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Synthesis of functionalized glucose units for use in glycan assembly

Master's thesis in Materials chemistry

GABRIEL SIGFRIDSSON

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
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Department of Chemistry and Chemical Engineering
Division of Chemistry and Biochemistry
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Gabriel Sigfridsson

Supervisor: Gunnar Westman, Assoc. Prof.
Co-supervisor: Jelka Feldhusen, PhD
Examiner: Jerker Mårtensson, Prof.

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Department of Chemistry and Chemical Engineering
Division of Chemistry and Biochemistry
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone +46 31 772 1000

Gothenburg, Sweden 2024

Abstract

Within the fields of chemistry and materials science, much effort is currently being dedicated to the study of cellulose. Due to the promising physico-chemical properties displayed by the material and its abundance in nature, many hold hope it may one day come to replace petroleum based chemicals in material applications and play a key role in the transition towards a more sustainable society. However, a challenge in the study of cellulose is its inconsistent chemical purity which causes low reproducibility of experiments. Some of these issues may be resolved by instead performing experiments on oligomeric model compounds assembled from appropriately functionalized D-glucose units. Hence, the aim of following study is to develop experimental procedures that may be used for synthesis of such functionalized monomeric units. The work involves designing a synthetic pathway and performing a literature study to find suitable reaction protocols and reference data. Finally, the synthesis plan is performed in practice as an attempt to arrive at the target compounds. In the multistep synthesis of the glycosyl acceptor the intermediates 4,6-O-Benzylidene-D-glucopyranose, 4,6-O-Benzylidene-1,2,3-Tri-O-Acetyl-D-glucopyranose and 1,2,3-Tri-O-Acetyl-D-glucopyranose were reliably produced. However, the last step aiming to generate 6-O-Trityl-1,2,3-D-glucopyranose did likely not yield the intended results, but further analysis is needed. In the synthesis of the complementary glycosyl donor the intermediary 1,2,3,4,6-Penta-O-Acetyl-D-glucopyranose was produced successsfully. The subsequent chlorination may have produced the target, however conversion of starting material was poor and additional characterisation is required for further verification. 1,2:5,6-Cyclohexylidene-3-O-methyl-D-glucofuranose was also produced in good yield which may be further hydrolysed to act as a precursor for new monomers. Overall the synthesis strategy seems promising but require tuning of reaction parameters to produce the desired compounds in larger quantities. Finally, some suggestions for adjusted procedures and optimization are provided for future investigations.

Keywords: Organic chemistry, Synthesis, Carbohydrate chemistry, Saccharides, glucose

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Gabriel Sigfridsson, Gothenburg, June 2024

List of Acronyms

Ac	Acetate ester protective group
Bz	Benzoate ester protective group
d	Doublet
DCM	Dichloromethane
DMF	Dimethylformamide
dd	Doublet of doublet
Glc	Glucose group
m	Multiplet
NMR	Nuclear magnetic resonance spectroscopy
q	Quartet
Rf	Retention factor
s	Singlet
SIMS	Secondary ion mass spectrometry
t	Triplet
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tr	Triphenylmethyl ("trityl") protective group

Contents

1. Introduction

Cellulose is bio-polymer that is considered to be a potential future alternative to petroleum based components in a wide range of different material applications such as plastics, packaging materials, composites and insulators. One reason for its wide range of potential applications is its high versatility and the fact that its properties may be altered by producing it in various different forms. For instance it may be obtained in fiber and pulp form, through the Kraft process, which is suitable for manufacturing paper and cardboard. It may also be refined, through more recently discovered methods, into crystalline nanocellulose (CNC) which is suitable for casting films with desirable properties such as high tensile-strength and optical transmittance [1]. Apart from its favourable properties in material applications cellulose is also a renewable resource that may be extracted from several different bio-based raw materials such as wood, plants, agricultural wastes and algae [1].

In the ideal case the chemical structure of the cellulose polymer consists of repeating units of glucose which are coupled together via $\beta(1\rightarrow4)$ glycosidic bonds as illustrated in figure 1.1. However, in practice the material often displays significant heterogenity and a varying degree of chemical purity as a result of manufacturing conditions. Impurities may include domains of hemicellulose, chemically attached lignin residues and a variable length of the polymer chains. Such variations in composition represent a challenge in the study of cellulose as it may cause experimental results to become inconsistent and difficult to reproduce [2]. Hence, there exists an interest in producing small oligomeric fragments of cellulose via organic synthesis that may be used as model compounds in the study of cellulose. The benefit of such a bottom-up approach, is that a greater control over the homogenity can be exercised and it also offers the researcher the possibility of introducing any desired functional groups by tuning the synthetic pathway [1] [2]. A pre-requisite for this type of oligomeric assembly, however, is that selectively functionalized glucose monomers can be accessed. Unfortunately, such specialized glucose units are not readily available through conventional chemical suppliers. Therefore the following project aims to develop experimental procedures that can be used to synthesize such monomers.

How the functionalization should be performed depends on which procedure is used to assemble the oligomers. One of the simplest and most widely used procedures is the Koenigs-Knorr reaction. This reaction commonly utilizes a $Hg(CN)_2$ catalyst to couple two different types of functionalized glucose units, denoted as glycosyl donors and glycosyl acceptors as illustrated in figure 1.2. The glycosyl donor is a

glucose unit where all hydroxyl groups have been blocked with protective groups and the remaining position has been functionalized with a leaving group. The glycosyl acceptor has all hydroxyl groups protected except one which may interact with the leaving group of the glycosyl donor to form the desired glycosyl bond [3]. Due to the simplicity of the Koenigs-Knorr reaction the monomers synthesized in this project will be tailored primarily for use in this type of reactions.

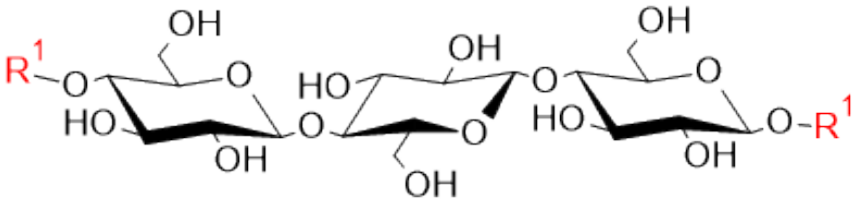

Figure 1.1: Illustration of the general repeating structure of cellulose.

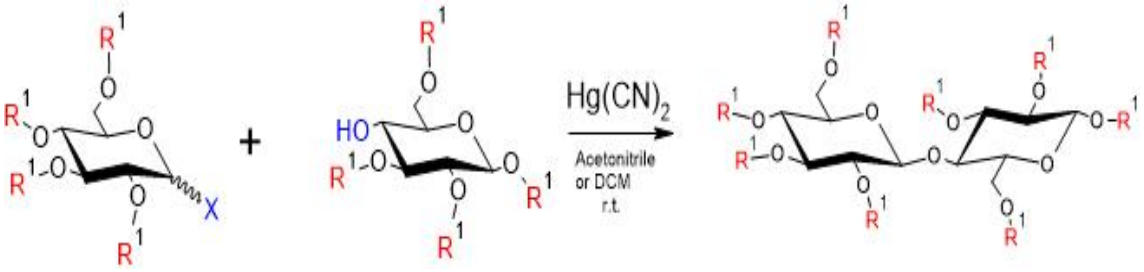

Figure 1.2: Reactants and products involved in the Koenig-Knorr reaction. X signifies a leaving group whereas R signifies a protective group.

Additionally, certain hydroxyl sites on the monomeric glucose units are of particular interest. For instance, it has been suspected that intermolecular hydrogen-bonding occurring from the hydroxyl at the C-3 position of the glucose unit plays a key role in establishing the characteristic stiffness associated with cellulose polymer chains [1]. Hence, there is interest to synthesize monomers where hydroxyl at C-3 has been modified into a non-hydrogen bonding functionality. Due to this interest, an attempt to synthesize glucose with C-3 substituted will be attempted as an additional challenge within this project.

1.1 Aims and boundaries

The following project will aim to conduct a literature study in order to investigate which functional and protective groups would be suitable in the synthesis of glucose monomers that are compatible with the Koenigs-Knorr reaction. Based on the knowledge obtained from these studies, synthesis pathways for the monomers will be developed. Finally, the pathways will be carried out in practice, via experiments, in an attempt to produce the desired compounds and to assess the suitability of the developed pathways.

To fit the work within the limited time frame of a thesis project, the aim will be to develop protocols for the synthesis of functionalized monomers only, whereas the

coupling of monomers into oligomers will not be attempted. The monomers will be tailored primarily for the Koenigs-Knorr reaction due to its simplicity relative to alternative assembly techniques.

The project will be carried out at the division for organic chemistry at Chalmers University of Technology. Experimental procedures will be performed only by utilizing equipment and facilities available at the institution.

Since experiments are performed at a relatively small scale, it has been assessed that the project should not be associated with any significant impacts on the environment and no further environmental analysis will be performed. Furthermore, the project should not cause any immediate ethical or moral dilemmas.

2. Theory

In the following section the theoretical foundations of the work are provided. Some characteristics and chemical properties of glucose and similar carbohydrates are explained. Notes are also given on the developed synthetic pathway and reactions that are considered to be of particular importance. Furthermore, the principles of NMR and mass spectrometry are described as these techniques were used extensively throughout the project for characterization.

2.1 Carbohyrate chemistry

Carbohydrate chemistry is a branch of organic chemistry concerned with studying the characteristics of saccharide molecules. The fundamental structure for such molecules is the cyclic, six-membered aldohexose ring with five hydroxyl groups attached so that the net molecular formula can be expressed as $C_6H_{12}O_6$. The carbons are conventionally assigned a number according to the illustration in figure 2.1 and some notable examples of monosaccharides that display these structural features are listed in figure 2.2. Since each of these molecules have the same atomic composition, the different classes of monosaccharides are diastereomers and are distinguished from one another solely based on the configuration at four of their respective chiral centers [4]. Furthermore, each type of monosaccharide exists in two anomeric states as determined by their configuration at C-1. The α-anomer is characterized by an axial configuration of the hydroxyl group whereas for the β-anomer the hydroxyl is directed equatorially. In protic solvents the interconversion between these anomeric states occurs freely since glucose may transition into its open-chain, linear form which has an aldehyde functionality and hence no chiral center at C-1, and then back into the pyranose ring which re-generates the chiral center and may cause the

chiral configuration to invert (see figure 2.3) [4]. While both anomeric states are always present in significant portions, the β-anomer is slightly favoured due to being less sterically hindered by neighbouring substituents. However, the more sterically strained α-anomer is still present in significant amounts due to a phenomena known as the anomeric effect. This is thought to be the result of interaction between the free electrons of the endocyclic oxygen atom in the ring and the empty, anti-bonding σ^* orbital from the C-O bond of the hydroxyl being more potent in the α-anomeric state [4] [5]. Furthermore, a small portion of the glucose equilibrates into yet another isomer, the five-membered furanose ring, however at ambient conditions around 99% of the material exists in the pyranose form [4].

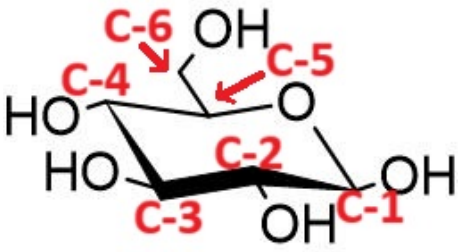

Figure 2.1: Structure of the D-glucose molecule in the hexapyranose configuration along with conventional carbon numbering.

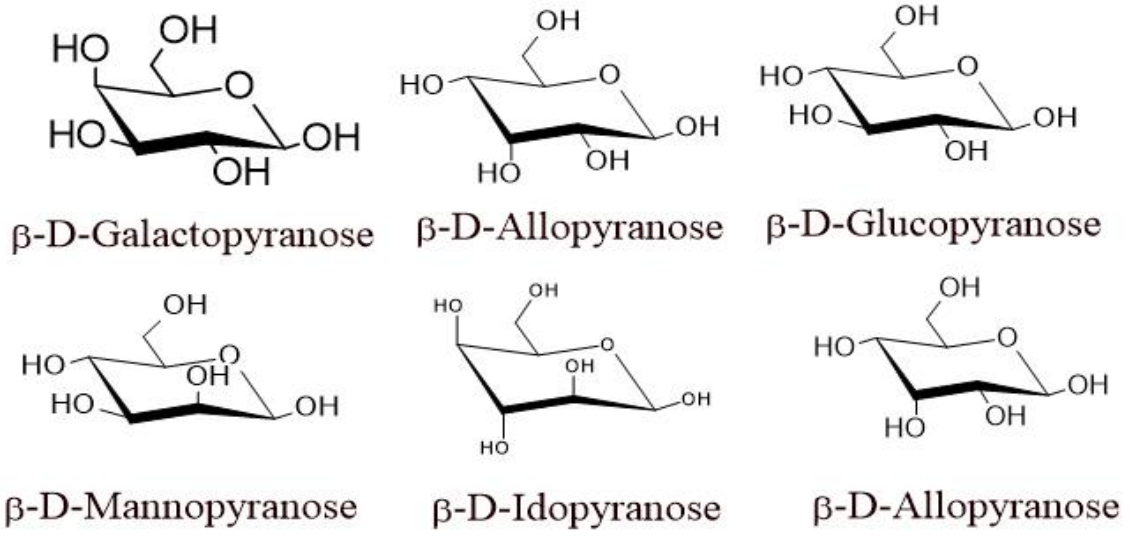

Figure 2.2: Structures of some common monosaccharides.

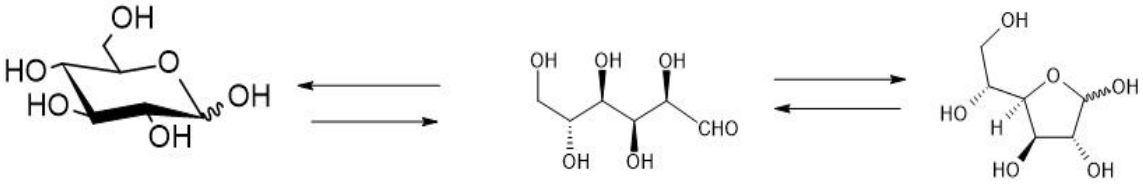

Figure 2.3: Scheme illustrating the equilibrium between the hexapyranose, hexafuranose and open-chain isomers of D-glucose.

Cellulose is a polymeric saccharide with the chemical structure indicated in figure

1.1. In the ideal case the structure of cellulose consists of repeating units of β-D-glucose units linked together from C-4 on one unit to C-1 on another through ether-bridges referred to as $\beta(1\rightarrow4)$ glycosidic bonds [1]. In order to obtain oligomeric model compounds with the same structure one may apply the Koenigs-Knorr reaction as illustrated in figure 1.2 [6]. The reactants involved in this procedure are called glycosyl donors and acceptors. A glycosyl donor is a monosaccharide that contains a good leaving group at the anomeric C-1 position, typically in the form of halides such as chlorides or bromides [3]. A glycosyl acceptor, on the other hand, is a monosaccharide that may react with such a leaving group through an unprotected hydroxyl. If the desired linkage is the $\beta(1\rightarrow4)$ glycosidic bond, this implies that all hydroxyls except the one at C-4 of the glycosyl acceptor must be blocked via use of protective groups [3]. Furthermore, to obtain the desired stereochemically defined $\beta(1\rightarrow4)$ bond (and not $\alpha(1\rightarrow4)$) the protective group at C-2 of the donor must be able to provide anchimeric assistance [7]. This is provided most effectively by acetate, benzoate or pivaloyl esters which usually result in a high selectivity towards $\beta(1\rightarrow4)$ glycosidic bonds whereas ether groups are less effective and tends to yield a mixture of $\alpha(1\rightarrow4)$ and $\beta(1\rightarrow4)$ linkages. Apart from these monomers, the coupling reaction requires a halide acceptor (often referred to as a promotor). The most well documented reagent for this purpose is mercury cyanide ($Hg(CN)_2$) but recent studies also indicate that silver trifluoromethanesulfonate (AgoTf) could be used as well. Commonly applied solvents for the reaction are acetonitrile and dichloromethane. [3].

2.2 The synthetic strategy

To obtain the type of glycosyl acceptors illustrated in figure 1.2 two strategies were developed and are displayed in figure 2.4. The first route relies on treating anhydrous D-glucose (1) with benzoyl chloride in pyridine to arrive directly at 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose (2). This method has been previously documented [8] and is attractive due to its simplicity. It relies on exploiting the fact that the hydroxyl group at C-4 is generally considered to have the lowest relative reactivity [8] [9]. Thus, by treating glucose with benzoyl chloride the other hydroxyl groups should react preferentially and leave C-4 unprotected and therefore give a monomer that can act as glycosyl acceptor in just one step starting from D-glucose [8].

An alternative strategy for the glycosyl acceptor is more laborious and involves more synthetic steps but is, on the other hand, not dependent on purely exploiting the reactivity order of the hydroxyl groups. In the first step 4,6-O-Benzylidene-D-glucopyranose (3) is formed. Several experimental procedures have been documented to lead to this structure [10] [11]. Most commonly D-glucose is treated with equimolar amounts of benzaldehyde dimethyl acetal to achieve a transacetalation reaction or alternatively with a large excess of benzaldehyde to perform a regular acetal reaction. Once this compound has been purified one may potentially apply an excess of acetic anhydride to achieve total protection of all the remaining hydroxyl groups without the need to take reactivity orders into consideration to arrive at (4) with the reported ^{1}H NMR data shown in table 2.1. The benzylidene-acetal may

then be removed via acidic hydrolysis [12] and C-6 may then possibly be selectively protected with a triphenylmethyl ("trityl") group, which has been documented to selectively engage C-6 even when other hydroxyl groups are unprotected [13].

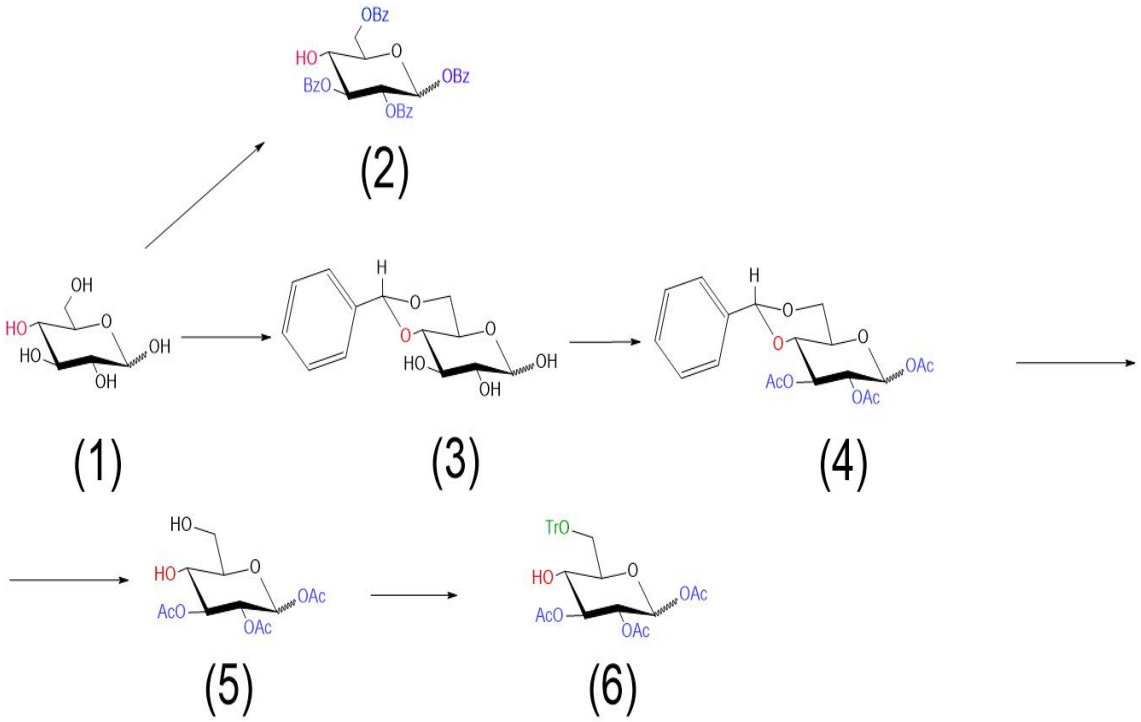

Figure 2.4: Proposed synthetic routes to monomers that may act as glycosyl acceptors in the Koenigs-Knorr reaction.

The monomer acting as a glycosyl donor may be obtained by the route illustrated in figure 2.5 by first protecting all the hydroxyl groups of the D-glucose molecule with acetate esters via reaction with acetic anhydride. It has been reported that several different acetate ester protected glucose species have been chlorinated at the C-1 position by reacting them with 1.5 molar equivalents of titanium tetrachloride for 3-4 hours in refluxing chloroform [14]. Reported yields for this procedure are generally high and it may therefore possibly be used to synthesize the product (8).

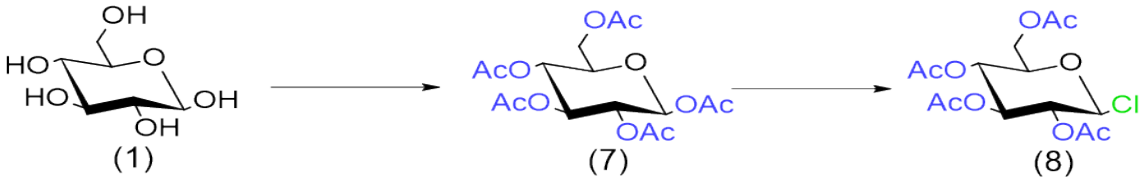

Figure 2.5: Suggested synthetic route to a monomer that may act as a glycosyl donor in the Koenigs-Knorr reaction.

As there exist a scientific interest in forming oligomeric chains of glucose units where the hydroxyl at C-3 has been exhanged for a methyl ether group, a strategy was devised for this as well as illustrated in figure 2.6. The compound 1,2:5,6-di-O-cyclohexylidene-D-glucofuranose (9) has been documented to be attained via

treating D-glucose with cyclohexanone and a catalytic amount of sulfuric acid. The methylated species (10) may then be obtained by treatment of (9) with sodium hydride and iodomethane [15]. The ketals should then be removable with acid hydrolysis leading to (11). It is then speculated that (11) may be protected similarily to (8) or (6) in order to create either glycosyl donors or acceptors that can be linked via the Koenigs-Knorr reaction [15].

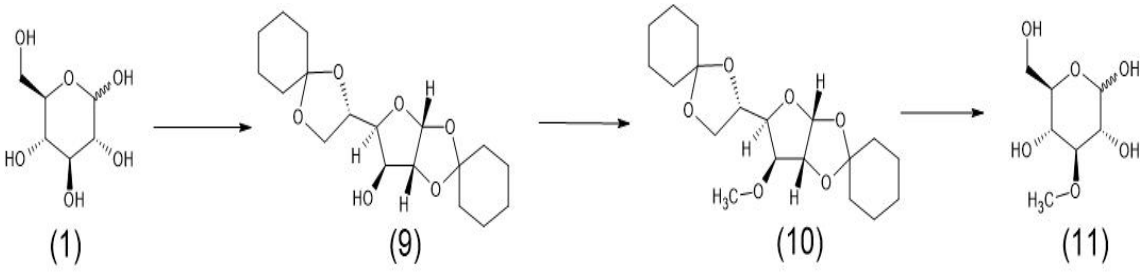

Figure 2.6: Suggested synthetic route to a methylated glucose monomer.

Table 2.1: Previously reported ^{1}H NMR chemical shifts for (4) [16].

^{1}H NMR signals for (4) in $CDCl_3$ (mixture of α- and β-anomers).		
Multiplet	Chemical shift, δ_H (ppm)	Integral
d	6.31	0.5H
d	5.79	0.5H
t	5.60	0.5H
s	5.52	0.5H
s	5.51	0.5H
t	5.37	0.5H
m	5.17-5.10	1.0H
dd	4.39	0.5H
dd	4.32	0.5H
m	4.08-4.00	0.5H
m	3.80-3.63	2.5H

2.2.1 1,2,3,6-tetra-O-benzoyl-D-glucopyranose (2)

According to reports [8] the compound denoted 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose (2) has previously been prepared by dissolving anhydrous D-glucose (1) in pyridine cooled to -30^{o}C before adding 4.2 molar equivalents of benzoyl chloride. The mixture is then slowly brought to room temperature and stirred for 36 hours. The crude product may then be purified through liquid-liquid extractions and recrystallizations from ethanol for a final yield of 50%.

The mechanism for this esterification reaction is a nucleophilic attack from the hydroxyl groups of the D-glucose towards the electrophillic carbonyl of the benzoyl

chloride to form a tetrahedral intermediate. The intermediate then rearranges into an ester while losing one unit of hydrochloric acid. Basic pyridine is used as a solvent in order to neutralize the hydrochloric acid formed and to catalyze the reaction by deprotonating the tetrahedral intermediate as per illustrated in figure 2.7 [4] [17].

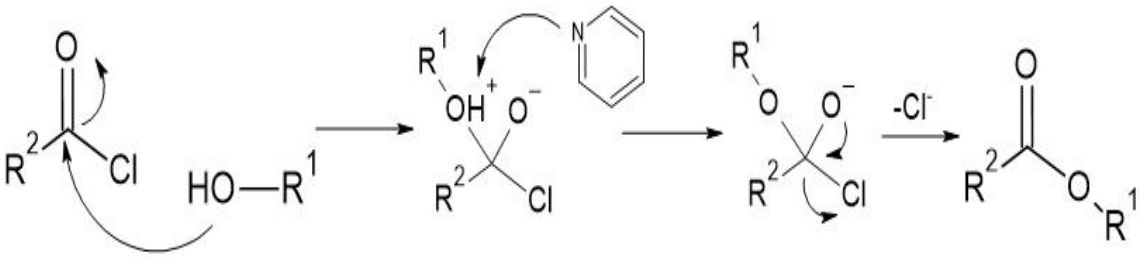

Figure 2.7: Scheme illustrating the reaction steps in base catalyzed nucleophilic substitution on acyl chlorides.

2.2.2 4,6-O-Benzylidene-D-glucopyranose (3)

Several synthetic routes to 4,6-O-Benzylidene-D-glucopyranose have been reported in scientific literature [10] [11]. A commonly used approach is to perform a reaction between equimolar amounts of benzaldehyde dimethyl acetal and D-glucose with DMF as solvent. The reaction mixtures are heated to moderate temperatures, often in the 50-60°C range since DMF degrades into dimethylamine at higher temperatures. In order to shift the equilibrium towards the products an inert gas is often bubbled through the reaction mixture to carry away the formed methanol and moisture. Purification may either be performed via column chromatography or recrystallization from water, ethyl acetate or a mixture of dichloromethane and hexanes. Reported yields are in the range of 50-90% of the target compound and the recorded chemical shifts for ^{1}H and ^{13}C NMR are shown in table 2.2 and 2.3.

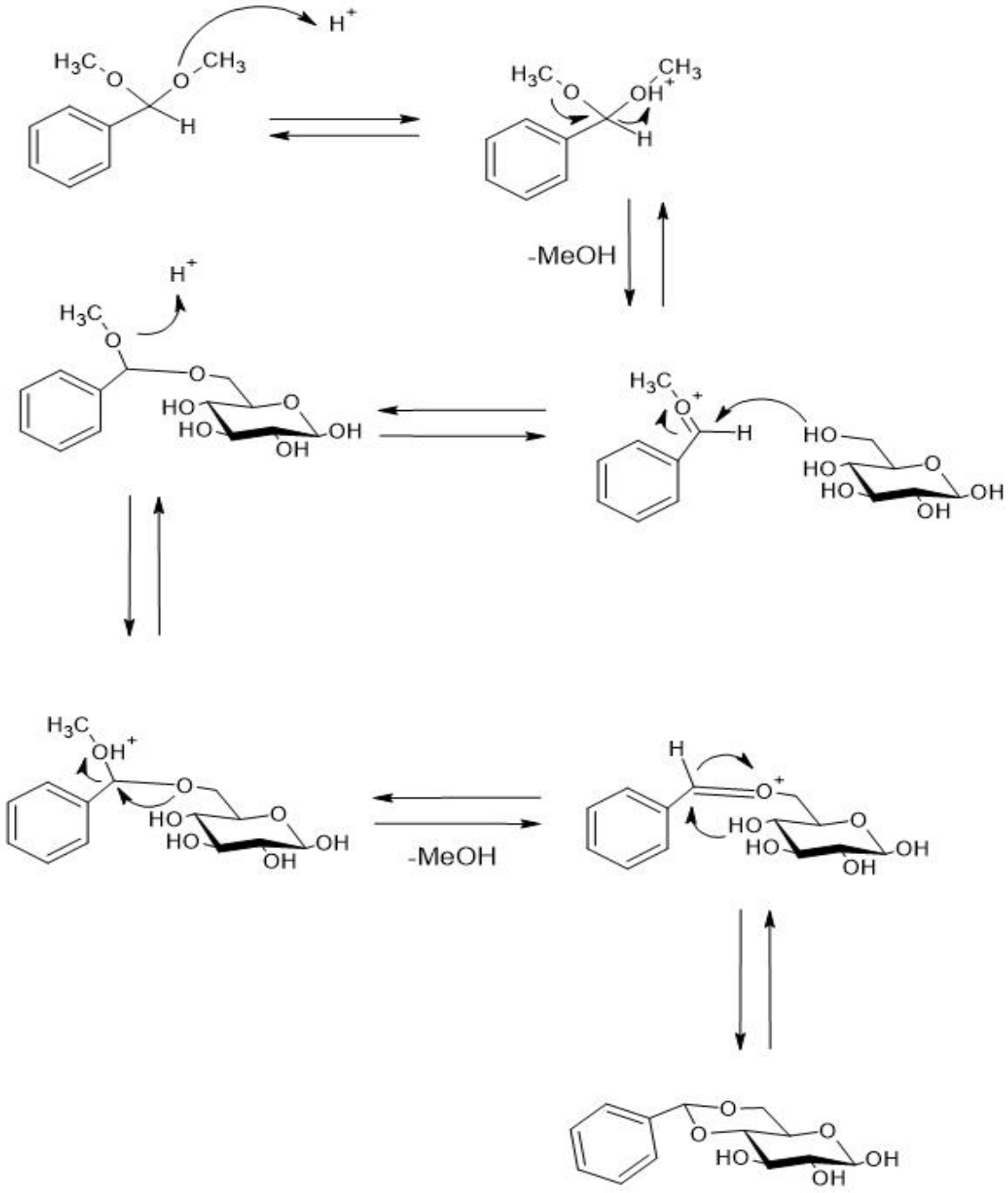

Figure 2.8: Scheme illustrating the equlilibria involved in a transacetalisation reaction.

From a theoretical perspective, the reaction proceeds according to a transacetalisation mechanism wherein the methoxy groups of the acetal are exchanged according to the scheme in figure 2.8. As can be seen, the reaction proceeds through several equilibrium steps in which methanol is produced. To ensure that the equilibirum is shifted towards the desired products, methanol should be removed continuously [4].

Another reported method is based on acetalation reaction of D-glucose with an excess of benzaldehyde in the presence of a $ZnCl_2$ catalyst under room temperature. Reaction times are generally longer between 5-24 hours and reported yields for are

somewhat lower in the range of 30-70% [11]. It can be seen that the formed acetal functionality in (3) constitutes a new six-membered ring, called a 1,3-dioxane, that is fused to the pyranose ring in an all-chair bicyclic system. Such a configuration is energetically favourable and (3) is therefore the thermodynamic product of this reaction [4] [18].

Table 2.2: Previously reported ^{1}H NMR chemical shifts for (3) [16].

^{1}H NMR Chemical shifts for (3) in CD_3OD, δ_H (ppm)	
α-anomer	β-anomer
5.56	5.56
5.13	4.60
4.17	4.26
3.97	3.75
3.87	3.65
3.72	3.48
3.48	3.46
3.43	3.26

Table 2.3: Previously reported ^{13}C NMR chemical shifts for (3) [16].

^{13}C NMR chemical shifts for (3) in CD_3OD, δ_C (ppm).	
α-anomer	β-anomer
94.7	98.9
83.0	82.3
74.4	77.1
71.8	74.6
70.2	69.7
63.5	67.7
103.0	103.0

2.2.3 1,2:5,6-cyclohexylidene-D-glucofuranose (9) and 1,2:5,6-cyclohexylidene-3-O-methyl-D-glucofuranose (10)

Documented preparations of 1,2:5,6-di-O-Cyclohexylidene-D-glucofuranose usually involve reacting anhydrous D-glucose in an excess of cyclohexanone catalyzed by an acid. When ketones are used to protect hydroxyls on glucose, the resulting ketals will have five-membered, 1,3-dioxolane structures which are formed from two adjacent hydroxyls, in cis-configuration, (1,2-cis diols) and one unit of the ketone. For ketones this is a more favourable than the six-membered, 1,3-dioxane structure due to the repulsive interactions between axially oriented substituents involved.

Furthermore, since the glucofuranose isomer presents the possibility of forming two ketals compared to the pyranose ring which has no adjacent 1,2-cis diols, the furanose diketal (9) is favoured on a thermodynamic basis [18] [19] [20].

Table 2.4: Reported ^{1}H NMR signals for (9) in $CDCl_3$ [21]

Chemical shift, δ_H (ppm)	Integral
5.96	1H
4.53	1H
4.34	2H
4.16	1H
4.10	1H
3.97	2H
1.68-1.35	19.8H

Since the suggested reaction leaves the hydroxyl at C-3 unprotected there is an opportunity to install the desired methyl group here. Reported procedures for O-alkylations at the C-3 position are most commonly based on using the analogous 1,2:5,6-di-O-isopropylidene-D-glucofuranose rather than (9) as starting material and are generally focused on installing long alkyl chains rather than methyl groups. Hence, reference data for characterization of the target compound is not available [15].

The documented reaction protocols for such analogous compounds usually involves first dissolving the starting material and an alkyl bromide in dry THF or DMF. NaH is then slowly added in excess to cause a deprotonation of the hydroxyl which may then engage in a nucleophilic S_n2 attack on the alkyl halide to yield the desired product [15]. Once the product has been obtained, the ketal protective groups may be selectively removed via acid hydrolysis to arrive at at 3-O-methyl-D-glucose which can be used as a precursor for new monomers [18].

2.3 Analytical techniques

In this section the theory for nuclear magnetic resonance and secondary ion mass spectrometry will be outlined as these are the main techniques used for characterization in this project.

2.3.1 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy is one of the most common techniques used to investigate the structure of organic molecules. The main components of a spectrometer used in this analysis consists of a magnet, a radiofrequency emitter and a detector. In standard experiments, a sample containing the analyte of interest is placed within the magnetic field of the instrument. For atomic nuclei that possesses a nuclear magnetic moment (for instance ^{1}H and ^{13}C) , this causes distinct energy levels to emerge associated with the alignment of the magnetic moments with or

against the applied external magnetic field [22].

By subsequently exposing the sample to pulses of electromagnetic radiation in the radiofrequency spectrum, excitations and relaxations between the energy states are induced which are recorded by the detector as characteristic signals of the different nuclei [22].

Furthermore, under the influence of the applied magnetic field electrons generate local magnetic fields in the opposite direction. These local fields may partially shield the nuclei from the applied field. Hence, depending on the electronic environment, which results from the chemical structure of a molecule, nuclei at different positions in a molecule will absorb electromagnetic radiation at slightly different frequencies. Such information is given by the chemical shifts in the NMR spectrum, where deshielded nuclei will have signals at high chemical shifts whereas signals of shielded nuclei will appear at low chemical shifts [22].

In ^{1}H NMR, signals generated by protons are also influenced by the presence of neighbouring proton nuclei bonded to adjacent carbons. Due to the different possible alignments of magnetic moments for these neighbouring nuclei, relative to the external field, several close energy levels emerge. Subsequently, in the recorded spectrum the resulting signal for a proton is split into n+1 signals, where n corresponds to the number of neighbouring protons. Therefore, through the analysis of such splitting patterns further information on the chemical structure of a molecule can be revealed. Due to the low presence of the ^{13}C-isotope, the likelyhood that two such nuclei would exist in neighbouring positions is low. Hence, such splitting patterns are not present in detectable amounts in ^{13}C NMR and each chemically different carbon is associated with one singlet signal [22].

By applying more sofisticated patterns of electromagnetic pulses and relaxations, couplings between proton signals at different chemical shifts can revealed via COSY NMR. This type of experiment generates a spectra in the form of a 2D plot where proton signals appear on a diagonal whereas coupled protons, at neighbouring positions in the molecule, appear off the diagonal at horizontal and vertical lines connecting to the diagonal. By employing similar techniques heteronuclear couplings between directly bonded ^{13}C and ^{1}H nuclei can be distinguished in a 2D HSQC spectrum. Herein, signals appearing at the intersection of proton and carbon chemical shifts indicate that the signals are coupled [22].

2.3.2 Secondary ion mass spectrometry (SIMS)

Secondary ion mass spectrometry is technique used to reveal structures of non-volatile organic molecules. The analysis is based on irradiating a solid-phase sample with a beam of accelerated ions (commonly Ar^{+} or Cs^{+}). The collision with the high-energy ions causes sample molecules to eject from the sample holder while simultaneously being ionized and fragmented. The fragments then passes through the mass analyzer where they are separated based on their mass-to-charge ratio (M/Z).

Of particular analytical value for structural characterization is the non-fragmented, molecular ion which is formed during the collision event by loss of a electron from the sample molecule. As desolvation follows desorption, the molecules (M) are usually ionized in a process of protonation to form $(M+H)^+$. Due to the small change in mass associated with the gain of a proton it is therefore possible to gain information of the molecular weight of the target molecule. Fragmentation of molecules usually occur in predictable patterns depending on the chemical structure. Investigating recorded spectra for expected fragments may therefore provide additional proof of a particular structure. Unfortunately, in SIMS it is common that molecules originating from the sample holder or matrix are ionized as well which produces extra signals in the mass spectra and may cause analysis of fragmentation patterns to be difficult [22].

3. Materials and Methods

The following section presents the practical experimental protocols used to synthesize the molecules outlined in the synthetic pathway. Reagents used were purchased from Sigma-Aldrich and Alfa Aesar. All NMR spectroscopy measurements were performed at 600MHz. TLC was carried out on silica plates and visualized with ultraviolet light, iodine vapours or by immersing the plates in a prepared solution of 3g $KMnO_4$, 10g K_2CO_3 and 200ml distilled water.

3.1 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose (2)

A mixture containing 300ml of dry pyridine and 5g (0.03mol) of anhydrous D-glucose was poured into a three-neck flask equipped with two rubber stoppers and an addition funnel that had been sealed off with a calcium chloride plug as illustrated in figure 3.1. The setup was placed in a cooling bath containing acetonitrile and solid carbon dioxide to ensure that the temperature does not exceed -20^{o}C as monitored with an analog mercury thermometer. The atmosphere of the reaction vessel was purged with pressurized nitrogen gas for 10 minutes while applying agitation via a magnetic stir bar. Once the preparatory measures were completed 13.90ml (16.80g, 0.12mol) of benzoyl chloride was added drop-wise via the addition funnel during 30 minutes. After the addition, the mixture was stirred for 2 hours with the temperature maintained at -20^{o}C by sporadically adding more solid carbon dioxide. After 2 hours no more carbon dioxide was added and the mixture was allowed to slowly reach room temperature. The mixture was kept agitated for a further 42 hours.

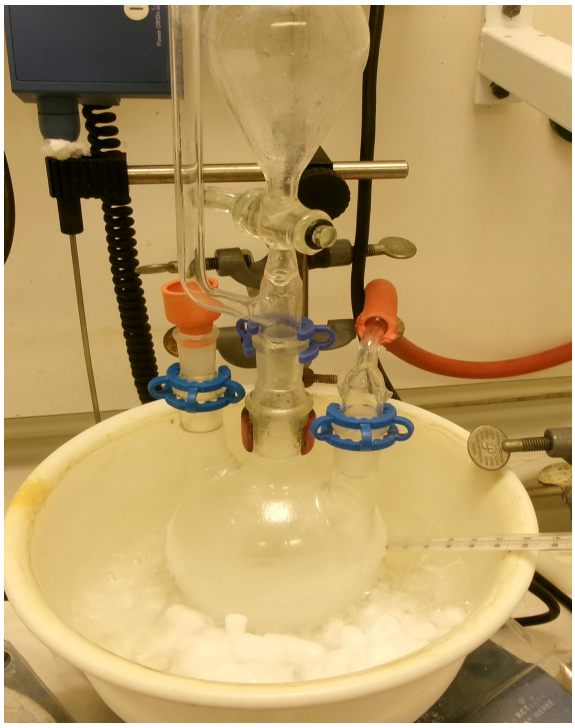

Figure 3.1: Setup for the reaction vessel used in the synthesis of 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose. Three-neck flask placed in cooling bath and equipped with an addition funnel, a nitrogen source and a rubber stopper.

The mixture was then poured into a round-bottom flask and most pyridine was evaporated with reduced pressure at 40°C on rotary evaporator. The small amount of remaining liquid was then diluted with 40ml dichloromethane and poured into a separatory funnel. The organic phase was washed three times with 30ml 1M hydrochloric acid to remove residual pyridine and unreacted glucose, twice with 30ml 10wt% Na_2CO_3 to neutralize remaining acids and finally two times with 30ml distilled water. The organic phase was then evaporated on a rotary evaporator at 40°C which yielded a yellow to orange viscous liquid. Dissolving this crude reaction product in dichloromethane and peforming TLC with 5 volume-% methanol in chloroform as eluent liquid revealed the presence of several UV-active spots (see figure 3.2). Flash chromatography was used in an attempt to purify the product, however only the spot with the highest retention factor (Rf=0.8) was successfully separated.

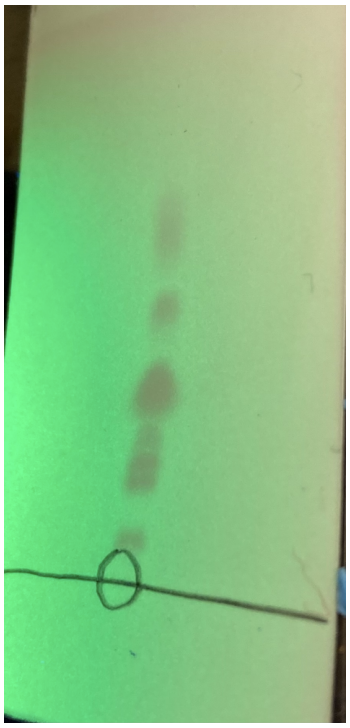

Figure 3.2: TLC plate for crude reaction product of 1,2,3,6-Tetra-O-benzoyl-D-glucopyranose visualized with ultraviolet light. Multiple components are visible.

3.2 4,6-O-Benzylidene-D-glucopyranose (3) (Procedure A)

9g (0.05mol) of anhydrous D-glucose and 7g (0.05mol) of $ZnCl_2$ were weighed out and dried for one hour at 50^oC before starting the reaction. 45ml (46.80g, 0.44mol) benzaldehyde was added to a 100ml round-bottom flask which was then connected to a rotary evaporator to remove moisture. This flask was briefly disconnected at regular intervals in order to add the $ZnCl_2$ in small portions to ensure total dissolution of the catalyst. Once the $ZnCl_2$ had dissolved, the D-glucose was added in a similar manner over the course of one hour. The reaction was then agitated for another three hours on the rotary evaporator while maintaining the negative pressure.

Once a total of 4 hours had passed the flask was disconnected and 50ml of petroleum ether was added in order to remove benzaldehyde. An orange oil precipitated and the flask was allowed to rest for half an hour in a freezer (-20^OC). The petroleum ether was then decanted off and 50ml of ice-cold water (0^OC) was subsequently added. The mixture was shaken vigourously causing white solid to form immediately. The mixture was then put into the freezer for another half an hour. Solids were filtered off in a membrane filter of the type illustrated in figure 3.4. Solids were then dispersed with 50ml petroleum ether in an erlenmeyer flask and agitated with a magnetic stir bar for half an hour. The liquid was discarded and the procedure was repeated with 50ml ice-cold (0^OC) water. These measures are taken to remove benzaldehyde and glucose. A resulting white solid of 3.8g was then obtained. This

crude mixture was leached with 300ml ethyl acetate which was subsequently washed twice with 60ml distilled water in a separatory funnel to further remove dispersed glucose. For each of the washes, the aqueous and organic layers were allowed to settle for 15 minutes to ensure that equilibrium was reached. The ethyl acetate was then evaporated under reduced pressure forming a solid of 2g (estimated 15% yield).

A retention factor of 0.3 on TLC plates was observed for the product, corresponding to the second spot from the bottom in figure 3.3 where pure ethyl acetate was used as eluent. ^{1}H NMR (CD_3OD): δ_H 7.6-7.3 (m, 7.4H) , 5.57 (d, 0.98H), 5.14 (d, 0.58H), 4.60 (d, 0.47H), 4.26 (dd, 0.61H), 4.18 (dd, 0.74H), 3.97 (td, 0.75H), 3.87 (t, 0.71H), 3.74 (dt, 1.15H), 3.63 (t, 0.53H), 3.49-3.41 (m, 2.0H), 3.25 (dd, 0.43H). ^{13}C NMR: δ_C 139.2, 139.1, 134.0, 130.7, 129.9, 129.9, 129.5, 129.0, 127.5, 126.1, 103.0, 102.9, 98.9, 94.7, 83.1, 82.4, 77.2, 74.7, 74.4, 71.8, 70.3, 69.8, 67.7, 63.5, 30.9; SIMS ($[M+H]^+$): 269.13.

Figure 3.3: TLC plate for crude reaction product of 4,6-O-Benzylidene-D-glucopyranose after a reaction time of 3 hours.

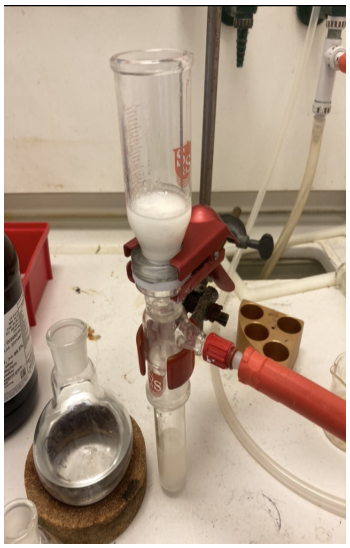

Figure 3.4: Photograph of the membrane filter used to recover the solid crude product.

3.3 4,6-O-Benzylidene-D-glucopyranose (3) (Procedure B)

2.7g (0.015mol) of anhydrous D-glucose was dried in an oven at 50^{o}C for 1 hour before the procedure started. The reagent was then added in small portions to 20ml anhydrous dimethyl formamide in a 100ml round bottom-flask heated to 60^{o}C while stirring vigorously to ensure that all D-glucose goes into solution. Once all glucose had dissolved 2.4ml (2.4g, 0.016mol) of benzaldehyde dimethyl acetal was added along with 0.024g (0.14mmol) of p-toluenesulfonic acid. The flask was then quickly attached to a rotary-evaporator with the water bath heated to 60^{o}C. The reaction was allowed to proceed for 1 hour before deactivating the reaction with 0.03ml (0.02g, 0.2mmol) triethylamine. The solvent was then evaporated by elevating the temperature of the water bath to 70-80^{o}C. This resulted in a crude product in the form of a viscous oil. 100ml of boiling ethyl acetate was poured onto the product with vigorous stirring for half an hour. The liquid was then decanted into a separatory funnel and washed once with 20ml deionized water. The ethyl acetate was then evaporated under reduced pressure affording ca. 100mg of a white solid. The compound may also be recovered by dissolving around 100mg of the oil in pyridine and loading on a silica gel column (column diameter 3.5cm, packing height 10 cm) using 10volume-% acetonitrile in ethyl acetate as eluent. After a dead volume of roughly 150ml the product may be collected. At around 180ml unreacted glucose starts eluting. Analysis via NMR gave the same results as for procedure A.

3.4 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose (4)

Into a 100ml round-bottom flask 1g (3.7mmol) of 4,6-O-Benzylidene-D-glucopyranose (obtained through procedure A) was dissolved in 45ml pyridine. To this mixture 25ml (25g, 0.25mol) of acetic anhydride was added slowly over the course of 30 minutes. The reaction vessel was then sealed with a rubber stopper and purged with pressurized nitrogen gas for 10 minutes. The reaction was then allowed to proceed at room temperature for 48 hours with agitation provided by a magnetic stir bar. After 48 hours had passed the reaction mixture was usually observed to assume a yellow to orange colour. 50ml chloroform was poured into the mixture and the liquid was decanted into a separatory funnel where it was washed two times with 50ml deionized water to remove residual acetic anhydride and the acetic acid byproduct. Furthermore it was washed twice with 50ml 1M HCl to partially remove pyridine and finally two times with 50ml 10wt% Na_2CO_3 to neutralize residual acids. The organic phase was then poured into a round bottom flask and evaporated. The remaining pyridine was co-evaporated with toluene yielding 1g solid (estimated yield 68%). ^{1}H NMR ($CDCl_3$): δ_H 6.30 (d, 1.04H), 5.78 (d, 0.86H), 5.58 (t, 1.16H), 5.49 (d, 1.97H), 5.36 (t, 0.95H), 5.11 (m, 2.04H), 4.36 (dd, 1.02H), 4.29 (dd, 1.38H), 4.09 (q, 3.55H), 4.02 (m, 1.85H), 3.73 (m, 6.85H). ^{13}C NMR: δ_C 101.6, 92.2, 89.6, 79.1, 78.6, 78.0, 71.7, 71.2, 69.9, 68.8, 68.5, 68.3, 67.0, 64.9, 60.4; ($[M+H]^+$): 395.11.

3.5 1,2,3-Tri-O-Acetyl-D-glucopyranose (5)

1g (2.5mmol) of solid 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose (obtained through the previously outlined procedure) was transferred to a 50ml round-bottom flask and 30ml of 20% aqueous acetic acid was added. The reaction vessel was placed in a water bath maintained at 80^{o}C. The reaction was allowed to proceed for 2 hours with agitation provided by a magnetic stir bar. The solution was then evaporated at a rotary evaporator set to 80^{o}C which yielded a crude product in the form of a highly viscous oil. Performing TLC by dissolving a small portion of the oil in DCM and using 10 volume-% methanol in chloroform as the elution liquid revealed the crude product to be a complex mixture of at least four components (see figure 3.5). The oil was dissolved in a small amount of DCM and loaded onto a silica column (2.5cm column diameter packed with 10cm silica) and chromatographed using the same elution liquid as for the TLC. The product starts eluting from the column after around 40ml liquid has passed through the column (Rf=0.6). The final solid weighs approximately 200mg (estimated yield 26%). ^{1}H NMR ($CDCl_3$): δ_H 6.26 (d, 1H), 5.69 (d, 0.56H), 5.32 (m, 1H), 5.10 (td, 0.6H), 5.01 (m, 0.6H), 4.97 (m, 0.9H), 4,09 (qd, 2.4H), 3.89 (dd, 0.8H), 3.84-3.74 (m, 5.4H), 3.55 (m, 1.1H). ^{13}C NMR: δ_C 92.0, 89.5, 76.6, 75.6, 74.1, 72.6, 70.6, 69.5, 68.64, 68.56, 61.6, 61.4, 60.6.

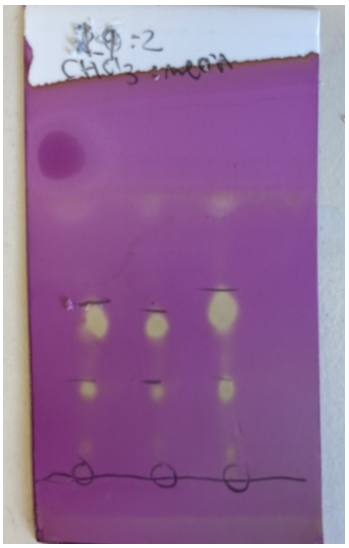

Figure 3.5: Photograph of the TLC plate for the crude product containing 1,2,3-tri-O-acetyl-D-glucopyranose. Elution liquid is 10 volume-% methanol in chloroform.

3.6 6-O-Trityl-1,2,3-Tri-O-Acetyl-D-glucopyranose (6)

Approximately 100mg (0.3mmol) 1,2,3-tri-O-acetyl-D-glucopyranose (5) obtained through previous procedures was mixed with 5ml anhydrous pyridine in a 10ml round bottom flask. 80mg (0.3mmol) trityl chloride was subsequently added and the flask was sealed and purged with nitrogen for 5 minutes. The mixture was agitated with a magnetic stir bar for 12 hours and then diluted with 10ml dichloromethane and poured into a separatory funnel. The organic phase was washed three times with 10ml 1M hydrochloric acid to remove pyridine, then with 10ml 10wt% Na2Co3 to neutralize residual acids and finally once with 10ml distillied water. The dichloromethane was removed on a rotary evaporator with reduced pressure and heating. 20ml toluene was added to co-evaporate remaining pyridine. A sample for NMR analysis was prepared directly from the resulting crude reaction mixture.

3.7 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (7)

A mixture of 1g (0.07mol) $ZnCl_2$ and 25ml (25g, 0.25mol) of acetic acid anhydride was poured into a 50ml round bottom flask which was submerged in a boiling water bath. With vigorous stirring facilitated by a magnetic stir bar 5g (0.03mol) of anhydrous D-glucose was added very slowly during half an hour due to the vigorous nature of the reaction. Once all glucose had been added the reaction was allowed to proceed for one hour. Then 100ml of ice-cold (0^{o}C) distilled water was added which caused a precipitate to form. The mixture was left for half an hour in a freezer (-20^{o}C). The solids where then filtered of with a Büchner funnel using paper filters. Re-crystallization of the solids was performed without problems by dissolving them in boiling 95% ethanol and then allowing the solution to reach room temperature. When the solids had formed the liquid was decanted and the crystals where washed

with water. The result was 8g of solid white material (estimated yield 34%). ^{1}H NMR ($CDCl_3$): 6.33 (d, 1H), 5.71 (d, 0.4H), 5.46 (t, 1H), 5.25 (t,0.4H), 5.15-5.08 (m, 3H), 4.30-4.24 (m, 1.5H), 4.12-4.08 (m, 2.7H), 3.85 (m, 0.4H), 3.73 (q, 1.3H)

Figure 3.6: Photograph showing the setup for the reaction vessel used in the synthesis of 1,2,3,4,5-penta-O-acetyl-D-glucopyranose.

3.8 2,3,4,5-Tetra-O-acetyl-D-glucopyranosyl chloride (8)

A mixture of 0.5g (1.3mmol) of 1,2,3,4,6-Penta-O-Acetyl-D-glucopyranose (obtained according to the previously outlined procedure) and 10ml chloroform was prepared in a 25ml round bottom flask. Using a syringe 2ml of 1M $TiCl_4$ in dichloromethane was carefully and slowly added to the solution. The round bottom flask was then submerged in a water bath maintained at 70^{o}C and connected to a reflux adapter. The solution was refluxed for 3 hours and then poured into a separation funnel and diluted with 50ml of dichloromethane. The solution was washed twice with 50ml distilled water and twice with 50ml 10wt% Na_2CO_3. The organic phase was evaporated on a rotary evporator under reduced pressure without heating. The obtained crude product was a very viscous oil.

3.9 1,2;5,6-di-O-cyclohexylidene-D-glucofuranose

A solution of 10g (0.06mol) anhydrous D-glucose was and 20ml cyclohexanone was prepared in a 50ml round-bottom flask and placed in a cooling bath with ice. Stirring was performed with a magnetic stir bar and 1.4ml of concentrated sulfuric acid was slowly added drop wise. Once the mixture had been prepared it was initially observed to slowly transition from yellow to orange in colour. The mixture was left

stirred over night. During the course of the reaction the mixture gradually became more viscous and eventually solidified into a brown mass. 50ml of diethyl ether was added and the brown solid was broken up with vigorous stirring and filtered through a Büchner funnel. The filtrate was washed twice with 50ml water and twice with 50ml 10wt% Na_2CO_3 causing the organic phase to become yellow.

The organic phase was then evaporated on a rotary evaporator into a viscous oil. Upon leaving the crude product in the freezer (-20^{o}C) for 24 hours the oil solidified. The target product may then be purified either by column chromatography (3.5cm column diameter, 10cm of packed silica) using a gradient of 20 volume-% diethyl ether in n-pentane and gradually increasing it towards 40 volume-% . After roughly 170ml two spots elute and the target elutes after roughly 300ml. The target may also be purified by heating the crude solid in petroleum benzin or n-heptane to 80^{o}C until the liquid turns transparent. The liquid is then decanted into a new vial and allowed to cool to room temperature which causes the solid to re-crystallize. Once enough solid has formed the liquid is decanted and the solid is washed with petroleum ether. The procedure may be repeated until TLC shows the pure product. A white solid of around 8g (estimated yield 39%) should be obtained. The product corresponds to the lowest spot on the TLC plate (Rf=0-0.3). ^{1}H NMR ($CDCl_3$): δ_H 5.95 (d, 1H), 4.52 (d, 1H), 4.33 (m, 2H), 4.15 (dd, 1H), 4.05 (dd, 1H), 3.96 (dd, 1H), 1.7-1.3 (m, 22.6H). ^{13}C NMR ($CDCl_3$): δ_C 112.5, 110.3, 104.9, 84.6, 81.2, 75.5, 73.3, 67.4, 36.5, 36.4, 35.7, 34.6, 25.1, 24.9, 24.0, 23.9, 23.8, 23.6.

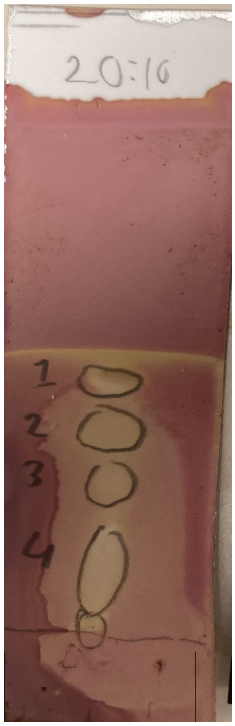

Figure 3.7: Photograph of the TLC plate for the crude product of 1,2:5,6-di-O-cyclohexylidene-D-glucofuranose. Elution liquid is 33 volume-% diethyl ether in n-pentane.

3.10 1,2;5,6-di-O-Cyclohexylidene-3-O-methyl-D-glucofuranose

0.25g (0.7mmol) of 1,2;5,6-di-cyclohexylidene-D-glucofuranose (as obtained by chromatography according to section 3.0.1.8) was dissolved in 10ml anhydrous tetrahydrofuran and poured into a 25ml round bottom flask. The flask is placed in an ice-bath and 0.125ml (0.284g, 2mmol) of methyl iodide is added. To the mixture 0.12g (5mmol) of sodium hydride is added slowly. The reaction vessel is then sealed with a rubber stopper and purged with nitrogen for 10 minutes. The vessel is left with stirring with a magnetic stir bar over night. The mixture is then diluted with 20ml ethyl acetate and washed with 20 ml distilled water. The organic phase is then evaporated yielding around 0.1g of crude product. Performing TLC with 20 volume-% diethyl ether in n-pentane and visualizing with iodine vapours, reveals two spots which may easily be separated via flash chromatography (2.5cm column diameter, 10cm packed silica) using the same eluent liquid as for the TLC. The spot closest to the solvent front (Rf=0.9) corresponds to the product. ^{1}H NMR ($CDCl_3$): δ_H 5.77 (d, 1H), 4.46 (d, 1H), 4.19 (m, 1H), 3.99 (m, 2H), 3.88 (dd, 1H), 3.7 (d, 1H), 3.38 (s,3H), 1.7-1.3 (m, 23.4H). ^{13}C NMR ($CDCl_3$): δ_C 112.3, 109.5, 104.8, 83.9, 81.7, 81.2, 77.4, 72.0, 67.0, 58.3, 36.5, 36.4, 35.7, 34.8, 25.2, 24.9, 24.1, 23.9, 23.8, 23.6.

Figure 3.8: Photograph of the TLC plate for the crude product of 1,2:5,6-di-O-Cyclohexylidene-3-O-Methyl-D-glucofuranose. Elution liquid is 20 volume-% diethyl ether in n-pentane.

4. Results and discussion

4.1 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose (2)

The reaction aiming to produce 1,2,3,6-Tetra-O-Benzoyl-D-glucopyranose did not yield results consistent with literature despite repeated attempts. TLC revealed that multiple different UV-active compounds had formed (see figure 3.2) in a complex mixture. This mixture could not be recrystallized from ethanol, but instead precipitated as an oil presumably due to the significant amount of impurities. Through flash chromatography it was possible to isolate the compounds corresponding to the spot on the TLC plate with the highest retention factor (Rf=0.8) while the rest co-eluted. However, even the isolated fraction generated very complicated ^{1}H NMR spectra with substantial overlap of signals (see figure A.1) which indicates that a mix of molecules is still obtained.

Although detailed analysis of the acquired spectrum is difficult, it may be noted that signals are present in the regions 6.0-5.0ppm and 8.0-7.5ppm which are characteristic for protons attached to glucose molecules and aromatic compounds respectively. Therefore, it may be speculated that the produced mixture consists of glucose molecules that have been partially protected with benzoate esters but possesses slightly different substitution patterns. However, since further purification via chromatography was unsuccessful this reaction pathway was deemed unsuitable and ultimately abandoned.

4.2 4,6-O-Benzylidene-D-glucopyranose (3)

Compound (3) was reliably produced through procedure A. The existence of the target molecule was verified by comparing chemical shifts for the spectra obtained through ^{1}H and ^{13}C NMR (see figure A.2 and A.3) with available reference data (see table 2.2 and 2.3). This comparison indicates that the measured chemical shifts had the expected values of the target molecule and the that compound is obtained as a mixture of α- and β-anomers. Mass spectrometry via SIMS was performed to further confirm that the target molecule had been produced. The measurement revealed a distinct fragment with M/Z=269.13 (see figure A.6) which likely corresponds to the molecular ion of 4,6-O-Benzylidene-D-glucopyranose which has a molecular weight of 268g/mol.

Assignment of proton signals through HSQC and COSY was carried out as well (see figure A.4 and A.5). From the COSY spectra it can be seen that the doublet at 4.60 ppm, likely corresponding to H-1 for one of the anomers, couples to a triplet at 3.25ppm which couples to another triplet at 3.63 corresponding to H-2 and H-3 respectively. Further couplings indicate that H-4 is part of a complex multiplet at 3.49-3.41 ppm which contains multiple different proton signals and therefore obscures any couplings between H-4 and H-5.

The only other doublet in the spectrum resides at 5.14 ppm and likely corresponds to H-1 of the other anomer. This signal couples directly to the complex multiplet at 3.49-31 ppm in the COSY spectrum making any connections between H-2 and H-3 difficult to spot. Hence, H-5 of this anomer is assumed to correspond to the signal found at 3.97 ppm due to having approximately the expected splitting pattern for this position. The expected splitting pattern is a doublet of doublet of doublet pattern and it is found in the form of an apparent triplet of doublets. The signal is assigned to this particular anomer due to similarity of integrals. The assumed H-5-signal couples further to one doublet of doublets at 4.18 ppm, a multiplet at 3.74 ppm and the multiplet at 3.49-3.41 ppm. According to HSQC (see figureA.5) the former two signals couple to the same carbon (70.3 ppm) and should therefore correspond to the two H-6 protons whereas H-4 exists in the multiplet at 3.49-3.41 ppm. The multiplet at 3.74 contains one more proton signal which couples to a carbon signal at 69.8ppm which is also associated with the doublet of doublet at 4.26ppm. Hence, these should correspond to the H-6 protons of the other anomer. In COSY both of these H-6 signals couple to H-5 which exists in the complex multiplet at 3.49-3.41 ppm as well. At this point only the H-3 of the second anomer remains unlocated which should then correspond to the only remaining signal at 3.87ppm.

Although it has been demonstrated that the target molecule was successfully produced through procedure A, the yields remain poor. Assuming the obtained product is pure (see figure A.2), the yield may be estimated to 15% based on the weight of the solid. Most likely some loss of product occurs during the purification steps. During the filtration step, for instance, it was visibly observed that a portion of solid material penetrated the filter and remained dispersed in the liquid phase. A potential solution to this problem would be to incorporate a centrifugation step to collect particles that are too small to be filtered off. Furthermore, it is possible that product is lost to the aqueous layer when the ethyl acetate phase is washed with water. Potentially, this washing step could be avoided if leaching is performed via soxhlet extraction. This technique is very slow and may therefore limit the amount of glucose dispersed in the organic phase. Hence, washing may not be necessary for glucose removal.

Procedure B, on the other hand, consistently failed to produce enough product to continue to the next step in the synthesis plan. Several experiments were conducted with adjusted reaction parameters in an attempt to resolve the issue. For instance a ten-fold increase in catalyst amount was tried, p-toluenesulphonic acid was ex-

changed for camphoric acid and the reaction time changed from one hour to four hours respecetively. However, each of these experiments failed to produce substantial amounts of product. Although most literature suggest an equimolar amount of D-glucose and benzaldehyde dimethyl acetal should be used in the reaction, it may be worthwile to attempt using an excess of the benzaldehyde derivative given that procedure A required a vast excess of benzaldehyde to yield sizeable results.

4.3 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose (4)

Existance of the target molecule was verified by comparing the measured ^{1}H and ^{13}C NMR spectra (see figure A.7 and A.8) with reference data (see table 2.1) which demonstrated a good agreement. Further confirmation that the target had been produced was obtained by performing mass spectrometry on the obtained solid. In the spectrum (see figure A.9) a fragment with M/Z=395.11 was identified which likely corresponds to the molecular ion of the desired molecule (molecular weight: 394g/mol).

Proton signals were assigned with HSQC and COSY. As seen from ^{13}C NMR 14 carbon signal occur in the "glucose region" of the spectrum indicating that an anomeric mixture has been produced. In the COSY spectrum the doublet at 5.78 ppm, diagnostic of the H-1 position couples to a multiplet at 5.11 ppm which couples further to a triplet at at 5.36 ppm. The next coupling leads to a complex multiplet at 3.73 ppm which makes further connections complicated to deduce. Hence, the positions of H-1, H-2, H-3 and H-4 have been located.

For the other anomer, H-1 should be the diagnostic doublet at 6.30 ppm. This signal couples to a multiplet at 5.11 ppm which couples to a triplet at 5.58 ppm. The next coupling goes to the complex multiplet at 3.73 ppm. Hereby, H-1, H-2, H-3 and H-4 have been identified. Since the outgoing couplings from the complex multiplet at 3.73 ppm are hard to assign due to overlap, H-5 of this anomer is assigned to the signal at 4.09 ppm since its splitting pattern could be the doublet of doublet of doublet expected from the H-5 position but in the form of an apparent triplet of doublets. This signal couples back to H-4 at 3.73 ppm and further to a doublet of doublets at 4.29 ppm which would correspond to one of the H-6 protons. In HSQC the H-6 proton shares a carbon with a signal within the multiplet at 3.73 indicating that the other H-6 proton is exists in this region.

1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose was reliably produced several times from the given procedure and yields where estimated to 68% by using the weight of the solid product when NMR spectra indicated that no contaminants were present. Hence, the yields are sufficient to proceed to the next step in the synthesis pathway. However, the success of this reaction is very dependent on the purity of the starting materials. If glucose has not been adequately removed in the preceding step, the result of this reaction will be a complex mixture of partially pro-

tected molecules which are difficult to separate. If the procedure for (3) has been carried out according to instructions in this report, however, the material should be sufficiently pure and no separation other than liquid-liquid-extractions is necessary.

4.4 1,2,3-Tri-O-Acetyl-D-glucopyranose (5)

The presence of 1,2,3-Tri-O-Acetyl-D-glucopyranose was verified by comparing the measured ^{1}H NMR (see figure A.10) to the literature data shown in table A.1. An overall good agreement for splitting patterns and chemical shifts can be observed for the α-anomer. However, since consistent reference data for the β-anomer is unavailable it could not be verified in this manner.

From the recorded ^{13}C NMR spectra it can be observed that the diagnostic signals for the benzylidene acetal that usually occurs above 100 ppm (see for instance figure A.3 and A.8 where they are located at 103 ppm and 101.6 ppm respectively) are absent. Likewise, the benzylidene acetal should be associated with a diagnostic singlet signal in the 7-4ppm region that does not couple to any neighbouring protons in the COSY spectrum (see for instance the signal at 5.57ppm in figure A.2 and 5.50ppm in figure A.7). However signals with this characteristic are missing. Hence, there are strong indications that the benzylidene acetal has been removed from the molecule.

Assignment of protons was performed with HSQC, COSY and HMBC. The doublet at 6.26 ppm, associated with H-1 with one of the anomers, couples to a doublet of doublets at 4.97 ppm which couples further to a multiplet at 5.32 ppm which then couples to a complex multiplet at 3.84-3.74 ppm. Hence, H-1, H-2, H-3 and H-4 have been located for one anomer. H-1 for the other anomer likely corresponds to the doublet at 5.69 ppm and couples to a triplet at 5.01 ppm which couples to a triplet at 5.10 ppm. The triplet at 5.10 ppm then couples the complex multiplet at 3.84-3.74 ppm. H-1, H-2, H-3 and H-4 of the other anomer has thus been assigned.

The multiplet at 3.84-3.74 ppm have no outgoing couplings except for those to H-3 of both anomers. This indicates that the multiplet contains complex overlap of H-4, H-5 and the two H-6-protons from both anomers (7 different protons). This is consistent with the observation that this region is associated with six different carbons in HSQC.

In HMBC it is observed that all of the proton signals in the region 6.30-4.90 ppm display long range couplings to carbons at approximately 170 ppm indicating that acetate esters are present at C-1, C-2, and C-3. Giving further evidence that the target molecule has been synthesized successfully.

Although there are multiple indicators that the target molecule has been produced, the estimated yield is low. As can be seen in figure 3.5, TLC indicates that byproducts were formed during reaction which partially explains why yields are low. The byproducts, although not characterized, may possibly form due to partial hydrol-

sysis of the acetate esters. Hence, it may be speculated that lower concetration of acid, reduced the reaction times or lower the temperatures may yields better results in this step.

4.5 6-O-Trityl-1,2,3-Tri-O-Acetyl-D-glucopyranose (5)

The obtained product was analyzed with ^{1}H NMR (see figure A.15). However, the reaction was ran with very small amounts of starting material since losses had occurred earlier in the synthetic pathway partly due to non-optimal yields and partly because some material had been sacrificed in each step to carry out characterizations. Therefore not enough product was acquired from the reaction to perform adequate separations and the NMR sample had to be prepared directly from the crude product which contained significant impurities. Hence, it is difficult to compare the results to reference data since the large amounts of impurities cause large overlaps in the NMR spectra. Therefore it is difficult to declare if the the target compound has been successfully generated and results of the experiment are inconclusive. In order to resolve these issues the reaction should be carried out again at a larger scale so that adequate separation and a full characterization can be performed, preferably by incorporating mass spectrometry and 2D NMR techniques.

4.6 1,2,3,4,6-Penta-O-Acetyl-D-glucopyranose (7)

The product obtained was analyzed with ^{1}H NMR (see figure A.16) and compared to the reference data in table A.3. These datasets were in good agreement indicating that compound (7) had been produced. The yields were low but sufficient to proceed to the next step in the synthesis plan. Loss of product was observed during both the filtration and the recrystallization. To improve yields paper filters should be exchanged for a membrane filter or sintered glass filters. The mother liquor from the recrystallization contained a significant amount of contaminated product which could potentially be further purified.

4.7 2,3,4,6-Tetra-O-Acetyl-D-glucopyranosyl chloride (8)

Conversion of compound (7) to (8) yielded inconclusive results. According to the recorded ^{1}H NMR spectrum most of crude product consists of unreacted starting material (see figure A.17) which causes a problem with overlapping signals and makes the spectrum difficult to accurately interpret. A few signals, which are consistent with literature (see table A.4), are observable which indicates that the target may be present. However, these signals are of very low intensity relative to signals corresponding to the starting material. A more complete characterization is thus necessary in order to confidently conclude if the target product is present or not.

Alternative chlorination agents such as $SOCl_2$ and $SnCl_2$ should also be investigated in order to obtain higher yields.

4.8 1,2;5,6-di-O-Cyclohexylidene-D-glucofuranose (9)

The purified product was analyzed primarily with ^{1}H, ^{13}C but a COSY NMR spectrum was collected as well. The 1D NMR spectra (see figures A.18 and A.19) were compared to available reference data (see table 2.4) and demonstrated a good agreement in terms of splitting patterns and chemical shifts. This gives a clear indication that the synthesis has succeeded. The obtained yields where estimated to 39% with excellent purity which is sufficient to proceed to the following reaction steps without difficulty.

4.9 1,2;5,6-di-O-Cyclohexylidene-3-O-Methyl-D-glucofuranose (10)

The purified product was analyzed with ^{1}H, ^{13}C, COSY, HSQC and HMBC NMR since reference data is unavailable for this molecule.

By comparing the recorded ^{1}H NMR spectra (see figure A.21) with the spectra for the starting material (see figure A.18) a close resemblance can be observed indicating a similarity in structure. However, for compound (10) a distinct signal at 3.38 ppm can be observed with an integral corresponding to 3H which is indicative of the protons on the added methyl group. Furthermore, this signal does not display any couplings in the COSY spectra (see figure A.23) indicating that it has no immediately neighbouring protons, which is to be expected. On the other hand HMBC reveals that a coupling exists between the proton signal and a carbon within the glucofuranose structure (see figure A.25) which gives further confirmation that the methyl group has attached to the hydroxyl at C-3.

5. Conclusions

Synthetic pathways to glycosyl donors and acceptors were developed based on theoretical knowledge from literature. When the pathway towards the glycosyl acceptor was attempted in practice most reactions where successful in generating target compounds as per verified by NMR and mass spectrometry. The yields were generally low and therefore future efforts should be dedicated to tuning the reaction parameters in order to make the pathway more convenient. It was speculated that compound (3) may be obtained in larger quantities if soxhlet extraction and centrifugations are applied. The hydrolysis reaction aiming to produce compound (5) may also benefit from shorter reaction times, lower temperatures and a lower concentration of acid in order to produce less byproducts. The final tritylation step in this pathway yielded inconclusive results due to incomplete characterization and the reaction should therefore be repeated with sufficient starting material for such measurements. An alternative reagent to trityl chloride that may be suitable to try is t-butyldiphenylslilyl chloride. This agent should offer similar selectivity as the trityl for the unprotected primary hydroxyl and should therefore be further investigated.

The pathway towards the glycosyl donor generated the intended product in the first step whereas the second step gave inconclusive results. Some indicators in ^{1}H NMR hint that the target may be present but overlapping signals from residual starting material make this conclusion difficult to verify. A more complete characterization should be applied to better evaluate the results and efforts should focus on increasing conversion of starting material. It was suggested that changing the chlorination agent to either $SnCl_2$ or $SOCl_2$ may produce better results. Furthermore, using a larger excess of $TiCl_4$ and longer reaction times should also be investigated as methods to improve conversion.

A synthetic pathway to 3-O-methyl-D-glucopyranose was also developed due to particular interest. The first and second step proceeded with sufficient yields and good purities. However, the last step aiming to convert (10) to (11) could not be realized due to time constraints. This step should be trivial, however, and could most likely be achieved with acidic hydrolysis to remove the cyclohexylidene ketals.

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A. Appendix 1

In this appendix some of reference data that was used in the analysis of measured NMR spectra is presented in tabular form. Furthermore, all of the measured NMR spectra for the synthesized compounds are presented in graphical form. For 1D spectra the chemical shifts and integrals have been highlighted. For certain compounds mass spectra are presented as well, where the fragment corresponding to the suspected molecular ion has been highlighted.

Table A.1: Previously reported ^{1}H NMR chemical shifts for (5) in α-anomeric form [23].

multiplet	Chemical shift, δ_H	Integral
d	6.29	1H
t	5.32	1H
dd	5.01	1H
m	3.86-3.80	4H

Table A.2: Previously reported ^{13}C NMR chemical shifts for (5) α-anomeric form [23].

Chemical shift, δ_C
89.5
74.0
72.9
69.4
69.1
61.7

Table A.3: Reported ^{1}H NMR signals for (7) in $CDCl_3$ (α-anomer) [24]

Multiplet	Chemical shift, δ_H (ppm)	Integral
d	6.33	1H
t	5.47	1H
t	5.14	1H
dd	5.10	1H
m	4-29-4.24	1H
m	4.15-4.05	2H

Table A.4: Reported ^{1}H NMR signals in for (8) in $CDCl_3$ [25]

Multiplet	Chemical shift, δ_H (ppm).	Integral
d	6.25	1H
t	5.53	1H
d	5.11	1H
dd	4.99	1H
m	4.32-4.25	2H

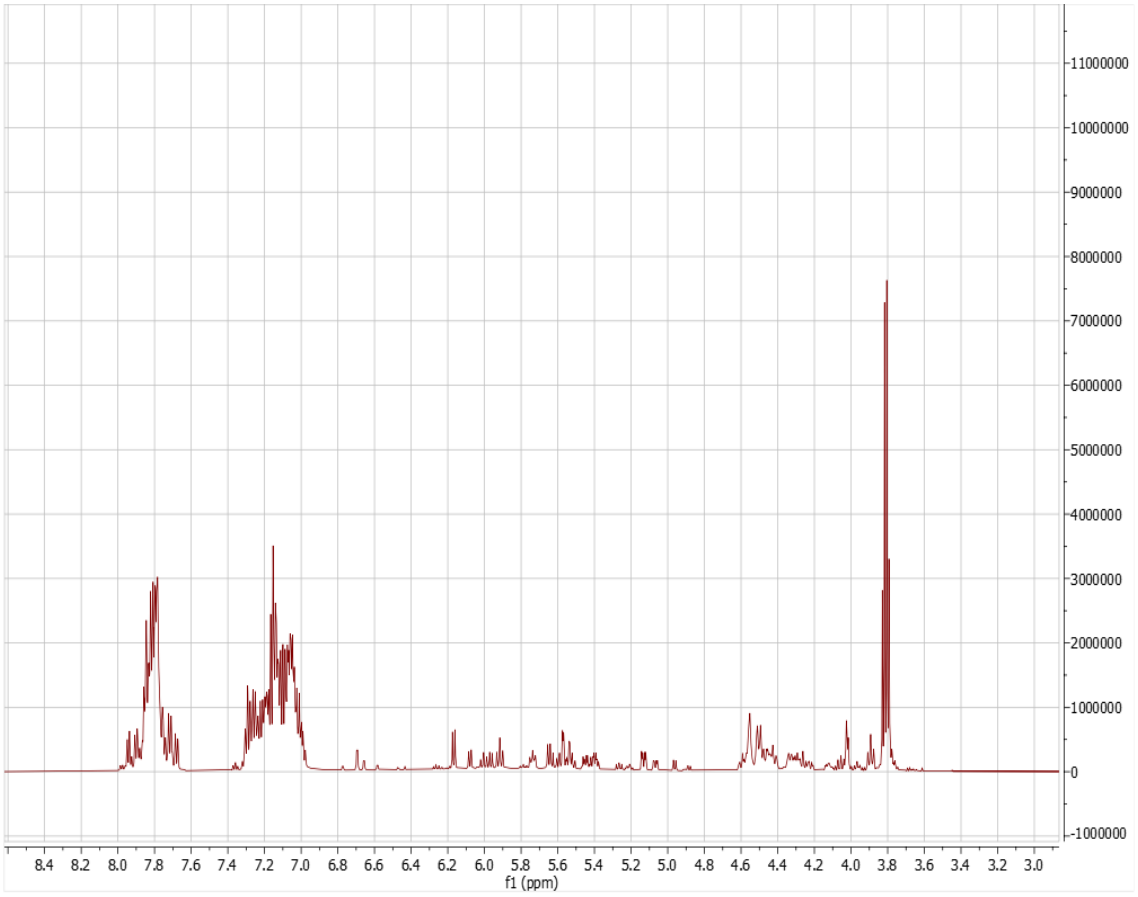

Figure A.1: ^{1}H NMR spectrum for fraction isolated via chromatography in the synthesis of 1,2,3,4,6-Tetra-O-Benzoyl-D-glucopyranose

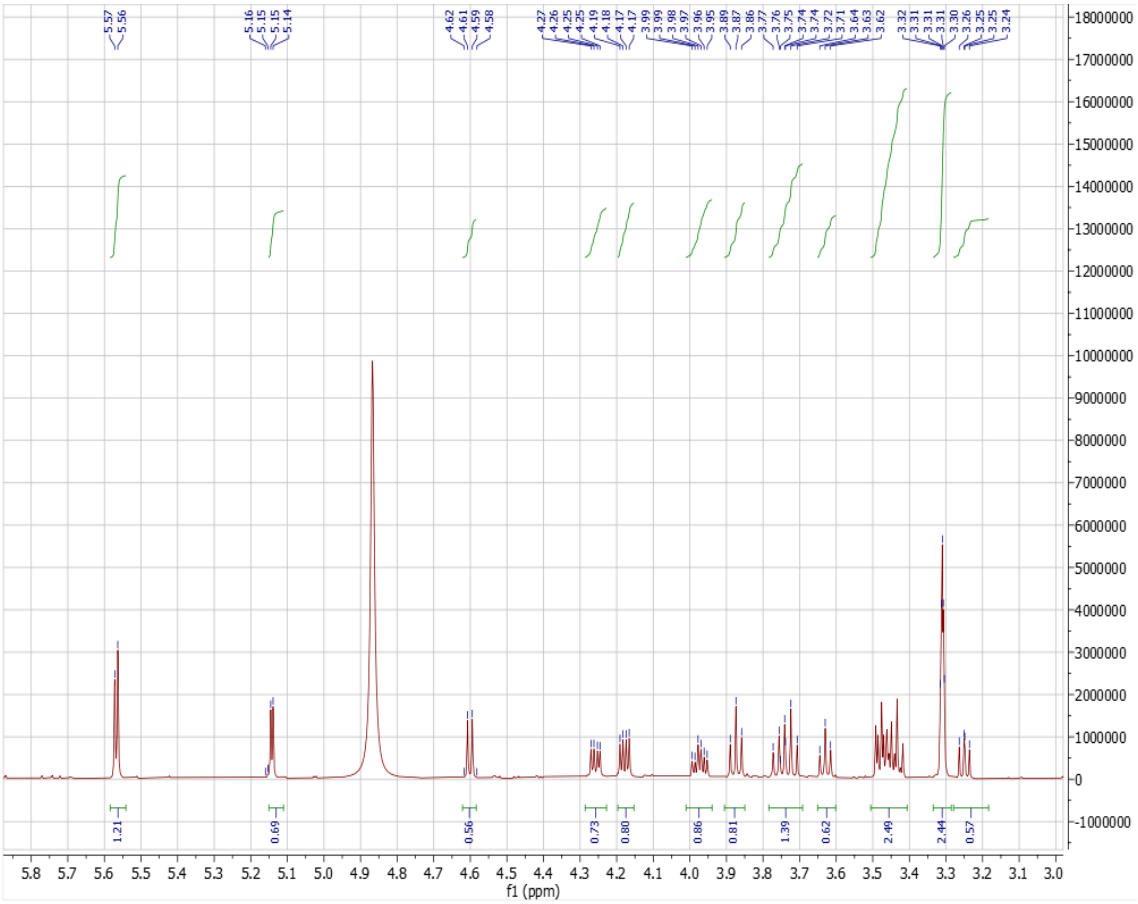

Figure A.2: ^{1}H NMR spectrum for 4,6-O-Benzylidene-D-glucopyranose.

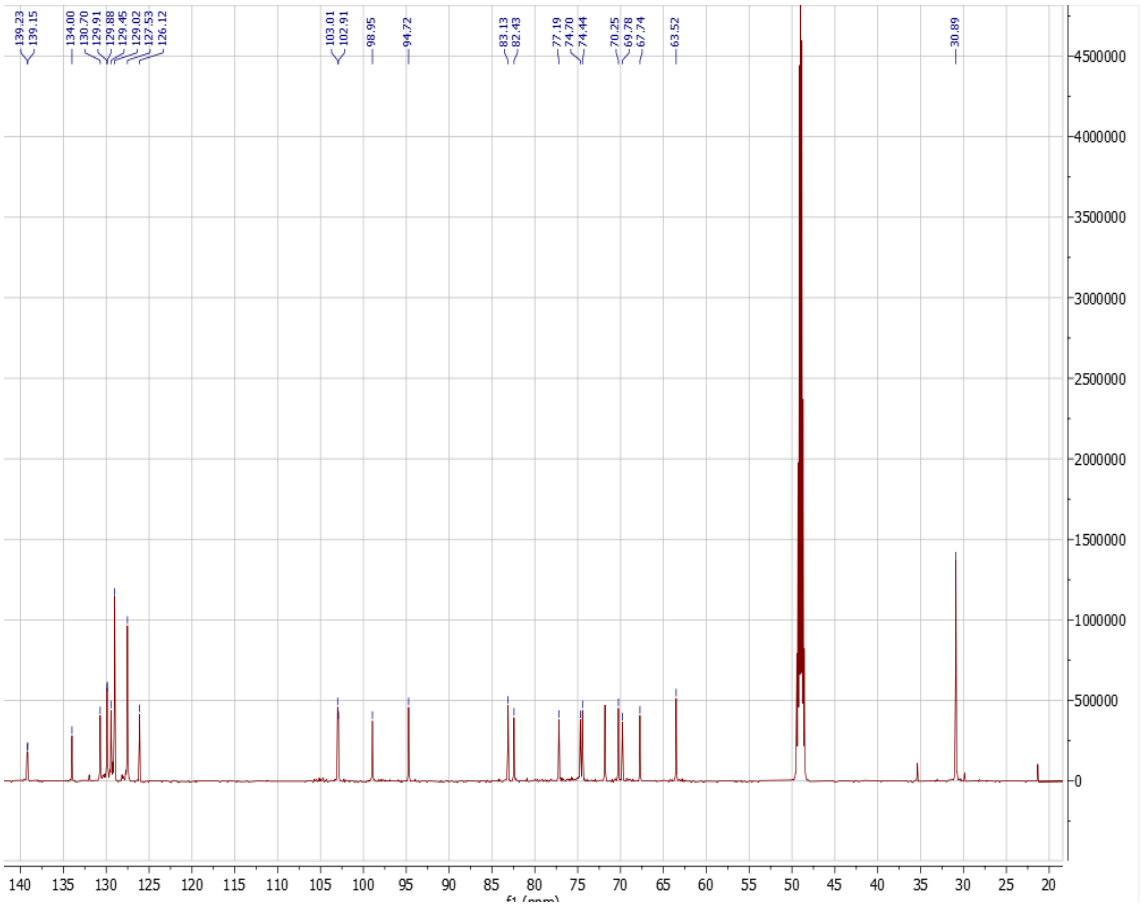

Figure A.3: ^{13}C NMR spectrum for 4,6-O-Benzylidene-D-glucopyranose.

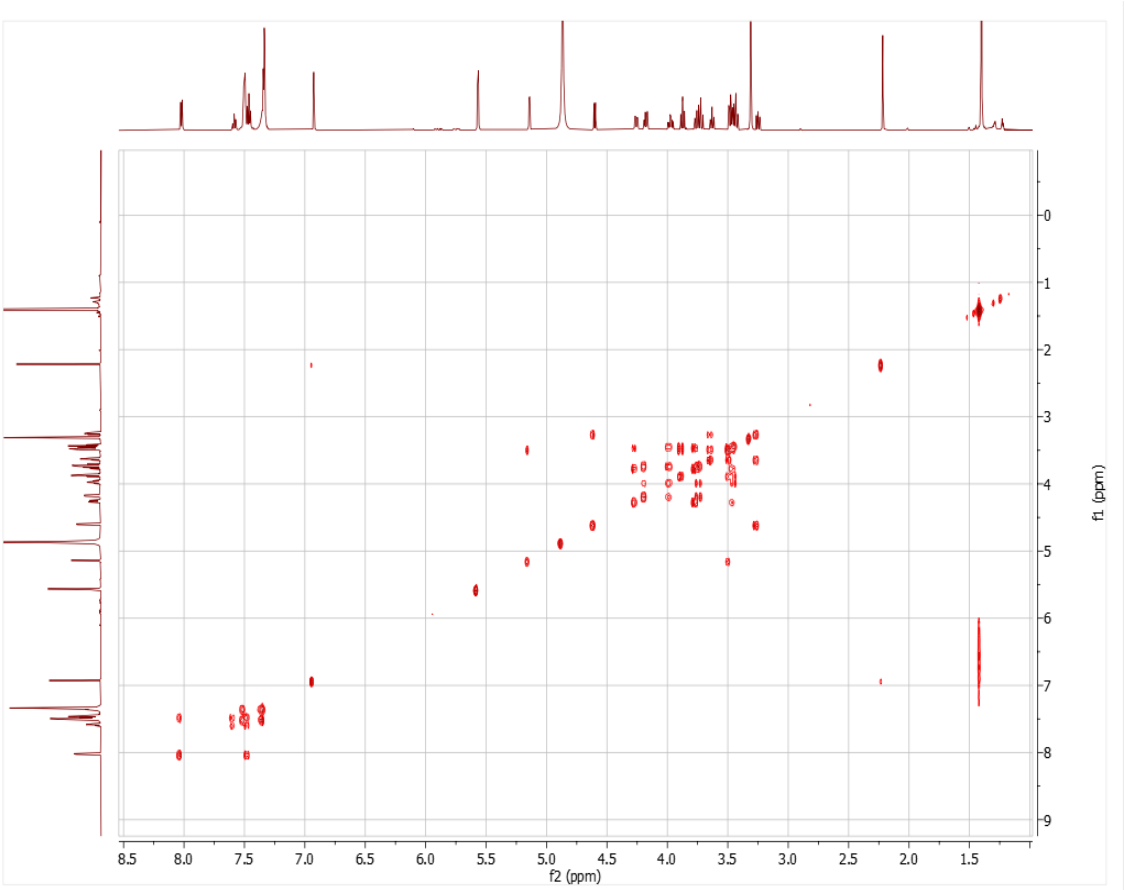

Figure A.4: COSY spectrum for 4,6-O-Benzylidene-D-glucopyranose.

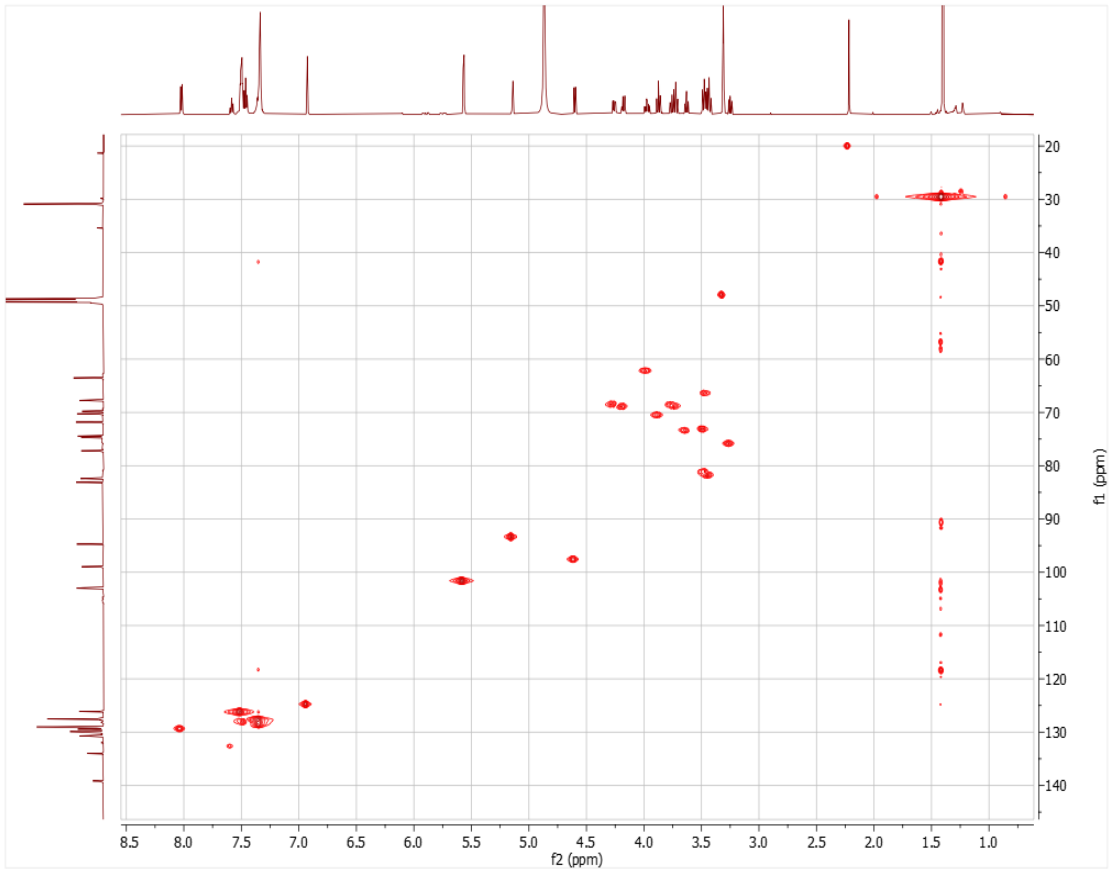

Figure A.5: HSQC NMR spectrum for 4,6-O-Benzylidene-D-glucopyranose.

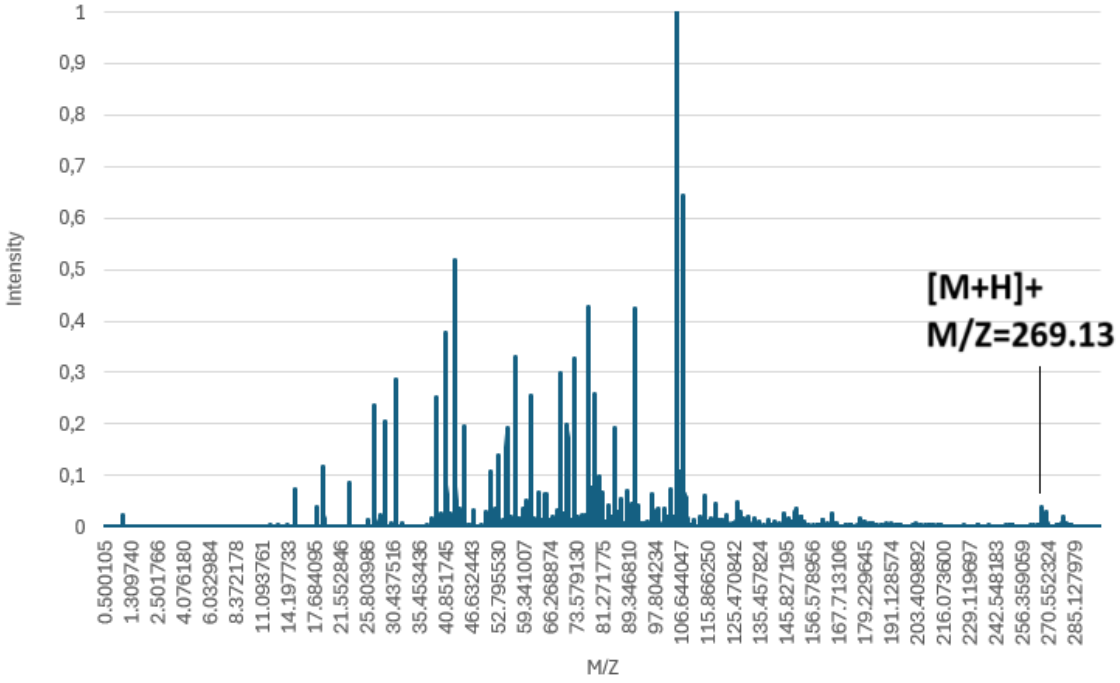

Figure A.6: SIMS mass spectrum for 4,6-O-Benzylidene-D-glucopyranose.

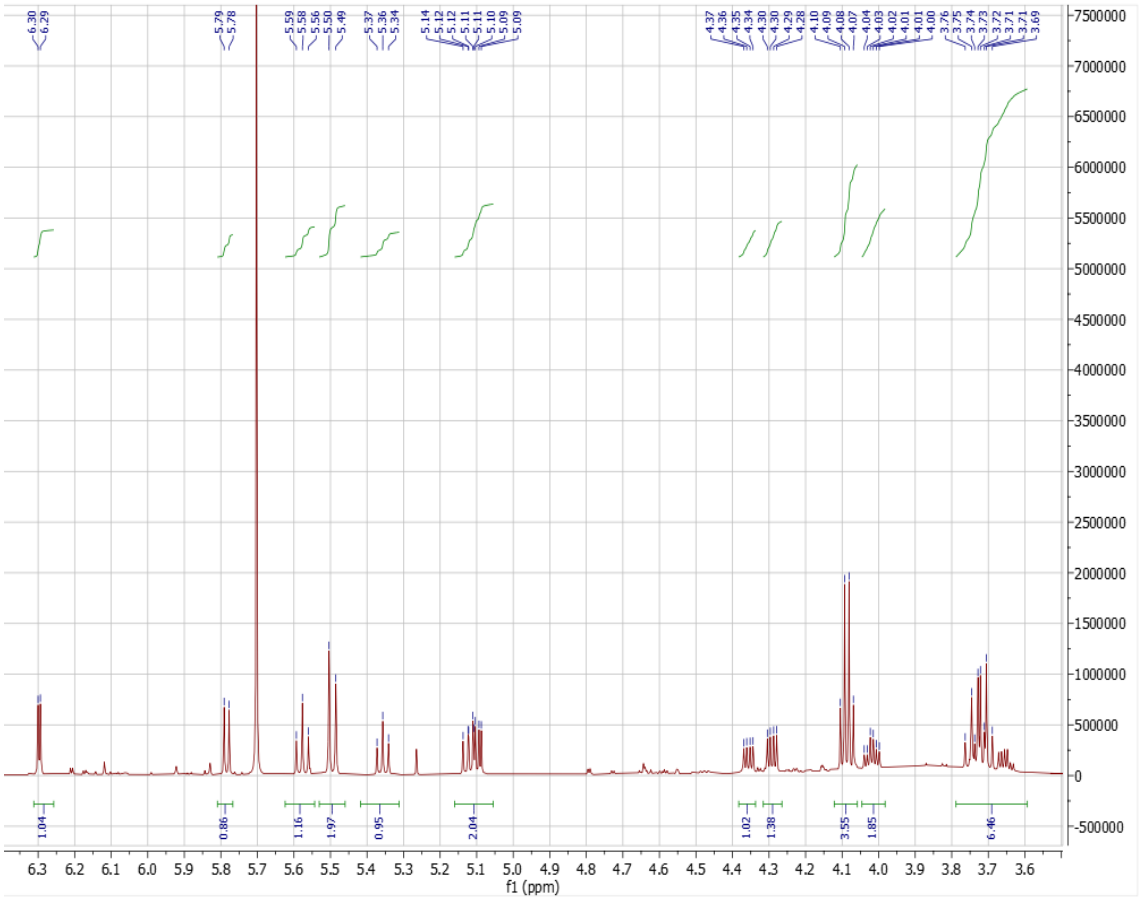

Figure A.7: ^{1}H NMR spectrum for 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose.

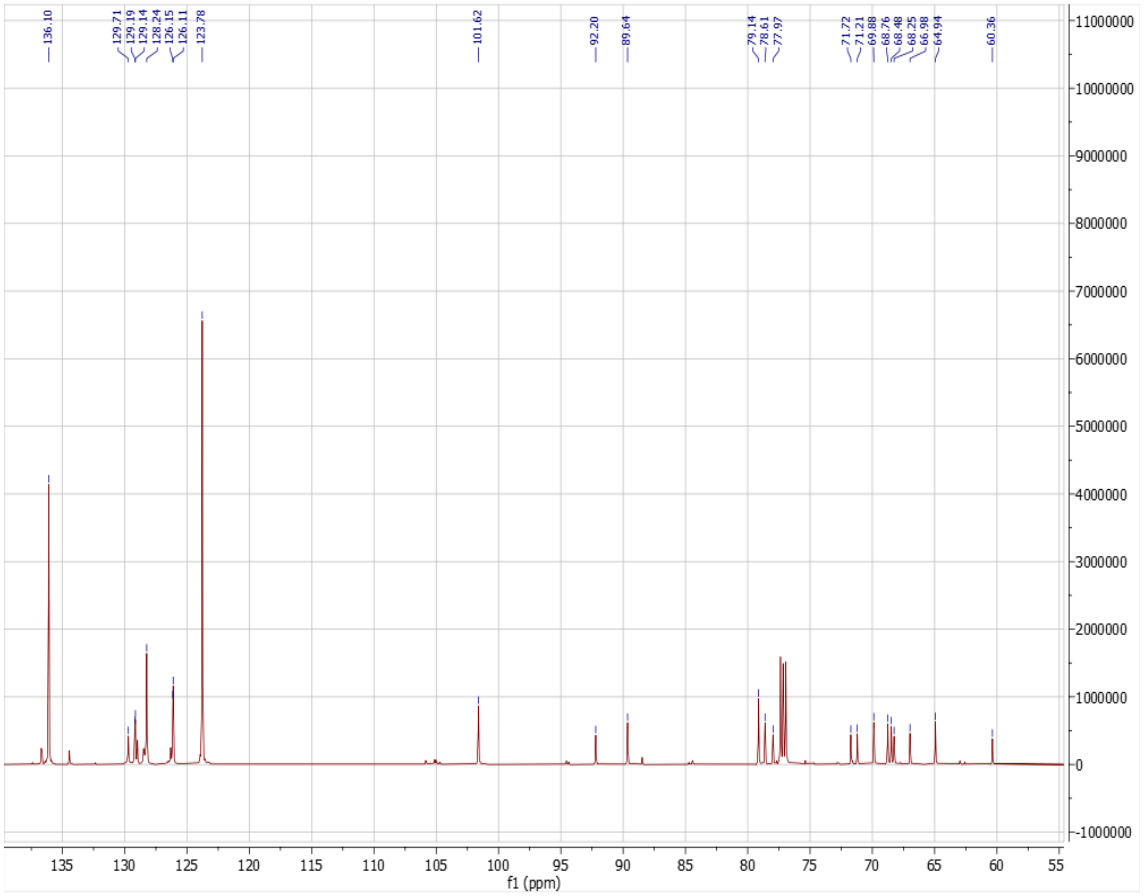

Figure A.8: ^{13}C NMR spectrum for 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose.

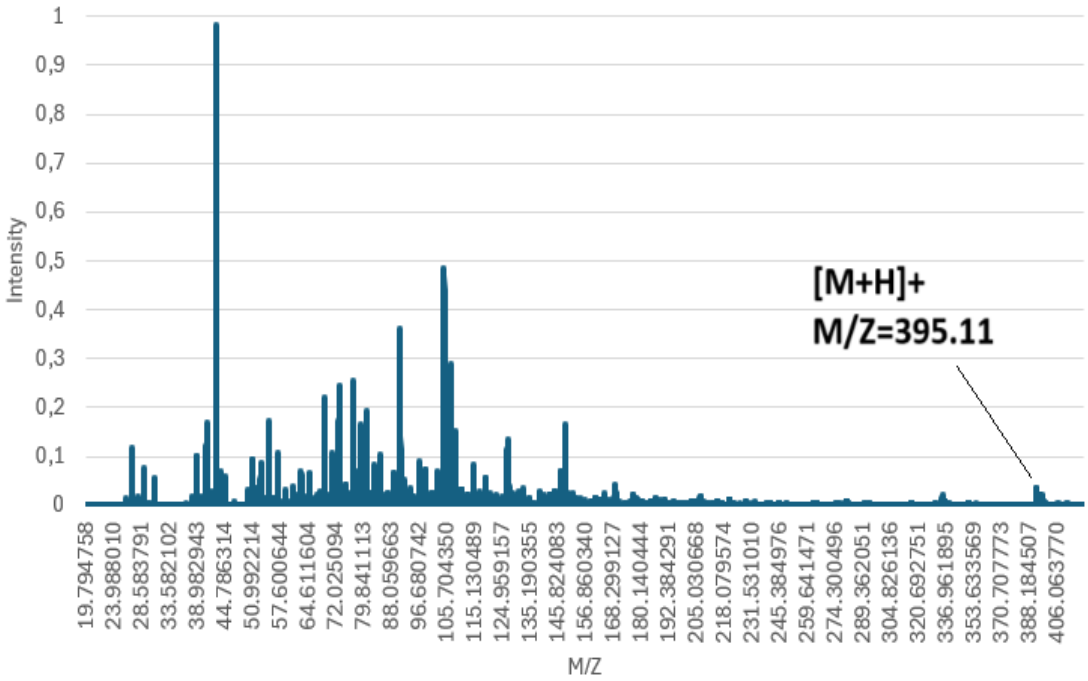

Figure A.9: SIMS mass spectrum for 1,2,3-Tri-O-Acetyl-4,6-O-Benzylidene-D-glucopyranose.

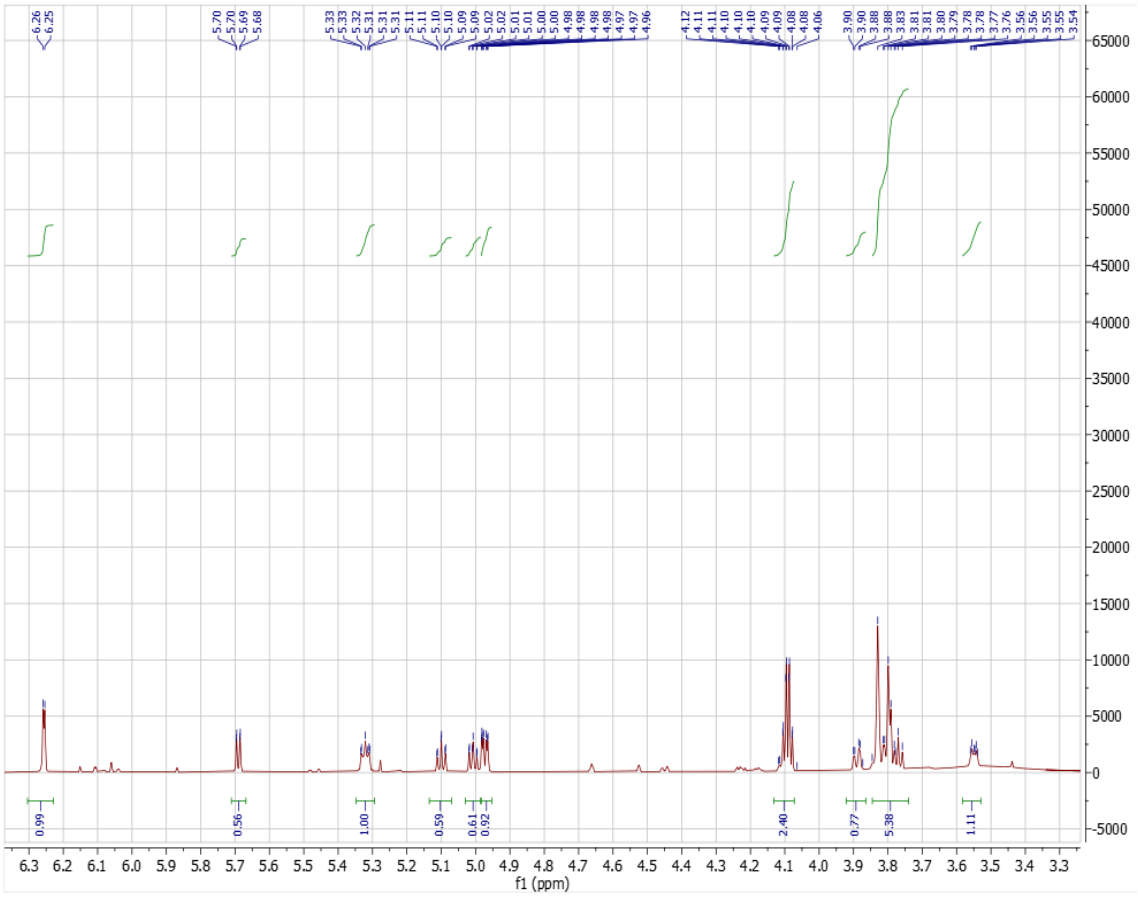

Figure A.10: ^{1}H NMR spectrum for 1,2,3-Tri-O-Acetyl-D-glucopyranose.

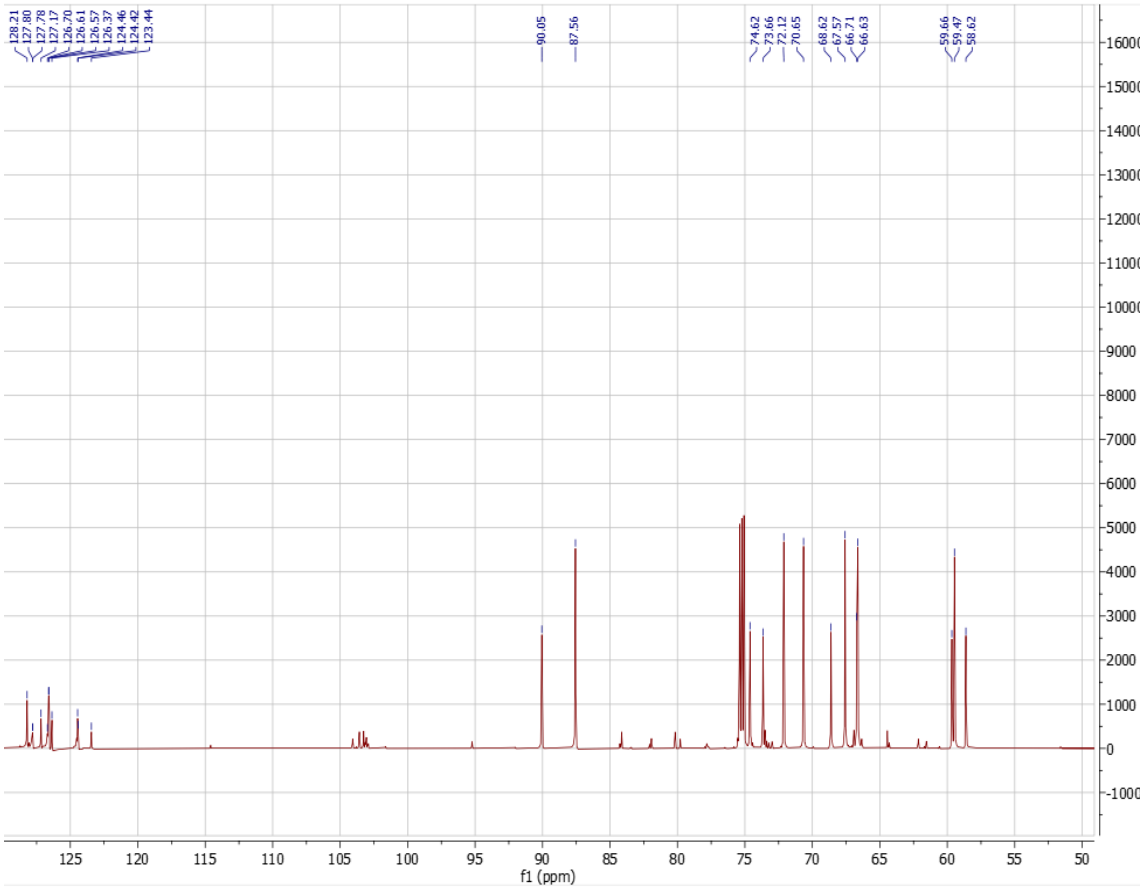

Figure A.11: ^{13}C NMR spectrum for 1,2,3-Tri-O-Acetyl-D-glucopyranose.

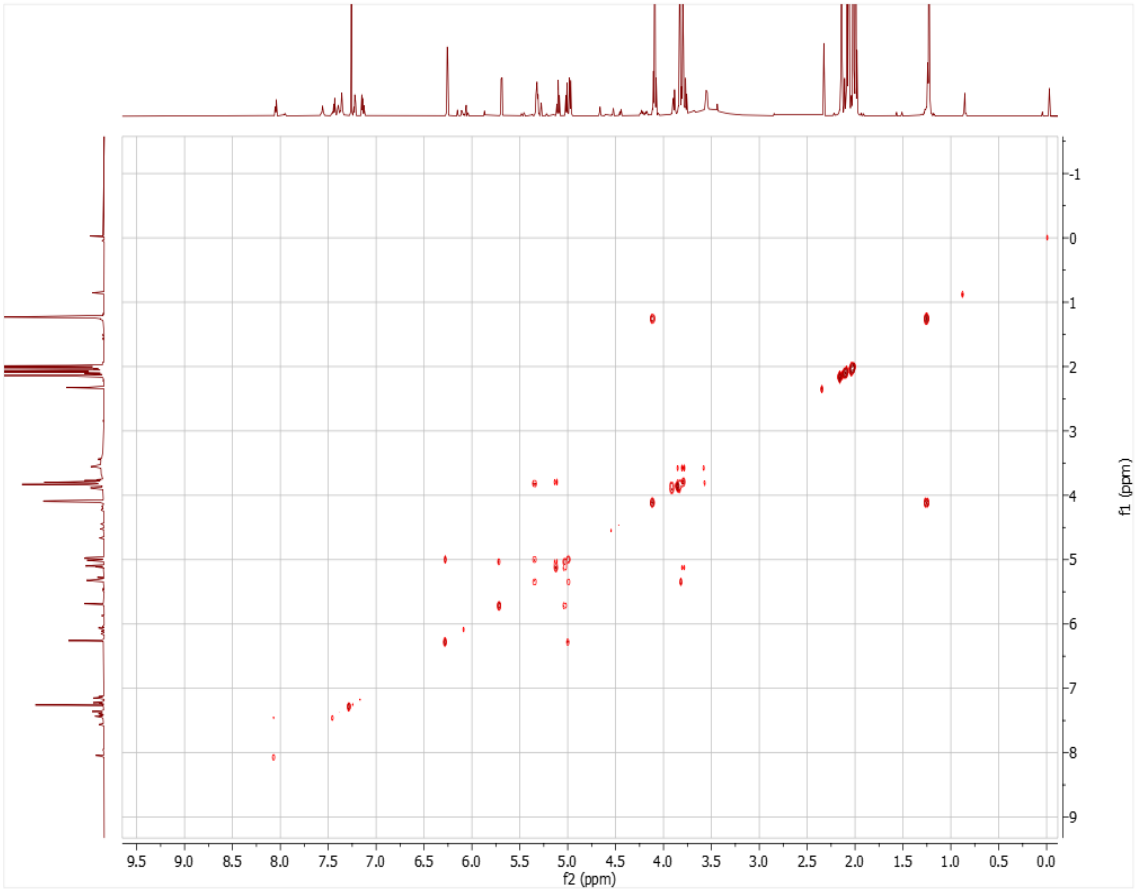

Figure A.12: COSY NMR spectrum for 1,2,3-Tri-O-acetyl-D-glucopyranose.

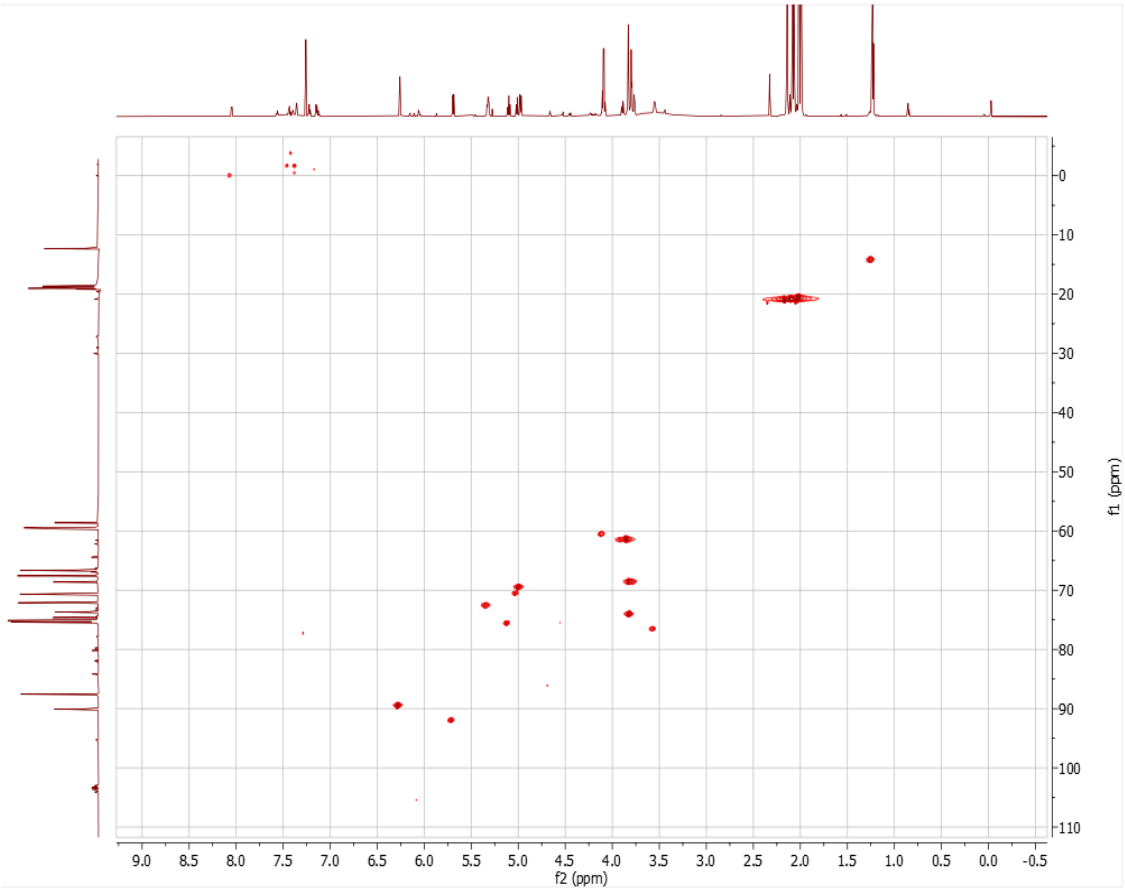

Figure A.13: HSQC NMR spectrum for 1,2,3-Tri-O-Acetyl-D-glucopyranose.

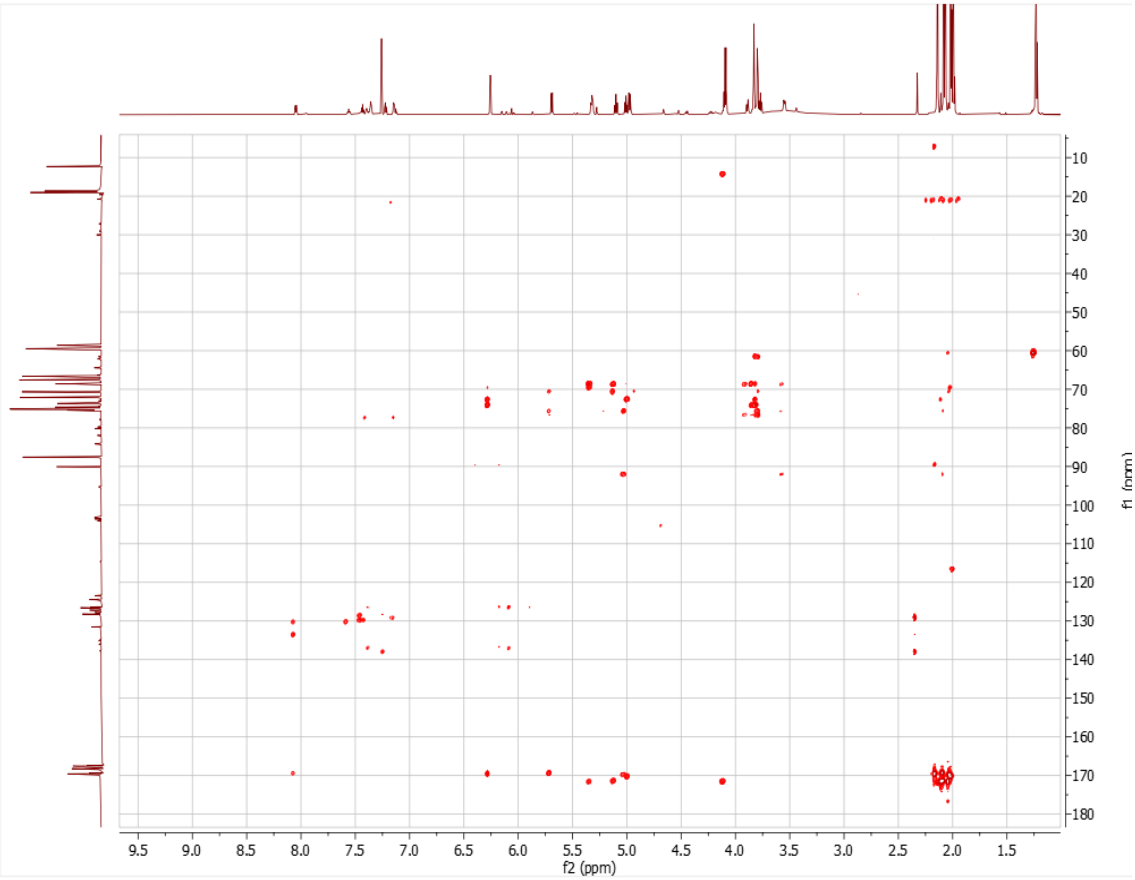

Figure A.14: HMBC NMR spectrum for 1,2,3-Tri-O-Acetyl-D-glucopyranose.

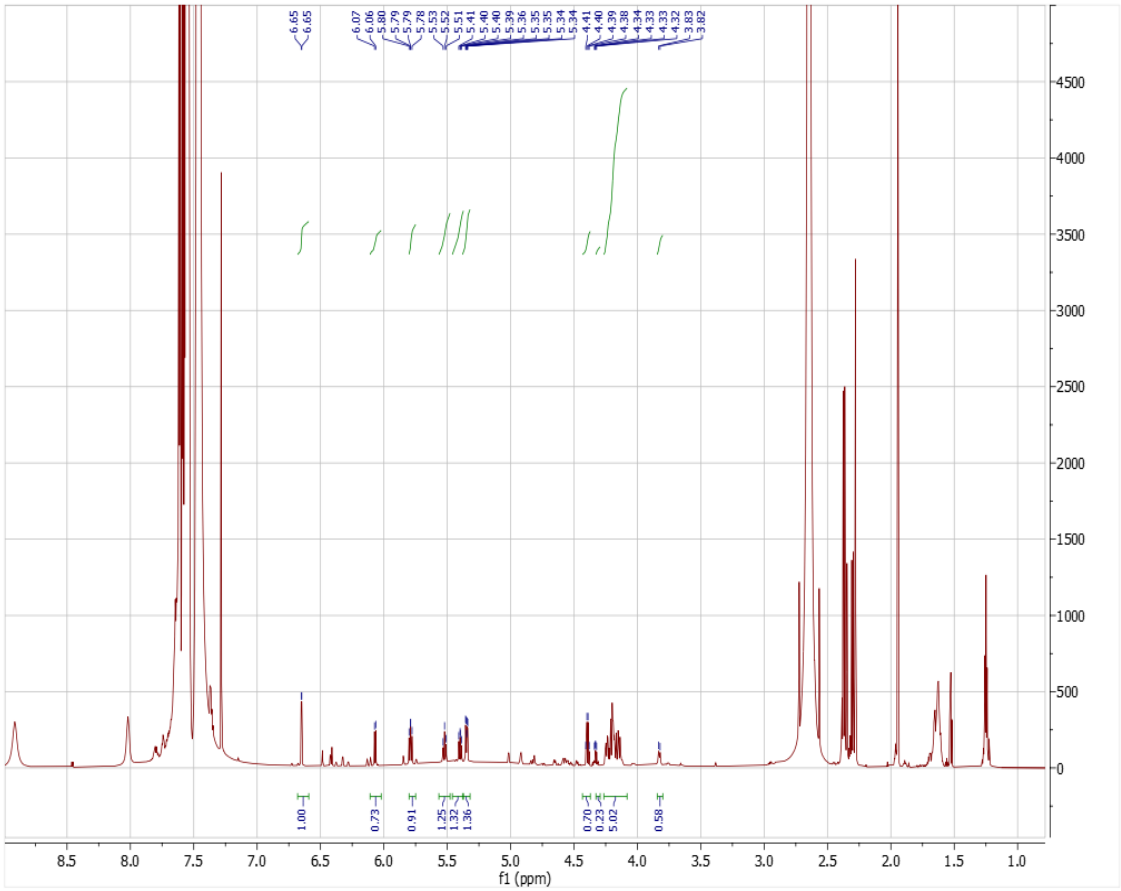

Figure A.15: 1H NMR spectrum for 6-O-Trityl-1,2,3-Tri-O-Acetyl-D-glucopyranose.

Figure A.16: ^{1}H NMR spectrum for 1,2,3,4,6-Penta-O-Acetyl-D-glucopyranose.

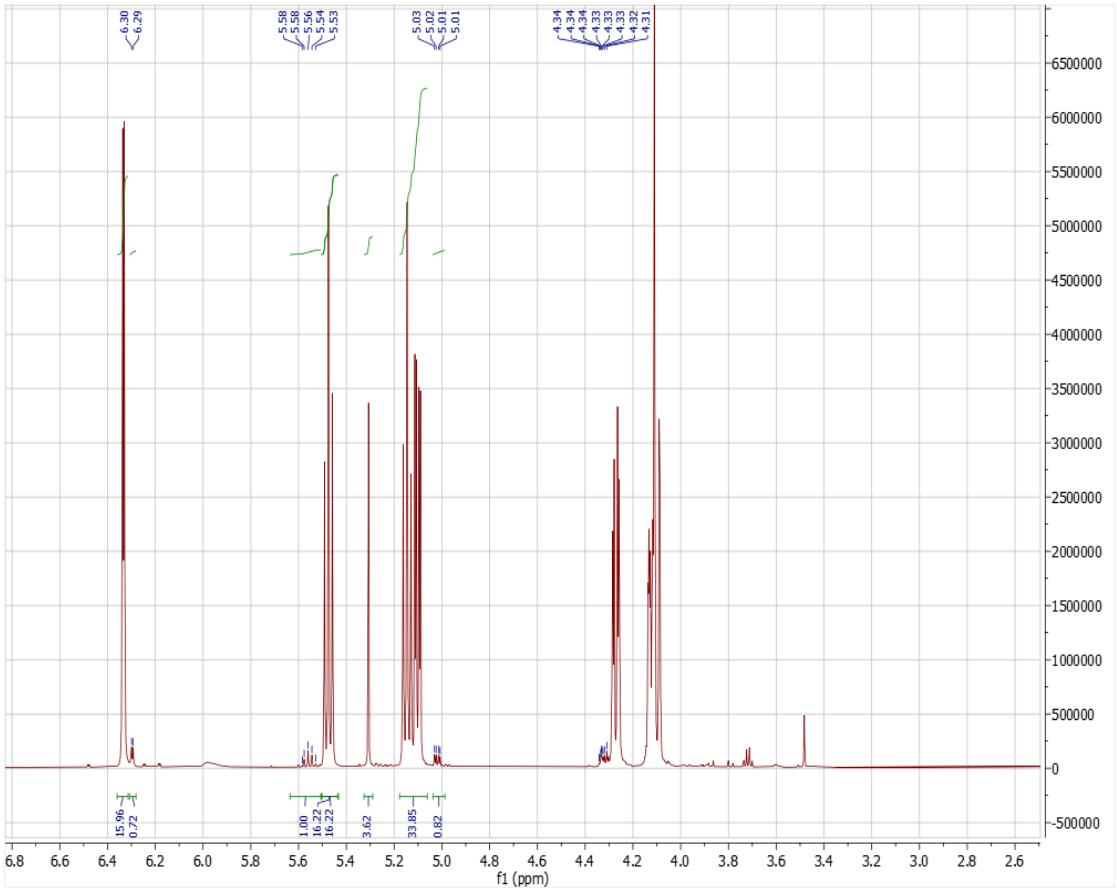

Figure A.17: ^{1}H NMR spectrum for 2,3,4,6-Tetra-O-Acetyl-D-glucopyranosyl chloride

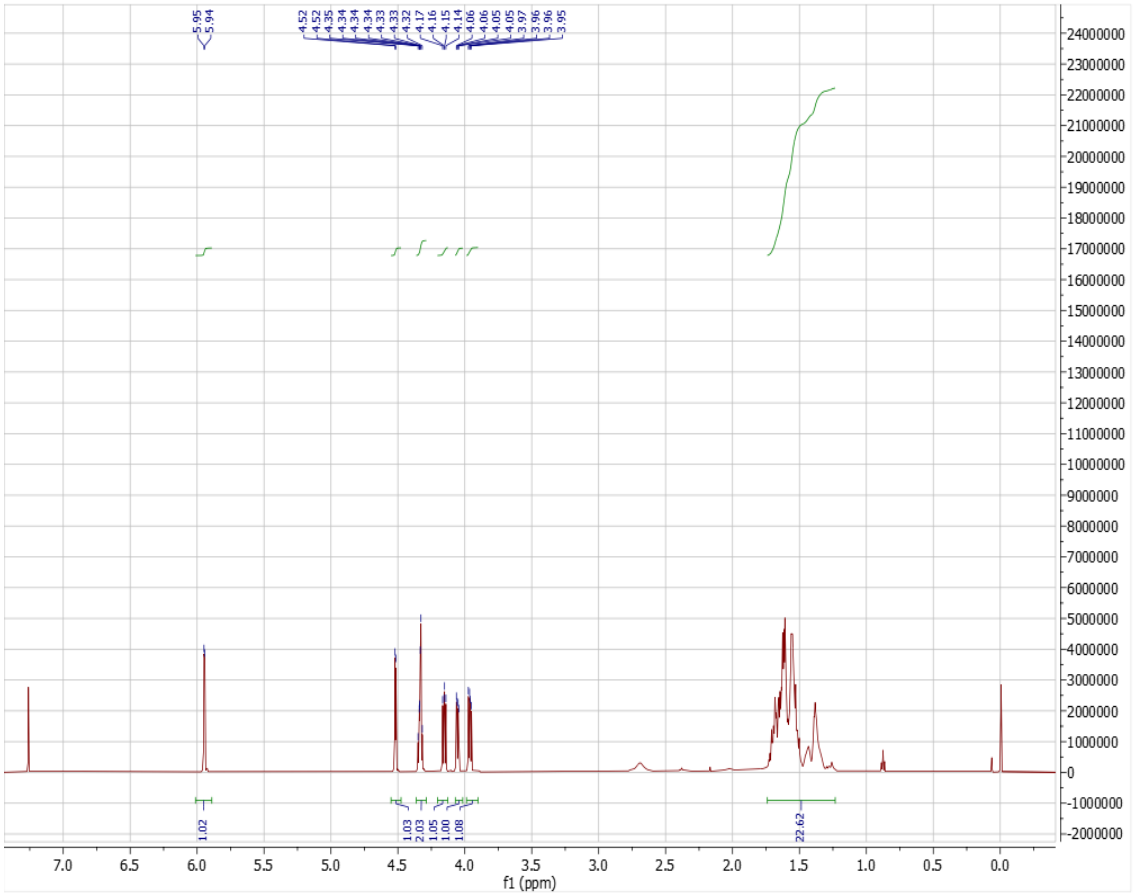

Figure A.18: ^{1}H NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-D-glucofuranose.

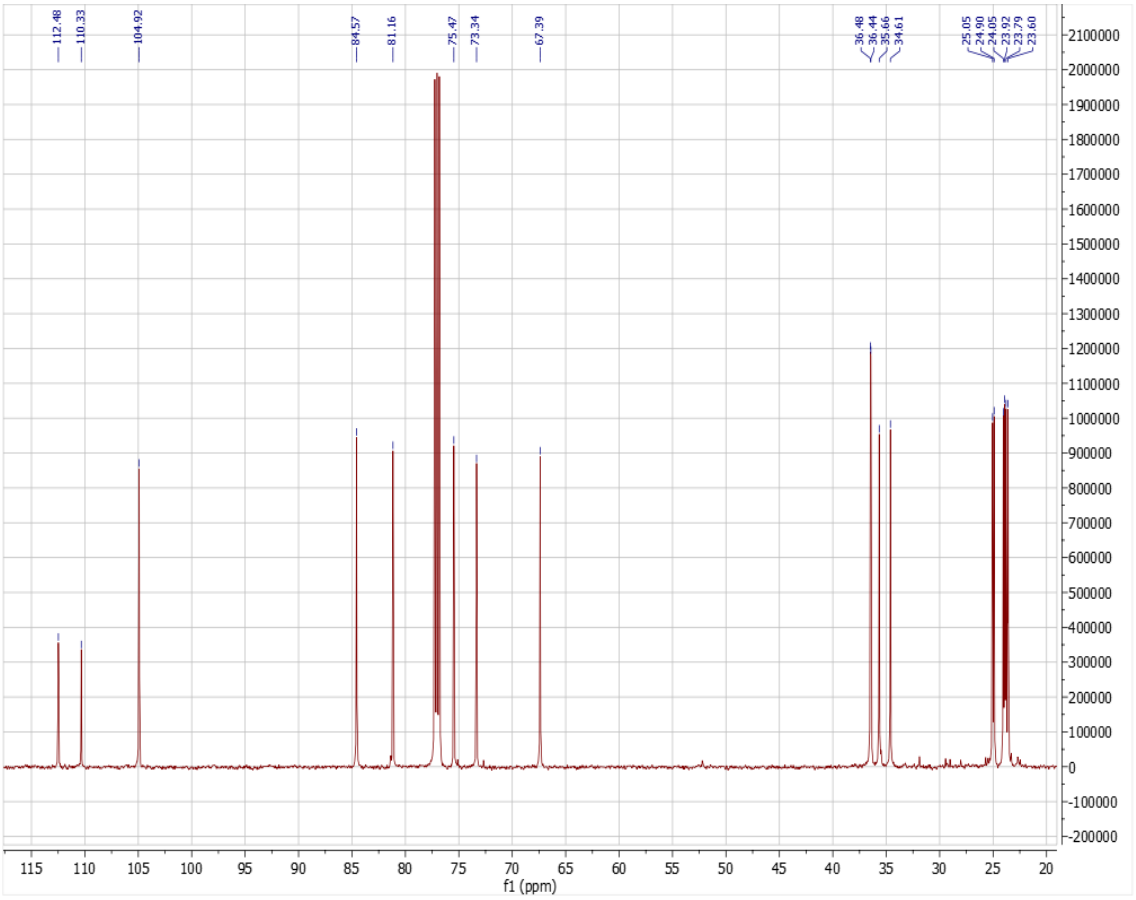

Figure A.19: ^{13}C NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-D-glucofuranose.

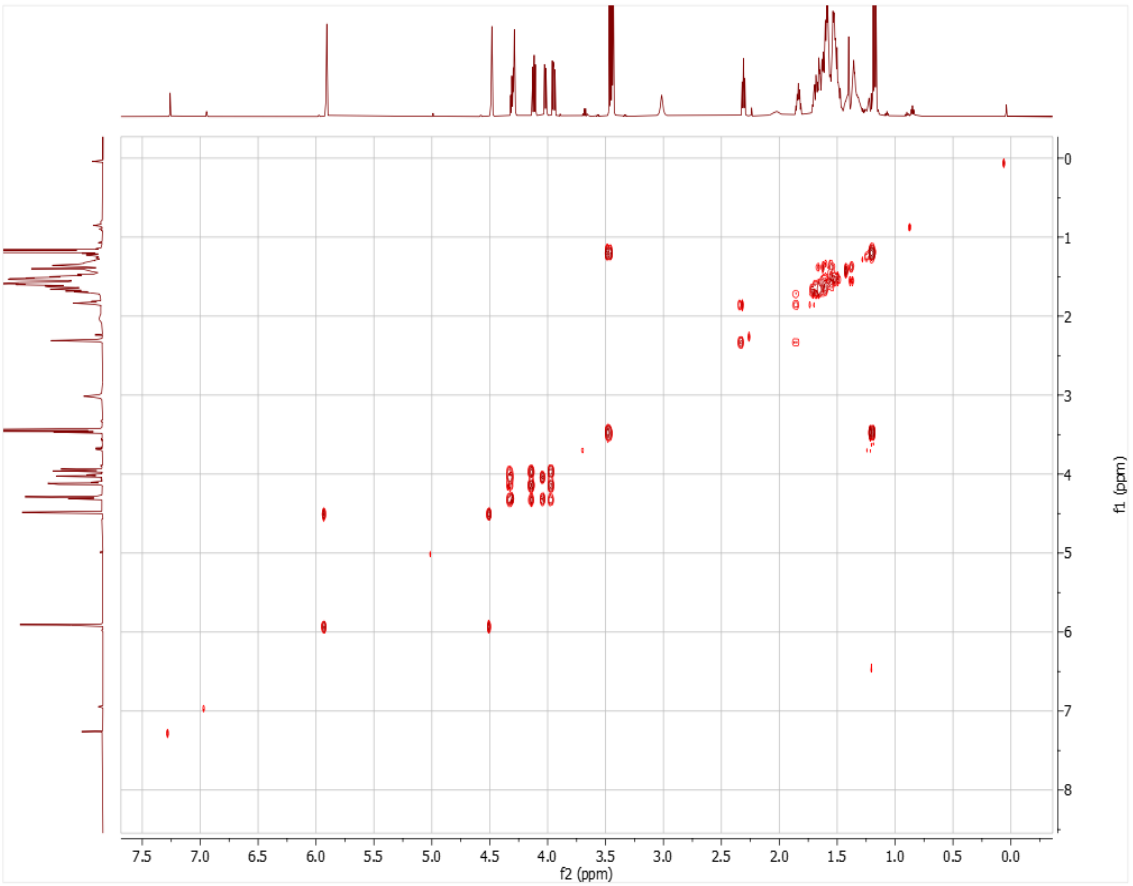

Figure A.20: COSY NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-D-glucofuranose.

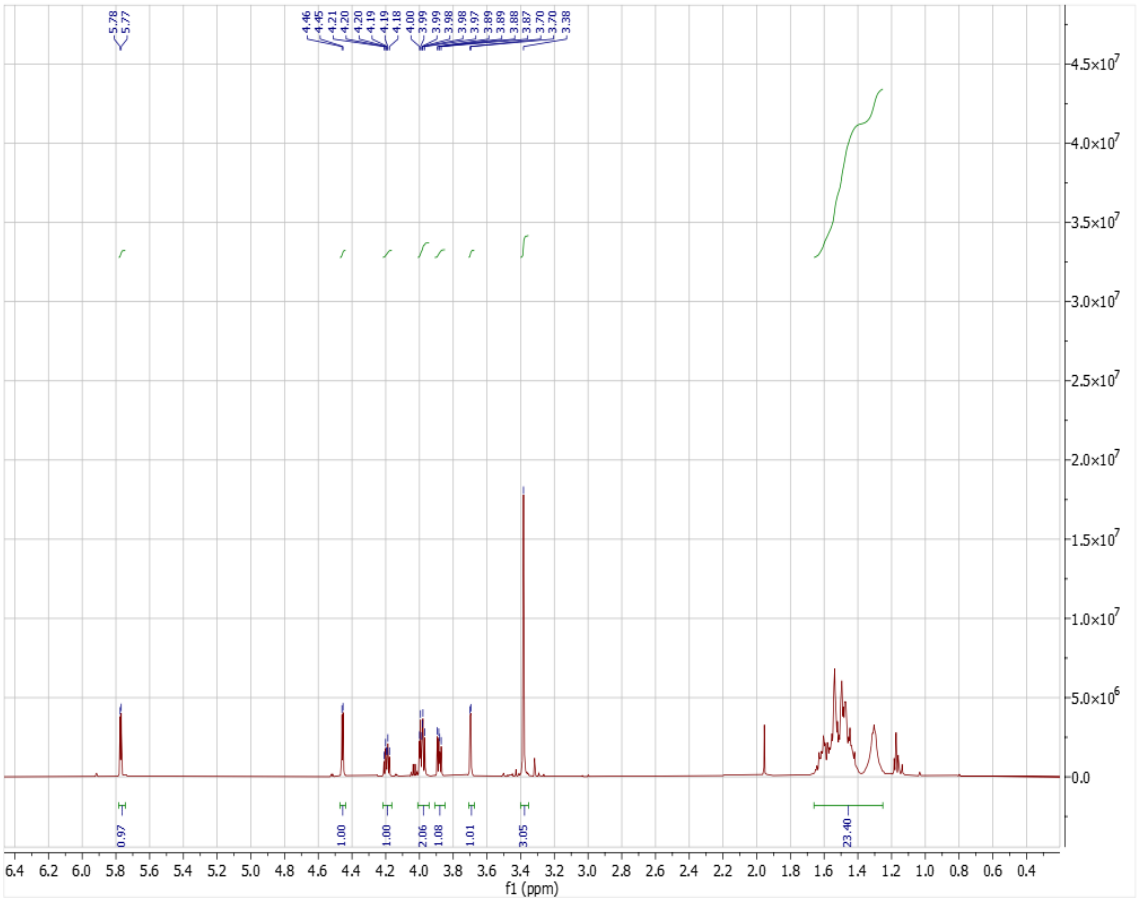

Figure A.21: ^{1}H NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-D-glucofuranose (methylated).

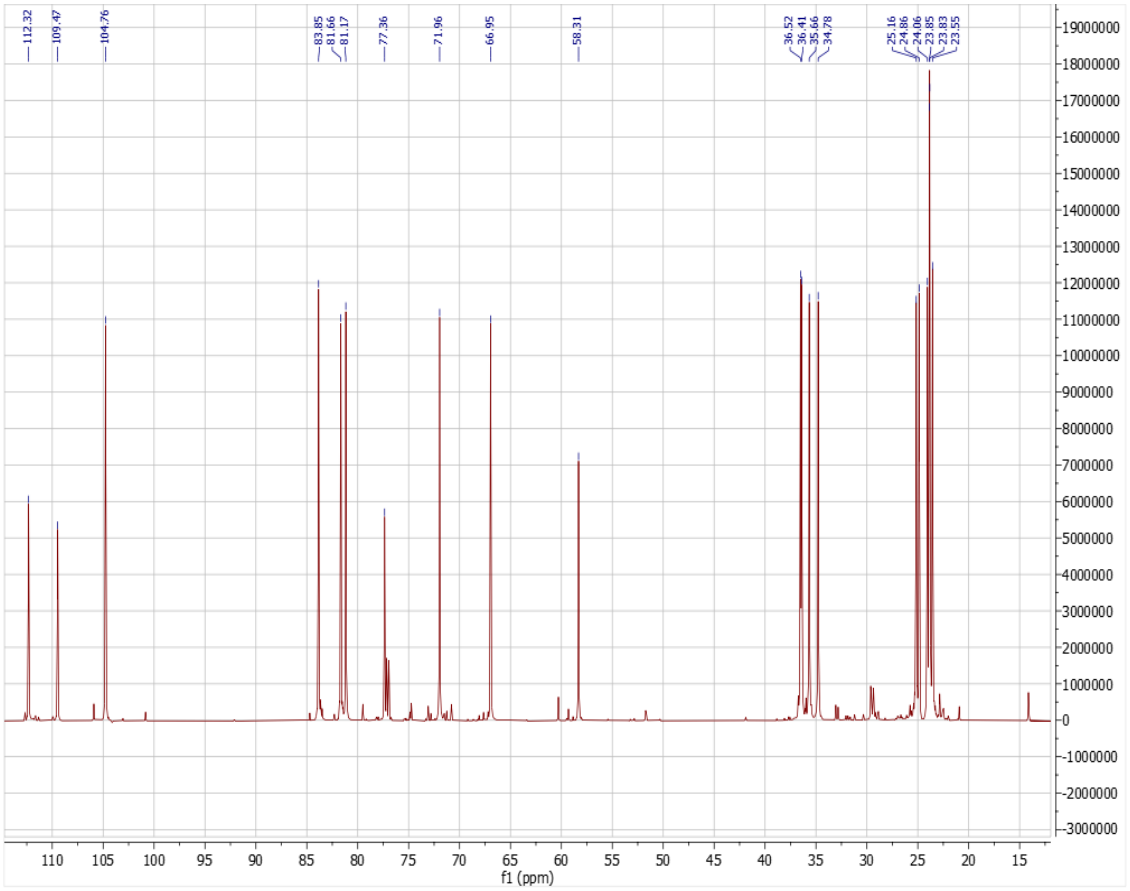

Figure A.22: ^{13}C NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-3-O-Methyl-D-glucofuranose.

Figure A.23: COSY NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene–3-O-Methyl-D-glucofuranose.

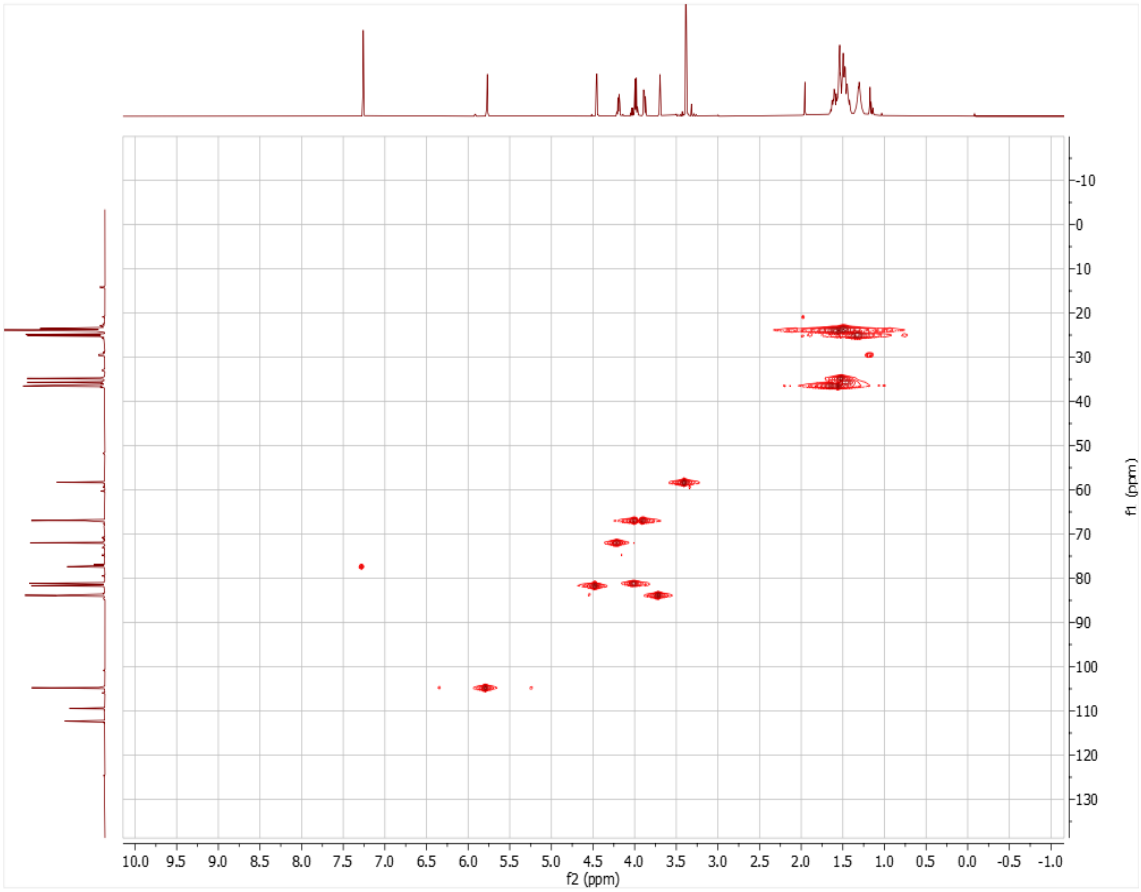

Figure A.24: HSQC NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-3-O-Methyl-D-glucofuranose.

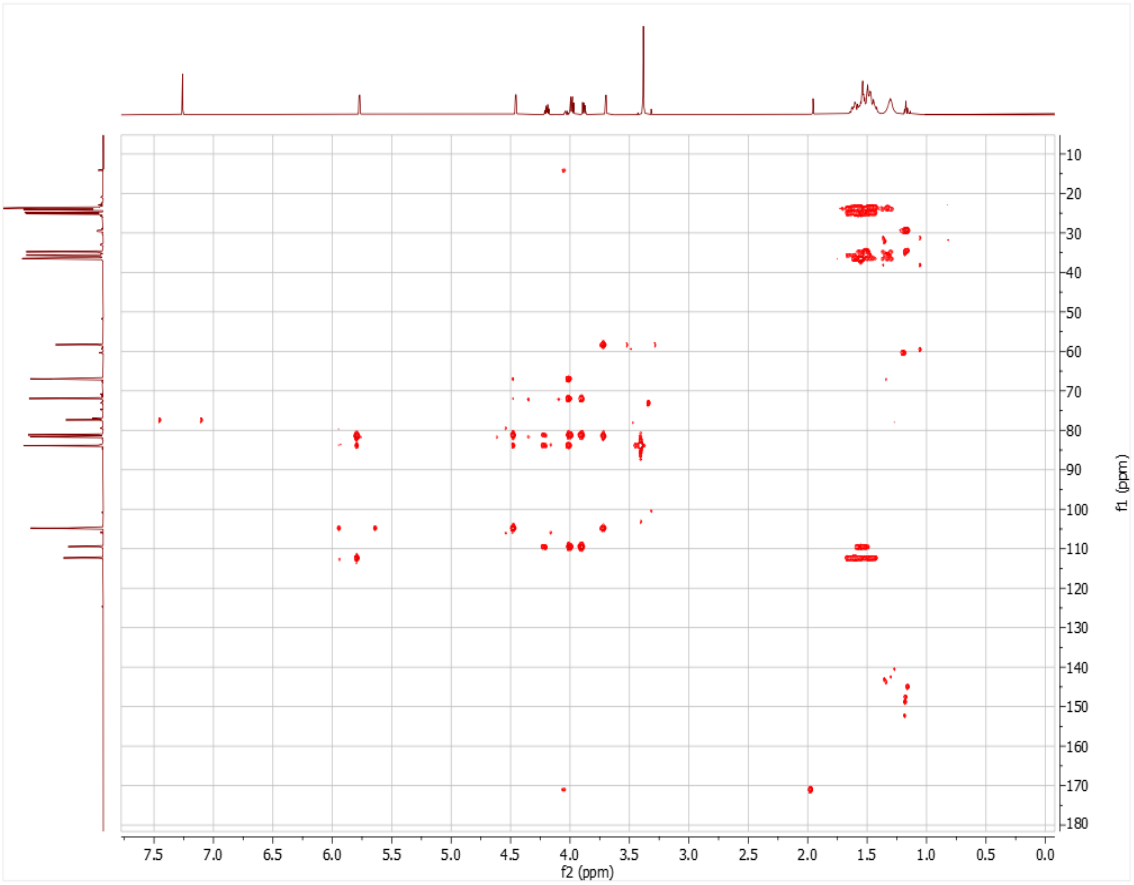

Figure A.25: HMBC NMR spectrum for 1,2:5,6-di-O-Cyclohexylidene-3-O-D-glucofuranose.

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