Abstract

This work explores modified dextran as a drug delivery system from two angles. The first aims to provide an in-depth structural characterization of substitution affinity and heterogeneity of Ac-Dex. The second is an exploration of novel triggers to create an H_2S sensitive solubility switching material. This work aims to provide two perspectives into methods of optimization of drug delivery systems based on the polysaccharide dextran.

Acetal-modified dextran (Ac-Dex) is a solubility switching polymer. Historically it has been used to form nanoparticles which can encapsulate drugs for targeted release vaccine delivery. It is insoluble in water, but under acidic conditions the acetals hydrolyze, converting it into water soluble dextran. Ac-Dex is prepared through modification of dextran with 2-methoxypropene, generating a heterogeneously modified polymer. Each monomer subunit in dextran has three possible hydroxyls for modification; additionally, the acetals can be either cyclic or linear, meaning there are 12 different possible modifications per monomer. The different types of modification affect the degradation rate of the polymer. It would be ideal to understand the conditions that favor each type of modification so that a more homogenous polymer can be synthesized consistently. This study details the synthesis and use of 1,6-dimethyl glucose for use as a monomeric model compound for dextran, in which the methyl caps simulate the glycosidic bonds of a dextran backbone. We report here on progress toward measuring and synthetically controlling the distribution of acetal modifications on dextran in the formation of Ac-Dex.

Modified dextran as a solubility switching polymer has shown promise with a variety of capping mechanisms. H_2S has been found to act as a gasseotransmitter within the body. As such it is subject to upregulation in the body in response to certain disease states. It would be desirable to have a targeted delivery method available for H_2S associated diseases. In an effort to create an H_2S triggered solubility switching polymer based on dextran, an aryl azide was selected as a capping group. This group has shown efficacy in H_2S sensing fluorophores and was therefore adopted as a capping group on dextran. This body of work reports on the synthesis and characterization of such a material, as well as its prospects for further adoption.

Exploring Optimization of Dextran Based Drug Delivery through Substitutional Investigation and Trigger Modification

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A thesis presented to the faculty of Mount Holyoke College in partial fulfillment of the requirements for the degree of Bachelor of Arts with Honors

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May 2025

This thesis was prepared under the direction of Dr. Kyle Broaders for 8 credits of independent study

Acknowledgements

I would like to thank my research advisor, Professor Kyle Broaders. The impact you have had on my life is bigger than I can begin to state. I am so grateful to have had the opportunity to learn from you. My first semester of organic chemistry with you was what first inspired my passion in chemistry, and my time in lab is what cemented my love for it. Thank you for supporting me as I developed both my skills as a researcher and myself as a person. My time spent in lab was truly the most valuable part of my education at Mount Holyoke, and I am forever grateful.

I would like to thank Professor Alan van Giessen; your presence in my education has been such a beacon of light. I am grateful for all the time I spent in your office hours where you encouraged my curiosity and helped to guide me as I learned how to navigate chemistry along with the rest of my life. I would like to thank my final committee member Professor Supraja Balasubramanian for your encouragement and support as I learned physics and for your willingness to support me by serving on my committee. I must extend a huge thanks to the Mount Holyoke Chemistry Department; I have received so much knowledge, encouragement and support from each of my professors, and so much guidance and inspiration from my PLUMs, peers, and students. I am grateful to you all for serving in my education. During my time at MHC this department became (occasionally more than it should have been) my home and I am so thankful for all of my time here.

I want to thank my lab mates Ran, Illia, and Mariana for our wonderful summer together. Staring lab and learning with you all is what made lab such a wonderful place to be. I don't know how I would have done it all without your constant input and support. All of our experiments and adventures both in and out of lab made me feel like I had a place where I truly fit in and felt comfortable. To Echo, Aaditi, and Isabela I want to thank you all for helping to show me the ropes in lab and encouraging me as I started. I also want to thank Venus for laying the groundwork on my project and helping me to get started; it was so wonderful to learn from you. To Ashh, Fati, Davida, Abby, Gwyn, Eliza, and Max, thank you so much for bringing so much energy and passion into your work, you all helped me to remember all the joys that research brings.

To my friends Lora, Mayan, Wenqi, Evelena, Mako, and so many more; thank you so much for all of your support during my senior year as I took on my graduate school applications and thesis. You gave me the strength and inspiration to continue pushing forwards. I am so grateful for all the times we spent together. Your support in this transitional period of my life has meant the world to me.

A special thanks to Sophie without whom most of this work would not have been completed. Thank you for being my eternal safety buddy and for all our evening "lab parties" spent doing research in parallel. Without your constant presence this work would have taken much longer to complete. Thank you for your support and friendship as we completed our classes, grad school applications, and thesis writing. It has been a pleasure to get to go through life with you.

Thank you to my Mom and to Gabe for your constant support as so much of my life has changed. Your unwavering support is what encouraged me to be free to learn and explore as I pleased. Thank you to SueSue and Popo, you both inspired me so much as I grew and helped me to feel like I could do anything. I am eternally grateful for your support of my education. I am so excited to walk down a path you helped lay for me. To Nana and Zedo, thank you for all your love and support, your presence in my life is so special, your encouragement allowed me to become who I am today.

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1 List of Abbreviations

2-MP	2-Methoxypropene
Ac-Dex	Acetal Modified Dextran
ArAz-Dex	Aryl Azide Capped Dextran
CAM	Ceric Ammonium Molybdate
CDI	Carbonyl Diimidazole
COSY	Correlated Spectroscopy
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDS	Drug Delivery System
DLS	Dynamic Light Scattering
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
Et_2O	Diethyl Ether
EtOH	Ethanol
EtOAc	Ethyl Acetate
FDA	Food and Drug Administration

HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
IR	Infrared
LNP	Lipid Nanoparticle
MeOH	Methanol
mRNA	Messenger Ribonucleic Acid
NMR	Nuclear Magnetic Resonance Spectroscopy
PBS	Phosphate Buffered Saline
PVOH	Polyvinyl Alcohol
Rf	Retention Factor
TLC	Thin Layer Chromatograph
TMS	Tetramethylsilane
UV	Ultraviolet

2 Introduction

2.1 Targeted Drug Delivery Systems

2.1.1 Pharmaceuticals and their Administration

There are a variety of ways to administer pharmaceuticals. Common routes are oral ingestion and injection[1]; others include nasal sprays[2], eye drops[3], topical ointments[4], subdermal implants[5], intravaginal application[6], nebulization[7] and many more.

When choosing a drug administration route, it is important to consider pharmacokinetics. Pharmacokinetics encompasses absorption, metabolites, distribution and circulation time within the body.[8] Absorption refers to the process of the drug moving from administration into circulation throughout the body.[9] Factors affecting drug absorption include drug solubility, permeability of intestinal membranes in the case of oral ingestion, and the rate of in vivo dissolution and diffusivity.[9] Metabolites are the digestion products of metabolism.[10] Considerations for drug metabolites include whether or not they are biologically active, and how easily the body can expel them.[10] It is preferable to have benign metabolites. Distribution refers to drug concentrations within the body which in turn affects the magnitude of response to the drug.[8] Circulation time within the body is a critical consideration as a drug must remain in circulation long enough to reach the target site. Additionally, certain drugs are liable to hysteresis effects meaning the biological response may change in reaction to the drug depending on how long it has been in the system.[8] The impact that drug structural properties and administration tactics have on pharmacokinetics must be considered when designing a new drug or drug delivery system.

2.1.2 Stimuli Responsive Polymers

Polymers have been used extensively in domestic and industrial settings. Over the past few decades, an emerging class of polymers has been noted for its increased utility. Stimuli responsive polymers are polymers that react to different environmental conditions to change their properties.[11] Stimuli responsive polymers, otherwise known as 'smart materials'[12], differ from generic polymers in that they have properties that change in response to external stimuli.[11] Triggers for these polymers commonly include light[13,14], temperature[15,16], electric[17] and magnetic[18] fields, pH[16,19], ultrasound[20], and redox[21]. Much of the recent attention to these materials is for their potential for use in drug delivery.[11] Some popular formats for drug delivery systems (DDS) are micelles[22], lipid nanoparticles, microparticles, metal organic frameworks, and coated nanoparticles[12], many of which have stimuli responsive derivatives.

The field of stimuli responsive polymers has gained more attention relatively recently for its potential use in biomedicine.[11,23,24] Many of the early stimuli responsive polymers were materials that switch between an expanded and contracted state in solution in response to heat and pH changes.[23] This is achieved by the polymer changing its affinity for water based on how much energy is in the system.[23]

Within the body there are a variety of pH states.[25] These can be leveraged for DDS purposes.[26] Acidic pH is associated with a variety of disease states including bacterial infections[27] and certain cancers.[25] Additionally, there is a pH drop once cargo is endocytoced.[28] These are situations that could be utilized as functional handles for drug delivery. Some examples of pH induced property changes and potential uses are shown in

Figure 1. Both changes in solubility and hydrogel volume have been reported for use in targeted drug delivery.[13,29–31]

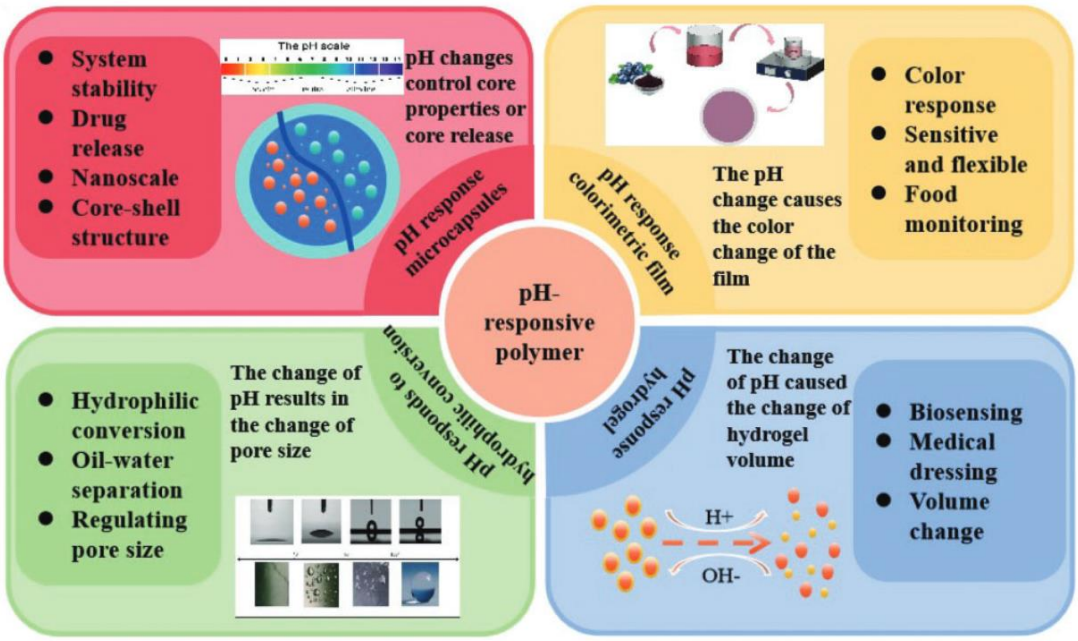


Figure 1. Examples of pH induced response in polymeric materials, reproduced from Yang et al.[26]

The goal of a drug delivery system is to encapsulate or otherwise protect medication within the body until it reaches the active site where it can be released.[32] Drug delivery systems can stabilize unstable drugs during administration, increase payload efficiency, and decrease side effects.[32] Developing biocompatible materials with these abilities is of great interest.

One potential mechanism of action for drug delivery is a solubility switching material.[11,26,33] A polymer that is insoluble in water could encapsulate and protect a drug from being released until it reached a target location at which point the polymer can

resolubilize and release the drug. This would require a polymer that is responsive to some disease state and that would resolubilize into a safe biocompatible material.

2.1.3 Polysaccharides for DDS

One way that is commonly used to make solubility switching materials is to modify a preexisting polymer backbone with functional groups.[32] A popular family of backbones for medicinally relevant materials are polysaccharides.[29] This is due to their high abundance and low cost as well as high biocompatibility.[32] Additionally, the abundance of free hydroxyls makes them easy to functionalize.[32] Because polysaccharides are naturally derived, they are inherently biodegradable.[32]

The main ways that polysaccharides are used as DDS are though polysaccharide-based microparticle encapsulation, the coupling of polysaccharides to drug molecules through cleavable bonds, and the encapsulation of drugs in polysaccharide based hydrogels.[32] Some examples of polysaccharides that have been modified for DDS are cellulose, mannose, pullulan, dextran, starch and cyclodextrin.[29,32] Though there have been in vivo and in vitro studies of polysaccharide DDS, there has not been rigorous testing of any polysaccharide DDS in relation to human pharmacokinetics.[32]

2.2 Physical Properties of DDS Materials

There are many ways to evaluate material; common ones for bulk polymers are: biocompatibility, thermal stability, mechanical properties, crystallinity, and solubility.[34] Because polymers are used for such a wide variety of applications there are many factors to consider when designing a new polymer.

International standards for biocompatibility encompass a wide array of factors. The main considerations are the inherit toxicity of the material, the interactions of the material with blood, local effects of the material on surrounding tissues, the type and amount of degradation products produced and whether it leeches chemicals or nanomaterials.[35] Evaluations for these measures change based on where in the body the material is intended for use.[35] There have been attempts to use models to predict cytotoxicity of materials, but there has not been much success in broad material predictions due to the limits in quantity of experimental data.[36] However, there has been promising work done in models referencing specific material classes such as liquid crystal monomers.[37] Additionally, there has been some success in predicting material impacts on specific biological pathways.[38] Despite recent progress, the most accurate assessments of biocompatibility are still empirical in vitro studies.[36]

The main concerns for applications are processability and stability.[34] Processing techniques for polymers generally involve either melting for molding or solution processing.[34] It is necessary to have a polymer that is cost effective to process and stable to the environment it will be used in.[34]

2.3 Dextran

2.3.1 Properties

Dextran has been found to be an especially proficient polymer for DDS. Dextran is a polysaccharide made by bacteria.[39] The most commonly produced strain is made from *Leuconostoc mesenteroides*.[39] Dextran is an α (1,6) D glucan. It is mainly linear but occasionally branches on the α (1,3) position and less frequently on the α (1,2) and

α (1,4) positions.[39] Its production is relatively cheap and, given that it is grown from bacteria, it is renewable. Dextran is notable in its high degree of biocompatibility.[40] It has received the designation "Generally regarded as safe" from the United States Food and Drug Administration (FDA).[41] One testament to its biocompatibility is its longstanding use in medicine. Since as early as the 1950s it has been used as a plasma volume expander[42], this is due to its slight heparin-like anticoagulant properties.[40]

2.3.2 Acetal Modified Dextran

Dextran has been studied with the intent of use as a DDS.[30,31,33,43,44] A successful example of this is acetalated dextran (Ac-Dex). The most common synthesis of this material is done with the addition of 2-methoxy propene (2-MP) and an acid catalyst to solubilized dextran.[33] The capping of dextran's polar, hydrophilic hydroxyls with less polar, hydrophobic acetals converts it into a hydrophobic polymer and makes it insoluble in water.[33] Acetals are resistant to a wide variety of chemical environments including strong bases, nucleophiles, reductive and oxidative conditions.[45] It is only upon exposure to acidic environments that the acetals will hydrolyze.[45] Once the acetals hydrolyze and fall off the dextran, they release acetone and methanol as byproducts.[33] Both products are relatively biocompatible. Acetone is a secondary metabolite and produced by the human body.[46] Methanol (MeOH), while generally toxic, is harmless in the concentrations released by Ac-Dex degradation.[33] Once the acetals have been hydrolyzed the dextran it will be in its pre-modified state and will resolubilize in water.[31] This type of environmental responsiveness makes Ac-Dex a good candidate for a potential drug delivery system.

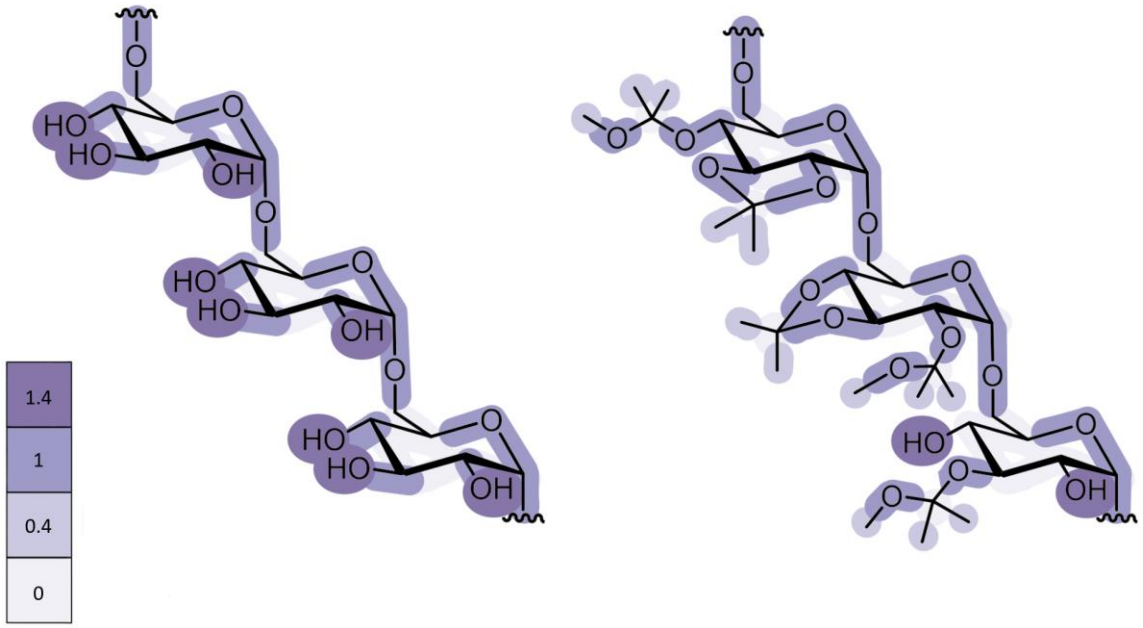


Figure 2. Dextran (left) and Ac-Dex (right) with bonds highlighted by electronegativity difference.

Figure 2 displays the bond polarities of the dextran chain. A polar bond is a special type of covalent bond which experiences a permanent dipole as a result of the electronegativity difference between the two atoms in the bond.[47] Generally, a bond with an electronegativity difference of greater than 0.4 and less than 1.7 is regarded as a polar bond.[47] A larger difference in electronegativity implies a more polar bond. On the dextran and Ac-Dex polymer chains, the C-O bonds and OH bonds are polar. Bonds that are more polar will interact more favorably with water which is a polar solvent.[47] When dextran is modified to Ac-Dex its most polar substituents are replaced with less polar acetals. This disrupts the favorable interactions between the dextran backbone and water and decreases the solubility of the polymer in water.

The solubility switching and biocompatibility of Ac-Dex make it a strong candidate for targeted drug delivery. Additionally, Ac-Dex does not burn, melt, or otherwise degrade at human body temperature. Ac-Dex has shown potential for degradation rate tunability[33], but its heterogeneity in substitution placement hinders the efficacy and precision of this tunability.

3 Substitutional Investigation with Model Monomer

3.1 Introduction

Dextran is a biopolymer most commonly derived from *leuconostoc meserentoidies.*[39] Dextran has a long history of use in the medical field and has been evaluated by the FDA to have the rating Generally Regarded As Safe.[41] Dextran has previously been modified into a solubility switching polymer for the purpose of targeted drug delivery.[31,33,43] This is achieved through capping the hydroxyls of dextran with acetals. The hydroxyls on dextran impart hydrophilicity due to their polar nature. By capping them with hydrophobic acetals, the entire polymer becomes insoluble in water. This reaction is convenient because of its rapid kinetics. After just 2 minutes it is possible to isolate Ac-Dex, but the acetal composition will continue to change for hours if the reaction is left running.[33] Additionally, this reaction runs at room temperature and preliminary studies by past group members indicate that the reaction can be run neat. Each of these factors — time, energy, and solvent use — are important considerations to optimize for the purposes of green chemistry[48], as well as financial feasibility of industrialization. Acetals are stable to a wide variety of extreme conditions including neutral and basic reductions, aqueous and non-aqueous bases, hydride reductions, most oxidants, and nucleophiles including organometallics.[45] The acetals will degrade under aqueous acidic conditions.[45] This is a good property for a drug delivery vehicle because it means that it will remain insoluble as long as the acetals are on the dextran and that the acetals will stay on until exposed to an acidic environment.

There are a wide variety of biological environments associated with decreased pH such as bacterial infections, being endocytosed into a cell, and certain cancers.[25] All of these are places that could benefit from targeted delivery. For example, one of the issues with antibiotics is that they kill all the bacteria in the body both good and bad. This leads to a wide variety of potential side effects such as nausea, diarrhea, and yeast infections.[49] If an antibiotic were to only be released at the site of infection, then many of those symptoms could be avoided. This is just one possibility for a way to use drug delivery vehicles. There are many other acid associated disease states that are ripe for exploitation.

Drug encapsulation for targeted delivery is a relatively new but effective procedure. One such example that has recently gained attention are the lipid nanoparticles (LNP) used in the COVID-19 vaccine.[22] The LNPs were able to safely transport the mRNA though the human body until it reached the desired location and protected it from the body's immune system en route.[22] One disadvantage of these LNPs was that they could not protect the mRNA under a wide variety of temperatures and had to be stored in the cold.[50] This caused problems in the distribution due to an incomplete cold chain globally.[50] Ac-Dex has been shown to form microparticles both with single and double emulsion techniques.[33] Ac-Dex microparticles differ from LNPs in that they are a continuous polymer rather than a membrane like the LNPs. Ac-Dex microparticles have been used to encapsulate drugs and have been shown to stabilize enzymes for delivery at room temperature.[44]

Ac-Dex has been fairly well characterized and was patented almost a decade ago in 2017.[51] Despite having been around so long there has yet to be a detailed study into the substitution type and rate of acetal addition. Disregarding intermonomer cyclizations,

there are 12 possible ways that each monomer can be acetal modified, with 0, 1, 2, or 3 hydroxyls capped and with either linear or cyclic acetals as shown in Figure 3. With an inability to directly favor certain modifications over others the polymer can become very heterogenous in its modifications. This means that under the same reaction conditions, slightly different polymers are made every time. Currently, there is no protocol for making a homogenous version of this polymer or even directing it to make strictly linear versus cyclic acetals. Additionally, there is no direct evidence of what modifications are occurring on a monomeric level, only reports of cyclic vs linear acetal degradation products on a polymer wide level. Linear acetals degrade into acetone and MeOH whereas cyclic acetals degrade into acetone. By measuring the relative rates of acetone to MeOH it is possible to determine the relative rates of cyclic to linear acetals.[33] This heterogeneity affects the polymer's physical properties, the most notable of which being its degradation rate. Ac-Dex has shown to degrade slower when there is a higher concentration of cyclic acetals and faster with a higher rate of linear acetal modification.[33] This is due to the fact that cyclic acetals are more stable than their linear counterparts.[52] There is interest in more direct investigation into the substitution of Ac-Dex with hopes that increased knowledge of substitutional affinity will lead to the development of more homogenous polymers.

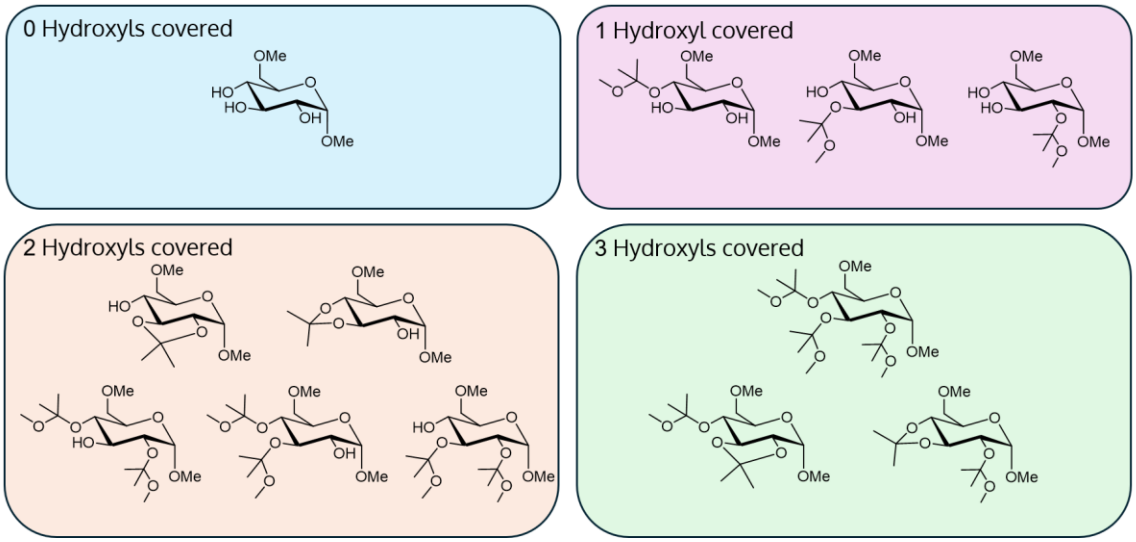


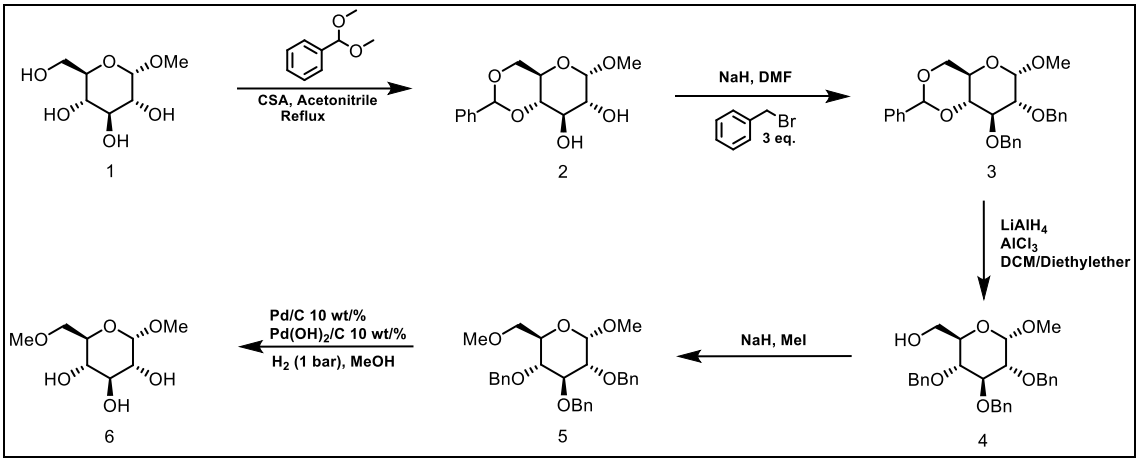
Figure 3. The 12 possible intramonomer acetal coverages.

In order to investigate which modifications were occurring, a model system was proposed. The model would be 1,6-dimethyl-α-glucose. Dextran is a 1,6 linked alpha glucoside, so the 1,6 methyl caps on the hydroxyls would simulate the glycosidic bonds. Theoretically, by running the acetalization reaction on the monomer rather than the polymer it would be possible to collect and separate all the different modifications that are occurring on the monomers and fully characterize each set of modifications by nuclear magnetic resonance (NMR). Following this, the different modification levels would be evaluated for relative abundance. Ultimately, the goal would be to test a variety of reaction conditions on a monomeric system in an attempt to determine whether a set of reaction conditions favors certain modifications over others to make a more uniformly modified polymer. Additionally, this investigation could potentially elucidate the relative reactivities of the hydroxyls in regards to 2-MP.

3.2 Results and Discussion

The model monomer compound used to simulate dextran for the purpose of an acetal modification reaction is not commercially available and therefore had to be produced in lab. The reaction sequence to prepare α-D-1,6 dimethyl glucose was adapted from Dibbert et al.[53] and is shown in Scheme 1.

Scheme 1.



It was necessary to synthesize α-D-1,6 dimethyl glucose **6** because it is not commercially available. Selective modification of monosaccharides is especially difficult due to the similarity of the hydroxyls. Hydroxyls are moderately reactive functional groups and act well as both acids and nucleophiles. There is not much sterically distinguishing the hydroxyls on the starting material, 1-methyl glucose **1**, from one another. Their chemical environments are incredibly similar; three are connected to a ring hydrocarbon and one is separated from the ring by a methylene unit. In this case stereochemistry is one element that helps to afford some distinguishability. The physical similarity of the hydroxyls regarding position and chemical environment is illustrated in Figure 4.

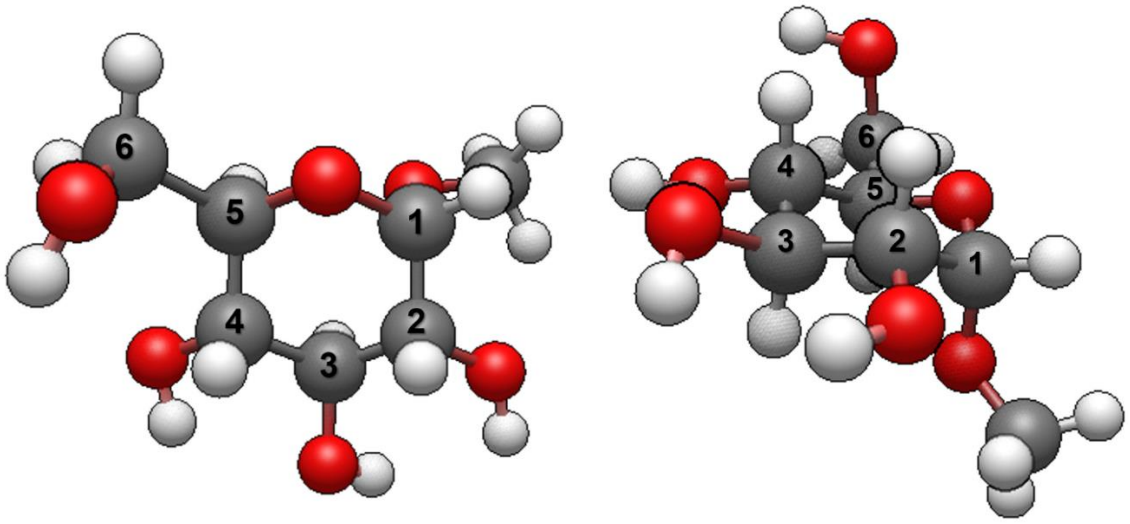


Figure 4. 1-methyl glucose from two angles where red atoms are oxygen, grey atoms are carbon, and white atoms are hydrogen. Made in Avogadro2 using GAFF for geometric optimization.

Due to the similarities in reactivity, it was necessary to cap the hydroxyls that were not to be modified before methylating the hydroxyl of interest to achieve the desired selectivity. For this purpose, α-D-1 methyl glucose **1** underwent an acetal exchange with benzaldehyde dimethyl acetal leading to the protection of the 4 and 6 hydroxyls yielding **2**. This was followed by alkylation with benzyl bromide by the Williamson ether synthesis (S_N2 type mechanism) to benzylate the remaining hydroxyls to give **3**. Product **4** was achived through a lewis acid promoted regioselective reductive cleavage by $LiAlH_4$ and $AlCl_3$. Literature suggests that these reagents tend to cleave acetals on the C-O bond preferential to the O on a less substituted carbon.[54] The free hydroxyl was methylated through an alkylation by MeI to give product **5**. This was followed by a global deprotection to give the selectively methylated sugar of interest **6**.

The deprotection step from **5** to **6** did not proceed smoothly. The relative amounts of Pd/C to Pd(OH)/C necessary for the transfomation have been rigorously investigated by previous group members and were not explored again in the optmimiation of this procedure. Initial attempts at hydrogenolysis yeilded only partial debenzylation due to a lack of sufficient H_2 to preform the transformation and catalyst poisioning. In order to force the reaction to proceed, it was necessary to regenerate the Pd/C catalyst. This was done by heating the catalyst at 60°C for an hour in chloroform and acetic acid in a 2:3 volumetric mixture. Following this, the solvent was filtered off and catalyst was dried at 110°C for 6 hours. The catalyst was then washed again in a fresh mixture of chloroform and acetic acid for 50 minutes at 60°C, followed by 15 minutes of sonication. Solvent was filtered off and the catalyst was rinsed in water and EtOH. It was then allowed to dry overnight at 110°C. Finally, all fully protected and partially deprotected intermediates were reacted with the regenerated Pd/C, Pd(OH)/C and excess H_2. This resulted in the full deprotection of all benzylated hydroxyls in solution.

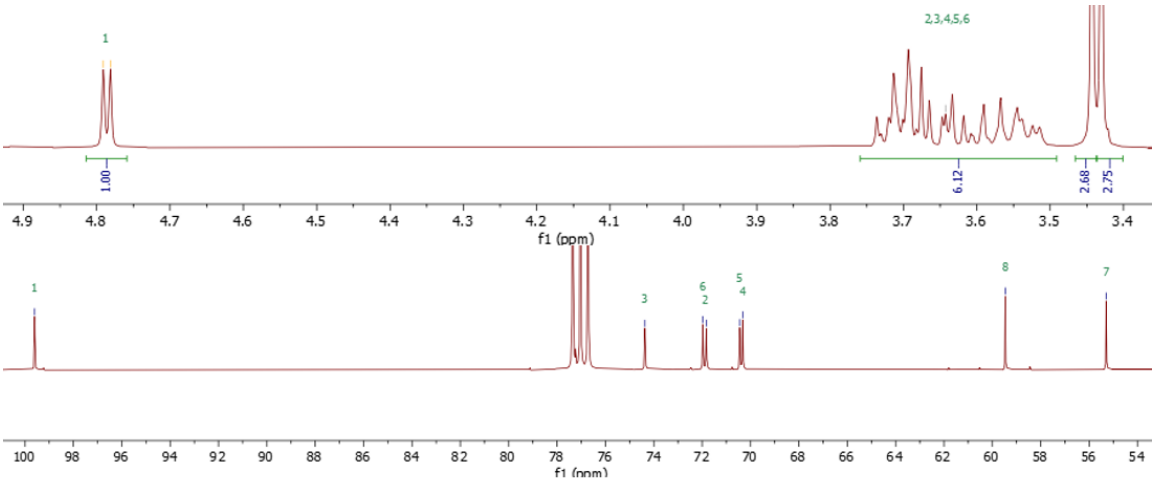


Figure 5. ^{13}C and ^{1}H NMR of Compound **6** in $CDCl_3$

Figure 5 displays the ^{13}C and ^{1}H NMR of Compound **6**. The peaks match literature values[53], and all expected atoms are accounted for. Unfortunately, a proton spectrum alone is not enough to confirm the synthesis of this compound. Heteronuclear single quantum coherance (HSQC) allows for the coupling of the proton and carbon spectra. This allows for more careful charactarizartion of the ring region of the sugar which had significant overlap when veiwed with ^{1}H alone. The HSQC of the synthesized comopund is displayed in Figure 6. Given the close match between compound **6**'s peaks and expected peaks, it is assumed that the compound collected is indeed of the expected structure.

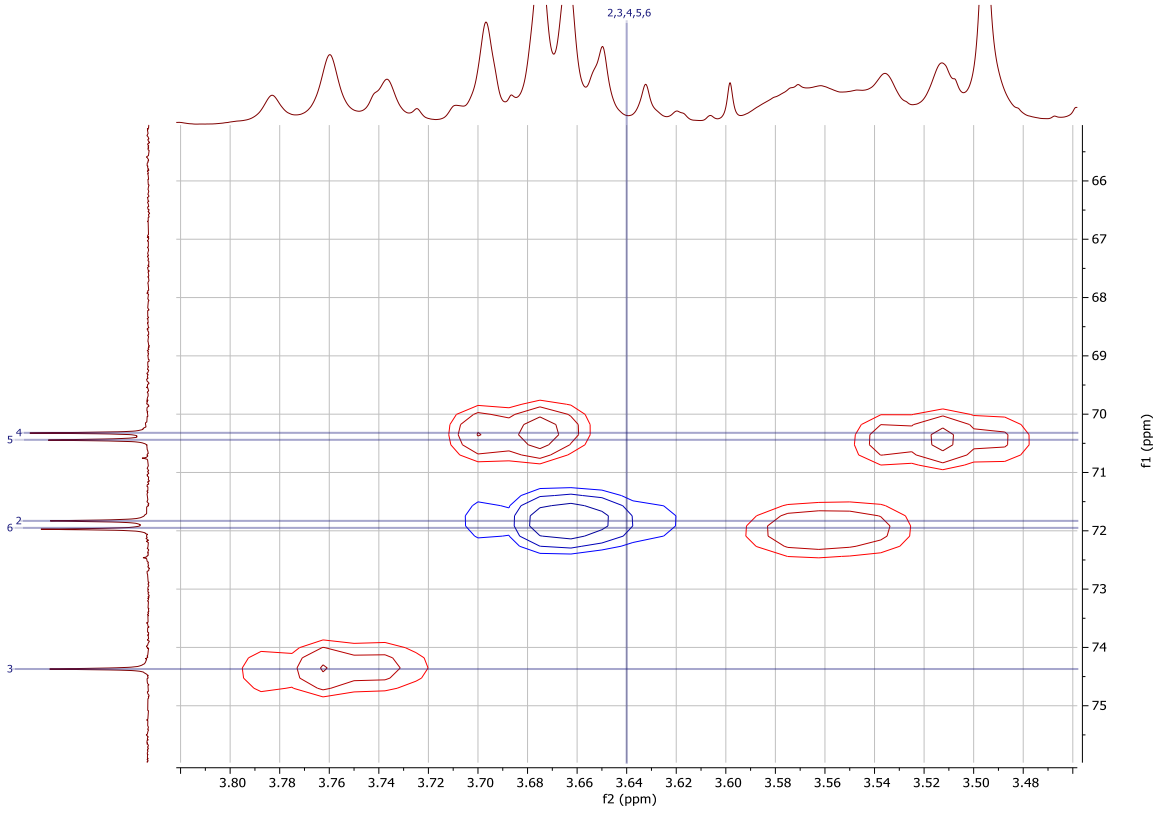
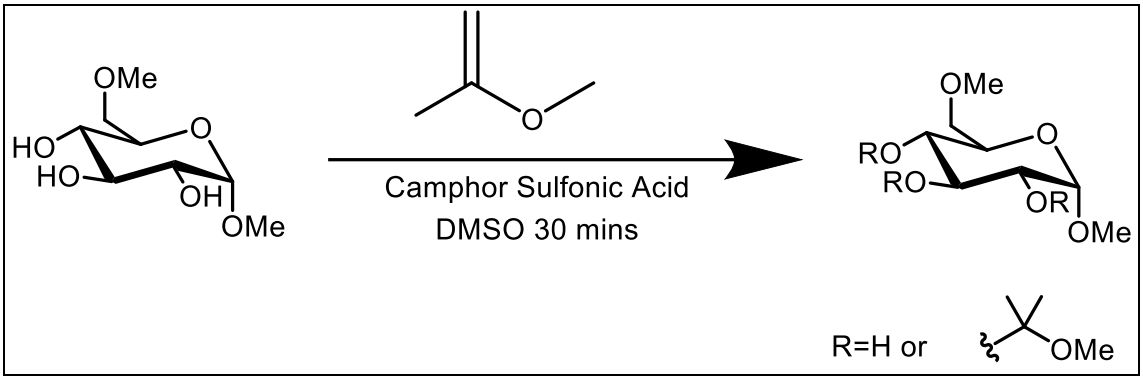


Figure 6. HSQC of α-D-1,6-dimethyl glucose ring section

The HSQC allows for clearer identification of the ring section of the NMR. In an HSQC the red and blue outlines on the graph represent areas of correlation between the proton and carbon spectra. The HSQC, as shown in Figure 6, correlates the carbon peaks in the range of 70.0 ppm through 75.0 ppm to the ring protons.

Scheme 2.



Once substrate **6** had been sucessfully prepared, the standard reaction for the synthesis of Ac-Dex was run using monomer **6** in place of dextran as shown in Scheme 2 which displays the acetalation reaction of 1,6-dimethyl glucose with 2-MP. There were many attempts to run this reaction, followed by a variety of work ups. Unlike polymeric Ac-Dex, the acetalated monomer could not be precipitated into water because it was too small. Because of this the reaction was instead combined with Ethyl Acetate (EtOAc) and the organic layer was washed with basic water. It was found that this work up degraded the newly formed product. In order to investigate conditions of potential stability for the monomer, the reaction was run and subsequently quenched with triethylamine. It was then partitioned into a variety of conditions designed to probe stability. The reaction solution was aliquoted into a panel of basic solvents while others were left in the

quenched reaction conditions with variation to light and temperature. Table 1 displays the conditions tested.

Table 1. Conditions aliqouts of quenched reaction solution were placed in and left for 24 hours where X indicates complete degradation occured over that period.

(a) In DMSO

	25°C	-20°C
Light	X	N/A
Dark	X	X

(b)With added K_2CO_3

	Light
D_2O	X
Et_2O	X
EtOAc	X

It was found that the product degraded in all the tested conditions, most notibly in basic reaction solution in a dark freezer. This condition was the lowest energy condition and was tested as an extreme to see if degradation could be prevented. One hypothesis for the observed instability was that the acid may not have been fully quenched by the triethylamine. In order to test this, the reaction was run again and quenched with DBU. Following this a 2D thin layer chromatorgaphy (TLC) was run to check if seperation by column chromatography would be possible. 2D TLC is shown in Figure 7. In a 2D TLC a square silica plate is used and the compund of interest is placed in a corner, the TLC is then run, following this the plate is dried and rotated 90 degrees and run again with the same eluent. If the compound remains stable throughout this test, then it should fall along the line diagonal through the TLC square, indicating that it moved up the same amount both times and that its polarity remained the same. If instead a compound degrades on the

silica plate, it will end up off the diagonal line. This is due to a change in polarity during the degradation, which results in a different retention factor (Rf) value in each of the TLCs. It was found that even with copius triethylamine degradation occurred.

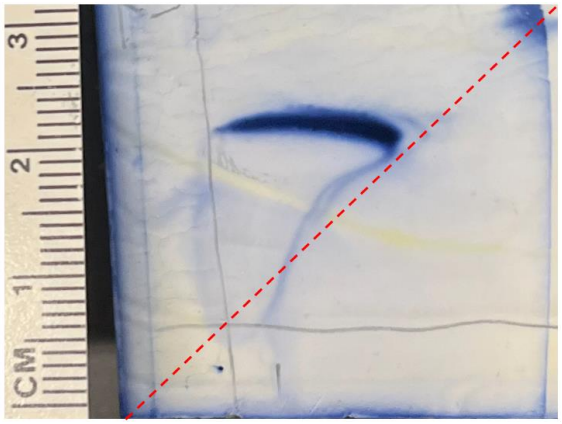


Figure 7. A 2D TLC of acetal modified compound **6** 1:9 MeOH:$CHCl_3$ with added triethylamine and stained with CAM. Red line added electronically to illustrate where a stable compound would appear.

Due to the inability to isolate the material of interest there began to be doubt as to whether the expected transformation was occuring. In order to get an in depth look at the reaction, a kinetics NMR of the reaction was run. The reaction was run for 24 hours in an NMR with a spectral recording 10 times per hour.

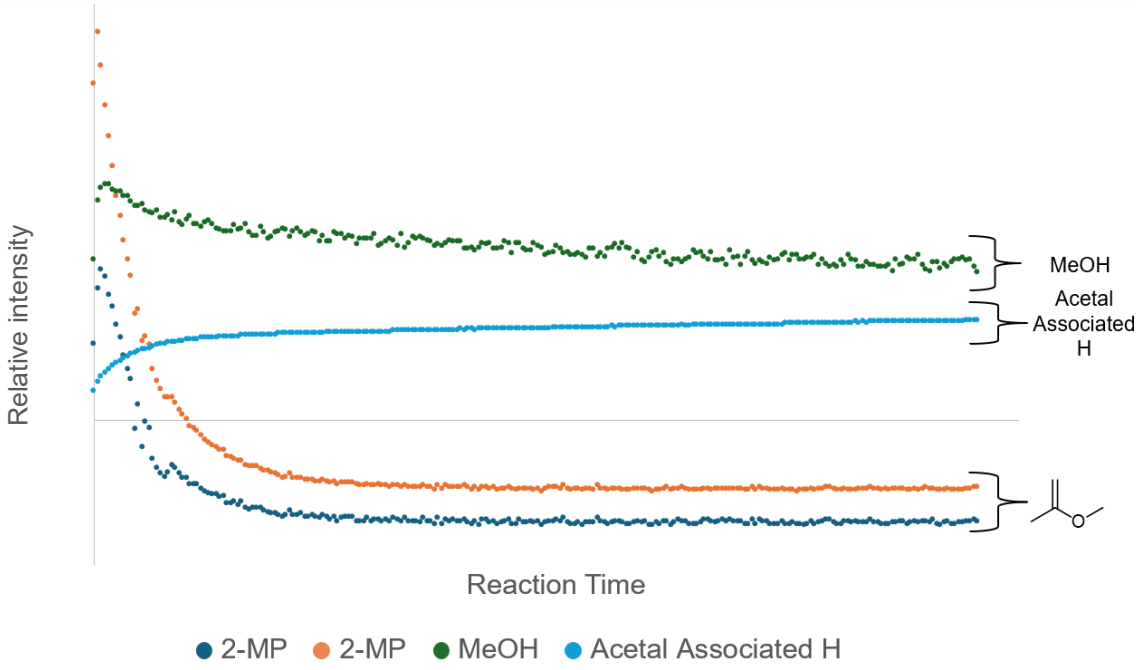


Figure 8. Change in peak area over the time of reaction measured by kinetics NMR, MeOH peak area reduced by a factor of 10, acetal associated peak area reduced by factor of 100.

The kinetics NMR confirmed, as shown in Figure 8, that the 2-MP reagent was reacting and that peaks were being formed in the region that would be associated with acetals. Many side reactions occurred in the kinetics NMR. Usually, these products are removed during purification; but because the kinetics NMR is of the crude reaction, all side reactions present were visible on the spectrum. To identify which peaks were associated with the main reaction and which were associated with side reactions it was necessary to anticipate likely side reactions and check if they appear on the spectra. 2-MP is a reactive nucleophile, this means that it is liable to a wide variety of side reactions such as an addition of water to form a hemiacetal or a self-polymerization. There was no side reaction of polymerization by the 2-MP observed. MeOH was another side product

that occurred. The production of MeOH is a result of acetals cyclizing but can also be due to acetal degradation.

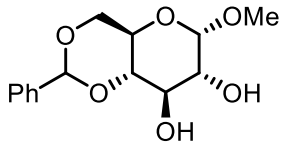
Given the general instability of the monomer relative to the polymer, it was deemed not an accurate representation of the polymeric material. One of the most favorable properties of Ac-Dex is that it is robust and only degrades under highly specific conditions. Additionally, with no way to isolate the monomers it would not be possible to characterize individual modified monomer variations. Due to these factors, it was determined that this monomeric compound was not an accurate or effective model for Ac-Dex.

Recent work has investigated the possibility of using this monomer to model other possible dextran modifications such as tetrahydrofuran and tetrahydropyran capped dextran. This work shows promise as these modifications are stable enough to be separable. Another avenue for improvement to this system would be a change in the monomer to a commercially available starting material. α-D-1 methyl xylopyranoside is of a similar structure to α-D-1,6 dimethyl glucopyranoside, but is missing the 6 carbon and associated methyl capped hydroxyl. This starting material would be preferable because it is commercially available, and this system does not seek to investigate steric effects of the glycosidic bonds or the reactivity of the 6-position hydroxyl so the missing 6 carbon should not negatively impact the results.

3.3 Materials and Methods

Materials: Chemicals were purchased from Sigma Aldrich, Oakwood Chemicals, and Acros Organics without further purification unless otherwise specified. Nuclear magnetic resonance experiments were performed on a Bruker Avance III 400 MHz spectrometer. Lyophilization was done on a FreeZone 4.5 (Labconco, USA). Reactions run under argon atmosphere unless otherwise specified.

Synthesis of Compound **2**

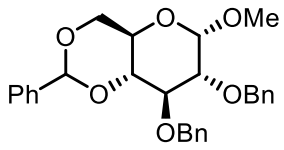


α-D-methyl glucopyranoside **1** (10 g, 51.5 mmol, 1.0 eq.) was dissolved in acetonitrile (250 mL). Benzaldehyde dimethyl acetal (13.9 mL, 92.7 mmol, 1.8 eq.) and camphor sulfonic acid (1.196 g, 5.15 mmol, 0.1 eq.) were added to solution. Reaction was heated to reflux for an hour to ensure all solids had dissolved. Following dissolution, the reaction was cooled to room temperature and quenched with a drop of triethylamine. About 150 ml of solvent was removed in vacuo. EtOAc (200 mL) was added, and the organic layer was washed with water (3x 50 mL) and dried over $MgSO_4$. Solvent was removed and crude product was recrystallized (CH_2Cl_2/petroleum ether= 1:10) producing capped compound **2** (5.15 g, 18.2 mmol, 35.4%) as cottony white crystals. Product **2** was found to be stable in ambient conditions for over a year.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 2H), 7.42 – 7.31 (m, 3H), 5.53 (s, 1H), 4.79 (d, *J* = 3.9 Hz, 1H), 4.29 (dd, 1H), 3.92 (td, *J* = 9.3, 1.9 Hz, 1H), 3.86 – 3.78 (m, 1H), 3.78 – 3.71 (q, 1H), 3.62 (td, *J* = 9.2, 3.9 Hz, 1H), 3.46 (t, 1H), 3.45 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.04, 129.25, 128.32, 126.34, 101.93, 99.80, 80.92, 72.81, 71.60, 68.92, 62.35, 55.53.

Synthesis of Compound **3**

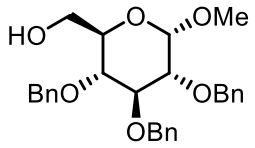


Compound **2 (**5.15 g, 18.24 mmol, 1.0 eq.) was dissolved in DMF and cooled to 0°C. NaH (60% dispersion in mineral oil, 3 g, 72.97 mmol, 4.0 eq.) was added portionwise to the reaction and the solution was allowed to stir for 30 minutes. Benzyl bromide (6.5 mL, 54.72 mmol, 3.0 eq.) was added dropwise at 0°C and the reaction was left to stir at room temperature for 20 hours. Reaction was quenched with 1 mL of MeOH. Solvent was removed in vacuo and crude product was run through a hot filtration in EtOH then crystallized. Compound **3** (5.084 g, 11.81 mmol, 64.7%) was recovered as a colorless solid. Product **3** was found to be stable when stored in a desiccator for over a year.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.45 (m, 2 H), 7.42 – 7.27 (m, 13 H), 5.55 (s, 1H), 4.95 – 4.80 (m, 3H), 4.74 – 4.64 (m, 1H), 4.60 (d, *J* = 3.7 Hz, 1H), 4.27 (dd, *J* = 10.0, 4.6 Hz, 1H), 4.05 (t, *J* = 9.3 Hz, 1H), 3.83 (td, *J* = 10.0, 4.9 Hz, 1H), 3.71 (t, *J* = 10.2 Hz, 1H), 3.60 (t, *J* = 9.4 Hz, 1H), 3.56 (dd, *J* = 9.3, 3.7 Hz, 1H), 3.40 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.77, 138.18, 137.45, 128.92, 128.47, 128.32, 128.23, 128.13, 128.04, 127.93, 127.60, 126.06, 101.25, 99.23, 82.15, 79.21, 78.61, 75.34, 73.78, 69.06, 62.34, 55.35.

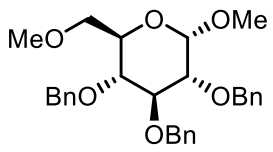
Synthesis of Compound **4**



Product **3** (5.084 g, 11.817 mmol, 1.0 eq) was dissolved into a 1:1 solution of DCM:Et_2O (250 mL) and heated to 50°C. This was followed by a slow addition of $LiAlH_4$ (3.134 g, 82.72 mmol, 7.0 eq.). Once everything was dissolved $AlCl_3$ (7.18 g, 53.18 mmol, 4.5 eq.) was slowly added to solution. The reaction was stirred at 50°C for 1 hour and 15 minutes after which the solution was cooled to room temperature and quenched with a potassium sodium tartrate solution. EtOAc was added to solution and the aqueous layer was washed with EtOAc (3x 250 mL). The organic layers were combined, rinsed with brine and dried over $MgSO_4$. Solvent was removed in vacuo leaving product **4** (4.59 g, 9.88 mmol, 83.61%) as a colorless oil. Product was characterized by ^{1}H NMR which closely matched literature values.[53]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 15H), 4.99 (d, *J* = 10.9 Hz, 1H), 4.94 – 4.76 (m, 3H), 4.66 (d, *J* = 9.6 Hz, 1H), 4.64 (d, *J* = 8.6 Hz, 1H), 4.56 (d, *J* = 3.6 Hz, 1H), 4.01 (t, *J* = 9.2 Hz, 1H), 3.83 – 3.73 (m, 1H), 3.73 – 3.61 (m, 2H), 3.57 – 3.46 (m, 2H), 3.36 (s, 3H), 1.26 (s, 1H).

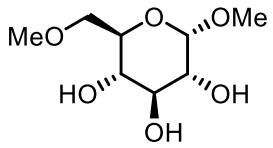
Synthesis of Compound **5**



Product **4** (4.59 g, 9.88 mmol, 1.0 eq.) was dissolved in DMF cooled to 0°C. The solution was then treated with NaH (60% dispersion in mineral oil, 0.95 g, 24.7 mmol, 2.5 eq) and reacted for 60 minutes at 0°C. Following this MeI (1.54 mL, 24.7 mL, 2.5 eq.) was added to the cooled solution, which was then allowed to warm to room temperature and reacted for 22 hours. The reaction was quenched with MeOH and solvent was removed in vacuo. Water was added (100 mL) to crude product and the aqueous solution was washed with DCM (3x 100 mL). The organic layers were combined and washed with brine and dried over $MgSO_4$. Solvent was removed in vacuo. Product **5** (5.175 g, 10.813 mmol, 109.44%) was recovered as a yellow oil. Product was characterized by ^{1}H NMR which closely matched literature values.[53]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 15H), 4.98 (d, *J* = 10.9 Hz, 1H), 4.90 – 4.74 (m, 3H), 4.69 – 4.55 (m, 3H), 3.98 (t, *J* = 9.3 Hz, 1H), 3.70 (ddd, *J* = 10.1, 3.6, 2.2 Hz, 1H), 3.63 – 3.50 (m, 4H), 3.37 (s, 3H), 3.33 (s, 3H).

Synthesis of Compound **6**



Compound **5** (5.175 g, 10.813 mmol, 1.0 eq.) was added to a flask with an argon atmosphere. To a separate flask 10% Pd/C (0.065 g, 0.64 mmol, 0.1 eq.) and 10% Pd(OH)/C (0.0899 g, 0.64 mmol, 0.1 eq.) were added. A solution of MeOH (100 mL) and acetic acid (5 mL) were used to dissolve compound **5** and cannulate it to the flask with the palladium catalyst. The flask was backfilled with hydrogen (3x) and the solution was sparged with hydrogen. Solution was reacted with hydrogen gas (1 bar) for 24 hours. Reaction was monitored by TLC. Reaction was filtered through a pad of celite, and solvent was removed in vacuo. Compound **6** (2g, 9.6 mmol, 88.8%) was recovered through column chromatography to yield a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (d, *J* = 3.9 Hz, 1H), 3.76 – 3.49 (m, 6H), 3.44 (s, 3H), 3.43 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 99.73, 74.49, 72.10, 71.95, 70.57, 70.45, 59.59, 55.42.

4 Synthesis and Characterization of H_2S Sensitive Modified Dextran

4.1 Introduction

H_2S has recently been found to be an important biomolecule that acts as a gasseotransmitter, or signaling molecule within the body.[55] H_2S is carefully regulated in the body due to its high toxicity, so when considering using it as a trigger, it is important to think about using it in ways that don't negatively impact the body by its consumption or release.[55] Because H_2S levels are usually on the mM scale within the body[55], it would be beneficial to create a material that is highly sensitive to H_2S. Additionally, in the interest of maintaining the body's initial H_2S levels it would increase biocompatibility if the polymer could replace the H_2S it removes from the system so that the H_2S levels are not changing drastically due to polymer interference.

Aryl azide capped dextran was synthesized as a H_2S sensitive counterpart to Ac-Dex. Recent literature has shown aryl azides to be H_2S sensitive caps and triggers.[56] The mechanism of action is that the azide is liable to reduction by the H_2S into an amine.[57] This changes the behavior of the functional group from an electron withdrawing group to an electron donating group. This change in the electronic structure of the molecule allows the capping group to remove itself from the dextran. Figure 9 displays the proposed mechanism of degradation by H_2S.

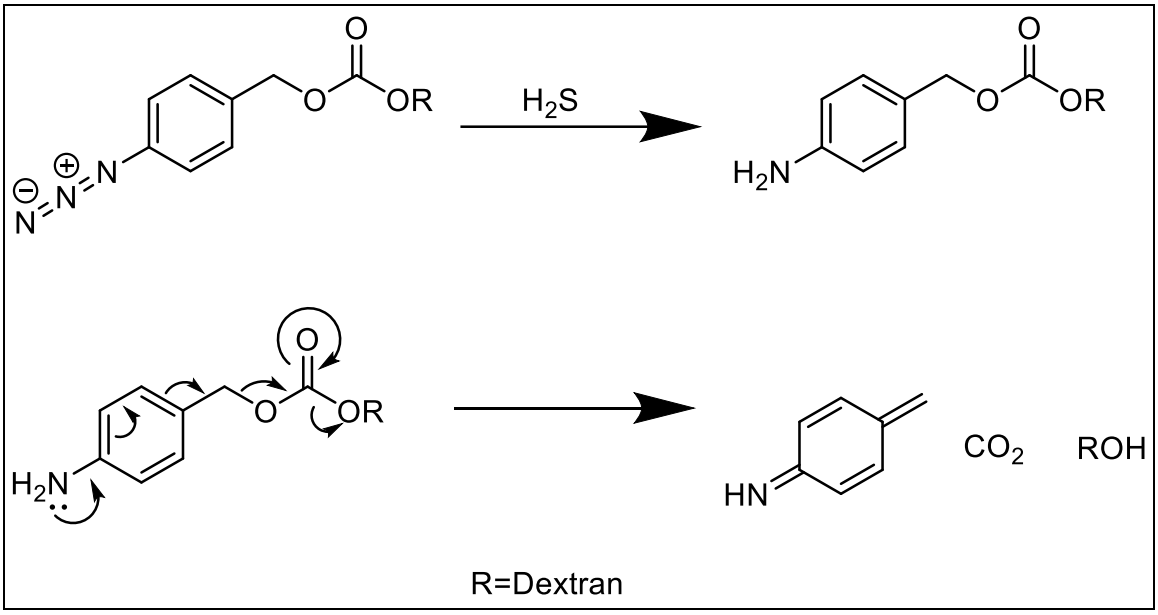


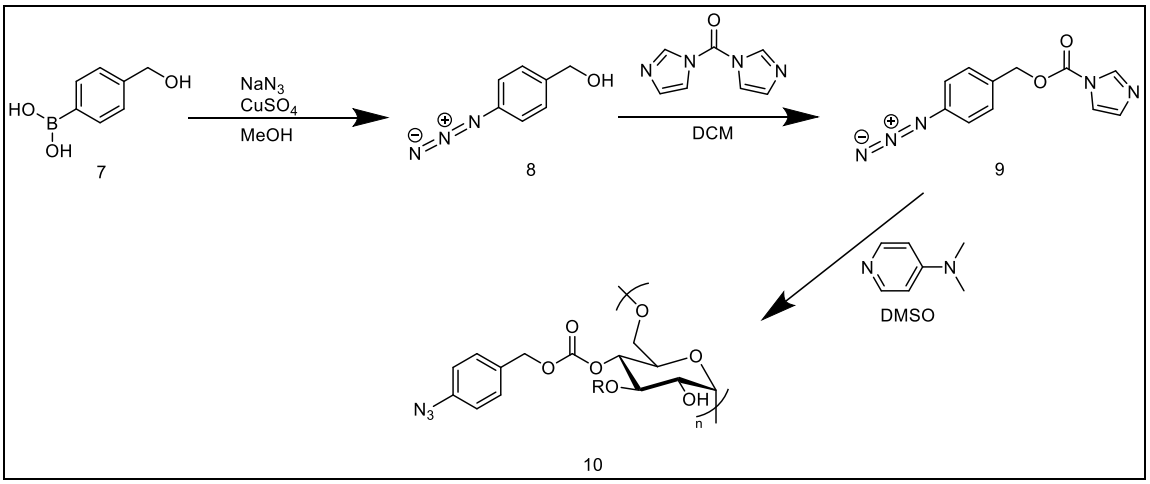
Figure 9. A proposed mechanism of removal of the aryl azide after reduction by H_2S, where R represents dextran and proposed degradation products are aza-p-quinone methide, CO_2 and dextran.

The degradation products based on this mechanism would be CO_2, dextran and aza-p-quinone methide. This proposed behavior of capping and release from the dextran makes ArAz-Dex a good candidate for potential solubility switching material applications. Given that there are a variety of disease states associated with increased H_2S production including certain colon cancers and liver cirrhosis[55], it would be favorable to have targeted drug delivery options based on this trigger.

4.2 Results and Discussion

Aryl azide **8** was synthesized using a method adapted from Tao et al.[58] In this method the copper catalyzed coupling between the boronic acid **7** and azide proceeded under mild conditions in the air. Three different literature methods were attempted with Tao et al.[58] being the only one to yield the desired product. This method of azidonation was favorable because it did not require long reaction times and was not air sensitive. Throughout the reaction and purification, the solution exhibited many colors including opaque brick orange, clear orange-black, opaque green and clear golden yellow. The change in color can be attributed to the copper in solution changing oxidation states. Product **8**'s was confirmed through NMR and infra red spectroscopy (IR). Compound **8** was found to be unstable over long periods of time with exposure to light, so it was stored in the dark at -23°C. The aryl azide carbonyl diimidazole (CDI) adduct **9** was prepared through nucleophilic substitution of the aryl azide **8** to CDI. The reaction was run overnight and monitored by TLC. TLC spots were visualized through exposure to ultraviolet (UV) light, but the spots quickly degraded upon UV light exposure. Aryl azide capped dextran (ArAz-Dex) was successfully prepared through a DMAP-catalyzed addition of dextran to **9.** Typical procedure for Ac-Dex involves precipitating into basic water. Because this is standard practice for Ac-Dex precipitation, ArAz-Dex was precipitated into basic water as well, even though it was almost certainly not necessary. Though almost certainly innocuous, the solution the reaction was precipitated in to quench the reaction was basic water with added triethylamine. It is believed the results would be similar if the reaction was precipitated into standard deionized water.

Scheme 3.



ArAz-Dex substitution was measured via ^{1}H NMR integration to be between 2-3 modifications per monomer. Broad overlapping peaks commonly associated with polymers lowered the precision of this measurement. ArAz-Dex was found to have solubility differing from that of unmodified dextran which is notably soluble in water. ArAz-Dex was soluble in dimethyl sulfoxide (DMSO) and dichloromethane (DCM), partially soluble in acetone and EtOAc. It was found to be insoluble in chloroform, isopropanol, hexanes, Et_2O, and water. Microparticles were made from ArAz-Dex as a single emulsion. Microparticles were characterized by dynamic light scattering (DLS) and found to be fairly monodisperse with a hydrodynamic diameter of 164 nm. Degradation experiments were run on the microparticles to determine the necessary concentration of H_2S to trigger degradation as well as the time scale.

The degradation was evaluated qualitatively though visual inspection of whether the microparticles had resolubilized after exposure to a variety of conditions. The first preliminary test was H_2S (100 μM) in a pH 14 solution to ensure full deprotonation of H_2S. Microparticles were placed in both this solution and a similar H_2S free solution as a

control. After 12 hours the particles in the H_2S solution had degraded. After 48 hours, particles in both solutions had fully degraded. In an effort to investigate more biologically relevant conditions the following degradation experiments were run in a phosphate buffered solution. Solutions were prepared with 10 eq H_2S at pH 10, 11, and 12. As shown in Figure 10, the microparticles in pH 12 solution experienced degradation in 5 days, the pH 11 solution showed microparticle degradation in over a week, and the microparticles in pH 10 solution did not fully degrade after 30+ days. Three solutions at pH 8 were subject to 1 eq, 5 eq and 10 eq of H_2S. As displayed in Figure 11, none of these solutions degraded the microparticles within 30+ days. Given the lack of degradation in solutions that approached but were still quite far from reasonably expectable biological conditions, ArAz-Dex was deemed unsuitable for drug delivery purposes.

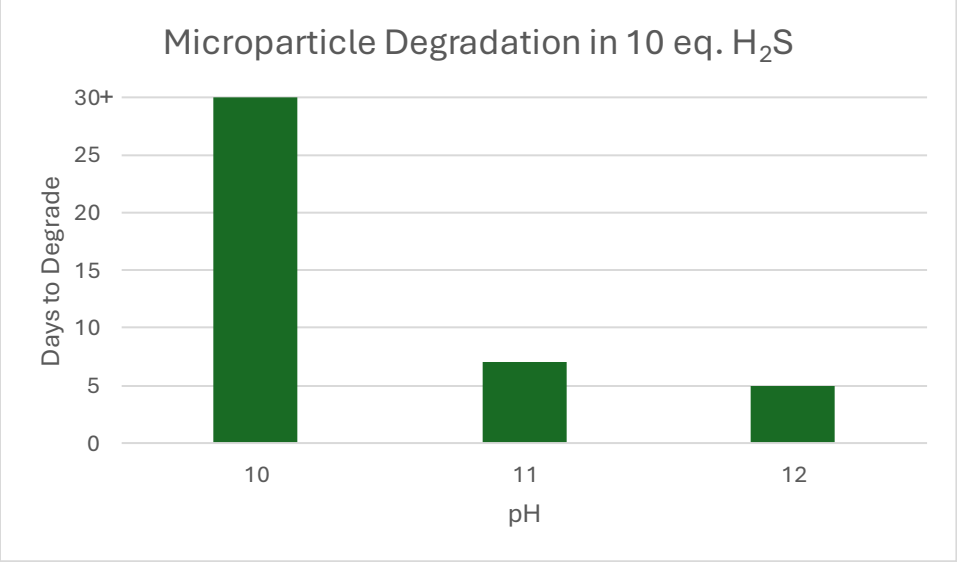


Figure 10. Time necessary for complete degradation of ArAz-Dex microparticles in aqueous solution of H_2S (10 eq.) with varied pH.

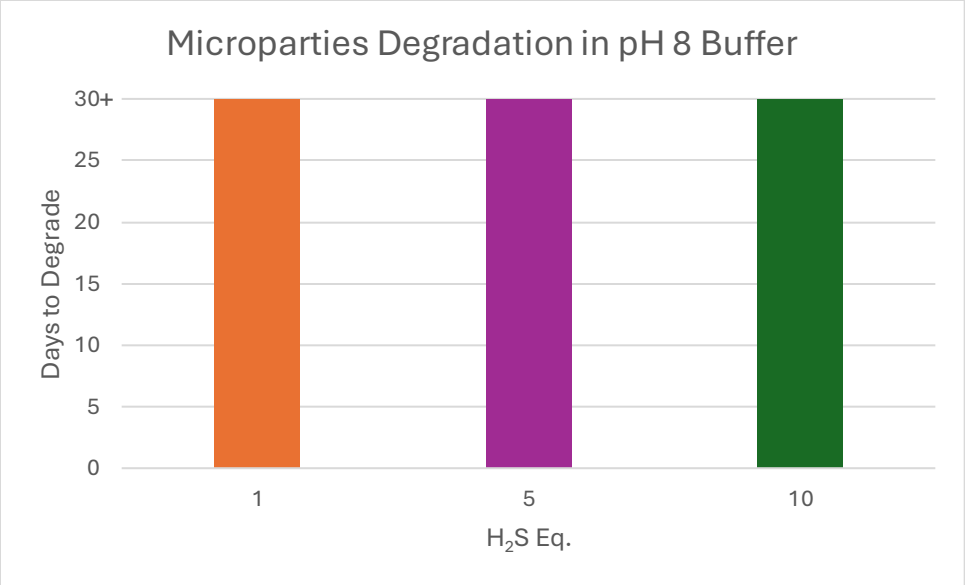


Figure 11. Time necessary for complete degradation of ArAz-Dex microparticles in phosphate buffered pH 8 solution with varying equivalencies of H_2S.

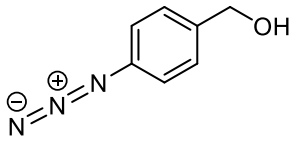
Due to the material's poor degradation kinetics, a similar structure was suggested that would be autocatalytic under biological conditions. If the oxygen of the carbonyl were to be replaced by a sulfur then the byproduct of the reaction would be COS rather than CO_2. There is a biological enzyme called carbonic anhydrase[56] that is responsible for maintaining the pH of blood. Carbonic anhydrase will take COS and produce CO_2 and H_2S.[56] This would mean that for each H_2S used in the reduction of the azide to free the cap from the dextran, one equivalent of H_2S would be released back into the system via conversion by carbonic anhydrase. This would be dually beneficial in that it would maintain H_2S levels in the body, and it would allow the degradation to proceed more rapidly. This project was briefly pursued and then discontinued due to an inability to synthesize the compound with safe reagents. The proposed cap has since been synthesized by the Pluth group at the University of Oregon and used to cap fluorescent

probes.[56] While the field of aryl azide caps for H_2S detection shows promise, ArAz-Dex has been found to be unsuitable for rapid release drug delivery and therefore was not further pursued.

4.3 Materials and Methods

Materials: Chemicals were purchased from Sigma Aldrich, Oakwood Chemicals, and Acros Organics without further purification unless otherwise specified. Nuclear magnetic resonance experiments were performed on a Bruker Avance III 400 MHz spectrometer. Infrared Spectroscopy was performed on Bruker Alpha FTIR-ATR. Dynamic Light Scattering was performed on Malvern Zetasizer ZS. A SFX550 Ultrasonicator (Branson Ultrasonics, USA) was used for particle preparation via single emulsion. Lyophilization was performed on a FreeZone 4.5 (Labconco, USA).

Synthesis of aryl azide **8**



Boronic ester **7** (3.039g, 20 mmol, 1.00 eq.) was dissolved in MeOH (120 mL) and treated with NaN_3 (1.556 g, 24 mmol, 1.2 eq.) which was allowed to dissolve for 15 minutes. Copper (II) Sulfate (0.449 g, 2 mmol, 0.1 eq.) was added to the reaction. The reaction was stirred and monitored by TLC. MeOH was removed in vacuo. Reaction was resolubilized into Et_2O then the organic phase was washed with water (x3 50mL). Solvent was removed from organic phase in vacuo. Addition of the azide was confirmed through IR as shown in Figure 12. Reaction proceeded in high yield (2.81 g, 18.84 mmol, 94%) to produce a dark golden transparent yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (d, 2H), 7.06 – 6.98 (d, 2H), 5.07 (s, 2H).

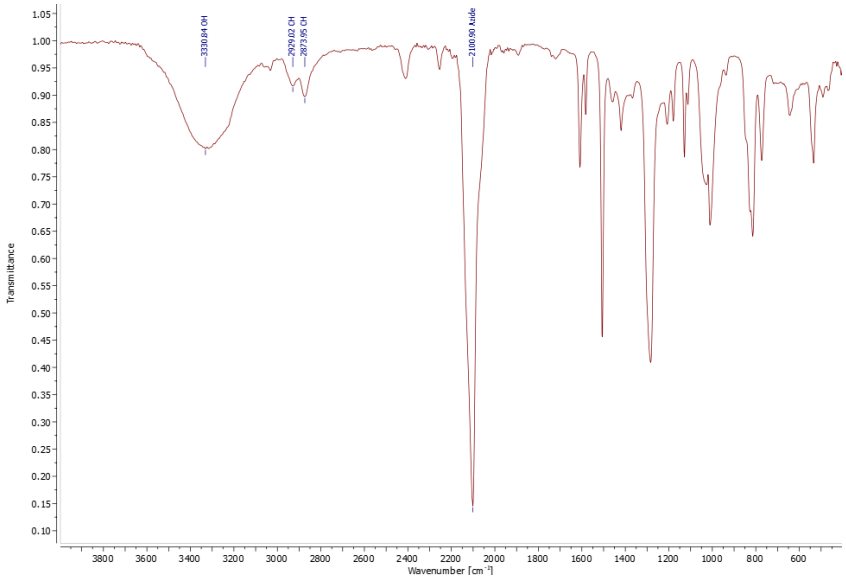
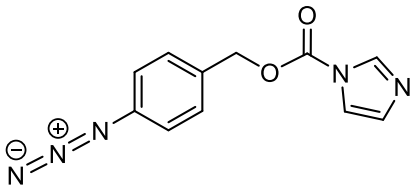


Figure 12. IR of Compound **8** confirming presence of OH (3330 cm^{-1}) and azide (2100 cm^{-1})

Synthesis of aryl azide CDI adduct **9**



Aryl azide **8** (0.745 g, 5 mmol, 1.0 eq.) was dissolved into 60 mL of DCM in a round bottom flask. CDI (2.51 g, 15 mmol, 3.0 eq.) was added to the reaction. The reaction was stirred overnight. Reaction was monitored by TLC. Solvent was removed in vacuo and reaction was transferred to a separatory funnel for purification. Reaction mixture was dissolved in EtOAc and washed with water twice, aqueous $KHSO_4$, and brine then dried over $MgSO_4$. The solvent was removed in vacuo and the product **9** (1.09 g, 4.48 mmol, 89.6%) was recovered as a golden oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.43 (s, 3H), 7.11 – 7.04 (m, 3H), 5.39 (s, 2H).

Synthesis of ArAz-Dex **10**

Dextran (0.242 g, 1.49 mmol, 1.0 eq.) was dissolved in 2 mL DMSO under argon. Once the dextran was fully dissolved the TCI adduct **9** (1.09g, 4.48 mmol, 3.0 eq) and DMAP (0.547 g, 4.48 mmol, 3.0 eq.) were added to the reaction. Solution was allowed to stir at room temperature for 48 hours. Upon reaction completion a drop of triethylamine was added, and the product was precipitated dropwise into pH 8.8 deionized water. Product was vortexed, centrifuged and decanted (3x). Product was lyophilized to remove water. Crude product was recrystallized by precipitation from concentrated DCM solution into hexanes in to give compound **4** (0.4 g) as a tan solid.

^{1}H NMR (400 MHz, CDCl3) δ 6.95 (m, 3H), 4.86 (m, 6H), 3.45 (m, 6H).

Preparation of Microparticles:

Capped dextran **10** (100 mg) was dissolved in CH_2Cl_2 (1 mL). An aqueous solution of 3% w/w poly(vinylalcohol) (PVOH, MW = 13 000 – 23 000 g/mol, 87-89% hydrolyzed) in phosphate buffered solution was added to the dextran solution as a 1.5 mL aliquot. This combined solution was then emulsified by sonicating for 30s on ice using a probe sonicator with a 0.7 s on 0.3 s off cycle at 40% power. The emulsion was added dropwise to a 0.3% w/w PVOH solution and allowed to stir for 4 hours. The particles were isolated

by centrifugation (10,000 x *g*, 10 mins) and washed with deionized water (3 x 50 mL) by vortexing, centrifugation and decantation of the supernatant. Lyophilized to yield a tan solid (16.17 mg). DLS, as shown in Figure 13, confirmed microparticle formation showing particles with a hydrodynamic diameter of 164 nm.

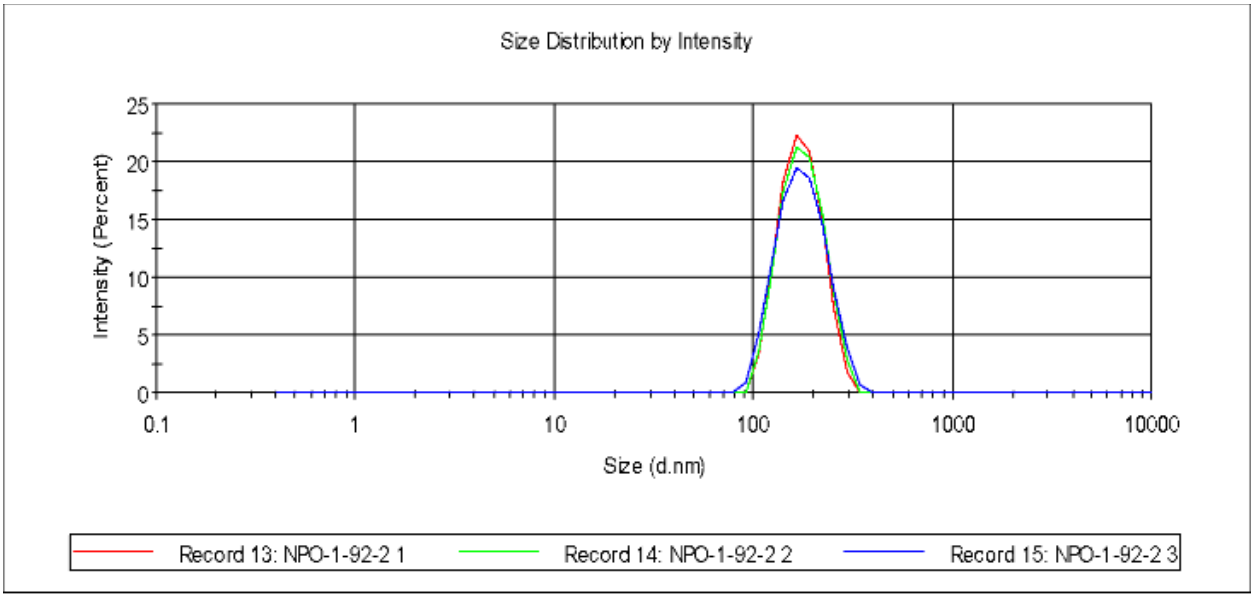


Figure 13. DLS of ArAz-Dex microparticles showing hydrodynamic diameter of 164 nm and a fairly monodisperse set of sizes.

5 Conclusion

Although 1,6-dimethyl-α-glucose was a poor model for Ac-Dex, it showed promise with other forms of modified dextran. It is possible that this method could elucidate the relative reactivities of the hydroxyls on dextran. The model monomer acetalation reaction is just one step taken in a long line of investigation of the structure of Ac-Dex. Substitution heterogeneity in Ac-Dex will continue to be a subject of investigation though likely not through continued use of this monomer.

Dextran is a system with lots of possibilities for modification and improvement. Being a natural product and highly modifiable makes it a competitive choice for further investigation. Ac-Dex is just one example of a modified dextran that works well. 2MP acetal caps remain a good modification but, in regards to synthesizing a more homogenous polymer, it would be more sensible to choose an acetal that can only add in one type of way.

Although aryl azides have shown to be a reliable sensing material for H_2S in literature, the same results were not found with solubility switching. Initially, the material showed promise in material properties because it could degrade under high equivalency H_2S and could form microparticles. While ArAz-Dex did exhibit the expected solubility switching upon exposure to H_2S, the concentrations necessary to trigger degradation were not ones that would be found in biologically relevant conditions. In conditions of H_2S abundance a rapidly responding material is usually more useful, whereas ArAz-Dex would end up as a slow release delivery system with a release window on the span of months.

6 Appendix

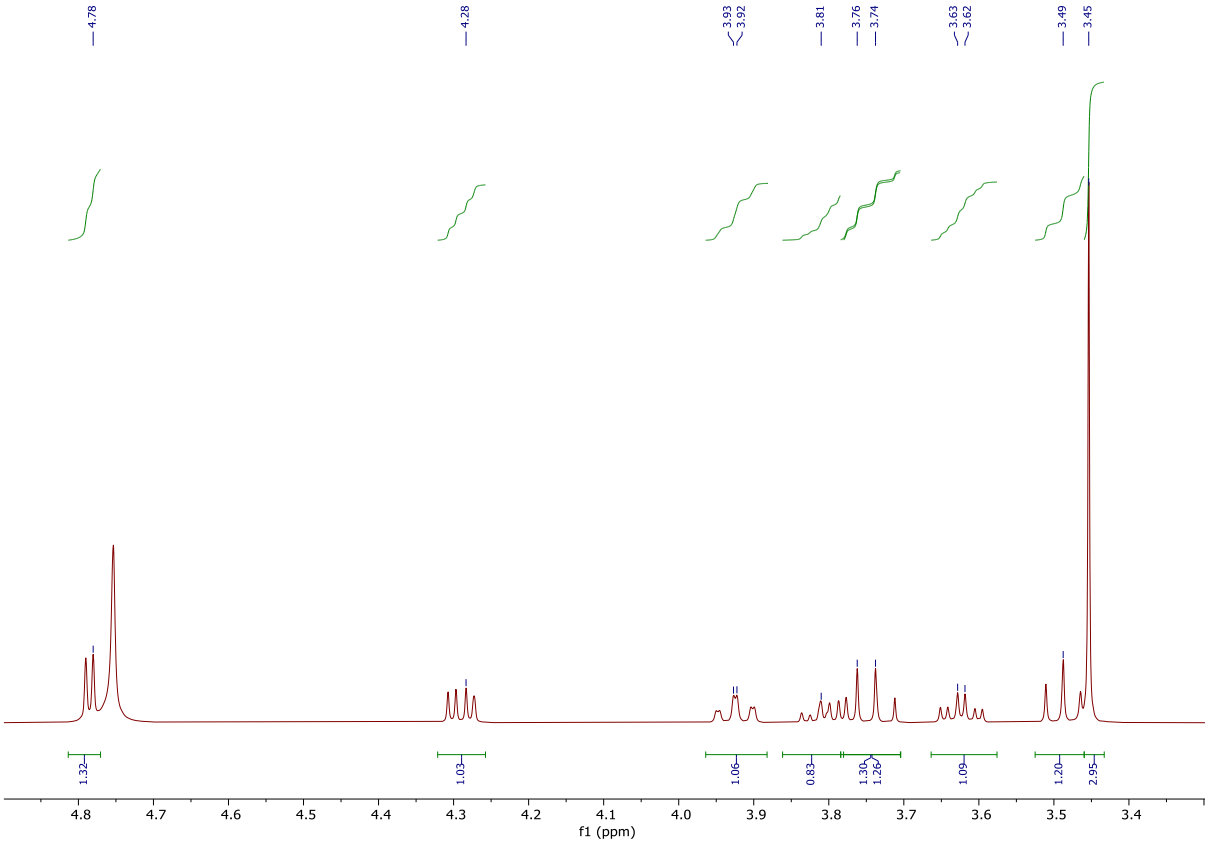


Figure A1. ^{1}H NMR of Compound **2** in $CDCl_3$

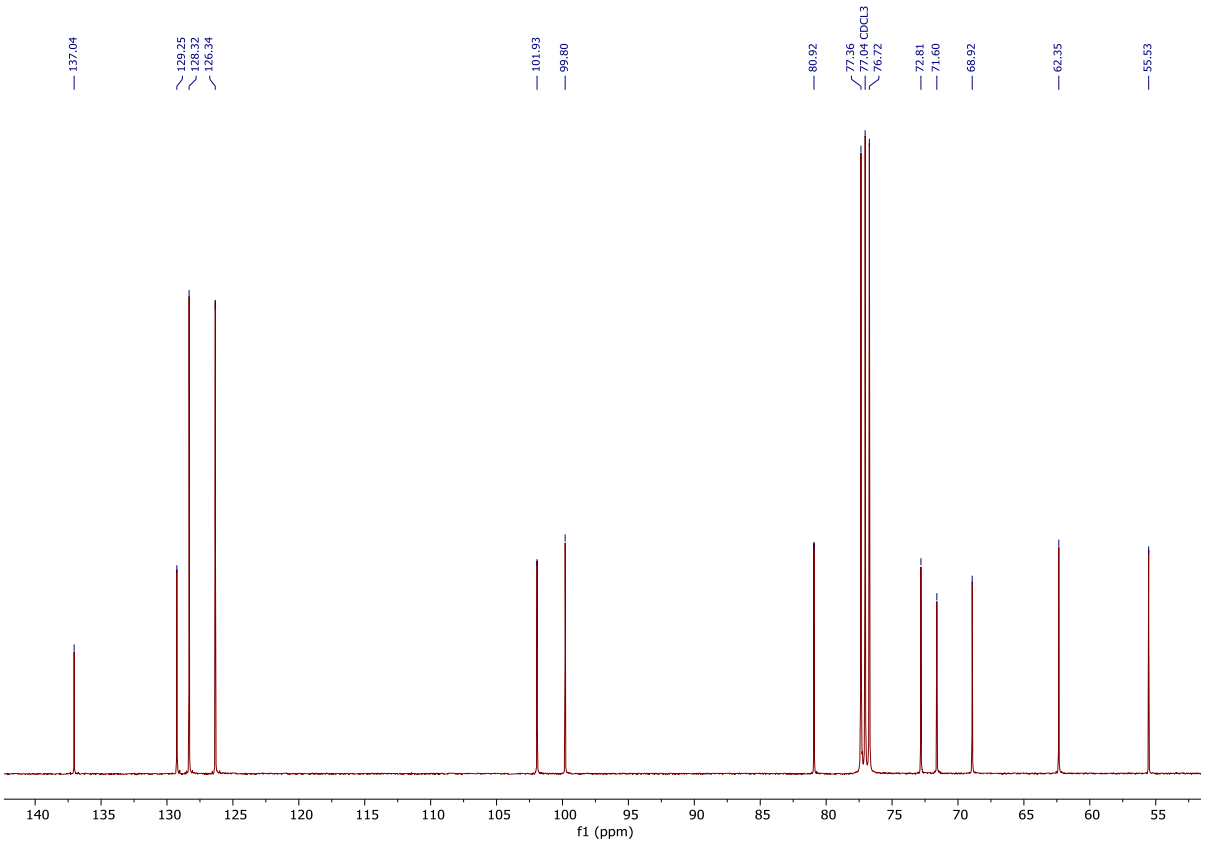


Figure A2. ^{13}C NMR of Compound **2** in $CDCl_3$

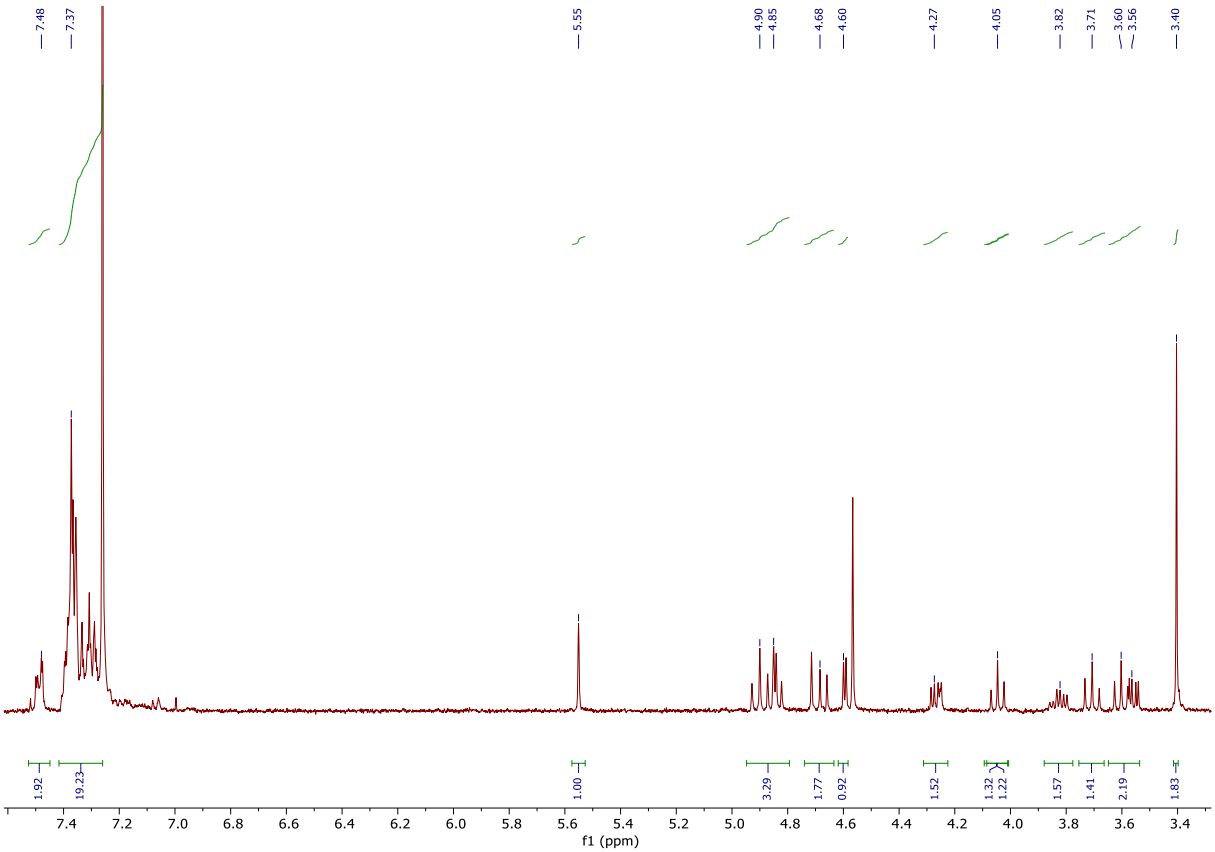


Figure A3a. ^{1}H NMR of Compound **3** in $CDCl_3$

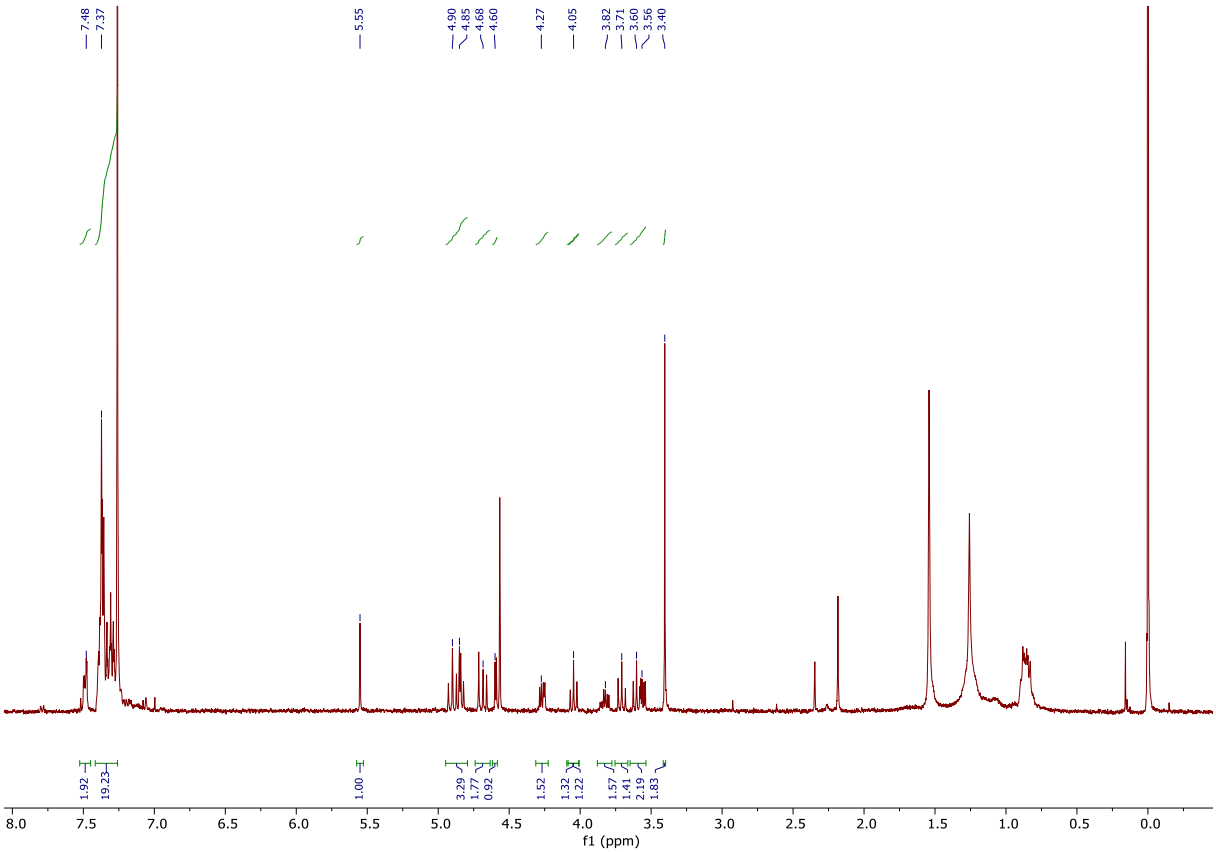


Figure A3b. ^{1}H NMR of Compound **3** in $CDCl_3$ full spectrum

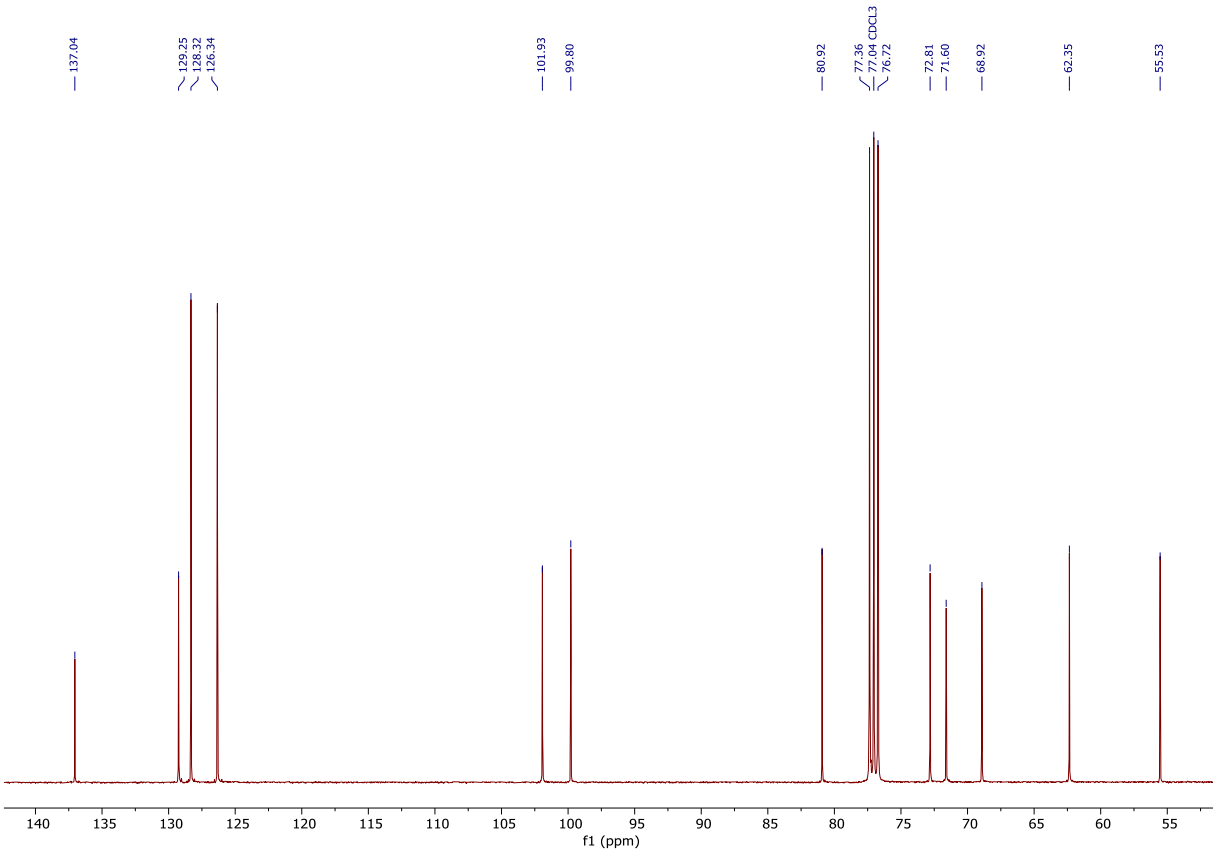


Figure A4a. ^{13}C NMR of Compound **3** in $CDCl_3$

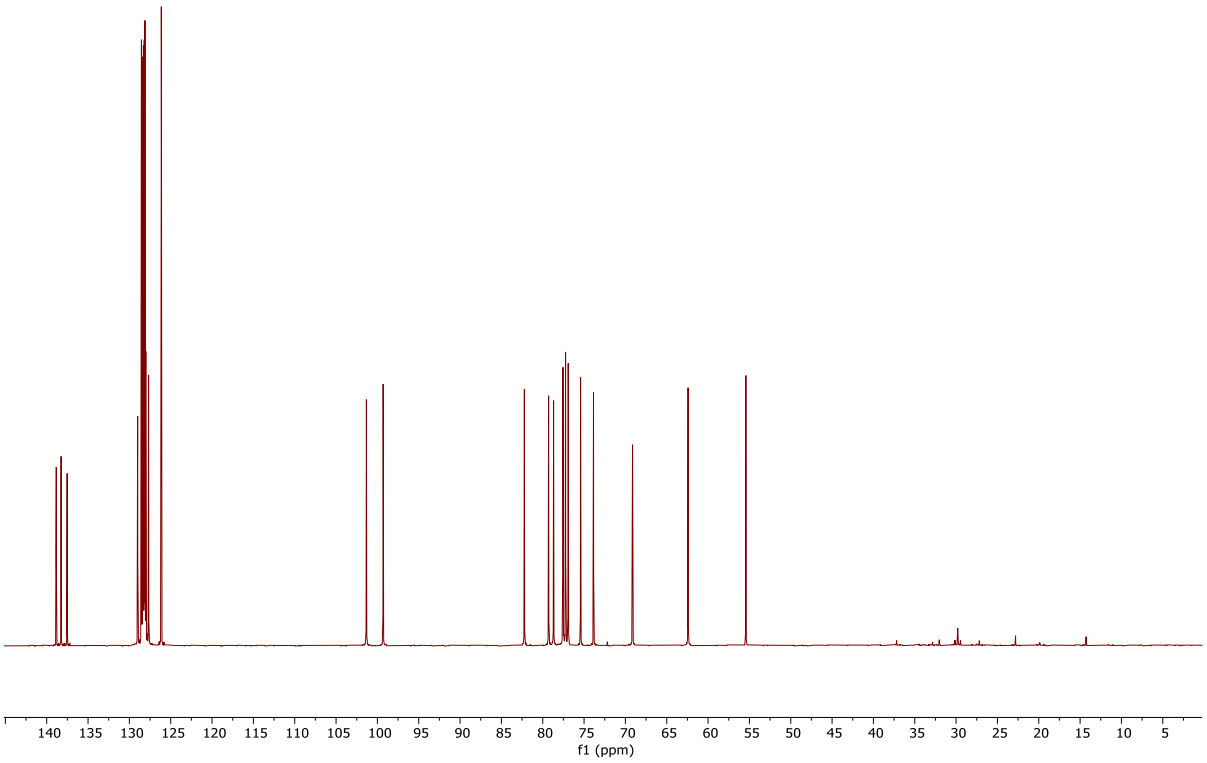


Figure A4b. ^{13}C NMR of Compound **3** in $CDCl_3$ full spectra with impurities below 40 ppm

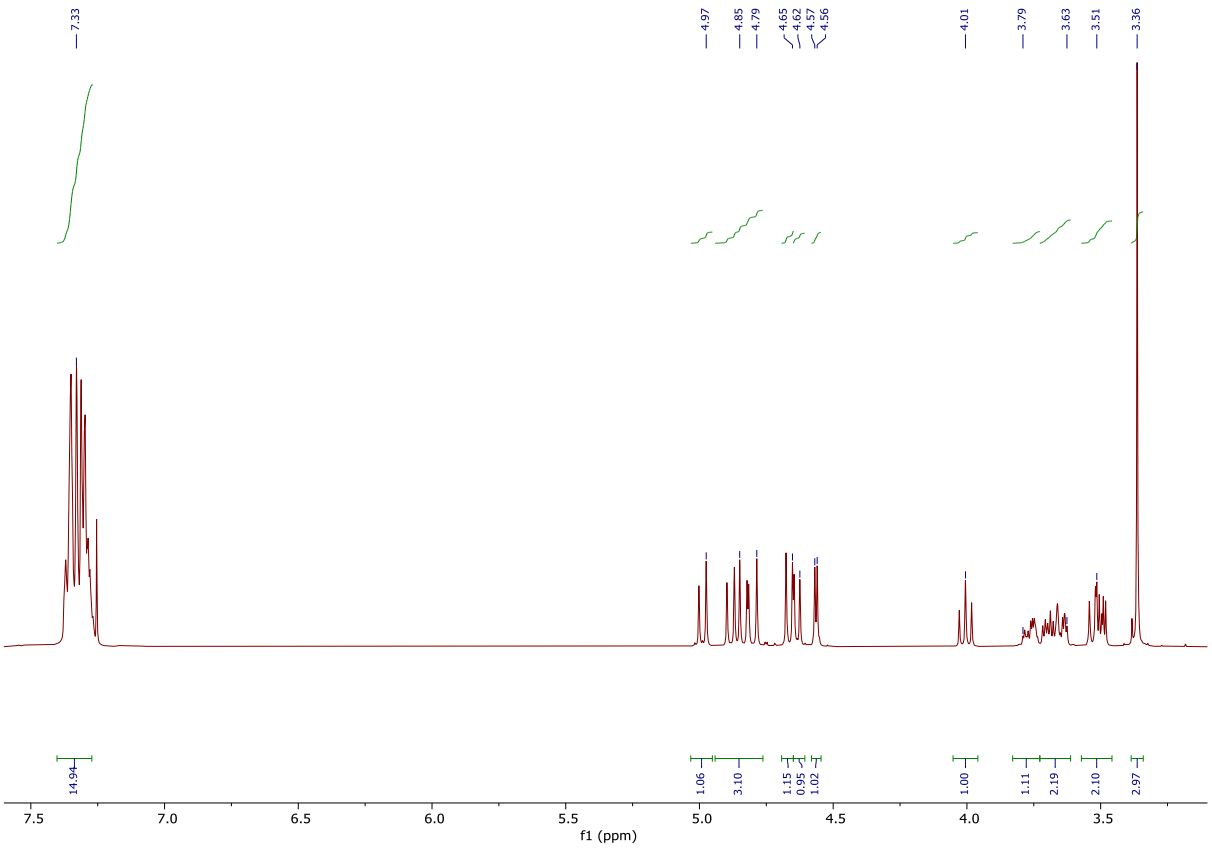


Figure A5a. ^{1}H NMR of Compound **4** in $CDCl_3$

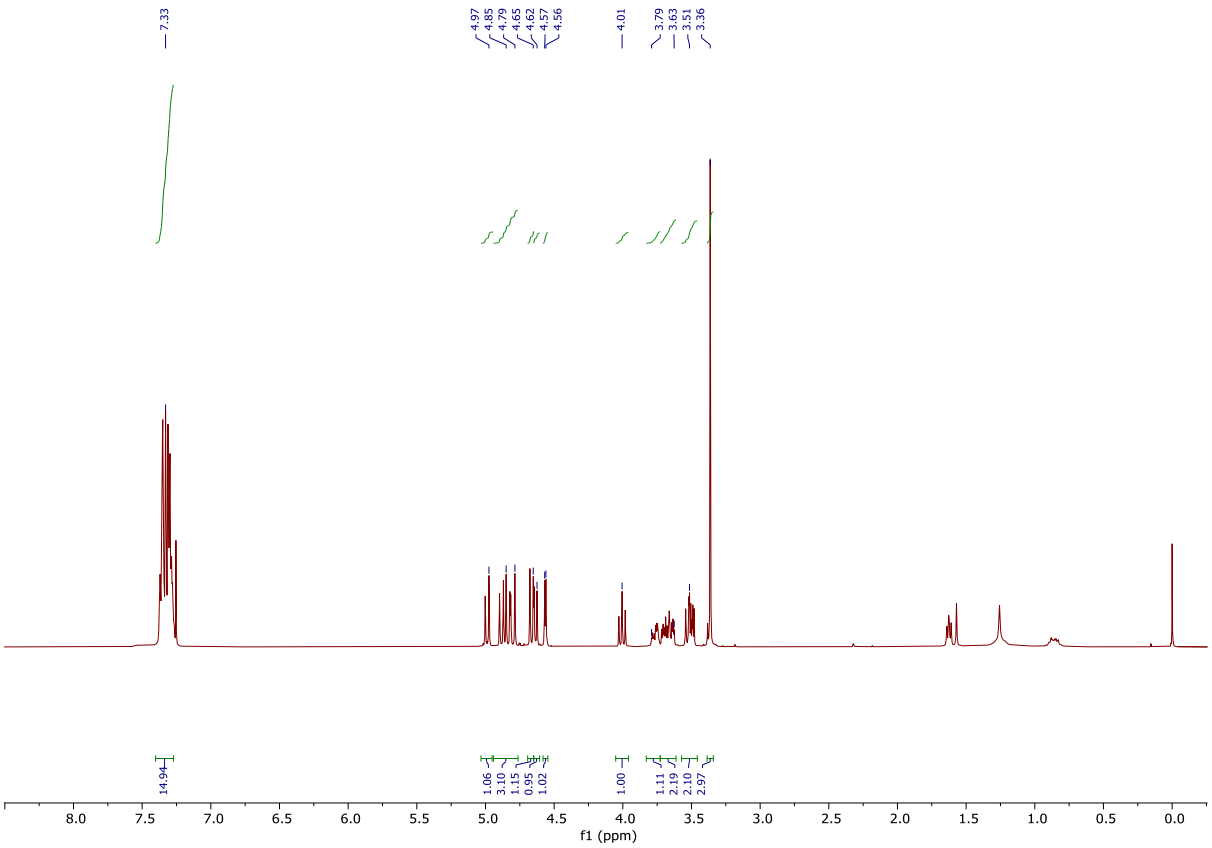


Figure A5b. ^{1}H NMR of Compound **4** in $CDCl_3$ full spectrum

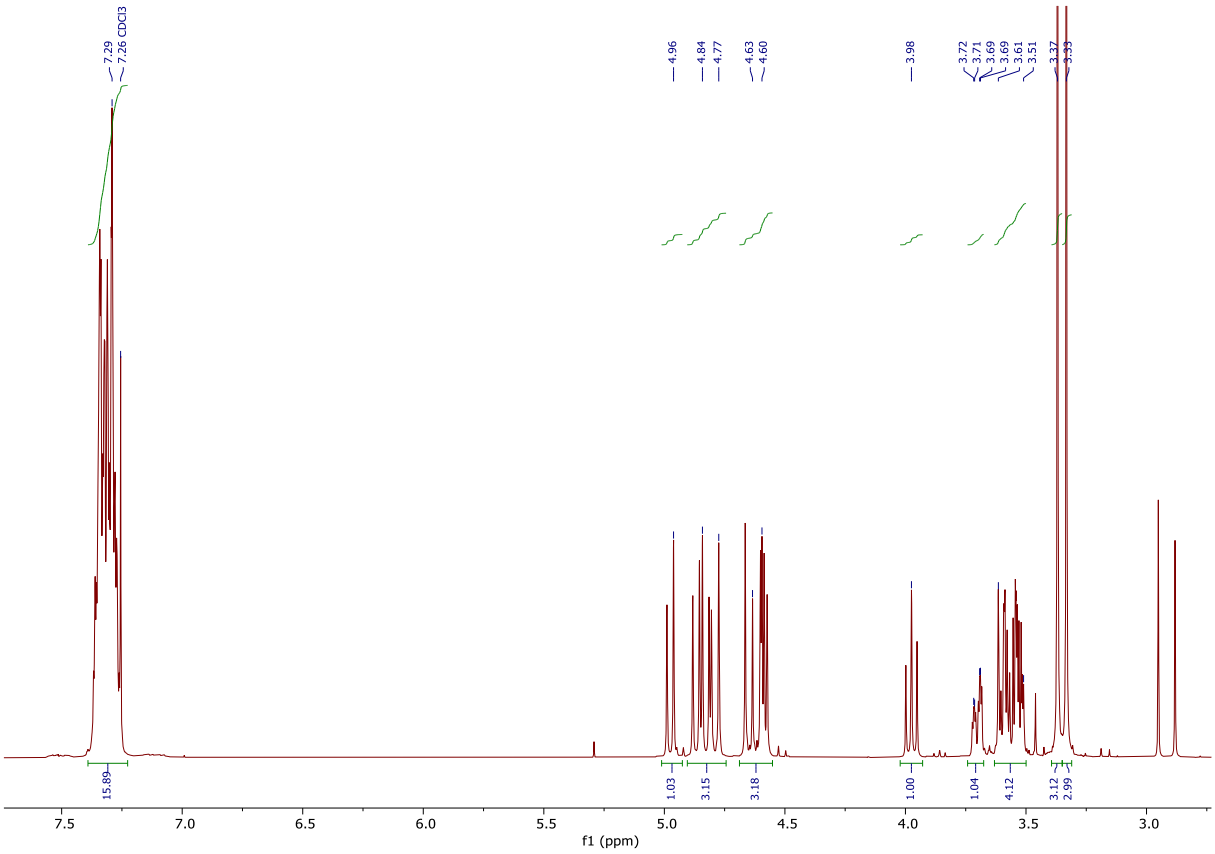


Figure A6a. ^{1}H NMR of Compound **5** in $CDCl_3$

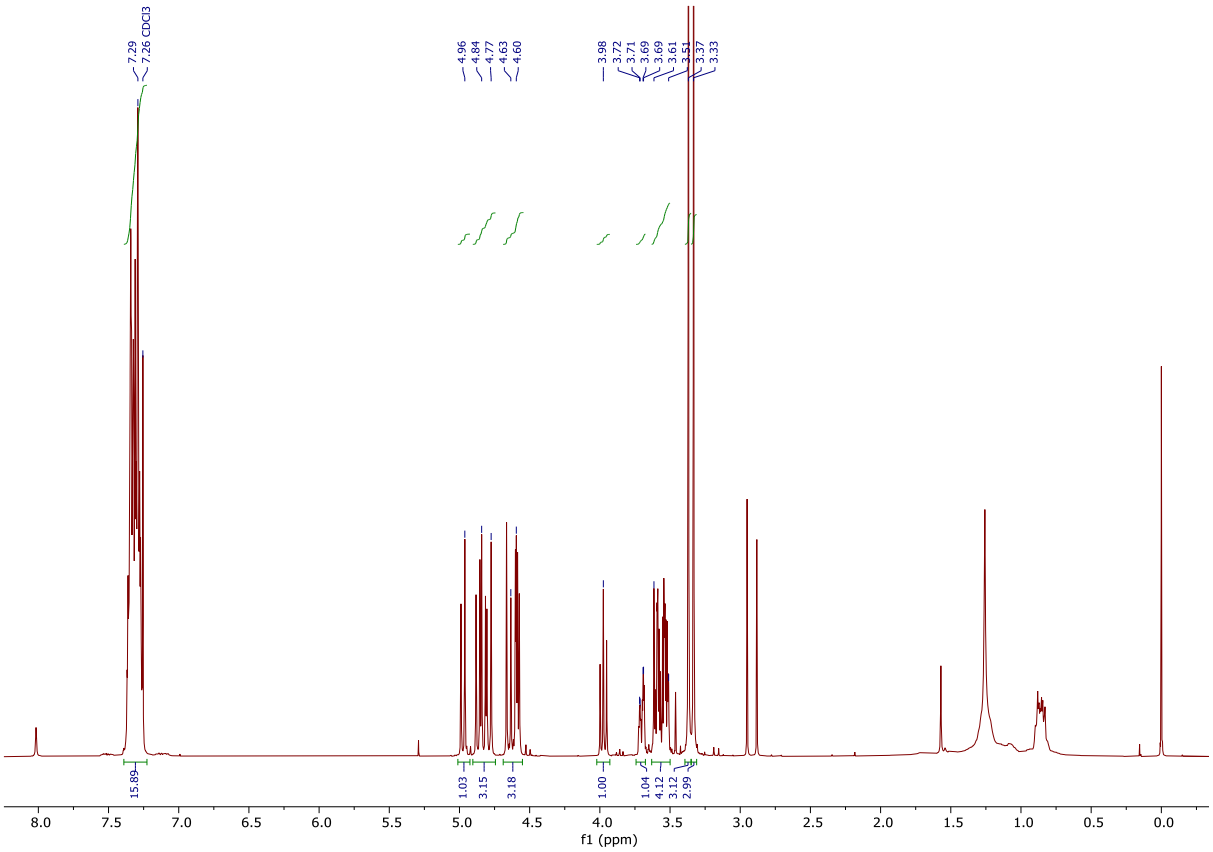


Figure A6b. ^{1}H NMR of Compound **5** in $CDCl_3$ full spectrum

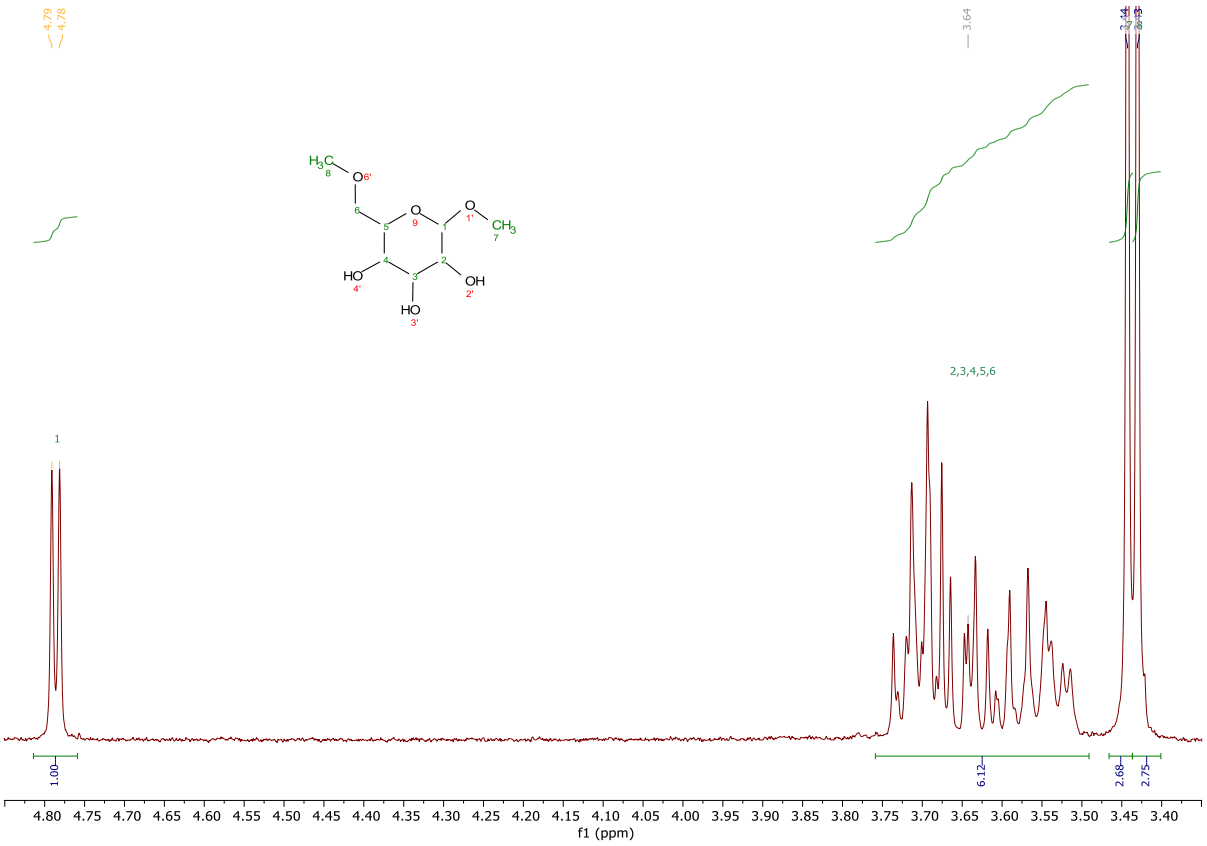


Figure A7a. ^{1}H NMR of Compound **6** in $CDCl_3$

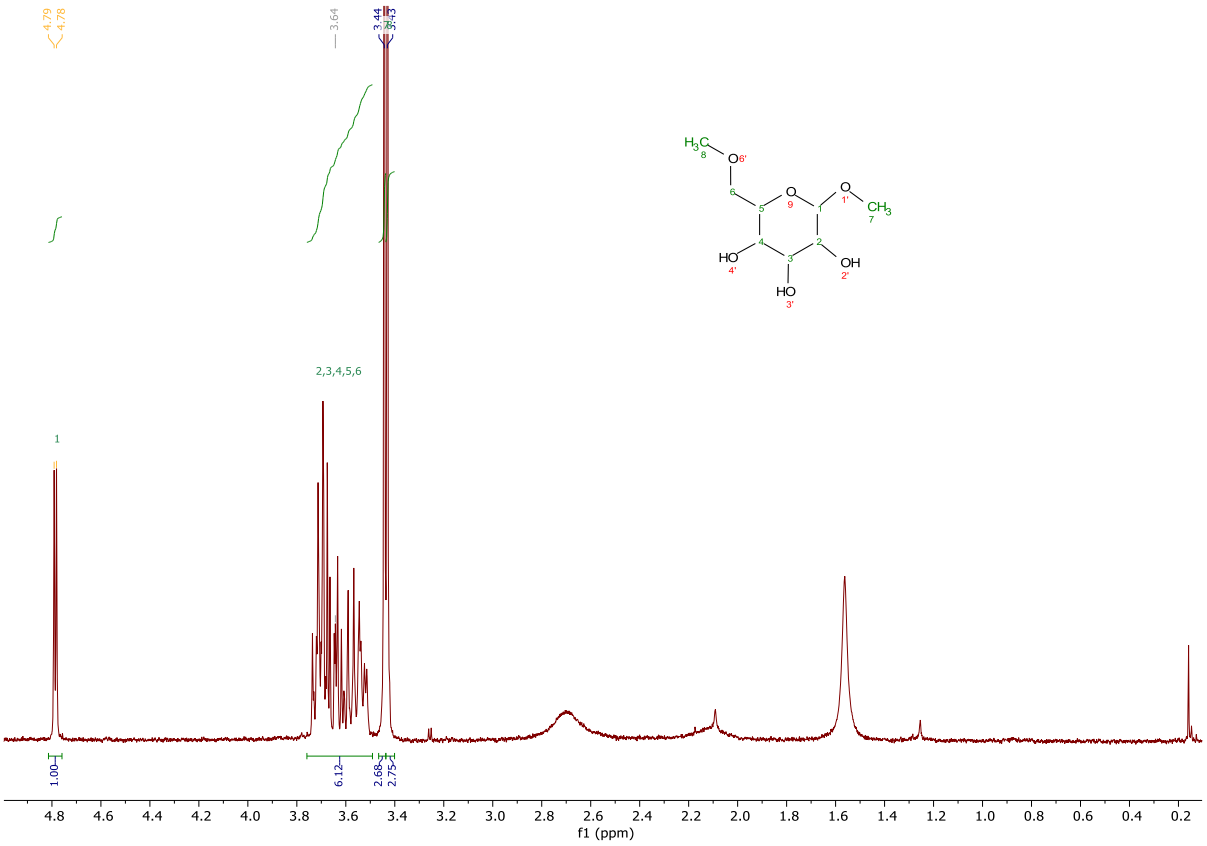


Figure A7b. ^{1}H NMR of Compound **6** in $CDCl_3$ full spectra with impurities

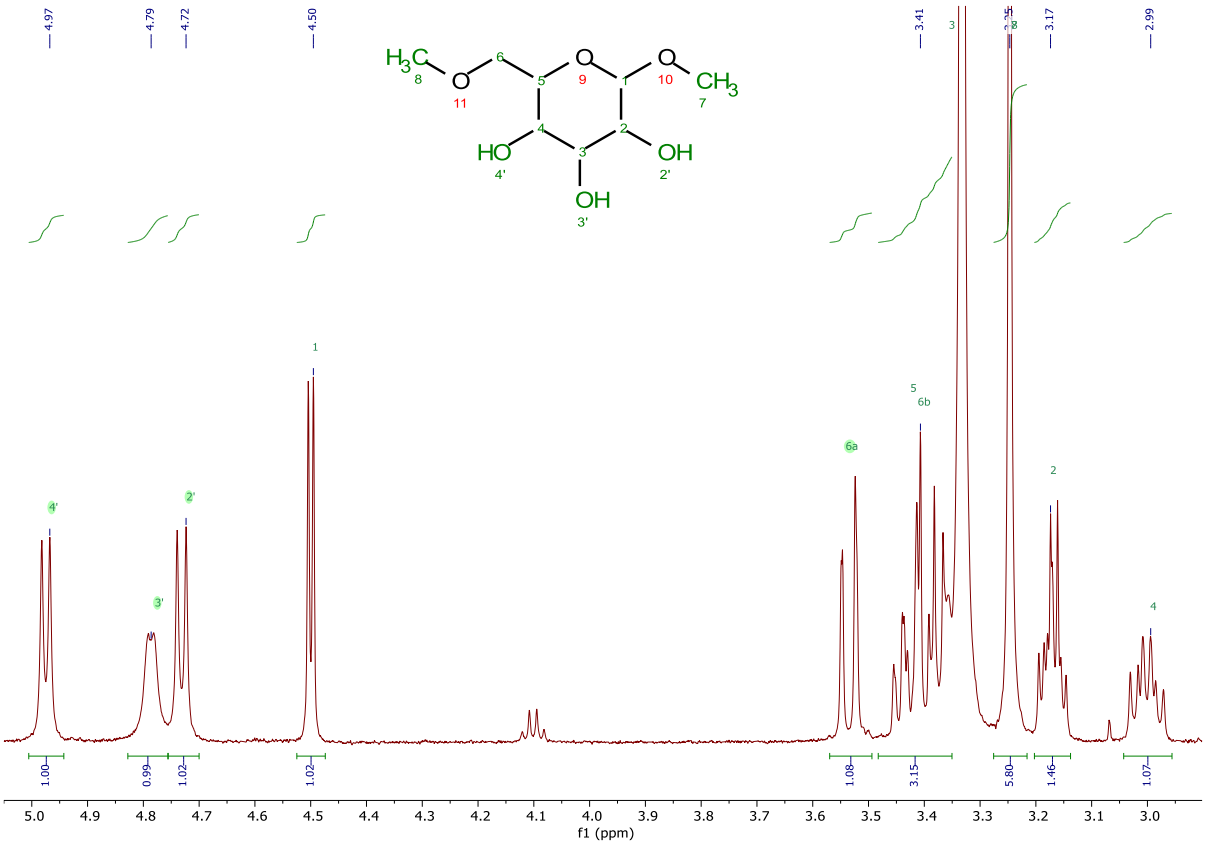


Figure A8. ^{1}H NMR of Compound **6** in DMSO-d_6

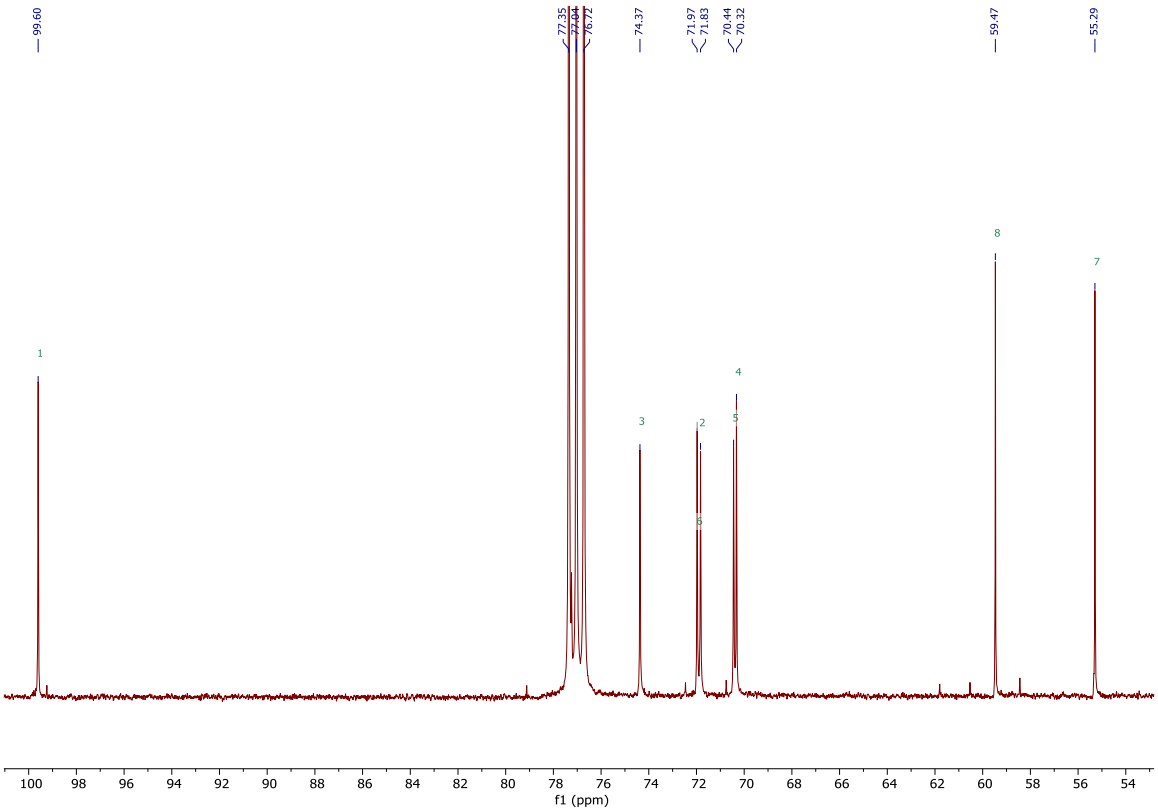


Figure A9a. ^{13}C NMR of Compound **6** in $CDCl_3$, assignments assisted by Mestrenova and NMRium

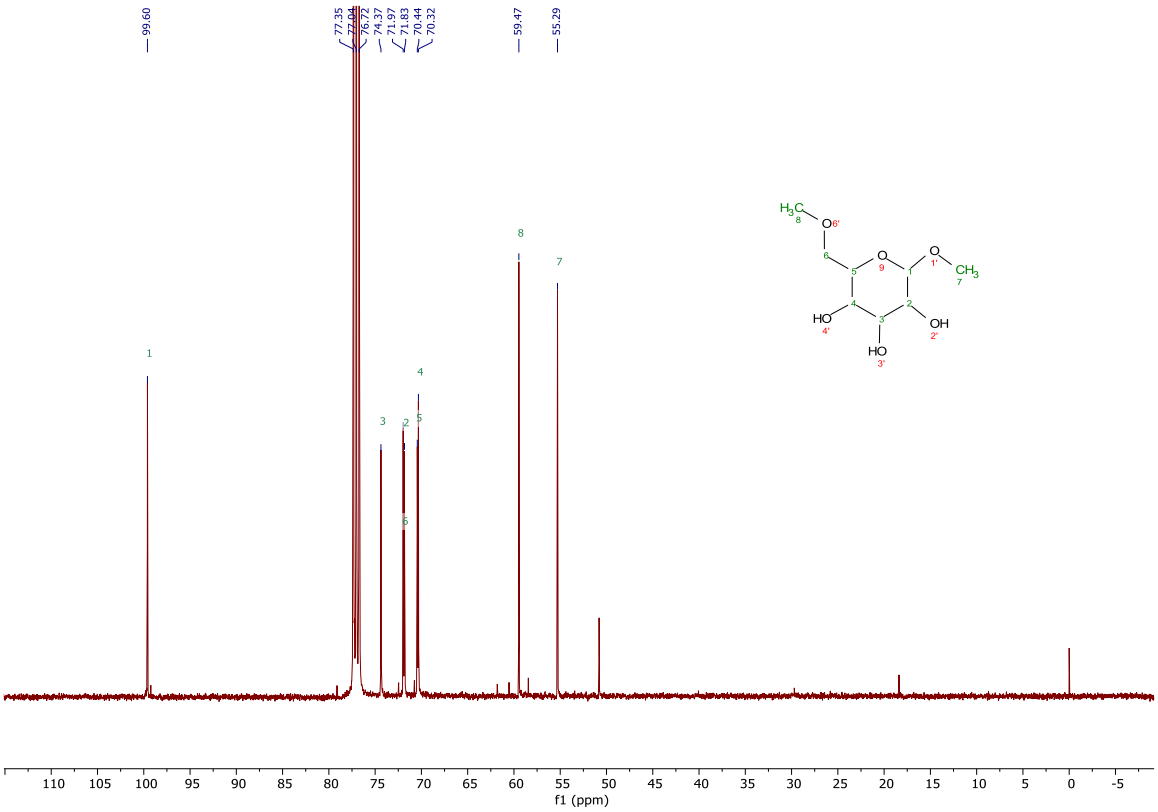


Figure A9b. ^{13}C NMR of Compound **6** in $CDCl_3$ full spectrum

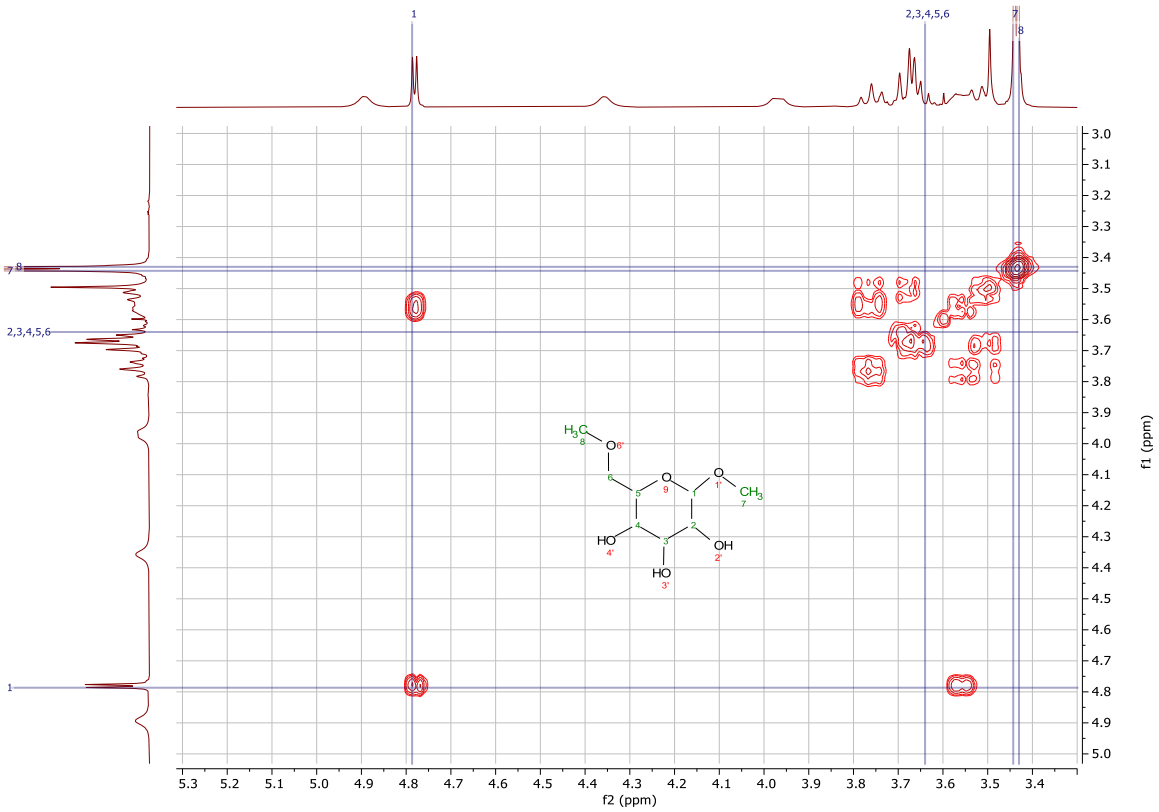


Figure A10a. ^{1}H COSY NMR of Compound **6** in $CDCl_3$

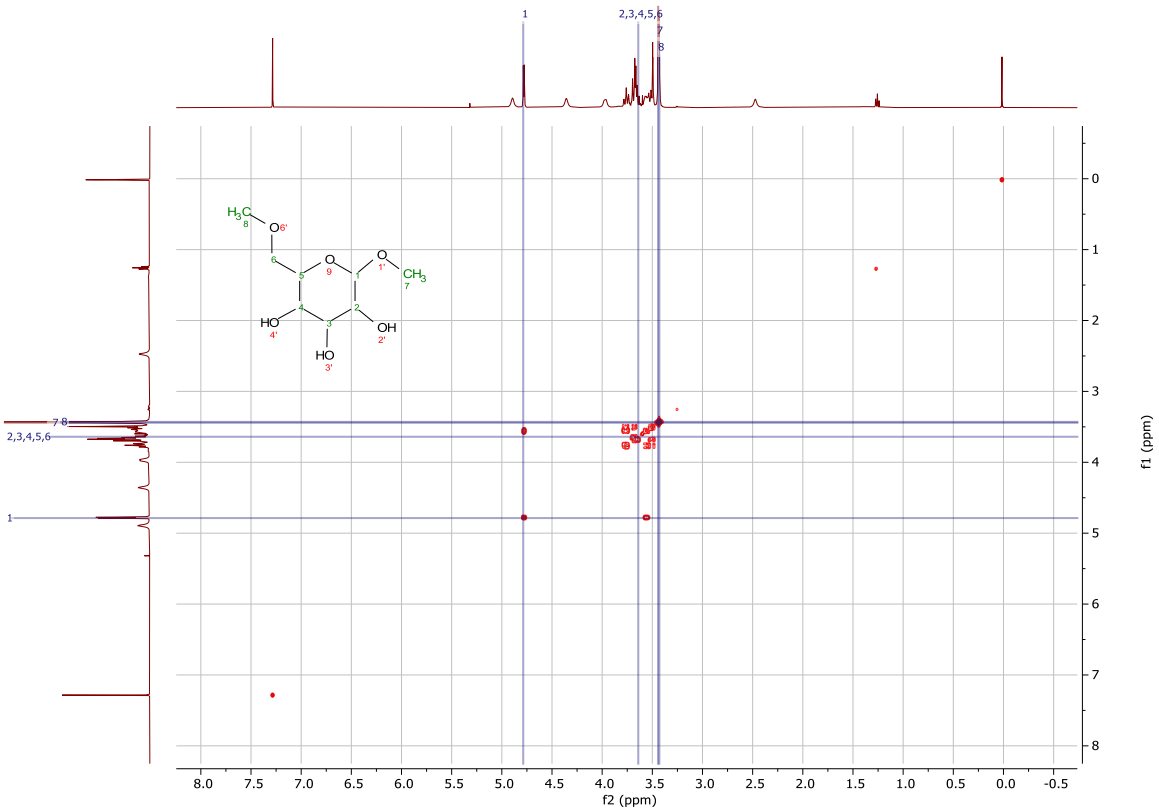


Figure A10b. ^{1}H COSY NMR of Compound **6** in $CDCl_3$ full spectrum

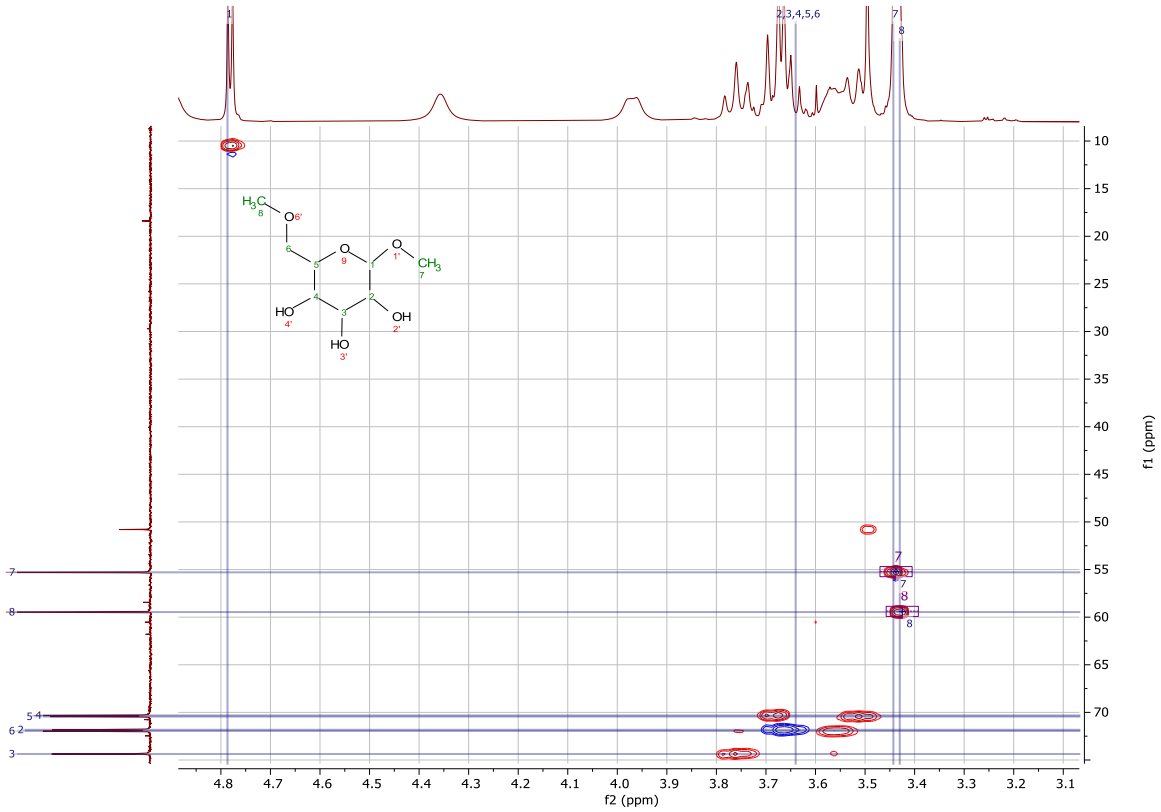


Figure A11a. HSQC NMR of Compound **6** in $CDCl_3$, assignments assisted by Mestrenova and NMRium

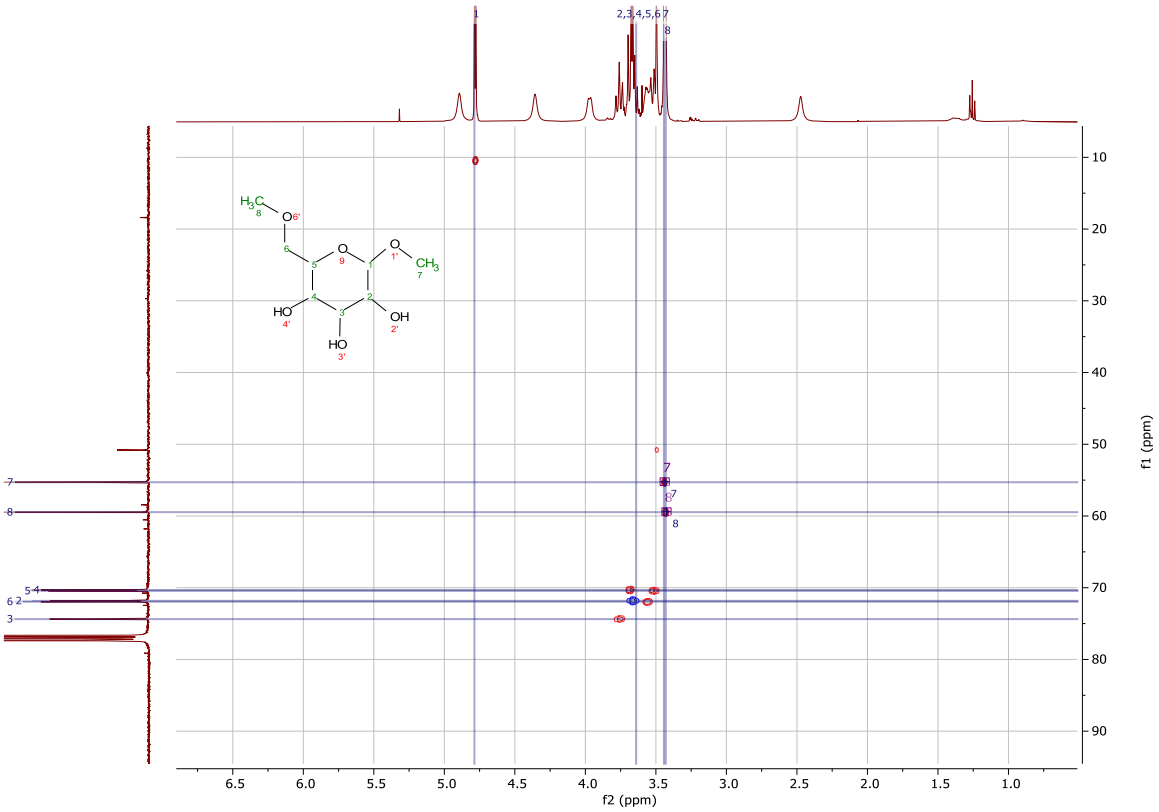


Figure A11b. HSQC NMR of Compound **6** in $CDCl_3$ full spectrum, when the carbon reading does not go to a high enough ppm to collect all carbon peaks on an HSQC the peak at the high carbon will occasionally wrap back around and show up towards the bottom of the spectra this is seen with the correlation under the anomeric peak here. The carbon peak would be expected around 100 ppm but the sweep width only went up to 90 ppm. This caused an “off the charts” reading where the correlated peak wrapped around the spectrum and appeared at 10 ppm.

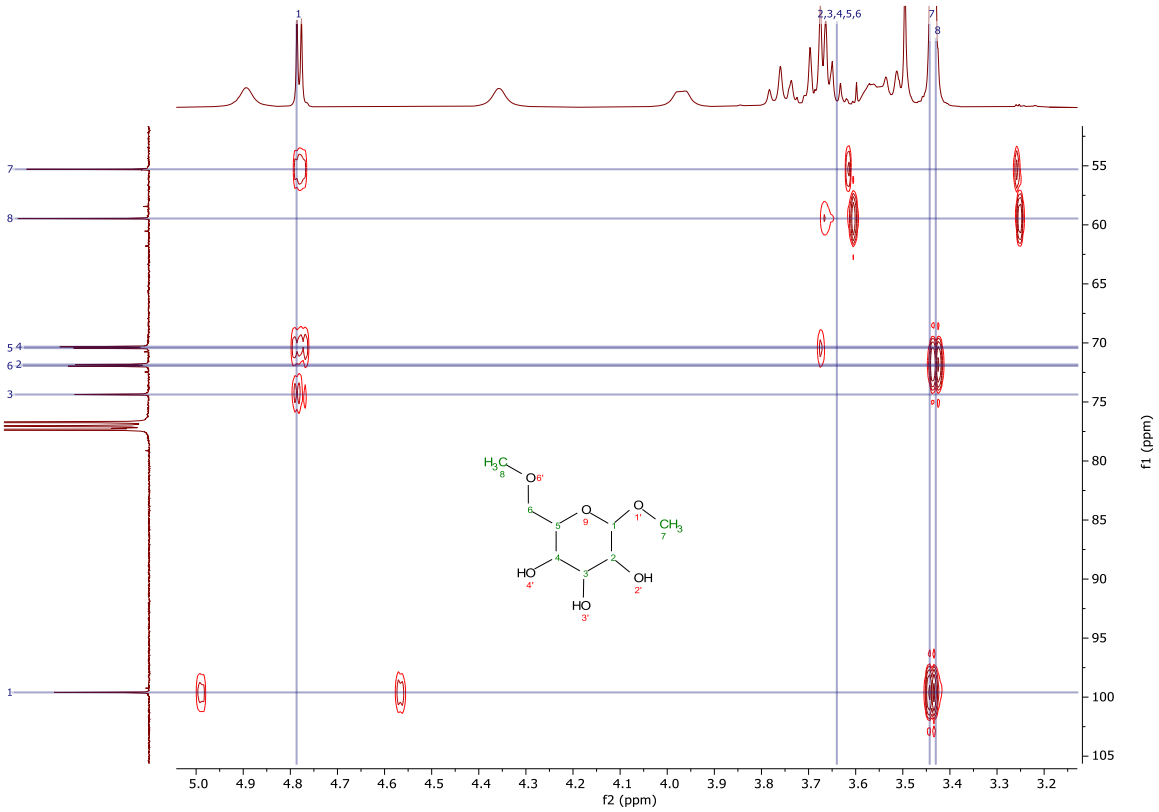


Figure A12a. HMBC NMR of Compound **6** in $CDCl_3$, assignments assisted by Mestrenova and NMRium

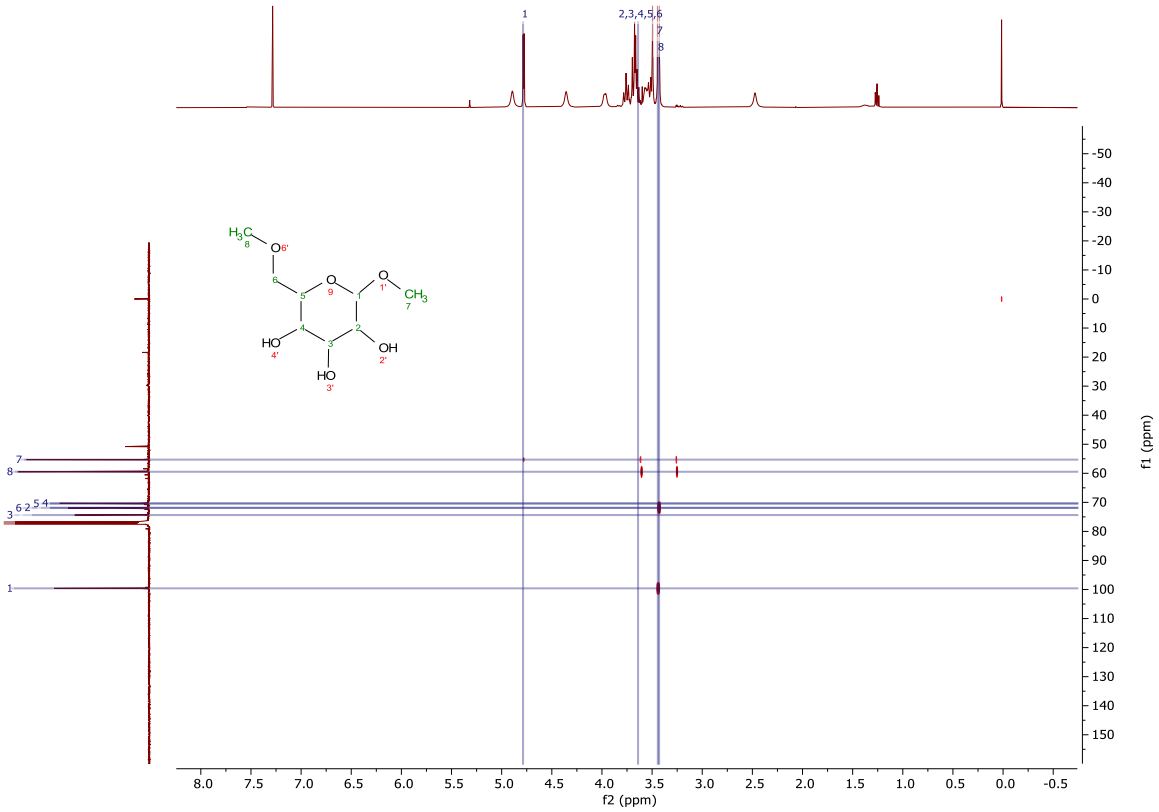


Figure A12b. HMBC NMR of Compound **6** in $CDCl_3$ full spectrum

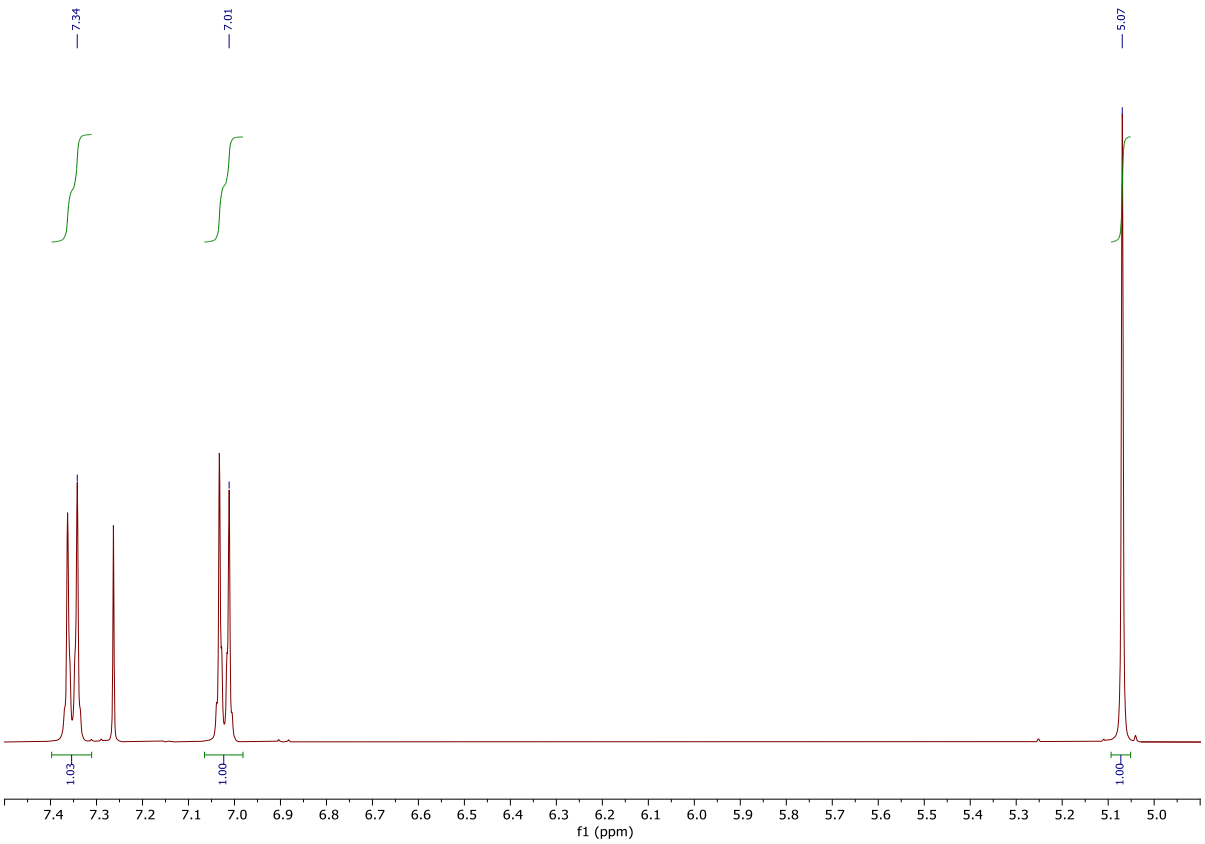


Figure A13a. ^{1}H NMR of Compound **8** in $CDCl_3$

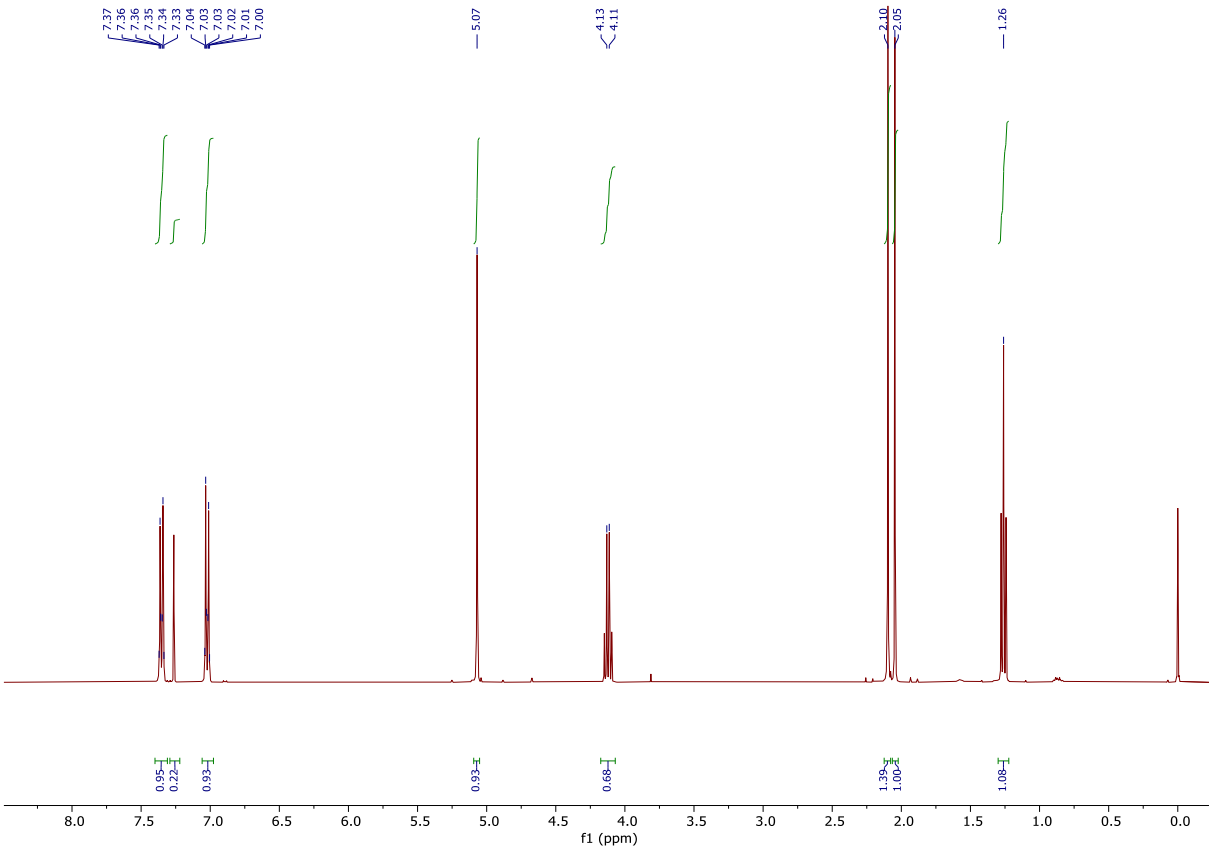


Figure A13b. ^{1}H NMR of Compound **8** in $CDCl_3$ full spectra with solvent impurities

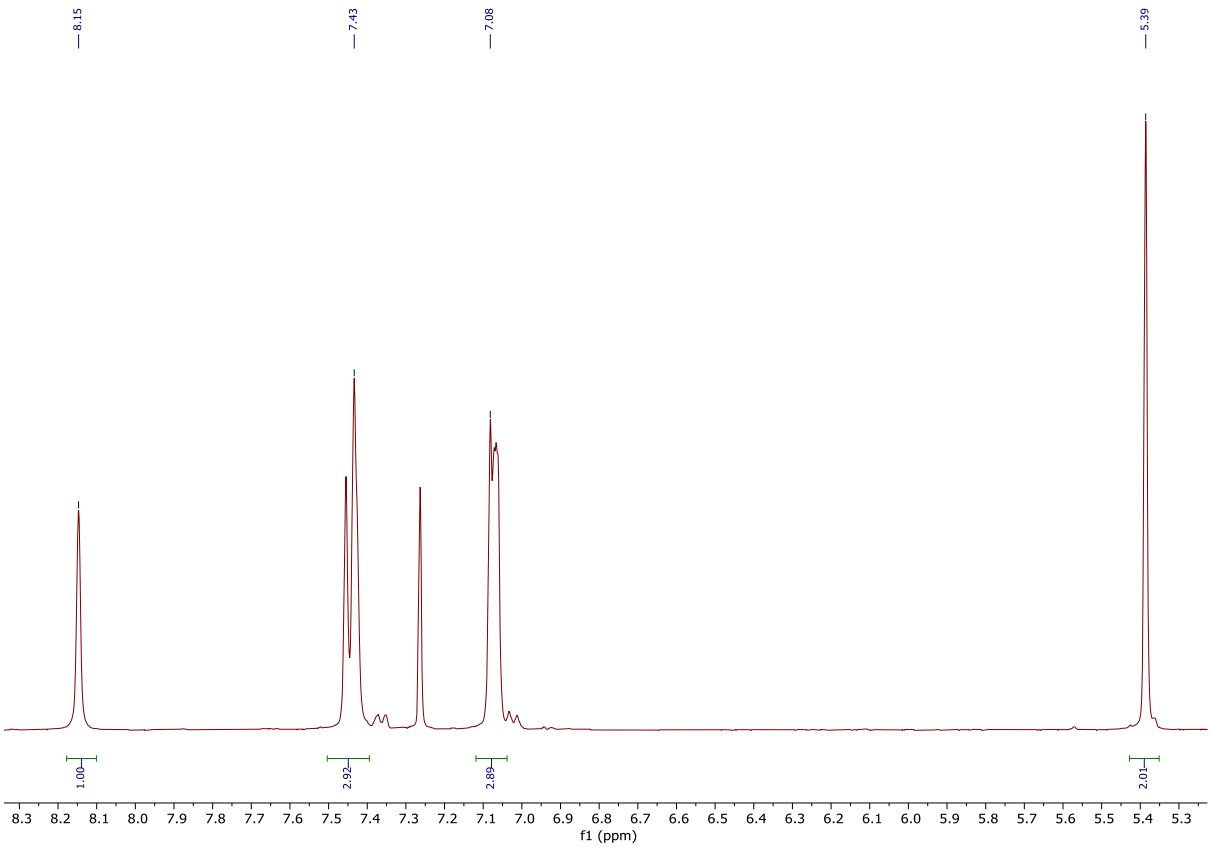


Figure A14a. ^{1}H NMR of Compound **9** in $CDCl_3$

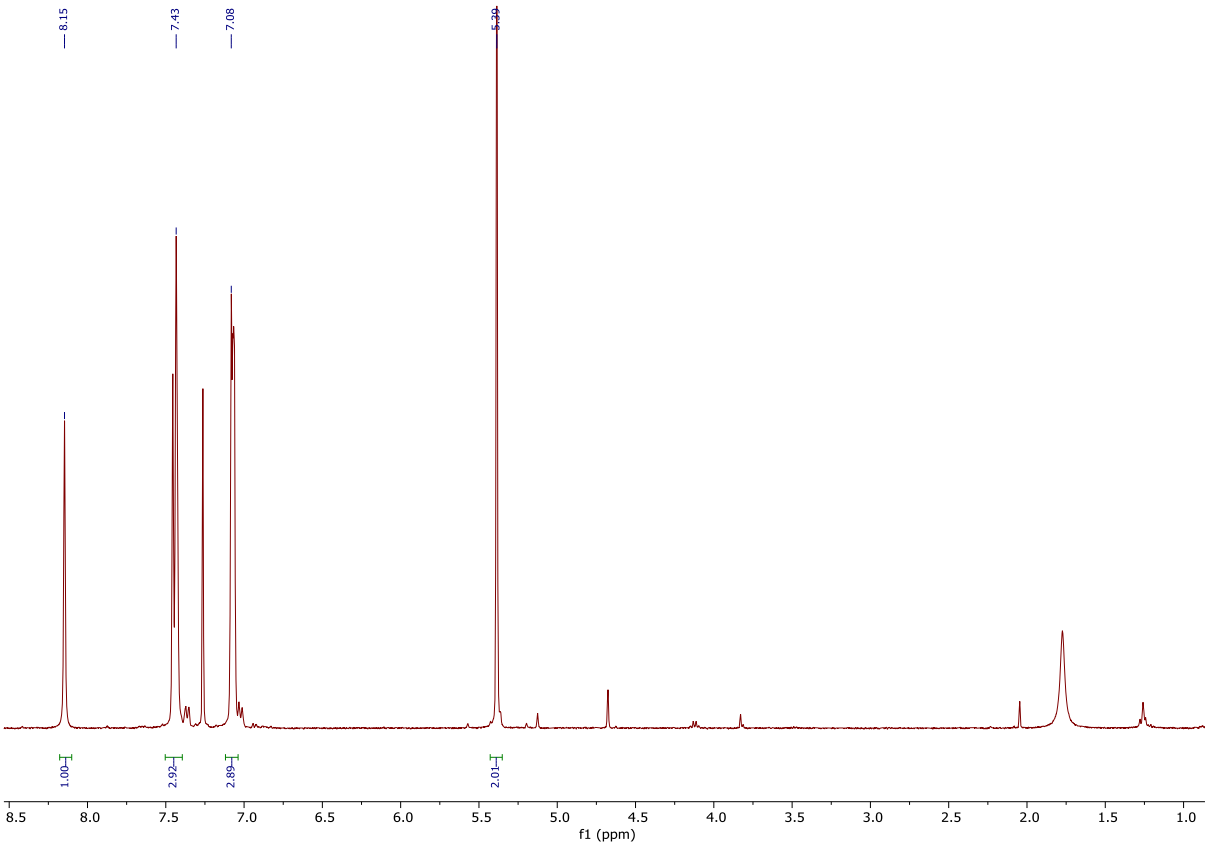


Figure A14b. ^{1}H NMR of Compound **9** in $CDCl_3$ full spectra showing impurities

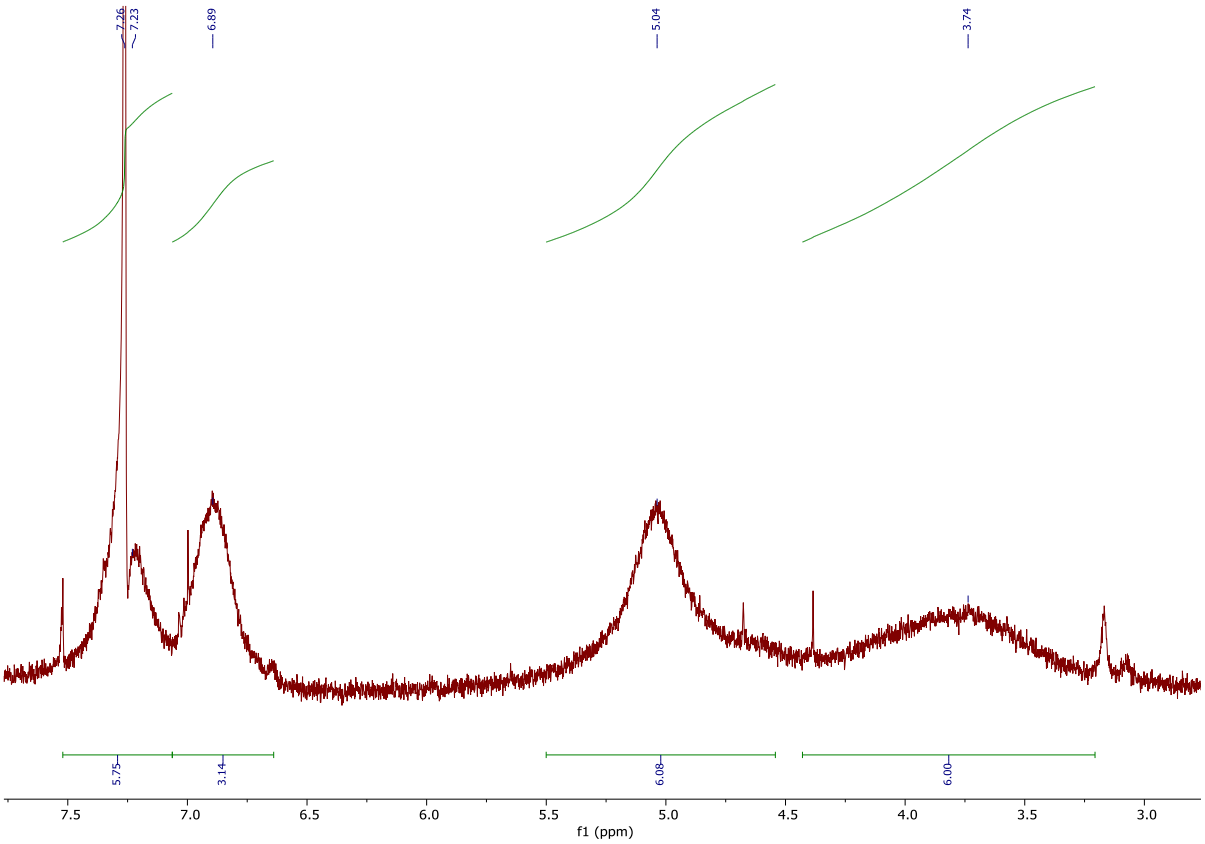


Figure A15a. ^{1}H NMR of Compound **10** in $CDCl_3$

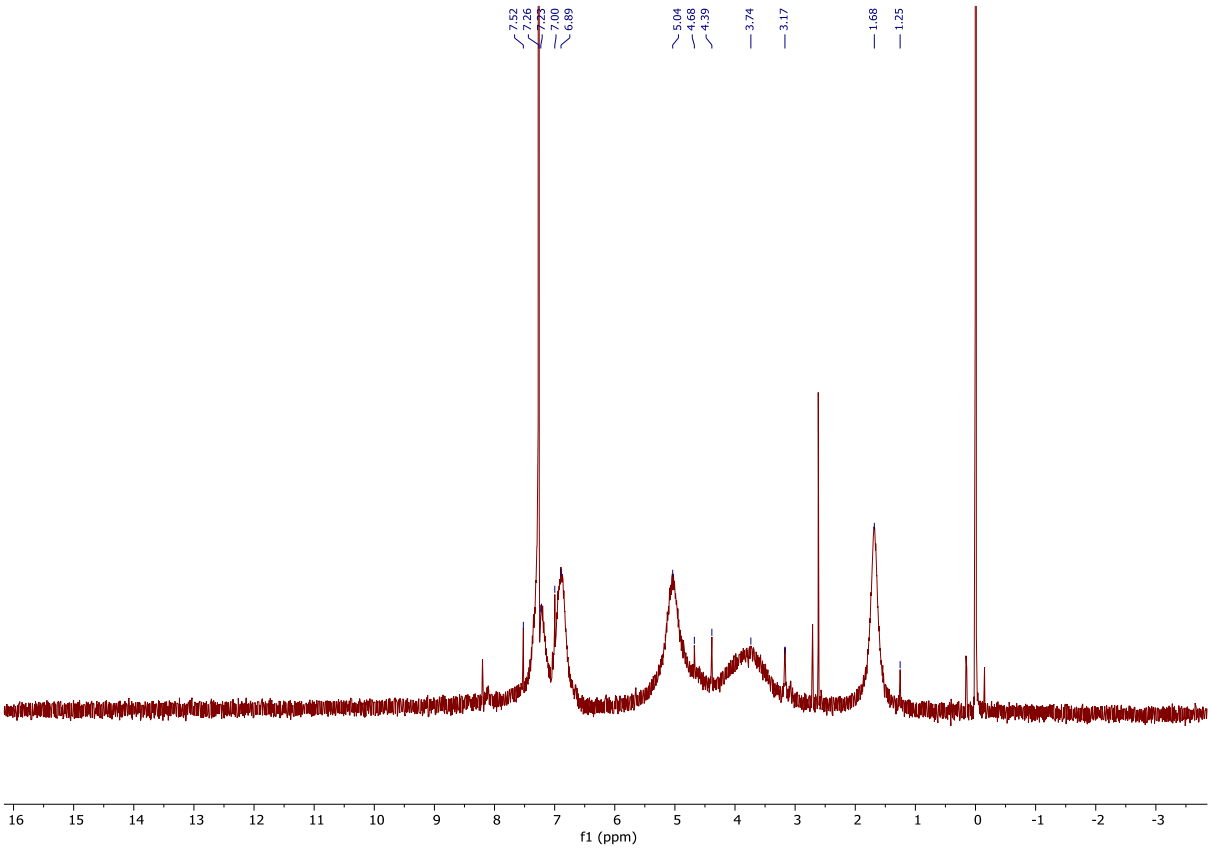


Figure A15b. ^{1}H NMR of Compound **10** in $CDCl_3$ full spectrum

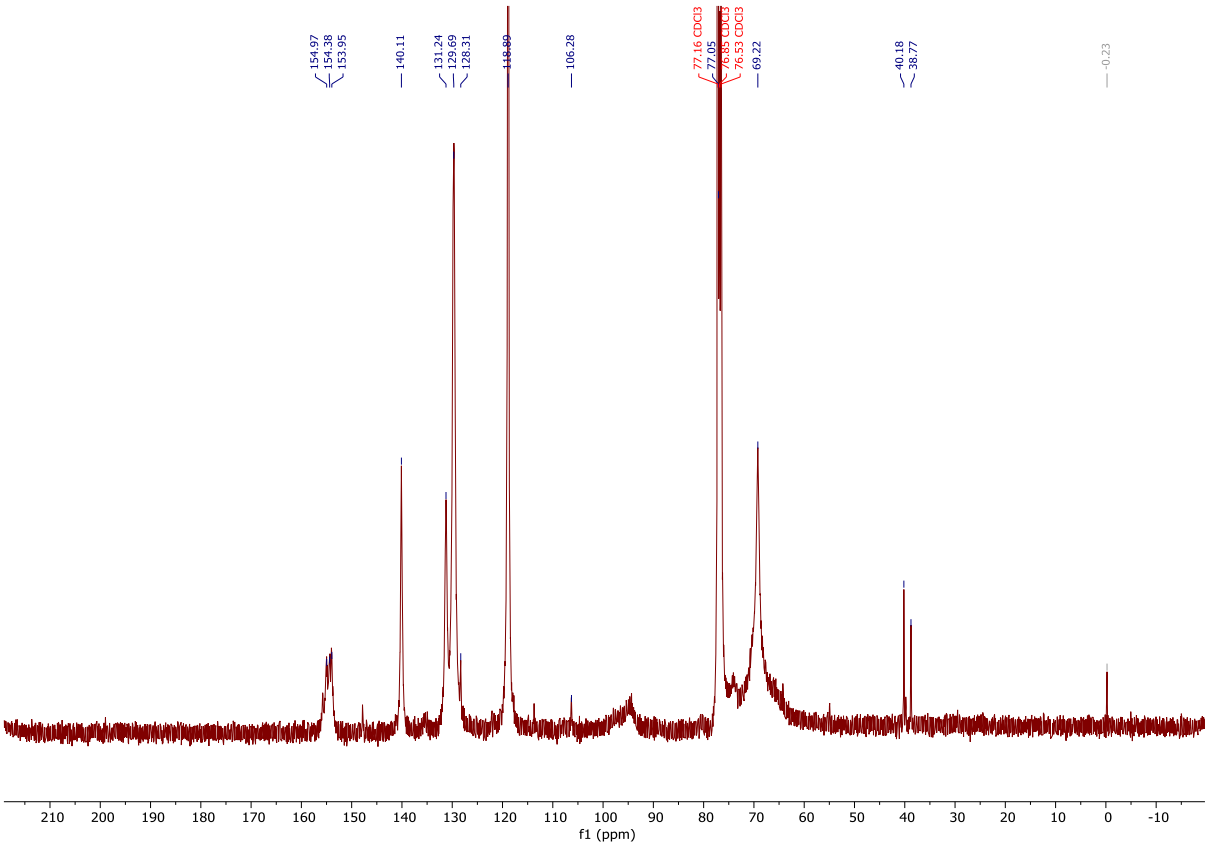


Figure A16. ^{13}C NMR of Compound **10** in $CDCl_3$

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