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# Synthetic standards for mass spectrometry-based carbohydrate sequencing and the automated solution-phase syntheses of beta-glucans

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# Synthetic standards for mass spectrometry-based carbohydrate sequencing and the automated solution-phase syntheses of beta-glucans

by

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| Ac | Acetyl |
| AcCl | Acetyl chloride |
| AcOH | Acetic acid |
| Bn | Benzyl |
| BnBr | Benzyl bromide |
| CSA | 10-Camphorsulfonic acid |
| DCC | *N,N'*-Dicyclohexylcarbodiimide |
| DIC | *N,N'*-Diisopropylcarbodiimide |
| DMAP | 4-(Dimethylamino)pyridine |
| DMF | *N,N*-Dimethylformamide |
| ESI-MS | Electrospray ionization mass spectrometry |
| FSPE | Fluorous solid-phase extraction |
| HPLC | High-performance liquid chromatography |
| Lev | Levulinyl (4-oxo-pentanoyl) |
| LevOH | Levulinic acid |
| MALDI | Matrix-assisted laser desorption ionization |
| NaH | Sodium hydride |
| Piv | Pivaloyl |
| PivCl | Pivaloyl chloride |
| Pyr | Pyridine |
| TBAB | Tetrabutylammonium bromide |
| TBAI | Tetrabutylammonium iodide |
| TEA | Triethylamine |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TMSOTf | Trimethylsilyl trifluoromethanesulfonate |

# ABSTRACT

This dissertation describes 1) the synthesis of disaccharide standards for mass spectrometry-based carbohydrate sequencing and 2) the automated iterative solution-phase synthesis of beta-glucans. Two libraries of mass-identical disaccharides were synthesized. Varying in linkage position and the identity of the non-reducing end monosaccharide residue, these compounds served as synthetic standards for systematic study by tandem mass spectrometry. These disaccharides were analyzed by mass spectrometry in the laboratories of Professors Edward Yeung and Young-Jin Lee. The varying degrees of fragmentation observed in the MS-MS spectra of several of these disaccharides were used to produce classification functions that were capable of correctly classifying the linkage position and identity of the non-reducing end monosaccharide residue. These results provide insight that will ultimately contribute to the development of faster carbohydrate sequencing methods.

The second portion of this dissertation describes the automated solution-phase syntheses of branched and linear beta-glucans. A fluorocarbon-based tag on the growing sugar chain allows for facile purification of intermediates by automated fluorous solid-phase extraction (FSPE), and also provides a means of noncovalent attachment to a fluorinated glass slide for the direct formation of carbohydrate microarrays. Our synthetic approach allows for traditional solution-phase kinetics, reaction monitoring, and chromatographic purification, techniques that are not possible with solid-phase oligosaccharide synthesis. Several new glucosyl trichloroacetimidate building blocks were synthesized and subsequently utilized for the automated synthesis of branched and linear beta-glucan fragments. Finally, conditions were developed to fully deprotect our

synthetic glucans, rendering them suitable for NMR binding studies and biological assays. These studies established automation protocols that can be used for the synthesis of larger, more complex beta-glucan structures.

# CHAPTER 1

# General Introduction

## 1. Dissertation Organization

This dissertation is composed of five chapters. Chapter 1 is a general introduction to the chemistry discussed in Chapters 2-4. Chapter 2 discusses the synthesis of a library of mass-identical disaccharides varying in linkage and monosaccharide composition. A select number of these compounds were analyzed by MALDI mass spectrometry by Hui Zhang and Ning Fang in the labs of Prof. Edward Yeung, and preliminary results were published in *Rapid Communications in Mass Spectrometry* in 2008.[1] Chapter 3 discusses the development of methodology for the automated fluorous solution-phase synthesis of beta-glucan fragments, culminating in the synthesis of a 3,6-branched glucose tetrasaccharide. This work demonstrates the ability to assemble branched oligosaccharides by automated iterative solution-phase synthesis. Chapter 4 discusses the automated solution-phase synthesis of other branched and linear beta-glucan fragments. Chapter 5 provides general conclusions and future directions for the entire dissertation.

## 2. Introduction to Carbohydrates

Carbohydrates are the most widely abundant class of organic compounds found on Earth.[2] All cells have oligosaccharides, or glycans, covalently linked to their surfaces. These glycans mediate cell-cell, cell-matrix, and cell-molecule interactions in multicellular organisms.[3] Carbohydrates also mediate interactions between host organisms and parasites.[4] The recognition of bacterial, fungal, and viral oligosaccharides

by specific immune system receptors is a key step in the immune system's process by which pathogens can be targeted and destroyed, as well as in the development of protective immunity.[5] A number of carbohydrate-based therapeutic agents and vaccine formulations have been developed and commercialized.[6] The difficulty in obtaining sufficient quantities of pure oligosaccharides, combined with the lack of technology for the rapid determination of oligosaccharide structure, have limited efforts toward the development of other carbohydrate-based therapeutic agents.

**3. Carbohydrate Structure Analysis by Mass Spectrometry**

Carbohydrates represent one of the three major classes of biopolymers, the other two being peptides and nucleotides. The field of proteomics was revolutionized during the 1990s when the development of rapid mass spectrometry-based methods for peptide sequencing displaced the more laborious and time-intensive Edman degradation.[7] Similarly, the pioneering work of Maxam and Gilbert[8] and Sanger and Coulson[9] led to methods for the sequencing of DNA. The sequencing of DNA and proteins has been automated,[10] and automated DNA and protein sequencing instruments are commercially available from companies such as Applied Biosystems. However, automated sequencing technology for carbohydrates has yet to be developed. Several factors have contributed to the lag in development of carbohydrate sequencing methods. Unlike DNA and polypeptides, which are primarily linear polymeric chains, oligosaccharides often contain branches. Oligosaccharide linkages can be formed through one of several hydroxyl groups found on each monosaccharide residue. Additionally, each linkage contains stereochemical information, with each linkage being either $\alpha$ or $\beta$. Clearly, the structural

complexity of carbohydrates presents a significant challenge for the development of sequencing methods.

Many techniques have been used for carbohydrate structure determination. Among these, mass spectrometry has emerged as one of the most valuable.[11] Soft ionization techniques, such as MALDI, can be used to generate high-mass molecular ions, which can then be fragmented using techniques such as collision-induced dissociation.[12] The fragmentation patterns observed can provide valuable information about the structure of carbohydrates. Ideally, technology could be developed for the automated structural determination of underivatized carbohydrates. However, the robustness and scope of any such technologies will depend on the ability to obtain and test a wide variety of well-defined carbohydrate structures not commercially available.

## 4. Iterative Chemical Synthesis of Oligosaccharides

The iterative approach to the synthesis of oligosaccharides relies on two key steps. First, a suitably protected glycosyl donor must be activated in the presence of a suitable nucleophile to generate a new glycosidic linkage of the desired stereochemical configuration. Second, a temporary protecting group must be selectively cleaved to generate a reactive hydroxyl group for further extension of the growing sugar chain (Figure 1.1). This process of glycosylation and deprotection steps can be repeated until the desired sugar chain length is achieved.

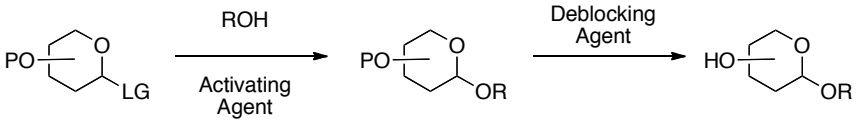


**Figure 1.1.** General strategy for iterative oligosaccharide synthesis. P = protecting group; LG = leaving group

The challenge of carbohydrate synthesis is thus two-fold. First, an orthogonal protecting group strategy must be developed that allows for the selective unmasking of reactive hydroxyl groups for chain elongation. Second, the choice of the anomeric leaving group in the glycosyl donor and conditions for its activation must be compatible with the protecting groups chosen. Fortunately, several types of synthetically useful glycosylating agents exist, as will be discussed below.

**4.1 Glycosyl Donors**

Several anomeric leaving groups have been developed for use in glycosylation reactions. Glycosyl halides were first introduced by Koenigs and Knorr.[13] Glycosyl halides, typically the chlorides and bromides, are activated by a halophilic promoter, such as $Ag_2O$ or AgOTf. Many variations of the classical Koenigs-Knorr method, including the use of glycosyl fluorides[14] and iodides,[15] have been developed. Thioglycosides can also act as glycosyl donors, and are typically activated by a Lewis acid, such as $BF_3{\cdot}OEt_2$,[16] or iodonium systems such as NIS/AgOTf.[17] One drawback to the thioglycosides, however, is the requirement of substantial quantities of highly reactive reagents needed for their activation. The 1,2-orthoesters of aldose sugars have also gained utility as glycosyl donors, especially the *n*-pentenyl orthoesters developed by Fraser-Reid and coworkers.[18]

Activated by various Lewis acid/NIS combinations, these donors result in the formation of 1,2-*trans* glycosidic linkages. The *n*-pentenyl orthoesters can be converted to *n*-pentenyl glycosides, another useful type of glycosyl donor.[19] Perhaps the most popular glycosyl donor developed to date is the glycosyl trichloroacetimidate, introduced by

Schmidt in 1980.[20] Glycosyl trichloroacetimidates are readily obtained by the addition of trichloroacetonitrile ($Cl_3CCN$) to a free anomeric hydroxyl group in the presence of a base, typically NaH, 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), or $Cs_2CO_3$. The ease of formation of glycosyl trichloroacetimidates, as well as their reactivity and broad applicability, have contributed to their widespread use in synthetic carbohydrate chemistry. Glycosyl trichloroacetimidates are generally activated by catalytic amounts of Lewis acids, such as $BF_3{\cdot}OEt_2$ or TMSOTf. Trivalent lanthanide triflates have also been used.[21-22] More recently, the use of glycosyl trifluoroacetimidates as glycosyl donors has been explored.[23-24] Many other types of glycosyl donors have been developed in recent years, providing chemists with a number of tools for the synthesis of complex oligosaccharides.

**4.2 Automated Synthesis of Oligosaccharides**

The development of solid-phase synthetic methodologies has led to the commercialization of automated peptide and nucleic acid synthesizers. Researchers in need of a specific nucleic acid or peptide sequence can purchase a custom-made molecule from a number of online vendors. Unfortunately, commercial custom oligosaccharide synthesis is not yet a reality.

Bruce Merrifield's development of solid-phase peptide synthesis[25] in the 1960s provided a means for the rapid synthesis of peptides. Other researchers began to apply solid-phase approaches to carbohydrate synthesis. The first report of solid-phase carbohydrate synthesis was made by Frechet and Schuerch in 1971.[26] Three decades later, a modified automated peptide synthesizer was used to synthesize

oligosaccharides.[27] However, solid-phase synthesis presents a number of challenges to carbohydrate synthesis. To overcome the kinetic limitations of a biphasic reaction system, large excesses of sugar building blocks (10-20 equivalents per coupling cycle) must be used to ensure high coupling efficiencies. Since these building blocks may require four or as many as fourteen or more steps to prepare, this represents a very significant cost in terms of both time and money. Additionally, the difficulty in monitoring reaction progress on the solid phase is another limitation of solid-phase synthesis. Clearly, further efforts must be undertaken to develop a robust, efficient method for the automated synthesis of oligosaccharides.

**5. Beta-Glucans**

Glucose and its polymers constitute the largest percentage of carbohydrates on Earth and therefore are important targets for the development of any automated synthesis protocols. For example, glucans are naturally occurring polysaccharides composed of repeating D-glucopyranose units connected by β-glycosidic linkages. These compounds can be obtained from a number of natural sources, including cereal grains, plants, algae, yeasts, and fungi. β-Glucans consist of linear (1→3)-β-linked backbones with (1→6) or (1→4)-linked side chains. The β-glucans vary considerably in terms of primary structure, degree of branching, molecular weight, and solution conformation, all of which play a role in determining the biological activity associated with these compounds.

**5.1. Sources and Structures of Naturally Occurring Beta-Glucans**

β-Glucans can be found in a wide variety of organisms. A number of β-glucans have been discovered in prokaryotes, including the capsular glucans from *Streptococcus pneumoniae*[28] and the (1→3)-β-linked glucan called curdlan, produced by fermentation of *Agrobacterium.*[29] Algae are also rich sources of β-glucans. Laminarans, obtained from *Laminaria* species of brown algae, are low-molecular weight storage polysaccharides comprised of (1→3)-β-D-glucopyranose chains with some (1→6)-branching. The laminarans can be likened to starch found in plants. The (1→3, 1→6)-β-glucans are major components of the cell walls of yeasts and fungi. These cell surface oligosaccharides play important roles in the formation of symbiotic and pathogenic relationships with plants and animals, and are important targets for the immune system.[30] Fungal and yeast glucans all share a similar structure: a (1→3)-linked β-D-glucopyranose backbone with some side chains of β-D-glucopyranosyl residues connected to the backbone by β-(1→6) linkages. For example, a β-glucan called HEP3, isolated from the fruiting bodies of the fungus *Hericium erinaceus*, was reported to be composed of a (1→3)-linked β-D-glucopyranose backbone, with one β-D-glucopyranosyl substituent at O-6 for, on average, every three backbone residues.[31] Linear β-glucans containing both (1→3)- and (1→4)-β-glucosidic linkages can be found in the endosperm walls of barley, wheat, and other cereal grains. β-Glucans can form complex tertiary structures stabilized by interchain hydrogen bonds, ranging from random coils to highly organized triple helices.[32] Clearly, the β-glucans are a structurally diverse family of oligosaccharides.

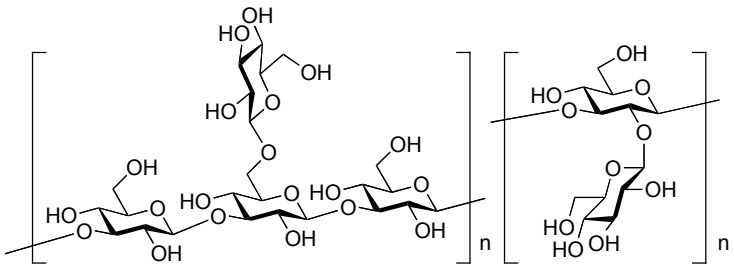


**Figure 1.2.** Examples of naturally occuring β-glucans

### 5.2 The Immune Response to Beta-Glucans

The β-glucans have been shown to exhibit a wide range of medicinal and bioactive properties, including anti-inflammatory, antimicrobial, anti-tumoral, cholesterol-lowering, and immunomodulatory activity.[32] However, the precise activity of β-glucan preparations can vary considerably based on the source of the glucan, and its method of isolation, structure, and purity. Hot water extracts from mushrooms and tree fungi have been used as folk remedies in Asia and Russia for centuries.[32] Currently, these preparations are used for both general health-stimulating effects and for specific therapeutic purposes.[33] The protective effects of β-glucans are thought to arise from non-specific immune stimulation via a number of immune pathways, including macrophage activation, activation of natural killer cells, activation of complement pathways, and increased antibody production.[32]

The β-glucans have emerged as a major target for the recognition of pathogens by the innate immune system. Recognition of fungal β-glucan structures by a number of immune system receptors triggers responses that protect the host from the pathogen. Several β-glucan receptors have been identified in vertebrates, including scavenger receptors, complement receptor 3, lactosylceramide, and Dectin-1.[34] In particular, the β-

glucan receptor Dectin-1 has been studied extensively over the past decade. Dectin-1 is a type II transmembrane receptor comprised of four main portions: a lectin-like carbohydrate recognition domain, a stalk region, a transmembrane domain, and an immunoreceptor tyrosine-based activation motif (ITAM) (Figure 1.3).[35,36] Dectin-1 recognizes particulate and soluble glucans from fungi, bacteria, and plants, and serves as the primary receptor for these carbohydrates on white blood cells.[37] Dectin-1 can induce a number of cellular responses to fungal pathogens, including phagocytosis and killing and the induction of cytokines and chemokines. The ITAM motif of Dectin-1 induces the intracellular signaling pathways that result in these responses.[36] Studies carried out *in vivo* have demonstrated the protective effects of Dectin-1. For example, the blockage of Dectin-1 function using a soluble inhibitor resulted in a reduced inflammatory response and increased fungal burdens in the lungs of mice infected with *A. fumigatus*.[38]

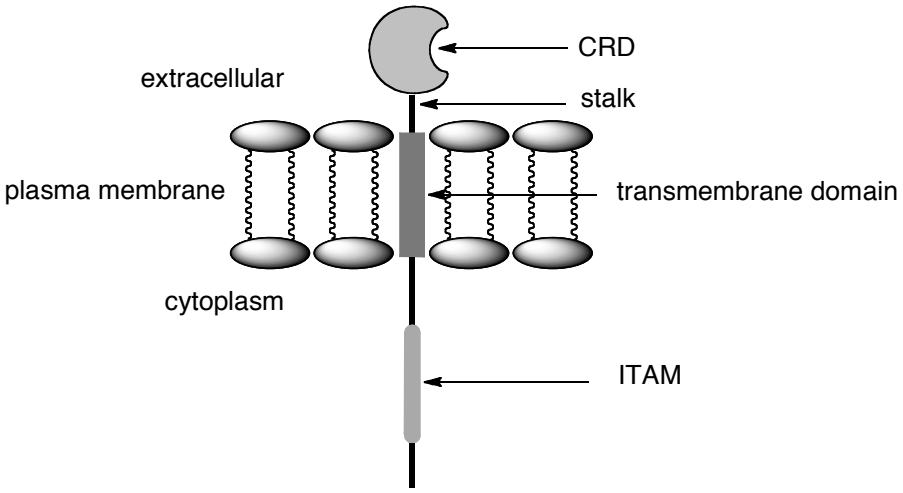


**Figure 1.3.** Schematic illustration of the structure of Dectin-1. Dectin-1 is composed of a carbohydrate-recognition domain (CRD), a stalk region, transmembrane domain, and a cytoplasmic region containing the immunoreceptor tyrosine-based activation motif (ITAM)

The discovery and characterization of the β-glucan receptor Dectin-1 has provided a wealth of information regarding the innate immune response to pathogens. Research has also shown that Dectin-1 plays a significant role in antifungal immunity. However, further efforts must be taken to continue to elucidate the molecular mechanisms involved in the action of Dectin-1. Access to pure, structurally defined β-glucans would allow researchers to study in detail the ligand specificity of Dectin-1 as a function of chain length and branching pattern in the carbohydrate ligand.

## 6. Research Aims

The aims of this research can be divided into two main areas: 1) the synthesis of a small library of mass-identical disaccharides, varying in monosaccharide composition and linkage position, for systematic structural analysis by mass spectrometry, and 2) the development of methods for the automated solution-phase synthesis of branched and linear β-glucan fragments.

### 6.1. Synthesis of Disaccharides for Mass Spectrometry Studies

The goal of this work was to synthesize a number of disaccharides with different monosaccharide residues and linkage positions, but with identical masses. These compounds could then be systematically studied by tandem mass spectrometry to obtain structural information based on fragmentation patterns. Initially, disaccharides bearing a propyl group at the reducing end were synthesized to avoid the presence of anomeric mixtures. Later, a second library of reducing disaccharides was prepared. In both cases, appropriately protected glucosyl acceptors were synthesized to facilitate the formation of

1,2-, 1,3-, 1,4-, and 1,6-glycosidic linkages. The acceptors were then glycosylated with the well-known peracetylated glycosyl donors derived from D-glucose, D-galactose, and D-mannose. Following global deprotection, the disaccharides could then be screened by mass spectrometry.

### 6.2. Automated Solution-Phase Synthesis of Beta-Glucan Fragments

The goal of this project was to develop a strategy for the synthesis of both linear and branched β-glucan fragments using an automated solution-phase approach. Several key challenges were met in the course of this work. First, a number of glucose-based trichloroacetimidate building blocks were synthesized to allow for the selective formation of various glycosidic linkages. In particular, building blocks were developed for the introduction of branch point glucose residues that would allow for either simultaneous or independent chain extension from the branch point. Second, protocols were established to transfer the many tasks involved in benchtop oligosaccharide synthesis to a new automated solution-phase synthesis platform. Finally, these new automation protocols were applied to the synthesis of a number of β-glucan fragments.

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# CHAPTER 2

## Synthesis of Disaccharides for Linkage and Residue Determination by Tandem Mass Spectrometry and Linear Discriminant Analysis*

*Portions of the work in this chapter were published in Zhang, H.; Brokman, S.M.; Fang, N.; Pohl, N.L.; Yeung, E.S. *Rapid Communications in Mass Spectrometry* **2008**, 22, 1579-1586.

Abstract

The development of rapid mass spectrometry-based techniques for the sequencing of nucleic acids and peptides sparked growth in the fields of genomics and proteomics. Unfortunately, no such technology currently exists for the sequencing of carbohydrates. The structural complexity of carbohydrates presents a considerable challenge to researchers seeking to develop oligosaccharide-sequencing tools. Mass spectrometry is perhaps the most powerful technique for determining the structure of oligosaccharides. The lack of access to a diverse library of well-defined oligosaccharides has limited the development of carbohydrate sequencing technologies. In this research, two small libraries of mass-identical disaccharides varying in linkage position and identity of the non-reducing end monosaccharide residue were synthesized. A number of these synthetic standards were subjected to tandem mass spectrometry in the laboratory of Prof. Edward Yeung. Preliminary results showed that differences in the MS-MS spectra of the synthetic disaccharides could be used to generate classification functions that were able to predict the linkage type as well as the identity of the non-reducing end monosaccharide residue present in each disaccharide.

Introduction

Carbohydrates represent one of the three major classes of biopolymers, the other two being nucleotides and proteins. Carbohydrates play a number of important roles in living systems, including structural roles, mediating cell-cell and cell-pathogen interactions, and acting as regulatory switches for cellular processes.[1] The structural complexity of carbohydrates represents a significant challenge for the development of carbohydrate sequencing methods. Unlike proteins and nucleotides, which are almost exclusively linear polymers, oligosaccharides often possess branched structures. Additionally, glycosidic linkages contain stereochemical information- each linkage may be either α or β. Since each monosaccharide is a polyfunctional molecule, multiple linkage types are possible for each monosaccharide residue. Despite their biological significance, development of technology for the structural determination of carbohydrates has not kept pace with that of nucleotides and proteins.

Of the many techniques explored for the structural characterization of carbohydrates, mass spectrometry has emerged as a dominant technique.[2] Soft ionization techniques such as MALDI allow the generation of high-mass molecular ions, making them well suited for carbohydrate analysis. Collision-induced dissociation (CID) of molecular ions generated by MALDI has been used to analyze underivatized oligosaccharides.[3] Electrospray ionization (ESI) has been used to analyze carbohydrates, but best results are achieved with permethylated oligosaccharides.[4] The ability to analyze underivatized oligosaccharides, without the need for permethylation of other forms of derivatization, would be a significant advancement in the area of oligosaccharide sequencing.

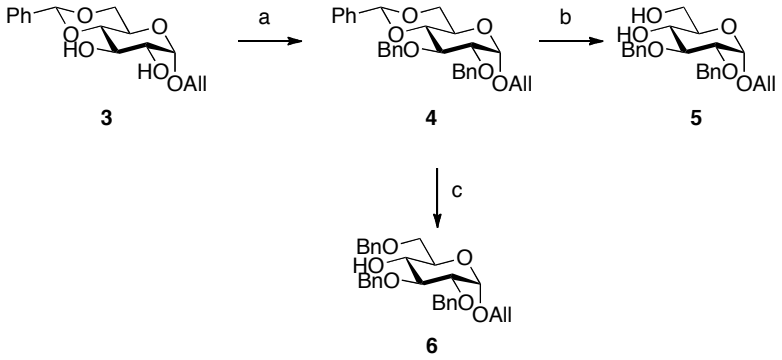
In order to develop a systematic approach to the determination of oligosaccharide sequence by mass spectrometry, we must explore the ability of mass spectrometry to differentiate between various linkage types and the identities of individual monosaccharide residues, even when those residues share identical masses. A library of disaccharides with various linkage types and differing but mass-identical monosaccharide residues could be systematically explored by mass spectrometry, in order to observe the influence of structure on the fragmentation patterns that are observed. Molecules containing the monosaccharides glucose, galactose, and mannose are ideal test cases as all three residues are commonly found in natural structures and all share the same mass. Unfortunately, only a limited number of disaccharides containing these monomeric sugars are available from commercial sources. Fortunately, chemical synthesis can provide ready access to a wide variety of disaccharides for mass spectrometry studies.

Results and Discussion

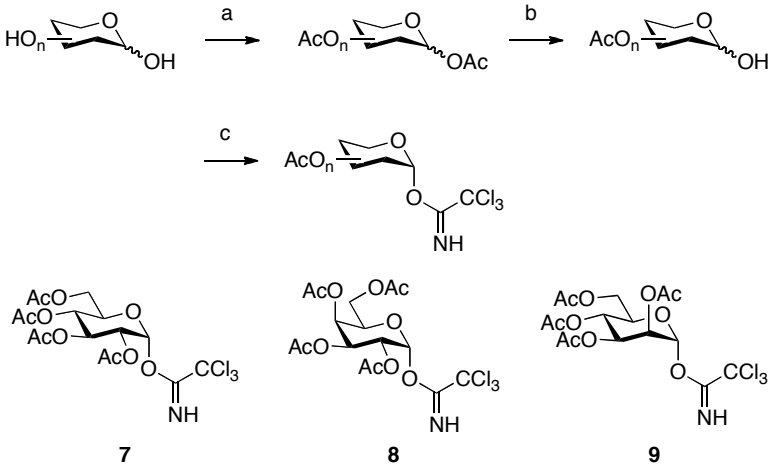
In order to supplement the commercially available disaccharides, we synthesized two small libraries of disaccharides bearing a glucose residue at the reducing end and either glucose, galactose, or mannose at the nonreducing end. One library of disaccharides would bear a propyl group at the reducing end to limit the complications provided by mutarotation of free anomers in solutions, and the other a free hydroxyl group. First, the library of propyl-tagged disaccharides was constructed. The known glycosyl acceptors **1**[5], **2**[6], **5**[7], and **6**[8], were selected. Acceptors **1** and **2** were synthesized from D-glucose following published procedures.[5,6] Acceptors **5** and **6** were synthesized from the known diol **3**[9](Scheme 1). Diol **3** was benzylated to provide the known 2,3-di-

*O*-benzyl derivative **4**.[8] Cleavage of the benzylidene acetal from **4** gave the 4,6-diol **5**. Regioselective reductive benzylidene ring opening[10] of **4** gave only the 6-*O*-benzyl derivative **6**.

With the glycosyl acceptors in hand, the known peracetylated glycosyl donors **7**[11], **8**[12], and **9**[11] were synthesized in three steps from D-glucose, D-galactose, and D-mannose, respectively, via peracetylation, selective anomeric deacetylation, and trichloroacetimidate formation (Scheme 2). The very reactive trichloroacetimidate-activated glycosyl donors[13] were expected to readily provide disaccharides with not only the less hindered primary alcohol nucleophiles, but also the various secondary alcohol nucleophiles. Additionally, through neighboring group participation of the 2-*O*-acetyl group, these donors can provide the 1,2-*trans* glycosides as the sole product.

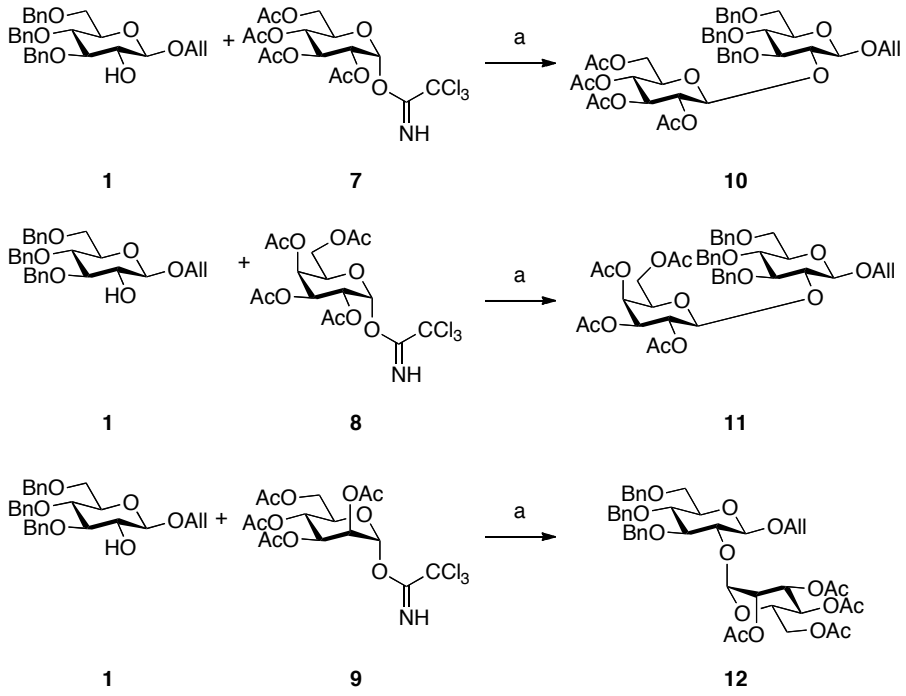



**Scheme 1**. Synthesis of acceptors **3** and **4**. a) BnBr, NaH, $Bu_4NI$, DMF, rt, 2.5 h, 87%; b) 60% TFA (aq.), $CH_2Cl_2$, rt, 45 min, 89%; c) $Et_3SiH$, TfOH, 4 A MS, $CH_2Cl_2$, -78 °C, 130 min, 67%.

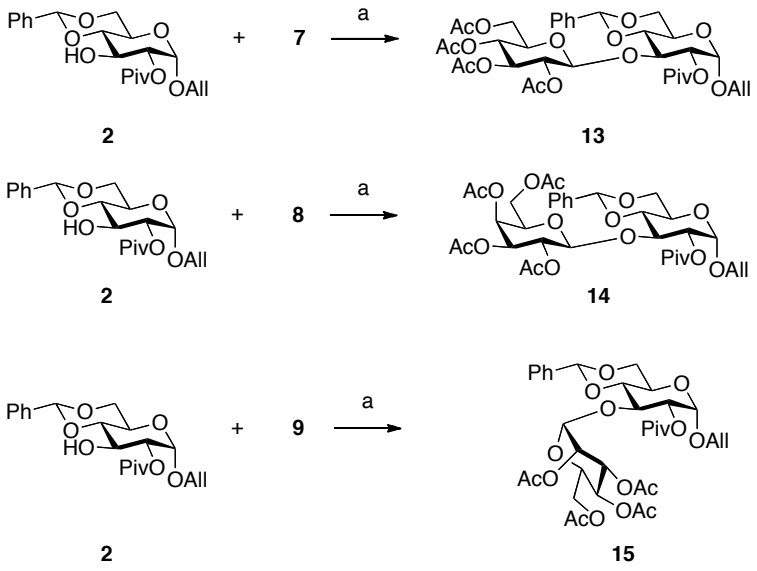



**Scheme 2**. General route for the synthesis of peracetylated monosaccharide donors. a) $Ac_2O$, $I_2$, rt, 3 h; b) $H_2NNH_2{\cdot}HOAc$, DMF, 60 $^oC$, 1h; c) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt.

With the requisite donors and acceptors in hand, the assembly of disaccharides was commenced. Glycosylation of the 2-hydroxyl glucose acceptor **1** with each of the three donors gave the desired disaccharides in excellent yields (Scheme 3). In a similar fashion, the 1,3-linked disaccharides **13**, **14**, and **15** were prepared by the glycosylation of acceptor **2** with the aforementioned donors **7**, **8**, and **9**, respectively (Scheme 4). The somewhat lower yields in these cases are likely a result of deactivation of the glycosyl acceptors by the sterically large and electron-withdrawing pivaloate[14] at the neighboring hydroxyl.



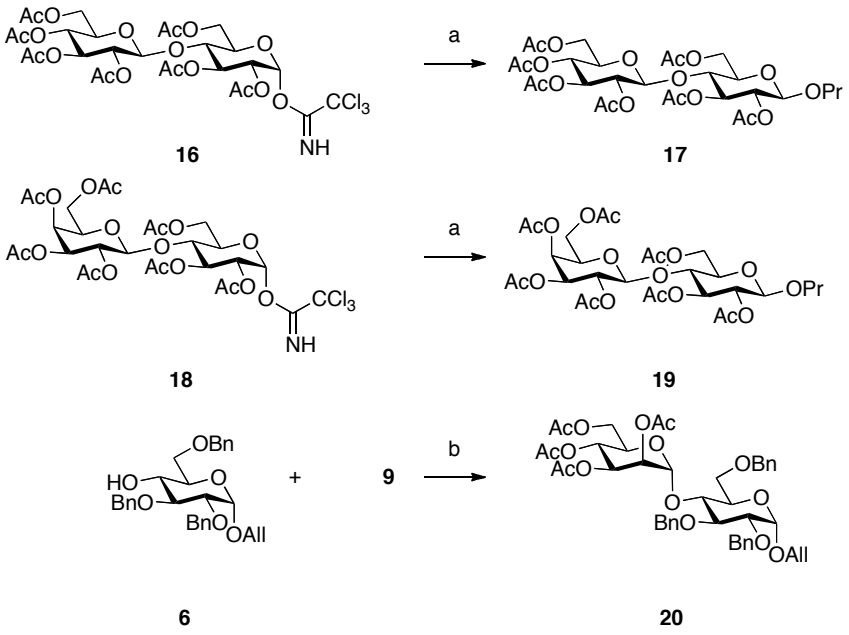

**Scheme 3.** Synthesis of allyl tagged 1,2-linked disaccharides. a) TMSOTf, $CH_2Cl_2$, 0 °C, 20 min (92% for **10**, 94% for **11**, 98% for **12**).



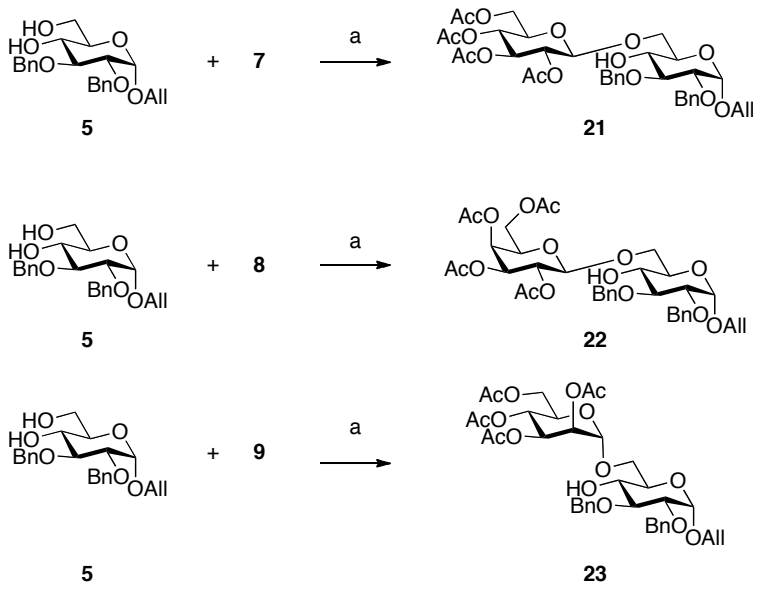

**Scheme 4**. Synthesis of allyl tagged 1,3-linked disaccharides. a) TMSOTf, $CH_2Cl_2$, -15 °C, 30 min (58% for **13**, 60% for **14**, 62% for **15**).

Next, the 1,4-linked disaccharides were synthesized. The commercially available disaccharides cellobiose and lactose were used to prepare their known peracetylated trichloroacetimidate derivatives **16**[15] and **18**.[16] Condensation of **16** and **18** with n-

propanol gave the desired propyl glycosides **17** and **19** in modest yields. To prepare the mannosyl analog, acceptor **6** was glycosylated with mannose donor **9** to furnish disaccharide **20** (Scheme 5). Finally, the 1,6-linked disaccharides **21**, **22**, and **23** were prepared by selective glycosylation at the O-6 position of diol **5**. By using limiting amounts (0.5 – 1.0 equiv.) of donors **7**, **8**, and **9**, the desired disaccharides could be obtained (Scheme 6). The structures of **21**, **22**, and **23** were confirmed by $^{1}$H-$^{1}$H COSY NMR experiments, which for each compound showed coupling of H-4 on the reducing-end glucose residue (δ 3.44 ppm) to the hydroxyl proton on O-4 (δ 2.3 ppm).

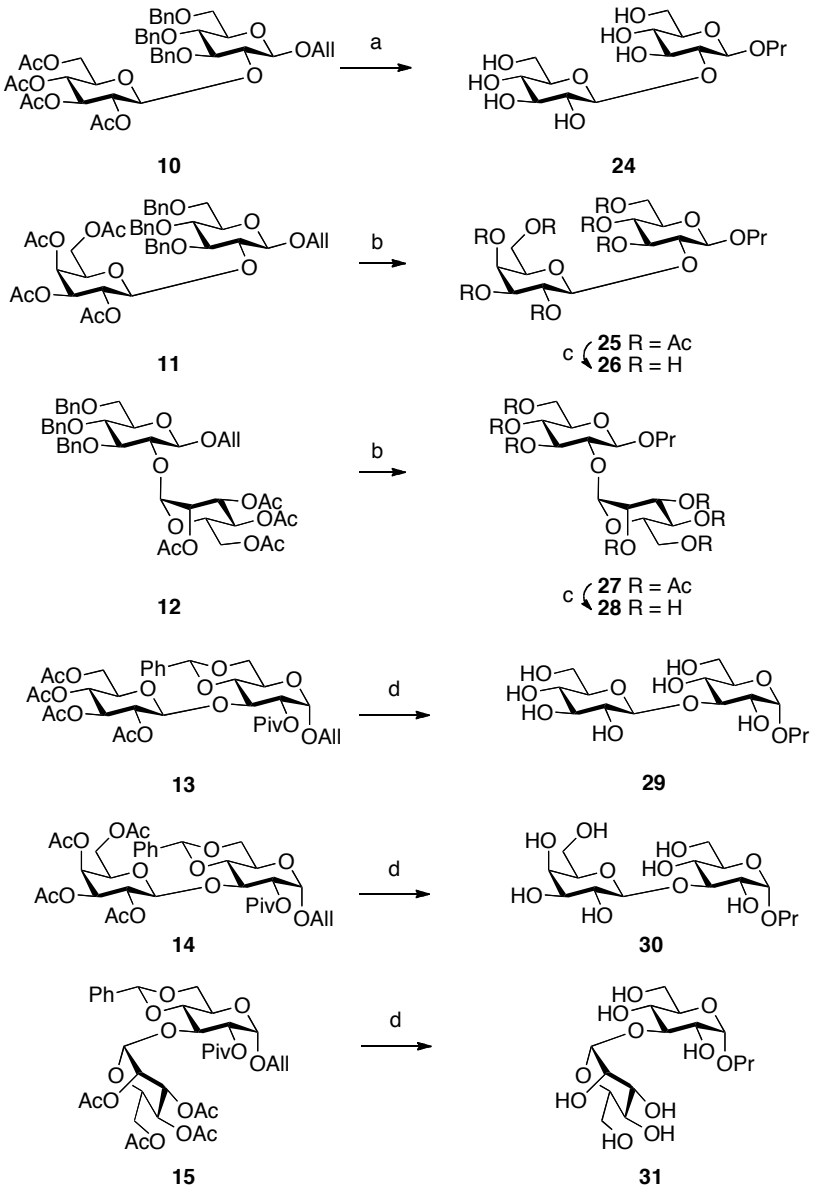



**Scheme 5.** Synthesis of 1,4-linked disaccharides. a) *n*-propanol, TMSOTf, $CH_2Cl_2$, 0 °C, 15-20 min (53% for **17**, 23% for **19**); b) TMSOTf, $CH_2Cl_2$, 0 °C, 30 min, 61%.



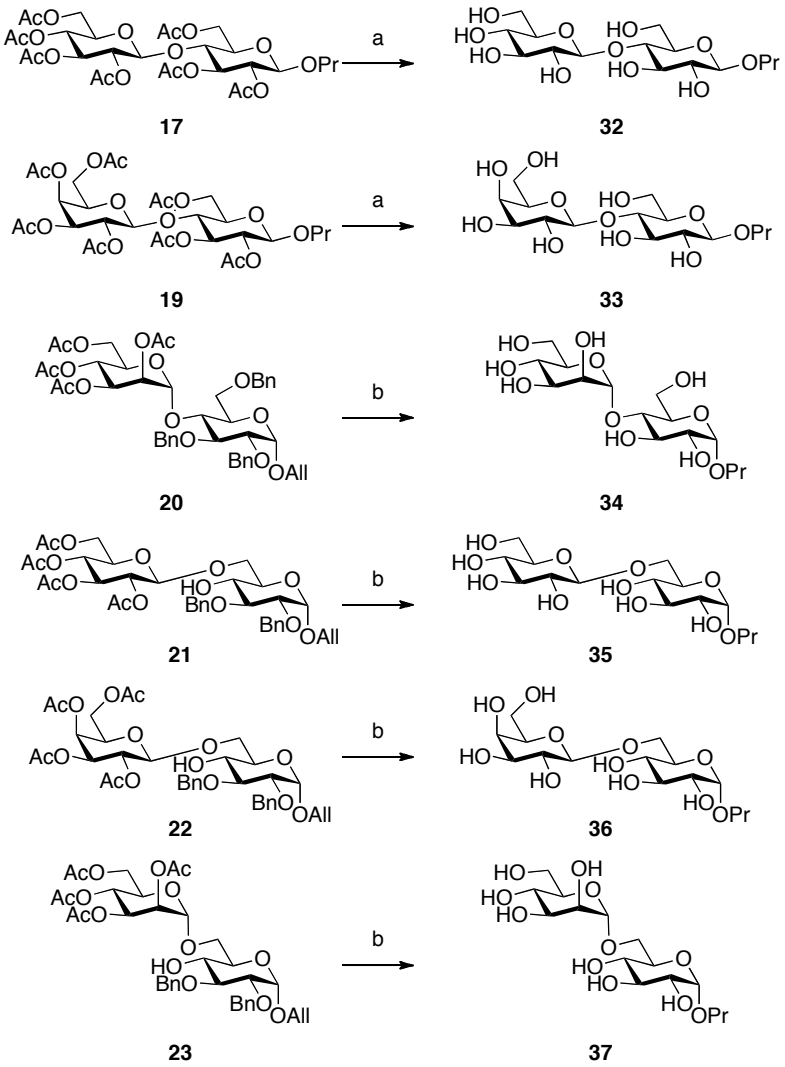

**Scheme 6.** Synthesis of allyl tagged 1,6-linked disaccharides. a) TMSOTf, $CH_2Cl_2$, 0 °C, 15 min (28% for **21**, 30% for **22**, 26% for **23**).

Having obtained each of the desired disaccharides, global deprotection was carried out to unmask the hydroxyl groups and yield the disaccharides as the propyl glycosides. The 1,2-linked disaccharides **10**, **11**, and **12** were subjected to catalytic hydrogenation to cleave the benzyl ethers and reduce the double bond in the allyl group, followed by deacetylation[17] under Zemplen conditions. The galactosyl and mannosyl analogs **11** and **12** were then purified as the corresponding peracetylated derivatives **25** and **27** prior to final deacetylation to give compounds **26** and **28**. The 1,3-linked disaccharides **13**, **14**, and **15** were subjected to acid-catalyzed debenzylation, catalytic hydrogenation, and Zemplen deacetylation to furnish the fully deprotected derivatives **29**, **30**, and **31** (Scheme 7).




**Scheme 7.** Deprotection of 1,2- and 1,3-linked disaccharides. a) $H_2$, 10% Pd/C, MeOH or EtOH, rt; then NaOMe, MeOH, rt, 95%; b) $H_2$, 10% Pd/C, MeOH, rt; then NaOMe, MeOH, rt; then $Ac_2O$, pyr, DMAP, rt, 77% for **25**, 58% for **27**; c) NaOMe, MeOH, rt, 96% for **26**, 99% for **28**; d) 60% TFA (aq.), $CH_2Cl_2$, rt; then $H_2$, 10% Pd/C, MeOH or EtOH, rt; then NaOMe, MeOH, rt, 90% for **29**, 96% for **30**, 94% for **31**.

The cellobiose derivative **17** and lactose derivative **19** were deacetylated to furnish the propyl glycosides **32** and **33** in 90 and 98% yields, respectively. The allyl glycosides **20**, **21**, **22**, and **23** were hydrogenated and deacetylated to furnish the corresponding propyl glycosides **34**, **35**, **36**, and **37** (Scheme 8).



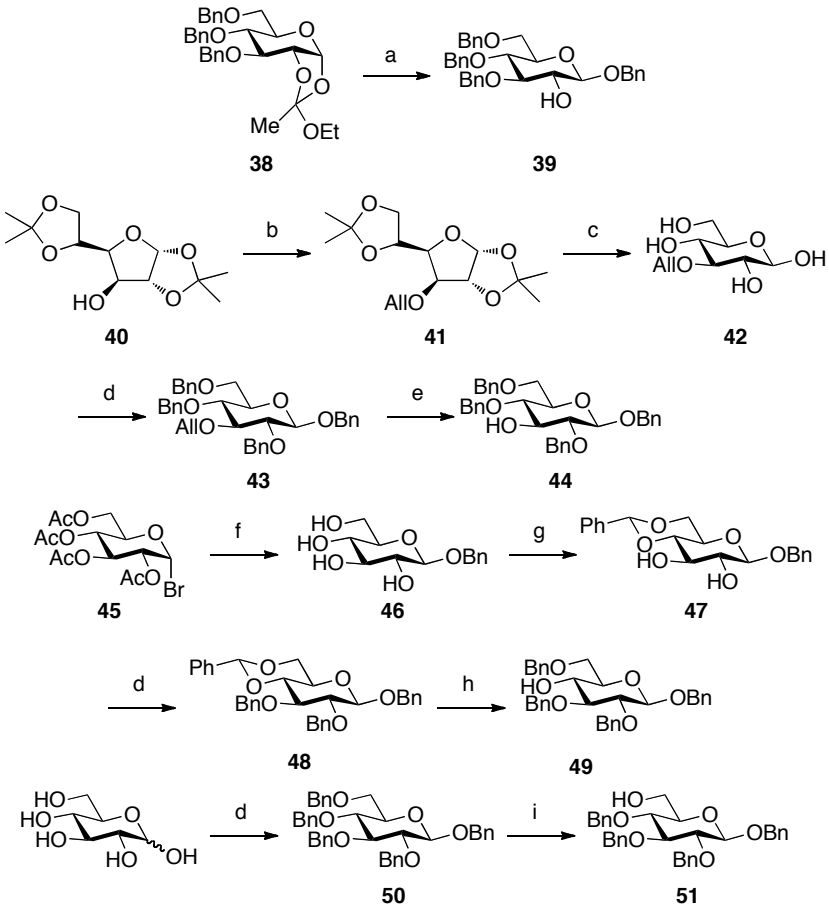

**Scheme 8.** Deprotection of 1,4- and 1,6-linked disaccharides. a) NaOMe, MeOH, rt, 90% for **32**, 98% for **33**; b) $H_2$, 10% Pd/C, MeOH, rt, then NaOMe, MeOH, rt, 75% for **34**, 96% for **35**, 97% for **36**, 99% for **37**.

Having successfully prepared a number of propyl-tagged disaccharides, our efforts then focused on the synthesis of disaccharides bearing a free reducing end. To this end, we chose to prepare glucose acceptors protected only by benzyl ethers and couple them with the aforementioned peracetylated glycosyl trichloroacetimidates **7**, **8**, and **9**. The resulting disaccharides would then be fully deprotected through a

straightforward two-step sequence consisting of catalytic hydrogenation followed by Zemplen deacetylation to furnish the desired reducing disaccharides.

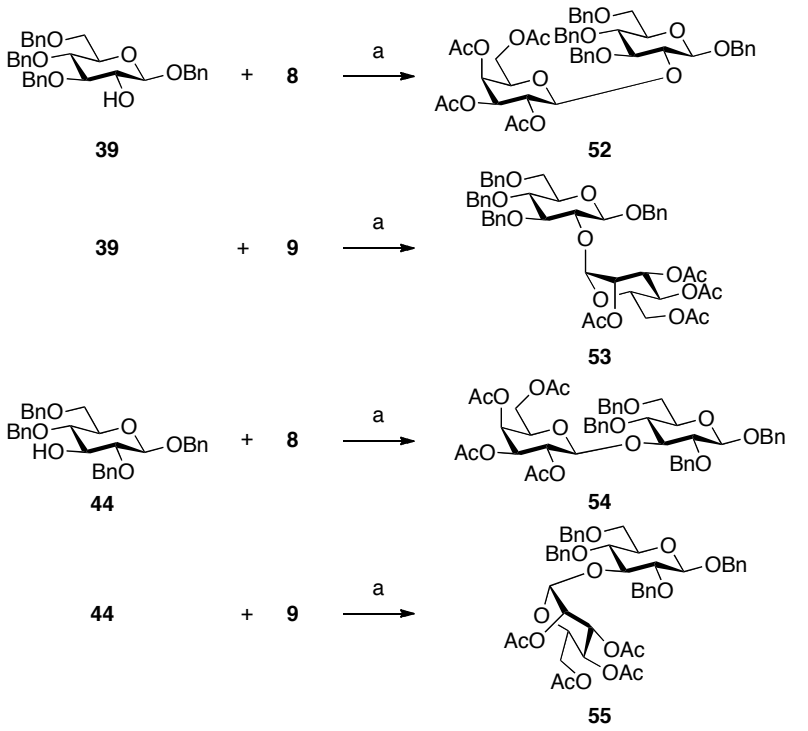
First, the four necessary acceptor molecules were synthesized, each bearing a single free hydroxyl group (Scheme 9). The synthesis of 2-OH acceptor **39**[18] was accomplished by treating the known 1,2-orthoester **38**[19] with benzyl alcohol and catalytic TMSOTf. Following cleavage of the 2-*O*-acetyl group from the crude product, a considerable amount of benzyl alcohol remained. The benzyl alcohol was converted to its trityl ether to facilitate the purification of benzyl glucoside **39**. The synthesis of 3-OH acceptor **44**[20] commenced from diacetone-D-glucose **40**.[21] The 3-OH group was protected as the allyl ether **41**.[22] Acidic hydrolysis of the ketal groups gave pyranose **42**,[23] which was benzylated to give the fully protected compound **43**. The allyl ether in **43** was cleaved to give the 3-OH acceptor **44**.[24]

For the production of acceptor **49**,[25] known glucosyl bromide **45**[26] was treated with benzyl alcohol and silver oxide to furnish benzyl glucoside **46**.[25] Formation of the 4,6-*O*-benzylidene acetal and benzylation of the two remaining hydroxyl groups yielded **48**.[25] Selective benzylidene ring opening of **48** furnished the 4-OH acceptor **49**. To construct the 6-OH acceptor, the procedure of Lu and coworkers[27] was followed. Perbenzylation of D-glucose yielded compound **50**.[27] Selective acetolysis of the 6-*O*-benzyl group in **50**, followed by deacetylation of the crude product, afforded the 6-OH acceptor **51**.[27,28]

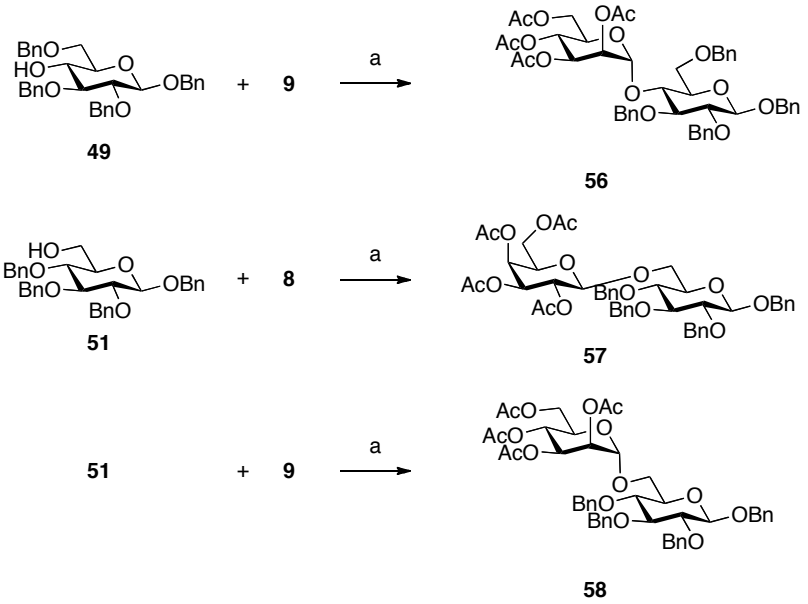


**Scheme 9**. Synthesis of benzylated glucosyl acceptors. a) BnOH, TMSOTf, 0 °C, 20 min, then NaOMe, MeOH, rt, 18 h, then TrCl, DMAP, DMF, 70 °C, 40 min, 54%; b) Allyl bromide, NaH, $Bu_4NI$, THF, rt, 2 h, 54%; c) Dowex 50 ($H^+$), $H_2O$, 70 °C, 4 h, 46%; d) BnBr, NaH, DMF, rt (44% for **43**, 94% for **48**, 50% for **50**); e) $[Ir(COD)(PMePh_2)_2]PF_6$, $H_2$, THF, rt, 16 h, then $HgCl_2$, HgO, acetone, $H_2O$, rt, 1 h, 86%; f) BnOH, $Ag_2O$, $CH_2Cl_2$, rt, 4 h, then NaOMe, MeOH, rt, 3 h, 66%; g) $PhCH(OMe)_2$, $CH_3CN$, CSA, reflux, 75 min, 72%; h) $Et_3SiH$, TfOH, 4A MS, $CH_2Cl_2$, -78 °C, 1 h, 53%; i) $ZnCl_2$, $Ac_2O$, AcOH, 0 °C to rt, 1 h, then NaOMe, MeOH, rt, 2 h, 37%

Acceptors **39** and **44** were successfully glycosylated with donors **8** and **9** to generate disaccharides **52**, **53**, **54**, and **55** (Scheme 10). Similarly, acceptor **49** was glycosylated with donor **9** to give disaccharide **56**, and acceptor **51** was glycosylated with donors **8** and **9** to give disaccharides **57** and **58**, respectively (Scheme 11).



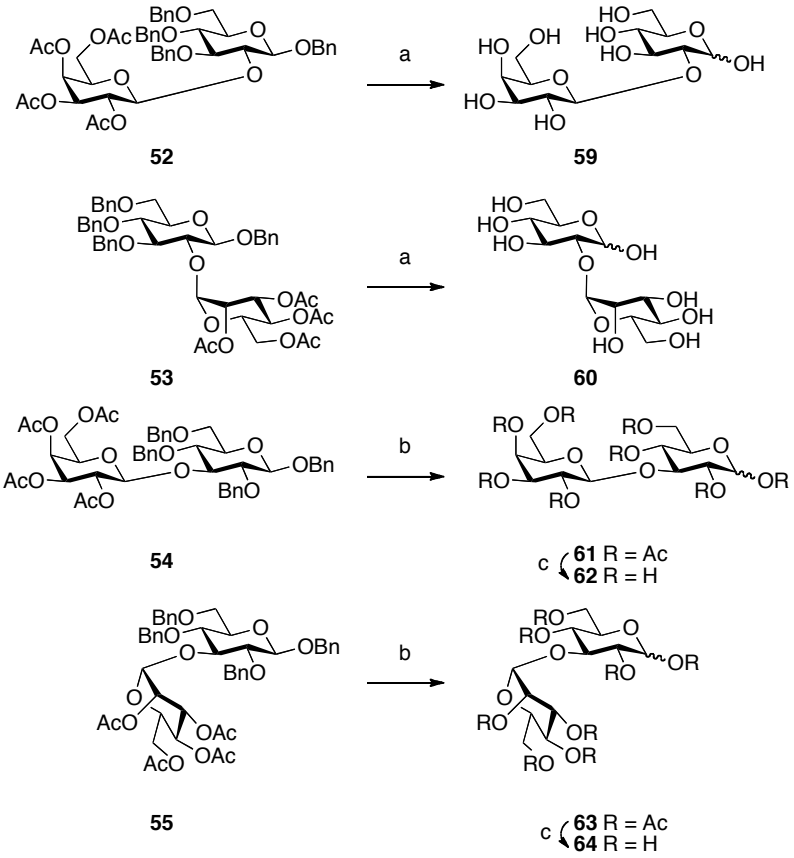

**Scheme 10.** Synthesis of 1,2- and 1,3-linked disaccharides. a) TMSOTf, $CH_2Cl_2$, rt, 30 min (48% for **52**, 88% for **53**, 60% for **54**, 88% for **55**).



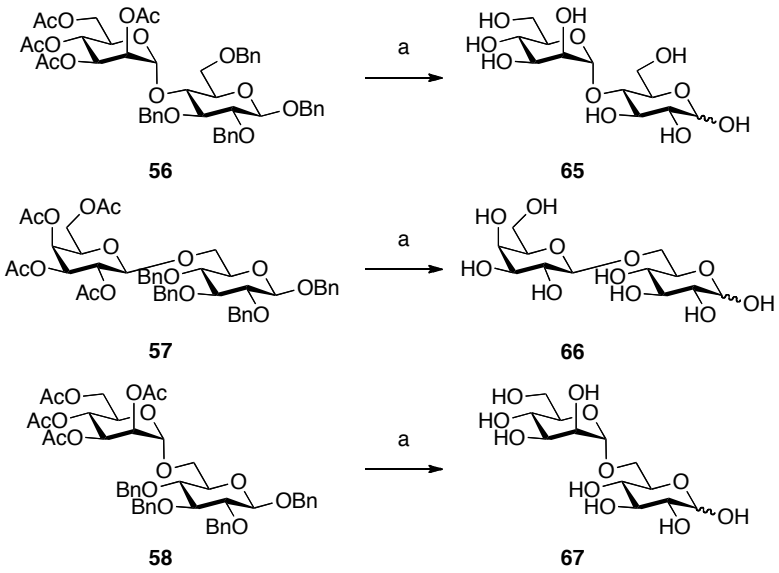

**Scheme 11.** Synthesis of 1,4- and 1,6-linked disaccharides. a) TMSOTf, $CH_2Cl_2$, rt, 30 min (85% for **56**, 51% for **57,** 75% for **58**).

The deprotection sequences for disaccharides **52-58** were then carried out (Schemes 12 and 13). Each disaccharide was subjected to debenzylation followed by

deacetylation to yield the corresponding deprotected reducing disaccharide. In the case of the deprotection reactions for compounds **54** and **55**, the debenzylated intermediates were purified as the peracetylated derivatives **61** and **63** prior to final deprotection.




**Scheme 12.** Deprotection of 1,2- and 1,3-linked disaccharides. a) $H_2$, 10% Pd/C, EtOH, rt, then NaOMe, MeOH, rt (92% for **59**, 96% for **60**); b) $H_2$, 10% Pd/C, EtOH, rt, then $Ac_2O$, pyr, rt (87% for **61**, 84% for **63**), c) NaOMe, MeOH, rt (93% for **62**, 95% for **64**).




**Scheme 13.** Deprotection of 1,4- and 1,6-linked disaccharides. a) $H_2$, 10% Pd/C, EtOH, rt, then NaOMe, MeOH, rt (97% for **65**, 99% for **66**, 97% for **67**).

The synthetic disaccharides **24**, **26**, **28**, **29**, **30**, **31**, **32**, **33**, and **34** served as standards for preliminary mass spectrometry studies carried out in the laboratory of Prof. Edward Yeung. The compounds were analyzed using MALDI mass spectrometry using an acidic fullerene matrix. The relative intensities of the fragments observed in the MS/MS spectra of each synthetic disaccharide were used as input for linear discriminant analysis (LDA). Following linear discriminant analysis, classification functions were produced. These functions were used to successfully determine not only the saccharide linkage type (1,2-, 1,3- or 1,4-) but also the identity of the non-reducing end monosaccharide residue (glucose, galactose, or mannose).[29] MALDI MS/MS analyses of the remaining synthetic disaccharides prepared in this work are in progress in the labs of Prof. Young-Jin Lee to determine the generality of this new approach to sequencing carbohydrates using mass spectrometry.

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## Conclusion

A library of synthetic disaccharides was prepared by coupling suitably protected glucosyl acceptors with peracetylated glycosyl trichloroacetimidate donors. After chromatographic purification, the protecting groups were removed to furnish the deprotected disaccharides, either as propyl glycosides or as free reducing sugars. Preliminary studies showed that the synthetic disaccharides exhibited differences in the fragmentation observed in their MS/MS spectra, and that these results could be used to classify each disaccharide by its linkage position as well as the identity of the nonreducing end monosaccharide residue. The results of this study will likely improve our ability to determine the structure of oligosaccharides and provide insight that will ultimately contribute to the development of faster carbohydrate sequencing methods.

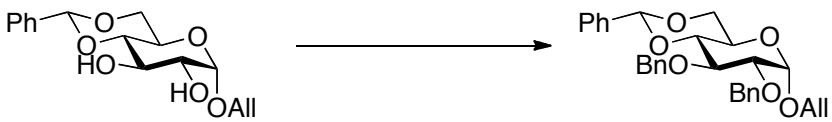
## Experimental Section

### General Materials and Methods

Reaction solvent dichloromethane was obtained from a commercial solvent purification tower or distilled from calcium hydride. Dowex 50W X-8 ($H^+$) resin was washed repeatedly with MeOH prior to use. Powdered 4Å molecular sieves were purchased from Aldrich Chemical Company and were activated by heating in a furnace at 250 °C overnight prior to use. All other reagents were used as received without further purification. Reactions were monitored and the $R_f$ values determined using analytical TLC with Sorbent Technologies silica gel plates (60F-254). Developed TLC plates were

visualized by immersion in 10% ethanolic sulfuric acid solution, or in acidic *p*-anisaldehyde solution, followed by heating on a hot plate. Moisture-sensitive reactions were run in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature for reactions performed at elevated or sub-ambient temperatures. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Manual flash chromatography was performed using Sorbent Technologies silica gel (60F-254), and the solvent systems outlined in the experimental procedures. Automated flash chromatography was carried out on a Biotage SP-1 chromatography workstation fitted with either a Biotage 12M or 25M silica gel cartridge, using ethyl acetate/hexane gradients as outlined in the experimental procedures. $^{1}$H NMR and $^{13}$C NMR spectra were obtained with a Bruker DRX400 at 400 MHz and 101 MHz, respectively. $^{1}$H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$, $CD_3OD$, $D_2O$, or TMS (7.27 ppm, 4.84 ppm, 4.81 ppm, and 0.00 ppm, respectively) as internal references. $^{13}$C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ or $CD_3OD$ (77.23 ppm or 49.15 ppm, respectively). For $^{13}$C NMR spectra obtained in $D_2O$, chemical shifts are reported relative to acetone as an internal standard (215.94 and 30.89 ppm).

Synthetic Procedures



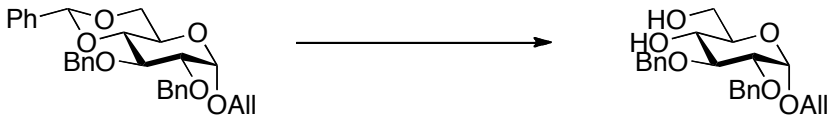

**3** **4**

**Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (4).** Allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**3**[9]) (778 mg, 2.52 mmol), benzyl bromide (0.66 mL,

5.5 mmol), and tetrabutylammonium iodide (20 mg) were dissolved in DMF (20 mL). To this solution, sodium hydride [60% (w/w) dispersion in mineral oil, 300 mg, 7.57 mmol] was added portionwise over 15 minutes. The mixture was stirred at ambient temperature for 2.5 hours, then quenched with water (20 mL). The mixture was extracted with dichloromethane (50 mL). The organic extract was washed with 2N aqueous HCl (2×40 mL) and brine (40 mL), and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel using 10% ethyl acetate in hexanes as the eluant. The product **4** was obtained as a white crystalline solid (1.07 g, 2.18 mmol, 87%). The $^1$H NMR spectrum was consistent with the reported data.[8] Full proton assignments are presented here.

***R**f*: 0.90 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.51-7.25 (m, 15H, Ar-H), 5.99-5.88 (m, 1H, $OCH_2CH$=$CH_2$), 5.55 (s, 1H, benzylidene *CH*), *5*.36-5.30 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.25-5.22 (m, 1H, $OCH_2CH$=$CH_aH_b$), 4.92 (d, 1H, $J$ = 11 Hz, PhC*H*), 4.86-4.82 (m, 2H, 2×PhC*H*), 4.80 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.68 (s, 1H, $J$ = 12 Hz, PhC*H*) 4.28-4.24 (dd, 1H, $J_{5,6}$ = 4.8 Hz, $J_{6,6'}$ = 10 Hz, H-6), 4.22-4.16 (m, 1H, $OCH_aH_bCH$=$CH_2$), 4.08 (t, 1H, $J_{2,3}$= $J_{3,4}$ = 9.2 Hz, H-3), 4.08-4.01 (m, 1H, $OCH_aH_bCH$=$CH_2$), 3.93-3.85 (m, 1H, H-5), 3.70 (t, 1H, $J_{5,6}$ = $J_{6,6'}$ = 10 Hz, H-6'), 3.61 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.59-3.55 (dd, 1H, $J_{1,2}$ = 4 Hz, $J_{2,3}$ = 9.2 Hz, H-2).

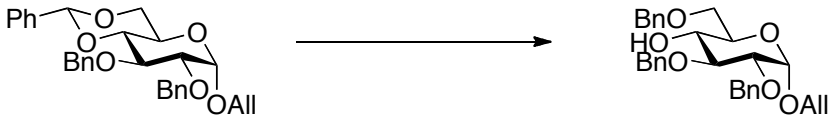



**4** **5**

**Allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (5).** Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**4**) (323 mg, 0.66 mmol), was dissolved in dichloromethane (4 mL). To this solution trifluoroacetic acid (60% v/v in water, 1 mL) was added, and the mixture stirred at ambient temperature. After 45 minutes, the mixture was diluted with dichloromethane (40 mL), washed with saturated $NaHCO_3$ solution (2 × 25 mL), and dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure. Purification of the residue by flash chromatography on silica gel, using 10% ethyl acetate in hexanes as the eluant, provided the product **5** as a syrup (237 mg, 0.59 mmol, 89%). The $^1$H NMR spectrum was in agreement with the reported data.[7] Full proton assignments are presented here.

***R**f*: 0.26 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.26 (m, 10H, Ar-H), 5.99-5.88 (m, 1H, $OCH_2CH$=$CH_2$), *5*.36-5.29 (m, 1H, $OCH_2CH$=C$H_a$H$_b$), 5.24-5.22 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.02 (d, 1H, *J* = 12 Hz, PhC*H*), 4.80 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.74-4.69 (m, 2H, 2×PhC*H*), 4.62 (d, 1H, *J* = 12 Hz, PhC*H*), 4.17-4.12 (m, 1H, $OCH_aH_bCH$=$CH_2$), 4.02-3.96 (m, 1H, $OCH_aH_bCH$=$CH_2$), 3.82 (t, 1H, $J_{2,3}$= $J_{3,4}$ = 9.6 Hz, H-3), 3.78-3.72 (m, 2H, H-6. H-6'), 3.68-3.64 (m, 1H, H-5), 3.53 (apparent t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9 Hz, H-2), 3.51-3.47 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 2.65 (br s, 1H, -O*H*), 2.16 (br s, 1H, -O*H*).



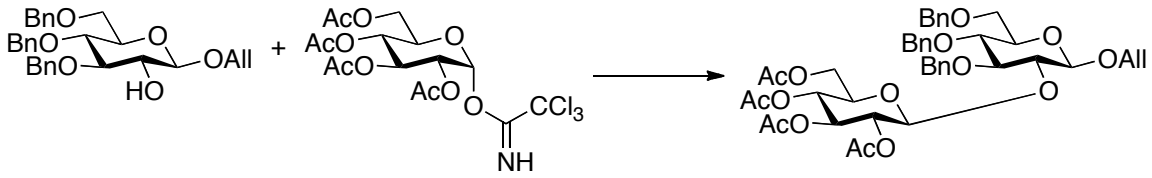

**4** **6**

**Allyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (6).** Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**4**) (449 mg, 0.92 mmol) was dissolved in dichloromethane (10 mL). Powdered activated 4Å molecular sieves (1 g) were added, and the mixture cooled to -78 °C. To this mixture, triethylsilane (0.44 mL, 2.8 mmol) and TfOH (0.28 mL, 3.1 mmol) were added. The mixture was stirred at -78 °C for 70 minutes, then additional TfOH ( 0.10 mL, 1.1 mmol) was added. After 60 additional minutes at -78 °C, the mixture was poured into a separatory funnel containing saturated $NaHCO_3$ solution and dichloromethane. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and filtered through Celite. The solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel, using a gradient of 10→30% ethyl acetate in hexanes. First, unreacted starting material (59 mg, 0.12 mmol, 13%) was eluted, followed by the product **6** as a colorless oil (302 mg, 0.62 mmol, 67%). The $^1$H NMR spectrum was in agreement with the literature data.[8]

***R**$_f$*: 0.58 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.25 (m, 15H, Ar-H), 5.94-5.89 (m, 1H, $OCH_2CH$=$CH_2$), 5.36-5.29 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.23-5.19 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.00 (d, 1H, $J$ = 11.6 Hz, PhC*H*), 4.83 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.74 (d, 2H, 2PhC*H*) 4.64, 4.59, 4.53 (3 d, each 1H, 3 PhC*H*), 4.19-4.13 (m, 1H,

OC$H_a$H$_b$CH=CH$_2$), 4.04-3.98 (m, 1H, OCH$_a$$H_b$CH=CH$_2$), 3.82 (t, 1H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), 3.79-3.74 (m, 1H, H-5), 3.70-3.59 (m, 3H, H-4, H-6, H-6') 3.56-3.52 (dd, 1H, $J_{2,3} =$ 9.6Hz, $J_{1,2} = 3.6$ Hz, H-2), 2.40-2.30 (br s, 1H, -O*H*).



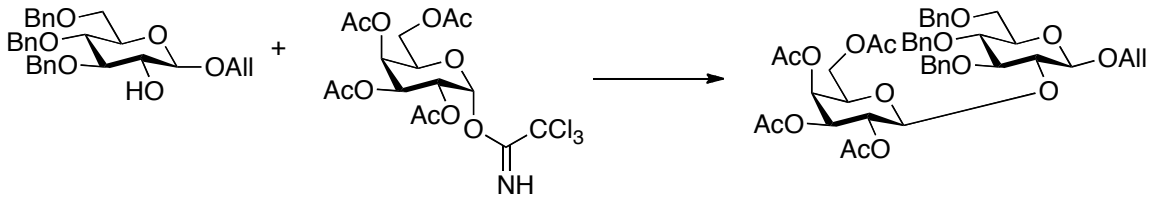

**1** **7** **10**

**Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (10)**. Allyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**1**[5]) (85 mg, 0.18 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**7**[11]) (175 mg, 0.35 mmol) were combined in a 25 mL round bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (1.5 mL) and cooled to 0 °C. TMSOTf (60 μL of a solution of 100 μL TMSOTf in 1 mL dichloromethane) was added, and the mixture stirred at 0 °C. After 20 minutes, the reaction was quenched by the addition of triethylamine (0.1 mL) and the solvent removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (3:7 EtOAc/hexanes) to give the product **10** as a colorless syrup (131 mg, 0.16 mmol, 92%).

***R**$_f$*: 0.77 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.07 (m, 15H, Ar-H), 5.90-5.88 (m, 1H), 5.33 (m, 1H), 5.20-5.11 (m, 3H), 5.03-4.98 (m, 2H), 4.82-4.76 (m, 3H), 4.63-4.52 (m, 3H), 4.47-4.42 (m, 2H), 4.24-4.22 (dd, 1H), 4.12-4.06 (m, 2H), 3.74-3.60 (m, 6H), 3.46-3.39 (m, 1H), 2.08 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.78 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.9, 170.5, 169.7, 169.5, 138.6, 138.2, 138.0, 128.6, 128.5, 128.0, 128.0, 127.9, 127.7, 116.9, 101.6, 100.4, 84.3, 81.6, 78.1, 75.8, 75.2, 74.9, 73.7, 73.5, 72.3, 71.9, 70.2, 68.8, 68.2, 62.1, 21.0, 20.8, 20.6.




**1** **8** **11**

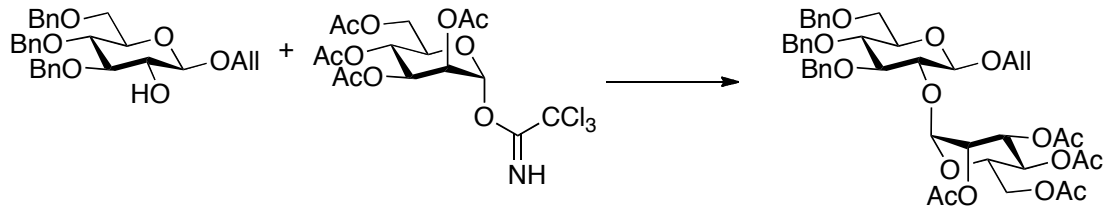
**Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (11).** Allyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**1**[5]) (72 mg, 0.15 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**[12]) (139 mg, 0.28 mmol) were combined in a 25 mL round bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The reactants were dried under high vacuum ($10^{-4}$ torr) for 1 h. Dichloromethane (1.5 mL) was added, and the solution cooled to 0 °C. TMSOTf (50 μL of a solution of 100 μL TMSOTf in 1 mL dichloromethane) was added, and the mixture stirred at 0 °C. After 20 minutes, the reaction was neutralized with triethylamine (0.1 mL) and the solvent evaporated. The residue was subjected to flash chromatography on

silica gel (3:7 EtOAc/hexanes) to give the product **11** as a pale yellow syrup (113 mg, 0.14 mmol, 94%).

***R**f*: 0.81 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.07 (m, 15H, Ar-H), 6.02-5.92 (m, 1H), 5.37-5.33 (m, 2H), 5.26-5.19 (m, 2H), 5.01-4.95 (m, 2H), 4.85-4.75 (m, 3H), 4.63-4.43 (m, 5H), 4.19-4.08 (m, 3H), 3.88 (t, 1H), 3,72-3.63 (m, 5H), 3.57-3.49 (m, 1H), 2.16 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.76 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.49, 170.46, 170.4, 170.0, 138.7, 138.2, 138.0, 134.2, 128.6, 128.4, 128.1, 128.0, 127.9, 127.64, 127.60, 117.0, 101.8, 100.8, 84.3, 82.0, 78.1, 75.9, 75.2, 74.8, 73.7, 71.6, 70.9, 70.4, 70.0, 68.8, 67.3, 61.5, 20.91, 20.86, 20.80, 20.7.




**1** **9** **12**

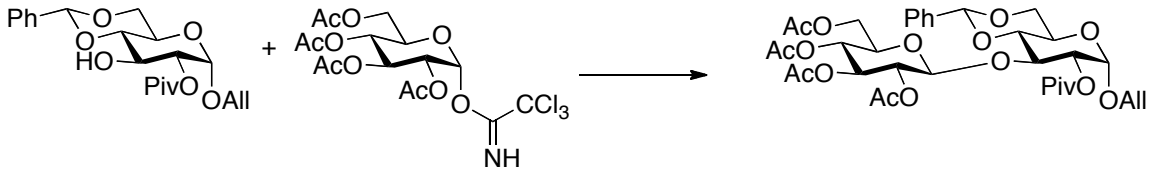
**Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (12)**. Allyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**1**[5]) (77 mg, 0.16 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**[11]) (147 mg, 0.30 mmol) were combined in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each

addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. Dichloromethane (1.5 mL) was added, and the solution cooled to 0 °C. TMSOTf (50 μL of a solution of 100 μL TMSOTf in 1 mL dichloromethane) was added, and the mixture stirred at 0 °C. After 20 minutes, the reaction was quenched with triethylamine (0.1 mL) and the solvent removed under reduced pressure. Flash chromatography on silica gel (3:7 EtOAc/hexanes) gave the product **12** as a pale yellow syrup (126 mg, 0.15 mmol, 98%).

***R**f*: 0.76 (50% EtOAc/hexanes)

**$^{1}$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.09 (m, 15H), 5.92-5.84 (m, 1H), 5.46 (s, 1H), 5.29-5.16 (m, 5H), 5.0-4.52 (m, 6H), 4.42 (d, 1H), 4.37-4.32 (m, 1H), 4.16-4.06, (m, 2H), 3.83-3.58 (m, 7H), 3.47-3.42 (m, 1H), 2.14 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.84 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.9, 170.2, 170.1, 169.7, 138.3, 138.1, 137.9, 133.8, 128.61, 128.58, 128.5, 128.12, 128.07, 127.9, 127.7, 127.0, 118.1, 102.2, 96.9, 83.2, 78.6, 76.2, 75.6, 75.2, 75.1, 73.7, 70.5, 69.5, 68.6, 68.3, 65.6, 61.9, 21.1, 20.95 (2), 20.8.




**2** **7** **13**

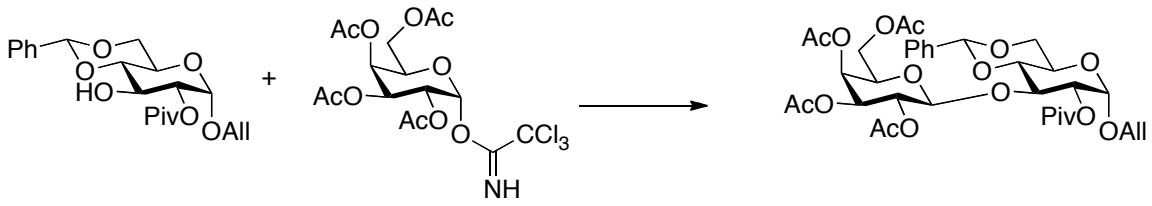
**Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (13)**. Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-

D-glucopyranoside (**2**[6]) (50mg, 0.13 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**7**[11]) (82 mg, 0.17 mmol) were combined in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The reactants were dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (3 mL) and cooled to -15 °C. TMSOTf (20μL) was added, and the mixture stirred at -15 °C. After 30 min, the reaction was quenched by the addition of triethylamine (0.1 mL) and the solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel (20→50% ethyl acetate in hexanes) to give the disaccharide product **13** as a syrup (53 mg, 0.073 mmol, 58%).

***R**f*: 0.75 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.50-7.32 (m, 5H), 5.91-5.81 (m, 1H), 5.57 (s, 1H), 5.32-5.27 (m, 1H), 5.24-5.20 (m, 1H), 5.11-5.01 (m, 4H), 4.83 (dd, 1H), 4.76 (d, 1H), 4.32-4.20 (m, 3H), 4.19-4.14 (m, 1H), 4.09-4.05 (dd, 1H), 3.96-3.87 (m, 2H), 3.77 (dd as t, 1H), 3.67 (dd as t, 1H), 3.62-3.57 (m, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.28 (s, 9H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 177.8, 171.0, 170.5, 169.5, 169.4, 137.5, 133.3, 129.1, 128.3, 126.2, 118.8, 101.2, 100.4, 95.5, 79.0, 75.7, 73.5, 73.1, 71.8, 71.3, 69.2, 68.9, 68.5, 62.7, 62.3, 39.0, 27.4, 20.9, 20.8 (3).



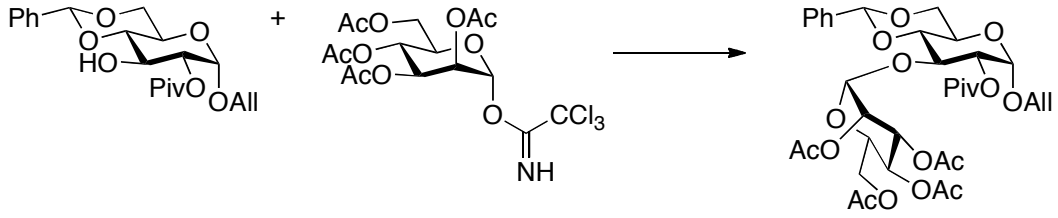

**2** **8** **14**

**Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (14)**. Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-D-glucopyranoside (**2**[6]) (55 mg, 0.14 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**[12]) (105 mg, 0.21 mmol) were combined in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2 mL) and cooled to -15 °C. TMSOTf (15 μL) was added, and the mixture stirred at -15 °C. After 30 minutes, the reaction was quenched with triethylamine (0.1 mL) and the solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel (15→35% ethyl acetate in hexanes) to give the **14** as a syrup (60 mg, 0.083 mmol, 60%).

***R**f*: 0.74 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.52-7.32 (m, 5H), 5.90-5.82 (m, 1H), 5.58 (s, 1H), 5.35-5.20 (m, 4H), 5.04 (d, 1H), 4.92 (dd, 1H), 4.83 (dd, 1H), 4.73 (d, 1H), 4.32-4.23 (m, 2H), 4.18-4.09 (m, 3H), 3.96-3.74 (m, 4H), 3.67 (dd as t, 1H), 2.12 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.27 (s, 9H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 177.8, 170.6, 170.5, 170.4, 169.6, 137.5, 133.3, 129.1, 128.3, 126.2, 118.7, 101.0, 100.9, 95.5, 79.0, 75.7, 73.5, 71.2, 70.6, 69.2, 69.1, 68.9, 67.1, 62.7, 61.3, 39.0, 27.4, 21.0, 20.9 (2), 20.8.



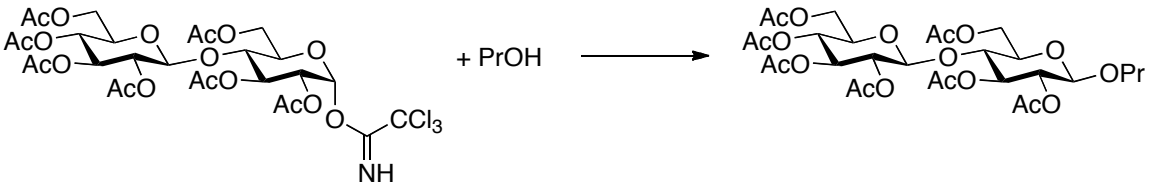

**2** **9** **15**

**Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (15).** Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-D-glucopyranoside (**2**[6] ) (50 mg, 0.13 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**[11]) (125 mg, 0.26 mmol) were combined in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The mixture was dissolved in dichloromethane (2 mL) and cooled to -15 °C. TMSOTf (15 μL) was added, and the mixture stirred at -15 °C. After 30 minutes, the reaction was quenched with triethylamine (0.1 mL). The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (15→35% ethyl acetate in hexanes) to give provide **15** as a syrup (57 mg, 0.079 mmol, 62%).

***R**f*: 0.73 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.41-7.30 (m, 5H), 5.88-5.79 (m, 1H), 5.56 (s, 1H), 5.44 (d, 1H), 5.33-5.30 (m, 2H), 5.27-5.25 (m, 2H), 5.23-5.19 (m, 1H), 5.14 (d, 1H), 4.80 (dd, 1H), 4.39 (dd as t, 1H), 4.31-4.15 (m, 4H), 4.07 (dd, 1H), 3.96-3.88 (m, 2H), 3.80-3.74 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.24 (s, 9H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 178.0, 170.9, 170.0, 169.9, 169.7, 136.9, 133.4, 129.2, 128.3, 126.3, 118.4, 101.5, 97.4, 95.5, 82.6, 72.3, 71.6, 69.4, 69.1, 69.0, 68.9, 68.6, 66.1, 62.3, 62.2, 38.9, 27.3, 21.0 (2), 20.9, 20.8.




**16** **17**

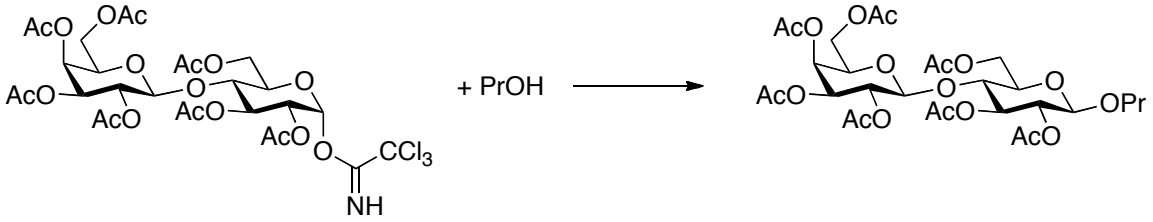
**Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (17).** The glycosyl donor 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl trichloroacetimidate (**16**[13]) (156 mg, 0.20 mmol) was placed in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The donor was dissolved in dichloromethane (3 mL) and cooled to 0 °C. To this solution, *n*-propanol (44 μL, 0.60 mmol) was added, followed by TMSOTf (11 μL, 0.06 mmol). The reaction was stirred at 0 °C for 15 minutes, then quenched by the addition of triethylamine (0.2 mL). The solvents were removed under reduced pressure. The residue was purified by flash column

chromatography on silica gel (20→60% ethyl acetate in hexanes) to afford propyl glycoside **17** as a white solid (72 mg, 0.11 mmol, 53%).

***R**f*: 0.79 (70% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.18-5.03 (m, 3H), 4.94-4.87 (m, 2H), 4.52 (m, 2H), 4.44 (d, 1H), 4.36 (dd, 1H), 4.11-4.02 (m, 2H), 3.82-3.74 (m, 2H), 3.67-3.64 (m, 1H), 3.61-3.56 (m, 1H) 3.54-3.47 (m, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.62-1.54 (m, 2H), 0.88 (t, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.6, 170.5, 170.4, 170.0, 169.8, 169.5, 169.2, 100.9, 100.8, 76.7, 73.1, 72.8, 72.7, 72.1, 72.0, 71.8, 68.0, 62.1, 61.7, 22.8, 21.0, 20.8, 20.7, 10.4.




**18** **19**

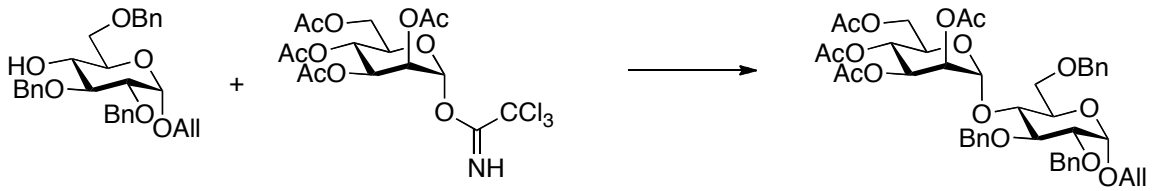
**Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (19).** The glycosyl donor 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glalactopyranosyl)-α-D-glucopyranosyl trichloroacetimidate (**18**[14]) (153 mg, 0.19 mmol) was placed in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The donor was dissolved in dichloromethane

(3 mL). The solution was cooled to 0 °C. To this solution, *n*-propanol (43 μL, 0.58 mmol) was added, followed by TMSOTf (10 μL, 0.06 mmol). The reaction was stirred at 0 °C for 20 minutes, then quenched by the addition of triethylamine (0.2 mL). The solvents were removed under vacuum, and the residue purified by flash column chromatography on silica gel (40→50% ethyl acetate in hexanes) to afford propyl glycoside **19** as a colorless syrup (30 mg, 0.040 mmol, 23%).

$R_f$: 0.80 (70% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.35 (d, 1H), 5.20 (t, 1H), 5.13-5.08 (dd, 1H), 4.97-4.94 (dd, 1H), 4.89 t, 1H), 4.49-4.45 (m, 3H), 4.16-4.05 (m, 3H), 3.87 (t, 1H), 3.83-3.77 (m, 2H), 3.62-3.58 (m, 1H), 3.45-3.38 (m, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 1.97 (s, 3H), 1.61-1.55 (m, 2H), 0.89 (t, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.6 (2), 170.4, 170.3, 170.0, 169.8, 169.3, 101.3, 100.8, 76.5, 73.0, 72.7, 72.0, 71.9, 71.2, 70.8, 69.3, 66.8, 62.2, 61.0, 22.9, 21.1, 21.0, 20.9, 20.8, 20.7, 10.5.



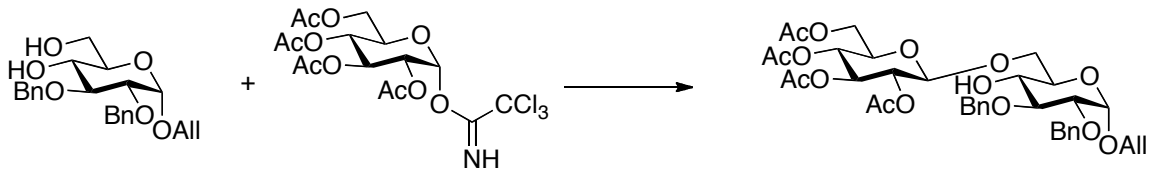

**6** **9** **20**

**Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (20).** A solution of allyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**[8])

(105 mg, 0.21 mmol) in dichloromethane (3 mL) was added to 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**[11]) (211 mg, 0.43 mmol). The solution was cooled to 0 °C. TMSOTf (11 μL, 0.064 mmol) was added. The reaction mixture was stirred at 0 °C for 30 minutes, then quenched by the addition of triethylamine (0.1 mL). The solvent was removed under reduced pressure. The residue was subjected to purification using a Biotage SP-1 flash chromatography system fitted with a Biotage 12M cartridge. Gradient elution from 6%-60% EtOAc/hexanes over 12 column volumes gave the desired product ($R_f$ = 0.59, 1:1 EtOAc/hexanes) along with a small amount of an impurity of slightly lower $R_f$. Fractions containing the desired product were concentrated and subjected to careful flash chromatography on silica gel using 30% EtOAc/hexanes as eluant to give **20** as a colorless syrup (107 mg, 0.013 mmol, 61%).

$R_f$: 0.59 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.20 (m, 15H), 6.00-5.90 (m, 1H), 5.41-5.18 (m, 6H), 5.05 (d, 1H, $J$ = 11.2 Hz), 4.80 (d, 1H, $J$ = 3.2 Hz), 4.70-4.52 (m, 5H), 4.21-4.11 (m, 2H), 4.03-3.63 (m, 8H), 3.56-3.52 (dd, 1H), 2.02 (2s, 2×3H), 1.95 (s, 3H), 1.94 (s, 3H).



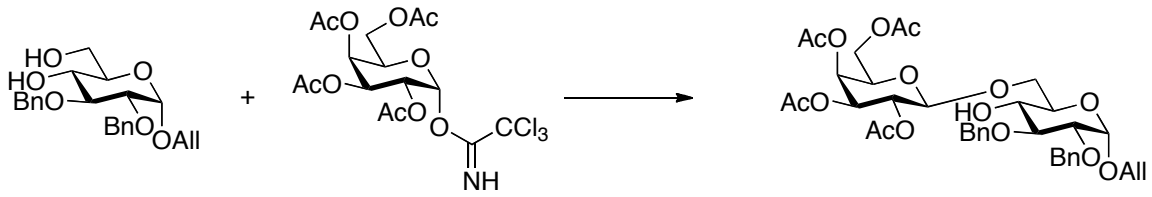

**5** **7** **21**

**Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (21).** A mixture of allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**5**[7]) (98 mg, 0.24 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**7**[11]) (65 mg, 0.13 mmol) was placed in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The mixture was dissolved in dichloromethane (2 mL), and the solution was cooled to 0 °C. TMSOTf (13 μL, 0.07 mmol) was added. The reaction mixture was stirred at 0 °C for 15 minutes, then quenched by the addition of triethylamine (0.1 mL). The solvent was removed under reduced pressure. The residue was purified using a Biotage SP-1 flash chromatography system (Biotage 12M cartridge, gradient elution from 30→50% ethyl acetate in hexanes, to furnish the product **21** as a syrup (27 mg, 37 μmol, 28% based on donor). The remaining unconsumed **5** was also recovered.

***R**f*: 0.40 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.28 (m, 10H), 5.94-5.87 (m, 1H), 5.36-5.31 (m, 1H), 5.25-5.16 (m, 2H), 5.10-4.99 (m, 3H), 4.80 (d, 1H, *J* = 3.2 Hz), 4.73-4.70 (m, 2H), 4.65-4.62 (d, 1H), 4.59 (d, 1H, *J* = 8.0 Hz), 4.26-4.22 (dd, 1H), 4.17-4.11 (m, 2H), 4.05-3.95 (m, 2H), 3.79 (t, 1H), 3.74-3.66 (m, 3H), 3.52-3.42 (m, 2H), 2.30 (br d, *J* = 2.4 Hz, -OH), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (2s, each 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 171.0, 170.5, 169.6, 169.5, 139.0, 138.2, 133.8, 128.8, 128.7, 128.3, 128.1(2), 128.0, 118.7, 101.2, 95.6, 81.4, 79.7, 75.5, 73.1, 72.8, 72.0, 71.3, 70.4, 70.0, 68.6, 68.5, 68.2, 62.0, 20.9, 20.8(3).



**5** **8** **22**

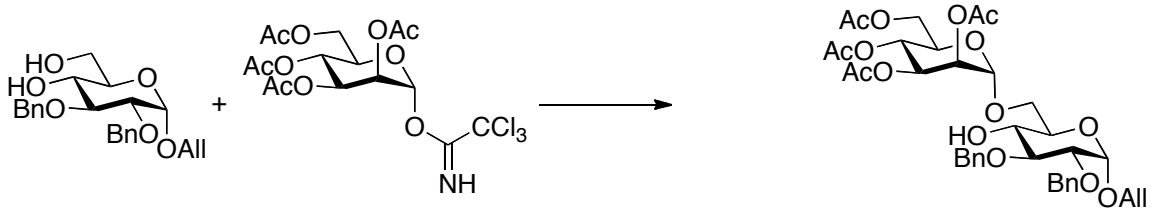
**Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (22).** A mixture of allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**5**[7]) (85 mg, 0.21 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**[12]) (50 mg, 0.10 mmol) was placed in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The mixture was dissolved in dichloromethane (2 mL), and the solution was cooled to 0 °C. TMSOTf (12 μL, 0.06 mmol) was added. The reaction mixture was stirred at 0 °C for 15 minutes, then quenched by the addition of triethylamine (0.1 mL). The solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel, using 40→50% ethyl acetate in hexanes as eluant, to furnish the product **22** as a syrup (23 mg, 31 μmol, 30% based on donor). The remaining unconsumed acceptor **5** (45 mg, 0.11 mmol) was also recovered.

***R**f*: 0.61 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.28 (m, 10H), 5.94-5.88 (m, 1H), 5.38-5.32 (m, 2H), 5.26-5.21 (m, 2H), 5.05-4.99 (m, 2H), 4.81 (d, 1H, *J* = 3.6 Hz, H-1$^I$), 4.75-4.71

(m, 2H), 4.64 (d, 1H, $J$ = 12 Hz), 4.54 (d, 1H, $J$ = 8.0 Hz, H-1$^{II}$), 4.10-4.03 (m, 4H), 4.01-3.96 (m, 1H), 3.90 (t, 1H), 3.80 (t, 1H), 3.78-3.71 (m, 2H), 3.53-3.44 (m, 2H), 2.27 (br d, 1H, $J$ = 1.2 Hz, -OH), 2.14 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.5, 170.3, 170.2, 169.4, 138.9, 138.0, 133.7, 128.6, 128.5, 128.1, 128.0, 127.9(2), 118.4, 101.5, 95.4, 81.3, 79.6, 75.3, 73.0, 71.0, 70.8, 70.2, 70.0, 68.8, 68.6, 68.1, 67.0, 61.3, 20.8, 20.7(2), 20.6.




**5** **9** **23**

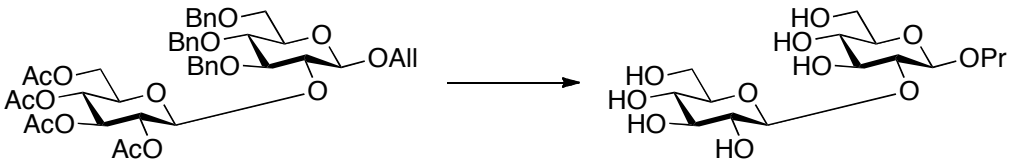
**Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (23).** A mixture of allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**5**[7]) (86 mg, 0.21 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannoopyranosyl trichloroacetimidate (**9**[11]) (106 mg, 0.21 mmol) was placed in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The mixture was dissolved in dichloromethane (2 mL), and the solution cooled to 0 °C. TMSOTf (40 μL of a 10% (v/v) solution in dichloromethane) was added. The reaction mixture was stirred at 0 °C for 15 minutes, then quenched by the addition of triethylamine (0.1 mL). The solvent was removed under reduced pressure. Purification of the residue by flash chromatography on silica gel, using 40% ethyl acetate in hexanes as eluant, furnished the

6-*O*-glycosylated product **23** as a syrup (40 mg, 55 μmol, 26%). The unconsumed acceptor **5** was also recovered.

***R**f*: 0.60 (50% EtOAc/hexanes)

**$^{1}$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.39-7.28 (m, 10H), 6.00-5.89 (m, 1H), 5.38-5.23 (m, 5H), 5.05 (d, 1H, *J* = 11.6 Hz), 4.86 (d, 1H, *J* = 3.6 Hz, H-1$^{I}$), 4.84 (2, 1H, *J* = 0.8 Hz, H-1$^{II}$), 4.76-4.66 (m, 3H), 4.25-4.12 (m, 3H), 4.09-4.00 (m, 2H), 3.92-3.88 (dd, 1H), 3.83-3.76 (m, 2H), 3.69-3.66 (dd, 1H), 3.60-3.57 (dd, 1H), 3.52 (t, 1H), 2.34 (br s, 1H, -OH), 2.15 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 171.0, 170.1, 170.0, 169.9, 138.8, 138.2, 133.8, 128.9, 128.7, 128.3(2), 128.2(2), 118.7, 97.6, 95.6, 81.6, 80.1, 75.7, 73.1, 70.1, 69.7(2), 69.2, 68.5, 66.5, 66.4, 62.7, 21.1, 20.9(3).




**10** **24**

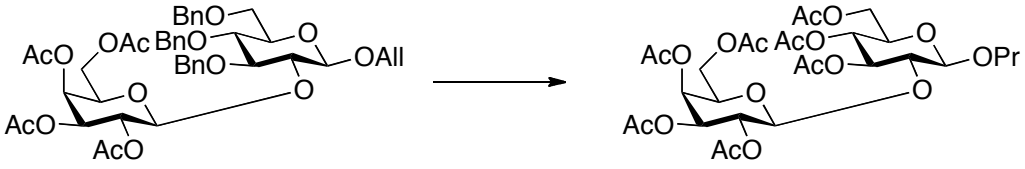
**Propyl 2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (24).** Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (**10**) (47 mg, 57 μmol) was dissolved in EtOH (2 mL). To this solution, 10% palladium on carbon (5 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 2 days, and filtered on Celite. The solvent was evaporated under reduced pressure. The residue was dissolved in MeOH (2 mL). Sodium metal (~5 mg) was

added. The mixture was allowed to stir overnight at ambient temperature. The solution was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was removed under reduced pressure to give **24** as a white solid (21 mg, 55 μmol, 95%).

***R**f*: 0.22 (1:2:7 $H_2O$/MeOH/EtOAc)

**$^1$H NMR ($CD_3OD$, 400 MHz)** δ (ppm) 4.60 (d, 1H, *J* = 7.6 Hz, H-1$^{II}$), 4.40 (d, 1H, *J* = 7.6 Hz, H-1$^{I}$), 3.88-3.80 (m, 3H), 3.68-3.63 (m, 2H), 3.58-3.52 (m, 2H), 3.45-3.30 (m, 3H), 3.28-3.19 (m, 4H), 1.66-1.60 (m, 2H), 0.95 (t, 3H).

**$^{13}$C NMR ($CD_3OD$, 101 MHz)** δ (ppm) 105.1, 103.1, 83.0, 78.4, 78.0, 77.9, 77.8, 76.1, 72.7, 71.6, 62.8, 24.1, 11.0.




**11** **25**

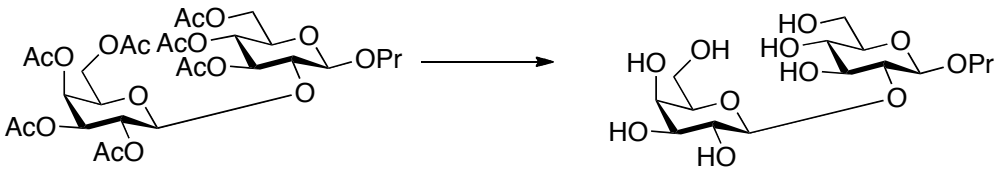
**Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (25).** Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**11**) (22 mg, 0.027 mmol) was dissolved in MeOH (2 mL). To this solution, 10% palladium on carbon (10 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 5.5 h. The palladium catalyst was removed by filtration on Celite. The solvent was evaporated under reduced pressure. The residue was dissolved in MeOH (2 mL). Sodium metal (~5 mg) was added. The mixture was stirred overnight at ambient temperature. The solution

was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was removed under reduced pressure. As proton NMR showed the presence of small amount of impurities at this stage, the crude product was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL). 4-dimethylaminopyridine (1 mg) was added, and the mixture stirred for 12 hours at ambient temperature. The solvent was removed under reduced pressure, and the residue dissolved in dichloromethane (10 mL). The organic solution was washed with water and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue subjected to flash chromatography on silica gel (1:1 EtOAc/hexane) to give the peracetylated product **25** as a colorless syrup (14 mg, 0.021 mmol, 77% over 3 steps).

***R*$_f$**: 0.35 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.35 (d, 1H), 5.21-5.12 (m, 2H), 5.00-4.93 (m, 2H), 4.76 (d, 1H, $J$ = 8.0 Hz, H-1$^{II}$), 4.46 (d, 1H, $J$ = 7.6 Hz, H-1$^{I}$), 4.29-4.25 (dd, 1H), 4.14-4.08 (m, 3H), 3.89-3.84 (m, 2H), 3.73-3.64 (m, 2H), 3.55-3.51 (m, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.68-1.60 (m, 2H), 0.98 (t, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.9, 170.6, 170.5, 170.4, 170.2, 170.0, 169.7, 102.0, 101.0, 78.1, 74.3, 72.1, 71.6, 71.3, 70.9, 69.6, 68.9, 67.1, 62.3, 61.4, 23.2, 21.0, 20.95, 20.90 (2), 20.84, 20.83, 20.79, 10.7.



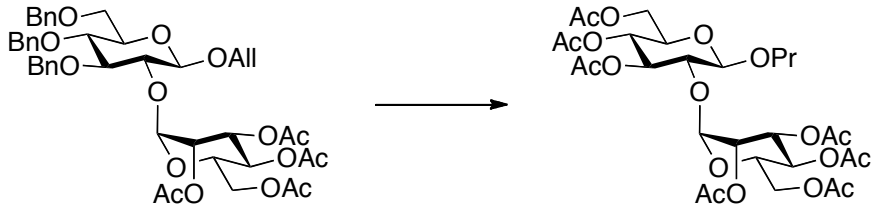
**25** **26**

**Propyl 2-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (26).** Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**25**) (12.0 mg, 18 μmol) was dissolved in MeOH (1 mL). Sodium metal (5 mg) was added and the mixture stirred at ambient temperature for 2 hours. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated to give the disaccharide **26** as a white solid (6.5 mg, 0.017 μmol, 96%).

***R**f*: 0.24 (1:2:7 $H_2O$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.71 (d, 1H, *J* = 7.6 Hz, H-1$^{II}$), 4.56 (d, 1H, *J* = 7.6 Hz, H-1$^{I}$), 3.91-3.32 (m, 14H), 1.62 (m, 2H), 0.90 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 103.9, 101.5, 81.1, 76.6, 76.4, 75.9, 73.2, 72.8, 72.2, 70.1, 69.2, 61.5, 61.3, 22.9, 10.3.



**12** **27**

**Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (27).** Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**12**) (23.5 mg, 0.0286 mmol) was dissolved in MeOH (3 mL). 10% palladium on carbon (10 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 19 h, then filtered on Celite. The solvent was evaporated under reduced pressure. The residue was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL). The mixture was stirred for 18 h at ambient temperature. The solvent was removed under reduced pressure, then dissolved in dichloromethane. The organic solution was washed successively with water, $NaHCO_3$ solution, and brine, then dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue subjected to flash chromatography on silica gel (1:1 EtOAc/hexane) to give the peracetylated product **27** as a colorless syrup (11.4 mg, 0.0168 mmol, 58% over 2 steps).

***R**f*: 0.33 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.30-5.15 (m, 5H), 5.00 (t, 1H), 4.47 (d, 1H, *J* = 7.6 Hz, H-1[1]), 4.31-4.24 (m, 2H), 4.14-4.08 (dd, 2H), 3.98-3.94 (m, 1H), 3.87-3.81 (m, 1H), 3.73-3.65 (m, 2H), 3.53-3.45 (m, 1H), 2,14, (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.03 (s, 6H), 1.98 (s, 3H), 1.64-1.55 (m, 2H), 0.87 (t, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.88 (2), 170.0 (2), 169.97, 169.91, 169.8, 103.0, 97.6, 75.7, 73.3, 72.3, 71.8, 69.3, 69.2, 69.1, 69.0, 65.9, 62.4, 62.2, 22.9, 21.0(3), 20.9(4), 10.6.

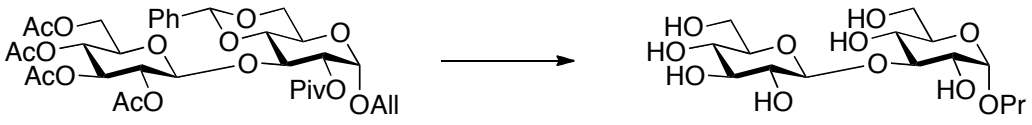
**27** **28**

**Propyl 2-*O*-(α-D-mannopyranosyl)-β-D-glucopyranose (28).** Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranose (**27**) (10.5 mg, 0.015 mmol) was dissolved in MeOH (1mL). Sodium metal (5 mg) was added and the mixture stirred at ambient temperature for 12 hours. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated to give **28** as a white solid (6.0 mg, 0.015 mmol, 99%).

***R**f*: 0.30 (1:2:7 $H_2O$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.28 (s, 1H, H-1$^{II}$), 4.57 (d, 1H, *J* = 8.0 Hz, H-1$^{I}$), 3.99-3.40 (m, 14H), 1.64 (m, 2H), 0.93 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 103.3, 100.4, 78.3, 76.5, 75.1, 73.4, 73.1, 71.1, 70.8, 70.5, 67.2, 61.5 (2), 23.0, 10.6.



**13** **29**

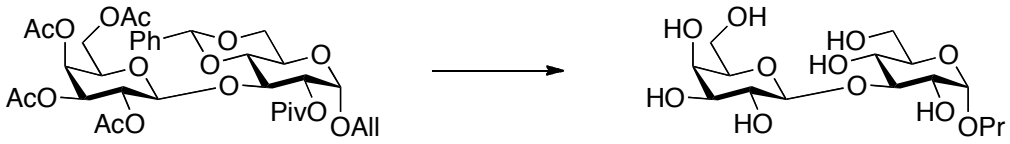
**Propyl 3-*O*-(β-D-glucopyranosyl)-α-D-glucopyranose (29).** Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside

(**13**) (25 mg, 35 μmol) was dissolved in dichloromethane (1 mL). To this solution, 60% aqueous TFA (0.2 mL) was added. The mixture was stirred at ambient temperature for 20 minutes, then diluted with dichloromethane (10 mL) and washed with $NaHCO_3$ solution, water, and brine. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was then dissolved in MeOH (2 mL). Palladium (10 wt.% on activated carbon, 10 mg) was added to the solution. The mixture was placed under an atmosphere of hydrogen and stirred overnight at ambient temperature. The mixture was filtered and the solvent removed under reduced pressure. The residue was then dissolved in MeOH (2 mL). Sodium metal (5 mg) was added to the solution. The mixture was stirred for 24 hours at ambient temperature. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to give the product **29** as a white solid (12 mg, 31 μmol, 90%).

***R**f*: 0.83 (19:1 MeOH/$H_2O$)

**$^1$H NMR ($CD_3OD$, 400 MHz)** δ (ppm) 4.80 (d, 1H, *J* = 3.6 Hz, H-1$^I$), 4.51 (d, 1H, *J* = 7.6 Hz, H-1$^{II}$), 3.87 (dd, 1H), 3.82-3.75 (m, 2H), 3.71-3.57 (m, 5H), 3.45-3.33 (m, 4H), 3.29-3.24 (m, 2H), 1.69-1.63 (m, 2H), 0.96 (t, 3H).

**$^{13}$C NMR ($CD_3OD$, 101 MHz)** δ (ppm) 105.3, 99.9, 85.8, 78.3, 78.0, 75.6, 73.6, 72.9, 71.7, 70.9, 70.3, 62.7, 23.9, 11.2.



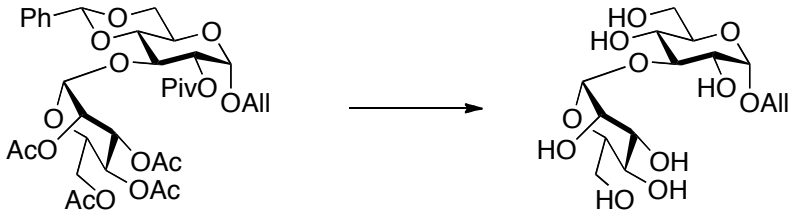

**14** **30**

**Propyl 3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranose (30).** Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**14**) (27 mg, 37 μmol) was dissolved in dichloromethane (1 mL). To this solution, 60% aqueous TFA (0.2 mL) was added. After stirring at ambient temperature for 30 minutes, the solution was diluted with dichloromethane (10 mL) and washed with $NaHCO_3$ solution, water, and brine. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure to give the debenzylidenated product (21 mg, 33 μmol, 89%). A portion of the 4,6-diol (11 mg, 17 μmol) was dissolved in EtOH (1 mL). Palladium (10 wt.% on activated carbon, 5 mg) was added to the solution. The mixture was stirred overnight at ambient temperature under an atmosphere of hydrogen, and filtered. The solvent was removed under reduced pressure. The residue was then dissolved in MeOH (0.7 mL). Sodium metal (11 mg) was added to the solution. The mixture was stirred for 48 hours at ambient temperature. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to yield the deprotected disaccharide **30** as a white solid (6.4 mg, 17 μmol, 96%).

***R**<sub>f</sub>*: 0.80 (19:1 $MeOH/H_2O$)

**$^{1}$H NMR ($CD_3OD$, 400 MHz)** δ (ppm) 4.80 (d, 1H, $J$ = 3.6Hz, H-$1^{I}$), 4.47 (d, 1H, $J$ = 7.6 Hz, H-$1^{II}$), 3.82-3.39 (m, 14H), 1.70-1.61 (m, 2H), 0.97 (t, 3H).

**$^{13}$C NMR ($CD_3OD$, 101 MHz)** δ (ppm) 105.8, 100.0, 85.7, 77.2, 74.9, 73.6, 73.2, 72.9, 70.8, 70.4, 70.3, 62.7, 23.9, 11.2.




**15** **31**

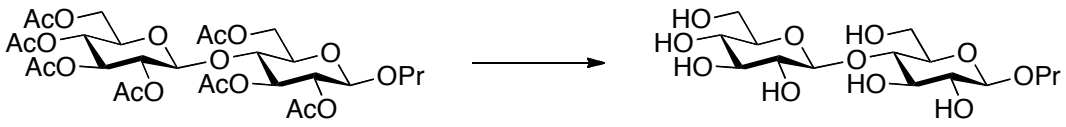
**Propyl 3-*O*-(α-D-mannopyranosyl)-α-D-glucopyranose (31).** Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**15**) (28 mg, 39 μmol) was dissolved in dichloromethane (1 mL). A solution of TFA (0.2 mL, 60% (v/v) in water) was added. After stirring at ambient temperature for 40 minutes, the solution was diluted with dichloromethane (10 mL) and washed with $NaHCO_3$ solution, water, and brine. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (1 mL). Palladium (10 wt.% on activated carbon, 10 mg) was added to the solution, and the mixture stirred overnight at ambient temperature under an atmosphere of hydrogen. The reaction mixture was filtered on Celite, and the solvent removed under reduced pressure. The residue was then dissolved in MeOH (1 mL), and sodium metal (5 mg) added. The mixture was stirred for 4 days at ambient temperature. The mixture was neutralized with Amberlyst 15 ($H^+$) resin and filtered. The filtrate was concentrated

under reduced pressure to furnish the deprotected disaccharide **31** as a white solid (14 mg, 36 μmol, 94%).

***R**f*: 0.82 (19:1 MeOH/$H_2O$)

**$^1$H NMR ($CD_3OD$, 400 MHz)** δ (ppm) 5.20 (s, 1H, H-$1^{II}$), 4.77 (d, 1H, *J* = 3.6 Hz, H-$1^{I}$), 3.99-3.55 (m, 11H), 3.44-3.38 (m, 3H), 1.70-1.62 (m, 2H), 0.96 (t, 3H).

**$^{13}$C NMR ($CD_3OD$, 101 MHz)** δ (ppm) 102.8, 100.2, 82.2, 74.6, 73.7, 72.6, 72.5, 72.2, 70.9, 68.9, 63.1, 62.6, 23.9, 11.2.



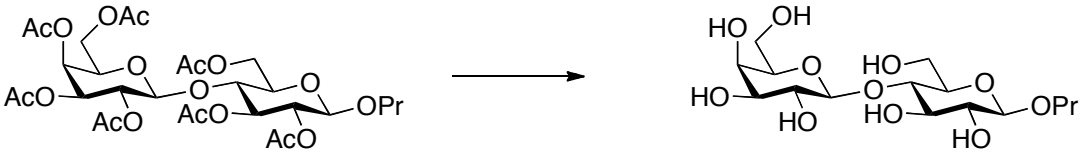

**17** **32**

**Propyl 4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (32).** Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**17**) (40 mg, 59 μmol) was dissolved in MeOH (2 mL). Sodium metal (5 mg) was added to the solution. The mixture was stirred for 72 hours at ambient temperature. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to give the product **32** as a syrup (20.5 mg, 53 μmol, 90%).

***R**f*: 0.40 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.53-4.48 (apparent t, 2H, *J* = 8.0 Hz, overlapping H-$1^{I}$ and H-$2^{II}$), 4.00-3.29 (m, 14H), 1.66-1.60 (m, 2H), 0.92 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 103.3, 102.6, 79.4, 76.7, 76.2, 75.4, 75.0, 73.8, 73.6, 73.0, 70.1, 61.2, 60.7, 22.9, 10.3.



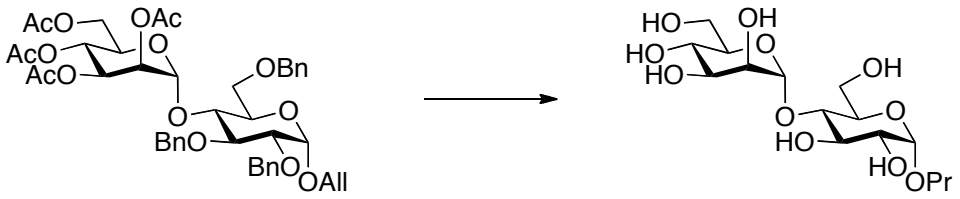

**19** **33**

**Propyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (33).** Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**19**) (18 mg, 27 μmol) was dissolved in MeOH (1 mL). Sodium metal (2 mg) was added, and the mixture stirred for 19 hours at ambient temperature. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was evaporated under reduced pressure to furnish disaccharide **33** as a syrup (10 mg, 26 μmol, 98%).

***$R_f$***: 0.24 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.50 (d, 1H, *J* = 7.6 Hz, H-$1^{II}$), 4.45 (d, 1H, *J* = 7.6 Hz, H-$1^{I}$), 4.00-3.51 (m, 13H), 3.34-3.28 (m, 1H), 1.68-1.58 (m, 2H), 0.92 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 104.2, 103.3, 79.7, 76.7, 76.1, 75.8, 74.2, 73.8, 73.6, 72.3, 69.9, 62.3, 61.4, 23.5, 10.9.



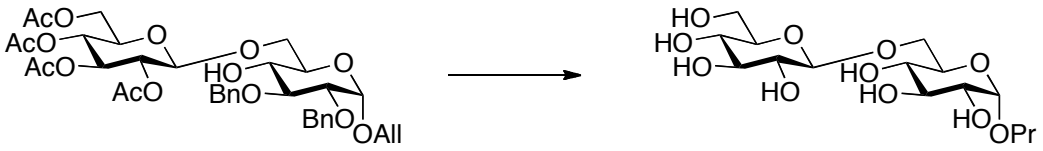

**20** **34**

**Propyl 4-*O*-(α-D-mannopyranosyl)-α-D-glucopyranoside (34).** Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**20**) (41 mg, 50 μmol) was dissolved in MeOH (2 mL). Palladium (10 wt.% on carbon, 2 mg) was added, and the mixture stirred at ambient temperature under a hydrogen atmosphere for 20 hours. The mixture was filtered on Celite and the solvent evaporated. The residue was dissolved in MeOH (2 mL), and sodium metal (2 mg) was added. The mixture was stirred for 6 hours at ambient temperature, then neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was removed under reduced pressure to furnish disaccharide **34** as a pale yellow syrup (14 mg, 37 μmol, 75%).

***R**f*: 0.17 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.29 (d, 1H, *J* = 2.0 Hz, H-$1^{II}$), 4.92 (2, 1H, *J* = 3.6 Hz, H-$1^{I}$), 4.06-4.03 (m, 1H), 3.90-3.43 (m, 13H), 1.68-1.58 (m, 2H), 0.92 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 102.7, 99.0, 77.6, 75.0, 74.9, 72.6, 71.6(3), 71.2, 67.8, 62.2, 61.9, 23.3, 11.2.



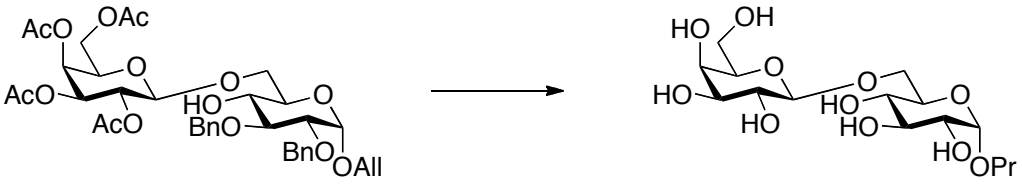
**21** **35**

**Propyl 6-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside (35)** Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**21**) (20 mg, 27 μmol) was dissolved in MeOH (2 mL). Palladium (10 wt.% on carbon, 10 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 19 hours, then filtered on Celite. The solvent was evaporated under reduced pressure, and the residue dissolved in MeOH (1.5mL). Sodium metal (5 mg) was added to the solution. The mixture was stirred for 4.5 hours at ambient temperature, then neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to give the disaccharide **35** as a syrup (10 mg, 26 μmol, 96%).

***R**f*: 0.23 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.92 (d, 1H, *J* = 3.6 Hz, H-$1^I$), 4.50 (d, 1H, *J* = 7.6 Hz, H-$1^{II}$), 4.18-4.13 (d, 1H), 3.95-3.82 (m, 3H), 3.76-3.64 (m, 3H), 3.58-3.29 (m, 7H), 1.70-1.60 (m, 2H), 0.93 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 103.3, 98.7, 76.5, 76.3, 73.8, 73.7, 72.0, 71.3, 70.7, 70.3, 70.0, 68.9, 61.4, 22.7, 10.6.



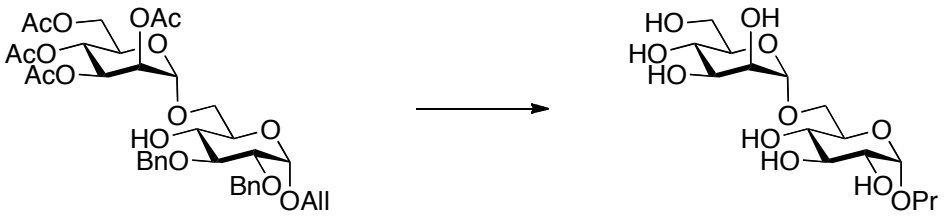

**22** **36**

**Propyl 6-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside (36).** Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**22**) (5.5 mg, 7.5 μmol) was dissolved in MeOH (1 mL). Palladium (10 wt.% on carbon, 1 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 4.5 hours, then filtered on Celite. The filtrate was concentrated under reduced pressure, and the residue dissolved in MeOH (1 mL). Sodium metal (1 mg) was added to the solution. The mixture was stirred for 30.5 hours at ambient temperature, then neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to give the disaccharide **36** as a white solid (2.8 mg, 7.3 μmol, 97%).

***R**f*: 0.18 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.92 (d, 1H, *J* = 3.6 Hz, H-$1^I$), 4.43 (d, 1H, *J* = 7.6 Hz, H-$1^{II}$), 4.20-4.16 (dd, 1H), 3.95-3.43 (m, 13H), 1.70-1.60 (m, 2H), 0.93 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 103.9, 98.7, 75.8, 73.7, 73.4, 71.9, 71.4, 71.3, 70.7, 69.9, 69.3, 68.8, 61.7, 22.7, 10.6.



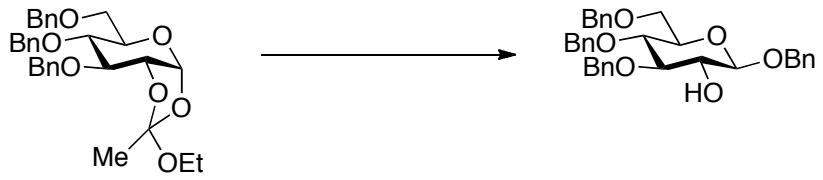

**23** **37**

**Propyl 6-*O*-(α-D-mannopyranosyl)-α-D-glucopyranoside (37).** Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**23**) (9.2 mg, 13 μmol) was dissolved in MeOH (1 mL). Palladium (10 wt.% on carbon, 1 mg) was added. The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 4.5 hours, and filtered on Celite. The filtrate was concentrated under reduced pressure, and the residue dissolved in MeOH (1 mL). Sodium metal (1 mg) was added to the solution. The mixture was stirred for 30.5 hours at ambient temperature, then neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The filtrate was concentrated under reduced pressure to give the disaccharide **37** as a white solid (4.8 mg, 12.5 μmol, 99%).

***R**f*: 0.17 (1:2:7 $H_20$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 4.92 (d, 1H, *J* = 3.6 Hz, H-$1^I$), 4.89 (d, 1H, *J* = 2.4 Hz, H-$1^{II}$), 3.99-3.46 (m 14H), 1.70-1.60 (m,2H), 0.94 (t, 3H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm)




**38** **39**

**Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (39)**. 1,2-*O*-(1-ethoxyethylidene)-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**38**[19]) (1.27 g, 2.43 mmol) was dissolved in benzyl alcohol (3 mL). The solution was cooled to 0 °C, and TMSOTf (90 μL, 0.49 mmol) was added. The mixture was stirred for 20 minutes at 0 °C, then quenched with triethylamine (1 mL). The reaction mixture was diluted with dichloromethane (100 mL), washed with water (2×20 mL) and brine (50 mL), and dried over $Na_2SO_4$. The solvent was removed under reduced pressure to give a mixture of the 2-OH and 2-OAc derivatives and residual benzyl alcohol. The crude product was dissolved in MeOH (10 mL). Sodium metal (50 mg) was added, and the mixture stirred for 18 hours at ambient temperature. TLC showed that the 2-OAc derivative had been consumed. The reaction was neutralized by the addition of Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was removed under reduced pressure. The excess benzyl alcohol from the first step was difficult remove by evaporation at this stage. To facilitate purification of the desired product, the benzyl alcohol was converted to its trityl ether. The crude product was dissolved in DMF (30 mL). Trityl chloride (8.9 g, 31.9 mmol) and DMAP (3.53 g) were added. The mixture was heated to 70 °C in a CEM Discover microwave reactor for 40 minutes. TLC showed the disappearance of benzyl alcohol ($R_f$ 0.40, 2:8 EtOAc/hexanes) and the appearance of benzyl trityl ether ($R_f$ 0.80, 2:8 EtOAc/hexanes). The reaction mixture was poured into water and extracted with dichloromethane (2×80 mL). The

organic layer was washed successively with saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography using a Biotage SP-1 chromatography worksation (Biotage 25M cartridge, gradient of 7→60% ethyl acetate in hexanes, to provide the product **39** as a white solid (716 mg, 1.32 mmol, 54%). An analytical sample was prepared by recrystallization from $CHCl_3$/hexanes. The $^{13}C$ NMR spectrum was consistent with that reported in the literature.[18]

***R**f*: 0.25 (20% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.40-7.24 (m, 18H, Ar-H), 7.19-7.15 (m, 2H, Ar-H), 4.96-4.90 (2 overlapping d, 2H, 2×PhC*H*), 4.85-4.80 (2 overlapping d, 2H, 2×PhC*H*), 4.65-4.60 (2d, 2H, 2×PhC*H*), 4.57-4.52 (2d, 2H, 2×PhC*H*), 4.35 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 3.78-3.74 (dd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$= 10.8 Hz, H-6), 3.73-3.69 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$= 10.8 Hz, H-6'), 3.65-3.55 (m, 3H, H-2, H-3, H-4), 3.50-3.45 (ddd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$= 10.8 Hz, H-5), 2.37 (d, *J* = 0.8 Hz, -O*H*).

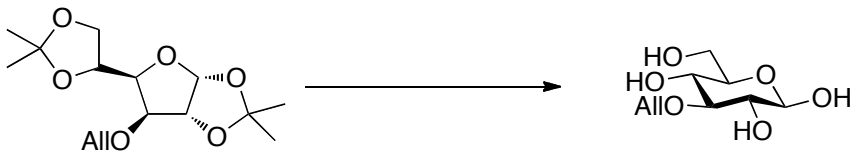
**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.8, 138.3(2), 137.3, 128.7, 128.6(2), 128.3, 128.1(2), 128.0(2), 127.9, 127.8, 101.9, 84.7, 77.7, 75.4, 75.3, 75.2, 74.9, 73.7, 71.2, 69.0.


**40** **41**

**3-*O*-Allyl-1,2-5,6-di-*O*-isopropylidene-D-glucofuranose (41).** Diacetone-D-glucose (**40**) was prepared according to the literature procedure.[21] To a solution of diacetone-D-glucose (3.00 g, 11.5 mmol) in THF (15 mL) was added sodium hydride [60% (w/w) dispersion in mineral oil, 552 mg, 13.8 mmol] and tetrabutylammonium iodide (425 mg, 1.15 mmol). To this mixture, allyl bromide (1.09 mL, 12.65 mmol) was added, and the mixture stirred under a nitrogen atmosphere for two hours at ambient temperature. The mixture was poured into water and extracted with ethyl acetate (2× 100 mL). The organic extract was washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, using a gradient of 10→20% ethyl acetate in hexanes, to provide the product **41** as a colorless oil (3.46 g, 11.5 mmol, 99%). The $^1$H NMR spectrum was consistent with the data reported in the literature.[22]

***R**f*: 0.61 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.96-5.84 (m, 2H), 5.34-5.28 (m, 1H), 5.22-5.18 (m, 1H), 4.55 (d, 1H, *J* = 3.6 Hz), 4.34-4.29 (dt, 1H, *J* = 7.6Hz, 6.0 Hz), 4.18-4.07 (m, 4H), 4.02-3.98 (dd, 1H, *J* = 8.4 Hz, 5.6 Hz), 3.94 (d, 1H, *J* = 3.2 Hz), 1.50 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.32 (s, 3H).

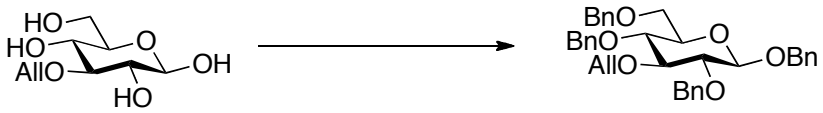



**41** **42**

**3-*O*-Allyl-β-D-glucopyranose (42).** A modification of the procedure of Takeo and coworkers[23] was followed. 3-*O*-allyl-1,2-5,6-di-*O*-isopropylidene-D-glucofuranose (**41**) (3.43 g, 11.4 mmol) and Dowex 50W X-8 ($H^+$) resin (7.0 g) were suspended in water (25 mL). The mixture was stirred for 4 hours at 70°C. The resin was filtered off and washed with water. The solvent was evaporated under reduced pressure, and the solid residue recrystallized from ethanol and hexanes to afford **42** as a white powdery solid (1.17 g, 5.31 mmol, 46%).

***R**f*: 0.34 (10% MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 6.05-5.95 (m, 1H, $OCH_2CH$=$CH_2$), 5.38-5.32 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.27-5.23 (m, 1H, $OCH_2CH$=$CH_aH_b$), 4.64 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.34 (d, 2H, $OCH_2CH$=$CH_2$), 3.91-3.86 (d, 1H, $J_{6,6'}$ = 12.0 Hz, H-6), 3.73-3.69 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$= 12.0 Hz, H-6'), 3.51-3.39 (m, 3H), 3.29 (t, 1H, $J$ = 8.8 Hz).




**42** **43**

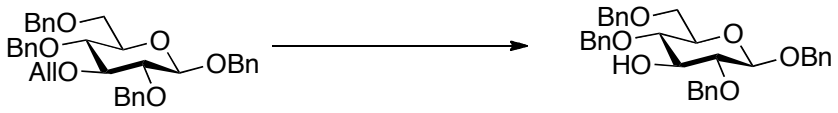
**Benzyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (43).** 3-*O*-allyl-β-D-glucopyranose (**42**) (500 mg, 2.27 mmol) was dissolved in DMF (10 mL). To this

solution, sodium hydride [60% (w/w) dispersion in mineral oil, 363 mg, 9.1 mmol] and benzyl bromide (1.08 mL, 9.1 mmol) were added, and the mixture stirred for 20 hours at ambient temperature. Additional sodium hydride (363 mg) and benzyl bromide (1.08 mL) were added, and the mixture stirred for an additional 22 hours at ambient temperature. The reaction mixture was then poured into water, and extracted with dichloromethane (2 × 75 mL). The combined organic extracts were washed with water and brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, using a gradient of 0→20% ethyl acetate in hexanes, to provide a white solid. The solid was recrystallized from ethyl acetate and hexanes to furnish **43** as fine white needles (583 mg, 1.0 mmol, 44%).

***R**f*: 0.70 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.39-7.20 (m, 20H, Ar-H), 5.99-5.89 (m, 1H, $OCH_2CH$=$CH_2$), 5.28-5.23 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.16-5.13 (m, 1H, $OCH_2CH$=$CH_aH_b$), 4.98-4.53 (m, 8H, 4×$PhCH_2$), 4.47 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 4.41-4.36 (m, 1H, $OCH_aH_bCH$=$CH_2$), 4.29-4.24 (m, 1H, $OCH_aH_bCH$=$CH_2$), 3.77-3.74 (dd, 1H, $J_{5,6}$ = 1.6 Hz, $J_{6,6'}$ = 10.8 Hz, H-6), 3.71-3.67 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$ = 10.8 Hz, H-6'), 3.58-3.41 (m, 4H, H-2, H-3, H-4, H-5).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.7, 138.5, 138.4, 137.8, 135.4, 128.6(2), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8(2), 116.9, 102.8, 84.6, 82.4, 78.0, 75.2, 75.1, 74.7, 73.7, 71.4, 69.2.



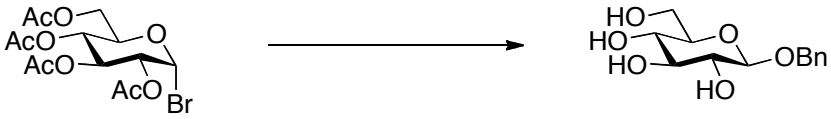

**43** **44**

**Benzyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (44).** Benzyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**43**) (309 mg, 0.53 mmol) was dissolved in THF (8 mL). To this solution, (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) (4.5 mg, 5.3 μmol) was added. The resulting red suspension was degassed by brief exposure of the reaction flask to vacuum. The mixture was then stirred under a balloon of hydrogen until the red suspension became a colorless solution (approximately 30 seconds). The mixture was degassed and the flask flushed with nitrogen. The resulting mixture was stirred for 16 hours at ambient temperature. Proton NMR analysis of a small aliquot (~50 μL) of the reaction mixture indicated that conversion to the 1-propenyl ether was complete. The solvent was removed under reduced pressure. The crude 1-propenyl ether was dissolved in 10:1 acetone/water (5 mL). To this solution, red mercuric oxide (173 mg, 0.80 mmol) was added, followed by the dropwise addition of a mercuric chloride solution (216 mg, 0.80 mmol, in 10:1 acetone/water, 3 mL). The reaction was complete after 1 hour at ambient temperature. The reaction mixture was filtered through a pad of Celite to remove the mercuric oxide. The solvent was partially evaporated under reduced pressure, and diethyl ether (75 mL) added. The organic layer was washed with a potassium iodide solution (2×30 mL), water (30 mL), and brine. After drying over $Na_2SO_4$, the solvent was evaporated. The residue was purified by flash chromatography on silica gel, using a gradient of 15→20% ethyl acetate in hexanes, to provide **44** as a colorless syrup (246 mg, 0.46 mmol, 86%).

***R**f*:** 0.39 (20% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.39-7.21 (m, 20H, Ar-H), 5.00-4.95 (d, 2H, 2×PhC*H*), 4.84 (d, 1H, *J* = 11.2 Hz, PhC*H*), 4.68-4.61 (m, 3H, 3×PhC*H*), 4.59-4.54 (m, 2H, 2×PhC*H*), 4.49 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 3.80-3.68 (m, 3H, H-3, H-6, H-6'), 3.52 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-4), 3.49-3.44 (m, 1H, H-5), 3.37-3.32 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 8.8 Hz, H-2), 2,43 (d, 1H, *J* = 2.0 Hz, -O*H*).

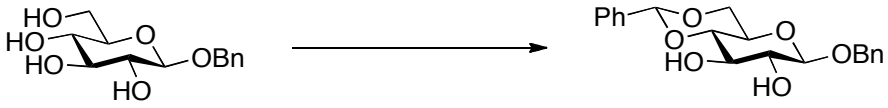
**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.5, 138.4, 137.6, 128.7, 128.6(2), 128.4, 128.2, 128.0, 127.9, 127.8, 102.4, 81.5, 77.6, 75.0, 74.7, 74.6, 73.7, 71.2, 69.1.




**45** **46**

**Benzyl β-D-glucopyranoside (46).** Commercial silver(II) oxide (Alfa Aesar, 1.72 g, 7.4 mmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**45**[26]) (2.04 g, 5.0 mmol) and benzyl alcohol (1.54 mL, 14.9 mmol) in dichloromethane (20 mL). The mixture was stirred for 4 hours at ambient temperature, then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the residue dissolved in MeOH (20 mL). Sodium metal (50 mg) was added to the solution. The mixture was stirred at ambient temperature for 3 hours, neutralized with Dowex 50W X-8 ($H^+$) resin, filtered, and concentrated. The residue was purified by flash

chromatography on silica gel, using a gradient of 10→20% methanol in dichloromethane, to provide the product **46** as a white crystalline solid (890 mg, 3.3 mmol, 66%).

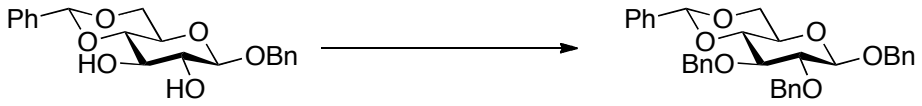



**46** **47**

**Benzyl 4,6-*O*-benzylidene-β-D-glucopyranoside (47).** Benzyl β-D-glucopyranoside (**46**) (880 mg, 3.26 mmol) was dissolved in acetonitrile (10 mL). Benzaldehyde dimethyl acetal (0.98 mL, 6.51 mmol) and 10-camphorsulfonic acid (38 mg, 0.16 mmol) were added, and the mixture heated to reflux for 75 minutes. The mixture was neutralized by the addition of triethylamine (0.5 mL), and the solvent evaporated. The residue was dissolved in dichloromethane (75 mL), washed successively with saturated $NaHCO_3$ solution, water, and brine, dried over $Na_2SO_4$, and concentrated. The solid residue was recrystallized from ethanol to afford **47** as a white solid (844 mg, 2.35 mmol, 72%). The $^1$H NMR was in agreement with the literature.[25]

***R**f*: 0.42 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.56-7.27 (m, 10H, Ar-H), 5.49 (s, 1H, benzylidene Ph-CH), 5.02-3.22 (m, 10H), 2.44 (bs, 2H, -OH).

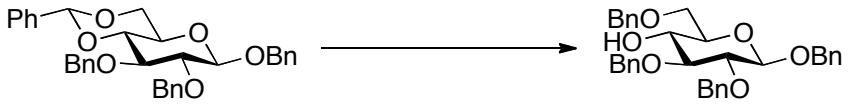



**47** **48**

**Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (48).** Benzyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**47**) (844 mg, 2.35 mmol) was dissolved in DMF (10 mL). To this solution, benzyl bromide (0.62 mL, 5.17 mmol) and sodium hydride [60% (w/w) dispersion in mineral oil, 280 mg, 7.0 mmol] were added, and the mixture stirred at ambient temperature. After 60 minutes at ambient temperature, the reaction was quenched by the addition of methanol (1 mL), and diluted with dichloromethane. The organic solution was washed with water and brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash chromatography on silica gel, using a gradient of 0→20% ethyl acetate in hexanes, to provide the product **48** as a white solid (1.19 g, 2.21 mmol, 94%). The $^1$H NMR spectrum was consistent with the partial assignments reported in the literature.[25] Full proton assignments are given below.

***R**f*: 0.74 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.50-7.23 (m, 20H, Ar-H), 5.57 (s, 1H, benzylidene C*H*), 4.95-4.87 (m, 3H, 3×PhC*H*), 4.79-4.74 (m, 2H, 2×PhC*H*), 4.66 (d, 1H, $J$ = 11.6 Hz, PhC*H*), 4.62 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.39-4.35 (dd, 1H, $J_{5,6}$ = 5.2 Hz, $J_{6,6'}$ = 10.2 Hz, H-6), 3.80 (t, 1H, $J_{5,6'} = J_{6,6'}$ = 10.0 Hz, H-6'), 3.74 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 3.69 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.52 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.0 Hz, H-2), 3.44-3.37 (m, 1H, H-5).

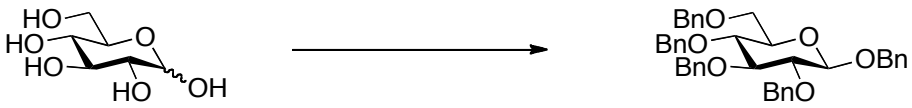



**48** **49**

**Benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (49).** Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**48**) (508 mg, 1.07 mmol) was dissolved in dichloromethane (10 mL). Activated powdered 4Å molecular sieves (1.0 g) were added, and the mixture stirred at ambient temperature for 1 hour. The mixture was cooled to -78 °C. Triethylsilane (0.51 mL, 3.2 mmol) and TfOH (0.32 mL, 3.7 mmol) were added. After stirring for 90 minutes at -78 °C, the reaction mixture was poured into a mixture of saturated aqueous $NaHCO_3$ solution and dichloromethane. The mixture was filtered through a pad of Celite to remove the molecular sieves. The filtrate was poured into a separatory funnel and the layers separated. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash chromatography on silica gel, using a gradient of 0→20% ethyl acetate in hexanes, to give **49** as an oil (308 g, 0.57 mmol, 53%). The $^1$H NMR spectrum was consistent with the partial assignments reported in the literature.[25] Full proton assignments are given below.

***R**f*:** 0.43 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.22 (m, 20H, Ar-H), 4.97-4.91 (apparent t, 3H, 3×PhC*H*), 4.73-4.64 (m, 3H, 3×PhC*H*), 4.62 (d, 1H, $J$ = 12 Hz, PhC*H*), 4.58 (d, 1H, $J$ = 12 Hz, PhC*H*), 4.52 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 3.81-3.77 (dd, 1H, $J_{5,6}$ = 4.0 Hz, $J_{6,6'}$ = 10.2 Hz, H-6), 3.74-3.70 (dd, 1H, $J_{5,6'}$ = 5.2 Hz, $J_{6,6'}$ = 10.2 Hz, H-6'), 3.61 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 3.52-3.42 (m, 3H, H-2, H-4, H-5), 2.50 (br s, 1H, -O*H*).



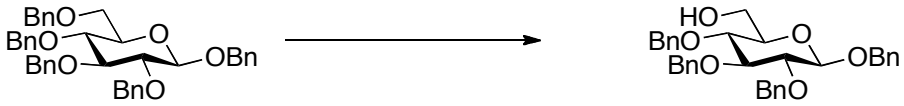
**50**

**Benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (50).** Benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (**50**) was prepared from D-glucose according to the procedure of Lu and coworkers.[27] D-glucose (2.00 g, 11.1 mmol) was suspended in DMF (50 mL). Sodium hydride [60% (w/w) dispersion in mineral oil, 1.33 g, 33.3 mmol] was added, and the mixture stirred for 30 minutes at 0 °C. To this mixture, benzyl bromide (4.6 mL, 38.9 mmol) was added. After 10 minutes, the ice bath was removed and the mixture stirred for 3 hours at ambient temperature. The procedure of additions of sodium hydride and benzyl bromide was repeated, using the same quantities as the first addition. After 12 hours at ambient temperature, additional sodium hydride (60% (w/w) in mineral oil, 891 mg) and benzyl bromide (3,1 mL, 26.0 mmol) were added, and the mixture stirred for 90 minutes at ambient temperature. The excess sodium hydride was destroyed by the addition of methanol (1 mL). The reaction mixture was diluted with water (75 mL) and extracted with dichloromethane (3×75 mL). The combined organic extracts were washed with water and brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash chromatography on silica gel, using 10% ethyl acetate in hexanes as the eluant, to afford the product **50** as a white solid (3.52 g, 5.6 mmol, 50%). The $^1H$ and $^{13}C$ NMR spectra were consistent with the reported data.[27]

***R**f*: 0.64 (20% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.40-7.14 (m, 25H, Ar-H), 5.00-4.50 (m, 11H, 10×PhCH, H-1), 3.78-3.75 (dd, 1H, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 10.2 Hz, H-6), 3.72-3.68 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$ = 10.2 Hz, H-6'), 3.67-3.59 (m, 2H, H-3, H-4), 3.53 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.4 Hz, H-2), 3.48-3.44 (m, 1H, H-5).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.8, 138.6, 138.4, 138.3, 137.7, 128.6(2), 128.5, 128.4, 128.1(2), 128.0, 127.8(3), 102.8, 84.9, 82.5, 78.1, 75.9, 75.2, 75.1, 73.7, 71.3, 69.1.



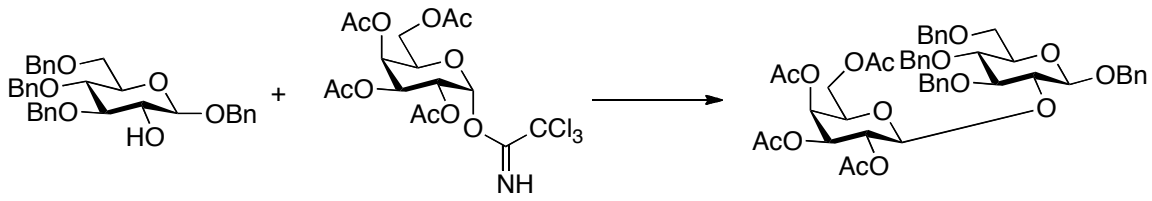

**50** **51**

**Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (51).** Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**51**) was prepared following the literature procedure.[27] Freshly fused zinc chloride (1.33 g, 9.76 mmol) was dissolved in 5:1 $Ac_2O$/AcOH (8 mL). The solution was cooled to 0 °C. A solution of benzyl-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside **50** (1.23 g, 1.95 mmol, in 5:1 $Ac_2O$/AcOH (8 mL)) was added dropwise to the cold zinc chloride solution. The reaction mixture was allowed to warm to ambient temperature with stirring. After 1 hour, ice water (60 mL) was added. The resulting white precipitate was washed with water and then filtered. The solid product was dissolved in dichloromethane (100 mL). The organic solution was washed with water (40 mL), saturated $NaHCO_3$ solution (50 mL), and brine (50 mL), dried over $Na_2SO_4$, and concentrated. The crude 6-*O*-acetyl product was suspended in methanol (20 mL), and sodium metal (50 mg) added. The mixture was stirred at ambient temperature for 2 hours, with complete dissolution of the starting material after 30 minutes. The solvent

was evaporated, and the residue partitioned between water (50 mL) and dichloromethane (50 mL). The aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were washed with water and brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash chromatography on silica gel, using 25% ethyl acetate in hexanes as the eluant, to afford a white solid. The solid was recrystallized from chloroform and hexanes to furnish **51** as fine white needles (391 mg, 0.72 mmol, 37%). The $^1$H NMR spectrum was identical to that reported in the literature.[28]

***R**f*: 0.39 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.25 (m, 20H, Ar-H), 4.95 (d, 1H, *J* = 11 Hz, PhC*H*), 4.93 (d, 1H, *J* = 11 Hz, PhC*H*), 4.92 (d, 1H, *J* = 12 Hz, PhC*H*), 4.86 (d, 1H, *J* = 11 Hz, PhC*H*), 4.81 (d, 1H, *J* = 11 Hz, PhC*H*), 4.73 (d, 1H, *J* = 11 Hz, PhC*H*), 4.69 (d, 1H, *J* = 12 Hz, PhC*H*), 4.63 (d, 1H, *J* = 11 Hz, PhC*H*), 4.57 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 3.90-3.85 (ddd, 1H, $J_{6,6'}$ = 12 Hz, $J_{5,6}$ = 2.8 Hz, $J_{6,\text{-OH}}$ = 6.4 Hz, H-6), 3.73-3.68 (m, 1H, H-6'), 3.67 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 3.57 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.49 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$= 9 Hz, H-2), 3.38-3.34 (m, 1H, H-5), 1.82-1.79 (dd, 1H, *J* = 6.0 Hz, 7.6 Hz, -O*H*).



**39** **8** **52**

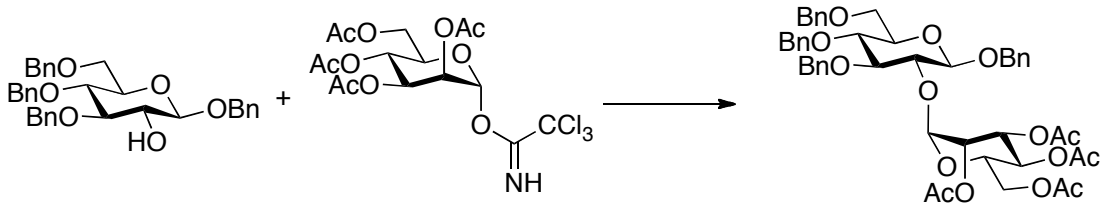
**Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (52).** Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**39**) (74 mg, 0.14 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**) (153 mg, 0.31 mmol) were combined in a 25 mL tear drop flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL), and TMSOTf (1 drop from a 1-mL syringe) was added. After stirring at ambient temperature for 30 minutes, the reaction was quenched by the addition of triethylamine (0.1 mL) and the solvent removed under reduced pressure. The residue was subjected to flash chromatography, using a gradient elution of 10-30% ethyl acetate in hexanes, to give the product **52** as a colorless syrup (57 mg, 65 μmol, 48%).

***R**f*: 0.71 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.42-7.23 (m 18H), 7.12-7.08 (m, 2H), 5.30 (d, 1H), 5.25-5.21 (dd, 1H), 5.05-5.01 (d, 1H), 4.99 (d, 1H, $J$ = 8.0 Hz, H-$1^{II}$), 4.95-4.91 (dd, 1H), 4.84-4.76 (m, 3H), 4.64-4.60 (d, 2H), 4.57-4.53 (m, 3H), 4.13-4.06 (m, 2H), 3.91-

3.86 (dd, 1H), 3.80-3.58 (m, 5H), 3.48-3.44 (m, 1H), 2.14 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.80 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.5, 170.4(2), 170.0, 138.6, 138.2, 138.0, 137.9, 128.6(2), 128.5, 128.1, 128.0(2), 127.9, 127.8, 127.7, 127.3, 101.7, 100.8, 84.6, 81.3, 78.2, 77.5, 76.0, 75.2, 75.0, 73.7, 71.5, 71.2, 70.8, 70.0, 68.8, 97.2, 61.2, 20.9, 20.8(3).



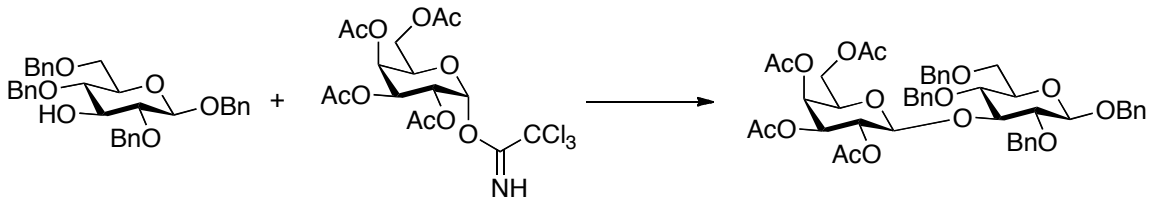

**39** **9** **53**

**Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (53)**. Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**39**) (70 mg, 0.13mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**) (144 mg, 0.29 mmol) were combined in a 25 mL tear drop flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL). To this solution, TMSOTf (1 drop from a 1-mL syringe) was added. The mixture was stirred at ambient temperature for 30 minutes, then quenched by the addition of triethylamine (0.1 mL). The solvent was evaporated, and the residue subjected to flash chromatography, using 30% ethyl acetate in hexanes as eluant, to afford **53** as a colorless syrup (98 mg, 0.11 mmol, 88%).

$R_f$: 0.70 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.22 (m, 18H), 7.12-7.08 (m, 2H), 5.44 (d, 1H, $J$ = 1.6 Hz, H-1$^{II}$), 5.34-5.32 (m, 1H), 5.25-5.17 (m, 2H), 5.99 (d, 1H), 4.87 (d, 1H), 4.81-4.76 (m, 2H), 4.65 (d, 1H), 4.59-4.54 (m, 3H), 4.46 (d, 1H, $J$ = 8.0 Hz, H-1$^{I}$), 4.17-4.11 (m, 1H), 3.81-3.68 (m, 6H), 3.59 (t, 1H), 3.48-3.43 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.82 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.9, 170.2, 170.0(2), 138.3, 138.2, 137.8, 137.0, 128.6, 128.5, 128.2, 128.0(2), 127.7, 127.0, 102.2, 96.9, 83.2, 78.7, 77.5, 75.9, 75.6, 75.2(2), 73.8, 71.5, 69.5, 69.2, 68.6, 68.3, 65.5, 61.8, 21.1, 21.0, 20.9, 20.8.




**44** **8** **54**

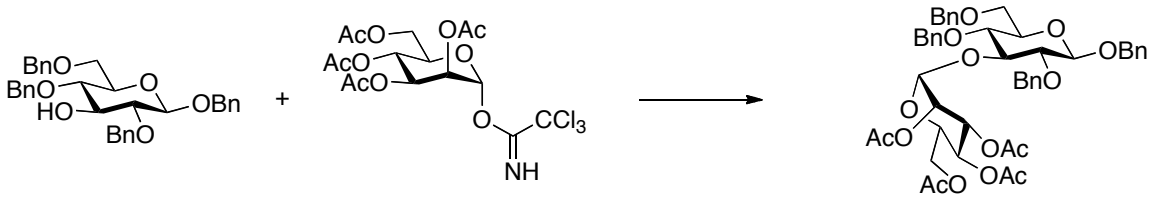
**Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (54)**. Benzyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**44**) (49 mg, 91 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**) (103 mg, 210 μmol) were combined in a 25 mL round-bottom flask. Toluene (3 x 3 mL) was added to the sugars and the solvent was removed under reduced pressure after each addition. The flask was dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL). TMSOTf (1 drop from a 1-mL syringe) was added to the solution. The mixture was stirred at ambient temperature for 30 minutes, quenched by the addition of triethylamine (0.1 mL), and the solvent removed under

reduced pressure. The residue was purified by flash chromatography on silica gel, using a gradient of 10-30% ethyl acetate in hexanes as the eluant, to furnish disaccharide **54** as a colorless syrup (48 mg, 55 μmol, 60%).

$R_f$: 0.65 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.40-7.25 (m, 20H), 5.34 (d, 1H), 5.28-5.23 (dd, 1H), 5.15 (d, 1H, $J$ = 8.0 Hz, H-1$^{II}$), 5.02-4.95 (m, 4H), 4.67-4.62 (m, 2H), 4.59-4.55 (m, 2H), 4.47 (d, 1H, $J$ = 8.0 Hz, H-1$^{I}$), 4.45-4.42 (d, 1H), 4.16-4.09 (dd, 1H), 4.02 (t, 1H), 3.98-3.93 (dd, 1H), 3.78-3.69 (m, 3H), 3.57 (t, 1H), 3.49-3.40 (m, 2H), 2.10 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.5, 170.4, 170.3, 169.5, 138.4, 138.3, 138.0, 137.4, 128.9, 128.7(2), 128.6, 128.4(2), 128.3, 128.1, 128.0, 127.9, 102.3, 100.5, 83.0, 80.9, 77.6, 75.8, 75.2, 75.1, 74.7, 73.7, 71.4, 71.2, 70.6, 69.8, 68.9, 67.3, 61.0, 21.2, 20.8(3).




**44** **9** **55**

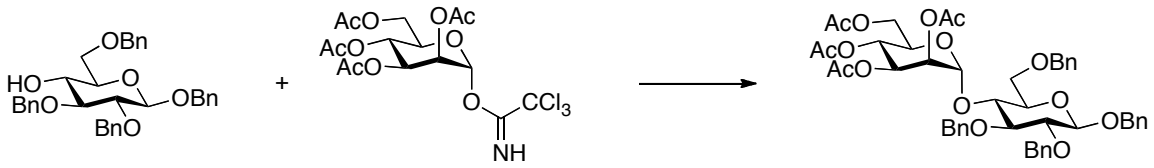
**Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (55).** Benzyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**44**) (42 mg, 78 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**) (79 mg,

160 μmol) were combined in a 25 mL round-bottom flask and dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL). After the addition of TMSOTf (1 drop from a 1-mL syringe), the mixture was stirred at ambient temperature for 20 minutes. The reaction was quenched by the addition of triethylamine (0.1 mL), and the solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel, using a Biotage SP-1 chromatography system (Biotage 12M cartridge, 12→50% ethyl acetate in hexanes over 16 column volumes), to furnish a white solid. The solid was recrystallized from hot ethyl acetate and hexanes to yield disaccharide **55** as fine white needles (60 mg, 69 μmol, 88%).

***R**f*: 0.76 (50% EtOAc/hexanes)

**$^{1}$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.21 (m, 18H), 7.12-7.08 (m, 2H), 5.34-5.32 (m, 1H), 5.32 (br s 1H, H-$1^{II}$), 5.27-5.24 (dd, 1H), 5.16 (t, 1H), 5.07 (d, 1H), 4.97 (d, 1H), 4.70-4.64 (m, 2H), 4.61-4.55 (m, 4H), 4.50 (d, 1H, $J$ = 7.6 Hz, H-$1^{I}$), 4.28-4.24 (m, 1H), 3.82-3.69 (m, 4H), 3.61 (d, 2H), 3.46-3.41 (m, 2H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.8, 170.2, 170.0, 169.6, 138.1(2), 137.7, 137.3, 128.7, 128.6, 128.5, 128.4, 128.3(2), 128.1, 128.0(2), 127.9, 127.8, 127.5, 102.6, 98.0, 79.6, 79.4, 78.5, 77.5, 74.9, 74.7, 74.6, 73.8, 71.2, 69.6, 69.5, 68.6, 68.2, 65.6, 61.6, 20.9(4).



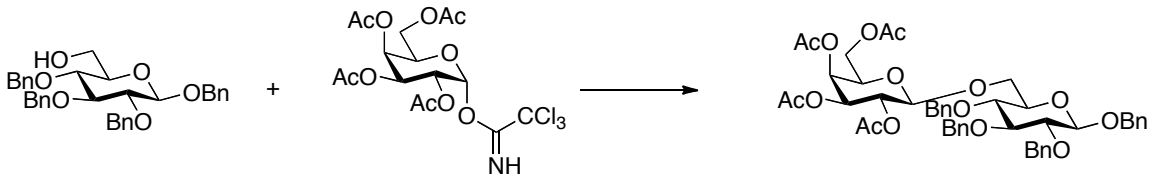
**49** **9** **56**

**Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (56).** Benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**49**) (47 mg, 87 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**) (91 mg, 185 μmol) were combined and dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL). TMSOTf (1 drop from a 1-mL syringe) was added to the solution. The mixture was stirred at ambient temperature for 30 minutes, then quenched by the addition of triethylamine (0.1 mL), The solvent removed under reduced pressure and the residue purified by flash chromatography on silica gel, using a gradient of 10→40% ethyl acetate in hexanes as eluant, to yield **56** as a syrup (65 mg, 74 μmol, 85%).

***R**f*: 0.57 (50% EtOAc/hexanes)

**$^{1}$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.16 (m, 20H), 5.40 (br s, 1H, H-$1^{II}$), 5.26-5.16 (m, 3H), 5.02-4.92 (m, 3H), 4.71-4.57 (m, 5H), 4.54 (d, 1H, $J$ = 8.0 Hz, H-$1^{I}$), 4.19-4.14 (dd, 1H), 3.97-3.94 (m, 1H), 3.89-3.78 (m, 3H), 3.75-3.71 (dd, 1H), 3.66 (t, 1H), 3.57-3.50 (m, 2H), 2.04 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.8, 170.2, 169.7(2), 138.3(2), 137.4, 128.6(2), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.5, 127.3, 102.6, 98.8, 84.6, 82.4, 77.5, 75.6, 75.1, 74.8, 74.4, 73.7, 71.4, 69.5, 96.4, 69.3, 65.9, 62.5, 20.9(2), 20.8(2).




**51** **8** **57**

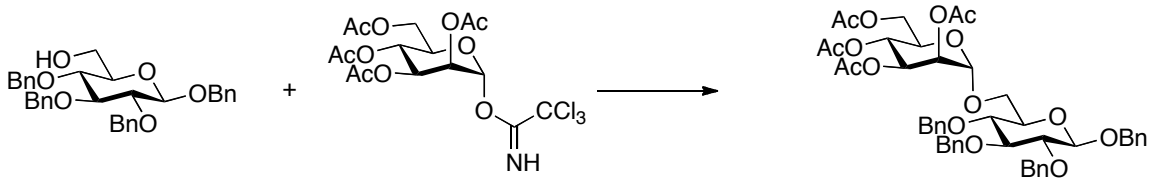
**Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (57).** Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**51**) (77 mg, 143 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**8**) (138 mg, 280 μmol) were combined and dried under high vacuum ($10^{-4}$ torr) for 1 h. The reactants were dissolved in dichloromethane (2.0 mL). TMSOTf (1 drop from a 1-mL syringe) was added to the solution, and the mixture stirred at ambient temperature for 20 minutes. The reaction was quenched by the addition of triethylamine (0.1 mL), and the solvent removed under reduced pressure. The residue was purified using a Biotage SP-1 chromatography system (Biotage 12M cartridge, 10→90% ethyl acetate in hexanes over 12 column volumes). The first compound to elute was the transacetylated acceptor benzyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside[27], a white solid ($R_f$ = 0.60 in 50% EtOAc/hexanes, 25 mg, 43 μmol, 30%). The second compound to elute was disaccharide **57**, which was recrystallized from hot ethyl acetate and hexanes to yield fine white needles (64 mg, 73 μmol, 51%).

***R**f*: 0.26 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.40-7.22 (m, 20H), 5.37 (d, 1H), 5.29-5.24 (dd, 1H), 4.98-4.91 (m, 4H), 4.85 (d, 1H), 4.79-4.68 (m, 3H), 4.58 (d, 1H, $J$ = 8.0 Hz, H-1$^{II}$),

4.55 (d, 1H), 4.50 (d, 1H, $J$ = 7.6 Hz, H-1[1]), 4.17-4.10 (m, 3H), 3.82 (t, 1H), 3.68-3.62 (m, 2H), 3.54-3.47 (m, 2H), 3.40 (t, 1H), 2.15 (s, 3H), 2.02 (s, 3H), 1.98 (s, 6H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.6, 170.5, 170.4, 169.4, 138.6, 138.4, 138.1, 137.5, 128.7, 128.7, 128.4, 128.2, 128.1(2), 128.0, 127.9(2), 102.6, 101.4, 84.8, 82.4, 78.2, 76.0, 75.1, 75.0, 71.2(2), 70.8, 69.0, 68.6, 67.2, 61.5, 21.0, 20.9(2), 20.8.



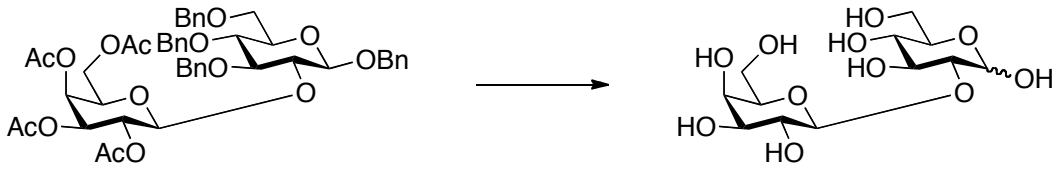

**51** **9** **58**

**Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (58).** Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**51**) (79 mg, 146 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**) (141 mg, 286 μmol) were combined and dried under vacuum ($10^{-4}$ torr) for 1 hour, then dissolved in dichloromethane (2.0 mL). TMSOTf (1 drop from a 1-mL syringe) was added to the solution, and the mixture stirred at ambient temperature for 30 minutes. The reaction was quenched by the addition of triethylamine (0.1 mL), and the solvent evaporated. The residue was purified using a Biotage SP-1 chromatography system (Biotage 12M cartridge, 12→50% ethyl acetate in hexanes over 12 column volumes) to provide disaccharide **58** as a white foam (95 mg, 109 μmol, 75%).

***R**$_f$*: 0.68 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.40-7.25 (m, 20H), 5.40-5.36 (dd, 1H), 5.32-5.26 (m, 2H), 4.98-4.87 (m, 5H), 4.76 (d, 1H), 4.72 (d, 1H), 4.66 (d, 1H), 4.58 (d, 1H), 4.51 (d, 1H, $J$ = 7.6 Hz, H-1$^I$), 4.25-4.29 (dd, 1H), 4.10-4.03 (m, 2H), 3.75 (d, 2H), 3.67 (t, 1H), 3.53-3.41 (m, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.8, 170.2, 170.0, 169.9, 138.6, 138.5, 138.1, 137.5, 128.7, 128.6(2), 128.4, 128.2, 128.1, 128.0(2), 127.9(2), 102.4, 97.6, 84.9, 82.5, 78.1, 75.9, 75.1(2), 74.2, 71.2, 69.6, 69.3, 68.7, 66.8, 66.2, 62.5, 21.1, 21.0, 20.9, 20.8.



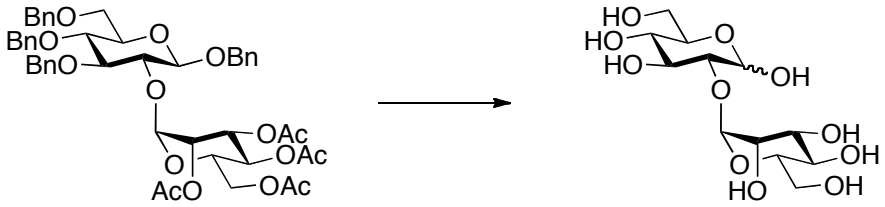

**52** **59**

**2-*O*-(β-D-galactopyranosyl)-D-glucopyranose (59).** Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**52**) ( 72 mg, 82 μmol) was suspended in EtOH (10 mL). Palladium (10% on carbon, 20 mg) was added, and the mixture stirred under an atmosphere of hydrogen at ambient temperature. After 14.5 hours, the starting disaccharide had completely dissolved and TLC indicated that debenzylation was complete. The reaction mixture was filtered through Celite, and the solvent removed under reduced pressure. The residue was dissolved in MeOH (5 mL), and sodium metal (10 mg) was added. The mixture was stirred for 4 hours at ambient temperature, then neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was evaporated under vacuum to furnish disaccharide **59**, a mixture of anomers, as a syrup (26 mg, 76 μmol, 92%).

$R_f$: 0.63 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.41 (d, 0.6H, *J* = 3.2Hz), 4.68 (d, 0.4H, *J* = 8.0 Hz), 4.52 (d, 0.6H, *J* = 7.6Hz), 3.90-3.45 (m, 12 H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 105.2, 103.9, 95.4, 92.5, 81.9, 81.4, 76.6, 76.5, 76.0, 75.7, 73.3, 73.2, 72.4, 72.0, 71.8, 71.7, 70.0, 69.3, 69.2, 61.8, 61.6, 61.3, 61.1.



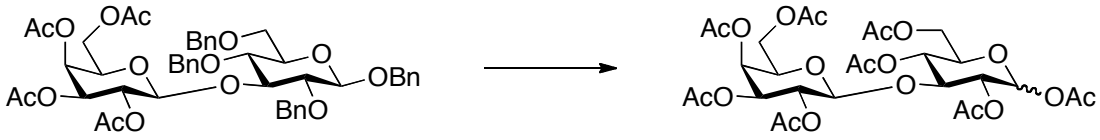

**53** **60**

**2-*O*-(α-D-mannopyranosyl)-D-glucopyranose (60).** Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**53)** (23 mg, 26 μmol) was dissolved in EtOH (2 mL). Palladium (10% on carbon, 10 mg) was added, and the mixture stirred under an atmosphere of hydrogen at ambient temperature. After 23 hours, the reaction mixture was filtered through a pad of Celite. The solvent was evaporated, and the residue dissolved in MeOH (2 mL). To this solution, sodium metal (10 mg) was added. The mixture was stirred for 22 hours at ambient temperature, neutralized with Dowex 50W X-8 ($H^+$) resin, and filtered. The solvent was evaporated under reduced pressure to furnish disaccharide **60**, a mixture of anomers, as a syrup (8.5 mg, 25 μmol, 96%).

$R_f$: 0.60 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.46 (d, 0.5H, *J* = 3.2 Hz), 5.27 (s, 0.5H), 5.03 (s, 0.5H), 5.73 (d obscured by solvent peak, *J* = 8.0 Hz), 4.00-3.36 (m, 12H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 100.4, 97.8, 96.8, 89.7, 79.6, 76.5, 75.5, 75.0, 73.4, 71.9(2), 71.0, 70.9, 70.3, 70.1, 67.2(2), 61.5, 61.4, 61.2.



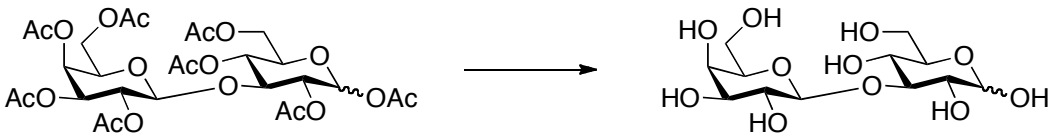

**54** **61**

**1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose (61).** Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**54**) (68 mg, 78 μmol) was dissolved in EtOH (3 mL). Palladium (10% on carbon, 40 mg) was added, and the mixture stirred under an atmosphere of hydrogen at ambient temperature. After 30 hours, the reaction mixture was filtered through a pad of Celite and concentrated. The crude product was acetylated with a mixture of acetic anhydride (2 mL) and pyridine (2 mL) at ambient temperature. After stirring for 24 hours, the volatiles were evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel, using 50% ethyl acetate in hexanes as eluant, to furnish the peracetylated disaccharide **61**, a 1:1 mixture of anomers, as a white foam (46 mg, 68 μmol, 87%).

$R_f$: 0.28 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 6.25 (d, 0.5H, *J* = 4.0 Hz, H-1$^{II}$), 5.62 (d, 0.5H, *J* = 8.4 Hz, H-1$^{II}$), 5.36 (t, 1H), 5.15-5.00 (m, 3H), 4.97-4.92 (m, 1H), 4.60 (d, 0.5H, *J* = 8.0 Hz, H-1$^{I}$), 4.56 (d, 0.5H, *J* = 8.0 Hz, H-1$^{I}$), 4.28-3.87 (m, 7H), 3.82-3.78 (m, 0.5H), 2.21 (s, 1.5H), 2.16 (s, 1.5H), 2.15 (s, 1.5H), 2.11-2.05 (m, 13.5H), 2.03 (s, 1.5H), 1.99 (s, 1.5H), 1.97 (2 s, 3H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 171.0, 170.9, 170.7, 170.6, 170.5(2), 170.4(2), 169.6, 169.5, 169.4, 169.3(2), 169.0(2), 101.4, 101.3, 92.0, 89.4, 78.6, 77.6, 75.9, 73.0, 72.1, 71.4, 71.2, 70.8, 70.5, 70.1, 69.0, 68.9, 68.0, 67.8, 67.0, 66.9, 62.0, 61.9, 61.1, 60.9, 21.2, 21.0, 20.9, 20.8, 20.6.



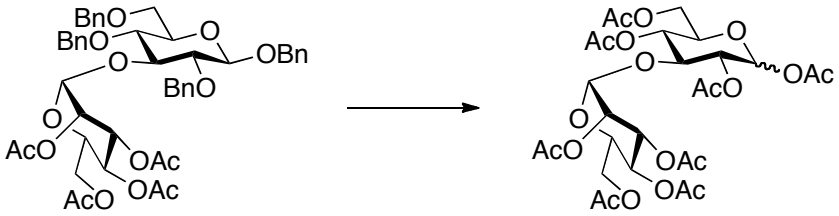

**61** **62**

**3-*O*-(β-D-galactopyranosyl)-D-glucopyranose (62)**. 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose (**61**) (11 mg, 16 μmol) was dissolved in MeOH (4 mL). Sodium metal (0.5 mg) was added, and the mixture stirred for 90 minutes at room temperature. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin, filtered, and concentrated to give the deprotected disaccharide **62** as a syrup (5 mg, 15 μmol, 93%).

***R**$_f$*: 0.53 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.24 (d, 0.4H, *J* = 3.6 Hz), 4.68 (d, 0.6H, *J* = 8.4 Hz), 4.64 (d, 0.6H, *J* = 7.2 Hz), 4.57 (d, 0.2H, *J* = 8.0 Hz), 3.95-3.41 (m, 11.5H), 3.25-3.15 (m, 0.5H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 104.1, 104.0, 96.3, 92.6, 85.4, 83.2, 76.2, 76.0, 75.8, 74.4, 73.2, 71.9(2), 71.8, 71.6, 69.2, 68.9, 61.7, 61.4, 61.2.



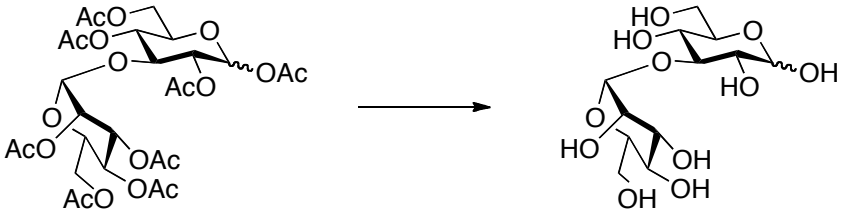

**55** **63**

**1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glucopyranose (63).** Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**55**) (120 mg, 138 μmol) was dissolved in EtOH (5 mL). Palladium (10% on carbon, 50 mg) was added. The mixture was stirred under an atmosphere of hydrogen at ambient temperature for 18 hours, then filtered through a pad of Celite. The solvent was evaporated, and the crude product acetylated with a mixture of acetic anhydride (2 mL) and pyridine (2 mL) at ambient temperature. After stirring for 12 hours, the volatiles were evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel, using 50% ethyl acetate in hexanes as eluant, to furnish the peracetylated disaccharide **63** as a syrup (79 mg, 116 μmol, 84%). The product was isolated as a 1:1 mixture of anomers.

***R**f*:** 0.32 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 6.25 (s, 0.5H, *J* = 4.0 Hz, H-1$^{II}$), 5.71-5.69 (dd, 0.5H), 5.54 (t, 0.5H), 5.30-5.20 (m, 3H), 5.02-4.96 (m, 3H), 4.46 (t, 1H), 4.26-4.17 (m, 2H), 4.08-4.03 (m, 2.5H), 3.87 (t, 1H), 3.79-3.74 (m, 0.5H), 2.20-1.98 (m, 24H).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 170.8, 170.7(2), 170.3(2), 170.1(2), 170.0, 169.8, 169.1(2), 100.1, 100.0, 91.6, 89.2, 77.5(2), 77.4, 74.3, 73.6, 71.3, 70.9, 70.8, 70.1(2), 70.0, 69.8, 68.6, 68.5, 66.0(2), 62.6, 62.5, 62.3(2), 21.1, 21.0(2), 20.9(2), 20.8(2), 20.7.



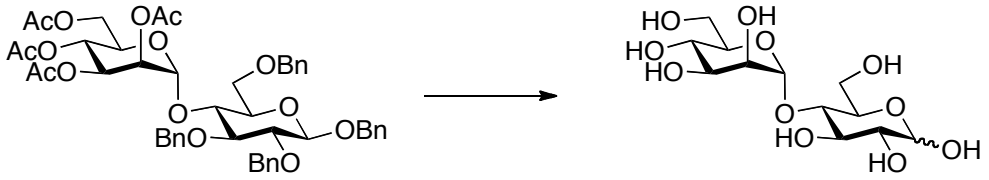

**63** **64**

**3-*O*-(α-D-mannopyranosyl)-D-glucopyranose (64).** 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glucopyranose (**63**) (14.5 mg, 16.6 μmol) was dissolved in methanol (2 mL). Sodium metal (10 mg) was added, and the mixture stirred at ambient temperature for 24 hours. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin, filtered, and concentrated to give the deprotected disaccharide **64** as a syrup (5.4 mg, 15.8 μmol, 95%).

***R**f*:** 0.55 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.25-5.21 (m, 1H), 4.65 (d, 0.6H, *J* = 8.0 Hz, H-1$^I$(β)), 4.05-3.25 (m, 12H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 101.6, 101.5, 96.6, 94.7, 92.9, 82.7, 80.1, 76.3, 73.5, 73.4, 71.9, 71.0, 70.9, 70.8, 70.7, 67.5, 67.2, 67.1, 61.6, 61.5, 61.3, 61.2, 61.0.



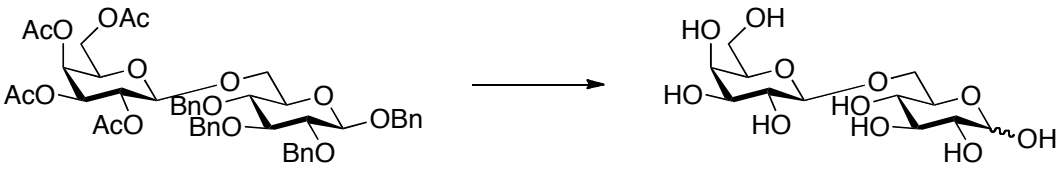

**56** **65**

**4-*O*-(α-D-mannopyranosyl)-D-glucopyranose (65).** Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glucopyranose (**56**) (53 mg, 78 μmol) was dissolved in EtOH (3 mL). Palladium (10 wt% on carbon) was added. The mixture was stirred at ambient temperature under a hydrogen atmosphere for 48 hours. The mixture was filtered through Celite and the solvent evaporated under reduced pressure. The residue was dissolved in MeOH (3 mL). Sodium metal (10 mg) was added, and the mixture stirred at ambient temperature for 3 hours. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin, filtered, and concentrated to give the deprotected disaccharide **65** as a syrup (26 mg, 76 μmol, 97%).

***R**$_f$*: 0.58 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.27 (d, 1H, *J* = 1.6 Hz, H-1$^{II}$), 5.21 (d, 0.5H, *J* = 3.6 Hz, H-1$^{I}$(α)), 4.62 (d, 0.5H, *J* = 8.0 Hz, H-1$^{I}$(β)), 4.07-4.02 (m, 1H), 3.90-3.51 (m, 10.5H), 3.25-3.21 (m, 0.5H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 102.1, 102.0, 96.4, 92.5, 77.0, 76.9, 76.8, 75.2, 74.9, 74.4, 73.9, 72.1, 71.0, 70.9, 70.7, 67.2, 61.5(2), 61.3.



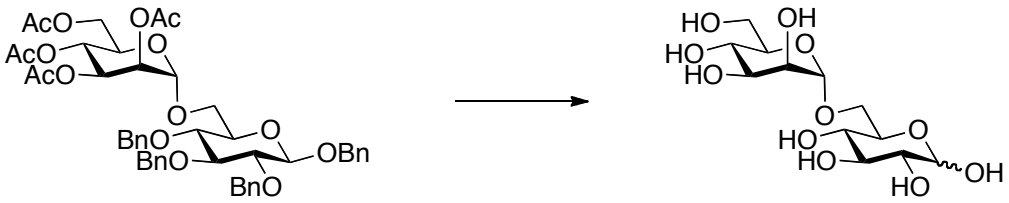

**57** **66**

**6-*O*-(β-D-galactopyranosyl)-D-glucopyranose (66).** Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**57**) (51 mg, 59 μmol) was dissolved in EtOH (10 mL). Palladium (10% on carbon, 20 mg) was added to the solution. The mixture was stirred at ambient temperature under a hydrogen atmosphere for 48 hours, then filtered through a pad of Celite. The solvent was evaporated under reduced pressure, and the residue dissolved in MeOH (10 mL). Sodium metal (5 mg) was added, and the mixture stirred at ambient temperature for 1 hour. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was evaporated under reduced pressure to yield disaccharide **66** as a syrup (20 mg, 59 μmol, 99%).

***R**<sub>f</sub>*: 0.58 (2:8 $H_2O$/MeOH)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.22 (d, 0.47H, *J* = 3.2 Hz, H-1$^1$(α)), 4.65 (d, 0.53H, *J* = 8.0 Hz), 4.43 (apparent t, 2 overlapping doublets, 1H), 4.22-4.14 (dd, 1H), 4.01-3.43 (m, 10.4H), 3.27-3.22 (m, 0.6H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 104.0, 96.6(2), 92.8, 76.3, 75.8(2), 75.5, 74.7, 73.3, 72.1, 71.4, 71.1, 70.1, 70.0, 69.3, 69.2, 61.6.




**58** **67**

**6-*O*-(α-D-mannopyranosyl)-D-glucopyranose (67).** Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**58**) (31 mg, 36 μmol) was dissolved in MeOH (2 mL) and EtOAc (0.5 mL). Palladium (10% on carbon, 10 mg) was added to the solution. After stirring at ambient temperature under a hydrogen atmosphere for 48 hours, the mixture was filtered through a pad of Celite. The solvent was evaporated under reduced pressure, and the residue dissolved in MeOH (2 mL). Sodium metal (5 mg) was added, and the mixture stirred at ambient temperature for 48 hours. The mixture was neutralized with Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was evaporated under reduced pressure to yield disaccharide **67** as a syrup (12 mg, 35 μmol, 97%).

***R**f*: 0.19 (1:1:3 $H_2O$/MeOH/EtOAc)

**$^1$H NMR ($D_2O$, 400 MHz)** δ (ppm) 5.07 (d, 0.4H, *J* = 3.2 Hz), 4.74-4.72 (d, 1H), 4.49 (d, 0.6H, *J* = 8.0 Hz), 3.85-3.03 (m, 12H).

**$^{13}$C NMR ($D_2O$, 101 MHz)** δ (ppm) 100.1(2), 96.6, 92.7, 76.4, 74.6, 74.5, 73.5, 73.2, 71.9, 71.1, 70.6, 70.4, 70.0, 67.2, 61.4.

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(1→4)-O-(2-O-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-O-sulfo-D-glucopyranose decasodium salt, a Heparin Fragment Having High Affinity for Antithrombin III. *Carbohydr. Res.* **1986**, 147, 221-236.

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# CHAPTER 3

## Development of Automation Protocols for the Solution-Phase Synthesis of Beta-Glucan Fragments

Abstract

Beta-glucans are oligosaccharides composed of repeating D-glucose units connected by beta glycosidic linkages. Found in plants, bacteria, and fungi, the beta-glucans vary considerably in terms of branching patterns between species. Beta-glucans have been shown to possess a number of interesting biological properties, including general stimulation of the innate immune system. The exact molecular determinants responsible for these properties remain unclear. Access to a library of structurally well-defined beta-glucan fragments would facilitate the systematic study of the structure-function relationships of this class of oligosaccharides. Iterative chemical synthesis offers the advantage of providing pure samples of beta-glucan fragments. Furthermore, automation of this chemistry has the potential to provide rapid access to structural diversity by assigning the many rote chemical operations involved in iterative synthesis to a robotic synthesizer platform. This chapter describes the development of methods for the automated iterative solution-phase synthesis of branched beta-glucan fragments from monosaccharide building blocks. Three new glucose building blocks were synthesized and used to construct a 3,6-branched beta-glucan tetrasaccharide. Through this work, a general strategy for the automated synthesis of beta-glucan fragments has been established. This strategy can be applied to the synthesis of a library of other linear and branched glucan structures.

Introduction

Beta-glucans are oligomers of D-glucose residues joined by beta glycosidic bonds. These compounds can be obtained from a number of natural sources, including plants, bacteria, and fungi. The structures and physical properties of beta-glucans vary considerably based on the source species. Beta-glucans have been shown to possess a number of biological properties, including cholesterol-lowering effects, anti-inflammatory properties, and immunomodulatory activity.[1] Beta-glucans have emerged as a major target for the recognition of pathogens by the innate immune system. A number of beta-glucan receptors have been discovered in vertebrates.[2] The transmembrane protein Dectin-1, in particular, has been studied extensively in recent years. Dectin-1 recognizes particulate and soluble glucans from plants, fungi, and bacteria, and serves as the primary receptor for these carbohydrates on white blood cells.[3] Dectin-1 can induce a number of cellular responses to fungal pathogens, including phagocytosis and killing and the induction of cytokines and chemokines. Further research is required to develop a more detailed understanding of the roles Dectin-1 plays in antifungal immunity. Access to a structurally defined library of beta-glucan fragments would greatly facilitate the study of the specificity of Dectin-1 with respect to the chain length and branching pattern of its beta-glucan ligand. Although beta-glucans can be obtained from natural sources, they are often isolated as heterogeneous mixtures. Chemical synthesis offers the advantage of producing pure, structurally well-defined samples of beta-glucan fragments.

A number of syntheses of beta-glucan structures have been reported in the literature. Larsson and coworkers prepared tetra- and pentasaccharides related to the capsular polysaccharide of *S. pneumoniae*.[4] Kong and coworkers have published several syntheses of 3,6-branched β-D-glucose oligosaccharides.[5a-e] Similarly, He and coworkers synthesized branched glucose tetra-, penta-, and hexasaccharides, relying on a key 3,6-branched trisaccharide trichloroacetimidate donor for the installation of branch points.[6] These syntheses rely upon convergent approaches using di-, tri- and tetrasaccharide building blocks. In contrast to these convergent approaches, Jamois and coworkers reported the synthesis of linear β-(1,3)-glucans by an iterative approach using a single monosaccharide building block.[7]

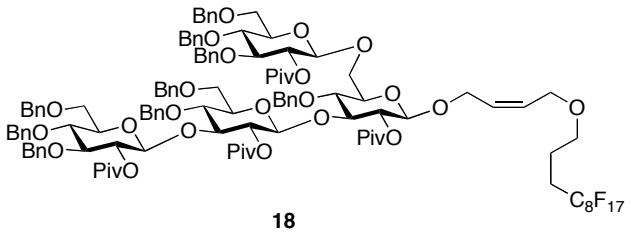
The main advantage of the iterative approach to oligosaccharide synthesis is the ability to incorporate any desired monosaccharide residue into a growing oligosaccharide chain. By relying on the use of monosaccharide building blocks, chemists can avoid using complex synthetic intermediates such as di- and trisaccharide building blocks as glycosyl donors. Since glycosyl donors must be used in excess to ensure high glycosylation yields, considerable amounts of these building blocks end up as waste. This is especially problematic when the building blocks are extremely costly to prepare, as is generally the case with di-, tri-, and higher oligosaccharide donors. Despite its advantages, iterative oligosaccharide synthesis involves multiple cycles of time-consuming and labor-intensive glycosylation and deprotection steps. The automation of rote, repetitive tasks such as these would greatly reduce the amount of manual labor involved in oligosaccharide synthesis.

The automation of peptide and nucleic acid synthesis based on solid-phase synthetic methodologies has fueled the development of the fields of proteomics and genomics. Custom-made nucleic acid or peptide sequences can be purchased from a number of online vendors. Unfortunately, commercial custom oligosaccharide synthesis is not yet a reality. Bruce Merrifield's development of solid-phase peptide synthesis in the 1960s provided a means for the rapid synthesis of peptides.[8] Shortly thereafter, other researchers began to apply this methodology to oligosaccharide synthesis. The first report of solid-phase carbohydrate synthesis was made by Frechet and Schuerch presented the first report of solid-phase carbohydrate synthesis in 1971.[9] After a prolonged lull in activity in this area, Plante and coworkers reported the automated solid-phase synthesis of oligosaccharides in 2001.[10] However, solid-phase synthesis has a number of limitations that have prevented its widespread use in oligosaccharide synthesis. For example, large excesses of sugar building blocks (10-20 equivalents per coupling cycle) must be used to ensure that high coupling efficiencies are obtained. Furthermore, reaction monitoring on the solid phase is often difficult. The development of an alternative phase-switching approach that offers the purification advantages of solid phase synthesis with the advantages of solution-phase chemistry and reaction monitoring would facilitate the advancement of automated oligosaccharide synthesis.

Results and Discussion

The primary goal of this work was to develop a reliable method for the automated solution-phase synthesis of branched and linear beta-glucan fragments. The synthesis of 3,6-branched beta-glucan structures such as those found on fungal cell walls was of

particular importance. For example, synthetic glucan fragments could be assayed for binding with Dectin-1 in order to determine the ligand specificity of this glucan-binding protein. With this in mind, the 3,6-branched tetrasaccharide **18** was chosen as our initial synthetic target (Figure 3.1). The synthesis of compound **18** via an iterative approach would require differentiation of O-3 and O-6 of the branch point glucosyl residue to allow for subsequent independent chain extension from these positions. The protocols developed for the synthesis of **18** could then be applied to other branched beta-glucan structures.




**18**

**Figure 3.1.** Initial synthetic target tetrasaccharide **18**

The first task in the course of this work was the synthesis of a set of suitable protected glucose building blocks. Retrosynthetic analysis shows that three monosaccharide building blocks and one fluorocarbon-based aglycon are needed to construct β-(1→3)-glucan fragments containing β-(1→6) branches using an iterative approach (Figure 3.2). The alkene-functionalized fluorous tag **A** provides a handle for the purification of intermediates by fluorous solid-phase extraction (FSPE) during the synthesis. The C-C double bond provides a functional handle for the cleavage of the fluorous tag from the sugar chain following completion of the synthesis. The monosaccharide building blocks **B**, **C**, and **D** share three common features. First, they possess an anomeric leaving group (LG) that, upon activation, will generate an

electrophilic species that can be attacked by a nucleophilic hydroxyl group to form a glycosidic bond. Second, each building block features a protecting group $P_1$ at the O-2 position. The use of an ester protecting group at the O-2 position is necessary to provide stereochemical control of each newly formed glycosidic linkage through neighboring group participation (Figure 3.3). Third, each building block contains permanent protecting groups (R) that will remain in place for the duration of the synthesis. The temporary protecting groups $P_2$, $P_3$, and $P_4$ must be carefully selected. In particular, the groups $P_2$ and $P_3$ must be orthogonal in order to allow for independent chain extension from the O-3 and O-6 positions of the branch point glucose unit.

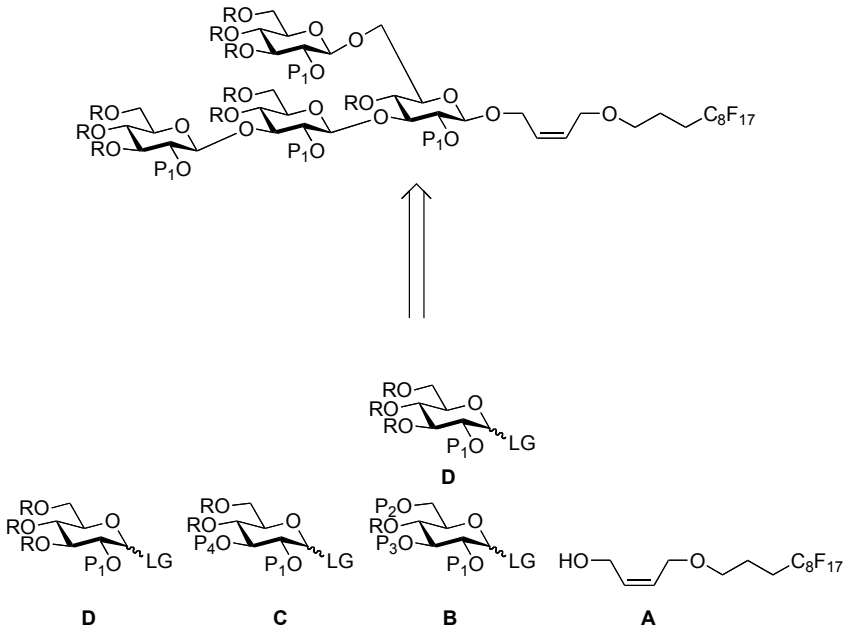



**Figure 3.2.** Retrosynthesis of a branched 1,3-glucan tetrasaccharide fragment. R = permanent protecting group; $P_1$ = semi-permanent group capable of neighboring group participation during glycosylation reactions; $P_2$, $P_3$, $P_4$ = temporary protecting groups.

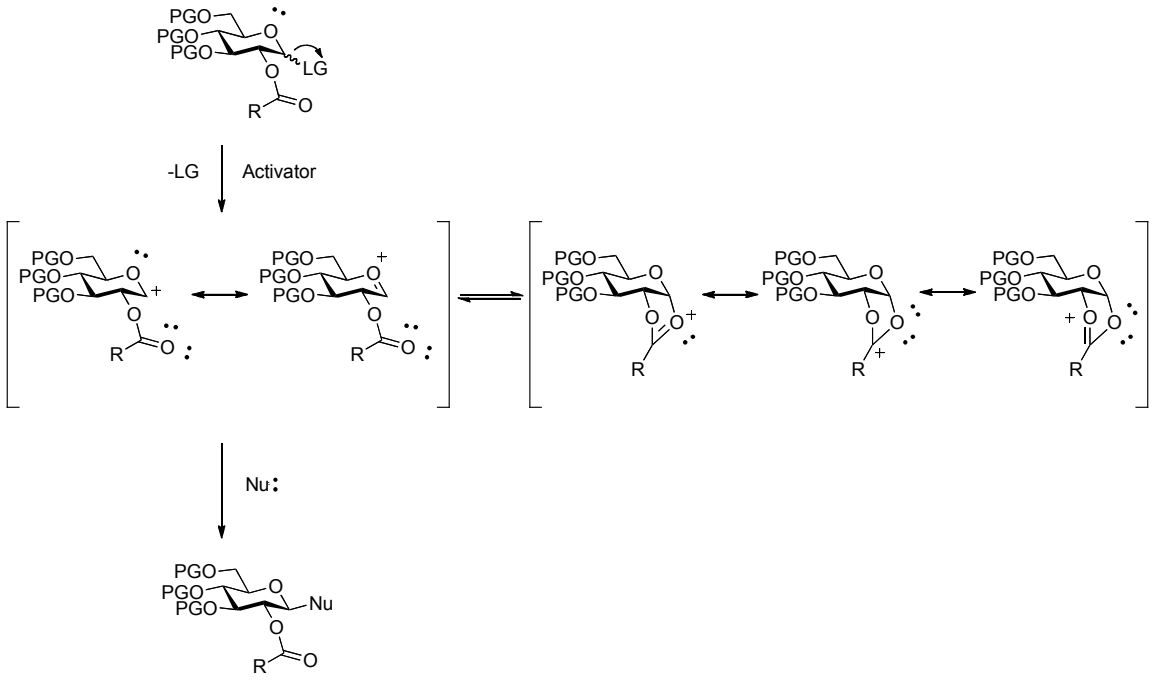



**Figure 3.3.** Neighboring group participation from an acyl substituent at O-2 of the sugar ring results in the selective formation of 1,2-*trans* glycosidic linkages.

Based on the considerations developed from our retrosynthetic plan, the synthesis of three monosaccharide building blocks **8**, **11**, and **15** was carried out (Schemes 1 and 2). The requisite ester functionality at the O-2 position of each building block was fulfilled using the trimethylacetyl group, commonly referred to as pivaloate (Piv). The sterically bulky pivaloate group was chosen because it is less likely to cause transacylation of the glycosyl acceptor, a common side reaction during glycosylation reactions.[11] Benzyl ethers were chosen as permanent protecting groups, and can be cleaved by catalytic hydrogenation or alkali metal reduction at the end of the synthesis. The selection of temporary protecting groups required careful consideration. Since each building block contains a pivaloate group, any ester group we choose as a temporary protecting group must be removed under conditions that will not result in the cleavage of the pivaloate

group. One such ester group that has gained popularity in oligosaccharide synthesis in recent years is the levulinate (4-oxopentanoate, Lev) group. The levulinate ester can be formed efficiently by reaction of an alcohol with levulinic acid in the presence of the coupling reagent *N,N'*-Dicyclohexylcarbodiimide (DCC). More importantly, levulinate esters are commonly cleaved using hydrazine in pyridine and acetic acid, conditions that are compatible with acetate and pivaloate groups. Lastly, the acetate group was chosen as a temporary protecting group on the O-6 position of building block **11**. We hypothesized that cleavage of the acetate ester at O-6 using sodium methoxide in methanol would occur much more rapidly than the cleavage of a sterically hindered O-2 pivaloate group. This difference in reaction rate would allow us to selectively deblock the O-6 position for incorporation of a second sugar chain from the branch point glucose unit.

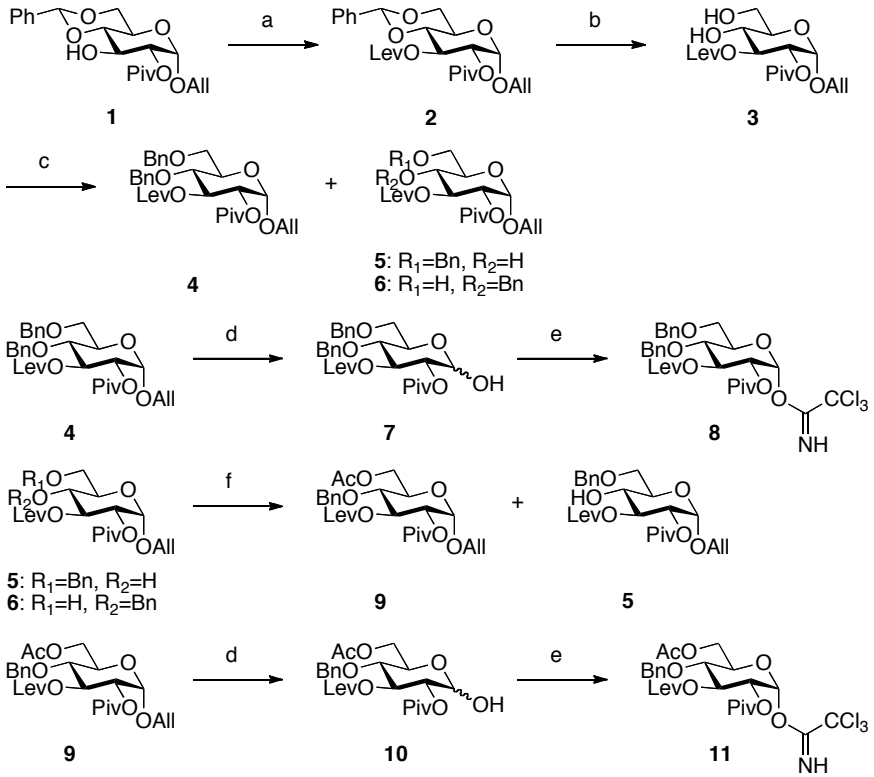
The syntheses of **8** and **11** commenced from the known glucose derivative **1**, which was prepared in three steps from D-glucose following published procedures.[12] Esterification of the O-3 hydroxyl group with levulinic acid and DCC gave rise to the crystalline derivative **2** in 87% yield. The benzylidene acetal in **2** was cleaved using aqueous TFA in dichloromethane to furnish diol **3** in nearly quantitative yield. The conversion of diol **3** to the 4,6-di-*O*-benzyl derivative **4** proved to be quite challenging. Exposure of **3** to strongly basic conditions (BnBr, NaH, DMF) resulted in an intractable mixture of products, likely due to ester group migration. Treatment of **3** with excess benzyl trichloroacetimidate and catalytic TfOH[13] did result in the formation of **4**, but low yields and time-consuming chromatographic purification steps prevented this from being a viable synthetic option. Treating **3** with Dudley's benzylating reagent, *N*-methyl 2-

benzyloxypyridinium triflate,[14] in toluene gave no reaction, even after prolonged heating. Treatment of **3** with excess benzyl bromide and silver (I) oxide in dichloromethane at ambient temperature reproducibly gave a mixture of the dibenzyl derivative **4** and monobenzyl derivatives **5** and **6**. Flash chromatography was used to separate compound **4** from the monobenzyl derivatives **5** and **6**. In this manner, the desired dibenzyl derivative **4** could be obtained in 35-40% yield. The monobenzyl derivatives **5** and **6** were isolated as a mixture, generally in 55-60% yield. The mixture of **5** and **6** could be resubjected to the benzylation conditions to produce additional **4** in low yield. Attempts to facilitate complete conversion of **3** to **4** by using more forcing conditions ($Ag_2O$, BnBr, DMF, heat) resulted in significant decomposition of the starting material and were not pursued further. Nevertheless, gram quantities of **4** could be obtained by the mild silver oxide-mediated benzylation of **3**. Allyl ether **4** was subjected to iridium-catalyzed olefin isomerization[15] followed by hydrolysis of the resulting vinyl ether to give the reducing sugar **7** in 70% yield. Lactol **7** was treated with trichloroacetonitrile in the presence of $Cs_2CO_3$ to generate the trichloroacetimidate **8**, solely the α isomer, in 82% yield.

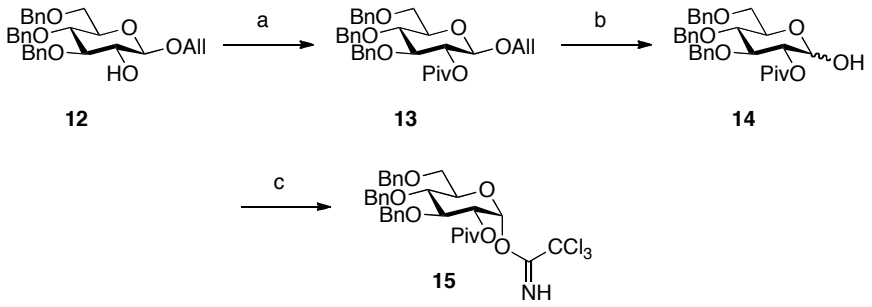
The mixture of mono-*O*-benzylated derivatives **5** and **6** obtained from the benzylation of **3** were difficult to separate chromatographically. Rather than performing this separation, the mixture of **5** and **6** was treated with acetyl chloride and 2,6-lutidine in dichloromethane at -78 °C, conditions that selectively acetylate primary alcohols in the presence of secondary alcohols.[16] Following this reaction, the 6-*O*-acetyl derivative **9** was easily separated from the unreacted secondary alcohol **5** by flash chromatography. The allyl ether in **9** was cleaved via olefin isomerization and hydrolysis of the resulting 1-

propenyl ether to furnish reducing sugar **10** in 91% yield. Lactol **10** was then converted to its trichloroacetimidate derivative **11**, only the α anomer, in 84% yield.

Next, the capping building block **15** was synthesized. The known alcohol **12**[17] was protected as its pivaloate ester **13** in 86% yield. The allyl ether was cleaved to give lactol **14**. Treatment of **14** with trichloroacetonitrile and $Cs_2CO_3$ provided the desired trichloroacetimidate **15** in 82% yield.

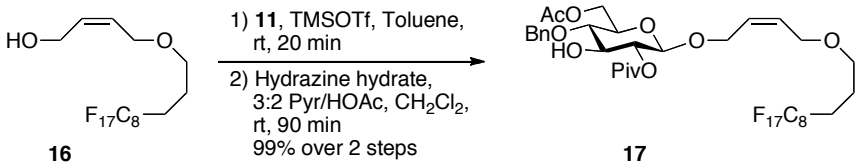



**Scheme 1.** Synthesis of linear 1,3-glucan building block **8** and 3,6-branch point building block **11**. a) Levulinic acid, DCC, DMAP,$CH_2Cl_2$, 0 °C to rt, overnight, 87%; b) 60% TFA (aq.), $CH_2Cl_2$, 0 °C to rt, 1.5 h, 97%; c) BnBr, $Ag_2O$, $CH_2Cl_2$, rt, 40 h, 37% (**4**), 60% (**5** & **6**); d) $[Ir(COD)(PMePh_2)_2]PF_6$, $H_2$, THF, rt, then $HgCl_2$, HgO, acetone, $H_2O$, rt, 70% for **7**, 91% for **10**; e) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt, 82% for **8**, 84% for **11**; f) AcCl, 2,6-lutidine, $CH_2Cl_2$, -78 °C, 1h, 40% (**9**), 56% (**5**).




**Scheme 2.** Synthesis of capping building block **15**. a) PivCl, pyr, DMAP, rt, 42 h, 86%; b) $[Ir(COD)(PMePh_2)_2]PF_6$, $H_2$, THF, rt, 18 h, then $HgCl_2$, HgO, acetone, $H_2O$, rt, 2 h, 88%; c) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt, 82%.

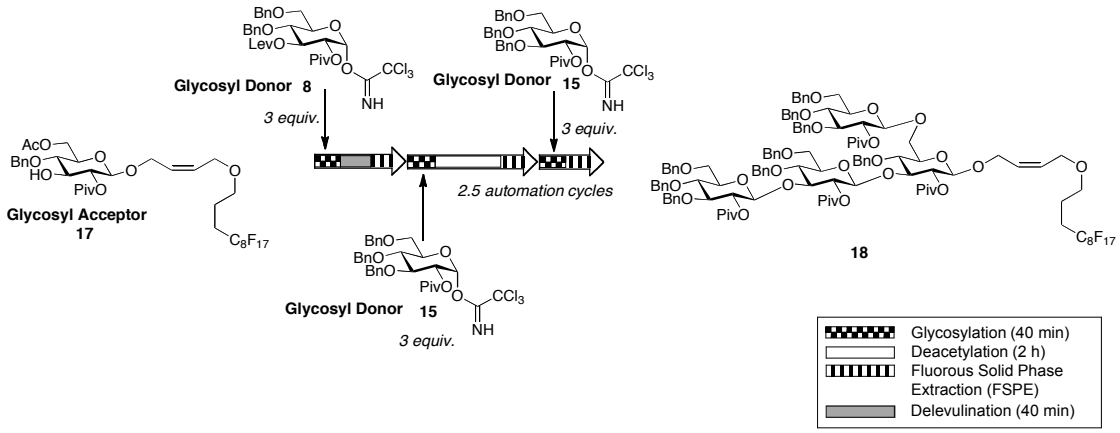
Fluorous tagged monosaccharide **17** was synthesized as an initial glycosyl acceptor for further chain elongation on the Chemspeed ASW-1000 synthesizer (Scheme 3). The fluorous tag provides a handle for the facile purification of synthetic intermediates by fluorous solid-phase extraction (FSPE). The alkene-containing fluorous alcohol **16**[18] was glycosylated with branch point glucosyl donor **11** in toluene in the presence of TMSOTF. The crude product was directly treated with hydrazine hydrate in pyridine and acetic acid to cleave the 3-*O*-levulinoyl group, and the product purified by fluorous solid-phase extraction to provide alcohol **17** in 99% yield over 2 steps.




**Scheme 3.** Synthesis of fluorous-tagged branch point glucose acceptor **17.**

With the fluorous-tagged acceptor **17** and the requisite monosaccharide building blocks **8** and **15** in hand, we set out to develop an automated synthesis of the branched β-glucan tetrasaccharide fragment **18** on the Chemspeed ASW-1000 automated synthesis workstation (Scheme 4). As shown in Scheme 4, a typical automated synthesis cycle consists of three main components: a glycosylation task, a deprotection task, and a

fluorous solid-phase extraction (FSPE) task. In the first glycosylation task, solutions of the acceptor and donor are transferred to a reaction vial, followed by the addition of a solution of the activating agent, TMSOTf. Following the designated reaction period, the reaction is quenched by the addition of triethylamine and the volatiles evaporated. Initially, we thought it would be necessary to purify the glycosylation product by FSPE prior to the deprotection step. However, the presence of spent glycosyl donor and any salts present in the crude glycosylation product did not affect the deprotection reactions. Thus, the crude product following the glycosylation cycle is directly subjected to the deprotection reagent, obviating an FSPE purification between the glycosylation and deprotection cycles. Following the deprotection cycle, the crude product is purified by FSPE. The eluate containing the desired fluorous compound is then returned to a reaction vial, where the solvent is evaporated prior to the next glycosylation-deprotection cycle. Using the basic synthetic protocols developed by other group members for the synthesis of linear mannose and rhamnose oligomers as a starting point, a program was developed to perform the synthesis of tetrasaccharide **18**.




**Scheme 4.** Automated synthesis of branched tetrasaccharide **18**.

The synthesis of **18** was then carried out on the ASW-1000 platform. To complete the synthesis, 2.5 automated cycles were necessary. In the first glycosylation cycle, toluene solutions of acceptor **17** and donor **8** were delivered to a clean reaction vial. The reactor block was cooled to 0 °C. A solution of TMSOTf in $CH_2Cl_2$ was added to the reaction mixture, which was vortexed for 40 minutes at 0 °C. A small aliquot was withdrawn from the reaction mixture and dispensed to a 96-well plate for analysis by TLC. The reaction was quenched by the addition of triethylamine, and the solvents were evaporated from the reaction mixture. In order to avoid excessive bumping of the solvent during the evaporation cycle, the ASW-1000 was programmed to incrementally reduce the pressure over several specified time intervals. Next, the delevulination cycle was carried out. The initial optimization of the ASW-1000 carried out by other group members was done using acetate protecting groups. As a result, the deprotection cycle was modified to reflect the shorter time required for levulinate cleavage as compared to that for deacetylation. In addition, the evaporation temperature was increased from 40 °C to 60 °C to facilitate complete evaporation of the less volatile pyridine/acetic acid mixture. A 1.0M solution of hydrazine hydrate in a 3:2 (v/v) mixture of pyridine and acetic acid was delivered to the crude glycosylation product, along with $CH_2Cl_2$ as a cosolvent. The mixture was vortexed for 40 minutes, and a sample of the reaction mixture delivered to the 96-well plate for TLC analysis. The excess hydrazine was destroyed by the addition of acetone, and the solvents removed under reduced pressure at 60 °C. DMF was added, and the mixture vortexed to dissolve the crude product. The solution of the product was loaded onto a 2 gram FSPE cartridge located in the SPE rack. The nonfluorous compounds were eluted to waste using 80% aqueous MeOH. Next, the

desired fluorous product was eluted into a vial located directly below the SPE block by using 100% MeOH. Because the ASW-1000 is equipped with a bevel-tipped septum-piercing needle for solution transfers, we initially experienced problems with incomplete transfer of the purified product from the vial below the SPE rack to the next reaction vial. By switching from a flat-bottomed SPE collection vial to a conical-bottomed vial, we were able to achieve more complete transfer of the purified product to the next reaction vial. Toluene was added to the reaction vial, and the solvents evaporated. Two additional toluene addition/evaporation cycles were carried out to facilitate the azeotropic removal of water from monosaccharide acceptor prior to the next glycosylation reaction.

The second synthetic cycle was carried out in a similar fashion as the first cycle. Glucosyl donor **15** was used to install the chain-terminating glucose unit. During the deprotection step, the 6-*O*-acetyl group on the reducing-end glucose unit was cleaved using 0.5M NaOMe in MeOH for 2 hours at room temperature. No evidence of cleavage of the 2-*O*-pivaloyl groups was observed based on TLC analysis. Following FSPE and evaporation of the solvent, the final glucose monomer was introduced using building block **15**. After evaporation of the solvent and a final FSPE purification, the crude product was subjected to silica gel flash chromatography to yield tetrasaccharide **18** in 9.4% overall yield over 5 chemical steps, resulting in an average per step yield of 62%.

## Conclusion

The automated synthesis of tetrasaccharide **18** described in this chapter provides experimental support for a number of hypotheses proposed at the onset of this work. First, the C-3 and C-6 hydroxyl groups of branch point glucose unit could be successfully

differentiated by using a levulinate protecting group at O-3 and an acetate group at O-6. A sugar chain could be extended from the O-3 position of the branch point sugar unit, and capped using glucose building block **15**. Second, the 6-*O*-acetyl group on the branch point sugar unit could be selectively cleaved in the presence of several 2-*O*-pivaloyl groups, providing a reactive hydroxyl group for further chain extension. The synthetic utility of the monosaccharide building blocks **8**, **11**, and **15** was also demonstrated in terms of both their reactivity and stereoselectivity as glycosyl donors. Furthermore, the general automation protocols developed during initial optimization of the ASW-1000 platform proved to be applicable to the synthesis of branched glucan fragments. Only slight modifications of the deprotection cycles were needed to accommodate the additional deprotection reagents and conditions needed for the synthesis of branched structures. A single set of conditions for the automated fluorous solid-phase extraction cycle could be used regardless of the oligosaccharide being produced, demonstrating that fluorous-fluorous interactions are indeed strong enough for reliable phase separation of organic and fluorinated compounds.

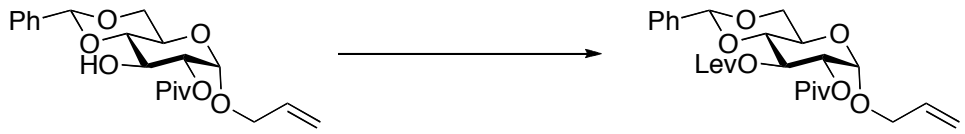
## Experimental Section

### General Materials and Methods

Reaction solvent dichloromethane was obtained from a commercial solvent purification tower or distilled from calcium hydride. Reaction solvents tetrahydrofuran and toluene were obtained from a commercial solvent purification tower. Dowex 50W X-8 ($H^+$) resin was washed repeatedly with MeOH prior to use. Powdered 4Å molecular sieves were

purchased from Aldrich Chemical Company and were activated by heating in a furnace at 250 °C overnight prior to use. All other reagents were used as received without further purification. Reactions were monitored and the $R_f$ values determined using analytical TLC with Sorbent Technologies silica gel plates (60F-254). Developed TLC plates were visualized by immersion in 10% ethanolic sulfuric acid solution, or in acidic *p*-anisaldehyde solution, followed by heating on a hot plate. Moisture-sensitive reactions were run in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature for reactions performed at elevated or sub-ambient temperatures. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Manual flash chromatography was performed using Sorbent Technologies silica gel (60F-254), and the solvent systems outlined in the experimental procedures. Automated flash chromatography was carried out on a Biotage SP-1 chromatography workstation fitted with either a Biotage 12M or 25M silica gel cartridge, using ethyl acetate/hexane gradients as outlined in the experimental procedures. $^{1}$H NMR and $^{13}$C NMR spectra were obtained with a Bruker DRX400 at 400 MHz and 101 MHz, respectively. $^{1}$H NMR spectra were reported in parts per million (δ) relative to $CDCl_3$, $CD_3OD$, $D_2O$, or TMS (7.27 ppm, 4.84 ppm, 4.81 ppm, and 0.00 ppm, respectively) as internal references. $^{13}$C NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ or $CD_3OD$ (77.23 ppm or 49.15 ppm, respectively). For $^{13}$C NMR spectra obtained in $D_2O$, chemical shifts are reported relative to acetone as an internal standard (215.94 and 30.89 ppm).

Synthetic Procedures




**1** **2**

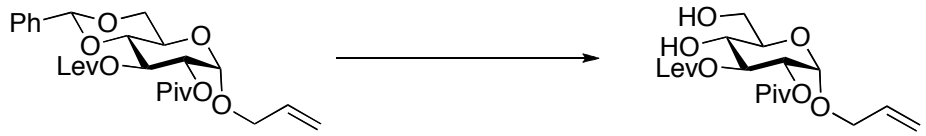
**Allyl 4,6-*O*-benzylidene-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (2).** Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-D-glucopyranoside[12] (**1**) (4.083 g, 10.4 mmol) was dissolved in dichloromethane (75 mL) and cooled to 0 °C. *N,N'*-dicyclohexylcarbodiimide (2.57 g, 12.48 mmol) and levulinic acid (1.59 mL, 15.6 mmol) were added to the solution. The reaction mixture was then allowed to warm to ambient temperature. After 2 h, additional *N,N'*-dicyclohexylcarbodiimide (400 mg, 1.9 mmol) and levulinic acid (0.5 mL, 5.0 mmol) were added. The mixture was stirred overnight at ambient temperature, then filtered over Celite. The filtrate was diluted with dichloromethane and washed successively with $NaHCO_3$, water, and brine, then dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel (2:8 EtOAc/hexanes) to give the product contaminated with a small amount of starting material (4.71 g). Recrystallization from 1:10 EtOAc/hexanes gave the product **2** as long colorless needles (3.92 g, 77%). The mother liquor was concentrated under reduced pressure and recrystallized under the same conditions to give a second crop of crystals (508 mg, 10%).

**$R_f$**: 0.72 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.5-7.3 (m, 5H, Ar-H), 5.88-5.79 (m, 1H, $CH_2$=C*H*$CH_2O$), 5.66 (t, $J_{3,2}$=$J_{3,4}$ 10.0 Hz, 1H, H-3), 5.51 (s, 1H, PhC*H*), 5.32 (m, 1H, C$H_a$$H_b$=$CHCH_2O$), 5.28 (m, 1H, $CH_a$$H_b$=$CHCH_2O$), 5.10 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1), 4.84-4.80 (dd, $J_{2,1}$= 3.6, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.31-4.27 (dd, $J_{6,6'}$= 10.0, $J_{6,5}$= 5.2 Hz, 1H, H-6), 4.22-4.17 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 4.00-3.93 (m, 2H, $CH_2$=$CHCH_a$$H_b$O and H-5), 3.76 (t, $J_{6',6}$=$J_{6',5}$ 10.0 Hz, 1H, H-6'), 3.65 (t, $J_{4,5}$=$J_{4,3}$ 10.0 Hz, 1H, H-4), 2.80-2.65 (m, 2H, $CH_3COCH_2CH_2COO$), 2.63-2.52 (m, 2H, $CH_3COCH_2CH_2COO$), 2.13 (s, 3H, $CH_3COCH_2CH_2COO$), 1.19 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.2, 178.1, 171.7, 137.2, 133.4, 129.2, 128.4, 126.4, 118.1, 101.7, 95.8, 79.4, 71.6, 69.3, 69.0, 62.7, 39.0, 38.0, 30.0, 28.0, 27.1.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 513.2101; Found: 513.1945.




**2** **3**

**Allyl 3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (3).**

Allyl 4,6-*O*-benzylidene-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**2**) (1.96 g, 4.00 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. Aqueous trifluoroacetic acid (60% v/v, 5.0 mL) was added dropwise, the ice bath removed, and the mixture was allowed to warm to ambient temperature with stirring. After 1.5 h, saturated aqueous $NaHCO_3$ solution (20 mL) was carefully added. The layers separated, and the organic layer was diluted with dichloromethane, washed successively with saturated

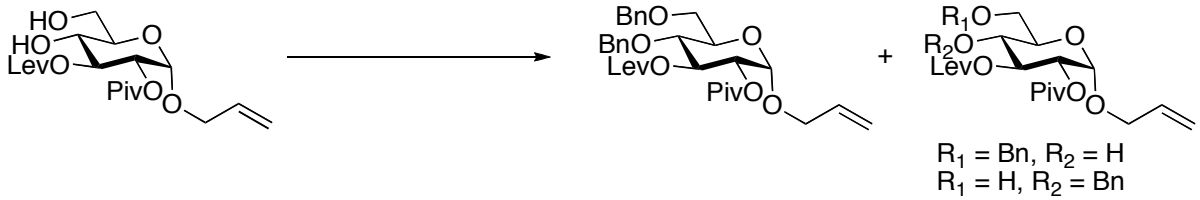
$NaHCO_3$ solution, water, and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue subjected to flash chromatography on silica gel (30% EtOAc/hexanes→100% EtOAc). The product **3** was obtained as a colorless syrup (1.56 g, 3.88 mmol, 97%).

**$R_f$**: 0.46 (100% EtOAc)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.88-5.79 (m, 1H, $CH_2$=C*H*$CH_2O$), 5.47-5.42 (m, 1H, H-3), 5.32-5.27 (m, 1H, C*H*$_a$H$_b$=CH$CH_2O$), 5.20-5.16 (m, 1H, $CH_a$*H*$_b$=CH$CH_2O$), 5.07 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.76-4.73 (dd, $J_{2,1}$= 3.6, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.21-4.17 (m, 1H, $CH_2$=CHC*H*$_a$H$_b$O), 4.00-3.94 (m, 1H, $CH_2$=CHCH$_a$*H*$_b$O), 3.87 (m, H-6, 2H, H-6') 3.78 (m, 2H, H-4, H-5), 3.30-2.90 (br s, 2H, 2×O*H*), 2.89-2.70 (m, 2H, $CH_3COCH_2CH_2COO$), 2.65-2.44 (m, 2H, $CH_3COCH_2CH_2COO$), 2.19 (s, 3H, $CH_3COCH_2CH_2COO$), 1.17 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 208.0, 178.0, 173.0, 133.5, 117.8, 95.1, 73.6, 71.4, 70.8, 69.6, 68.7, 62.0, 38.9, 38.3, 30.0, 28.2, 27.1.

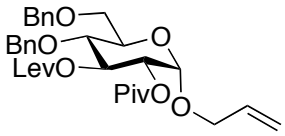
**HRMS-ESI (m/z)**: [*M*+Na]$^+$ calcd: 425.1788; found: 425.1781.




**3** **4** **5** **6**

**Allyl 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (4).**

Commercial silver (I) oxide (6.00 g, 25.8 mmol) was added to a solution of allyl 3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**3**) (2.60 g, 6.46 mmol) and benzyl bromide (3.07 mL, 25.8 mmol) in dichloromethane (50 mL). The reaction vessel was wrapped in aluminum foil to exclude light, and the mixture was stirred for 40 h at ambient temperature. After this reaction period, TLC analysis (30% EtOAc in hexanes) showed the presence of the di-*O*-benzylated derivative **4** ($R_f$= 0.54) and two mono–*O*-benzylated derivatives **5** and **6** ($R_f$= 0.18 and 0.26, respectively). The reaction mixture was filtered over Celite and the solvent removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (10% EtOAc/hexanes → 100% EtOAc). Unreacted benzyl bromide was eluted first, followed by compound **4** as a colorless syrup (1.38 g, 2.36 mmol, 37%). Lastly, a mixture of compounds **5** and **6** was eluted. This mixture, a white semisolid (1.91 g, 3.88 mmol, 60%), was carried on to the next step without further purification efforts.




**4**

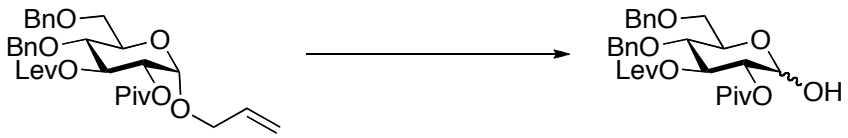
**$R_f$**: 0.54 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.14 (m, 10H, Ar-H), 5.86-5.77 (m, 1H, $CH_2$=C*H*$CH_2O$), 5.61 (t, $J_{3,2}$=$J_{3,4}$ 9.6 Hz, 1H, H-3), 5.30-5.23 (m, 1H, C$H_a$$H_b$=$CHCH_2O$), 5.17-5.12 (m, 1H, $CH_a$$H_b$=$CHCH_2O$), 5.08 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.77-4.73 (dd, $J_{2,1}$= 3.6, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.70-4.45 (m, 4H, 2×Ph$CH_2$), 4.18-4.11 (m, 1H, $CH_2$=$CHCH_a$$H_b$O), 3.97-3.90 (m, 1H, $CH_2$=$CHCH_a$$H_b$O), 3.90-3.85 (m, 1H, H-6), 3.81-3.74 (m, 2H, H-4, H-6'), 3.69-3.64 (dd, $J_{5,4}$= 11.0, $J_{5,6}$= 2.0 Hz, 1H, H-5), 2.64-2.60 (m,

2H, $CH_3COCH_2CH_2COO$), 2.46-2.41 (m, 2H, $CH_3COCH_2CH_2COO$), 2.14 (s, 3H, $CH_3COCH_2CH_2COO$), 1.16 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.3, 178.0, 171.8, 138.1, 138.0, 133.6, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 127.2, 117.7, 95.1, 76.1, 74.6, 73.8, 72.6, 71.4, 70.3, 68.6, 68.3, 65.5, 38.9, 37.8, 30.1, 28.1, 27.1.

**HRMS-ESI (m/z)**: calcd: 605.2727; found: 605.2805 $[M+Na]^+$.




**4** **7**

**4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (7).**

To a mixture of allyl 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**4**) (337 mg, 0.58 mmol) and (1,5-cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (5 mg, 5.8 μmol) was added anhydrous THF (3.0 mL) under nitrogen atmosphere. The red suspension was degassed under vacuum, then placed under hydrogen atmosphere for a few minutes until the mixture became a colorless, homogeneous solution. The solution was degassed under vacuum once more, then stirred at room temperature under nitrogen for 24 h. The solvent was removed in vacuo and the crude vinyl ether was dissolved in 10:1 acetone/water (6 mL). Red mercuric oxide (157 mg, 0.72 mmol) was added, followed by the dropwise addition of a solution of mercuric chloride (157 mg, 0.58 mmol, in 2 mL 10:1 acetone/water). The mixture was stirred

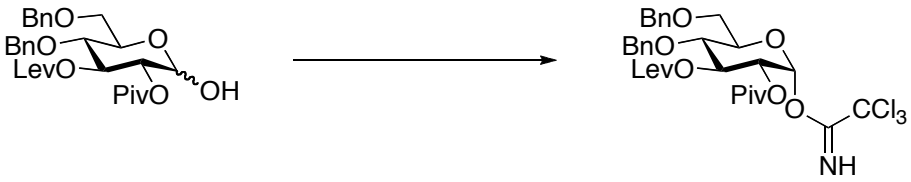
overnight at ambient temperature, then filtered on Celite. The filtrate was partially concentrated under reduced pressure, then diluted with dichloromethane. The solution was washed successively with saturated aqueous KI solution (30 mL) and brine (30 mL), and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (1:2 EtOAc/hexanes) to provide **7**, a ~6:1 mixture of α- and β-anomers, as a white solid (219 mg, 0.40 mmol, 70% from allyl ether).

**$R_f$**: 0.30 and 0.25 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz, assignments for the major isomer)** δ (ppm) 7.38-7.12 (m, 10H, Ar-H), 5.61 (t, $J_{3,2}$=$J_{3,4}$ 10.0 Hz, 1H, H-3), 5.41 (t, $J_{1,2}$= $J_{1,\text{-OH}}$=3.6 Hz, 1H, H-1), 4.78-4.74 (dd, $J_{2,1}$= 3.2, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.69-4.45 (m, 4H, 2×PhC$H_2$), 4.12-4.09 (m, 1H, H-5), 3.80-3.53 (m, 3H, H-4, H-6, H-6'), 3.10 (d, $J_{1,\text{-OH}}$= 3.2Hz, 1H, -O*H*), *2*.62-2.56 (m, 2H, $CH_3COCH_2CH_2COO$), 2.49-2.40 (m, 2H, $CH_3COCH_2CH_2COO$), 2.14 (s, 3H, $CH_3COCH_2CH_2COO$), 1.16 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.3, 178.0, 171.8, 138.0, 137.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 90.5, 76.3, 74.6, 73.8, 72.2, 71.7, 70.1, 68.5, 38.9, 37.9, 30.1, 28.3, 27.1.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 565.2414; Found: 565.2485.




**7** **8**

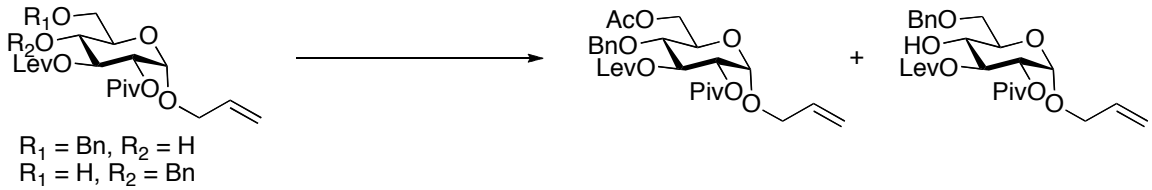
**4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (8).** Trichloroacetonitrile (0.38 mL, 3.8 mmol) and cesium carbonate (340 mg, 1.05 mmol) were added to a solution of 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**7**) (515 mg, 0.95 mmol) in dichloromethane (6 mL). The reaction mixture was stirred at ambient temperature for 21 h, then filtered over Celite. The solvent was removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (100:30:1 EtOAc/hexanes/$Et_3N$) to give the product, almost exclusively the α-anomer, as a light yellow syrup (535 mg, 0.78 mmol, 82%).

**$R_f$**: 0.58 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.60 (s, 1H), 7.36-7.17 (m, 10H, Ar-H), 6.54 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 5.67 (t, $J_{2,3}$= $J_{3,4}$= 10.0 Hz, 1H, H-3), 5.07-5.03 (dd, $J_{2,3}$= 10.0 Hz, $J_{1,2}$= 3.6 Hz, 1H, H-2), 4.66-4.47 (m, 4H, PhC$H_2$ ×2), 4.08-4.04 (m, 1H, H-5), 3.94 (t, $J_{3,4}$= $J_{4,5}$= 9.6 Hz, 1H, H-4), 3.83-3.79 (dd, $J_{6,6'}$ = 11.2 Hz, $J_{6,5}$ = 2.8 Hz, 1H, H-6), 3.71-3.68 (dd, $J_{6,6'}$ = 11.2 Hz, $J_{6',5}$ = 2.0 Hz, 1H, H-6'), 2.65-2.60 (m, 2H, $CH_3COCH_2CH_2COO$), 2.46-2.43 (m, 2H, $CH_3COCH_2CH_2COO$), 2.16 (s, 3H, $CH_3COCH_2CH_2COO$), 1.12 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.0, 177.6, 171.7, 160.9, 137.8, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 93.5, 75.3, 75.0, 73.7, 73.2, 72.1, 70.2, 67.8, 38.9, 37.7, 30.0, 27.9, 27.0.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 710.1568; Found: 710.1480.

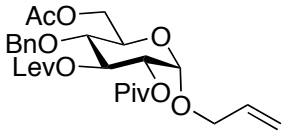



**5** **9** **6**
**6**

**Allyl 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (9).**

A mixture of allyl 4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**6**) and allyl 6-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**5**) (1.91 g, 3.88 mmol, obtained from the synthesis of **4**) was dissolved in dichloromethane (40 mL). 2,6-Lutidine (0.54 mL, 4.7 mmol) was added, and the mixture cooled to -78 °C. Acetyl chloride (0.17 mL, 2.3 mmol) was added, and the reaction mixture stirred at -78 °C. After 1h, TLC analysis showed the complete conversion of **6** to the 6-*O*-acetyl product **9**, and **5** remained unchanged. The reaction was quenched by the addition of MeOH (1 mL), and the reaction mixture allowed to warm to ambient temperature. The solvent was removed under reduced pressure, and the residue partitioned between EtOAc (100 mL) and water (30 mL). The organic layer was washed successively with aqueous 2N HCl, water, saturated aqueous $NaHCO_3$, water, and brine. The organic solution was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was subjected to flash

chromatography on silica gel (30→50% EtOAc/hexanes) to give **9** as a colorless syrup (832 mg, 1.56 mmol, 40%) and unreacted **5** as a syrup (1.081 g, 2.19 mmol, 56%).




**9**

**$R_f$**: 0.67 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.26 (m, 5H, Ar-H), 5.87-5.77 (m, 1H, $CH_2$=C*H*$CH_2$O), 5.65 (dd, $J_{3,2}$=$J_{3,4}$ 10.0 Hz, 1H, H-3), 5.30-5.25 (m, 1H, C$H_a$$H_b$=CH$CH_2$O), 5.19-5.15 (m, 1H, $CH_a$$H_b$=CH$CH_2$O), 5.03 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.75-4.72 (dd, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, 1H, H-2), 4.66 (d, $J$ = 11.2 Hz, 1H, PhC$H_a$$H_b$O), 4.55 (d, $J$ = 11.2 Hz, 1H, Ph$CH_a$$H_b$O), 4.33-4.29 (dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 2.4 Hz, 1H, H-6), 4.27-4.23 (dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.0 Hz, 1H, H-6') 4.18-4.12 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 3.98-3.91 (m, 2H, $CH_2$=CH$CH_a$$H_b$O and H-5), 3.63 (t, $J_{3,4}$=$J_{4,5}$= 9.6 Hz, 1H, H-4), 2.72-2.59 (m, 2H, $CH_3$COC$H_2$$CH_2$COO), 2.51-2.47 (m, 2H, $CH_3$CO$CH_2$C$H_2$COO), 2.16 (s, 3H, C$H_3$CO$CH_2$$CH_2$COO), 2.07 (s, 3H, C$H_3$COO), 1.17 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.1, 178.0, 171.7, 170.8, 137.6, 133.4, 128.6, 128.3, 128.2, 117.9, 95.0, 75.9, 74.6, 72.5, 71.2, 68.8, 68.7, 62.8, 38.9, 37.8, 30.0, 28.1, 27.1, 21.0.

**HRMS-ESI (m/z)**: [*M*+Na]$^+$ Calcd: 557.2363; Found: 557.2417.


**9** **10**

**6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (10).**

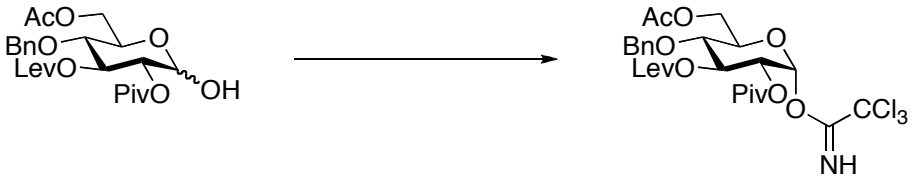
To a mixture of allyl 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**9**) (564 mg, 1.05 mmol) and (1, 5-cyclooctadiene)bis (methyldiphenylphosphine)iridium(I) hexafluorophosphate (9 mg, 10.5 μmol) was added anhydrous THF (8.0 mL) under nitrogen atmosphere. The red suspension was degassed under vacuum, then placed under hydrogen atmosphere until the mixture became a colorless, homogeneous solution (~1 minute). The solution was degassed under vacuum once more, then stirred at room temperature under nitrogen for 18 h. The solvent was removed in vacuo and the crude vinyl ether was dissolved in 9:1 (v/v) acetone/water (10 mL). Red mercuric oxide (341 mg, 1.58 mmol) was added, followed by the dropwise addition of a solution of mercuric chloride (313 mg, 1.15 mmol, in 3 mL 9:1 (v/v) acetone/water). After stirring for 1 h at ambient temperature, additional mercuric chloride (35 mg, in 2 mL 9:1 (v/v) acetone/water) was added. The mixture was stirred for an additional 1.5 h at ambient temperature, then filtered on Celite The filtrate was partially concentrated under reduced pressure, then diluted with dichloromethane (75 mL). The solution was washed successively with aqueous KI solution and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (30→50% EtOAc/hexanes) to give the product **10**, a ~2:1 mixture of α- and β-anomers, as a syrup (472 mg, 0.95 mmol, 91% from allyl ether).

**$R_f$**: 0.55 and 0.45 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.25 (m, 5H, Ar-H), 5.69-5.64 (t, $J_{2,3}$= $J_{3,4}$= 9.6 Hz, 0.65H, H-3), 5.38-5.33 (m, 1H), 4.79-4.54 (m, 3.35H), 4.35-4.32 (m, 1H), 4.23-4.14 (m, 1.65H), 3.77 (d, $J$ = 8.4 Hz, 0.35H), 3.67-3.61 (m, 1.35H), 3.39 (br d, $J$ = 3.6 Hz, 0.65H), 2.72-2.60 (m, 2H, $CH_3COCH_2CH_2COO$), 2.50-2.46 (m, 2H, $CH_3COCH_2CH_2COO$), 2.16 (s, 3H, $CH_3COCH_2CH_2COO$), 2.06 (s, 3H, $CH_3COO$), 1.12 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.3, 206.1, 178.1, 171.8, 171.0, 137.6, 137.4, 128.7, 128.6, 128.3, 128.2, 95.8, 90.3, 75.8(2), 74.7, 74.6, 74.4, 73.6, 73.3, 72.1, 71.5, 68.5, 62.8, 39.0, 38.9, 37.8, 37.7, 30.0(2), 28.1, 28.0, 27.1, 27.0, 21.0.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 517.2050; Found: 517.2155.




**10** **11**

**6-*O*-Acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (11).** Trichloroacetonitrile (0.43 mL, 4.3 mmol) and cesium carbonate (420 mg, 1.29 mmol) were added to a solution of 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**10**) (426 mg, 0.86 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at ambient temperature for 17 h, then filtered on Celite. The solvent was removed under reduced pressure. The residue was subjected to

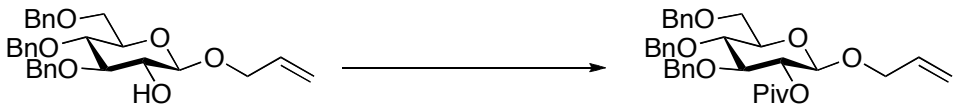
flash chromatography on silica gel (25:75:1 EtOAc/hexanes/$Et_3N$) to give the product **11**, exclusively the α-anomer, as a colorless syrup that solidified upon standing (459 mg, 0.72 mmol, 84%).

**$R_f$**: 0.39 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.63 (s, 1H, N-H), 7.37-7.26 (m, 5H, Ar-H), 6.50 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.72 (t, $J_{3,2}$=$J_{3,4}$=9.6 Hz, 1H, H-3), 5.06-5.03 (dd, $J_{2,1}$= 3.6, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.71 (d, $J$ = 11.2 Hz, 1H, PhC$H_a$H$_b$O), 4.58 (d, $J$ = 11.2 Hz, 1H, PhCH$_a$$H_b$O), 4.35-4.32 (dd, $J_{6,6'}$= 12.4, $J_{6,5}$= 2.0 Hz, 1H, H-6), 4.25-4.21 (dd, $J_{6,6'}$= 12.4, $J_{6,5}$= 4.0 Hz, 1H, H-6') 4.15-4.13 (m, 1H, H-5), 3.77 (t, $J_{3,4}$=$J_{4,5}$= 9.6 Hz, 1H, H-4), 2.72-2.61 (m, 2H, $CH_3COCH_2CH_2COO$), 2.54-2.47 (m, 2H, $CH_3COCH_2CH_2COO$), 2.17 (s, 3H, $CH_3COCH_2CH_2COO$), 2.04 (s, 3H, $CH_3COO$), 1.13 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 206.0, 177.7, 171.7, 170.7, 160.9, 137.3, 128.8, 128.6, 128.4, 93.1, 75.1, 75.0, 72.2, 71.5, 70.1, 62.3, 39.0, 37.7, 30.0, 28.0, 27.1, 21.0.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 662.1116; Found: 662.1225.




**12** **13**

**Allyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (13).** Allyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside[17] (**12**) (1.69 g, 3.45 mmol), was dissolved in pyridine (15 mL). Pivaloyl chloride (0.47 mL, 3.80 mmol) was added, and the mixture stirred for 17 hours at ambient temperature. After this period, TLC indicated the reaction was not yet

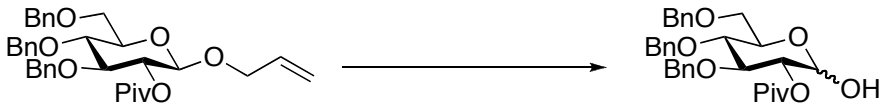
complete. To the mixture, 4-dimethylaminopyridine (DMAP, 20 mg) and pivaloyl chloride (0.47 mL) were added. After another 5.5 h, another aliquot of pivaloyl chloride (0.47 mL) was added. The mixture was stirred for an additional 20 h at ambient temperature. After this period, TLC indicated that the reaction was complete. The reaction was quenched by the addition of MeOH (3 mL). The solvent was removed under reduced pressure. The residue was partitioned between EtOAc and water. The organic layer was washed with 2N HCl, water, saturated aqueous $NaHCO_3$, water, and brine. The organic solution was dried over $Na_2SO_4$, and the solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel (20% EtOAc/hexanes) to give **13** as a colorless syrup (1.70 g, 2.97 mmol, 86%).

**$R_f$**: 0.86 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.13 (m, 15H, Ar-H), 5.86-5.80 (m, 1H, $CH_2$=C*H*$CH_2O$), 5.28-5.23 (m, 1H, C$H_a$$H_b$=$CHCH_2O$), 5.17-5.14 (m, 1H, $CH_a$$H_b$=$CHCH_2O$), 5.11-5.08 (m, 1H, H-2), 4.78-4.51 (m, 6H, 3×Ph$CH_2$) 4.43 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.37-4.32 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 4.06-4.01 (m, 1H, $CH_2$=$CHCH_a$$H_b$O), 3.77-3.68 (m, 4H, H-3, H-4, H-6, H-6'), 3.52-3.50 (m, 1H, H-5), 1.19 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 176.9, 138.3, 138.3, 138.1, 133.8, 128.5 (3), 128.1, 127.9 (2), 127.8, 127.7, 127.6, 117.4, 100.3, 83.4, 78.0, 75.4, 75.1 (2), 73.7, 73.2, 70.0, 68.9, 39.0, 27.3.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 597.2828; Found: 597.2945.




**13** **14**

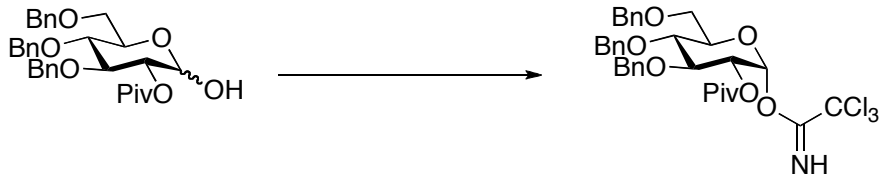
**3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-D-glucopyranose (14).** To a mixture of allyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**13**) (1.65 g, 2.88 mmol) and (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (24 mg, 28.8 μmol) was added anhydrous THF (15.0 mL) under nitrogen atmosphere. The red suspension was degassed under vacuum, then placed under hydrogen atmosphere until the mixture became a colorless, homogeneous solution (~1 minute). The solution was degassed under vacuum once more, and stirred at ambient temperature under nitrogen for 18 h. The solvent was removed in vacuo, and the residual crude vinyl ether was dissolved in 10:1 (v/v) acetone/water (30 mL). Red mercuric oxide (873 mg, 4.03 mmol) was added, followed by the dropwise addition of a solution of mercuric chloride (860 mg, 3.17 mmol, in 10 mL 10:1 (v/v) acetone/water). The mixture was stirred for 2 h at ambient temperature, and filtered on Celite. The filtrate was partially concentrated under reduced pressure, then diluted with dichloromethane (80 mL). The solution was washed successively with aqueous KI solution (15 mL), water (30 mL), saturated sodium thiosulfate solution (20 mL), water (30 mL), and brine (30 mL), and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The residue was subjected to flash chromatography on silica gel (25% EtOAc/hexanes) to give the compond **14**, a ~3:1 mixture of α- and β-anomers, as a semisolid (1.35 g, 2.53 mmol, 88% from allyl ether).

$\mathbf{R}_f$: 0.49 and 0.42 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.32-7.09 (m, 15H, Ar-H), 5.42 (m, 0.75H), 4.83-4.73 (m, 3.75H), 4.62-4.47 (m, 3.5H), 4.10-4.05 (m, 1.5H), 3.40-3.70 (m, 1.5H), 3.65-3.59 (m, 2H), 3.55-3.49 (m, 0.25H), 3.41-3.37 (m, 0.75H), 1.21 and 1.20 (2s, 9H total, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 178.0, 138.6, 138.2, 138.0, 137.9, 128.6, 128.5, 128.1(2), 128.0(2), 127.9(2), 127.8, 127.6, 127.5, 96.3, 90.5, 82.7, 79.8, 77.9, 75.9, 75.5, 75.4, 75.2, 74.1, 73.7, 73.6, 70.3, 68.9, 68.6, 39.1, 38.9, 27.3(2).

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 557.2515; Found: 557.2613.




**14** **15**

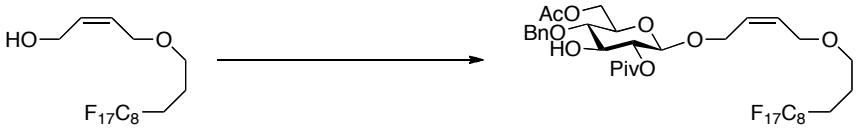
**3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (15).** Trichloroacetonitrile (1.24 mL, 12.3 mmol) and cesium carbonate (1.21 g, 3.70 mmol) were added to a solution of 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-D-glucopyranose (**14**) (1.32 g, 2.47 mmol) in dichloromethane (20 mL). The reaction mixture was stirred at ambient temperature for 17 h, followed by filtration through a pad of Celite. The solvent was removed under reduced pressure, and the residue was subjected to flash chromatography on silica gel (20:80:1 EtOAc/hexanes/$Et_3N$) to give **15**, exclusively the α-anomer, as a colorless syrup (1.37 g, 2.02 mmol, 82%).

$\mathbf{R_f}$: 0.74 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.59 (s, 1H, N-H), 7.33-7.12 (m, 15H, Ar-H), 6.58 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 5.14-5.10 (dd, $J_{2,1}$ = 3.6, $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.87-4.48 (m, 6H, 3×PhC$H_2$), 4.14-4.11 (t, $J$ = 9.6 Hz, 1H, H-3), 4.08-4.04 (m, 1H, H-5), 3.92-3.86 (t, $J$ = 10.0 Hz, 1H, H-4), 3.82-3.78 (dd, $J$ = 11.2 Hz, 2.0Hz, 1H, H-6) 3.71-3.65 (m, 1H, H-6'), 1.16 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 177.6, 160.9, 138.3, 138.0, 128.6(2), 128.3, 128.1(2), 127.9, 127.8, 127.6, 115.5, 93.9, 79.9, 75.6, 73.7, 73.5, 72.5, 68.0, 56.9, 39.0, 27.3.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 702.1582; Found: 702.1730.




**16** **17**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl-6-*O*-acetyl-4-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (17).** *Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl alcohol (**16**[18]) (44.4 mg, 0.081 mmol) and 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**11**) (72.0 mg, 0.113 mmol) were combined in a round-bottomed flask. Toluene (3 x 3 mL) was placed in the flask and evaporated under reduced pressure. The flask was then placed under vacuum for 90 min. Anhydrous toluene (3 mL) was then added to the flask, which was stirred for 10 min at ambient temperature to allow for complete dissolution of

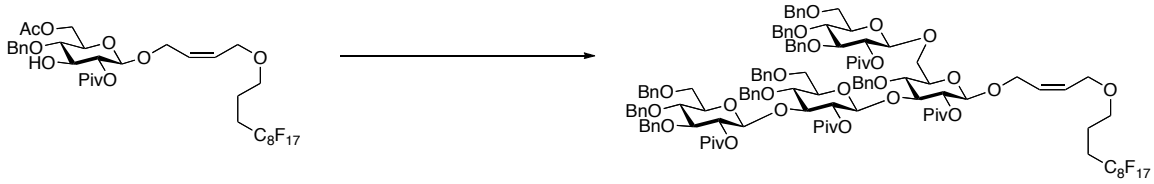
the reagents. TMSOTf (1 drop, approximately 10 μL) was then added to the reaction mixture. After 20 min, the reaction was quenched with triethylamine (0.l mL). The solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (1 mL). To this solution, hydrazine hydrate (3.20 mL of a 0.5 M solution in 3:2 pyridine/acetic acid, 1.62 mmol) was added. The mixture was stirred for 90 min at ambient temperature, then quenched by the addition of acetone (1 mL). The solvent was removed under reduced pressure. The residue was dissolved in DMF (0.8 mL) and adsorbed onto a fluorous solid-phase extraction cartridge (5 g). Non-fluorous compounds were eluted with 80% MeOH/water (24 mL) and the desired product was eluted with 100% MeOH (24 mL). The fraction containing the fluorous product was concentrated under reduced pressure to give pure **17** as a colorless syrup (75 mg, 0.080 mmol, 99%).

**$R_f$:** 0.47 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.37-7.28 (m, 5H), 5.72-5.62 (m, 2H, vinylic H), 4.86 (d, $J$ = 11.2 Hz, 1H, PhC$H_a$H$_b$O), 4.77-4.72 (dd, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 4.68 (d, $J$ = 11.2 Hz, 1H, PhCH$_a$$H_b$O), 4.45 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.40-4.31 (m, 2H, H-6 and sugar-OC$H_a$H$_b$=CHCH$_2$O-(fluoroalkane)), 4.25-4.16 (m, 2H, H-6' and sugar-OCH$_a$$H_b$=CHCH$_2$O-(fluoroalkane)), 4.02-4.00 (m, 2H, sugar-OCH$_a$H$_b$=CHC$H_2$O-(fluoroalkane), 3.80 (t, $J_{2,3}$= $J_{3,4}$=10.0 Hz, 1H, H-3), 3.68 (t, $J_{4,5}$= $J_{3,4}$=9.2 Hz, 1H, H-3), 3.55-3.49 (m, 4H, H-4, H-5, OC$H_2$CH$_2$CH$_2$CF$_2$), 2.66 (br d, $J$ = 3.2 Hz, O-$H$), 2.26-2.10 (m, 2H, OCH$_2$CH$_2$C$H_2$CF$_2$), 2.05 (s, 3H, C$H_3$COO), 1.90-1.83 (m, 2H, OCH$_2$C$H_2$CH$_2$CF$_2$), 1.22 (s, 9H, $t$-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 178.9, 170.9, 137.9, 130.2, 128.8, 128.5, 128.3(2), 99.7, 77.9, 76.7, 75.0, 74.4, 73.0, 69.0, 66.6, 64.8, 63.1, 39.1, 27.2, 21.0.

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 949.2220; Found: 949.2339.




**17** **18**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl-4-O-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-3-*O*-((4,6-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranosyl)-β-D-glucopyranoside (18).** The β-glucan fragment tetrasaccharide (**18**) was synthesized from fluorocarbon-tagged glucose monosaccharide acceptor (**17**) via three coupling and two deprotection cycles using the Chemspeed ASW1000 synthesizer. The specific reaction conditions are outlined in the table:

| Step | Task | Reagent/Operation | Time |
|---|---|---|---|
| 1 | Glycosylation | 3 eq. donor **8** (150 μmol) in 1.0 mL toluene, 1 eq. F-tagged acceptor **17** (50 μmol) in 0.5 mL toluene, 0.1 eq. TMSOTf (0.027 M in DCM), 0 $^oC$ | 40 min |
| 2 | TLC Sample | 20 μL of crude reaction mixture withdrawn | |
| 3 | Quenching | 0.5 mL triethylamine | |
| 4 | Evaporation | 40 $^oC$ | 45 min |
| 5 | Delevulinoylation | 20 eq. Hydrazine hydrate (1.0 M in 3:2 pyr/HOAc), DCM, rt | 40 min |
| 6 | TLC Sample | 20 μL of crude reaction mixture withdrawn | |
| 7 | Quenching | 0.5 mL acetone | |

| | | | |
|---|---|---|---|
| 8 | Evaporation | 50 °C | 45 min |
| 9 | FSPE Sample Preparation | 0.4 mL DMF transferred to reaction vial | |
| 10 | Sample Loading | 0.7 mL crude sample transferred to F-SPE cartridge | |
| 11 | Elution | 4.7 mL 80% methanol wash | |
| 12 | Elution | 1.5 mL methanol wash (repeated 3 times) | |
| 13 | Transfer | 4.7 mL collected sample to clean reaction vial | |
| 14 | Transfer/ Evaporation | 2 mL toluene added, then evaporated at 50 °C (repeated 3×) | 45 min × 3 |
| 15 | Glycosylation | Donor **15** (150 μmol) in 1.0 mL toluene, F-tagged acceptor from cycle 1, 0.1 eq. TMSOTf (0.027 M in DCM), 0 °C | 40 min |
| 16 | TLC Sample | 20 μL of crude reaction mixture withdrawn | |
| 17 | Quenching | 0.5 mL triethylamine | |
| 18 | Evaporation | 40 °C | 45 min |
| 19 | Deacetylation | 0.5 M NaOMe in MeOH, MeOH, rt | 2 h |
| 20 | TLC Sample | 20 μL of crude reaction mixture withdrawn | |
| 21 | Quenching | 0.5 M AcOH in MeOH | |
| 22 | Evaporation / FSPE | Same as steps 8-14 | |
| 23 | Glycosylation | Donor **15** (150 μmol) in 1.0 mL toluene, F-tagged acceptor from cycle 2, TMSOTf (0.027 M in DCM), 0 °C | 40 min |
| 24 | TLC Sample | 20 μL of crude reaction mixture withdrawn | |
| 25 | Quenching | 0.5 mL triethylamine | |
| 26 | Evaporation / FSPE | Same as steps 8-12 | |

**Table 3.1**. Synthetic procedure for the automated synthesis of tetrasaccharide (**18**).

Following the final F-SPE procedure, the conical-bottomed extraction vial containing the crude tetrasaccharide product was removed from the ASW1000. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (15→40% EtOAc/hexanes) to yield tetrasaccharide **8** (11.0 mg, 4.7 μmol, 9.4% overall yield) as a colorless syrup.

**$R_f$**: 0.45 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.11 (m, 45H, Ar-H), 5.68-5.63 (m, 1H, vinylic H), 5.56-5.51 (m, 1H, vinylic H), 5.10-4.88 (m, 5H), 4.82-4.40 (m, 20H), 4.25-3.98 (m, 8H), 3.80-3.40 (m, 18H), 3.24 (t, 1H, $J$ = 8.6 Hz), 2.22-2.04 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.86-1.78 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.25 (s, 9H, (*t*-BuCO), 1.17 (s, 9H, *t*-BuCO), 1.15 (s, 9H, *t*-BuCO), 1.12 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 177.5, 176.8, 176.6, 176.4, 138.6, 138.5, 138.4, 138.3, 138.2(2), 138.1, 138.0, 130.6, 128.8, 128.7, 128.6(2), 128.4(2), 128.3, 128.1(2), 128.0, 127.9, 127.8, 127.7, 101.2 (C-1), 99.6 (C-1), 99.3 (C-1), 99.0 (C-1), 83.6, 83.4, 78.4, 77.9, 76.5, 76.4, 75.8, 75.7, 75.4, 75.2, 75.1, 74.7, 74.5, 73.9, 73.7, 73.6, 73.5, 73.0, 69.3, 68.9, 68.8, 68.3, 66.7, 64.3, 39.0(2), 38.9, 38.8, 29.9, 28.2, 27.6, 27.4, 27.3(2).

**HRMS-ESI (m/z)**: $[M+Na]^+$ Calcd: 2365.9181; Found: 2365.7170.

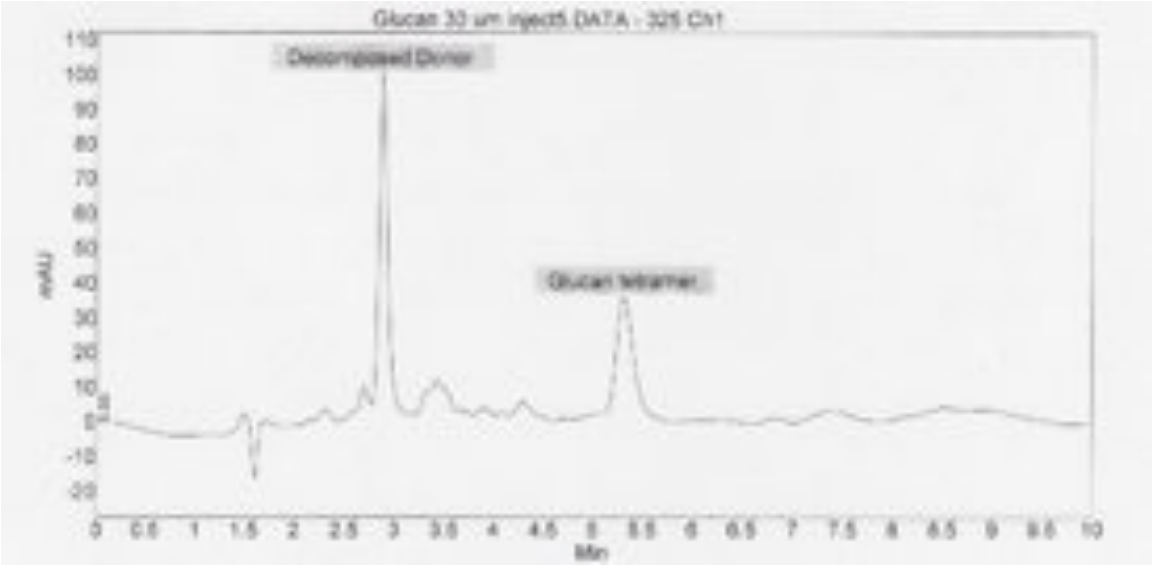



**Figure 3.4**. Analytical HPLC chromatogram of crude glucan tetramer (**18**) (1 mL/min flowrate, 20% ethyl acetate/hexane, 10 minute run).

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# CHAPTER 4

## Automated Solution-Phase Synthesis of Branched and Linear Beta-Glucan Fragments

Abstract

Beta-glucans are oligosaccharides made up of repeating beta-linked D-glucose monomer units. These compounds are found in many living systems, including bacteria, plants, and fungi. A considerable degree of variation is observed in terms of branching pattern, molecular weight, solubility, and solution conformation among beta-glucans isolated from different species. The beta-glucans have garnered increasing attention in recent years due to their interesting biological properties. The immune-stimulatory properties of beta-glucans have been studied considerably in recent years. However, the lack of availability of homogeneous, structurally well-characterized beta-glucans has limited the elucidation of the structure-function relationships of these compounds. Iterative chemical synthesis provides a means of generating pure, well-characterized samples of beta-glucans for structure-function studies. By its very nature, iterative oligosaccharide synthesis lends itself well to automation, as a computer-controlled robot is capable of executing repetitive, rote operations quite successfully. This chapter discusses the automated solution-phase syntheses of branched and linear beta-glucan fragments via an iterative approach using solely monosaccharide building blocks. The presence of a single perfluorinated tag provides a handle for the facile purification of synthetic intermediates by automated fluorous solid-phase extraction (FSPE). Three new glucosyl trichloroacetimidate building blocks were synthesized in the course of this work.

The synthetic utility of each building block was demonstrated by assembling a branched glucan trisaccharide and three linear glucan trisaccharides on the Chemspeed ASW1000 automated synthesis workstation.

Introduction

Beta-glucans, oligosaccharides comprised of repeating D-glucose units joined by beta-glycosidic linkages, occur naturally in a number of living things, including plants, bacteria, yeasts, and fungi. This structurally diverse class of biomolecules has been shown to possess antitumor activity, cholesterol-lowering properties, and the ability to stimulate the innate immune system.[1] Several beta-glucan receptors have been discovered in vertebrates, including the transmembrane protein Dectin-1.[2] Dectin-1 is the primary glucan receptor present on white blood cells.[3] This protein can induce a number of cellular responses to fungal pathogens. Despite the many discoveries made about Dectin-1, further research is needed to gain an increased understanding of role this glucan receptor plays in the immune response to fungal pathogens. Chemical synthesis provides a means of preparing a library of structurally diverse beta-glucans that can be screened for binding with Dectin-1 in order to determine its ligand specificity.

Many examples of syntheses of beta-glucan structures have been reported in the literature. For example, Larsson and coworkers prepared tetra- and pentasaccharides related to the capsular polysaccharide of *S. pneumoniae*.[4] Kong and coworkers have reported a number of syntheses of 3,6-branched β-D-glucose oligosaccharides.[5a-e] Similarly, He and coworkers synthesized branched glucose tetra-, penta-, and hexasaccharides, relying on a key 3,6-branched trisaccharide trichloroacetimidate donor

for the installation of branch points.[6] For the most part, these syntheses rely upon convergent approaches for the synthesis of branched glucans. In contrast, Jamois and coworkers published the assembly of linear β-(1,3)-glucans by an iterative approach using a single monosaccharide building block.[7]

The iterative approach to oligosaccharide synthesis offers the freedom to incorporate any desired monosaccharide residue into a growing oligosaccharide chain at any point in the synthesis. Since glycosyl donors must be used in excess to ensure high glycosylation yields, considerable amounts of these building blocks end up in the waste bin. This is especially problematic when the building blocks are extremely costly to prepare, as is generally the case with di-, tri-, and higher oligosaccharide donors. Despite its advantages, iterative oligosaccharide synthesis involves multiple cycles of time-consuming and labor-intensive glycosylation and deprotection steps. By automating these rote, repetitive tasks, the amount of manual labor involved in oligosaccharide synthesis could be greatly reduced.

The fields of proteomics and genomics have progressed significantly as a resulting of the automation of peptide and nucleic acid synthesis based on solid-phase synthetic methodologies. Researchers in need of a particular peptide or nucleic acid sequence can access the Internet and purchase these compounds from a number of online vendors. However, commercial custom oligosaccharide synthesis is not yet a reality. Following Bruce Merrifield's development of solid-phase peptide synthesis in the 1960s,[8] other researchers began to apply this approach to the synthesis of oligosaccharides. The Frechet and Schuerch reported the first example of solid-phase carbohydrate synthesis in 1971.[9] Decades later, Plante and coworkers reported the automated solid-phase synthesis

of oligosaccharides using a modified peptide synthesizer.[10] However, several key limitations have prevented the widespread use of solid-phase oligosaccharide synthesis. Large excesses of sugar building blocks (10-20 equivalents per coupling cycle) must be used to overcome the kinetic limitations of biphasic glycosylation reactions. Furthermore, reaction monitoring on the solid phase and cleavage of the sugar chain from the solid support are often difficult. The development of an alternative phase-switching approach that combines the ease of purification associated with solid phase synthesis with the advantages of solution-phase kinetics and reaction monitoring would represent a significant advancement in automated oligosaccharide synthesis.

Results and Discussion

The primary objective of this study was to prepare a small library of branched and linear beta-glucan fragments for NMR binding studies with a soluble form of the glucan-binding protein Dectin-1. In addition to the 3,6 branched beta-glucans such as those found in fungal species, we are also interested in studying the binding affinity of linear beta-glucan fragments of varying linkage types. Our synthetic targets in this work were the 3,6-branched trisaccharide **1** and the linear 1,3-, 1,4-, and 1,6-glucans **2**, **3**, and **4** (Figure 4.1). Retrosynthetic analysis of branched glucan **1** shows that this trisaccharide can be assembled from the capping building block **5,** the branch point building block **6**, and the alkene-containing fluorous alcohol **7**[11] (Figure 4.2). The linear glucans **2**, **3**, and **4** can be assembled using monosaccharide building blocks **8**, **9**, and **10**, respectively (Figure 4.3). Each glucosyl trichloroacetimidate building block possesses a pivaloate ester substituent at O-2 to provide stereochemical control of each newly formed glycosyl

linkage through neighboring group participation. Building blocks **6**, **8**, and **9** feature levulinate esters as temporary masking groups. The levulinate ester

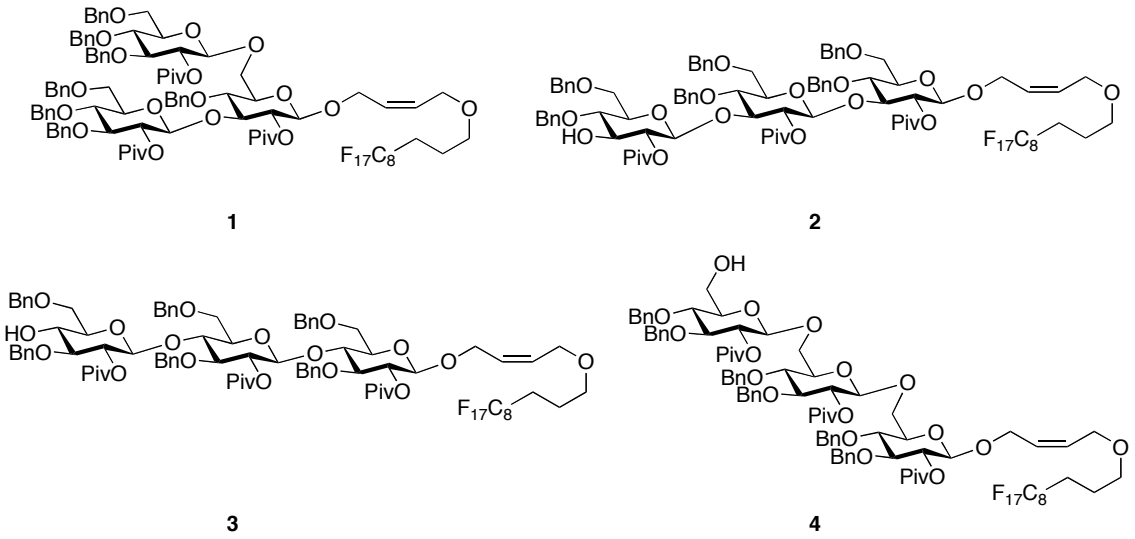



**Figure 4.1.** Beta-Glucan Synthetic Targets

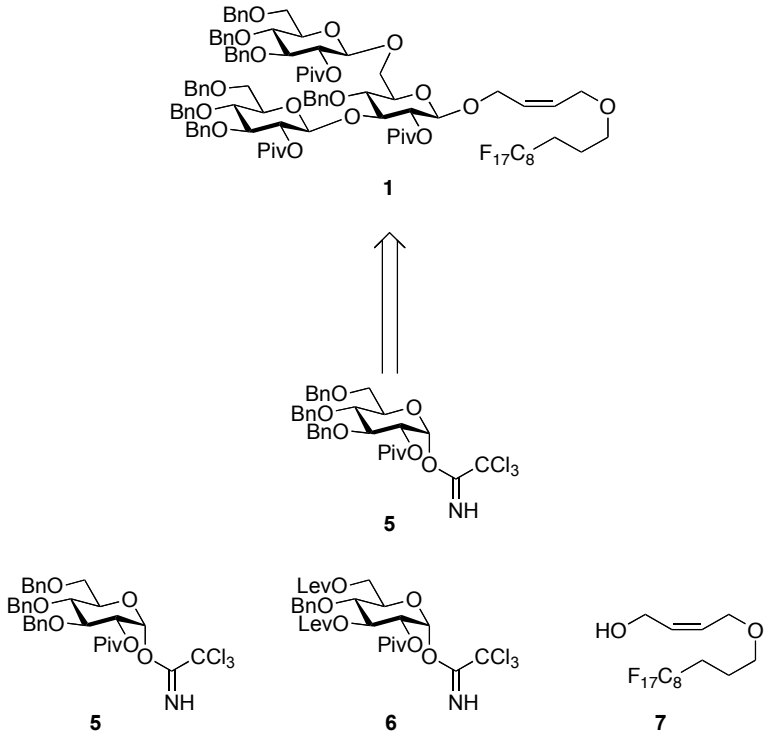



**Figure 4.2.** Retrosynthesis of branched 1,3-glucan trisaccharide **1**.

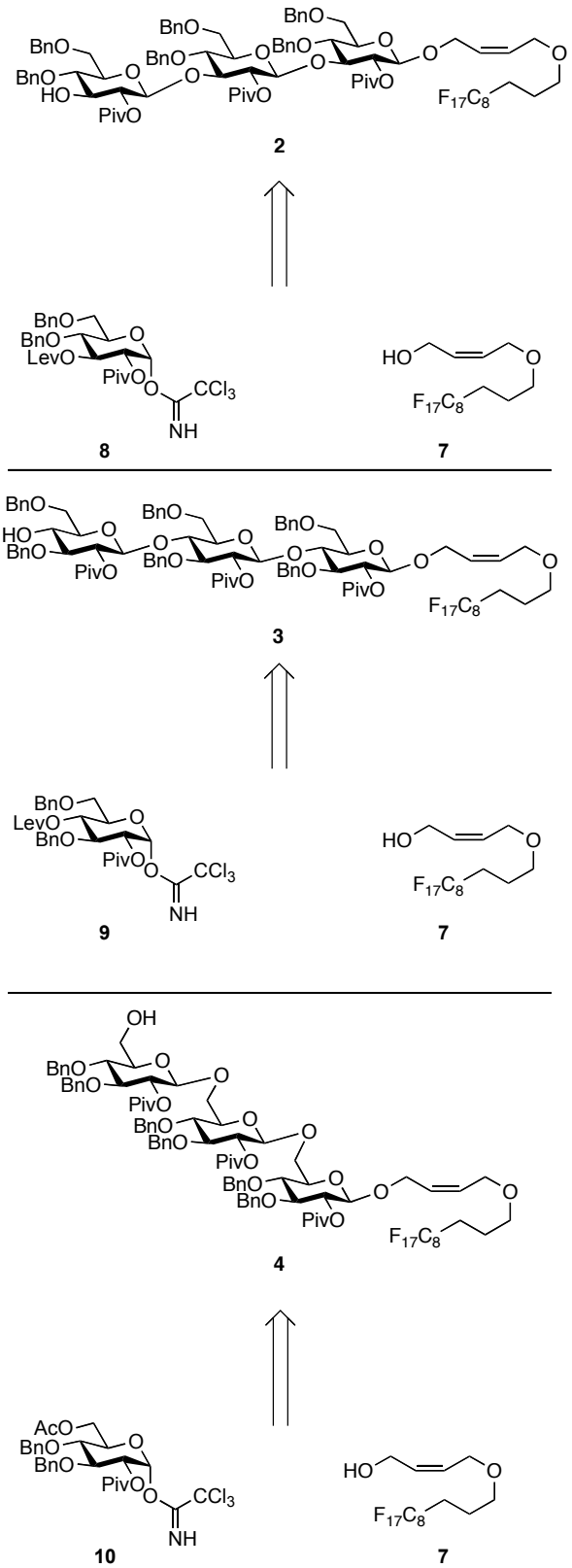
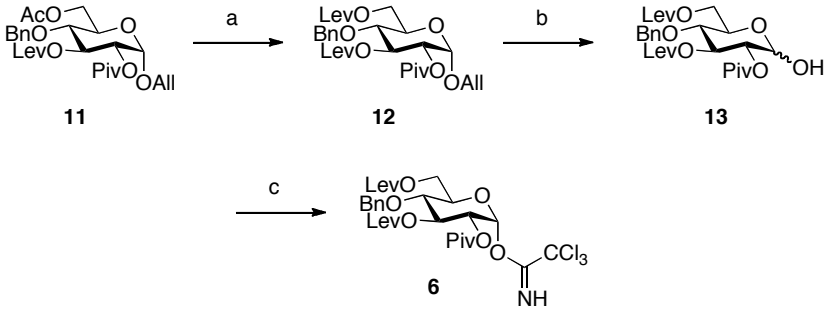



**Figure 4.3.** Retrosynthesis of Linear Glucans **2**, **3**, and **4**.

can be cleaved selectively in the presence of other ester groups using hydrazine hydrate in pyridine and acetic acid at ambient temperature. Building block **10** possesses an acetyl group at the O-6 position. We postulated that the rate of cleavage of an acetate ester at the O-6 position using sodium methoxide in methanol would be much faster than the rate of cleavage of the sterically less accessible pivaloate ester at the O-2 position. This difference in reaction rate would allow us to selectively cleave the acetate ester to generate the desired primary alcohol for further extension of the saccharide chain.

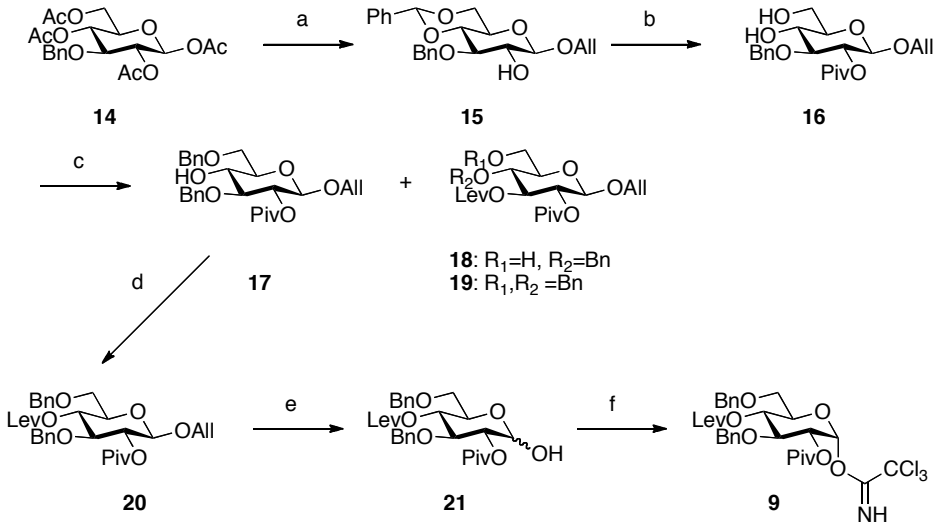
The syntheses of building blocks **5** and **8** were outlined in Chapter 3 of this dissertation. The synthesis of branch point building block **6** commenced from the 6-*O*-acetyl derivative **11**, a building block intermediate discussed in Chapter 3 (Scheme 1). Selective cleavage of the 6-*O*-acetyl group in **11**, followed by esterification of the resulting alcohol with levulinic acid in the presence of *N,N'*-Dicyclohexylcarbodiimide (DCC), gave rise to the crystalline 3,6-di-*O*-levulinoyl derivative **12** in 72% yield over two steps. A two-step, one-pot procedure was used for the cleavage of the allyl ether in **12**. Compound **12** was converted to the corresponding 1-propenyl ether via iridium-catalyzed olefin isomerization.[12] Water and iodine were added directly to the reaction mixture, resulting in hydrolysis of the 1-propenyl ether to give compound **13** in 80% yield over two steps. The reducing sugar **13** was then treated with trichloroacetonitrile in the presence of cesium carbonate to generate trichloroacetimidate **6**, solely as the α-anomer, in 77% yield.




**Scheme 1.** Synthesis of 3,6-branch point building block **6**. a) NaOMe, MeOH, rt, 2 h, then levulinic acid, DCC, DMAP,$CH_2Cl_2$, rt, 1 h, 72%; b) $[Ir(COD)(PMePh_2)_2]PF_6$, $H_2$, THF, rt, 23 h, then $I_2$, THF, $H_2O$, rt, 40 min, 80%; c) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt, 77%.

The synthesis of linear 1,4-glucan building block **9** (Scheme 2) began from 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose **14**[13], which was prepared in 4 steps from D-glucose. Treatment of **14** with allyl alcohol in the presence of $BF_3{\cdot}OEt_2$ provided the crude allyl glucoside, which was directly deacetylated using NaOMe in MeOH, and subsequently benzylidenated to provide the crystalline derivative **15** in 53% yield over three steps. The hydroxyl group in **15** was protected as its pivaloate ester. The crude product was directly subjected to debenzylidenation using aqueous TFA in $CH_2Cl_2$, resulting in the formation of 4,6-diol **16**. Diol **16** was subjected to benzylation using benzyl bromide (3.0 equiv.) and commercially obtained $Ag_2O$ (3.0 equiv.) to generate a mixture of the desired 3,6-di-*O* benzyl derivative **17**, 3,4-di-*O*-benzyl derivative **18**, and 3,4,6-tri-*O*-benzyl derivative **19.** Compounds **17**, **18**, and **19** were easily separated by flash column chromatography on silica gel. The use of commercial $Ag_2O$ is critical to the success of this reaction. When freshly prepared $Ag_2O$ is used, the yield of **17** is reduced considerably due to the increased formation of tri-*O*-benzyl derivative **19**. The C-4 hydroxyl group in **17** was esterified with levulinic acid and DCC to give the fully protected intermediate **20**. The allyl ether in **20** was cleaved using the same one-pot procedure described for the synthesis of **13** resulting in the formation of the reducing

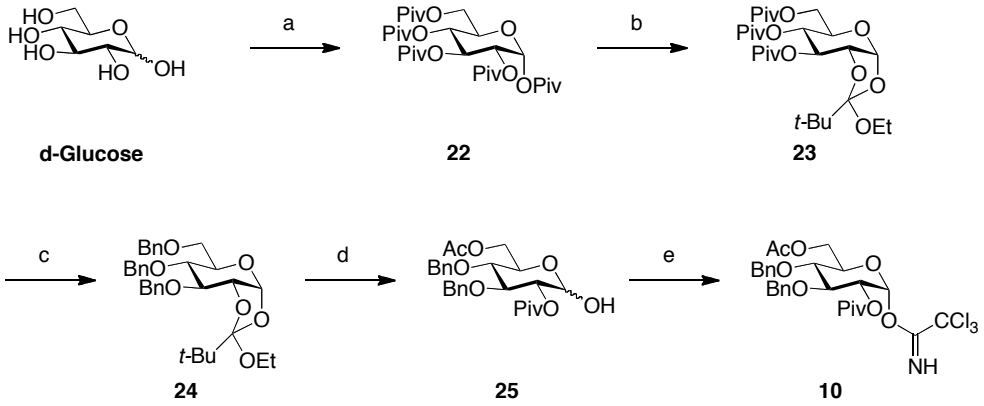
sugar **21** in 76% yield over two steps. Finally, lactol **21** was converted to the desired α-linked trichloracetimidate **9**.




**Scheme 2.** Synthesis of linear 1,4-glucan building block **9**. a) allyl alcohol, $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, 0 °C, 2.5 h, then NaOMe, MeOH, rt, 16.5 h, then $PhCH(OMe)_2$, CSA, $CH_3CN$, rt, 1 h, 53%; b) PivCl, DMAP, $Et_3N$, $CH_2Cl_2$, rt, 24 h, then 60% TFA (aq.), $CH_2Cl_2$, rt, 40 min, 78% over 2 steps; c) BnBr, $Ag_2O$, $CH_2Cl_2$, rt, 3 h, 78% for **17**, 6% for **18**, 10% for **19**; d) LevOH, DCC, DMAP, $CH_2Cl_2$, rt, 1 h, 84%; e) $[Ir(COD)(PMePh_2)_2]PF_6$, $H_2$, THF, rt, 9 h, then $I_2$, $H_2O$, THF, rt, 30 min, 76% over 2 steps; e) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt, 2.5 h, 78%.

The synthesis of linear 1,6-glucan building block **10** (Scheme 3) began from D-glucose, which was converted to the penta-*O*-pivaloate derivative **22** following the literature procedure.[14] Compound **22** was treated with HBr to yield the corresponding crude glycosyl bromide, which was treated with EtOH in the presence of $Et_3N$ and TBAB to form orthopivaloate **23**. The pivaloate esters in **23** were cleaved, and the resulting crude triol treated with BnBr and NaH in DMF to generate the 3,4,6-tri-*O*-benzyl derivative **24**. Orthoester **24** was subjected to acetolysis in the presence of a stochiometric amount of $ZnCl_2$, conditions known to selectively acetolyze a 6-*O*-benzyl group.[15] As anticipated, acetolysis of the orthoester in **24** also took place under these conditions, resulting in the formation of an α/β mixture of anomeric acetates. This crude

mixture was treated with hydrazine acetate in DMF to generate lactol **25** in good yield. Finally, the reducing sugar **25** was converted to the crystalline α-linked trichloroacetimidate **10**. This synthetic route, aided in large part by the tandem benzyl ether acetolysis and orthoester cleavage transformation used to convert **24** to **25**, provided an efficient means of generating gram quantities of building block **10**.




**Scheme 3.** Synthesis of linear 1,6-glucan building block **10**. a) PivCl, $Et_3N$, DMAP, $CH_2Cl_2$, 0 °C to rt, 16 h, 88%; b) 33% HBr in HOAc, $CH_2Cl_2$, 0 °C to rt, then EtOH, TBAB, $Et_3N$, $CH_2Cl_2$, reflux, 18 h, 75% over 2 steps; c) NaOEt, EtOH, rt, 24 h, then BnBr, NaH, DMF, 0 °C to rt, 17 h, 94% over 2 steps; d) $ZnCl_2$, $Ac_2O$, AcOH, 0 °C to rt, 3 h, then hydrazine acetate, DMF, rt, 20 h, 76% over 2 steps; e) $Cl_3CCN$, $Cs_2CO_3$, $CH_2Cl_2$, rt, 2.5 h, 64%.

Having prepared sufficient quantities of the requisite monosaccharide building blocks **5**, **6**, **8**, **9**, and **10**, our efforts were then focused on the synthesis of our target trisaccharides **1, 2**, **3**, and **4** on the Chemspeed ASW-1000 automated synthesis workstation. The ASW-1000 consists of a reactor block that contains sixteen individual reaction vials. The reactor block can be heated or cooled to achieve a desired reaction temperature, blanketed with inert gas for air- and moisture-sensitive reactions, and vortexed to provide mixing of the reagents. The reactor block is also connected to a vacuum pump to facilitate the evaporation of solvents from reaction mixtures. A robot arm fitted with a septum-piercing needle executes all liquid handling tasks on the

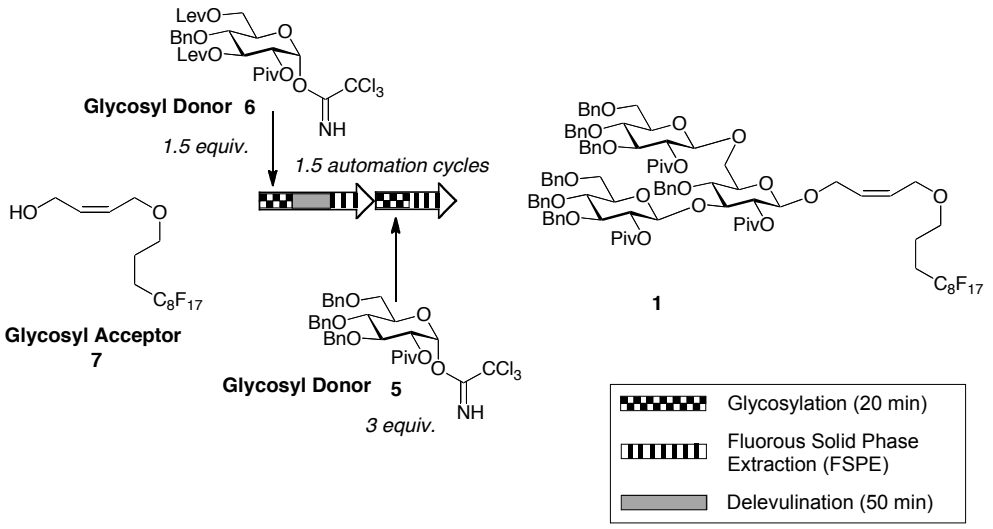
workstation. An SPE rack holds the fluorous solid-phase extraction cartridges that are used to purify our synthetic oligosaccharides after each synthetic cycle. Finally, reagent racks, including one blanketed by inert gas, provide storage for the reagent solutions needed to carry out the synthesis. Multiple parameters exist for every operation carried out by the ASW-1000, including reactor block temperature, vortexing speed, vacuum, volume of reagents transferred, and reaction time. Each of these parameters require careful attention when preparing an automated synthesis program.

The automated iterative solution-phase synthesis of oligosaccharides can be broken down into three basic tasks. Each cycle consists of a glycosylation task, a deprotection task, and a fluorous solid-phase extraction (FSPE) task. In the glycosylation task, the acceptor molecule is combined with a solution of the glycosyl trichloroacetimidate donor and vortexed to ensure that the reagents are adequately mixed. At this time, the reactor block can be heated or cooled to the desired reaction temperature. A solution of the activating reagent, trimethylsilyl trifluoromethanesulfonate (TMSOTf), is a added to initiate the glycosylation reaction. After vortexing for the desired reaction time, an aliquot of the reaction mixture is dispensed into a 96-well plate for analysis by thin-layer chromatography. The reaction is quenched by the addition of triethylamine, and the solvents evaporated. At this point, the crude glycosylation product could be purified by FSPE prior to the deprotection step. However, we have observed that the delevulination and deacetylation steps carried out in this study can be performed directly on the crude reaction mixtures present following each glycosylation step. Thus, a solution of the deprotection reagent is added to the crude glycosylation product, and the mixture vortexed for the desired reaction time. By

doing so, we can eliminate an FSPE purification step between glycosylation and deprotection cycles, thus eliminating unnecessary material transfers and reducing the amount of time needed to complete each synthesis. Following completion of the deprotection reaction, an aliquot of the reaction mixture is removed for TLC analysis. The appropriate quenching reagent is added to the reaction mixture, and the solvents removed by evaporation under reduced pressure. At this time, a small amount of solvent, typically DMF or a mixture of DMF and water, is added to the reaction vial containing the crude product. The reactor block is vortexed to dissolve the crude product, and the resulting solution loaded onto a 2-gram FSPE cartridge. The nonfluorous compounds are eluted to waste by washing with 8:2 (v/v) MeOH:$H_2O$, and then the desired fluorous-tagged product is eluted into a vial located below the FSPE cartridge using a fluorophilic solvent such as MeOH or THF. The eluate containing the fluorous compounds is transferred to a clean vessel in the reactor block, where the solvent is evaporated prior to the next synthetic cycle. These synthetic cycles are repeated in an iterative fashion until the desired sugar chain length has been achieved.

By combining the proper number of glycosylation, deprotection, and FSPE tasks, programs were written for the synthesis of each target glucan. First, the synthesis of branched glucan trisaccharide **1** was executed (Scheme 4). In contrast with the automated synthesis of the branched glucan tetrasaccharide discussed in Chapter 3, the initial glycosylation of fluorous alcohol **7** was carried out on the ASW-1000 platform, eliminating several hours of manual benchtop chemistry. Additionally, we have chosen to construct a branched structure by simultaneous extension of sugar chains from the hydroxyl groups on C-3 and C-6 of the branch point monomer unit. To begin the

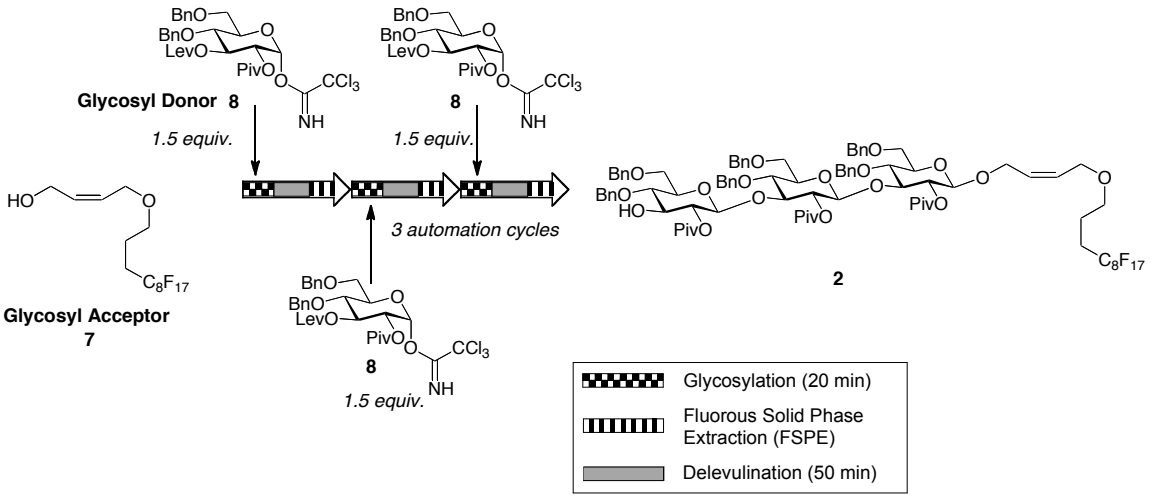
automated synthesis, toluene solutions of the flourous alcohol **7** and 3,6-di-*O*-levulinoyl building block **6** (1.5 equiv.) were delivered to the first reaction vial. Following addition of a stock solution of TMSOTf (0.1 equiv.) in $CH_2Cl_2$, the reaction was vortexed for 20 minutes at 20 °C. TLC indicated that the glycosylation reaction was complete. After evaporation of the solvents under reduced pressure at 40 °C, the delevulination reagent, 1.0 M hydrazine hydrate in 3:2 (v/v) pyridine/acetic acid, was added, along with $CH_2Cl_2$ as a cosolvent. After the fifty-minute reaction period, TLC showed that deprotection was complete. The solvents were removed in vacuo, with the temperature increased to 60 °C to facilitate evaporation of the higher-boiling solvents used in the delevulination reaction. The crude product was then dissolved in DMF and transferred to the FSPE cartridge for purification by automated fluorous solid-phase extraction. Following FSPE purification, the eluate containing the intermediate 3,6-diol monosaccharide was transferred to the next reaction vial. Toluene (0.5 mL) was added, and the solvent evaporated. Following two additional toluene addition-evaporation cycles, the acceptor was ready for the second glycosylation cycle. A toluene solution of the chain-terminating glucose building block (3.0 equiv.) was added to the reaction vial, and the mixture vortexed to ensure complete dissolution of the acceptor. The remainder of the second glycosylation cycle was carried out in the same way as the first. Again, TLC revealed that the glycosyl acceptor was completely consumed. Following evaporation of the solvent, a final FSPE cycle was carried out. The crude product was purified by column chromatography to provide the desired branched glucan **1** in 18% overall yield using only 4.5 total equivalents of monosaccharide building blocks. The average yield per step was 56%, based on the overall yield, and the synthesis was completed in only nine hours of machine time.



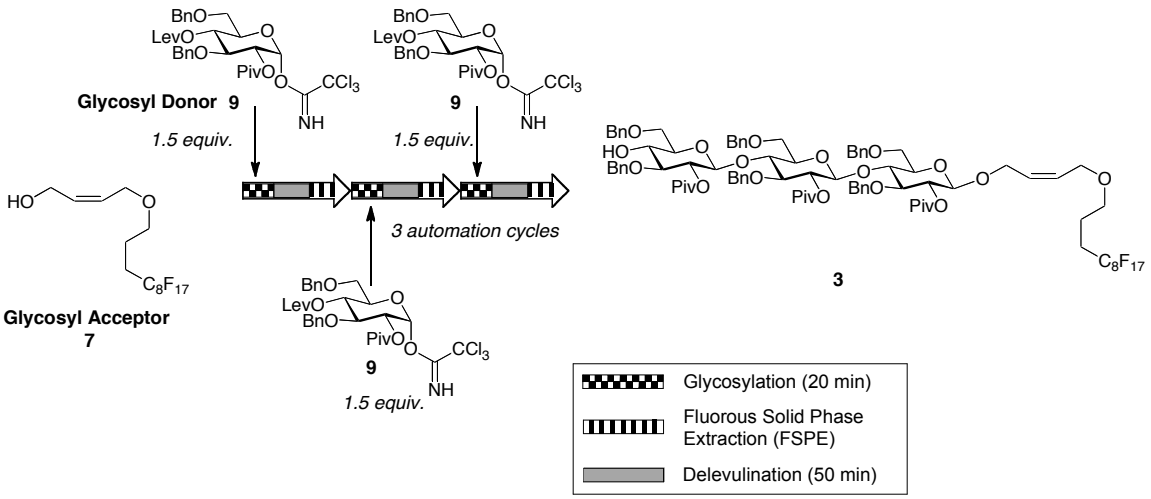

**Scheme 4.** Automated synthesis of branched trisaccharide **1**.

Following the successful synthesis of branched trisaccharide **1**, we then set out to construct the linear 1,3-glucan trisaccharide **2** (Scheme 5). The program for this synthesis was easily prepared by combining three iterations of the first synthetic cycle used for the synthesis of trisaccharide **1**, and using the linear 1,3-glucan donor **8** in place of donor **6** (Scheme 5). After three automated glycosylation/delevulination/FSPE cycles, the crude product was purified by careful silica gel column chromatography to furnish the desired 1,3-linked glucan **2** in 9.5% overall yield. Based on the overall yield, the calculated average step yield was 67%.

The program used for the synthesis of **2** was used to synthesize the 1,4-linked trisaccharide **3** (Scheme 6). The only modification was the use of building block **9** instead of building block **8**. Following completion of the synthesis, TLC analysis showed the presence of the desired trisaccharide and a small amount of the corresponding disaccharide. The mixture was purified by column chromatography and preparative TLC to furnish the desired trisaccharide **3** in 3.7% overall yield.

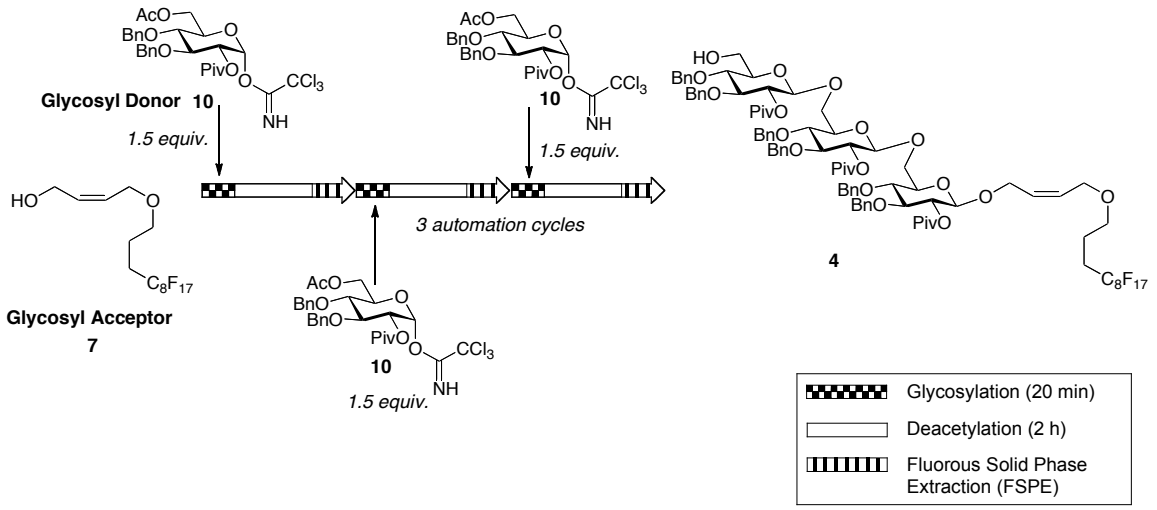



**Scheme 5.** Automated synthesis of branched trisaccharide **2**.



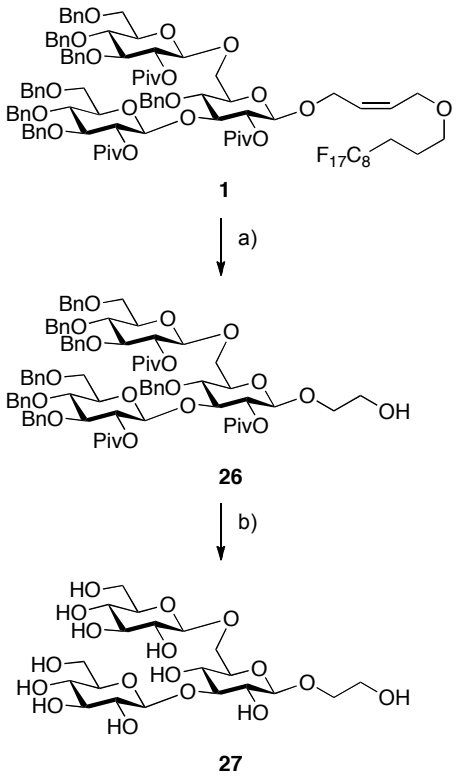

**Scheme 6.** Automated synthesis of branched trisaccharide **3**.

Next, the 1,6-linked trisaccharide **4** was synthesized (Scheme 7). The program used to synthesize trisaccharides **3** and **4** was modified to carry out acetate deprotection rather than the levulinate deprotection, and to use glycosyl donor **10**. Following three synthetic cycles, TLC analysis of the crude product showed the presence of trisaccharide **4** along with a lesser amount of the corresponding disaccharide. The compounds were separated by preparative TLC to provide the desired trisaccharide **4** in 3.8% overall yield.




**Scheme 7.** Automated synthesis of branched tetrasaccharide **4**.

Having successfully synthesized each of the target trisaccharides **1**, **2**, **3**, and **4**, our efforts then focused on the global deprotection of our synthetic glucans to generate compounds suitable for NMR binding studies. We chose to carry out the deprotection of branched trisaccharide **1** in order to demonstrate the ability to fully deprotect our synthetic glucans (Scheme 8). The fluorous tag in **1** was cleaved by ozonolysis of the alkene and subsequent reduction with sodium borohydride to generate the 2-hydroxyethyl glycoside **26** in 40% yield. The benzyl ethers were cleaved from compound **26** by catalytic hydrogenation. The crude hydrogenation product was treated with NaOMe in MeOH to cleave the three pivaloate esters, and generate the fully deprotected 2-hydroxyethyl glycoside **27** in 95% yield over two steps. These results clearly demonstrated that global deprotection of our synthetic glucans can be carried out in a straightforward fashion. In particular, the sterically bulky pivaloate ester groups could be cleaved without complications.




**Scheme 8.** Global deprotection of branched trisaccharide **1**. a) $O_3$, MeOH, $CH_2Cl_2$, -78 °C, 2 min, then $NaBH_4$, MeOH, $CH_2Cl_2$, -78 °C to rt, 14 h, 40%; b) $H_2$, Pd/C, EtOH, rt, 5 h, then NaOMe, MeOH, rt, 21.5 h, 95%.

## Conclusion

Through the completion of the research described in the chapter, a number of important discoveries have been made. First, viable synthetic routes to the monosaccharide building blocks **6**, **9**, and **10** were developed. The automated synthesis of branched trisaccharide **1** demonstrated the synthetic utility of building block **6** as a precursor to a 3,6-diol glycosyl acceptor for the simultaneous extension of two sugar chains from a single glucose residue. Furthermore, the automated syntheses of trisaccharides **3** and **4** demonstrated the synthetic utility of building blocks **9** and **10** in terms of both their reactivity and stereoselectivity in glycosylation reactions. The

synthesis of trisaccharide **4** provided support for our hypothesis that an acetate group at O-6 of the glucose ring could be selectively cleaved in the presence of an O-2 pivaloate group, as no evidence of pivaloate cleavage was observed. Additionally, only slight modifications of a computer program were required to carry out the syntheses of all four target trisaccharides. A single protocol for the automated fluorous solid-phase extraction cycle was used for all four syntheses discussed in this chapter, demonstrating that fluorous-fluorous interactions are robust enough to provide reliable phase separation of organic and fluorous compounds on the automation platform. Lastly, through the preparation of compound **27**, we have shown that global deprotection of our synthetic glucans can be achieved. The synthetic strategies presented in this chapter will provide access to libraries of larger and more complex branched and linear beta-glucan fragments for binding assays other studies that will aid in the determination of the structure-function relationships of these compounds.

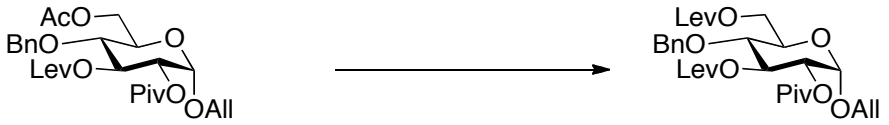
## Experimental Section

### General Materials and Methods

Reaction solvent dichloromethane was obtained from a commercial solvent purification tower or distilled from calcium hydride. Reaction solvents tetrahydrofuran and toluene were obtained from a commercial solvent purification tower. Dowex 50W X-8 ($H^+$) resin was washed repeatedly with MeOH prior to use. Powdered 4Å molecular sieves were purchased from Aldrich Chemical Company and were activated by heating in a furnace at 250 °C overnight prior to use. All other reagents were used as received without further

purification. Reactions were monitored and the $R_f$ values determined using analytical TLC with Sorbent Technologies silica gel plates (60F-254). Developed TLC plates were visualized by immersion in 10% ethanolic sulfuric acid solution, or in acidic *p*-anisaldehyde solution, followed by heating on a hot plate. Moisture-sensitive reactions were run in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature for reactions performed at elevated or sub-ambient temperatures. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Manual flash chromatography was performed using Sorbent Technologies silica gel (60F-254), and the solvent systems outlined in the experimental procedures. Automated flash chromatography was carried out on a Biotage SP-1 chromatography workstation fitted with either a Biotage 12M or 25M silica gel cartridge, using ethyl acetate/hexane gradients as outlined in the experimental procedures. $^{1}H$ NMR and $^{13}C$ NMR spectra were obtained with a Bruker DRX400 at 400 MHz and 101 MHz, respectively. $^{1}H$ NMR spectra were reported in parts per million (δ) relative to $CDCl_3$, $CD_3OD$, $D_2O$, or TMS (7.27 ppm, 4.84 ppm, 4.81 ppm, and 0.00 ppm, respectively) as internal references. $^{13}C$ NMR spectra were reported in parts per million (δ) relative to $CDCl_3$ or $CD_3OD$ (77.23 ppm or 49.15 ppm, respectively). For $^{13}C$ NMR spectra obtained in $D_2O$, chemical shifts are reported relative to acetone as an internal standard (215.94 and 30.89 ppm).

Synthetic Procedures




**11** **12**

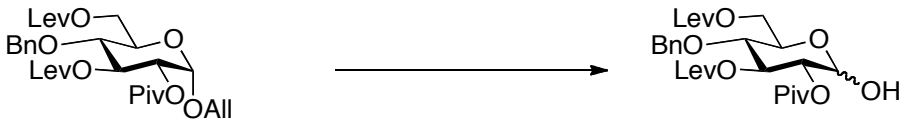
**Allyl 4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (12).** Allyl 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranose (**11**) (2.15 g, 4.02 mmol) was dissolved in MeOH (50 mL) and $CH_2Cl_2$ (50 mL). A solution of NaOMe (0.25M in MeOH) was added until the solution reached a pH of 10 as measured by pH paper. The reaction mixture was stirred for 2 h at ambient temperature, after which TLC analysis showed completion conversion of the starting material ($R_f$ 0.68, 1:1 EtOAc/hexanes) to the deacetylated product ($R_f$ 0.62, 1:1 EtOAc/hexanes). The reaction mixture was neutralized by the addition of Dowex 50W X-8 ($H^+$) resin and filtered. The solvent was evaporated under reduced pressure. The crude product was dissolved in $CH_2Cl_2$ (20 mL). To this solution, *N,N*-dicyclohexylcarbodiimide (1.66 g, 8.04 mmol), levulinic acid (0.933 g, 8.04 mmol), and 4-dimethylaminopyridine (40 mg) were added. The resulting mixture was stirred for 1 hour at room temperature. The mixture was diluted with hexanes (20 mL) and filtered through a pad of Celite to remove the precipitated dicyclohexylurea byproduct. The filtrate was diluted with $CH_2Cl_2$, washed successively with water and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The residue was dissolved in a 3:7 mixture of EtOAc and hexanes (50 mL) and filtered through a pad of silica gel (4 cm deep in a conical glass funnel) to remove the residual insoluble dicyclohexylurea. The solvent was evaporated

to give the crude product as a solid, which was recrystallized from EtOH and hexanes to afford **12** as colorless needles (1.72 g, 2.91 mmol, 72% over 2 steps).

***R**<sub>f</sub>*: 0.81 (7:3 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.26 (m, 5H, Ar-H), 5.88-5.77 (m, 1H, $CH_2$=C*H*$CH_2O$), 5.65 (dd, $J_{3,2}$=$J_{3,4}$ 10.0 Hz, 1H, H-3), 5.30-5.24 (m, 1H, C$H_a$$H_b$=$CHCH_2O$), 5.19-5.15 (m, 1H, $CH_a$$H_b$=$CHCH_2O$), 5.04 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.75-4.70 (dd, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, 1H, H-2), 4.66 (d, $J$ = 11.2 Hz, 1H, PhC$H_a$$H_b$O), 4.57 (d, $J$ = 11.2 Hz, 1H, Ph$CH_a$$H_b$O), 4.37-4.31 (dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.0 Hz, 1H, H-6), 4.30-4.25 (dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 2.4 Hz, 1H, H-6') 4.18-4.12 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 3.98-3.90 (m, 2H, $CH_2$=$CHCH_a$$H_b$O and H-5), 3.64 (t, $J_{3,4}$=$J_{4,5}$= 9.6 Hz, 1H, H-4), 2.80-2.72 (m, 2H, $CH_3COCH_2CH_2COO$), 2.60-2.48 (m, 4H, $CH_3COCH_2CH_2COO$, $CH_3COCH_2CH_2COO$), 2.52-2.46 (m, 2H, $CH_3COCH_2CH_2COO$), 2.20 (s, 3H, $CH_3COCH_2CH_2COO$), 2.16 (s, 3H, $CH_3COCH_2CH_2COO$), 1.16 (s, 9H, *t*-BuCO)

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 206.5, 206.2, 178.1, 172.6, 171.7, 137.8, 133.4, 128.7, 128.3, 128.1, 118.0, 95.0, 76.1, 74.8, 72.5, 71.3, 68.8, 62.9, 38.9, 38.1, 37.8, 30.1, 28.2, 28.0, 27.1.




**12** **13**

**4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (13).** Allyl 6-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**12**) (558 mg, 0.94 mmol) was dissolved in tetrahydrofuran (10 mL). To this solution, (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) ( 8 mg, 9.5 μmol) was added. The resulting red suspension was degassed by brief exposure of the reaction flask to vacuum. The mixture was then stirred under a balloon of hydrogen until the red suspension became a colorless solution (approximately 30 seconds). The mixture was degassed and the flask flushed with nitrogen. The resulting mixture was stirred for 17 hours at ambient temperature. Proton NMR analysis of a small aliquot (~100 μL) of the reaction mixture indicated that conversion to the 1-propenyl ether was ~ 70% complete. Additional (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) (10 mg, 12 μmol) was added. The mixture was again stirred under a balloon of hydrogen until the red color disappeared, then the flask flushed with nitrogen and stirred for another 6 hours at ambient temperature. At this time, proton NMR analysis indicated that conversion to the 1-propenyl ether was complete. To the crude reaction mixture, water ( 1.5 mL) and iodine ( 477 mg, 1.88 mmol) were added. The reaction mixture immediately turned dark brown. After stirring for 40 minutes, saturated sodium thiosulfate solution was added to destroy the excess iodine. The mixture was partitioned between water and dichloromethane, and the layers separated. The aqueous layer was extracted twice with dichloromethane. The combined organic extracts were washed successively with

saturated aqueous $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 30→60% EtOAc in hexanes as the eluant to afford **13**, a ~7:3 mixture of α- and β-anomers, as an amber colored syrup (415 mg, 0.75 mmol, 80% over 2 steps).

***R**f*: 0.29 and 0.19 (1:1 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.25 (m, 5 H, Ar-H), 5.65 (t, 0.7H, $J_{3,2}$=$J_{3,4}$ 9.6 Hz, H-3), 5.37-5.27 (m, 1H), 4.77-4.54 (m, 3.3H), 4.36-4.30 (m, 1H), 4.27-4.1 (m, 1.7H), 3.72-3.56 (m, 1.7H), 3.22 (br d, 0.7H, *J*= 2.4 Hz, -O*H*), 2.77-2.73 (m, 2H, $CH_3COCH_2CH_2COO$), 2.65-2.54 (m, 4H, $CH_3COCH_2CH_2COO$, $CH_3COCH_2CH_2COO$), 2.48-2.44 (m, 2H, $CH_3COCH_2CH_2COO$), 2.17 (s, 3H, $CH_3COCH_2CH_2COO$), 2.13 (s, 3H, $CH_3COCH_2CH_2COO$), 1.16 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 207.3, 207.0, 206.2, 206.1, 179.1, 178.1, 172.6(2), 171.8(2), 137.8, 137.6, 128.7(2), 128.3, 128.2, 128.1, 95.8, 90.3, 76.3, 76.0, 74.9, 74.5, 74.4, 73.7, 73.4, 72.1, 71.6, 68.5, 63.1, 62.8, 39.1, 39.0, 38.3, 38.1, 37.8(2), 30.1, 30.0, 28.2, 28.1(2), 28.0, 27.1(2).


**13** **6**

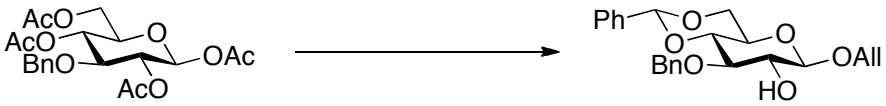
**4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (6).** 4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-D-

glucopyranose (**13**) (395mg, 0.72 mmol) was dissolved in dichloromethane (10 mL). Cesium carbonate (350 mg, 1.07 mmol) and trichloroacetonitrile (0.36 mL, 3.59 mmol) were added, and the mixture stirred at ambient temperature for 24 h. The mixture was filtered through a pad of Celite and the solvents removed under reduced pressure. The residue was purified by column chromatography on silica gel, using ethyl acetate/hexanes/triethylamine 30:70:1) as the eluant, to afford **6** as a yellow syrup (385 mg, 0.55 mmol, 77%).

$R_f$: 0.49 (4:6 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.62 (s, 1H, NH), 7.36-7.24 (m, 5H, Ar-H), 6.51 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.71 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 5.05-5.02 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 4.70 (d, 1H, $J$ = 11 Hz, PhC$H_a$H$_b$O), 4.61 (d, 1H, $J$ = 11 Hz, PhCH$_a$$H_b$O), 4.37-4.28 (m, 2H, H-6, H-6'), 4.16-4.11 (m, 1H, H-5), 3.78 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 2.78-2.72 (m, 2H, $CH_3COCH_2CH_2COO$), 2.70-2.56 (m, 4H, $CH_3COCH_2CH_2COO$, $CH_3COCH_2CH_2COO$), 2.53-2.47 (m, 2H, $CH_3COCH_2CH_2COO$), 2.20 (s, 3H, $CH_3COCH_2CH_2COO$), 2.17 (s, 3H, $CH_3COCH_2CH_2COO$), 1.13 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 206.6, 206.0, 177.7, 172.5, 171.7, 160.9, 137.5, 128.7, 128.5, 128.4, 128.3, 93.2, 91.0, 75.3, 75.1, 72.1, 71.6, 70.1, 62.3, 39.0, 38.0, 37.7, 30.0(2), 28.0, 27.1.




**14** **15**

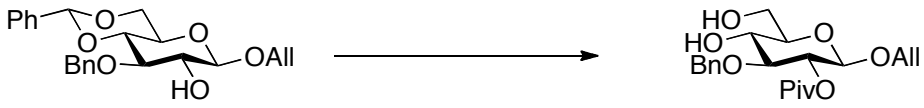
**Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (15).** A solution of 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose[13] (**14**) (981 mg, 2.23, mmol) and allyl alcohol (0.79 mL, 11.61 mmol) in dichloromethane (10 mL) was cooled to 0°C. To this solution, boron trifluoride diethyl etherate (2.93 mL, 23.2 mmol) was added. The mixture was stirred for 2.5 hours at 0°C, then diluted with ethyl acetate. The organic solution was washed successively with saturated $NaHCO_3$ and brine, and dried over $Na_2SO_4$. The solvents were evaporated to give the crude allyl glycoside as a syrup. An additional quantity of the crude allyl glycoside was prepared by treating **14** (5.91 g, 13.48 mmol) with allyl alcohol (4.6 mL, 67.4 mmol) and boron trifluoride diethyl etherate (17.1 mL, 134.8 mmol) in dichloromethane (60 mL) at 0°C for 2.5 hours. Following workup as described previously, the two batches of the crude allyl glycoside were combined and dissolved in methanol (100 mL). Sodium metal (200 mg) was added, and the resulting mixture stirred for 16.5 hours at ambient temperature. The solution was neutralized by the addition of Dowex-50 ($H^+$) resin and filtered. The solvent was removed by rotary evaporation. The residual crude triol was dissolved in acetonitrile (30 mL). To this solution, benzaldehyde dimethyl acetal (4.71 mL, 31.4 mmol) and 10-camphorsulfonic acid (729 mg, 3.14 mmol) were added. Following stirring for 1 hour at ambient temperature, hexanes (50 mL) and water (50 mL) were added to the reaction mixture, resulting in the precipitation of **15** as a white solid. The liquids were filtered off and the solid product dissolved in ethyl acetate. The solution was washed successively with

water and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The crude product was recrystallized from ethyl acetate and hexanes to furnish **15** as fine needles (3.36 g, 8.43 mmol, 53% over three steps).

***R**f*: 0.57 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.50-7.25 (m, 10H, Ar-H), 5.97-5.89 (m, 1H, $OCH_2CH$=$CH_2$), 5.57 (s, 1H, $PhCHO_2$), 5.36-5.31 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.25-5.22 (m, 1H, $OCH_2CH$=$CH_aH_b$), 4.96 (d, 1H, $J$ = 11.6 Hz, $PhCH_aH_bO$), 4.80 (d, 1H, $J$ = 11.6 Hz, $PhCH_aH_bO$), 4.45 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.40-4.33 (m, 2H, $OCH_aH_bCH$=$CH_2$ and H-6), 4.18-4.12 (m, 1H, $OCH_aH_bCH$=$CH_2$), 3.80 (t, 1H, $J_{5,6}$ = $J_{6,6'}$ = 10.2 Hz, H-6'), 3.73-3.64 (m, 2H, H-3 and H-4), 3.60-3.57 (br dt, 1H, $J_{2,3} \approx J_{1,2}$ = 9.2 Hz, $J_{H\text{-}2,OH}$ = 2.4 Hz, H-2), 3.47-3.41 (m, 1H, H-5), 2.51 (d, 1H, $J$ = 2.4 Hz, -O*H*).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.5, 138.4, 133.7, 129.2, 128.6, 128.5, 128.2, 128.0, 126.2, 118.4, 102.4, 101.4, 81.5, 80.4, 74.8, 74.4, 70.7, 68.9, 66.6.




**15** **16**

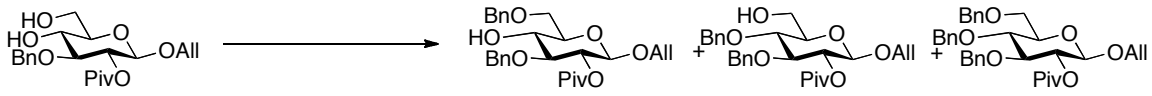
**Allyl 3-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (16).** A solution of allyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**15**) (2.23 g, 5.60 mmol), trimethylacetyl chloride (1.38 mL, 11.2 mmol), 4-dimethylaminopyridine (70 mg), and triethylamine (2.34 mL, 16.8 mmol) in dichloromethane (10 mL) was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with dichloromethane and

washed successively with water, saturated $NaHCO_3$, and brine, then dried over $Na_2SO_4$. The solvents were evaporated and the residue dissolved in dichloromethane (20 mL). An aqueous solution of trifluoroacetic acid (60% v/v, 8 mL) was added. Following stirring for 40 minutes at ambient temperature, dichloromethane (50 mL) and saturated $NaHCO_3$ solution (50 mL) were added. The mixture was stirred until the evolution of $CO_2$ subsided. The mixture was poured into a separatory funnel and the layers separated. The organic layer was washed with water and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel, using a gradient elution of 25% EtOAc in hexanes to 100% EtOAc, to furnish diol **16** as a syrup that solidified upon standing (1.72 g, 4.36 mmol, 78% over 2 steps).

***R**f*: 0.27 (7:3 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.25 (m, 5H, -Ph), 5.85-5.78 (m, 1H, $OCH_2CH$=$CH_2$), 5.28-5.23 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.18-5.15 (m, 1H, $OCH_2CH$=$CH_aH_b$), 5.02 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ =9.2 Hz, H-2), 4.72 (d, 1H, $J$ = 11.6 Hz, $PhCH_aH_bO$), 4.64 (d, 1H, $J$ = 11.6 Hz, $PhCH_aH_bO$), 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.33-4.29 (m, 1H, $OCH_aH_bCH$=$CH_2$), 4.05-4.00 (m, 1H, $OCH_aH_bCH$=$CH_2$), 3.88-3.85 (dd, 1H, $J_{6,6'}$ = 12.0 Hz, $J_{6,5}$ = 2.8 Hz, H-6), 3.80-3.75 (dd, 1H, $J_{6,6'}$ = 12.0 Hz, $J_{6',5}$ = 4.4 Hz, H-6'), 3.69 (t, 1H, $J_{3,4}$ =$J_{4,5}$ = 9.2 Hz, H-4), 3.55 (t, 1H, $J_{2,3}$ =$J_{3,4}$ = 9.2 Hz, H-3), 3.38-3.32 (m, 1H, H-5), 3.06-2.97 (br s, 1H, -OH), 2.60-2.45 (br s, 1H, -OH), 1.20 (s, 9H, *t*-Bu).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 177.1, 138.2, 133.7, 128.7, 128.0, 127.8, 117.7, 100.5, 82.8, 75.4, 74.6, 72.9, 70.4, 62.4, 39.0, 27.3.




**16** **17** **18** **19**

**Allyl 3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (17).** Allyl 3-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**16**) (2.21 g, 5.60 mmol) was dissolved in dichloromethane (50 mL). To this solution, commercial silver (I) oxide (Alfa Aesar, 3.89 g, 16.8 mmol) and benzyl bromide (2.0 mL, 16.8 mmol) were added. The mixture was stirred for 3 hours at ambient temperature, and the solvents evaporated. The residue was purified by flash column chromatography on silica gel using a gradient of 10% to 40% ethyl acetate in hexanes to give **17** as a syrup (2.14 g, 4.41 mmol, 78%). Additionally, small amounts of the 3,4-di-*O*-benzyl derivative **18**, a light yellow solid (157 mg, 0.32 mmol, 6%), and 3,4,6-tri-*O*-benzyl derivative **19**, a syrup (337 mg, 0.59 mmol, 10%), were also isolated.

Spectral Data for **17:**

***R**f*: 0.60 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.25 (m, 10H, ArH), 5.87-5.79 (m, 1H, C*H*=$CH_2$), 5.28-5.22 (m, 1H, CH=C$H_a$$H_b$), 5.17-5.14 (m, 1H, CH=$CH_a$$H_b$), 5.08-5.04 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.0 Hz, H-2), 4.74-4.66 (AB dd, 2H, *J* = 11.6 Hz, PhC$H_2$O), 4.62-4.55 (AB dd, 2H, *J*= 12.0 Hz, PhC$H_2$O), 4.52-4.43 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.34-4.30 (m, 1H, OC$H_a$$H_b$CH=$CH_2$), 4.05-4.00 (m, 1H, OC$H_a$$H_b$CH=$CH_2$), 3.79-3.70 (m, 3H, H-4, H-6, H-6'), 3.57-3.53 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.50-3.46 (m, 1H, H-5), 2.71 (d, 1H, *J* = 2.4, Hz, -O*H*), 1.20 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 177.0, 138.4, 138.0, 133.8, 128.7, 128.6, 128.0, 127.9, 127.8, 127.2, 117.5, 82.8, 74.5, 74.4, 73.9, 72.7, 72.0, 70.5, 70.1, 39.0, 27.3.

Spectral Data for **18**:

***R**$_f$*: 0.44 (3:7 EtOAc/hexanes)

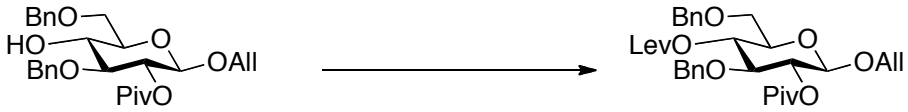
**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.38-7.25 (m, 10H, ArH), 5.89-5.80 (m, 1H, C*H*=$CH_2$), 5.28-5.23 (m, 1H, CH=C$H_a$$H_b$), 5.19-5.17 (m, 1H, CH=C$H_a$$H_b$), 5.08-5.04 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.0 Hz, H-2), 4.83-4.56 (m, 4H, 2×PhC$H_2$O), 4.48 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.34-4.30 (m, 1H, OC$H_a$$H_b$CH=$CH_2$), 4.07-4.02 (m, 1H, OC$H_a$$H_b$CH=$CH_2$), 3.90-3.87 (br d, 1H, H-4), 3.76-3.65 (m, 3H, H-3, H-6, H-6'), 3.45-3.38 (m, 1H, H-5), 2.05-1.80 (br s, 1H, -OH), 1.20 (s, 9H, *t*-BuCO).

Spectral Data for **19**:

***R**$_f$*: 0.86 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.13 (m, 15H, Ar-H), 5.86-5.80 (m, 1H, $CH_2$=C*H*$CH_2$O), 5.28-5.23 (m, 1H, C$H_a$$H_b$=C$HCH_2$O), 5.17-5.14 (m, 1H, C$H_a$$H_b$=C$HCH_2$O), 5.11-5.08 (m, 1H, H-2), 4.78-4.51 (m, 6H, 3×PhC$H_2$) 4.43 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.37-4.32 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 4.06-4.01 (m, 1H, $CH_2$=CHC$H_a$$H_b$O), 3.77-3.68 (m, 4H, H-3, H-4, H-6, H-6'), 3.52-3.50 (m, 1H, H-5), 1.19 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 176.9, 138.3, 138.3, 138.1, 133.8, 128.5 (3), 128.1, 127.9 (2), 127.8, 127.7, 127.6, 117.4, 100.3, 83.4, 78.0, 75.4, 75.1 (2), 73.7, 73.2, 70.0, 68.9, 39.0, 27.3.




**17** **20**

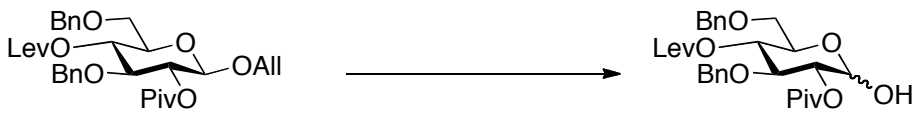
**Allyl 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-β-D-glucopyranoside (20).** Allyl 3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**17**) (2.09 g, 4.30 mmol) was dissolved in dichloromethane (30 mL). To this solution, *N,N*-dicyclohexylcarbodiimide (1.78 g, 8.60 mmol), levulinic acid (1.00 g, 8.60 mmol), and 4-dimethylaminopyridine (100 mg) were added. The resulting mixture was stirred for 1 hour at ambient temperature. The mixture was then filtered on Celite to remove the precipated dicyclohexylurea. The filtrate was diluted with dichloromethane and washed successively with saturated aqueous $NaHCO_3$ solution, water, and brine. The organic extract was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 25% ethyl acetate in hexanes to give **20** as a colorless syrup that solidified upon standing (2.09 g, 3.59 mmol, 84%).

***R**$_f$*: 0.43 (3:7 EtOAc/hexanes)

**$^{1}$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.36-7.21 (m, 10H, ArH), 5.87-5.79 (m, 1H, C*H*=$CH_2$), 5.28-5.22 (m, 1H, CH=C$H_a$H$_b$), 5.18-5.07 (m, 3H, CH=CH$_a$$H_b$, H-2, H-4), 4.64-4.51 (m, 4H, 2×PhC$H_2$O), 4.49-4.47 (d, 1H, $J_{1,2}$= 8.0 Hz, H-1), 4.37-4.32 (m, 1H,

OC$H_a$H$_b$CH=CH$_2$), 4.07-4.02 (m, 1H, OCH$_a$$H_b$CH=CH$_2$), 3.78-3.73 (t, 1H, $J_{2,3} = J_{3,4} =$ 9.2 Hz, H-3), 3.62-3.58 (m, 3H, H-5, H-6, H-6'), 2.65-2.49 (m, 2H, CH$_3$COC$H_2$CH$_2$COO), 2.44-2.25 (m, 2H, CH$_3$COCH$_2$C$H_2$COO), 2.11 (s, 3H, C$H_3$COCH$_2$CH$_2$COO), 1.20 (s, 9H, *t*-BuCO).

**$^{13}$C NMR (CDCl$_3$, 101 MHz)** δ (ppm) 206.4, 176.8, 171.7, 138.2(2), 133.7, 128.5(2), 128.0, 127.8(2), 127.7(2), 100.2, 80.5, 73.8, 73.7, 73.5, 72.5, 70.9, 70.1, 69.8, 39.0, 37.9, 30.0, 28.0, 27.3.




**20** **21**

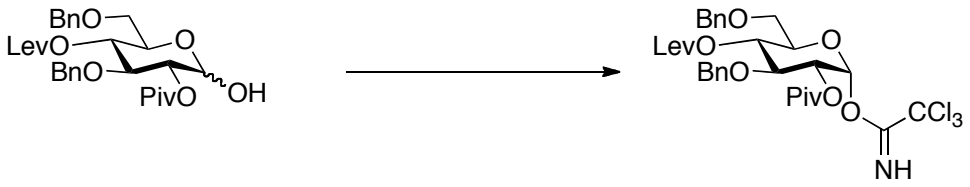
**3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (21).** Allyl 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-β-D-glucopyranoside (**20**) (2.08 g, 3.57 mmol) was dissolved in tetrahydrofuran (30 mL). To this solution, (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) (30 mg, 35.7 μmol) was added. The resulting red suspension was degassed by brief exposure of the reaction flask to vacuum. The mixture was then stirred under a balloon of hydrogen until the red suspension became a colorless solution (approximately 30 seconds). The mixture was degassed and the flask filled with nitrogen. The resulting mixture was stirred for 9 hours at ambient temperature. Proton NMR analysis of a small aliquot (~100 μL) of the reaction mixture indicated that isomerization of the allyl ether was complete. To the crude reaction mixture, water (2 mL) and iodine (1.36 g, 5.35 mmol) were added. The reaction mixture immediately turned dark brown. After 30 minutes, saturated sodium

thiosulfate solution was added until all traces of brown color disappeared. The mixture was diluted with water and dichloromethane, and the layers separated. The aqueous layer was extracted twice with dichloromethane. The combined organic extracts were washed successively with saturated aqueous $NaHCO_3$ solution and brine. The organic extract was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of 30% to 40% ethyl acetate in hexanes to give **21**, a 1:3 mixture of α- and β-isomers, as a white solid. (1.47 g, 2.71 mmol, 76% over 2 steps).

***R**f*: 0.20 and 0.27 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.23 (m, 10H, Ar-H), 5.49 (t, 0.25H, $J$ = 3.6 Hz, H-1α), 5.18 (t, 0.75H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4 β), 5.08 (t, 0.25H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4 α), 4.94-4.89 (dd, 0.75H, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 8.4 Hz, H-2 β), 4.84-4.81 (dd, 0.25H, $J_{2,3}$ = 8.8 Hz, $J_{1,2}$ = 3.6 Hz, H-2 α), 4.78-4.50 (m, 4.75H, 2× PhC$H_2$O and H-1 β), 4.18-4.12 (m, 0.25H), 4.08 (t, 0.25H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3 α), 3.79 (t, 0.75H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3 β), 3.66-3.52 (m, 3.5H), 2.87 (br s, 0.25H, -OH), 2.68-2.48 (m, 2H, $CH_3COCH_2CH_2COO$), 2.43-2.22 (m, 2H, $CH_3COCH_2CH_2COO$), 2.12(s, 3H, $CH_3COCH_2CH_2COO$), 1.23 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 206.5, 206.4, 179.2, 177.9, 171.7(2), 138.5, 138.1, 138.0(2), 128.6, 128.5, 128.3, 127.9(2), 127.8(2), 96.3, 90.3, 80.0, 75.4, 75.0, 74.4, 73.9, 73.8(2), 73.7, 70.9(2), 69.3, 69.2, 69.0, 39.1, 38.9, 38.0(2), 30.0, 28.0(2), 27.3(2).



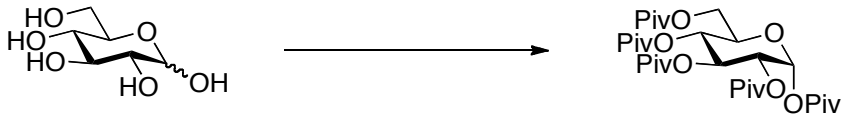

**9** **21**

**3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (9).** 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**21**) (1.46 g, 2.69 mmol) was dissolved in dichloromethane (25 mL). Cesium carbonate (1.31 g, 4.03 mmol) and trichloroacetonitrile (1.94 g, 1.34 mL, 13.45 mmol) were added, and the resulting mixture stirred at ambient temperature for 2.5 h. The mixture was filtered through a pad of Celite and the solvents removed under reduced pressure. The residue was purified by column chromatography on silica gel, using ethyl acetate/hexanes/triethylamine (25:75:1) as the eluant, to afford **9** as a colorless syrup (1.44 g, 2.09 mmol, 78%).

***R**f*: 0.52 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.64 (s, 1H, NH), 7.33-7.24 (m, 10H, Ar-H), 6.59 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.30 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 5.13-5.10 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 4.73 (d, 1H, $J$ = 11.6 Hz, PhC$H_a$H$_b$O), 4.63 (d, 1H, $J$ = 11.6 Hz, PhCH$_a$$H_b$O), 4.53-4.46 (2 d, 2H, $J$ = 11.6 Hz, PhC$H_2$O), 4.16-4.10 (m, 2H, H-3, H-5), 3.62-3.58 (dd, 1H, $J_{6,6'}$ = 11.2 Hz, $J_{6,5}$ = 3.2 Hz, H-6), 3.56-3.52 (dd, 1H, $J_{6,6'}$ = 11.2 Hz, $J_{6',5}$ = 4.4 Hz, H-6'), 2.65-2.50 (m, 2H, $CH_3COCH_2CH_2COO$), 2.44-2.25 (m, 2H, $CH_3COCH_2CH_2COO$), 2.11 (s, 3H, $CH_3COCH_2CH_2COO$), 1.17 (s, 9H, *t*-BuCO).**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 206.4, 177.4, 171.5, 160.7, 138.1, 138.0, 128.5, 128.4,

128.1, 127.9, 127.8, 93.5, 91.2, 77.0, 74.7, 73.7, 72.1, 72.0, 70.0, 68.5, 39.0, 37.9, 30.0, 28.0, 27.3.



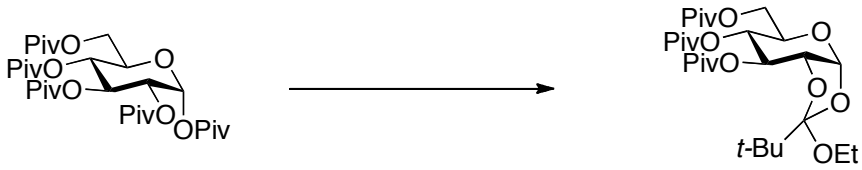
**22**

**α-D-Glucose pentapivaloate (22).** A solution of triethylamine (18.6 mL, 133.2 mmol), 4-dimethylaminopyridine (150 mg), and trimethylacetyl chloride (16.5 mL, 133.2 mmol) in dichloromethane (60 mL) was cooled to 0°C. To this solution, D-glucose (3.00 g, 16.7 mmol) was added in small portions over 30 minutes. The ice bath was then removed and the mixture slowly warmed to room temperature. After 16 h, the mixture was diluted with dichloromethane and washed successively with 2N HCl, water, $NaHCO_3$, water, and brine. Following drying over $Na_2SO_4$, the solvent was evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel (1:9 EtOAc/hexanes) to give the product **22** as a white solid (8.86 g, 14.74 mmol, 88%).

***R**f*: 0.32 (1:9 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 6.32 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.54 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 5.19 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 5.14-5.10 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.16-4.08 (m, 3H, H-5, H-6, H-6'), 1.28 (s, 9H, *t*-Bu), 1.21 (s, 9H, *t*-Bu), 1.18 (s, 9H, *t*-Bu), 1.13 (s, 9H, *t*-Bu), 1.12 (s, 9H, *t*-Bu).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 178.2, 177.3, 177.2, 176.7, 176.1, 88.8, 70.5, 69.8, 69.6, 67.5, 61.8, 39.4, 39.1, 39.0 (2), 38.9, 27.4, 27.3 (3).



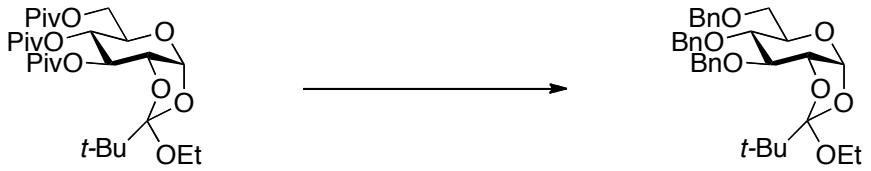

**22** **23**

**1,2-*O*-(1-ethoxy-2,2,2-trimethylethylidene)-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranose (23).** A solution of α-D-Glucose pentapivaloate (**22**) (4.45 g, 7.40 mmol) in dichloromethane (50 mL) was cooled to 0°C. To this solution, 20 mL of a 33% (w/w) solution of HBr in acetic acid was added via syringe. The mixture was stirred overnight and allowed to warm to room temperature. The mixture was poured into ice water and extracted with dichloromethane. The organic extracts were washed successively with saturated $NaHCO_3$ and brine, then dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure to give the crude glucosyl bromide as a white solid. This solid was dissolved in dichloromethane (40 mL). To this solution, ethanol (0.47 mL, 8.14 mmol), tetrabutylammonium bromide (1.19 g, 3.70 mmol), and triethylamine (3.1 mL, 22.2 mmol) were added. The mixture was heated to reflux for 18 h, then cooled to room temperature. The mixture was diluted with dichloromethane, washed successively with water and brine, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel using 1:9:0.1 EtOAc/hexanes/$Et_3N$ as eluant to give the product **23** as a white solid (3.03 g, 5.56 mmol, 75%).

***R**f*: 0.41 (1:9 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 5.78 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 5.23 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 6.0 Hz, H-3), 4.98 (dd, 1H, $J_{4,3}$ = 6.0 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.29 (dd, 1H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 5.6 Hz, H-2), 4.28-4.10 (m, 3H, H-5, H-6, H-6'), 3.59 (m, 2H, $OCH_2CH_3$), 1.22 (s, 9H, *t*-Bu), 1.20 (s, 9H, *t*-Bu), 1.18 (s, 9H, *t*-Bu), 1.17 (t, 3H, $J$ = 7.2 Hz, $OCH_2CH_3$), 1.11 (s, 9H, *t*-Bu).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 178.2, 177.0, 176.9, 125.1, 97.6, 76.1, 72.2, 68.9, 67.2, 62.8, 60.3, 39.5, 39.0, 38.9, 38.8, 27.3, 27.2 (2), 25.8, 15.5.




**23** **24**

**1,2-*O*-(1-ethoxy-2,2,2-trimethylethylidene)-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (24).** A solution of 1,2-*O*-(1-ethoxy-2,2,2-trimethylethylidene)-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranose (**23**) (4.03 g, 7.39 mmol) was suspended in absolute ethanol (100 mL). To this suspension sodium metal (60 mg) was added. The mixture was stirred at room temperature. The starting material slowly dissolved as the reaction proceeded. After ~20 h, additional sodium metal (50 mg) was added to the reaction mixture. Following stirring for an additional 4 h, the solvents were evaporated under reduced pressure. The resulting crude triol was dissolved in DMF (40 mL). Benzyl bromide (3.96 mL, 33.3 mmol) was added, and the mixture cooled to 0°C. Sodium hydride (1.33 g of a 60% dispersion in mineral oil) was added, and the mixture allowed to slowly warm to room temperature while stirring. After 17 h, the excess sodium hydride was destroyed by the addition of

methanol (2 mL). The reaction mixture was partitioned between ethyl acetate (100 mL) and water (60 mL). The layers separated and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic extracts were washed with brine and dried over $Na_2SO_4$. After removal of solvents under reduced pressure, the residue was purified by flash chromatography on silica gel using a gradient elution of 5-10% ethyl acetate in hexanes to give tribenzyl orthoester **24** as a light yellow syrup (3.91 g, 6.94 mmol, 94%).

***R**f*: 0.63 (2:8 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.41-7.19 (m, 15H, Ar-H), 5.89 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1) 4.86, 4.80, 4.70, 4.52, 4.48, 4.41 (6 d, each 1H, $J$ = 12.0 Hz, 3×$PhCH_aH_bO$), 4.36 (t, 1H, $J_{2,3} = J_{1,2}$ = 5.2 Hz, H-2), 3.89-3.73 (m, 3H, H-3, H-4, H-5), 3.66 (dd, 1H, $J_{6,6'}$ = 10.4 Hz, $J_{6,5}$ = 1.6 Hz, H-6), 3.59 (dd, 1H, $J_{6',6}$ = 10.4 Hz, $J_{6',5}$ = 2.8 Hz, H-6'), 3.51 (q, 2H, $J$ = 7.2 Hz, $OCH_2CH_3$), 1.18 (t, 3H, $J$ = 7.2 Hz, $OCH_2CH_3$), 1.05 (s, 9H, *t*-Bu).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 138.4, 138.3, 138.2, 128.6, 128.5, 128.3, 128.2, 128.0 (2), 127.9 (2), 126.7, 98.3, 82.1, 81.5, 74.5, 74.3, 73.6, 73.5, 72.5, 69.8, 58.7, 39.1, 25.7, 15.4.

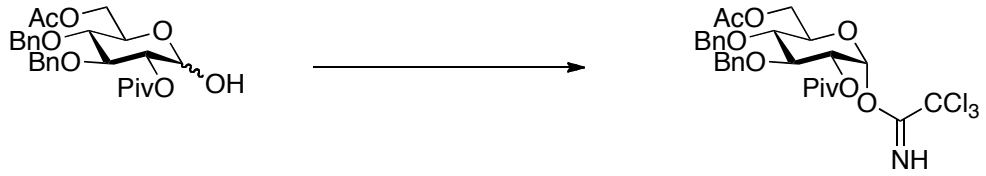

**24** **25**

**6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-pivaloyl-D-glucopyranose (25).** A solution of freshly fused zinc chloride (4.66 g, 34.2 mmol) was dissolved in 24 mL of 5:1 acetic anhydride/acetic acid (v/v) and cooled to 0°C. A solution of orthoester (**24**) (3.85 g, 6.84 mmol) in 24 mL of 5:1 acetic anhydride:acetic acid (v/v) was added dropwise to the cooled zinc chloride solution. The ice bath was removed and the reaction warmed to room temperature. After 3 h, the mixture was poured into water (100 mL) and extracted with dichloromethane (3×80 mL). The combined organic extracts were washed successively with saturated $NaHCO_3$ (2×100 mL) and brine (100 mL), and dried over $Na_2SO_4$. The solvents were removed under reduced pressure. The resulting residue was dissolved in DMF (20 mL). To this solution, hydrazine acetate (755 mg, 8.21 mmol) was added and the mixture stirred at room temperature. After 20 h, the mixture was diluted with water (60 mL) and extracted with dichloromethane (4×80 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using 1:3 EtOAc/hexanes as eluant to furnish the product **25**, a 7:3 mixture of α- and β-anomers, as a light yellow syrup (2.52 g, 5.18 mmol, 76% over 2 steps).

***R**f*: 0.28 and 0.39 (3:7 EtOAc/Hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.23 (m, 10H, Ar-H), 5.42 (d, 0.7H, $J_{1,2}$ = 3.6 Hz, H-1α), 4.87-4.79 (m, 4H), 4.62-4.55 (m, 1.3H), 4.35-4.32 (m, 1H), 4.25-4.21 (m, 1H), 4.14-4.09 (m, 1.3H), 3.79 (t, 0.3H, $J$ = 9.6 Hz), 3.62-3.56 (m, 1.3 H), 2.05 (s, 3H, C*H*$_3$CO), 1.22 (br s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 179.4, 178.2, 171.1, 171.0, 138.3, 138.0, 137.9, 137.6, 128.7 (2), 128.6, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 96.2, 90.4, 82.8, 79.8, 75.8, 75.6, 75.5, 75.2, 74.0, 73.4, 68.8, 63.0, 39.1, 39.0, 27.3, 27.2, 21.1.




**25** **10**

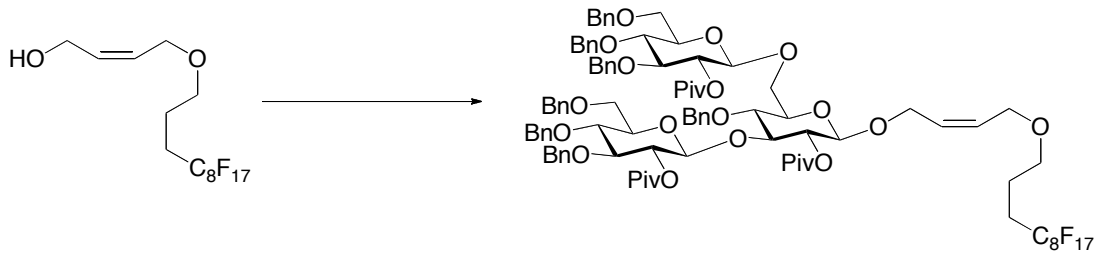
**6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (10).** To a solution of lactol (**25**) (2.52 g, 5.18 mmol) in dichloromethane (50 mL) was added cesium carbonate (2.53 g, 7.77 mmol) and trichloroacetonitrile (3.73 g, 2.6 mL, 25.9 mmol). The mixture was stirred at room temperature for 18 h, then filtered on Celite an concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using a gradient elution of 10-70% ethyl acetate in hexanes to give trichloroacetimidate **10** as a white crystalline solid (2.08 g, 3.29 mmol, 64%).

***R**$_f$*: 0.73 (3:7 EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 8.62 (s, 1H, N*H*), 7.36-7.24 (m, 10H, Ar-H), 6.52 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 5.10 (dd, 1H, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.86-4.83 (m,

3H, PhC$H_aH_b$, PhC$H_a$'$H_b$'), 4.58 (d, 1H, J = 10.8 Hz, PhC$H_a$'$H_b$'), 4.32 (dd, 1H, $J_{6,6'}$ = 12.0 Hz, $J_{6,5}$ = 2.0 Hz, H-6), 4.24 (dd, 1H, $J_{6',6}$ = 12.0 Hz, $J_{6',5}$ = 4.0 Hz, H-6'), 4.16 (t, 1H, $J_{3,2} = J_{3,4}$ = 9.6 Hz, H-3), 4.09 (ddd, 1H, $J_{5,6}$ = 2.0 Hz, $J_{5,6'}$ = 4.0 Hz, $J_{5,4}$ = 10.0 Hz, H-5), 3.71 (t, 1H, $J_{4,3} = J_{4,5}$ = 9.6 Hz, H-4), 2.02 (s, 3H, C$H_3$CO), 1.17 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 101 MHz)** δ (ppm) 177.7, 170.8, 160.9, 138.1, 137.5, 128.8, 128.7, 128.6, 128.4, 128.0, 127.6, 93.6, 91.2, 80.0, 76.7, 75.7, 75.5, 72.4, 71.7, 62.5, 39.0, 27.3, 21.0.




**7** **1**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl 4-O-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranoside (1).**

The branched β-glucan trisaccharide **1** was synthesized from fluorocarbon-tagged alcohol **7** via 1.5 automated synthesis cycles using the Chemspeed ASW1000 synthesizer. The specific reaction conditions are outlined in the table below:

| Step | Task | Reagent/Operation | Time |
|---|---|---|---|
| 1 | Glycosylation | 1.5 eq. donor **6** (112 μmol) in 1.5 mL toluene, 1 eq. F-tagged acceptor **7** (75 μmol) in 0.5 mL toluene, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | 20 min |
| 2 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 3 | Quenching | 0.2 mL triethylamine | |
| 4 | Evaporation | 40 °C | 45 min |
| 5 | Delevulinoylation | 10 eq. Hydrazine hydrate (1.0 M in 3:2 pyr/HOAc), DCM, rt | 50 min |
| 6 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 7 | Quenching | 0.5 mL acetone | |
| 8 | Evaporation | 60 °C | 45 min |
| 9 | FSPE Sample Preparation | 0.8 mL DMF transferred to reaction vial | |
| 10 | Sample loading | crude sample transferred to F-SPE cartridge | |
| 11 | Elution | 4.7 mL 80% methanol wash | |
| 12 | Elution | 1.5 mL THF wash (repeated 3 times) | |
| 13 | Transfer | 4.7 mL collected sample to clean reaction vial | |
| 14 | Transfer / Evaporation | 0.5 mL toluene added, then evaporated at 40 °C (repeated 3×) | 45 min × 3 |
| 15 | Glycosylation | Donor **5** (225 μmol) in 1.5 mL toluene, F-tagged acceptor from cycle 1, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | |
| 16 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 17 | Quenching | 0.2 mL triethylamine | |
| 18 | Evaporation | 40 °C | 45 min |
| 19 | FSPE | Same as steps 9-12 | |

**Table 4.1**. Synthetic procedure for the automated synthesis of trisaccharide (**1**).

Following the final F-SPE procedure, the conical-bottomed extraction vial containing the crude trisaccharide product was removed from the ASW1000. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography on

silica gel (0→20% EtOAc/hexanes) to yield trisaccharide **1** (25.4 mg, 13.2 μmol, 18% overall yield) as a colorless syrup.

**$R_f$**: 0.49 (20% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.11 (m, 35H, Ar-H), 5.68-5.63 (m, 1H, vinylic H), 5.57-5.51 (m, 1H, vinylic H), 5.10-4.97 (m, 3H), 4.87 (t, 1H, $J$ = 9 Hz), 4.80-4.62 (m, 8H), 4.60-4.43 (m, 8H), 4.41 (d, 1H, $J$ = 8.0 Hz, H-1), 4.26-4.18 (m, 3H), 4.17-4.00 (m, 4H), 3.80-3.40 (m, 16H), 3.30 (t, 1H, $J$ = 8.8 Hz), 2.22-2.08 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.86-1.78 (m, 2H, $OCH_2CH_2CH_2CH_2CF_2$), 1.21 (s, 9H, (*t*-BuCO), 1.19 (s, 9H, *t*-BuCO), 1.16 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 177.5, 176.8, 176.2, 138.5(2), 138.3(2), 138.2, 128.7(2), 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8(2), 127.7(2), 127.5(2), 101.3, 99.6, 99.4, 83.6, 83.5, 78.5, 78.0, 76.2, 75.8, 75.5, 75.4, 75.2, 75.1, 74.8, 73.8, 73.7, 73.4, 73.1, 69.0(2), 68.9, 68.4, 66.7, 64.3, 39.0, 38.9(2), 29.9, 28.2, 28.0, 27.4(2), 27.3, 21.0.

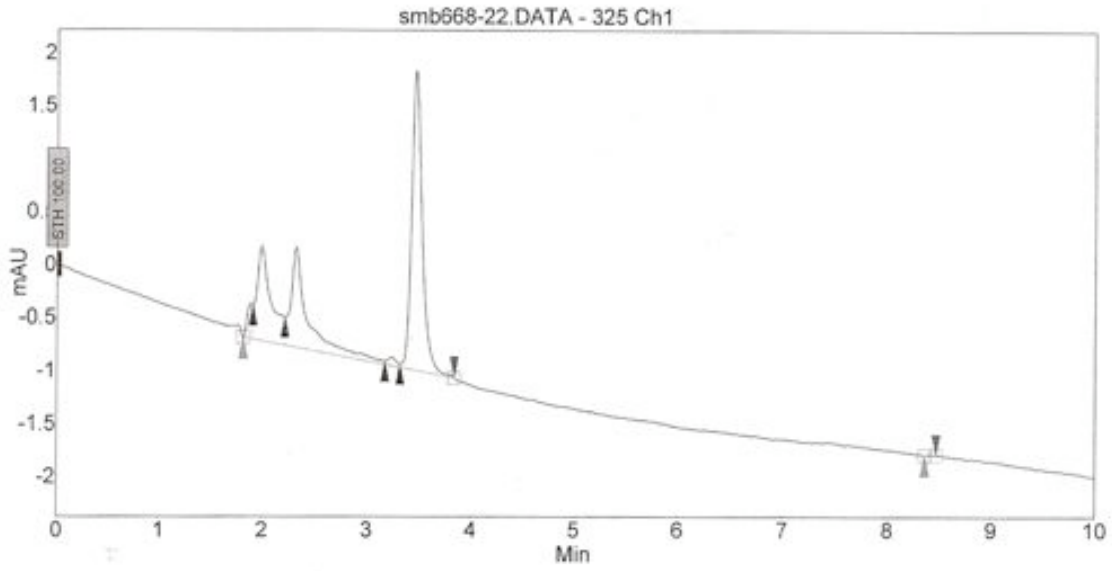
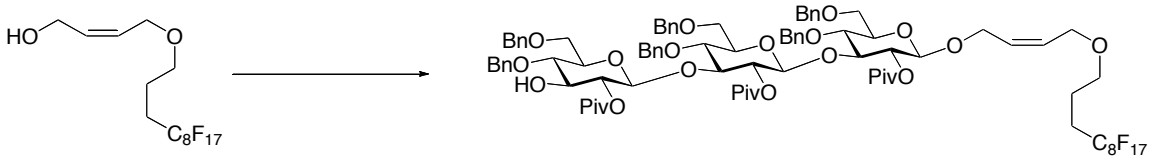



**Figure 4.4**. Analytical HPLC chromatogram of crude **1** obtained from the Chemspeed ASW-1000 workstation. T = 1.9, 2.2 min: decomposed donor; T = 3.4 min; trisaccharide **1**. (Flow rate 1 mL/min, 20% EtOAc in hexanes)



**7** **2**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl 4,6-di-O-benzyl-3-*O*-(4,6-di-*O*-benzyl-3-*O*-(4,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranoside (2).**

The linear 1,3-linked β-glucan trisaccharide **2** was synthesized from fluorocarbon-tagged alcohol **7** and glucosyl donor **8** via three automated synthesis cycles using the Chemspeed ASW1000 synthesizer. The specific reaction conditions are given in the table below:

| Step | Task | Reagent/Operation | Time |
|---|---|---|---|
| 1 | Glycosylation | 1.5 eq. donor **8** (112 μmol) in 1.5 mL toluene, 1 eq. F-tagged acceptor **7** (75 μmol) in 0.5 mL toluene, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | 20 min |
| 2 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 3 | Quenching | 0.2 mL triethylamine | |
| 4 | Evaporation | 40 °C | 45 min |
| 5 | Delevulinoylation | 10 eq. Hydrazine hydrate (1.0 M in 3:2 pyr/HOAc), DCM, rt | 50 min |
| 6 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 7 | Quenching | 0.5 mL acetone | |
| 8 | Evaporation | 60 °C | 45 min |
| 9 | FSPE Sample Preparation | 0.8 mL DMF transferred to reaction vial | |
| 10 | Sample loading | crude sample transferred to F-SPE cartridge | |
| 11 | Elution | 4.7 mL 80% methanol wash | |
| 12 | Elution | 1.5 mL THF wash (repeated 3 times) | |
| 13 | Transfer | 4.7 mL collected sample to clean reaction vial | |
| 14 | Transfer / Evaporation | 0.5 mL toluene added, then evaporated at 40 °C (repeated 3×) | 45 min × 3 |
| 15 | Glycosylation | Donor **8** (225 μmol) in 1.5 mL toluene, F-tagged acceptor from cycle 1, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | |
| 16 | TLC / Delevulinoylation Evaporation / FSPE | Same as steps 2-12 | |
| 17 | Transfer / Evaporation | Same as steps 13-14 | |
| 18 | Glycosylation / Delevulinoylation / FSPE | Same as steps 15-16 | |
| 19 | FSPE | Same as steps 9-12 | |

**Table 4.2**. Synthetic procedure for the automated synthesis of trisaccharide (**2**).

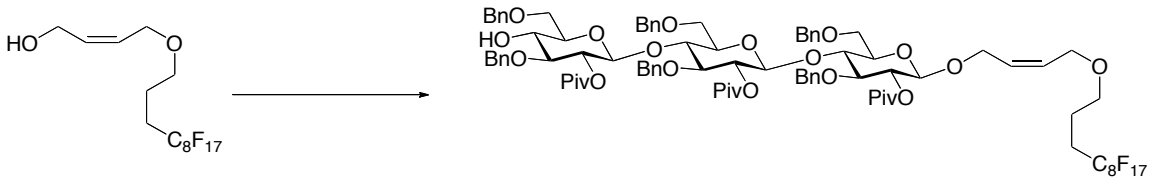
Following the final automated FSPE cycle, the conical-bottomed extraction vial containing the crude trisaccharide product was removed from the ASW1000. The solvent was removed under reduced pressure. The crude product (30 mg) was purified by flash

chromatography on silica gel (1 cm × 15 cm column, 0→20% EtOAc/hexanes) only partially separated trisaccharide **2** from a small amount of the corresponding disaccharide product. Careful column chromatography of the mixed fractions (1 cm × 20 cm column, 10→20% EtOAc/hexanes) gave additional pure **2.** (total yield 13.0 mg, 7.11 μmol, 9.5% overall yield) as a colorless syrup.

**$R_f$**: 0.31 (20% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.18 (m, 30H, Ar-H), 5.68-5.60 (m, 1H, vinylic H), 5.59-5.53 (m, 1H, vinylic H), 4.99-4.90 (m, 3H), 4.87-4.72 (m, 4H), 4.64-4.60 (m, 2H), 4.53-4.40 (m, 8H), 4.28-4.06 (m, 5H), 3.94 (d, 2H, *J*= 5.6 Hz, allylic $CH_2$), 3.78-3.72 (m, 2H), 3.70-3.40 (m, 11H), 3.57 (t, 2H, *J* = 5.6 Hz, $OCH_2CH_2CH_2CF_2$), 2.46 (br d, 1H, *J* = 4.0 Hz, -OH), 2.20-2.02 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.84-1.76 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.22 (s, 9H, (*t*-BuCO), 1.19 (s, 9H, *t*-BuCO), 1.11 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 179.3, 176.6, 176.4, 138.7(2), 138.4, 138.3, 138.1, 130.1, 128.8, 128.7(2), 128.6, 128.5(2), 128.4(2), 128.3, 128.2, 128.0, 99.6, 99.3, 98.9, 78.9, 78.0, 76.6(2), 76.0, 75.7, 75.5, 75.3, 75.2, 75.0, 74.7, 74.5, 74.0, 73.7, 73.6, 39.2, 39.0, 38.8, 29.9, 28.2, 27.6, 27.3(2), 21.0.




**7** **3**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl 3,6-di-O-benzyl-4-*O*-(3,6-di-*O*-benzyl-4-*O*-(3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-*2*-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranoside (3).**

The linear 1,4-linked β-glucan trisaccharide **3** was synthesized from fluorocarbon-tagged alcohol **7** and glucosyl donor **9** via three automated synthesis cycles using the Chemspeed ASW1000 synthesizer. The specific reaction conditions are outlined in the table below:

| Step | Task | Reagent/Operation | Time |
|---|---|---|---|
| 1 | Glycosylation | 1.5 eq. donor **9** (112 μmol) in 1.5 mL toluene, 1 eq. F-tagged acceptor **7** (75 μmol) in 0.5 mL toluene, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | 20 min |
| 2 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 3 | Quenching | 0.2 mL triethylamine | |
| 4 | Evaporation | 40 °C | 45 min |
| 5 | Delevulinoylation | 10 eq. Hydrazine hydrate (1.0 M in 3:2 pyr/HOAc), DCM, rt | 50 min |
| 6 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 7 | Quenching | 0.5 mL acetone | |
| 8 | Evaporation | 60 °C | 45 min |
| 9 | FSPE Sample Preparation | 0.8 mL DMF transferred to reaction vial | |
| 10 | Sample loading | crude sample transferred to F-SPE cartridge | |
| 11 | Elution | 4.7 mL 80% methanol wash | |
| 12 | Elution | 1.5 mL THF wash (repeated 3 times) | |
| 13 | Transfer | 4.7 mL collected sample to clean reaction vial | |
| 14 | Transfer / Evaporation | 0.5 mL toluene added, then evaporated at 40 °C (repeated 3×) | 45 min × 3 |

| | | | |
|---|---|---|---|
| 15 | Glycosylation | Donor **9** (225 μmol) in 1.5 mL toluene, F-tagged acceptor from cycle 1, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | |
| 16 | TLC / Delevulinoylation Evaporation / FSPE | Same as steps 2-12 | |
| 17 | Transfer / Evaporation | Same as steps 13-14 | |
| 18 | Glycosylation / Delevulinoylation / FSPE | Same as steps 15-16 | |
| 19 | FSPE | Same as steps 9-12 | |

**Table 4.3**. Synthetic procedure for the automated synthesis of trisaccharide (**3**).

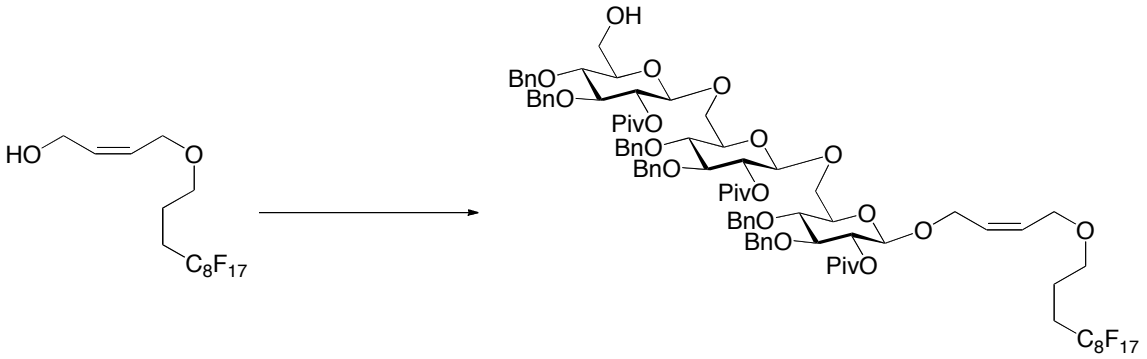
Following the final automated FSPE cycle, the conical-bottomed extraction vial containing the crude trisaccharide product was removed from the ASW1000. The solvent was removed under reduced pressure. The crude product (27 mg) was purified by column chromatography on silica gel (1 cm × 20 cm column, 10→30% EtOAc/hexanes), followed by a second silica gel column (1 cm × 20 cm column, 10→24% EtOAc/hexanes). However, these chromatographic steps failed to remove the presumed disaccharide impurity present. The remaining mixed product (8.0 mg) was further purified by preparative TLC (10 cm × 20 cm plate, eluted once with 30% EtOAc in hexanes), to provide pure **3** as a colorless syrup (5.1 mg, 2.8 μmol, 3.7% overall yield).

**$R_f$**: 0.59 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.34-7.12 (m, 30H, Ar-H), 5.64-5.57 (m, 2H, vinylic H), 5.12-4.90 (m, 5H), 4.77 (apparent t, 2H, $J$ = 12.8 Hz), 4.58 (d, 1H, $J$ = 11.2 Hz), 4.50 (d, 1H, $J$ = 11.6 Hz), 4.44-4.38 (m, 2H), 4.38-4.28 (m, 4H), 4.25 (d, 2H, $J$ = 6.0 Hz, allylic $CH_2$), 4.18-4.12 (dd, 1H), 4.07-3.72 (m, 6H), 3.80-3.52 (m, 6H), 3.43-4.34 (m,

4H), 3.30-3.12 (m, 5H), 2.96 (s, 1H, -OH), 2.22-2.06 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.86-1.77 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.11 (s, 9H, (*t*-BuCO), 1.08 (s, 9H, *t*-BuCO), 1.07 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm)




**7** **4**

***Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl 3,4-di-O-benzyl-6-*O*-(3,4-di-*O*-benzyl-6-*O*-(3,4-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-*2*-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranoside (4).**

The linear 1,6-linked β-glucan trisaccharide **4** was synthesized from fluorocarbon-tagged alcohol **7** and glucosyl donor **10** via three automated synthesis cycles using the Chemspeed ASW1000 synthesizer. The specific reaction conditions are outlined in the table below:

| Step | Task | Reagent/Operation | Time |
|---|---|---|---|
| 1 | Glycosylation | 1.5 eq. donor **10** (112 μmol) in 1.5 mL toluene, 1 eq. F-tagged acceptor **7** (75 μmol) in 0.5 mL toluene, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | 20 min |
| 2 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 3 | Quenching | 0.2 mL triethylamine | |
| 4 | Evaporation | 40 °C | 45 min |
| 5 | Deacetylation | 0.25 M NaOMe in MeOH, rt | 2 h |

| | | | |
|---|---|---|---|
| 6 | TLC Sample | 40 μL of crude reaction mixture withdrawn | |
| 7 | Quenching | 0.5 M AcOH in MeOH | |
| 8 | Evaporation | 40 °C | 45 min |
| 9 | FSPE Sample Preparation | 0.8 mL DMF transferred to reaction vial | |
| 10 | Sample loading | crude sample transferred to F-SPE cartridge | |
| 11 | Elution | 4.7 mL 80% methanol wash | |
| 12 | Elution | 1.5 mL THF wash (repeated 3 times) | |
| 13 | Transfer | 4.7 mL collected sample to clean reaction vial | |
| 14 | Transfer / Evaporation | 0.5 mL toluene added, then evaporated at 40 °C (repeated 3×) | 45 min × 3 |
| 15 | Glycosylation | Donor **10** (225 μmol) in 1.5 mL toluene, F-tagged acceptor from cycle 1, 0.1 eq. TMSOTf (0.1 M in DCM), 20 °C | |
| 16 | TLC / Deacetylation Evaporation / FSPE | Same as steps 2-12 | |
| 17 | Transfer / Evaporation | Same as steps 13-14 | |
| 18 | Glycosylation / Deacetylation / FSPE | Same as steps 15-16 | |
| 19 | FSPE | Same as steps 9-12 | |

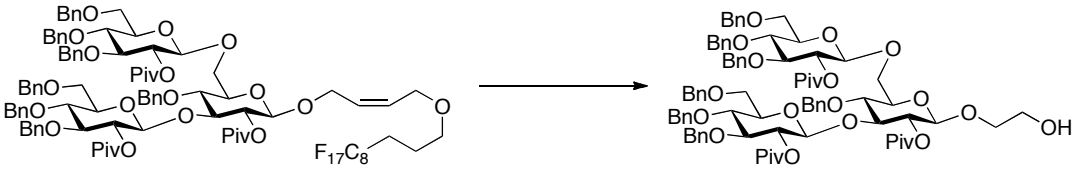
**Table 4.4**. Synthetic procedure for the automated synthesis of trisaccharide (**4**).

Following the final automated FSPE cycle, the conical-bottomed extraction vial containing the crude trisaccharide product was removed from the ASW1000. The solvent was removed under reduced pressure. TLC of the crude product showed the presence of two products, the desired trisaccharide ($R_f$ = 0.55, 3:7 EtOAc/hexanes) and some unreacted disaccharide acceptor ($R_f$ = 0.43, 3:7 EtOAc/hexanes). The ratio of trisaccharide to disaccharide was approximately 3:1 based on TLC. The crude product (16.5 mg) was purified by preparative TLC (20 cm × 20 cm plate, eluted once with 30% EtOAc in hexanes), to provide pure **4** as a colorless syrup (5.2 mg, 2.8 μmol, 3.8% overall yield).

**$R_f$**: 0.55 (30% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.13 (m, 30H, Ar-H), 5.70-5.56 (m, 2H, vinylic H), 5.06-4.97 (m, 3H), 4.80-4.55 (m, 13H), 4.39 (d, 2H, $J$ = 7.6 Hz), 4.32-4.15 (m, 1H), 4.18-4.10 (m, 1H), 4.10 (d, 2H, allylic $CH_2$), 3.90-3.53 (m, 14H), 3.50-3.40 (m, 3H), 3.37-3.30 (m, 1H), 2.22-2.00 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.88-1.77 (m, 2H, $OCH_2CH_2CH_2CF_2$), 1.16 (s, 9H, (*t*-BuCO), 1.15 (s, 9H, *t*-BuCO), 1.14 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 177.4, 176.8, 176.7, 138.3(2), 138.2, 138.1, 138.0, 130.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2(2), 128.1, 127.9, 127.8, 127.6, 127.5(2), 102.2, 101.5, 100.0, 83.4(2), 83.2, 76.0, 75.1(2), 75.0, 73.3, 73.1, 73.0, 69.0, 66.8, 64.7, 62.0, 39.0(2), 29.9, 27.4(2), 27.3.




**1** **26**

**2-hydroxyethyl 4-O-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-2-*O*-pivaloyl-β-D-glucopyranoside (26).** A solution of branched trisaccharide **1** (20.0 mg, 10.4 μmol) was dissolved in MeOH (2 mL) and $CH_2Cl_2$ (1 mL). The solution was cooled to -78 °C. Ozone was bubbled through the solution until a pale blue color persisted (~ 2 minutes). Nitrogen was then bubbled through the solution until it became colorless. To this solution, $NaBH_4$ (12 mg, 313 μmol) was added, and the mixture allowed to warm to ambient temperature. After 9 hours, additional $NaBH_4$ (4

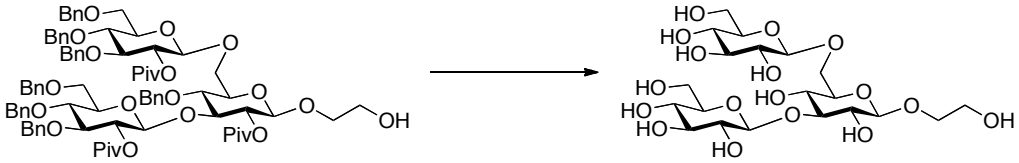
mg, 106 μmol) was added, and the mixture stirred at ambient temperature for an additional 5 hours. The solvents were evaporated using a stream of nitrogen. The residue was partitioned between $CH_2Cl_2$ (10 mL) and saturated aqueous $NH_4Cl$. The aqueous layer was extracted with $CH_2Cl_2$ (5 mL). The combined organic extracts were washed successively with water (5 mL) and brine (5 mL), and dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure. Silica gel flash chromatography of the residue gave a white, waxy syrup (12.2 mg), but proton NMR showed the presence of the cleaved fluorous alcohol compound, which wasn't visible upon staining the TLC plate. The product was then loaded onto a 2-gram fluorous solid-phase extraction cartridge with MeOH (0.2 mL). The desired product was eluted using 80% aqueous MeOH. However, some of the desired product was retained on the FSPE column until the fluorophilic solvent, MeOH, was eluted through the cartridge. The fraction containing the fluorophilic eluant was concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane (0.2 mL) and loaded onto a 2-gram FSPE cartridge. This time, the desired product was completely eluted by the fluorophobic solvent. The combined fluorophobic eluate from the two FSPE purifications was concentrated under reduced pressure to provide **26** as a colorless syrup (5.9 mg, 4.12 μmol, 40%).

**$R_f$**: 0.69 (50% EtOAc/hexanes)

**$^1$H NMR ($CDCl_3$, 400 MHz)** δ (ppm) 7.35-7.10 (m, 35H, Ar-H), 5.08-5.02 (m, 2H), 4.97 (d, 1H, $J$ = 11.2 Hz), 4.86 (t, 1H, $J$ = 8.8 Hz), 4.78-4.62 (m, 7H), 4.58-4.42 (m, 8H), 4.24 (2, 1H, $J$ = 8.0 Hz, H-1), 4.16 (t, 1H, $J$ = 8.8 Hz), 4.01 (d, 1H, $J$ = 10 Hz), 3.78-3.44 (m,

16H), 3.30 (t, 1H, *J* = 8.0 Hz), 3.14-3.08 (m, 1H), 2.40-2.30 (br s, 1H, -OH), (1.21 (s, 9H, (*t*-BuCO), 1.17 (s, 9H, *t*-BuCO), 1.14 (s, 9H, *t*-BuCO).

**$^{13}$C NMR ($CDCl_3$, 100 MHz)** δ (ppm) 177.4, 177.0, 176.6, 138.5, 138.4, 138.3(2), 138.2(2), 137.9, 128.7, 128.6(2), 128.4(2), 128.2, 128.1, 128.0, 127.9, 127.8(2), 127.7 (2), 127.5(2), 101.3, 101.1, 99.7, 83.5, 83.4, 78.5, 78.0, 77.5, 76.0, 75.9, 75.4, 75.3, 75.1, 75.0, 74.8, 74.2, 73.7, 73.6, 73.4, 73.0, 72.0, 69.0, 68.9, 68.0, 62.0, 46.2, 39.1, 39.0, 29.9, 27.4(3).




**26** **27**

**2-hydroxyethyl 3,6-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (27).** A solution of the 2-hydroxyethyl glycoside **26** (5.5 mg, 3.8 μmol) was dissolved in EtOH (1 mL) To this solution, palladium (10 wt.% on activated carbon, 10 mg) was added. The reaction flask was evacuated and purged with hydrogen gas three times, then stirred under an atmosphere of hydrogen for 5 hours at ambient temperature. The reaction mixture was filtered through a pad of Celite, and the solvents evaporated. The residue was dissolved in MeOH (0.5 mL). To this solution, a 0.25M solution of NaOMe in MeOH was added until the pH of the reaction mixture was ~10 as measured by pH paper. After stirring at ambient temperature for 22.5 hours, the mixture was neutralized with Dowex 50W X-8 ($H^{+}$) resin and filtered. The solvent was evaporated to yield the deprotected trisaccharide **27** as a colorless syrup.

**$^{1}$H NMR ($CD_3OD$, 400 MHz)** δ (ppm) 4.57 (d, 1H, *J* = 8.0 Hz), 4.34 (d, 2H, *J* = 8.0 Hz), 4.26-4.19 (m, 1H), 3.90-3.10 (m, 21H).

**$^{13}$C NMR ($CD_3OD$, 100 MHz)** δ (ppm) 105.3, 105.1, 104.3, 87.7, 78.3, 78.1(2), 78.0, 76.9, 75.7, 75.3, 74.7, 73.2, 71.7, 70.2, 70.1, 62.9, 62.8, 62.6.

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# CHAPTER 5

## Conclusions and Future Directions

In this dissertation, two libraries of mass-identical disaccharides varying in linkage position and the identity of the reducing-end monosaccharide residue were synthesized. The initial results obtained from the MS-MS spectra of the initial test group of nine synthetic disaccharides bearing a propyl group at the reducing end revealed subtle differences in fragmentation that were used to generate classification functions. These classification functions could be used to correctly assign the linkage position and non-reducing end monosaccharide residue of each disaccharide. However, the method must be carried out on larger and more complex oligosaccharides to show its true potential. Since only a limited number of simple trisaccharides are commercially available, a library of synthetic trisaccharides is needed to provide additional standards for analysis by mass spectrometry. The Chemspeed ASW1000 automated synthesis workstation provides an excellent platform for parallel oligosaccharide synthesis, and early efforts are underway to prepare a library of trisaccharides using this approach.

Secondly, we demonstrated that branched and linear beta-glucans can be rapidly synthesized using an automated solution-phase approach. Synthetic routes to several new glucosyl trichloroacetimidate building blocks were developed. These building blocks reliably generate the desired 1,2-*trans* linkage during each coupling reaction. Fluorous solid-phase extraction provides a robust means of purifying intermediates during the course of each synthesis. This study culminated in the syntheses of two branched glucans

and three linear glucans. To fully exploit the utility of the automation protocols developed in this work, larger and more elaborate beat-glucans can be synthesized. Additionally, each synthetic glucan will be subjected to global deprotection in preparation for NMR binding studies with the glucan-binding protein Dectin-1.

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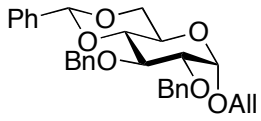
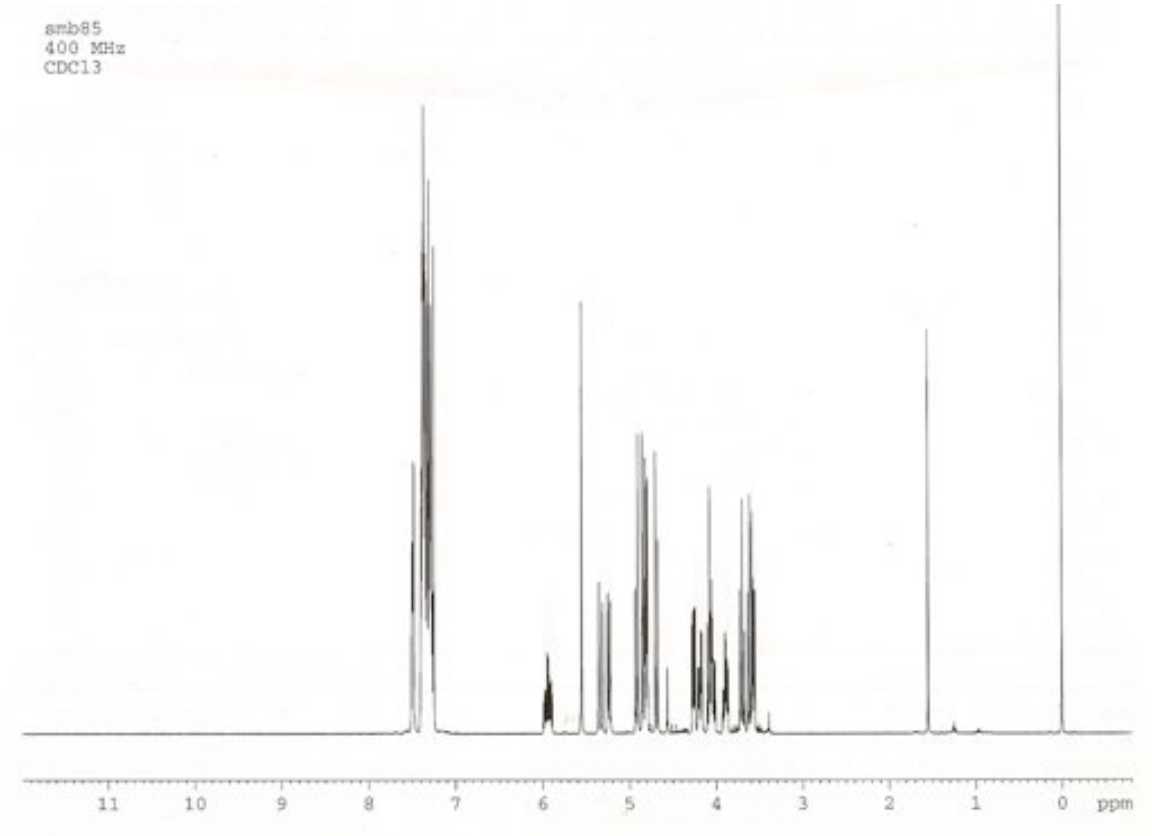
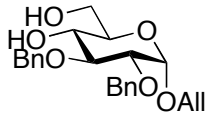
# APPENDIX A: CHAPTER 2 $^{1}H$ AND $^{13}C$ NMR SPECTRA

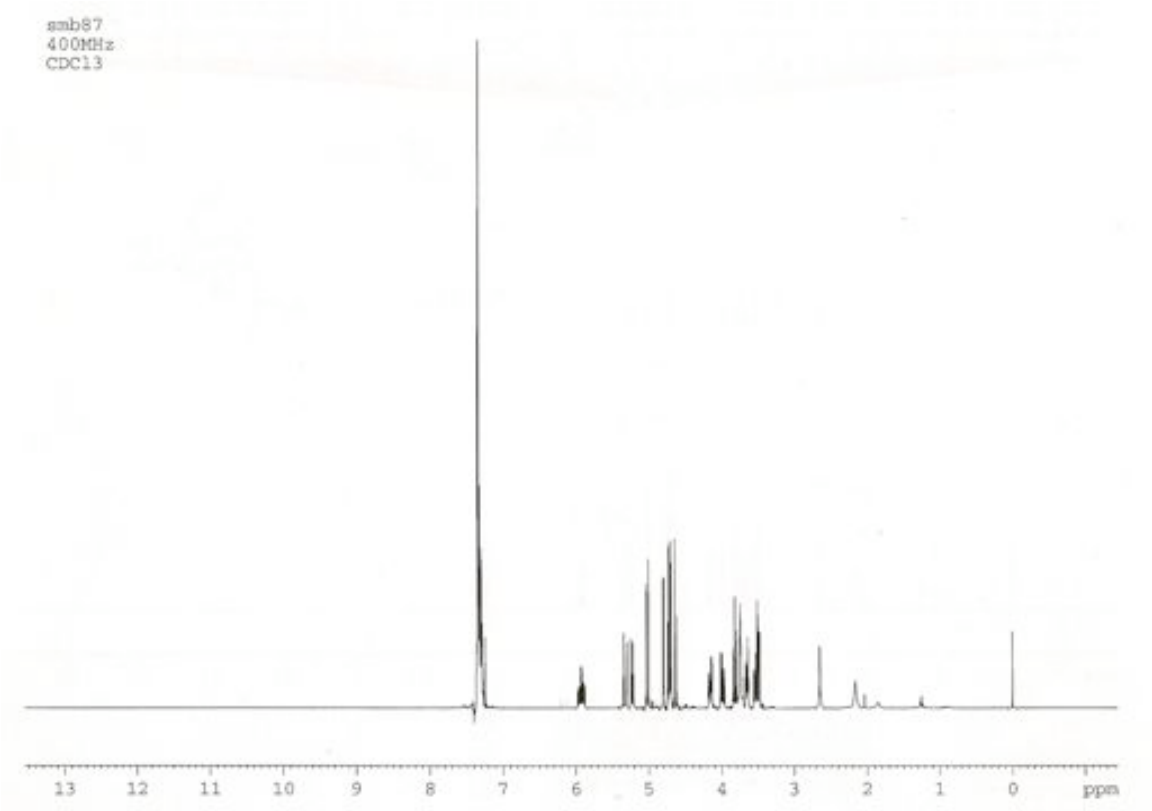
## TABLE OF CONTENTS



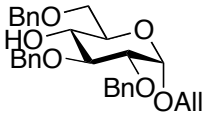



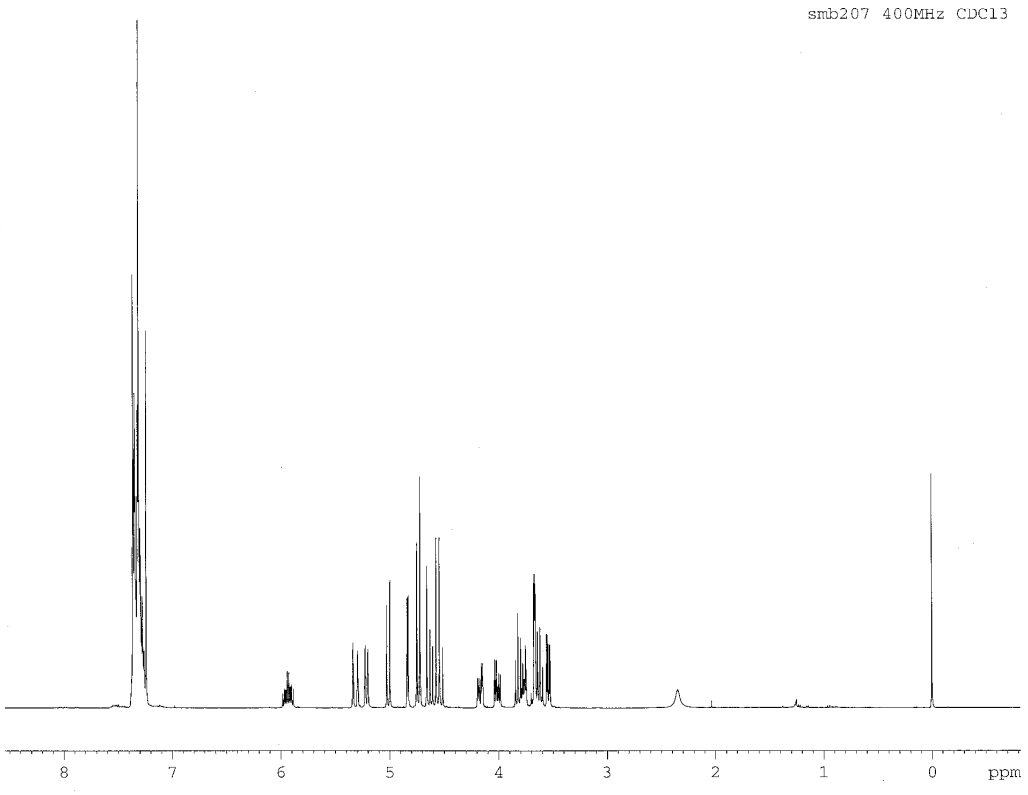

$^{1}$H NMR Spectrum of Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**4**).



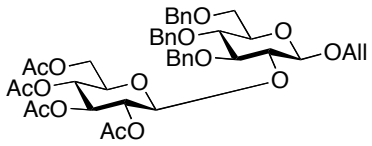



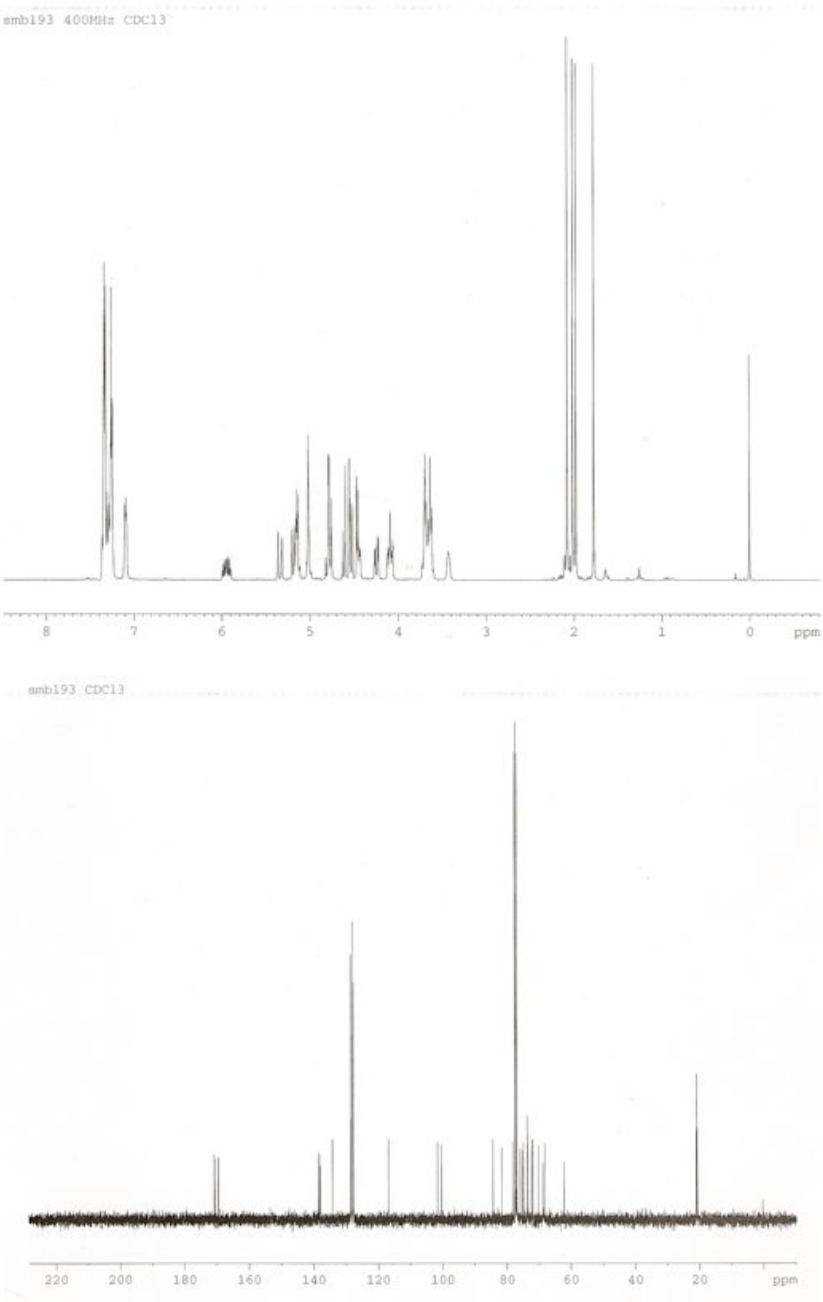

$^{1}$H NMR Spectrum of Allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**5**).



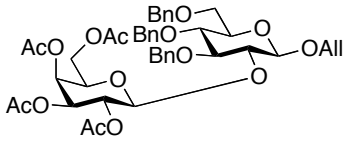



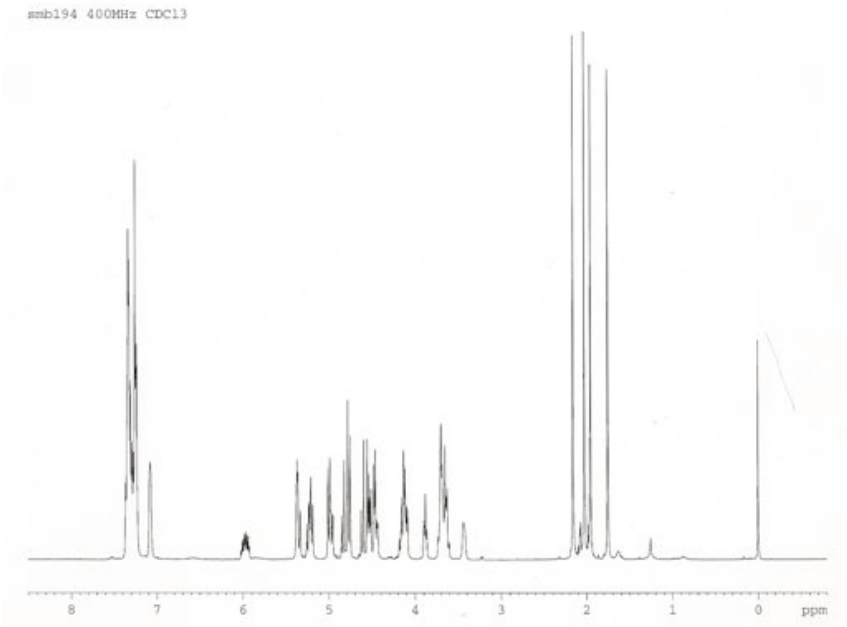

$^{1}$H NMR Spectrum of Allyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**).

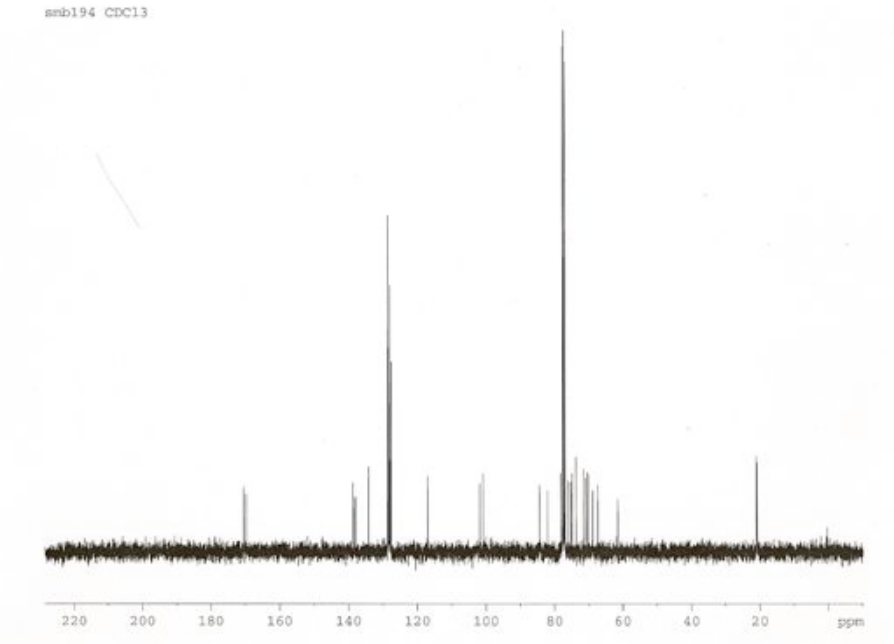


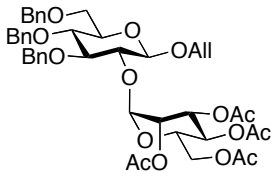

$^{1}$H and $^{13}$C NMR Spectra of Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**).

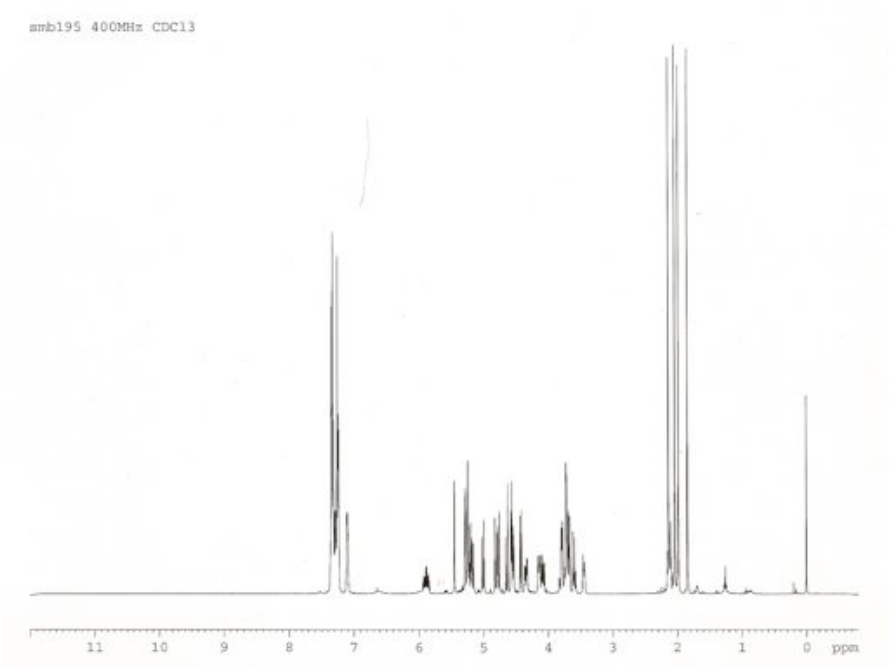


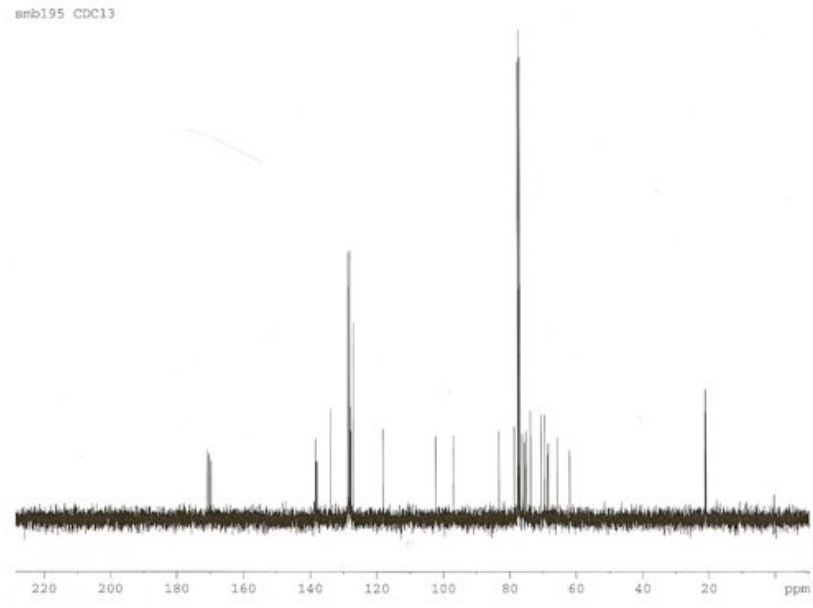



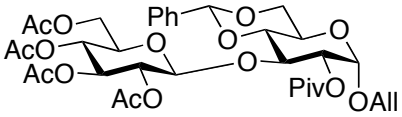

$^{1}$H and $^{13}$C NMR Spectra of Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**11**).

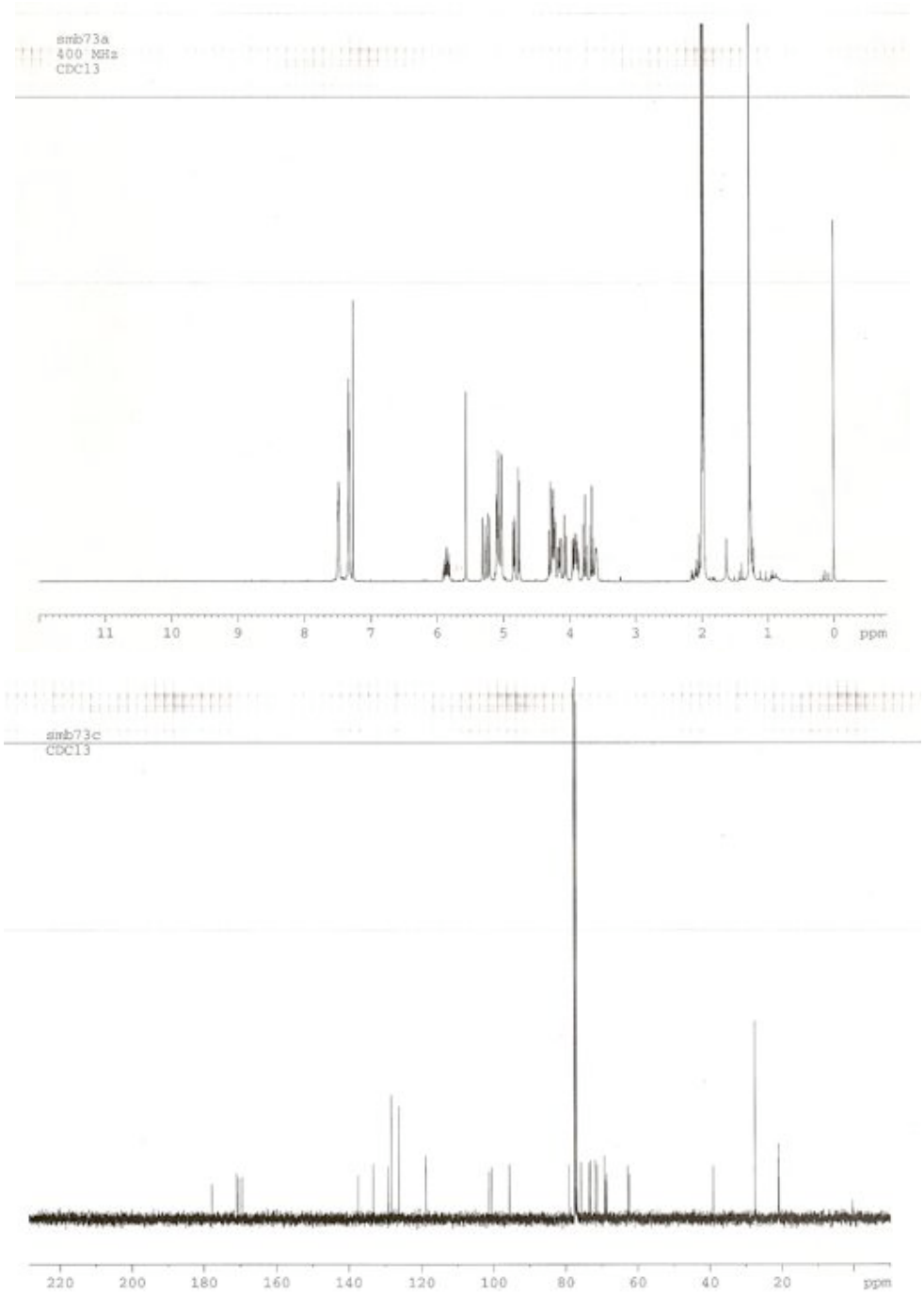


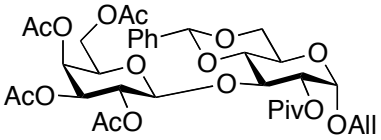



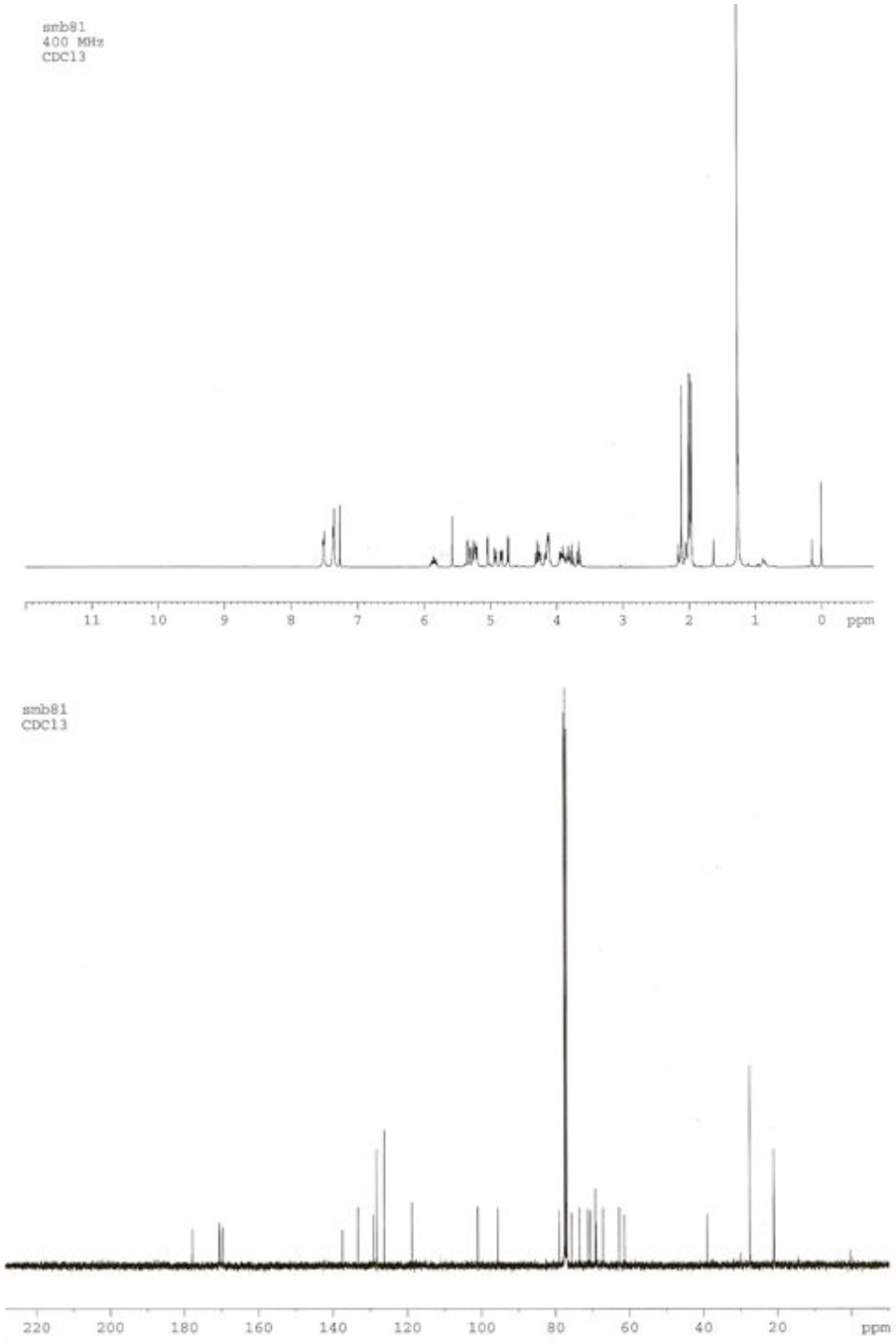

$^{1}$H and $^{13}$C NMR Spectra of Allyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**12**).



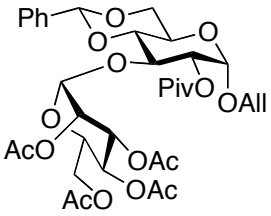



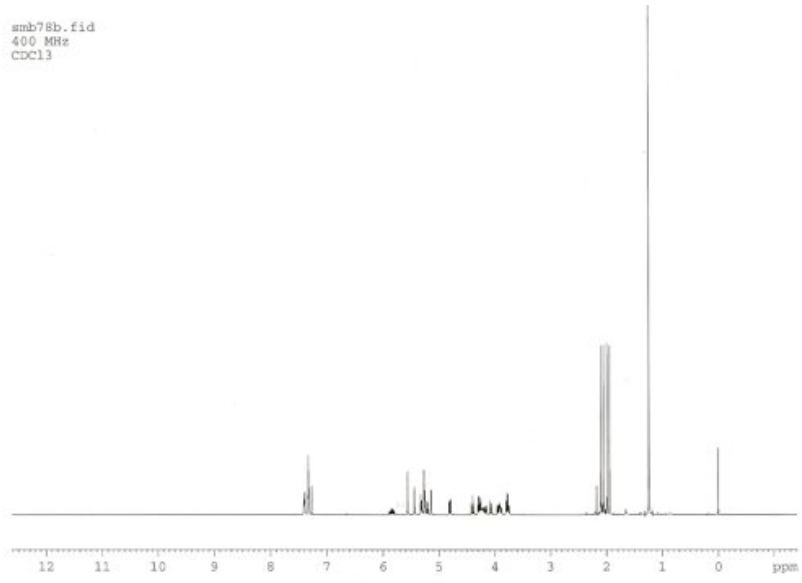

$^{1}$H and $^{13}$C NMR Spectra of Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**13**).

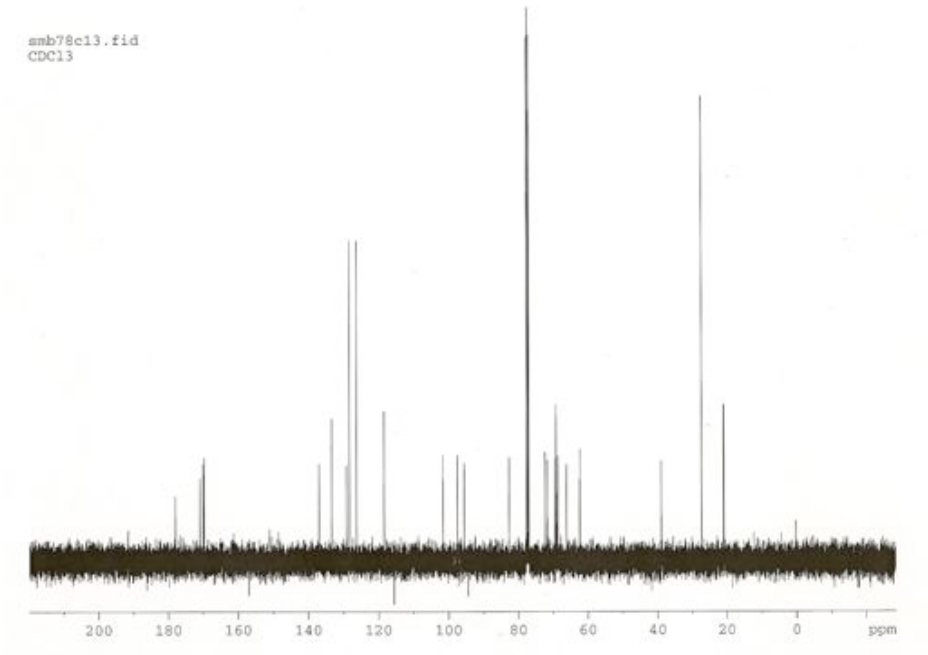


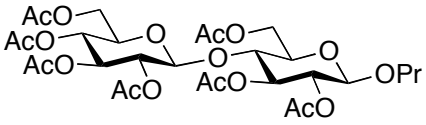

$^{1}$H and $^{13}$C NMR Spectra of Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**14**).

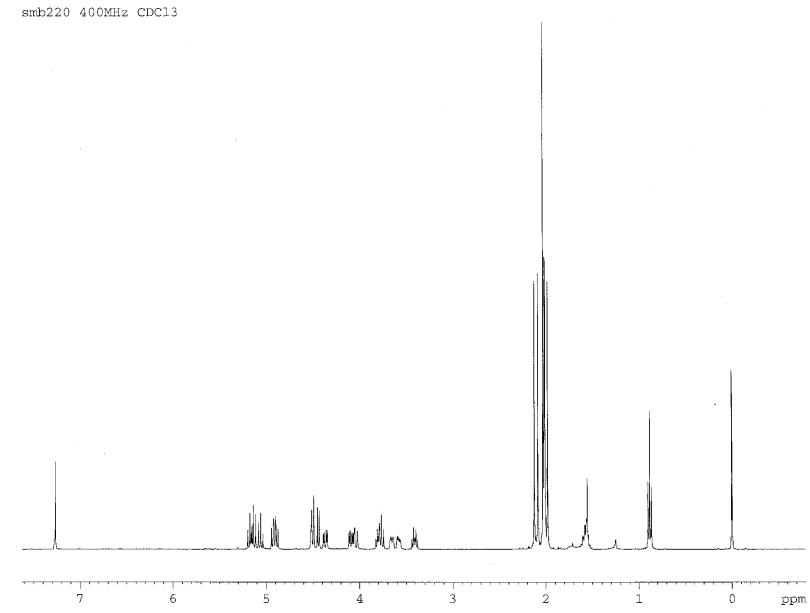


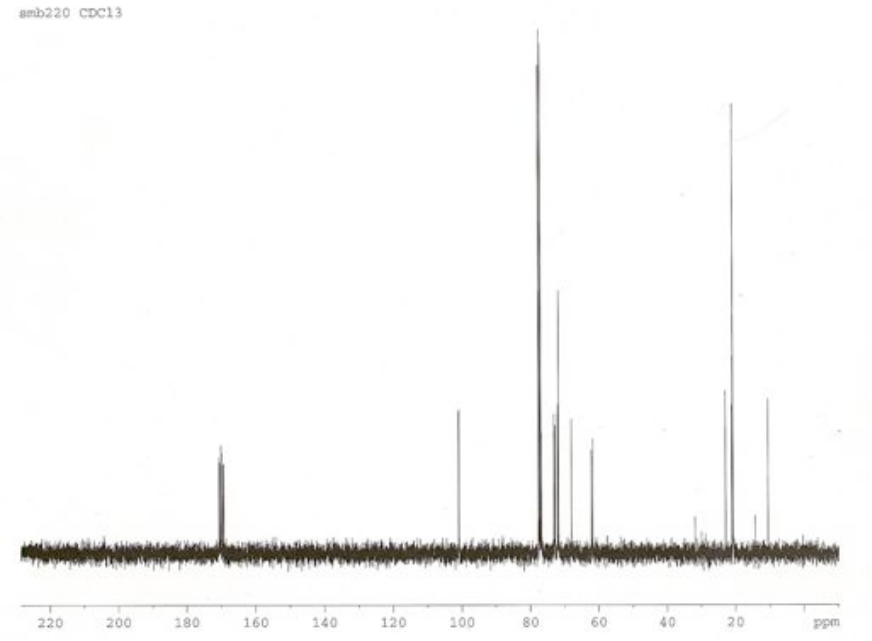



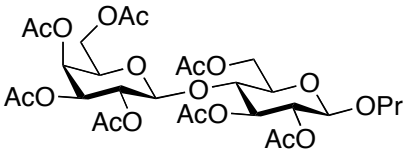

$^{1}$H and $^{13}$C NMR Spectra of Allyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**15**).

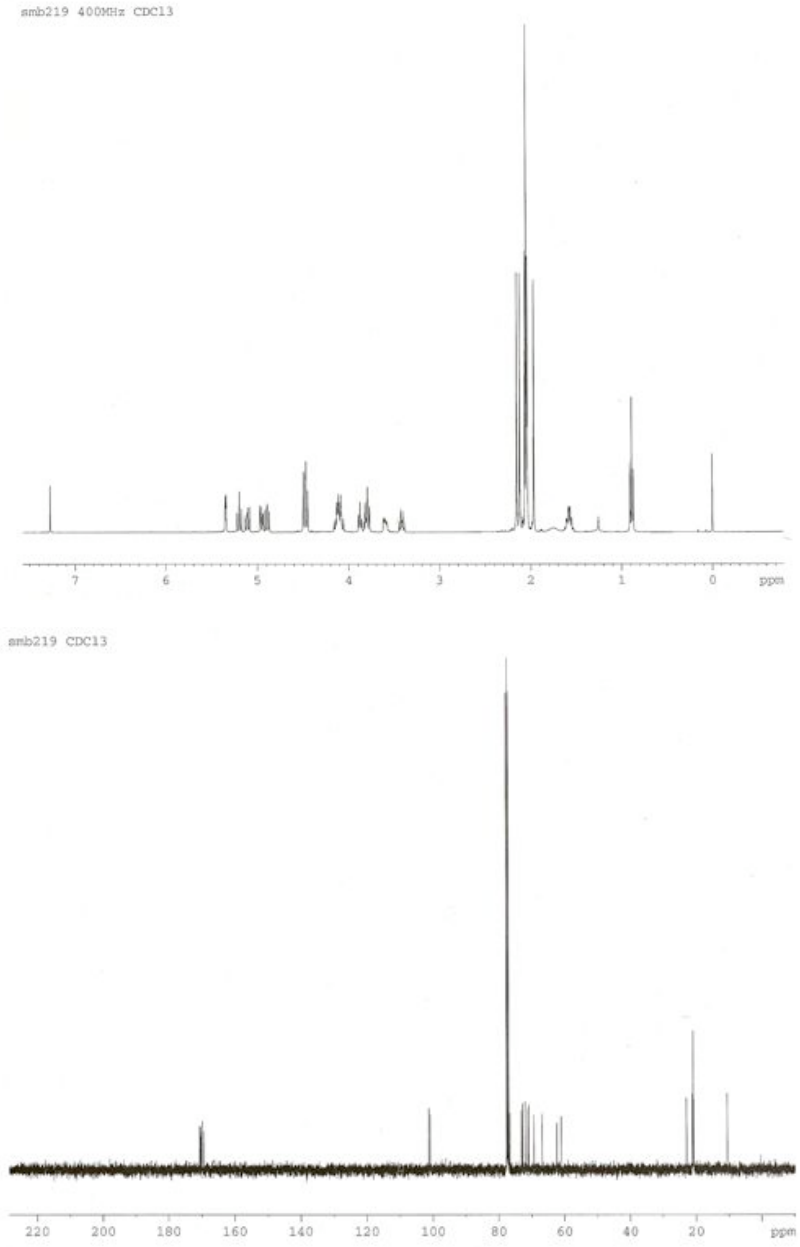


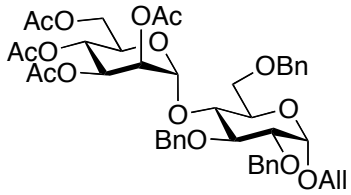



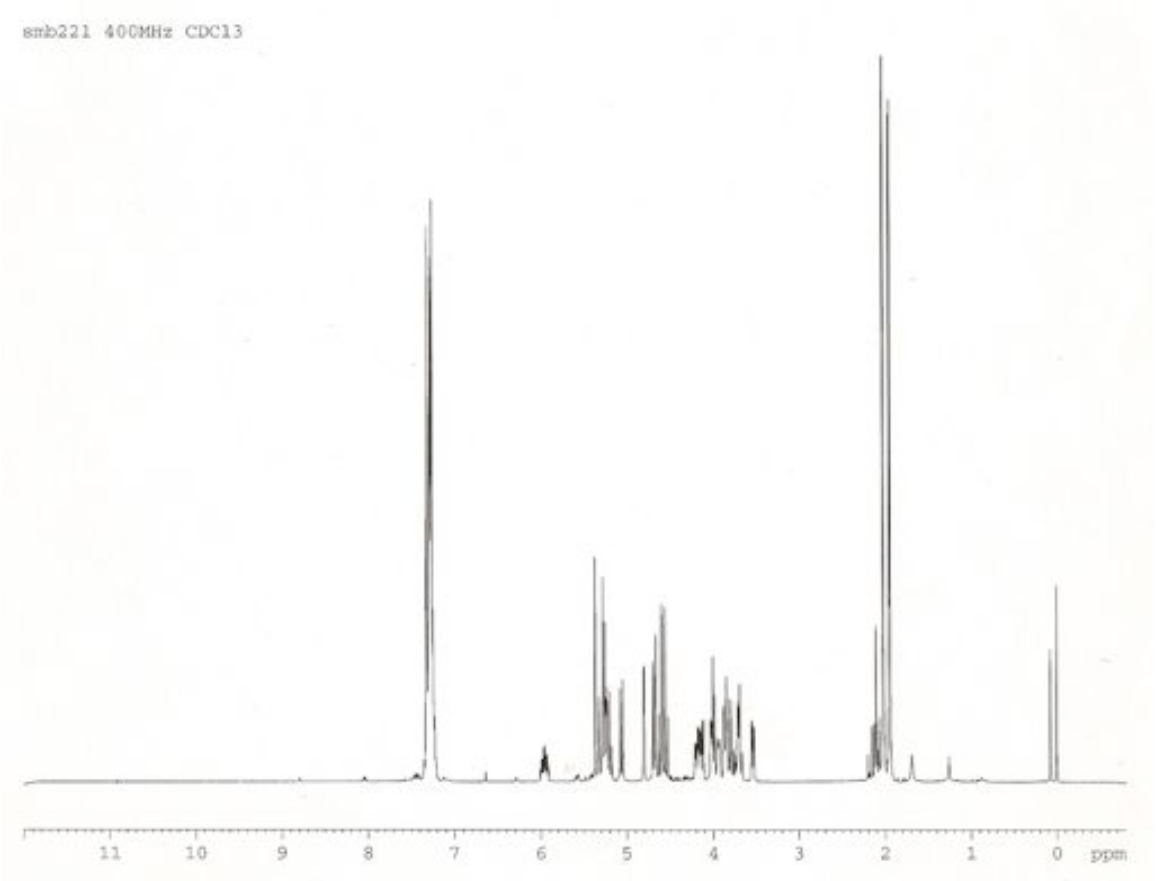

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- β-D-glucopyranoside (**17**).



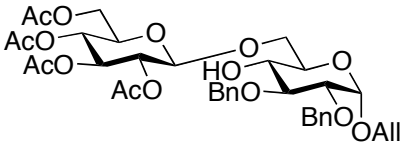



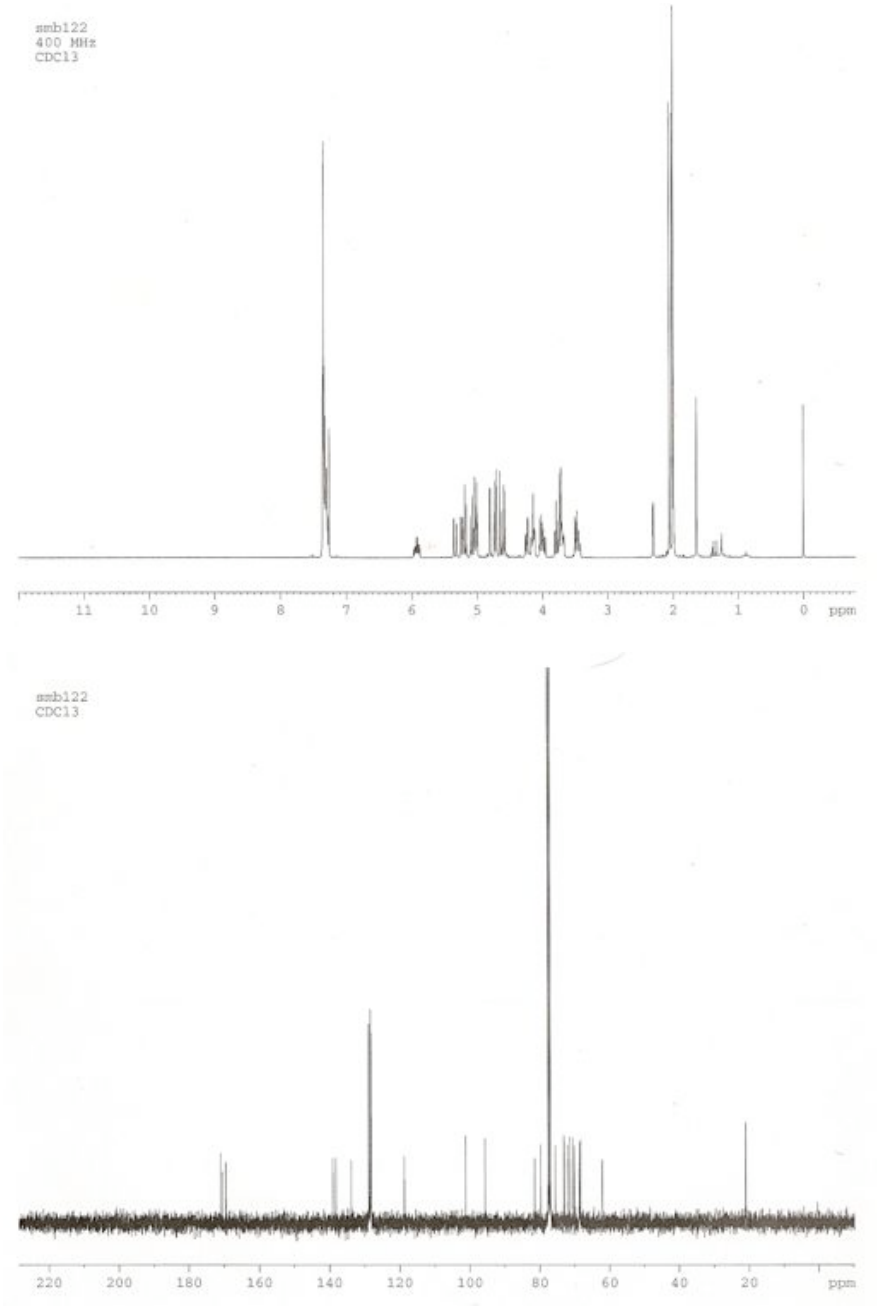

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)- β-D-glucopyranoside (**19**).



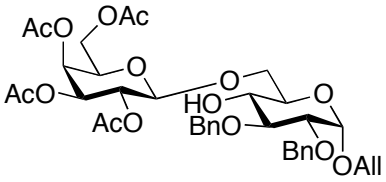



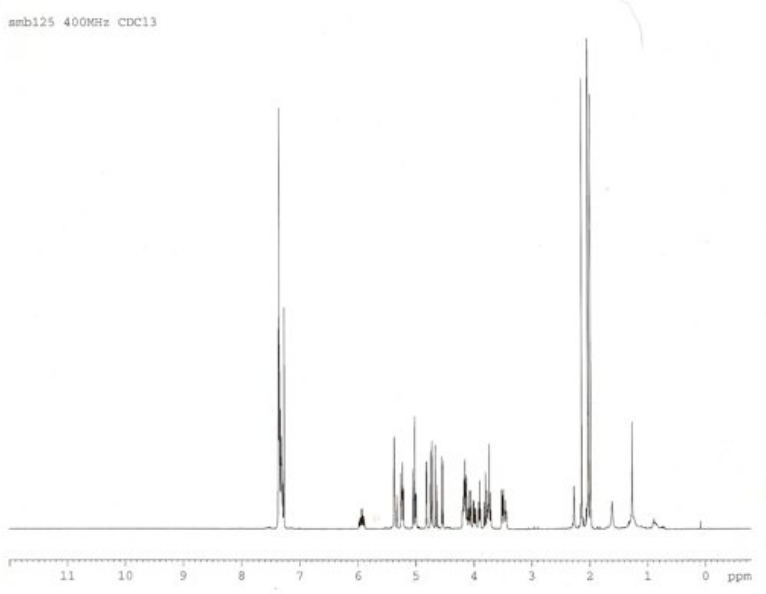

$^{1}$H NMR Spectrum of Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**20**).

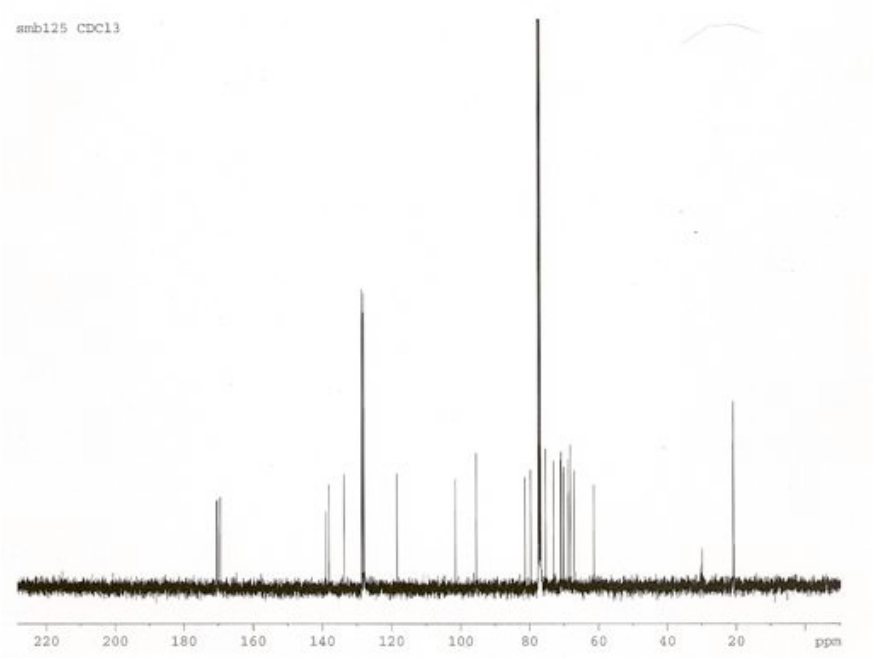


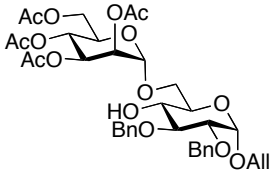

$^{1}$H and $^{13}$C NMR Spectra of Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**21**).

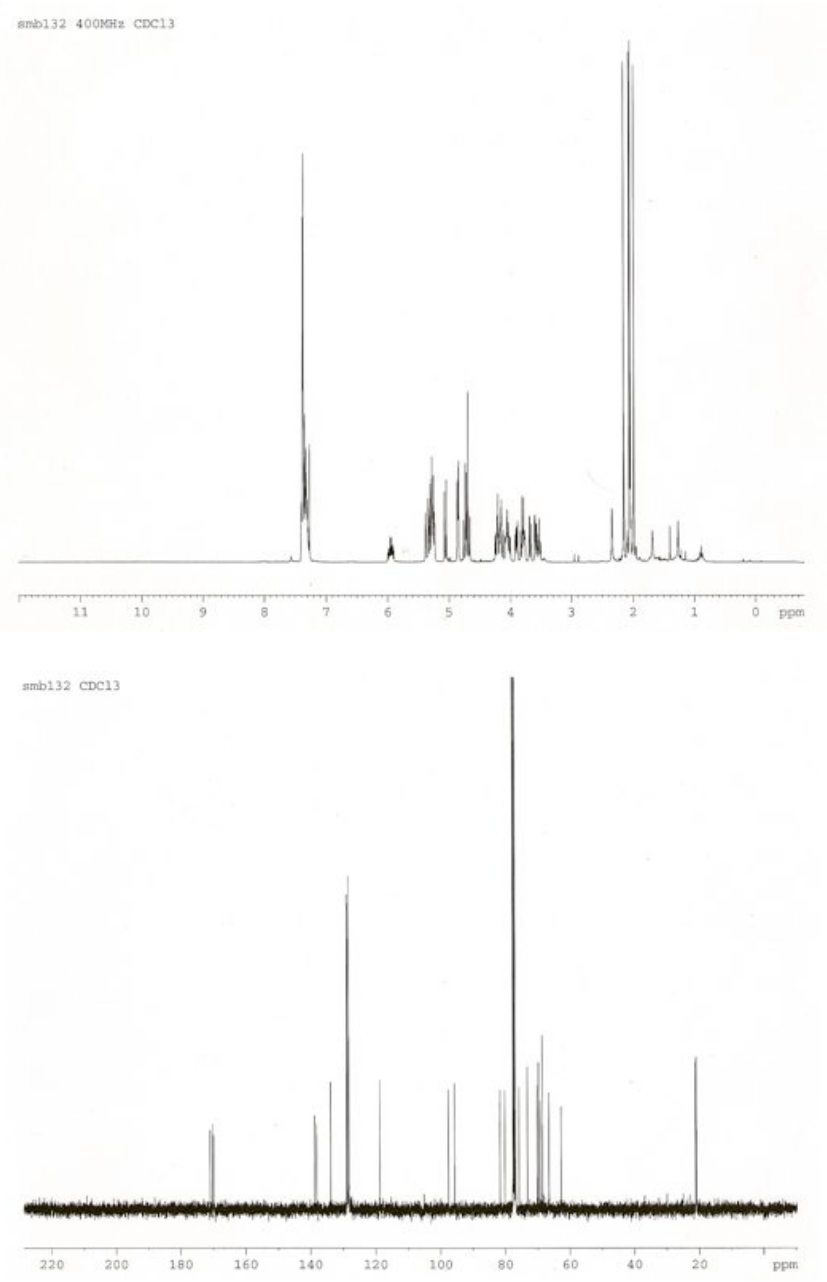


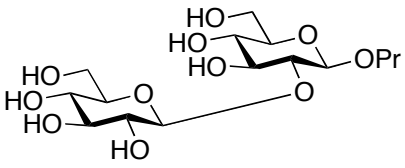



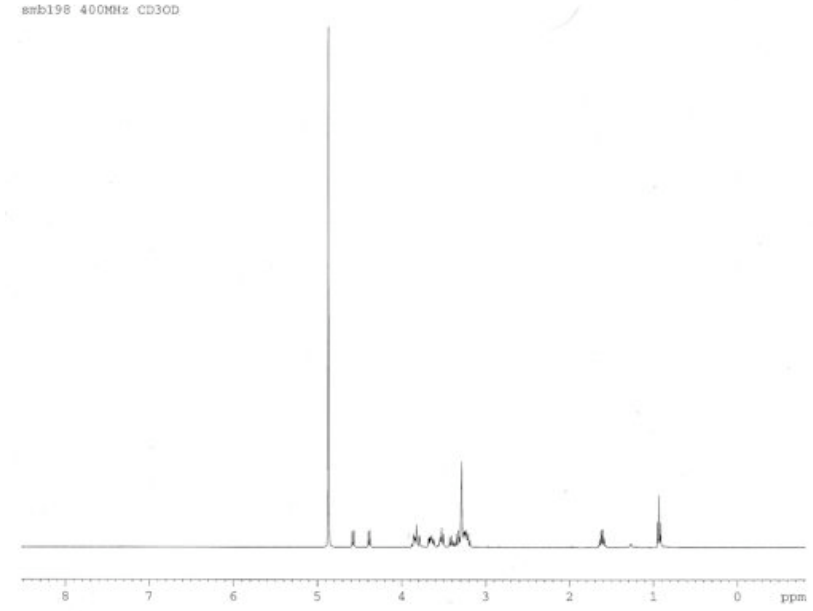

$^{1}$H and $^{13}$C NMR Spectra of Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**22**).

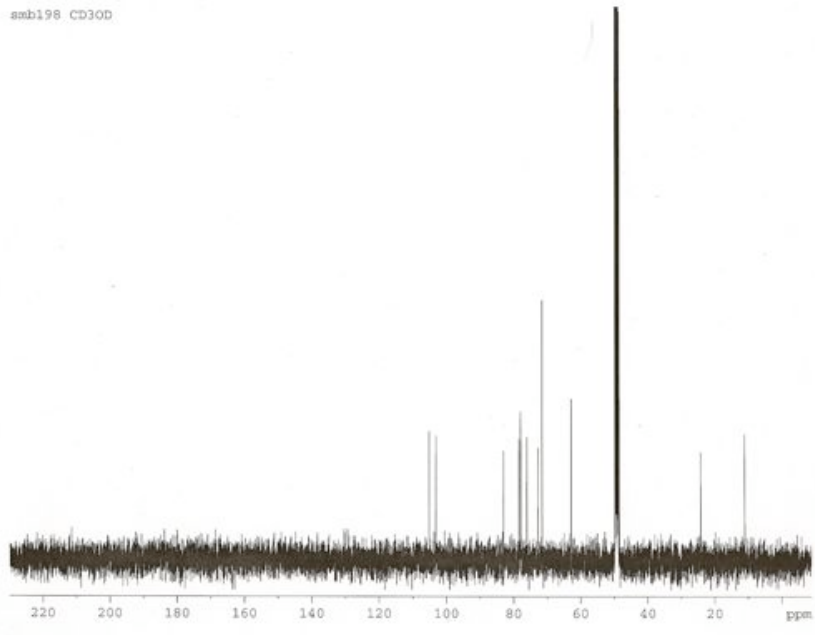


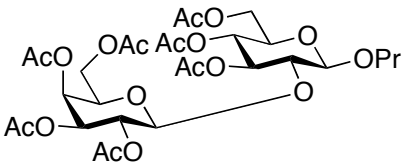

$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 2,3-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**23**).

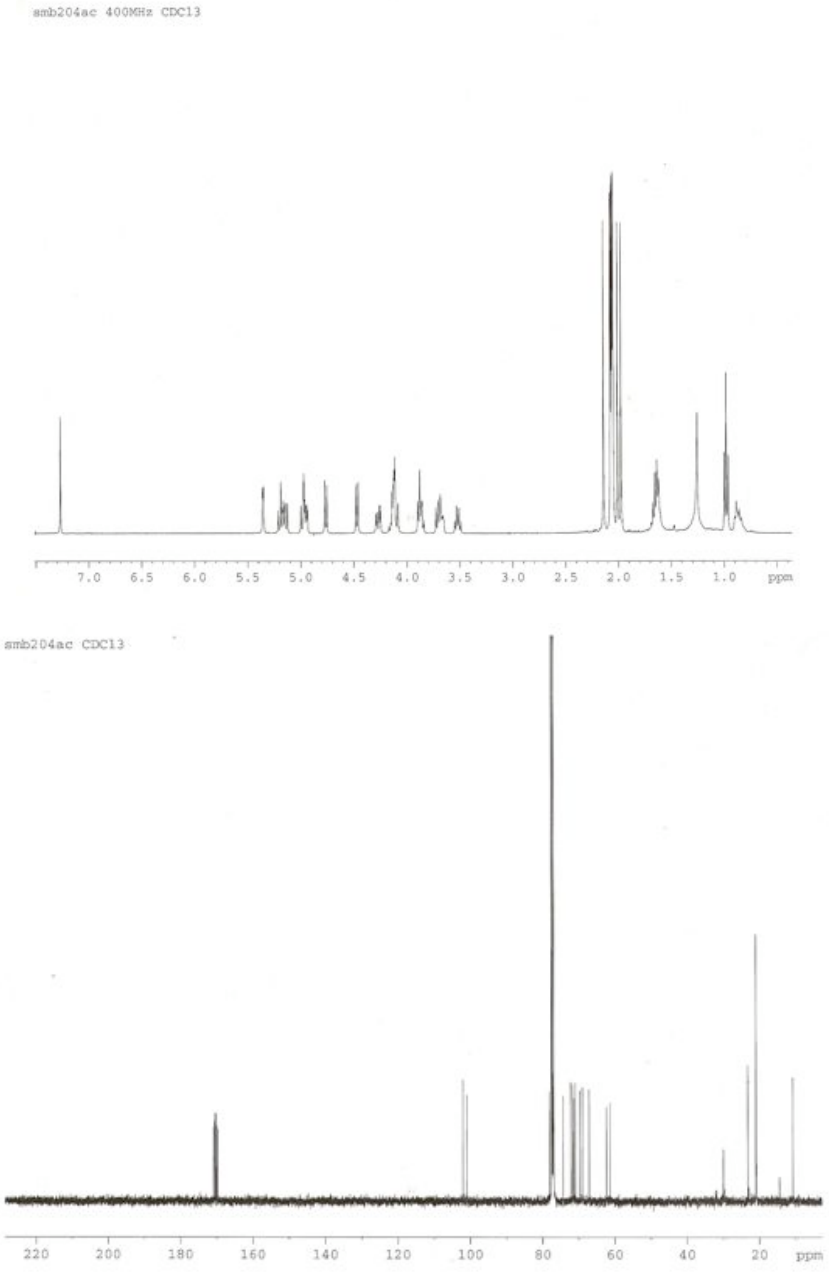


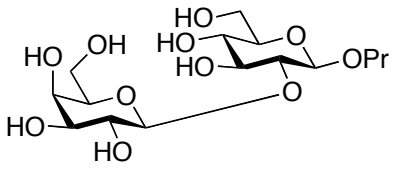



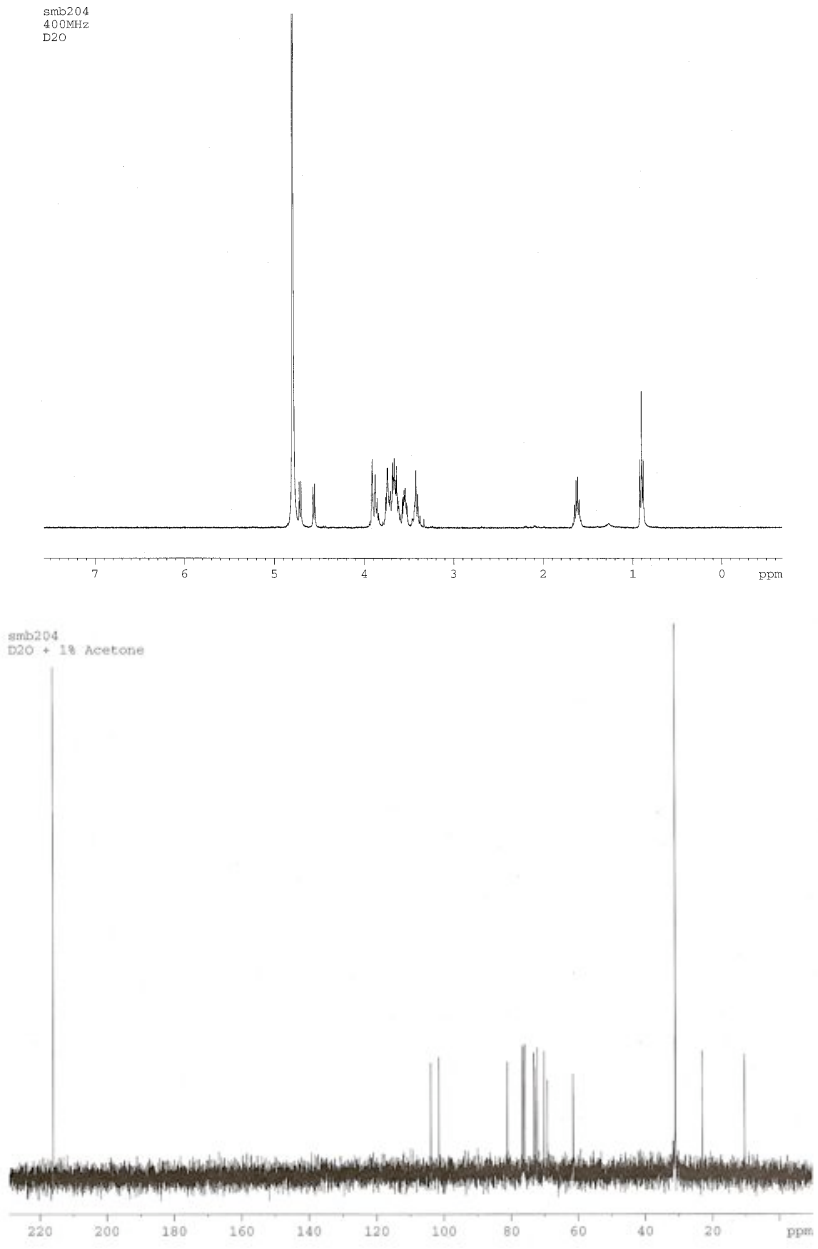

$^{1}$H and $^{13}$C NMR Spectra of Propyl 2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (**24**).



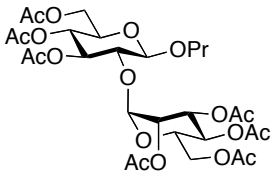



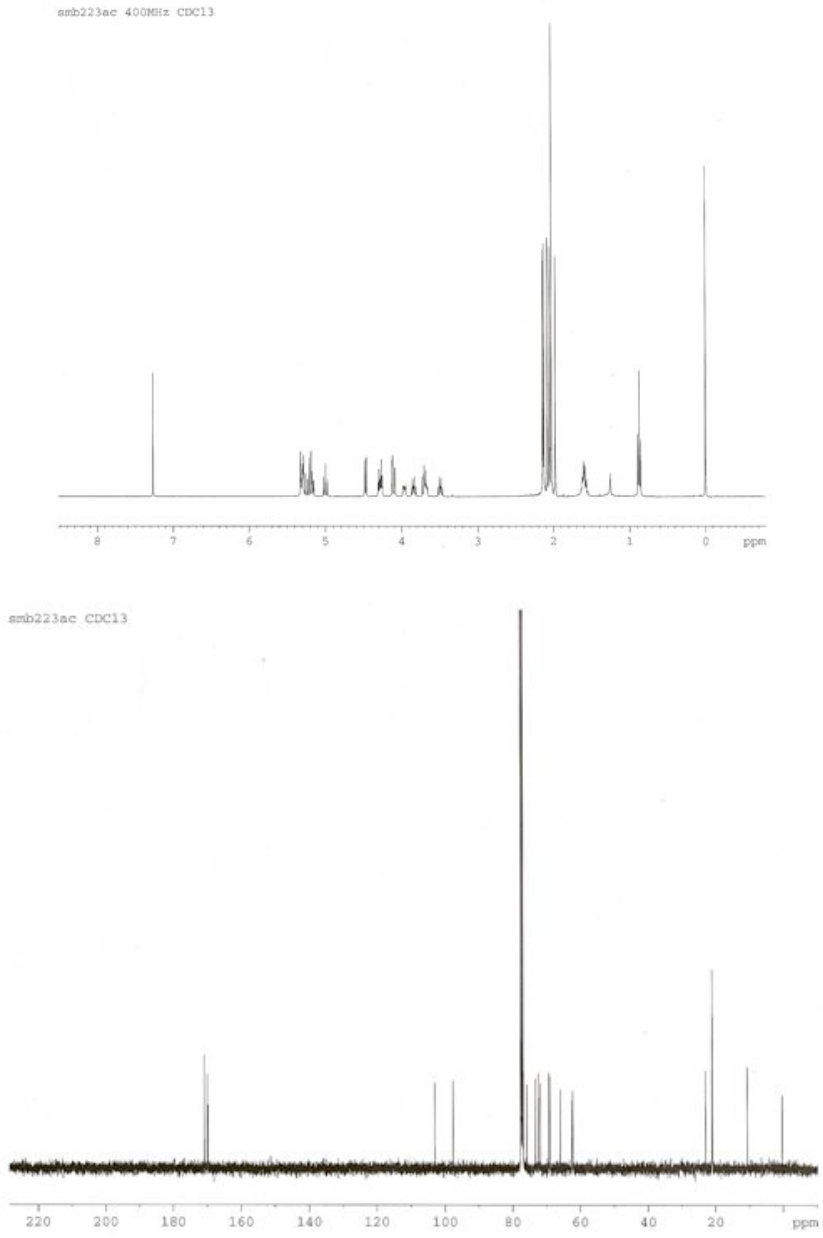

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**25**).



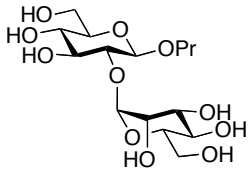



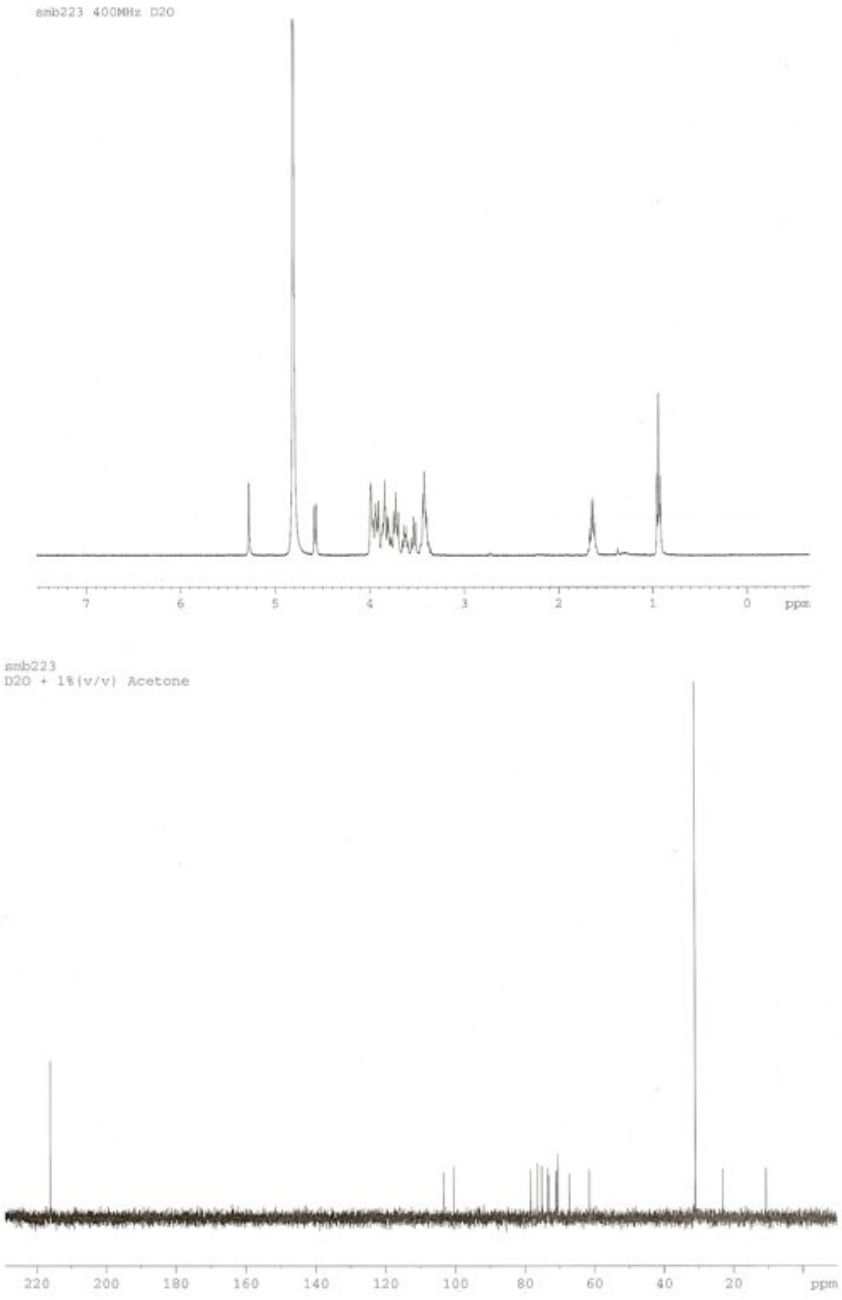

$^{1}$H and $^{13}$C NMR Spectra of Propyl 2-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**26**).



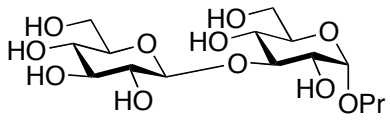



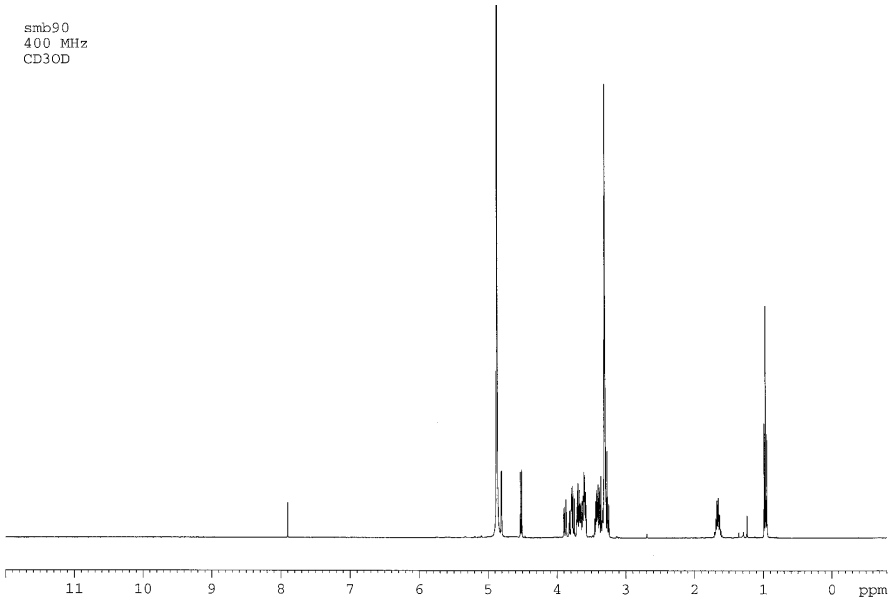

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**27**).

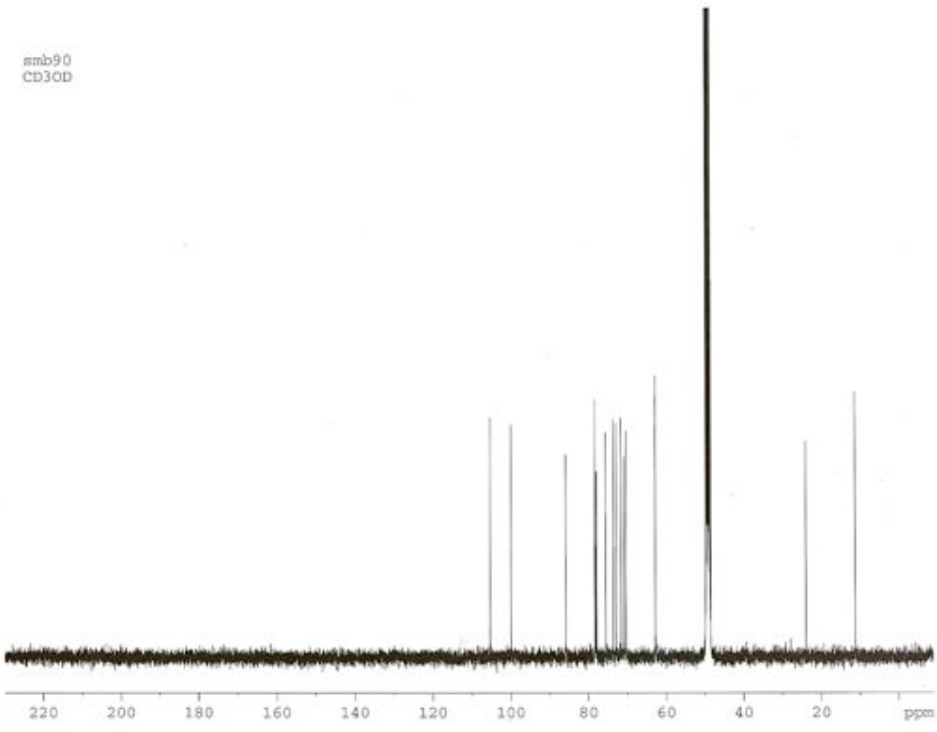


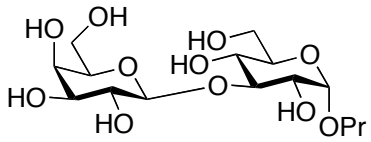

$^{1}$H and $^{13}$C NMR Spectra of Propyl 2-*O*-(α-D-mannopyranosyl)-β-D-glucopyranose (**28**).

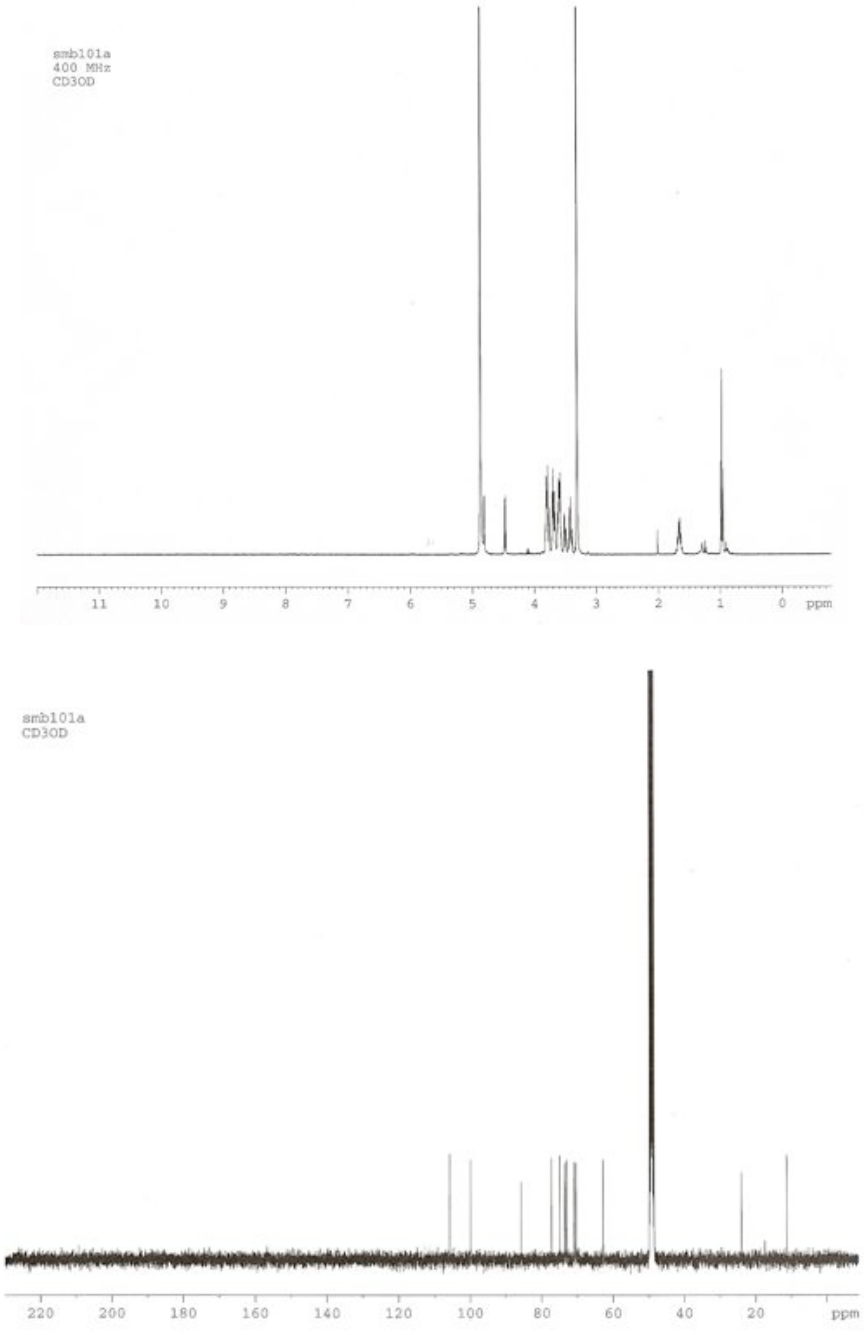


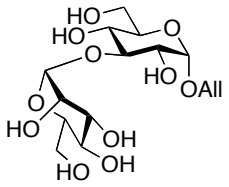



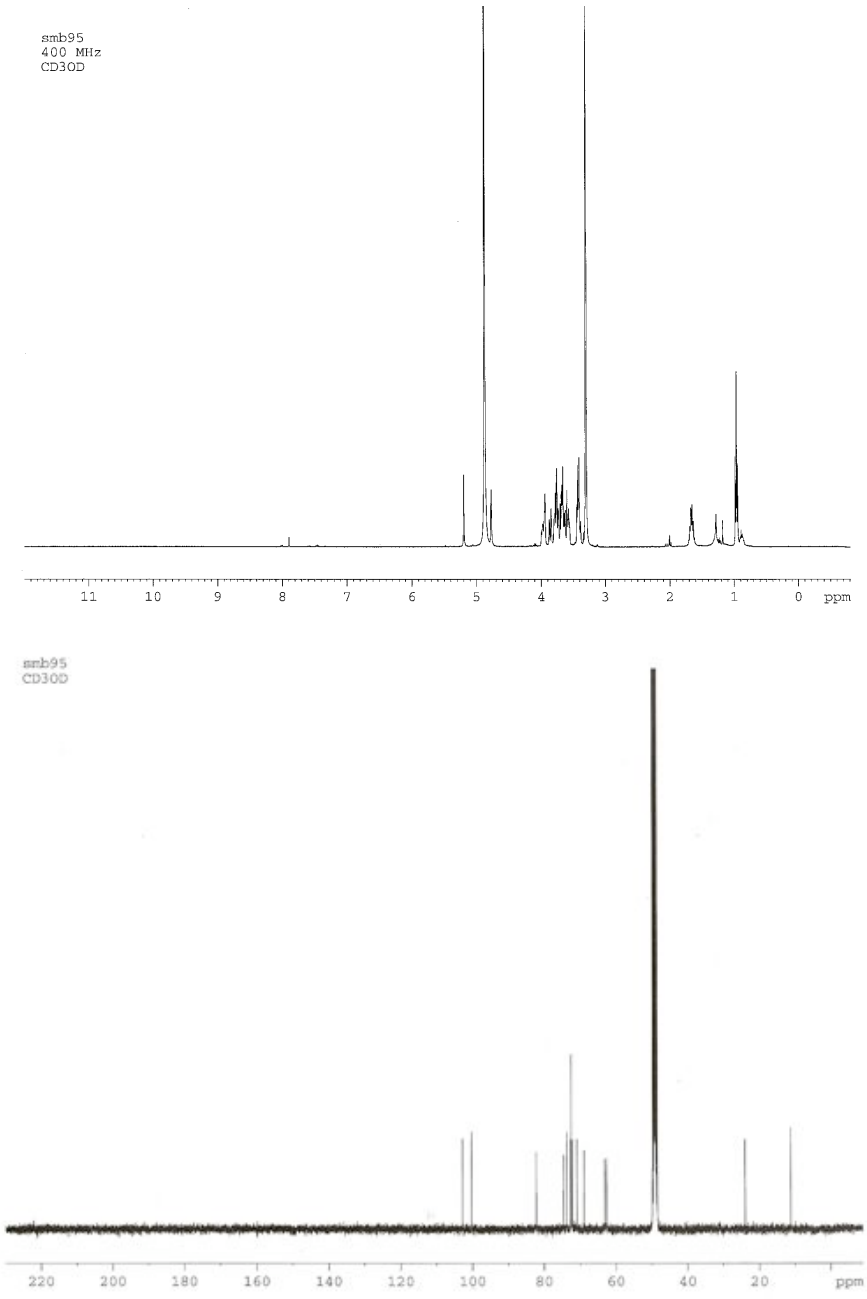

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 3-*O*-(β-D-glucopyranosyl)-α-D-glucopyranose (**29**).



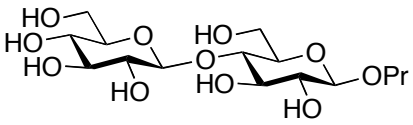



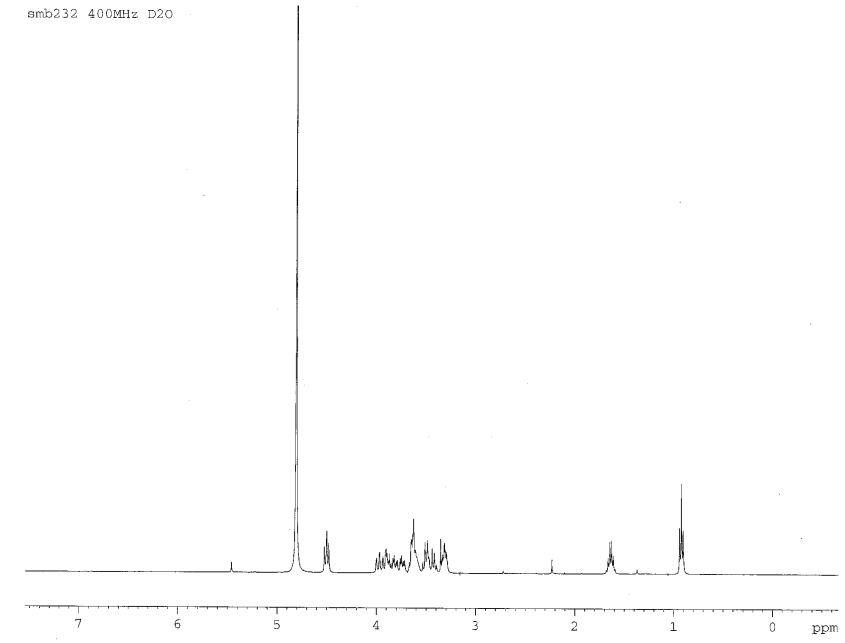

$^{1}$H and $^{13}$C NMR Spectra of Propyl 3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranose (**30**).

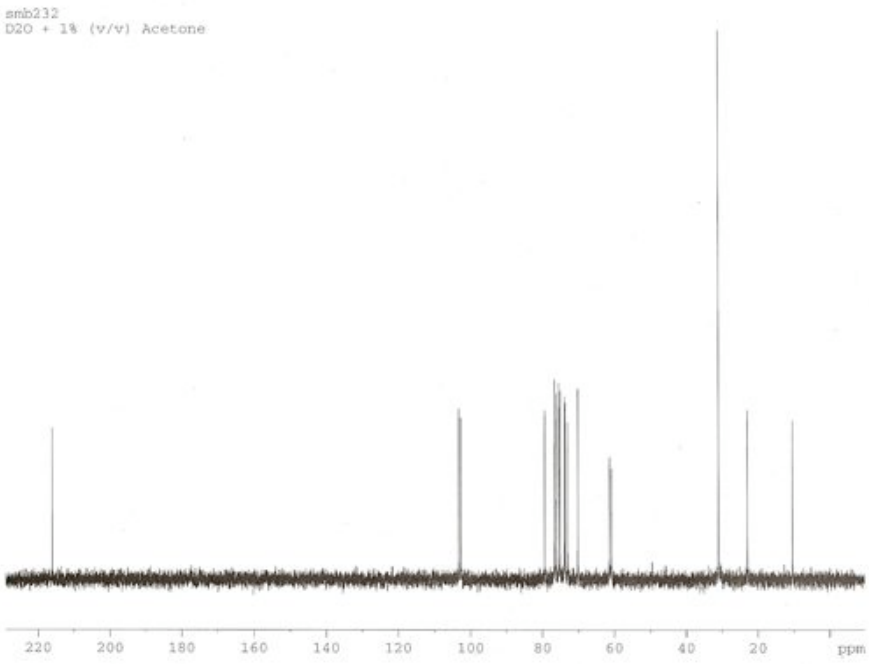


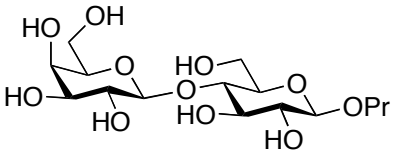

$^{1}H$ and $^{13}C$ NMR Spectra of Propyl 3-*O*-(α-D-mannopyranosyl)-α-D-glucopyranose (**31**).

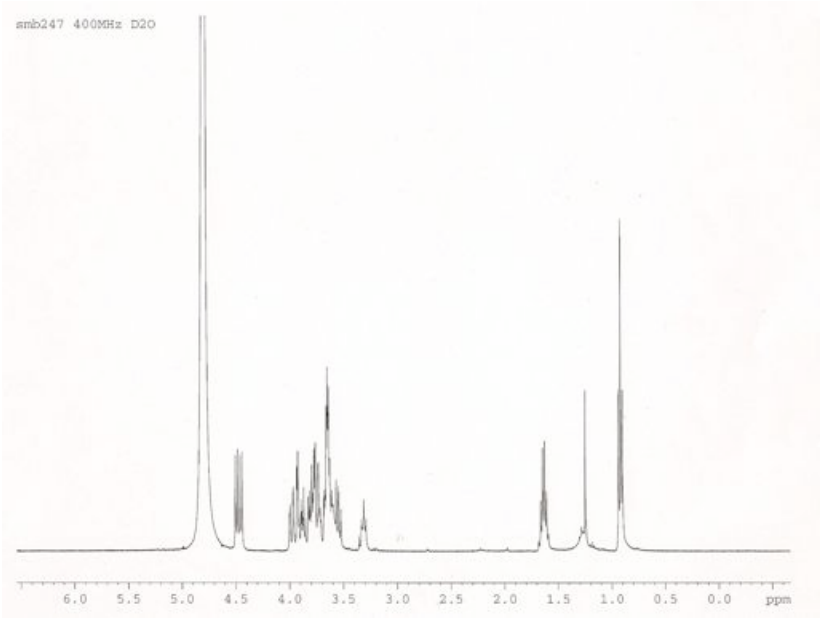


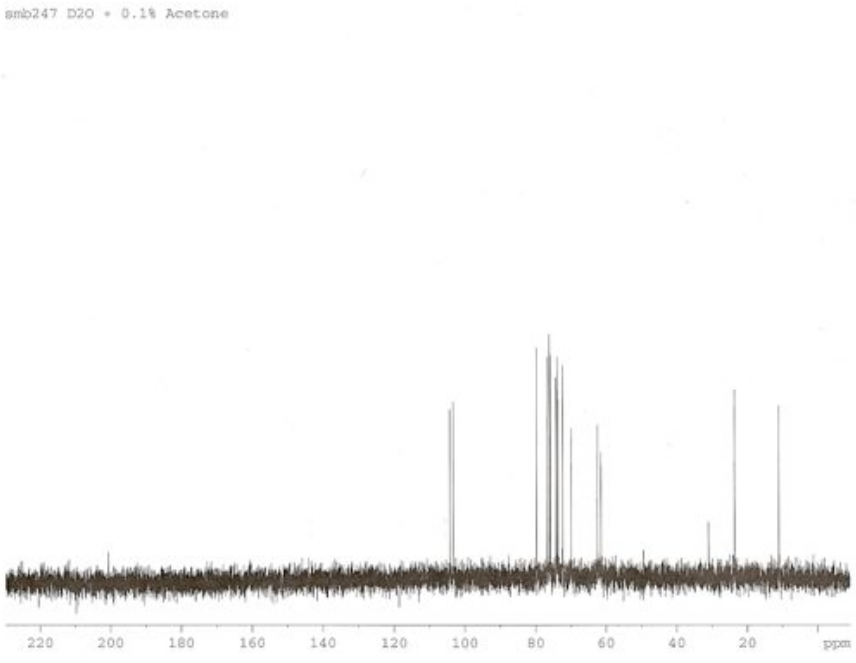



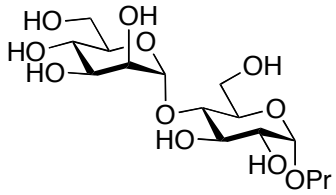

$^{1}$H and $^{13}$C NMR Spectra of Propyl 4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside (**32**).

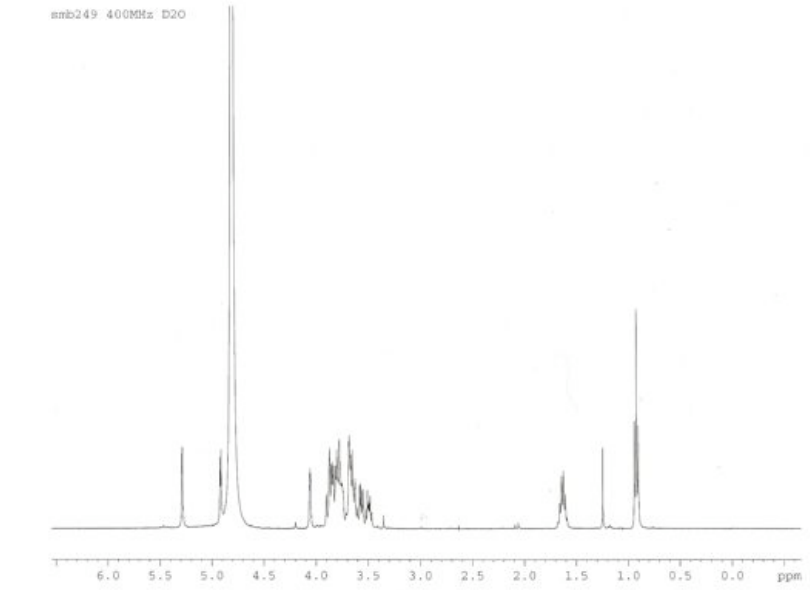


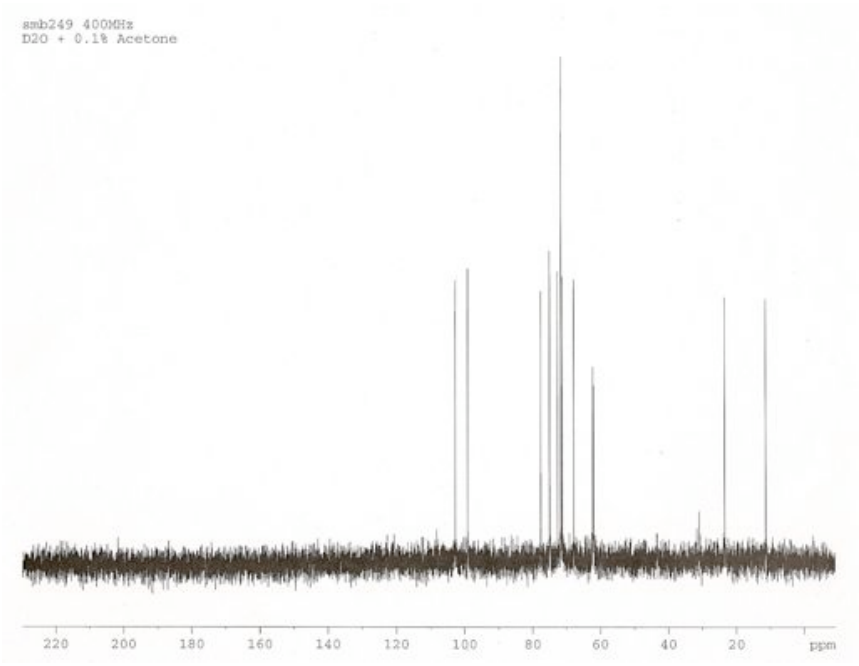



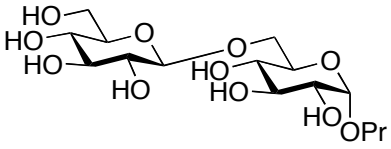

$^{1}$H and $^{13}$C NMR Spectra of Propyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**33**).

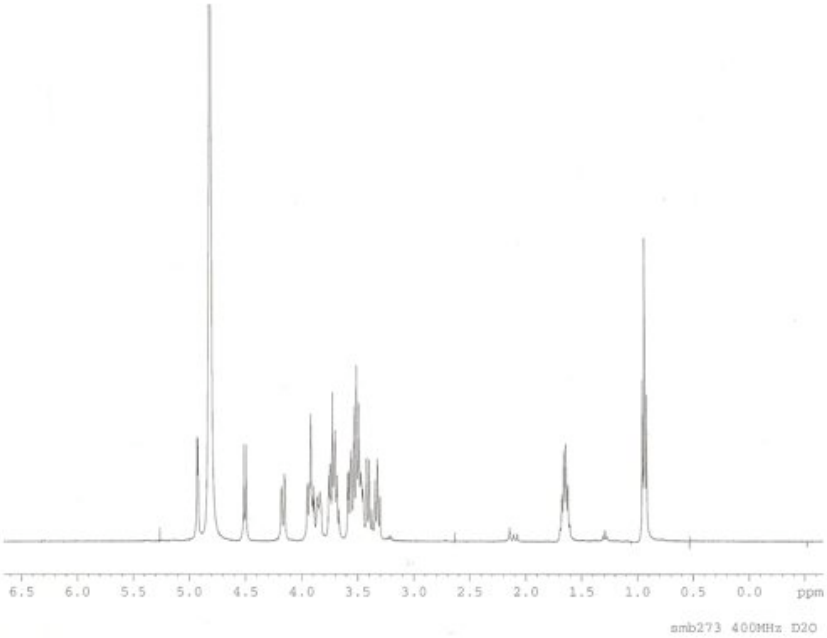


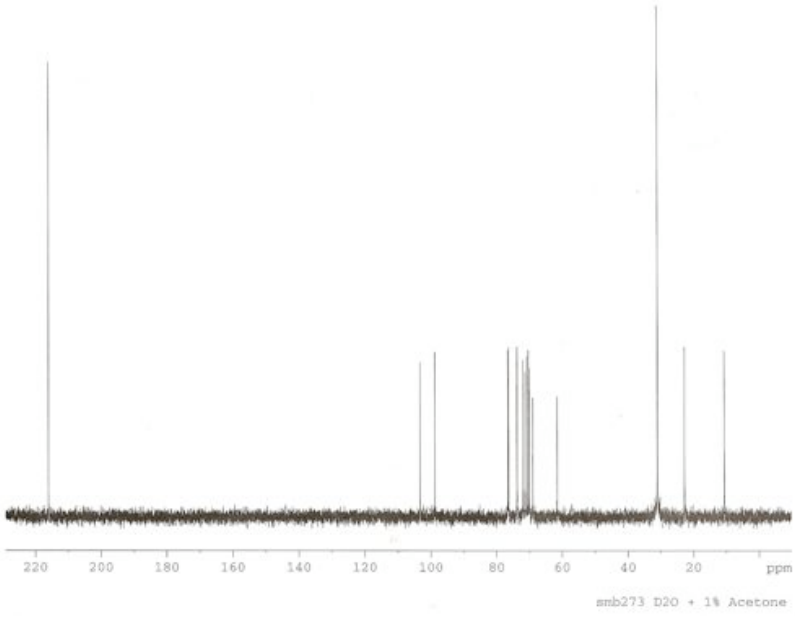



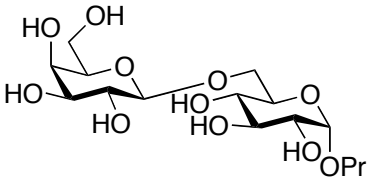

$^{1}$H and $^{13}$C NMR Spectra of Propyl 4-*O*-(α-D-mannopyranosyl)-α-D-glucopyranoside (**34**).

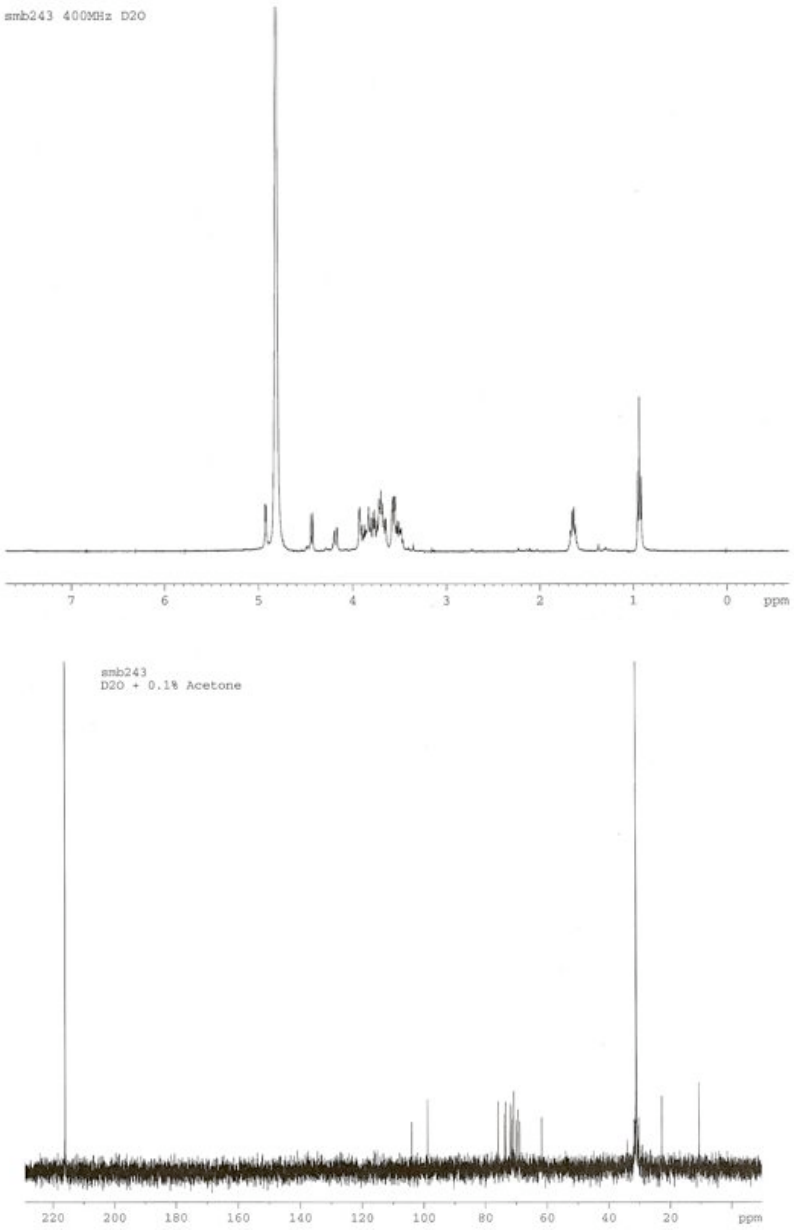


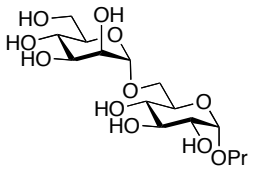



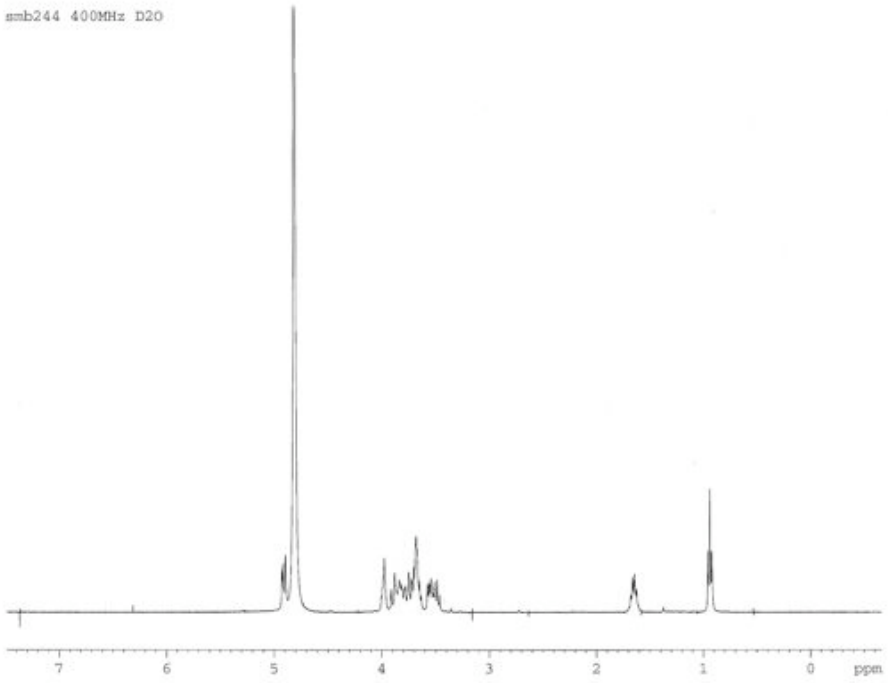

$^{1}$H and $^{13}$C NMR Spectra of Propyl 6-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside (**35**).



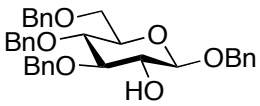



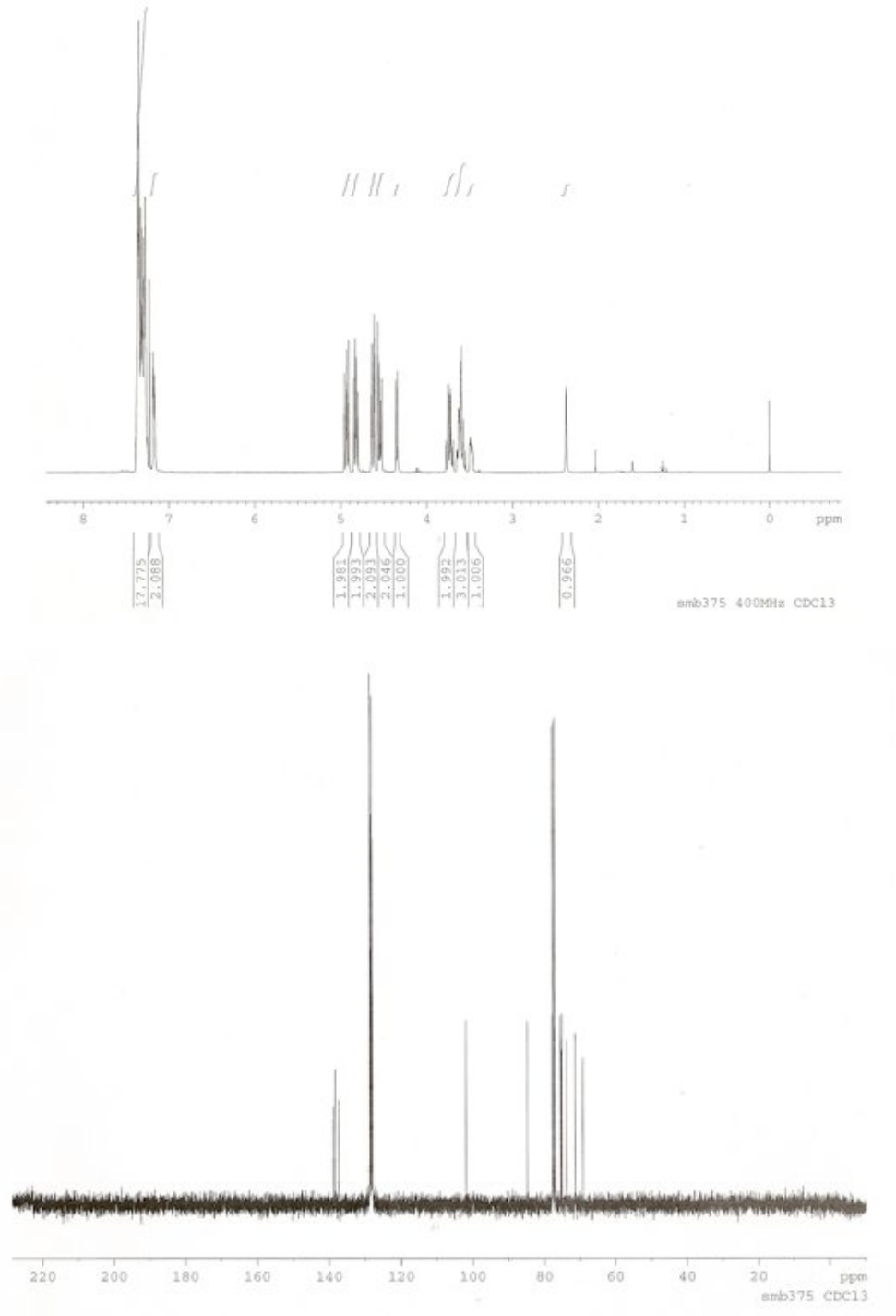

$^{1}$H and $^{13}$C NMR Spectra of Propyl 6-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside (**36**).



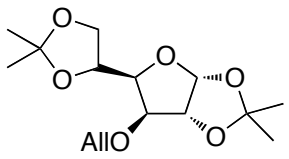



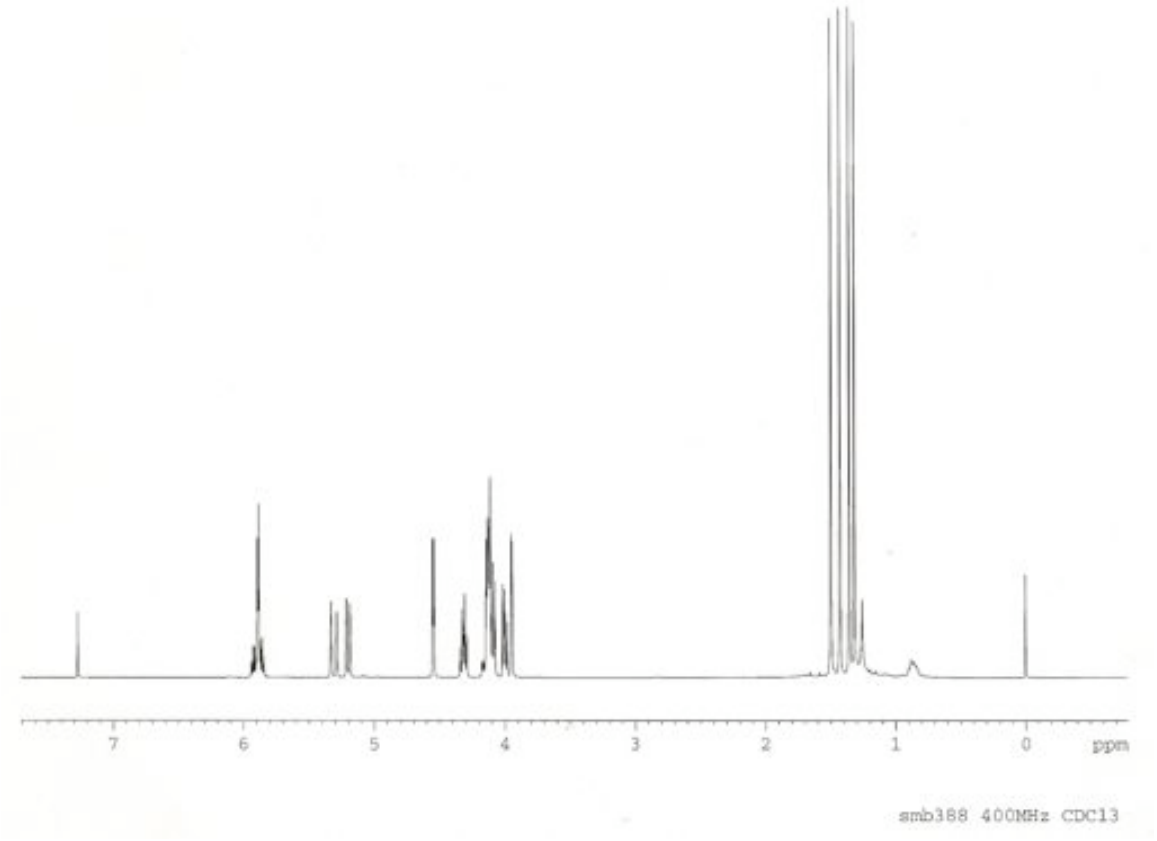

$^{1}$H NMR Spectrum of Propyl 6-*O*-(α-D-mannopyranosyl)-α-D-glucopyranoside (**37**).



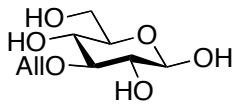



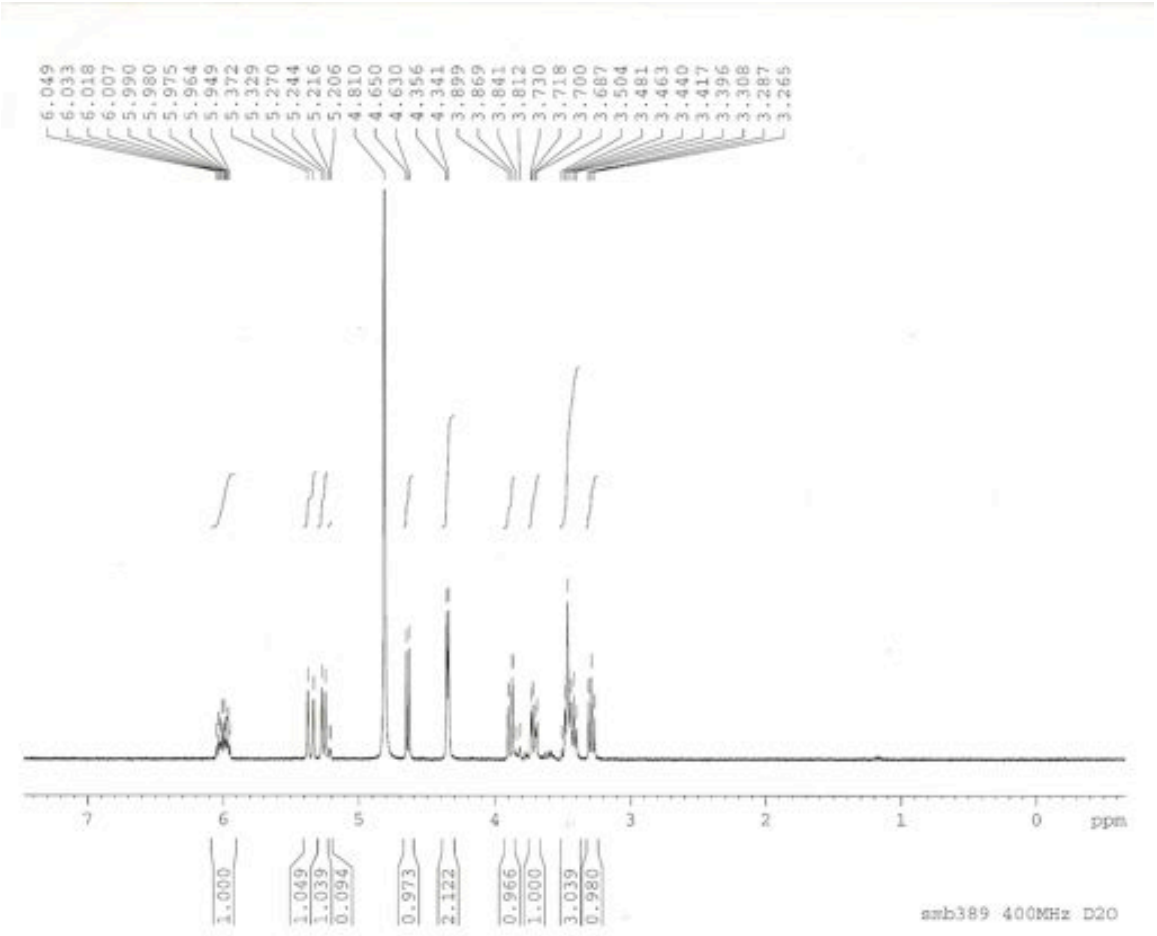

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**39**).



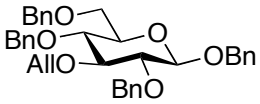



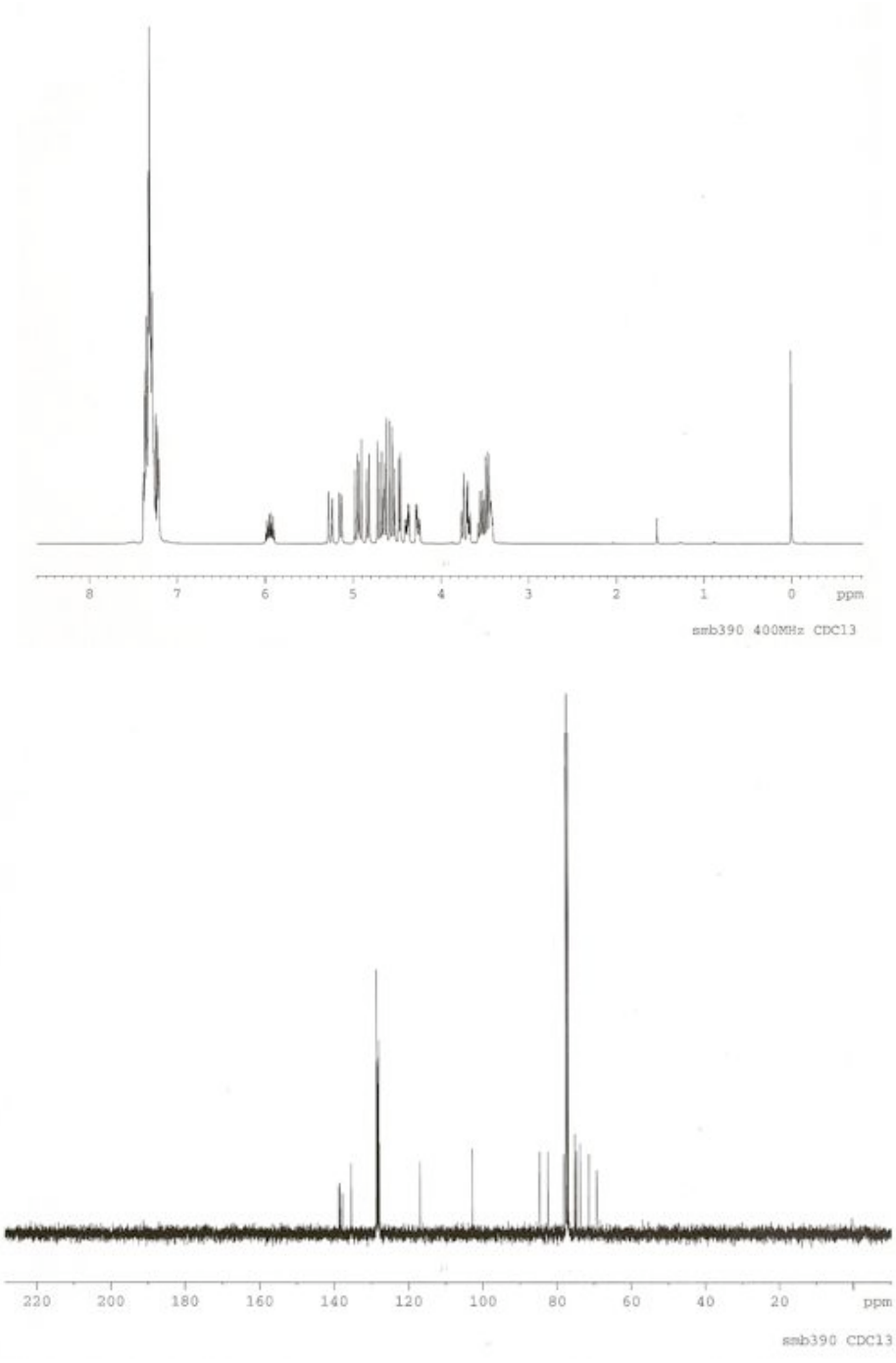

$^{1}$H NMR Spectrum of 3-*O*-allyl-1,2-5,6-di-*O*-isopropylidene-D-glucofuranose (**41**).



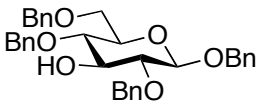



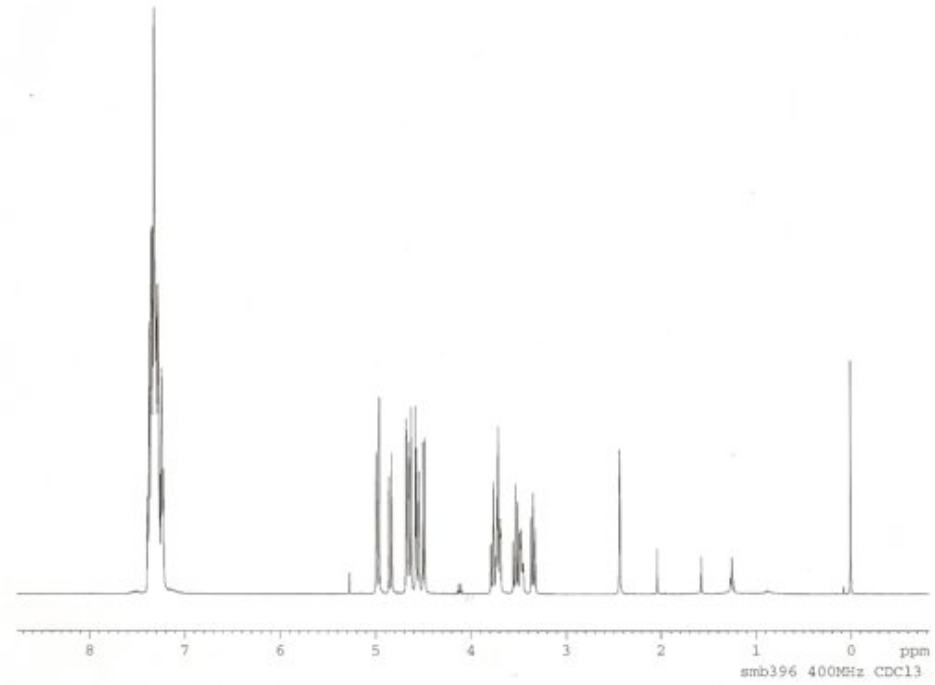

$^{1}$H NMR Spectrum of 3-*O*-allyl-β-D-glucopyranose (**42**).

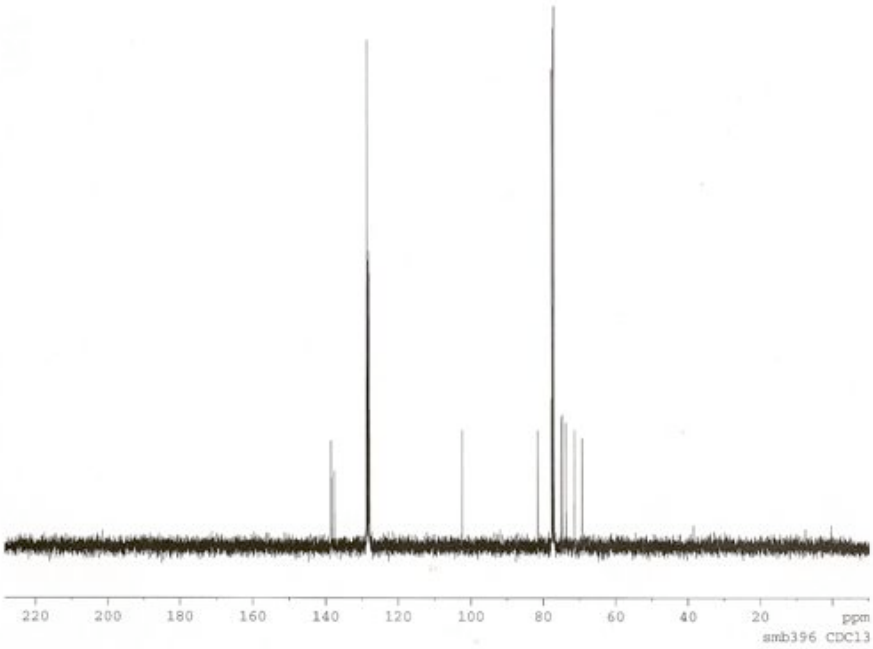


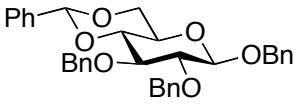

$^{1}$H and $^{13}$C NMR Spectra of Benzyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**43**).

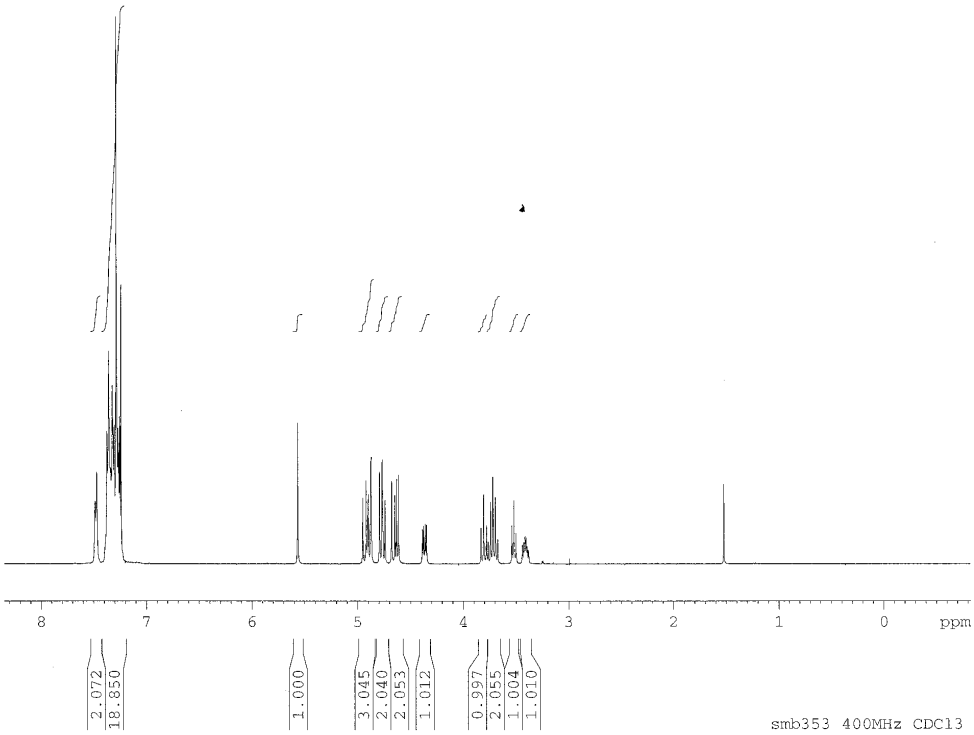


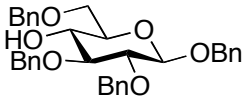



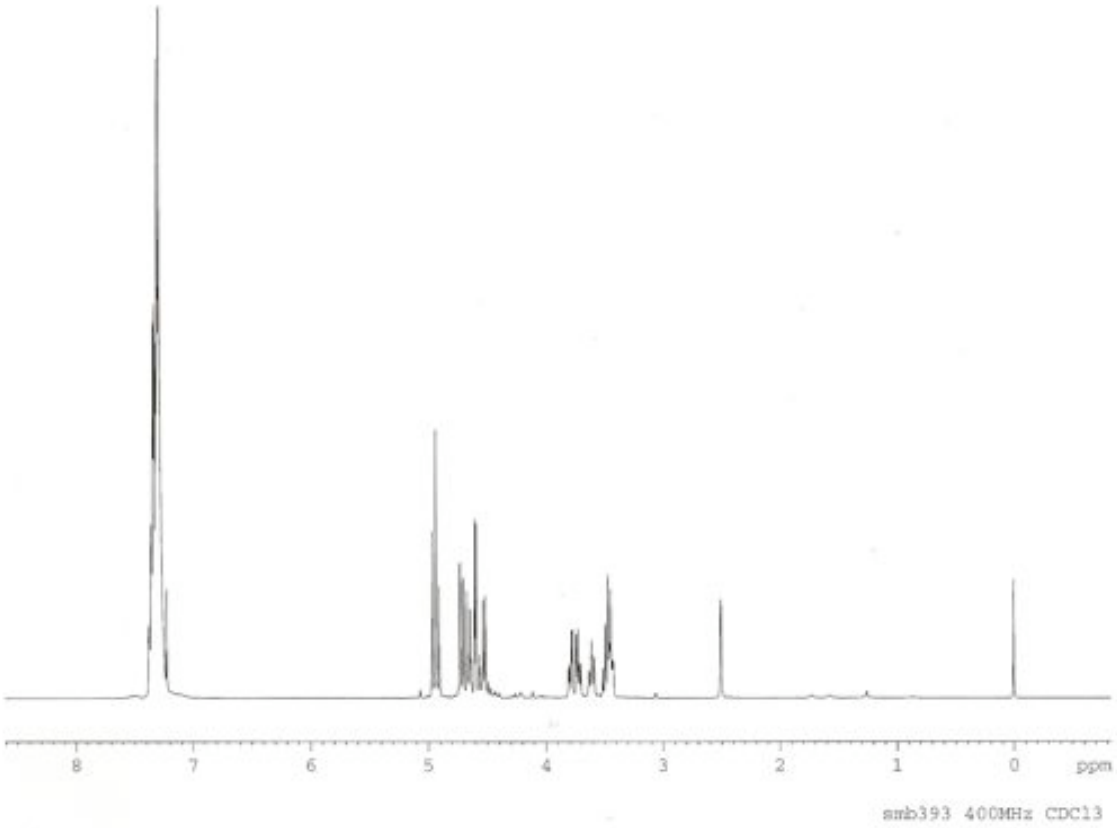

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**44**).



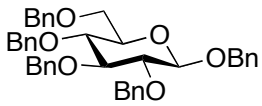



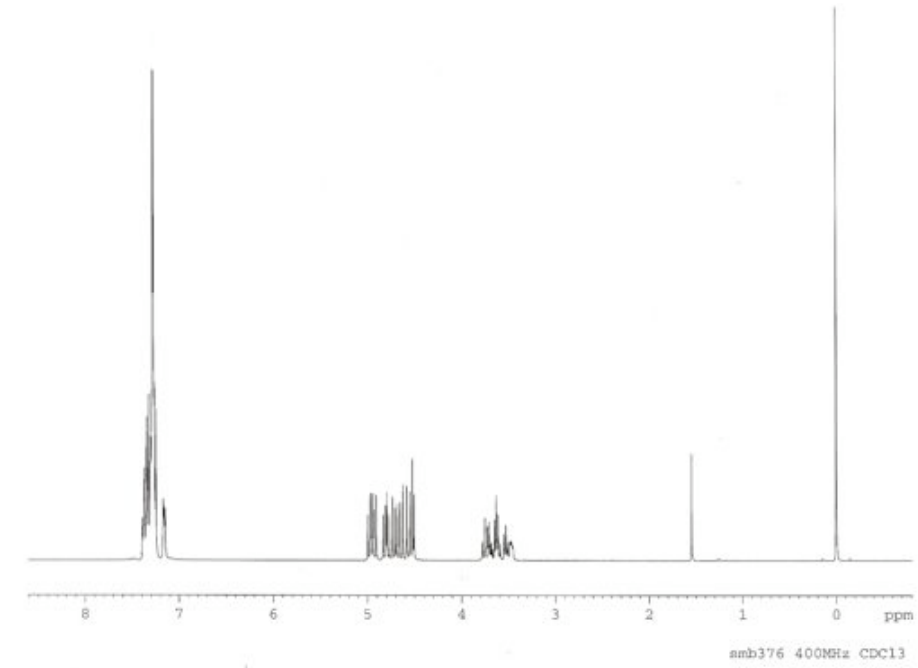

$^{1}$H NMR Spectrum of Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**48**).

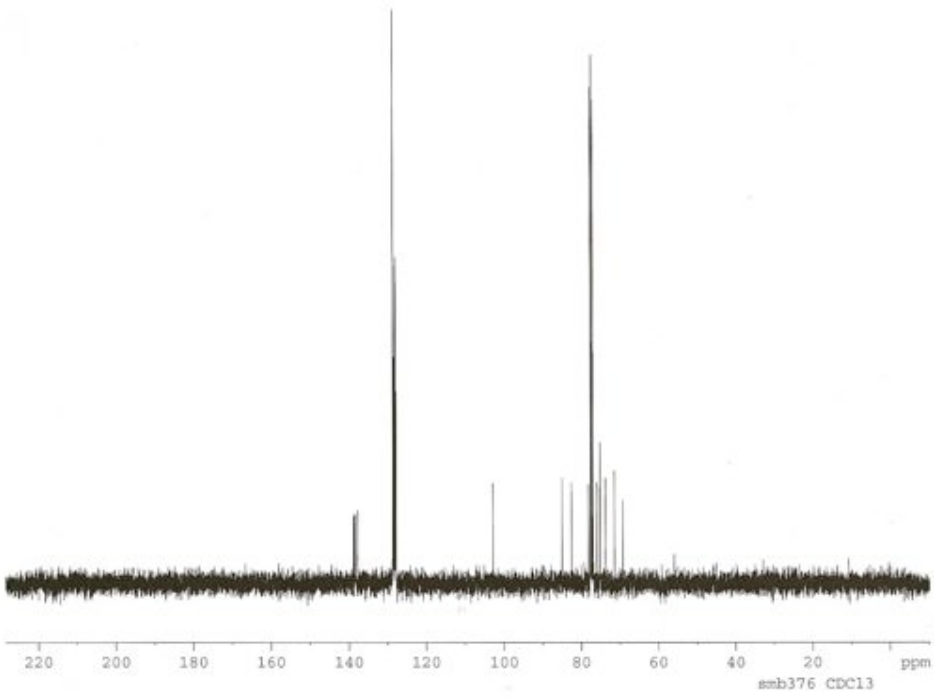


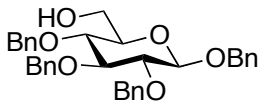

$^{1}$H NMR Spectrum of Benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**49**).

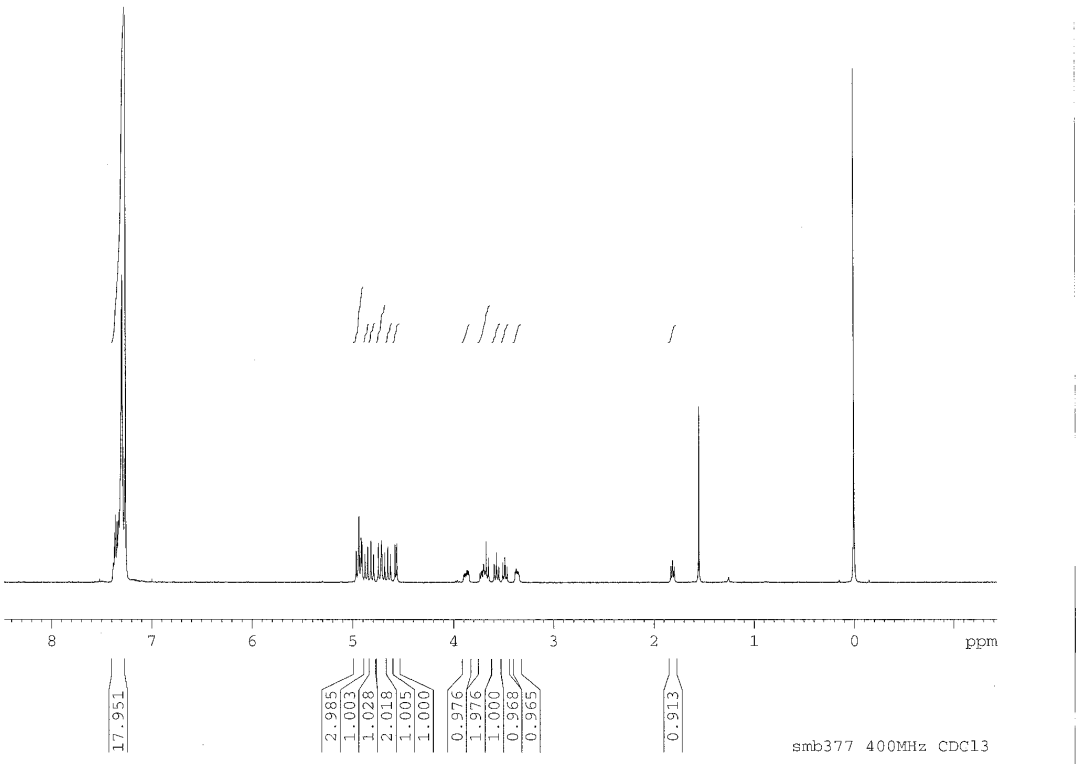


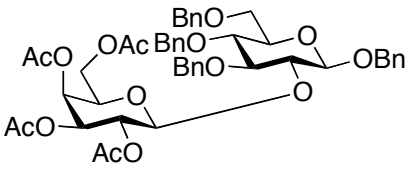



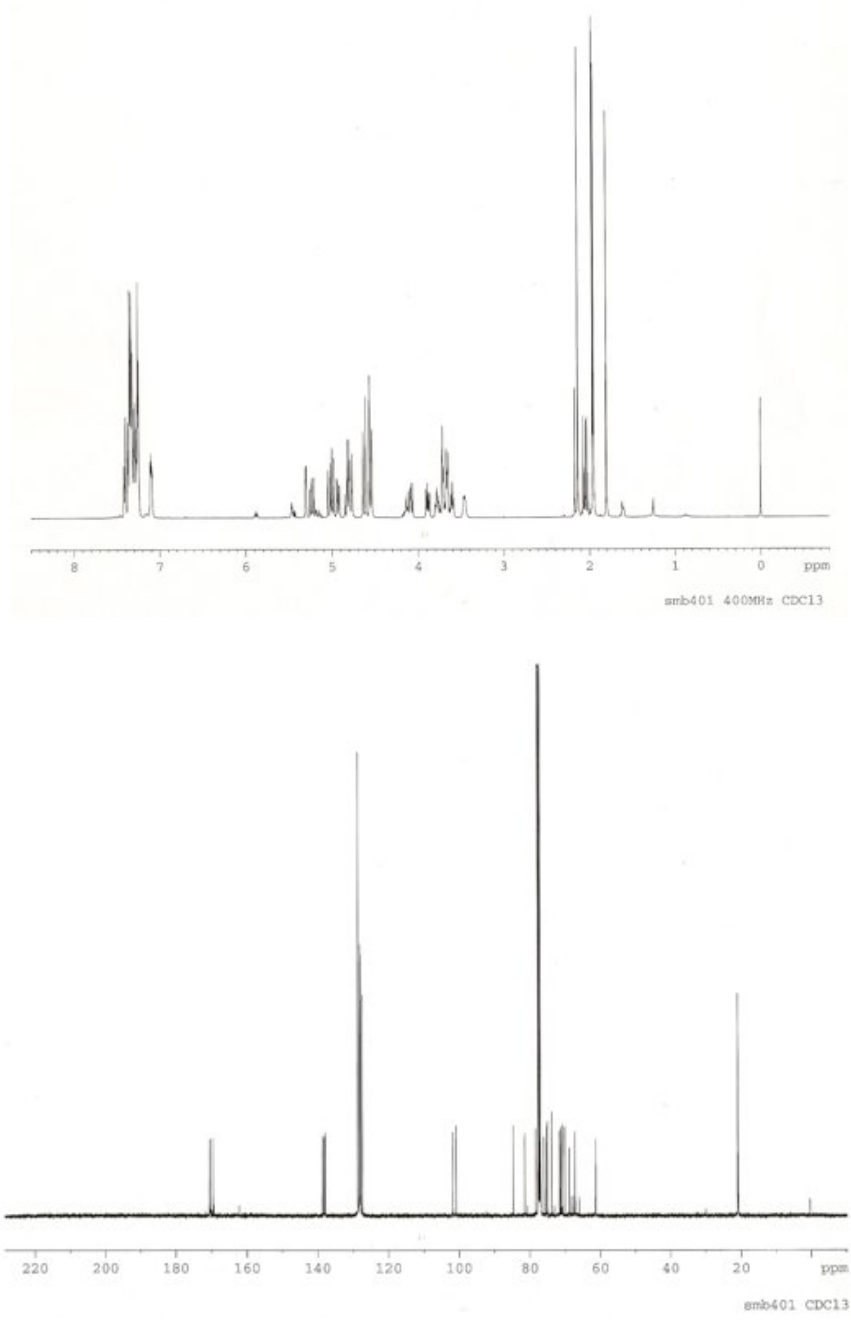

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (**50**).



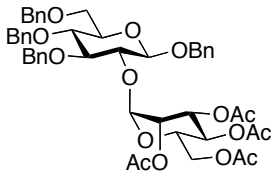



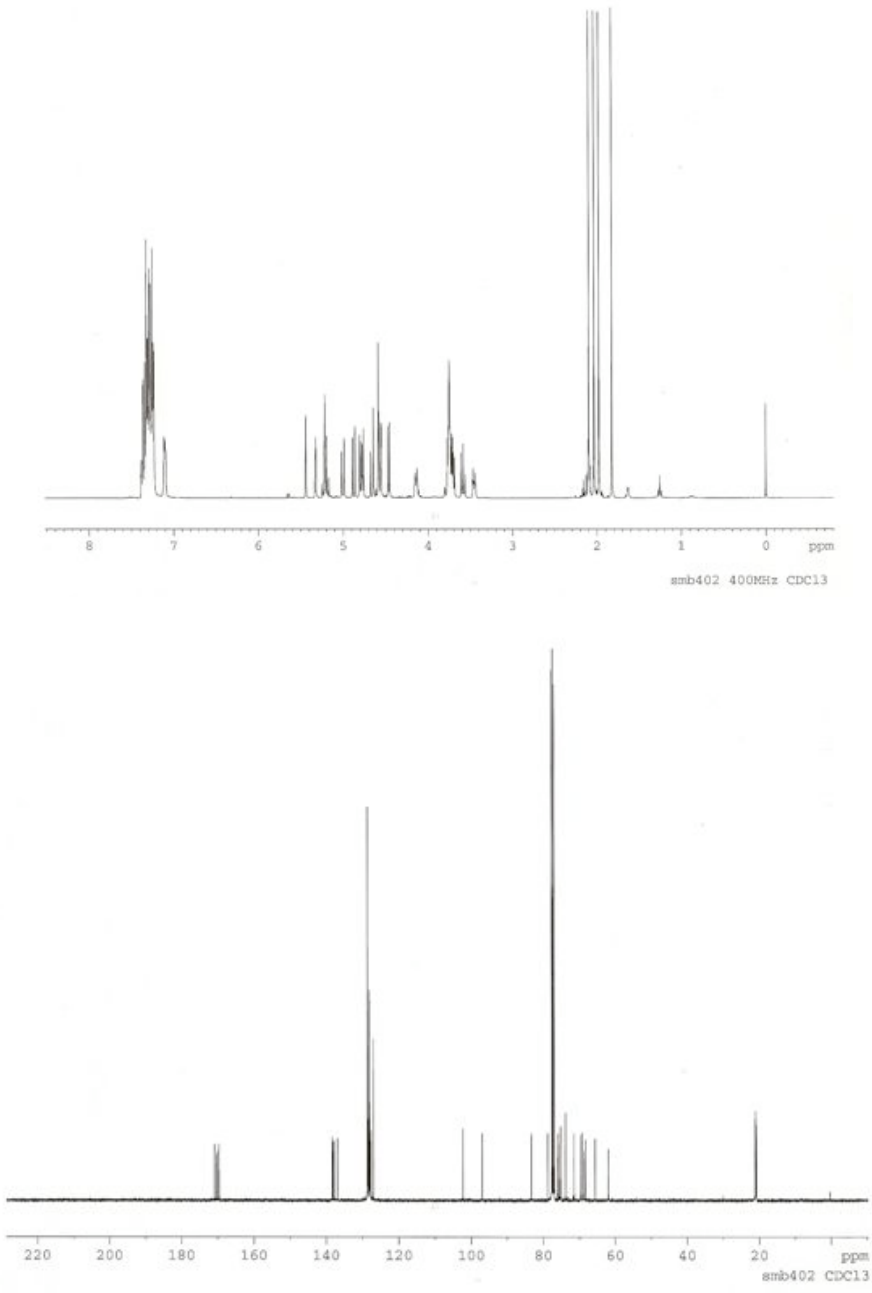

[1]H NMR Spectrum of Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**51**).



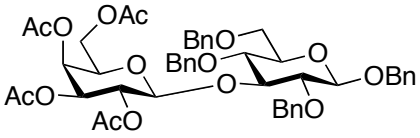



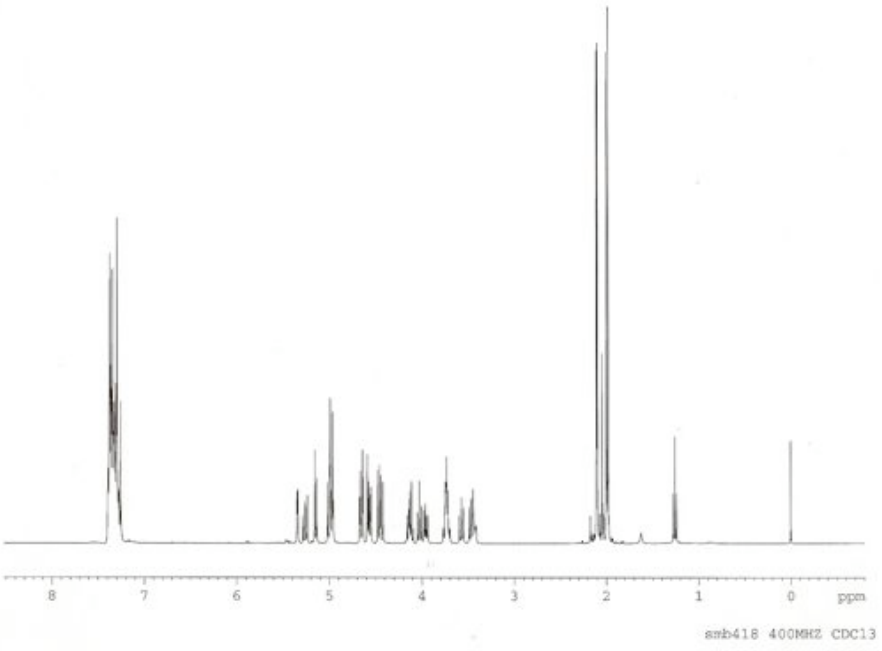

$^{1}$H and $^{13}$C NMR Spectra of Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**52**).

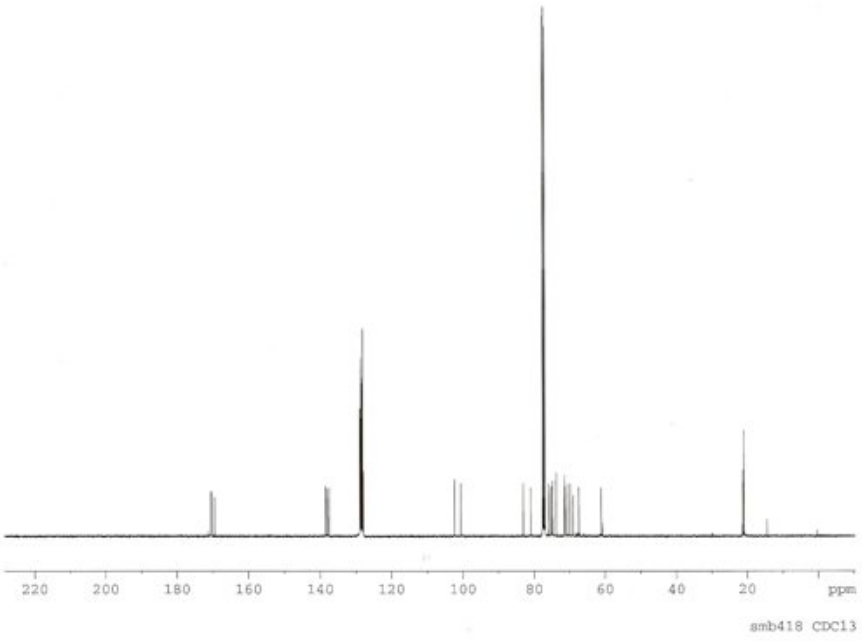


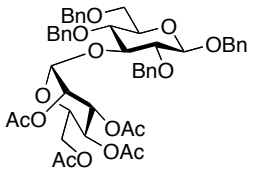

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**53**).

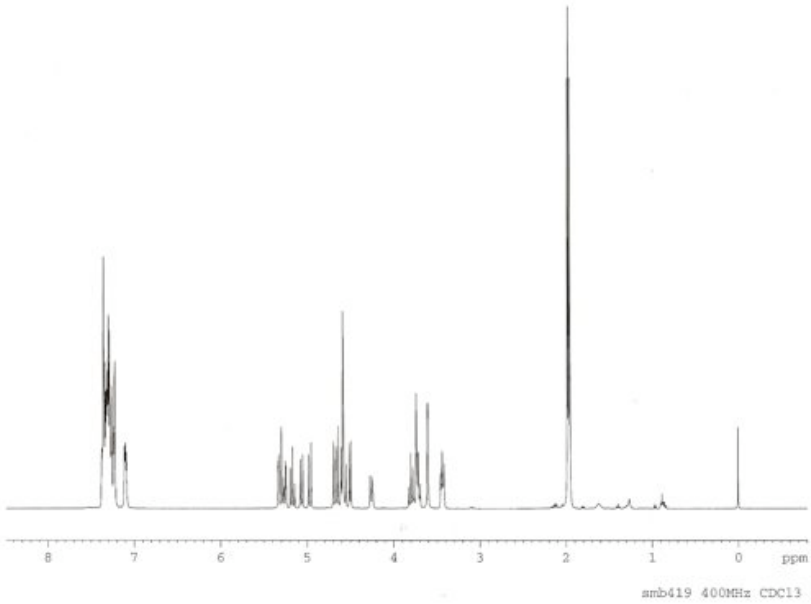


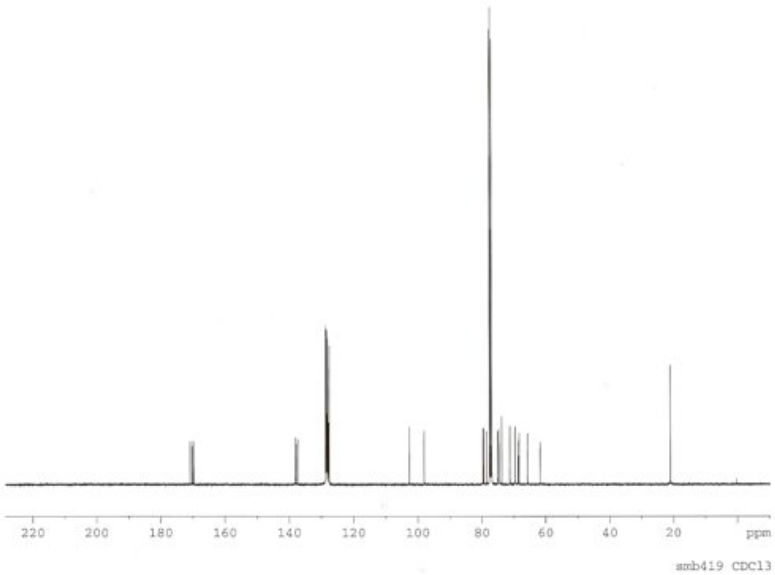



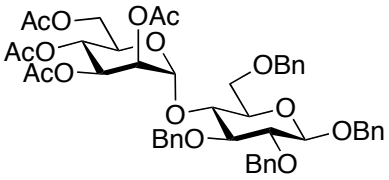

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**54**).

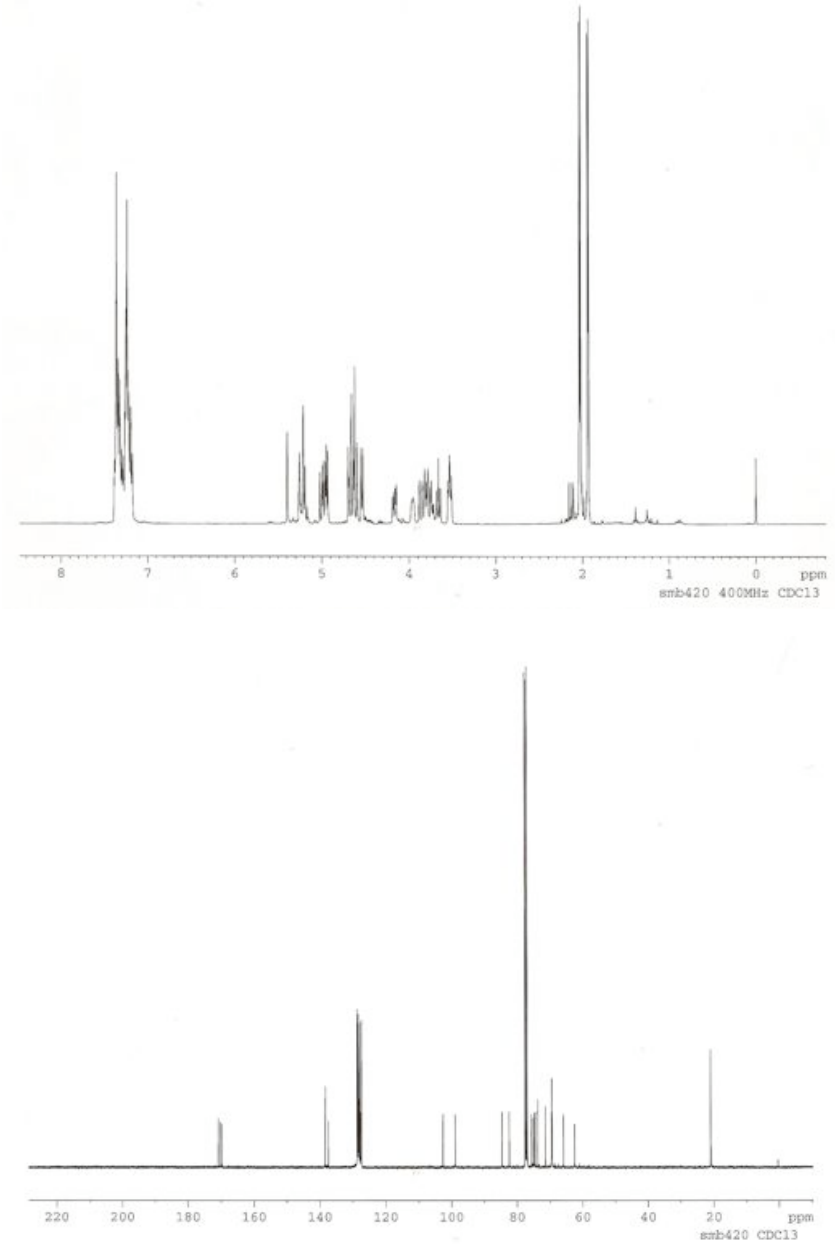


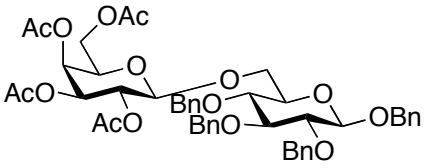



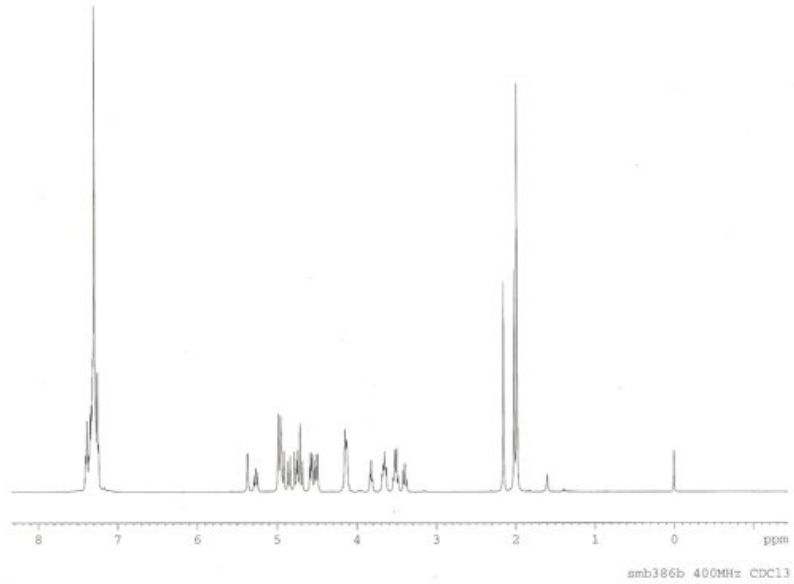

$^{1}$H and $^{13}$C NMR Spectra of Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**55**).

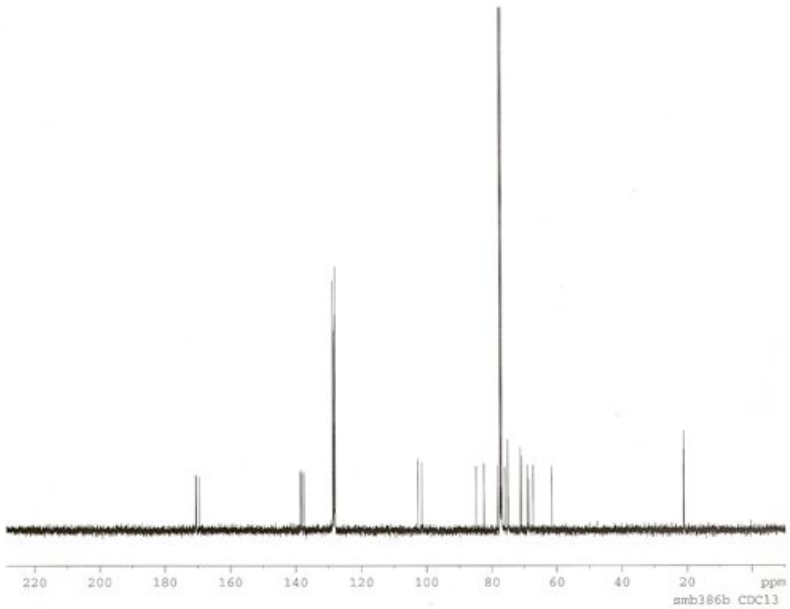


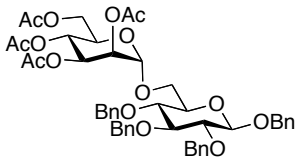

$^{1}H$ and $^{13}C$ NMR Spectra of Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**56**).

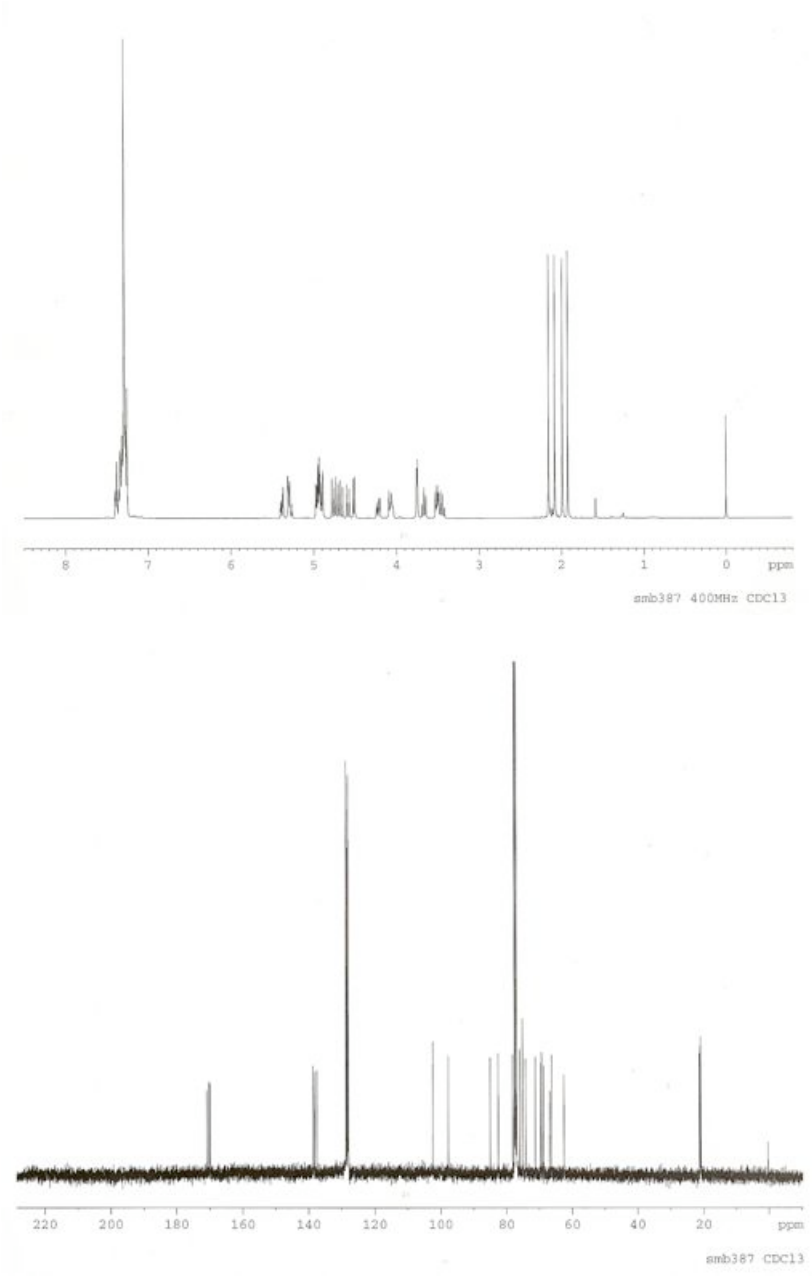


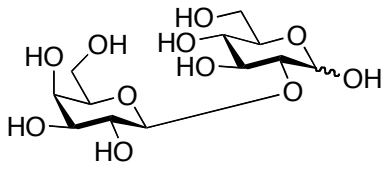



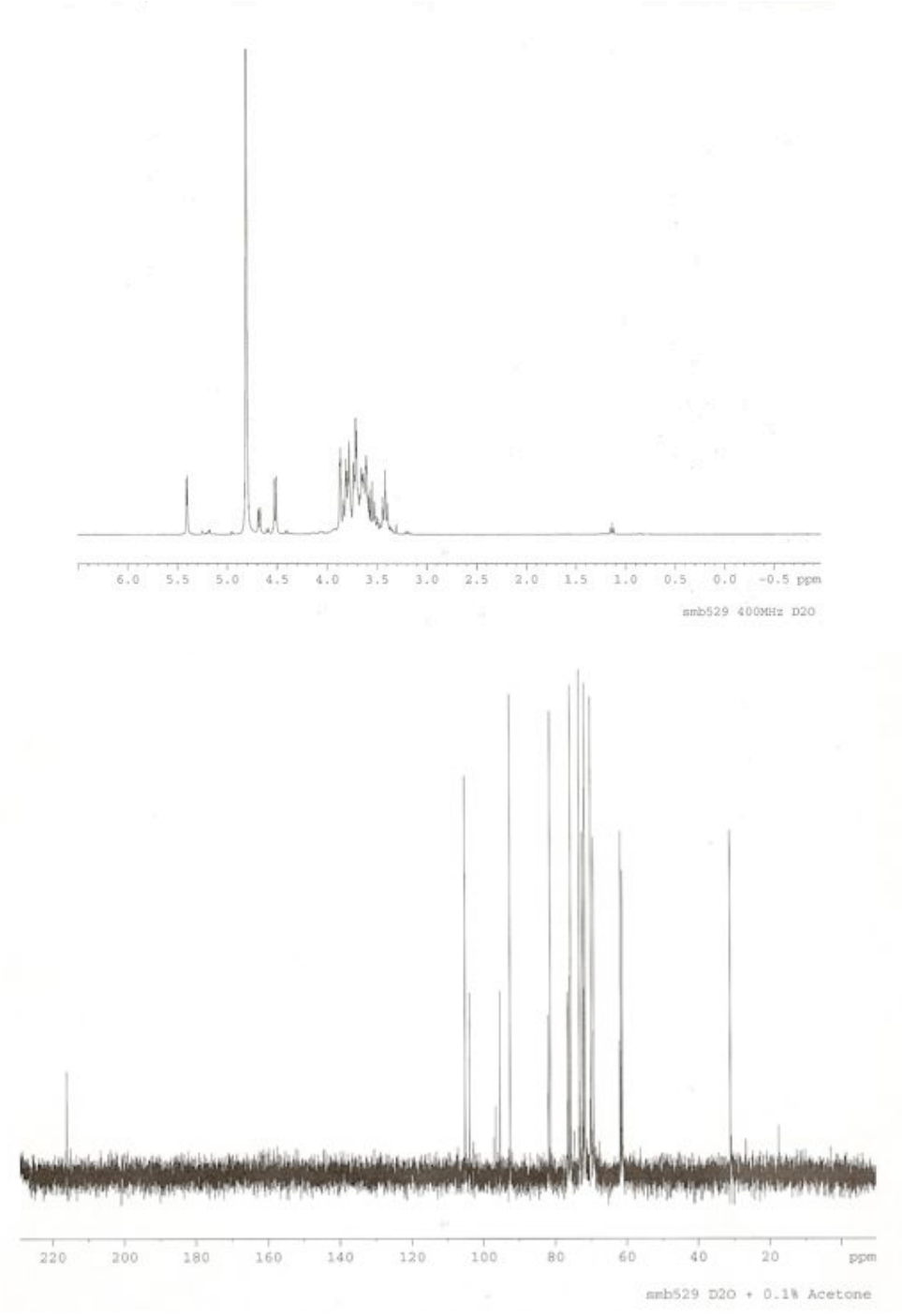

$^{1}$H and $^{13}$C NMR Spectra of Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-**β**-D-galactopyranosyl)-β-D-glucopyranoside (**57**).



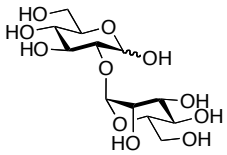



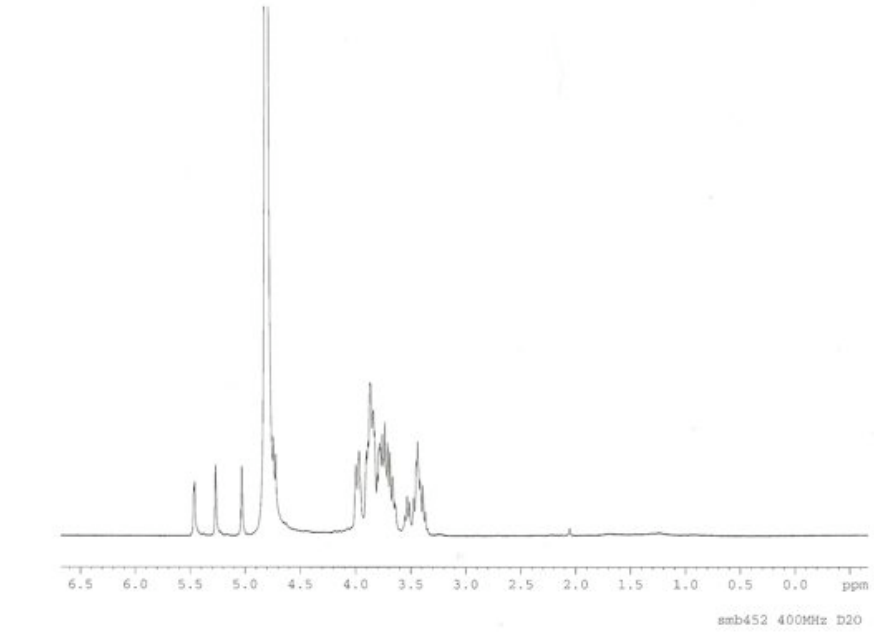

$^{1}$H and $^{13}$C NMR Spectra of Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranoside (**58**).

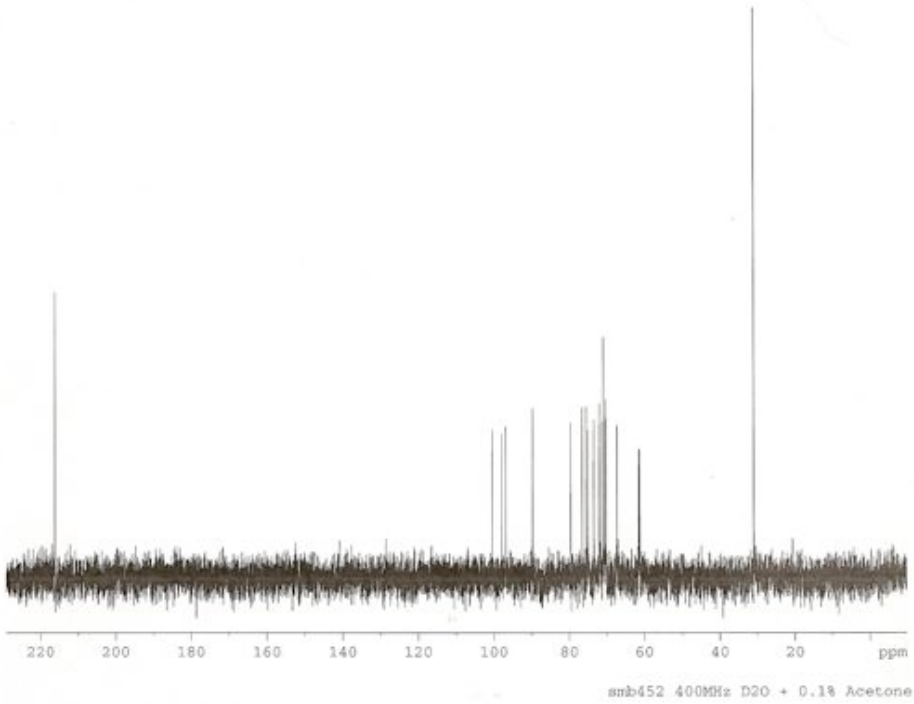


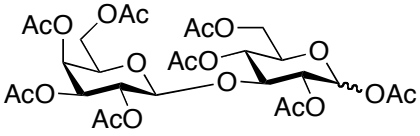

$^{1}$H and $^{13}$C NMR Spectra of 2-*O*-(β-D-galactopyranosyl)-D-glucopyranose (**59**).

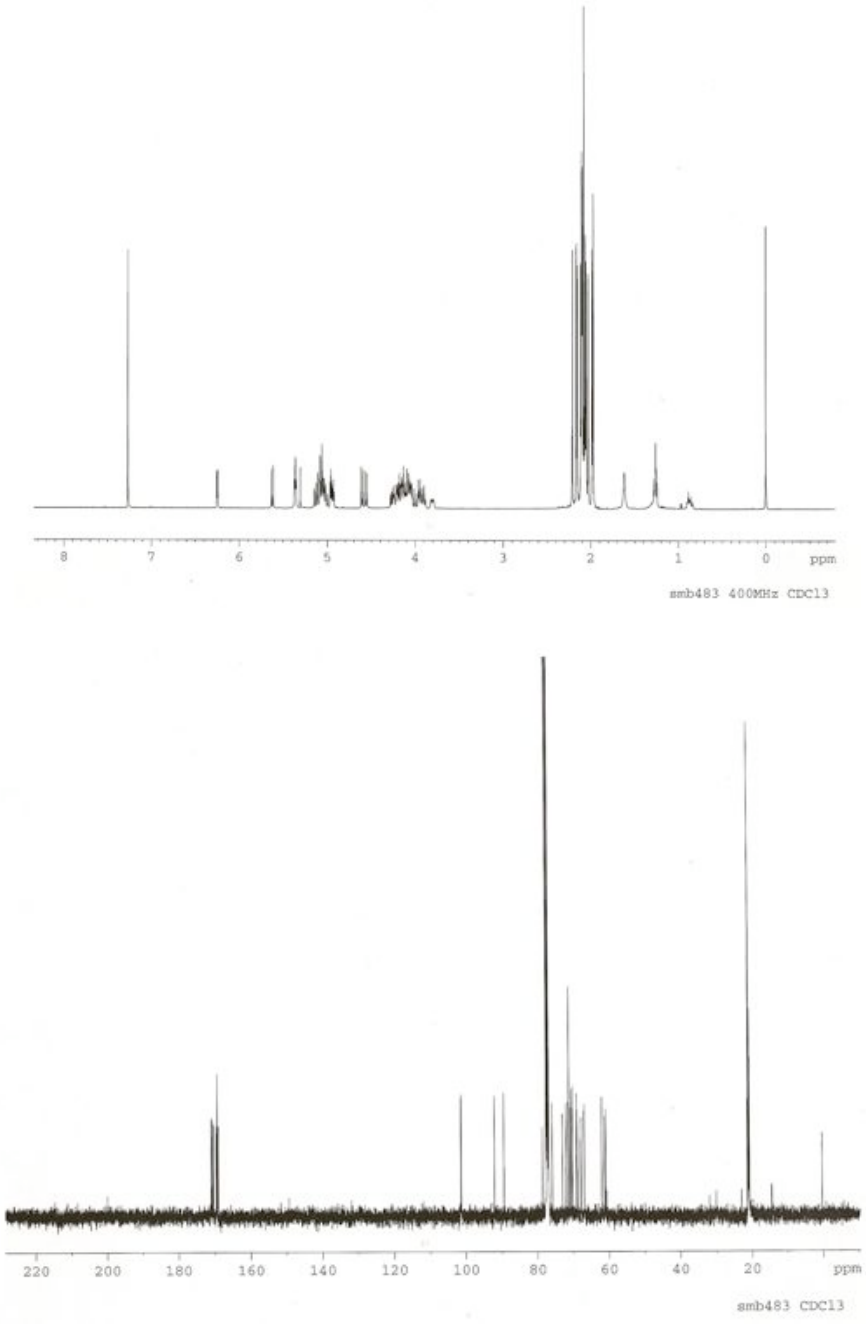


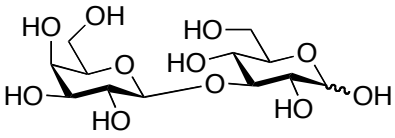



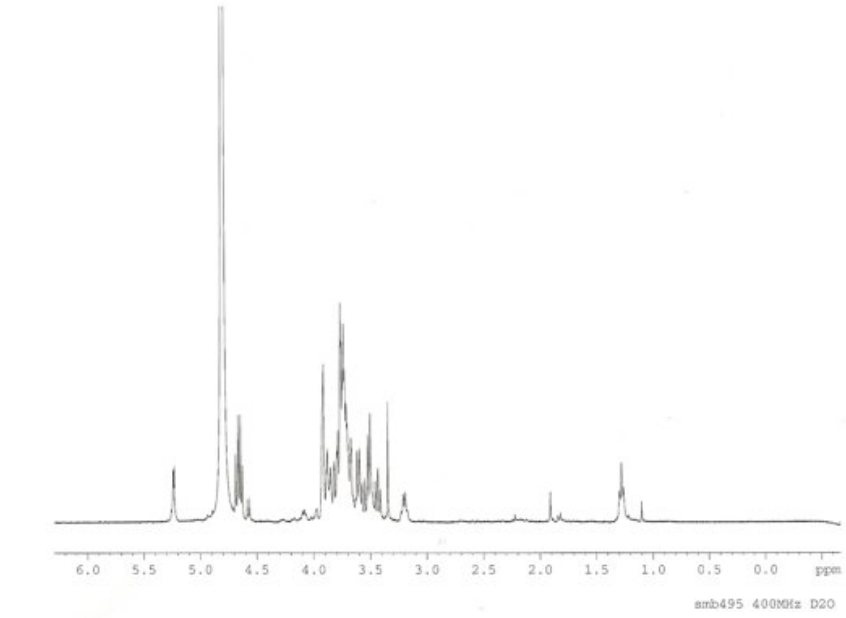

$^{1}$H and $^{13}$C NMR Spectra of 2-*O*-(α-D-mannopyranosyl)-D-glucopyranose (**60**).

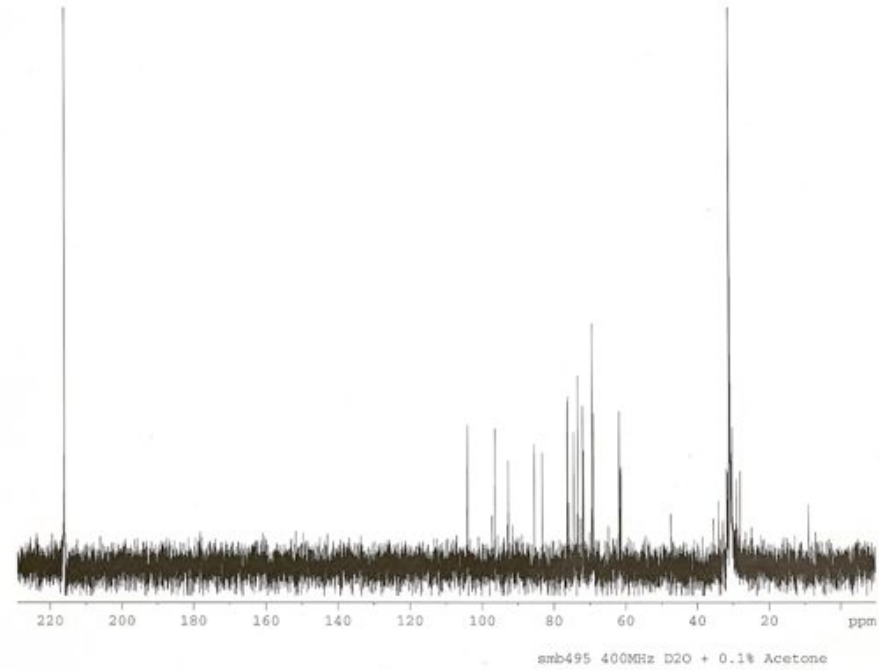


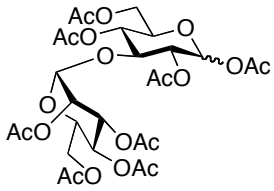

$^{1}$H and $^{13}$C NMR Spectra of 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose (**61**).

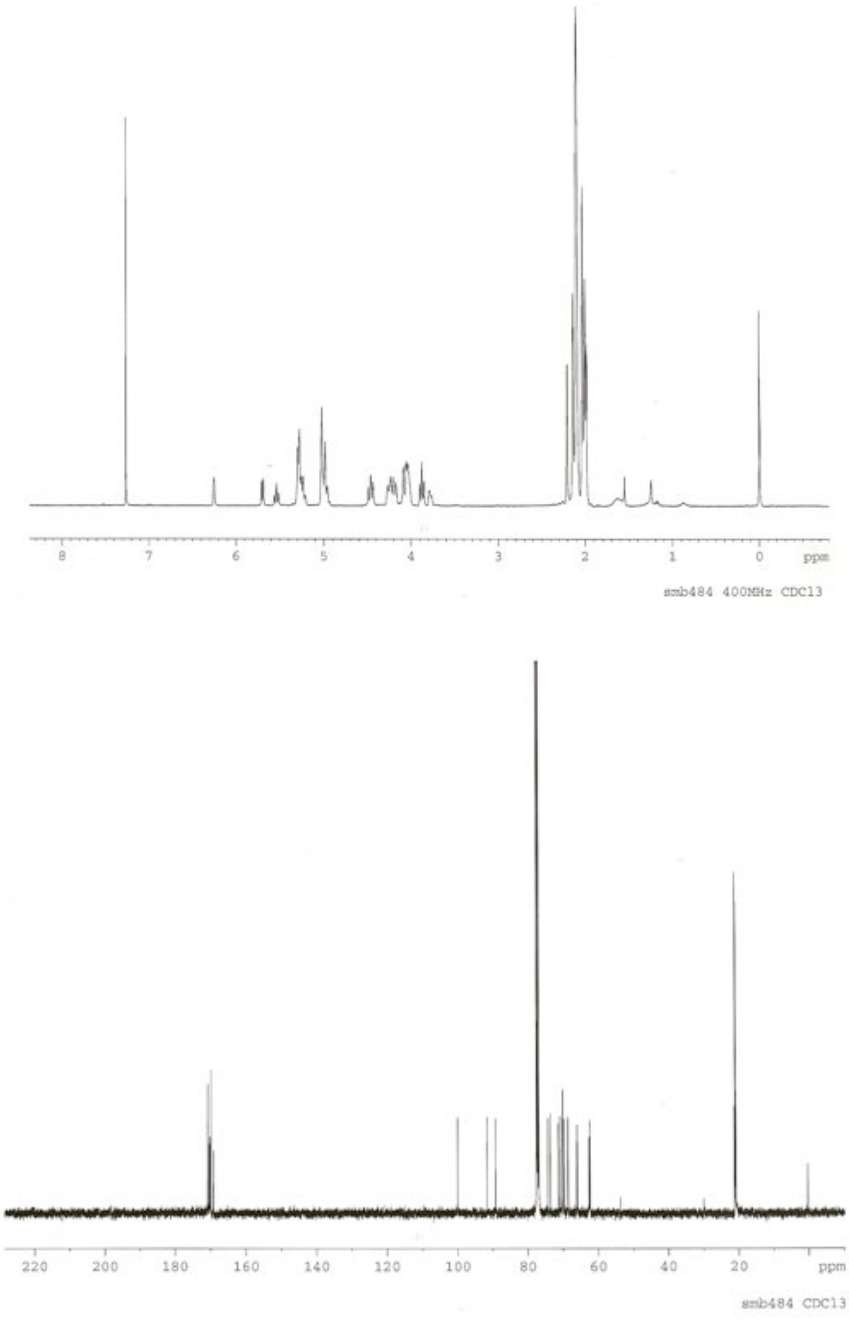


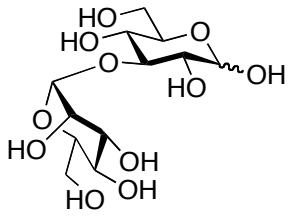



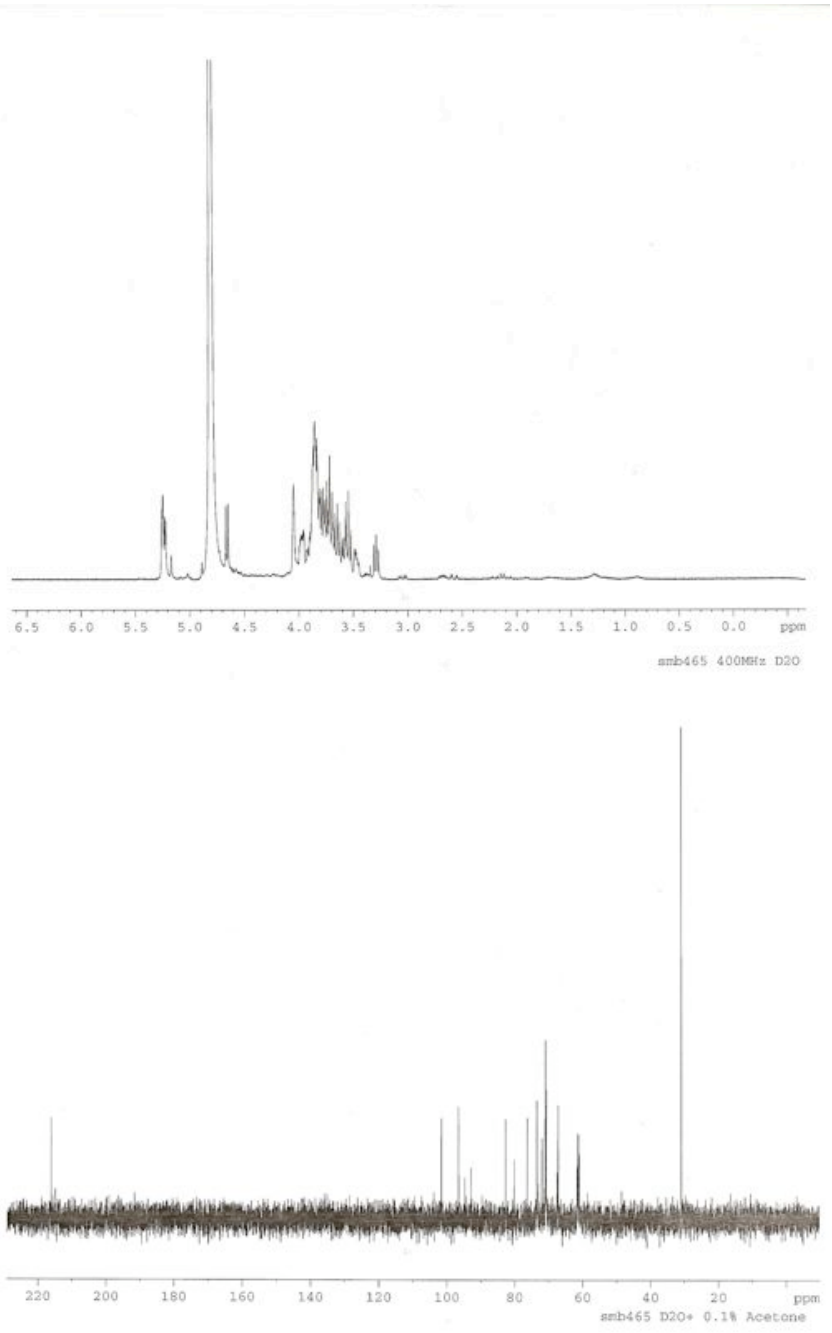

$^{1}$H and $^{13}$C NMR Spectra of 3-*O*-(β-D-galactopyranosyl)-D-glucopyranose (**62**).



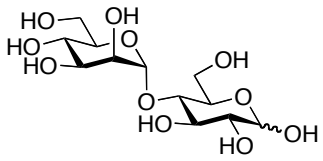



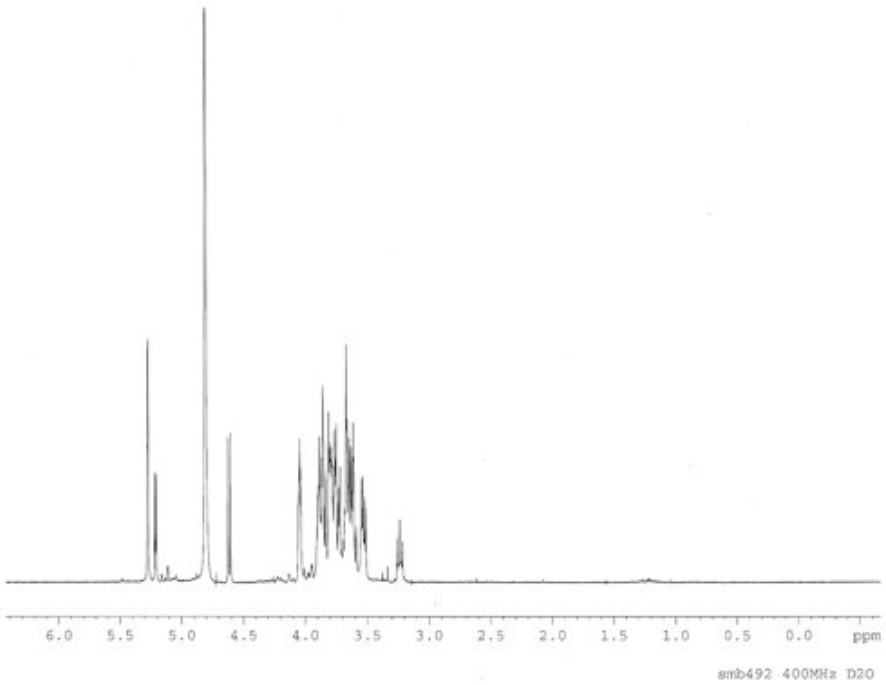

$^{1}$H and $^{13}$C NMR Spectra of 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glucopyranose (**63**).

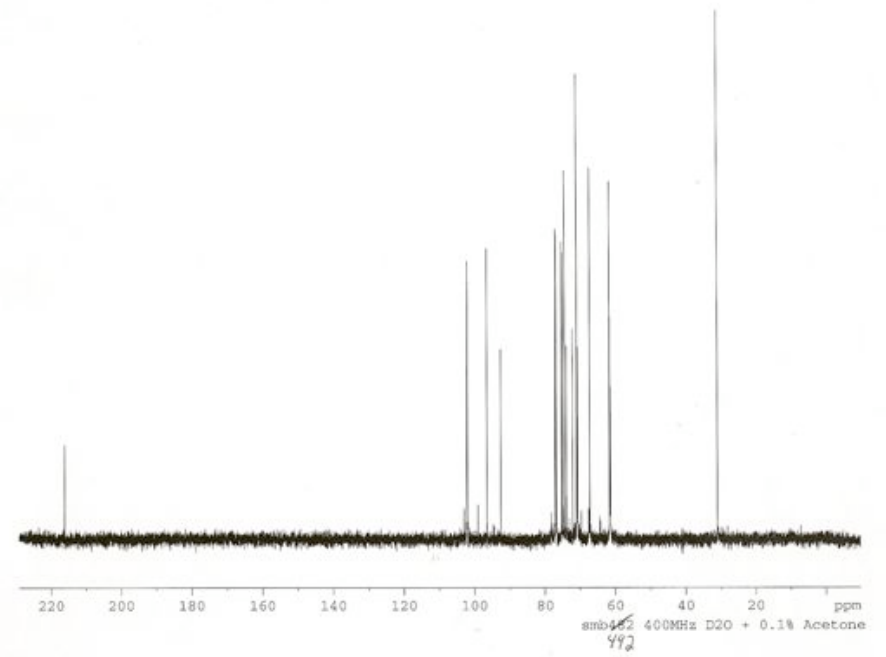


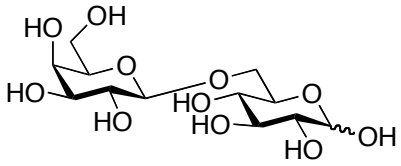

$^{1}$H and $^{13}$C NMR Spectra of 3-*O*-(α-D-mannopyranosyl)-D-glucopyranose (**64**).

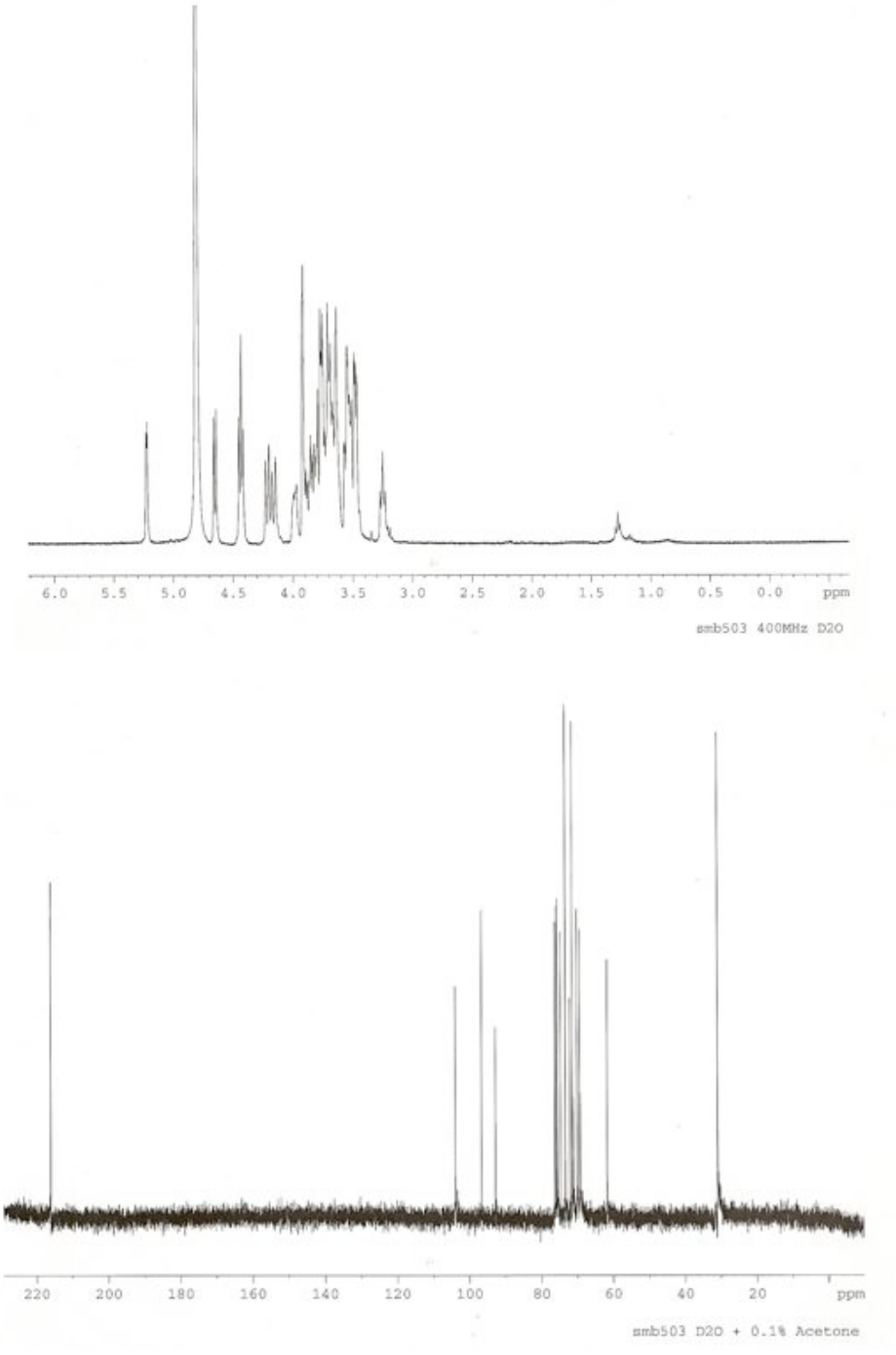


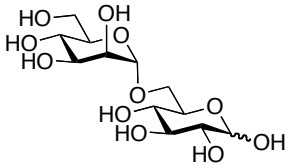



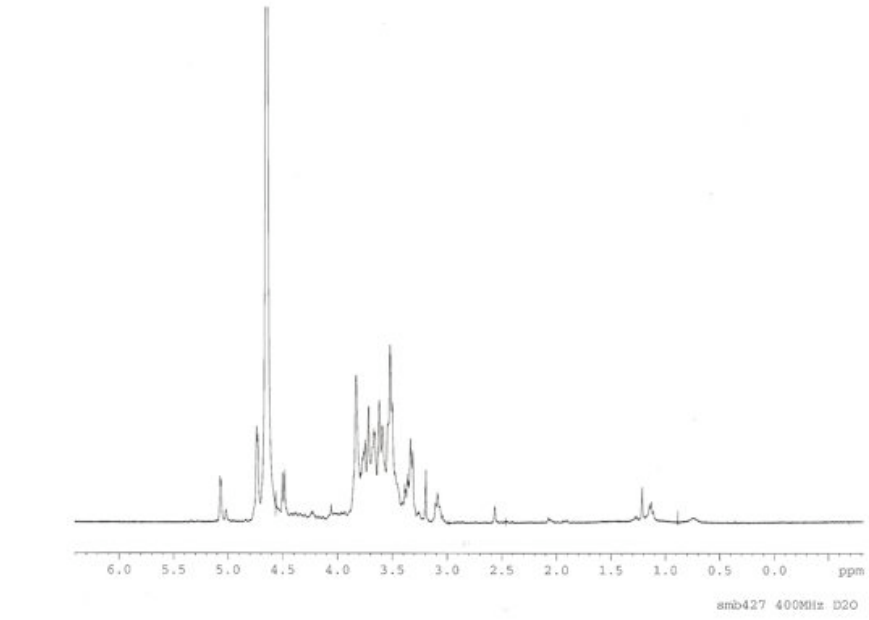

$^{1}$H and $^{13}$C NMR Spectra of 4-*O*-(α-D-mannopyranosyl)-D-glucopyranose (**65**).

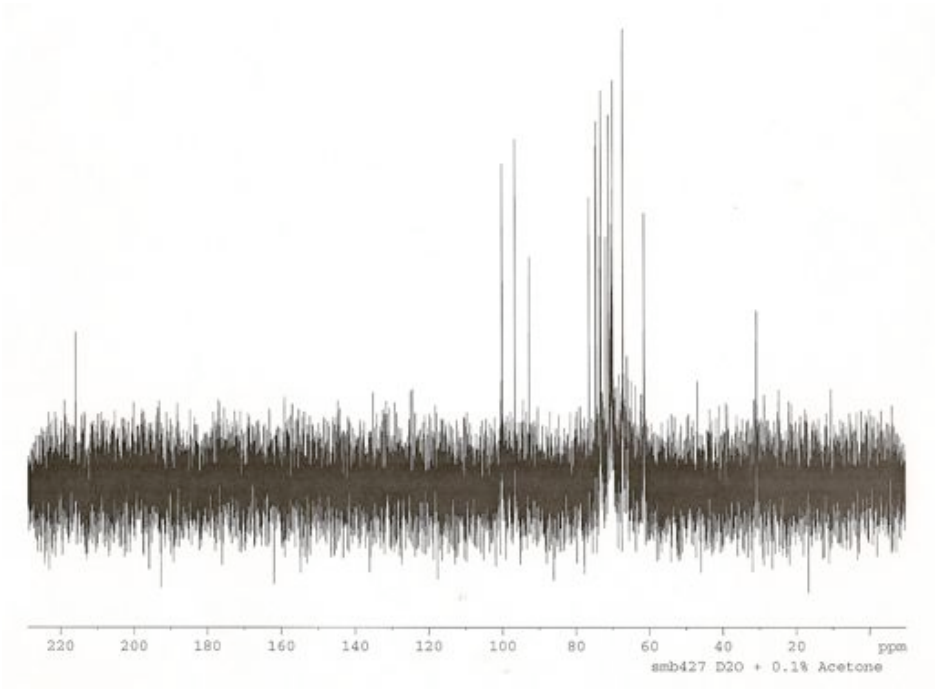



$^{1}$H and $^{13}$C NMR Spectra of 6-*O*-(β-D-galactopyranosyl)-D-glucopyranose (**66**).




$^{1}$H and $^{13}$C NMR Spectra of 6-*O*-(α-D-mannopyranosyl)-D-glucopyranose (**67**).

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

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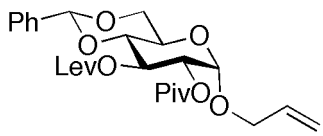
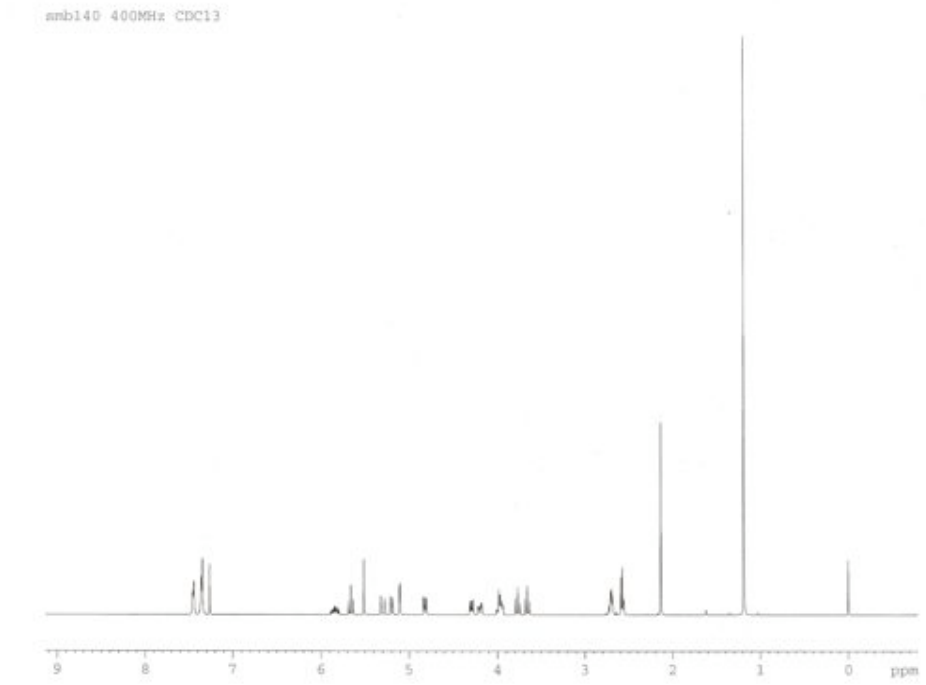
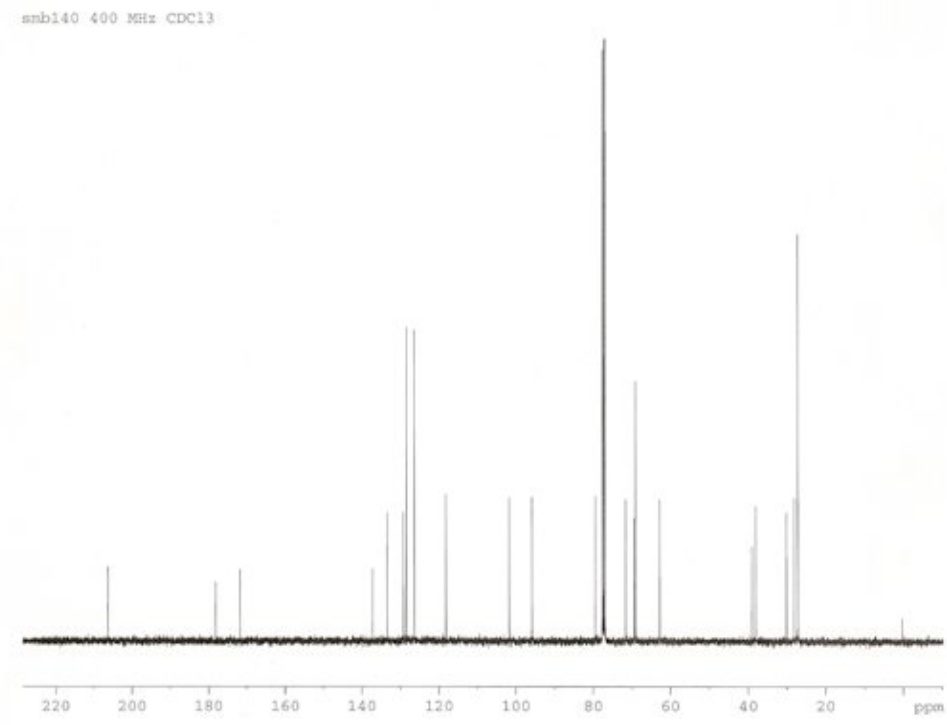
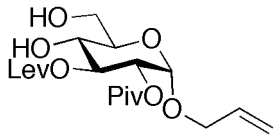
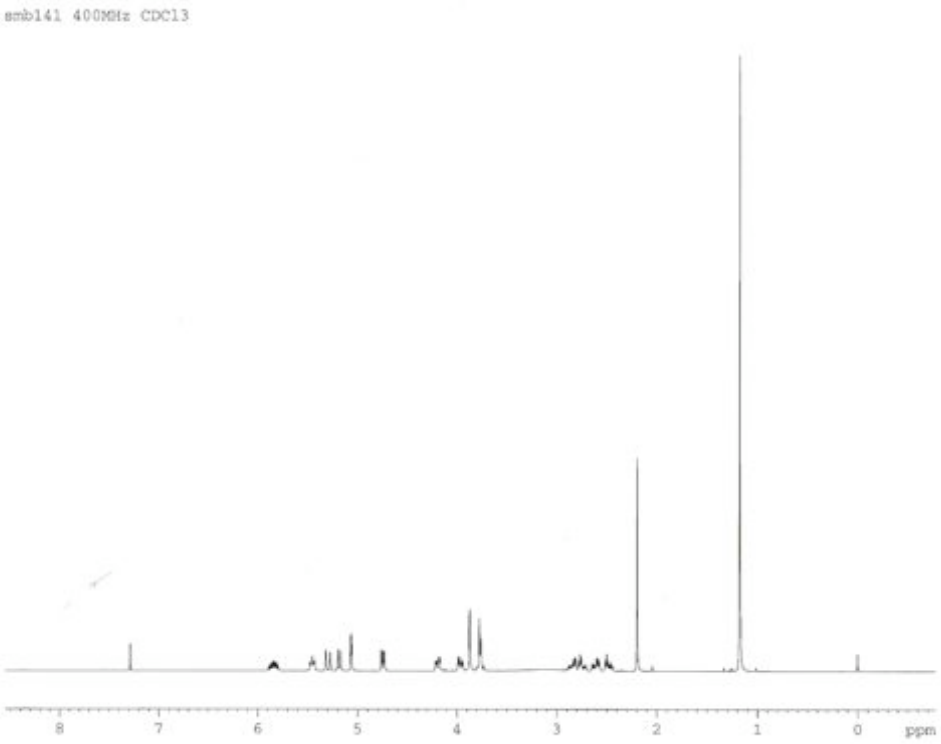


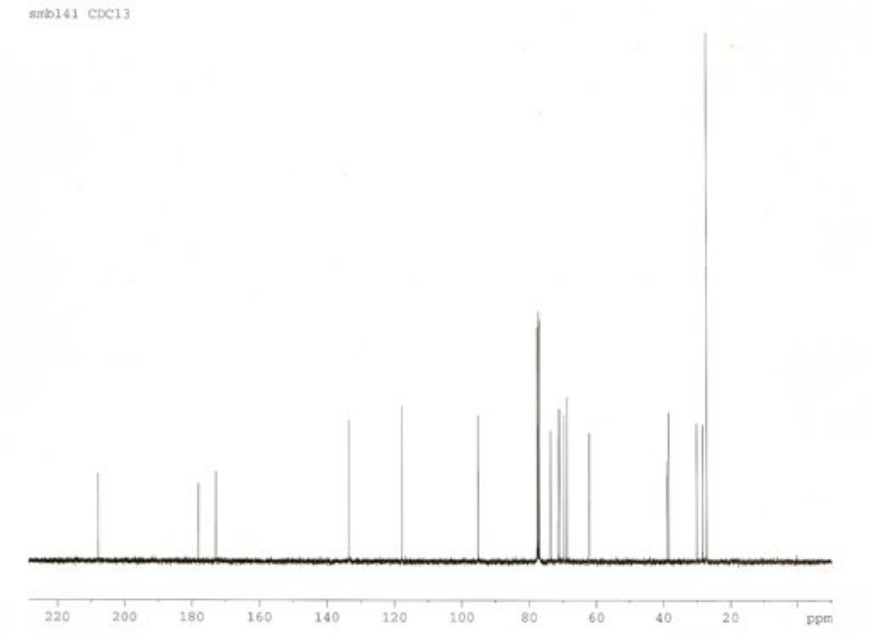



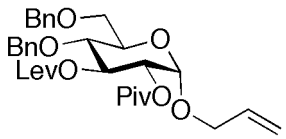

$^{1}$H and $^{13}$C NMR Spectra of Allyl 4,6-*O*-benzylidene-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**2**).

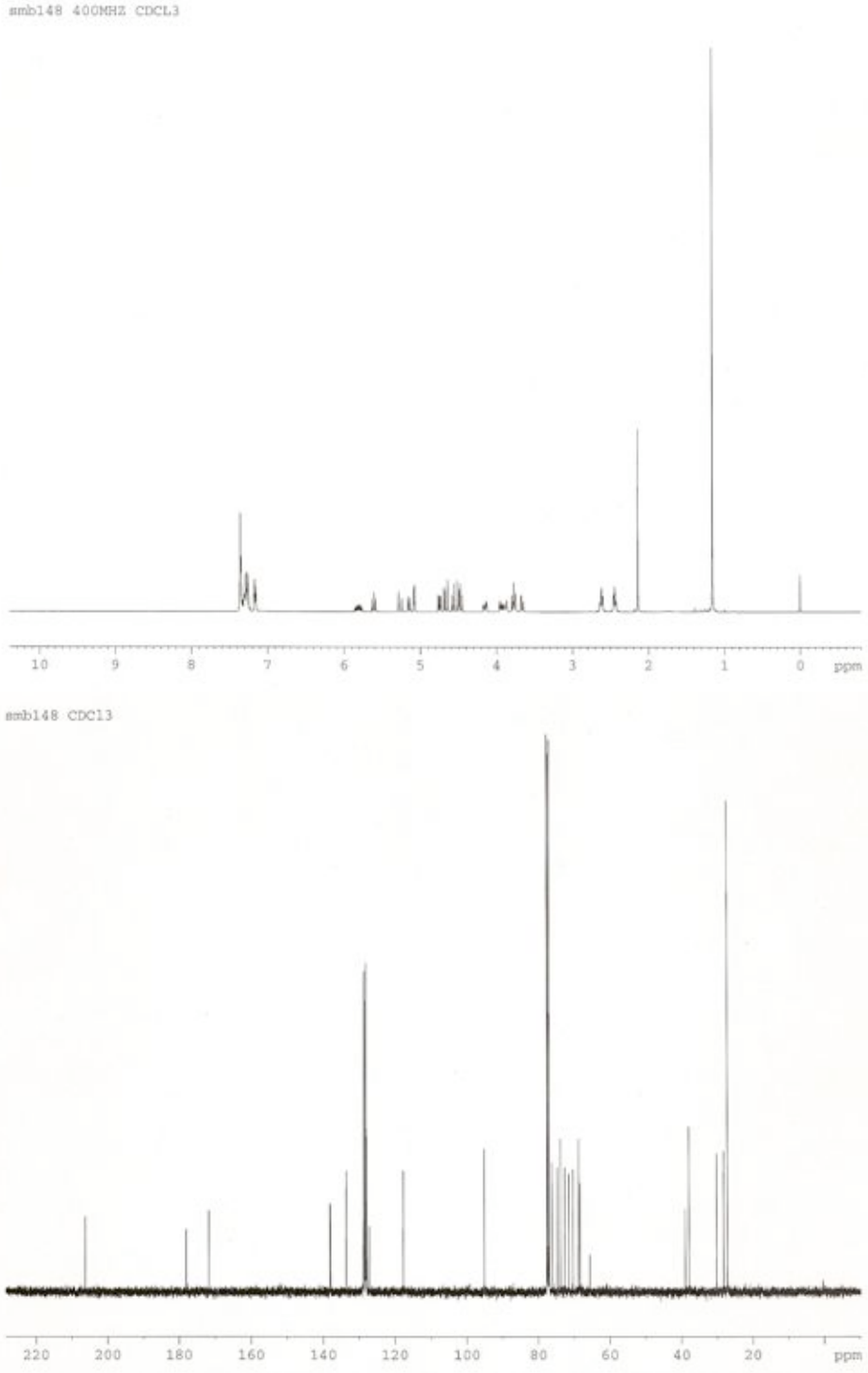


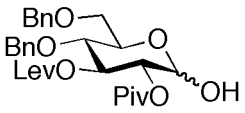



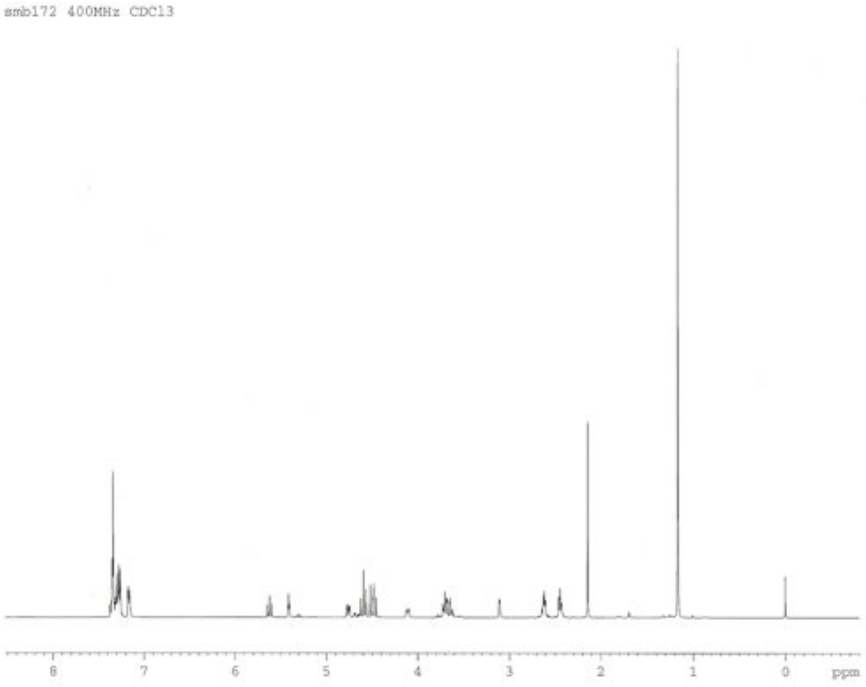

$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**3**).

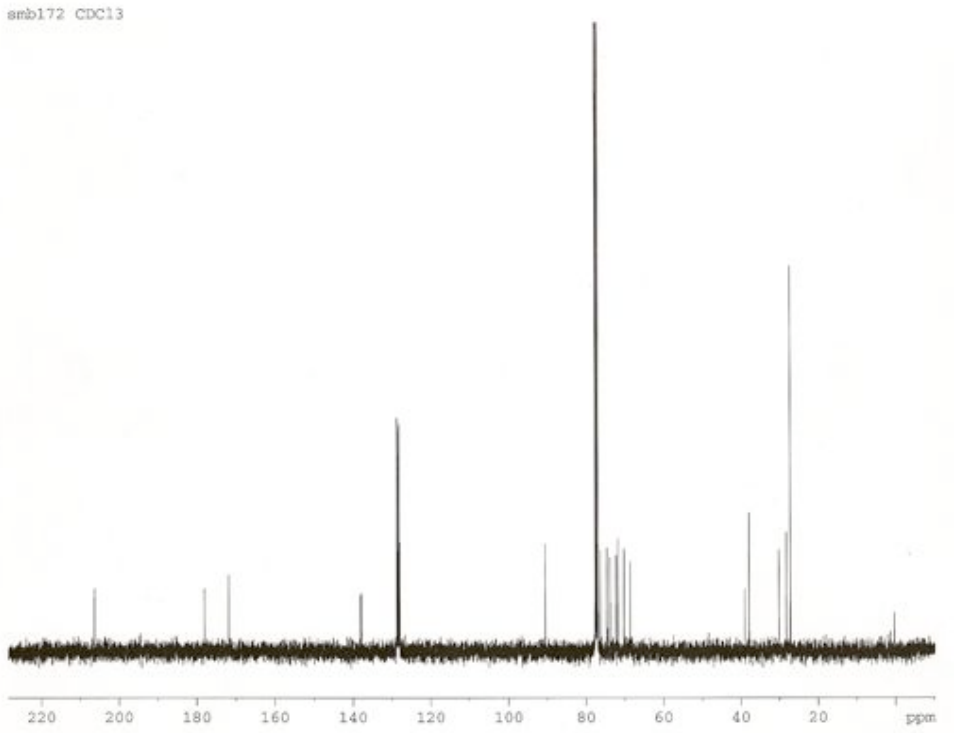


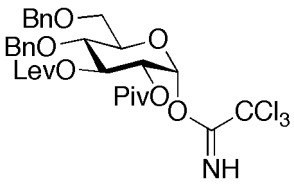

$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**4**).

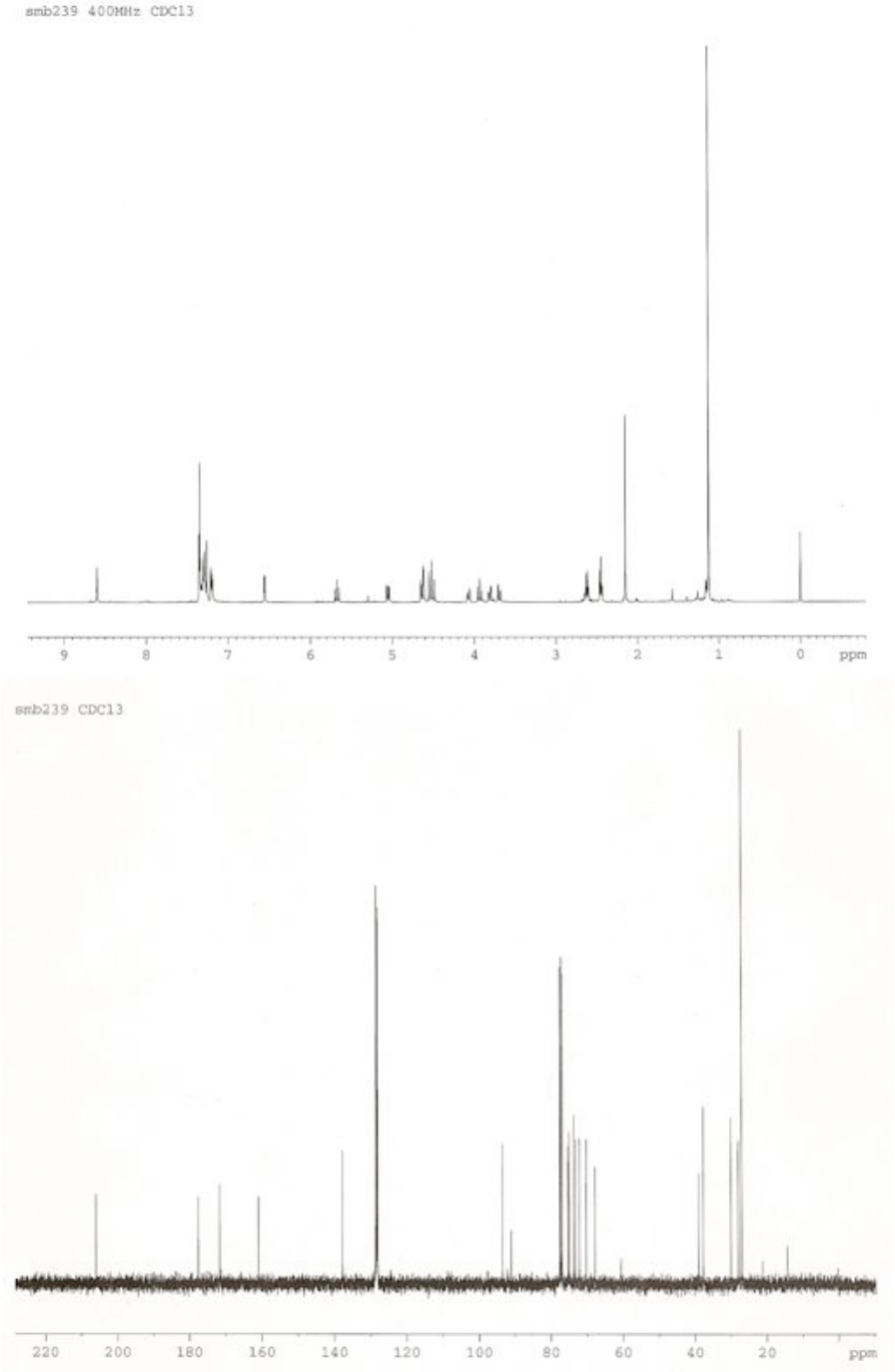


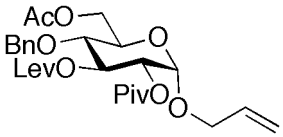



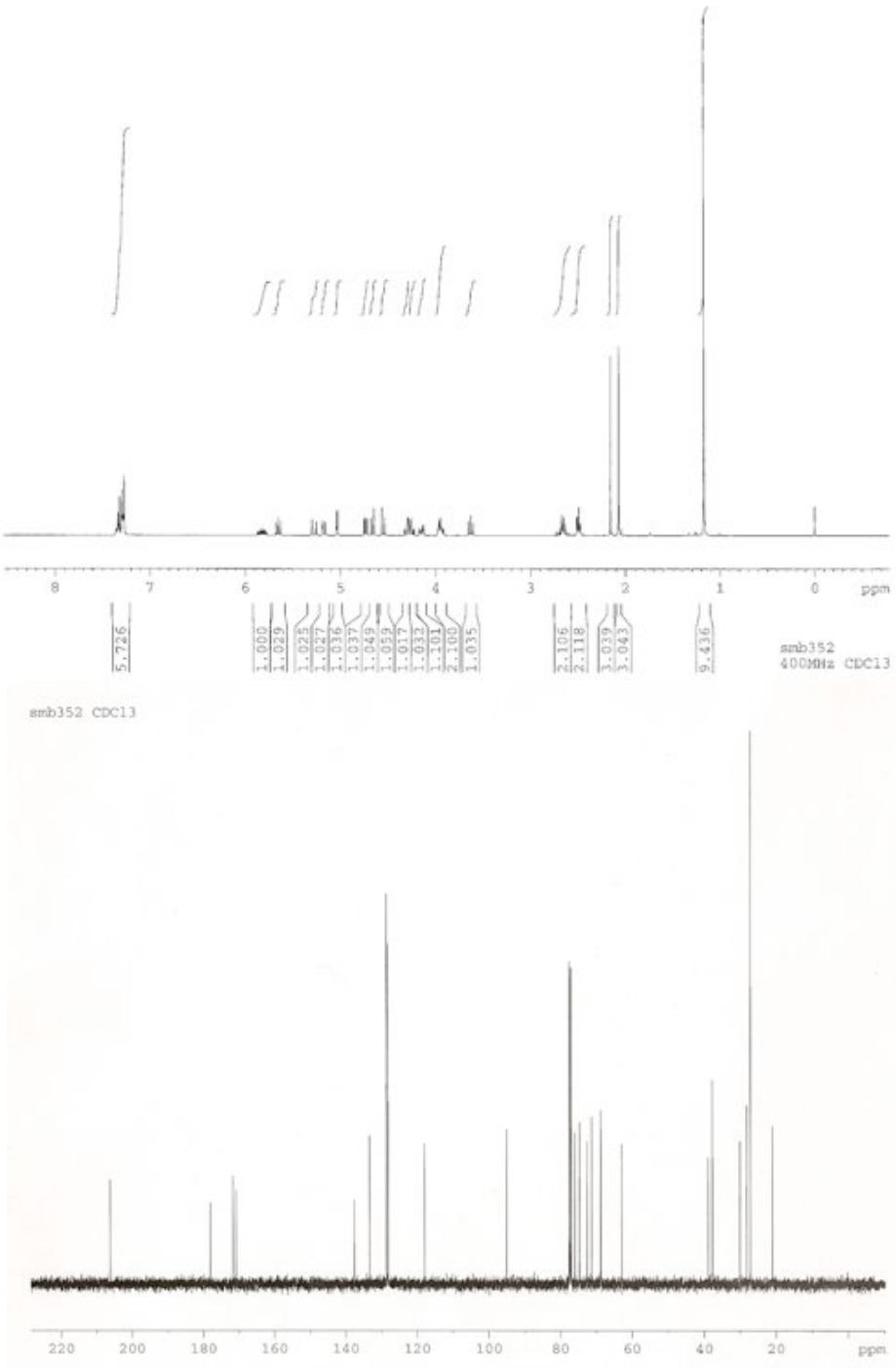

$^{1}H$ and $^{13}C$ NMR Spectra of 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**7**).



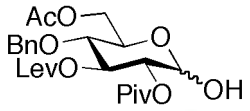



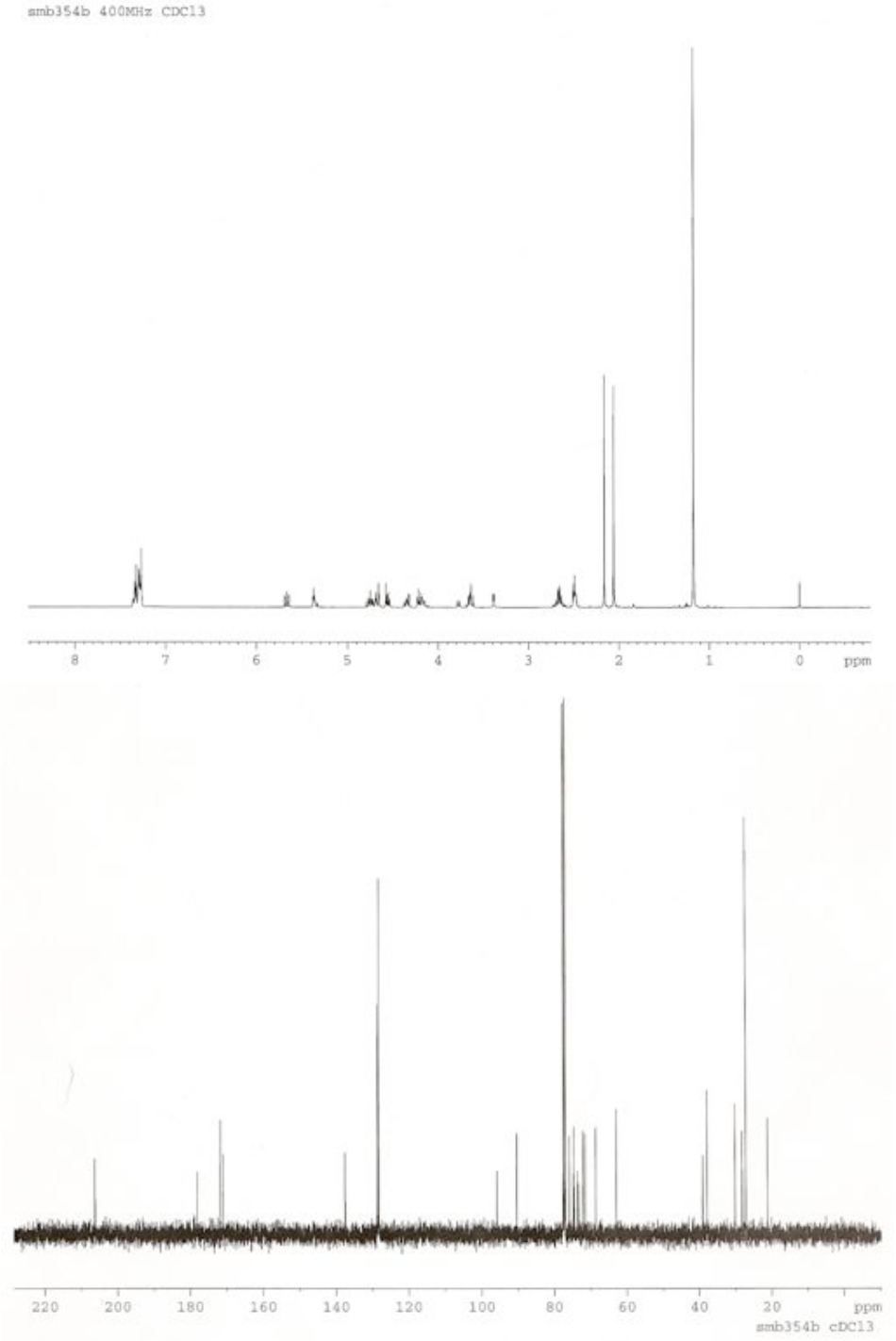

$^{1}$H and $^{13}$C NMR Spectra of 4,6-di-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**8**).



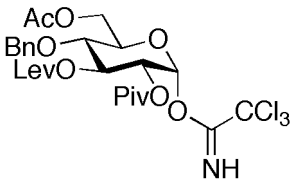



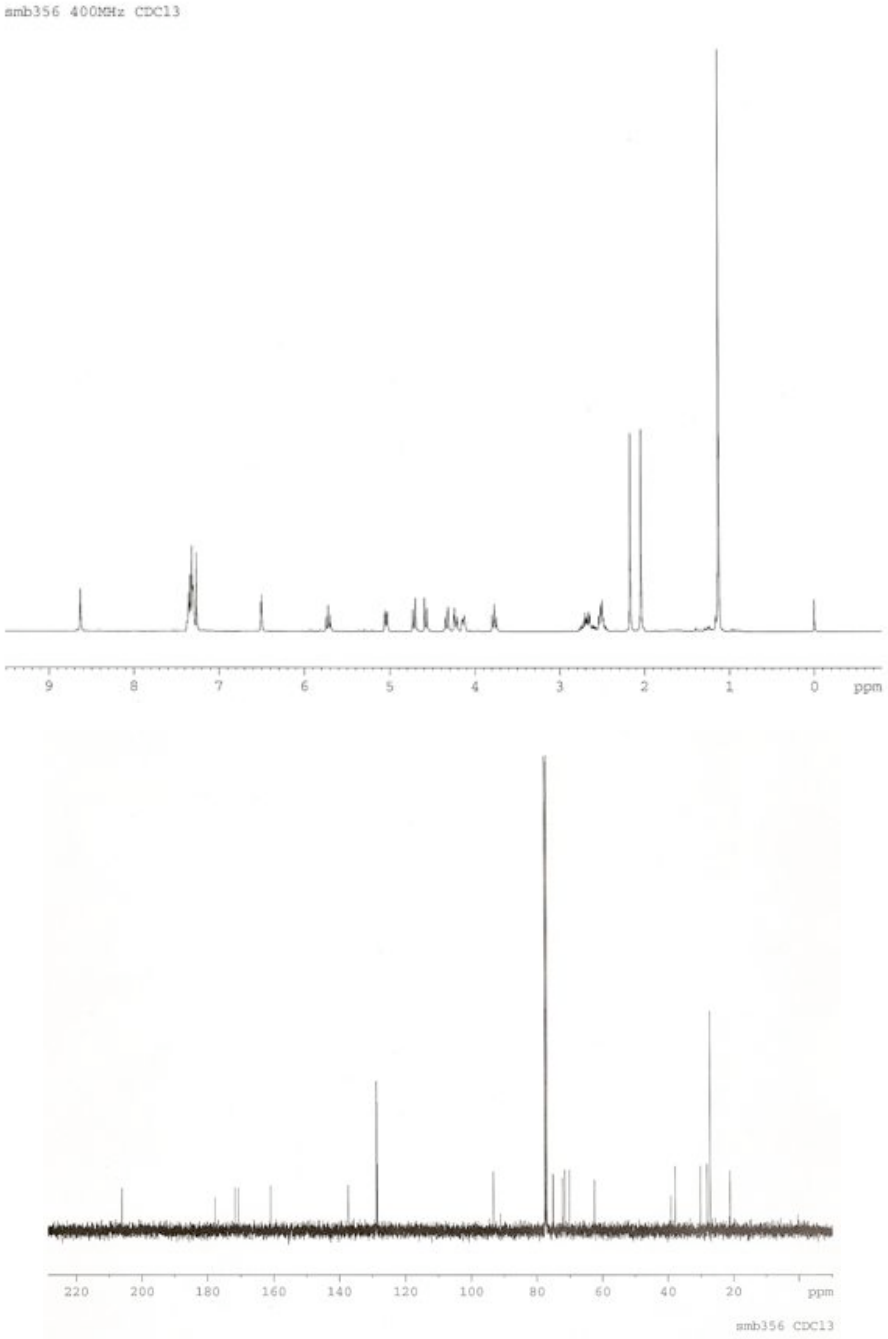

$^{1}$H and $^{13}$C NMR Spectra of Allyl 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**9**).



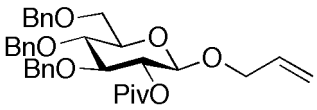



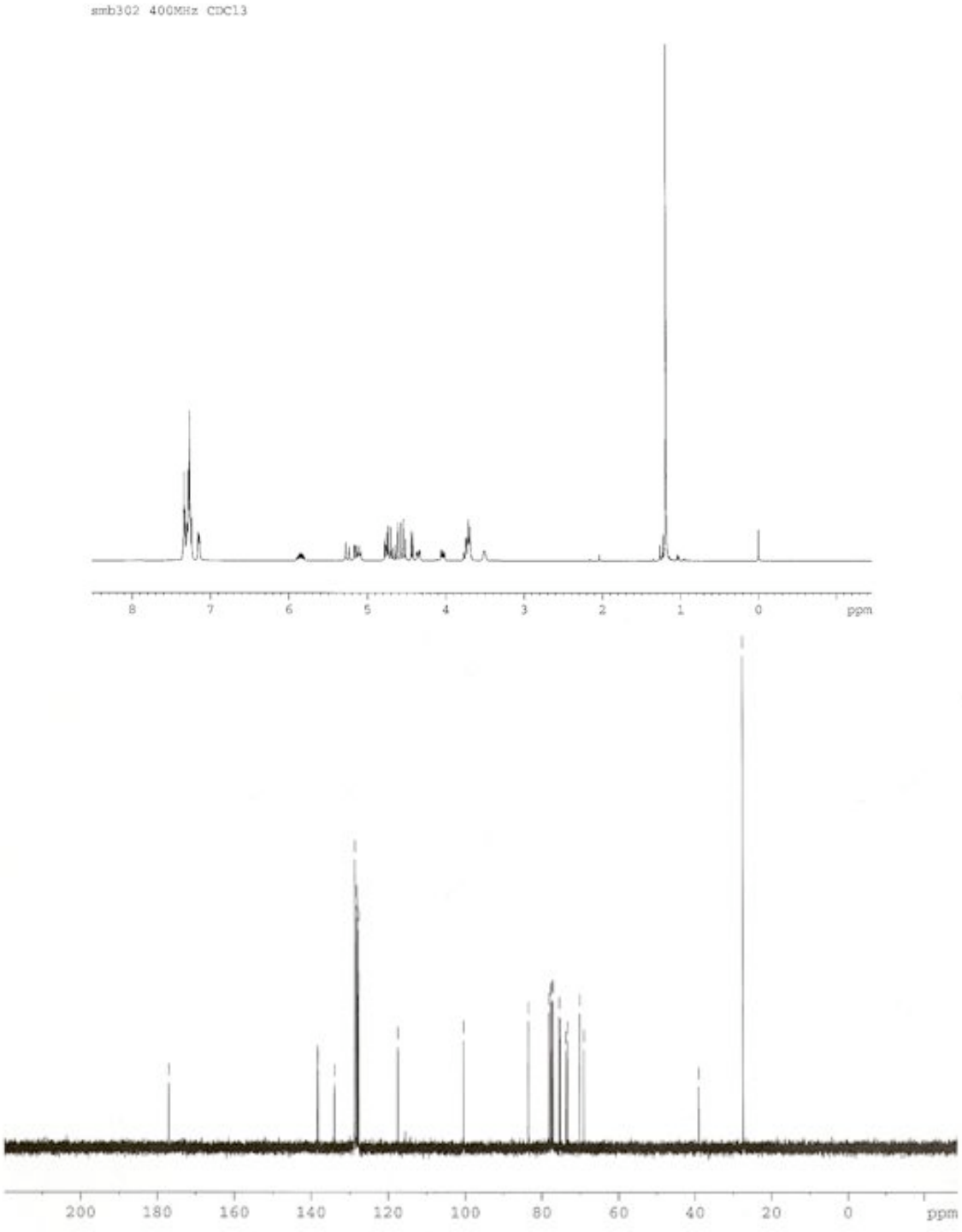

$^{1}$H and $^{13}$C NMR Spectra of 6-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**10**).



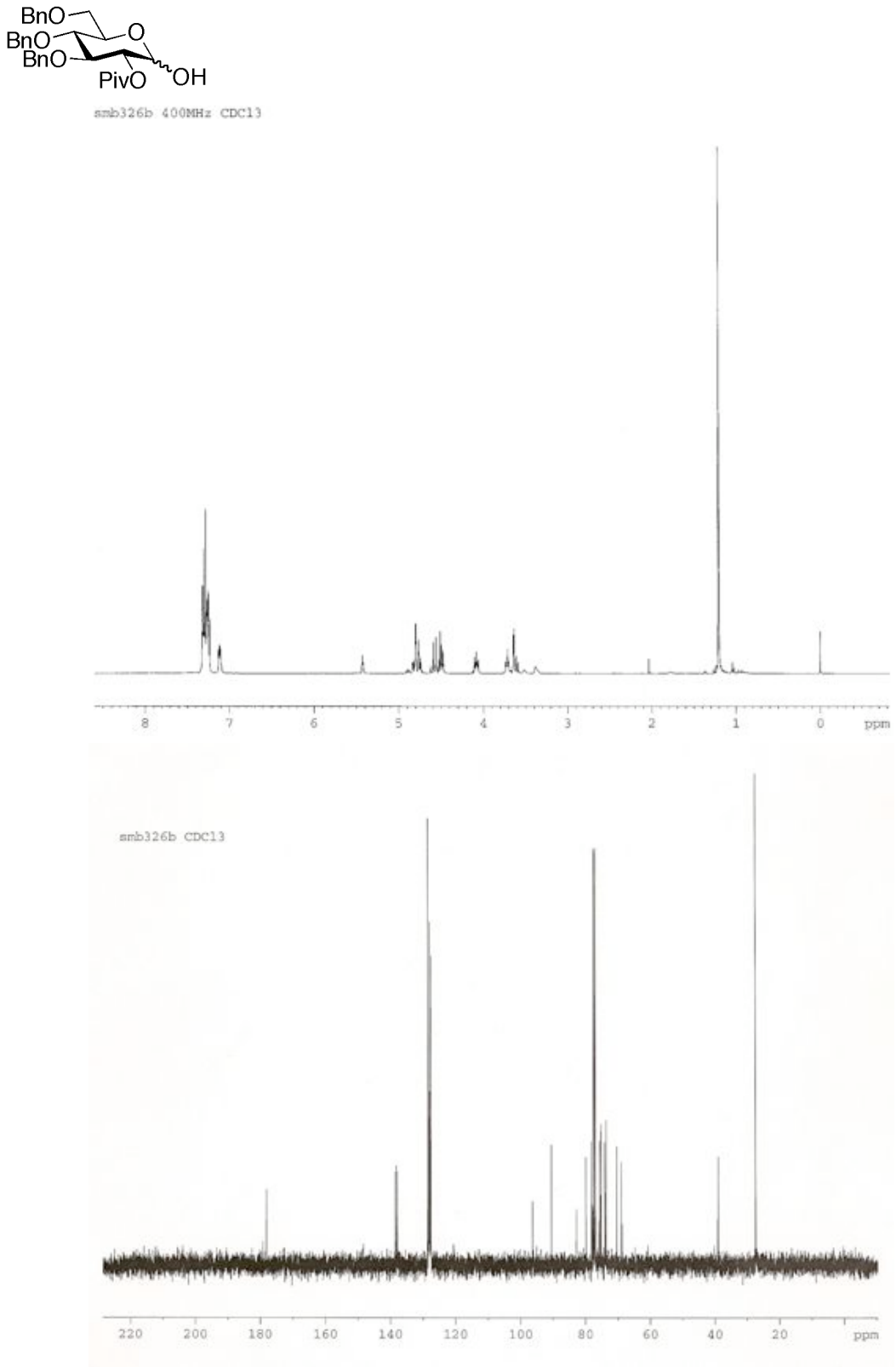




$^{1}H$ and $^{13}C$ NMR Spectra of 6-*O*-Acetyl-4-*O*-benzyl-3-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**11**).

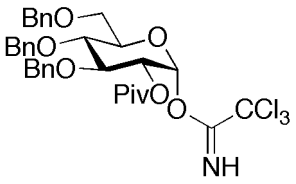


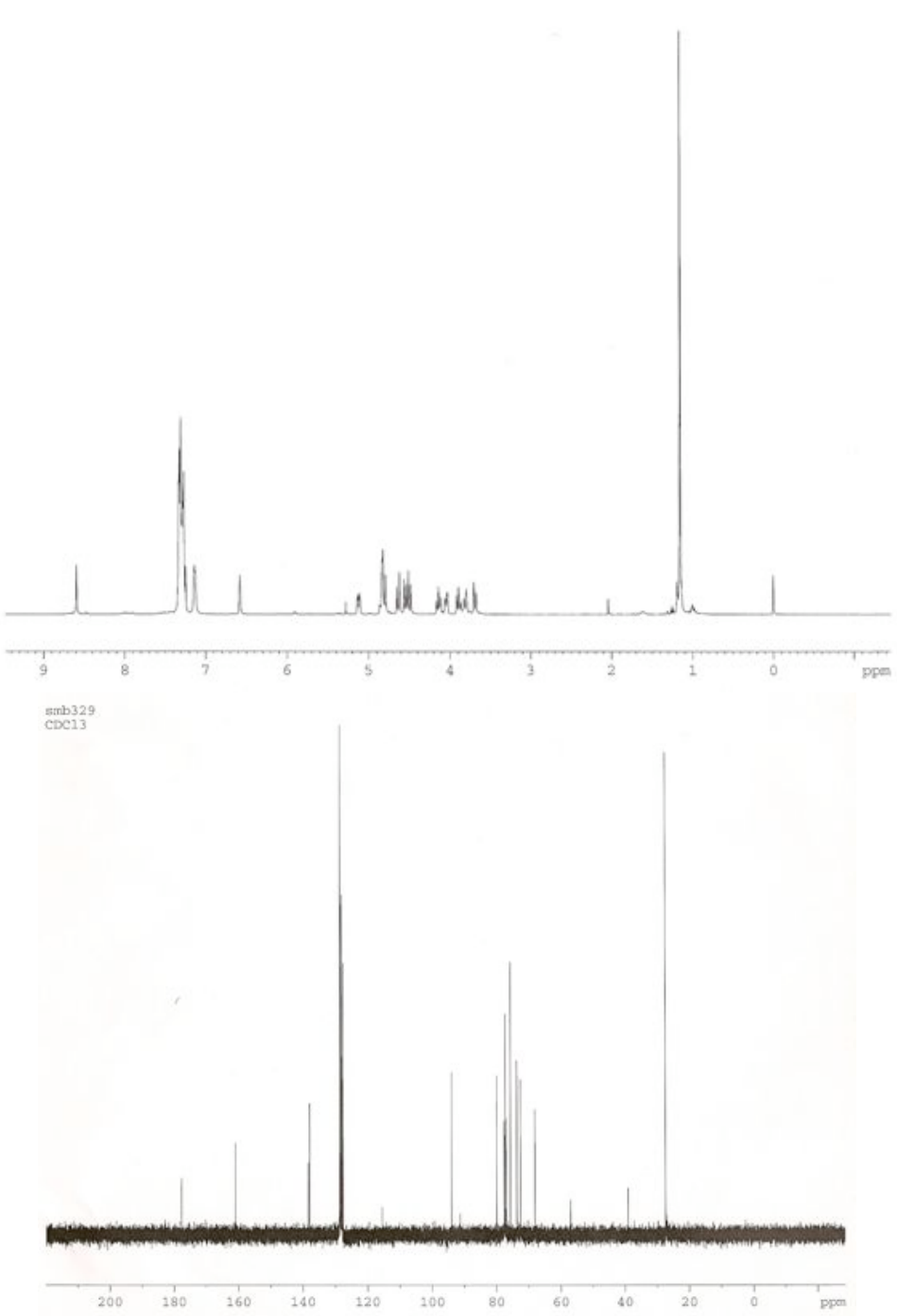




$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**13**).

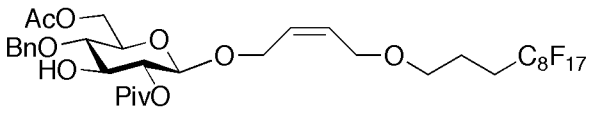


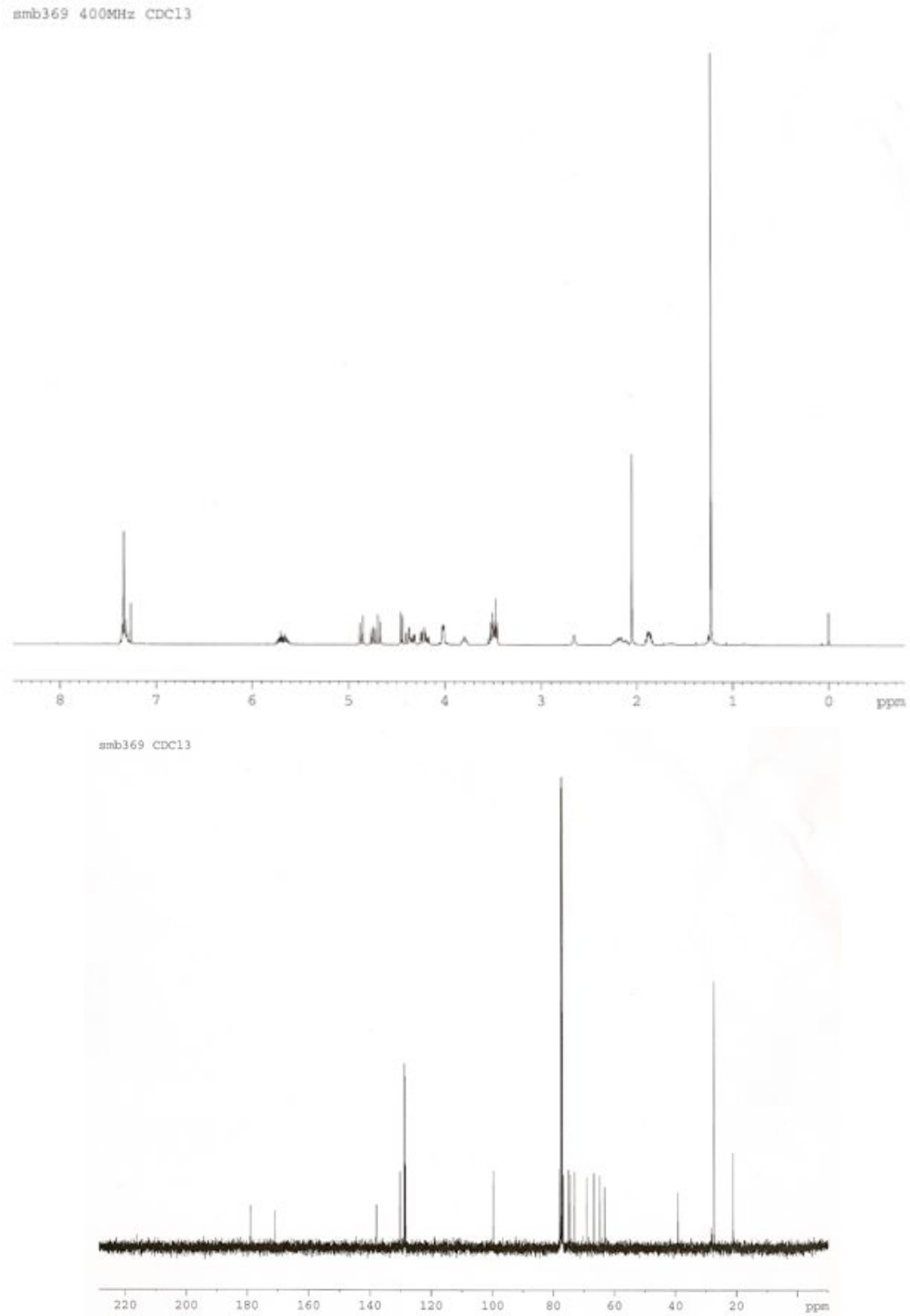

$^{1}H$ and $^{13}C$ NMR Spectra of 3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-D-glucopyranose (**14**).



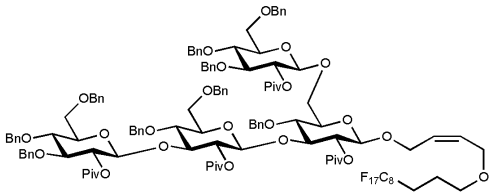



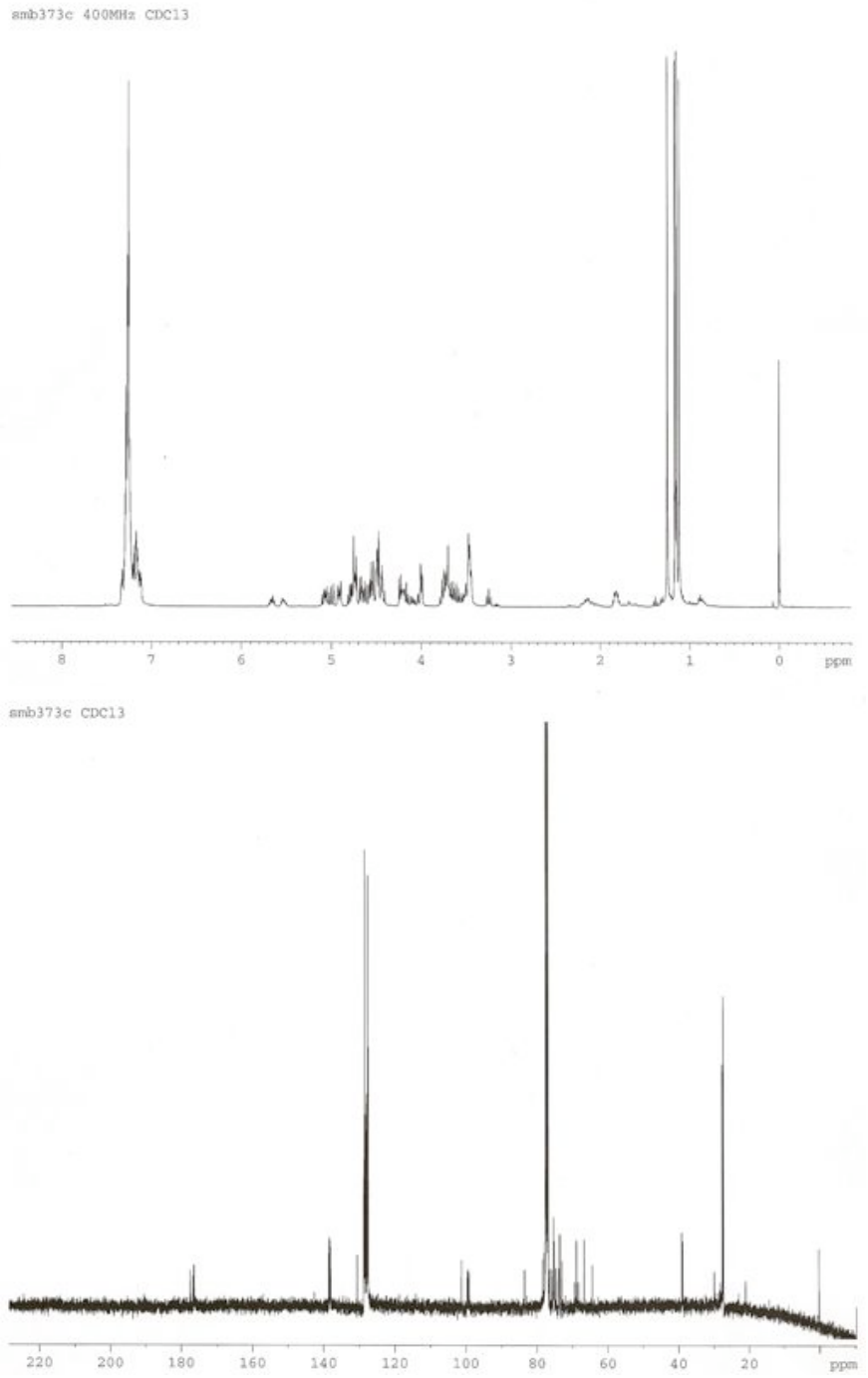

$^{1}$H and $^{13}$C NMR Spectra of 3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**15**).



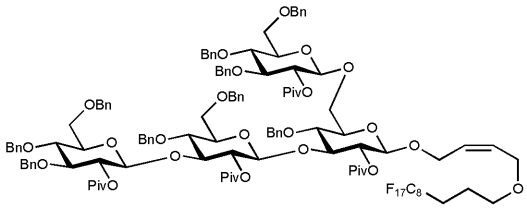



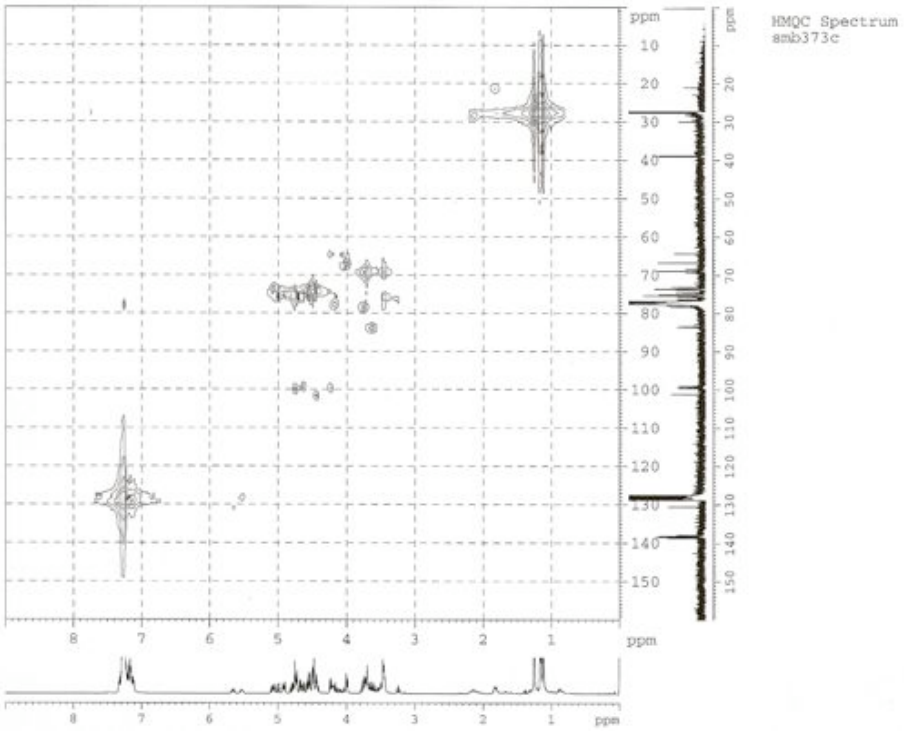

$^{1}H$ and $^{13}C$ NMR Spectra of *Cis*-4-(*1H,1H,2H,2H,3H,3H*-perfluoroundecyloxy)-2-butenyl-6-*O*-acetyl-4-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**17**).

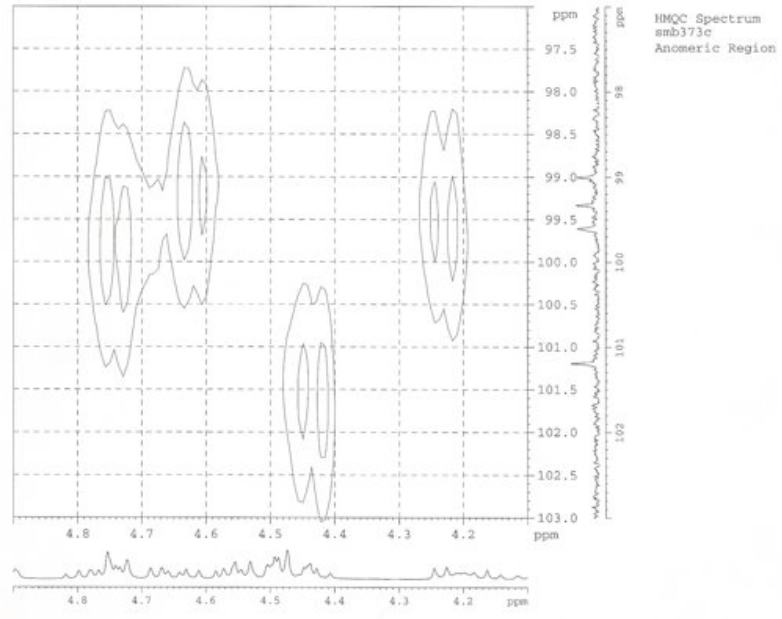



$^{1}H$ and $^{13}C$ NMR Spectra of tetrasaccharide **18**.




2-D HMQC NMR Spectra of tetrasaccharide **18**.

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

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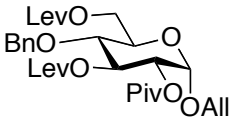
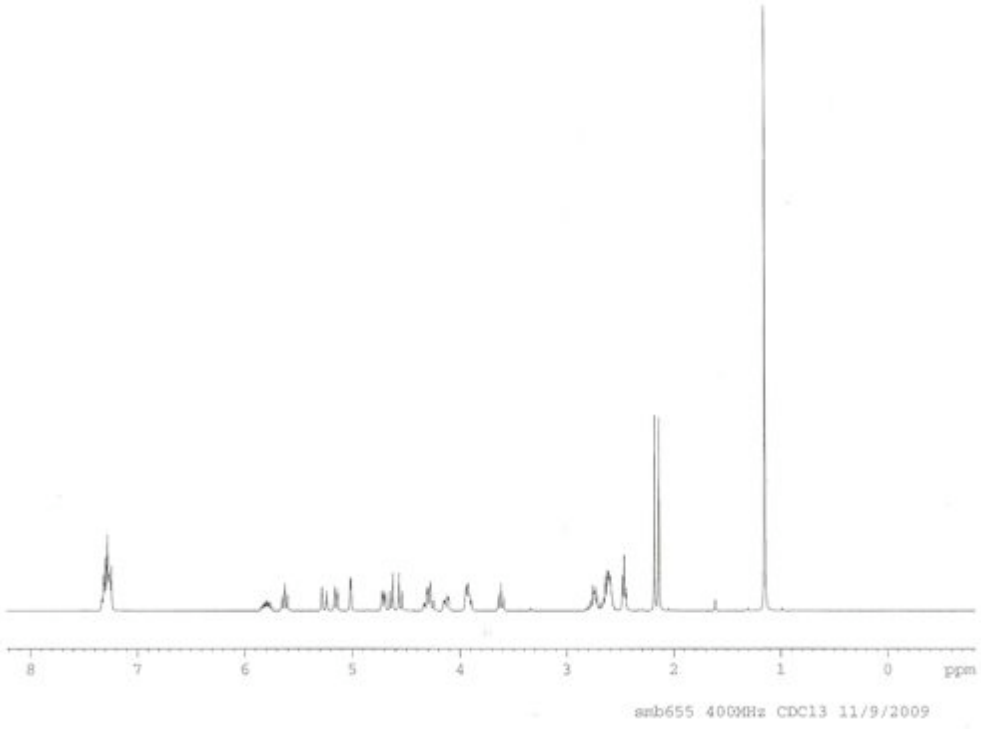
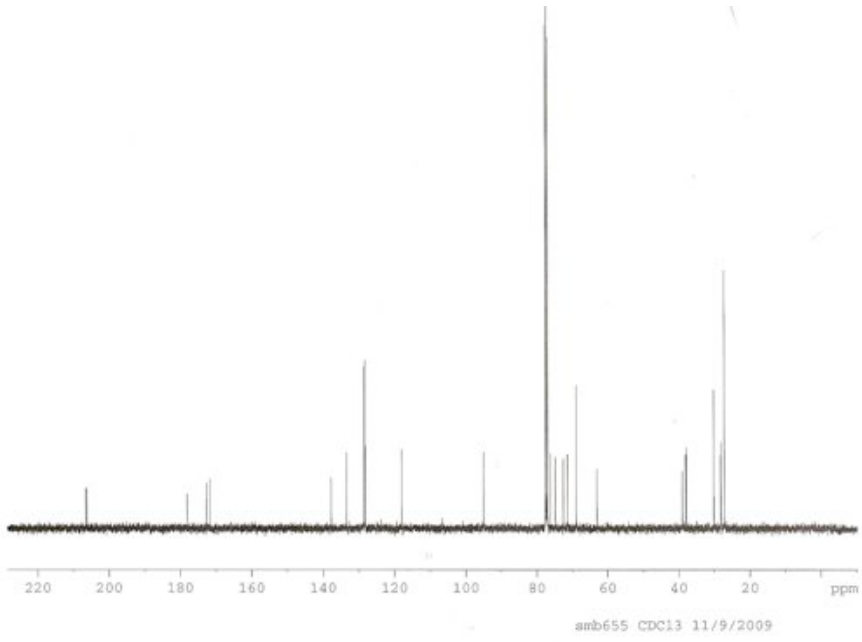
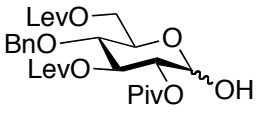
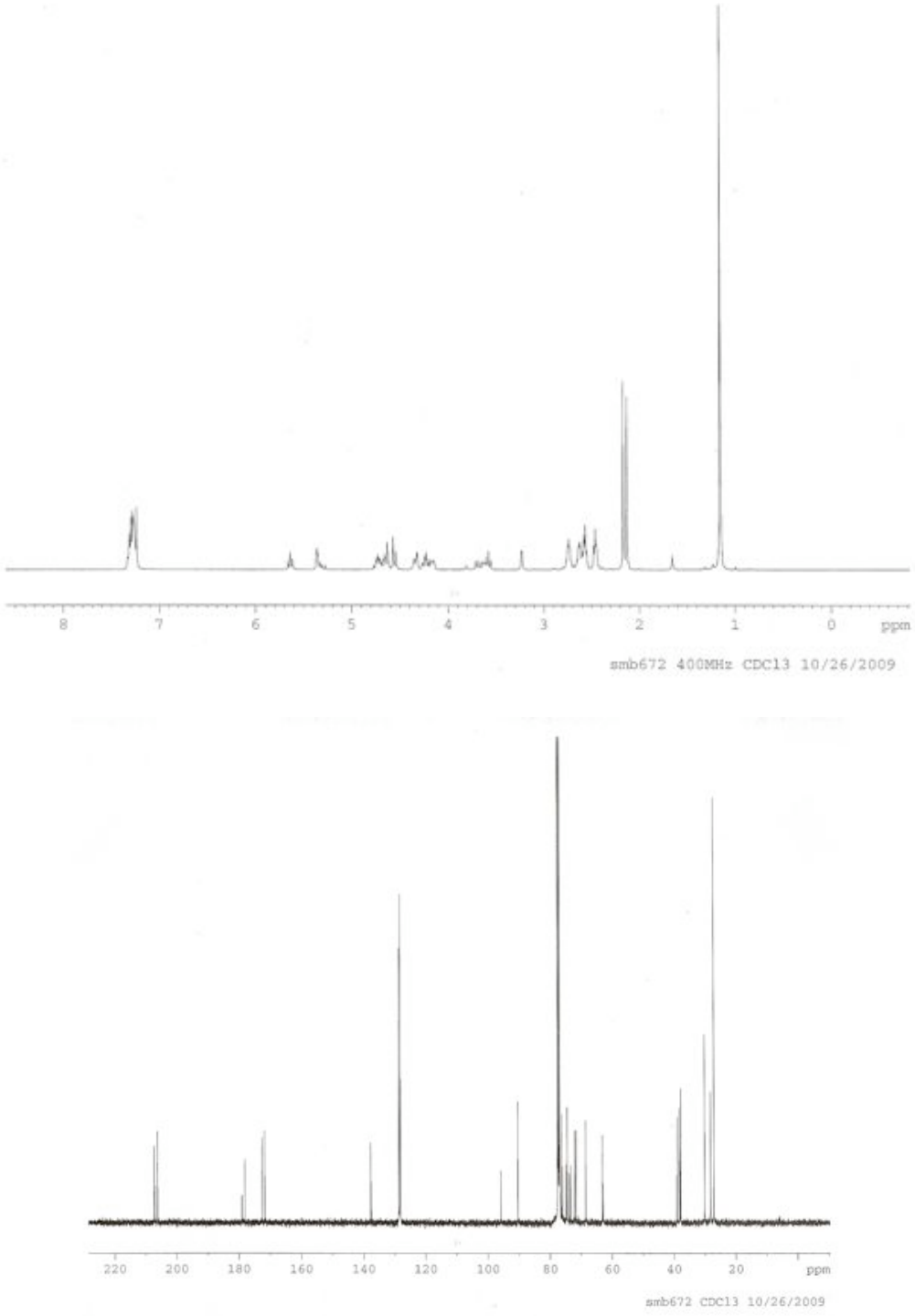


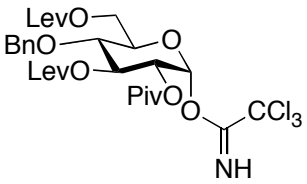



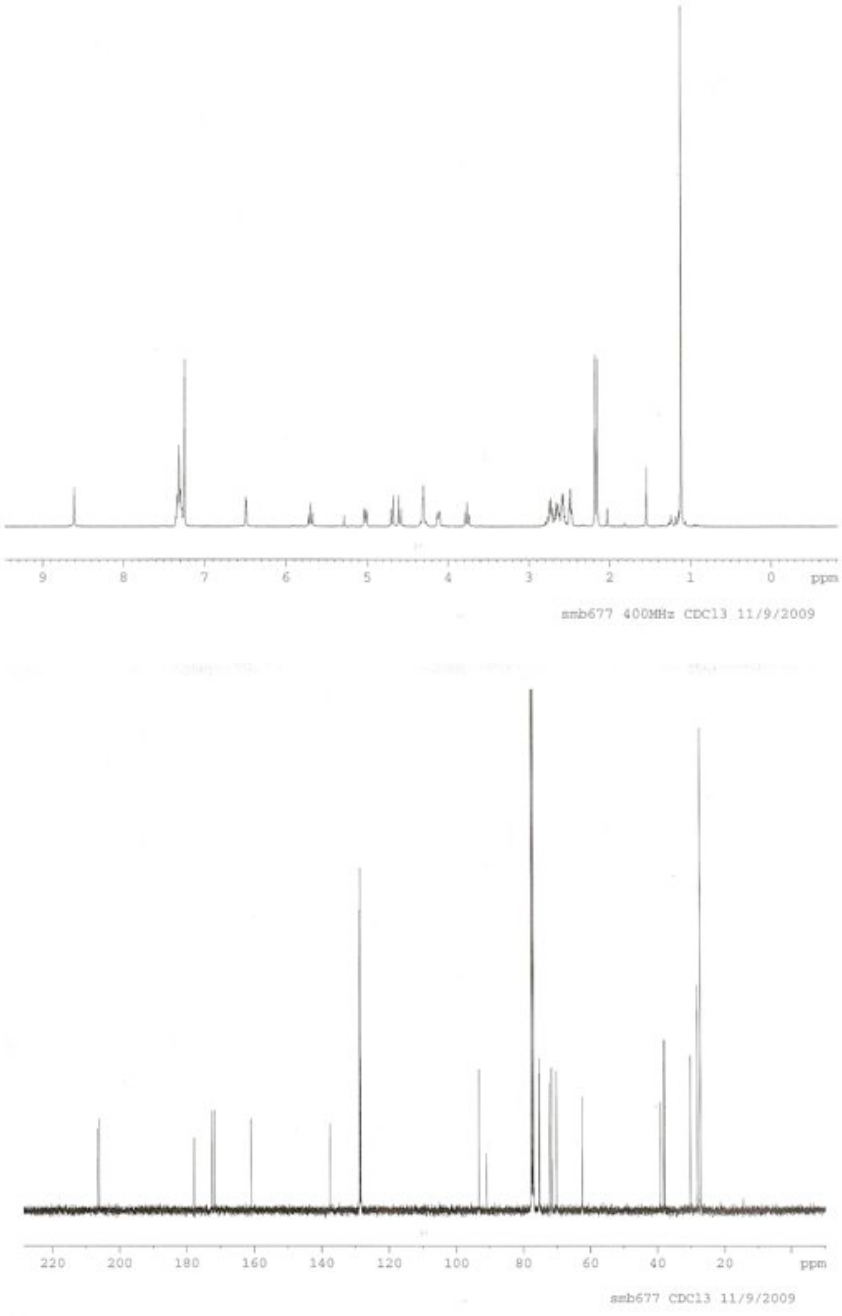

$^{1}$H and $^{13}$C NMR Spectra of Allyl 4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranoside (**12**).



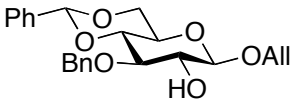



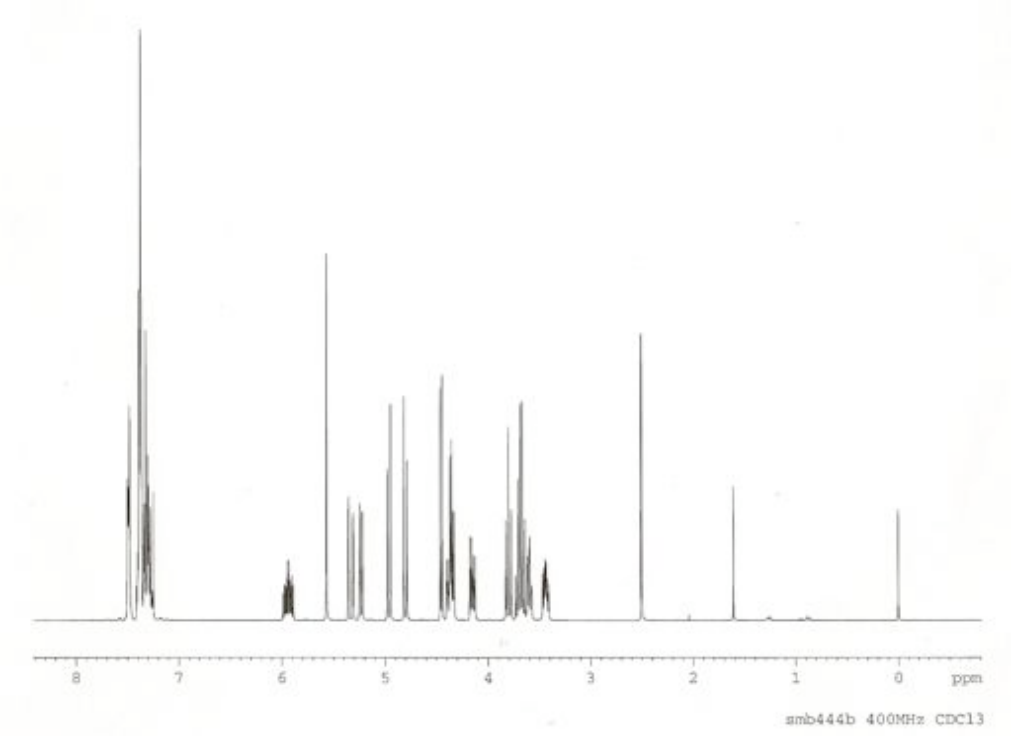

$^{1}$H and $^{13}$C NMR Spectra of 4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**13**).

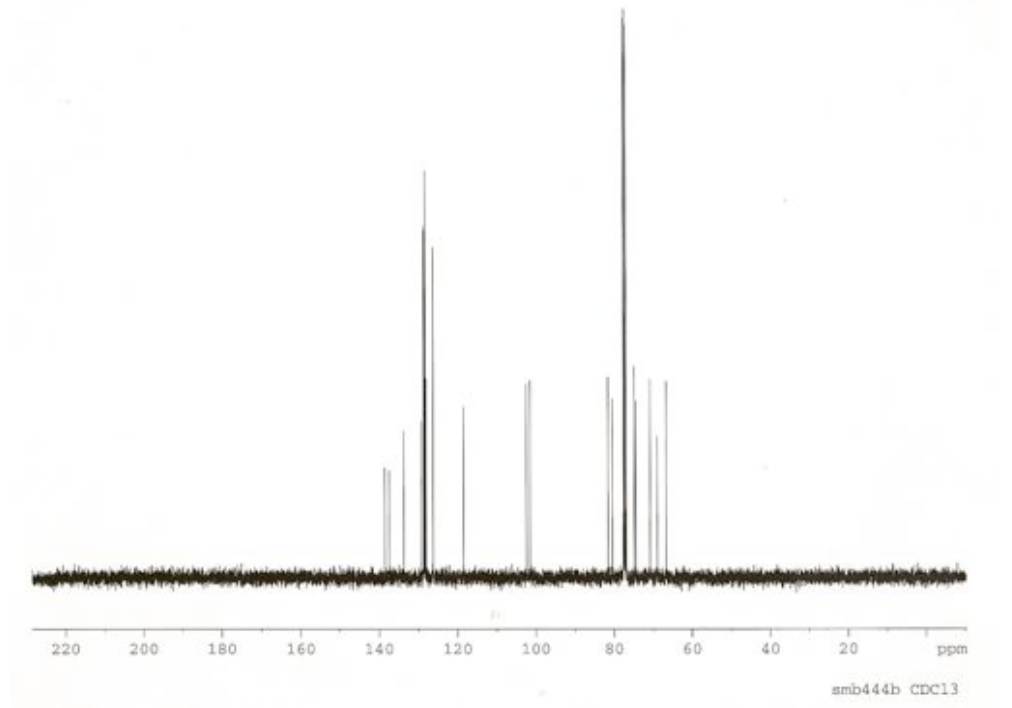


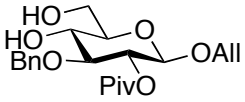

$^{1}$H and $^{13}$C NMR Spectra of 4-*O*-benzyl-3,6-di-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**6**).

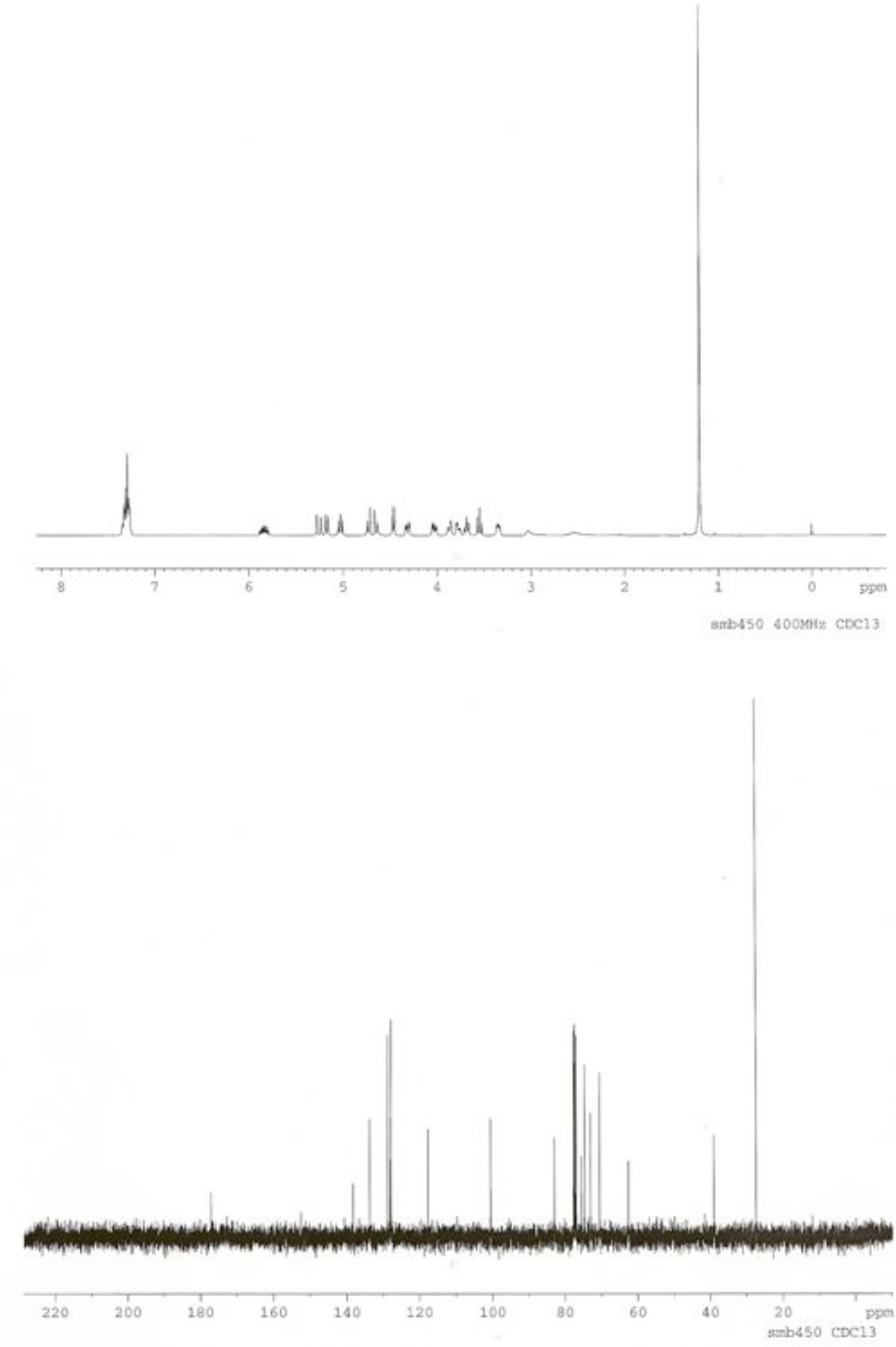


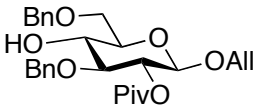



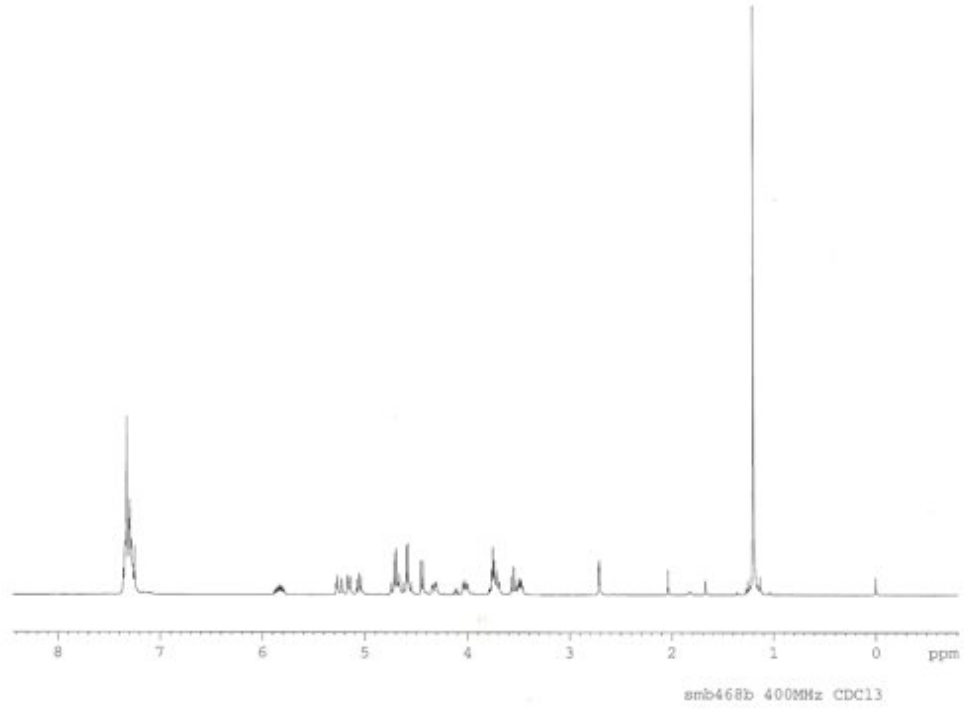

$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**15**).

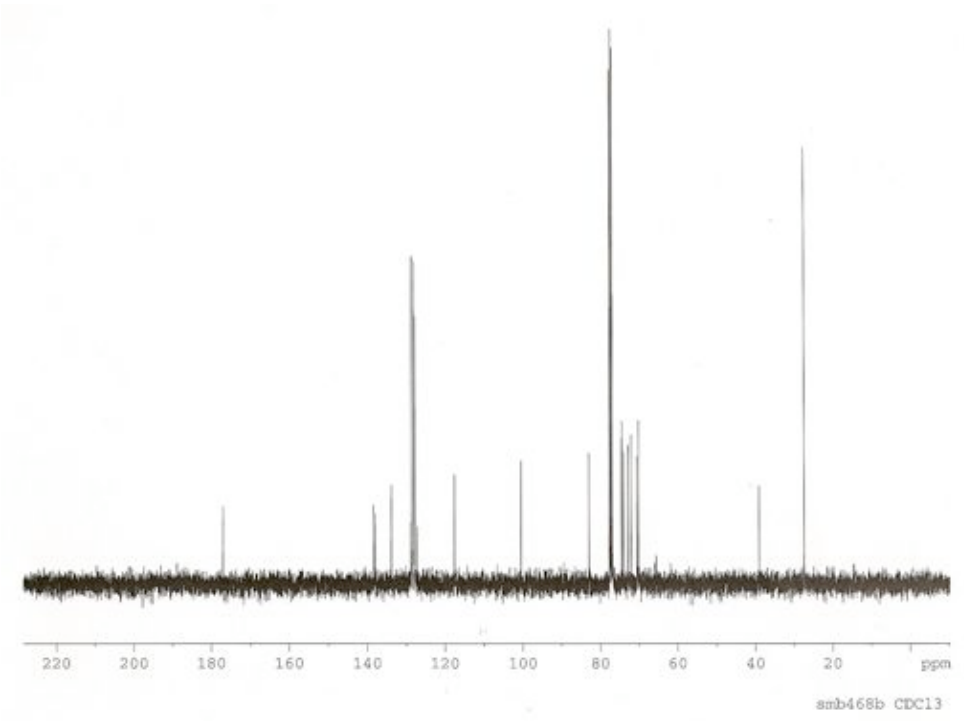


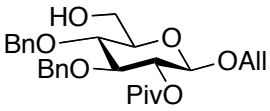

$^{1}$H and $^{13}$C NMR Spectra of Allyl 3-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**16**).

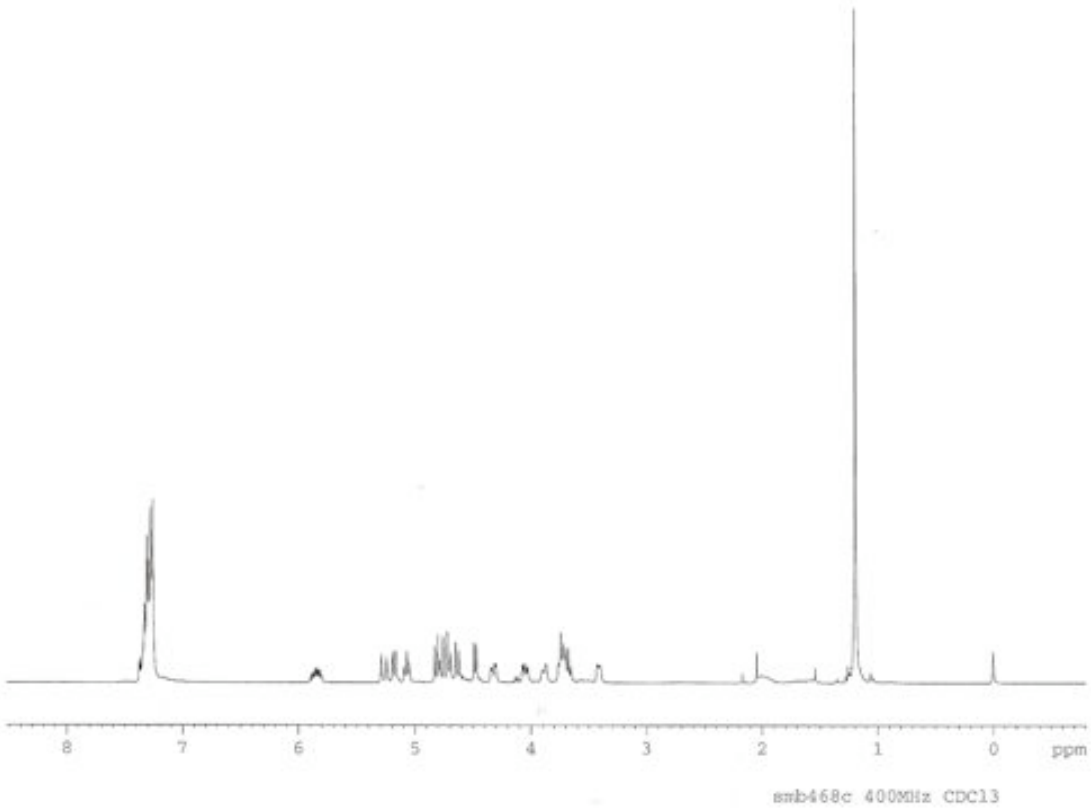


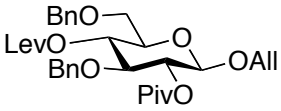



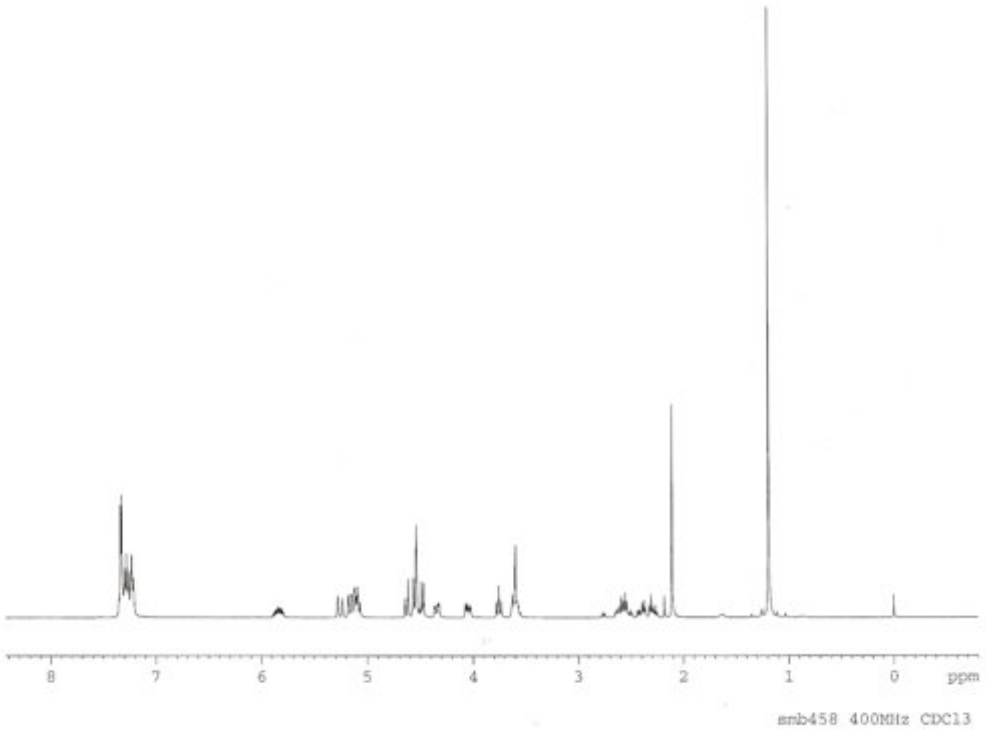

$^{1}H$ and $^{13}C$ NMR Spectra of Allyl 3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**17**).

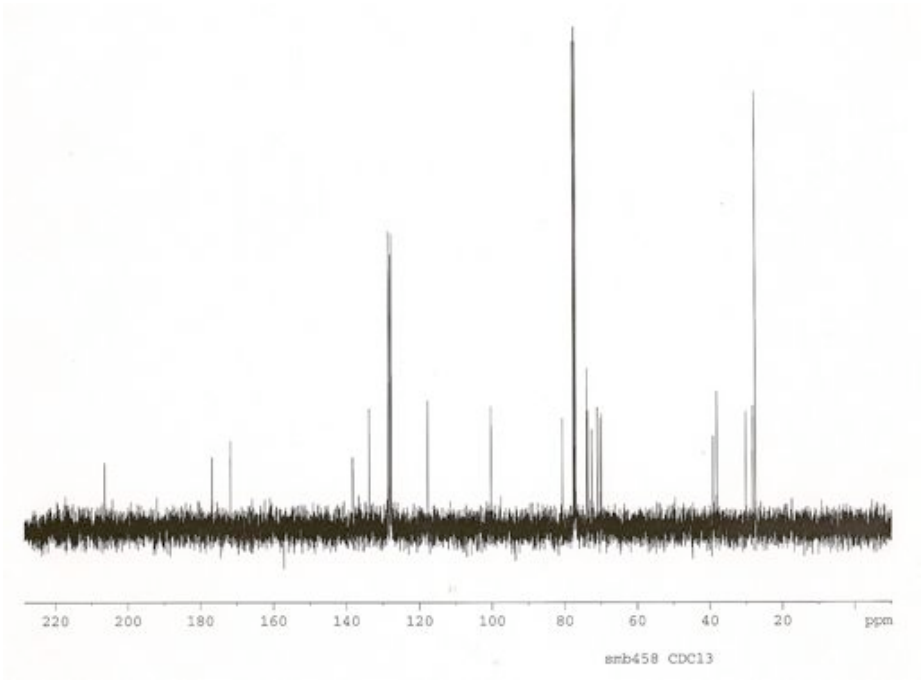


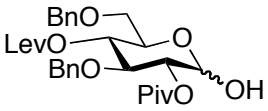

$^{1}$H NMR Spectrum of Allyl 3,4-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside (**18**).



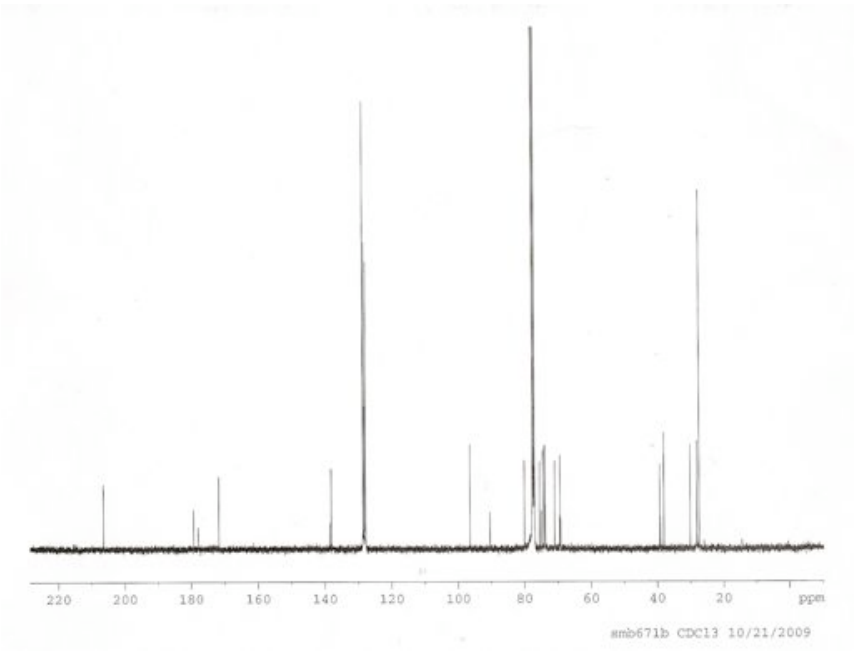



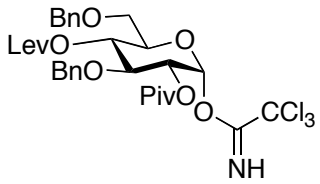

$^{1}$H and $^{13}$C NMR Spectra of Allyl 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-β-D-glucopyranoside (**20**).

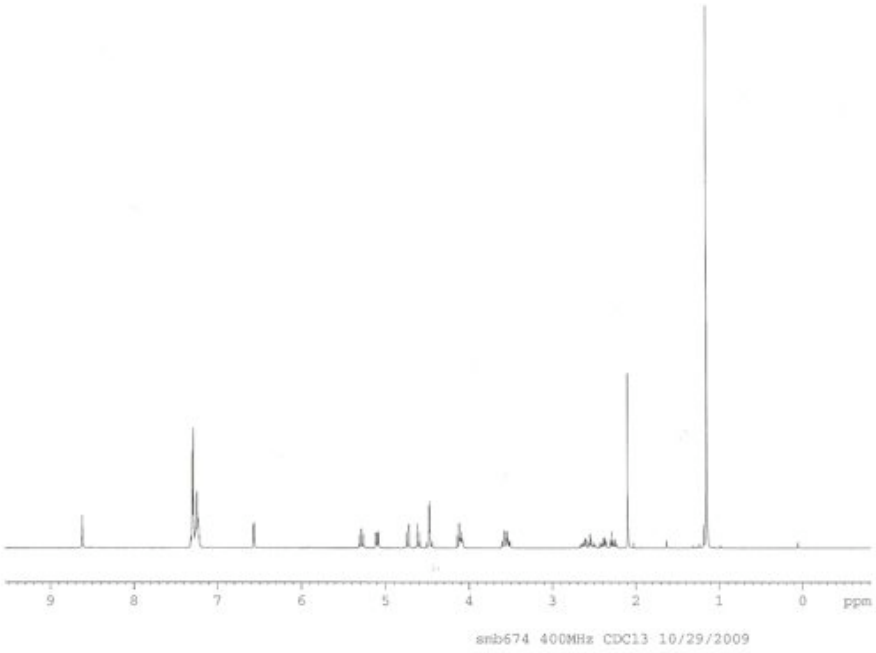


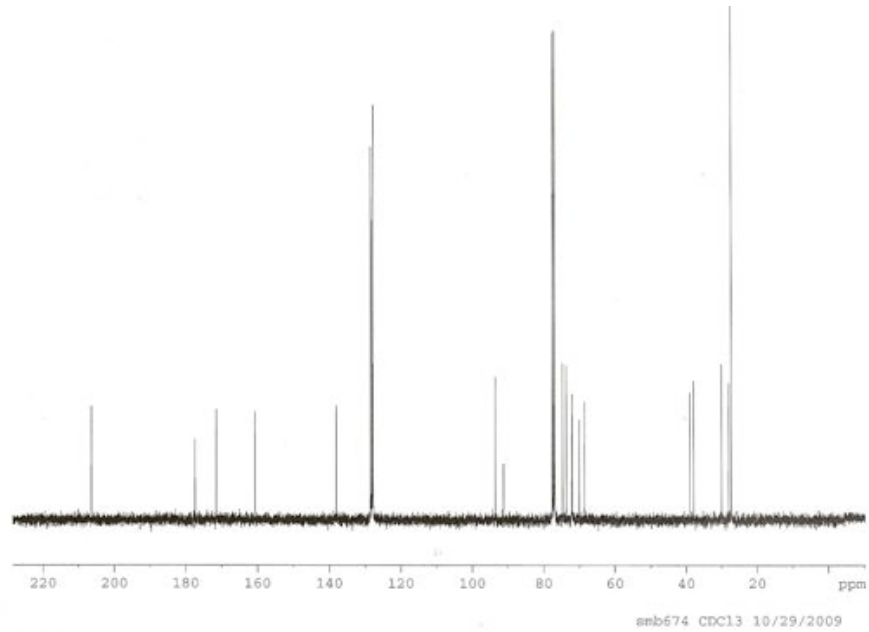




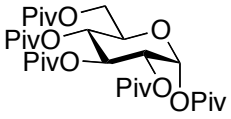

$^{1}$H and $^{13}$C NMR Spectra of 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-D-glucopyranose (**21**).

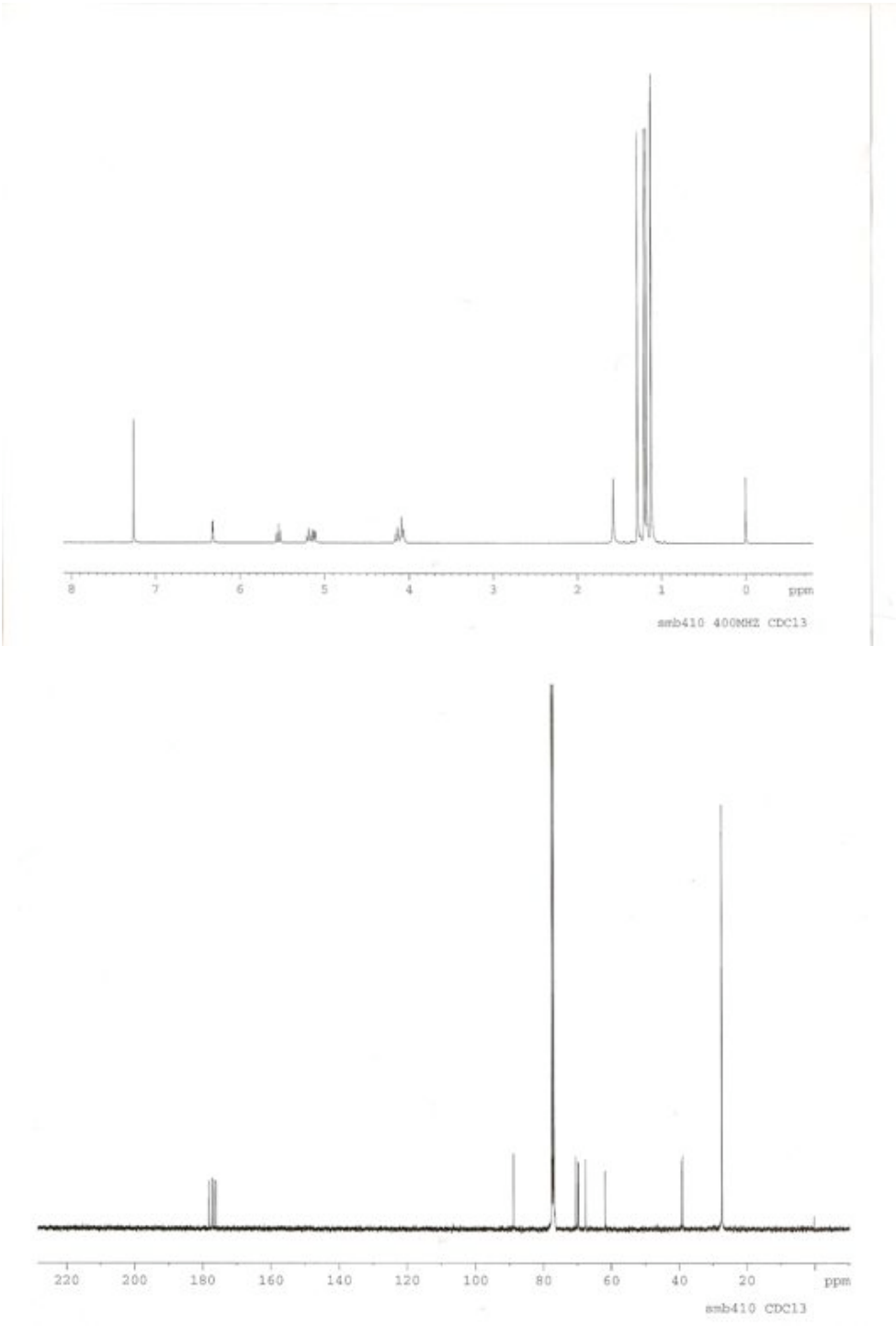


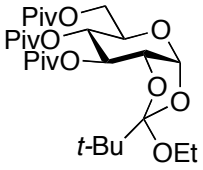



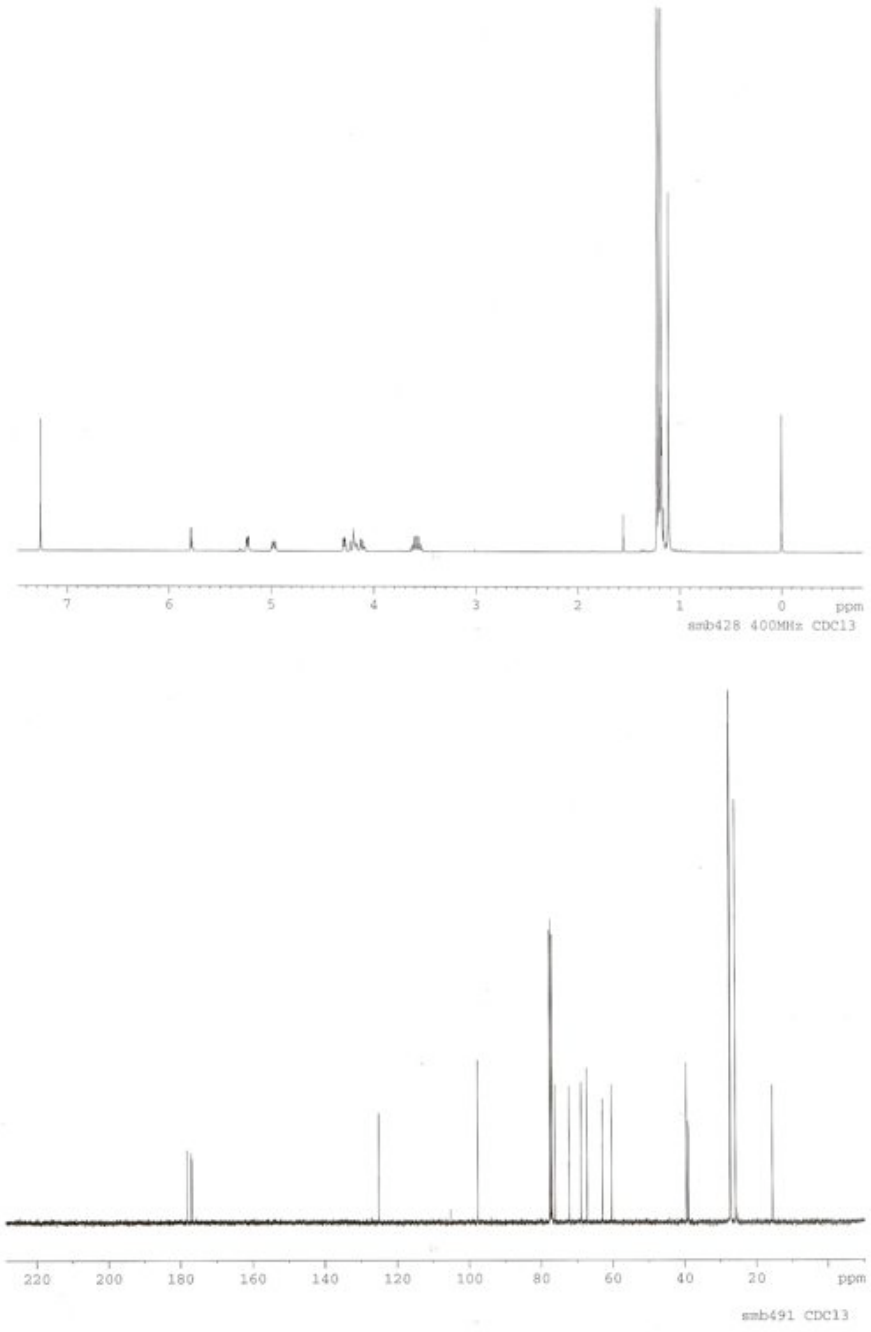

$^{1}$H and $^{13}$C NMR Spectra of 3,6-di-*O*-benzyl-4-*O*-levulinoyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**9**).



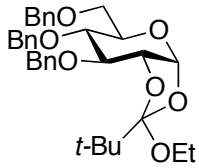



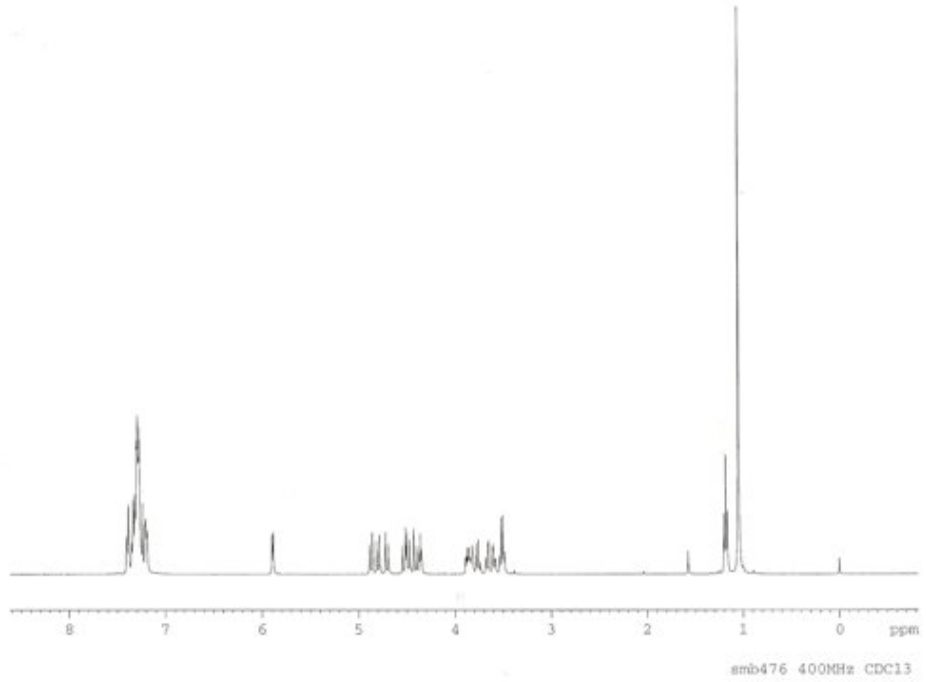

$^{1}H$ and $^{13}C$ NMR Spectra of α-D-Glucose pentapivaloate (**22**).

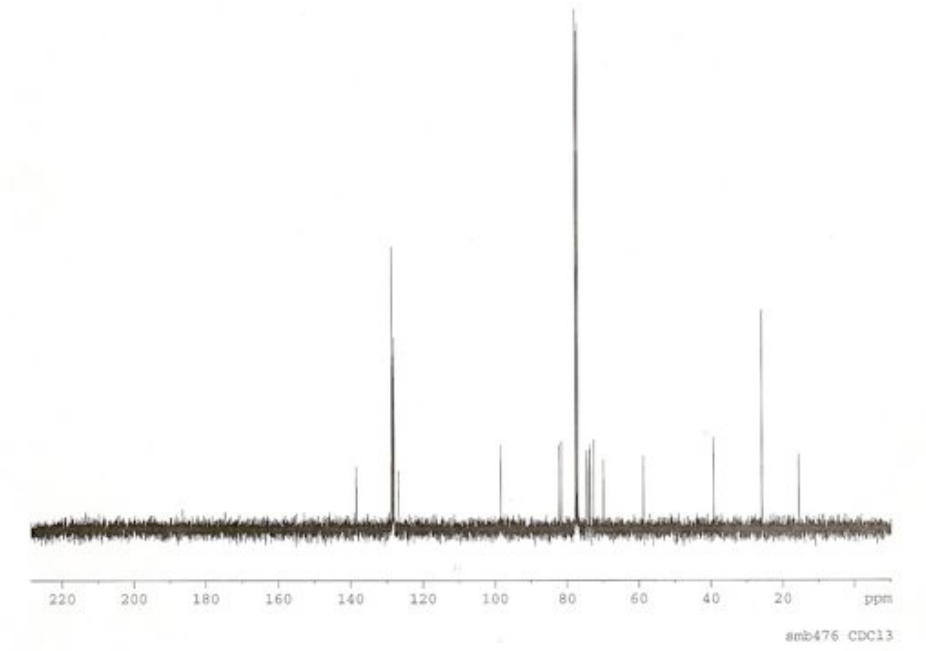


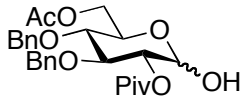

$^{1}$H and $^{13}$C NMR Spectra of 1,2-*O*-(1-ethoxy-2,2,2-trimethylethylidene)-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranose (**23**).

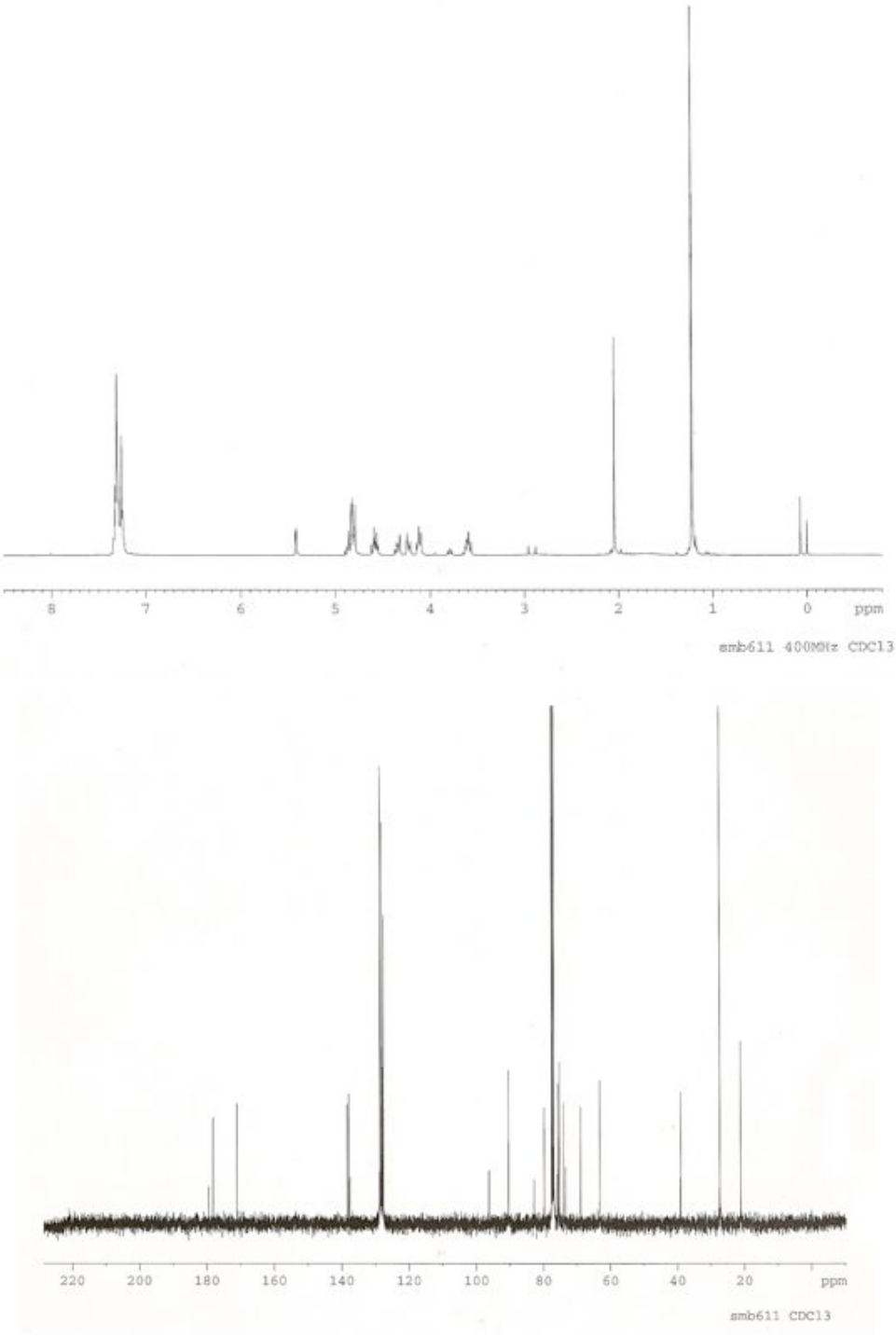


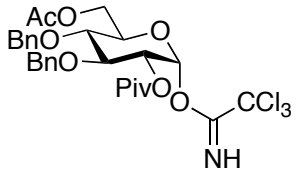



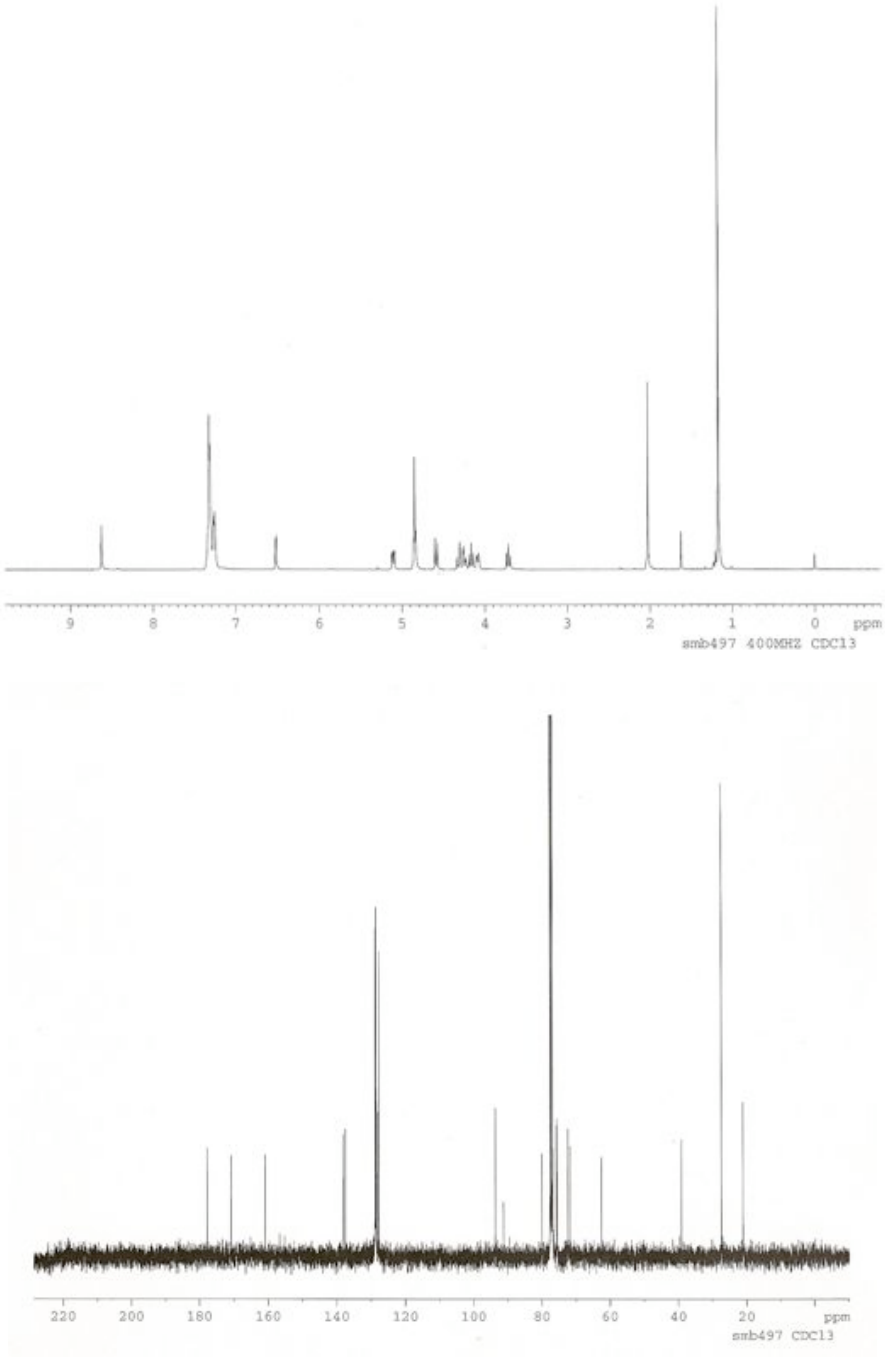

$^{1}$H and $^{13}$C NMR Spectra of 1,2-*O*-(1-ethoxy-2,2,2-trimethylethylidene)-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**24**).



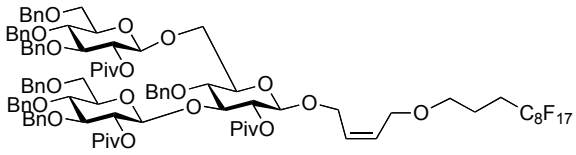



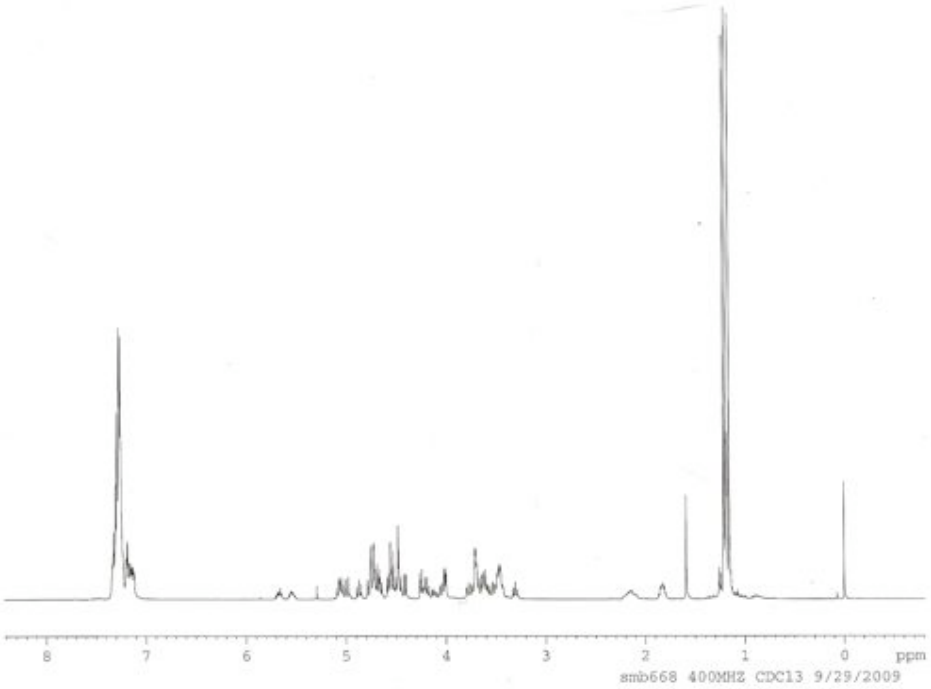

$^{1}H$ and $^{13}C$ NMR Spectra of 6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-pivaloyl-D-glucopyranose (**25**).

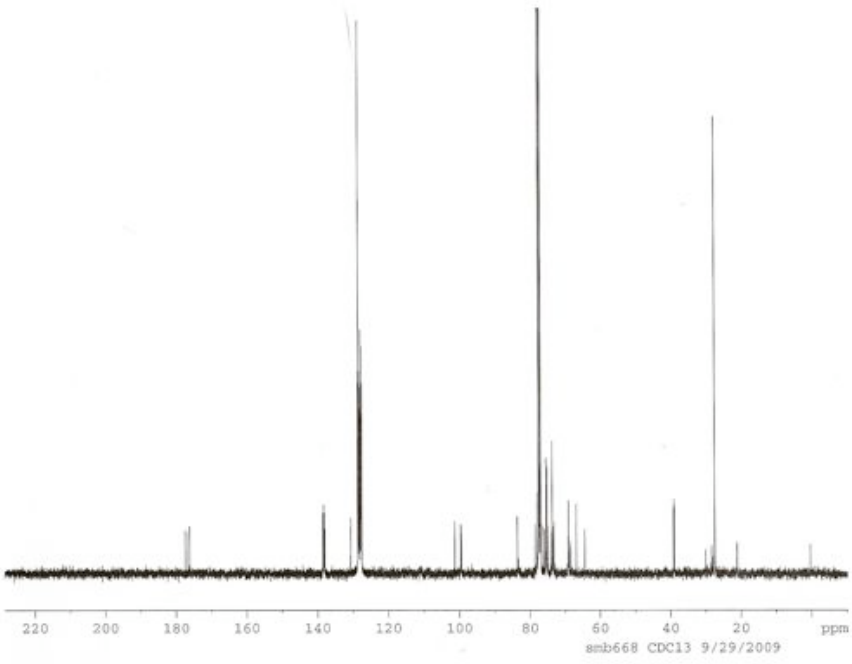


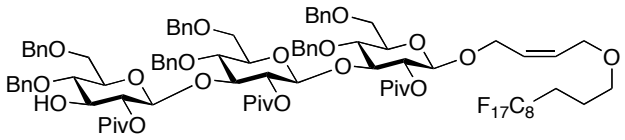

$^{1}$H and $^{13}$C NMR Spectra of 6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**10**).

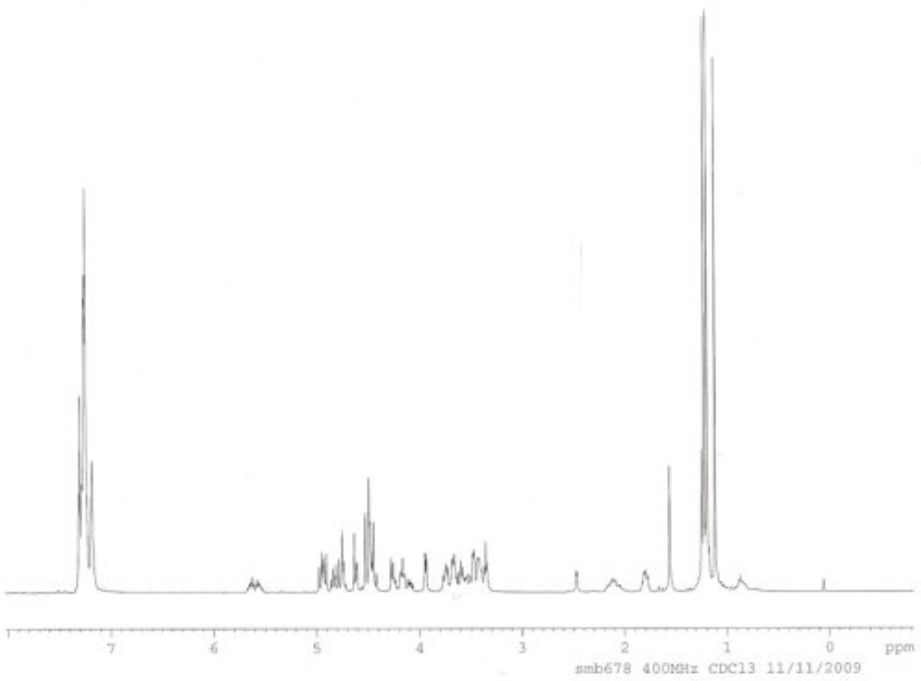


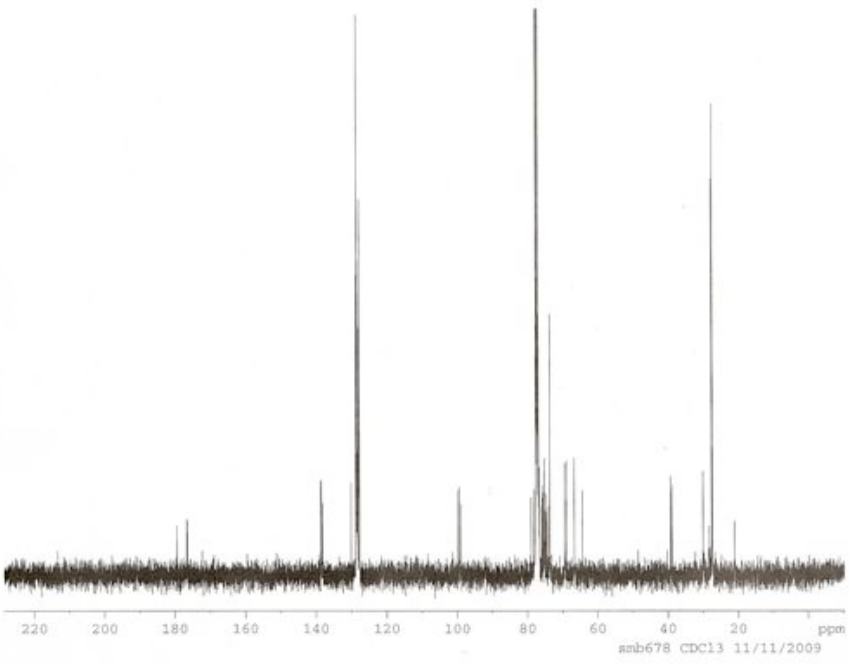



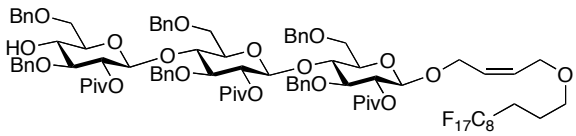

$^{1}H$ and $^{13}C$ NMR Spectra of trisaccharide (**1**).

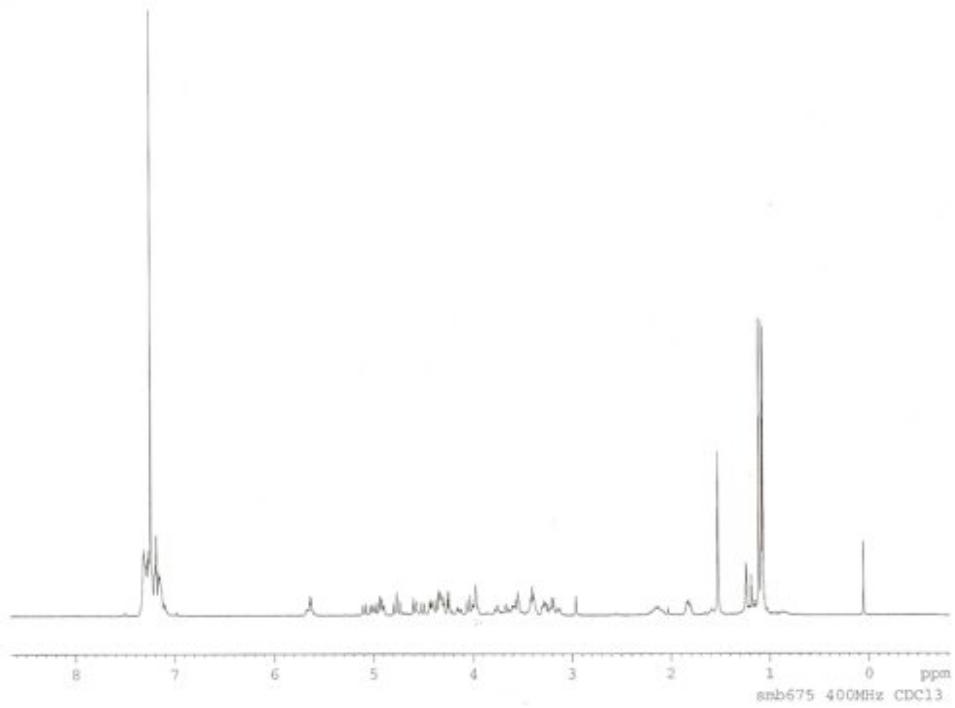


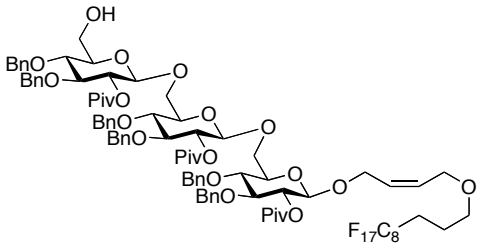



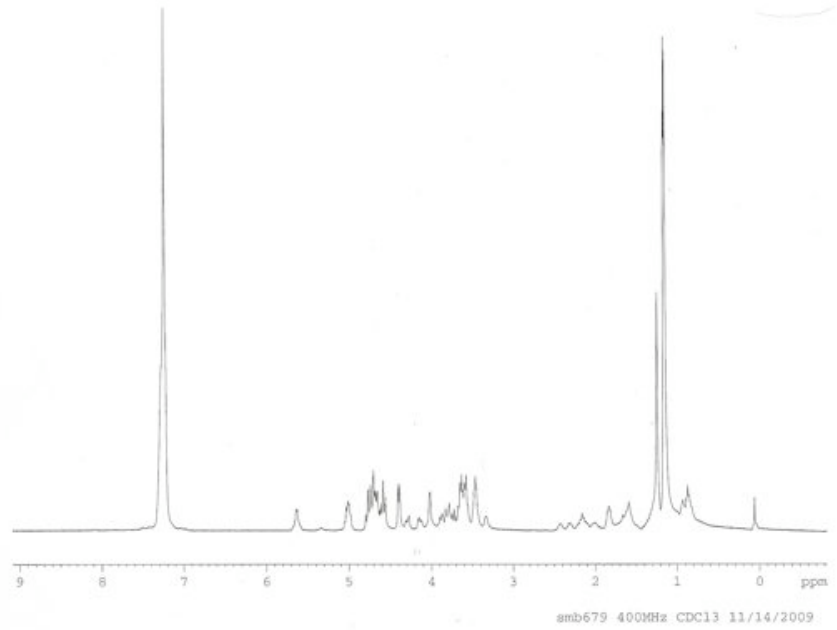

$^{1}H$ and $^{13}C$ NMR Spectra of trisaccharide (**2**).

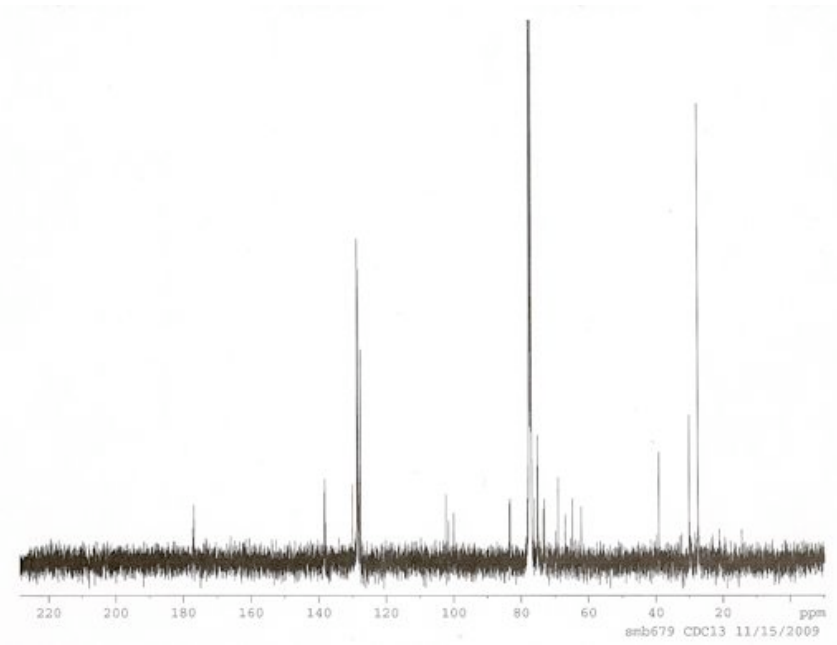


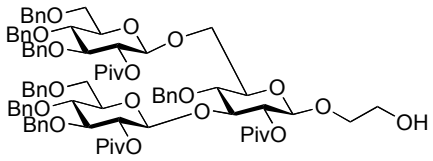

$^{1}$H NMR Spectrum of trisaccharide (**3**).

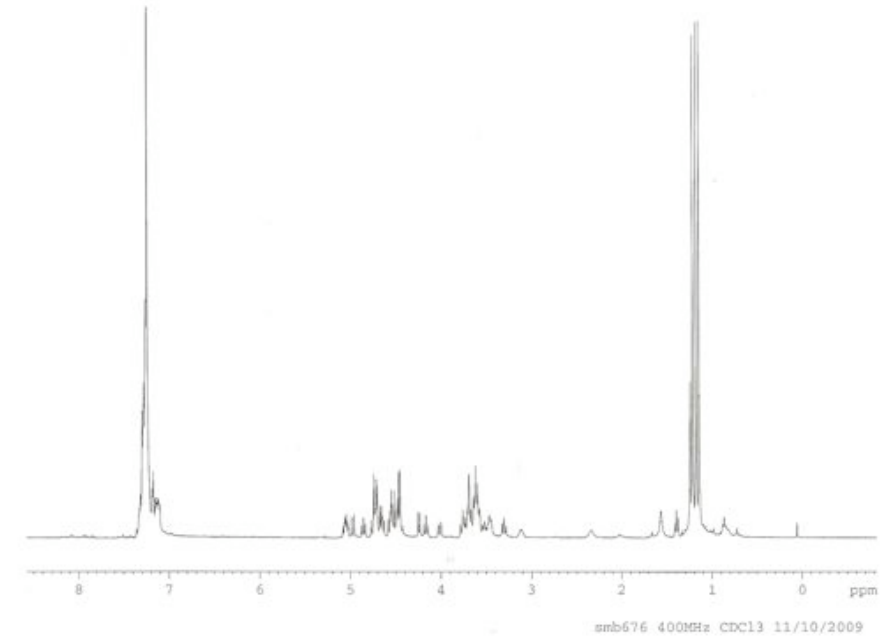


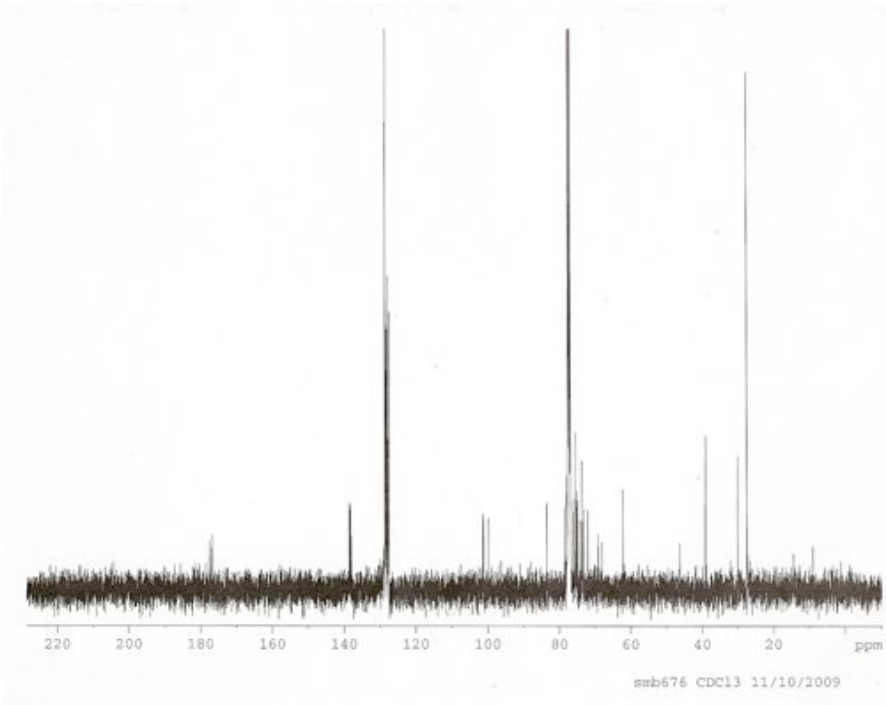



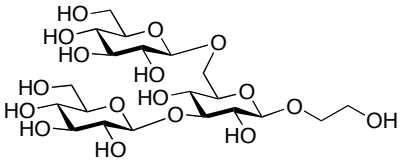

$^{1}H$ and $^{13}C$ NMR Spectra of trisaccharide (**4**).

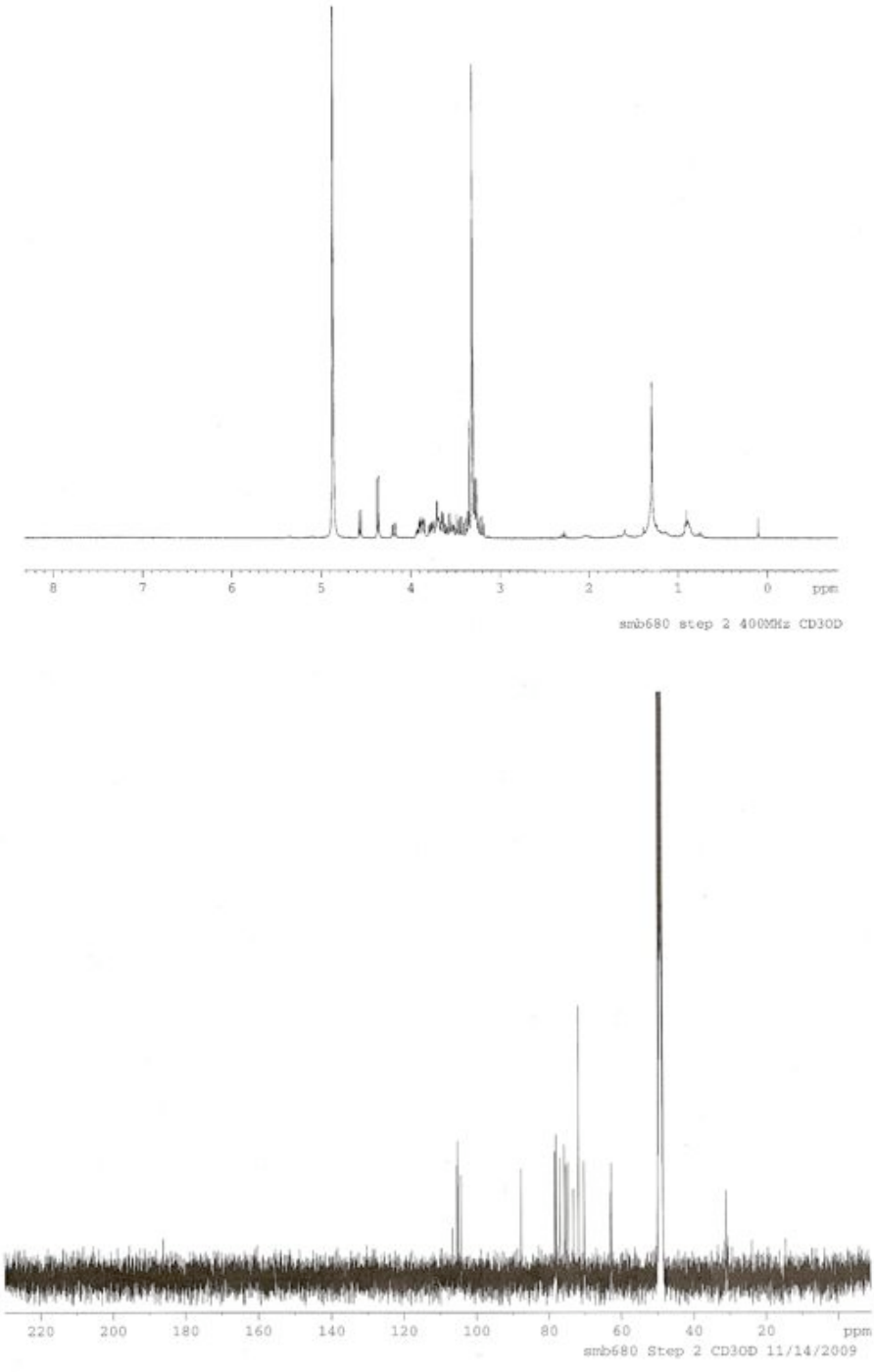



$^{1}H$ and $^{13}C$ NMR Spectra of trisaccharide (**26**).




$^{1}H$ and $^{13}C$ NMR Spectra of trisaccharide (**27**).