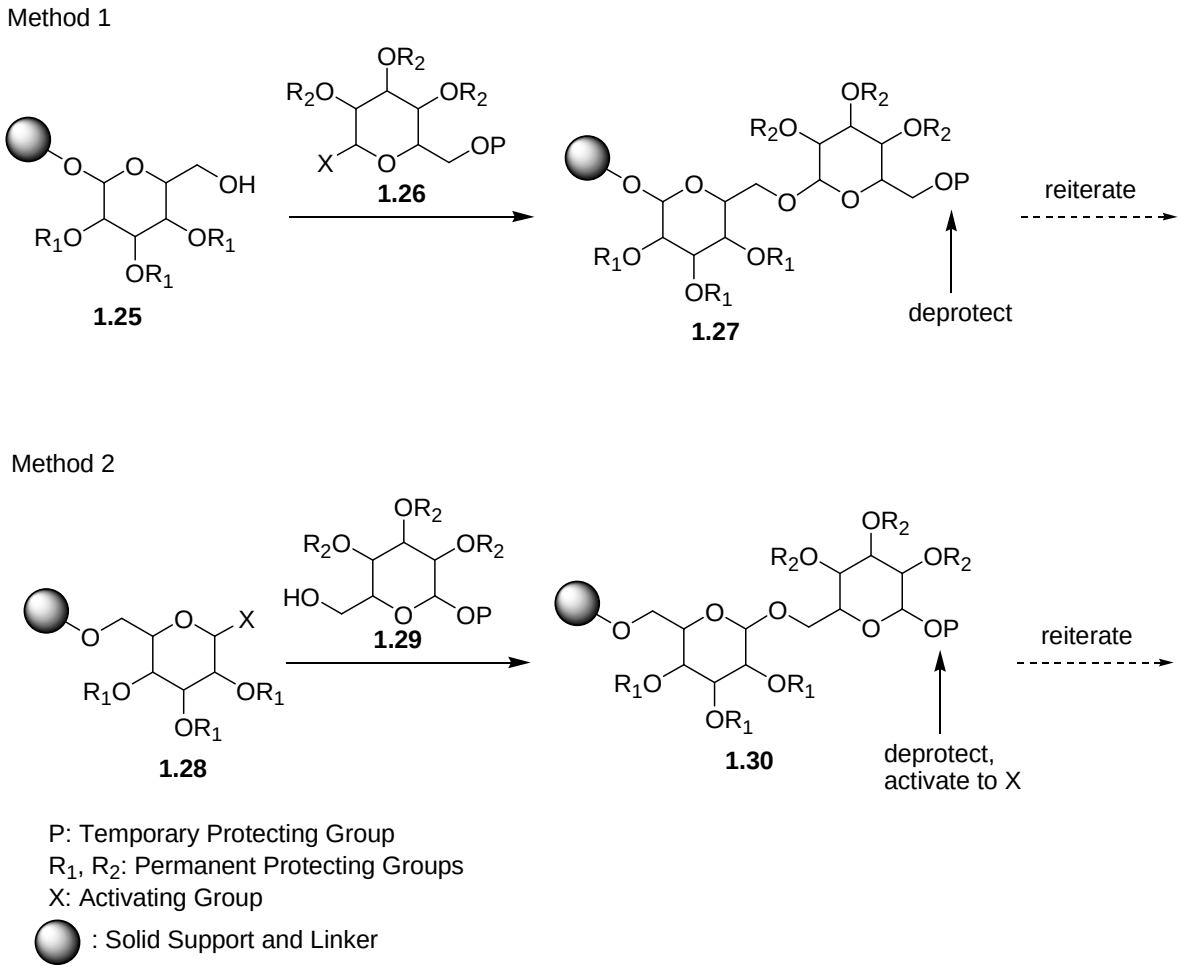
The pioneering work in solid-phase oligosaccharide synthesis provided the foundation for the rapid progress that several research groups have made in the area. These early approaches explored some of the important fundamental issues, including different synthetic strategies (donor- vs. acceptor-bound synthesis), various solid supports (soluble

and insoluble), and linker systems. Unfortunately, at that time this approach was not competitive with the more classical solution chemistry methods, due mainly to the lack of suitable glycosidation reactions (Fréchet 1980), that would meet the demands and conditions of solid-phase synthesis. The considerable loss of sugar donors during each coupling steps also compromised the advantages of solid-phase paradigm, since it was less effective and more expensive than synthesis in solution-phase. Major advances in solution-phase oligosaccharide synthesis with regard to donor reactivity, glycosylation selectivity, protecting group diversity, and analytical techniques were necessary before solid-phase oligosaccharide synthesis set the stage for the developments.

PEG and other soluble polymers have a clear advantage over resin-based solid supports in several respects (Gravert 1997). Firstly, most of the synthetic steps can be performed under homogeneous conditions, thereby avoiding the pseudo high dilution problem of solid-phase reactions. Secondly, the loading density of the monosaccharide primer, as well as the quality of the product, can easily be estimated after each step in a straightforward manner by means of conventional ^{1}H-NMR spectroscopy. PEG bound oligosaccharides can be easily recovered by precipitation from ethereal or alcoholic solvents. Synthesis on soluble polymers seems to have advantages associated with both solution-phase and solid-phase chemistry. This aspect was clearly demonstrated by Krepinsky and co-workers (Douglas 1995) and van Boom and co-workers (verduyn 1993) in their highly efficient synthesis of oligomannoside and the phytoalexine elicitor, respectively. Soluble support is likely to have less potential compared with solid support for utility in library synthesis using the split-mix method, a popular technique in combinatorial chemistry that is a powerful tool

for making libraries comprising large numbers of compounds, as well as in the future automation of oligosaccharide synthesis.

Two possible approaches immediately present themselves for the synthesis of oligosaccharides on solid support. These involve the decision as to the mode of attachment of the first carbohydrate to the matrix.



Scheme 1.7 Acceptor-bound and donor-bound glycosylation strategies for the synthesis of oligosaccharides.

In method 1, the first carbohydrate is anchored via its "reducing" end (Scheme 1.7). Here, the support-bound carbohydrate will function as acceptor in the coupling step to a solution-based donor. As the next cycle is contemplated, the temporary protecting group **P** must be removed in the now elongated, resin-bound carbohydrate construct.

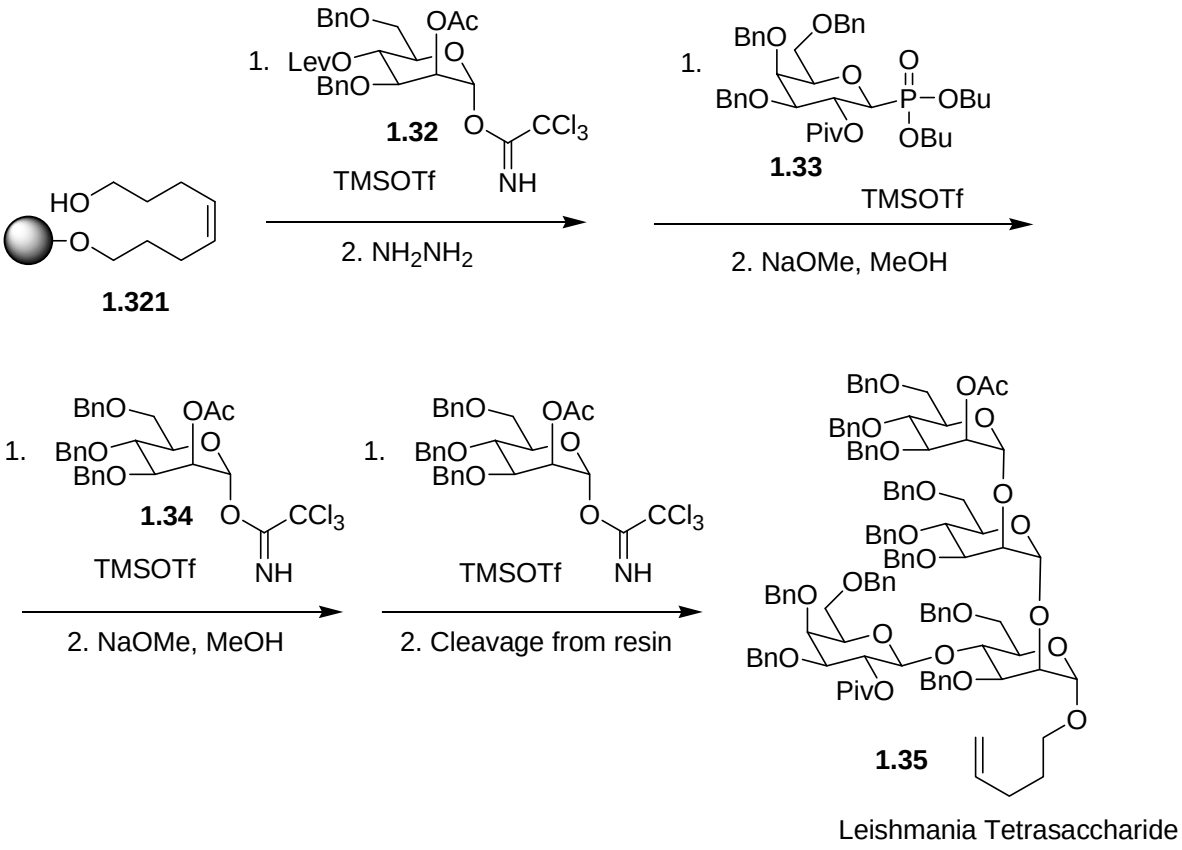
Connection of the glycosyl acceptor to the solid-support allows for the use of excess donor, a feature that can be used to drive reactions to completion. Nevertheless, this strategy requires a capping step to prevent the formation of deletion sequences, which would complicate final purification. Moreover, and perhaps most importantly for the broader context of glycoconjugate synthesis, the completed oligosaccharide construct would likely require retrieval from the solid matrix before conjugation to the peptide or lipid, unless this portion were already present as part of the linker to the solid support.

In method 2 (Scheme 1.7), the oligomer undergoing elongation is mounted to the solid support somewhere in the nonreducing region. In this case the reducing end (i.e., glycosyl donor portion) of the molecule is available for coupling to a solution-based acceptor. The main drawback of donor-bound strategy has to be considered. Most side reactions during glycosylations involve the glycosyl donor and thus result in termination of chain elongation. A reduction of the overall yield in the donor-bound strategy is the consequence. These inherent challenges not withstanding, an impressive array of complex oligosaccharide structures have been synthesized by Danishefsky and co-workers using the glycal assembly method under the donor-bound paradigm (Seeberger 1998).

Synthetic chemistry has finally advanced to the point that solid phase oligosaccharide synthesis is also feasible, and can now be automated. Seeberger and coworkers in 42%

yield in 9 h achieved automated synthesis of protected Leishmania antigen successfully (Scheme 1.8).

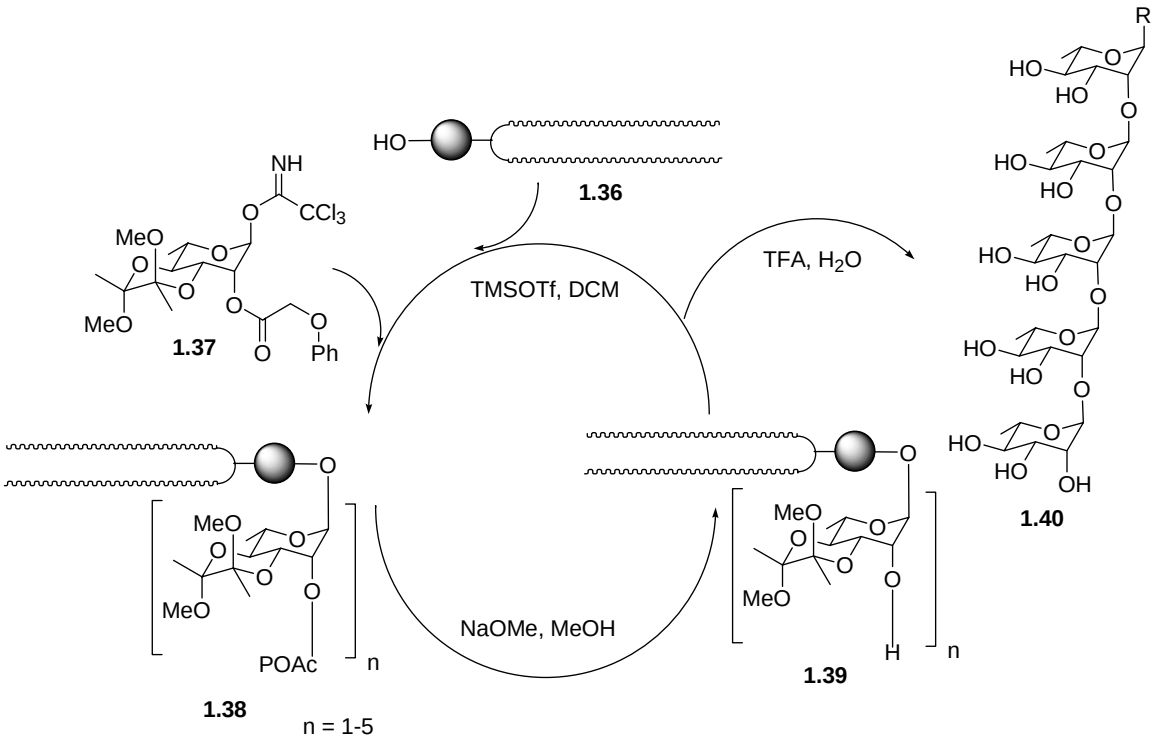
Automated solid phase synthesis is remarkably efficient and is often 10 times faster and higher yielding than corresponding solution phase syntheses. Automation makes synthetic methodology accessible to non-chemists. Even with automation, a bewildering number of specialized reactants are still required. This problem must be solved if automated synthesizers are to become commercially available.



Scheme 1.8 Automated synthesis of Leishmania tetrasaccharide.

Hydrophobically Assisted Switching Phase Synthesis

Hydrophobically assisted switching phase (HASP) synthesis is a concept that allows the choice between the advantages of solid-supported chemistry and those of solution-phase synthesis. In this method, sugar is attached to a sufficiently long hydrophobic anchor that can be adsorbed and desorbed quantitatively. The retention of single hydrocarbon chains (C_8-C_{20}) hydrocarbon chain depended strongly on the polarity and charge of the head group, rendering these molecules unsuitable as high-yielding and reversible tags. On the contrary, a sufficiently long, hydrophobic double chain anchor (C_{18}) was both adsorbed and desorbed quantitatively.



Scheme 1.9 Hydrophobically assisted switching phase synthesis of a library of rhamnolipids.

The doublechain HASP anchor 1.37 was glycosylated with rhamnosyl donor 1.38. RP-18 silica was added, the reaction concentrated and all unlabeled material was easily removed by washing with 80% MeOH/water. Deprotection of phenoxy acetate was performed while attached to the solid phase. MgSO4 was added to the solid support, and the clean products were released with DCM.

Fluorous Oligosaccharide Synthesis

Based on the difference in solubility of fluorous-tagged compounds from the non-fluorous compounds, a novel acyl fluorous protecting group (Bfp) was developed by Inazu and coworkers (Miura 2001). A natural synthetic oligosaccharide was synthesized using this method with minimal chromatography purification. After each step the reaction mixture was partitioned between the organic phase and the fluorous phase. Sugars containing fluorous protecting group were extracted in the fluorous phase and everything else in organic phase.

Chemoenzymatic Synthesis of Oligosaccharides

As an alternative to chemical synthesis, many biochemists and bioorganic chemists have explored the use of glycosidases and glycosyltransferases in the synthesis of oligosaccharides and glycoconjugates. The appeal of this approach is obvious: Nature has already figured out how to make all of the naturally occurring oligosaccharides, and if we could borrow from her toolbox we'd save a lot of time and effort. The use of glycosyltransferases and glycosidases has strengths and weaknesses that are in many ways

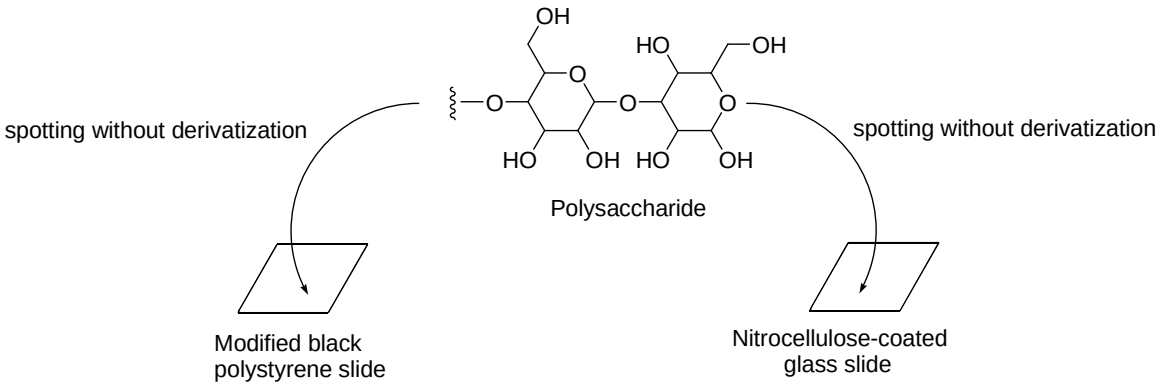
complementary to those of chemical synthesis. The main advantage of this method is that enzyme-catalyzed reactions are very stereo- and regio- selective and do not require protected donors for coupling reactions. The major drawback is that most requisite enzymes are not readily available, and those that are available are very expensive.

Carbohydrate Microarrays

While the importance of carbohydrates in biological systems is well documented, screening techniques to further elucidate their function are still needed. These arrays will facilitate screening of complex carbohydrate libraries to identify ligands and define specificity of carbohydrate-protein interactions. A better understanding of protein-carbohydrate interactions would greatly aid in the elucidation of intercellular signaling pathways, possibly leading to improved diagnostic and therapeutic tools.

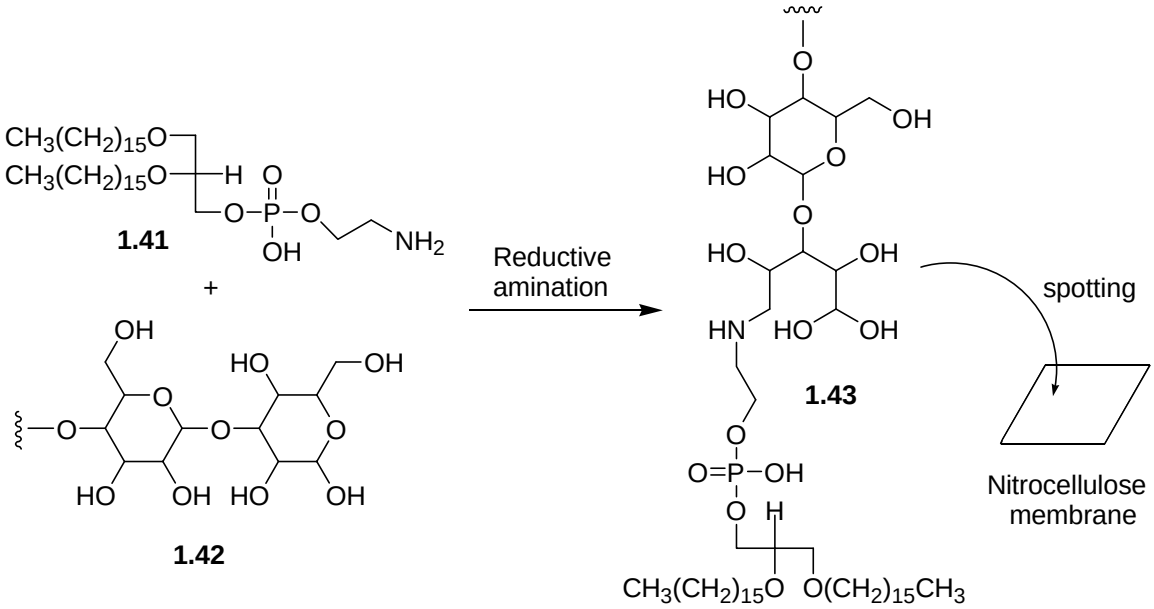
Wang and coworkers described microarrays of polysaccharides and glycoproteins on nitrocellulose-coated glass slides (Wang 2002). They used a high-precision robotic arrayer that was developed for cDNA and the spots were generated without derivatization (Scheme 1.10). These were air dried to allow adsorption (noncovalent immobilization) onto the hydrophobic surface. All were immobilized, although the efficiency of immobilization was dependent on molecular mass; the larger molecules were better retained than the smaller molecules. To overcome this limitation, Feizi and colleagues have established a microarray system in which the oligosaccharides are linked to lipid (Fukui 2002). The oligosaccharides, containing two to twenty monosaccharide residues, were linked by reductive amination to the amino phospholipid 1,2-dihexadecyl-sn-glycero-3-

phosphoethanolamine or its anthracene-containing fluorescent analogue (Scheme 1.11). These neoglycolipids were attached to the nitrocellulose membrane containing glass slide. It was shown that they could be robustly attached by noncovalent absorption.



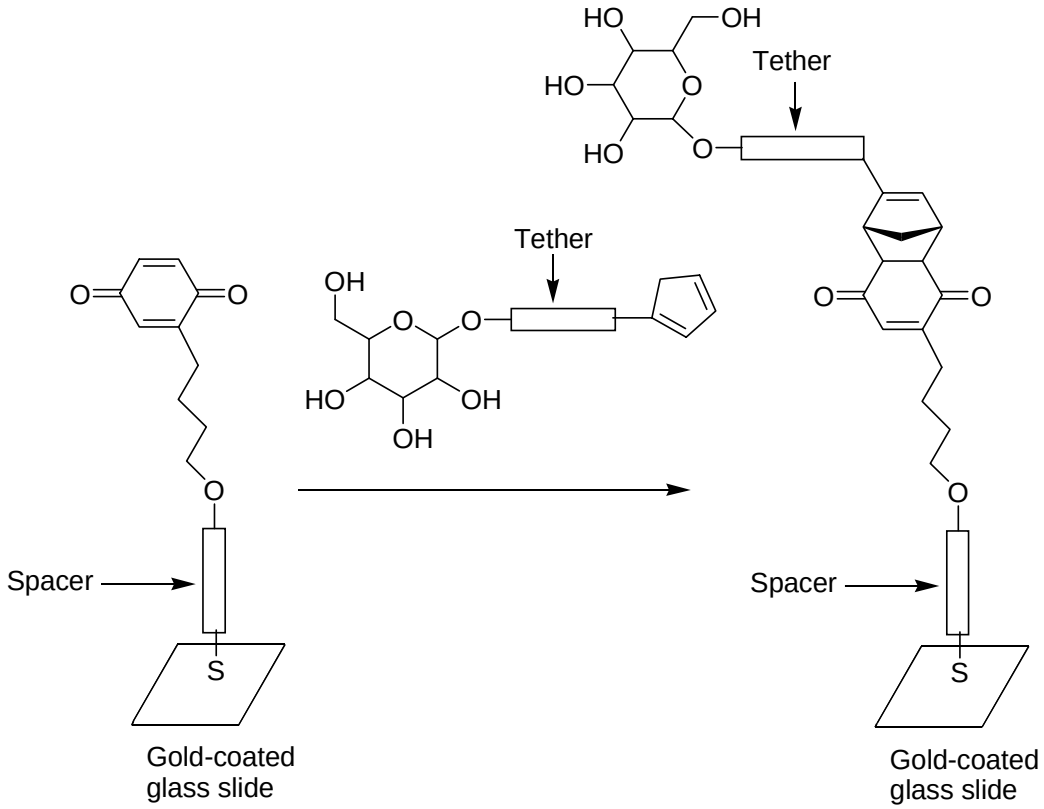
Scheme 1.10 Noncovalent immobilization of polysaccharides on slides for microarray formation.

Willats and coworkers used relatively hydrophobic black polystyrene produced with a physical surface modification that increased the surface area available for noncovalent adsorption (Scheme 1.10, Willats 2002).

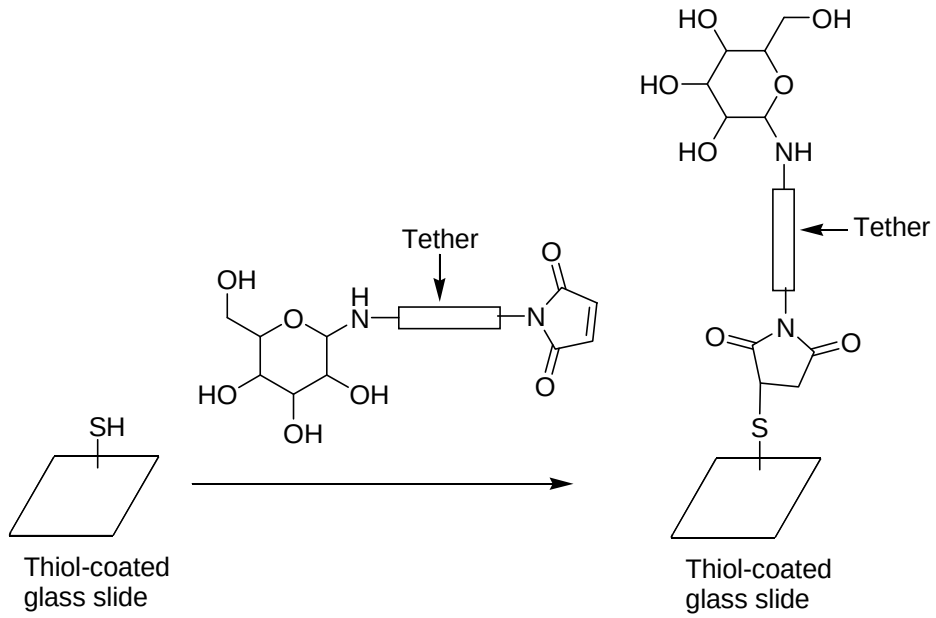


Scheme 1.11 Noncovalent immoblization of lipid modified oligosaccharides onto nitrocellulose membrane coated glass slide.

Several methods were used to covalently attach oligosaccharides onto the glass slide. Mrksich and colleagues using Diels-Alder reaction achieved covalent attachment of oligosaccharides onto the glass slide (Houseman 2002, Scheme 1.12). Shin's group has reported another approach another approach to carbohydrate microarray fabrication (Park 2002, 2004). They used hetero-Michael addition reaction to covalently attach maleimide-modified carbohydrates to thiol coated glass slide (Scheme 1.13).



Scheme 1.12 Covalent attachment on a gold surface by Diels-Alder reaction.



Scheme 1.13 Covalent Immobilization on a thiol-coated glass slide by hetero-Michael addition reaction.

The field of carbohydrate microarrays is in its infancy and further developments are expected, such as new fabrication strategies, new array surfaces, and printing methods. The results of the several 'proof of concept' experiments reported thus far have been very promising, and it is envisaged that microarrays of oligosaccharides and also glycoconjugates will revolutionize surveys of proteins for carbohydrate-binding activities. Various groups have used this technology to create microarrays of complex carbohydrates. Seeberger and coworkers used microarrays to study interactions of heparin-like glycosaminoglycans with fibroblast growth factors (FGF-1 and FGF-2), two important heparin-binding proteins (de Paz 2006). Recently Hseih-Wilson's group reported the first example of synthetic chondroitin sulfate (CS) microarrays and used these microarrays to identify a previously unknown interaction between chondroitin sulfate-E (CS-E) and tumor necrosis factor-α (TNF- α) (Tully 2006).

In summary complex oligosaccharides have been synthesized successfully using solid-phase synthesis and other methods. To study protein-carbohydrate interactions using microarray methods available, the oligosaccharides after cleavage from the solid support needs further modification. An easy approach would be to use a linker that would facilitate the synthesis of oligosaccharide and also could be used directly for microarrays without further modification.

CHAPTER 2: FLUOROUS-BASED CARBOHYDRATE MICROARRAYS

A paper published in the Journal of the American Chemical Society[1]

Kwang-Seuk Ko,[2,3] Firoz A. Jaipuri,[2] and Nicola L. Pohl[2,4]

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

Sreeman Mamidyala,[3] Kwang-Seuk Ko,[2] Firoz A. Jaipuri,[2] Gisun Park,[2] and Nicola L. Pohl[2,5]

Introduction

The success of microarrays, such as DNA chips, for biosample screening with minimal sample usage has led to a variety of technologies for assays on glass slides (Tomizaki 2005, Niemeyer 1999, Schena 1996). Unfortunately, for small molecules, such as sugars, these methods usually rely on covalent bond formation, which requires unique functional handles and multiple chemical steps (Shin 2005, Biskup 2005). Herein, we present a new simpler concept in microarray formation that is based on noncovalent fluorous-based

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[2] Graduate student, undergraduate student, and Assistant Professor, respectively, Department of Chemistry and the Plant Sciences Institute, Iowa State University

[3] Primary researcher and an author

[4] Author for correspondence

[5] Author for correspondence

interactions and demonstrate the strength of these interactions in the direct formation of carbohydrate microarrays for biological screening.

Unlike comparable lipid tails interacting with hydrophobic solid phases (Bauer 2005, Fang 2003, Fazio 2002), a single C_8F_{17} tail is sufficient to bind biomolecules, such as peptides, to fluorinated solid supports (Brittain 2005, Zhang 2004, Filippov 2002). The use of such fluorinated solid supports for affinity chromatography can substantially simplify the purification of synthetic carbohydrate intermediates in a process amenable to automation (Figure 2.1) (Manzoni 2004, Jing 2004, Curran 2001). An additional potential advantage of a fluorous-based approach is the direct formation of microarrays; the production of carbohydrate microarrays from compounds made on solid-phase still requires multiple solution-phase deprotection and derivatization steps (Adams 2004).

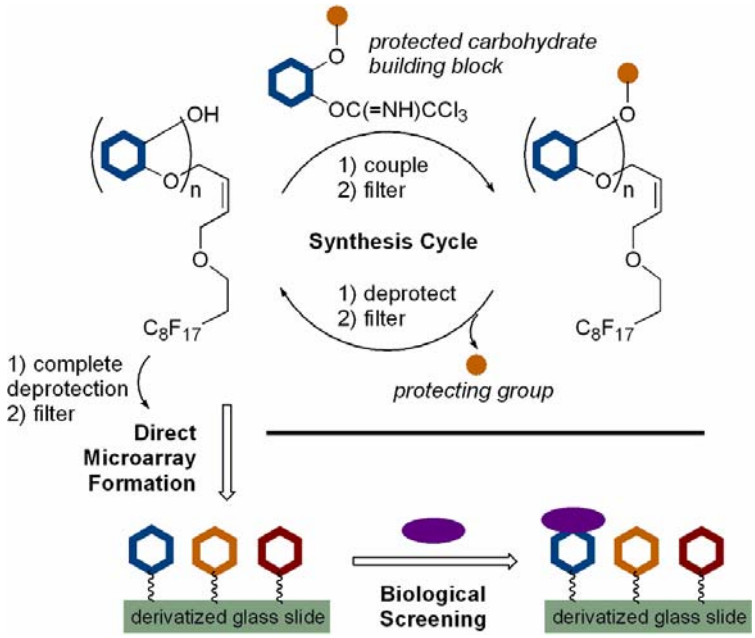
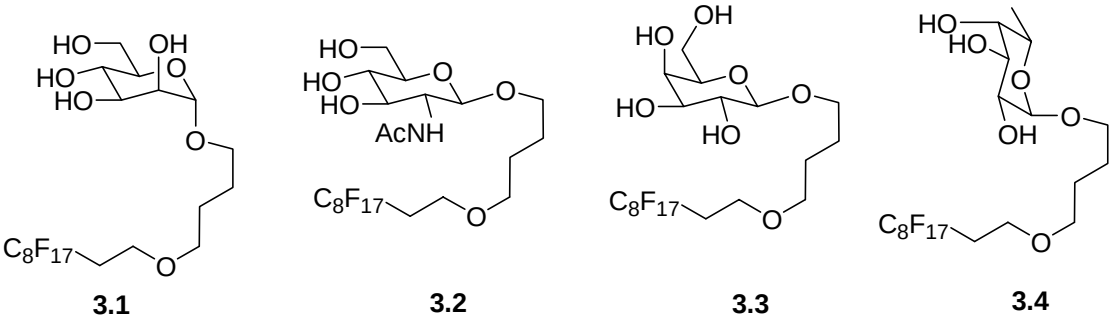


Figure 2.1 Strategy for the synthesis of carbohydrates and direct formation of fluorous-based carbohydrate microarrays (Taken with permission by The American Chemical Society from Ko 2005).

We began our studies with the design of a suitable fluorous tag for carbohydrate synthesis that could survive the necessary sequential reaction conditions and also be removed if desired. An allyl group would be orthogonal to the trichloroacetimidate coupling conditions and other deprotection conditions. Therefore, to create a fluorous allyl protecting group as well as potential anchor for microarray formation, a fluorous tail was synthesized in one step by reaction of *cis*-1,4-butenediol with substoichiometric amounts of 1*H*,1*H*,2*H*,2*H*-perfluorodecyl iodide to produce an alcohol for use in glycosylations (By Kwang).

Sugars commonly found in plants were chosen as initial targets to test the feasibility of a noncovalent fluorous-based array for protein-binding studies (Scheme 2.1). Genome sequencing projects have revealed that the number of genes related to carbohydrate metabolism is far greater in plants, such as *Arabidopsis thaliana*, than in animals or fungi (Henrissat 2001). In addition to providing an understanding of plant biology, the study of carbohydrate-protein interactions in plants could lead to the discovery of new sugar-binding lectins for use as glycobiology tools (Lis 1998). The known trichloroacetimidates of peracylated mannose (By Kwang) (Schmidt 1980), galactose (Schmidt 1991), and *N*-acetylglucosamine (By Kwang) (Schmidt 1994) were reacted with the fluorous-tagged allyl alcohol, and subsequent deacylation and hydrogenation of the double bond yielded the

initial array components 3.1-3.3.

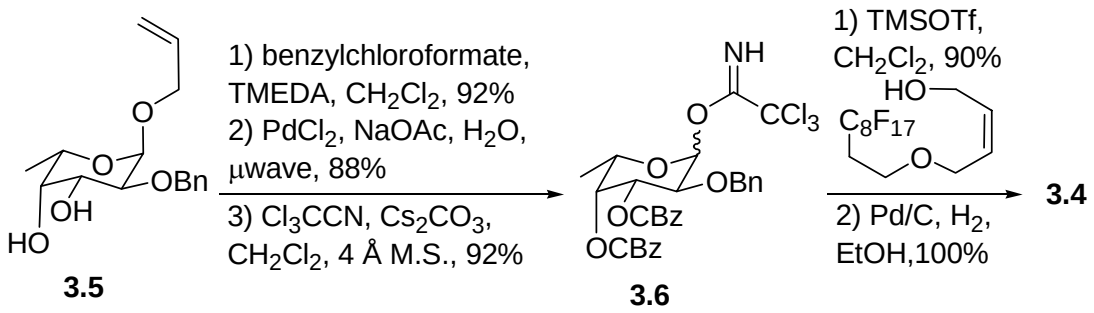


Scheme 2.1. Fluorous-tagged sugars (3.1-3.4) included in a microarray for screening with carbohydrate-binding proteins (Taken with permission by The American Chemical Society from Ko 2005).

Synthesis of Fucose Donor

Although neighboring-group participation could be used to control the stereochemistry of glycosylation for mannose, galactose, and *N*-acetylglucosamine, the synthesis of α-linked fucose required a different approach. Fucose glycosyl donors are usually built with nonparticipating benzyl protecting groups to provide predominantly the α-configured glycosylation products. Unfortunately, such electron-donating groups on a 6-deoxysugar also serve to make the resulting glycosidic linkage more acid sensitive. Several strategies alleviate this problem, for example, the use of more electron-withdrawing halobenzyl groups (Pohl 1997) or replacing the 3- and 4-OH protecting groups with an ester (Plante 2001, Amer 2003) that can potentially serve as a distant participating group. The latter approach is appealing, but requires an additional basic deprotection step. To avoid this extra step, we decided to test if a benzyl carbonate protecting group could serve the same purpose with the advantage of removal during the hydrogenation step. The requisite

glycosyl donor was synthesized from the known allylated compound (Khattuntseva 2000) 3.5 in three steps to provide the desired trichloroacetimidate 3.6 (Scheme 2.2). This donor was glycosylated with the fluorous-tagged alcohol using trimethylsilyl triflate to yield only the axially linked product in 90% yield. Indeed, the benzyl carbonate could serve to direct formation of the α-anomer and be removed by hydrogenation.



Scheme 2.2. Synthesis of Fucose Donor (Taken with permission by The American Chemical Society from Ko 2005).

Carbohydrate Microarrays

After synthesis of the requisite fluorous-tagged sugars, the next challenge was to find a suitable fluorinated surface. Solutions of each sugar were spotted onto a commercially available glass microscope slide coated with a Teflon/epoxy mixture employing a standard robot used for DNA arraying. The spots were dried, incubated with a solution of the fluorescein isothiocyanate-labeled jack bean lectin concanavalin A (FITC-ConA) for 20 min, rinsed repeatedly with assay buffer and distilled water, and then scanned with a standard fluorescent slide scanner. The scan clearly showed binding of FITC-ConA only to the mannose-containing spots (By Kwang). The anomeric position could be distinguished as the β-linked GlcNAc 3.2 was not recognized.

This lectin experiment demonstrated the ability of the C_8F_{17} tail to anchor the carbohydrates to the slide surface even after repeated washes. However, the slide was also intrinsically and unevenly fluorescent at 488 nM, a wavelength that is commonly used to detect labeled analytes. Clearly, a new approach was necessary to obtain an optically and fluorescently clear surface for the formation of compound microarrays. To this end, a glass microscope slide was reacted (Maoz 1984) with a fluoroalkylsilane to provide a clear coating on which water forms beads.

With the new microarray substrate in hand, we next probed the scope of a fluorous-based microarray approach for compound screening. Fluorous-tagged sugars were spotted on the coated slide using an arraying robot, and then the slides were incubated with FITC-labeled lectins (Figure 2.2). To test the reproducibility of the process, the same concentration of sugar was spotted repeatedly. In addition, several different concentrations of sugars were spotted. To test the ability of the array to withstand detergents often included in biological screens, the labeled plant lectin from the bush *Erythrina crystagalli* (FITC-ECA) was used to probe the microarrays with Tween-20. The array withstood the 20 min incubation time and repeated rinsing with this detergent-containing buffer.

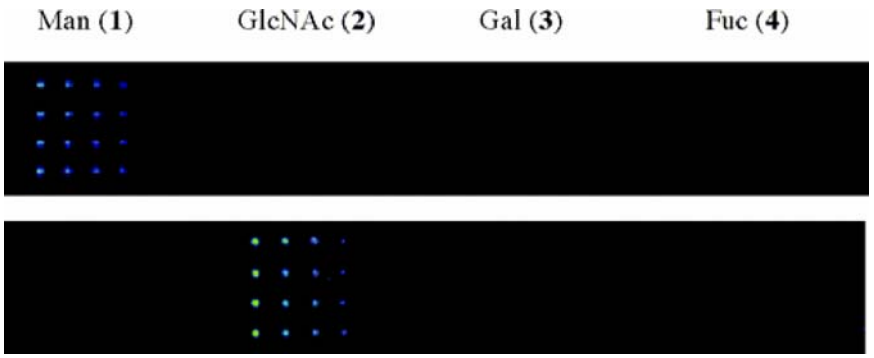


Figure 2.2. Fluorescence images of arrayed carbohydrates probed with FITC-labeled lectins. Columns of 4 spots each of 2, 1, 0.5, and 0.1 mM carbohydrates were incubated for

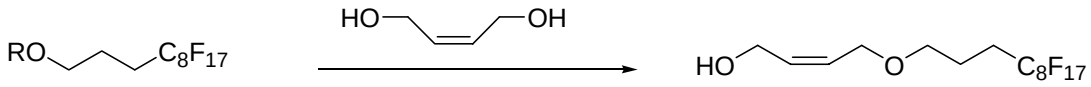
20 min with FITC-ConA (top) or FITC-ECA with 1%(Taken with permission by The American Chemical Society from Ko 2005).

A fluorous-based microarray method allows the facile formation of a range of carbohydrate chips for the plant and other sciences using synthetic sugars produced with the aid of fluorous-tagged synthesis. Efforts are underway to automate portions of the solution-phase fluorous-based synthetic process and to incorporate enzymatic steps to expand the scope of carbohydrates that can be easily arrayed for biological screening. Although not limited to carbohydrates, the approach should be especially valuable for the production of arrays containing compounds, such as glycosaminoglycan fragments, that contain nucleophiles that complicate current defined covalent attachment strategies.

These initial experiments, however, used only simple monosaccharides. Ideally, the method would be applicable to a range of synthetic carbohydrates. To expand the scope of these arrays, we report below the first syntheses of arabinose (synthesized by Gisun), rhamnose (synthesized by Gisun), lactose (synthesized by Kwang), maltose (synthesized by Kwang), and glucosamine tagged with a single C_8F_{17}-tail (synthesized by Sreeman); their incorporation into expanded carbohydrate microarrays; and screening of this microarray against two lectins to test the scope of a fluorous-based microarray method.

In order to anchor carbohydrates onto an array surface, a suitable fluorous tag is needed. This tag needs to survive the necessary sequential reaction conditions and also be removed if desired. Our previous work demonstrated that an allyl group works well with standard tricholoroacetimidate coupling conditions and other deprotection conditions. Reaction of *cis*-1,4-butenediol with 1*H*,1*H*,2*H*,2*H*-perfluorodecyl iodide produces an alcohol with the

requisite alkene spacer for use in glycosylations. The reliable synthesis of this tag on a larger scale proved difficult, however, as iodide elimination can compete with nucleophilic substitution. Therefore, a slightly modified tag was developed. An additional methylene spacer between the leaving group and the electron-withdrawing fluoroalkane was added to reduce the acidity of the hydrogen beta to the leaving group. To this end, commercially available 3-(perfluorooctyl)propanol 3.7 was mixed with methanesulfonyl chloride to provide mesylate derivative 3.8 quantitatively. This electrophile was then reacted with *cis*-1,4-butenediol to produce fluorous-tagged alcohol 3.9 in 70% yield for use in subsequent glycosylation reactions (Scheme 2.3).



3.7; R = H
3.8; R = Ms

3.9

Scheme 2.3. Synthesis of a new fluorous linker (Taken with permission by Journal of Fluorine Chemistry from Mamidyala 2006).

Sugars 3.10-3.17 were chosen as targets for glycosylation reactions with alcohol 3.9 (Figure 2.3). Screening of microarrays containing these sugars could lead to the discovery of new carbohydrate-binding specificity profiles by lectins for use as glycobiology tools (Lis 1998). Of particular interest are the two disaccharides (3.16, 3.17) and the charged amino-sugar (3.10) to test if such compounds are viable for inclusion in microarrays based on noncovalent fluorous–fluorous interactions.

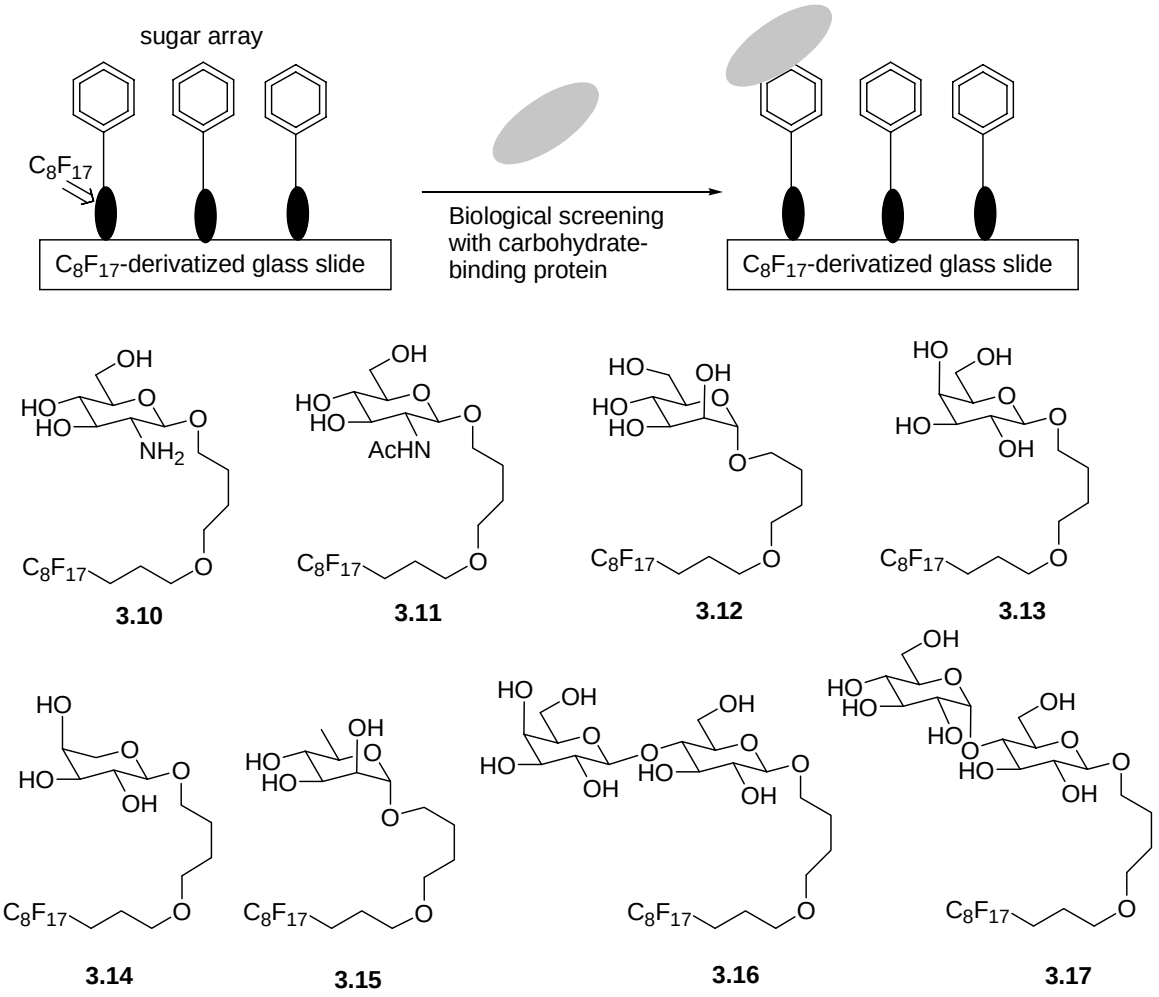


Figure 2.3. Fluorous-tagged sugars (3.10-3.17) included in a microarray for screening with carbohydrate-binding proteins (Taken with permission by Journal of Fluorine Chemistry from Mamidyala 2006).

With the fluorous-tagged carbohydrates in hand, we next probed the ability of the single C_8F_{17}-tail to anchor these mono- and disaccharides to a glass slide surface even after repeated washes. The fluorous-tagged carbohydrates 3.10-3.17 were dissolved in 80% methanol/water and were spotted onto fluoroalkylsilane-derivatized glass slides employing

a standard robot used for DNA arraying. The slides then were incubated for 30 min with the commercially available FITC (fluorescein isothiocyanate)-labeled lectins from *Triticum vulgaris* (wheat germ, FITC-WGA) (Szentkuti 1998) in phosphate buffered saline (PBS buffer) (0.25 M) and *Arachis hypogaea* (peanut, FITC-PNA) in PBS buffer (0.5 M) and rinsed repeatedly with the assay buffer followed by distilled water. After drying, the slide was scanned with a standard fluorescent slide scanner at 488 nm to reveal the carbohydrate-binding specificities of these two lectins. As expected (Szentkuti 1998) the wheat germ lectin WGA bound only to the fluorous-tagged *N*-acetylglucosamine (3.11) (Figure 2.4).

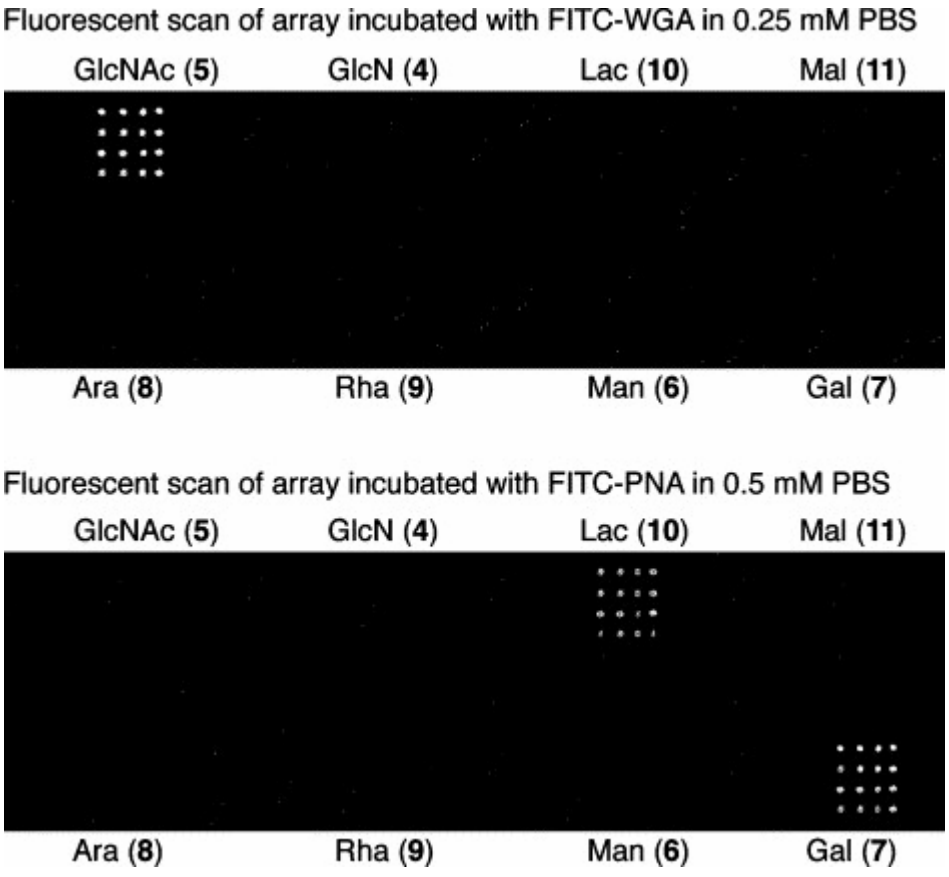


Figure 2.4. A scan at 488 nm of glass slides arrayed with fluorous-tagged carbohydrates after incubation with fluorescently-labeled lectins (Taken with permission by Journal of Fluorine Chemistry from Mamidyala 2006).

In contrast, the peanut lectin PNA bound to both galactose (3.13) and lactose (3.16) in the array experiment. This lectin is known to bind to galactose both alone and in the context of the lactose disaccharide (Szentkuti 1998) and (Caffarena 2002). This experiment vividly demonstrates that the disaccharide is retained on the fluorous-derivatized glass slides under the conditions required for screening of proteins for their carbohydrate-binding specificities.

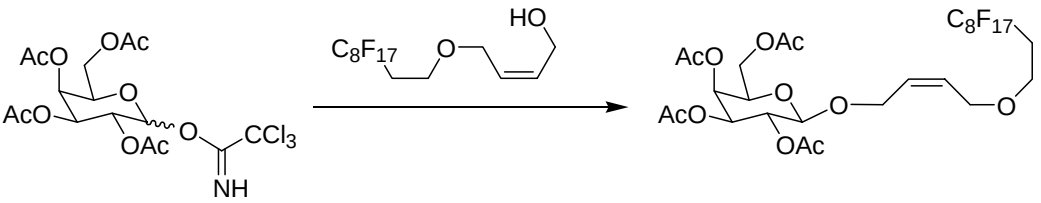
Conclusions

In conclusion, our new method for the facile fabrication of carbohydrate microarrays by using the noncovalent immobilization of carbohydrates on fluorous derivatized glass slides clearly can be extended beyond monosaccharides. Screening of these carbohydrate microarrays against two lectins demonstrates that the noncovalent fluorous–fluorous interaction is sufficient to retain not only mono- but also disaccharides under biological assay conditions. As the fluorous tag also facilitates purification during synthesis, this microarray approach should be particularly valuable for screening of synthetic carbohydrate libraries. Future work will test the scope of using noncovalent fluorous interactions for the production of microarrays that contain additional charged compounds such as glycosaminoglycan fragments (Kovensky 1999) and (Rele 2004) and larger saccharides as well as other classes of biomolecules such as peptides and nucleic acids.

Experimental

All reagents were purchased from Aldrich. Dichloromethane was distilled over calcium hydride THF and toluene was used from solvent purification towers. Amberlyst 15 ion-exchange resin was washed repeatedly with methanol before use. All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the R_f values determined using analytical thin layer chromatography (tlc) with 0.25 mm EM Science silica gel plates (60F-254). The developed TLC plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Flash chromatography was performed with Selecto Scientific silica gel, 32-63 μm particle sizes. Fluorous phase chromatography was performed using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies Inc.; Pittsburgh, PA). All other Fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated.

^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker DRX400 and Varian VXR300. ^{1}H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (7.27 ppm) or CD_3OD (4.80) as an internal reference. ^{13}C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (77.23 ppm) or CD_3OD (49.15 ppm). A Shimadzu LCMS 2010 quadrupole mass spectrometer (Shimadzu Scientific Instruments, Columbia, MD) equipped with and electronspray ionization (ESI) source was used in positive ion mode.



Synthesis of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside.

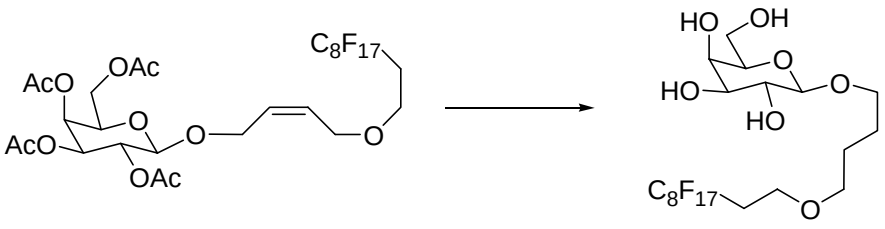
2,3,4,6-tetra-*O*-acetyl-α/β-D-galactopyranoside trichloroacetimidate[8] (77.0 mg, 0.16 mmol) and 1H,1H,2H,2H-perfluorodecyloxybutenyl alcohol (69.0 mg, 0.13 mmol) were dissolved in dichloromethane (2 mL) and the mixture was cooled down to –15 °C. TMSOTf (12 μL, 0.065 mmol) was added and the reaction mixture was stirred at –15 °C for 30 min. The reaction was quenched with triethylamine (0.1 mL) and concentrated. The crude product was purified by solid-phase extraction using fluoro flash column. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. (1H,1H,2H,2H-perfluorodecyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside as a colorless oil, 98.8 mg, 0.11 mmol, 90%).

R_f: 0.52 (1:1 EtOAc/hexane)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.69 – 5.74 (m, 2H), 5.38 (d, 1H, *J* = 3.3 Hz, H-1), 5.21 (dd, 1H, *J* = 10.5, 2.7 Hz), 5.01 (dd, 1H, *J* = 10.5, 3.3 Hz), 4.48 (d, 1H, J = 7.8 Hz), 4.36 – 4.41 (m, 1H), 4.04 – 4.26 (m, 6H), 3.86 – 3.91 (m, 1H), 2.14 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.5, 170.4, 170.3, 169.6, 129.4, 128.9, 100.3, 71.1, 70.9, 68.9, 67.2, 66.5, 64.9, 62.1, 62.1, 61.4, 20.9, 20.8(2), 20.7.

MS (ESI) m/z = 887 $[M + Na]^+$



Synthesis of 1H,1H,2H,2H-perfluorodecyloxybutanyl-β-D-galactopyranoside.

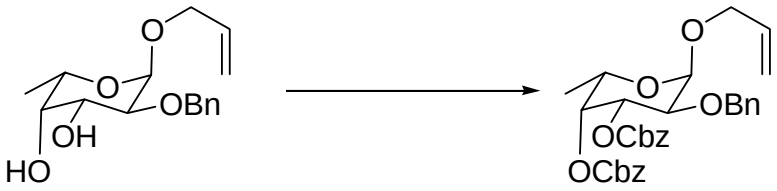
1H,1H,2H,2H-perfluorodecyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (50 mg, 0.058 mmol) was dissolved in ethanol (3 mL) and 5% Pd/C (10 mg) was added. The reaction mixture was put under hydrogen atmosphere and stirred for 1 h. It was then filtered over Celite and ethanol was removed under reduced pressure to give the hydrogenated product in quantitative yield. To a solution of the resulting compound (45 mg, 0.052 mmol) in methanol (3 mL) was added K_2CO_3 (4 mg, 0.026 mmol) and the reaction mixture was stirred for 2 h. The mixture was then neutralized using Amberlyst-15 ion-exchange resin and filtered. The solvent was removed under reduced pressure and 1H,1H,2H,2H-perfluorodecyloxybutanyl-β-D-galactopyranoside was obtained as white solid (0.058 mol, 40 mg, 100%).

R_f: 0.23 (methanol/ethyl acetate/hexane/2/2/3).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.17 (d, 1H, *J* = 5.4 Hz), 3.44 – 3.91 (m, 12H), 2.01 (m, 2H), 1.60 – 1.70 (m, 4H).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 103.6, 75.2, 73.7, 71.2, 69.2, 69.1, 68.9, 61.4, 61.3, 28.8 (2), 25.9(2).

MS (ESI) m/z = 721 $[M + H]^+$



Synthesis of Allyl-2-O-benzyl-3,4-di-O-carboxybenzyl-α-D-fucopyranoside.

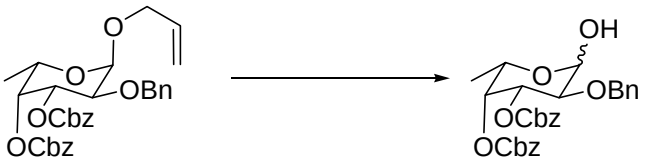
Allyl-2-O-benzyl-α-D-fucopyranoside (917 mg, 3.12 mmol) was dissolved in methylene chloride (15 mL) and cooled to 0 °C. TMEDA (434 mg, 3.74 mmol) and benzylchloroformate (1.20 g, 6.86 mmol) were added. The reaction mixture was stirred at 0 °C for 45 min and quenched with water (10 mL). The organic layer separated and the aqueous layer was extracted with methylene chloride (2 x 40 mL), washed with HCl (2N, 20 mL), brine (20 mL), and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel using 25% EtOAc/hexane as eluent. The product was obtained as clear gel (1.7 g, 3.02 mmol, 92%).

R_f: 0.48 (1:1 EtOAc/hexane)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.25 – 7.40 (m, 15 H), 5.88 – 5.94 (m, 1 H), 5.15 – 5.36 (m, 8H), 4.82 (d, 1H, *J* = 4 Hz, H-1), 4.70 (d, 1H, J = 12 Hz), 4.53 (d, 1 H, *J* = 12 Hz), 4.13 – 4.17 (m, 2H), 3.98 (dd, 1H, *J* = 12.8, 6.4 Hz), 3.90 (dd, 1H, *J* = 9.6, 3.6 Hz), 1.17 (d, 3H, *J* = 6.8 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.3, 154.4, 138.2, 135.3, 135.1, 133.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.1, 127.9, 127.8, 117.9, 96.3, 75.6, 74.3, 73.7, 73.4, 69.9, 69.9, 68.6, 64.3, 15.8.

MS (ESI) m/z = 585$[M + Na]^+$



Synthesis of 2-*O*-benzyl-3,4-di-*O*-carboxybenzyl-α-D-fucopyranose.

Palladium chloride (685 mg, 3.87 mmol) and sodium acetate (635 mg, 7.74 mmol) were dissolved in AcOH/water mixture (5:1, 36 mL). The mixture was heated in commercial microwave oven at 80 °C for 5 min. The reaction was then put on an 80 °C oil bath and compound 2 (1.45 g, 2.58 mmol) in AcOH was added dropwise. The reaction mixture was heated for 5 min and allowed to cool down to rt. It was poured into water and extracted with ether (2 x 60 mL). The combined organic layer was washed with $NaHCO_3$ (2 x 40 mL), water (40 mL), and brine (20 mL) and dried over $MgSO_4$.The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 32% EtOAc/hexane as eluent. The product of 2-O-benzyl-3,4-di-O-carbobenzyl-α/β-D-fucopyranose was obtained as mixture of anomers.

Yield = 88%

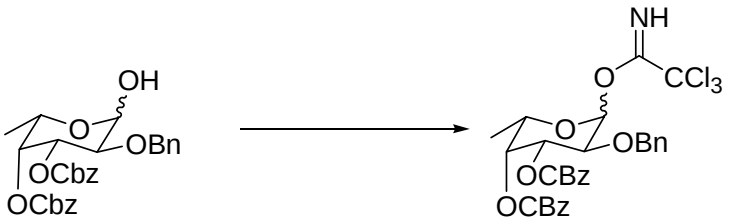
R_f: 0.44 (1:1 EtOAc/hexane)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.15 – 7.36 (m, 19H), 5.12 – 5.22 (m, 8H), 4.81 – 4.90 (m, 1H), 4.50 – 4.74 (m, 3H), 4.31 – 4.49 (m, 1H), 3.82 – 4.24 (m, 3H), 1.21 (d, 1H, *J* = 6.4 Hz), 1.15 (t, 3H, *J* = 6.4 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 137.7, 135.2, 129.2, 129.2, 128.7, 128.7, 128.7, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 97.8, 91.8, 75.6, 75.5, 75.5, 75.2, 74.7,

74.5, 74.3, 74.2, 74.1, 73.9, 73.6, 73.6, 72.7, 70.2, 70.2, 70.2, 70.1, 69.1, 65.0, 64.7, 64.6, 62.6, 43.4, 26.8, 21.9, 16.1, 16.0.

MS (ESI) m/z = 523 $[M + H]^+$



Synthesis of 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α/β-D-fucopyranoside trichloroacetimidate.

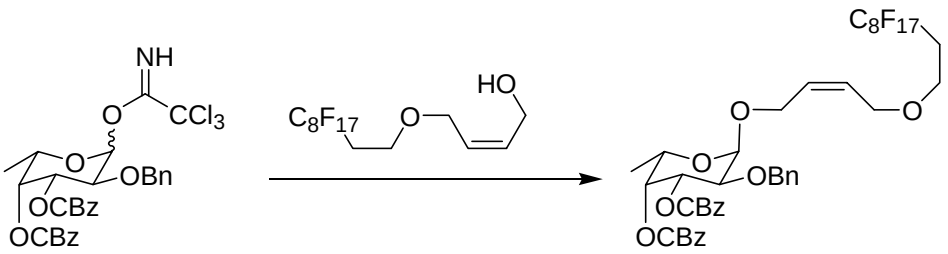
To a solution of 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranose (1.17 g, 2.24 mmol) in dichloromethane (10 mL) were added powdered 4Å molecular sieves (100 mg) and trichloroacetonitrile (2.2 mL, 22.4 mmol). The reaction was stirred for 30 min and Cs_2CO_3 (802 mg, 2.46 mmol) was added. The reaction mixture was further stirred for 45 min and filtered over Celite. The eluent was concentrated and the crude product was purified by flash column chromatography on silica gel using 28% EtOAc/hexane as eluent to provide 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α/β-D-fucopyranoside trichloroacetimidate as a white solid (1.43 g, 2.1 mmol, 92%).

R_f: 0.46 (1:1 EtOAc/hexane)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.68 (s, 1H), 7.19 – 7.39 (m, 15H), 5.78 (d, 1H, *J* = 8.1 Hz), 5.15 – 5.29 (m, 6H), 4.96 (dd, 1H, *J* = 9.9, 3.3 Hz), 4.83 (d, 1H, *J* = 11.0 Hz), 4.67 (d, 1H, *J* = 11.0 Hz), 3.94 – 4.00 (m, 1H), 1.27 (d, 3H, J = 6.3 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 161.3, 155.4, 154.5, 137.9, 135.2, 135.1, 128.8, 128.8, 128.8, 128.81, 128.8, 128.5, 128.5, 128.4, 127.9, 127.9, 98.1, 77.1, 75.6, 75.4, 74.5, 70.4, 70.3. 69.9,16.2.

MS (ESI) m/z = 667 $[M + H]^+$



Synthesis of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranoside.

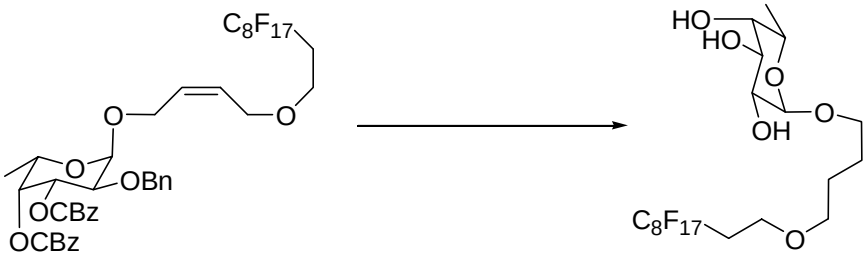
To a solution of 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α/β-D-fucopyranoside trichloroacetimidate (185.0 mg, 0.28 mmol) and 1H,1H,2H,2H-perfluorodecyloxybutenyl alcohol (123.0 mg, 0.23 mmol) in dichloromethane (5 mL) were added powdered 4Å molecular sieves (10 mg) and the mixture was cooled down to -15 ^{o}C. TMSOTf (42 µL, 0.23 mmol) was added and the reaction mixture was stirred at –15 °C for 30 min. The reaction was quenched with triethylamine (0.1 mL) and concentrated. The crude product was purified by solid-phase extraction using fluoro flash column. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH (1H,1H,2H,2H-perfluorodecyloxybutenyl-2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranoside as a colorless oil, 215.3 mg, 20.7 mmol, 90%).

R_f: 0.26 (ethyl acetate/hexane/2/3).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26 – 7.36 (m, 15H), 5.72 – 5.84 (m, 2H), 5.15 – 5.23 (m, 6H), 4.44 – 4.81 (m, 4H), 4.05 – 4.23 (m, 6H), 3.66 – 3.78 (m, 1H), 1.15 – 1.27 (dd, 3H, J = 6.4 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.3, 154.4, 138.1, 135.2, 135.1, 128.9, 128.7, 128.6, 128.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.7 (2), 96.5, 73.3, 75.5, 74.2, 73.6, 73.5, 70.0, 69.9, 66.4, 64.4, 63.2, 61.9 (2), 26.12), 15.7.

MS (ESI) m/z = 1061 $[M + Na]^+$



Synthesis of 1H,1H,2H,2H-perfluorodecyloxybutenyl-α-D-fucopyranoside.

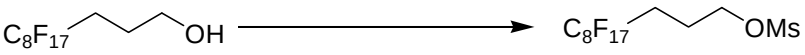
1H,1H,2H,2H-perfluorodecyloxybutenyl-2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranoside (81.0 mg, 0.078 mmol) was dissolved in ethanol (3 mL) and 5% Pd/C (20 mg) was added. The reaction mixture was put under hydrogen atmosphere and stirred overnight. It was then filtered over celite and ethanol was removed under reduced pressure to give the hydrogenated product in quantitative yield.

Yield = 100%

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.15 – 4.31 (m, 1H), 3.45 – 3.98 (m, 11H), 1.62 – 1.68 (m, 4H), 1.23 (dd, 3H, J = 16.8, 6.4 Hz).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 99.1, 72.3, 70.6, 70.3, 68.6, 67.4, 66.1, 64.3 (2), 26.1(2), 15.2.

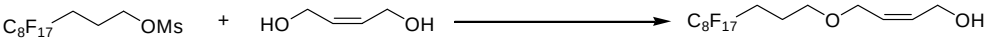
MS (ESI) m/z = 705 $[M+ Na]^+$



Synthesis of 3-(perfluorooctyl)propanyl methyl sulfonate

To a solution of 3-(perfluorooctyl)propanol (2.0 g, 4.2 mmol) in dichloromethane (20 mL) was added triethylamine (1.20 mL, 8.36 mmol) and the mixture was cooled to 0 °C. Mesyl chloride (0.64 mL, 8.4 mmol) was added drop wise over 5 min and the reaction mixture was allowed to warm to ambient temperature over 2 h. The reaction mixture was diluted with dichloromethane (100 mL) and the organic layer was washed with water (40 mL), HCl (2N, 40 mL), and brine (30 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to provide mesylated product (2.32 g, 100%) as a solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05–2.13 (m, 2H), 2.20–2.36 (m, 2H), 3.04 (s, 3H), 4.31 (t, J = 6 Hz, 2H).



Synthesis of 4-[3-(perfluorooctyl)propyloxy]-*cis*-2-butenyl alcohol

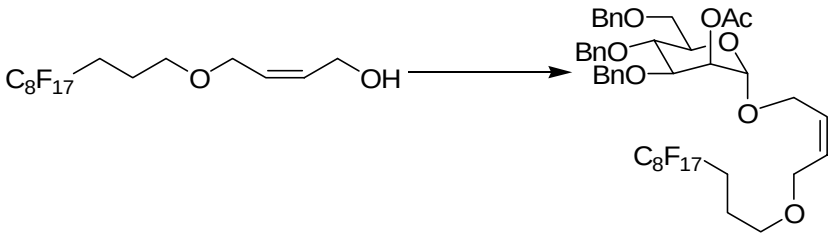
To a solution of *cis*-1,4-butenediol (0.48 g, 5.4 mmol), 3-(perfluorooctyl)propanyl methyl sulfonate (2.3 g, 4.16 mmol), and tetrabutylammonium bromide (0.27 g, 0.83 mmol) in DMF (20 mL) was added powdered KOH (0.47 g, 8.32 mmol). The reaction mixture was heated at 70 °C for 1 h and then poured into water (20 mL). The aqueous layer was

extracted with ethyl acetate (2 mL × 60 mL) and the organic layer was washed with water (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 27% EtOAc/hexane as eluent to provide fluorous alcohol (1.57 g, 70%) as a yellow oil.

R_f: 0.64 (ethyl acetate/hexane, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.78–1.88 (m, 2H), 2.04–2.19 (m, 2H), 2.99 (s, 1H), 3.45 (t, J = 6.0 Hz, 2H), 4.0 (d, J = 6.3 Hz, 2H), 4.13 (d, J = 6.3 Hz, 2H), 5.57–5.65 (m, 1H), 5.70–5.78 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.8, 28.0, 58.4, 66.4, 68.9, 127.9, 132.3.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside

To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetimidate (370 mg, 0.58 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (210 mg, 0.38 mmol) in dichloromethane (4 mL) was added TMSOTf (14 μL, 0.077 mmol) at 5 ºC. The reaction mixture was stirred at 5 ºC for 30 min. The reaction mixture was quenched with triethylamine (0.05 mL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction using a fluorous solid-phase extraction cartridge.

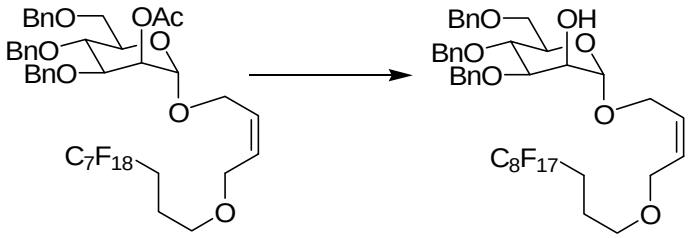
Nonfluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide fluorous-tagged mannose (360 mg, 0.352 mmol, 92%) as viscous yellow oil.

R_f: 0.74 (ethyl acetate/DCM, 1:9).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.80–1.88 (m, 2H), 2.11–2.27 (m, 2H) (s, 3H), 3.43 (t, *J* = 6.0 Hz, 2H), 3.75–3.92 (m, 4H), 4.01–4.04 (m, 3H), 4.07–4.15 (m, 1H), 4.19–4.26 (m, 1H), 4.45–4.58 (m, 3H), 4.70 (dd, *J* = 12.0 Hz, 2H), 4.88 (dd, *J* = 6.6 Hz, 8.4 Hz, 2H), 5.35–5.41 (m, 1H), 5.64–5.77 (m, 2H), 7.15–7.36 (m, 15H).

^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.1, 61.7, 65.4, 67.6, 67.7, 67.9, 70.5, 70.7, 72.4, 73.3, 74.2, 77.2, 95.8, 126.6, 126.7, 126.8, 126.8, 126.9, 126.9, 127.0, 127.3, 127.4, 129.3, 136.9, 137.1, 137.2, 169.4.

MS (ESI) = 1023 $[M+ H]^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (360 mg, 0.352 mmol) in methanol (4 mL) was added 0.5 M NaOMe (1.4 mL, 0.704 mmol) and the reaction mixture was stirred at ambient temperature for 30

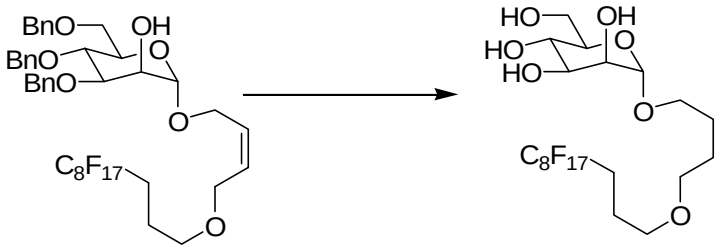
min. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide deacetylated mannose (350 mg, 100%) as yellow oil.

R_f: 0.42 (ethyl acetate/DCM, 1:9).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.78–1.87 (m, 2H), 2.08–2.23 (m, 2H), 2.53 (s, 1H), 3.40 (t, *J* = 6.0 Hz, 2H), 3.68–3.92 (m, 5H), 4.0–4.12 (m, 4H), 4.18–4.24 (m, 1H), 4.50 (d, *J* = 10.8 Hz, 1H), 4.53–4.65 (m, 2H), 4.68 (d, *J* = 2.4 Hz, 2 H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.92 (d, *J* = 1.6 Hz, 1H), 5.60–5.75 (m, 2H), 7.15–7.34 (m, 15H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.8, 28.0, 62.5, 66.4, 68.3, 68.7, 69.0, 71.2, 72.0, 73.5, 74.3, 75.2, 80.2, 98.3, 127.6, 127.7, 127.8, 128.0, 128.1, 128.3, 128.4, 128.6, 130.2, 137.9, 138.2.

MS (ESI) m/z = 981 $[M+ H]^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (60 mg, 0.061 mmol) in methanol (3 mL) was added 10% Pd/C (20 mg).

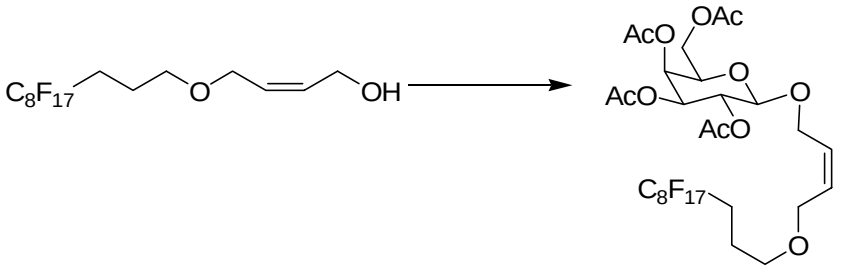
The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 12 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide fully deprotected mannose (44 mg, 100%) as a solid.

R_f: 0.75 (methanol/DCM, 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.60–1.69 (m, 4H), 1.80–1.89 (m, 2H), 2.18–2.33 (m, 2H), 3.40–3.88 (m, 12H), 4.74 (m, 1H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.8, 26.2, 26.4, 27.6, 61.6, 67.1, 67.4, 68.8, 70.5, 71.1, 71.4, 73.5, 101.2.

MS (ESI) m/z = 713 $[M+ H]^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside

To a solution of 2,3,4,6-tetra-*O*-acetyl-α/β-d-galactopyranosyl trichloroacetimidate (100 mg, 0.20 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (**3**) (75 mg, 0.14 mmol) in dichloromethane (3 mL) was added TMSOTf (5 µL, 0.027 mmol) at −15 °C. The reaction mixture was stirred at −15 °C for 30 min. The reaction mixture was quenched with triethylamine (0.5 mL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction using a fluorous solid-phase

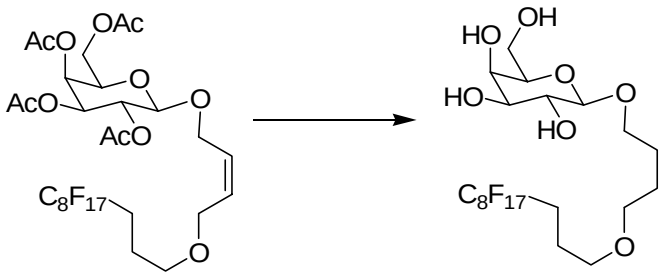
extraction cartridge. Nonfluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide fluorous-tagged galactose (97 mg, 80%) as a syrup.

R_f: 0.67 (ethyl acetate/hexane, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$,): δ (ppm) 1.82–1.89 (m, 2H), 1.95, 2.02, 2.03 (3s, 9H), 2.08–2.24 (m, 2H) (s, 3H), 3.46 (t, *J* = 6.0 Hz, 2H), 3.86 (t, *J* = 5.2 Hz, 1H), 4.01 (t, *J* = 5.2 Hz, 1H), 4.10–4.23 (m, 4H), 4.34 (dd, *J* = 5.6, 10.0 Hz, 1H), 4.75 (d, *J* = 8 Hz, 1H), 4.99 (dd, *J* = 3.6, 10.4 Hz, 1H), 5.19 (dd, *J* = 8.0, 10.4 Hz, 1H), 5.36 (d, *J* = 3.6 Hz, 1H), 5.60–5.76 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 20.7, 20.8, 20.9, 61.2, 64.7, 66.5, 67.0, 68.7, 68.8, 70.7, 70.9, 76.7, 100.1, 127.8, 130.2, 169.4, 170.1, 170.2, 170.3.

MS (ESI) m/z = 879 $[M+ H]^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-β-D-galactopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-d-galactopyranoside (97 mg, 0.110 mmol) in methanol (3 mL) was added 5% Pd/C (25 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was then filtered over Celite and the solvent was removed under

reduced pressure. The product was used directly in the next step. To a solution of 3-(perfluorooctyl)propanyloxybutanyl-2,3,4,6-tetra-*O*-acetyl-β-d-galactopyranoside (97 mg, 0.110 mmol) in methanol (3 mL) was added NaOMe (15 mg) and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was neutralized with Amberlyst-15 ion-exchange resin and filtered. The solvent was removed under reduced pressure to provide fully deprotected galactose (75 mg, 98%) as a solid.

R_f: 0.70 (methanol/DCM, 1:9).

^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.62–1.71 (m, 4H), 1.79–1.87 (m, 2H), 2.11–2.36 (m, 2H), 3.22–3.34 (m, 1H), 3.41–3.63 (m, 6H), 3.70–3.74 (m, 3H), 3.81–3.99 (m, 2H), 4.20 (d, J = 6.9 Hz, 1H).

^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 26.1, 26.2, 26.3, 53.3, 61.9, 69.2, 69.3, 69.4, 71.1, 72.6, 74.1, 76.2, 114.4.

MS (ESI) m/z = 713 $[M+ H]^+$

Appendix: NMR Spectra

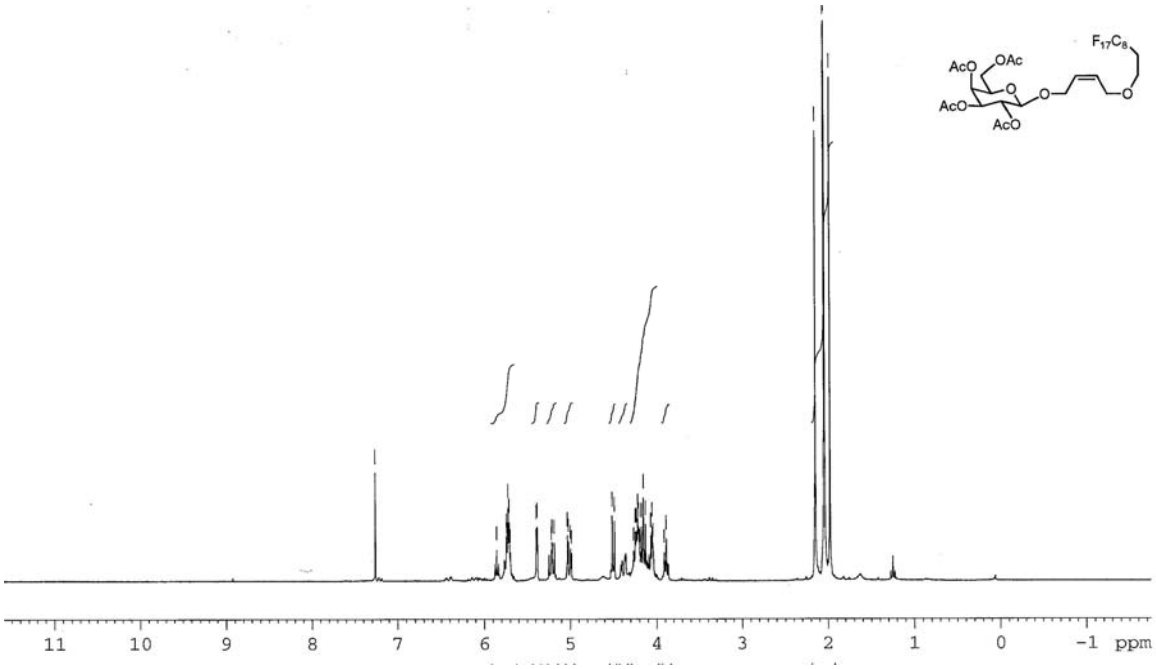


Figure 2.1: ^{1}H NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside.

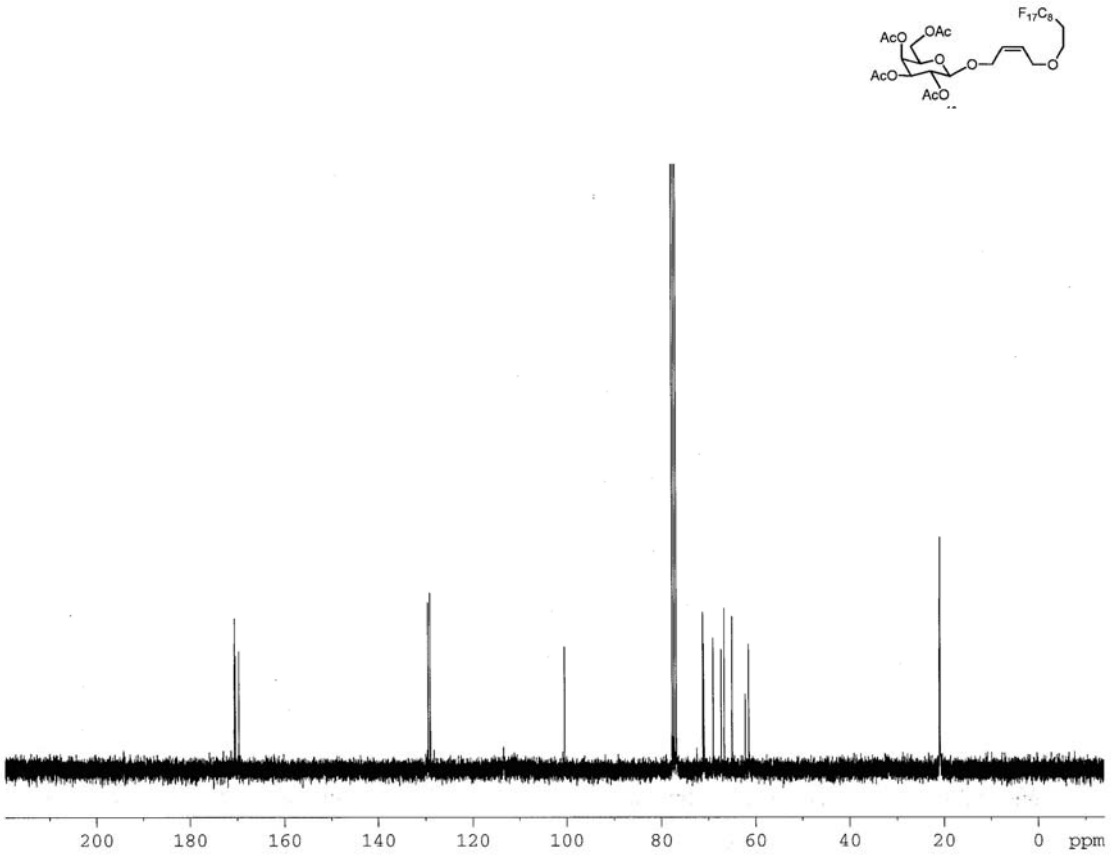


Figure 2.2: ^{13}C NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside.

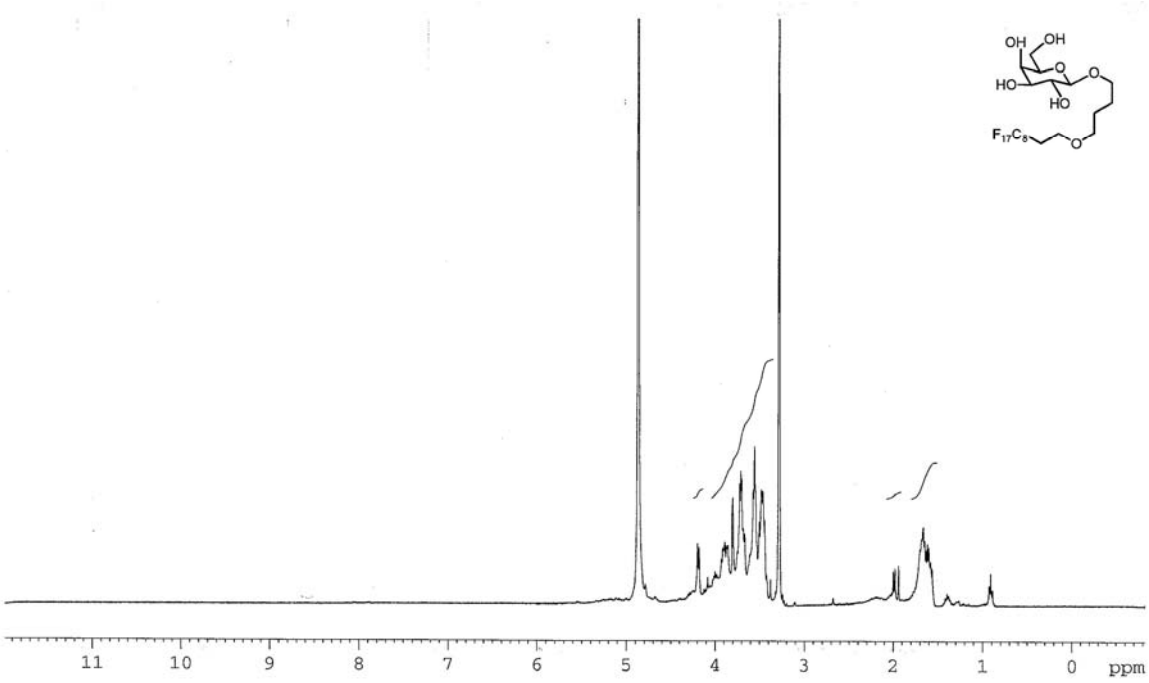


Figure 2.3: ^{1}H NMR of 1H,1H,2H,2H-perfluorodecyloxybutanyl-β-D-galactopyranoside.

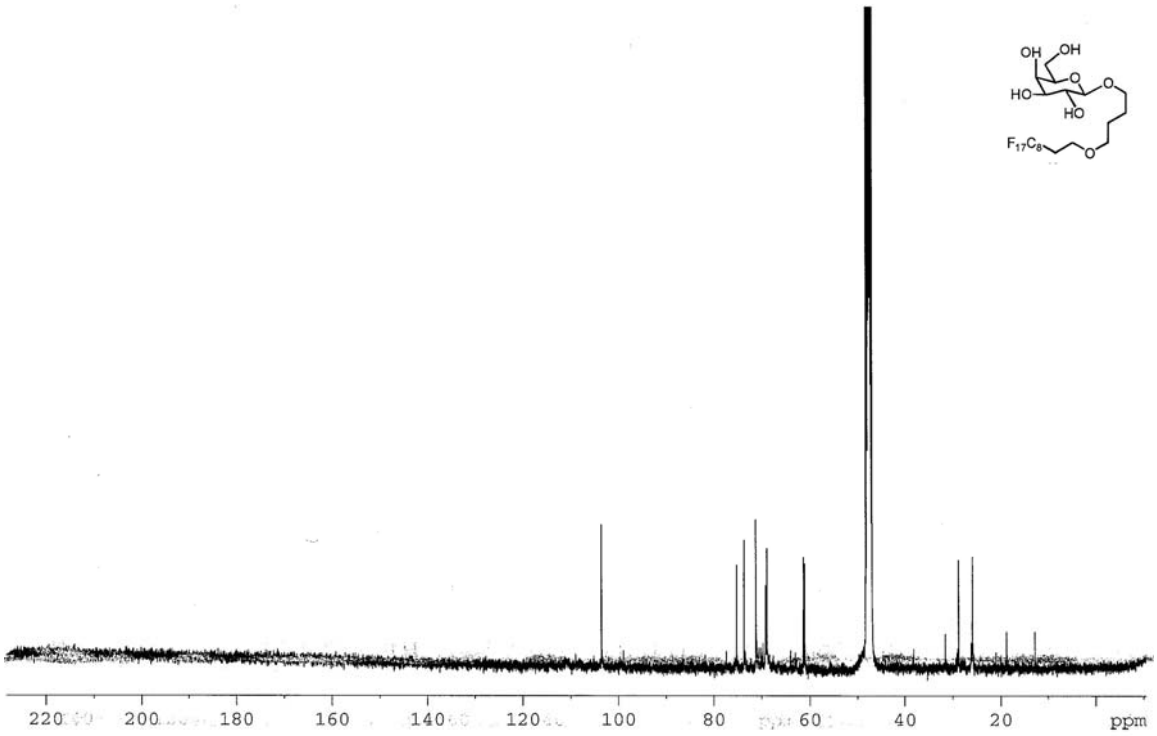


Figure 2.4: ^{13}C NMR of 1H,1H,2H,2H-perfluorodecyloxybutanyl-β-D-galactopyranoside.

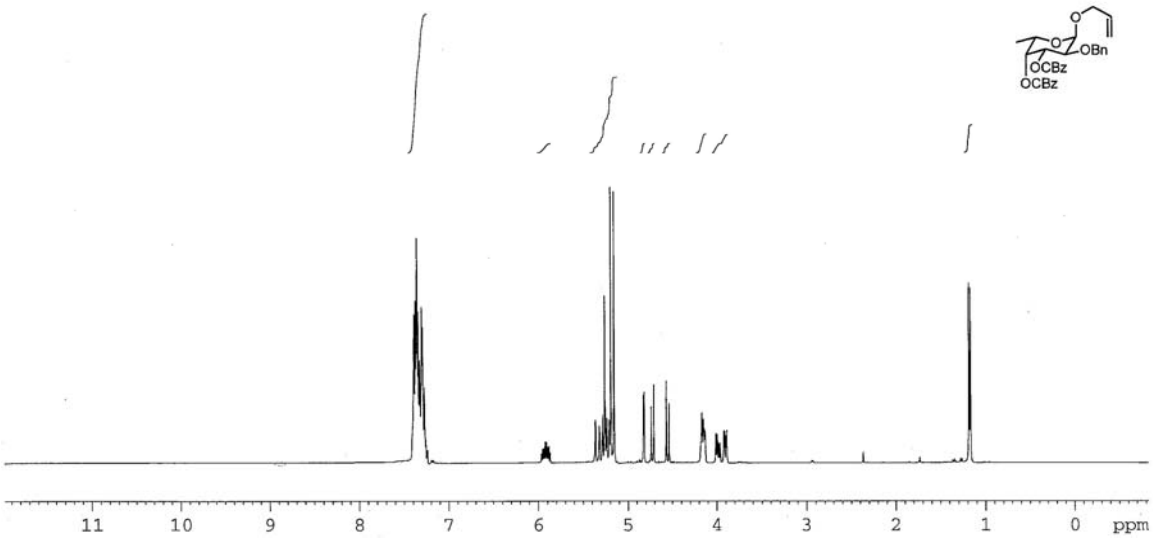


Figure 2.5: ^{1}H NMR of allyl-2-O-benzyl-3,4-di-O-carboxybenzyl-α-D-fucopyranoside.

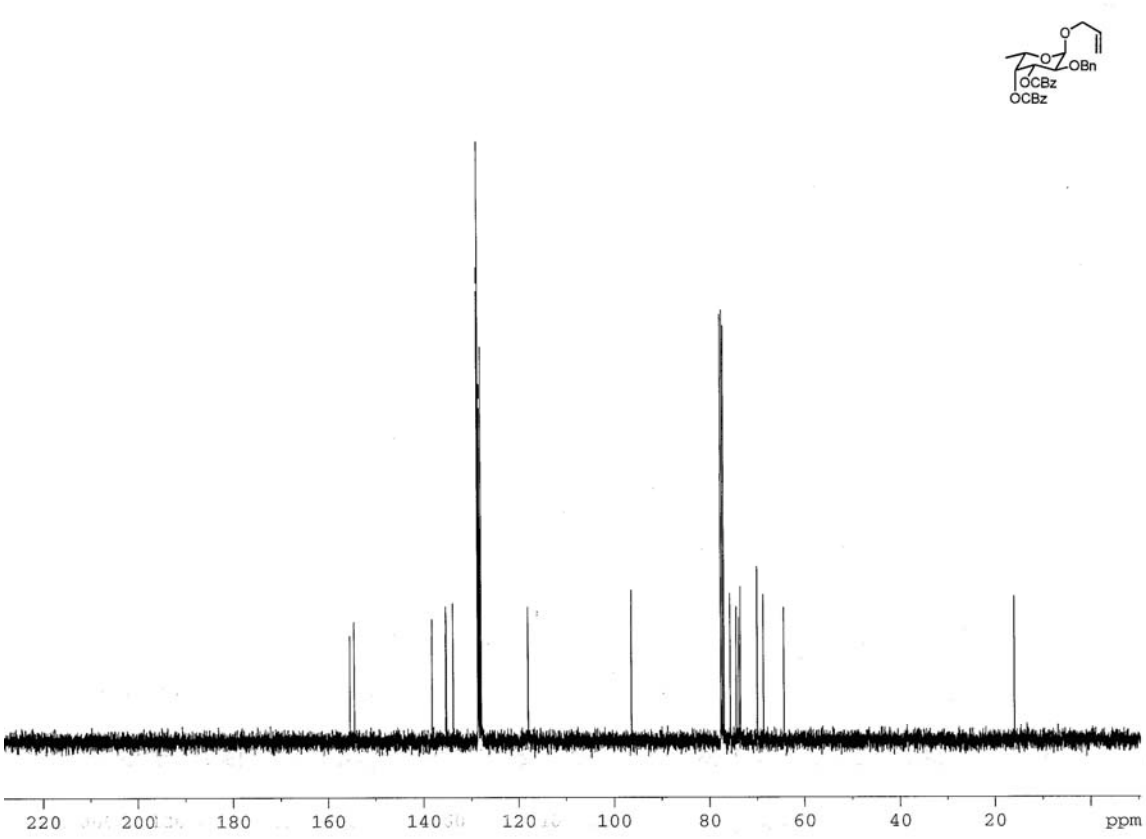


Figure 2.6: ^{13}C NMR of allyl-2-O-benzyl-3,4-di-O-carboxybenzyl-α-D-fucopyranoside.

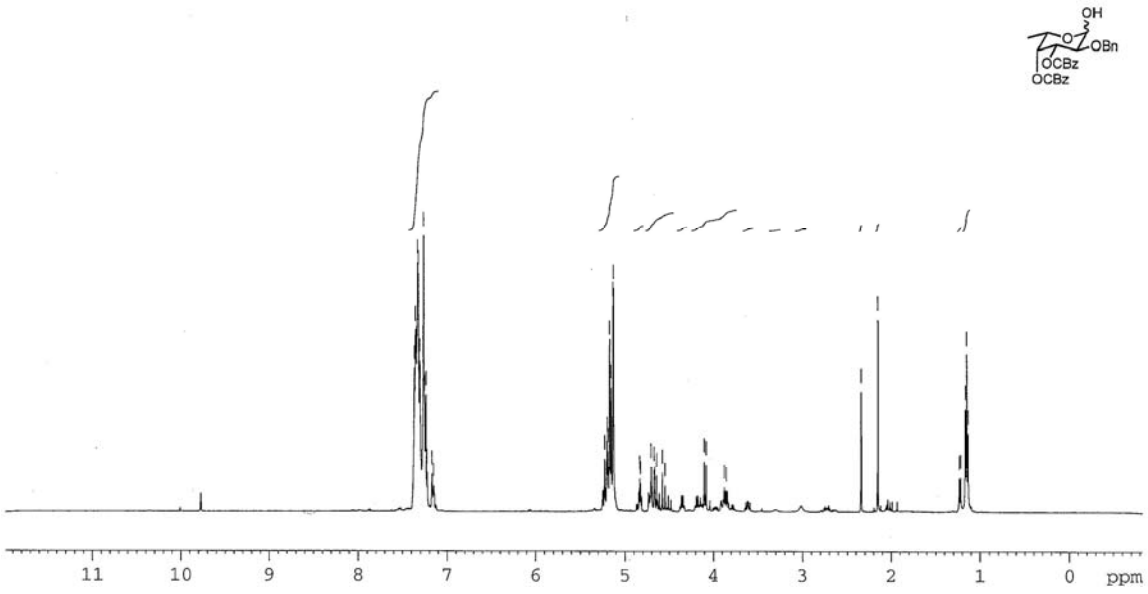


Figure 2.6: ^{1}H NMR of 2-*O*-benzyl-3,4-di-*O*-carboxybenzyl-α-D-fucopyranose.

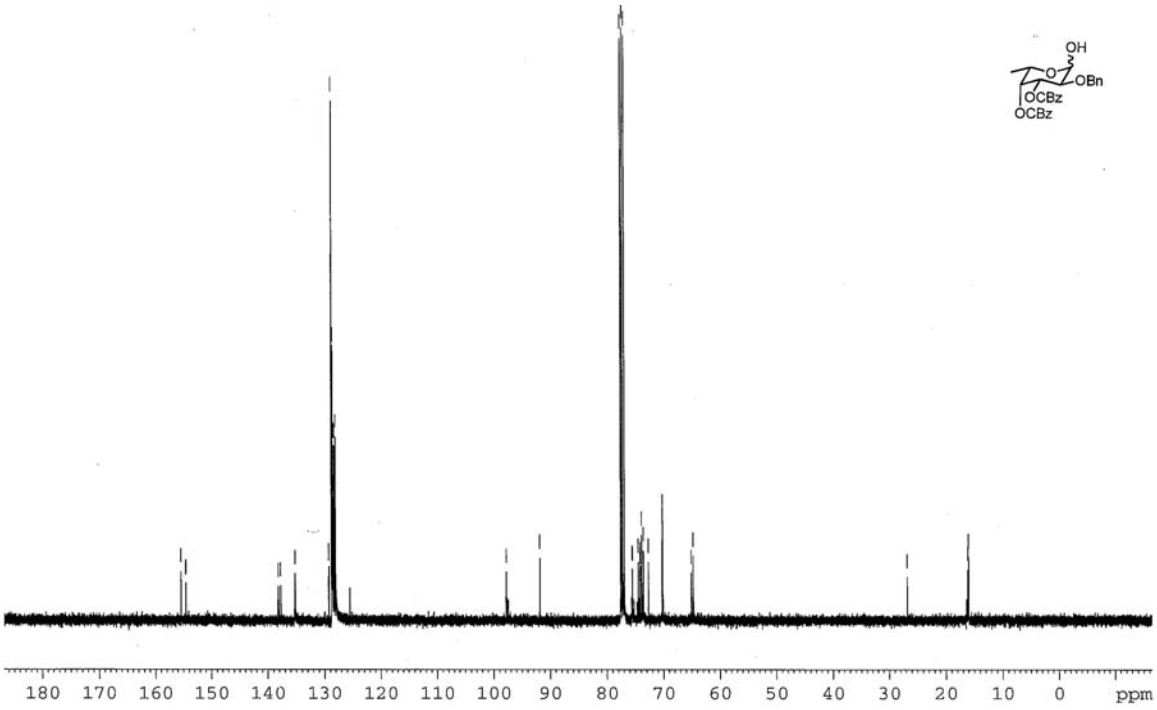


Figure 2.7: ^{13}C NMR of 2-*O*-benzyl-3,4-di-*O*-carboxybenzyl-α-D-fucopyranose.

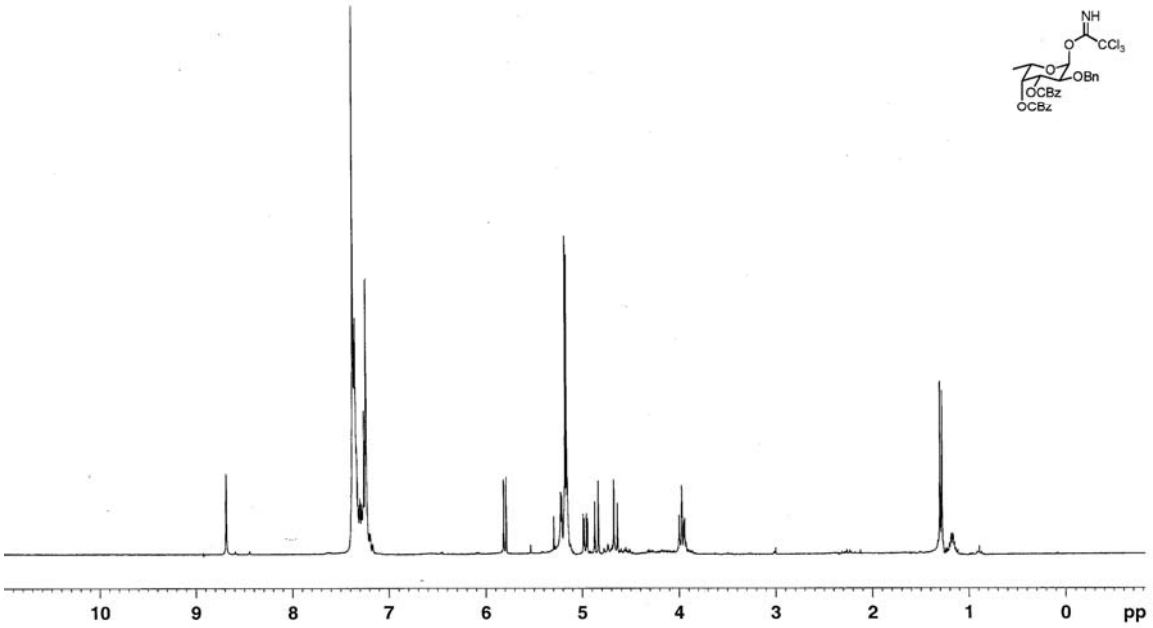


Figure 2.8: ^{1}H NMR of 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α/β-D-fucopyranoside trichloroacetimidate.

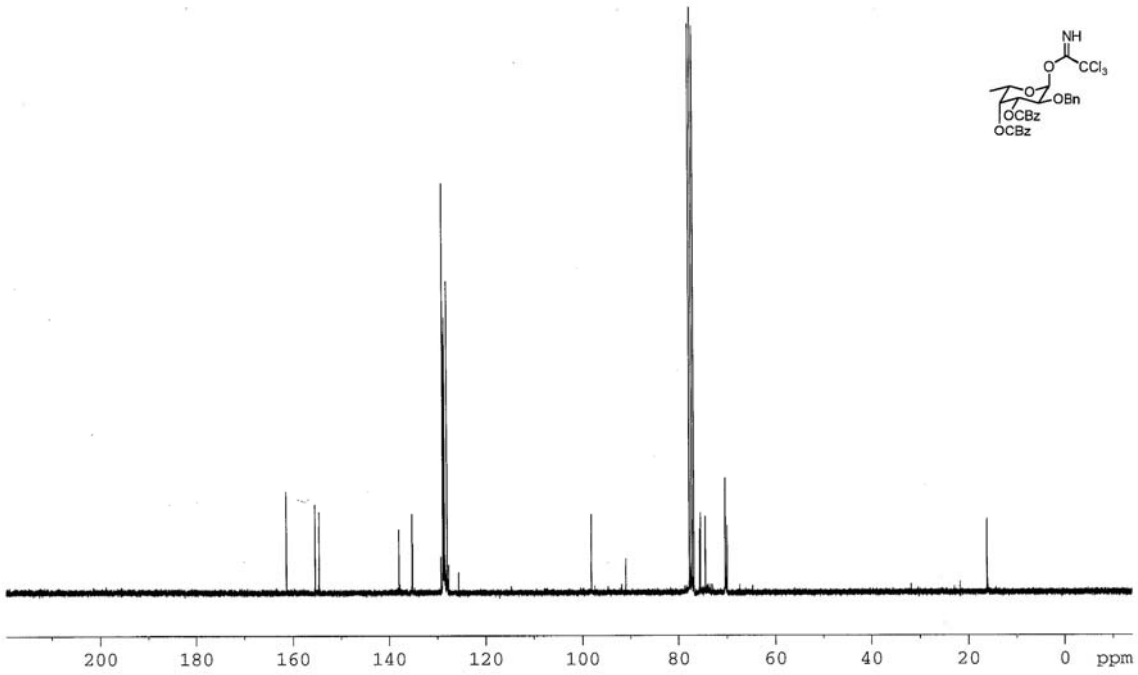


Figure 2.9: ^{13}C NMR of 2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α/β-D-fucopyranoside trichloroacetimidate.

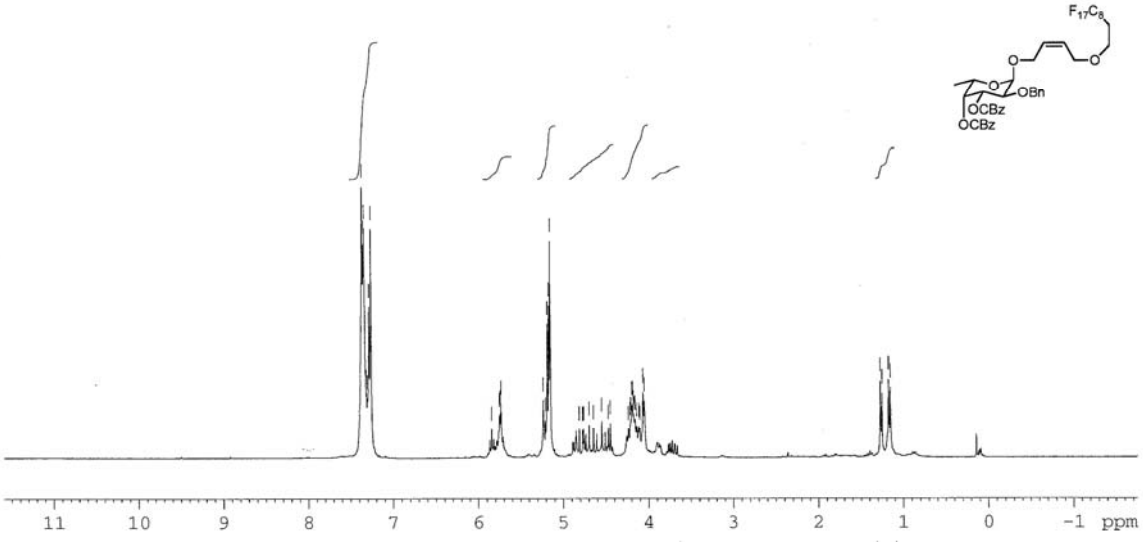


Figure 2.10: ^{1}H NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranoside.

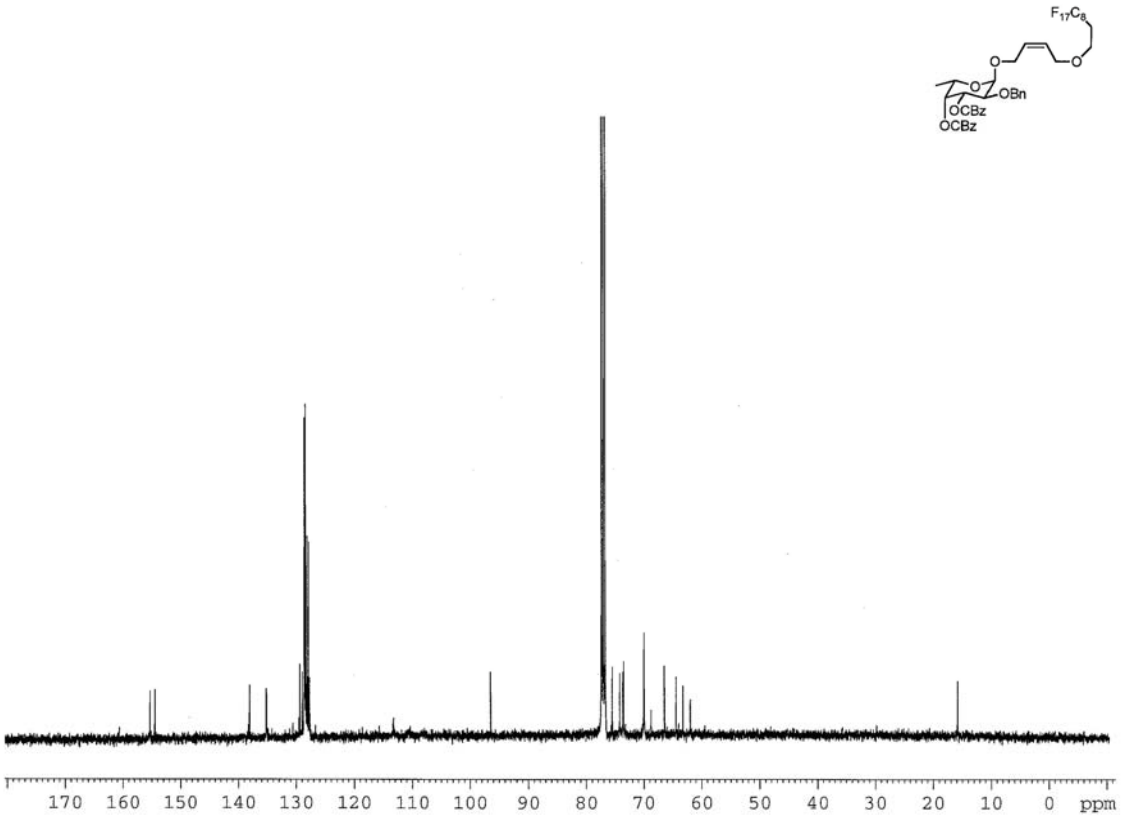


Figure 2.11: ^{13}C NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-2-*O*-benzyl-3,4-di-*O*-carbobenzyl-α-D-fucopyranoside.

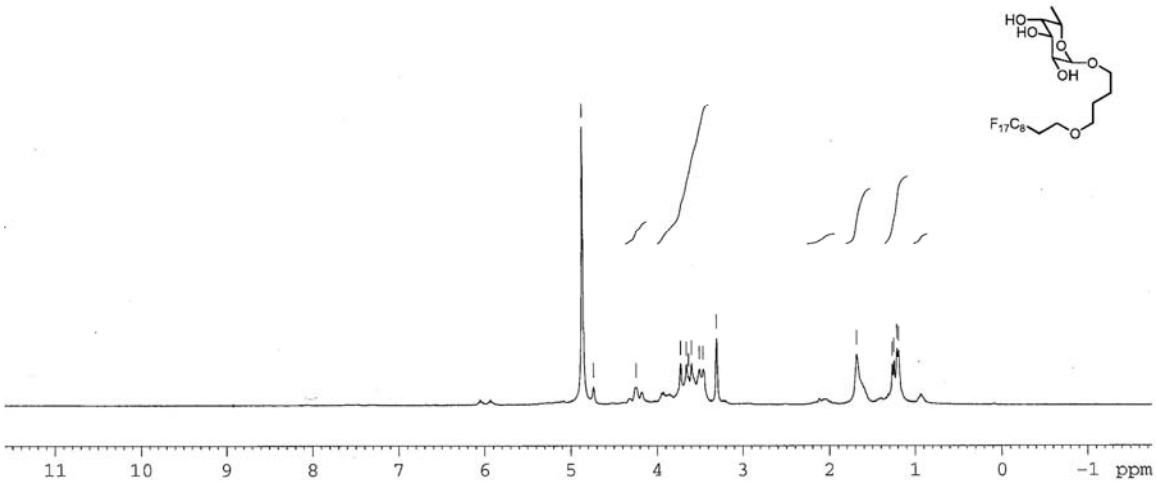


Figure 2.12: ^{1}H NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-α-D-fucopyranoside.

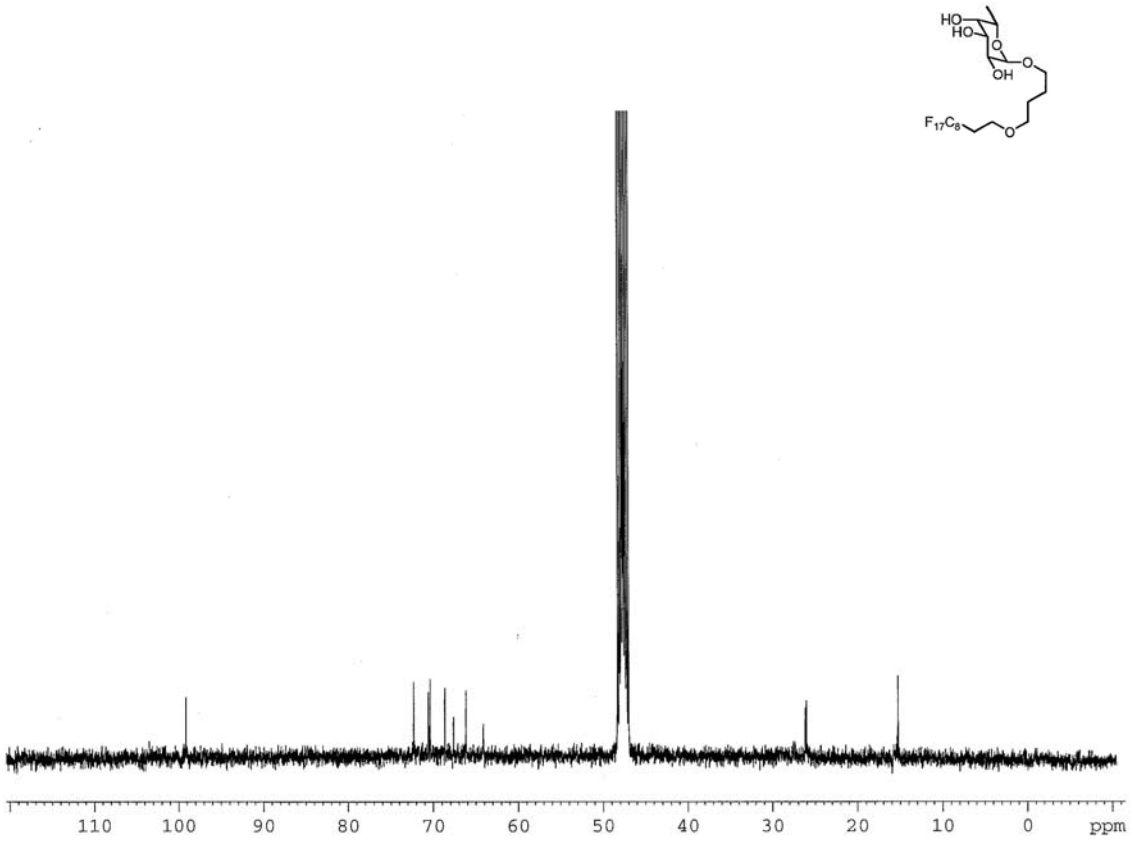


Figure 2.13: ^{13}C NMR of 1H,1H,2H,2H-perfluorodecyloxybutenyl-α-D-fucopyranoside.

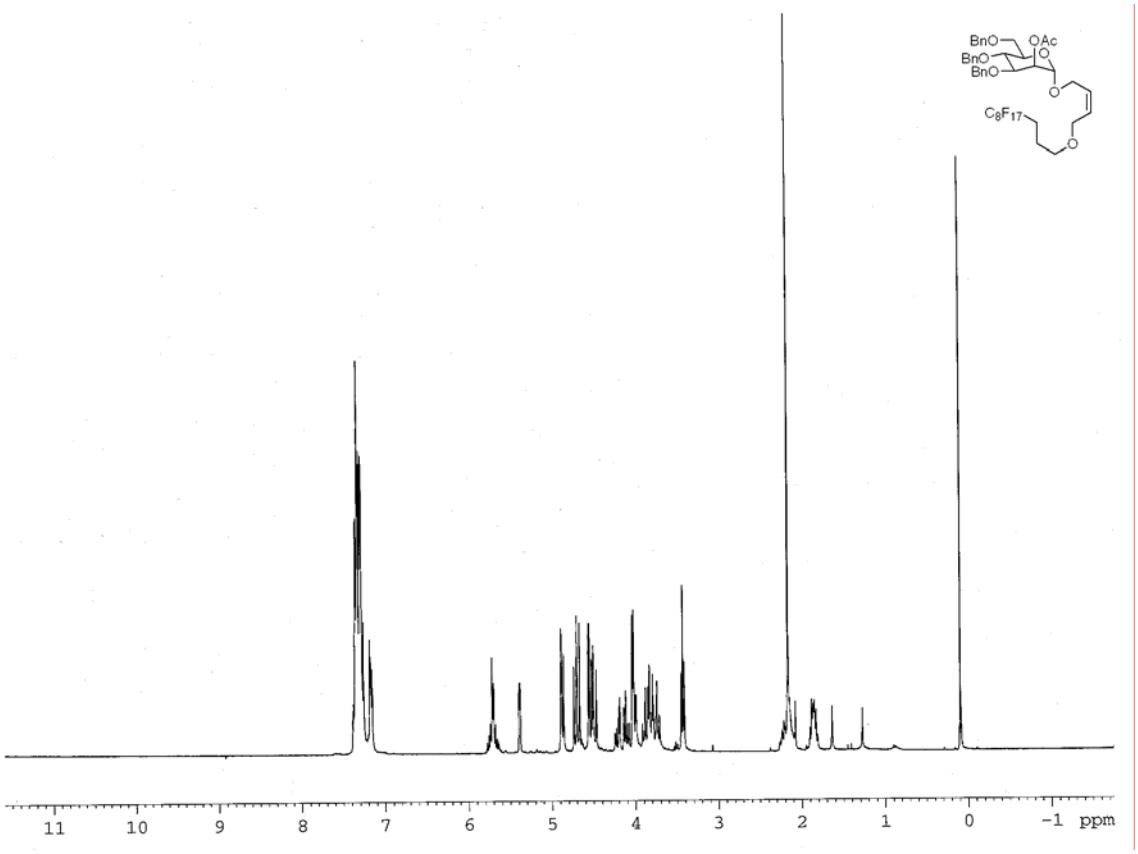


Figure 2.14: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside.

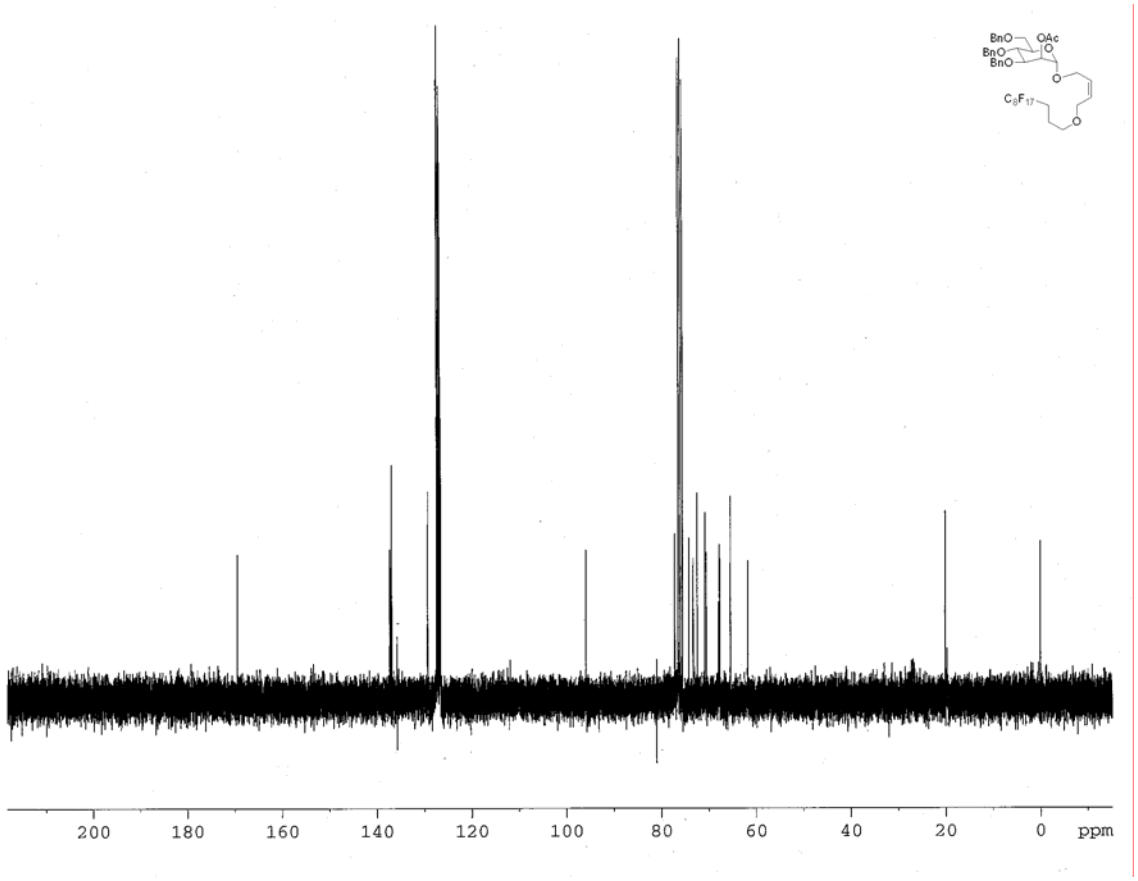


Figure 2.15: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside.

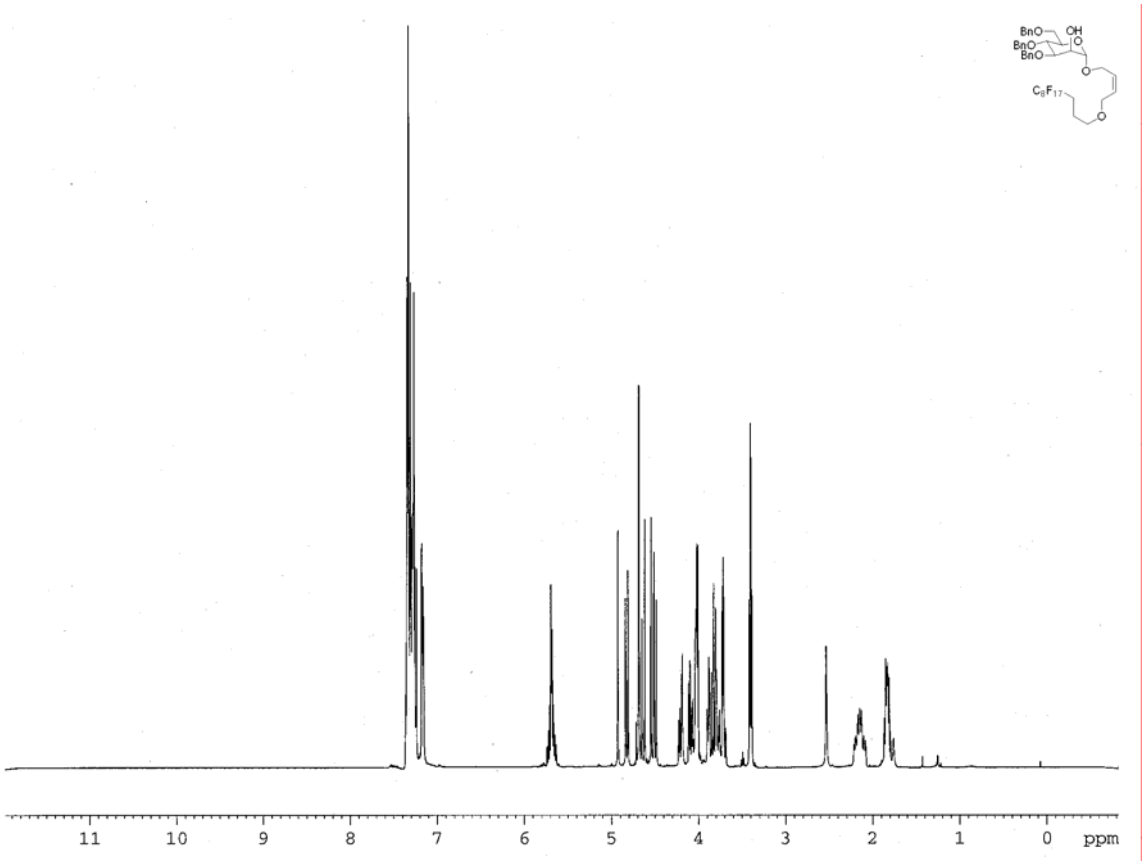


Figure 2.16: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside.

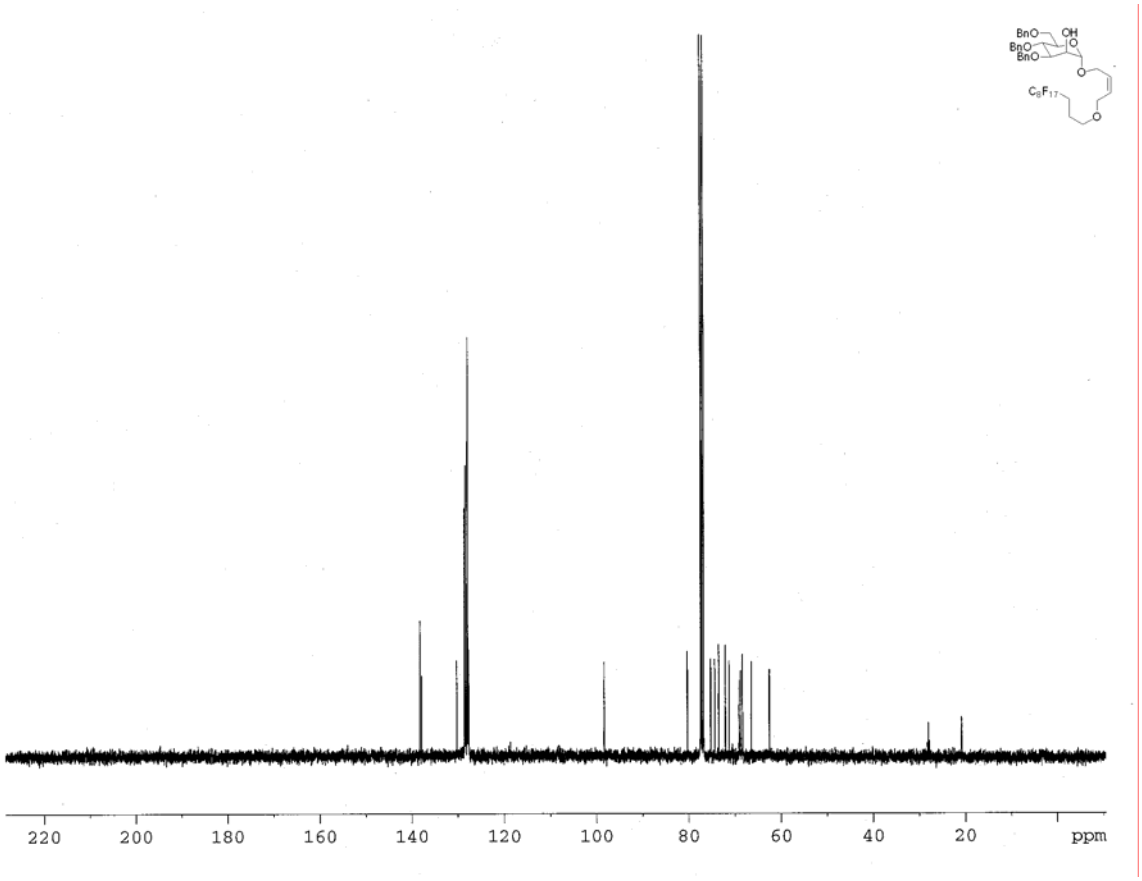


Figure 2.17: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside.

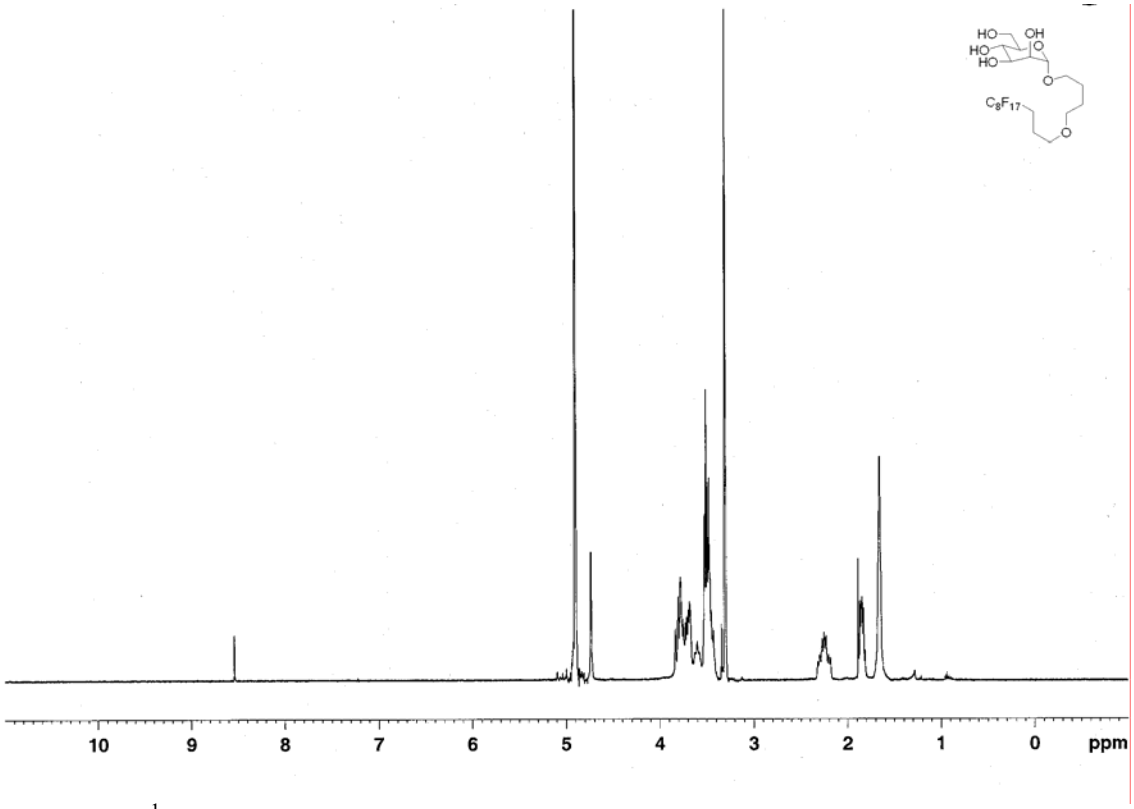


Figure 2.18: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-α-D-mannopyranoside.

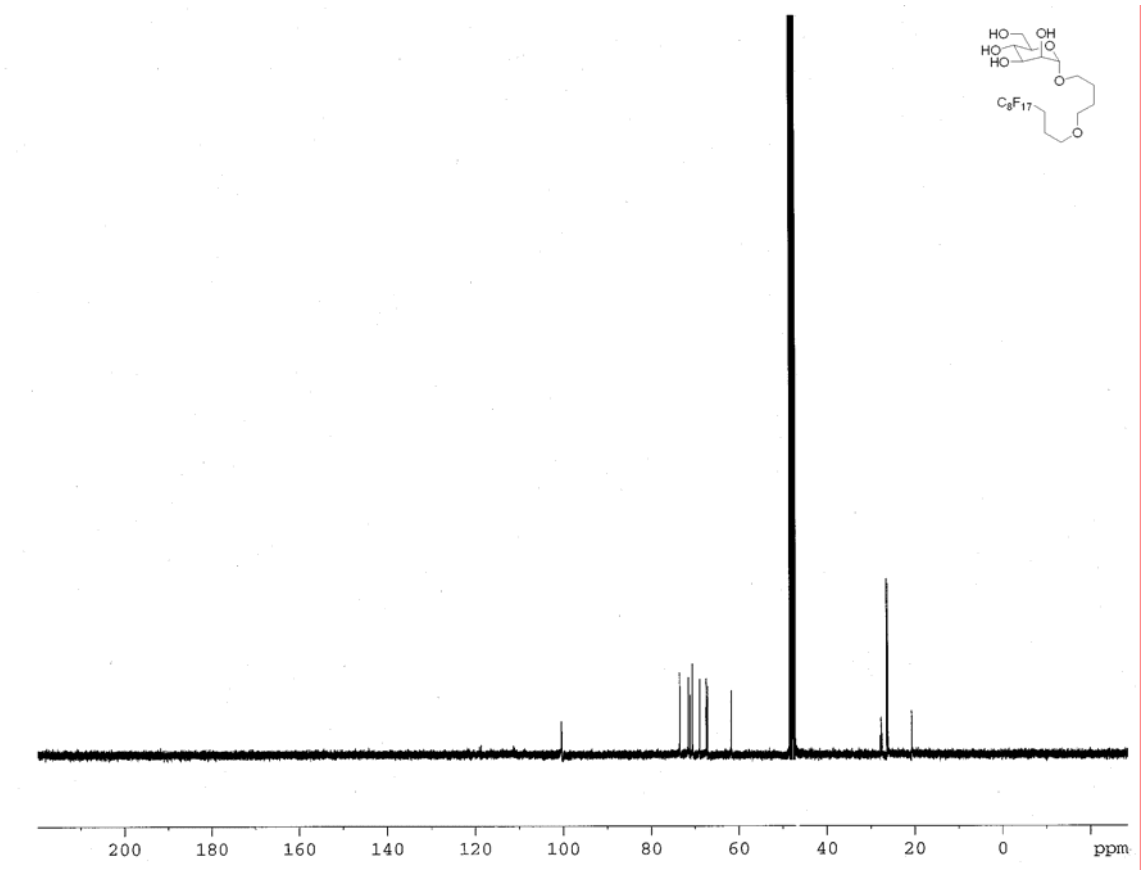


Figure 2.19: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-α-D-mannopyranoside.

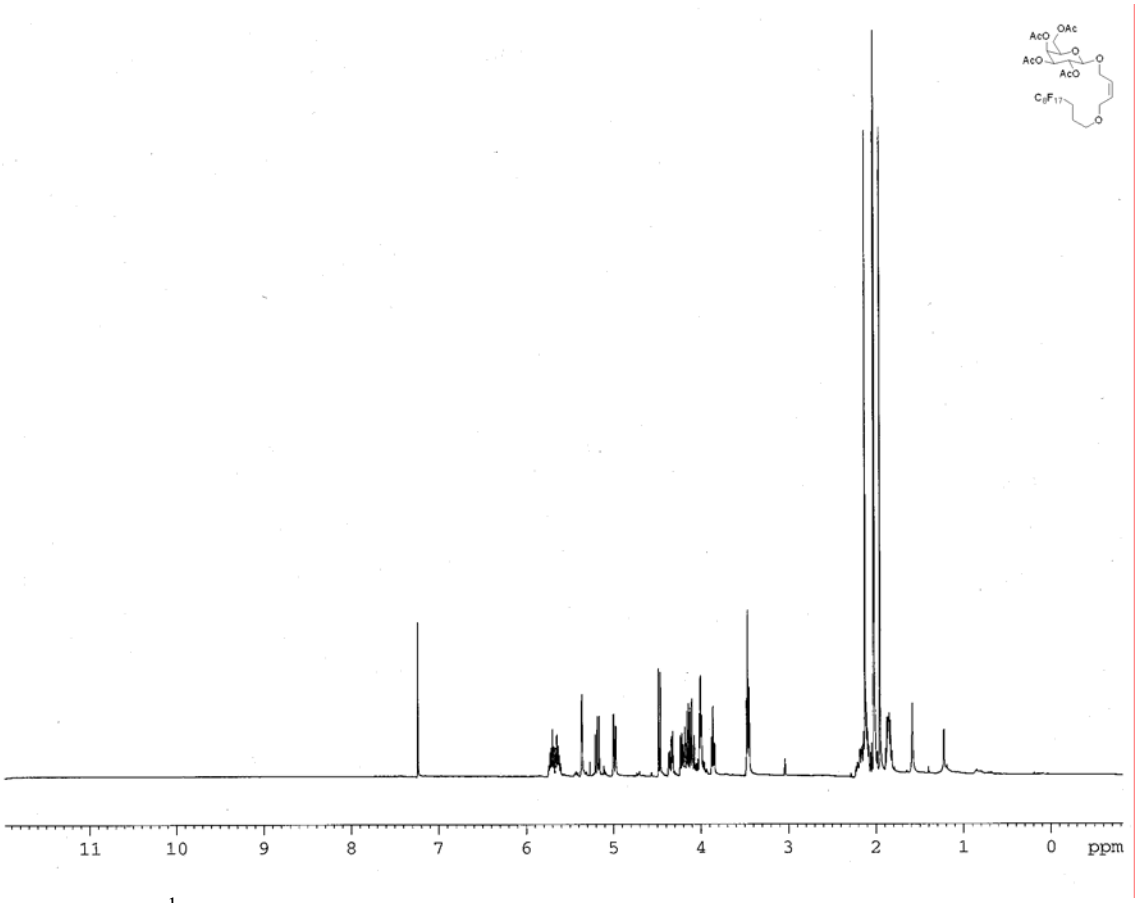


Figure 2.20: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside.

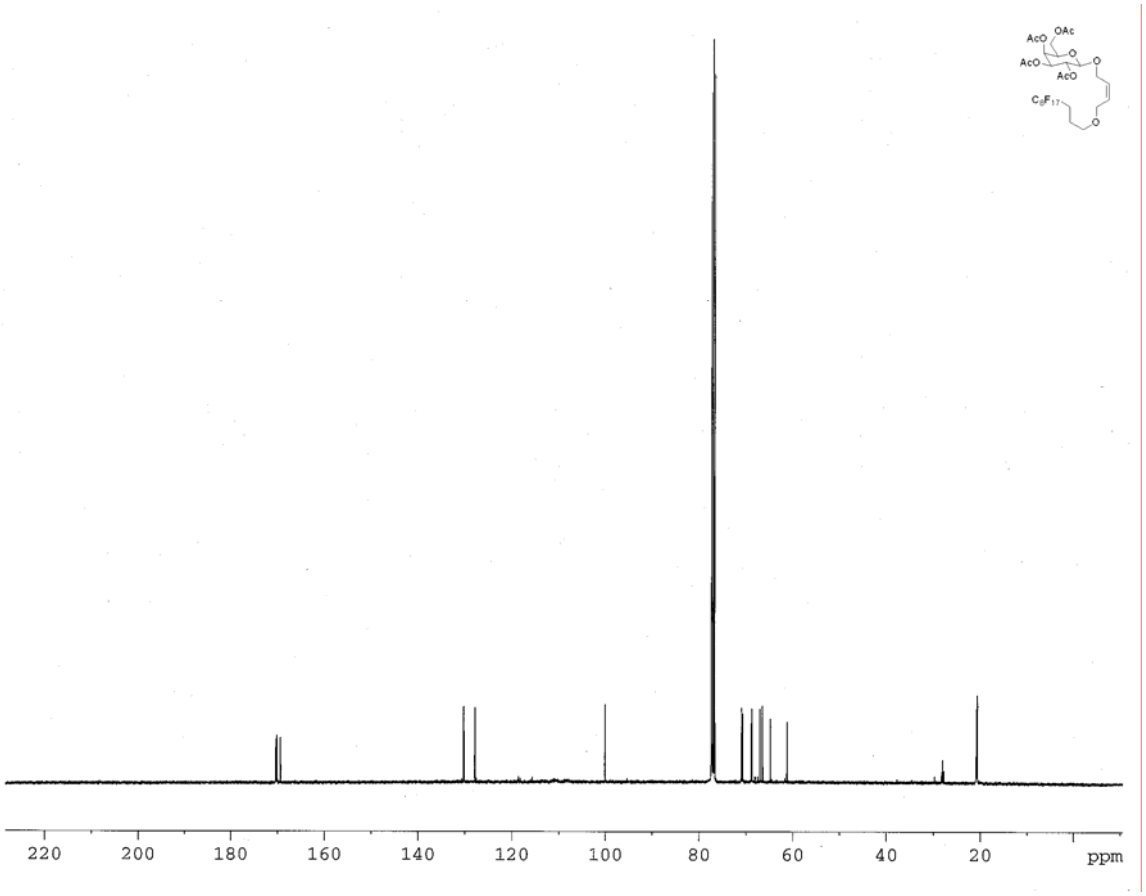


Figure 2.21: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside.

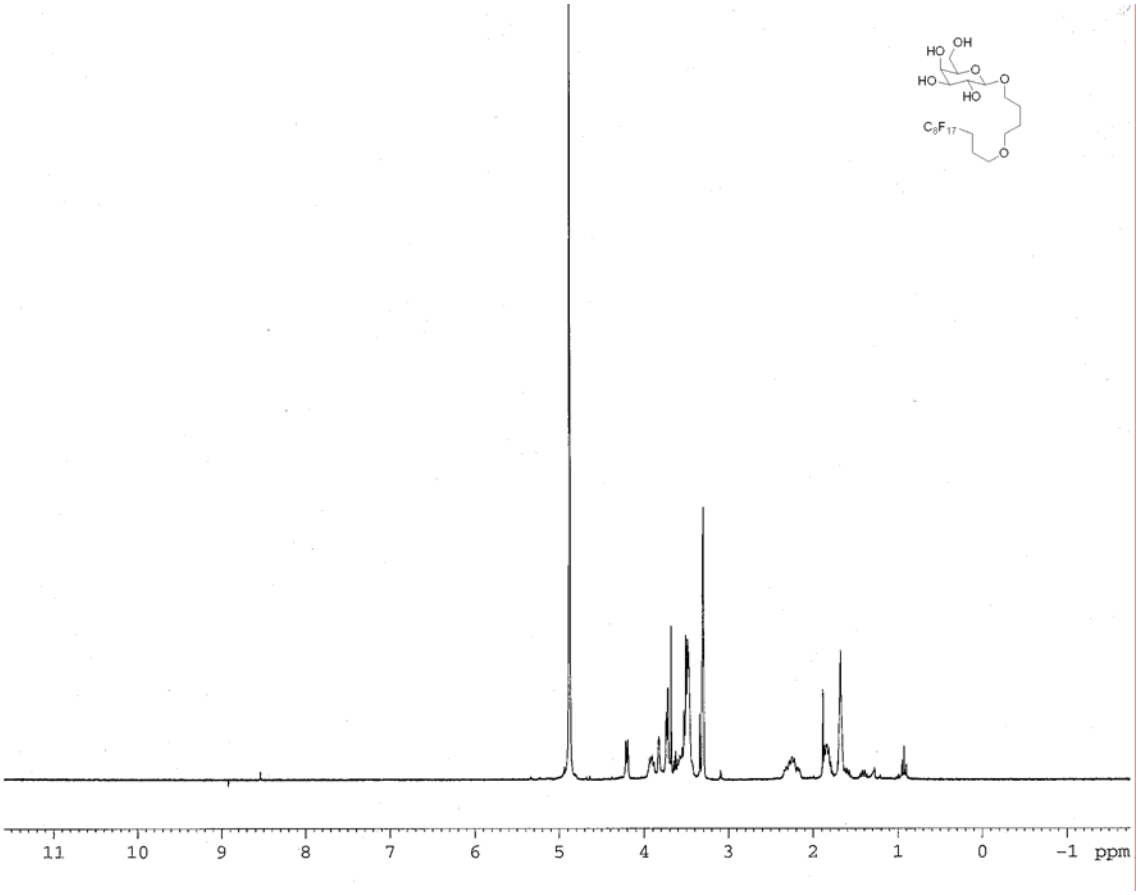


Figure 2.22: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-β-D-galactopyranoside.

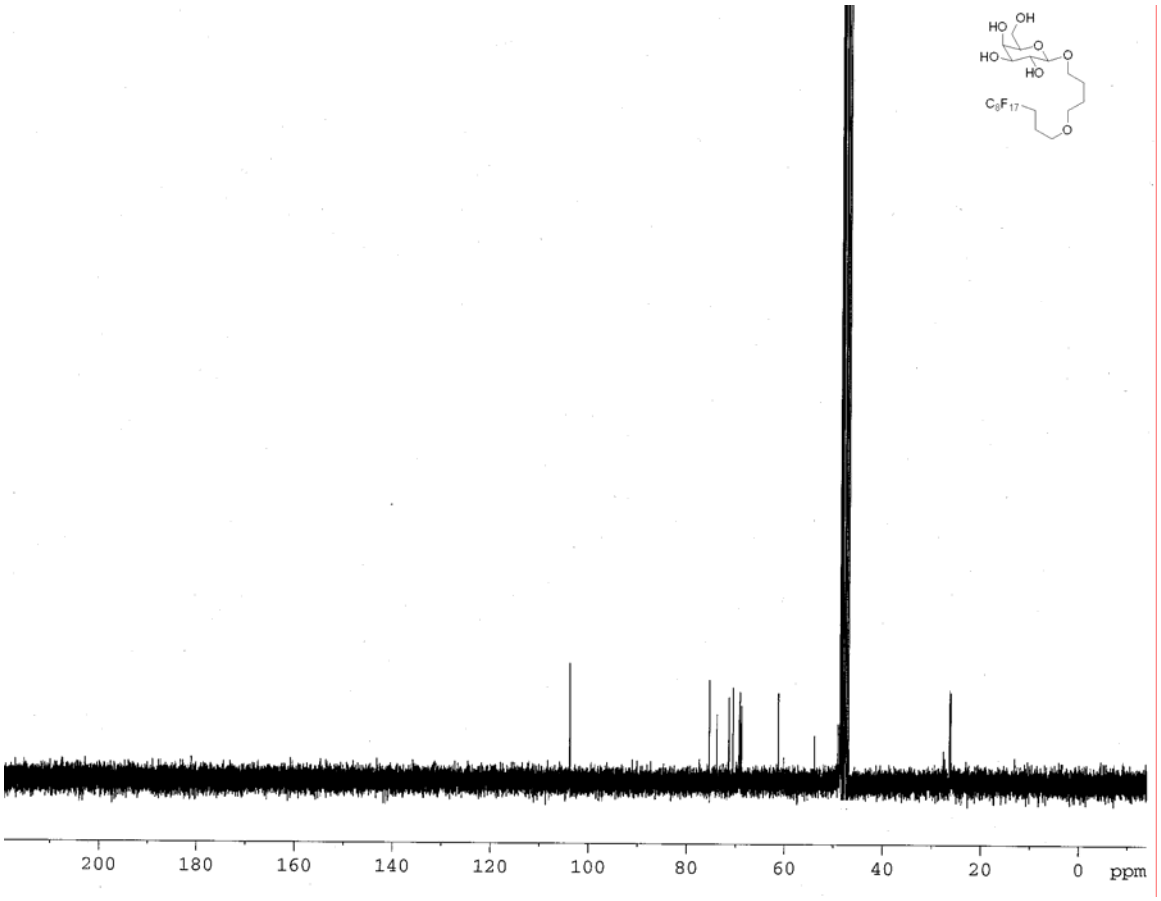


Figure 2.23: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-β-D-galactopyranoside.

CHAPTER 3: FLUOROUS-TAG ASSISTED SOLUTION PHASE SYNTHESIS OF MANNOSE OLIGOSACCHARIDES AND *GLYCERO*-D-*MANNO* HEPTOSES

Introduction

The emerging understanding of the critical role of oligosaccharides in biological processes and the promise of therapeutics based on oligosaccharides create an urgent need for an efficient synthetic methodology for oligosaccharides. In spite of the achievements in the chemical synthesis of oligosaccharides based on the development of highly reactive sugar donors (Schmidt 1986, 1994), glycosylation strategies and advanced protective group chemistry in recent years (Schmidt 1992, Ogawa 1994, Barresi 1995), one of the future tasks is to combine this knowledge with the advantages of the solid phase synthesis and support glycobiology studies with a variety of well defined oligosaccharides and glycoconjugates.

Oligosaccharides of D-mannose (Figure 3.1) are found in nature as essential substructures of many bioactive glycoconjugates, such as N-glycans, fungal cell wall mannans (Kobayashi 1992) and GPI anchors (Pekari 2001), and as high affinity ligands for various mannose binding proteins, for example, Con A (Sanders 2001) and Cyanovirin N (Botos 2002).

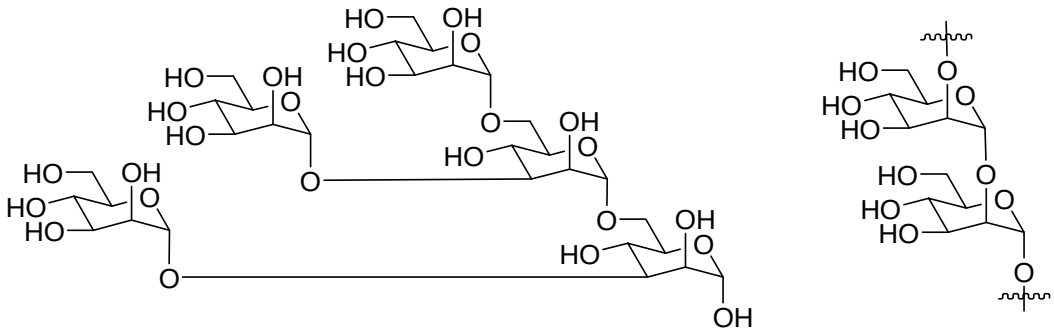


Figure 3.1. High-mannose oligosaccharide targets

Several research groups have developed very effective strategies for the conventional step-wise solution synthesis of manno-oligosaccharides (Ogawa 1981, 1985, 1986, Ning 1999, Zhu 2000, Zheng 2003, Grice 1995, 1996, Green 1998, Mayer 1994, Ratner 2002). High yields were achieved in relatively short reaction sequences from monosaccharide building blocks by prudent choice of protecting groups and/or fine-tuning of mannosyl donors. Such syntheses, involving selective protection, followed by glycosylation and deprotection sequences, are nevertheless time consuming, as they require chromatographic purification of all intermediates. Over the last two decades efforts have been made to develop protocols for the construction of oligosaccharides on polymer supports (Osborne 1995, Krepinsky 2000, Seeberger 2000, Sears 2001) that simplify the isolation and purification of intermediates, since nonsupported reagents can be removed merely by washing the insoluble products with suitable solvents. Chromatography is required only once, after cleavage of the end product from the support. The automation of oligosaccharide synthesis has lagged decades behind those of the oligopeptides and oligonucleotides, but significant developments have now been reported (Sears 2001, Plante 2003).

In spite of its considerable merits, progress in polymer supported oligosaccharide synthesis has, on the whole, been slow because glycosidic bond formation does not, in general, proceed with the high yield and stereoselectivity necessary for successful application of this technique. α-Mannosylation is one of the few exceptions, and the polymer supported synthesis of α-linked oligomannosides has therefore seemed a feasible alternative to classical solution synthesis. Thus, α-(1→2)-linked manno-tri- (Rademan 1997), tetra- (Rademan 1997), hexa- (Grathwohl 2001) and hepta (Andrade 1999) -ose derivatives have been prepared on Merrifield's resin with isolated yields of cleaved products of 54%, 34%, 19% and 9%, respectively, and when syntheses of such compounds were carried out under automation, higher yields were achieved (Plante 2003).

A distinct disadvantage of synthesis on the most common, insoluble supports, for example, polystyrene resins or controlled-pore glass, is the difficulty of monitoring the progress of reactions. This problem can be overcome by the use of soluble polymer supports, in particular polyethylene glycol ω-monomethyl ether [$HOCH_2$-$CH_2(OCH_2CH_2)_nOMe$ (MPEG)], introduced by Krepinsky and co-workers (Douglas 1995, Krepinsky 2001).

As core structures, the 3,6-branched trimannosaccharide unit (Manαl-6Man3-lαMan) and pentamannoside unit [(Manαl-6Man3-lαMan)αl-6Man3-lαMan)] is widely expressed on a lot of glycoproteins. They exist in all asparagines-linked oligosaccharides (N-glycans) and the major Con A binding epitope on oligosaccharide-type carbohydrate (Wang 1999). Recently, it has been shown that these core trisaccharide and pentasaccharide is highly expressed on gp120 of HIV (Figure 3.2) (Hadar 2001). It is selectively bound to DC-SIGN, leading to the HIV migration from mucous membrane to the lymph system (Carole 2002).

Consequently, the syntheses of these core trisaccharide and pentasaccharide are of great interest (Zhang 2002).

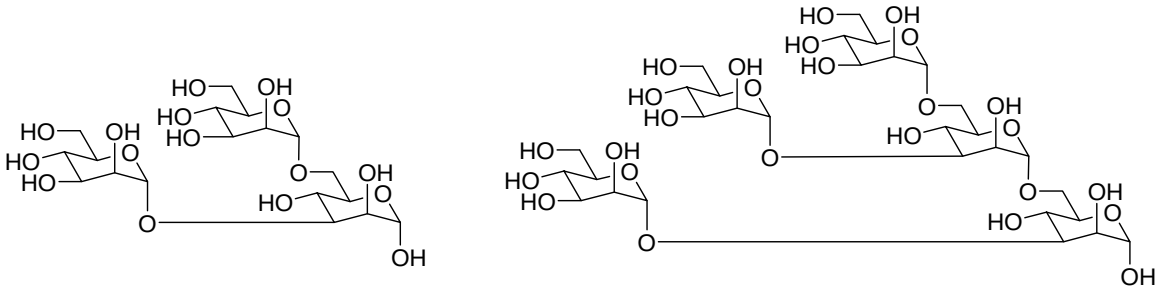


Figure 3.2 3- and 6-Modified branched mannose oligosaccharides.

Structurally complex lipopolysaccharides (LPS) are amphipathic and microheterogeneous glycolipids and essential components of the outer membrane of Gram-negative bacteria (Raetz 2002). LPS mediate numerous immunological and pathophysiological effects in bacterial infections. The L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptoses, are common constituents of both the inner- and outer-core regions of LPS of many pathogenic bacteria, where they are present mostly in the α-anomeric configuration (Zamyatina 2003). The synthesis of higher-carbon sugars such as heptoses has been investigated for more than a century (Györgydeák 1998), and several methods have been developed (Györgydeák 1998, Zamyatina 2003). The synthesis of a large number of complex oligosaccharides containing α-L,D- or α-D,D-heptopyranosides (Hep*p*), has been achieved by Oscarson and co-workers (Oscarson 1994, Ekeloef 1995, Bernlind 1998, Oscarson 2001, Segerstedt 2004). Due to the increasing resistance of many bacterial strains against conventional antibiotics, a detailed understanding of the immunological responses is necessary in order

to develop novel drugs, which may prevent the assembly of a functionally intact bacterial cell wall by inhibition of its biosynthesis (Zamyatina 2003).

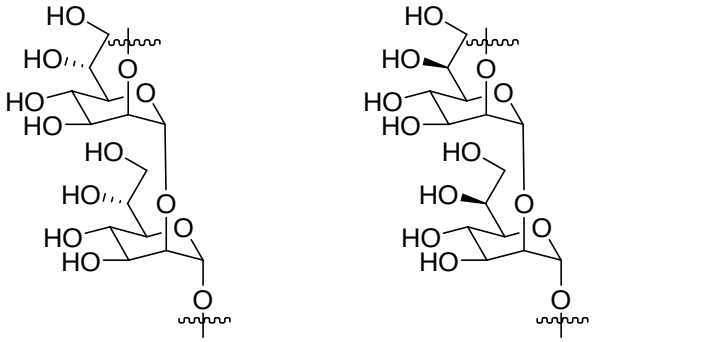


Figure 3.3. Structure of α-(1→2)-linked heptomannose oligosaccharides.

Thus, well-defined syntheses of complex oligosaccharides and glycoconjugates corresponding to the native bacterial structures are essential, as the synthetic derivatives can be employed to (i) locate immunodominant motifs, (ii) determine the specificity of the antibody response obtained from native structures, and (iii) investigate the processing of glycoproteins by the immune systems. Here, we describe the synthesis of fluorous-tagged L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptoses to study the binding interactions of these seven carbon sugars with mannose binding lectin, Con A. Con A has been widely used to identify mannose containing ligands. By replacing one of the hydrogen at C-6 position of mannose with a CH_2OH, a heptomannose is obtained. It will be interesting to know whether Con A will bind to heptomannose compounds.

Results and Discussion

There are several methods reported in literature for the synthesis of oligosaccharides. The most common method is the solution phase synthesis but it is very tedious and time consuming. Solution phase synthesis does not provide access of oligosaccharides to nonchemists. Application of solid-phase chemistry to carbohydrates provided access to complex oligosaccharides. As discussed in the introduction chapter, the major drawback of SPOS is the use of excess donor during coupling cycle to drive the reaction to completion. To circumvent this problem, Hindsgaul and coworkers came up with a novel method of combining solution-phase and solid-phase chemistry (Hindsgaul 1995). Rademann took this approach to a new level by developing hydrophobically assisted switching phase synthesis (HASP) of oliogosaccharides (Bauer 2005). It has huge promise for the synthesis of oligosaccharides. But like other methods, this does not provide direct access for the fabrication of carbohydrate microarrays to study protein-carbohydrate interactions.

We have already discussed the use of fluorous support for oligosaccharide synthesis and direct micrarray formation. In order to test if we can extend this method to the synthesis of polysaccharides, we decided to apply this method for the synthesis of linear α-(1→2)-linked mannose oligosaccharides and 3, 6-modified branched mannose oligosaccharides. Linear α-(1→2)-linkages in mannose oligosaccharides is easy to make and hence would be a good starting point to test the power of this method. It will further test the scope of fluorous-based carbohydrate microarrays to study the binding of several mannose oligosacharides with mannose binding lectin Con A. Concanavallin A (Con A), is a lectin isolated from jack bean meal, has been reported to bind with non-reducing α-D-glucose

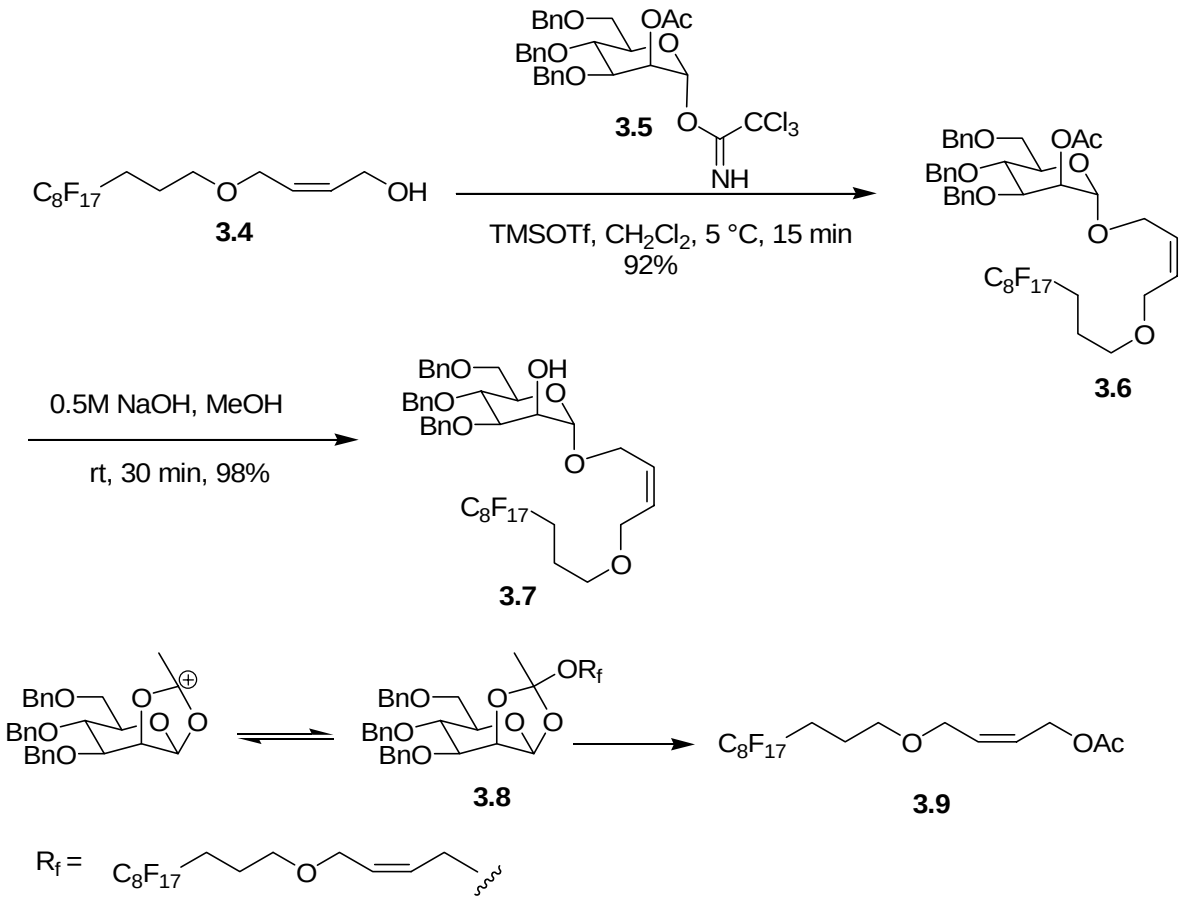
and α-D-mannose. Inhibition data suggest that unmodified hydroxyl groups at the C-3, C-4, and C-6 positions of the D-mannopyranose (Goldstein 1965).

As mentioned in the previous chapter, fluorous support could be used for the synthesis of complex oligosaccharides and has all advantages of solid-phase synthesis. The fluorous-tagged compounds can be easily separated from the non-fluorous compounds by passing the crude product through fluorous solid-phase extraction (FSPE) column and it is amenable to automation. All glycosylations and protective group manipulations clearly should be compatible with the fluorous linker. The chain was elongated from the reducing end (i.e. the terminal sugar residue whose C-l oxygen atom is not conneced to another sugar residue) to the non-reducing end (i.e. a terminal residue that is connected to other parts of the oligosaccharide chain via its C-l oxygen atom). Linear α(1→2)-linked mannose tetrasaccharide was synthesized using fluorous support. All intermediates were purified using FSPE column only.

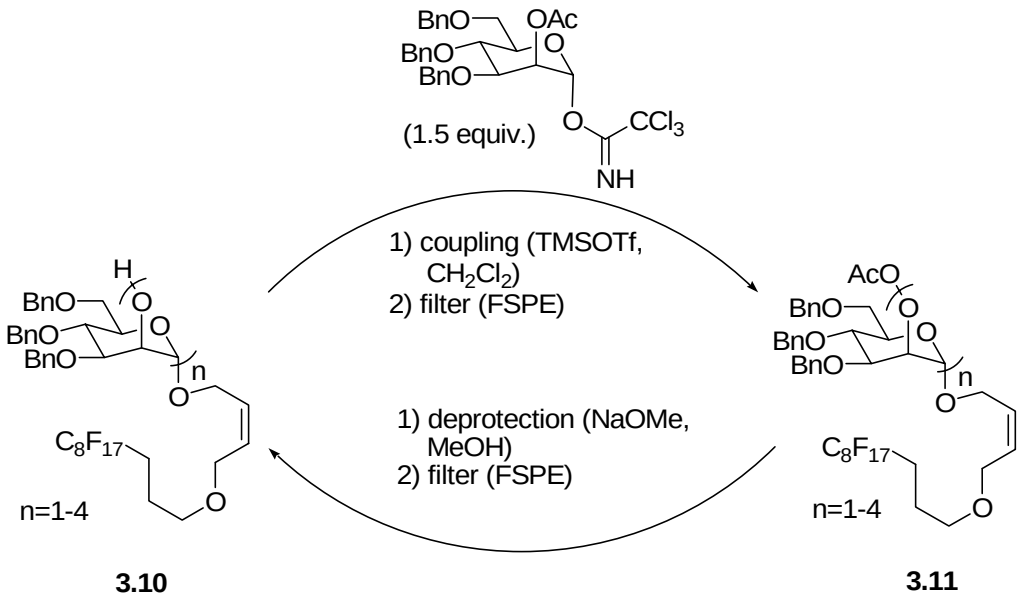
Synthesis of Mannose Oligosaccharides

The mannose trichloroacetimidate donor **3.5** was chosen as the donor building block because it can be prepared on a multigram scale, is activated at room temperature, and bears a C2-ester functionality to control the anomeric configuration of the fluorous linker. Initial experiments on coupling of the fluorous linker **3.4** with mannose donor **3.5** resulted in the formation of fluorous-tagged mannose **3.6** and some transacetylation product **3.9** (Scheme 3.1). It is reported in the literature that transacetylation is a very common side product in glycosylation reactions. It is proposed that the transacetylaed product is formed via orthoester formation (Scheme 3.1). The following solutions have been provided to

avoid the formation of transacetylation product: 1) use of pivaloyl group and 2) run the reactions concentrated. We chose to apply the second solution to our glycosylation reaction and found that the glycosylation reaction worked well and no transacetylation product was observed. Deprotection of temporary acetyl group with NaOMe/MeOH produced compound **3.7** in 98% yield.



Scheme 3.1 Glycosylation of fluorous linker with mannose trichloroactimidate donor.



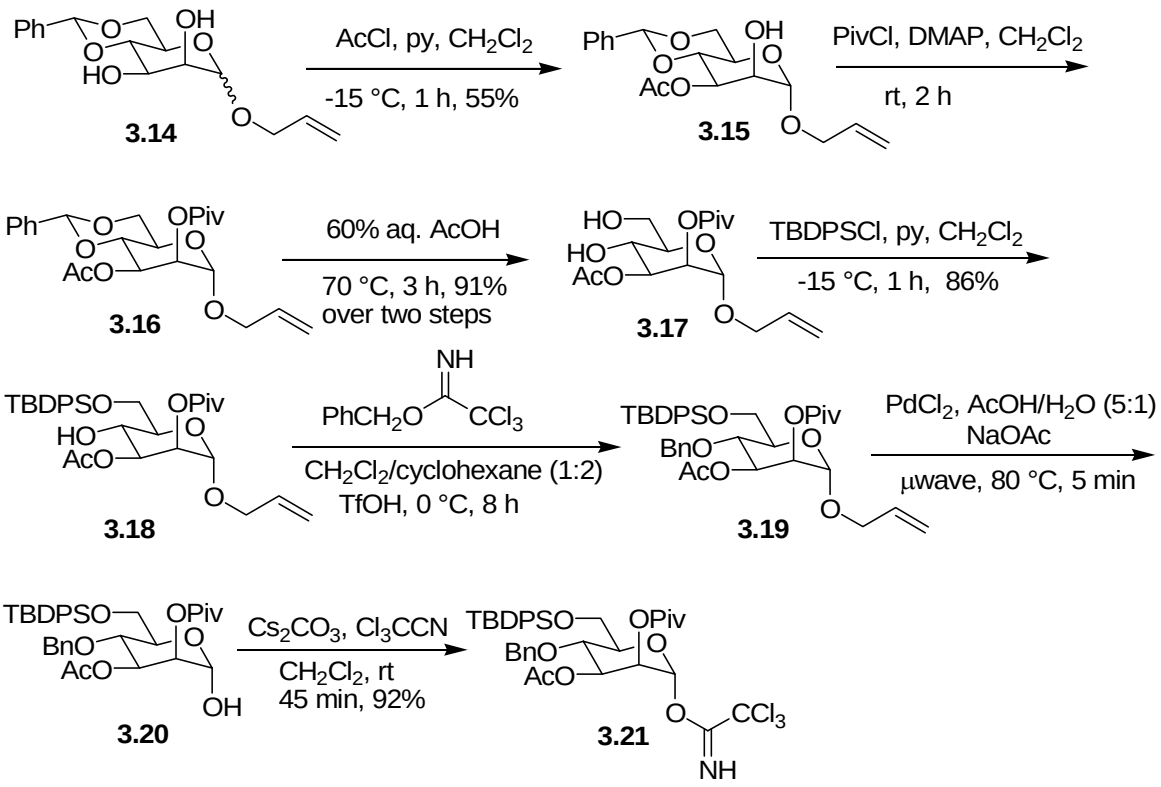
Scheme 3.2 Synthesis of mannose tetrasaccharide in a manner to mimic automated synthesizer.

Glycosylation and deprotection steps were repeated to obtain di-, tri- and tetrasaccharides respectively (Scheme 3.2). The HPLC trace of all the compounds obtained after FSPE purification shows high purity in these reactions.

After successfully achieving the synthesis of linear mannose tetrasaccharide, we put our attention towards synthesizing branched mannose oligosaccharides. To extend the scope of this method, we chose two 3- and 6-modified branched mannose oligosaccharides (Figure 3.2).

To synthesize the building block for 3- and 6-modified mannose oligosaccharides, various combinations of protecting groups were explored based on their orthogonality. It is reported in the literature that acetyl group can be selectively removed in presence of pivaloyl. We designed a scheme where we used acetyl group at 3-position and pivaloyl at

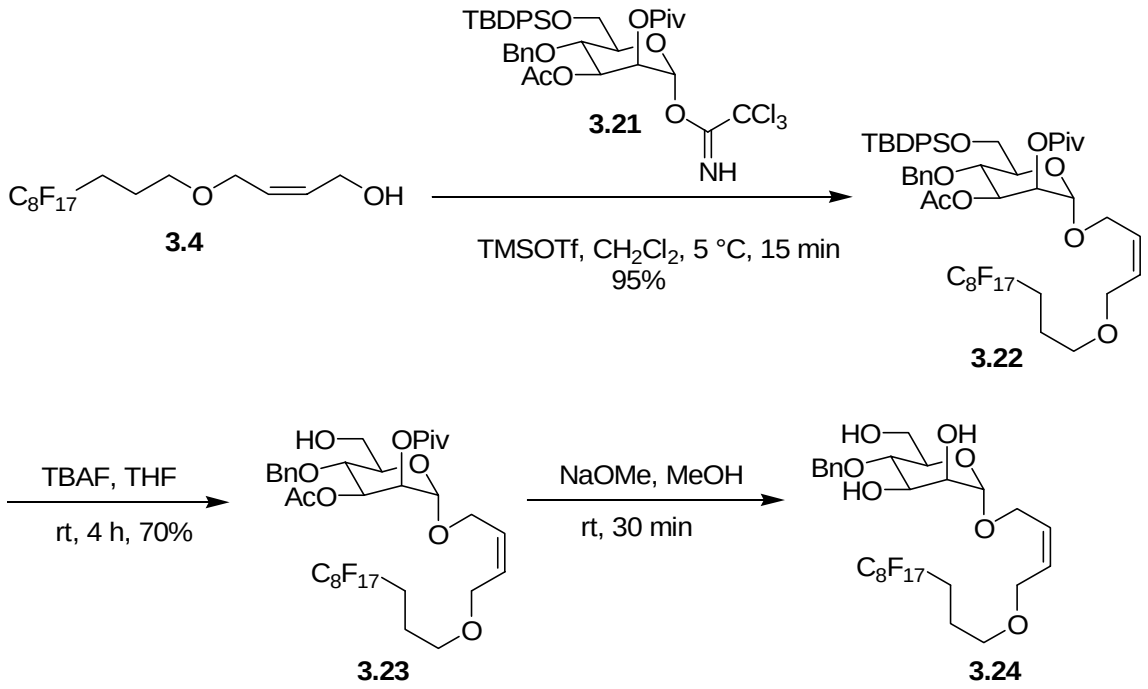
2-postion to construct the desired linkages (Scheme 3.3). Since equatorial hydroxyl group is more acidic than axial, the 3-position hydroxyl group in compound 3.14 was selectively acetylated. Compound 3.17 was obtained after performing standard protection and deprotection steps. At this stage 6-position hydroxyl group was selectively silylated with TBDPSCl to give compound 3.18. Acid-catalyzed benzylation, microwave-assisted cleavage of allyl group followed by trichloroacetimidate reaction gave the desired sugar donor 3.21.



Scheme 3.3 Synthesis of donor for 3, and 6-modified mannose oligosaccharides.

Now glycosylation of fluorous alcohol with donor 3.21 was performed in dichloromethane using catalytic amount of TMSOTf (Scheme 3.4). The coupling reaction worked well

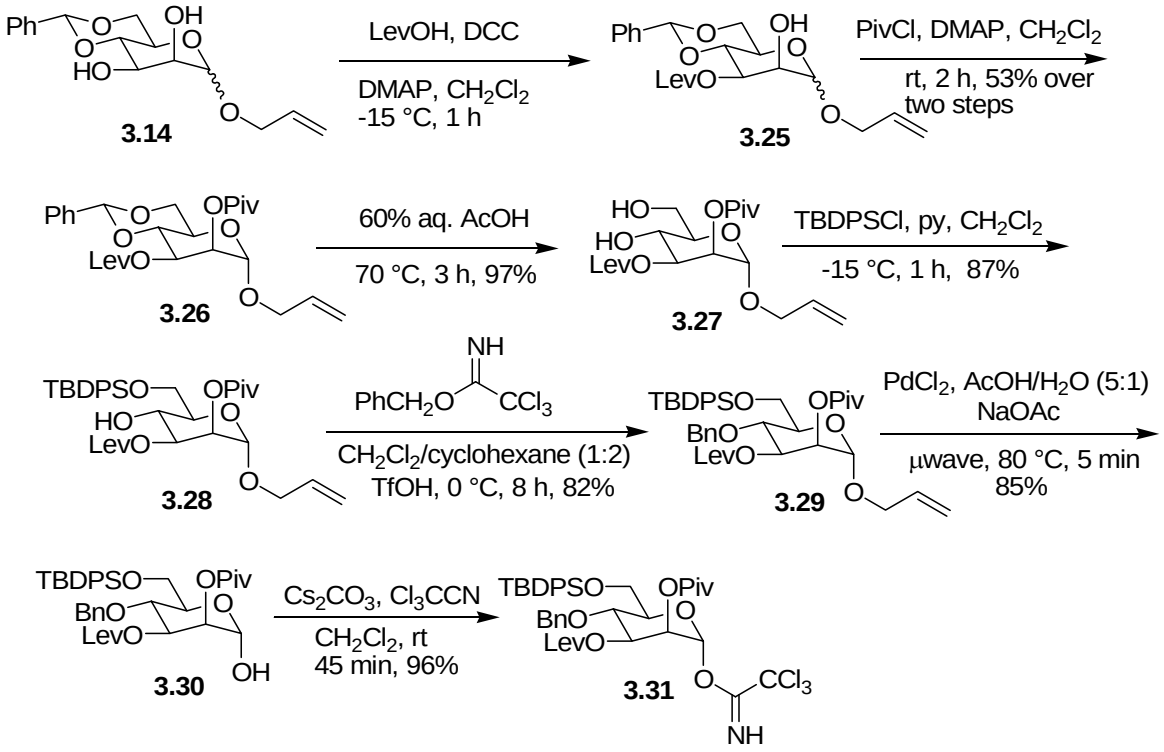
resulting in 95% yield of the glycosylated product. The product was purified by passing through FSPE column. Deprotection of TBDPS group with 1 M TBAF gave compound 3.23 in 70% yield. Recently Seeberger reported the synthesis of Leishmania cap tetrasaccharide where he selectively deprotects acetyl group in the presence of pivaloyl using NaOMe/MeOH. These conditions were tried on compound 3.23 to get selective deacetylation but after only 30 min the reaction resulted in the cleavage of both acetyl and pivaloyl group.



Scheme 3.4 Synthesis of fluorous-tagged 3- and 6-modified mannose oligosaccharide.

Numerous attempts to selectively remove acetyl group using $Mg(OMe)_2$, guanidine, and AcCl/MeOH were unsuccessful. So we decided to change the protecting group at 3-position to levulinyl (Scheme 3.5). Selective protection of 3-position hydroxyl group with

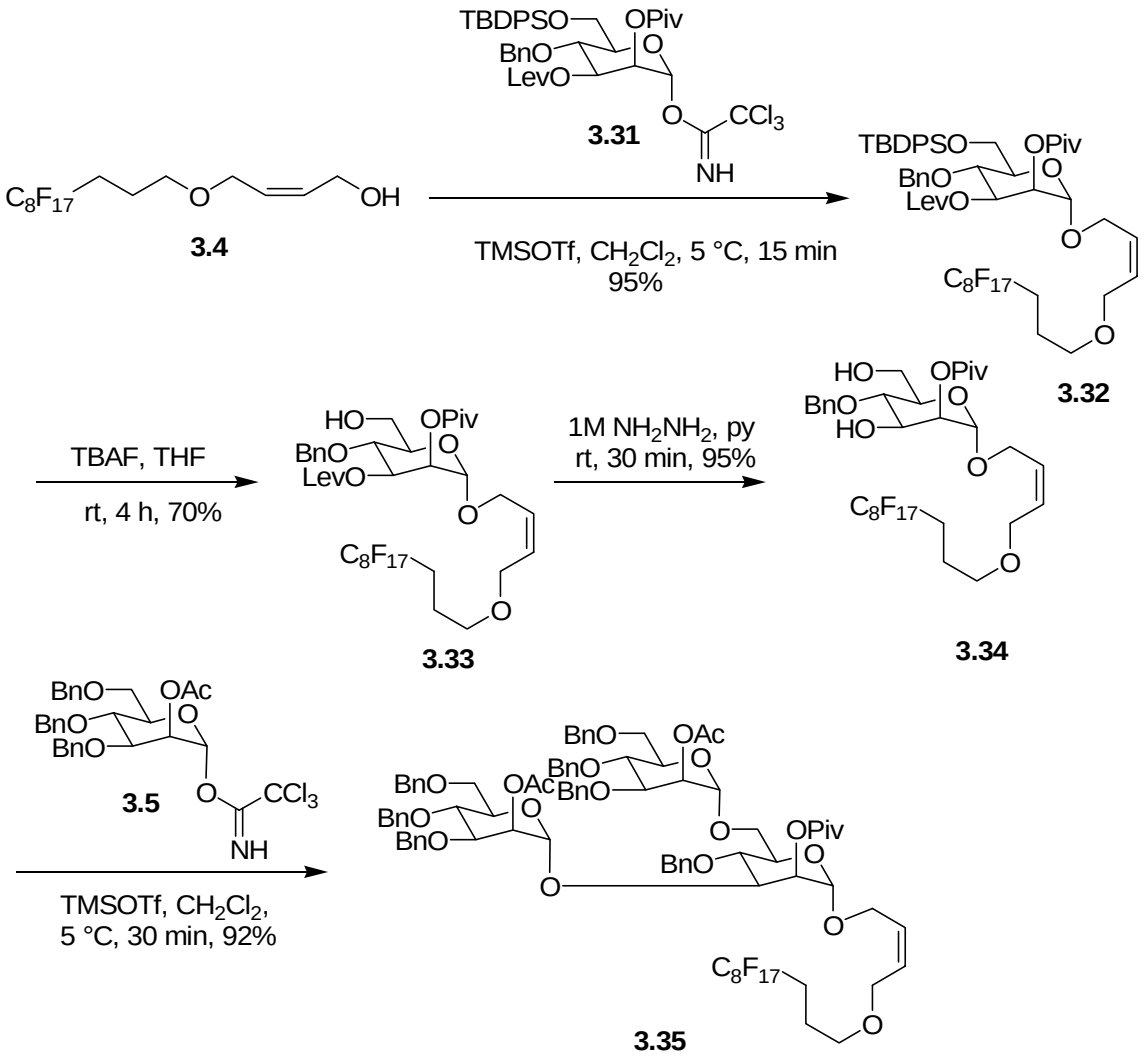
levulinic acid resulted in compound 3.25 in 90% yield as a mixture of anomers. Pivaloylation of 2-position hydroxyl group produced compound 3.26 in 75% yield. Cleavage of benzylidine followed by selective protection of 6-position hydroxyl group with TBDPS gave compound 3.28. Benzylation at 4-position, deprotection of allyl group followed by trichloroacetimidate reaction produced the desired mannose donor 3.31 in good yield.



Scheme 3.5 A new 3- and 6-modified mannose donor.

Coupling of the new donor and fluorous alcohol was performed as described earlier. The resulting product 3.32 was desilylated with 1 M TBAF to produce compound 3.33 in 70% yield. Treatment of compound 3.33 with NH_2NH_2 (1 M in pyridine/acetic acid, 3:2) in

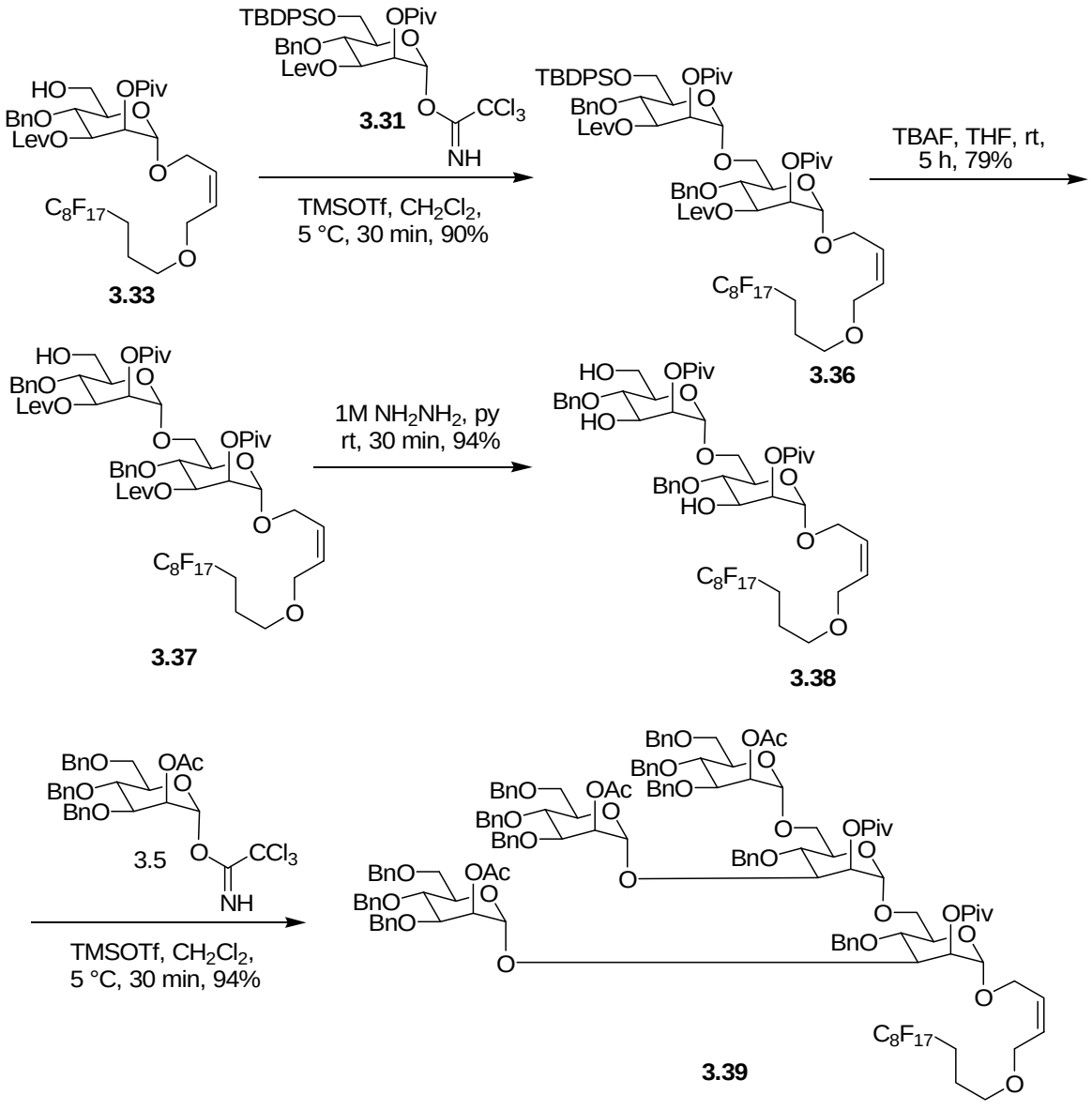
pyridine for 30 min gave the desired product 3.34 in 95% yield. Coupling of fluorous-tagged mannose 3.34 with mannose trichloroactimidate 3.5 gave trimannoside 3.35 in 92% yield (Scheme 3.6).



Scheme 3.6 Synthesis of 3,6-branched trimannosaccharide unit.

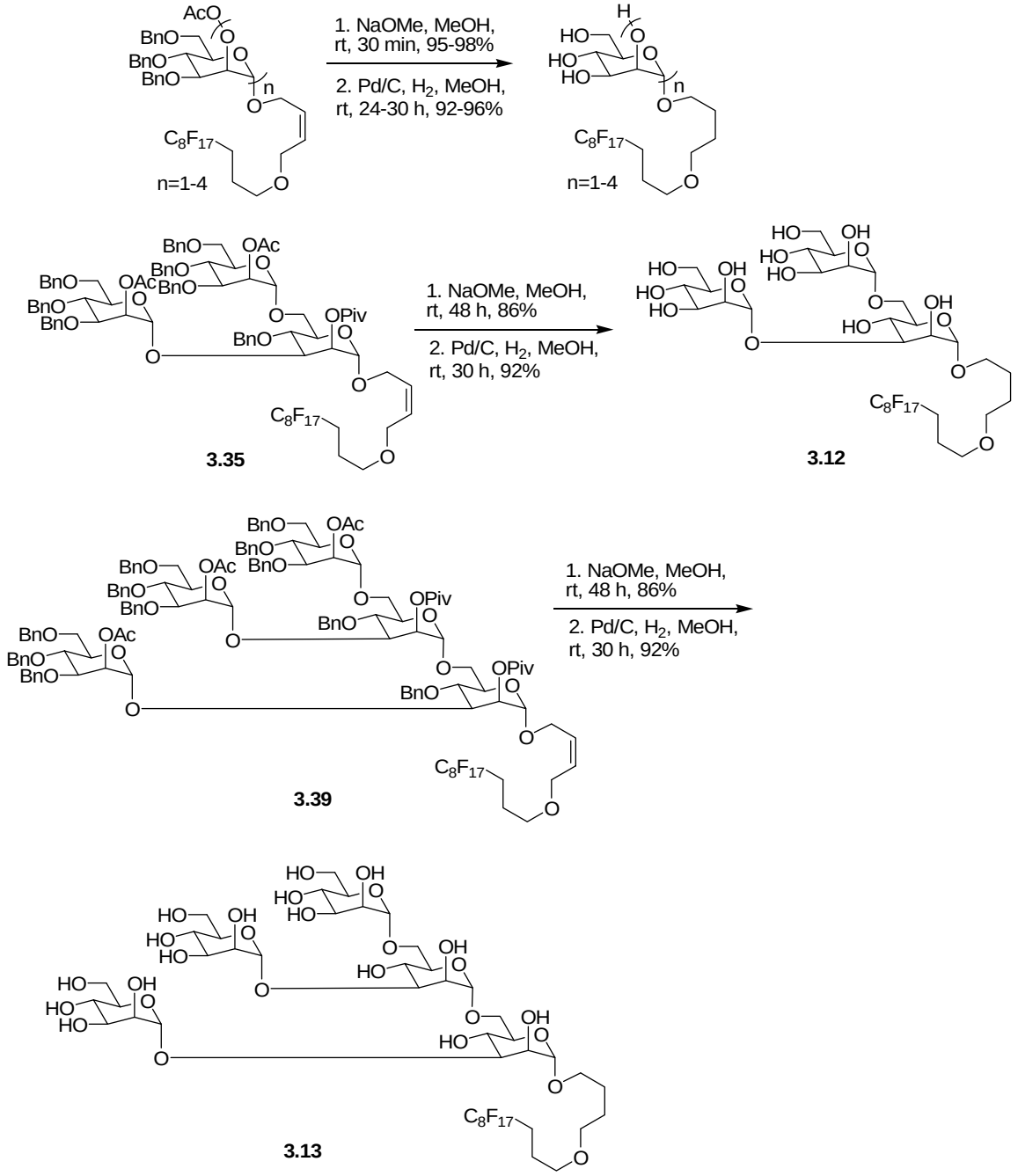
Mannose donor 3.31 was also used to synthesize pentamannoside (Scheme 3.7). Fluorous-tagged mannose 3.33 was coupled with another mannose unit to make mannose α-(1→6)-

disaccharide 3.36. Removal of 6-position TBDPS followed by deprotection of two levulinyl groups gave compound 3.38. Tri-glycosylation of compound 3.38 with mannose trichloroacetimidate 3.5 resulted in the formation of mannose pentasaccharide 3.39 in 90% yield.



Scheme 3.7 Synthesis of pentamannoside.

Deprotection of acetyl and pivaloyl groups followed by global deprotection of benzyl groups resulted in the formation of fully deprotected fluorous-tagged linear and branched mannose oligosaccharides (Scheme 3.8).



Scheme 3.8 Synthesis of fully deprotected linear and branched oligosaccharides.

Synthesis of Heptomannose

In order to synthesize both diastereomers of fluorous-tagged heptomannose compounds, we found several methods in the literature (Gurjar 2004, Van Boom 1989, Oscarson 2000, Crich 2005). We approached the synthesis of heptomannose building block using the method published by Crich and coworkers that gives a mixture of D-*glycero*-D-*manno* heptose and L-*glycero*-D-*manno* heptose in 3:1 ratio. The principal disconnections for the retrosynthetic analysis used are illustrated in figure 3.4.

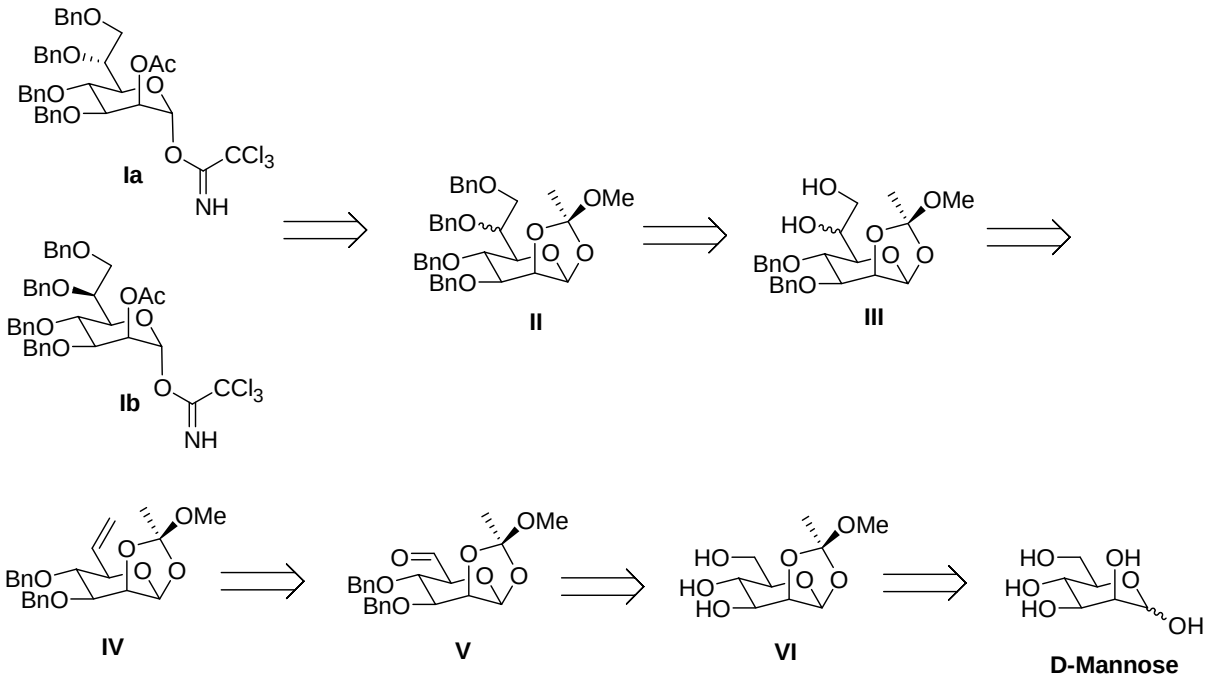
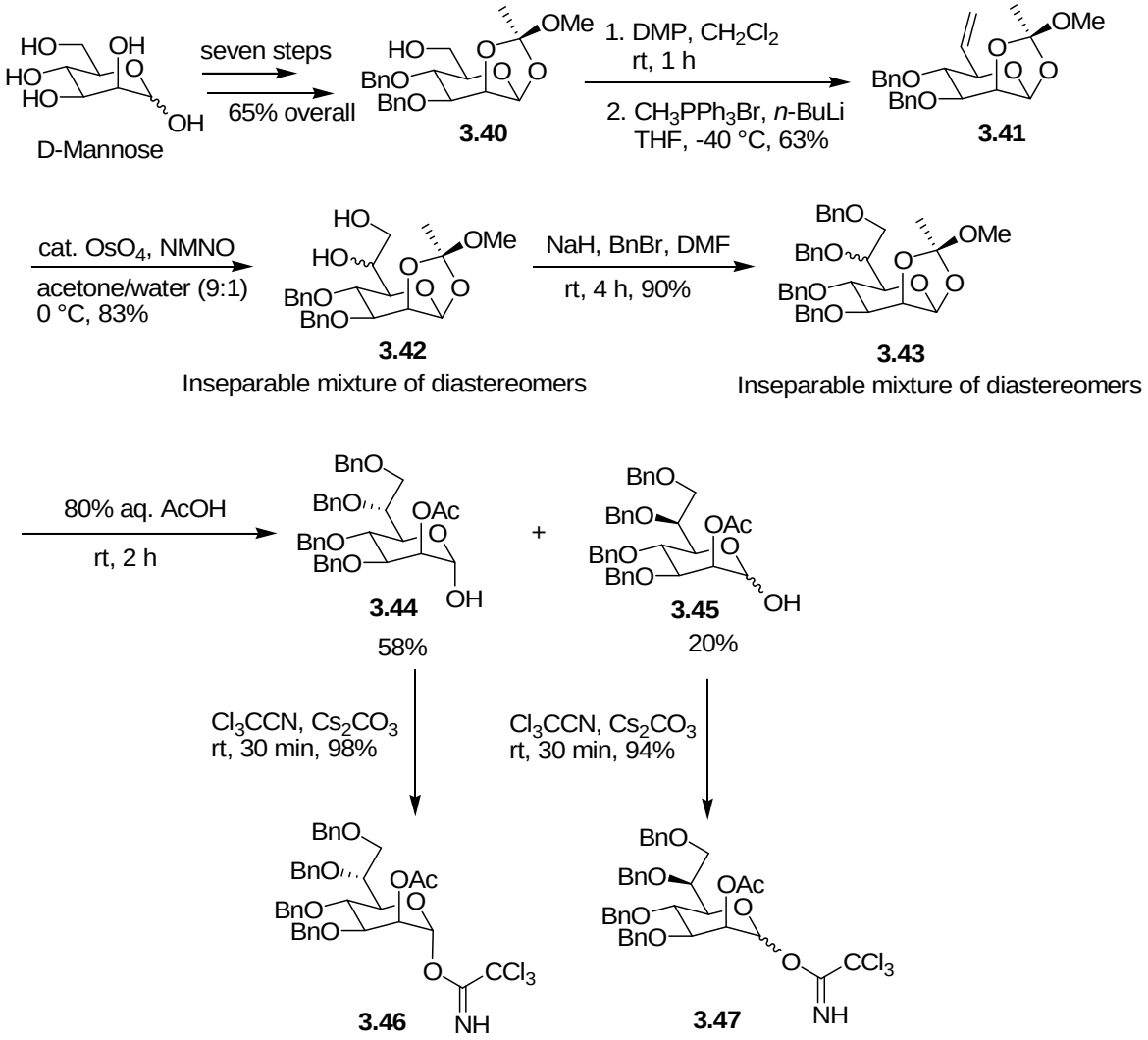


Figure 3.4 Retrosynthetic analysis for D-*glycero*-D-*manno* heptose and L-*glycero*-D-*manno* heptose.

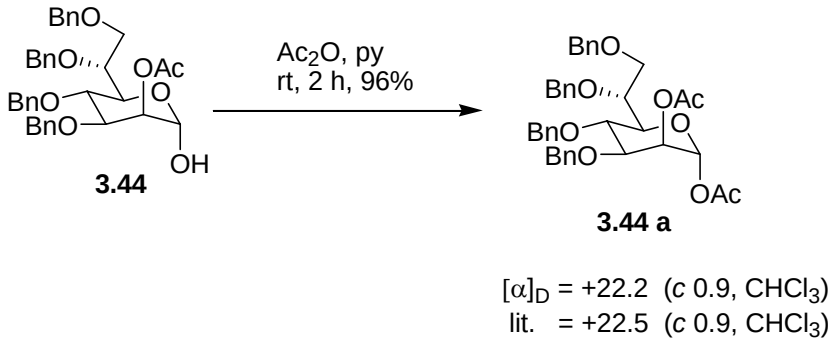
Trichloroacetimidate 1 could be obtained from orthoester 2 by opening of the orthoester, separation of diastereomers followed by trichloroacetimidate formation. Compound 2 could be made by benzylation of compound 3. The key reaction in this strategy is dihydroxylation of compound 4 to obtain compound 3 as a mixture of diastereomers. Compound 4 could be obtained by Wittig reaction of aldehyde 5, which could be made from D-mannose via orthoester 6.



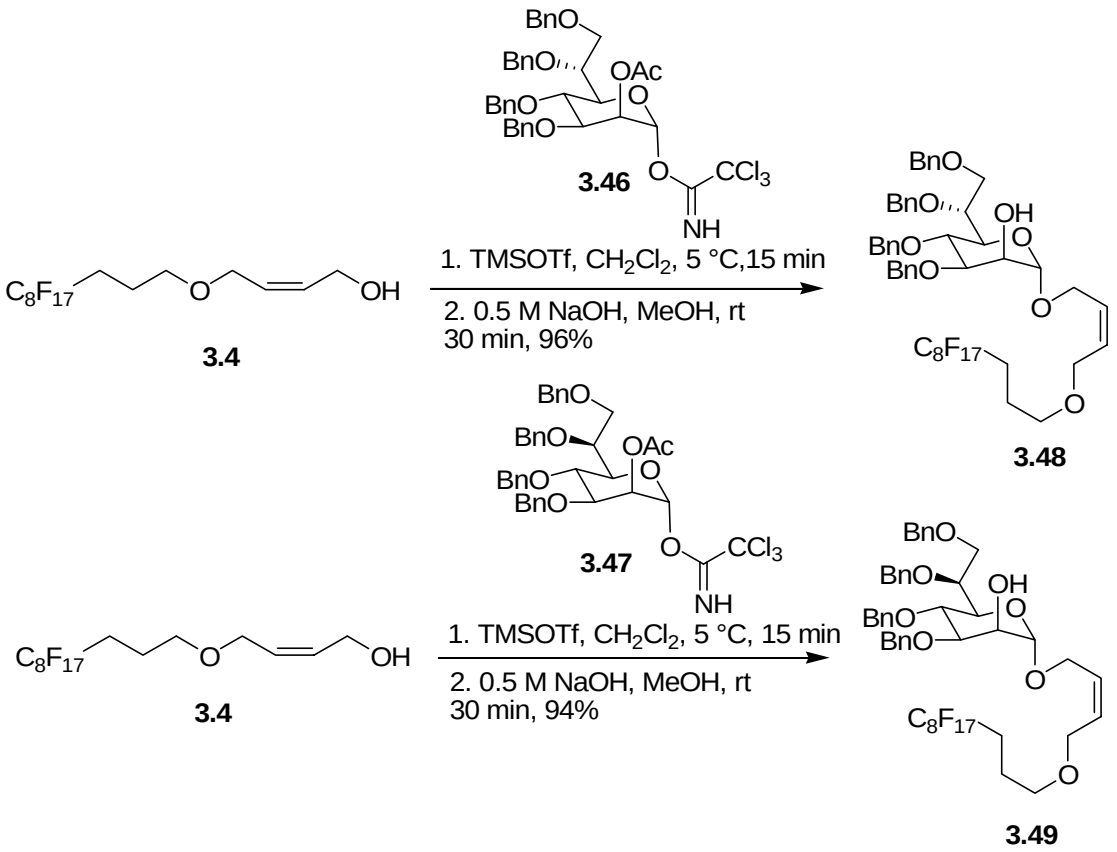
Scheme 3.9 Synthesis of *D*-glycero-*D*-manno heptopyranoside and *L*-glycero-*D*-manno heptopyranoside donors.

Compound 3.40 was synthesized from D-mannose in seven steps and 65% overall yield (Scheme 3.9). Various methods like PCC, Swern, Parikh-Doering were tried for the oxidation of 6-position hydroxyl groups but they all gave poor yield of the desired aldehyde. Dess-Martin reagent used for the oxidation gave good yield of the desired aldehyde. The crude aldehyde was used directly for Wittig reaction to give alkene 3.41 in 90% yield over two steps. Osmylation of alkene 3.41 gave a 3:1 mixture of diasteromers in favor of *R* configuration at the newly generated stereocenter, which is in accordance with Kishi's empirical rule. Benzylation of compound 3.42 followed by the opening of orthoester resulted in compounds 3.44 and 3.45 respectively. These two compounds were converted to the corresponding trichloroacetimidate donors 3.46 and 3.47 respectively.

To verify the stereochemistry of the major diastereomer **3.44**, it was converted into the known compound **3.48** {$[\alpha]_D$ = +22.2 (*c* 0.9, $CHCl_3$); lit. (Gurjar 2004) $[\alpha]_D$ = +22.5 (*c* 0.9, $CHCl_3$)} (Scheme 3.10). These studies indirectly ensured the stereochemical assignment of compound **3.45** as indicated.

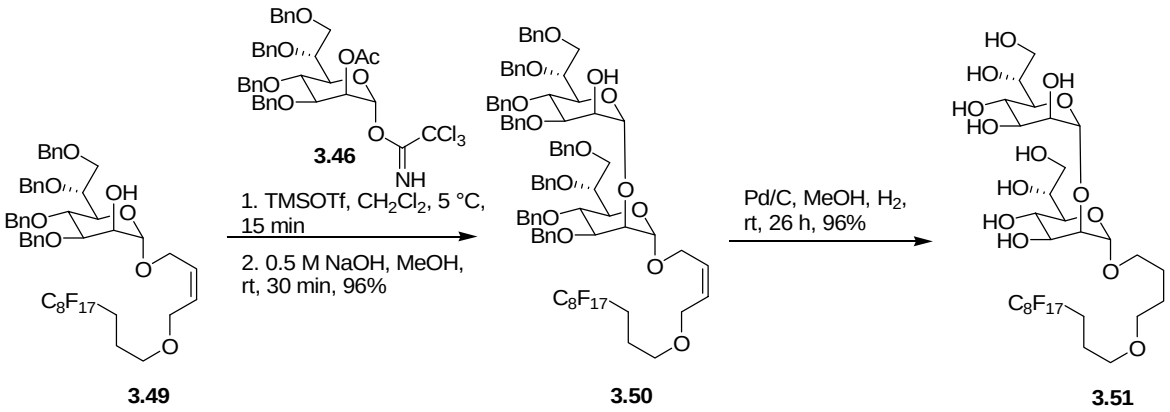


Scheme 3.10 Confirmation of stereochemistry at C-6 position.



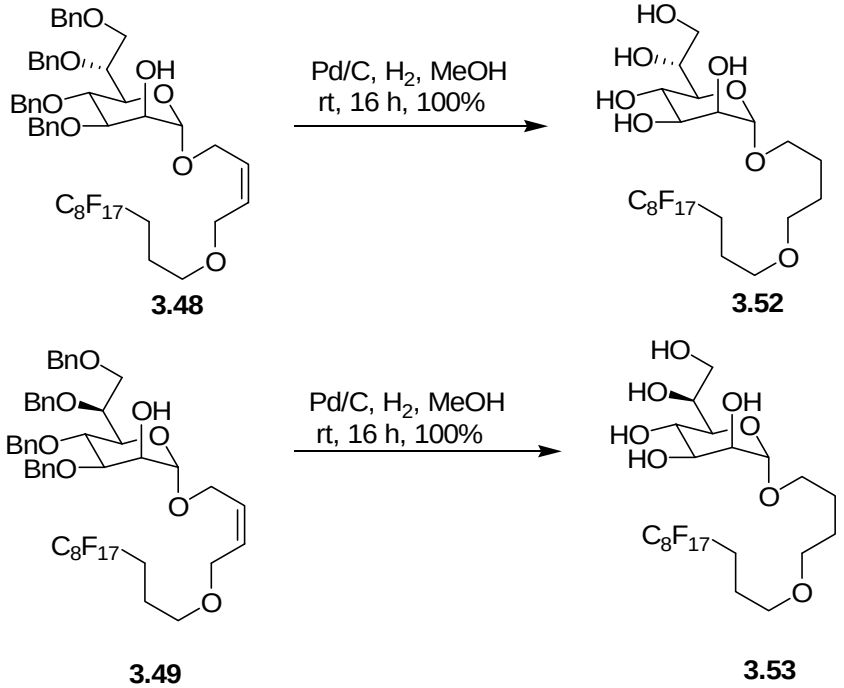
Scheme 3.11 Synthesis of fluorous-tagged D-*glycero*-D-*manno* heptopyranoside and L-*glycero*-*D*-*manno* heptopyranoside.

Next step was to try glycosylation to make fluorous-tagged heptomannose compounds. Both the donors were separately coupled with fluorous alcohol 3.4 to produce the desired fluorous-tagged heptomannose compounds (Scheme 3.11). To show that the chain could be elongated to make disaccharide, compound 3.48 was deacetylated and coupled with another heptomannose donor 3.46 to make linear α-(1→2) heptomannose disaccharide (Scheme 3.12).



Scheme 3.12 Synthesis of Heptomannose disaccharide.

Deprotection of acetyl groups followed by global deprotection to remove benzyl groups resulted in the formation of fully deprotected fluorous-tagged heptomannose compounds 3.52 and 3.53 respectively (Scheme 3.13).



Scheme 3.13 Synthesis of fully deprotected fluorous-tagged heptomannose.

Carbohydrate Microarrays

We have reported earlier that monosaccharides and disaccharides can be successfully immobilized on the fluorous-coated glass slides. Now we focused our attention to immobilize higher oligomers such as trisaccharides, tetrasaccharides, etc. onto a fluorous-coated glass slide and study their binding with Con A. Con A, the mannose-specific lectin has long been used as a model for carbohydrate-protein interactions. Its commercial availability and knowledge of 3D structure make it attractive for assessing and optimizing the functional parameters that affect its affinity for mannose. Understanding these key elements may facilitate the development of new therapeutic strategies based on specific recognition events such as targeting of drugs.

Fluorous-tagged mannose oligosaccharides and heptomannose monosaccharides and disaccharide were spotted onto a fluorous-coated glass slide using a microarrayer. The glass slide was dried and then incubated with FITC labeled Con A containing 1% BSA for 30 min. The glass slide was then washed with HEPES buffer containing 0.05% TWEEN-20, dried for 30 min in a dark chamber. The glass slide was scanned with a laser at 488 nm. The results of the scans are shown in figure 3.4.

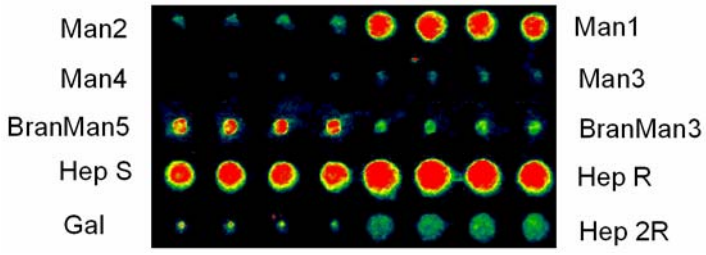


Figure 3.4 Scan of fluorous-tagged carbohydrate microarray incubated with FITC-labeled Con A at 488 nm (Data provided by Dr. Beatrice Collet).

It is evident from the scan that the noncovalent fluorous-fluorous interactions are strong enough to retain higher oligomers of mannose onto the glass slide. The most important information revealed from this experiment is that seven-carbon mannose also binds to Con A that was not known previously.

Conclusions

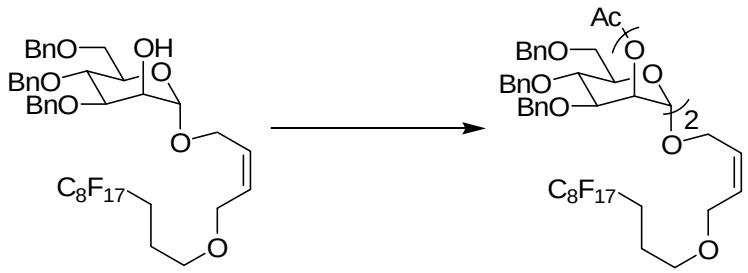
In summary a library of fluorous-tagged mannose and heptomannose oligosaccharides were synthesized using a C_8F_{17} fluorous support. Fluorous support was used in a manner as to mimic automated synthesis of oligosaccharides. Separation of fluorous-tagged compounds from non-fluorous was performed by simple passing the reaction mixture through fluorous solid-phase extraction column (FSPE). Direct formation of microarrays of the higher mannose oligosaccharides and heptomannose mono- and di-saccharides to test if noncovalent fluorous-fluorous interactions are strong enough to retain higher oligomers of mannose onto the glass slide were fruitful. The study of binding interactions between both diastereomers of *glycero*-D-*manno*-heptoses and Con A revealed that Con A has affinity towards C-6 modified mannose.

Experimental

All reagents were purchased from Aldrich. Dichloromethane was distilled over calcium hydride THF and toluene was used from solvent purification towers. Amberlyst 15 ion-exchange resin was washed repeatedly with methanol before use. All other commercial reagents and solvents were used as received without further purification. The reactions

were monitored and the R_f values determined using analytical thin layer chromatography (tlc) with 0.25 mm EM Science silica gel plates (60F-254). The developed TLC plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Flash chromatography was performed with Selecto Scientific silica gel, 32-63 μm particle sizes. Fluorous phase chromatography was performed using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies Inc.; Pittsburgh, PA). All other Fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated.

^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker DRX400 and Varian VXR300. ^{1}H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (7.27 ppm) or CD_3OD (4.80) as an internal reference. ^{13}C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ (77.23 ppm) or CD_3OD (49.15 ppm). A Shimadzu LCMS 2010 quadrupole mass spectrometer (Shimadzu Scientific Instruments, Columbia, MD) equipped with and electronspray ionization (ESI) source was used in positive ion mode.



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

A solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (150 mg, 0.153 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetimidate (146 mg, 0.229 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (6 µL, 0.031 mmol) was added. The reaction mixture was stirred for 30 min. The reaction mixture was quenched with triethylamine (30 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain mannose disaccharide (214 mg, 0.147 mmol, 96%) as a yellow gel.

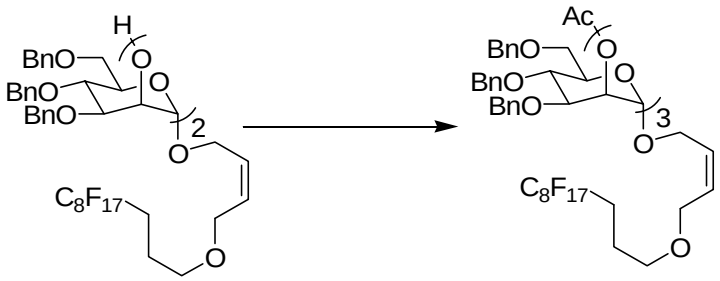
R_f: 0.77 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.79-1.86 (m, 2 H), 2.09-2.21 (m, 2 H), 2.16 (s, 3 H), 3.38 (t, 2 H, *J* = 6 Hz), 3.71-4.21 (m, 14 H), 4.43-4.70 (m, 12 H), 4.88 (dd, 1 H, *J* = 3.6, 10.8 Hz), 4.94 (s, 1 H), 5.11 (s, 1 H), 5.57 (s, 1 H), 5.62-5.69 (m, 2 H), 7.16-7.36 (m, 30 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.87, 21.27, 28.06, 62.77, 66.54, 68.72, 68.79, 62.84, 69.49, 69.57, 71.95, 71.97, 72.01, 72.19, 72.31, 73.32, 73.40, 73.44, 73.54, 74.31, 74.81, 74.91, 74.99, 75.04, 75.15, 75.22, 75.27, 77.35, 78.21, 79.62, 98.21, 100.74, 127.53, 127.55, 127.58, 127.61, 127.64, 127.68, 127.71, 127.77, 127.85, 127.93, 128.02, 128.06,

128.14, 128.17, 128.26, 128.30, 128.32, 128.34, 128.39, 128.42, 128.43, 128.51, 129.77, 138.11, 138.25, 138.34, 138.41, 138.44, 138.45, 138.52, 138.59, 138.62, 170.25.

MS (ESI): 1477 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*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

A solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (150 mg, 0.106 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetimidate (101 mg, 0.159 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (4 µL, 0.021 mmol) was added. The reaction mixture was stirred for 30 min. The reaction mixture was quenched with triethylamine (30 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under

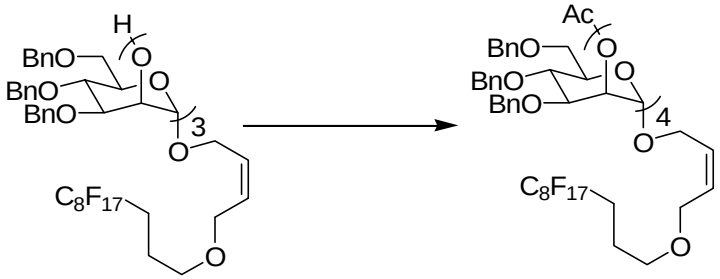
reduced pressure to obtain mannose trisaccharide (189 mg, 0.100 mmol, 94%) as a yellow gel.

R_f: 0.79 (EtOAc/hexane, 1/1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.79-1.86 (m, 2 H), 2.05-2.21 (m, 2 H), 2.15 (s, 3 H), 3.37 (t, 2 H, *J* = 6 Hz), 3.55 (d, 1 H, *J* = 10.8 Hz), 3.65-4.02 (m, 17 H), 4.13-4.17 (m, 2 H), 4.33 (d, 1 H, *J* = 12 Hz), 4.43-4.72 (m, 14 H), 4.83-4.90 (m, 3 H), 4.97 (s, 1 H), 5.08 (s, 1 H), 5.23 (s, 1 H), 5.26 (s, 1 H), 5.57 (s, 1 H), 5.62-5.65 (m, 2 H), 7.17-7.35 (m, 45 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.87, 21.27, 28.06, 62.77, 66.54, 68.72, 68.79, 62.84, 69.49, 69.57, 71.95, 71.97, 72.01, 72.19, 72.31, 73.32, 73.40, 73.44, 73.54, 74.31, 74.81, 74.91, 74.99, 75.04, 75.15, 75.22, 75.27, 77.35, 78.21, 79.62, 98.21, 100.74, 127.53, 127.55, 127.58, 127.61, 127.64, 127.68, 127.71, 127.77, 127.85, 127.93, 128.02, 128.06, 128.14, 128.17, 128.26, 128.30, 128.32, 128.34, 128.39, 128.42, 128.43, 128.51, 129.77, 138.11, 138.25, 138.34, 138.41, 138.44, 138.45, 138.52, 138.59, 138.62, 170.25.

MS (ESI): 1909 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[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)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside

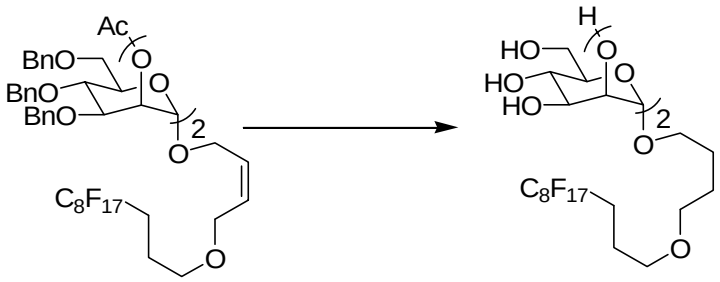
A solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (150 mg, 0.081 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetimidate (77 mg, 0.122 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (3 μL, 0.016 mmol) was added. The reaction mixture was stirred for 30 min. The reaction mixture was quenched with triethylamine (30 μL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain mannose trisaccharide (183 mg, 0.079 mmol, 97%) as a yellow gel.

R_f: 0.80 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.78-1.86 (m, 2 H), 2.05-2.21 (m, 2 H), 2.15 (s, 3 H), 3.36 (t, 2 H, *J* = 5.6 Hz), 3.49-3.79 (m, 11 H), 3.85-4.02 (m, 14 H), 4.12-4.19 (m, 4 H), 4.35 (d, 1 H, *J* = 2.4 Hz), 4.37 (d, 1 H, *J* = 3.2 Hz), 4.39-4.89 (m, 22 H), 4.99 (s, 1 H), 5.07 (s, 1 H), 5.20 (s, 1 H), 5.26 (d, 1 H, *J* = 3.6 Hz), 5.57-5.66 (m, 3 H), 7.17-7.38 (m, 60 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.81, 21.28, 29.79, 62.77, 66.54, 68.70, 68.75, 69.52, 69.56, 69.65, 71.62, 71.80, 71.87, 71.96, 71.99, 72.22, 72.34, 73.33, 73.43, 73.55, 74.35, 74.75, 74.91, 75.04, 75.15, 75.23, 75.30, 75.58, 77.34, 78.24, 79.48, 98.21, 99.49, 100.85, 101.27, 127.27, 127.52, 127.54, 127.60, 127.64, 127.67, 127.73, 127.77, 127.82, 127.93, 128.03, 128.12, 128.22, 128.26, 128.31, 128.37, 128.38, 128.41, 128.51, 128.64, 128.70, 129.69, 138.13, 138.21, 138.37, 138.41, 138.44, 138.48, 138.50, 138.56, 138.61, 138.68, 170.22.

MS (ESI): 2341 $(M+Na)^+$



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

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 (200 mg, 0.138 mmol) in methanol (4 mL) was added 0.5 M NaOMe (0.55 mL, 0.276 mmol) and the reaction mixture was stirred at ambient temperature for 30 min. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide deacetylated mannose (195 mg, 0.138 mmol, 100%) as yellow oil. 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 (195 mg, 0.138 mmol) in methanol (3 mL) was added 10% Pd/C (60 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 24 h. The reaction mixture was then filtered

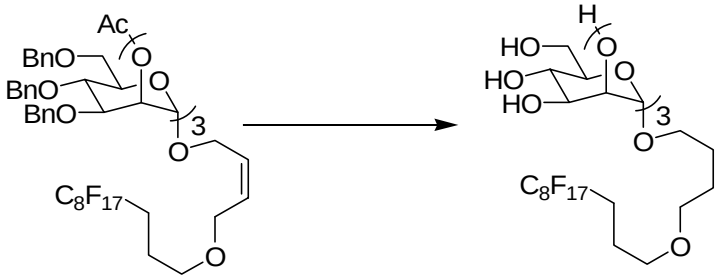
over Celite and the solvent was removed under reduced pressure to provide (121 mg, 0.138 mmol, 100%) as a white solid.

R_f: 0.68 (methanol/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.60-1.65 (m, 4 H), 1.78-1.86 (m, 2 H), 2.14-2.29 (m, 2 H), 3.45-3.79 (m, 17 H), 3.95 (s, 1 H), 4.93 (s, 1 H), 5.03 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 19.11, 20.50, 25.96, 26.23, 61.68, 66.99, 67.42, 67.67, 68.73, 70.34, 70.50, 70.83, 71.01, 73.24, 73.30, 73.63, 79.35, 98.55, 102.83.

MS (ESI): 875 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*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 (200 mg, 0.106 mmol) in methanol (4 mL) was added 0.5 M NaOMe (0.42 mL, 0.212 mmol) and the reaction mixture was stirred at ambient temperature for 30 min. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent

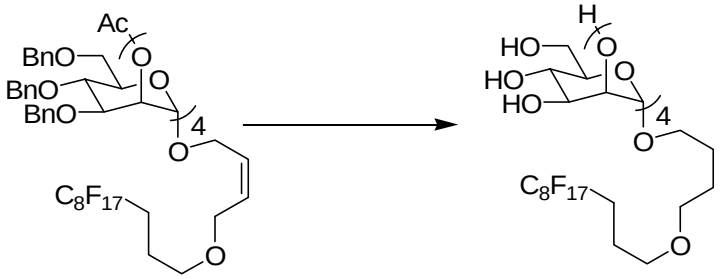
was removed under reduced pressure to provide deacetylated mannose (196 mg, 0.106 mmol, 100%) as yellow oil. To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (196 mg, 0.106) in methanol (3 mL) was added 10% Pd/C (60 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 30 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide (110 mg, 0.106 mmol, 100%) as a white solid.

R_f: 0.63 (methanol/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.60-1.65 (m, 4 H), 1.78-1.86 (m, 2 H), 2.14-2.25 (m, 2 H), 3.45-3.84 (m, 22 H), 3.95 (s, 1 H), 4.01 (s, 1 H), 4.95 (s, 1 H), 5.03 (s, 1 H), 5.25 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.50, 25.97, 26.23, 61.65, 61.75, 61.90, 67.01, 67.41, 67.66, 67.86, 68.73, 70.34, 70.51, 70.55, 70.83, 71.04, 73.25, 73.60, 78.95, 79.49, 98.51, 101.12, 102.70.

MS (ESI): 1037 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside

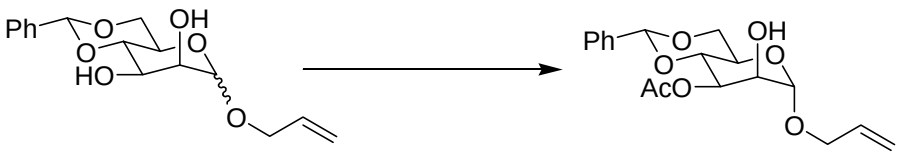
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (200 mg, 0.086 mmol) in methanol (4 mL) was added 0.5 M NaOMe (0.34 mL, 0.172 mmol) and the reaction mixture was stirred at ambient temperature for 30 min. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide deacetylated mannose (195 mg, 0.086 mmol, 100%) as yellow oil. To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (195 mg, 0.086 mmol) in methanol (3 mL) was added 10% Pd/C (60 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 30 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide (103 mg, 0.086 mmol, 100%) as a white solid.

R_f: 0.58 (methanol/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.60-1.65 (m, 4 H), 1.80-1.86 (m, 2 H), 2.16-2.25 (m, 2 H), 3.32 (s, 2 H), 3.44-3.79 (m, 26 H), 3.95-3.99 (m, 2 H), 4.96 (s, 1 H), 5.03 (s, 1 H), 5.25 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 12.88, 20.50, 25.97, 26.23, 27.24, 27.45, 27.64, 48.49, 60.05, 61.64, 61.86, 61.95, 67.02, 67.31, 67.67, 67.83, 68.73, 70.34, 70.51, 70.55, 70.84, 71.03, 73.26, 73.57, 73.64, 78.82, 79.09, 79.49, 98.50, 101.02, 102.67.

MS (ESI): 1199 $(M+H)^+$



Synthesis of Allyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-hydroxy-α-D-mannopyranoside

Allyl-4,6-*O*-benzylidene-2,3-di-*O*-hydroxy-α/β-D-mannopyranoside (5.18 g, 16.8 mmol) was dissolved in dichloromethane (60 mL) and cooled to –15 ºC and pyridine (2.0 mL, 25.2 mmol) was added followed by acetyl chloride (1.32 mL, 18.5 mmol). The reaction mixture was stirred at –15 ºC for 1 h and quenched with water. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic layer was washed with HCl (2N, 50 mL), brine (50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 38% ethyl acetate/hexane to give the desired product as α-anomer (3.21 g, 9.17 mmol, 55%).

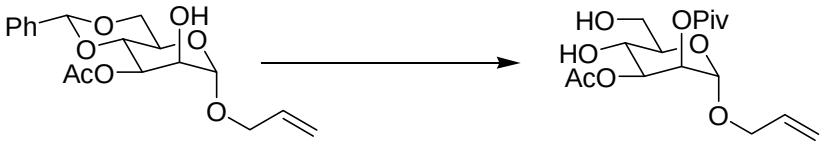
R_f: 0.37 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 2.08 (s, 3 H), 2.76 (br s, 1 H), 3.79-3.84 (m, 1 H), 3.91-4.00 (m, 2 H), 4.05-4.11 (m, 2 H), 4.15-4.4.20 (m, 1 H), 4.24 (dd, 1 H, *J* = 4.4, 10

Hz), 4.84 (s, 1 H), 5.20 (d, 1 H, J = 10.4 Hz), 5.26-5.33 (m, 2 H), 5.52 (s, 1 H), 5.82-5.91 (m, 1 H), 7.33-7.46 (m, 5 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 21.14, 63.94, 68.34, 68.82, 69.74, 70.83, 76.17, 99.57, 101.93, 118.03, 126.24, 128.30, 129.13, 133.40, 137.27, 170.18.

MS (ESI): 373 $(M+Na)^+$



Synthesis of Allyl-3-*O*-acetyl-4,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside

Allyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-hydroxy-α-D-mannopyranoside (2.24 g, 6.40 mmol) was dissolved in dichloromethane (40 mL) at room temperature. DMAP (1.56 g, 12.8 mmol) was added followed by pivaloyl chloride (0.95 mL, 7.68 mmol) and it was then stirred for 1 h. After the reaction was complete, ethyl acetate/hexane (1:3) (60 mL) was added. The white solid was filtered over celite and the filtrate was concentrated. A solution of crude Allyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-pivaloyl-α-D-mannopyranoside in 60% aq. AcOH (50 mL) was heated at 70 ºC for 3 h. It was then cooled down to room temperature and poured into water (50 mL). The water layer was extracted with ethyl acetate (2 x 200 mL). The combined organic layer was washed with sodium bicarbonate (3 x 80 mL) followed by brine (80 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column

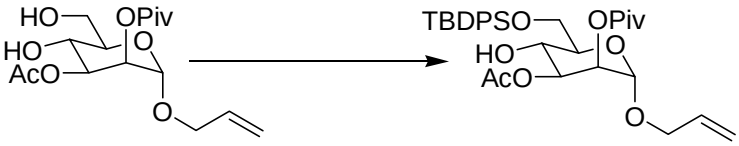
chromatography on silica gel using 45% ethyl acetate/hexane to yield of the desired product (1.81 g, 5.22 mmol, 91%) as white oil.

***R*$_f$** 0.15 (silica, 50% EtOAc/hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.19 (s, 9 H), 2.01 (s, 3 H), 2.18 (br s, 1 H), 2.74 (br s, 1 H), 3.69-3.73 (m, 1 H), 3.82-3.88 (m, 2 H), 3.94-4.00 (m, 2 H), 4.16 (dd, 1 H, *J* = 5 Hz, 13 Hz), 4.77 (s, 1 H, H-1), 5.16-5.30 (m, 4 H), 5.82-5.92 (m, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.88, 27.11, 39.04, 62.28, 66.42, 68.45, 69.47, 72.26, 72.39, 96.73, 118.12, 133.26, 171.01, 177.44.

MS (ESI): 348 $(M+H)^+$



Synthesis of Allyl-3-*O*-acetyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-2-*O*-pivaloyl-α-D-mannopyranoside

Allyl-3-*O*-acetyl-4,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside (1.47 g, 4.24 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 ºC. Imidazole (433 mg, 6.36 mmol) was added followed by TBDPSCl (1.10 mL, 4.24 mmol) and the reaction mixture was stirred at 0 ºC for 1 h. It was then diluted with ethyl acetate (200 mL) and the organic layer washed with water (30 mL), HCl (2N, 30 mL), and brine (30 mL). The solvent was removed under reduced pressure and the crude product was purified by flash column

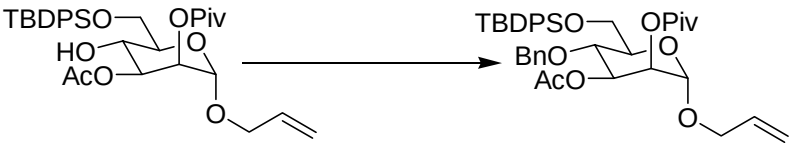
chromatography on silica gel using 6% ethyl acetate/dichloromethane to give the desired product (2.14 g, 3.66 mmol, 86%) as white oil.

R_f: 0.55 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.16 (s, 9 H), 2.05 (s, 3 H), 2.95 (br s, 1 H), 3.84-4.17 (m, 4 H), 4.28 (d, 1 H, *J* = 12 Hz), 4.45 (dd, 1 H, *J* = 3.6 Hz, 12 Hz), 4.81 (s, 1 H, H-1), 5.02-5.28 (m, 6 H), 5.79-5.88 (m, 1 H), 7.23-7.30 (m, 5 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.72, 27.01, 38.99, 63.09, 65.52, 68.54, 69.12, 70.03, 70.68, 75.78, 96.67, 118.11, 128.28, 128.33, 128.57, 128.59, 133.18, 135.00, 154.52, 171.40, 177.34.

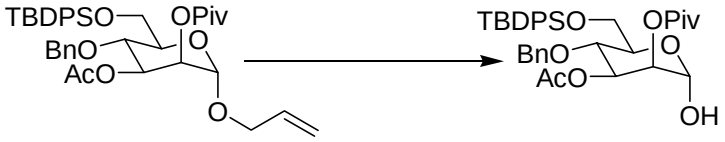
MS (ESI): 587 $(M+H)^+$



Synthesis of Allyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside

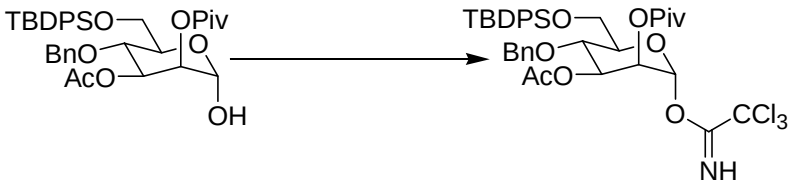
To a solution of allyl-3-*O*-acetyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-2-*O*-pivaloyl-α-D-mannopyranoside (1.67 g, 2.85 mmol) in dichloromethane (20 mL) and cyclohexane (10 mL) was added benzyltrichloroacetimidate (2.88 g, 11.4 mmol). The reaction mixture was cooled to 0 ºC and trfilic acid (0.13 mL, 1.43 mmol) was added. It was then stirred at the same temperature for 8 h and filtered over celite. The filtrate was washed with saturated sodium bicarbonate (30 mL), water (30 mL), and brine (30 mL), and dried over sodium

sulfate. The solvent was removed under reduced pressure and the crude product was subjected to the next step without further purification.



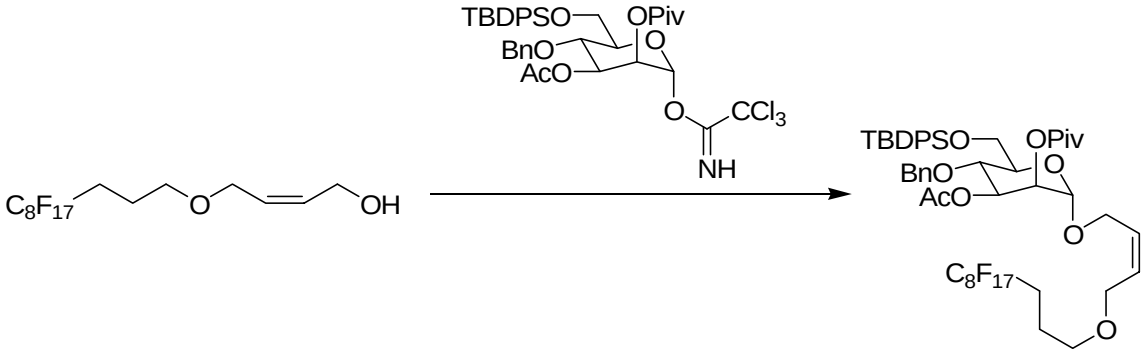
Synthesis of 3-*O*-Acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside

The crude allyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside (1.06 g, 1.57 mmol) was dissolved in a mixture of acetic acid (5 mL) and water (1 mL) (5:1). Sodium acetate (386 mg, 4.71 mmol) was added followed by palladium chloride (416 mg, 2.35 mmol) and the reaction mixture was heated in a commercial microwave oven at 100 W power and 80 ºC for 5 min. It was then filtered over celite and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with sodium bicarbonate (3 x 30 mL) followed by brine (50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 8% ethyl acetate/dichloromethane to give the desired lactol as a mixture of anomers.



Synthesis of 3-*O*-Acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α/β-D-mannopyranosyl trichloroacetimidate

To a solution of 3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside (229 mg, 0.360 mmol) in dichloromethane (4 mL) was added powdered 4 Å molecular sieves (100 mg) and trichloroacetonitrile (0.18 mL, 1.80 mmol) at room temperature. The reaction mixture was stirred for 10 min and cesium carbonate (129 mg, 0.396 mmol) was added and stirred further for 45 min. It was then filtered over celite and solvent was removed under reduced pressure. The crude product was directly used for glycosylation.



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of 3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α/β-D-mannopyranosyl trichloroacetimidate (165 mg, 0.212 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (77 mg, 0.141 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (13 µL, 0.071 mmol) was added. The reaction

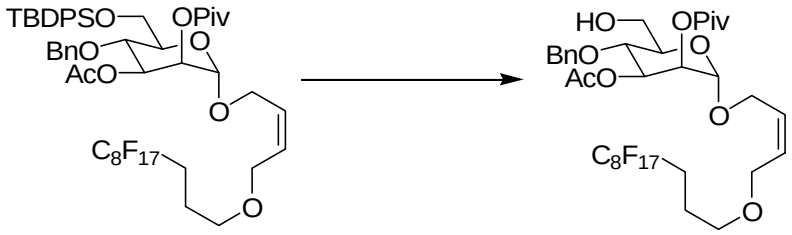
mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (40 μL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain Fluorous-tagged mannose (151 mg, 0.130 mmol, 92%) as a yellow gel.

R_f: 0.68 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.12 (s, 9 H), 1.26 (s, 9 H), 1.81-1.88 (m, 2 H), 1.93 (s, 3 H), 2.09-2.20 (m, 2 H), 3.40 (t, 2 H, *J* = 6 Hz), 3.78-4.18 (m, 7 H), 4.62-4.68 (m, 2 H), 4.81 (s, 1 H), 5.29 (d, 1 H, *J* = 1.6 Hz), 5.31-5.37 (m, 1 H), 5.68-5.72 (m, 2 H), 7.16-7.43 (m, 11 H), 7.73 (d, 2 H, *J* = 6.8 Hz), 7.77 (d, 2 H, *J* = 6.4 Hz).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 19.40, 20.86, 26.91, 27.16, 39.06, 62.66, 62.81, 66.56, 68.74, 69.86, 72.26, 72.52, 72.90, 74.96, 77.31, 96.76, 127.67, 127.78, 127.89, 128.45, 129.73, 129.80, 130.43, 133.13, 133.53, 135.67, 135.94, 138.10, 169.79, 177.60.

MS (ESI): 1189 $(M+Na)^+$

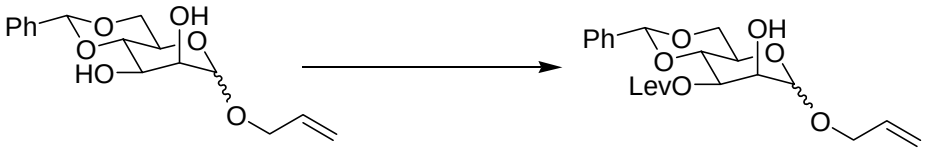


Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-hydroxy-2-*O*-pivaloyl-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside (137 mg, 0.118 mmol) in THF (3 mL) was added acetic acid (30 μL) followed by tetrabutylammonium fluoride (1.0 M in THF, 0.24 mL, 0.236 mmol). The reaction mixture was stirred for 10 h and then concentrated. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain the desilylated product (89 mg, 0.096 mmol, 82%) as a white gel.

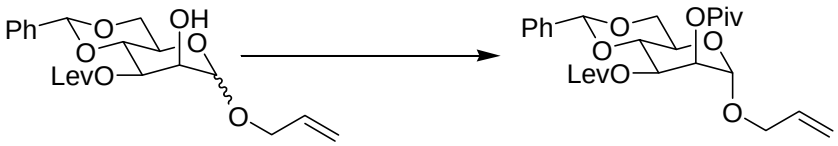
R_f: 0.47 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.21 (s, 9 H), 1.81-1.89 (m, 2 H), 1.56 (br s, 1 H), 1.90 (s, 3 H), 2.06-2.22 (m, 2 H), 3.46 (t, 2 H, *J* = 6 Hz), 3.73-3.90 (m, 4 H), 4.02 (d, 1 H, *J* = 4.8 Hz), 4.08 (dd, 1 H, *J* = 6, 13.8 Hz), 4.18 (dd, 1 H, *J* = 4.4, 12.4 Hz), 4.63 (s, 2 H), 4.74 (d, 1 H, *J* = 1.6 Hz), 5.19-5.20 (m, 1 H), 5.31 (dd, 1 H, *J* = 3.2, 9.6 Hz), 5.62-5.71 (m, 2 H), 7.23-7.31 (m, 5 H).



Synthesis of Allyl-4,6-*O*-benzylidene-2-hydroxy-3-*O*-levulinyl-α/β-D-mannopyranoside

Allyl-4,6-*O*-benzylidene-2,3-di-*O*-hydroxy-α/β-D-mannopyranoside (1.06 g, 3.44 mmol) was dissolved in dichloromethane (20 mL) and cooled to –15 ºC and DCC (779 mg, 3.78 mmol) was added followed by DMAP (84 mg, 0.688 mmol). Levulinic acid (0.39 mL, 3.78 mmol) was then added dropwise. The reaction mixture was stirred at –15 ºC for 1 h and filtered through celite. The filtrate was concentrated and the crude product was taken directly to the next step without further purification.



Synthesis of Allyl-4,6-*O*-benzylidene-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

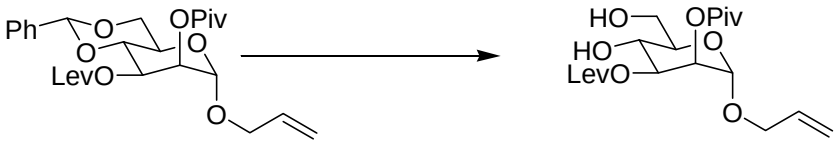
Allyl-4,6-*O*-benzylidene-2-*O*-hydroxy-3-*O*-levunilyl-α/β-D-mannopyranoside (1.0 g, 2.46 mmol) was dissolved in dichloromethane (20 mL) at room temperature. DMAP (600 mg, 4.92 mmol) was added followed by pivaloyl chloride (0.36 mL, 2.95 mmol) and it was then stirred for 1 h. After the reaction was complete, ethyl acetate/hexane (1:3) (60 mL) was added. The white solid was filtered over celite and the filtrate was concentrated. The crude product was purified by flash column chromatography on silica gel using 26% ethyl acetate/hexane to give the desired product as α-anomer (635 mg, 1.29 mmol, 53%).

R_f: 0.72 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.24 (s, 9 H), 2.06 (s, 3 H), 2.38-2.66 (m, 4 H), 3.73-3.81 (m, 1 H), 3.93-3.98 (m, 3 H), 4.12-4.25 (m, 2 H), 4.75 (d, 1 H, *J* = 1.2 Hz, H-1), 5.16 (dd, 1 H, *J* = 1.2 Hz, 10 Hz), 5.24-5.40 (m, 3 H), 5.54 (s, 1H), 5.81-5.88 (m, 1 H), 7.23-7.44 (m, 5 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 27.19, 27.85, 29.82, 37.87, 39.03, 63.85, 68.49, 68.83, 68.88, 69.80, 76.58, 97.66, 101.87, 118.12, 126.24, 128.25, 129.10, 133.17, 137.13, 171.70, 177.34, 206.24.

MS (ESI): 513 $(M+Na)^+$



Synthesis of Allyl-4,6-dihydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of allyl-4,6-*O*-benzylidene-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (635 mg, 1.29 mmol) in 60% aq. AcOH (20 mL) was heated at 70 ºC for 2 h. It was then cooled down to room temperature and poured into water (20 mL). The water layer was extracted with ethyl acetate (2 x 100 mL). The combined organic layer was washed with sodium bicarbonate (3 x 50 mL) followed by brine (50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 45% ethyl acetate/hexane to yield of the desired product (505 mg, 1.25 mmol, 97%) as white oil.

R_f: 0.22 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.14 (s, 9 H), 2.07 (s, 3 H), 2.30-2.73 (m, 4 H), 3.62-3.92 (m, 5 H), 4.10 (dd, 1 H, *J* = 4.4 Hz, 13 Hz), 4.69 (s, 1 H, H-1), 5.06-5.21 (m, 4 H), 5.71-5.80 (m, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 27.05, 27.87, 29.79, 37.99, 38.94, 62.14, 66.00, 68.18, 69.49, 72.28, 72.41, 96.64, 117.83, 133.30, 172.30, 177.43, 207.72.

MS (ESI): 403 $(M+H)^+$

Synthesis of Allyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

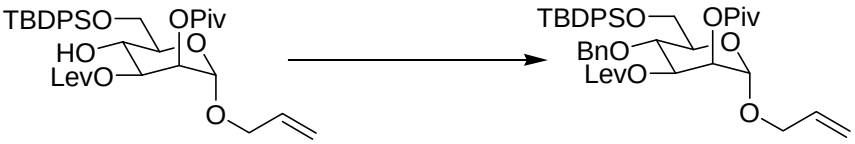
Allyl-4,6-dihydroxy-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (505 mg, 1.25 mmol) was dissolved in dichloromethane (8 mL) and cooled to 0 ºC. Imidazole (128 mg, 1.88 mmol) was added followed by TBDPSCl (0.35 mL, 1.38 mmol) and the reaction mixture was stirred at 0 ºC for 1 h. It was then diluted with ethyl acetate (100 mL) and the organic layer washed with water (30 mL), HCl (2N, 30 mL), and brine (30 mL). The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 6% ethyl acetate/dichloromethane to give the desired product (700 mg, 1.09 mmol, 87%) as white oil.

R_f: 0.52 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.07 (s, 9 H), 1.21 (s, 9 H), 2.11 (s, 3 H), 2.40-2.84 (m, 4 H), 3.16 (d, 1 H, *J* = 4 Hz), 3.75-3.77 (m, 1 H), 3.93-4.13 (m, 5 H), 4.81 (s, 1 H), 5.13 (d, 1 H, *J* = 10 Hz), 5.21-5.26 (m, 3 H), 5.79-5.85 (m, 1 H), 7.33-7.38 (m, 6 H), 7.72 (d, 4 H, *J* = 7 Hz).

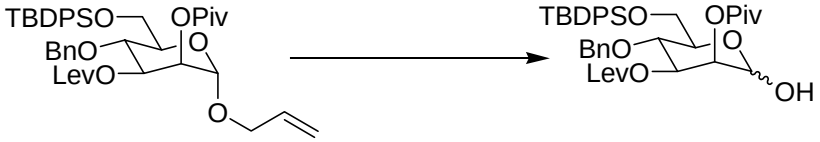
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 19.38, 26.91, 27.18, 28.04, 29.81, 38.11, 39.02, 63.72, 66.34, 67.98, 69.61, 72.57, 72.83, 96.65, 117.76, 127.76, 129.80, 133.29, 133.35, 133.53, 135.71, 135.76, 172.38, 177.56, 207.36.

MS (ESI): 643 $(M+H)^+$



Synthesis of Allyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

To a solution of allyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (1.69 g, 2.63 mmol) in dichloromethane (20 mL) and cyclohexane (10 mL) was added benzyltrichloroacetimidate (2.0 g, 7.89 mmol). The reaction mixture was cooled to 0 ºC and trfilic acid (0.12 mL, 1.32 mmol) was added. It was then stirred at the same temperature for 6 h and filtered over celite. The filtrate was washed with saturated sodium bicarbonate (30 mL), water (30 mL), and brine (30 mL), and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was subjected to the next step without further purification.

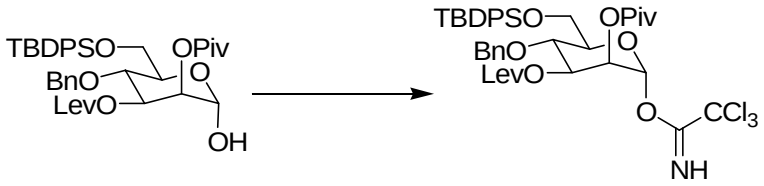


Synthesis of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α/β-D-mannopyranoside

To crude allyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (2.50 g, 3.42 mmol) in methanol (30 mL) was added palladium chloride (303 mg, 1.71 mmol). The reaction mixture was stirred for 2 h at room temperature and filtered over celite. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 8% ethyl acetate/dichloromethane to the desired product as a mixture of anomers (1.94 g, 3.37 mmol, 85%).

R_f: 0.32 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.09 (s, 9 H), 1.26 (s, 9 H), 2.13 (s, 3 H), 2.32-2.49 (m, 2 H), 2.61-2.78 (m, 2 H), 3.05 (d, 1 H, *J* = 4 Hz), 3.81-4.19 (m, 4H), 4.60-4.73 (m, 2H), 5.12 (s, 1H), 5.24 (s, 1H), 5.36-5.41 (m, 1H), 7.16-7.38 (m, 11H), 7.67-7.74 (m, 4H).



Synthesis of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α/β-D-mannopyranosyl trichloroacetimidate

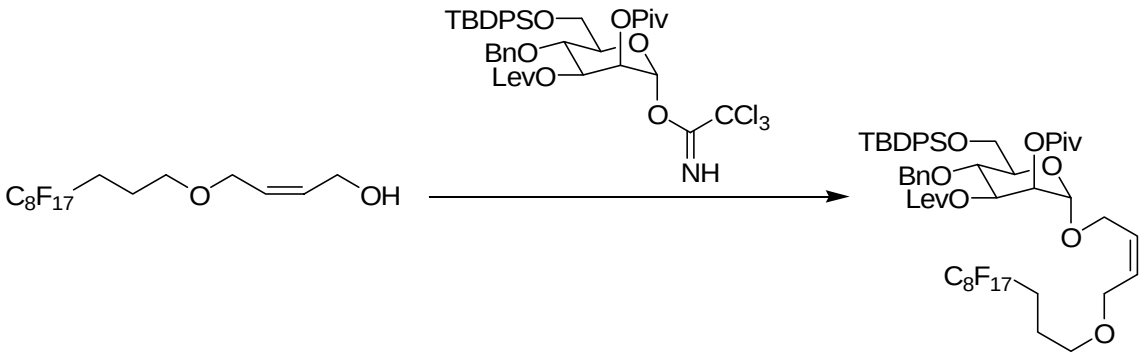
To a solution of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levunilyl-2-*O*-pivaloyl-α/β-D-mannopyranoside (735 mg, 1.06 mmol) in dichloromethane (8 mL) was added powdered 4 Å molecular sieves (200 mg) and trichloroacetonitrile (0.53 mL, 5.30 mmol) at room temperature. The reaction mixture was stirred for 10 min and cesium carbonate (380 mg, 1.17 mmol) was added and stirred further for 45 min. It was then filtered over celite and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 3% ethyl acetate/dichloromethane to yield the desired product (851 mg, 1.02 mmol, 96%)as a mixture of anomers.

R_f: 0.75 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.14 (s, 9H), 1.30 (s, 9H), 2.17 (s, 3H), 2.40-2.46 (m, 1H), 2.51-2.58 (m, 1H), 2.64-2.72 (m, 1H), 2.76-2.83 (m, 1H), 3.96-4.06 (m, 3H), 4.30 (t, 1H, *J* = 10 Hz), 4.69 (d, 1H, *J* = 10.8 Hz), 4.79 (d, 1H, *J* = 11.2 Hz), 5.47 (dd, 1H, *J* = 3.6, 10 Hz), 5.50-5.52 (m, 1H), 6.32 (s, 1H), 7.23-7.45 (m, 11H), 7.72-7.76 (m, 4H), 8.71 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.45, 27.02, 27.24, 27.82, 29.89, 37.87, 39.13, 62.35, 68.13, 72.07, 72.30, 75.04, 75.18, 90.89, 95.41, 127.77, 127.85, 127.96, 128.14, 128.52, 129.81, 129.87, 132.95, 133.51, 135.73, 135.97, 137.88, 160.11, 171.80, 177.34, 206.19.

MS (ESI): 837 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levunilyl-2-*O*-pivaloyl-α/β-D-mannopyranosyl trichloroacetimidate (200 mg, 0.239 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (87 mg, 0.159 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (15 µL, 0.80 mmol) was added. The reaction mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (30 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain Fluorous-tagged mannose (183 mg, 0.150 mmol, 95%) as a yellow gel.

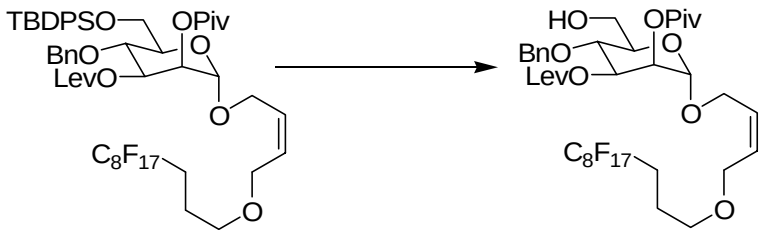
R_f: 0.71 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.10 (s, 9 H), 1.24 (s, 9 H), 1.77-1.86 (m, 2 H), 2.05-2.21 (m, 2 H), 2.15 (s, 3 H), 2.32-2.49 (m, 2 H), 2.61-2.78 (m, 2 H), 3.38 (t, 2 H, *J* = 6 Hz), 3.77 (d, 1 H, *J* = 9.6 Hz), 3.88-4.17 (m, 7 H), 4.61 (d, 1 H, *J* = 11.2 Hz), 4.70 (d, 1

H, J = 11.2 Hz), 4.79 (s, 1 H), 5.24 (s, 1 H), 5.35 (dd, 1 H, J = 3.2, 9.6 Hz), 5.61-5.71 (m, 2 H), 7.17-7.43 (m, 11 H), 7.73 (dd, 4 H, J = 6.8, 14.8 Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.37, 20.84, 26.90, 27.15, 27.89, 29.82, 37.86, 39.04, 62.65, 62.82, 66.53, 68.72, 69.81, 72.54, 72.65, 72.76, 74.93, 96.74, 127.64, 127.76, 127.87, 127.92, 128.41, 129.70, 129.77, 130.40, 133.15, 133.52, 135.66, 135.91, 138.05, 171.76, 177.65, 206.32.

MS (ESI): 1223 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (102 mg, 0.084 mmol) in THF (3 mL) was added acetic acid (30 μL) followed by tetrabutylammonium fluoride (1.0 M in THF, 0.17 mL, 0.168 mmol). The reaction mixture was stirred for 8 h and then concentrated. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent

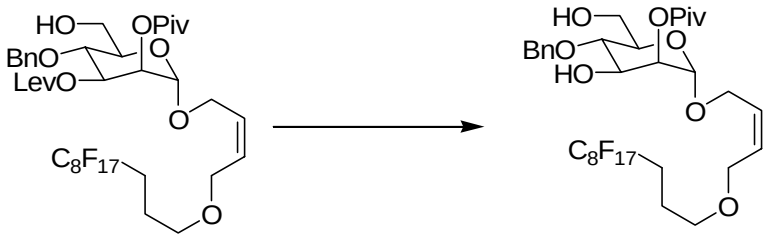
was removed under reduced pressure to obtain the desilylated product (57 mg, 0.058 mmol, 70%) as a yellow gel.

R_f: 0.54 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20 (s, 9 H), 1.80-1.88 (m, 2 H), 2.07-2.21 (m, 2 H), 2.13 (s, 3 H), 2.32-2.49 (m, 2 H), 2.61-2.78 (m, 2 H), 3.45 (t, 2 H, *J* = 6 Hz), 3.72-3.83 (m, 3 H), 3.90 (t, 1 H, 9.6 Hz), 4.01 (d, 2 H, *J* = 5.2 Hz), 4.07 (dd, 1 H, *J* = 6.4, 12.8 Hz), 4.17 (dd, 1 H, *J* = 5.6, 13.2 Hz), 5.17 (dd, 1 H, *J* = 2, 3.2 Hz), 5.33 (dd, 1 H, *J* = 3.2, 10 Hz), 5.63-5.71 (m, 2 H), 7.23-7.34 (m, 5 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.82, 27.11, 27.85, 29.84, 37.81, 39.02, 61.78, 63.16, 66.51, 68.86, 69.62, 71.95, 72.24, 72.35, 74.91, 96.90, 127.89, 128.02, 128.22, 128.51, 130.19, 137.76, 171.73, 177.42, 206.31.

MS (ESI): 983 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-hydroxy-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (42 mg, 0.043 mmol) in pyridine (3 mL) was added 1 M solution of $NH_2NH_2.H_2O$ in pyridine/acetic acid (3:2) (0.215 mmol, 0.22

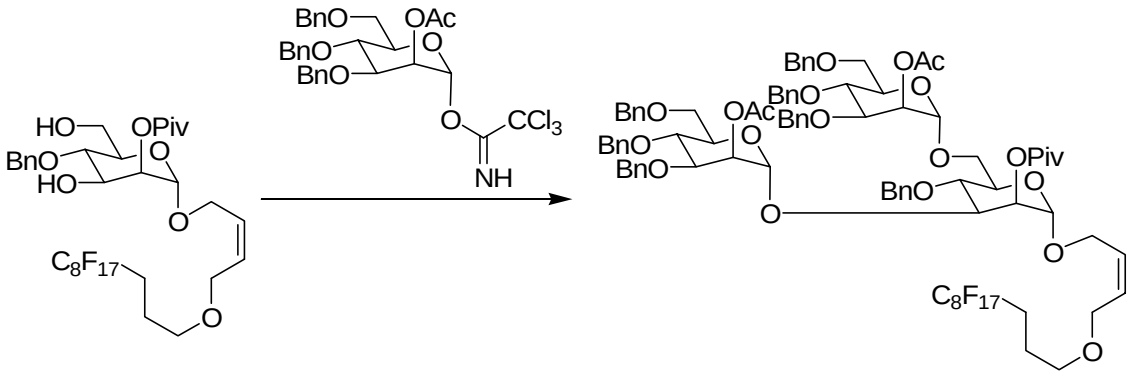
mL). The reaction mixture was stirred for 30 min and then concentrated. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain the desired product (36 mg, 0.041 mmol, 95%) as a yellow gel.

R_f: 0.38 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.21 (s, 9 H), 1.62 (br s, 1 H), 1.81-1.88 (m, 4 H), 2.09-2.18 (m, 2 H), 3.39 (br s, 1 H), 3.45 (t, 2 H, *J* = 6 Hz), 3.64-3.72 (m, 2 H), 3.76 (dd, 1 H, *J* = 3.6, 12 Hz), 3.84 (dd, 1 H, *J* = 2.4, 12 Hz), 4.00 (d, 1 H, *J* = 5.6 Hz), 4.05 (dd, 1 H, *J* = 6, 12.8 Hz), 4.11-4.19 (m, 2 H), 4.70 (d, 1 H, *J* = 11.2 Hz), 4.75 (d, 1 H, *J* = 1.2 Hz), 4.77 (d, 1 H, *J* = 11.2 Hz), 5.02-5.04 (m, 1 H), 5.62-5.73 (m, 2 H), 7.23-7.33 (m, 5 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 27.15, 39.11, 62.01, 63.15, 66.49, 68.88, 70.46, 71.77, 72.08, 74.99, 75.15, 96.96, 128.03, 128.18, 128.31, 128.34, 128.68, 130.08, 137.98, 178.11.

MS (ESI): 884 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside (30 mg, 0.034 mmol) and 2-*O*-acetyl-3,4,6-*O*-tribenzyl-α/β-D-mannopyranosyl trichloroacetimidate (65 mg, 0.102 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (15 µL, 0.80 mmol) was added. The reaction mixture was stirred for 30 min. The reaction mixture was quenched with triethylamine (30 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain mannose trisaccharide (58 mg, 0.032 mmol, 94%) as a yellow gel.

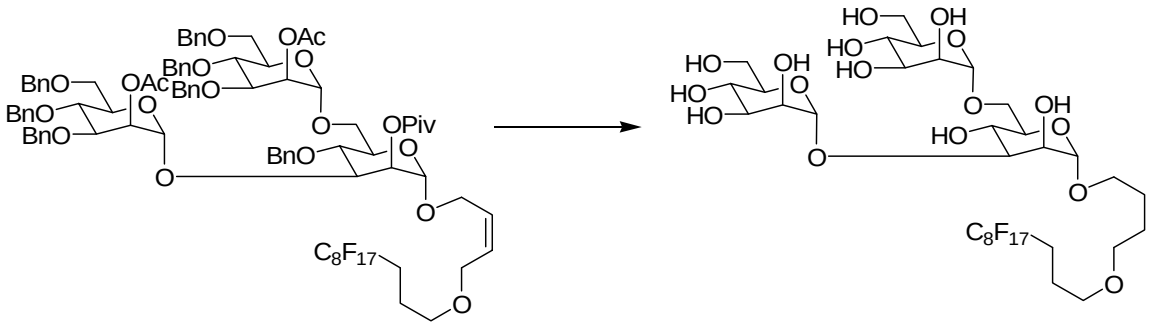
R_f: 0.78 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.17 (s, 9 H), 1.78-1.85 (m, 2 H), 2.09 (s, 3 H), 2.13 (s, 3 H), 2.08-2.18 (m, 2 H), 3.41 (t, 2 H, J = 6 Hz), 3.57-4.18 (m, 18 H), 4.40-4.49 (m, 7 H), 4.59-4.73 (m, 7 H), 4.83 (d, 2 H, J = 10.8 Hz), 4.96 (s, 1 H), 5.04 (s, 1 H), 5.13 (s, 1 H), 5.35 (s, 1 H), 5.44 (d, 1 H, J = 2 Hz), 5.56-5.68 (m, 2 H), 7.10-7.38 (m, 35 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.86, 21.10, 21.15, 27.20, 39.01, 63.18, 65.88, 66.52, 68.21, 68.40, 68.61, 68.79, 70.84, 71.69, 71.77, 71.82, 71.95, 72.12, 73.41, 73.47, 73.95, 74.16, 74.50, 74.62, 77.29, 77.59, 78.06, 78.57, 96.37, 98.29, 100.22, 127.27, 127.29, 127.32, 127.51, 127.60, 127.68, 127.72, 127.79, 127.87, 127.90, 127.96, 128.07,

128.13, 128.17, 128.27, 128.35, 128.36, 128.39, 128.51, 128.57, 130.11, 137.59, 137.75, 137.83, 138.11, 138.36, 138.38, 138.84, 170.27, 170.30, 177.57.

MS (ESI): 1835 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside (120 mg, 0.066 mmol) in methanol (4 mL) was added 0.5 M NaOMe (0.26 mL, 0.132 mmol) and the reaction mixture was stirred at ambient temperature for 48 h. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide deacetylated mannose (109 mg, 0.066 mmol, 86%) as yellow oil. 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 (109 mg, 0.066 mmol) in methanol (3 mL) was added 10% Pd/C (50 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 30 h. The reaction mixture was then filtered

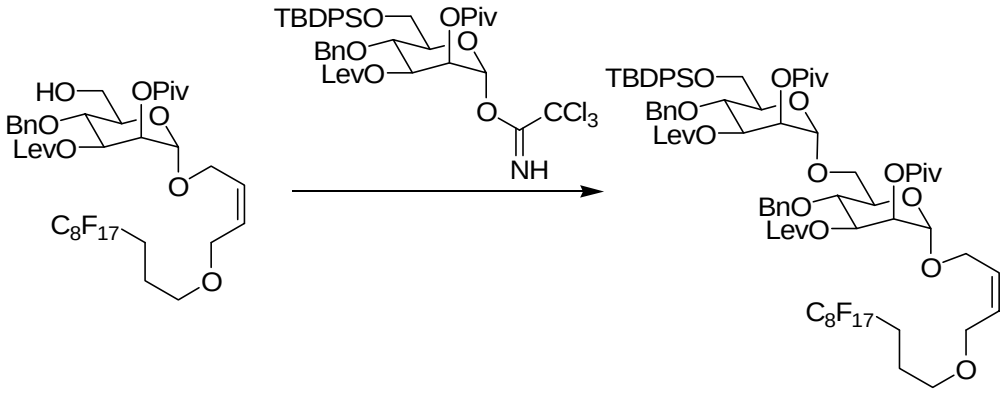
over Celite and the solvent was removed under reduced pressure to provide (63 mg, 0.061 mmol, 92%) as a solid.

R_f: 0.45 (methanol/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.61-1.66 (m, 4 H), 1.79-1.86 (m, 2 H), 2.16-2.28 (m, 2 H), 3.41-3.51 (m, 6 H), 3.56-3.82 (m, 14 H), 3.90 (dd, 1 H, *J* = 7, 11.2 Hz), 3.93-3.96 (m, 1 H), 4.01-4.03 (m, 1 H), 4.62 (s, 1 H), 4.67 (d, 1 H, *J* = 1.6 Hz), 4.79 (s, 1 H), 5.03 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.62, 26.27, 27.47, 28.25, 61.49, 65.86, 66.11, 67.10, 67.20, 67.38, 68.80, 70.09, 70.37, 70.74, 71.09, 71.26, 72.01, 72.99, 73.55, 79.55, 100.02, 100.38, 102.64.

MS (ESI): 1037 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-hydroxy-3-*O*-levunilyl-2-*O*-pivaloyl-α-D-mannopyranoside (78 mg, 0.079 mmol) and 4-*O*-benzyl-6-*O*-*t*-

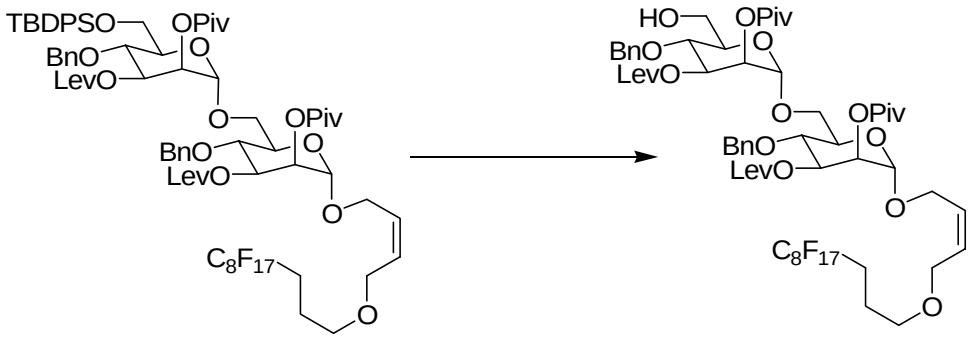
butyldiphenylsilyl-3-*O*-levunilyl-2-*O*-pivaloyl-α/β-D-mannopyranosyl trichloroacetimidate (86 mg, 0.103 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (7 µL, 0.40 mmol) was added. The reaction mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (20 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain mannose disaccharide (118 mg, 0.071 mmol, 90%) as a yellow gel.

R_f: 0.75 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.09 (s, 9 H), 1.19 (s, 9 H), 1,24 (s, 9 H), 1.79-1.86 (m, 2 H), 2.14 (s, 3 H), 2.16 (s, 3 H), 2.08-2.21 (m, 2 H), 2.37-2.50 (m, 4 H), 2.63-2.76 (m, 2 H), 3.44 (t, 2 H, *J* = 6 Hz), 3.70 (d, 2 H, *J* = 9.6 Hz), 3.81-3.91 (m, 5 H), 4.03-4.21 (m, 5 H), 4.56 (d, 1 H, *J* = 11.2 Hz), 4.61 (d, 1 H, *J* = 11.2 Hz), 4.69 (d, 1 H, *J* = 2.8 Hz), 4.71 (s, 2 H), 4.91 (s, 1 H), 5.16 (s, 1 H), 5.31-5.33 (m, 2 H), 5.37 (dd, 1 H, *J* = 3.6, 10 Hz), 5.56-5.68 (m, 2 H), 7.10-7.43 (m, 16 H), 7.70 (d, 2 H, *J* = 6.8 Hz), 7.75 (d, 2 H, *J* = 6.8 Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.37, 26.91, 27.12, 27.17, 27.89, 29.83, 29.86, 37.83, 37.88, 39.00, 62.60, 63.10, 66.59, 68.76, 69.44, 69.57, 71.09, 72.47, 72.61, 72.67, 74.76, 74.96, 77.30, 96.68, 98.04, 127.63, 127.75, 127.86, 127.87, 128.38, 128.41, 129.68, 129.76, 130.53, 133.13, 133.50, 135.69, 135.94, 137.75, 138.14, 171.40, 171.71, 177.38, 177.65, 206.27.

MS (ESI): 1679 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside

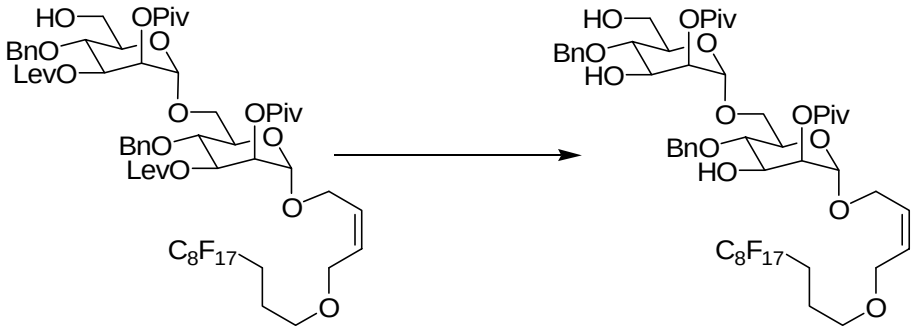
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside (96 mg, 0.058 mmol) in THF (3 mL) was added acetic acid (30 μL) followed by tetrabutylammonium fluoride (1.0 M in THF, 0.17 mL, 0.168 mmol). The reaction mixture was stirred for 10 h and then concentrated. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain the desilylated product (65 mg, 0.046 mmol, 79%) as a yellow gel.

R_f: 0.54 (EtOAc/DCM, 1:9)

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.20 (s, 9 H), 1,22 (s, 9 H), 1.79-1.86 (m, 3 H), 2.14 (s, 3 H), 2.16 (s, 3 H), 2.08-2.21 (m, 2 H), 2.36-2.49 (m, 4 H), 2.63-2.74 (m, 2 H), 3.45 (t, 2 H, J = 6 Hz), 3.68-4.16 (m, 14 H), 4.58 (d, 1 H, J = 11.2 Hz), 4.62 (d, 1 H, J = 11.2 Hz), 4.69-4.75 (m, 3 H), 4.89 (d, 1 H, J = 2 Hz), 5.15-5.17 (m, 1 H), 5.27-5.38 (m, 3 H), 5.61-5.72 (m, 2 H), 7.16-7.38 (m, 10 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.85, 27.12, 27.15, 27.83, 27.88, 29.84, 37.82, 39.00, 61.61, 63.17, 65.51, 66.56, 68.75, 69.25, 69.53, 71.07, 71.99, 72.07, 72.40, 72.43, 72.53, 74.81, 74.94, 96.71, 97.89, 127.66, 127.87, 127.98, 128.22, 128.47, 128.49, 130.47, 137.77, 137.82, 171.39, 171.72, 177.12, 177.61, 206.26, 206.28.

MS (ESI): 1419 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-hydroxy-6-*O*-(4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside

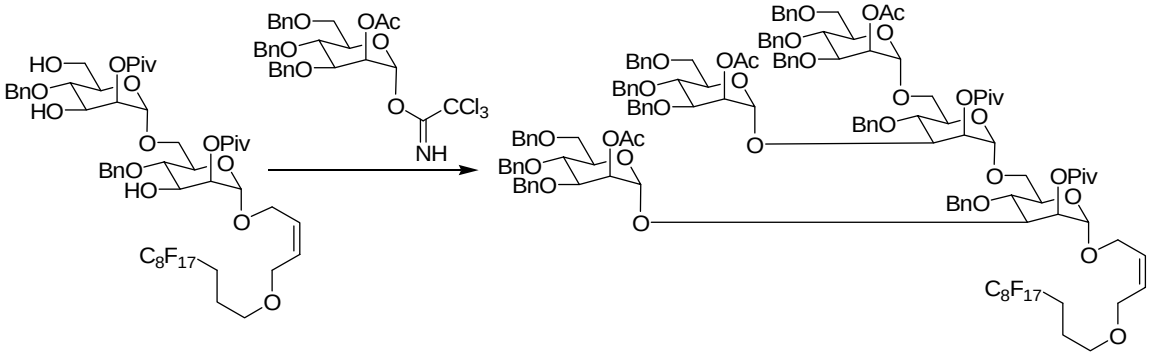
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside (65 mg, 0.046 mmol) in pyridine (3 mL) was added 1 M solution of $NH_2NH_2.H_2O$ in pyridine/acetic acid (3:2) (0.460 mmol, 0.46 mL). The reaction mixture was stirred for 30 min and then concentrated. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain the desired product (53 mg, 0.043 mmol, 94%) as a yellow gel.

R_f: 0.38 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.19 (s, 9 H), 1.21 (s, 9 H), 1.79-2.21 (m, 7 H), 3.44 (t, 2 H, *J* = 6 Hz), 3.58-3.86 (m, 9 H), 4.01-4.16 (m, 5 H), 4.62 (d, 1 H, *J* = 11.2 Hz), 4.69 (d, 1 H, *J* = 11.2 Hz), 4.73 (d, 1 H, *J* = 1.2 Hz), 4.76 (d, 1 H, *J* = 11.2 Hz), 4.82-4.85 (m, 2 H), 5.00-5.01 (m, 1 H), 5.08-5.09 (m, 1 H), 5.60-5.69 (m, 2 H), 7.23-7.33 (m, 10 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 27.13, 27.15, 39.08, 39.13, 61.80, 63.12, 66.11, 66.52, 68.82, 70.57, 70.72, 70.79, 71.79, 71.85, 72.17, 74.89, 74.91, 74.94, 75.62, 77.28, 96.68, 97.72, 127.90, 127.95, 128.04, 128.17, 128.29, 128.61, 128.66, 130.18, 138.00, 138.03, 177.95, 178.27.

MS (ESI): 1222 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-6-*O*-[4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside]-2-*O*-pivaloyl-α-D-mannopyranoside

A solution of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-hydroxy-6-*O*-(4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-

mannopyranoside (50 mg, 0.041 mmol) and 2-*O*-acetyl-3,4,6-*O*-tribenzyl-α/β-D-mannopyranosyl trichloroacetimidate (117 mg, 0.185 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (4 µL, 0.021 mmol) was added. The reaction mixture was stirred for 30 min. The reaction mixture was quenched with triethylamine (30 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a Fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain mannose pentasaccharide (58 mg, 0.032 mmol, 94%) as a yellow gel.

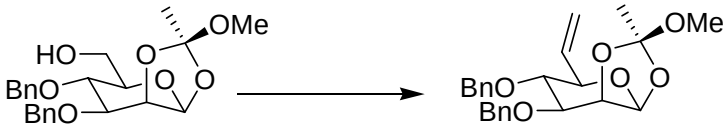
R_f: 0.82 (EtOAc/DCM, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.14 (s, 9 H), 1.18 (s, 9 H), 1.79-1.86 (m, 2 H), 2.06 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), 2.03-2.21 (m, 2 H), 3.36 (t, 2 H, J = 6 Hz), 3.53-3.58 (m, 3 H), 3.59-3.78 (m 12 H), 3.81-3.88 (m, 4 H), 3.90-4.00 (m, 6 H), 4.05-4.12 (m, 2 H), 4.14 (dd, 1 H, J =3.2, 8.8 Hz), 4.31-4.49 (m, 11 H), 4.52-4.76 (m, 11 H), 4.81-4.86 (m, 3 H), 4.96 (s, 1 H), 5.03 (s, 1 H), 5.09-5.14 (m, 3 H), 5.34-5.36 (m, 2 H), 5.46 (s, 1 H), 5.58-5.64 (m, 2 H), 7.09-7.28 (m, 55 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.85, 21.03, 21.07, 21.15, 27.16, 27.23, 29.76, 38.98, 63.13, 66.53, 68.13, 68.40, 68.55, 68.68, 68.98, 69.02, 70.72, 70.84, 71.24, 71.79, 71.85, 71.96, 72.13, 72.27, 73.41, 73.48, 73.84, 73.94, 74.11, 74.42, 74.59, 74.82, 75.20, 75.31, 75.45, 75.52, 77.28, 77.66, 78.19, 78.34, 78.60, 96.24, 97.01, 98.45, 100.17, 100.29, 127.26, 127.29, 127.49, 127.53, 127.62, 127.65, 127.68, 127.71, 127.75, 127.78, 127.81, 127.83, 127.86, 127.88, 127.96, 128.01, 128.04, 128.06, 128.09, 128.15, 128.26, 128.33,

128.36, 128.44, 128.46, 128.50, 128.53, 128.65, 130.49, 137.48, 137.73, 137.76, 137.82, 138.10, 138.30, 138.39, 138.89, 138.91, 170.18, 170.24, 177.18, 177.64.

MS (ESI): 2667 $(M+Na)^+$



Synthesis of 3,4-di-*O*-benzyl-6,7-dideoxy-1,2-*O*-(methoxyethylidene)-*α*-D-hept-6-enopyranoside

To a solution of 3,4-di-*O*-benzyl-6-hydroxy-1,2-*O*-(methoxyethylidene)-*α*-D-mannopyranoside (2.21 g, 5.31 mmol) in dichloromethane was added Dess-Martin reagent (4.51 g, 10.6 mmol). The mixture was stirred at room temperature for 30 min before it was quenched with aqueous solution of sodium sulfite (10 mL) and sodium bicarbonate (10 mL). The mixture was further stirred for 15 min and the aqueous layer was extracted with dichloromethane (2 x 50 mL). The combined organic layer was dried and concentrated to give the crude aldehyde which was taken forward without further purification. To a suspension of methyltriphenylphosphonium bromide (2.28 g, 6.37 mmol) in THF was cooled to –40 ºC and n-BuLi (2.55 mL, 6.37 mmol, 2.5 M in hexane) was added. The reaction mixture was stirred at the same temperature for 30 min and a solution of above aldehyde in THF was added. The reaction mixture was further stirred for 1 h. It was then allowed to warm up to room temperature over a period of 4 h before it was quenched with saturated a solution of ammonium chloride (15 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with water, brine, dried, and concentrated. The

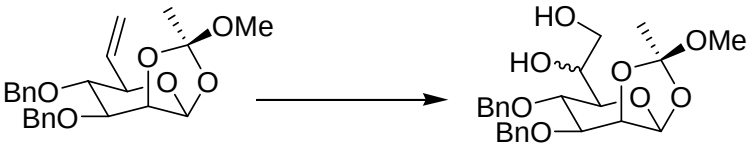
crude product was purified by flash column chromatography on silica gel using 22% ethyl acetate/hexane to give the desired product (1.38 g, 3.35 mmol, 63%) as a white solid.

R_f: 0.48 (EtOAc/hexane, 1:9)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.74 (s, 3H), 3.28 (s, 3H), 3.59 (d, 1H, *J* = 9.2 Hz), 3.70-3.74 (m, 2H), 4.37-4.38 (m, 1H), 4.66 (d, 1H, *J* = 10.8 Hz), 4.78 (d, 2H, *J* = 5.6 Hz), 4.85 (d, 1H, *J* = 10.8 Hz), 5.27 (d, 1H, *J* = 10.8 Hz), 5.34 (d, 1H, *J* = 2.4 Hz, H-1), 5.43 (d, 1H, *J* = 17.2 Hz), 5.93-6.04 (m, 1H), 7.23-7.40 (m, 10H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 24.54, 49.88, 72.61, 74.61, 75.40, 76.85, 78.40, 78.73, 97.57, 117.86, 123.95, 127.90, 128.08, 128.13, 128.47, 128.60, 134.54, 137.94, 138.19.

MS (ESI): 413 $(M+H)^+$



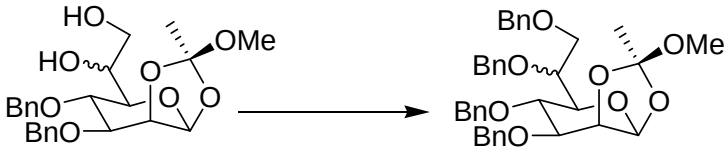
Synthesis of 3,4-di-*O*-benzyl-(D,L)-*glycero*-1,2-*O*-(methoxyethylidene)-*α*-D-*manno*-heptopyranoside

A solution of 3,4-di-*O*-benzyl-6,7-dideoxy-1,2-*O*-(methoxyethylidene)-*α*-D-hept-6-enopyranoside (1.38 g, 3.35 mmol) in acetone-water (9:1, 20 mL) was cooled to 0 ºC and NMNO (788 mg, 6.70 mmol) was added followed by 2.5 wt% of osmium tetroxide (2.1 mL, 0.168 mmol) solution. The reaction mixture was stirred at room temperature for 5 h and poured into water (30 mL). The aqueous layer was extracted with ethyl acetate (2 x 50

mL) and the combined organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 45% ethyl acetate/hexane to give the diol product (1.24 g, 2.78 mmol, 83%) as a mixture of diasteromers (3:1, by NMR).

R_f: 0.18 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.68 (s, 3H), 1.71 (s, 1H), 2.45 (br s, 1 H), 2.56 (br s, 1 H), 3.26 (s, 3 H), 3.27 (s, 2 H), 3.33-3.45 (m, 3 H), 3.61-4.02 (m, 9 H), 4.38-4.41 (m, 1 H), 4.66-4.78 (m, 5 H), 4.95-5.02 (m, 1 H), 5.32 (d, 1 H, J = 3.6 Hz), 5.35 (d, 0.5 H, J = 4 Hz), 7.25-7.41 (m, 15 H).



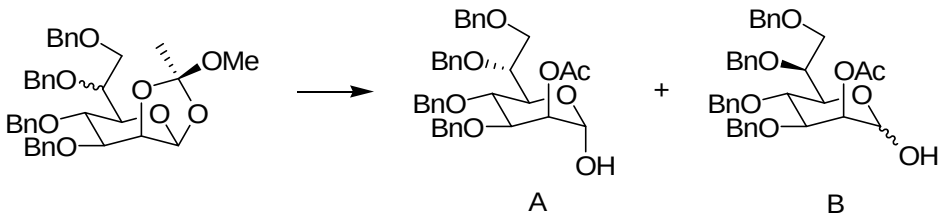
Synthesis of 3,4,6,7-tetra-*O*-benzyl-1,2-*O*-(methoxyethylidene)-(D,L)-*glycero*-*α*-D-*manno*-heptopyranoside

To a solution of 3,4-di-*O*-benzyl-(D,L)-*glycero*-1,2-*O*-(methoxyethylidene)-*α*-D-*manno*-heptopyranoside (1.24 g, 2.78 mmol) in DMF (10 mL) was added benzyl bromide (0.73 mL, 6.12 mmol), tetrabutylammonium iodide (205 mg, 0.556 mmol), and sodium hydride (334 mg, 8.34 mmol, 60% suspension). The reaction mixture was stirred at room temperature for 1 h and then poured into ice-water mixture. The aqueous layer was extracted with ethyl acetate (2 x 50 mL) and the combined organic layer was washed with water, brine, and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel

using 22% ethyl acetate/hexane to give the desired product (1.26 g, 2.01 mmol, 90%) as a gel.

R_f: 0.52 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.73 (s, 3 H), 1.78 (s, 2 H), 3.30 (s, 3 H), 3.31 (s, 2 H), 3.28 (s, 3H), 3.41-3.46 (m, 1 H), 3.54 (dd, 1 H, *J* = 1.6, 9.2 Hz), 3.71-3.84 (m, 5 H), 3.96-4.15 (m, 4 H), 4.36-4.38 (m, 2 H), 4.49-4.55 (m, 5 H), 4.66-4.88 (m, 9 H), 4.99 (d, 1 H, *J* = 10.8 Hz), 5.28 (d, 0.5 H, *J* = 2.4 Hz), 5.32 (d, 1 H, *J* = 2.4 Hz), 7.23-7.36 (m, 30 H).



Synthesis of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-(D,L)-*glycero*-*α*-D-*manno*-heptopyranoside

A solution of 3,4,6,7-tetra-*O*-benzyl-1,2-*O*-(methoxyethylidene)-(D,L)-*glycero*-*α*-D-*manno*-heptopyranoside (140 mg, 0.224 mmol) in 80% aq. AcOH (4 mL) was stirred at room temperature for 2 h and poured into ice-water mixture (20 mL). The aqueous layer was extracted with ethyl acetate (2 x 40 mL) and the combined organic layer was washed with sodium bicarbonate (30 mL), water (20 mL), brine (20 mL), and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 28% ethyl acetate/hexane to give *R* (80 mg, 0.131 mmol, 58%) product as single anomer and 32% ethyl acetate/hexane to give *S* product as 1:1 mixture of α:β anomers. (27 mg, 0.045 mmol, 20%).

Compound **A**

R_f: 0.39 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.11 (s, 3H), 3.68 (dd, 2H, *J* = 6.8 Hz, 10.8 Hz), 3.75 (dd, 1H, *J* = 4.4, 10.4 Hz), 3.85 (t, 1H, *J* = 9.6 Hz), 4.01-4.05 (m, 1H), 4.15 (d, 1H, *J* = 10 Hz), 4.16 (br s, 1H), 4.45-4.54 (m, 4H), 4.62-4.72 (m, 3H), 4.85 (d, 1H, *J* = 10.8 Hz), 5.12 (s, 1H), 5.35-5.36 (m, 1H), 7.15-7.33 (m, 20H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 21.20, 69.12, 70.36, 71.79, 71.92, 72.29, 73.34, 74.47, 74.77, 78.15, 92.38, 127.58, 127.66, 127.81, 128.06, 128.19, 128.37, 128.39, 128.42, 128.48, 137.98, 138.20, 138.27, 138.49, 170.49.

MS (ESI): 613 $(M+H)^+$

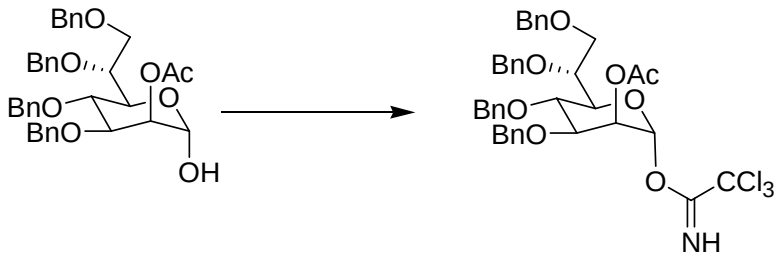
Compound **B**

R_f: 0.33 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.12 (s, 3H), 2.14 (s, 3H), 3.66-3.75 (m, 6H), 3.94-4.09 (m, 6H), 4.29 (d, 1H, J = 11.2 Hz), 4.42-4.88 (m, 16H), 5.17 (s, 1H), 5.35 (s, 1H), 5.65 (s, 1H), 7.15-7.31 (m, 40H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 21.22, 21.14, 67.11, 68.77, 69.82, 70.56, 71.17, 71.78, 71.94, 72.63, 73.06, 73.25, 73.33, 73.39, 73.86, 74.66, 74.69, 74.79, 76.47, 76.76, 78.08, 78.20, 81.48, 92.50, 92.53, 127.48, 127.51, 127.56, 127.60, 127.65, 127.72, 127.79, 127.83, 127.87, 127.91, 127.93, 127.95, 127.98, 128.07, 128.17, 128.31, 128.36, 128.37, 127.42, 128.48, 128.67, 137.37, 138.10, 138.32, 138.63, 169.12, 171.58.

MS (ESI): 613 $(M+H)^+$



Synthesis of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranosyl trichloroacetimidate

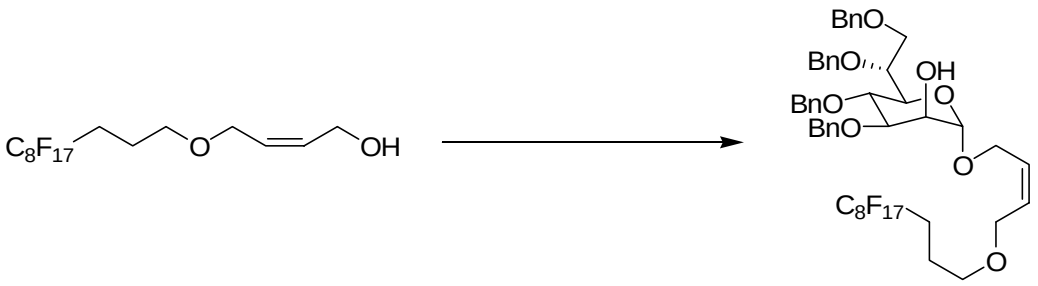
To a solution of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranoside (60 mg, 0.098 mmol) in dichloromethane (3 mL) was added powdered 4 Å molecular sieves (40 mg) and trichloroacetonitrile (0.05 mL, 0.49 mmol) at room temperature. The reaction mixture was stirred for 10 min and cesium carbonate (35 mg, 0.108 mmol) was added and stirred further for 45 min. It was then filtered over celite and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 24% ethyl acetate/hexane to yield the desired product (275 mg, 0.353 mmol, 98 %) as a white gel.

R_f: 0.62 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.15 (s, 3 H), 3.69-3.74 (m, 2 H), 4.04-4.14 (m, 4 H), 4.47 (d, 1 H, J = 3.2 Hz), 4.56-4.80 (m, 6 H), 4.88 (d, 1 H, J = 10.8 Hz), 5.49 (s, 1 H), 6.28 (d, 1 H, J = 1.2 Hz), 7.19-7.38 (m, 20 H), 8.68 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 21.01, 67.33, 70.80, 72.17, 72.79, 73.82, 74.92, 75.10, 77.81, 78.53, 90.86, 95.15, 127.50, 127.59, 127.75, 128.05, 128.08, 128.32, 128.41, 128.44, 128.56, 137.48, 138.18, 138.37, 138.77, 160.00, 170.00.

MS (ESI): 757 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside

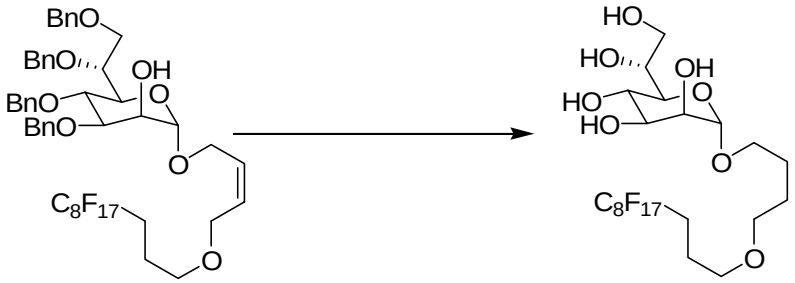
A solution of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranosyl trichloroacetimidate (73 mg, 0.097 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (44 mg, 0.076 mmol) in dichloromethane (3 mL) was cooled to 5 ºC and TMSOTf (3 µL, 0.016 mmol) was added. The reaction mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (10 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. To the methanol solution of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside was added 0.5 M NaOMe (0.29 mL, 0.146 mmol). The reaction mixture was stirred for 30 min and concentrated. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain deacetylated heptomannose (77 mg, 0.070 mmol, 96%) as a yellow gel.

R_f: 0.48 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.76-1.82 (m, 2 H), 2.05-2.18 (m, 2 H), 2.40 (br s, 1 H), 3.33 (t, 2 H, *J* = 6 Hz), 3.70 (dd, 1 H, *J* = 6.4, 10 Hz), 3.76 (dd, 1 H, *J* = 4.8, 10.4 Hz), 3.83-4.00 (m, 7 H), 4.07 (dd, 1 H, *J* = 6.4, 13.2 Hz), 4.16 (dd, 1 H, *J* = 5.6, 13.2 Hz), 4.47 (d, 2 H, *J* = 2.8 Hz), 4.58 (d, 1 H, *J* = 10.8 Hz), 4.66 (d, 2 H, *J* = 3.2 Hz), 4.71 (d, 2 H, *J* = 4.4 Hz), 4.80 (d, 1 H, *J* = 10.8 Hz), 4.87 (d, 1 H, *J* = 1.2 Hz), 5.62-5.69 (m, 2 H), 7.16-7.35 (m, 20 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 62.41, 66.48, 68.20, 68.68, 70.52, 71.96, 72.04, 72.54, 73.30, 74.33, 74.89, 77.88, 80.77, 97.96, 127.46, 127.55, 127.61, 127.69, 127.88, 127.94, 128.04, 128.29, 128.36, 128.42, 128.61, 130.42, 137.83, 138.29, 138.40.

MS (ESI): 1123 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-D-*glycero*-α-D-*manno*-heptopyranoside

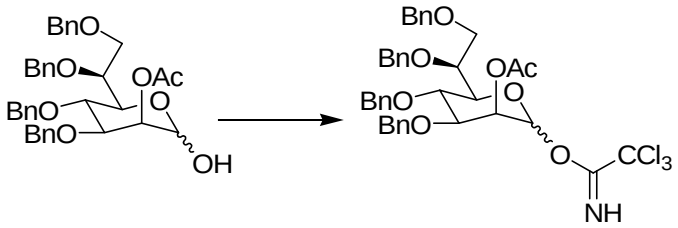
To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-hydroxy-D-*glycero*-α-D-*manno*-heptopyranoside (40 mg, 0.036 mmol) in methanol (2 mL) was added 10% Pd/C (10 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 12 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide fully deprotected fluorous-tagged heptomannose (27 mg, 0.036 mmol, 100%) as a white solid.

R_f: 0.56 (MeOH/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.61-1.64 (m, 4 H), 1.79-1.86 (m, 2 H), 2.16-2.28 (m, 2 H), 3.38-3.50 (m, 5 H), 3.56 (dd, 1 H, *J* = 4, 9.6 Hz), 3.62-3.66 (m, 2 H), 3.72-3.76 (m, 4 H), 3.88-3.89 (m, 1 H), 4.69 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.50, 25.93, 26.23, 27.46, 62.54, 66.92, 68.66, 68.71, 70.34, 70.63, 71.35, 72.34, 73.21, 100.13.

MS (ESI): 765 $(M+Na)^+$



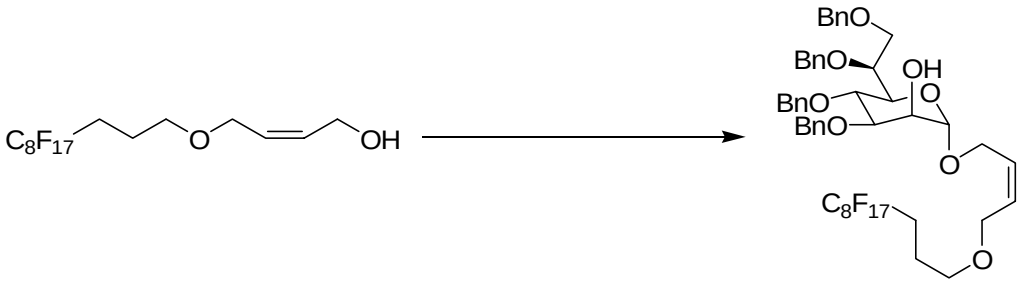
Synthesis of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α*/*β*-D-*manno*-heptopyranosyl trichloroacetimidate

To a solution of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α*/*β*-D-*manno*-heptopyranoside (20 mg, 0.033 mmol) in dichloromethane (2 mL) was added powdered 4 Å molecular sieves (10 mg) and trichloroacetonitrile (0.02 mL, 0.163 mmol) at room temperature. The reaction mixture was stirred for 10 min and cesium carbonate (12 mg, 0.036 mmol) was added and stirred further for 45 min. It was then filtered over celite and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 24% ethyl acetate/hexane to yield the desired product (23 mg, 0.030 mmol, 94 %) as a yellow gel.

R_f: 0.60 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.12 (s, 3H), 2.17 (s, 3H), 3.62-3.81 (m, 6H), 3.97-4.08 (m, 6H), 4.47-4.88 (m, 16H), 5.44-5.46 (m, 1H), 5.47-5.49 (m, 1H), 6.25 (d, 1H, J = 1.6 Hz), 6.29 (s, 1H), 7.16-7.32 (m, 40H), 8.63 (s, 1H), 8.65 (s, 1H).

MS (ESI): 757 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-L-*glycero*-*α*-D-*manno*-heptopyranoside

A solution of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α/β*-D-*manno*-heptopyranosyl trichloroacetimidate (23 mg, 0.030 mmol) and 3-(perfluorooctyl)propanyloxybutenyl alcohol (19 mg, 0.025 mmol) in dichloromethane (2 mL) was cooled to 5 ºC and TMSOTf (1 µL, 0.005 mmol) was added. The reaction mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (10 µL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. To this solution of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-acetyl-3,4,6,7-tetra-*O*-benzyl-L-*glycero*-*α*-D-*manno*-heptopyranoside in methanol was added 0.5 M NaOMe (0.13 mL, 0.064 mmol). The reaction mixture was stirred for 30 min and concentrated. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE)

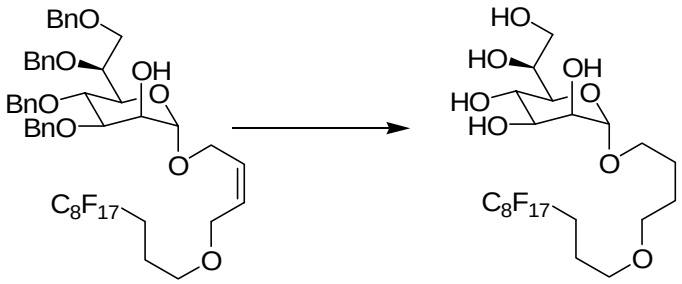
cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain deacetylated heptomannose (33 mg, 0.030 mmol, 94%) as a yellow gel.

R_f: 0.45 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.76-1.82 (m, 2 H), 2.05-2.18 (m, 3 H), 3.38 (t, 2 H, *J* = 6 Hz), 3.75-3.83 (m, 3 H), 3.89-4.11 (m, 8 H), 4.31 (d, 1 H, *J* = 11.2 Hz), 4.48-4.51 (m, 3 H), 4.61 (d, 1 H, *J* = 11.2 Hz), 4.67 (d, 1 H, *J* = 11.2 Hz), 4.77 (d, 1 H, *J* = 12 Hz), 4.83 (d, 1 H, *J* = 11.2 Hz), 4.92 (s, 1 H), 5.58-5.68 (m, 2 H), 7.18-7.30 (m, 20 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 62.55, 66.50, 68.09, 68.76, 69.94, 70.88, 71.76, 72.87, 73.44, 73.64, 74.73, 80.72, 98.43, 127.57, 127.60, 127.71, 128.00, 128.08, 128.39, 128.45, 128.59, 130.22, 137.83, 138.04, 138.37, 138.64.

MS (ESI): 1123 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-L-*glycero*-α-D-*manno*-heptopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-hydroxy-L-*glycero*-α-D-*manno*-heptopyranoside (33 mg, 0.030 mmol) in methanol (2 mL) was

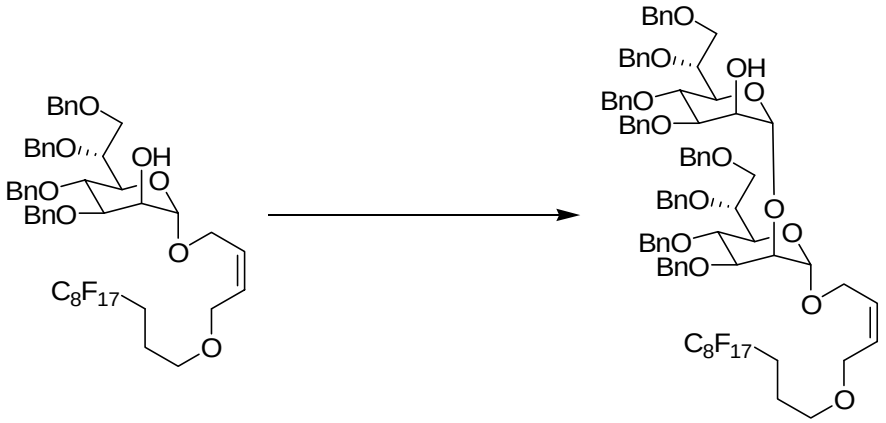
added 10% Pd/C (10 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 12 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide fully deprotected fluorous-tagged heptomannose (25 mg, 0.036 mmol, 100%) as a white solid.

R_f: 0.58 (MeOH/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.61-1.64 (m, 4 H), 1.79-1.86 (m, 2 H), 2.16-2.28 (m, 2 H), 3.37-3.40 (m, 1 H), 3.43-3.48 (m, 4 H), 3.50-3.81 (m, 7 H), 3.96 (s, 1 H), 4.72 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.50, 25.95, 26.23, 27.46, 63.10, 66.44, 66.94, 68.71, 69.28, 70.36, 70.90, 71.26, 71.48, 100.23.

MS (ESI): 765 $(M+Na)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-*O*-(3,4,6,7-tetra-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside)-D-*glycero*-α-D-*manno*-heptopyranoside

A solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside (20 mg, 0.018 mmol) and 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate (16 mg, 0.022 mmol) in

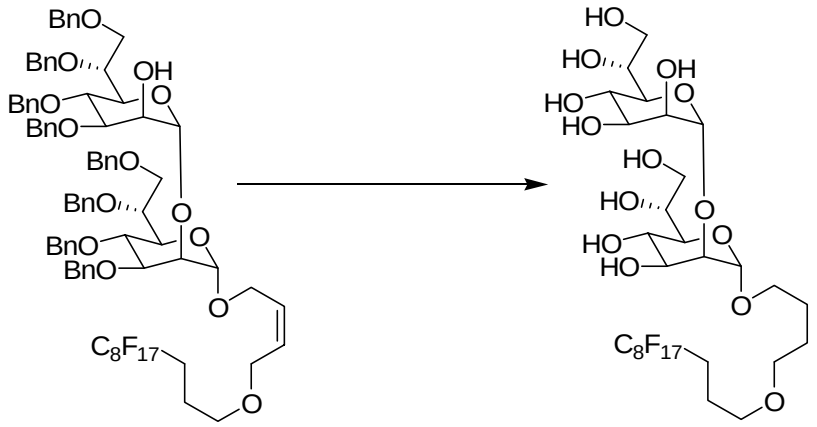
dichloromethane (2 mL) was cooled to 5 ºC and TMSOTf (1 μL, 0.004 mmol) was added. The reaction mixture was stirred for 15 min. The reaction mixture was quenched with triethylamine (10 μL) and then concentrated under reduced pressure. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. To the methanol solution was added 0.5 M NaOMe (0.07 mL, 0.034 mmol). The reaction mixture was stirred for 30 min and concentrated. The crude product was purified by solid-phase extraction by using a fluorous solid-phase extraction (FSPE) cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to obtain deacetylated disaccharide (27 mg, 0.016 mmol, 96%) as a yellow gel.

R_f: 0.67 (EtOAc/hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.75-1.81 (m, 2 H), 2.04-2.15 (m, 2 H), 3.33 (t, 2 H, J = 6 Hz), 3.74-4.15 (m, 19 H), 4.41-4.86 (m, 17 H), 5.58-5.68 (m, 2 H), 7.16-7.34 (m, 40 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.84, 28.02, 29.76, 62.41, 66.48, 68.19, 68.68, 70.51, 71.95, 72.03, 72.34, 72.53, 73.29, 74.32, 74.88, 77.87, 80.77, 97.95, 127.46, 127.54, 127.60, 127.68, 127.82, 127.88, 127.93, 128.04, 128.28, 128.35, 128.39, 128.42, 128.52, 128.61, 130.41, 137.82, 138.29, 138.40, 138.73.

MS (ESI): 1653 $(M+H)^+$



Synthesis of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-(D-*glycero*-*α*-D-*manno*-heptopyranosyl)-D-*glycero*-*α*-D-*manno*-heptopyranoside

To a solution of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-*O*-(3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranosyl)-D-*glycero*-*α*-D-*manno*-heptopyranoside (27 mg, 0.016 mmol) in methanol (2 mL) was added 10% Pd/C (15 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 24 h. The reaction mixture was then filtered over Celite and the solvent was removed under reduced pressure to provide fully deprotected fluorous-tagged disaccharide (14 mg, 0.015 mmol, 96%) as a white solid.

R_f: 0.44 (MeOH/DCM, 1:1)

^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.61-1.64 (m, 4 H), 1.79-1.86 (m, 2 H), 2.16-2.28 (m, 2 H), 3.37-3.40 (m, 1 H), 3.43-3.48 (m, 4 H), 3.50-3.81 (m, 7 H), 3.96 (s, 1 H), 4.72 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.50, 25.93, 26.23, 27.45, 62.53, 66.94, 68.65, 68.72, 70.34, 70.62, 71.33, 72.32, 73.20, 100.12.

MS (ESI): 957 $(M+Na)^+$

Microarray Preparation and Screening (Provided by Dr. Beatrice Collet)

Formation of carbohydrate microarrays. Flurous-tagged carbohydrate compounds were dissolved in MeOH/DMSO/water (40/40/20) and spotted on the fluorinated glass slide (Fluorous Technologies, Inc) using robot (Cartesian PixSys 5500 Arrayerand arrayer) at 30% humidity. The glass slide was dried in a humidifying chamber for 2 h.

Detection of protein-carbohydrate binding.[2,3] FITC-labeled Con A (20 μg/mL) in HEPES buffer (pH = 7.5, 10 mM) 1 mM $CaCl_2$, 1 mM $MnCl_2$, 100 mM NaCl, 1% BSA (w/v) was used for detection of protein-carbohydrate interactions. For protein incubation, 0.2 mL of protein solution was applied to the printed glass slide. The arrays was incubated by using a PC500 CoverWell incubation chamber and gently shaken to every 5 min for 30 min. It was then washed several times with HEPES buffer containing 0.05% TWEEN-20 until there is no water sticking on the slide. It was then dried for 30 min in a dark humidity chamber. Glass slide was scanned using fluorescence scanner at 488 nm.

Appendix: NMR Spectra

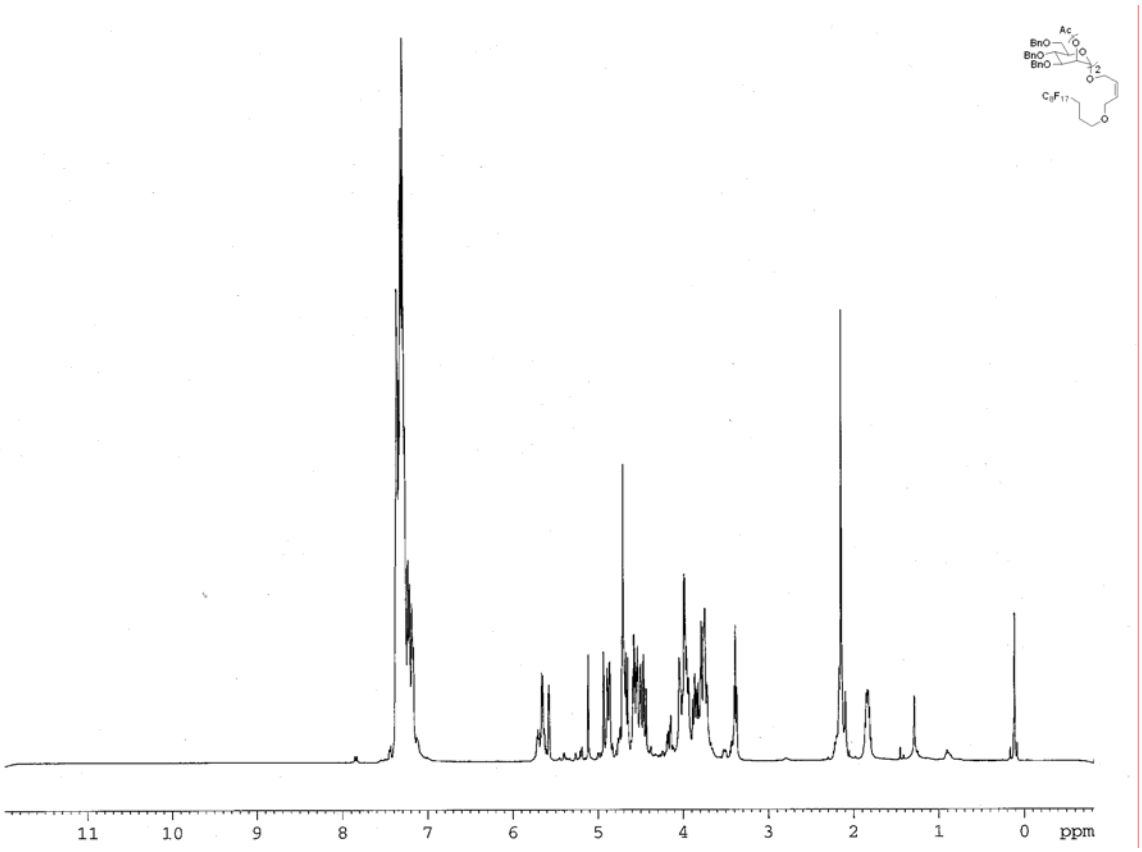


Figure 3.1: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside.

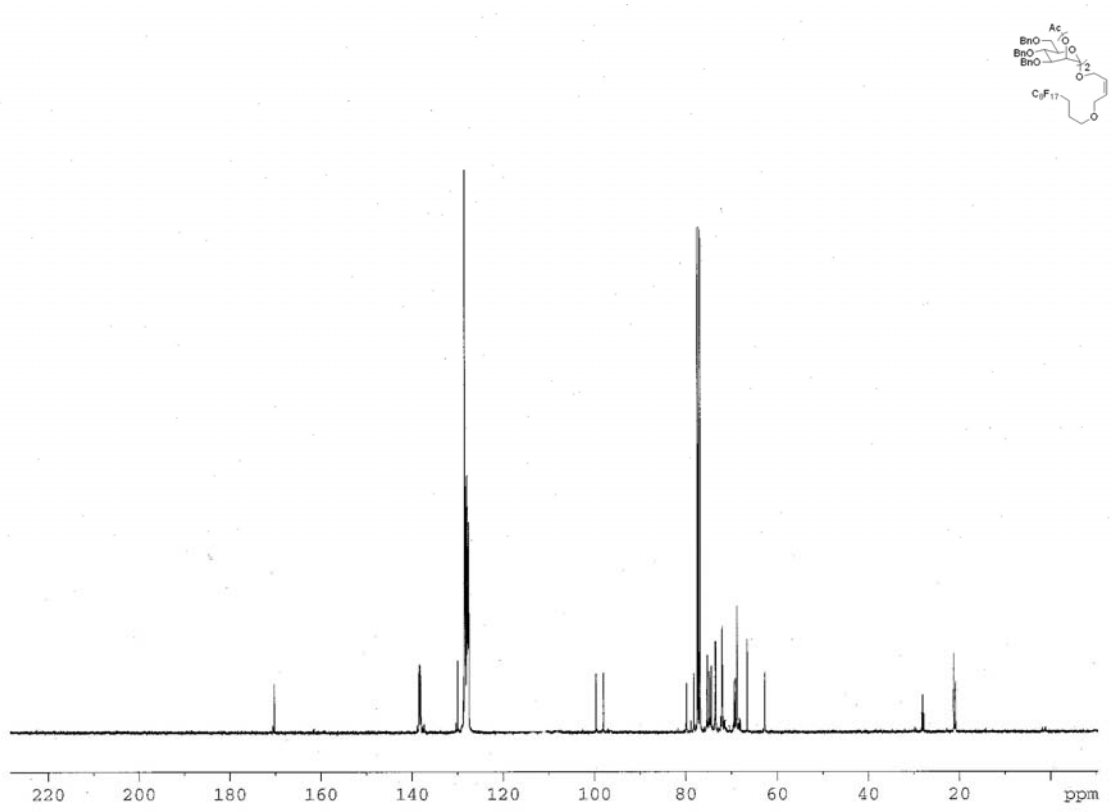


Figure 3.2: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside.

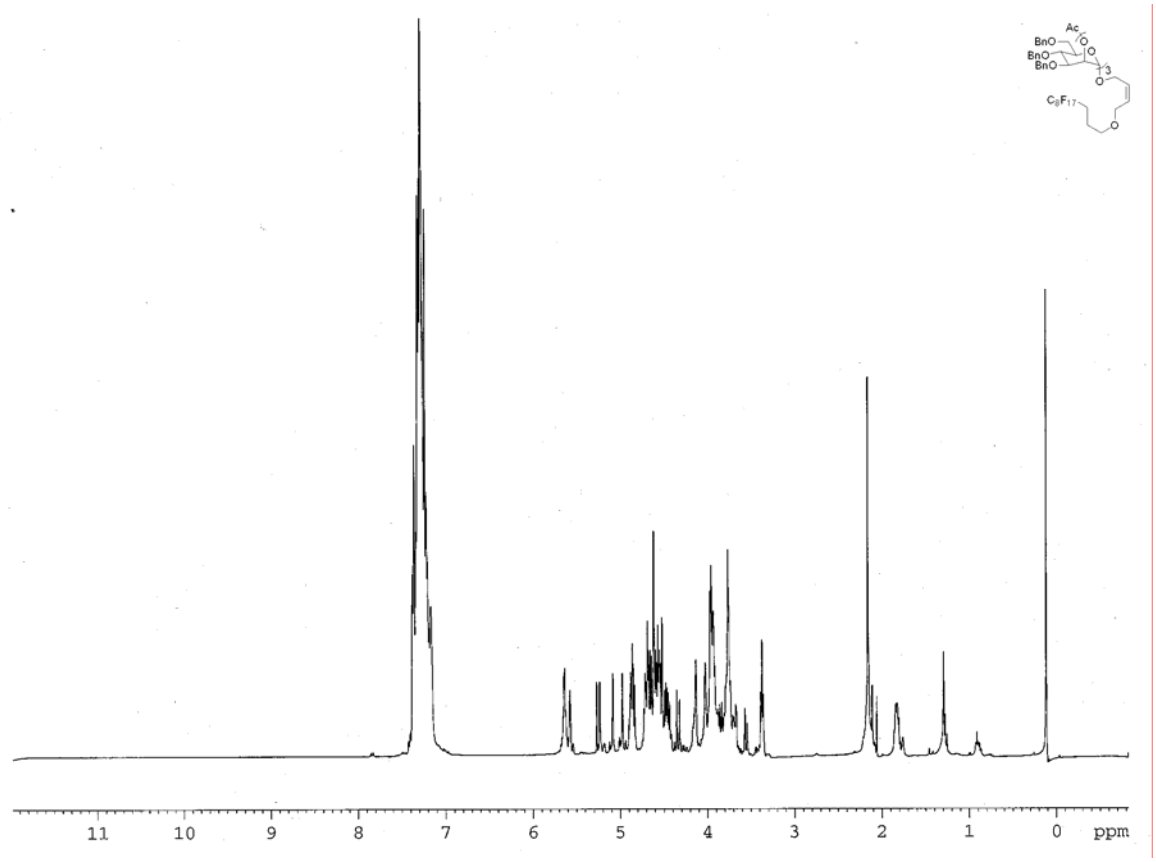


Figure 3.3: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside.

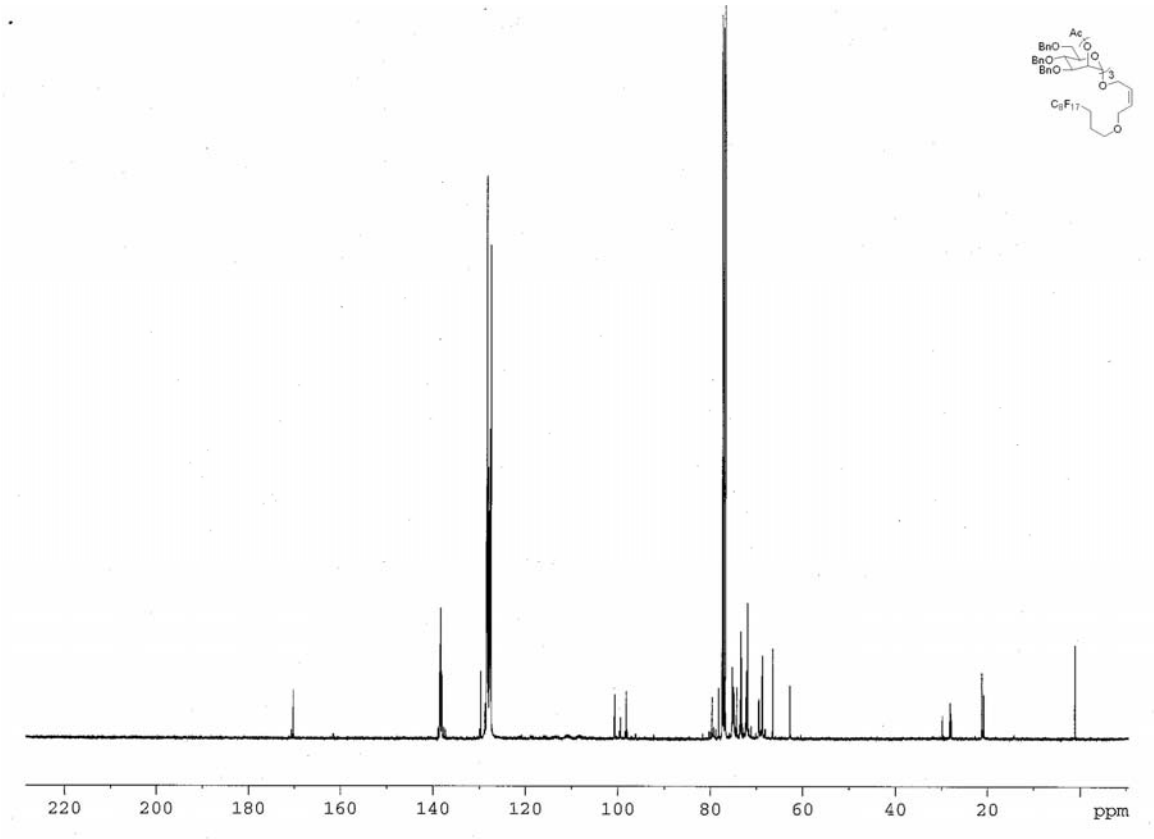


Figure 3.4: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside.

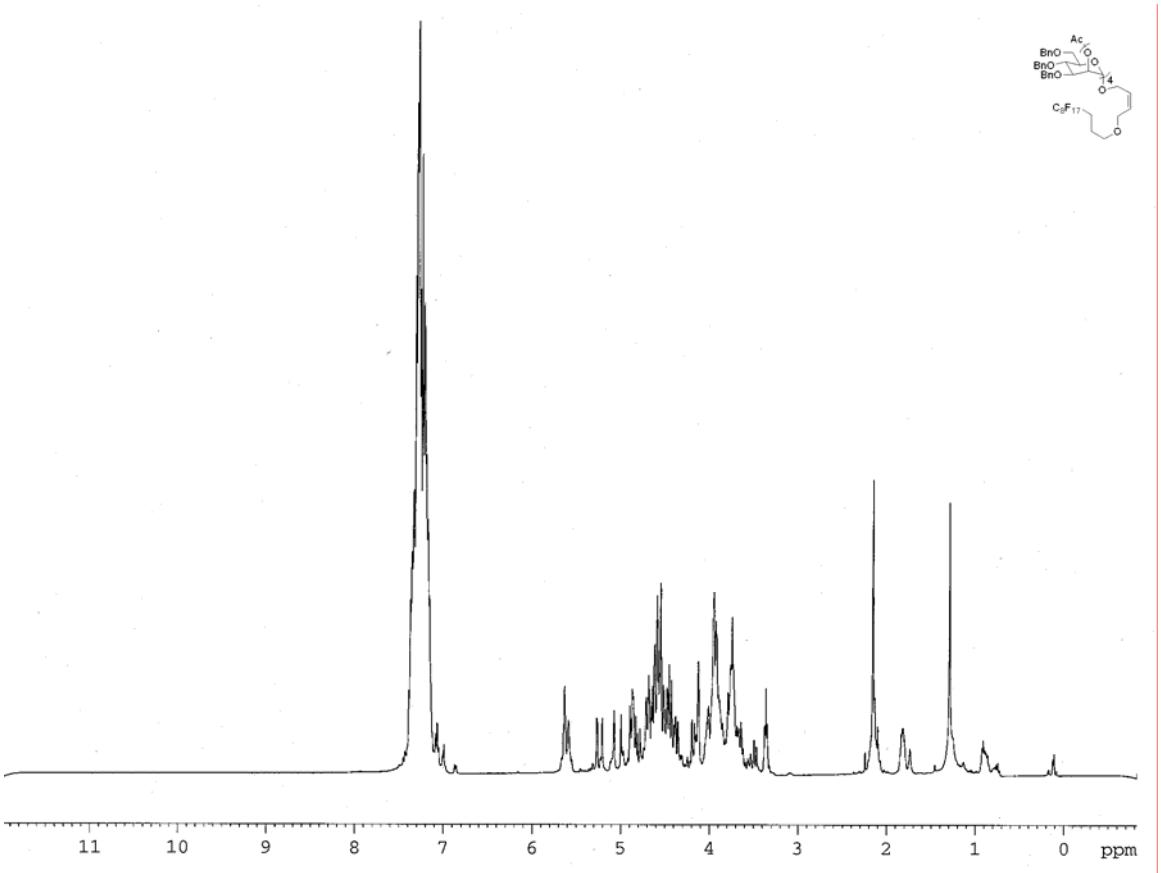


Figure 3.5: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside.

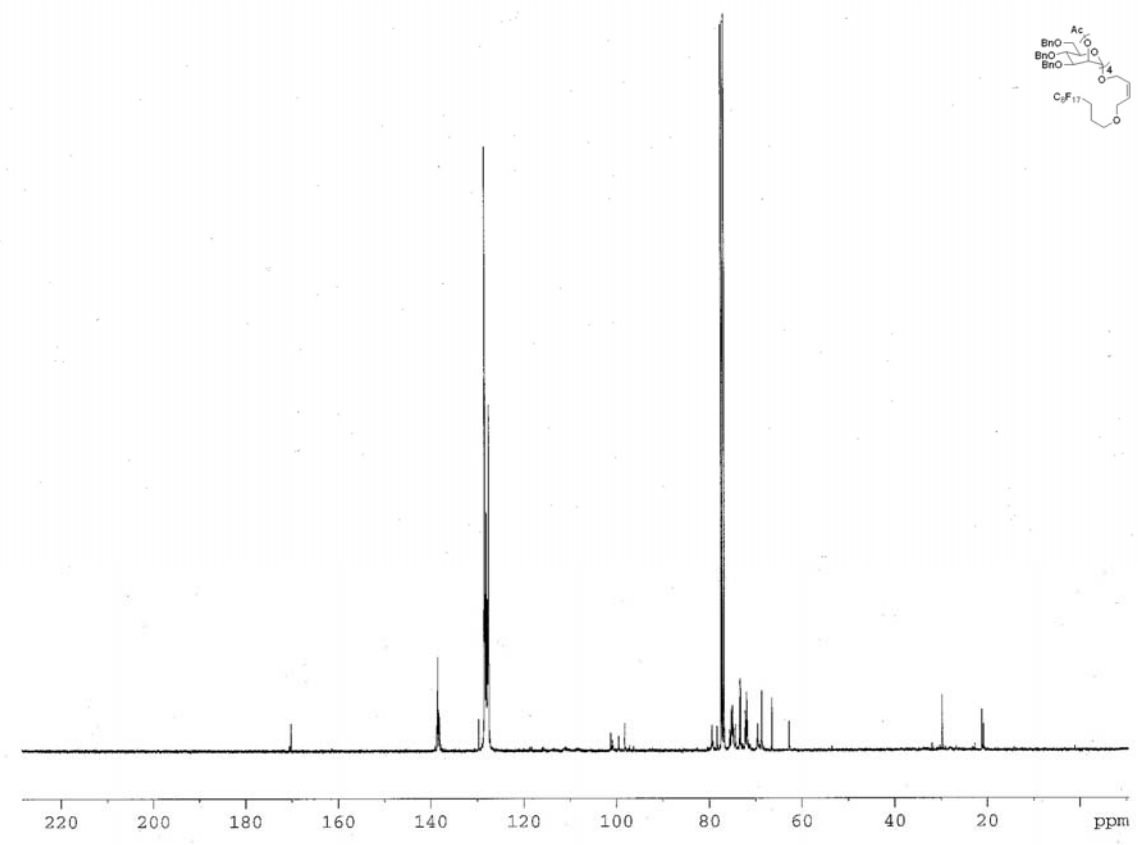


Figure 3.6: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6-tri-*O*-benzyl-2-*O*-[2-*O*-(2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside.

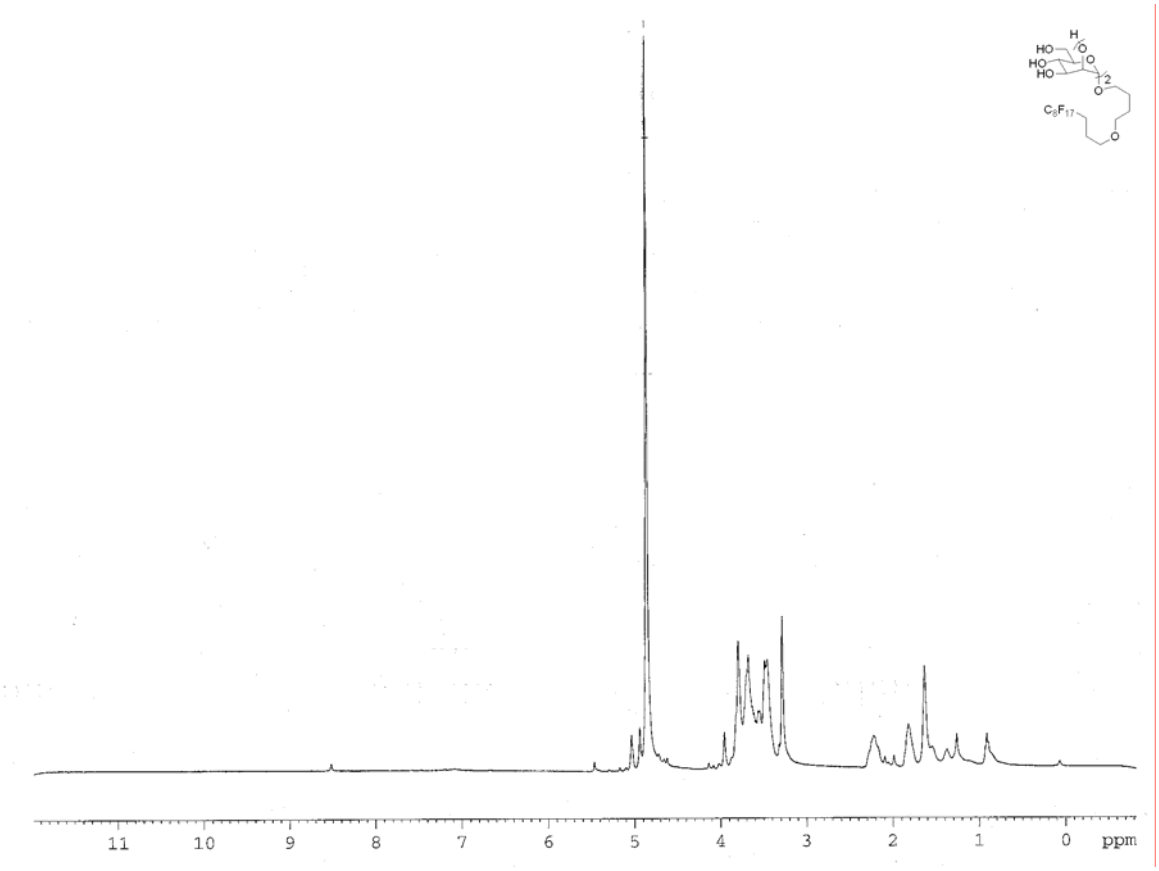


Figure 3.7: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside.

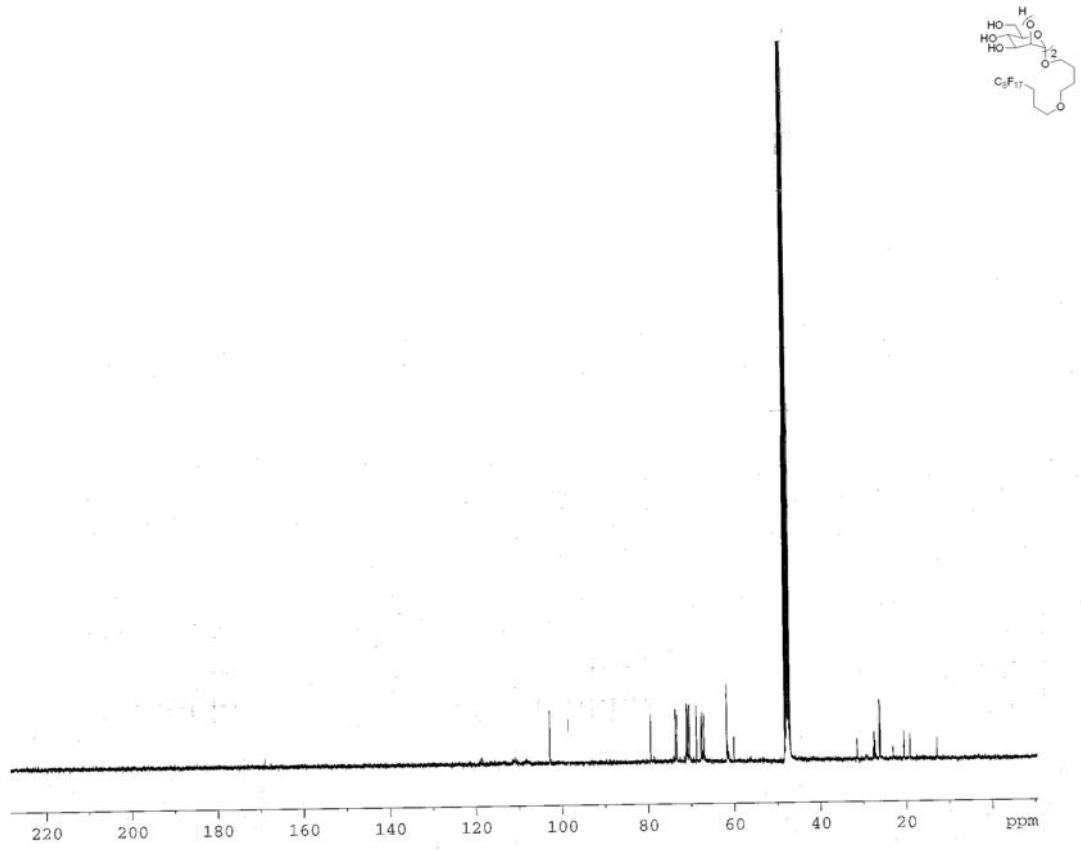


Figure 3.8: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside.

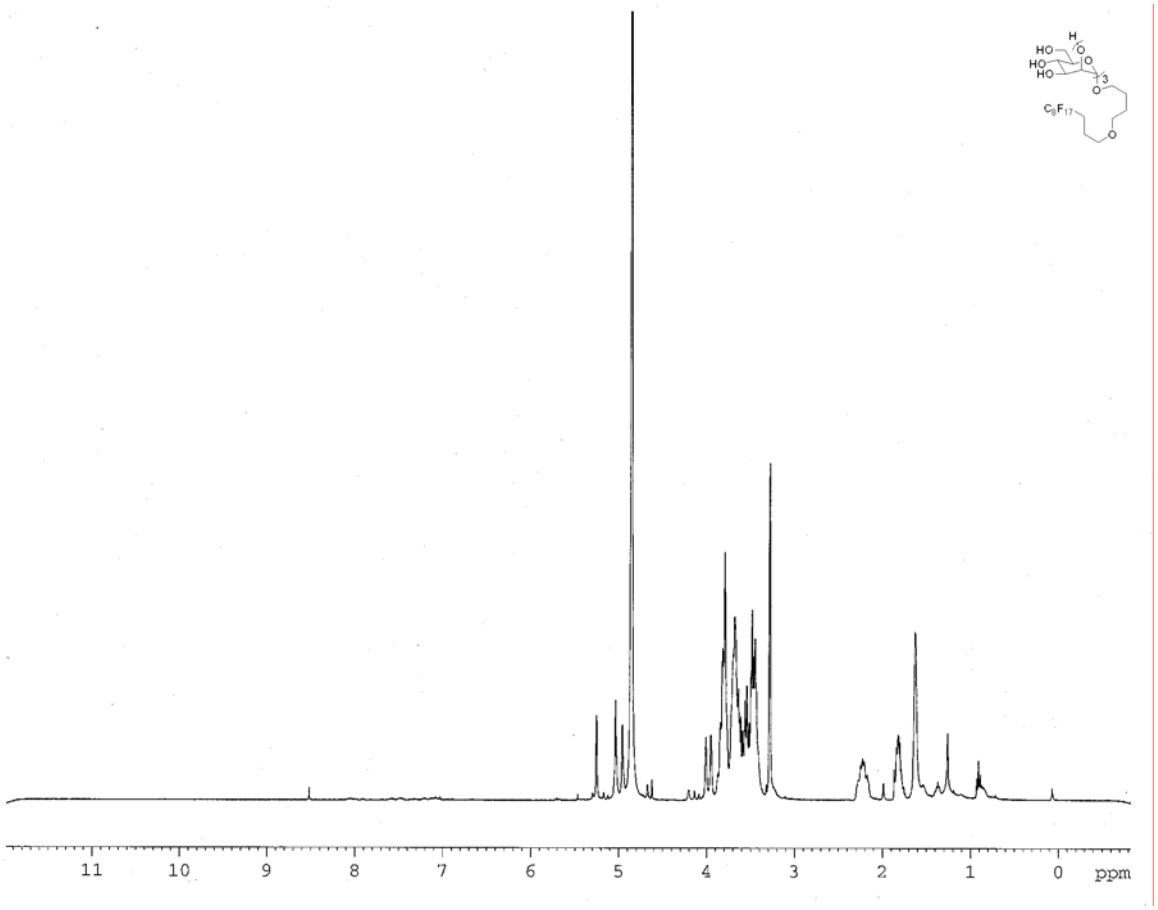


Figure 3.9: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside.

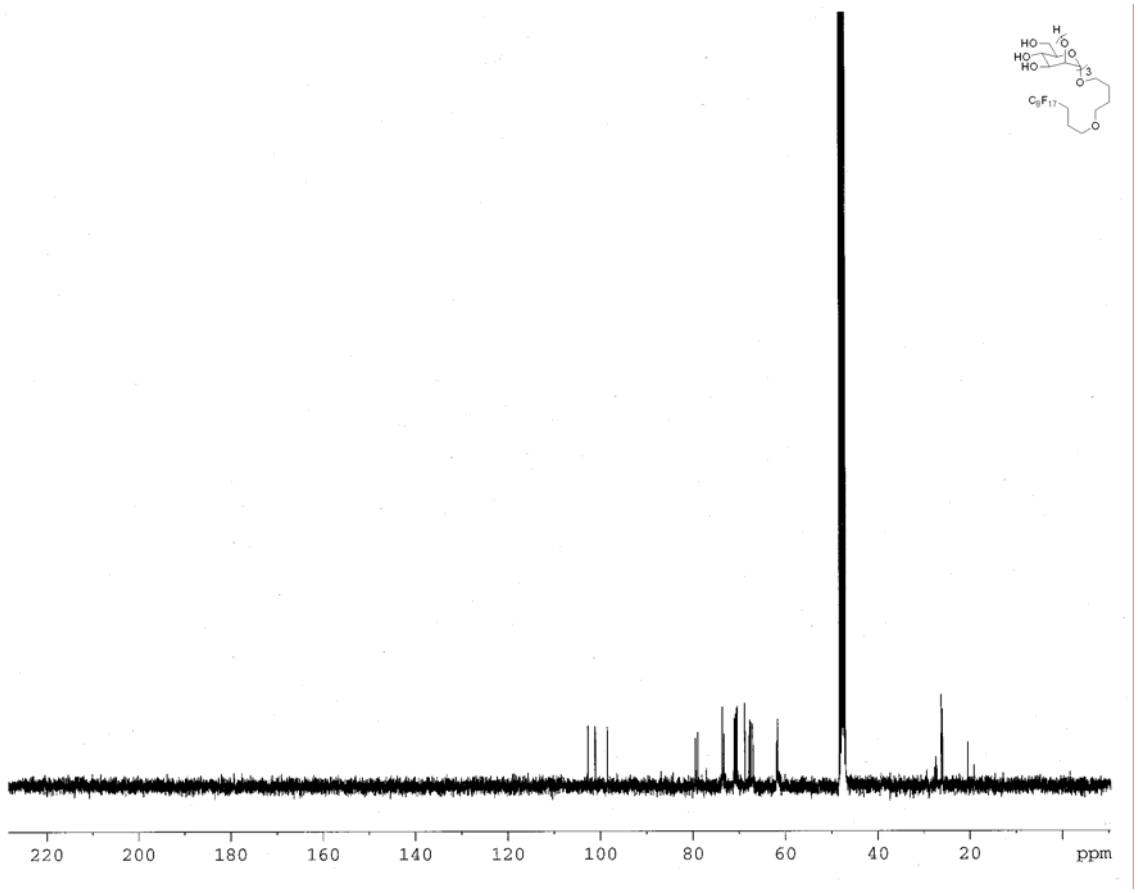


Figure 3.10: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside.

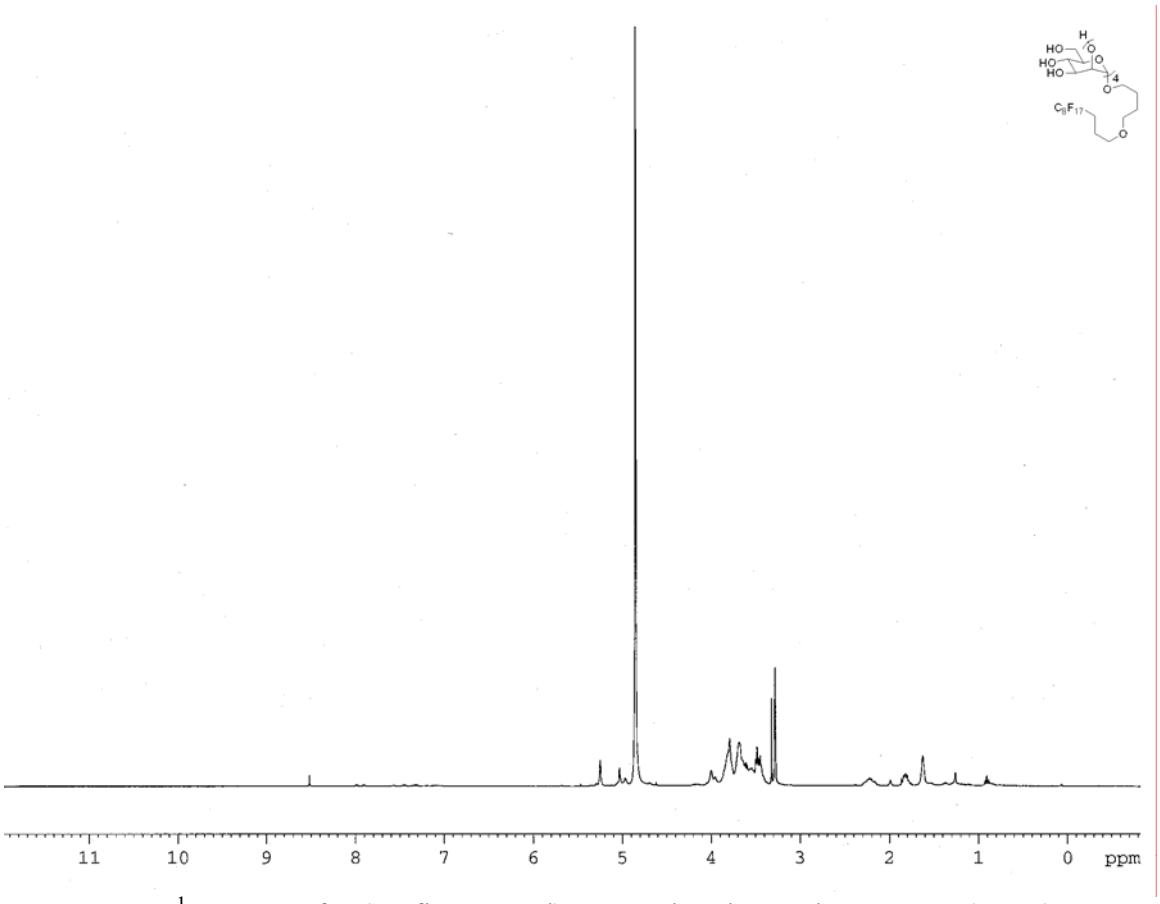


Figure 3.11: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside.

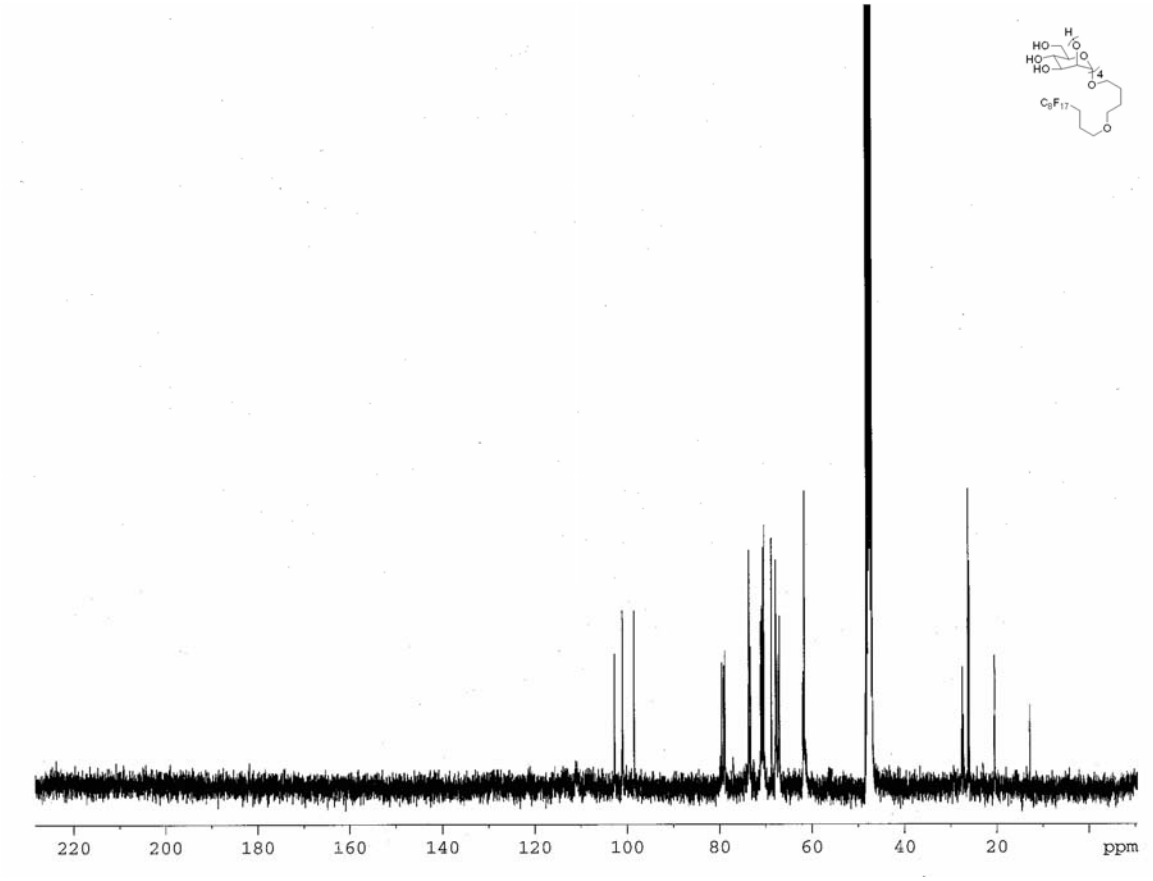


Figure 3.12: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-2-*O*-[2-*O*-(2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside.

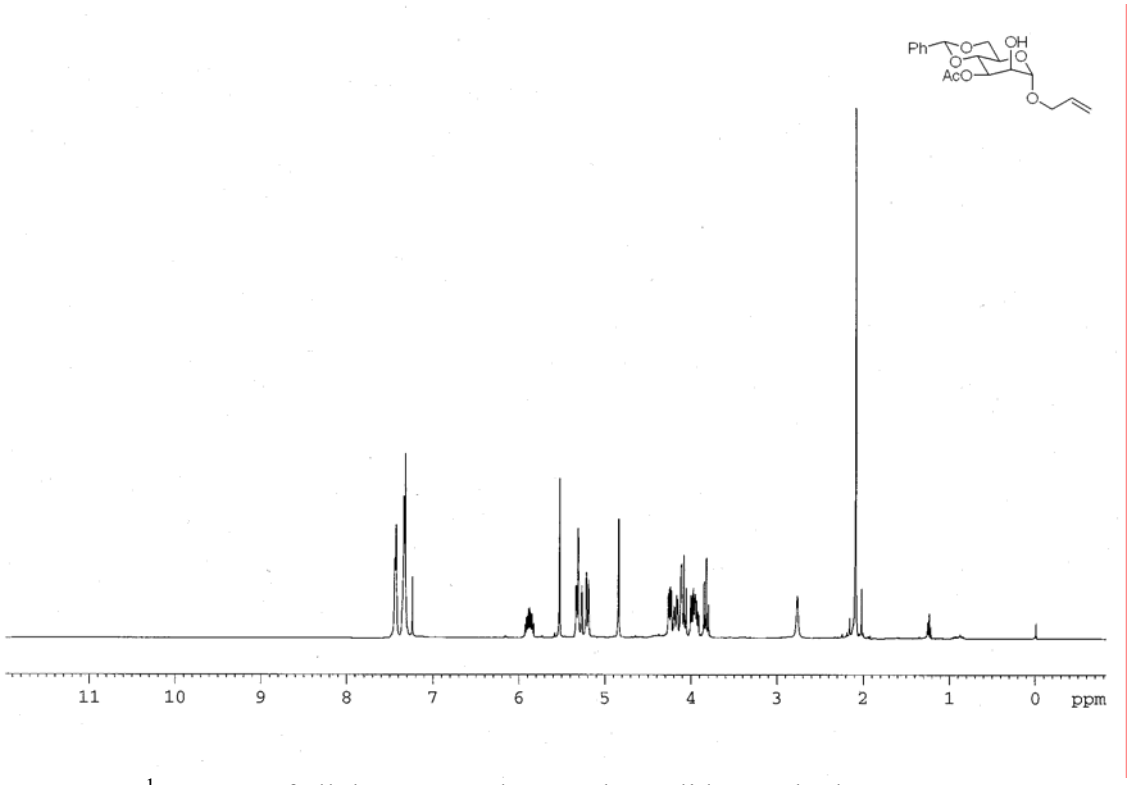


Figure 3.13: ^{1}H NMR of allyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-hydroxy-α-D-mannopyranoside.

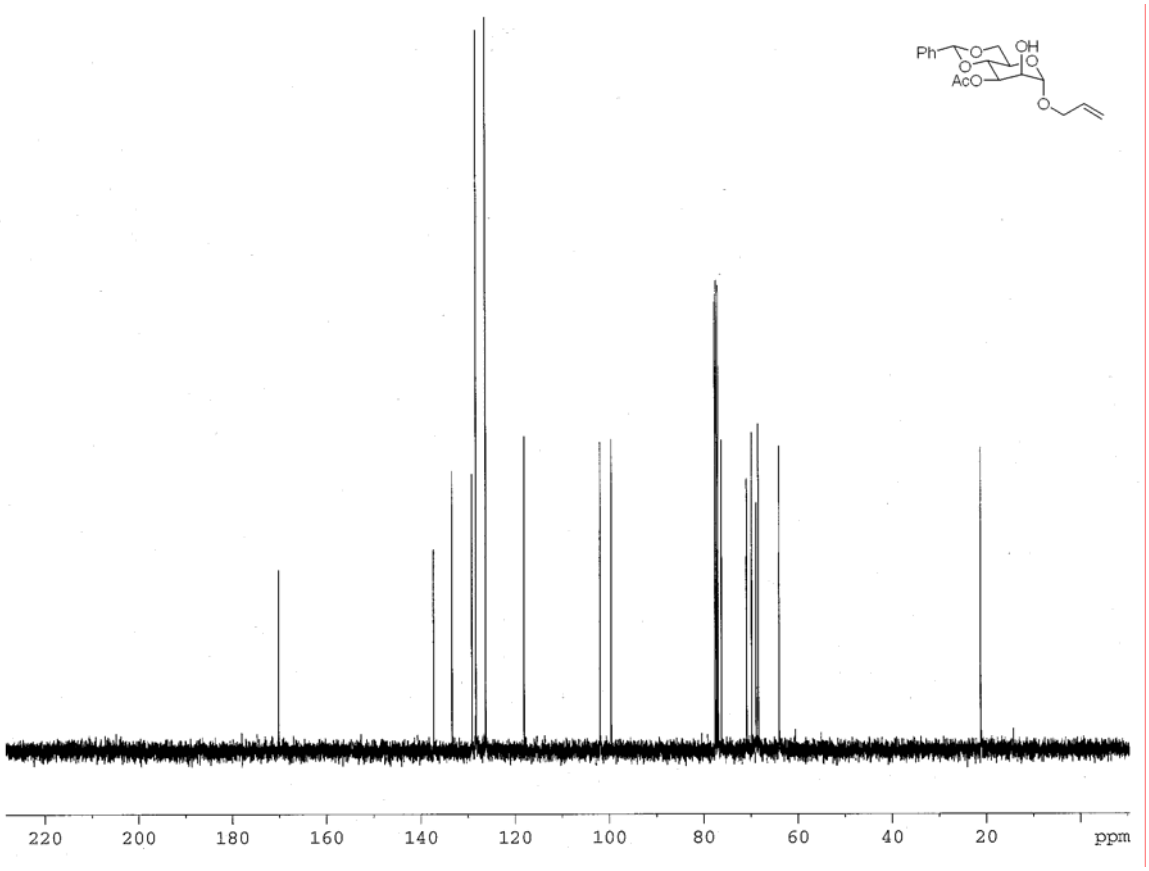


Figure 3.14: ^{13}C NMR of allyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-hydroxy-α-D-mannopyranoside.

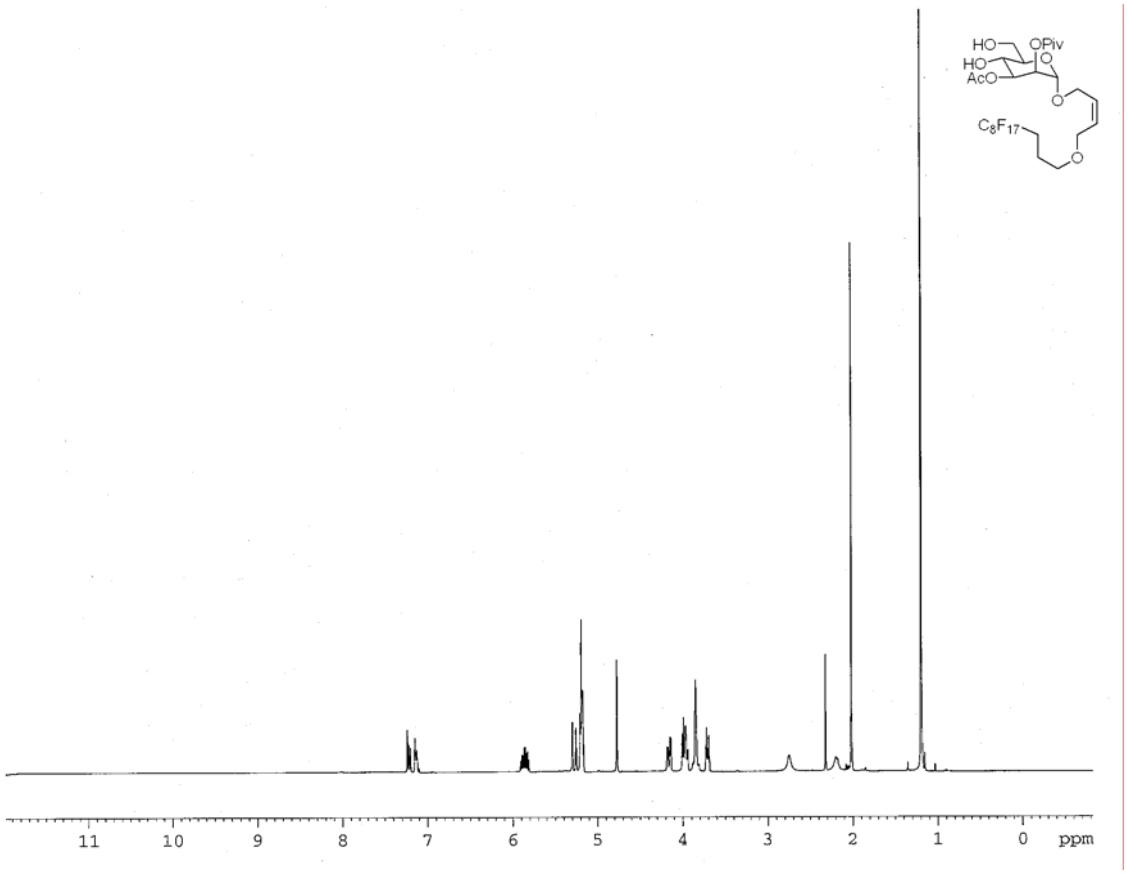


Figure 3.15: ^{1}H NMR of allyl-3-*O*-acetyl-4,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside.

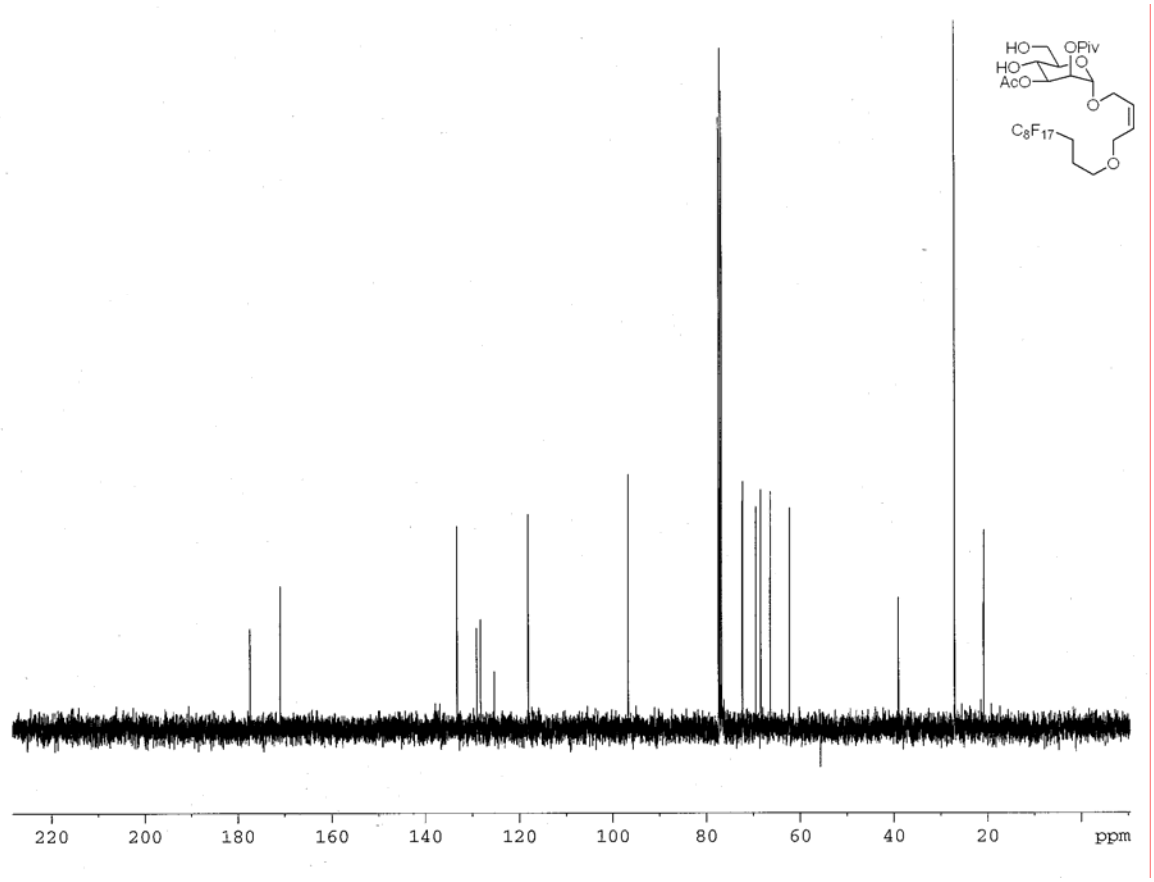


Figure 3.16: ^{13}C NMR of allyl-3-*O*-acetyl-4,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside.

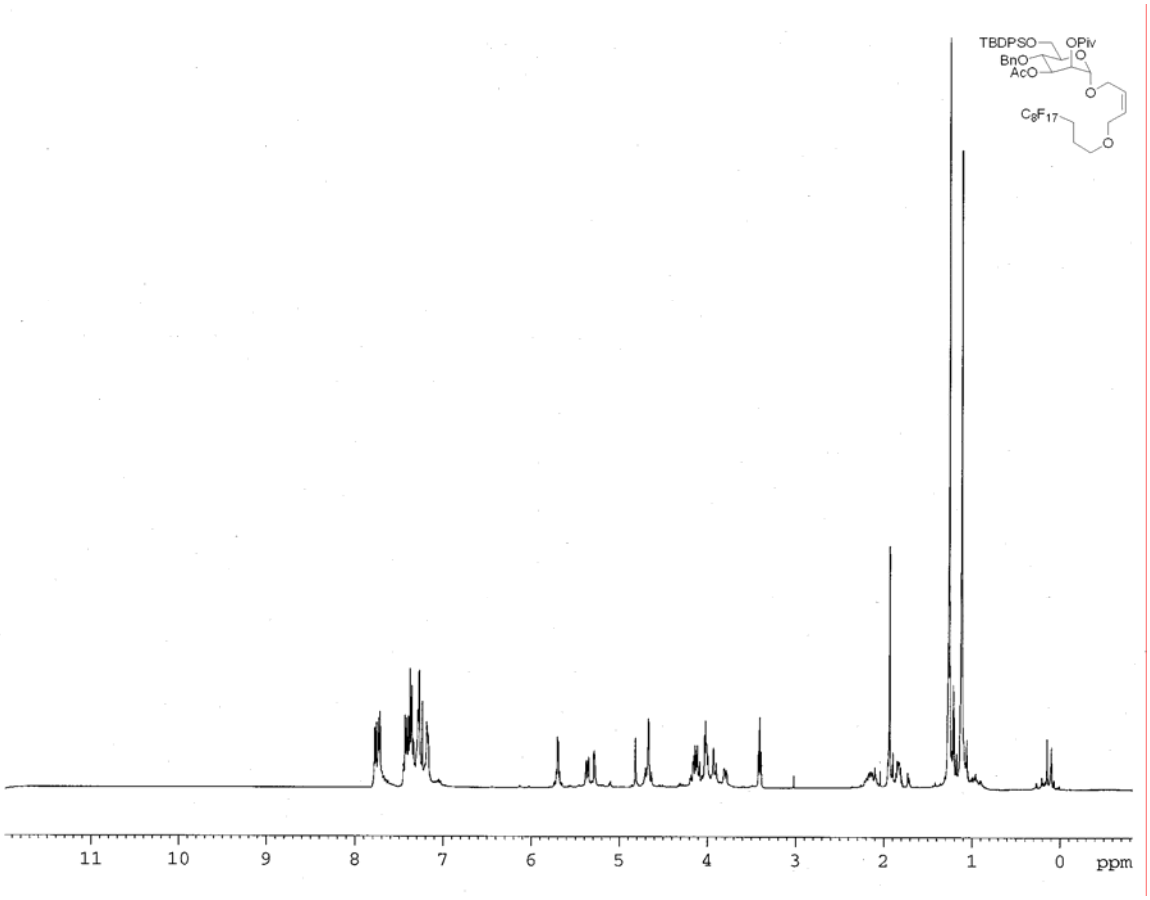


Figure 3.17: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside.

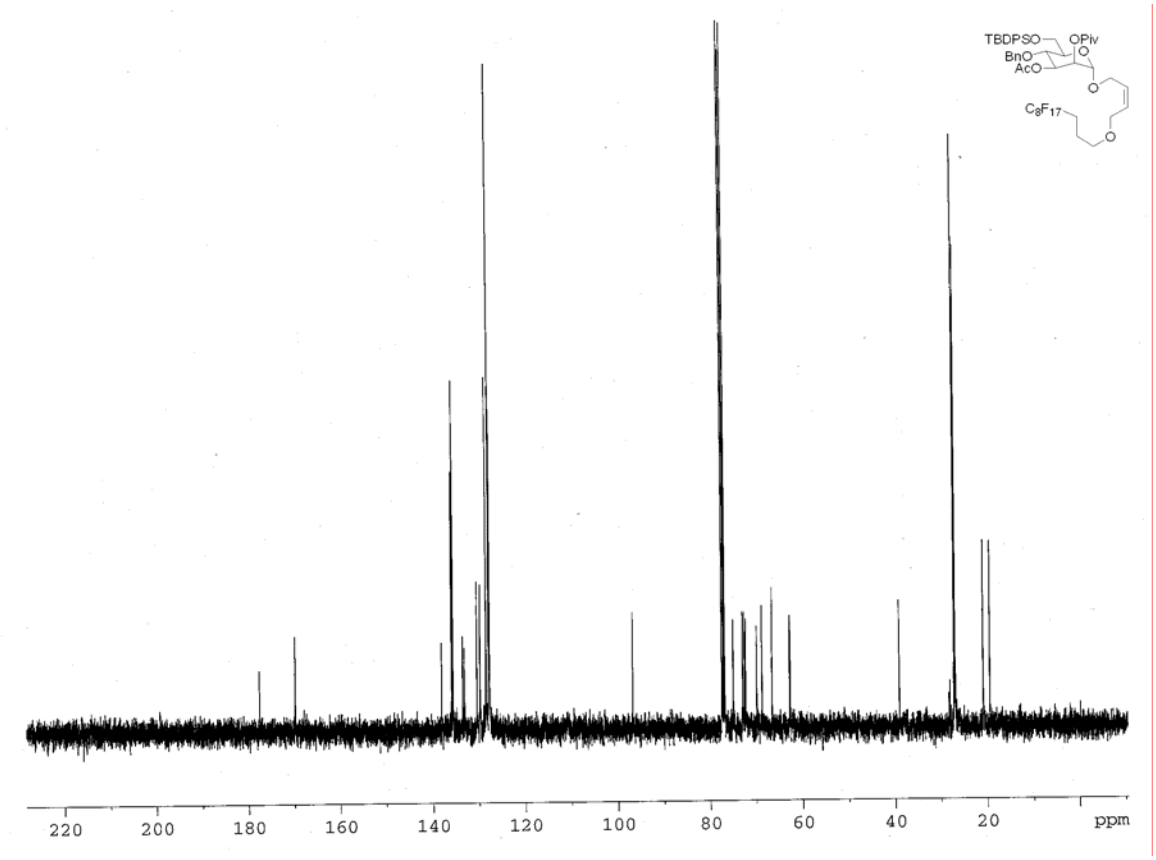


Figure 3.18: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-2-*O*-pivaloyl-α-D-mannopyranoside.

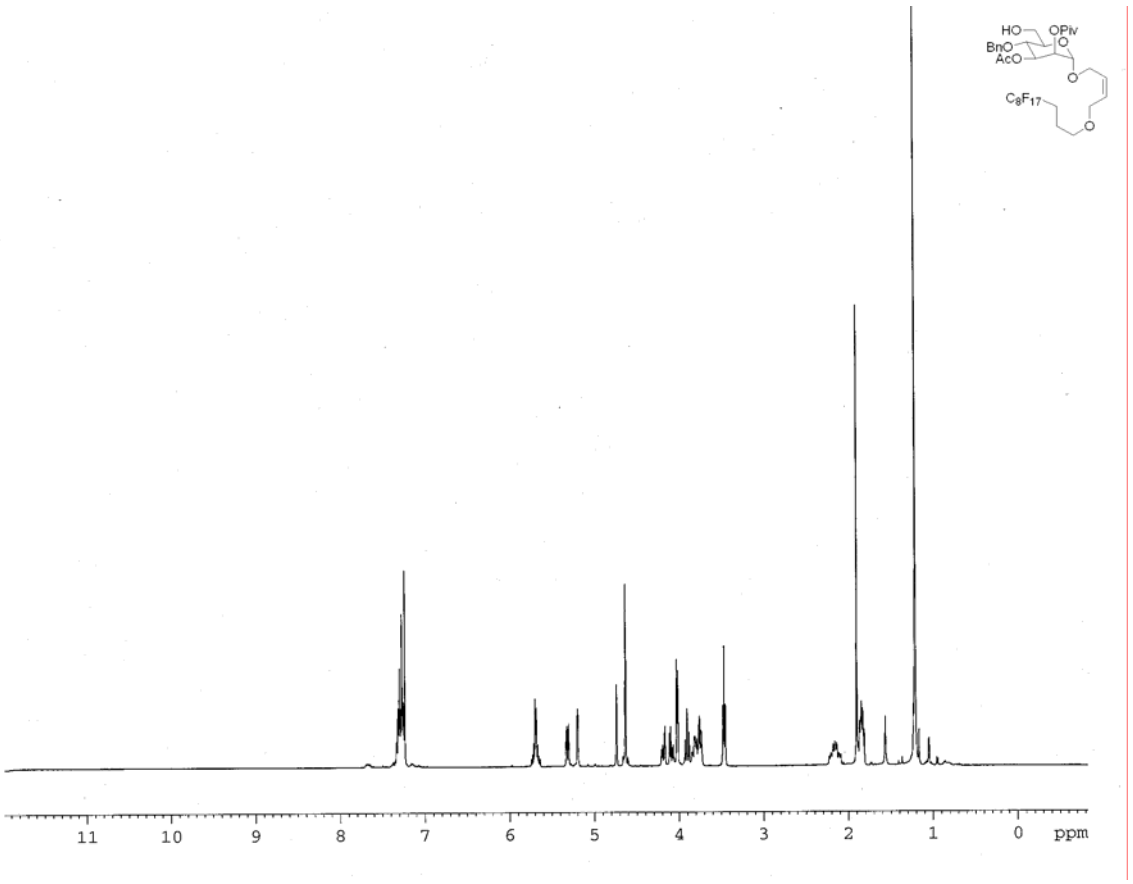


Figure 3.19: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3-*O*-acetyl-4-*O*-benzyl-6-hydroxy-2-*O*-pivaloyl-α-D-mannopyranoside.

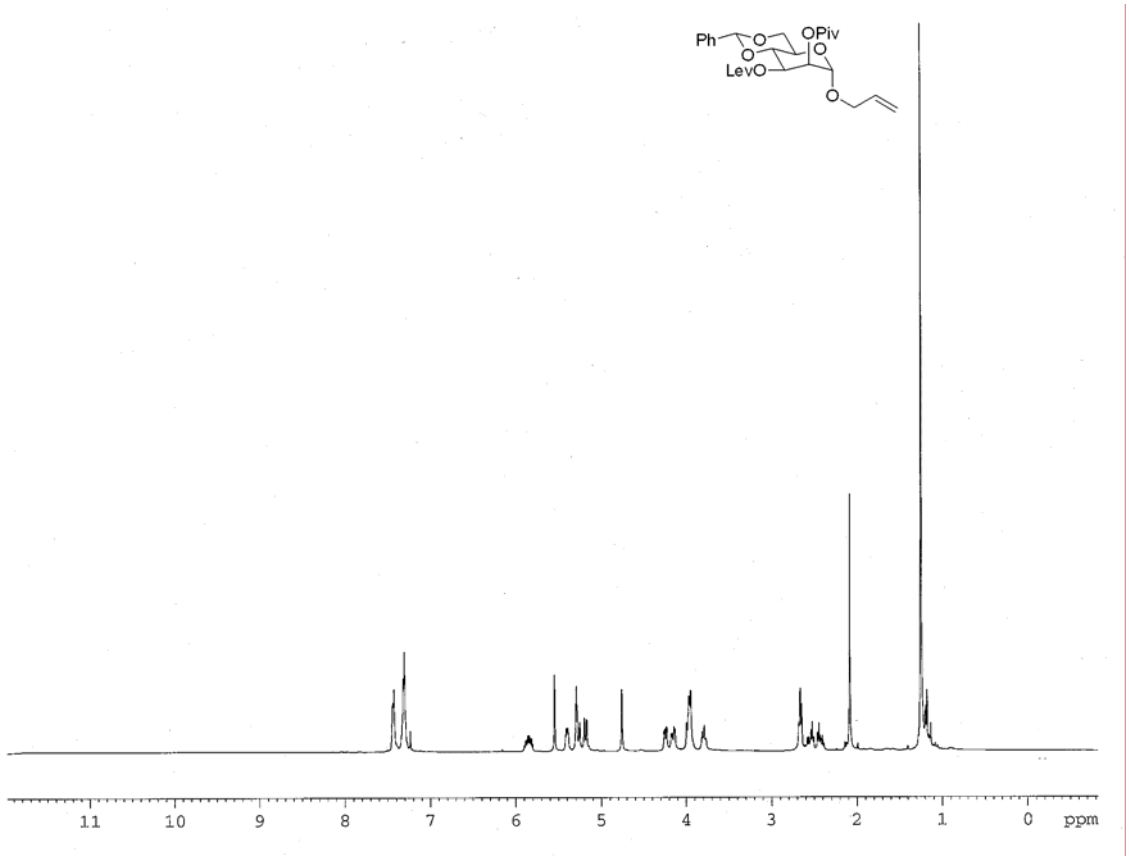


Figure 3.20: ^{1}H NMR of allyl-4,6-*O*-benzylidene-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

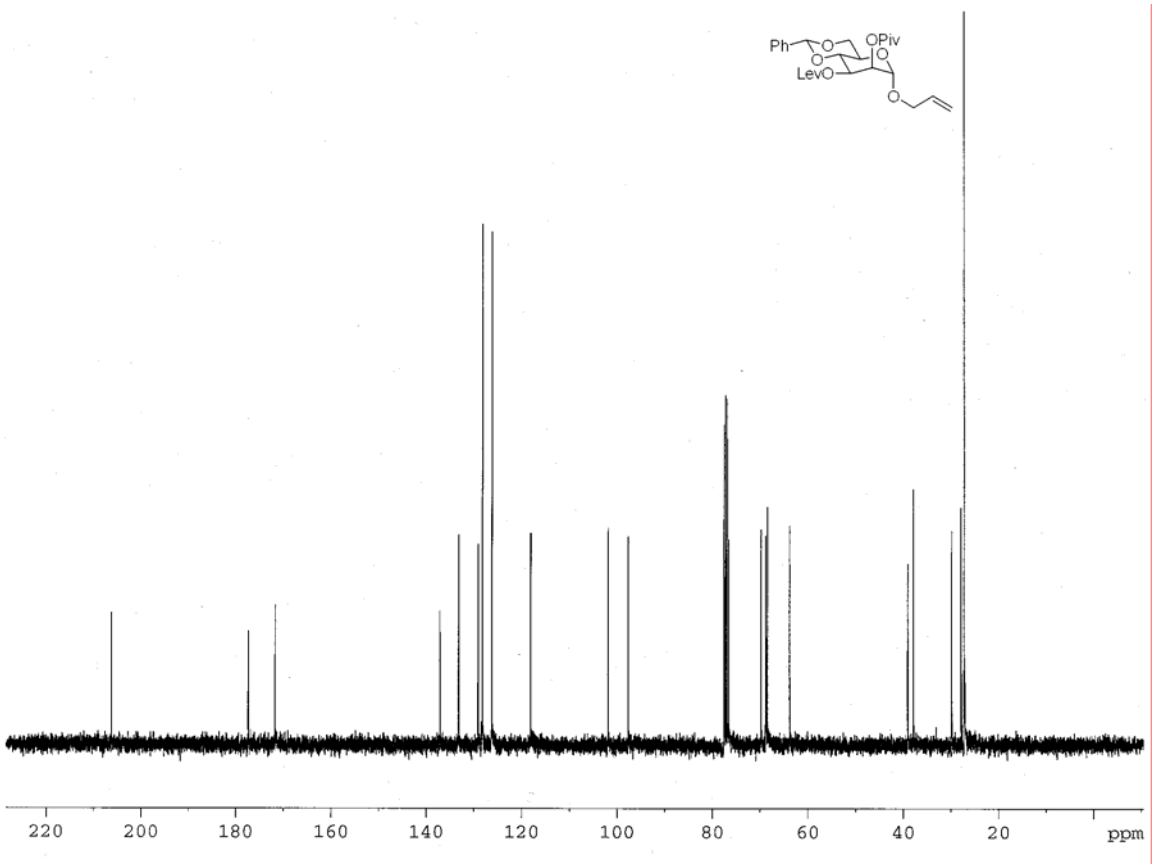


Figure 3.21: ^{13}C NMR of allyl-4,6-*O*-benzylidene-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

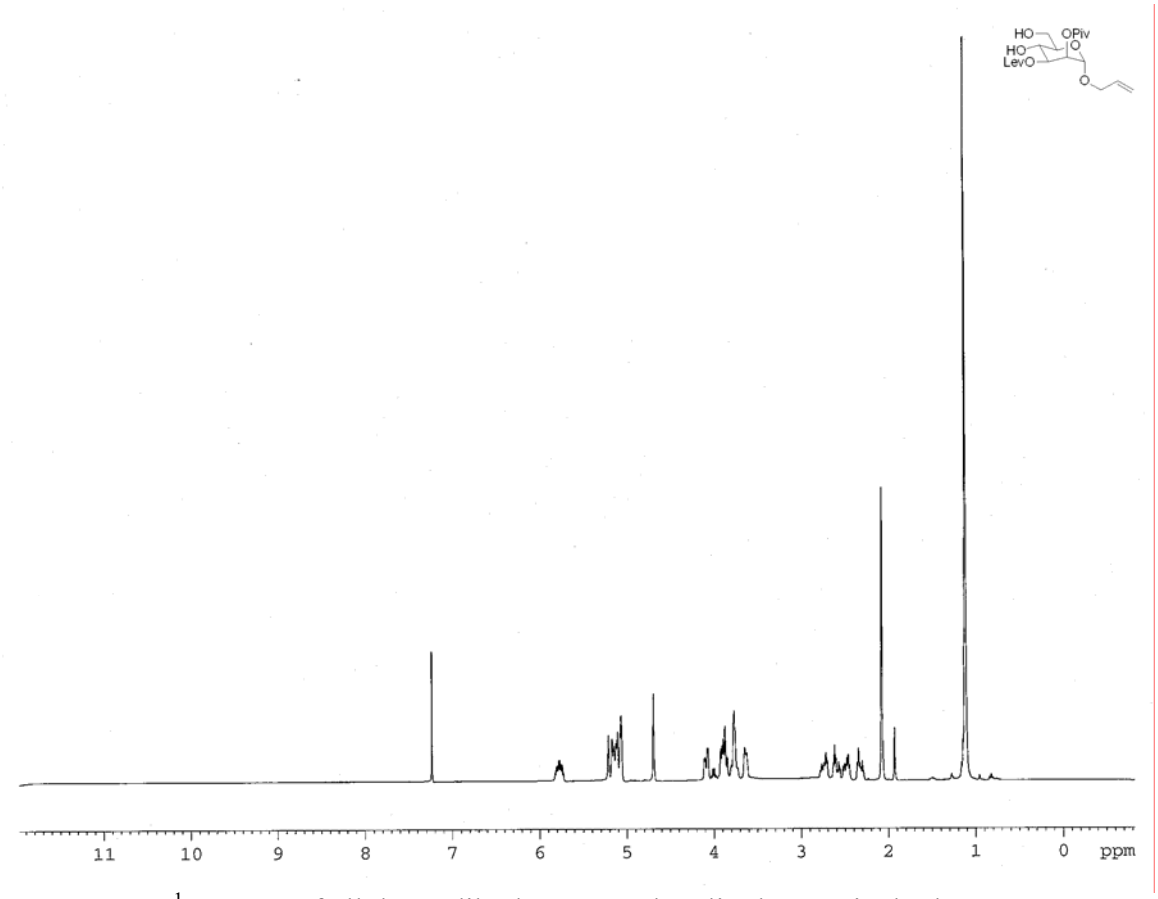


Figure 3.22: ^{1}H NMR of allyl-4,6-dihydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

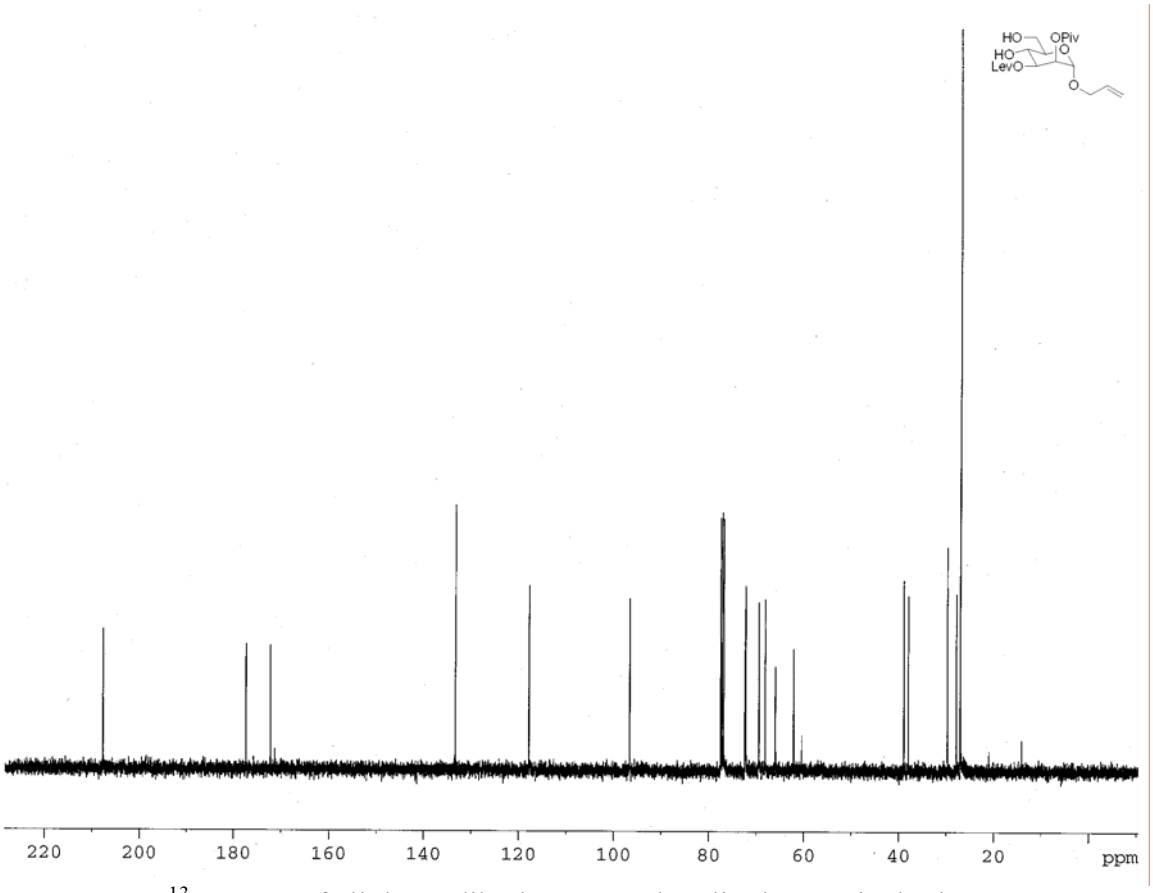


Figure 3.23: ^{13}C NMR of allyl-4,6-dihydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

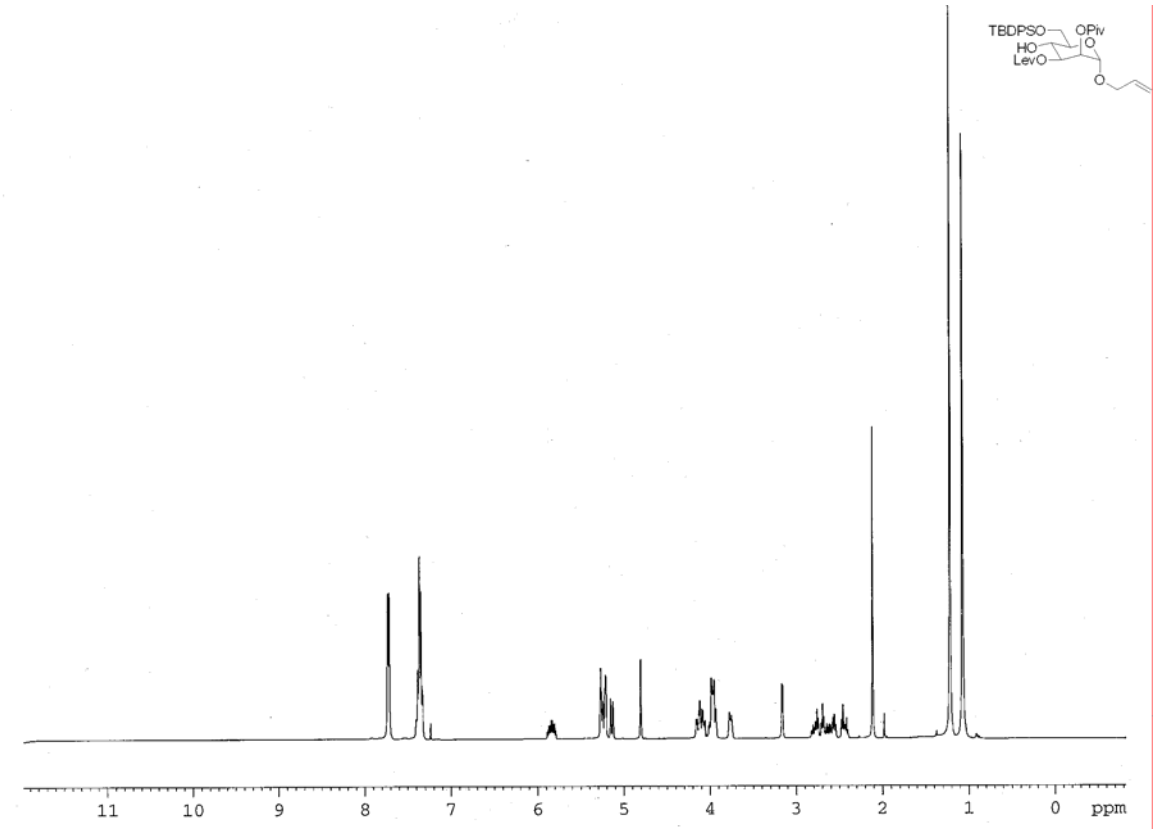


Figure 3.24: ^{1}H NMR of allyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

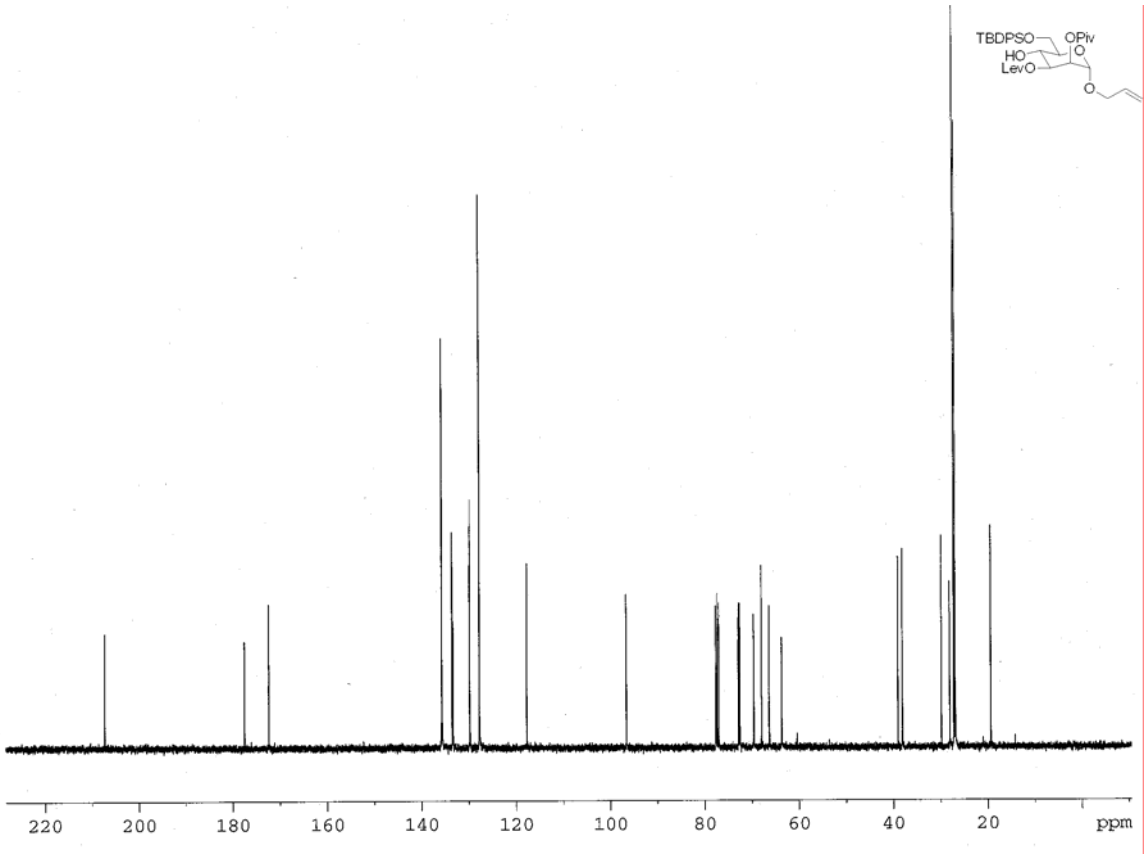


Figure 3.25: ^{13}C NMR of allyl-6-*O*-*t*-butyldiphenylsilyl-4-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

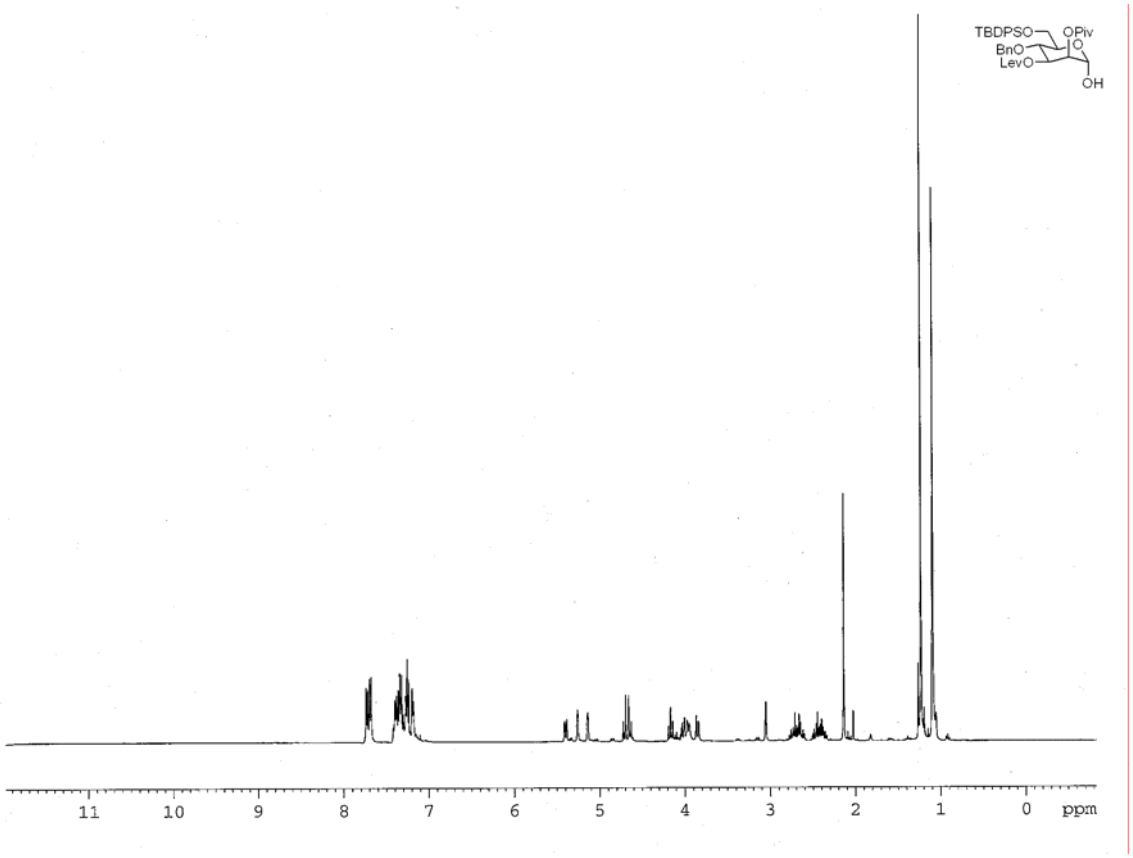


Figure 3.26: ^{1}H NMR of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

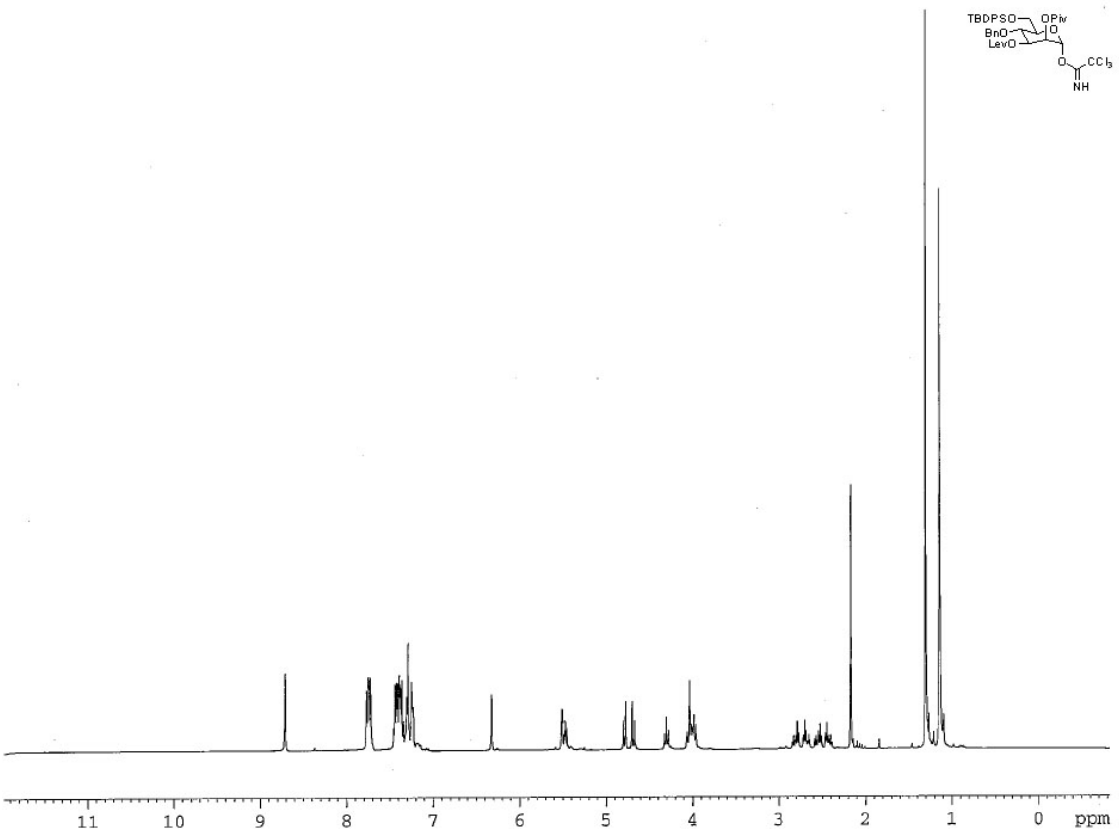


Figure 3.27: ^{1}H NMR of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranosyl trichloroacetimidate.

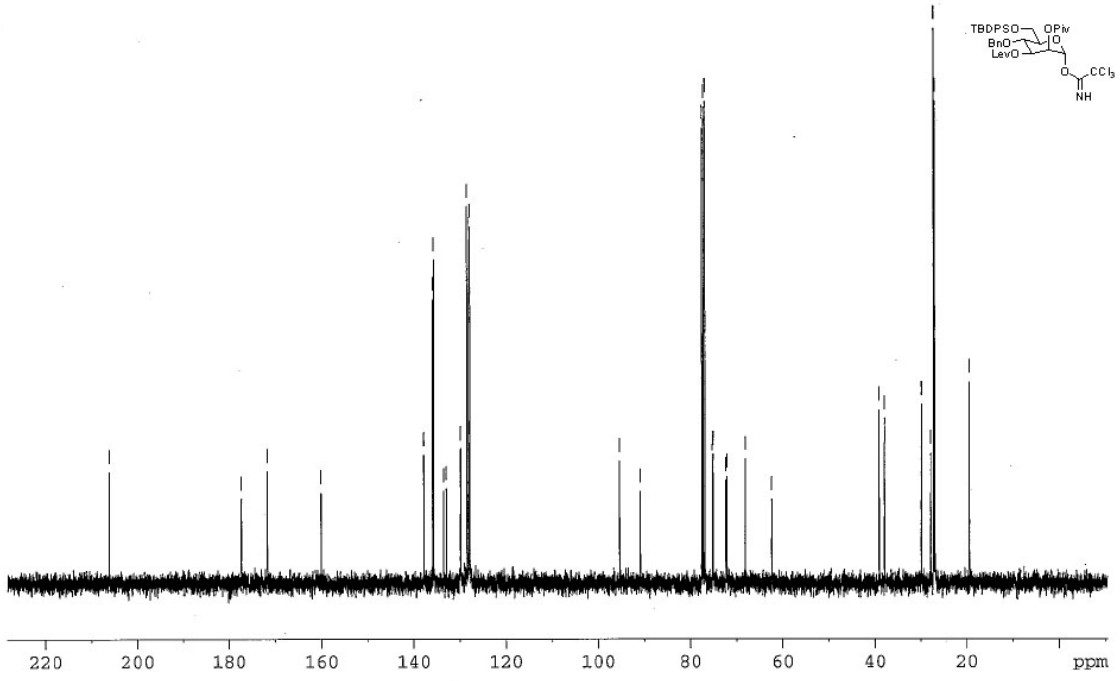


Figure 3.28: ^{13}C NMR of 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranosyl trichloroacetimidate.

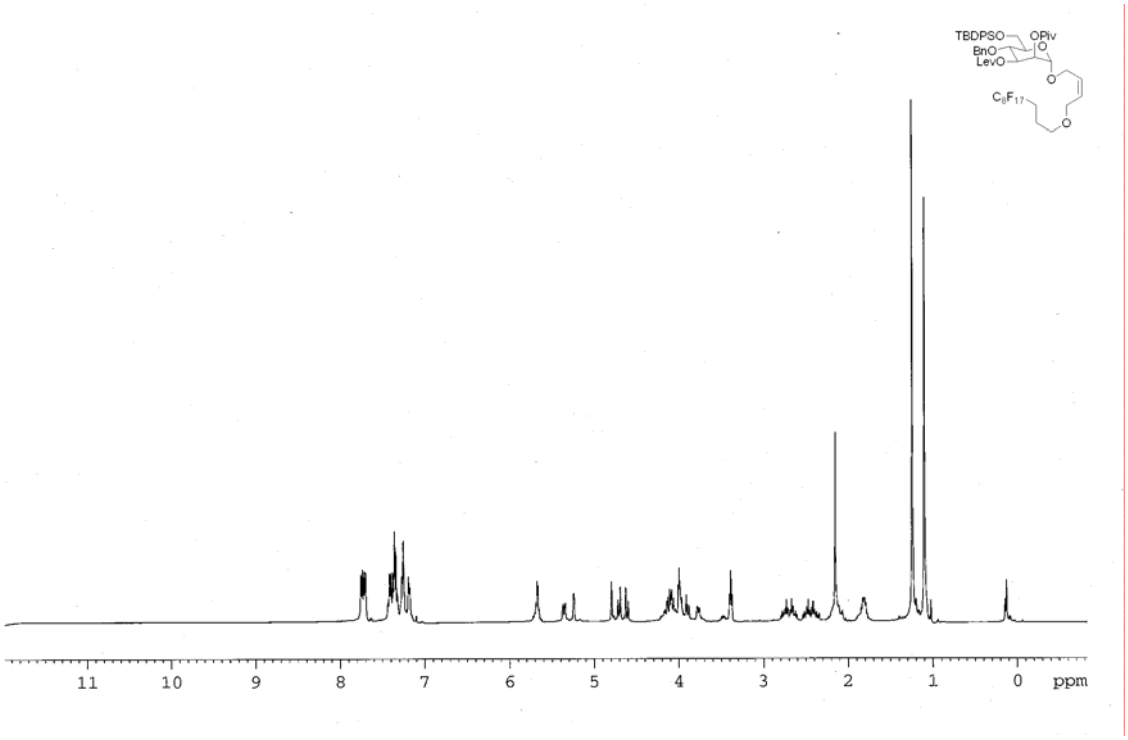


Figure 3.29: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

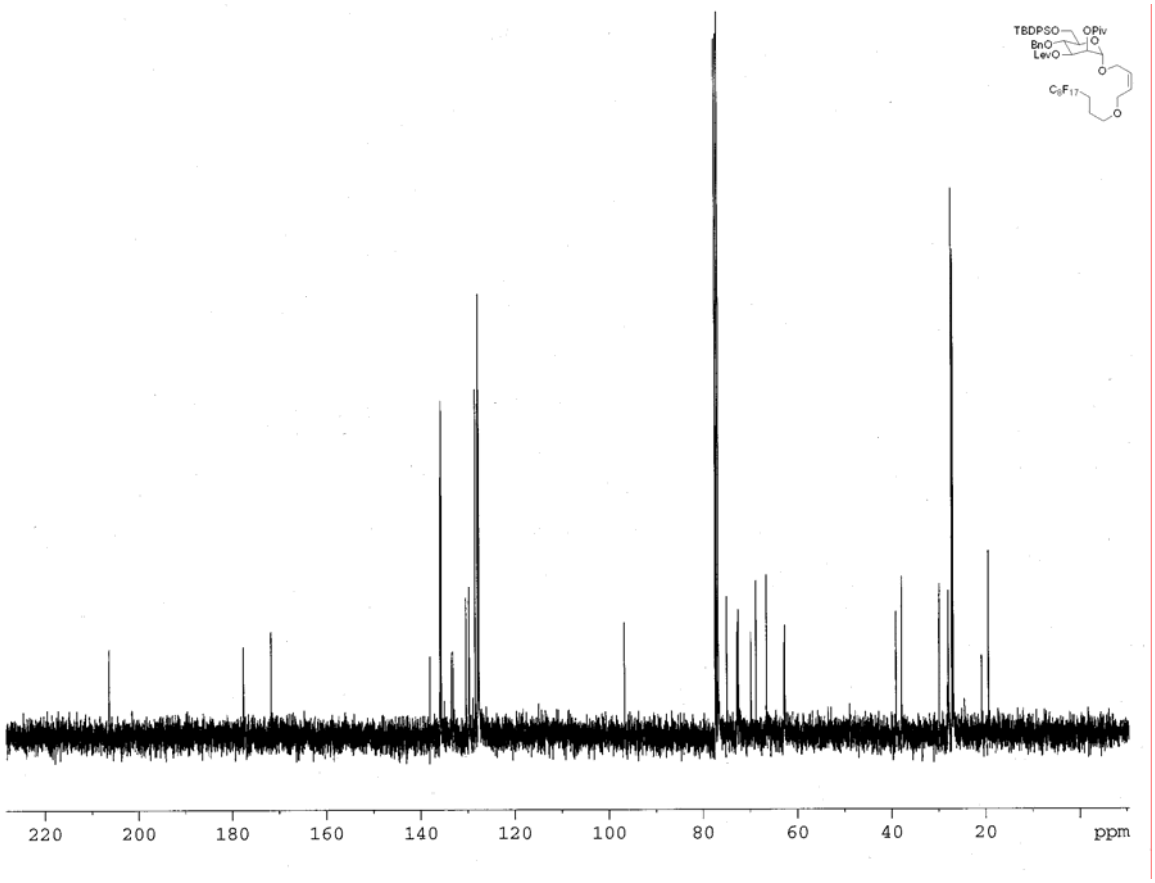


Figure 3.30: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

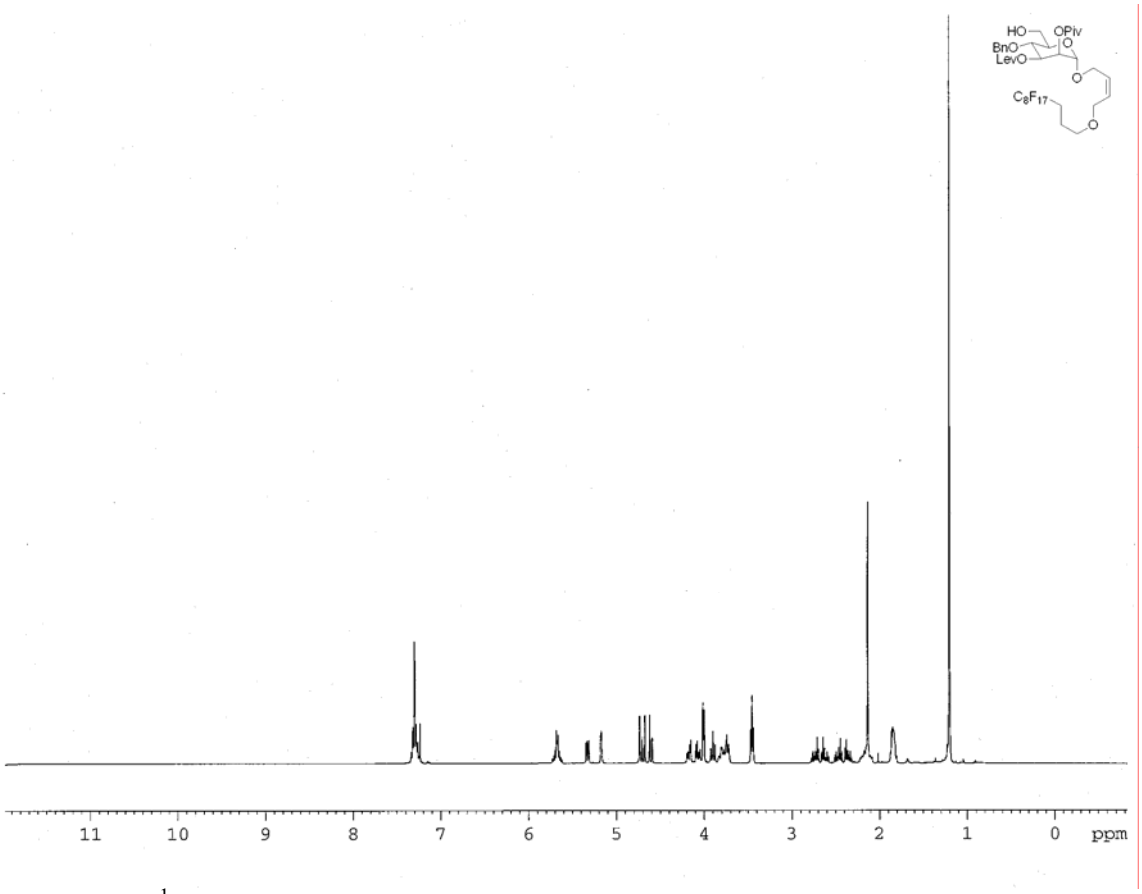


Figure 3.31: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

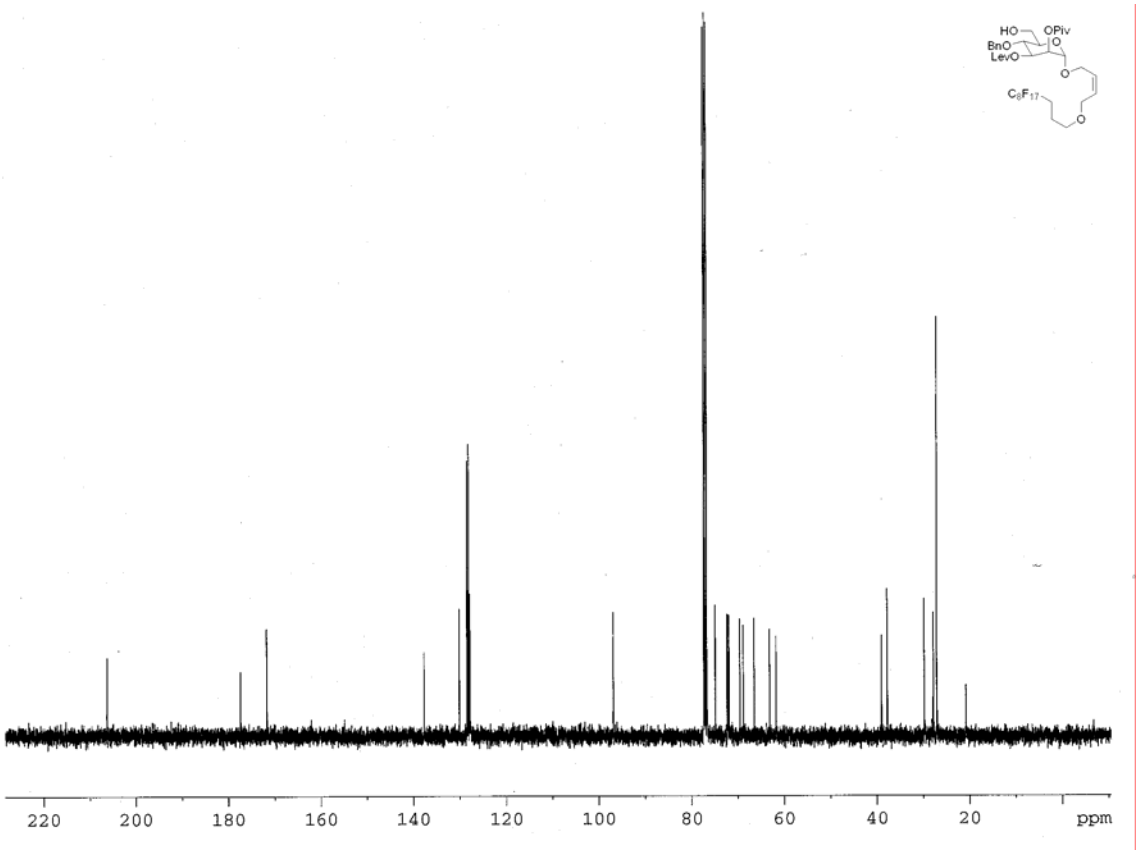


Figure 3.32: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside.

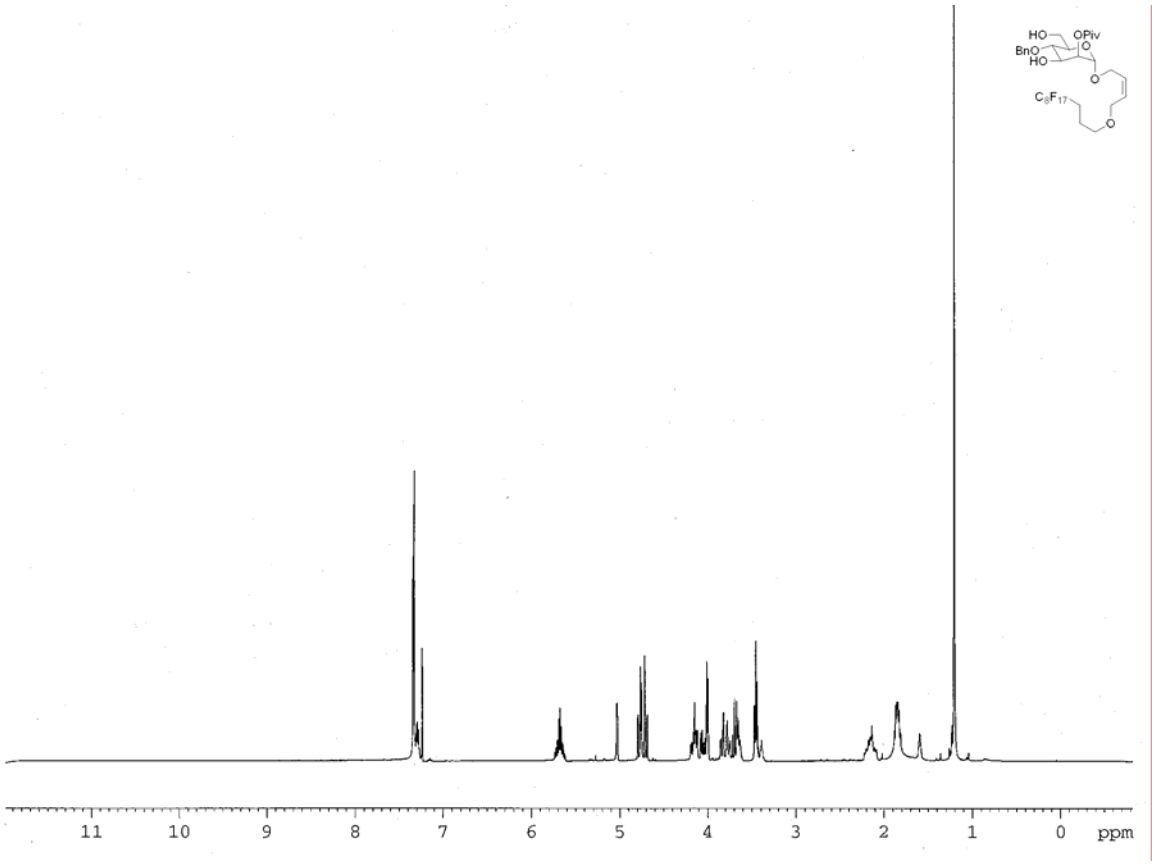


Figure 3.33: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside.

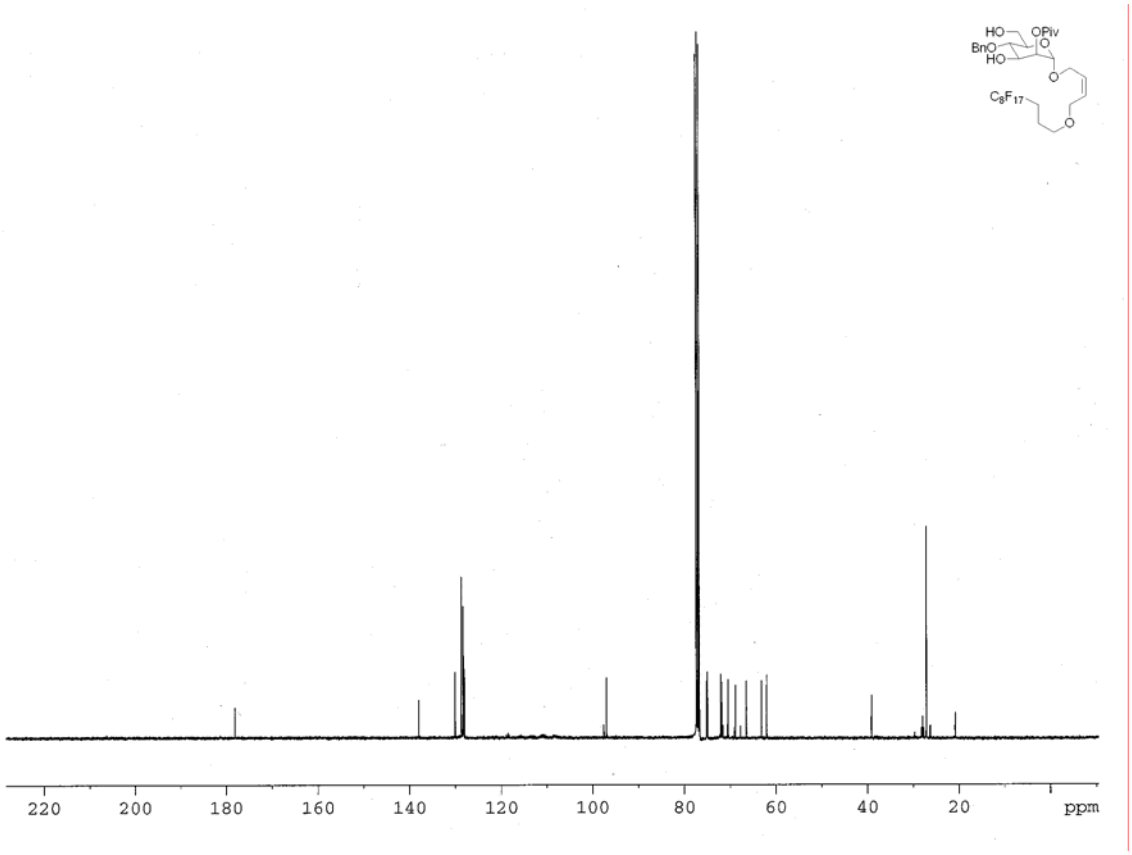


Figure 3.34: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside.

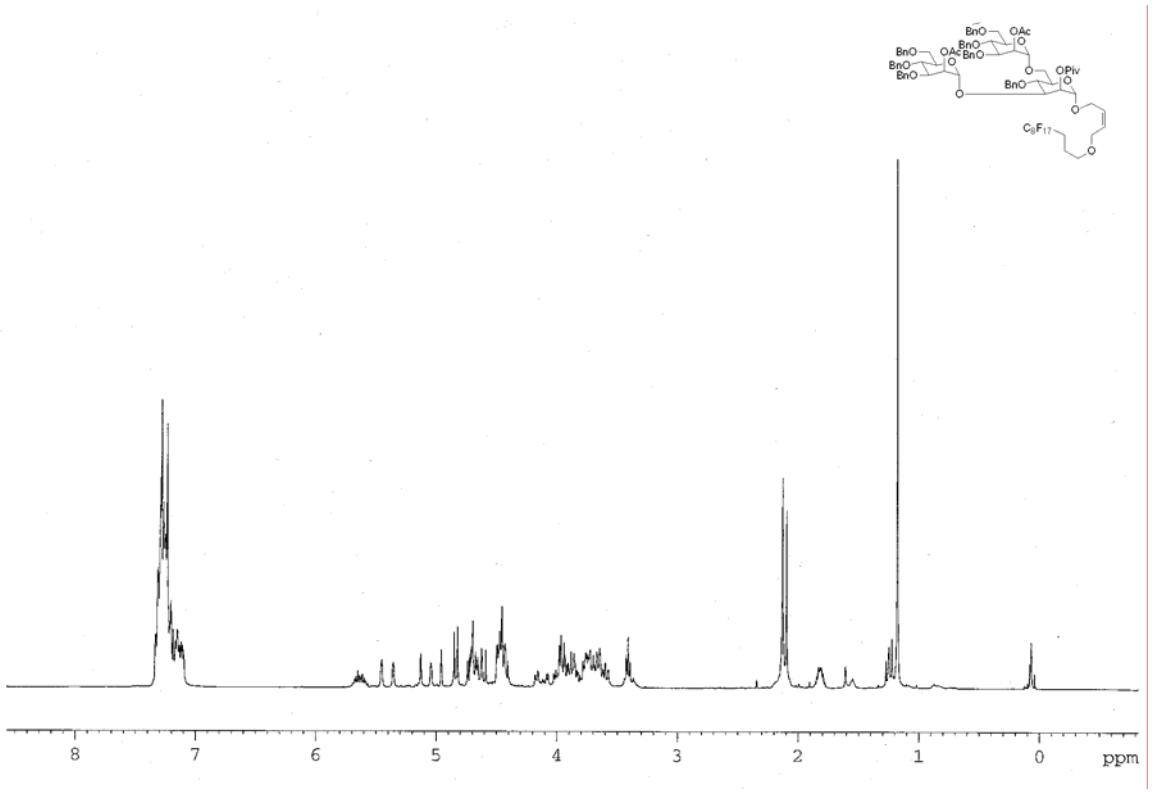


Figure 3.35: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

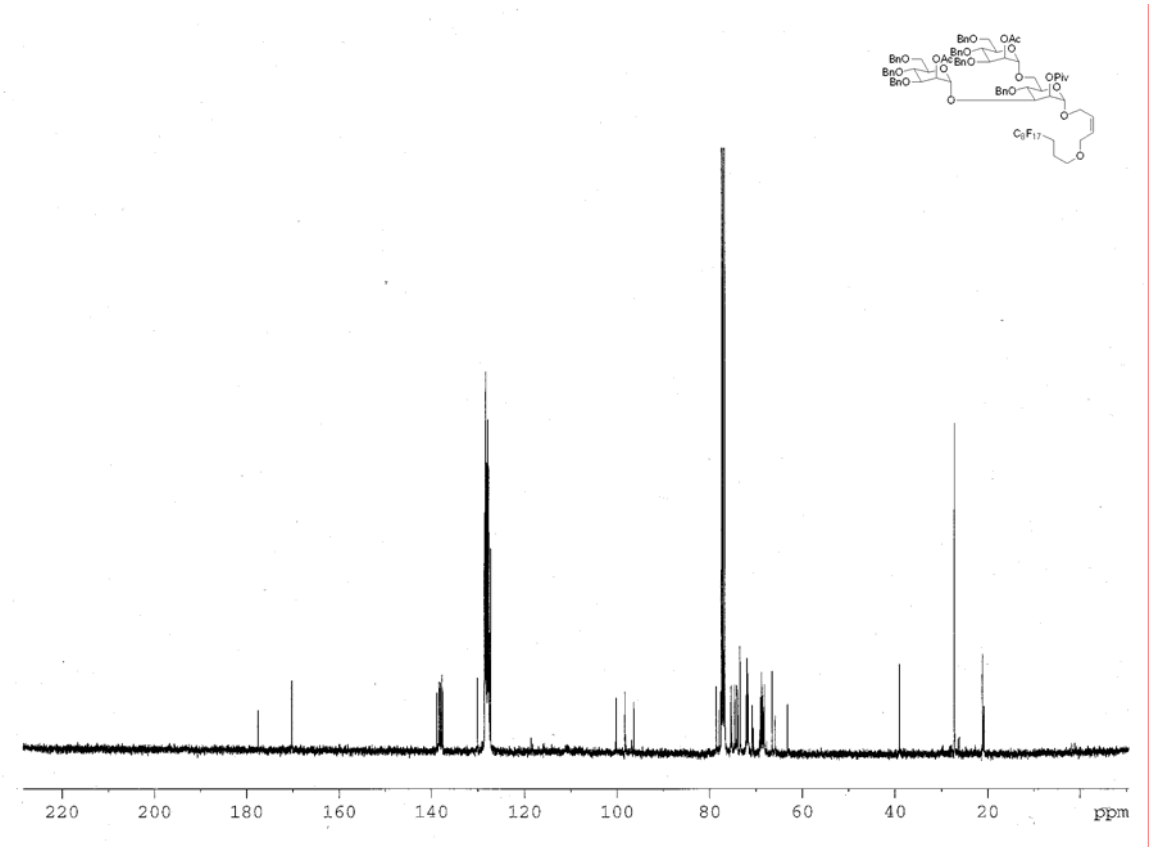


Figure 3.36: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

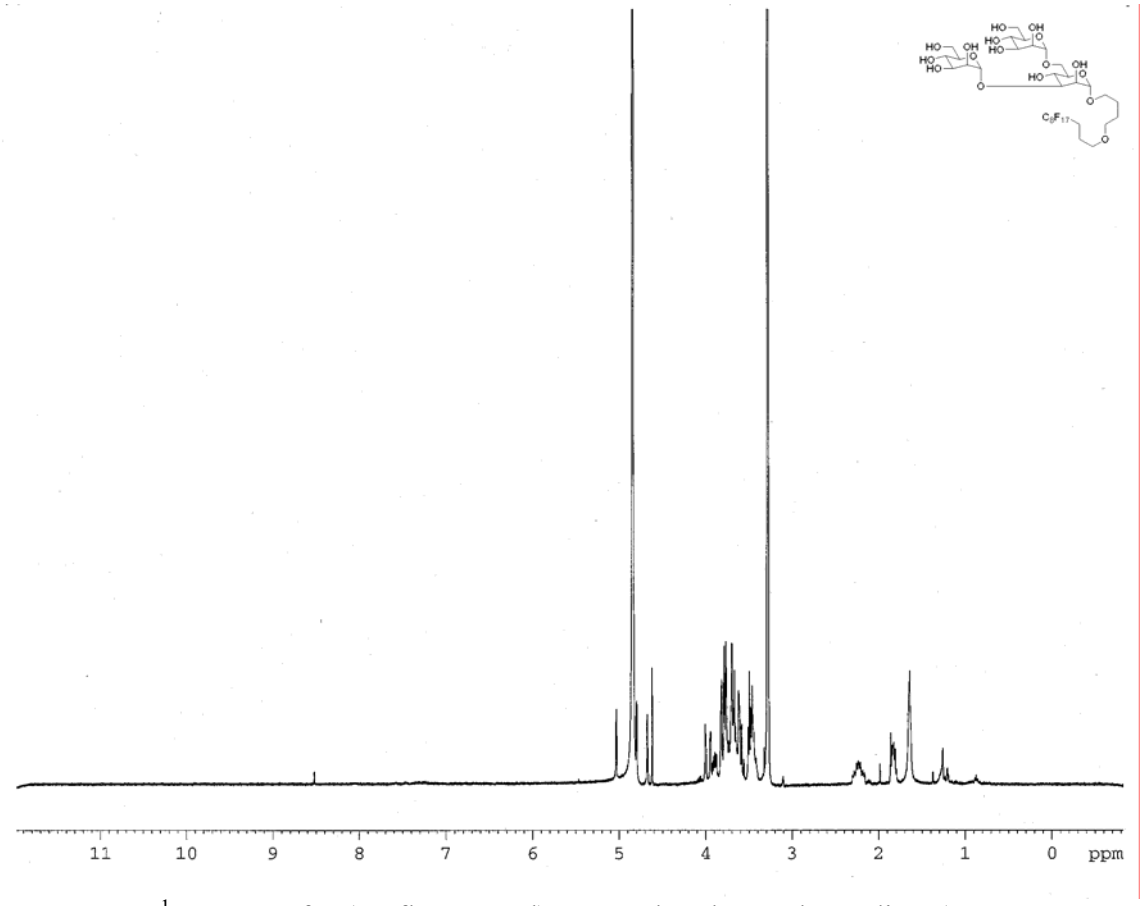


Figure 3.37: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside.

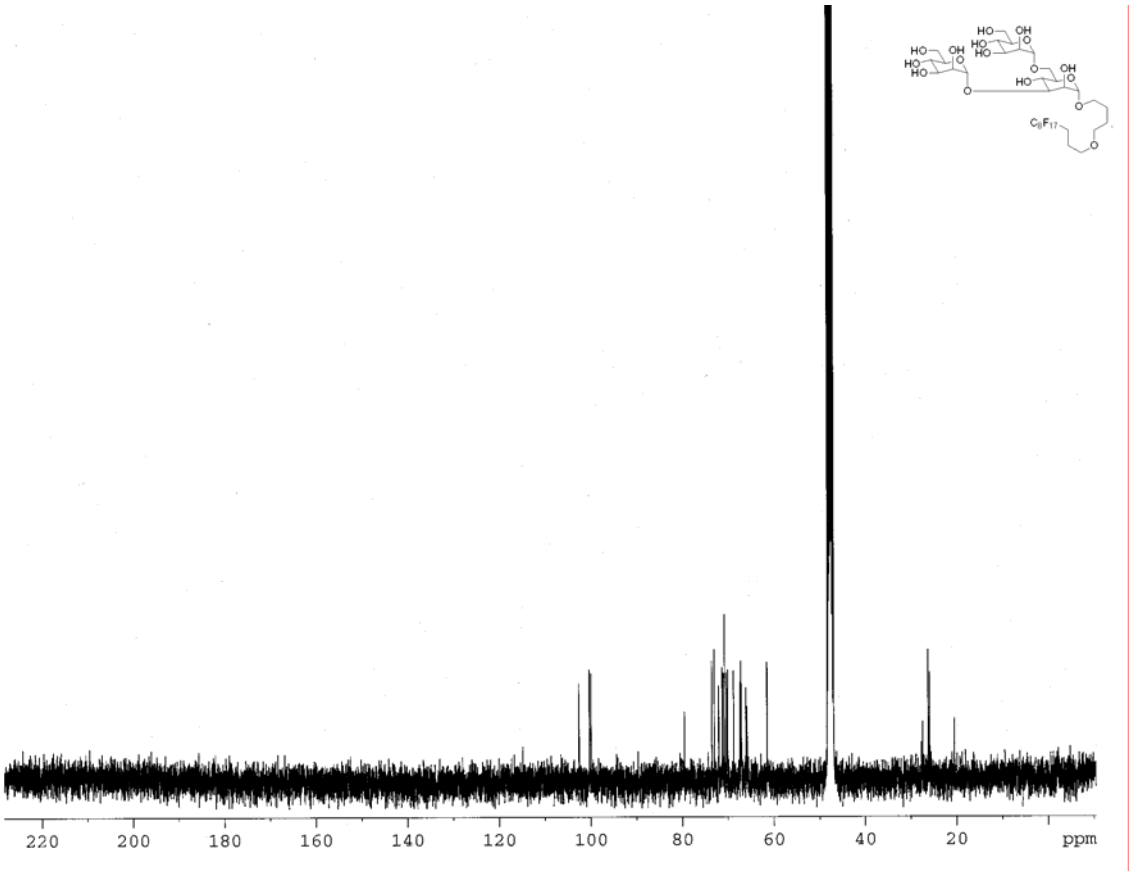


Figure 3.38: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside.

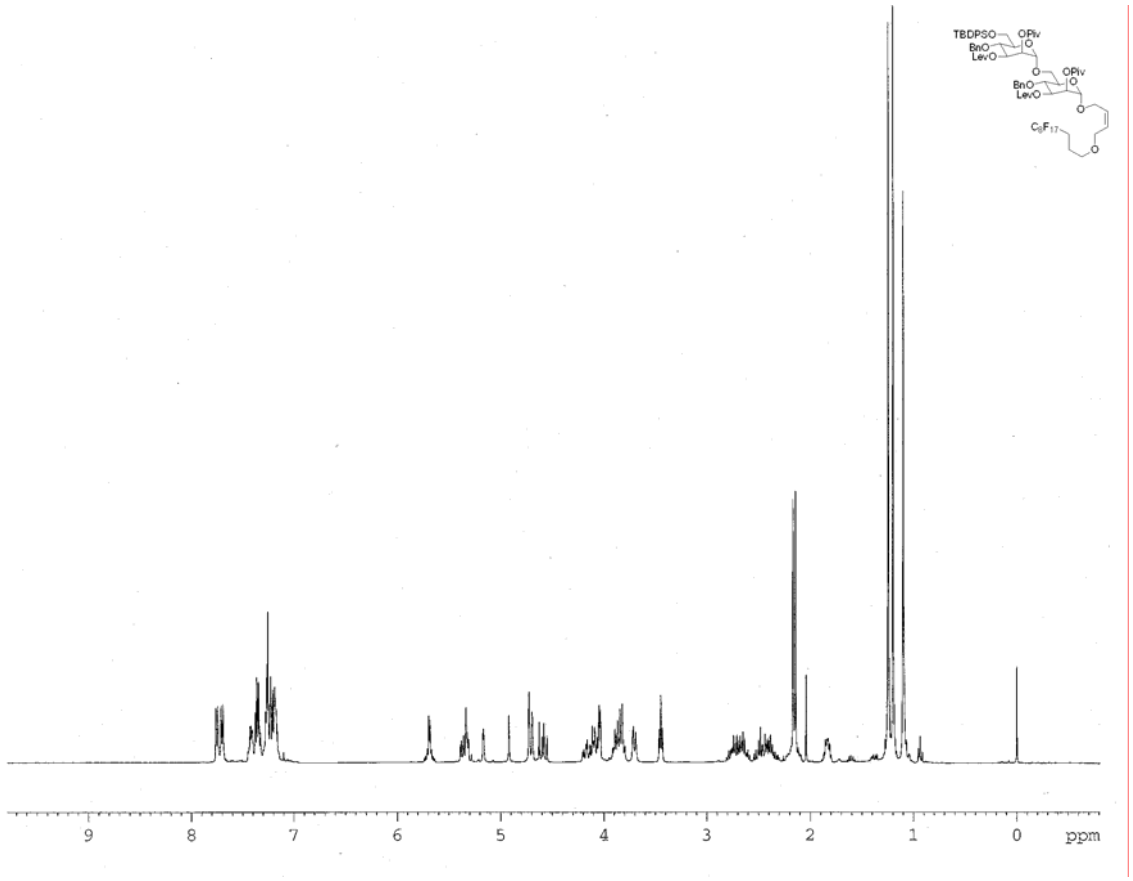


Figure 3.39: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

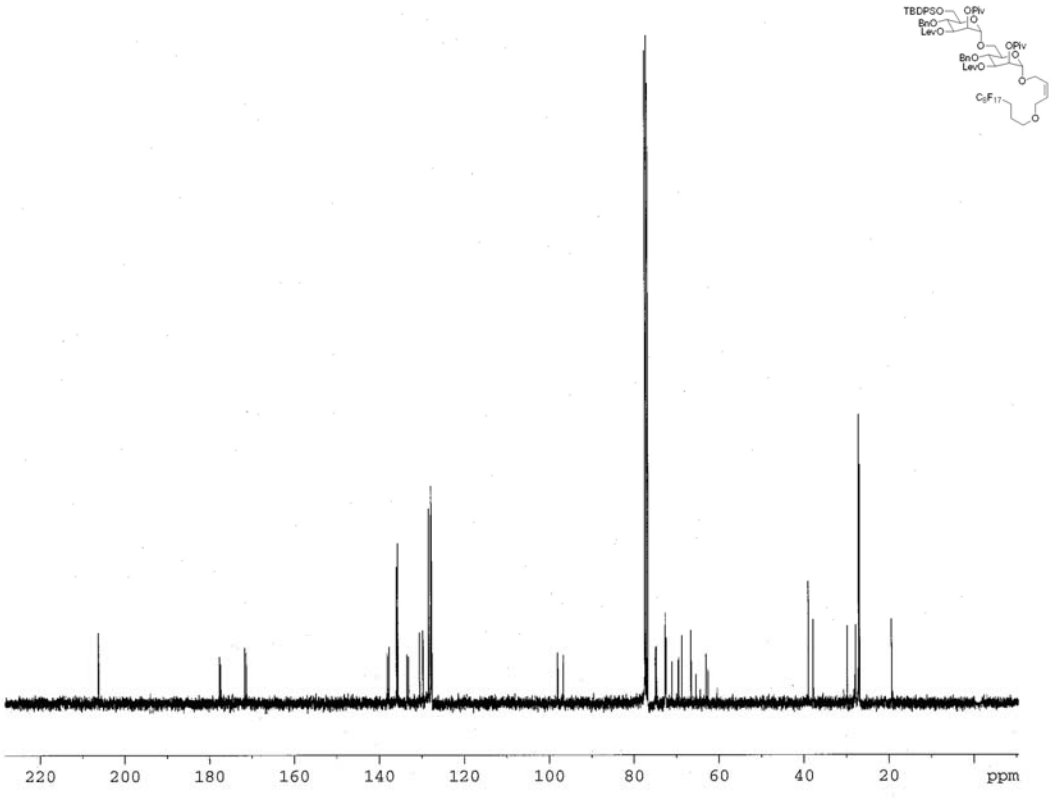


Figure 3.40: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

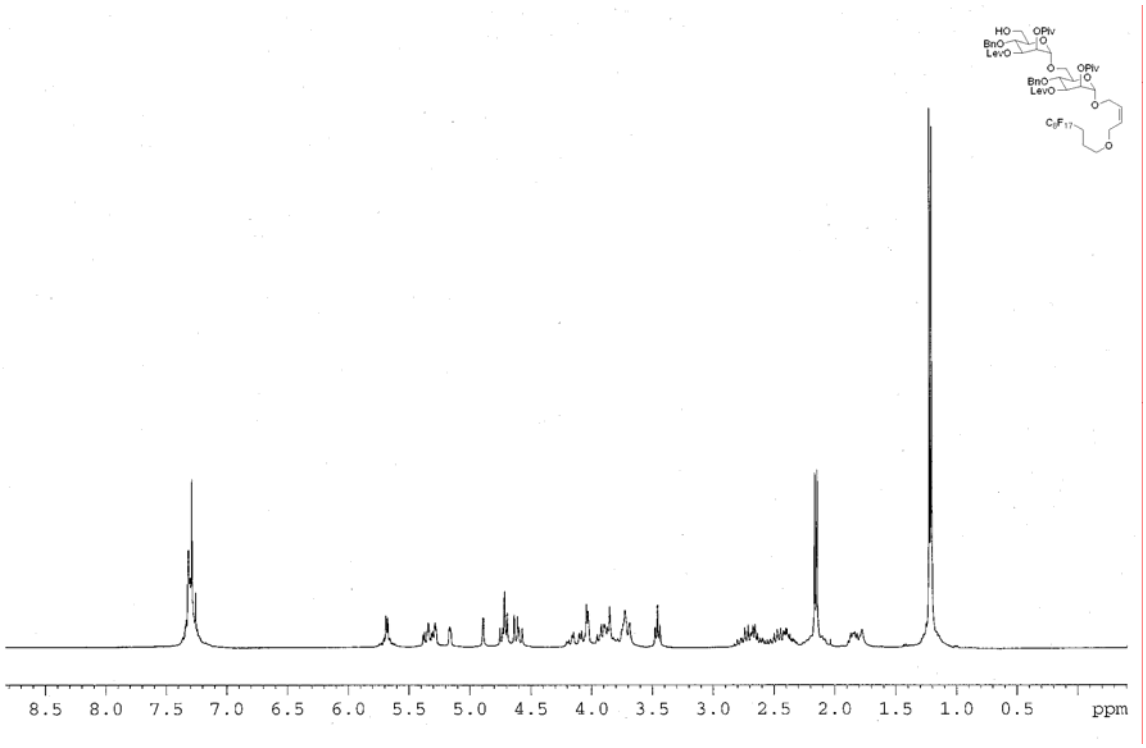


Figure 3.41: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

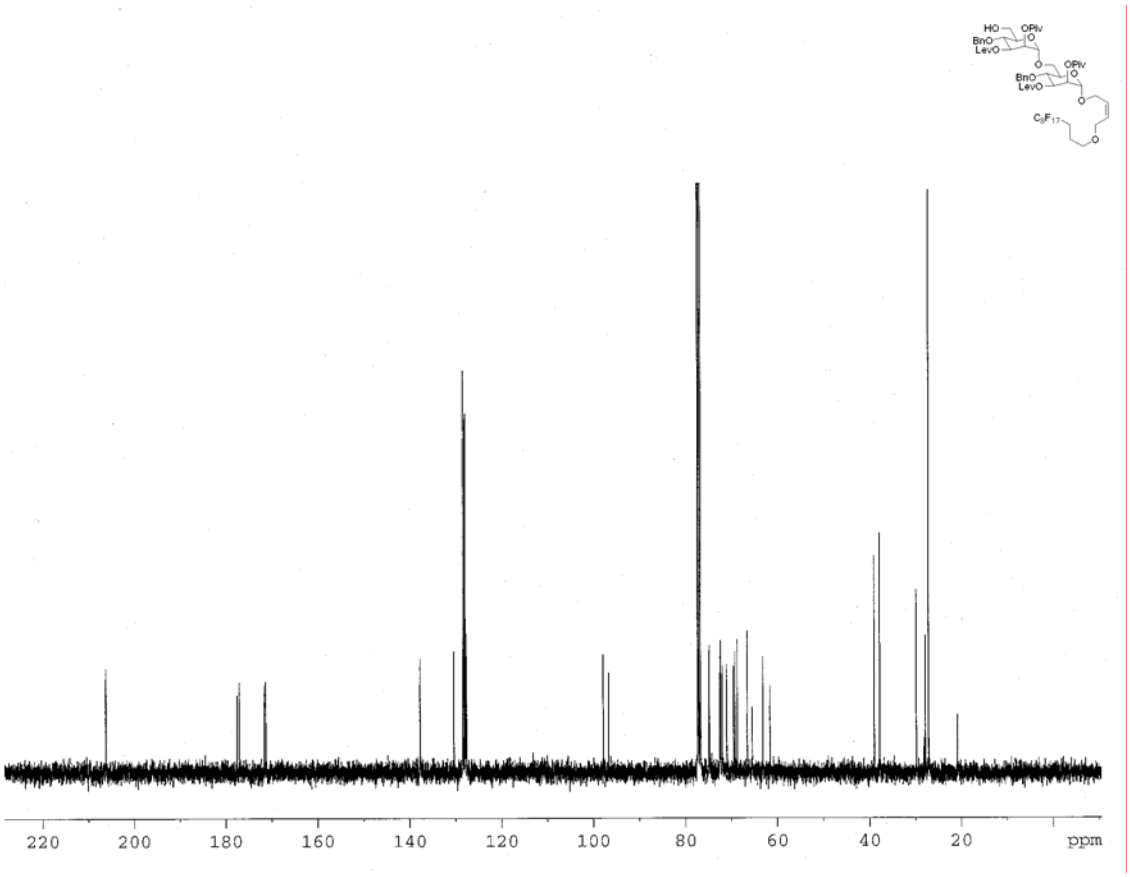


Figure 3.42: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-levulinyl-6-*O*-(4-*O*-benzyl-6-hydroxy-3-*O*-levulinyl-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

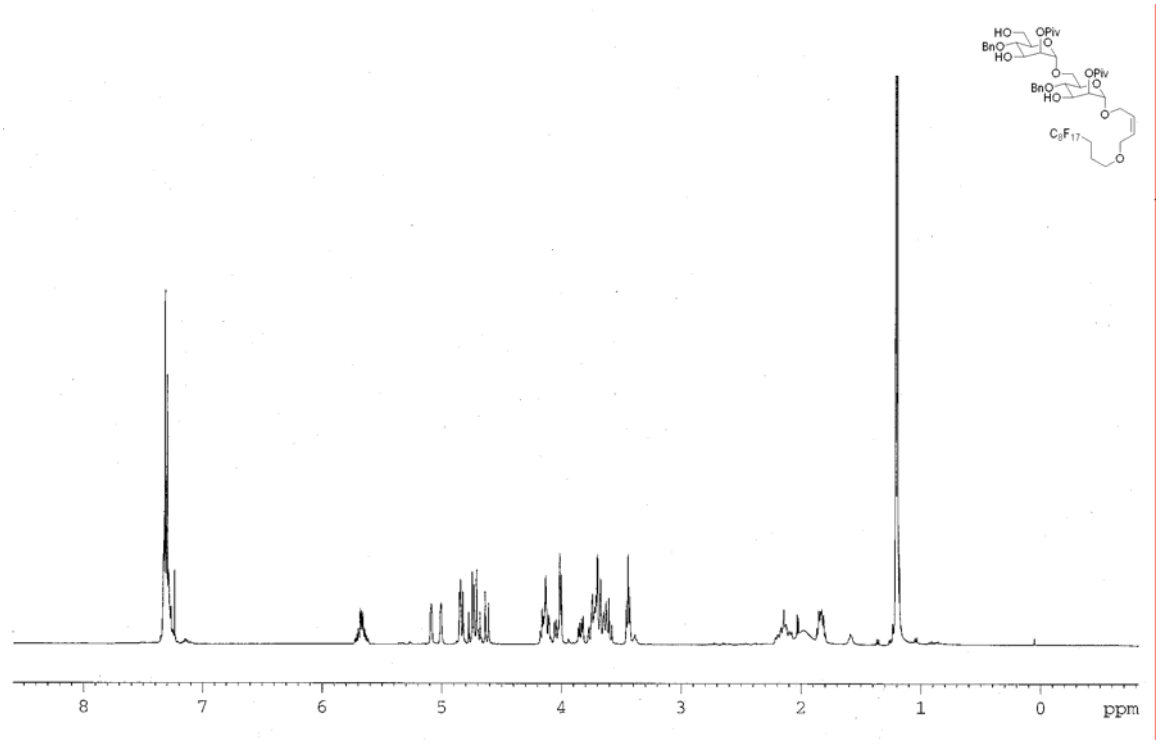


Figure 3.43: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-hydroxy-6-*O*-(4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

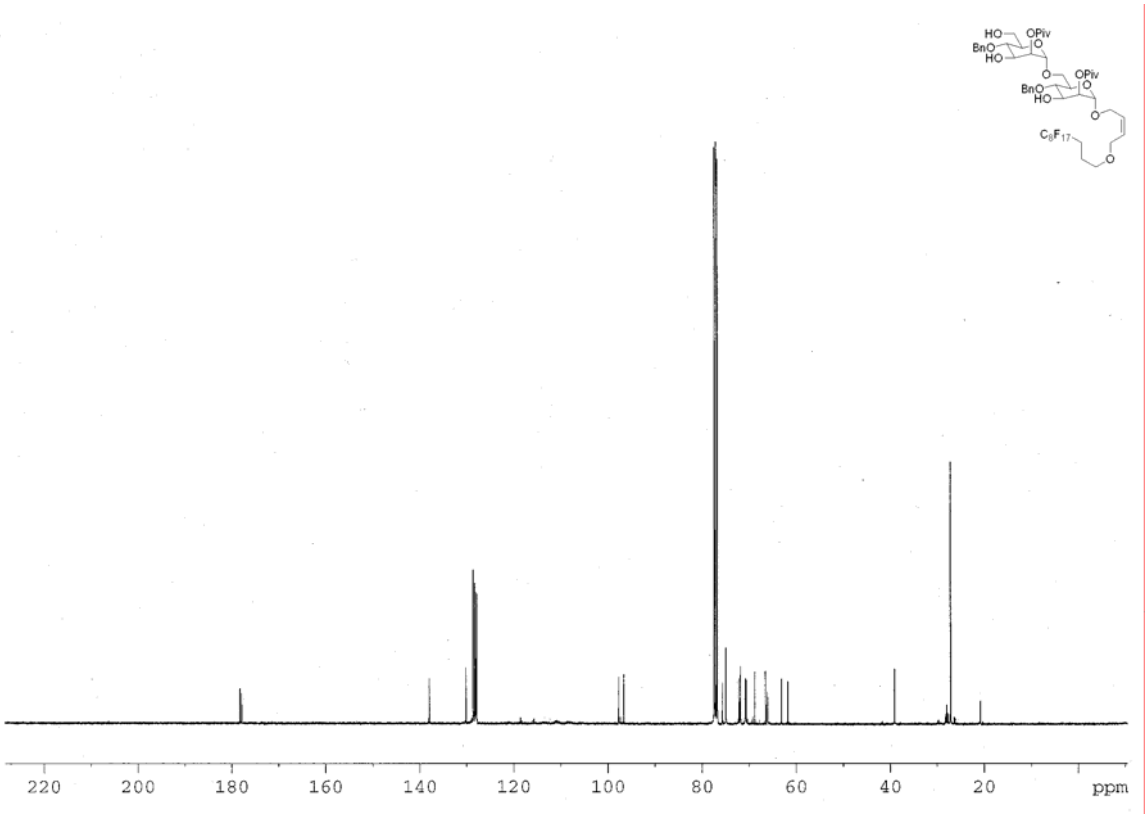


Figure 3.44: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-hydroxy-6-*O*-(4-*O*-benzyl-3,6-dihydroxy-2-*O*-pivaloyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside.

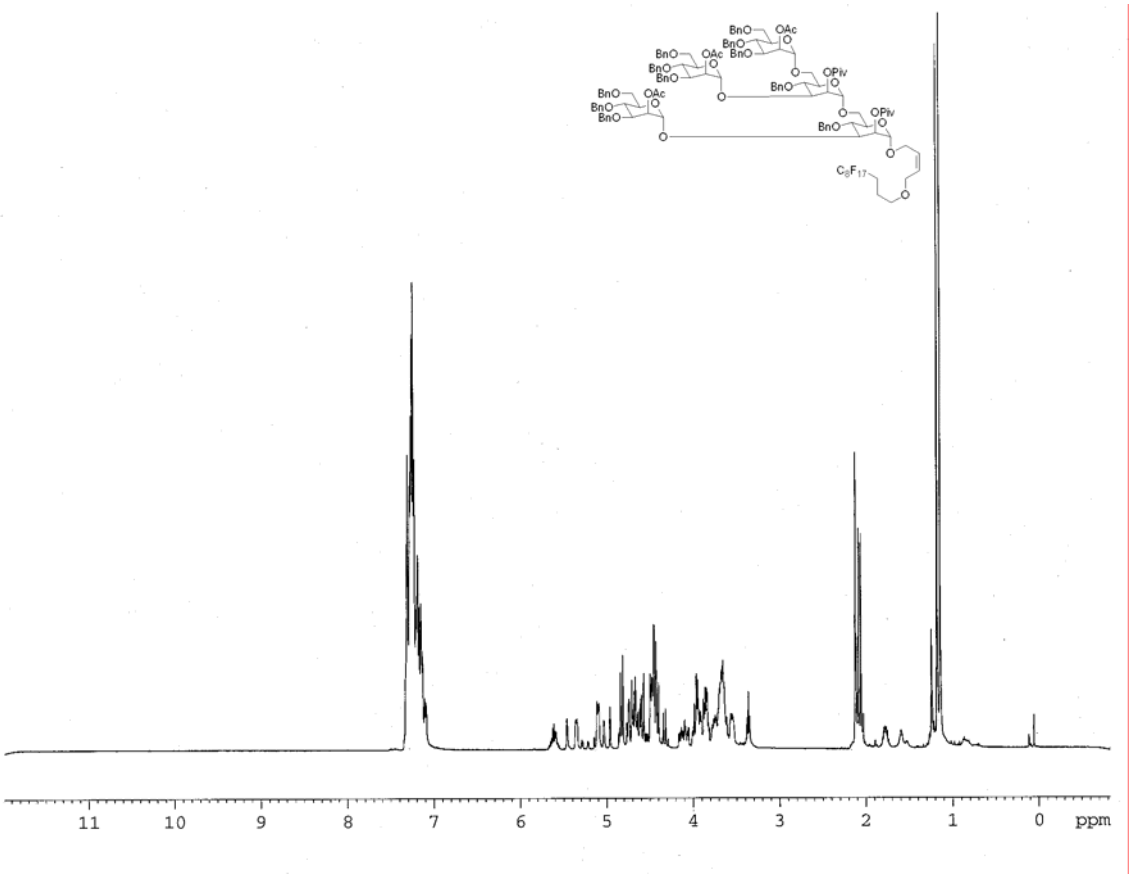


Figure 3.45: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-6-*O*-[4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside]-2-*O*-pivaloyl-α-D-mannopyranoside.

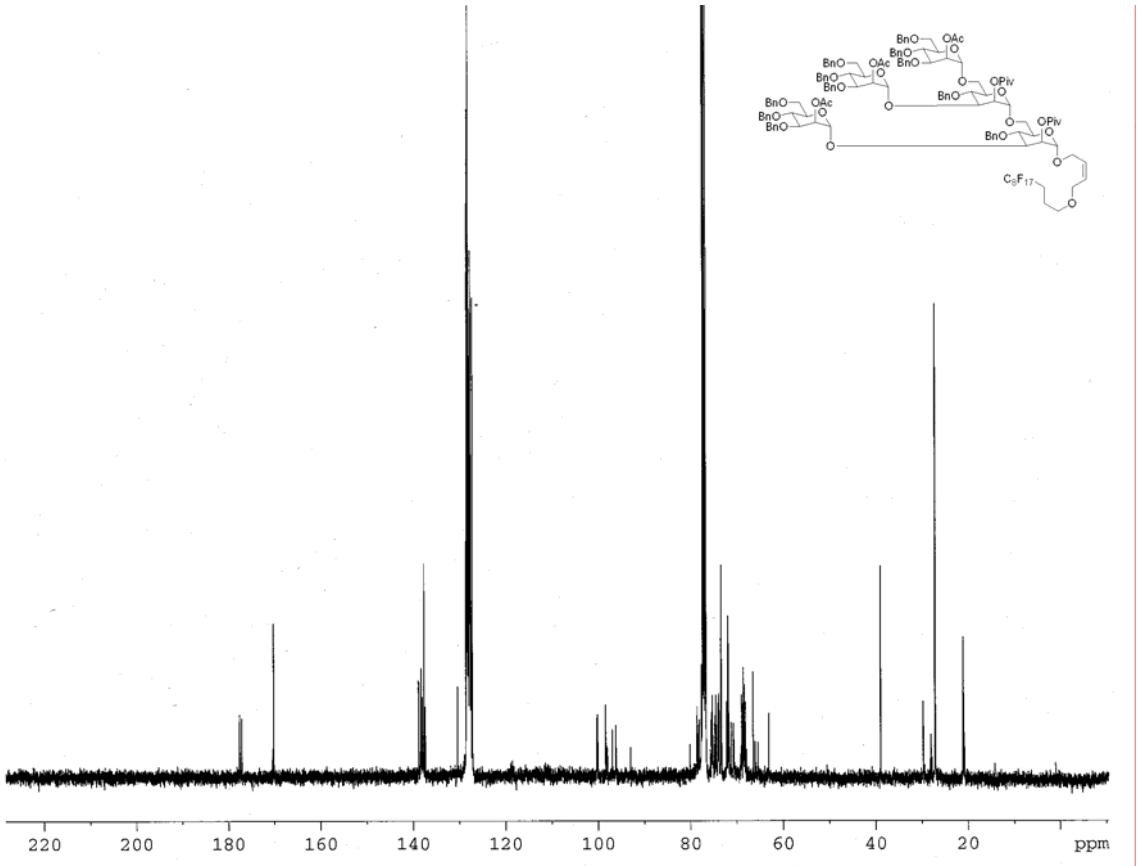


Figure 3.46: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-4-*O*-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-6-*O*-[4-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-*O*-tribenzyl-α-D-mannopyranoside)-2-*O*-pivaloyl-α-D-mannopyranoside]-2-*O*-pivaloyl-α-D-mannopyranoside.

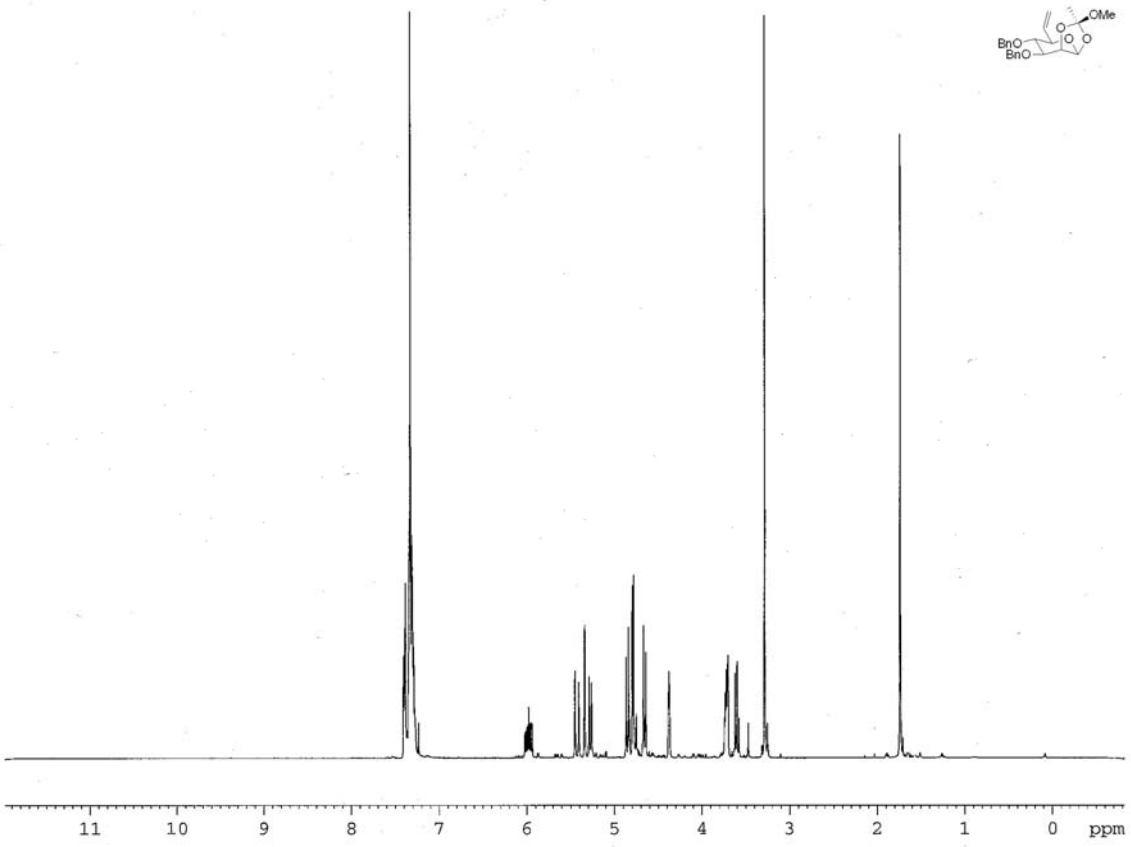


Figure 3.47: ^{1}H NMR of 3,4-di-*O*-benzyl-6,7-dideoxy-1,2-*O*-(methoxyethylidene)-*α*-D-hept-6-enopyranoside.

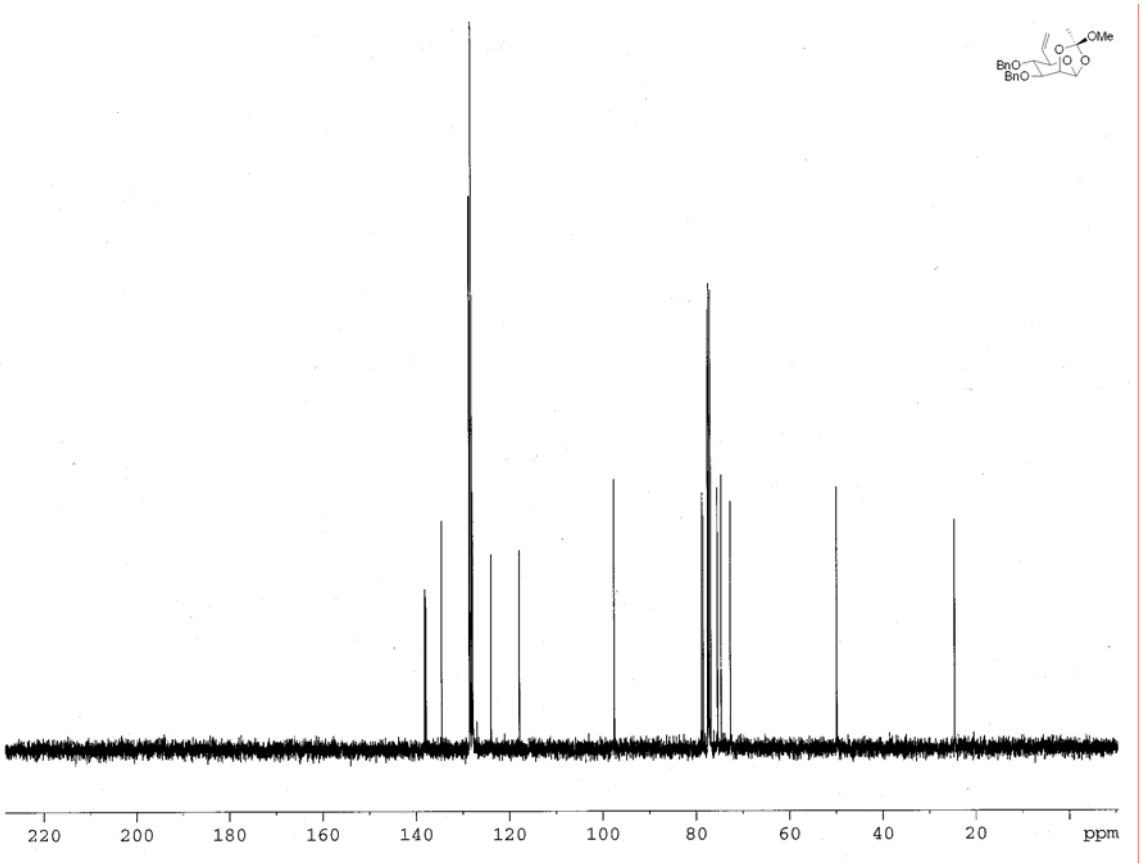


Figure 3.48: ^{13}C NMR of 3,4-di-*O*-benzyl-6,7-dideoxy-1,2-*O*-(methoxyethylidene)-*α*-D-hept-6-enopyranoside.

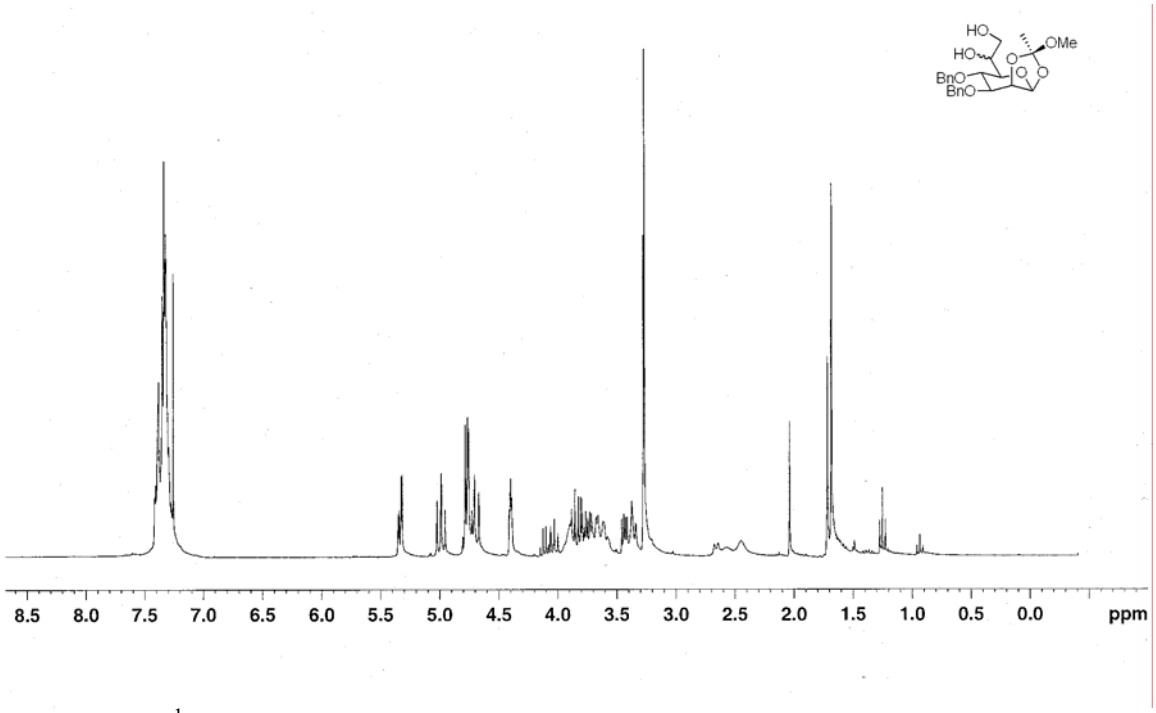


Figure 3.49: ^{1}H NMR of 3,4-di-*O*-benzyl-(D,L)-*glycero*-1,2-*O*-(methoxyethylidene)-*α*-D-*manno*-heptopyranoside.

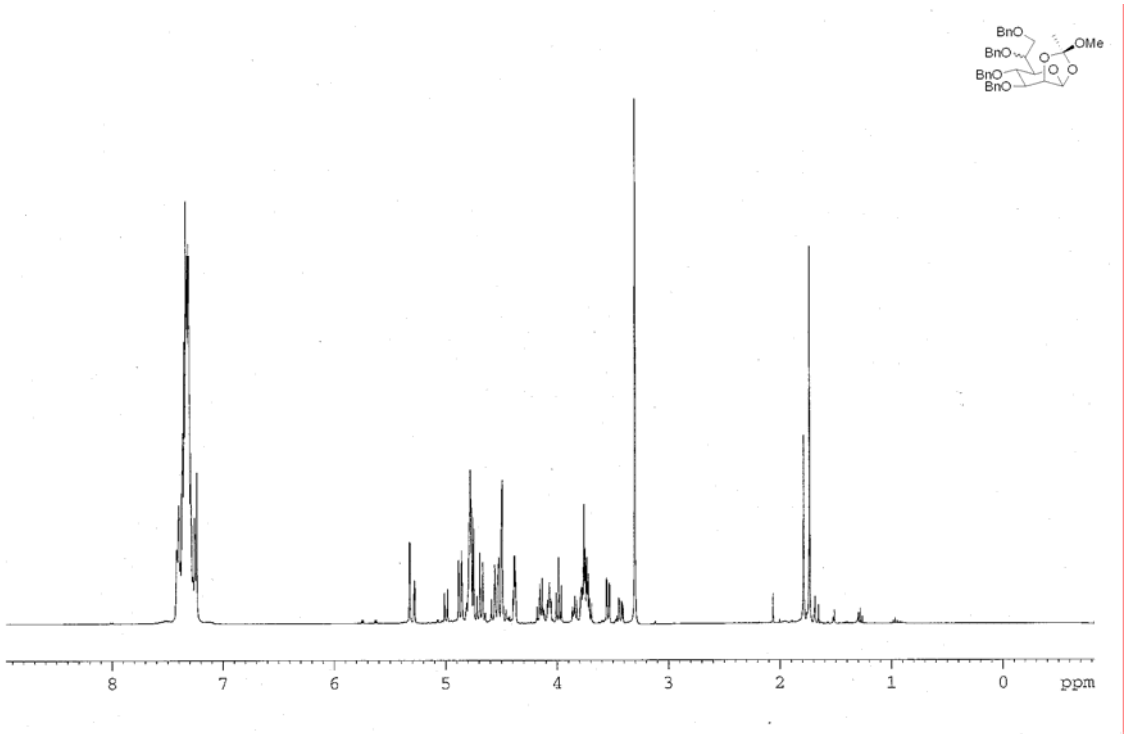


Figure 3.50: ^{1}H NMR of 3,4,6,7-tetra-*O*-benzyl-1,2-*O*-(methoxyethylidene)-(D,L)-*glycero*-*α*-D-*manno*-heptopyranoside.

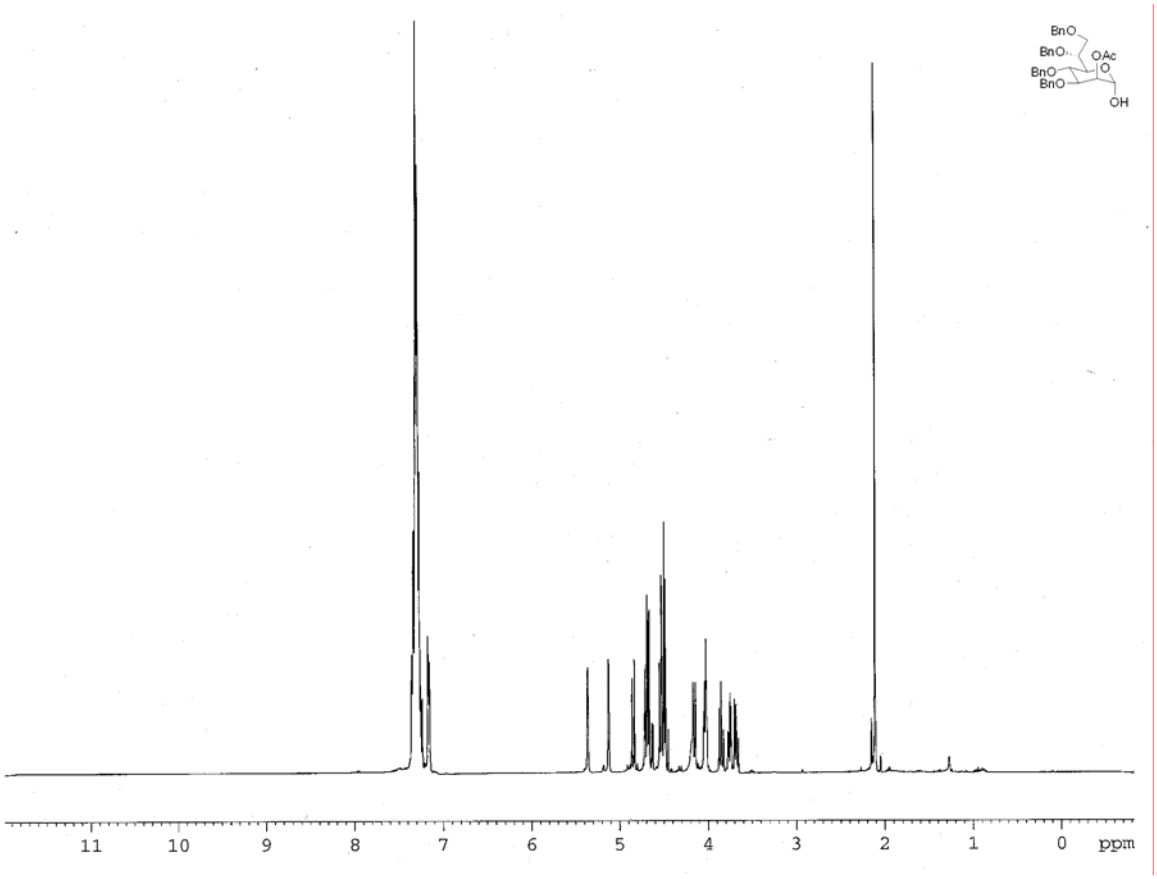


Figure 3.51: ^{1}H NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

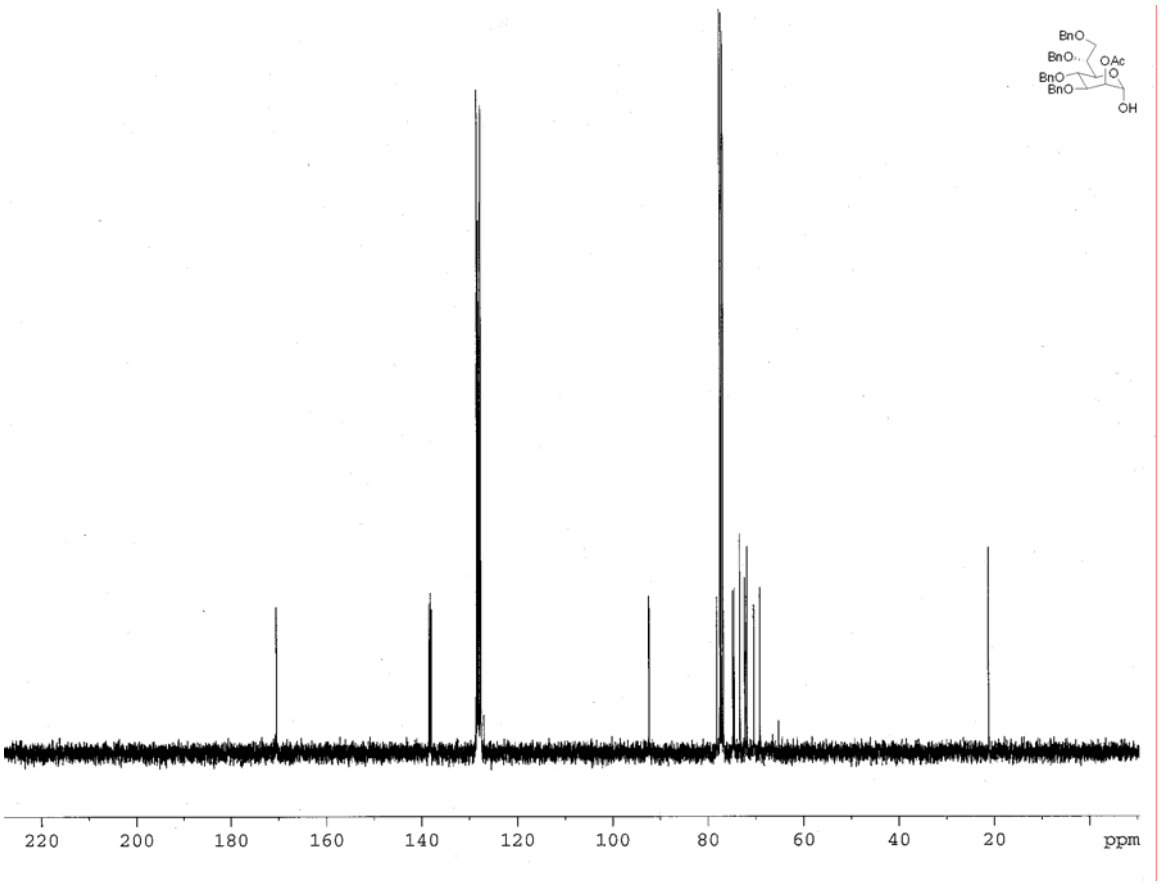


Figure 3.52: ^{13}C NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

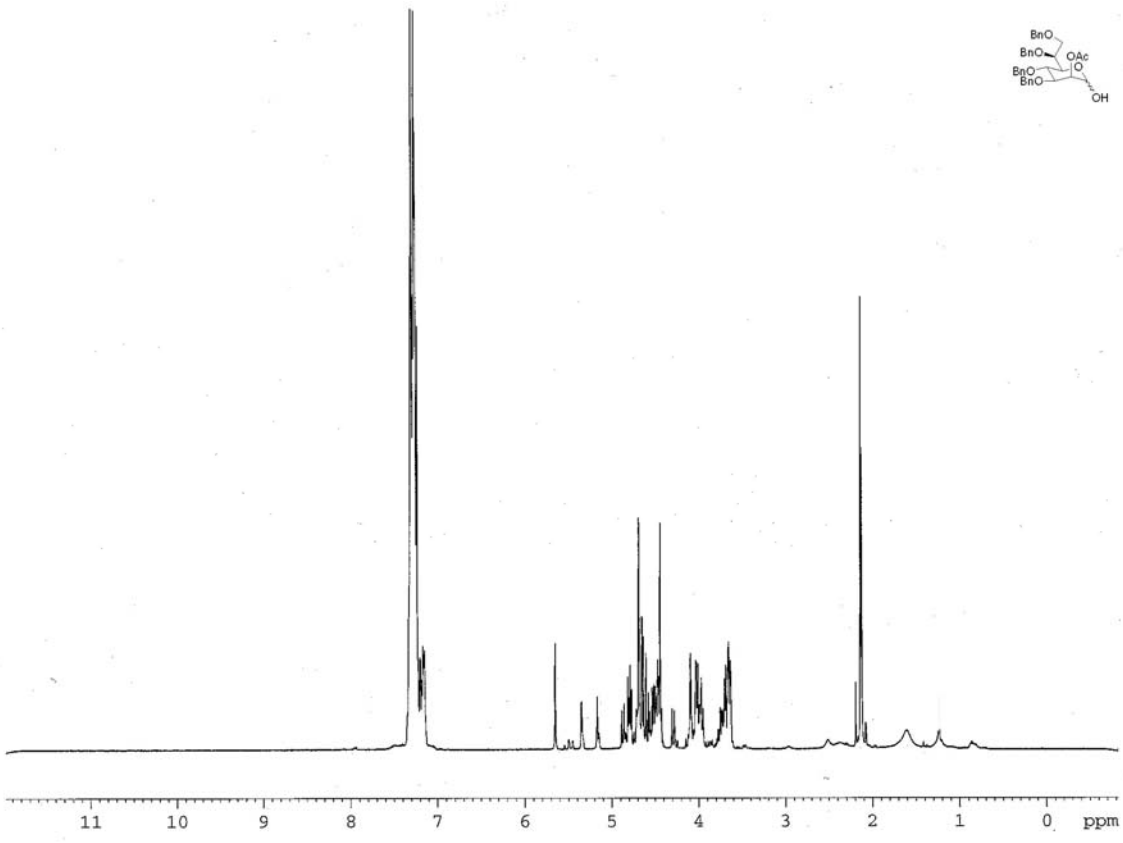


Figure 3.53: ^{1}H NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α*/*β*-D-*manno*-heptopyranoside.

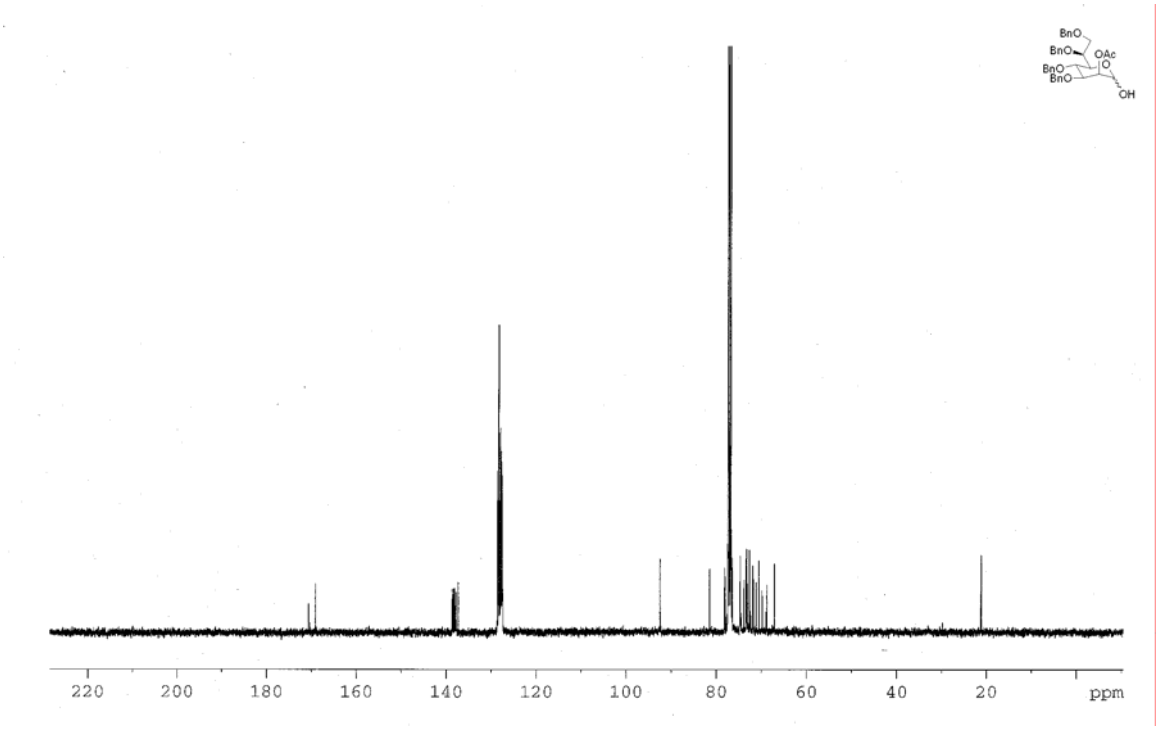


Figure 3.54: ^{13}C NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α/β*-D-*manno*-heptopyranoside.

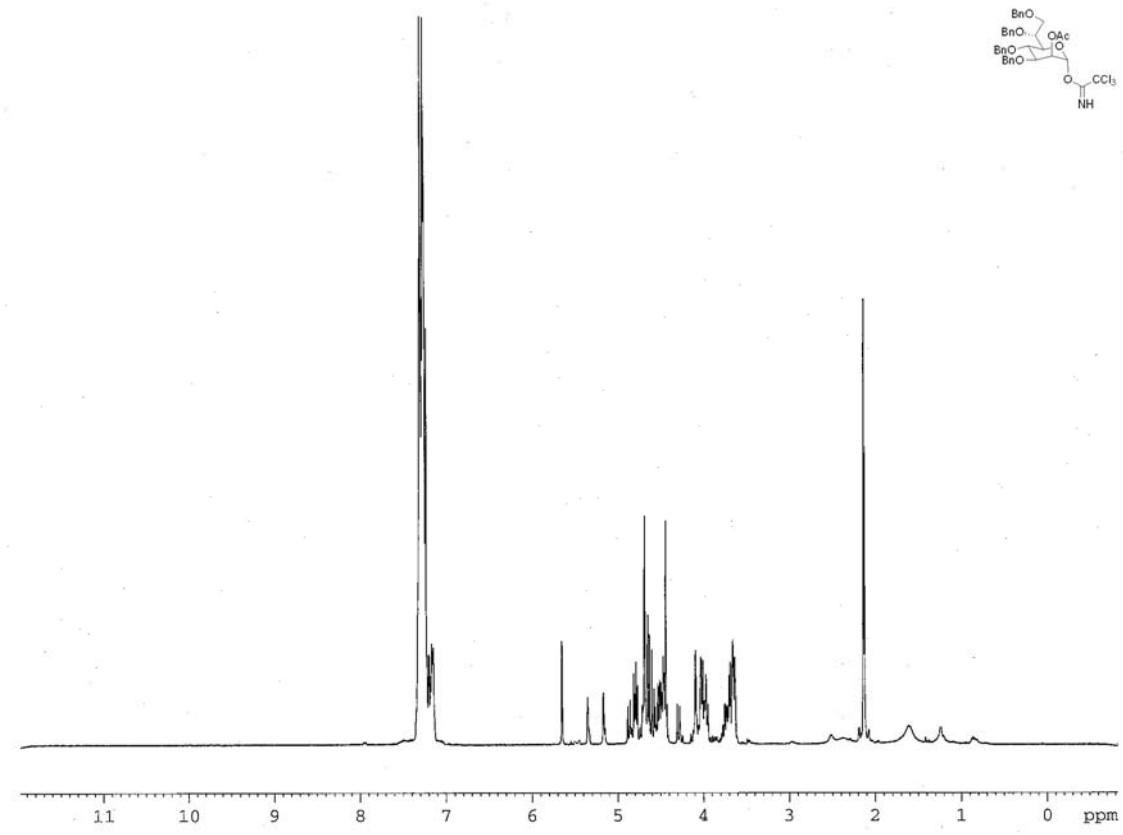


Figure 3.55: ^{1}H NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranosyl trichloroacetimidate.

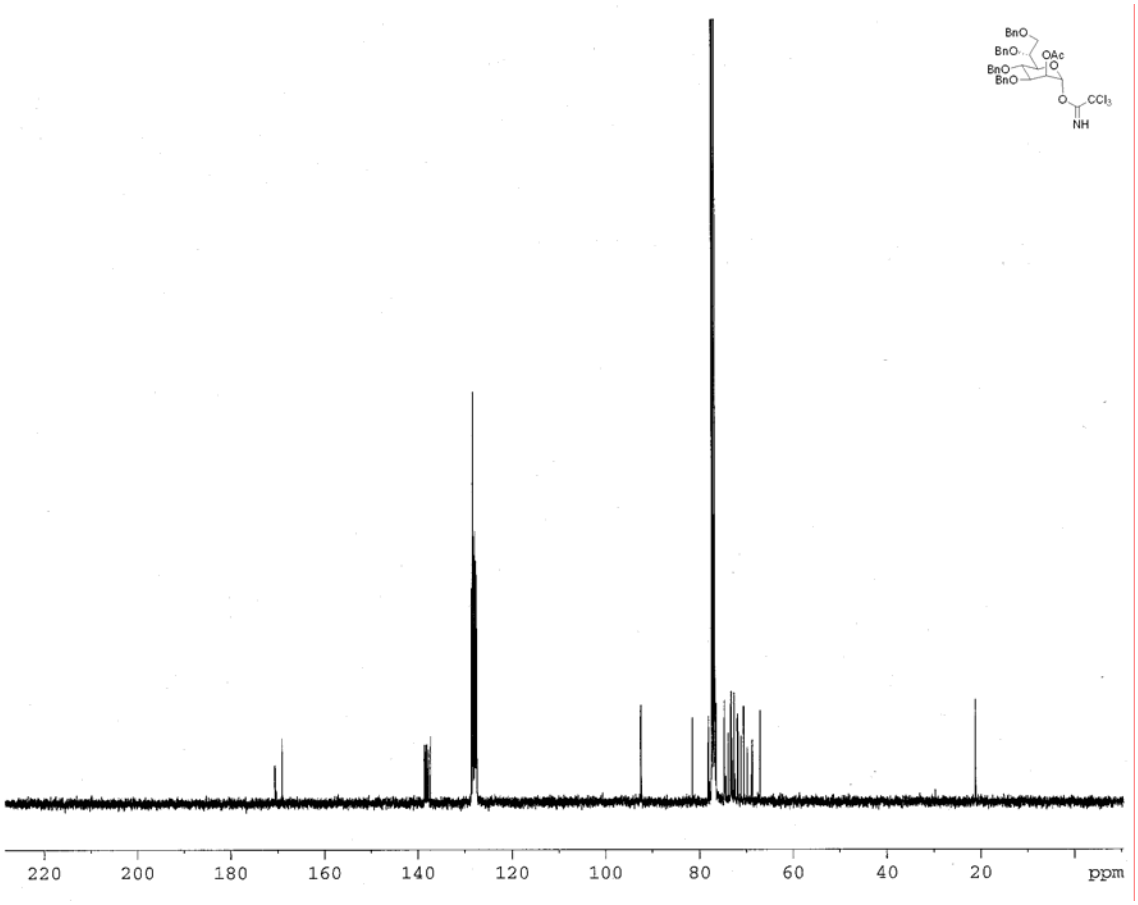


Figure 3.56: ^{13}C NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-D-*glycero*-*α*-D-*manno*-heptopyranosyl trichloroacetimidate.

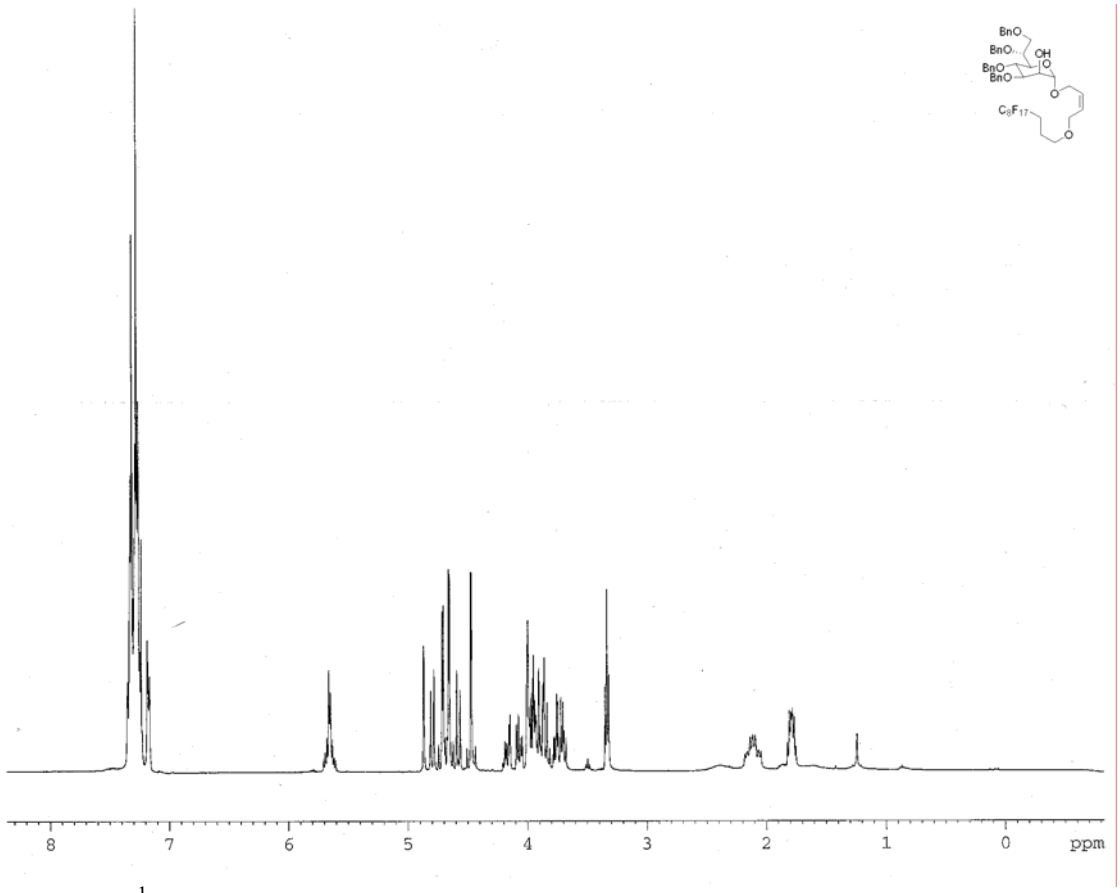


Figure 3.57: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

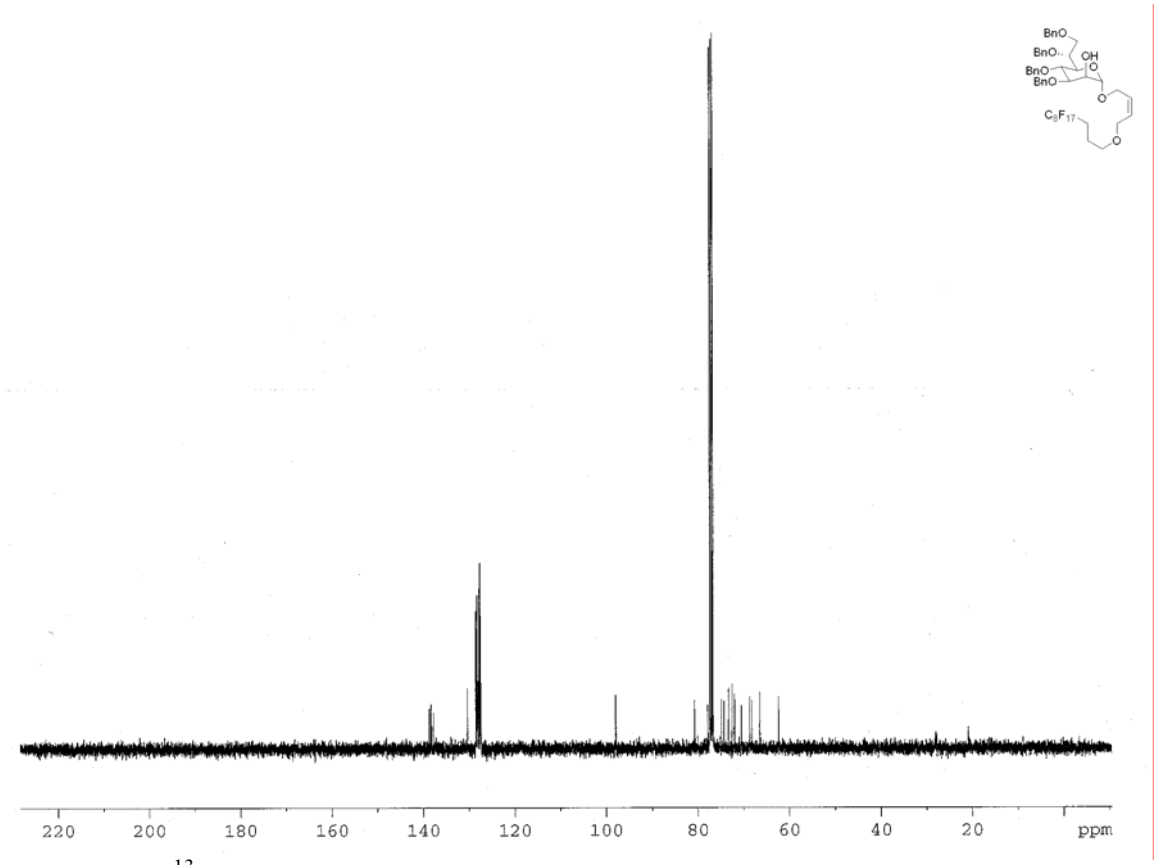


Figure 3.58: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

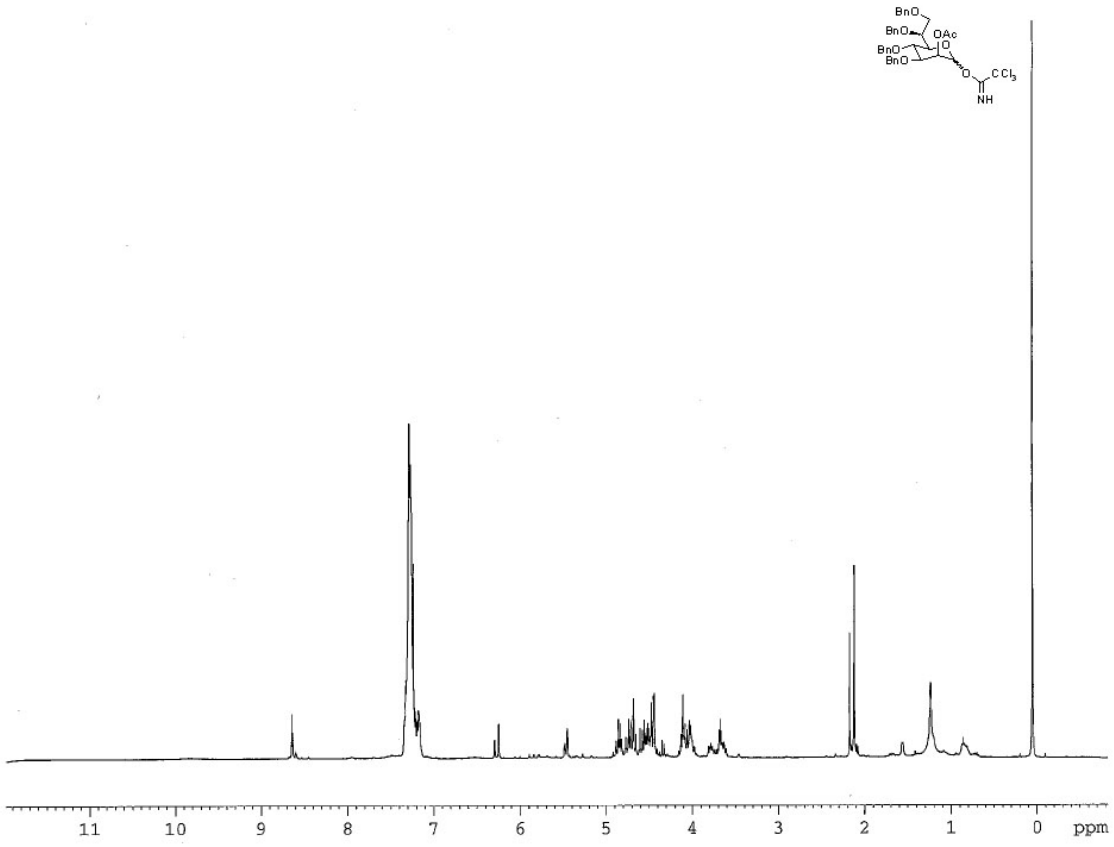


Figure 3.59: ^{1}H NMR of 3,4,6,7-tetra-*O*-benzyl-2-*O*-acetyl-L-*glycero*-*α*/*β*-D-*manno*-heptopyranosyl trichloroacetimidate.

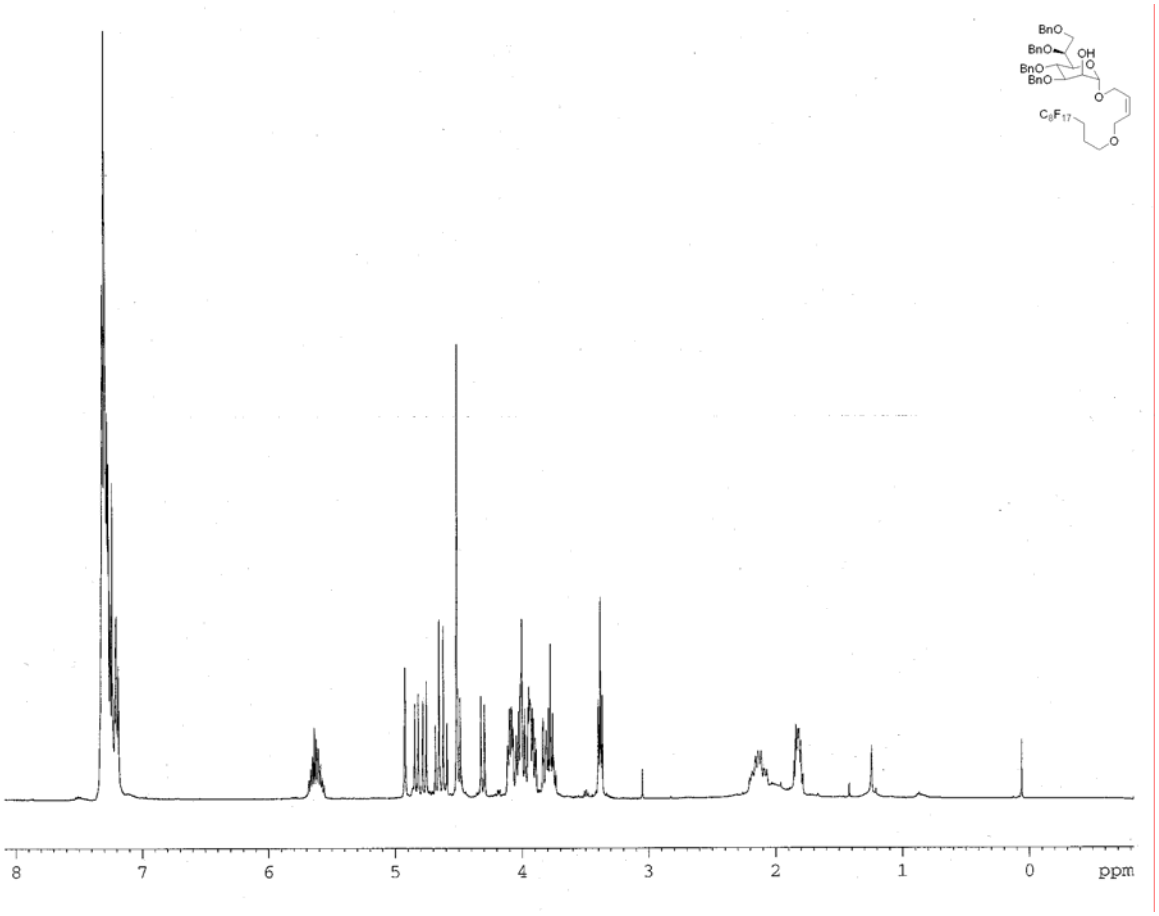


Figure 3.60: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-L-*glycero*-*α*-D-*manno*-heptopyranoside.

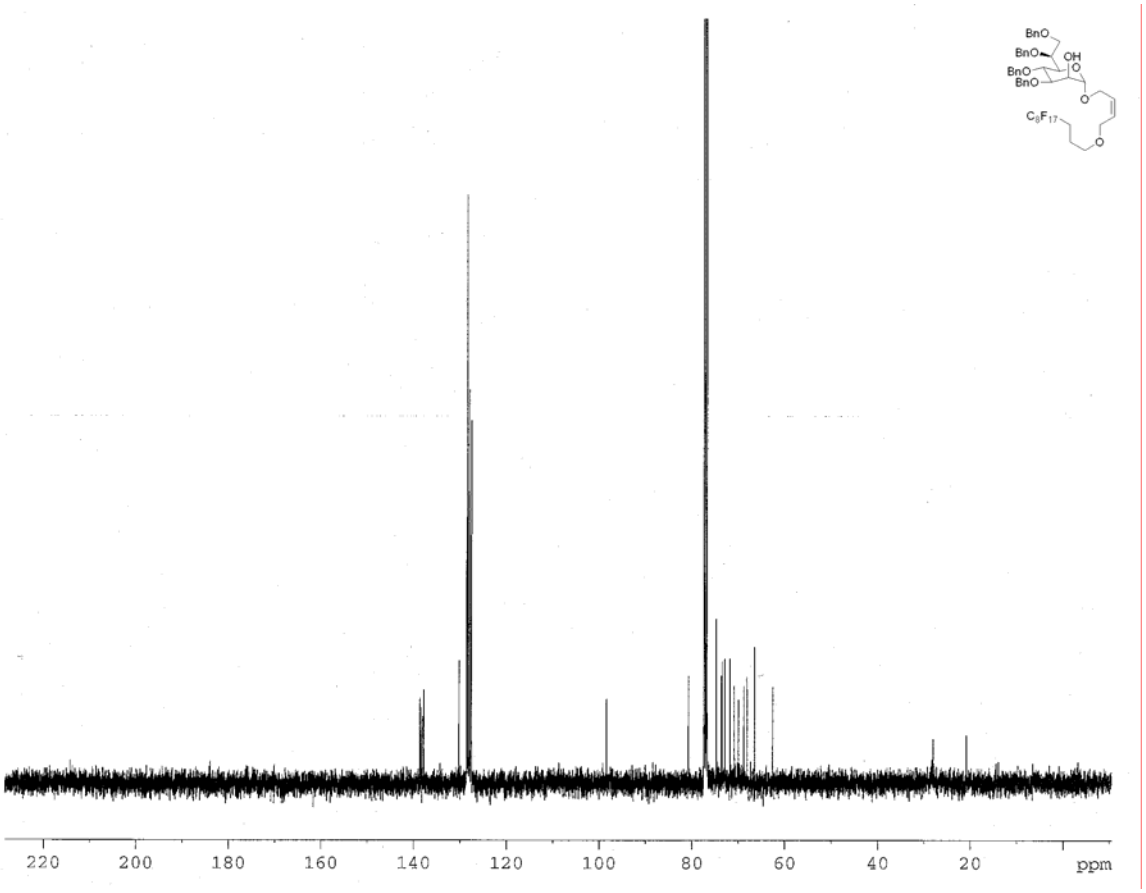


Figure 3.61: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside.

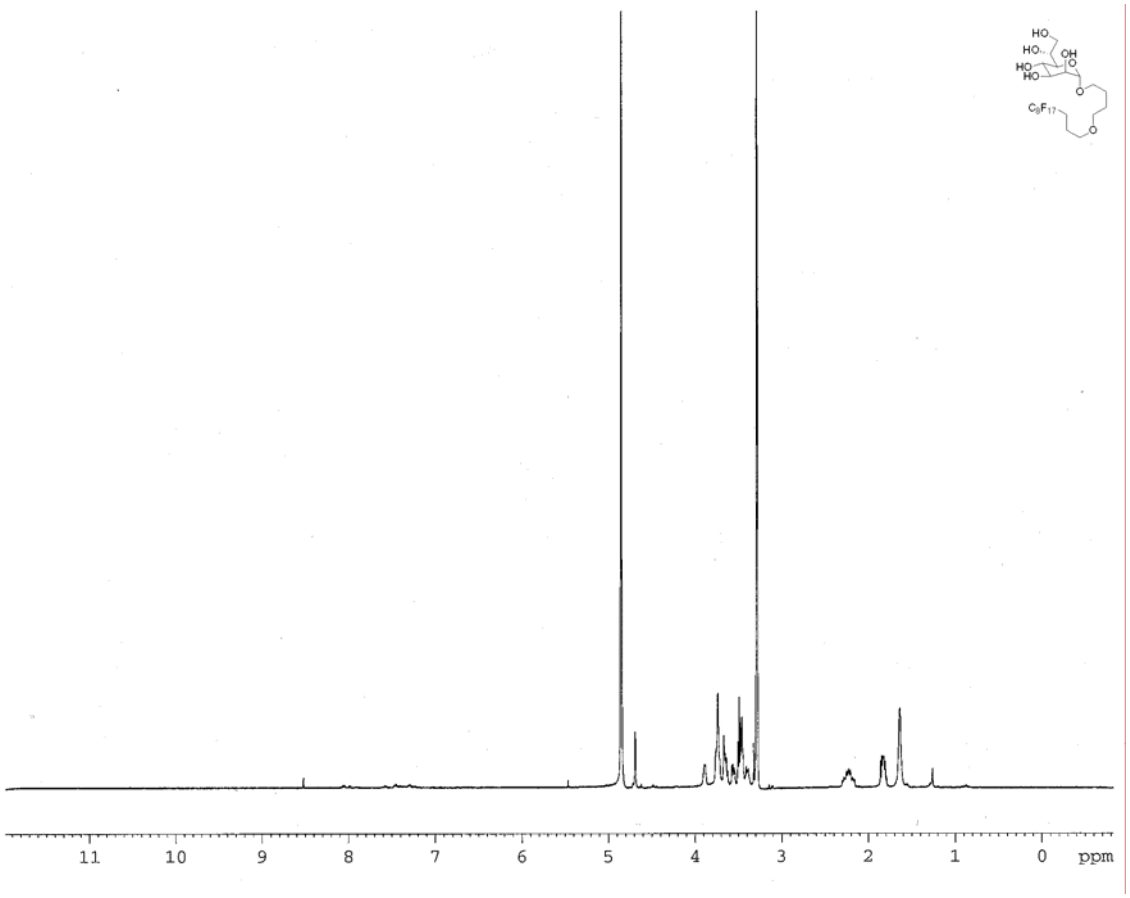


Figure 3.62: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

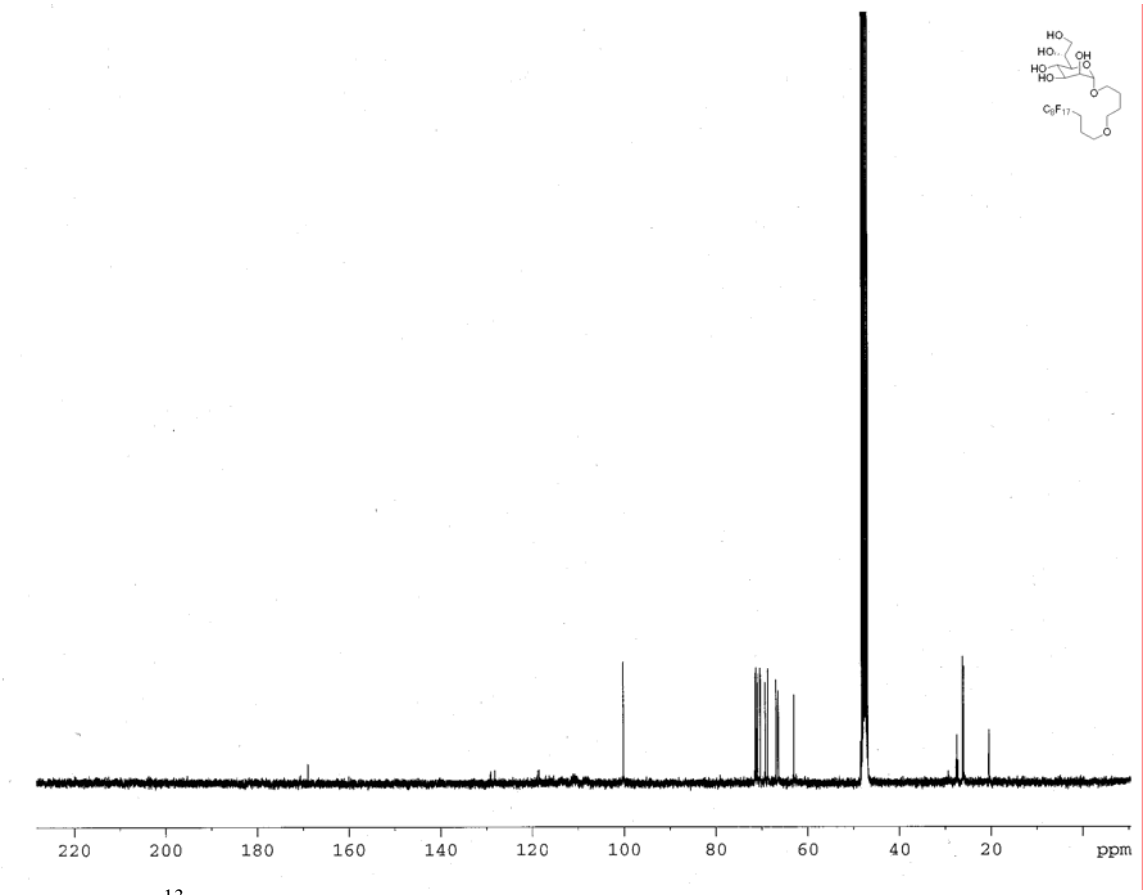


Figure 3.63: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-D-*glycero*-*α*-D-*manno*-heptopyranoside.

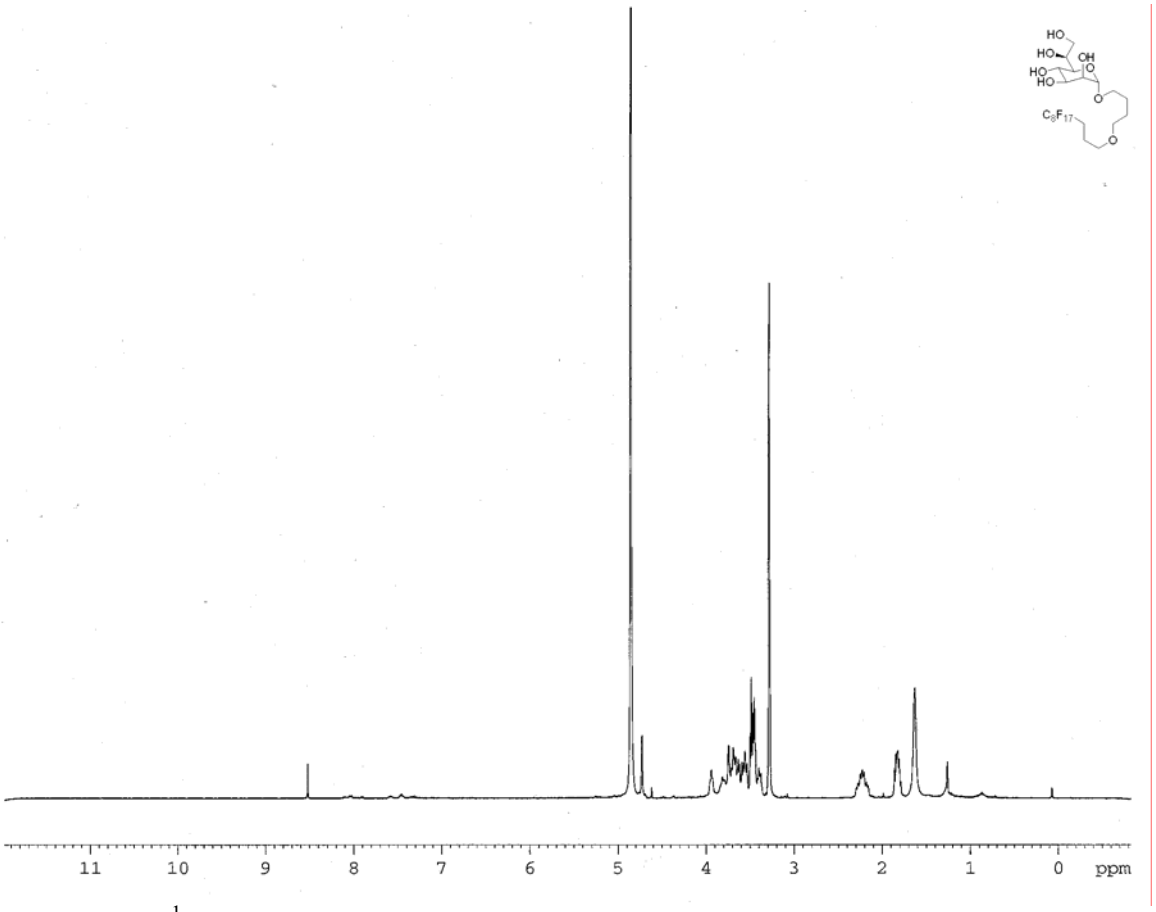


Figure 3.64: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-L-*glycero*-*α*-D-*manno*-heptopyranoside.

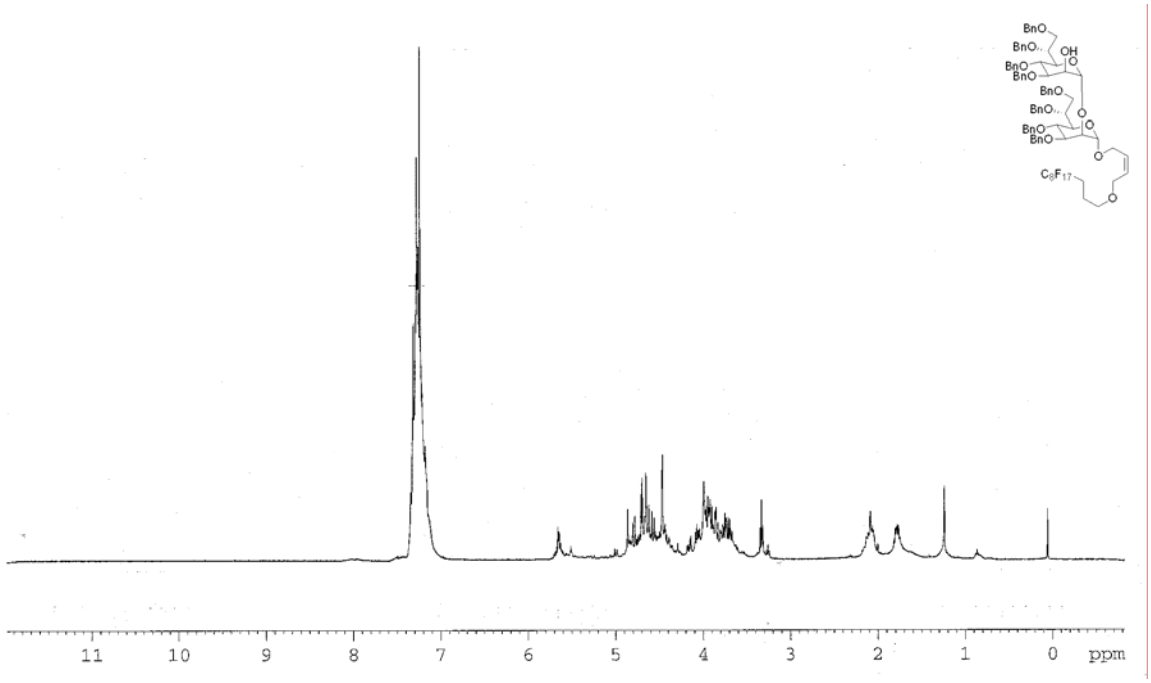


Figure 3.65: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-*O*-(3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside)-D-*glycero*-*α*-D-*manno*-heptopyranoside.

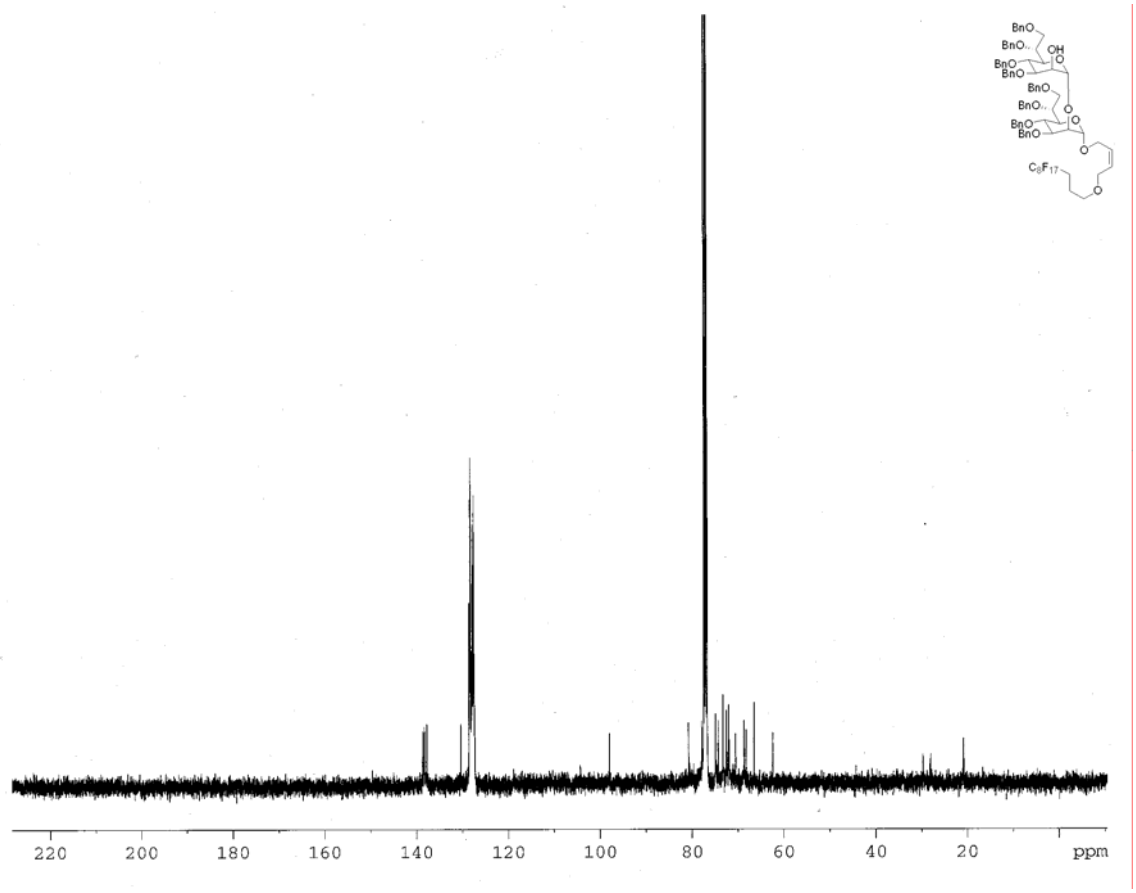


Figure 3.66: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutenyl-3,4,6,7-tetra-*O*-benzyl-2-*O*-(3,4,6,7-tetra-*O*-benzyl-D-*glycero*-*α*-D-*manno*-heptopyranoside)-D-*glycero*-*α*-D-*manno*-heptopyranoside.

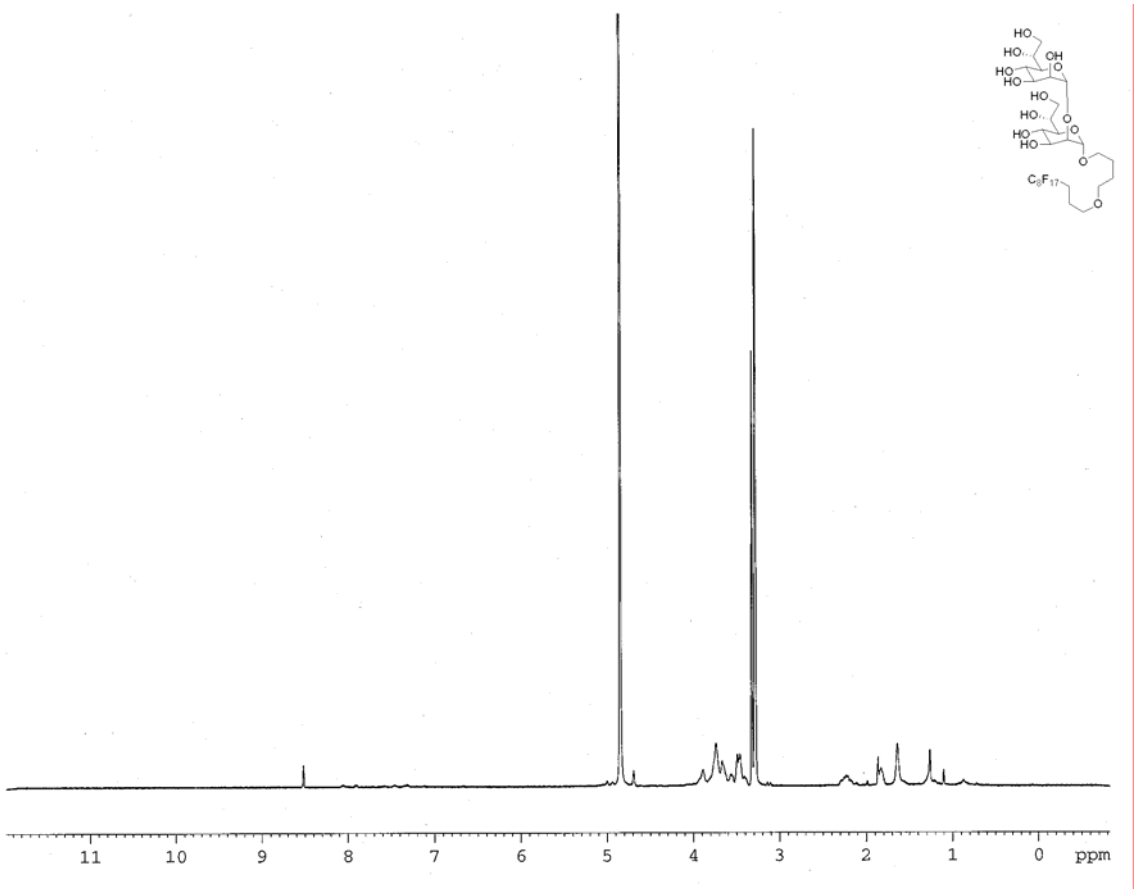


Figure 3.67: ^{1}H NMR of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-(D-*glycero*-*α*-D-*manno*-heptopyranosyl)-D-*glycero*-*α*-D-*manno*-heptopyranoside.

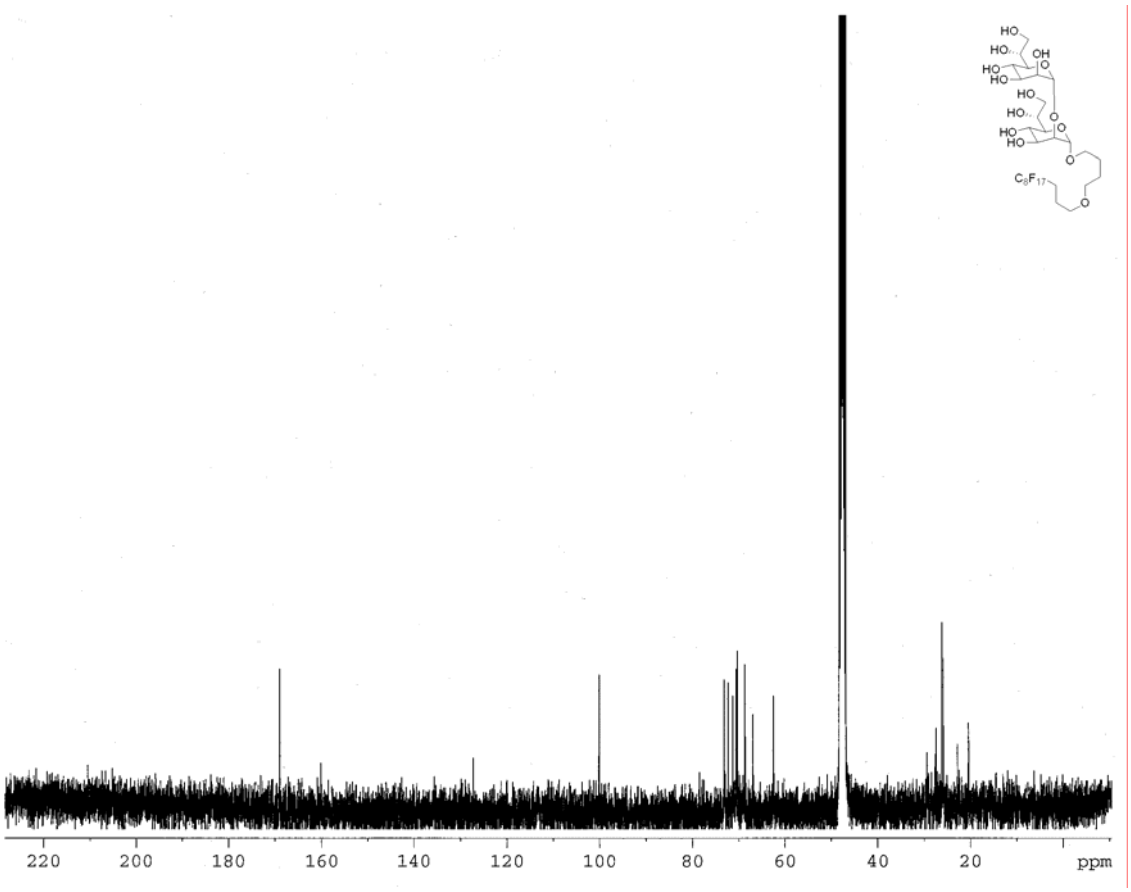


Figure 3.68: ^{13}C NMR of 3-(perfluorooctyl)propanyloxybutanyl-2-*O*-(D-*glycero*-*α*-D-*manno*-heptopyranosyl)-D-*glycero*-*α*-D-*manno*-heptopyranoside.

CONCLUSIONS

The goal of this thesis is to devise an efficient method for the synthesis of oligosaccharides to provide a handy solution to a practical problem. This was achieved by using an allyl fluorous linker that facilitates the synthesis of oligosaccharides as fluorous-tagged compounds can by easily separated by fluorous solid phase extraction columns (FSPE). Since the glycosylation reactions are done in solution, it requires only 1.5 equivalent of sugar donor for each coupling step. We have also shown that this whole process can be automated by synthesizing a linear α-(1→2)-linked mannose tetrasaccharide in a manner to mimic automated synthesizer.

A C_8F_{17} tail also allows direct formation of carbohydrate microarrays based on noncovalent fluorous-fluorous interactions. This was demonstrated by noncovalent immobilization of carbohydrates on fluorous derivatized glass slides. Several monosaccharides, and disaccharides were printed on a glass slide and screening of these carbohydrates against different lectins demonstrated that the noncovalent fluorous–fluorous interaction is sufficient to retain not only mono- but also disaccharides. Next, we prepared microarrays of mannose oligosaccharides and *glycero*-D-*manno* heptoses for the following purposes: 1) to test if longer chain mannose oligosaccharides will stick to the fluorous-coated glass slide, 2) to check if Con A has affinity towards C-6 modified mannose. Microarrays revealed that noncovalent fluorous interactions are strong enough to hold longer chain mannose oligosaccharides on to the fluorous-coated glass slide. It was

also shown that Con A has affinity for both D-*glycero*-D-*manno* heptose and L-*glycero*-D-*manno* heptose. This information will be very useful in understanding the role of these heptomannose sugars that are found in the core of LPS of several bacteria.

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