

**Development of a Glycoconjugate Tool Set for the Assembly
and Presentation of Carbohydrate Ligands on Surfaces**

A Dissertation

Presented for the Doctor of

Philosophy Degree

The University of Tennessee, Knoxville

Irene Esah Abia

December 2011

Dedication

I dedicate this dissertation to my parents Lucy and Lucas Abia who made sure my six siblings and I get the best education they could possibly afford. They never considered the money spent for the education of their female children a waste; for that I will forever be grateful.

I also dedicate this dissertation to all the little girls and grown women, especially those in developing countries, who have been told their only place in life is in the kitchen. There is so much more out there for all of you.

Acknowledgements

I would like to first of all thank Almighty God for giving me the strength and perseverance to go through this program. My sincere thanks go to my advisor Dr. David Baker for his tremendous support, advice, patience and for believing in me. You have truly given me the biggest opportunity and encouragement to become an Organic Synthetic Chemist and I will always be grateful for that. I would also like to thank Dr. Michael Best for teaching and assisting me to carry out biological analysis of my compounds. Thank you for introducing me to the world of Biological Chemistry. I am also grateful to Dr. Shawn Campagna, Dr. Ben Xue, and Dr. Elias Fernandez for being willing to serve on my committee and for their valuable inputs. I give thanks to the Department of Chemistry at University of Tennessee and National Science Foundation for all the financial support. I am also grateful to Dr. Hazari for the encouragements and introducing me to Chemistry outreach programs that appeal to local communities. Many thanks to Dr. Carlos Steren for assistance with NMR experiment setups and 2D NMR data analysis.

I would also like to thank past and present members of Baker research group: Dr. Christiane Barnes, Dr. Medhanit Bahta, Dr. Riyam Kafri, Dr. Samson Francis, Dr. Julio Guittierez and Dr. Chao Wang for their assistance with laboratory training; Costyl Njiojob, Chad LeCroix, Veronica Gantert, Steven Glass, Yundi Gan, Bo Meng, Robert Simion, Andy Hahn, Adnan Dervishi, Andrew Moss and Andrew Rowe for their support and friendship. I am grateful to Dr. Samson Francis for help with software and computer

repairs. My profound gratitude goes to Veronica and Silver Festin for assistance with the picking up Sandy and Kay from school so I could focus on research.

Many thanks to the past and present members of Best research group for their help and corporation with biological studies: Dr. Meng Rowland, Dr. Chi-Linh Do-Thanh, Heidi Bostic, Ritu Nandal, Andrew Bayer, Ben Smith, Sammy Eni Eni, and Yilin Wang. Meng and Heidi, you have been such great friends and wonderful aunties to my kids, thank you for everything. I will never forget you two taking turns to bring us dinner when we were all sick. This meant a great deal to us, we will always be grateful. Chi-Linh, you have been a true computer ‘wizard’; you solved many of my computer problems and taught me so much in the process, thank you.

I want to thank all the friends whose kind words and encouragement lifted my spirit on difficult days: Nan Chen, Marilyn Ownby, Arthur Pratt, Vighter Iberi, Lisa Bonds, Thomas Moore, Gail Cox and Rachel Rui. I would like to thank Bill Gurley, Johnny Jones and all the other staff members at the electronic shop for technical support and numerous computer repairs.

My deepest and sincere thanks go to my family: my parents Lucy and Lucas Abia for their loving support and prayers, my brother Dr. Jude Abia and sister-in-law Tomia Abia for being so encouraging and always being there through thick and thin; my sisters Naomi Mbetsi, Esther Amana, Prisca Omotayo and their families for all the long talks and cheer leading, my kid brothers Jonathan Abia and Solomon Abia for their love and genuine concern. I am grateful to my cousins, friends and in-laws for the encouragement

and moral support. Last but not the least, I am grateful to my husband Dr. Jerry Angeh for his patience and support; and my daughters Sandy-Sheryl Angeh and Kyra-Kay Angeh for being exceptionally patient and very entertaining; you all are my source of inspiration and joy. I am blessed and very fortunate to have you all in my life.

Abstract

Carbohydrate and protein interactions are often essential in viral and bacterial infection, the immune response, cell differentiation and development, and the progression of tumor cell metastasis. Therefore, an understanding of carbohydrate–protein interactions at the molecular level would lead to a better insight into the biological process of living systems and assist in the development of therapeutic and diagnostic strategies. Our goal was to synthesize different mannose derivatives, immobilize them on nano-patterned surfaces and carry out binding studies with mannose-binding lectins in order to characterize carbohydrate–protein interactions.

Different derivatives of D-mannose (monosaccharide, (1→2)-linked disaccharide, (1→3)-linked disaccharide, and (1→2, 1→3)-linked trisaccharide) with tethered –SH groups were synthesized. Alkyne-terminated D-mannose derivatives were synthesized to be immobilized via click chemistry on azide-functionalized glass slides. These molecules were constructed by glycosylation of appropriately protected glycosyl donors and acceptors, followed by free-radical addition to introduce the thiol terminals onto the aglycons. Subsequent deprotection afforded the corresponding free-OH saccharides. Standard robotic microarray printing technology was used to couple these thiol-terminated aglycons to epoxide-functionalized glass slides.

Using a fluorescence scanner, binding between carbohydrates and Con A were quantified and processed to obtain dissociation constants (K_D). The (1→2)-linked disaccharide **18** showed highest binding with Con A with dissociation constant of 58 nM

[nano molar]. The (1→3)-linked disaccharide **24** had a dissociation constant of 68 nM [nano molar] with Con A. The differences in binding constants seem to be greater at higher concentrations above 400 μ M [micro molar]. The monosaccharide **5** had an average surface dissociation constant of 76 nM [nano molar] and 91 nM [nano molar] for the trisaccharide **29**. In general, the disaccharides **18** and **24** showed enhanced binding interaction with Con A than the monosaccharide **5** and trisaccharide **29**.

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Abbreviations

Ac	Acetyl
AcOH	Acetic Acid
ADMA	Anisaldehyde dimethyl acetal
AgOTf	Silver trifluoromethanesulfonate
AIBN	Azoisobutyronitrile
All	Allyl
Bn	Benzyl
COSY	Correlated spectroscopy
CRD	Carbohydrate-recognition domain
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DMAP	4-(Dimethylamino)pyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	Dimethyl sulfoxide
ESI	Electrospray ionization
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum correlation

MALDI	Matrix-assisted laser-desorption ionization
MeOH	Methanol
Me	Methyl
MS	Mass spectrometry
MS	Molecular sieves
NMR	Nuclear magnetic resonance
PMB	<i>p</i> -Methoxybenzyl
SD	Standard deviation
TBAB	Tetrabutylammonium bromide
Et ₃ N	Triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOCSY	Totally correlated spectroscopy
TOF	Time of flight
TsOH	<i>p</i> -Toluenesulfonic acid

PART ONE
TOTAL SYNTHESIS OF MANNOSE DERIVATIVES

I. Introduction

1. Carbohydrates

The major classes of organic compounds common to living systems are lipids, proteins, nucleic acids, and carbohydrates.¹ Carbohydrates are very familiar to us; we call many of them “sugars”. They make up a substantial portion of the food we eat and provide most of the energy that keeps the human engine running. Carbohydrates are the structural components of the walls of plant cells and the wood of trees; they are also major components of the exoskeletons of insects, crabs and lobsters. Historically, carbohydrates were once considered to be the hydrates of carbon because their molecular formulas in many (but not all) cases correspond to $C_n(H_2O)_m$. It is more realistic to define a carbohydrate as a polyhydroxy aldehyde or ketone, a point of view closer to structural reality and more suggestive of chemical reactivity.²

1.1. Classification of carbohydrates

The Latin word for sugar is *saccharum*, and the derived term *saccharide* is the basis of a system of carbohydrate classification. A monosaccharide is a simple carbohydrate, one that on attempted hydrolysis is not cleaved to smaller carbohydrates. Glucose ($C_6H_{12}O_6$), for example is a monosaccharide. The other common six-carbon sugars (hexoses) are D-fructose, D-galactose and D-mannose. Monosaccharides rarely exist as open carbon chains, but more commonly found as cyclic ring structure of five or six atoms. The simple sugars are the building blocks of carbohydrates. A disaccharide on hydrolysis is

cleaved to two monosaccharides that may be the same or different. Sucrose (common table sugar) is a disaccharide which upon hydrolysis yields one molecule of glucose and one of fructose. There are two basic types of disaccharides, reducing and non-reducing. Reducing disaccharides are two monosaccharides linked via the anomeric center of one glycosyl unit to a hydroxyl group of another sugar, which leave free a hemiacetalic OH group at the anomeric center. Non-reducing disaccharides are linked at both the anomeric positions of the two monosaccharides. Sucrose is a non-reducing sugar, since both sugars are linked through their anomeric carbons.

An oligosaccharide (*oligos* is a Greek word that in its plural form means *few*) yields two or more monosaccharides on hydrolysis. Thus, the IUPAC classifies disaccharides, trisaccharides, and so on as subcategories of oligosaccharides. Polysaccharides are high-molecular-weight polymers built by repeated condensations of monosaccharides. Examples are starches, glycogen, cellulose, and carbohydrate gums. Recently, the IUPAC has chosen not to specify the number of monosaccharide components that separates oligosaccharides from polysaccharides.² This standard is a more practical one; it notes that an oligosaccharide is homogeneous. Polysaccharides are almost always mixtures of molecules having similar, but not necessarily the same, chain length. Cellulose, for example, is a polysaccharide that gives thousands of glucose molecules on hydrolysis but only a small fraction of the cellulose chains contains exactly the same number of glucose units. Carbohydrates are found on every cell surface; they are found both on eukaryotic and prokaryotic cell surfaces.

2. Eukaryotes and Prokaryotes

The cell is the basic functional unit of life and often called the building block of life. There are primarily two types of cells: eukaryotic and prokaryotic cells. Like the name implies, eukaryote mean *true nucleus* to the extent that, unlike prokaryotes, eukaryotes have a nucleus. Animals, plants, fungi, and protists are eukaryotes. They are organisms with complex cell or cells and where the genetic material is organized into a membrane-bound nucleus or nuclei. Animals, plants, and fungi are mostly multicellular, while many sorts of protists are unicellular.³ Eukaryotic cells also contain membrane-bound organelles such as mitochondria and chloroplasts. The prokaryote cell is simpler, and therefore smaller, than a eukaryote cell, lacking a nucleus and most of the other organelles of eukaryotes. There are two kinds of prokaryotes: bacteria and archaea; they share a similar structure. Nuclear material of prokaryotic cell consists of a single chromosome that is in direct contact with the cytoplasm. Prokaryotes contain only a single loop of stable chromosomal DNA stored in an area called the nucleoid. Mitochondria and chloroplasts are membrane-bound organelles in eukaryotes responsible in performing various metabolic processes; however, in prokaryotes similar processes occur across the cell membrane. The difference between the structure of prokaryotes and eukaryotes is so great that it is considered to be the most important distinction among the groups of organisms. The structures of eukaryotic and prokaryotic cells are shown in Figures 1 and 2, respectively.⁴

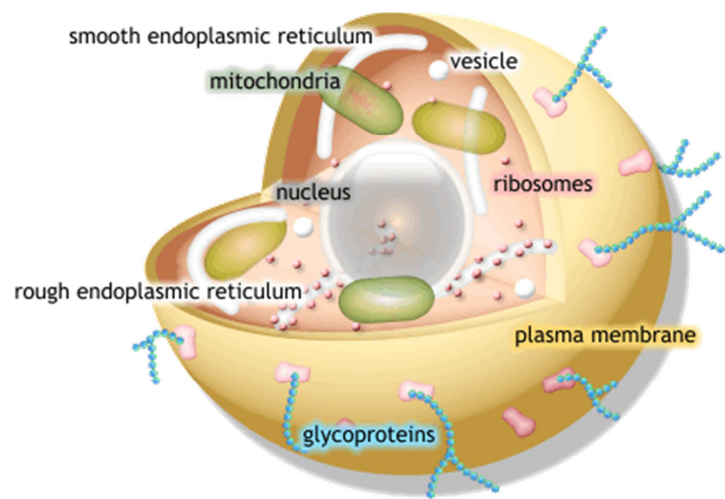


Figure 1. Eukaryotic cell.

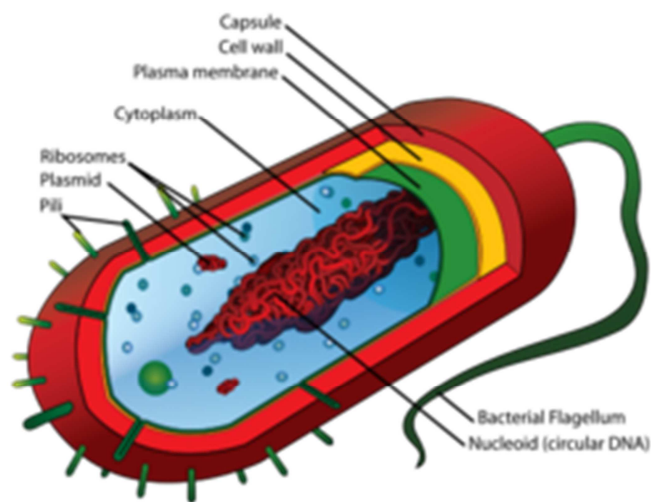


Figure 2. Prokaryotic cell.⁴

3. Glycoconjugates

The glycocalyx is a complex layer of glycoconjugates (carbohydrates linked to proteins and lipids) that forms an outer coat on both eukaryotic and prokaryotic cells (Figure 3).⁵ For a long time the role of the glycocalyx was ascribed to the protection of the cell against the outside environment by repulsive interactions. However, just this role cannot explain the complexity of glycoconjugates. Their location at the outer leaflet indicates that carbohydrates have to be involved in some way in cell-adhesion and recognition processes based on both interactive and repulsive interactions.⁶

The complexity of these conjugates has made their study difficult, and they have been kept aside for many years in comparison with other biomolecules such as nucleic acids or proteins. This complexity arises mostly due to the diversity in structure of carbohydrates.⁷

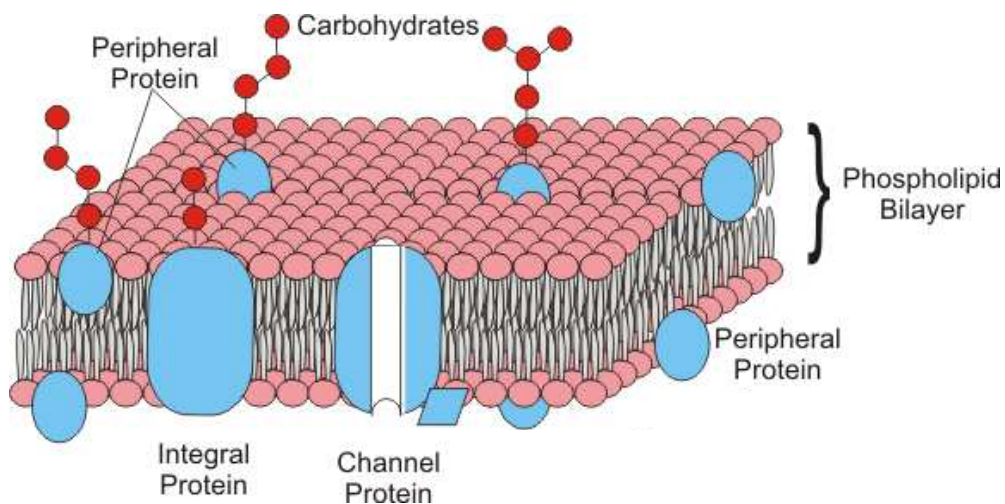


Figure 3. Glycocalyx.

The chain length of the saccharides can vary widely from monosaccharides up to branched oligosaccharides with more than 30 building blocks, or in the case of polysaccharides to several thousand building blocks. The nine common monosaccharides found in mammalian cells (Figure 4) can be combined in various ways to form structures more diverse than those accessible with the twenty naturally occurring amino acids or the four natural nucleotides. A comparison of the permutations of hexamer formation illustrates this point well. Whereas, DNA (with a basis set of 4) and amino acids (with a basis set of 20) may construct a biological language for information transfer of 4096 and 6.4×10^7 ‘words’, respectively, carbohydrates have access to greater than 1.05×10^{12} variations.⁸

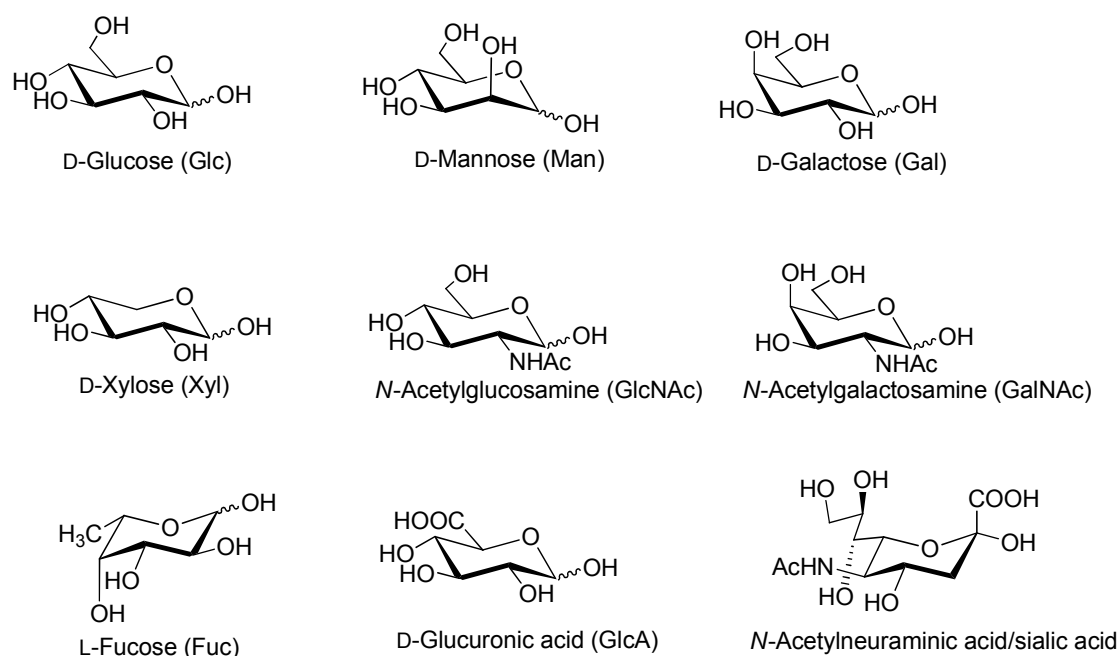


Figure 4. The nine common monosaccharides found in mammalian cells.

Added to this is the variety afforded by anomeric stereochemistry, ring size and sub-unit modification (*e.g.*, sulfation, phosphorylation or acylation), and it can be quickly seen that this greater variety of possible combinations gives the language of carbohydrates exquisite eloquence.⁸ This language has been christened ‘glycocode’—a term that well represents the potential level of complex information that carbohydrate structures are able to convey. The vast number of potential permutations represents a technological barrier and means that no longer can oligosaccharidic structures be made on an iterative basis since there are far too many possible synthetic targets. It is therefore crucial that the design of new carbohydrate-containing structures be guided by the identification of the associated functions of existing structures.

Cells, bacteria, toxins and other organisms interact with their receptors, which in most cases are glycoconjugates (glycolipids and/or glycoproteins), at the cell surface. Three models for glycoconjugate interactions have been suggested: (i) Carbohydrate Recognition Model—only single oligosaccharide motifs are ligands; (ii) Cluster Model—clusters of many carbohydrate motifs are ligands; (iii) Carbohydrate–Protein Recognition—the binding ligand is both the carbohydrate motif and a given region of protein that supports it.⁸

Since most glycans are on the outer surface of cellular and secreted macromolecules, they are in a position to modulate or mediate a wide variety of events. These interactions mediate cell adhesion, cell motility, and cell–cell communication and recognition of toxins (Figure 5). They are also in position to mediate interactions between

organisms, for example between host and parasite. Examples of important cell-surface recognition events include: (1) cell–cell adhesion in inflammatory processes involving lectin–carbohydrate interactions, which mediate leukocyte latching to inflamed tissues;⁹ (2) neutrophil penetration is thought to be mediated by CD177, a glycoprotein;¹⁰ (3) HIV infection is mediated by glycoprotein (GP 120) binding with cell-surface receptors;¹¹ and (4) the metastasis of certain cancers involves saccharide-mediated events.¹² Most recently, the precise binding of human sperm cells to the ovum has been defined as a protein–carbohydrate interaction.¹³

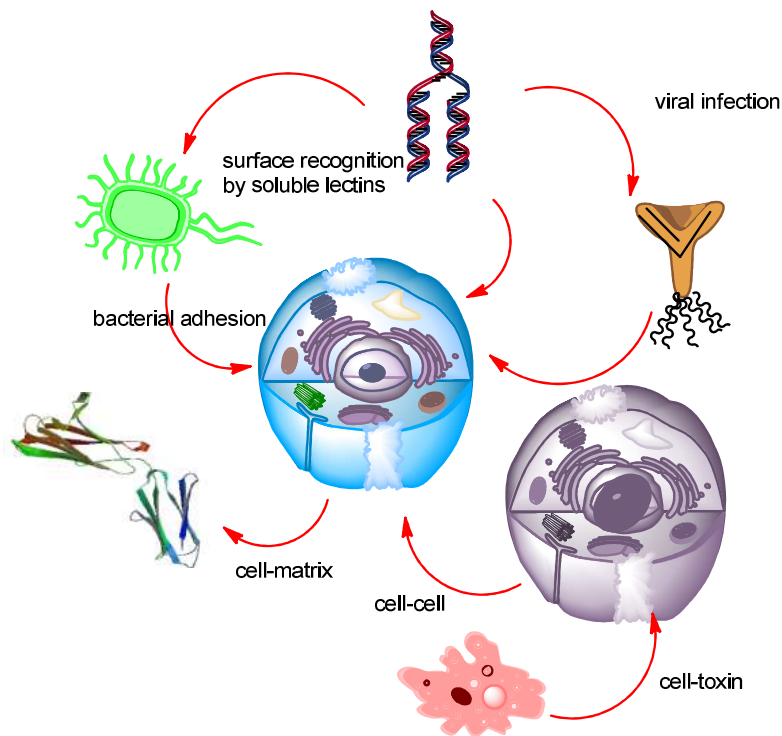


Figure 5. Cell–cell interactions, cell–matrix adhesion, cell–viral recognition and adhesion, and cell–bacterial adhesion.

In addition, many important biological interactions and functions mediated by glycans are potentially amenable to manipulation in vivo. For this reason alone, glycobiology and carbohydrate chemistry have become of increasing importance in modern biotechnology. Furthermore, several human diseases are characterized by changes in glycan biosynthesis that can be of diagnostic and/or therapeutic significance.

4. Carbohydrate–Protein Interactions

Among the interactions involving carbohydrates, one of the most important is the binding between carbohydrates and proteins. Proteins excluding enzymes and immunoglobulins that bind carbohydrates are generally called lectins.¹⁴ Simply put, lectins are proteins that bind carbohydrates.¹⁵ They are decipherers of the glycode, and despite their very shallow binding sites, show a remarkable specificity in their binding of multivalent complex carbohydrate structures.¹⁶ The term lectin, which owes its origin to the Latin word *legere* meaning *specific*, was first used by Boyd in 1954¹⁷ to describe proteins that show a potent and highly specific ability to bind glycosylated structures. However, they were first discovered in plants more than 100 years ago, and they are now known to be present throughout nature. The concept of glycans being specifically recognized by proteins dates back to Emil Fischer, who used the phrase “lock and key” (Figure 6) to refer to enzymes that recognize specific glycan substrates.^{18,19}

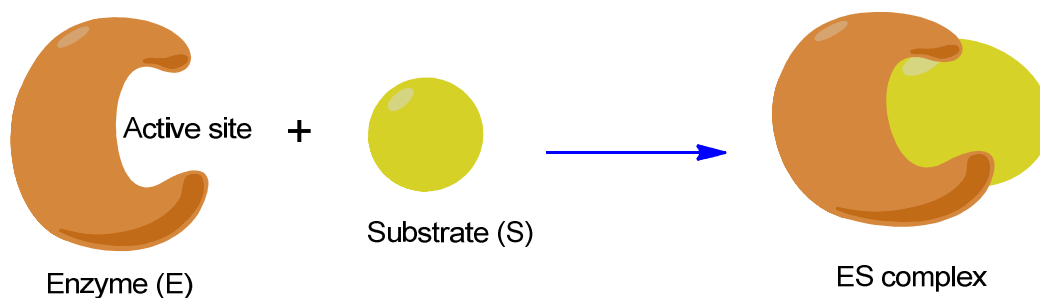


Figure 6. Lock and key mechanism

The characterization of specific interactions between sugars and proteins in three-dimensional space was realized by the determination of the crystal structure of lysozyme, which was the first “carbohydrate-binding protein” to be crystallized. Its structure was solved in a complex with a tetrasaccharide in an elegant series of studies by Phillips and co-workers in the late 1960s.

Lysozyme is an ellipsoidal protein that has a long cleft that runs for most of the length on one surface of the protein (Figure 7).¹⁸ This cleft is astonishingly large, considering that lysozyme has only 129 amino acids, and is capable of accommodating a hexasaccharide and cleaving it into a disaccharide product and a tetrasaccharide product.

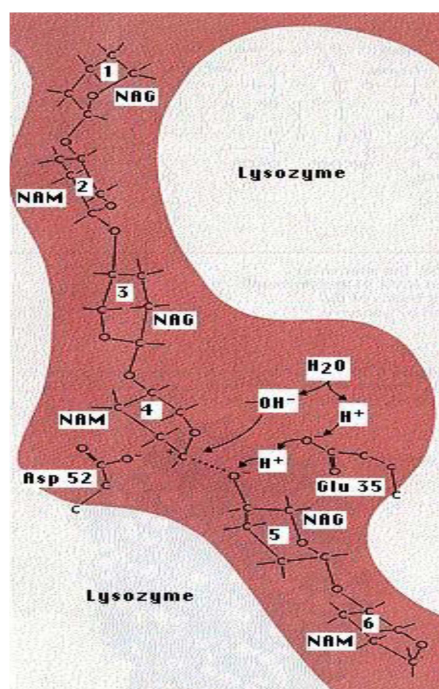


Figure 7. Lysozyme binding glycans¹⁸

Other glycan-binding proteins whose three-dimensional structures are of historical significance are concanavalin A (crystal structure reported in 1972) and influenza virus hemagglutinin (crystal structure reported in 1981). In addition, critical information to the development of this field was gathered by Lemieux and Kabat and co-workers in studies on the combining sites of lectins and antibodies toward specific blood-group antigens.¹⁸ Currently, many lectins have been isolated, characterized and classified into different groups.

4.1. Types of lectins

Lectins are cell agglutinating proteins of non-immune origin that are widely distributed in nature, being found in plants, microorganisms and animals.²⁰ Aside from this common trait, they differ greatly in structure, organization, and biological purpose. As more and more lectins are discovered and studied, a consistent classification of lectins has been developed based on amino acid sequence motifs in the carbohydrate-recognition domains (CRD) of two groups of lectins: one group requires calcium for recognition and is therefore called C-type lectins, and the other group requires “free” thiols for stability and is termed S-type lectins. Meanwhile, lectins of these two groups that recognize mannose-6-phosphate are found distinct from all others, thus justifying their recognition as P-type lectins.²¹

C-type lectins are also distinguished from other lectins by a unique protein fold.²² Two particular important features of C-type lectins that make them appealing for many studies are: a. *Hierarchical pattern recognition*. C-type lectins share the lectin hallmark of multimeric architecture. The prototypical ligand preferences of individual C-type lectin domains (CLDs) are typically for mannose (Man) (most vertebrate lectins) or galactose (Gal) (invertebrate lectins). Affinity for simple, free sugars (monosaccharides) is demonstrable but weak. Strong interactions (avidity) result from simultaneous binding to multiple ligands (multivalency). Because of the intermediacy of the calcium ions, C-type lectins make few direct protein–carbohydrate contacts, and binding occurs primarily through ligation of the calcium ion by the equatorial 3- and 4-hydroxyl groups on the

sugar (Figure 8). Because the protein and sugar are insulated by the calcium ion, recognition is somewhat loose and primarily involves a single sugar unit. Sugars can be recognized in alternative orientations, and structurally related sugars can be recognized (e.g., *N*-acetylglucosamine instead of mannose). Thus, how strongly a particular biological surface is recognized depends heavily on presentation.

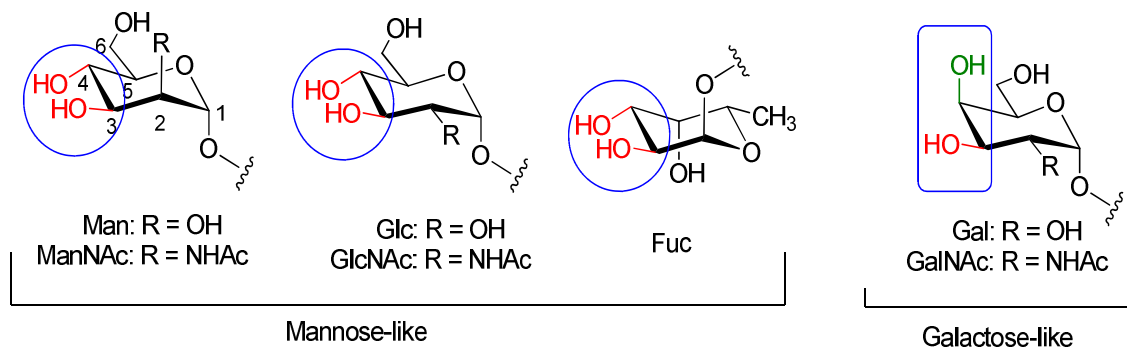


Figure 8. Some sugar motifs recognized by C-type lectins.

b. Highly diverse organization. Any protein containing at least one C-type lectin domain (CLD) is classified as a C-type lectin.²² This large group includes single-CLD, soluble proteins, and integral membrane proteins containing extracellular CLDs, and multi-domain proteins containing one or more CLDs. The organization of mannose-binding lectin (MBL) is shown in Figure 9. MBL assembles into a bouquet-shaped 18-mer (6 × 3).²³ Others adopt a variety of scaffolds for their presentation.

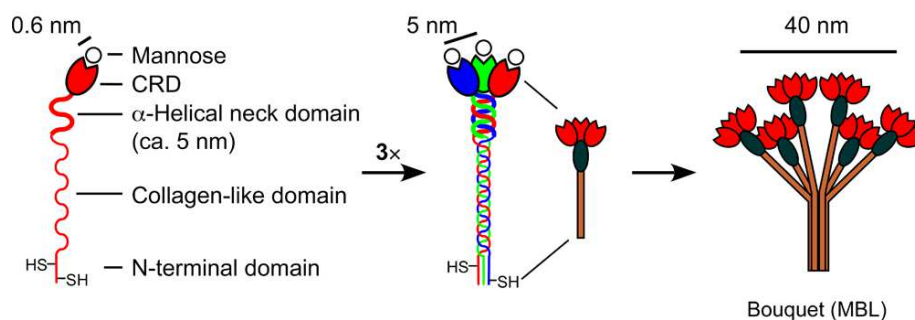


Figure 9. Architecture of soluble collectins, a group of mannose-binding lectins involved in innate immunity, showing hierarchical organization. (Illustration adapted from van de Wetering et al.²⁴)

The endocytic pathway involves lectin recognition of ligands at the cell surface, internalization via coated pits, and delivery of the complex to endosomal compartments where the low pH induces dissociation of ligand and lectin. The lectin recycles to the cell surface and repeats the process.²⁵ The selectins are a class of type I membrane-bound C-type lectins expressed in the vascular endothelium and on circulating leukocytes. At this time, there are only three selectins that have been indentified: L-selectin, expressed on all leukocytes; E-selectin, expressed by cytokine-activated endothelial cells; and P-selectin, expressed constitutively in granules of platelets or cells.

4.2. Multivalency in carbohydrate–protein interactions

It is frequently found that interactions between monovalent carbohydrate ligands and a single binding site (CRD) of a complementary receptor protein are weak with dissociation constants in the millimolar range.²⁶ Nature effects tight binding by multivalency which has been recognized as an important functional principle in

carbohydrate–protein interactions during recent years.^{27,28} Multivalent interactions provide the basis for mechanisms of both agonizing and antagonizing biological interactions that are fundamentally different from those available in monovalent systems. Additionally, the order of ligand specificities in monovalent interactions has been found to be greatly magnified in a multivalent presentation.²⁸ Multivalency in carbohydrate recognition is easily achieved, as the involved ligands are most frequently presented as multiple copies of recognition elements, and this usually finds an analogy in a cluster of receptor binding sites.

Enhancement of affinity upon multivalent binding requires that the change in Gibbs free energy ΔG upon binding of an N-valent ligand is greater than N times the change in Gibbs free energy upon binding of each of the constituent monomeric ligands to an individual lectin binding site.²⁰ In other words, multivalent effect is only observed when a higher binding energy is found in multivalent binding than it can be expected of the sum of individual binding energies. Multivalent interactions have several advantages and controls a wide variety of cellular processes including cell surface recognition events, the immune response, tumor metastasis, fertilization, and microbial adhesion. An understanding of the mechanistic principles that underlie multivalent binding events would facilitate the generation of new classes of carbohydrate-based therapeutic agents because the low affinity of saccharide ligands for their lectin receptors is, of course, one major impediment for the development of carbohydrate derivatives as therapeutics.

The first synthetic oligovalent glycoligands that showed enhanced affinities were introduced by Y.C. Lee and co-workers.^{14, 29,30} With these simple cluster glycosides, a logarithmic increase in affinity for multimeric hepatic lectins was observed in a hemagglutination assay upon a linear increase of scaffolded carbohydrate ligands that were varied from one to three (Figure 10).³¹

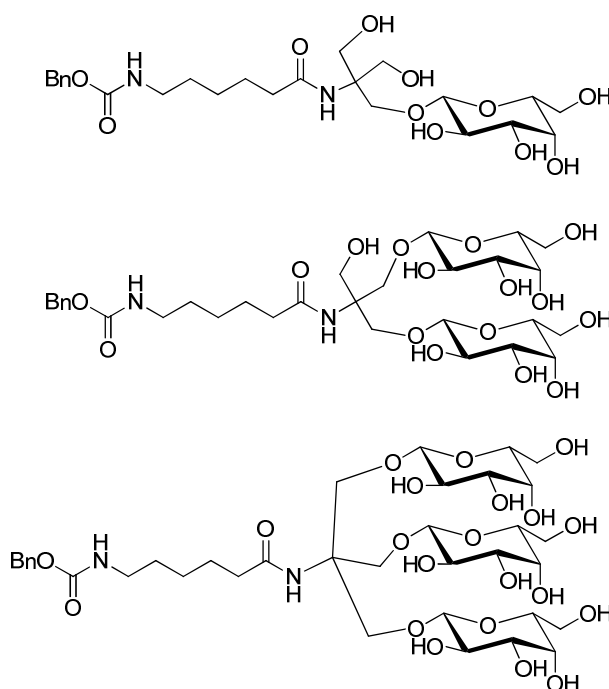


Figure 10. Structure of cluster glycosides with which the ‘cluster effect’ was first observed.

For this observation the term ‘cluster effect’ was coined. Currently, Y.C. Lee’s finding, together with a number of additional exciting observations, made in the context of multivalency, are a major incentive for the design and preparation of synthetic

multivalent ligands to serve for the exploration and modification of cell-surface interactions.³²

One important example of functional multivalency in biology is microbial infection, which requires an adhesion event as a prerequisite. Often microbial adhesion is mediated by multiple carbohydrate-protein interactions. Blocking of these initiating interactions may lead to an anti-adhesion therapy against microbial infections based on carbohydrates, a concept which was first suggested by Sharon et al.³² The best investigated microbial adhesion system is the adhesion of the influenza virus to its host cells. In the first step of infection, the influenza virus attaches to the surface of a bronchial epithelial cell (Figure 11).

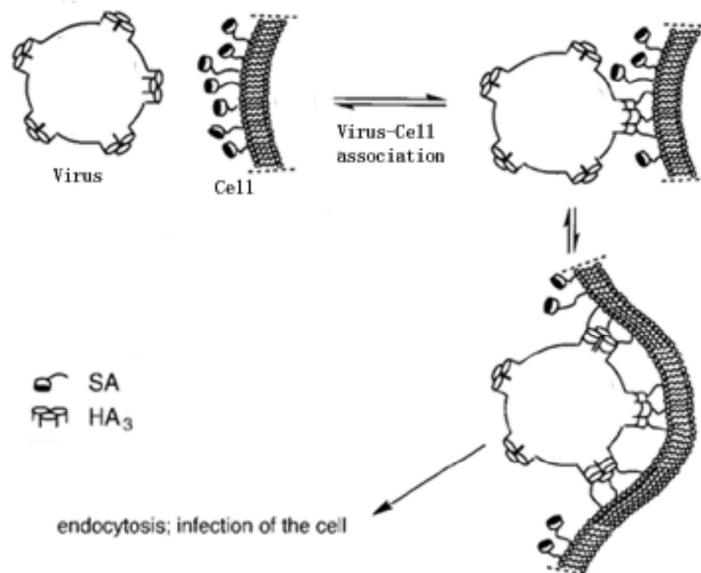


Figure 11. Influenza virus attaching to cell surface

This virus recognizes specific saccharides on the host's epithelial cells and utilizes these cellular glycans as receptors to initiate an infection.¹ The attachment occurs by interactions between multiple trimers of the hemagglutinin (HA, a lectin that is densely packed on the surface of the virus, about 2–4 per 100 nm²) and multiple moieties of sialic acid (SA, the terminal sugar on many glycoproteins and one that is also arranged densely on the surface of the target cell, about 50–200 per 100 nm²). HA variants adapted to humans recognize α -(2→6) linkage whereas strains specific for birds recognize α -(2→3) linkage. Recent glycan array analysis in conjunction with mutation studies have shown that specific mutations control the specificity of a given HA that selectively binds to a given linkage. These studies helped in understanding and predicting how pathogenic strains can become virulent towards humans.^{33,34,35}

5. Concanavalin A

In the past few decades concanavalin A (Con A) has become the most widely known and intensively investigated phytohemagglutinin. Much of this interest stems from the discovery that this lectin possesses several remarkable biological properties. In addition to its role as a hemagglutinin,³⁶ a structural probe, and a mitogen,³⁷ Con A has been shown to restore normal growth patterns to transformed fibroblasts, to serve as an anti-carcinogenic agent,³⁸ and to distinguish between malignant and normal cells.³⁹ It all started in 1919 when James B. Sumner at Cornell University, well known for being the first to crystallize an enzyme, urease (for which he was awarded a Nobel Prize 21 years later), isolated from jack bean (*Canavalia enformis*) a crystalline protein that he named

concanavalin A. In 1936, Sumner and Howell determined that the crystals of Con A were isoelectric at pH 5.5.³⁶ Concanavalin A is a metalloprotein composed of subunits with a molecular weight of 27,000 daltons; each subunit possesses one sugar-binding site. At pH 5.5 and below, the protein exists as a dimer, molecular weight 54,000 daltons. Above pH 5.5 the protein dimers begin to aggregate, forming a tetramer with four binding sites, each capable of interacting with specific, terminal glycosyl residues of polysaccharide or glycoprotein chain ends (Figure 12).⁴⁰

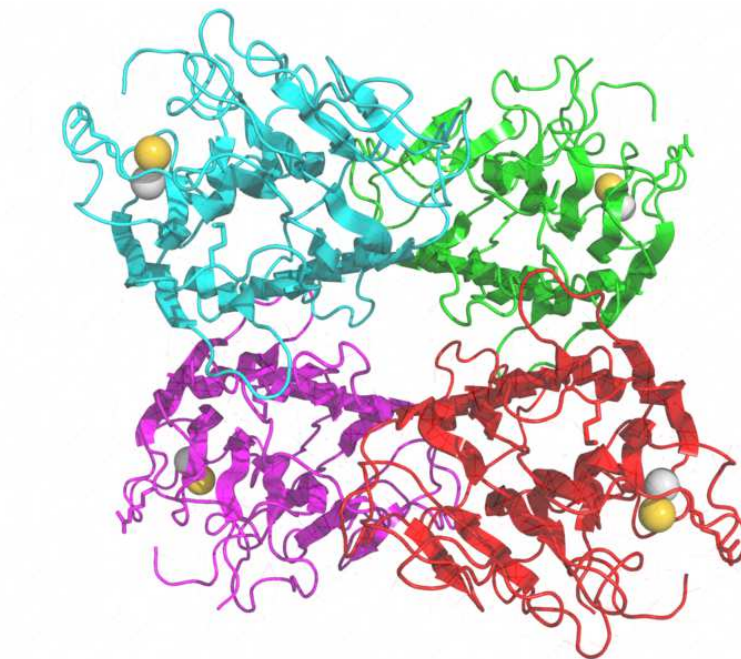


Figure 12. Tetrameric structure of Con A showing 4 binding sites.

Various mammalian cells bind from 10^6 to 10^7 Con A molecules per cell, presumably via surface glycoprotein receptors. This binding can be inhibited by α -D-

mannopyranosides and α -D-glucopyranosides.⁴⁰ In 1969, Goldstein and co-workers suggested that unmodified hydroxyl groups at the C-3, C-4, and C-6 positions of the D-glucopyranose (or D-mannopyranose) ring are essential for binding to the active sites of the protein. They found that the hydroxyl group at C-2 was not essential, but the D-manno configuration was bound more firmly than the D-gluco configuration. It was realized that the α -glycopyranosides of these sugars were much more active than the free sugars, whereas the corresponding β -glucosides were poor inhibitors. These results suggest that the interaction of Con A with polysaccharides involves the chain ends rather than intact inner branches.⁴¹

In 1972, Edelman and coworkers determined the three-dimensional structure of Con A with saccharide-binding pocket of approximately $6\text{\AA} \times 7.5\text{\AA} \times 18\text{\AA}$ and that the metal binding sites were at least $20\text{\AA}$ removed from the position at which saccharides are bound.⁴⁰ Di-, tri- and tetra-saccharides containing D-(1 $\rightarrow$ 2)-mannosidic linkages have been found to bind much more strongly to Con A than their corresponding monosaccharides.²⁰ The Con A carbohydrate binding site is quite clearly different from that found in lysozyme, which is a long cleft in the molecule containing up to six subsites for hexapyranose residues.⁴² The site in Con A is much smaller, perhaps only involving one sugar residue and is not a “groove” but only a shallow indentation in the surface where the pyranose ring lies parallel or almost parallel to the protein surface.⁴³

II. Statement of the Problem

1. Objectives

Despite the centrality of cell-surface carbohydrates in biological recognition, the mechanisms by which they are recognized remain poorly understood. An impediment to progress is the complex structure of the glycocalyx, which makes both chemical synthesis of the oligosaccharide structures involved and biochemical analysis of their interactions with recognition proteins highly challenging. Fortunately, advances in both carbohydrate synthesis⁴⁴ and nanoscale synthesis and characterization⁴⁵ have opened the possibility of emulating a critical subset of glycocalyx features in a context where they can be probed to elicit quantitative details on protein–carbohydrate recognition. In this project, a modular approach to creating carbohydrate structures will be implemented. This approach will be combined with nanoscale patterning to investigate the influence of spatial organization and site-specific ligand modifications on the recognition and binding of carbohydrates by proteins. This dissertation will focus on the developments, challenges and solutions in the following areas:

- A. *Synthesis of a collection of chemically defined, diversely presented carbohydrate monomers and multimers (ligand clusters).* The compounds produced will be novel chemical entities that mimic features of cell surfaces in recognition by proteins (lectins) and binding to entities such as other cells, toxins (peptides or small proteins), and pathogens. The structural entities will be readily adaptable to the study of a number of lectins that have a diverse set of structures and functions.

Initial studies will be carried out on mannose-binding lectin (MBL), a key protein in mammals associated with the recognition of fungi and yeasts.

B. *Refinement of techniques for the mapping and quantitative assessment of carbohydrate recognition on synthetic surfaces.* The techniques, using microarray analysis and fluorescent-tagged lectins will allow direct quantitation of recognition events and produce correlations between avidity, binding energies, and ligand organization. These proposed studies will add new dimensions to our understanding of the effects of valency and density of carbohydrate ligands in protein–carbohydrate binding.

2. Research Design

The multivalency of carbohydrate interactions is well-known, and several groups have pioneered the synthesis of compounds displaying multiple carbohydrate ligands,⁴⁶ primarily with the intent of blocking recognition events at cell surfaces. Parallel work has focused on the immobilization of carbohydrate ligands on solid supports, including nanoparticles and carbohydrate microarray surfaces. Interrogation of these surfaces has been carried out using both fluorescence and atomic force microscopies. Following some early work in which sugars were attached to the surface of gold,⁴⁷ Kataoka and co-workers,⁴⁸ in 2001 reported a lactose–lectin binding study with gold nanoparticles. Since that time, a number of papers have reported sugars (mono-, di- and more complex saccharides) incorporated onto the surfaces of nanostructures and related polymeric

substances, along with characterization and binding studies with a number of proteins. Laboratories involved include those of Barchi (gold nanoshells, cancer therapeutics),⁴⁹ Cloninger (dendrimers, probes for carbohydrate–protein interactions),⁵⁰ Coullierez and Seeberger (polymers, biological probes),⁵¹ Gervay-Hague (gold particles, viral adhesion),⁵² Haines and Field (gold nanoparticles, bioassays and detection),⁵³ Kamerling and Vliegthart (gold surfaces, pathogen (cell) recognition),⁵⁴ Mrksich (self-assembled monolayers, microarrays, on-chip synthesis),⁵⁵ Penadés (metal oxides, magnetic devices, biomedical applications),⁵⁶ Russell (toxin detection),⁵⁷ Turnbull (amide-linked arrays, SPR analysis),⁵⁸ and Wong (microarray analysis)⁵⁹. These works cited are representative, not exhaustive.

This project is designed to combine the advantages of clustered ligand polyvalency, with the development of combinatorial synthesis and surface analysis techniques, including fluorescence.⁶⁰ Features that distinguish our proposed work include (a) controlled ligand presentation via specification of cluster valency and surface density, and (b) rapid quantitative assessment of recognition events using fluorescence. These features will provide insight into the aspects of synthetic design that have the most significant impact on carbohydrate–lectin recognition.

Previous efforts to develop carbohydrate arrays have largely focused on arraying small spots of pure oligosaccharides on glass surfaces.⁶¹ In these designs, the creation and testing of individual carbohydrate ligands in a parallel manner is emphasized. Contrarily, our approach emphasizes the integration of spatial and chemical patterning.

The presentation of carbohydrates is a critical feature of cell recognition and is not fully addressed by approaches used to date.

A major difficulty in the construction of conventional arrays is the synthesis of a large collection of pure oligosaccharides. The synthesis of complex oligosaccharides is notoriously difficult. By emphasizing the presentation aspect of recognition, we will acquire more information from each carbohydrate derivative than is currently possible. Combinatorial synthesis is an efficient means of making large numbers of compounds simultaneously from different combinations of modular building blocks. It has been used in the synthesis of oligosaccharides intended for immobilization on surface arrays,⁵¹ and recently oligosaccharide synthesis has been carried out on the surface of biochips.^{55c}

Therefore, the first part of this project will involve synthesis of carbohydrate ligands. We set out to synthesize mono-, (1→2)-linked di-, (1→3)-linked di-, and (1→2, 1→3)-linked tri-saccharides of thiol-terminated mannose derivatives that will be immobilized on epoxide-functionalized glass slides via covalent bonds. Also, another set of mono-, (1→2)-linked di- and (1→2, 1→3)-tri-saccharides of alkyne-terminated mannose derivatives will be synthesized for immobilization via click chemistry onto azide-functionalized glass slides. These targets have features of glycans that are the most abundant on the glycocalyx and serve as ligands for most receptors.

Screening of carbohydrate arrays has traditionally been accomplished by interrogating them with fluorescently labeled proteins and measuring the fluorescence intensity localized at each spot.^{55b} This approach provides useful information regarding

relative affinities and distinctive patterns of recognition that are useful for characterizing and subsequently identifying proteins. Our array platform seeks to maintain the advantages of fluorescence interrogation regarding overall binding strength and the number and nature of carbohydrate ligands associated with each interaction. Therefore, the second part of the project will involve a preliminary microarray analysis of binding interactions between thiol-terminated mannose derivatives with Con A.

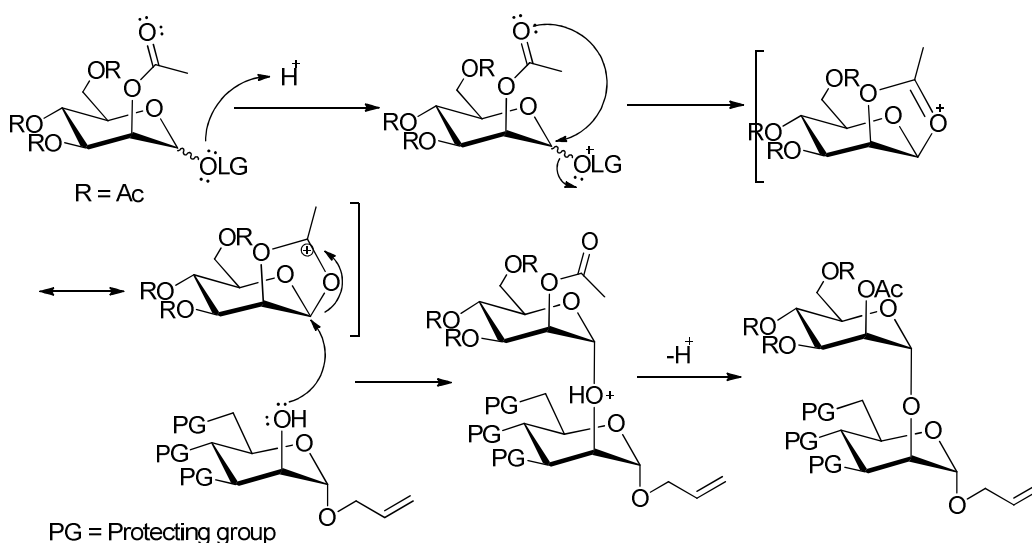
3. Retrosynthetic Analysis

3.1. Retrosynthetic analysis of thiol-terminated mannose derivatives

There are so many variables involved in the synthesis of oligosaccharide that each target compound requires that a particular strategy be developed. The strategy must take into consideration the monomers to be bonded, the types of inter-unit linkages to be made and the sequence in both the topological and chronological senses in which they must be linked.

Our plan is to install a thiol-functionalized spacer-arm into the reducing end of a mannose-bearing saccharide. The synthesis of mannose-bearing oligosaccharides is well documented in the Schmidt glycosylation that makes use of glycosyl trichloroacetimidate donors.⁶² In the Schmidt glycosylation, a trichloroacetimidate donor, prepared from a well-protected 1-OH-free unit can be used to glycosylate another sugar with a free OH group (acceptor). In this project, we are going to use protected D-mannosyl trichloroacetimidate to react with protected 2-OH-free or 3-OH-free D-mannose acceptors in order to establish glycosidic connections.

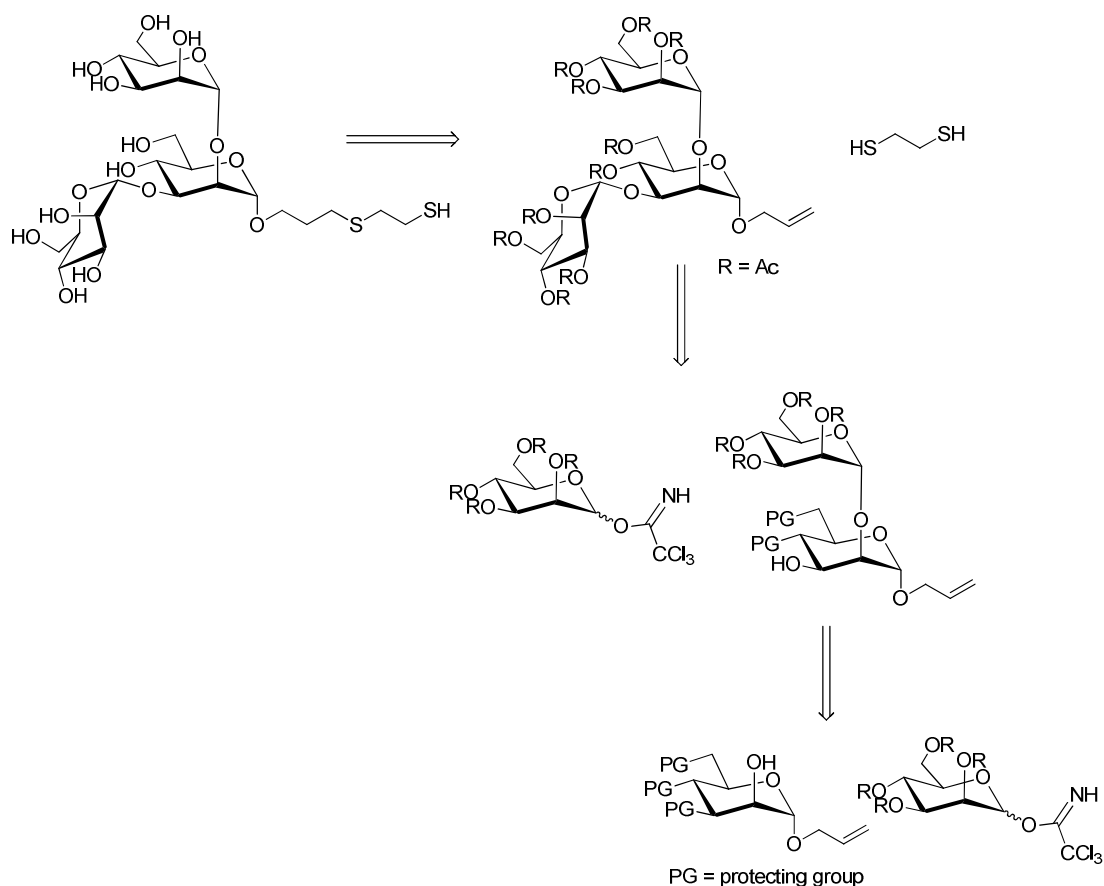
Normally, all hydroxyl groups of the acceptor other than that to be glycosylated must be protected. The hydroxyl groups can be protected as ethers, esters, or acetals. Carbohydrates can be easily condensed through their hydroxyl groups with a variety of both organic and inorganic acid derivatives to give esters. Carbohydrate acetates have been encountered in monomeric and polymeric natural materials, but they are known as synthetic products which often show favorable crystalline properties, and from which the parent alcoholic compounds can be recovered under basic or acidic conditions. Deacetylation can be effected by the well documented and convenient Zemplén procedure,⁶³ in which NaOMe (0.025 M) is employed in catalytic amounts in methanolic solution. Use of acetyl protecting group in this synthesis is appealing not only because of the ease to protect/deprotect but more importantly, its neighboring-group participation effect that will ensure α -anomeric selectivity of the glycosidic bond. Neighboring group participation (as shown in Scheme 1) occurs when a molecule that can undergo nucleophilic substitution also contains a substituent that can act as a nucleophile.⁶⁴



Scheme 1. Neighboring-group participation.

As a result, the rate and stereochemistry of the nucleophilic substitution are strongly affected. Since our desired stereochemistry is the α -linkage, the acetyl protective group will work in our favor. Now that the construction of oligosaccharide can be carried out by the Schmidt glycosylation, our attention will be drawn to the installation of thiol functionalized spacer-arm to the reducing end of the trisaccharide.

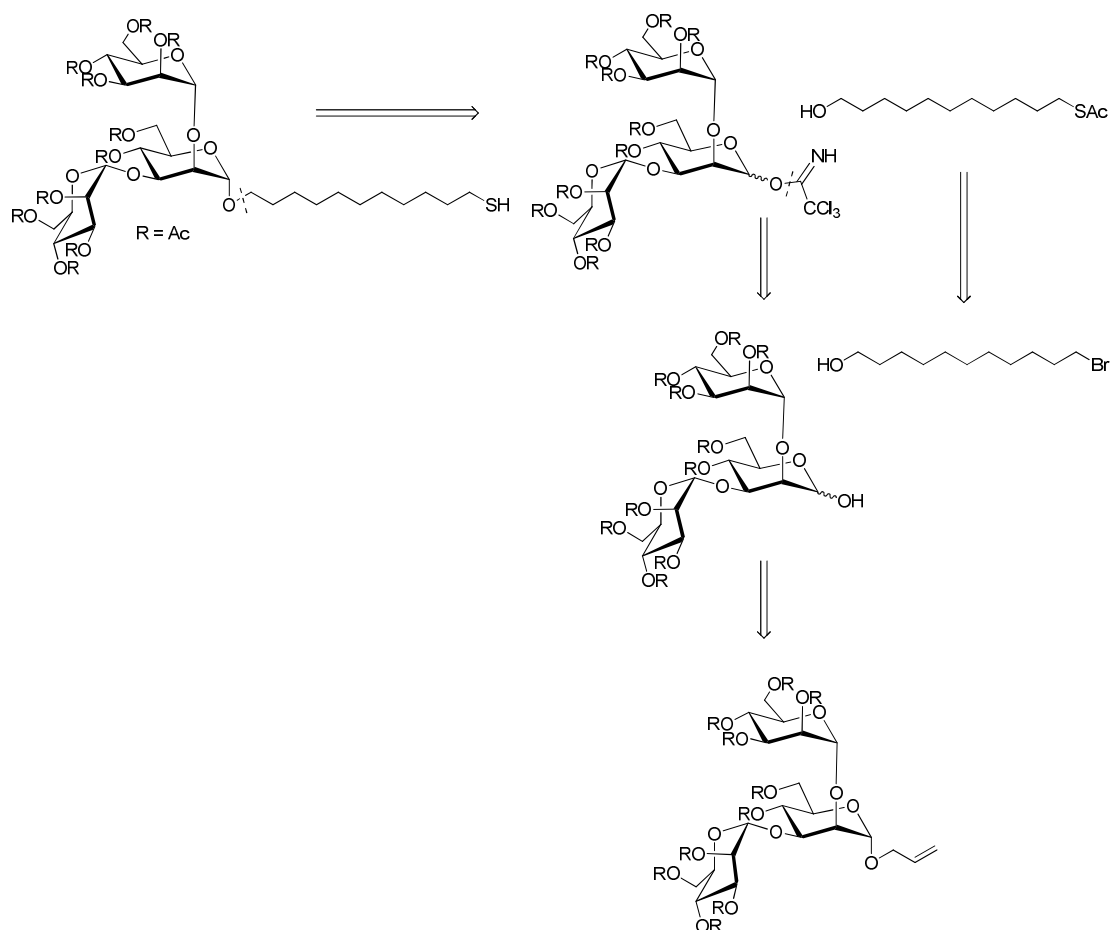
Currently in the literature, there are four major ways to introduce a thiol group into a sugar unit. In 1997, Vliegthart, Kamerling, and co-workers reported an improved method to introduce thiol functionality: thiol addition to protected allyl glycosides via a radical addition pathway as shown in Scheme 2.⁶⁵ The radicals initiated by azobisisobutyronitrile (AIBN) were able to activate a dithiol, and subsequently the activated dithiol reacted with various allyl glycosides in 1,4-dioxane at 75 °C to yield thiol spacer-armed glycosides.



Scheme 2. Vliegthart's method for thiol addition.

The second way to install the thiol spacer-arm was developed in 2003 by Penadés and co-workers. Again, Schmidt glycosylation is used to put together the saccharide units with an SH-protected alcohol as shown in Scheme 3.⁶⁶ While the Schmidt glycosylation can be carried out smoothly without any issues, our project itself doesn't need such a long spacer-arm as 11 carbons. Since we intend to use various linkers and adaptors to link different molecules to the nano-surface, the length of the carbohydrate spacer-arm must

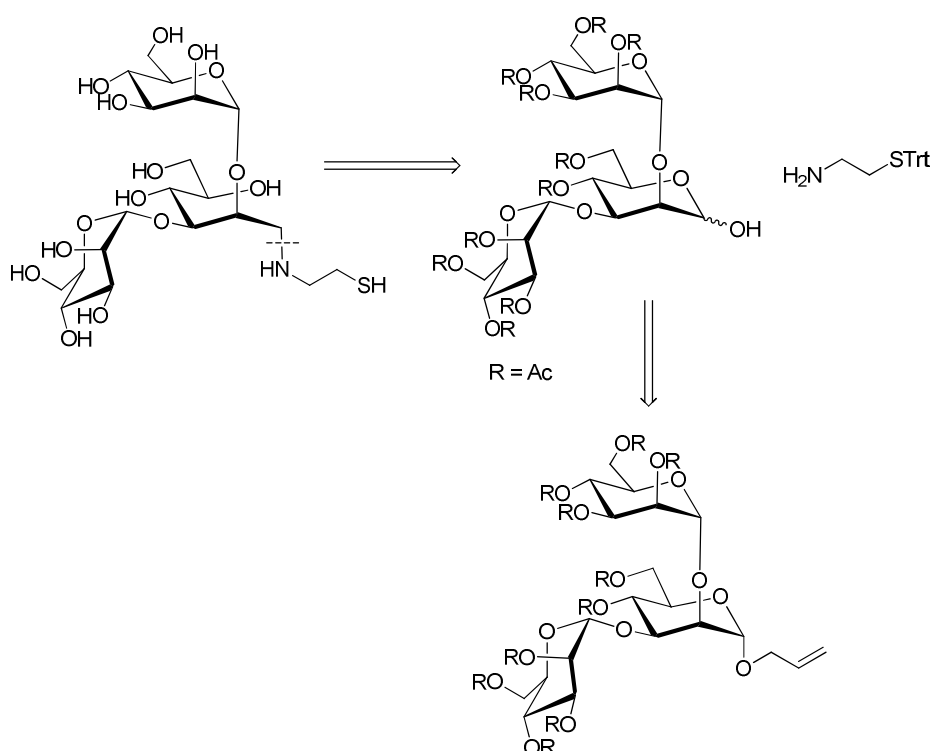
be kept within a certain range: an arm of 11 carbons is more suitable to be attached to the surface without use of any other linkers.



Scheme 3. Penadés' method for thiol addition.

The third approach, as reported by Kamerling and co-workers in 2005, took advantage of reductive amination to introduce the thiol functionality. They used a trityl-protected cysteamine (2-aminoethanethiol) to do reductive amination in presence of NaCNBH₃ in DMSO as shown in Scheme 4.⁶⁷ Reductive amination is a well-documented and robust chemical method widely used in various situations. There should

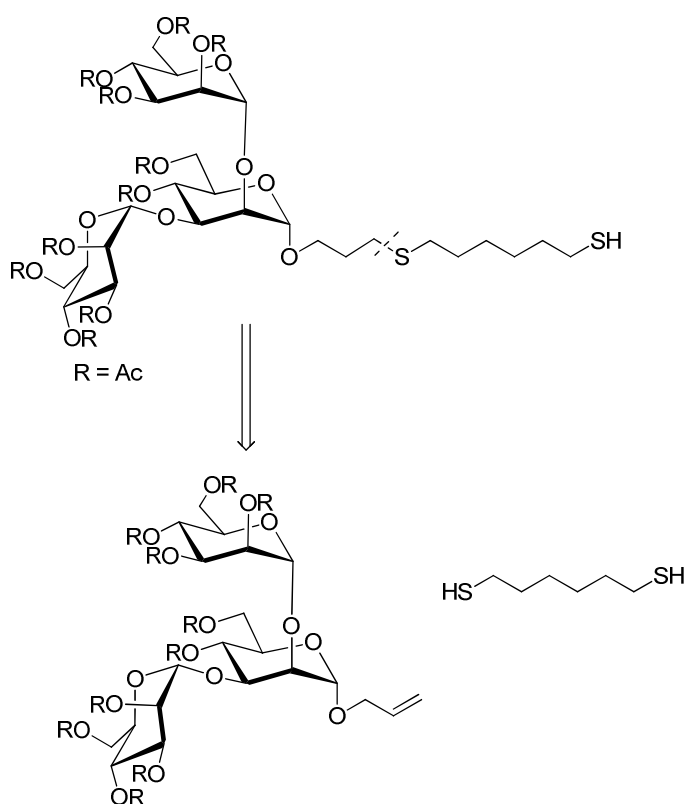
be no problem to apply this method to our syntheses. Since a number of oligosaccharide structures were evaluated in this paper, this would be considered an alternative approach to introduce a thiol terminus if the thiol addition method failed. The major drawback to this process is that reductive amination opens the reducing-end terminus to form an alditol derivative.



Scheme 4. Kamerling's method to introduce cysteamine into a free-hydroxy glycan.

The fourth major way for installation of thiol terminal is the photoaddition of a dithiol to a terminal allyl group. An example of this methodology was reported in 2004 by Kamerling, Vliegthart and co-workers. In this paper, the authors added 1,6-hexanedithiol photochemically to the double bond of an allyl group in MeOH under the

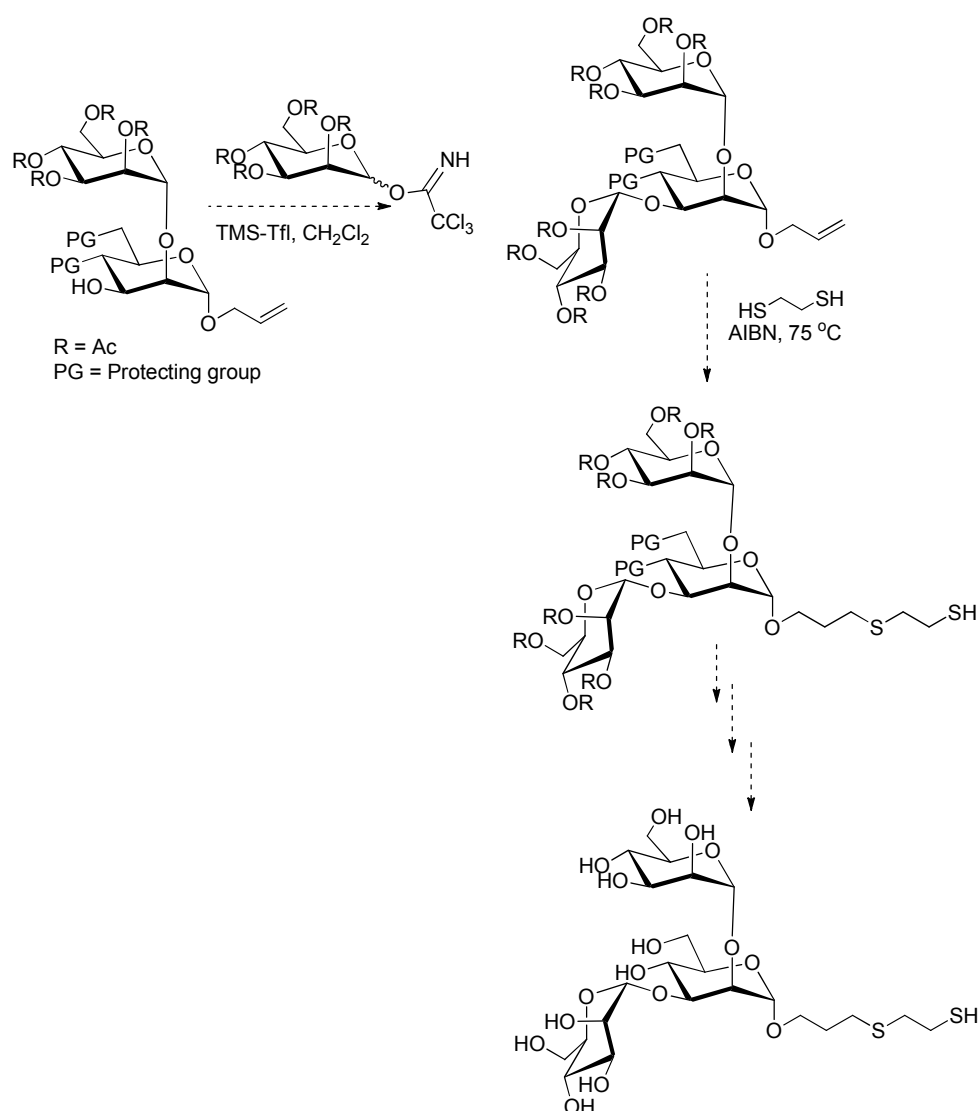
radiation of a UV light as shown in Scheme 5.⁶⁸ However, there were two drawbacks that make this approach not applicable in our project: (1) the length of spacer-arm obtained here was also longer than what we expected; (2) photochemistry is not in general a good way for large-scale synthesis in that it produces insoluble precipitates during UV radiation, and therefore, this method should be avoided if other approaches are possible.



Scheme 5. Kamerling's second method to introduce sulfide into a free-hydroxy glycan.

In conclusion, we will adopt Vliegthart's thiol addition method (Scheme 2) to add a dithiol by means of a free-radical reaction to the allyl group of our mannose-

bearing trisaccharide, since it is the most convenient and straightforward method to consider. Also, this method was successfully used in our research group by Chao Wang resulting in a high yield of 85%.⁶⁹ The proposed approach to synthesize a thiol-terminated mannose-bearing trisaccharide is shown in Scheme 6.



Scheme 6. Proposed synthesis of a trisaccharide.

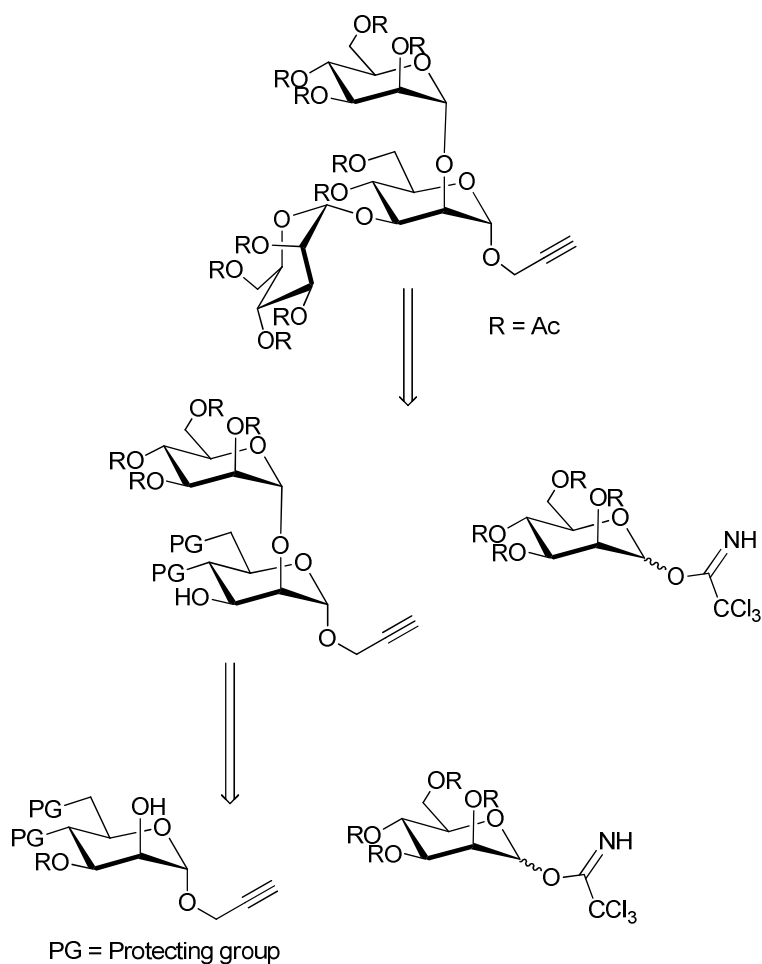
The same approach will be used for the synthesis of the thiol-terminated (1→2)-linked, (1→3)-linked disaccharides and also for the synthesis of the thiol-terminated monosaccharide.

3.2. Retrosynthetic analysis of alkyne-terminated mannose derivatives

Since we desired to evaluate the synthetic oligosaccharides for their biological activities on solid surfaces, the choice of the reducing end aglycon was very important. For our second set of sugars, we chose the propargyl glycoside since it is suitable for cycloaddition reaction with azide-functionalized surfaces.^{70,71} Due to the ease of installing and uninstalling propargyl-terminated aglycons to oligosaccharides, propargyl glycosides can be synthesized in two major ways. A stepwise approach in which one monosaccharide unit is added to the growing chain or a convergent block synthetic route can be used. In the stepwise method, it will eventually become necessary to perform protecting group manipulations on large molecules, which results in low-yielding reactions. One of the advantages of a convergent block synthesis is that these manipulations are kept to a minimum since they are instead performed on smaller fragments.

For the synthesis of the target alkyne-derivatized trisaccharide, a 2 + 1 disconnection was planned. The 3-OH free disaccharide will serve as the acceptor and could be coupled to the trichloroacetimidate donor following Schmidt glycosylation procedure (Scheme 7).⁶² The disaccharide could be obtained from a similar glycosylation

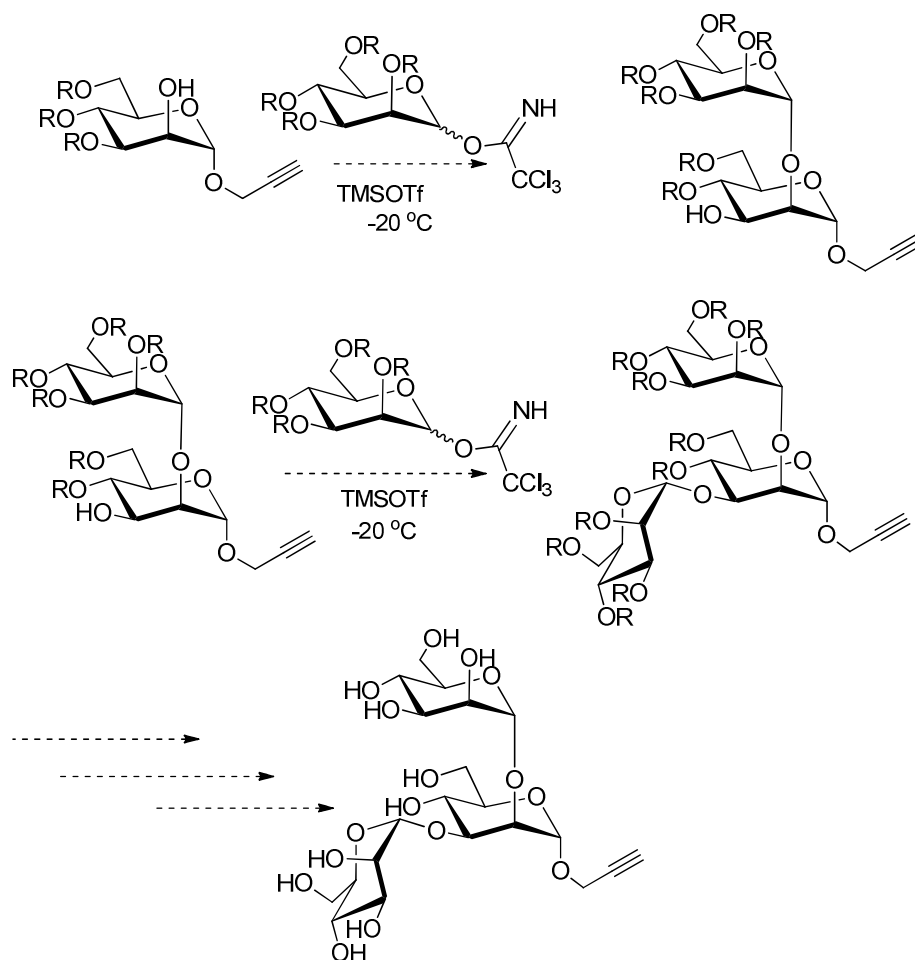
with a trichloroacetimidate donor and propargyl glycoside acceptor with a 2-OH free position.



Scheme 7. Retrosynthetic analysis for an alkyne-terminated trisaccharide.

A similar approach will be used for the synthesis of the (1→2)-linked disaccharide and the monosaccharide. In conclusion, we will adopt the Schmidt glycosylation between the trichloroacetimidate donor and the propargyl glycoside acceptor with TMSOTf as catalyst. Conventional Zemplén deacetylation will be applied

to remove acetyl groups, thus furnishing the alkyne-derivatized mannose derivatives. The proposed approach is as shown in Scheme 8.



Scheme 8. Proposed synthesis for a propargyl-terminated trisaccharide.

3.3. Retrosynthetic analysis of dimeric mannose derivative

Characterization of glycan clusters on cell surfaces requires detection methods of domains with high carbohydrate density.⁷² Recently, there has been great interest in the development of molecules that are efficient in carbohydrate recognition due to their

utility as research tools, especially in biomedical applications. As a result, many different approaches are being pursued for the design of carbohydrate sensors; the most promising being the use of boronic acids. The Best research group of Department of Chemistry at the University of Tennessee is one of such groups involved in the synthesis of boronic acids for the detection of carbohydrate clusters.^{72,73} Their strategy for detection of carbohydrate clustering employs carbohydrate-binding boronic acid receptors derivatized with FRET (Förster resonance energy transfer) pairs (Figure 13). FRET-tagged receptors upon binding will result in an increase in acceptor emission resulting from energy transfer.^{74,74b}

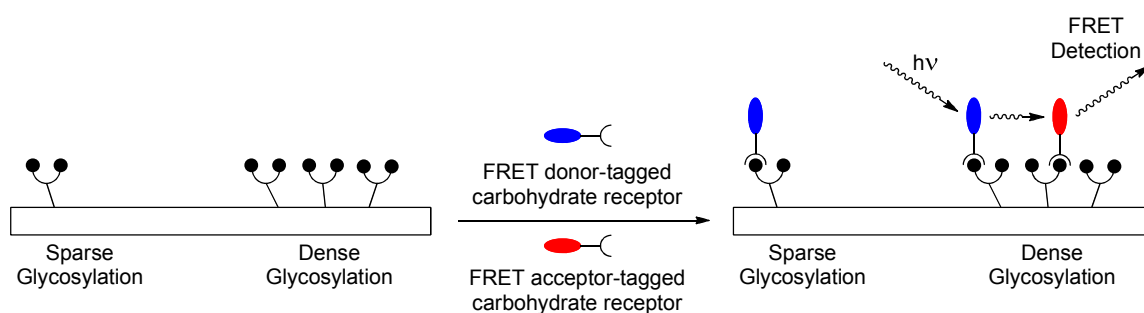
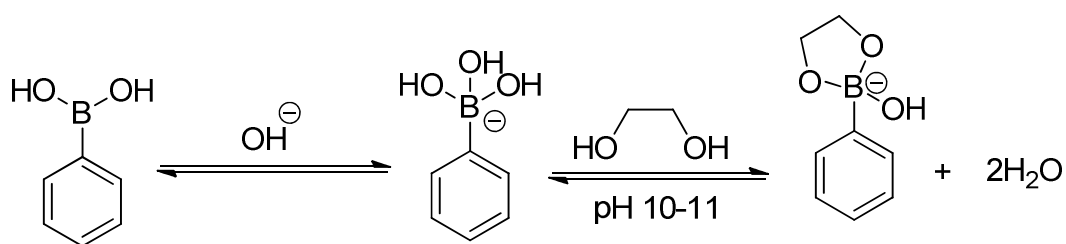


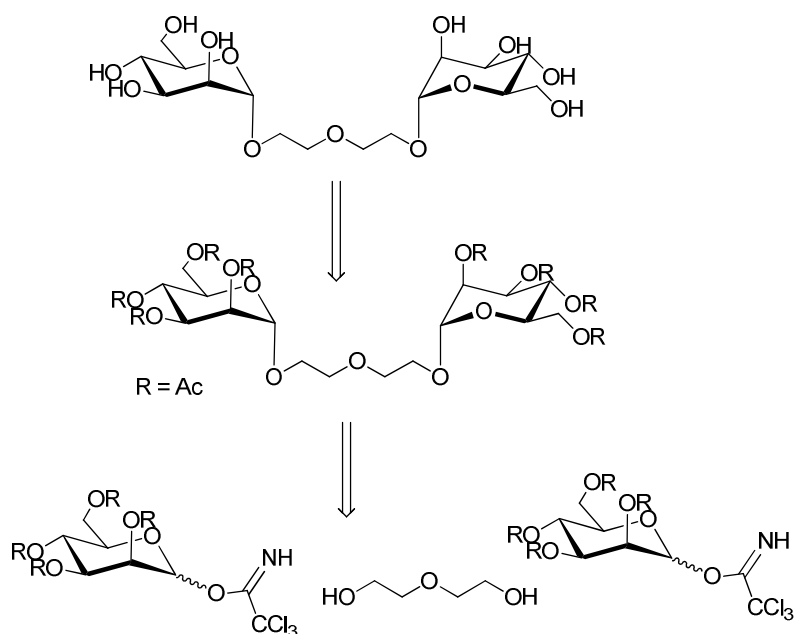
Figure 13. Diagrammatic representation of boronic acid FRET pairs employed for carbohydrate recognition.

Domains of sparse glycosylation should yield little background acceptor emission. Hence, regions of heavy glycosylation are expected to be identified with high resolution by employing this FRET-based detection assay. Boronic acids covalently react with 1,2- or 1,3-diols to form stable and reversible five- or six- membered cyclic boronate esters, as shown in Scheme 9.



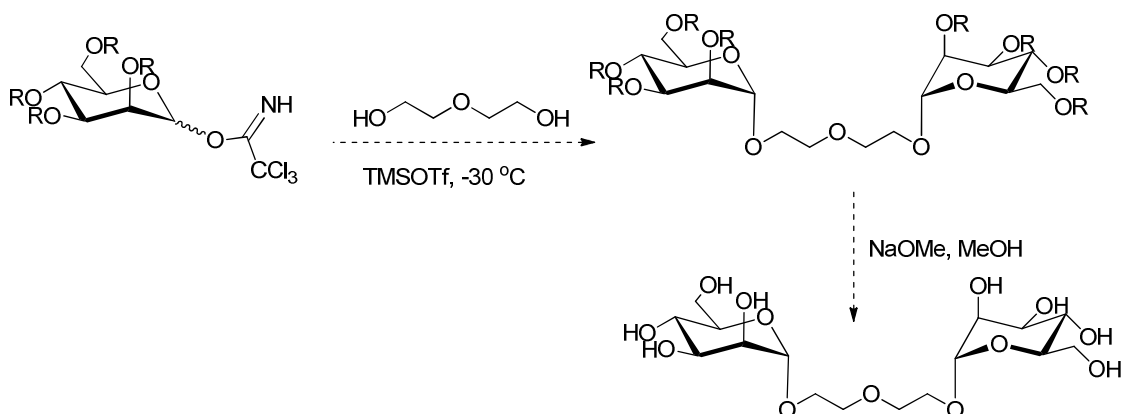
Scheme 9. The binding process between phenylboronic acid and a diol.

For the development of detection methods using boronic acids, a simple carbohydrate cluster was to be synthesized. The synthesis of a dimeric mannose derivative linked by dithylene glycol at their anomeric carbons was designed. The dimeric mannose derivative could be synthesized from a one-pot glycosylation of trichloroacetimidate donor and diethylene glycol as acceptor (Scheme 10). Deacetylation under standard Zemplén conditions will then yield the desired disaccharide.



Scheme 10. Retrosynthesis of dimeric mannose derivative.

In conclusion, the proposed synthesis of the dimeric mannose derivative will be carried out by a one pot glycosylation of trichloroacetimidate donor and diethylene glycol serving as acceptor (Scheme 11).



Scheme 11. Proposed synthesis for dimeric mannose derivative.

4. Significance

The chemistry described in this dissertation is novel and is the newest frontier at the interface of chemistry and biology. The synthesized disaccharides and trisaccharides are all new compounds. Compared to the synthesis of disaccharides, synthesis of trisaccharides seems to be more challenging due to steric hindrance of groups already present on disaccharide. Not many other examples of synthesis for thiol-terminated mannose derivatives exist in literature. The synthesis of propargyl functionalized mannose disaccharide and trisaccharide is new and refreshing and will make a great contribution to the new science of click chemistry.

These studies will offer insight into the important question of how cells interact with proteins and other chemicals in their environment, offering a rich, boundless area of research for different scientists. The insights gained will find applications in a wealth of diverse activities ranging from pathogen recognition in animals to biomineralization in barnacles. More importantly, the techniques developed will have broad implications for the construction of analytical platforms that display the complex arrangements of chemical epitopes commonly found in natural systems.

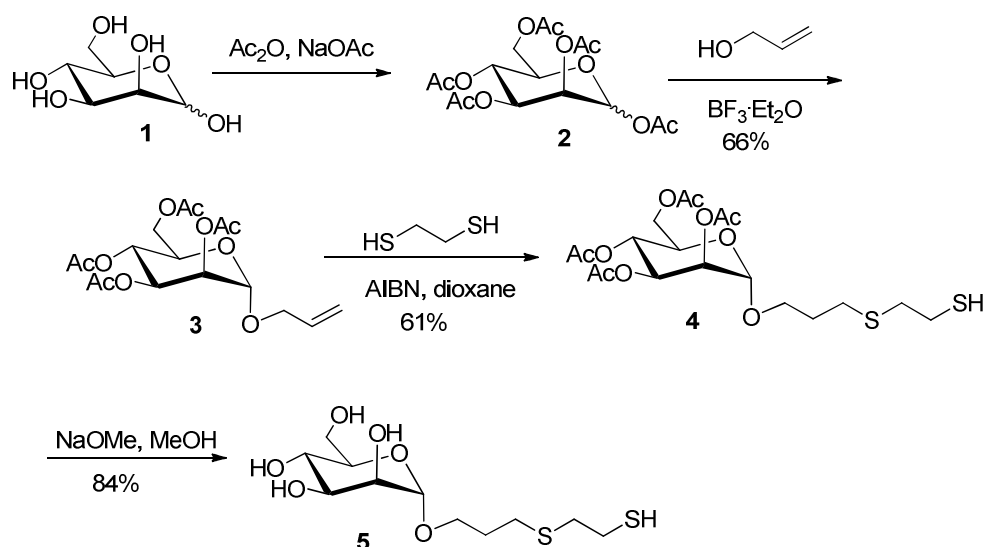
III. Results and Discussion

1. Synthesis of Thiol-Terminated Carbohydrates

1.1. Synthesis of thiol-terminated monosaccharide **5**

The thiol-terminated monosaccharide **5** had been previously synthesized by Brian Sanders and was used by Medhanit Bahta for preliminary binding studies with mannose-binding lectins.⁷⁵ A similar approach was used in this synthesis.

Since mannopyranose pentacetate is rather expensive and the pure anomers were not required, our synthesis commenced with the more affordable mixture of α - and β -D-mannose (**1**). The target thiol-terminated mannose derivative **5** was prepared in four steps from commercially available D-mannose (**1**) as illustrated in Scheme 12. Reaction of D-mannose (**1**) with acetic anhydride (AC_2O) and sodium acetate (NaOAc) at 90 °C gave a 90.1% yield of pentaacetate **2** with 65% α anomer and 35% β anomer. These anomers were assigned based on $J_{1,2}$ coupling constants. The α anomer had $J_{1,2} = 1.6$ Hz while the β anomer had $J_{1,2} = 3.5$ Hz. The treatment of penta-*O*-acetyl-D-mannopyranose (**2**) with freshly distilled allyl alcohol under catalysis with boron trifluoride diethyl ether complex (Lewis acid) produced allyl tetra-*O*-acetyl- α -D-mannopyranose (**3**) in 66% yield⁷⁶. Following the Vliegthart's procedure,⁶⁵ the monosaccharide **3** was treated with 1,2-dithiol in 1,4-dioxane, after free-radical initiation by AIBN and three hours of heating. The target thiol-terminated monosaccharide **4** was isolated in 61% yield.



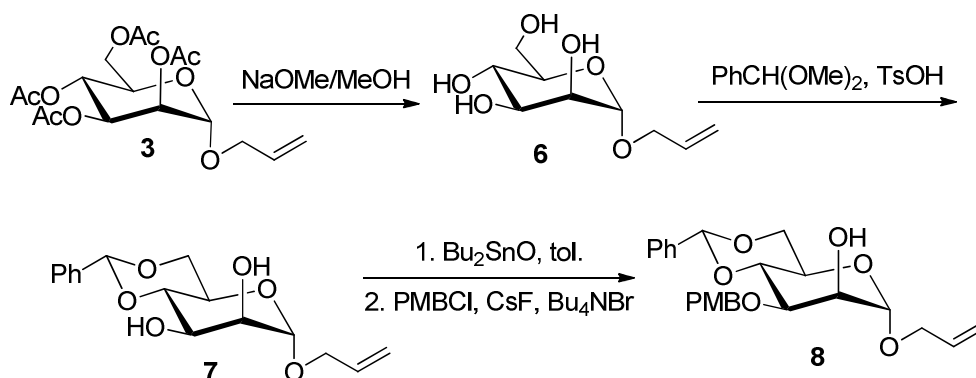
Scheme 12. Synthesis of thiol-terminated monosaccharide 5.

Since the 1,2-dithiol is volatile and carries very unpleasant odors, this reaction was conducted with traps for 1,2-ethanedithiol. Clorox[®] bleach solution was used to quench the smell immediately after the use of syringes and gloves to handle it. Finally, global deprotection using methanolic sodium methoxide (Zemplén conditions)⁶³ and purification using LH-20 gave the free thiol-terminated mannose-bearing monosaccharide 5 in 84% yield.

1.2. Synthesis of acceptor 8

The acceptor **8** required for glycosylation reaction was prepared by the following 4-step procedure starting from previously synthesized allylated monosaccharide **3** as shown in Scheme 13. Deacetylation of compound **3** under Zemplén conditions gave the allyl α -D-mannopyranoside (**4**) with a yield of 81.7%. The product was used without further purification. Selective protection of the 4,6-diol was achieved upon treatment of

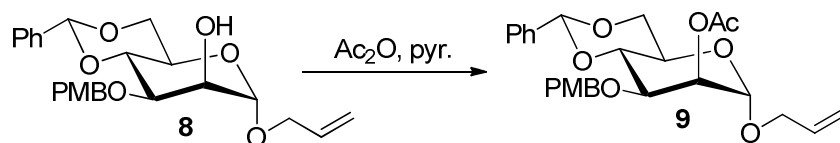
allyl α -D-mannopyranoside (**4**) with benzaldehyde dimethyl acetal under catalysis with *p*-toluenesulfonic acid, which gave compound **7** in 84% yield.⁷⁷ Selective protection of 3-OH (equatorial) over 2-OH (axial) was achieved with the use of dibutyltin oxide (Bu_2SnO) in the first step of the reaction.⁷⁸ A suspension of the acetal, allyl 4,6-O-benzylidene- α -D-mannopyranoside (**7**) and Bu_2SnO in toluene was refluxed for 3 h under an N_2 atmosphere to form a stannane intermediate. A Dean–Stark trap was used for removal of H_2O to improve the yield of the reaction. Tetrabutyl ammonium bromide (Bu_4NBr), cesium fluoride (CsF) and *p*-methoxybenzyl chloride (PMBCl) was added to the stannane intermediate to afford the desired acceptor (**8**) in 76% yield.⁷⁹



Scheme 13. Synthesis of acceptor 8.

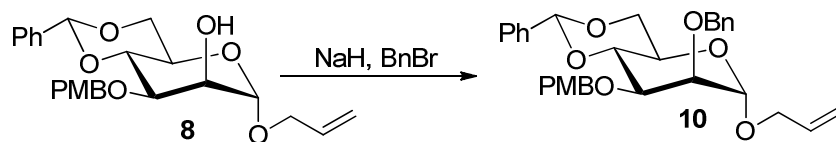
The regioselectivity of the *p*-methoxybenzyl group at the 3-*O*-position was further confirmed by adding protective groups on the 2-OH position and observing their electronic effects on the chemical shift of H-2. Acetylation of the free 2-OH gave the fully protected allyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside

(**9**) as shown in Scheme 14. The acetyl group is an electron-withdrawing group and caused the NMR signal of H-2 to move downfield from δ 4.11 to δ 4.91 ppm. The ^1H NMR signal of H-3 was not affected.



Scheme 14. Acetylation of allyl acceptor 8.

Following the procedure in Chao Wang's dissertation,⁶⁹ the acceptor was reacted with NaH and BnBr to give the benzylated product **10** (Scheme 15). The benzyl group is an electron donating group and caused the NMR signal of H-2 to shift upfield from δ 4.11 to δ 3.50.

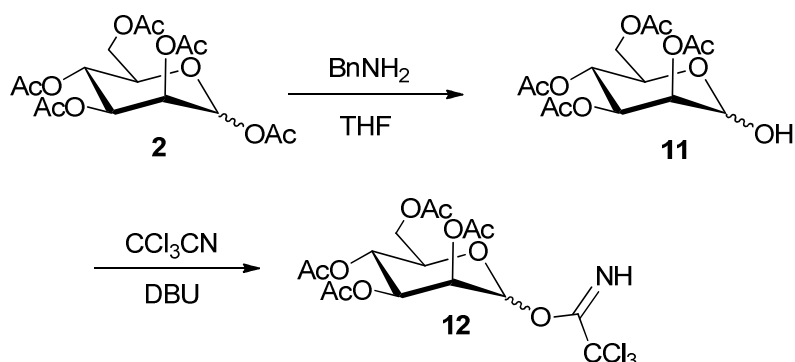


Scheme 15. Benzylation of allyl acceptor 8.

This careful analysis of the ^1H NMR spectrum confirmed that the 2-O-position was free and could be used for glycosylation to make the (1 $\rightarrow$ 2)-linked disaccharide. With the acceptor in hand, it was now time to synthesize the trichloroacetimidate donor.

1.3. Synthesis of trichloroacetimidate donor 12

According to Schmidt and co-workers,⁶² glycosyl trichloroacetimidates are such powerful glycosyl donors that they react with a broad range of glycosyl acceptors with very good stereochemical control. These donors have proved to be more versatile glycosylating agents because of their higher reactivity with nucleophiles under mild conditions. Trichloroacetimidate donors with participating groups at C-2 yield 1,2-trans-compounds, as would be expected. The mannosyl trichloroacetimidate donor was conveniently synthesized in three steps as shown in Scheme 16.



Scheme 16. Synthesis of trichloroacetimidate donor 12.

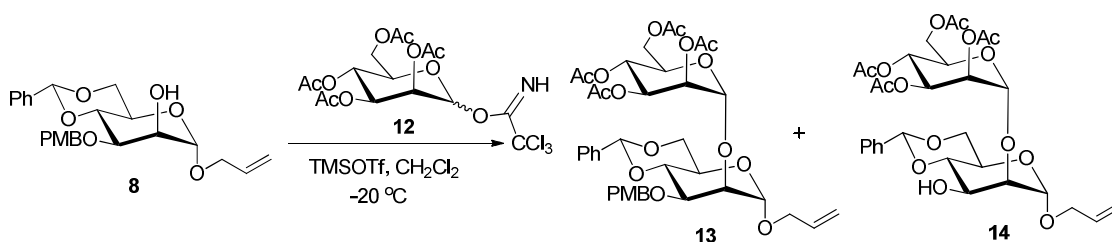
Following Tosin and Murphy's procedure,⁸⁰ penta-O-acetyl- α/β -D-mannoside (2) was anomerically deacetylated using BnNH_2 in THF in an overnight reaction. The anomeric-OH of 2,3,4,6-tetra-O-acetyl-D-mannopyranose (11) was readily converted into the corresponding trichloroacetimidate (12) by the well-documented Schmidt procedure.⁶² Having synthesized the acceptor and donor, the stage was now set for glycosylation.

1.4. Synthesis of α -(1 $\rightarrow$ 2)-linked disaccharides 13 and 14

The glycosylation step is always considered the biggest challenge throughout an oligosaccharide synthesis. There have been various methods for glycosylation reactions; however, the most widely used one is without a doubt Schmidt's trichloroacetimidate procedure.⁶² The typical reaction conditions involve the use of 4 Å molecular sieves as a drying agent before the moisture-sensitive trimethylsilyl triflate is applied. This treatment was critical to the success of our glycosylation reactions after various unsuccessful attempts. Since the triflate catalyst is very moisture sensitive, it was quenched by the traces of moisture in the flask shortly after addition, resulting in no glycosylation. After we realized the importance of an absolute moisture-free environment in this reaction, we took the effort to pulverize and activate the commercially packaged molecular sieves. Molecular sieves were heated in a flask at 250 °C for 4 h under vacuum. The reaction was more successful when the pulverized and dried molecular sieves were used. However, the yield remained low for some unknown reasons.

A premix–predry technique used by other chemists was also adopted, and the yield improved to a modest 44%. In this technique, the acceptor and donor are mixed and dried for some days before the glycosylation reaction to remove all traces of moisture. TMSOTf was commercially packaged and sold in a vial without septum, moisture crept into the vial each time it was opened for use and quenched the TMSOTf. It then became very important to distill TMSOTf and store it in a vial capped with a septum and protected from moisture. With all these treatments, a 54% yield was achieved.

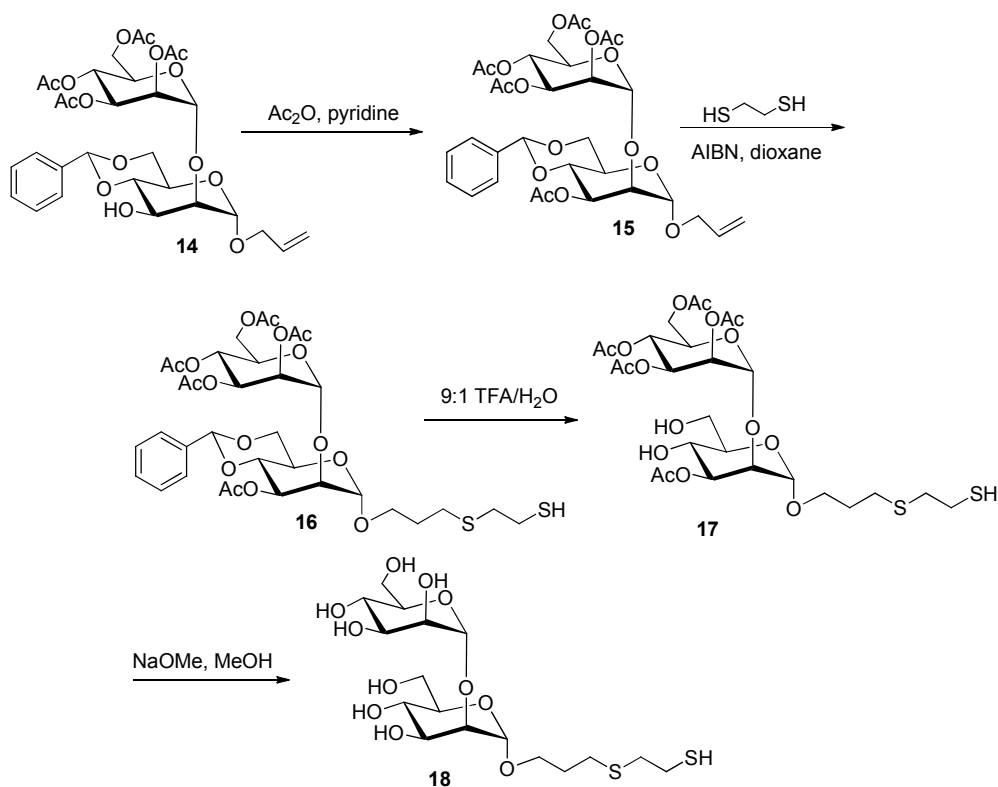
Glycosylation of trichloroacetimidate donor **12** and acceptor **8** was achieved following Schmidt's procedure (Scheme 17).⁶² Evidence from NMR spectroscopy and mass spectrometry showed that two main products **13** and **14** were formed. The expected fully protected disaccharide **13** was formed alongside a disaccharide with a free 3-OH group **14**. The *p*-methoxybenzyl (PMB) protective group was cleaved when the reaction mixture was warmed to room temperature during the glycosylation process to afford disaccharide **14**. To be sure that the PMB group was not cleaved prior to the glycosylation process, the reaction was repeated. This time, the reaction was closely monitored by TLC and upon complete consumption of the acceptor; the reaction was quenched at -20 °C with the addition of Et₃N. Evidence from NMR spectroscopy and mass spectrometry showed that the PMB protective group was not cleaved and that the fully protected disaccharide **13** was the only product formed.



Scheme 17. Synthesis of α -(1→2)-linked disaccharides **13 and **14**.**

In order to install the thiol functionality, it was important to protect the free hydroxyl group (3-OH). This is because there should be no active hydrogen on the compound as this will react with 1,2-ethanedithiol. Treatment of disaccharide **14** with

acetic anhydride in the presence of pyridine and 4-(dimethylamino)pyridine (DMAP) afforded the fully pentaacetylated disaccharide **15** in 81% yield (Scheme 18). Disaccharide **15** was fully characterized using 2D NMR techniques (gCOSY, HSQC, HMBC) to prove the α -(1 $\rightarrow$ 2)-linkage. Now that the free 3-OH of the disaccharide has been temporarily protected, the thiol functionality could be introduced by radical addition. Following procedure by Vliegthart and co-workers,⁶⁵ disaccharide **15** was treated with 1,2-ethanedithiol in 1,4-dioxane, and after free-radical initiation by AIBN and three hours heating, extended thiol compound **16** was produced in 78% yield.

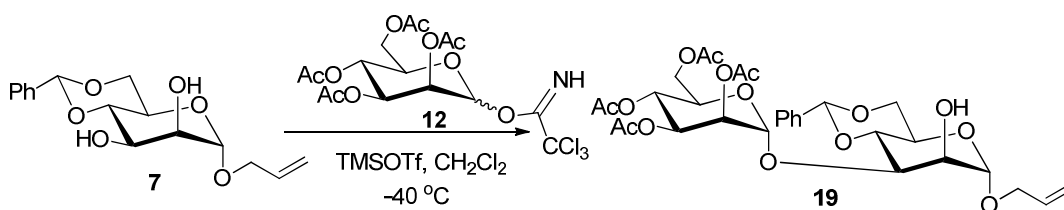


Scheme 18. Synthesis of the free α -(1 $\rightarrow$ 2)-linked disaccharide **18.**

The benzylidene protective group was removed by treatment of thiol-terminated disaccharide **16** with 90% of aqueous trifluoroacetic acid (TFA).⁸¹ Without further characterization, global deprotection of diol (**17**) under Zemplén conditions gave the thiol-terminated mannose-bearing disaccharide **18** in pure form after purification on an LH-20 column. ¹H and ¹³C NMR analyses of the product confirmed that it was 3-[(2-sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranoside (**18**), the desired disaccharide.

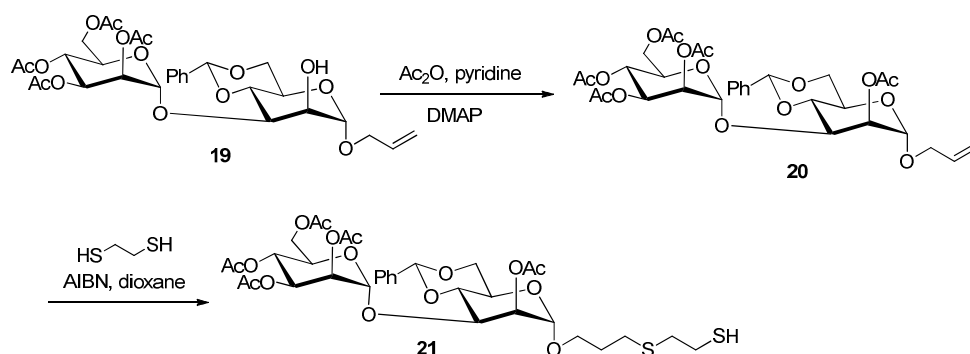
1.5. Synthesis of α -(1 $\rightarrow$ 3)-linked disaccharide **19**

The synthesis of (1 $\rightarrow$ 3)-linked disaccharide commenced with the diol **7** as the acceptor and trichloroacetimidate donor **12**. The 3-OH group of diol **7** was more accessible for glycosylation since it was oriented in the equatorial position and than the 2-OH that was axial. Using this to our advantage, a glycosylation reaction was carried out between the diol **7** and trichloroacetimidate donor **12** at -40 °C for 2 h under an N₂ atmosphere (Scheme 19). The diol **7** was not readily soluble in DCM, so it was sonicated for ~ 1 h prior to glycosylation.



Scheme 19. Synthesis of α -(1 $\rightarrow$ 3)-linked allyl disaccharide **19.**

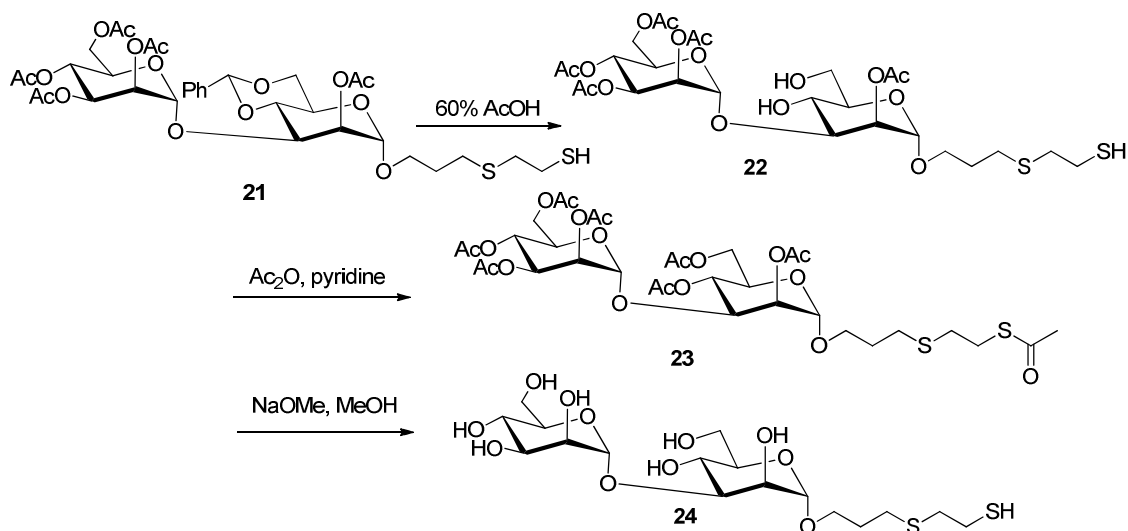
This afforded α -(1 $\rightarrow$ 3)-linked disaccharide **19** in 62% yield alongside a 10% yield of trisaccharide. The structure of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-4,6-*O*-benzylidene α -D-mannopyranoside (**19**) was confirmed by 1D and 2D NMR spectroscopy (gCOSY, gHSQC, TOCSY, HMBC). The disaccharide was acetylated using Ac₂O and pyridine to mask the active hydrogen on the hydroxyl group (Scheme 20). Following the procedure by Vliegthart and co-workers,⁶⁵ the thiol-terminus was installed on compound **20** using 1,2-ethanedithiol in a free-radical reaction with AIBN in 1,4-dioxane. This radical reaction led to the formation of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**21**) in 63% yield.



Scheme 20. Installation of thiol terminal on the (1 $\rightarrow$ 3)-linked disaccharide.

Deprotection of the benzylidene acetal was readily effected by treatment of thiol-terminated disaccharide **21** with 60% aqueous acetic acid at 65 °C to provide a diol intermediate. For ease of purification and characterization, the diol intermediate **22** was acetylated (Scheme 21). Transformation of the diol intermediate into the heptaacetate **23**

was carried out using Ac₂O in pyridine with DMAP catalyst. Finally, global deprotection through Zemplén deacetylation afforded final disaccharide **24** in 64% yield.



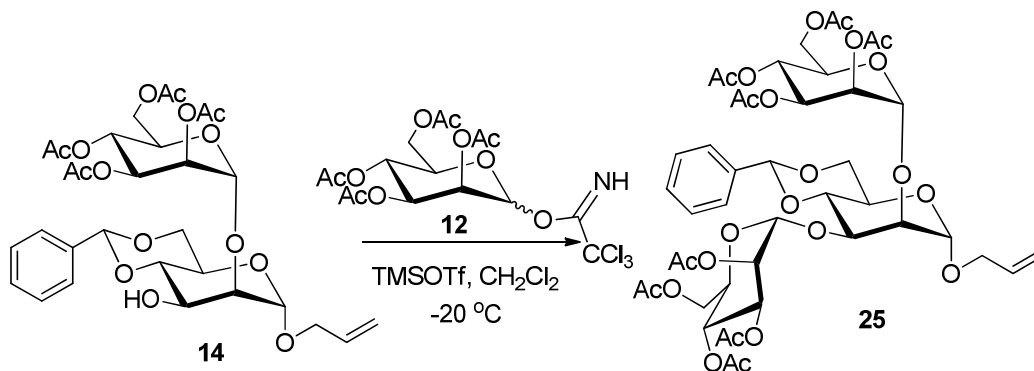
Scheme 21. Synthesis of the free α -(1→3)-linked disaccharide **24.**

Having synthesized both (1→2)-linked and (1→3)-linked disaccharides, we turned our attention to the synthesis of a trisaccharide with combined features of both disaccharides.

1.6. Synthesis of thiol-terminated trisaccharide **25**

The (1→2)-linked disaccharide **14** with open 3-OH served as the acceptor (for synthesis of acceptor refer back to Scheme 17) for the synthesis of the trisaccharide **25**. When a satisfactory amount of this disaccharide was obtained, glycosylation was carried out with a trichloroacetimidate donor **12** (Scheme 22) to afford the trisaccharide **25** in 59% yield. This thiol-terminated trisaccharide was characterized extensively via 1D (¹H and ¹³C) and

2D (gCOSY, HSQC, TOCSY, HMBC, NOESY) NMR spectroscopy (for spectra, refer to Appendix).



Scheme 22. Synthesis of allyl trisaccharide 25.

All ¹H and ¹³C chemical shifts were assigned based on collective 2D data. Heteronuclear single quantum correlation (HSQC) relates the chemical shift of a proton with the chemical shift of the directly bonded carbon. HSQC data was first used to account for all protonated carbons and their respective protons. Gradient correlation spectroscopy (gCOSY) data was next used to determine the protons that coupled with the anomeric protons, since the anomeric protons only have one neighboring proton with which they couple. A heteronuclear multiple bond correlation (HMBC) NMR experiment utilizes multiple-bond couplings over two or three bonds ($J = 2\text{--}15$ Hz). Cross peaks are between protons and carbons that are two or three bonds away (and sometimes up to four or five bonds away). Thus HMBC was the perfect method for confirming the (1→2) and (1→3)-linkages. The HMBC correlations depicted in Figure 14 were analyzed.

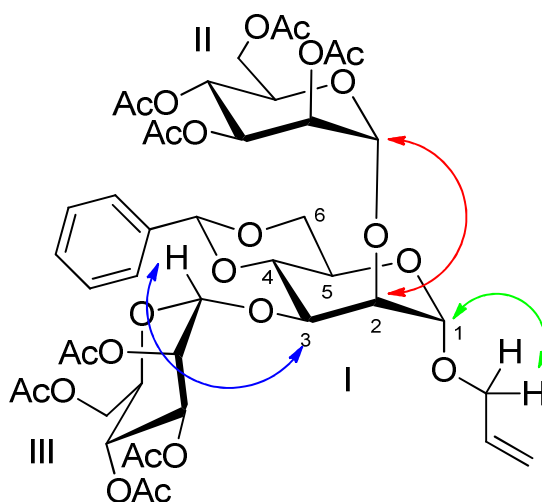


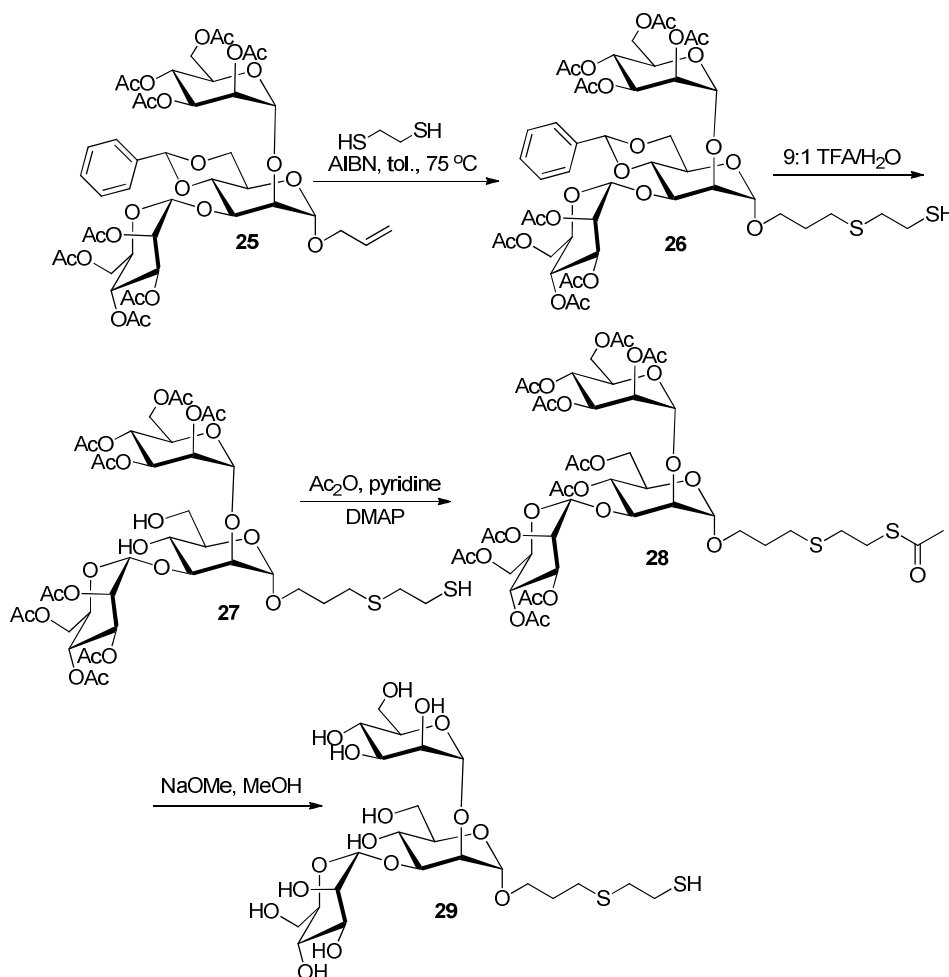
Figure 14. HMBC correlations of trisaccharide 25.

Table 1 is a summary of the correlations used to assign chemical shifts for the anomeric carbons and protons. The anomeric carbon of the reducing sugar (δ 98.65 ppm, C-1^I) was easily identified through correlation with the allylic protons (δ 4.16 and 3.99 ppm, OCH₂CH=CH₂). Following the same pattern, the other protons and carbons that could be assigned as correlations were followed around each sugar unit.

Table 1. HMBC correlations of trisaccharide 25

¹³ C	$\delta(^{13}\text{C})$ ppm	$\delta(^1\text{H})$ ppm	$\delta(^1\text{H})$ ppm
C-1 ^I	98.65	4.16 OCH ₂ aCH=CH ₂	3.99 OCH ₂ bCH=CH ₂
C-2 ^I	77.46	4.89 H-1 ^I	5.21 H-1 ^{II}
C-3 ^I	72.41	5.30 H-1 ^{III}	
C-1 ^{II}	99.48	4.06 H-2 ^I	

Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**25**) was treated with 1,2-ethanedithiol in 1,4-dioxane, after free-radical initiation by AIBN (Scheme 23) following the well-established Vliegthart method.⁶⁵ This led to the formation of the thiol-terminated trisaccharide **26** in a high yield of 78%.



Scheme 23. Synthesis of free thiol-terminated trisaccharide 29.

At room temperature, thiols easily oxidize to form dithiols; however, the MALDI-TOF mass spectrum (Figure 15) of the compound **26** did not indicate a dithiol as shown by m/z 1085.2979 corresponding to $[M+Na]^+$ (calcd for $[M+Na]^+$ m/z 1085.2970). Opening of the benzylidene acetal using 90% TFA at room temperature afforded the diol intermediate **27**. For easy purification and characterization, the diol was temporarily protected by acetylation with Ac_2O in pyridine. The reaction was started cold at -10 °C and slowly warmed up to room temperature. To improve the yield, DMAP was added to the reaction mixture to afford the trisaccharide **28** in a near quantitative yield of 82%.

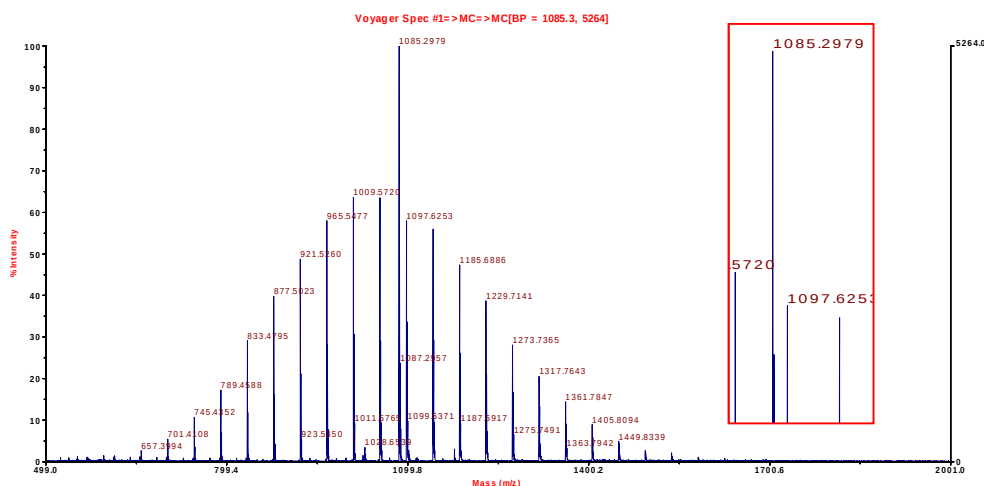


Figure 15. MALDI-TOFMS characterization of thiol-terminated trisaccharide 26.

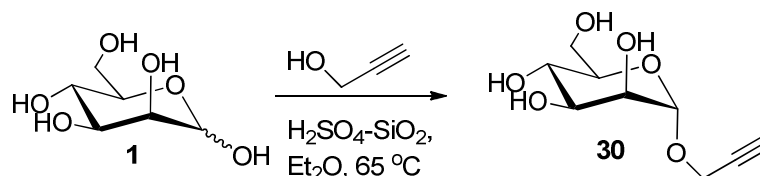
The thiol functionality was acetylated during the acetylation process, and this was confirmed by the 1H and ^{13}C NMR data showing an extra CH_3 peak at δ 30.77 ppm and

an extra carbonyl carbon at δ 195.47, respectively. Deacetylation of the resulting trisaccharide with NaOMe in 1:5 DCM–MeOH provided the target thiol-terminated trisaccharide **29**. The product was purified on an LH-20 column with water and methanol to afford pure compound **29** as a colorless oil.

2. Synthesis of Alkyne Derivatized Mannose Sugars

2.1. Synthesis of an alkyne-derivatized monosaccharide **30**

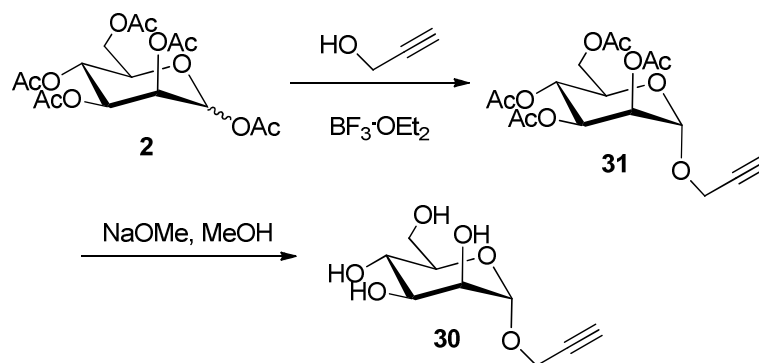
The propargyl-terminated monosaccharide **30** was synthesized in one step according to the procedure developed by Rajput and co-workers (Scheme 24).⁸² Commercially available mannose (**1**) was heated at 65 °C with propargyl alcohol in the presence of H₂SO₄-activated silica gel for 2.5 h. This produced a mixture of 65% α -anomer and 35% β -anomer and separation of these anomers was difficult since they ran too close to each other on TLC. The mixture was acetylated for easier purification.



Scheme 24. Synthesis of free alkyne-terminated monosaccharide **30.**

The yield after acetylation was lower than expected, so an alternate approach was used to synthesize the target monosaccharide as shown in Scheme 25. A solution of previously synthesized pentaacetate **2** in DCM was reacted with propargyl alcohol and BF₃·OEt₂ to afford propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**31**).⁸³

Deacetylation of **31** was achieved under Zemplén conditions to afford the known monosaccharide **30** with a high yield of 88%.

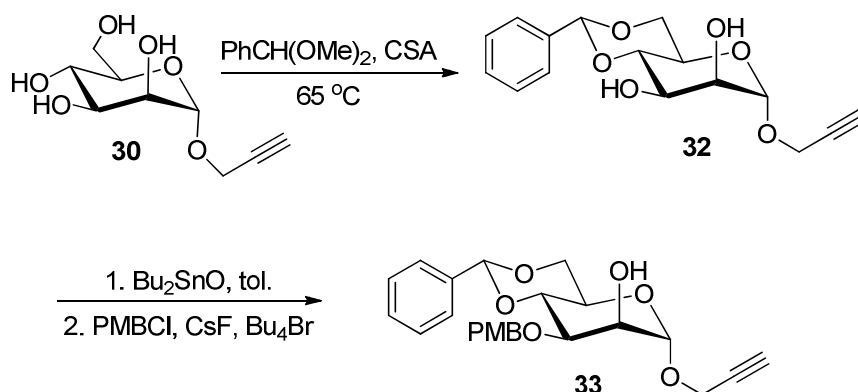


Scheme 25. Synthesis of free alkyne-terminated monosaccharide 30.

The product was recrystallized from EtOH to obtain pure colorless crystals of propargyl α -D-mannopyranoside (**30**). Having synthesized the monosaccharide, our attention was turned to the synthesis of the disaccharide.

2.2. Synthesis of an alkyne-derivatized acceptor **33**

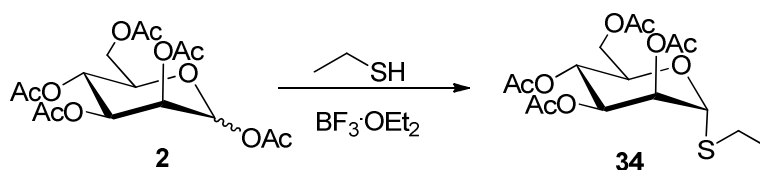
The synthetic methods used for the synthesis of allyl acceptor **8** (Scheme 13) were adopted for the synthesis of the propargyl acceptor **33**. The synthesis of the acceptor commenced with the installation of the benzylidene protecting group on the monosaccharide **30** (Scheme 26). The resulting propargyl 4,5-O-benzylidene- α -D-mannopyranoside (**32**) was selectively protected at the 3-O-position with a *p*-methoxybenzyl group to afford the required the 2-ol acceptor **33** in 65% yield as a viscous gel.



Scheme 26. Synthesis of alkyne-terminated acceptor 33.

2.3. Synthesis of thio-ether donor 34

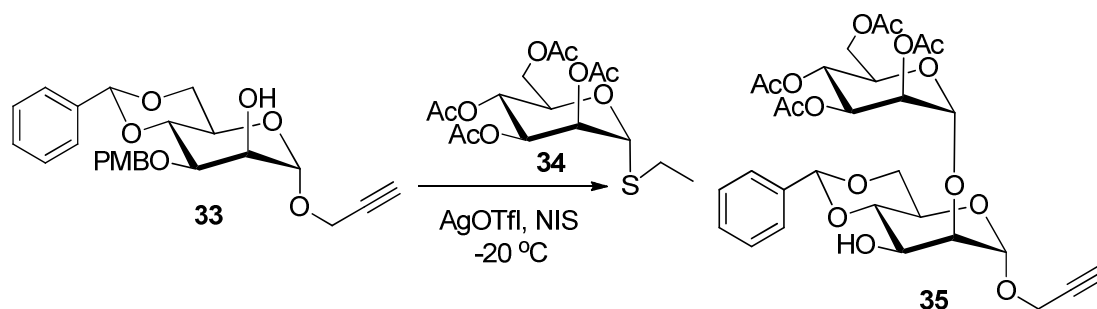
The propargyl acceptor was isolated as a very viscous oil that trapped moisture; it required a long time to dry even under high vacuum. The pre-mix and pre-dry technique with trichloroacetimidate prior to glycosylation was unsuccessful. The trichloroacetimidate is known to be unstable at room temperature and was not suitable for glycosylation with the propargyl acceptor. Contrarily, thioglycoside donors are more stable at room temperature. The thioglycosides differ from O-glycosides in their reactions with several electrophiles since they complex with a range of sulfur-specific reagents to form sulfonium cations which, as good leaving groups, are readily displaced. Consequently, thioglycosides have become valuable intermediates in a variety of transformations, notably glycosylations.⁸⁴ Thiophilic metal salts of mercury(II), lead(II), copper(II) and silver(I) were originally the most popular activators for these reactions.⁸⁴ In the presence of the Lewis acid $\text{BF}_3 \cdot \text{OEt}_2$ and ethanethiol, pentaacetate **2** was converted to the thioglycoside donor **34** in a 65% yield (Scheme 27).⁸⁵



Scheme 27. Synthesis of thioglycoside donor 34.

2.4. Synthesis of an α -(1 $\rightarrow$ 2)-linked alkyne-derivatized disaccharide 38

The propargyl acceptor **33** was coupled with known thioglycoside donor **34** using NIS in the presence of silver triflate (AgOTf) at -20 °C as shown in Scheme 28.⁸⁶ After complete conversion of the acceptor, the temperature of the solution was raised to room temperature, and the mixture was stirred for an additional hour, at which time the *p*-methoxybenzyl group was cleaved to furnish the target disaccharide **35** in 61% yield.



Scheme 28. Synthesis of an α -(1 $\rightarrow$ 2)-linked propargyl disaccharide

MALDI-TOF mass spectrometry data (Figure 16) confirmed that the *p*-methoxybenzyl group was cleaved at room temperature showing m/z 659.3515 corresponding to $[M+Na]^+$ and m/z 675.2224 corresponding to $[M+K]^+$.

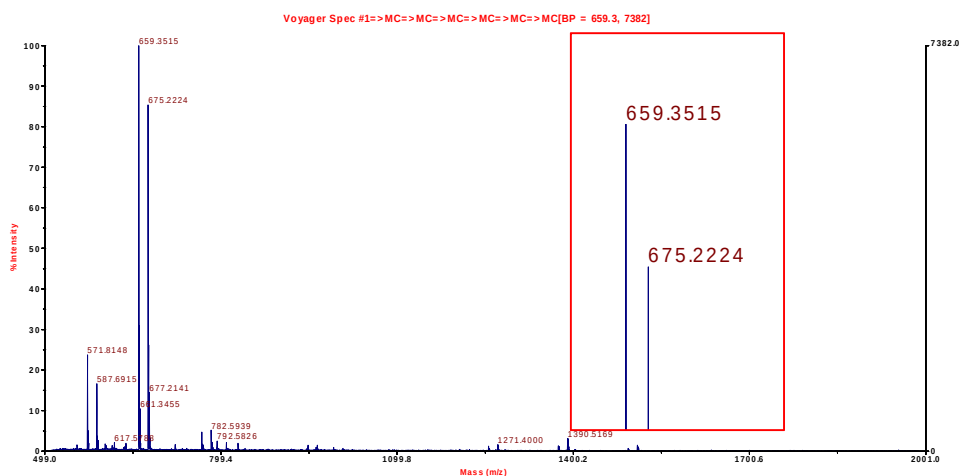
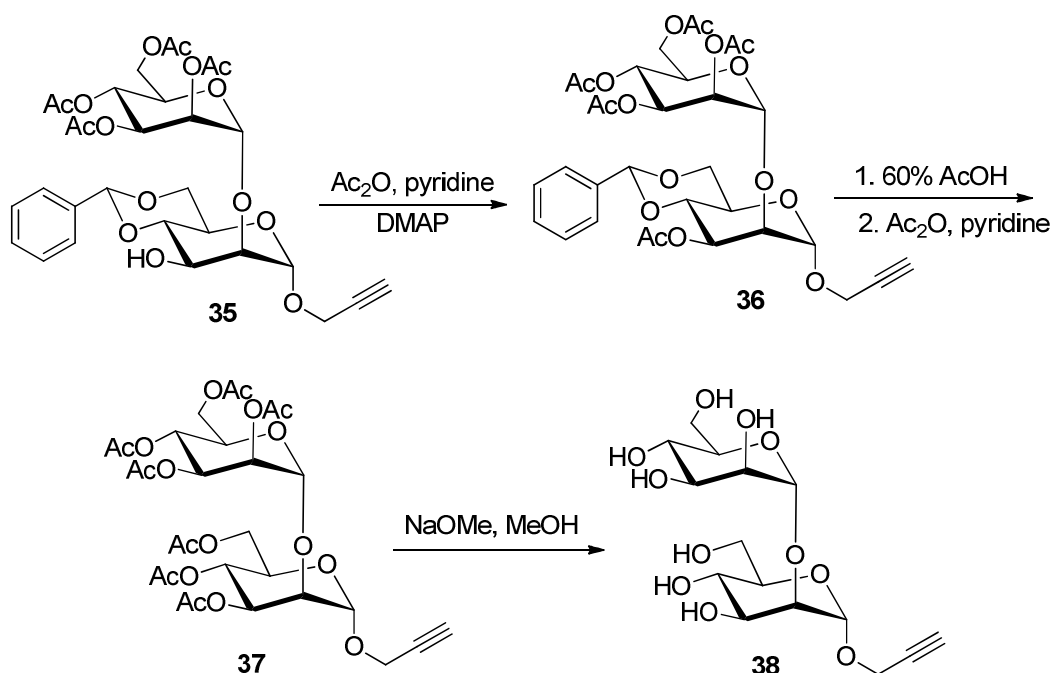


Figure 16. MALDI-TOFMS characterization of the alkyne-terminated disaccharide **35.**

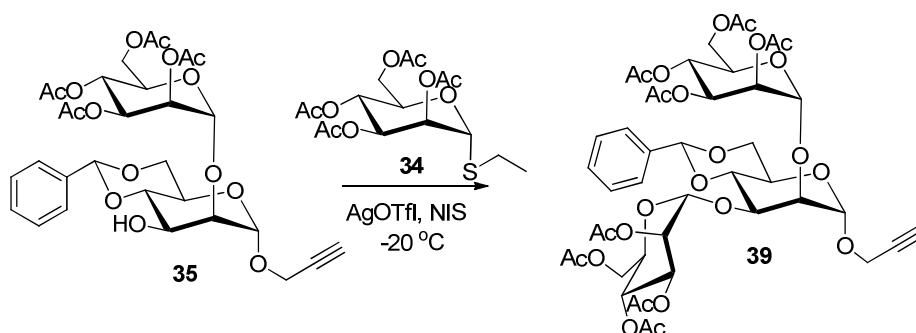
For easier characterization, the disaccharide **35** was acetylated with Ac_2O in the presence of pyridine to afford **36** in a nearly quantitative yield (Scheme 29). The benzylidene protecting group was removed by heating compound **36** in a solution of 60% aqueous acetic acid at 65 °C for 45 minutes. The resulting diol intermediate was acetylated for easier purification and characterization to afford compound **37**. There were now seven acetate protecting groups on the disaccharide as confirmed by their ^1H and ^{13}C NMR spectra. Finally, global deprotection of heptaacetate **37** furnished the target disaccharide **38** in 59% yield (as shown in scheme 29).



Scheme 29. Synthesis of the free (1→2)-linked alkyne-terminated disaccharide 38.

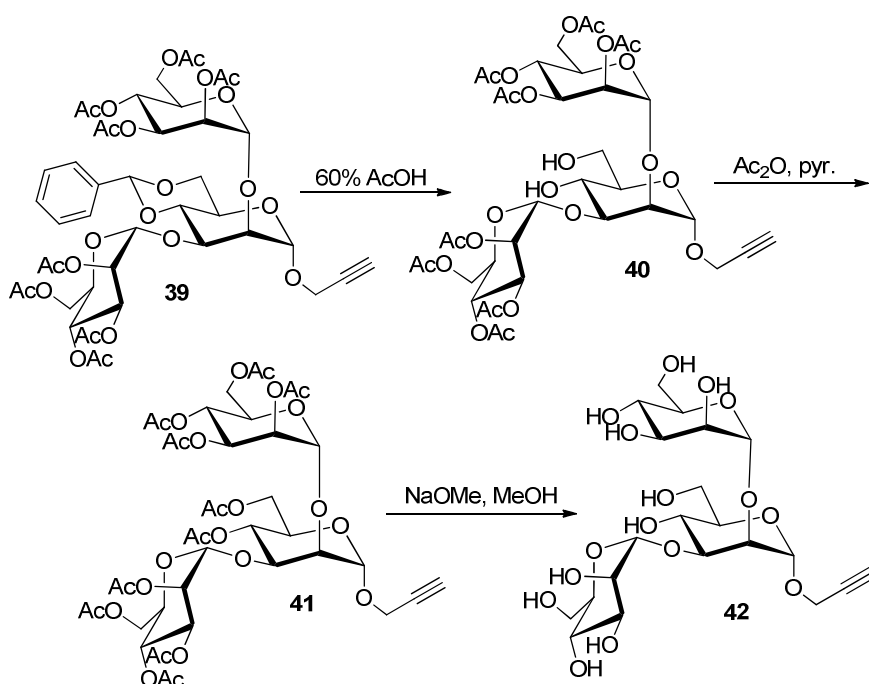
2.5. Synthesis of alkyne-derivatized trisaccharide 42

The synthesis of alkyne derivatized trisaccharide **39** began with 3-OH free disaccharide acceptor **35** (for synthesis of disaccharide, refer to Scheme 28) as shown in Scheme 30. Glycosylation of acceptor **35** and thioglycoside donor **34** was achieved through thioether activation with NIS and AgOTf at -20 °C for 2 h. The reaction proceeded smoothly to furnish the protected trisaccharide **39**. The desired α -(1→3)-link was the major product, but the β -anomer was also formed and had a smaller R_f than the α -anomer on the TLC plate. Purification by column chromatography led to the separation of products with an α : β ratio of 4:1. All ^1H and ^{13}C chemical shifts were assigned using extensive 2D NMR data.



Scheme 30. Synthesis of alkyne-terminated trisaccharide 39.

Opening of the benzylidene acetal was achieved by heating the trisaccharide **39** with 60% aqueous acetic acid at 65 °C to afford the diol intermediate **40** (Scheme 31). For ease of purification and characterization, the diol was acetylated with Ac₂O in the presence of pyridine and DMAP catalyst to furnish the trisaccharide **41**.



Scheme 31. Synthesis of the free alkyne-terminated trisaccharide 42.

The resulting acetylated trisaccharide was fully characterized by 1D and 2D (gCOSY, HSQC, HMBC, TOCSY) NMR analysis. However, it was very difficult to assign chemical shifts to the different protons and carbons due to extensive overlap of the NMR signals. To resolve this problem, an HSQC-TOCSY (Heteronuclear Single Quantum Coherence-Total Correlation Spectroscopy) NMR experiment was employed. HSQC-TOCSY is a 2D TOCSY NMR experiment that has been resolved into the carbon dimension. This is especially useful when overlap in the proton spectrum prevents analysis since often the corresponding carbons will be resolved. Cross peaks are seen between all *J*-coupled protons in a spin system and each carbon in that spin system.⁸⁷ Changing the mixing time for each experiment allows for a sequential acquisition of data. Hence three HSQC-TOCSY experiments were carried out with different mixing times of 24 ms, 50 ms and 80 ms. Previously acquired 2D NMR data and the newly acquired data (all NMR data as well as tables of HSQC and HMBC correlations are found in the Appendix) clearly showed three ring systems as shown with different colors in Figure 17.

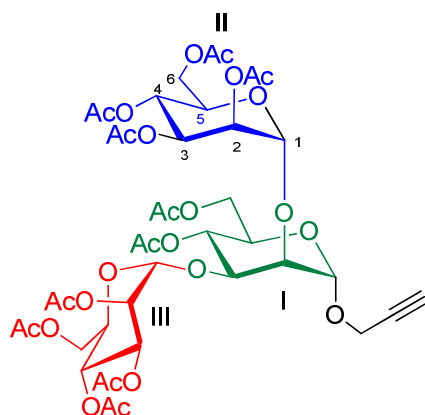


Figure 17. Three ring systems of the alkyne-terminated trisaccharide 41.

The first HSQC-TOCSY experiment (24 ms) showed correlations between anomeric protons and their neighboring protons (H-2). The second experiment (50 ms) showed more correlations extending to H-3 and H-4. The last experiment (84 ms) showed complete correlations from anomeric proton (H-1) to H-6 for each ring system. The HSQC-TOCSY correlations are shown in Table 2.

Table 2. HSQC-TOCSY correlations of trisaccharide 41.

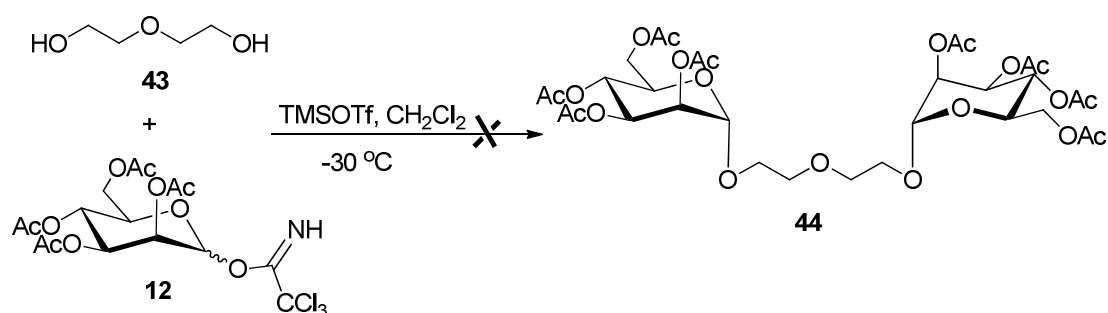
$\delta(^{13}\text{C})$ ppm	$\delta(^1\text{H})$ ppm		Type of carbon	CH_3
55.07	4.23	$\text{OCH}_2\text{C}\equiv\text{H}$	CH_2	
62.22	4.11, 4.20	H-6, H-6'	CH_2	2.14
62.50	4.24, 4.17	H-6, H-6'	CH_2	2.08
62.95	4.28, 4.03	H-6, H-6'	CH_2	2.06
66.21	5.21	H-4	CH	2.04
66.46	5.30	H-4	CH	2.03
66.69	5.38	H-4	CH	2.10
68.71	5.27	H-3	CH	1.96
68.73	5.40	H-3	CH	1.98
69.35	4.09	H-5	CH	
69.46	4.21	H-5	CH	
69.63	3.85	H-5	CH	
69.76	5.13	H-2	CH	2.11
69.76	5.30	H-2	CH	2.12
75.71	2.47	$\equiv\text{CH}$	CH	
77.36	4.10	H-3	CH	
78.48	4.07	H-2	CH	
96.78	5.25	H-1	CH	
99.34	5.09	H-1	CH	
99.47	4.97	H-1	CH	

Another advantage of using HSQC-TOCSY NMR is that even the CH_3 can be assigned to their specific ring systems (Table 1). With the structure of compound **41** now confirmed, it was deacetylated using methanolic NaOMe under Zemplén conditions to afford the target free alkyne derivatized trisaccharide **42** in 80% yield.⁶³

3. Synthesis of a Dimeric Mannose Derivative

3.1 First attempt to synthesize dimeric mannose derivative 48

Trichloroacetimidate donor **12** (three equivalents) was used in a glycosylation reaction with diethylene glycol (**43**) serving as the acceptor (Scheme 32).

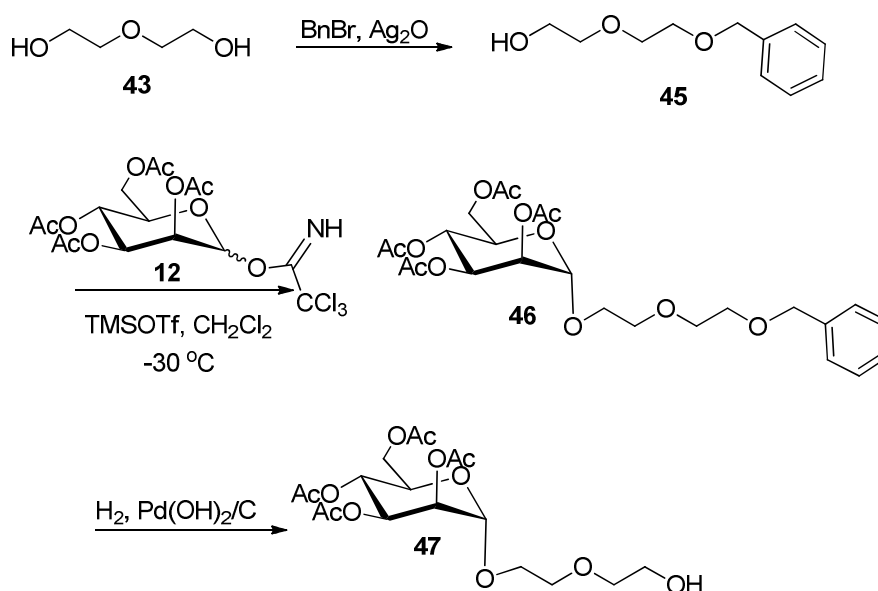


Scheme 32. First attempt to synthesize the dimeric mannose derivative 44.

The desired disaccharide **44** was not formed, and the isolated product turned out to be the hydrolyzed donor. This could be attributed to the quenching of the promoter TMSOTf by traces of water in the diethylene glycol. The pre-mix and pre-dry technique, addition of molecular sieves, did not completely get rid of all traces of moisture. Diethylene glycol is very viscous; it locks in moisture and impurities which are difficult to remove even under high vacuum. After all efforts to dry the sample for glycosylation failed, a new stepwise approach was used.

3.2 Second attempt to synthesize dimeric mannose derivative 48

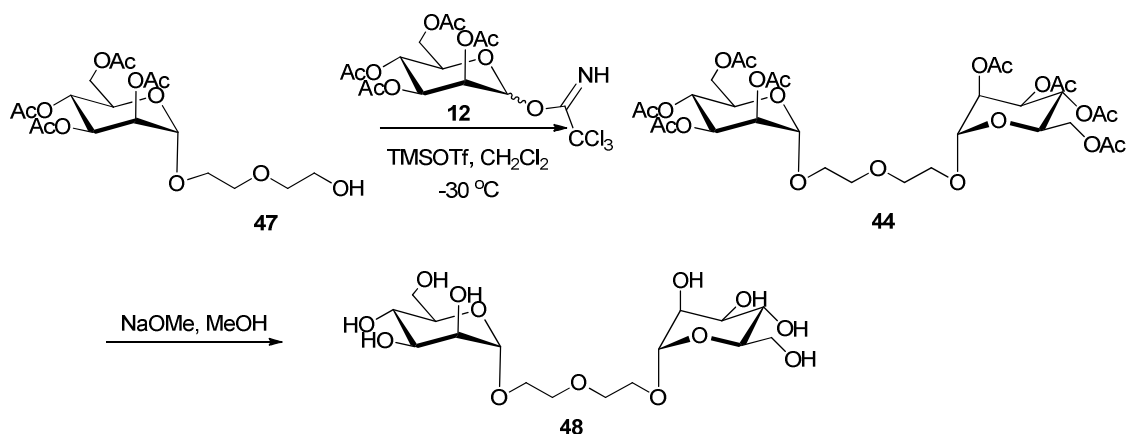
The step-wise approach used to synthesize dimeric mannose derivative commenced with mono-benylation of the commercially available diethylene glycol **43** (Scheme 33). A mixture of diethylene glycol (**43**), BnBr and Ag₂O (Lewis acid) in DCM was stirred at room temperature for 14 h according to the procedure developed by Sauv  and co-workers.⁸⁸



Scheme 33. Synthesis of ethylene glycol-linked moosaccharide 47.

The mono-benzylated diethylene glycol **45** was used in a glycosylation reaction with the trichloroacetimidate donor **12** in the presence of 4 Å molecular sieves and TMSOTf. This furnished the desired diethylene glycol-linked monosaccharide **46** in a 39% yield. Using 20% Pd(OH)₂/C,⁸⁹ the benzyl group was removed under an H₂

atmosphere to afford 2-(2-hydroxyethoxy)ethyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**47**) in an 80% yield. This newly deprotected monosaccharide **47** could serve as the acceptor for the formation of the dimeric mannose derivative **44** (Scheme 34). Acceptor **47** was coupled with trichloroacetimidate donor **12** in the presence of TMSOTf promoter to furnish dimeric sugar **44**. From the ^1H NMR spectrum, the $J_{1,2}$ coupling constant was determined to be 1.6 Hz confirming an α -(1 $\rightarrow$ 1)-linked dimeric sugar. The free dimeric sugar **48** was synthesized from compound **44** under Zemplén conditions using freshly made NaOMe from Na_(s) and MeOH.⁶³



Scheme 34. Synthesis of free dimeric mannose derivative 48.

IV. Conclusion and Future Work

A dimeric mannose derivative **48** was synthesized for use in the development of methods for detection of carbohydrate clusters. Thiol-terminal monosaccharide **5**, (1→2)-linked disaccharide **18**, (1→3)-linked disaccharide **24** and trisaccharide **29** were also synthesized for analysis of their binding properties with Con A. Alkyne derivatized monosaccharide **30**, (1→2)-linked disaccharide **38** and trisaccharide **42** were successfully synthesized for applications in click chemistry. These compounds (Figure 18) were obtained via multi-step synthesis from commercially available D-mannose (**1**).

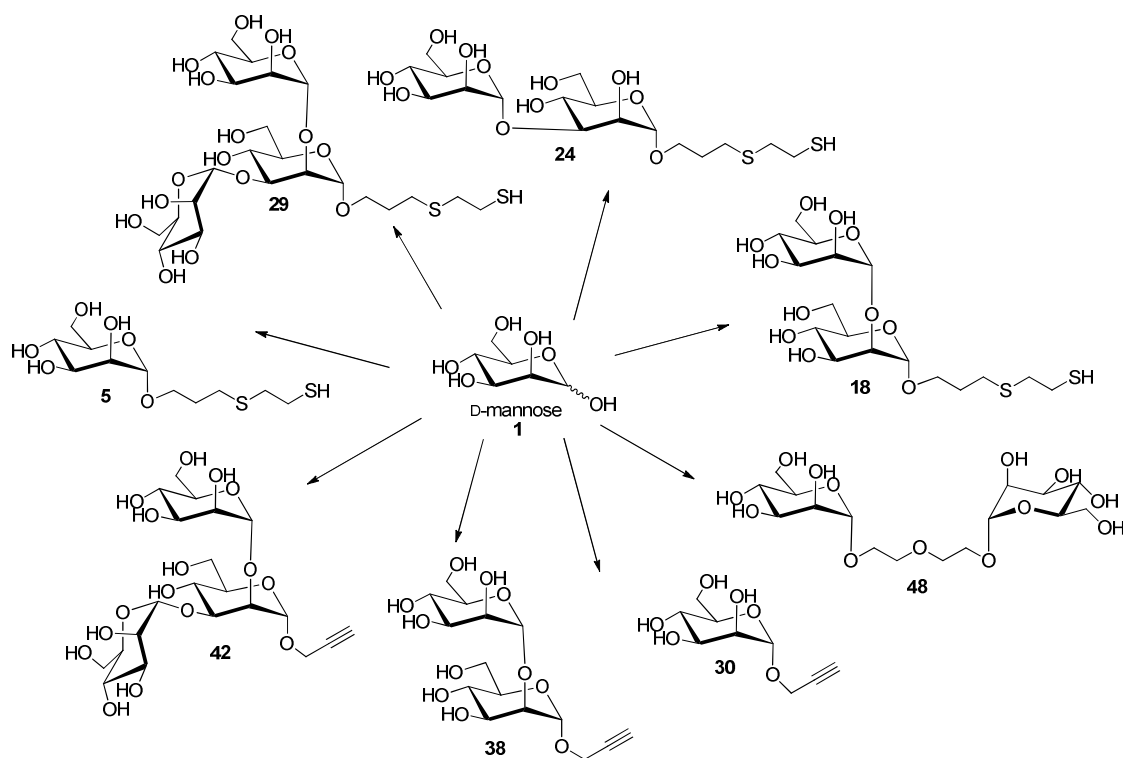


Figure 18. Final mannose derivatives.

Schmidt glycosylation⁶² was used for the coupling reactions, and thiol terminals were added by reaction of allyl group with ethanedithiol under free-radical conditions as developed by Vliegthart and coworkers.⁶⁵ These disaccharides and trisaccharides have two different branching patterns, the α -(1 $\rightarrow$ 2)-linkage and the α -(1 $\rightarrow$ 3)-linkage. Future work will include synthesis of alkyne-derivatized galactosides with similar linkages.

V. Experimental

General methods: ^1H (300 MHz, 500 MHz, 600 MHz) NMR spectra were recorded at room temperature with a Mercury 300, Varian 500 and Innova 600 instruments, respectively. ^{13}C spectra were recorded at 75 MHz, 126 MHz and 150 MHz, respectively, on the same instruments. Chemical shifts are reported in δ -units (ppm) relative to the internal standard tetramethylsilane (TMS) for ^1H (δ 0) and CDCl_3 for ^{13}C (δ 7.26), unless otherwise indicated. Multiplicities are first-order values in Hz: s, singlet; bs broad singlet; d, doublet; dd, doublet of doublet; t, triplet; dt, doublet of triplet; ddd doublet of doublet of doublet; m, multiplet. All two-dimensional experiments (gCOSY, gHSQC, gHMBC, NOESY and TOCSY) were recorded on either a Varian 500 or Inova 600 MHz instruments. All NMR resonance assignments were confirmed by 2D NMR techniques. MALDI-TOF mass spectra were obtained on a Voyager-DE PRO BioSpectrometry workstation. High-resolution DART mass spectra were obtained on a JEOL AccuTOF-DART workstation with an ESI source. All reactions were monitored by thin-layer chromatography (TLC) on aluminum-backed E. Merck Silica Gel 60 F₂₅₄ plates (0.25 mm). Detection was by UV irradiation using a 254 nm lamp and then charring with *p*-anisaldehyde–sulfuric acid stain reagent. Column chromatography was performed on 60 Å (63–200 μm) and 40 Å (40–60 μm) silica gel. All chemicals used were reagent grade and used as supplied unless noted otherwise. All reactions were performed in oven-dried glassware under an inert N_2 atmosphere unless noted otherwise. Reagent grade dichloromethane (DCM), tetrahydrofuran (THF), methanol (MeOH), *N,N*-

dimethylformamide (DMF) and toluene were obtained dry from the Pure Solv (Innovation Technologies) solvent system. Pyridine and Et₃N were distilled over CaH₂ prior to use. Organic extracts were dried over anhydrous magnesium sulfate (MgSO₄).

General procedure 1: acetylation⁶⁵

To a cooled mixture (0 °C) of mannose derivative in dry pyridine were added 2 molar equiv of Ac₂O and 0.1 molar equiv of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred overnight and allowed to warm up slowly to room temperature. The reaction was quenched by dilution with water, and the mixture was concentrated. The concentrate was extracted with EtOAc; the extract was washed with water and then brine. After separation and drying over MgSO₄, the organic phase was concentrated to dryness. Co-evaporation of the residue under vacuum with toluene afforded the dry product. The products were purified by flash chromatography on silica gel.

General procedure 2: deacetylation⁹⁰

To a solution of the acetylated mannose derivative in dry 1:5 CH₂Cl₂–MeOH was added 0.3 molar equiv of NaOMe (25 wt % in MeOH). The reaction was allowed to stir at room temperature for 3–5 h, after which time it was neutralized with Dowex-50 (H⁺ form), filtered, and concentrated.

General procedure 3: free-radical addition of thiol⁶⁵

To a stirred solution of allylated mannopyranoside in 1,4-dioxane was added 10 molar equiv of 2-mercaptoethanethiol and 3 molar equiv of AIBN. The mixture was stirred for 3 h at 75 °C under N₂ and quenched with 5 molar equiv of cyclohexene for 30 min. After cooling to room temperature, the mixture was concentrated.

General procedure 4: glycosylation A⁸¹

The glycosyl acceptor (1 molar equiv) and trichloroacetimidate donor (1.5 molar equiv) were premixed and predried under high vacuum for 1 day. The mixture was then dissolved in anhyd DCM (to make a 0.5 M solution) and activated powdered 4 Å molecular sieves were added. The solution was stirred at room temperature for 30–50 min under an N₂ atmosphere and then cooled to -20 °C. TMSOTf (0.1 molar equiv) was diluted into 0.5–1 mL of anhyd DCM and added dropwise to the solution. The reaction mixture was stirred for 2–3 h while monitoring by TLC for the complete consumption of the acceptor. Upon completion of reaction, the mixture was neutralized with Et₃N (0.1 molar equiv), filtered through a pad of Celite and concentrated under reduced pressure to dryness.

General procedure 5: glycosylation B

The glycosyl acceptor (1 molar equiv) and 2 molar equiv of trichloroacetimidate donor were premixed with dry DCM and pre-dried for a day under vacuum. To a stirred

solution of the donor and acceptor in dry DCM (0.5 M solution) was added powdered 4 Å molecular sieves, the mixture was stirred at room temperature for 30–50 min under an N₂ atmosphere. The mixture was cooled to -20 °C and 0.1 molar equiv of TMSOTf was added. The reaction was monitored by TLC and was warmed up to room temperature upon complete consumption of the acceptor. After stirring at room temperature for 1–2 h, the reaction was quenched with Et₃N (0.1 molar equiv). The solution was filtered through a pad of Celite and concentrated; the crude product was purified by silica gel column chromatography.

General procedure 6: glycosylation C⁸⁶

The glycosyl acceptor (1 molar equiv) and 2 molar equiv of thioglycoside donor were premixed with dry DCM and pre-dried for 2 days under vacuum. To a stirred suspension of donor and acceptor in dry DCM (0.5 M solution) was added powdered 4 Å molecular sieves and the mixture stirred at room temperature for 1 h under an N₂ atmosphere. The mixture was cooled to -20 °C and 0.1 molar equiv of AgOTf and 1 molar equiv of NIS were added. Reaction was monitored by TLC, and after complete consumption of the acceptor, the reaction was warmed up to room temperature. After stirring at room temperature for 1 h the reaction was quenched with TEA (0.1 molar equiv), filtered through Celite, concentrated and purified by silica gel column chromatography.

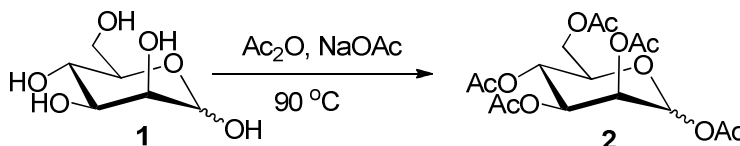
General procedure 7: removal of benzylidene protecting group A⁸¹

The mannose derivative was dissolved in 9:1 TFA–H₂O to make a 0.1 M solution. The mixture was stirred at room temperature for 1–2 h while monitoring by TLC. Upon completion of the reaction, the solution was concentrated to dryness and the residue was extracted with DCM.

General procedure 8: removal of benzylidene B⁸²

The mannose derivative was dissolved in 80% AcOH to make a 0.05 M solution. The mixture was heated at 80 °C for 2 h with monitoring by TLC. Upon completion of reaction, the solution was concentrated to dryness and extracted with DCM.

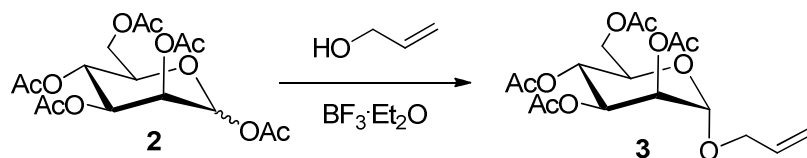
1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranose (2)



A mixture of NaOAc (5.02 g, 60.5 mmol) and Ac₂O (50.0 mL, 526 mmol) was heated under an N₂ atmosphere to 70 °C. D-Mannose (**1**, 10.0 g, 55.6 mmol) was slowly added to the mixture, and the temperature was raised to 90 °C and stirred for 8 h. The reaction was cooled to room temperature, poured into ice (1000 g) and quenched with 1 L of satd NaHCO₃. The aq phase was extracted three times with CH₂Cl₂. The organic extract was washed successively with water and brine, then dried over MgSO₄ and concentrated. Purification of the product by column chromatography on a silica gel column (2:1

hexane–EtOAc) gave compound **2** (19.5 g, 90.1%) as a viscous oily mixture of 65% α and 35% β anomers. ^1H NMR for α anomer (CDCl_3 , 300 MHz): δ 6.08 (d, $J_{1,2} = 1.52$ Hz, 1H, H-1), 5.34 (m, 2H, H-3, H-4), 5.26 (m, 1H, H-2), 4.27 (dd, $J_{6a,6b} = 12.38$ Hz, $J_{5,6} = 4.89$ Hz, 1H, H-6a), 4.10 (dd, $J_{6a,6b} = 12.38$ Hz, $J_{5,6b} = 2.35$ Hz, 1H, H-6b), 4.05 (m, 1H, H-5), 2.17, 2.16, 2.09, 2.05, 2.00 (5s, 15H, 5 COCH_3). ^{13}C NMR for α anomer (CDCl_3 , 125 MHz): δ 170.53, 169.88, 169.63, 169.43, 167.96, (5C, 5 COCH_3), 90.54 (C-1), 70.55 (C-5), 68.67 (C-3), 68.27 (C-2), 65.49 (C-4), 62.04 (C-6), 20.78, 20.68, 20.63, 20.58, 20.55 (5C, 5 COCH_3). The spectroscopic data matched that previously reported.⁹¹

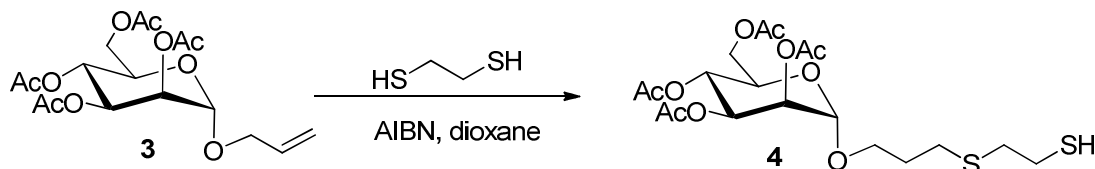
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**3**)



Under an N_2 atmosphere, 18.0 g (46.2 mmol) of the pentaacetate **2** was dissolved in 100 mL of dry CH_2Cl_2 and 19.0 mL of allyl alcohol (278 mmol) was added. While stirring, the mixture was cooled to -5°C . $\text{BF}_3\cdot\text{Et}_2\text{O}$ (119 mL, 884 mmol) was added dropwise into the solution. The solution was stirred at 0°C for 30 min, then warmed up to room temperature and stirred for 51 h. The reaction was quenched by pouring it into ice water, and the two phases were separated. The organic layer was washed with water, satd NaHCO_3 and brine; and then it was dried over anhyd MgSO_4 . The solution was filtered through a Celite bed and concentrated. The residue was passed through a short silica gel column with 2:1 hexane–EtOAc as the eluent to yield pure **3** as a colorless oil (11.86 g,

66%): ^1H NMR (300 MHz, CDCl_3): δ 5.98–5.76 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.34 (dd, $J_{3,4} = 10.01$ Hz, $J_{2,3} = 3.42$ Hz, 1H, H-3), 5.31–5.27 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.24 (m, 1H, H-4), 5.20 (m, 2H, H-2, $\text{OCH}_2\text{CH}=\text{CH}_2$), 4.83 (d, $J_{1,2} = 1.58$ Hz, 1H, H-1), 4.25 (dd, $J_{6a,6b} = 12.23$ Hz, $J_{5,6a} = 5.27$ Hz, 1H, H-6a), 4.20–4.11 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 4.07 (dd, $J_{6a,6b} = 12.22$ Hz, $J_{5,6b} = 2.43$ Hz, 1H, H-6b), 4.04–3.99 (m, 1H, $\text{OCH}_2\text{-CH}=\text{CH}_2$), 3.99–3.94 (m, 1H, H-5), 2.12, 2.07, 2.01, 1.96 (4s, 12H, 4 CH_3COO). ^{13}C NMR (75 MHz, CDCl_3): δ 170.50, 169.91, 169.74, 169.61 (4C, 4 CH_3COO), 132.83 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 118.33 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 96.49 (C-1), 69.53 (C-3), 68.98 (C-4), 68.55 (C-5), 68.46 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 66.10 (C-2), 62.37 (C-6), 20.78, 20.70, 20.63, 20.59 (4C, 4 COCH_3). The spectroscopic data was consistent with that previously reported.⁹²

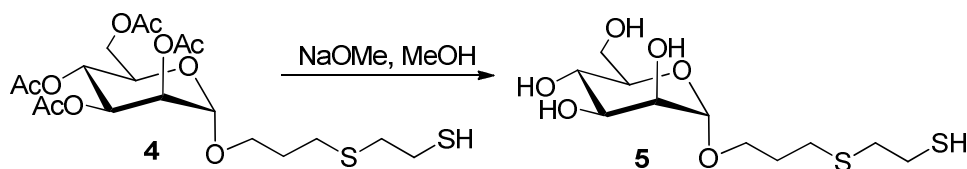
3-[(2-Sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**4**)



Following general procedure 3, a mixture of **3** (2.50 g, 6.44 mmol), ethanedithiol (5.40 mL, 64.4 mmol), dioxane (50 mL), and AIBN (5.28 g, 32.2 mmol) was stirred under an N_2 atmosphere at 75 °C for 2.5 h. Cyclohexene (3.30 mL, 32.2 mmol) was added to quench the reaction. The solution was concentrated and purified by column chromatography on silica gel with 2:1 hexane–EtOAc as the eluent to yield pure **4** (1.90 g, 61%). $[\alpha]_{\text{D}} = +38.8^\circ$ ($c = 1.0$ in CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.30 (dd, $J_{3,4} = 10.03$ Hz, $J_{2,3} = 3.36$ Hz, 1H, H-3), 5.25 (t, $J_{3,4} = J_{4,5} = 9.89$ Hz, 1H, H-4), 5.21 (dd, $J_{2,3}$

= 3.14 Hz, $J_{1,2}$ = 1.69 Hz, 1H, H-2), 4.79 (d, $J_{1,2}$ = 1.69 Hz, 1H, H-1), 4.25 (dd, $J_{6a,6b}$ = 12.23 Hz, $J_{5,6a}$ = 5.32 Hz, 1H, H-6a), 4.10 (dd, $J_{6a,6b}$ = 12.23 Hz, $J_{5,6b}$ = 2.29 Hz, 1H, H-6b), 3.97 (ddd, $J_{4,5}$ = 9.04 Hz, $J_{5,6a}$ = 5.21 Hz, $J_{5,6b}$ = 2.26 Hz, 1H, H-5), 3.80 (m, 1H, OCH₂CH₂CH₂S), 3.52 (m, 1H, OCH₂CH₂CH₂S), 2.72 (m, 4H, SCH₂CH₂SH), 2.63 (m, 2H, OCH₂CH₂CH₂S), 2.13, 2.08, 2.02, 1.97 (4s, 12H, 4 COCH₃), 1.87 (m, 2H, OCH₂CH₂CH₂S), 1.70 (m, 1H SH). ¹³C NMR, (125 MHz, CDCl₃): δ 170.53, 169.97, 169.80, 169.63 (4C, 4 COCH₃), 97.52 (C-1), 69.52 (C-2), 69.01 (C-3), 68.54 (C-5), 66.32 (OCH₂CH₂CH₂S), 66.13 (C-4), 62.43 (C-6), 36.13 (SCH₂CH₂SH), 29.03 (OCH₂CH₂CH₂S), 28.49 (OCH₂CH₂CH₂S), 24.61 (SCH₂CH₂SH), 20.81, 20.69, 20.63, 20.61 (4 COCH₃). MALDI-TOFMS: calcd for C₁₉H₃₀O₁₀Na [M+Na]⁺ m/z 505.1178; found m/z 505.1207.

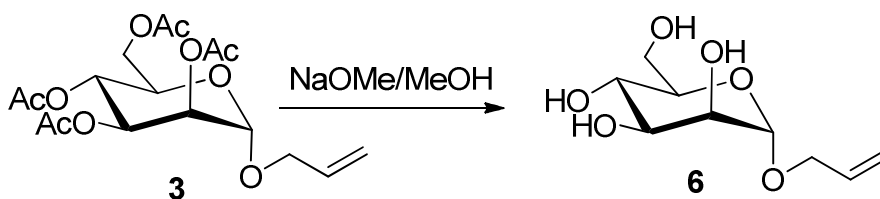
3-[(2-Sulfanylethyl)sulfanyl]propyl α-D-mannopyranoside (5)



Following general procedure 2, 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**4**, 1.90 g, 3.94 mmol) was deacetylated in dry CH₂Cl₂ (5 mL) and MeOH (25 mL) using NaOMe (0.06 g, 1.18 mmol). The crude product was purified on an LH-20 column to give pure product **5** as a colorless oil (1.04 g, 84%). $[\alpha]_D$ = +50.5° (c = 1 in H₂O). ¹H NMR (300 MHz, CD₃OD): δ 4.76 (d, $J_{1,2}$ = 1.56 Hz, 1H, H-1), 3.83 (m, 3H, H-2, H-3, H-5), 3.71 (m, 2H, H-6a,b), 3.62 (t, $J_{3,4}$ = $J_{4,5}$ = 9.25 Hz, 1H,

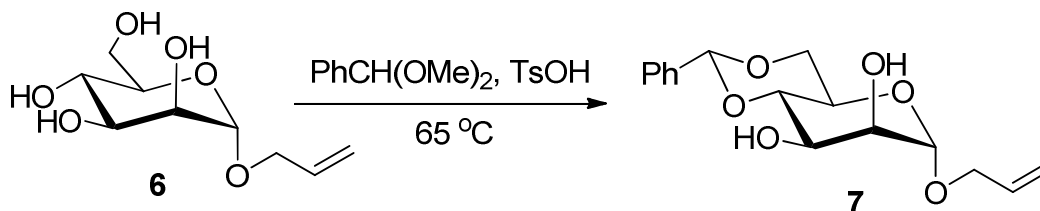
H-4), 3.53 (m, 2H, OCH₂CH₂CH₂S), 2.93 (ddd, 2H, SCH₂CH₂SH), 2.84 (m, 2H, SCH₂CH₂SH), 2.67 (t, 2H, OCH₂CH₂CH₂S), 1.89 (m, 2H, OCH₂CH₂CH₂S). ¹³C (75 MHz, CD₃OD): δ 101.63 (C-1), 74.66 (C-5), 72.69 (C-3), 72.21 (C-2), 68.59 (C-4), 66.93 (OCH₂CH₂CH₂S), 62.91 (C-6), 39.68 (SCH₂CH₂SH), 32.30 (OCH₂CH₂CH₂S), 30.74 (OCH₂CH₂CH₂S), 29.61 (SCH₂CH₂SH).

Allyl α-D-mannopyranoside (6)



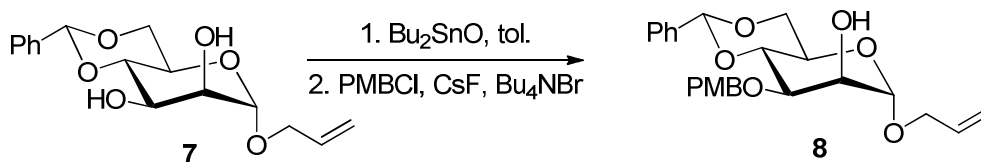
Following general procedure 2, allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**3**, 8.01 g, 20.6 mmol) in dry CH₂Cl₂ (20 mL) and MeOH (100 mL) was deacetylated using NaOMe (0.33 g, 6.2 mmol). The crude product was purified on an LH-20 column to give the pure product as a colorless oil (3.7 g, 81.7%). [α]_D = +69.8° (*c* = 1.0, MeOH). ¹H NMR (300 MHz, CD₃OD): δ 5.94 (m, 1H, OCH₂CH=CH₂), 5.29 (m, 1H, OCH₂CH=CH₂a), 5.17 (m, 1H, OCH₂CH=CH₂b), 4.80 (d, *J* = 1.50 Hz, 1H, H-1), 4.21 (m, OCH₂aCH=CH₂), 4.00 (m, 1H, OCH₂bCH=CH₂), 3.83 (m, 2H, H-6a, H-2), 3.72 (m, 2H, H-3, H-6b), 3.63 (t, *J*_{3,4} = *J*_{4,5} = 9.38 Hz, 1H, H-4), 3.53 (m, 1H, H-5). ¹³C NMR (75 MHz, CD₃OD): δ 135.39 (OCH₂CH=CH₂), 117.31 (OCH₂CH=CH₂), 100.59 (C-1), 74.57 (C-5), 72.55 (C-3), 72.04 (C-2), 68.76 (OCH₂CH=CH₂), 68.48 (C-4), 62.78 (C-6). The spectroscopic data matched that of the known compound.⁸⁶

Allyl 4,6-O-benzylidene- α -D-mannopyranoside (7)



A solution of allyl α -D-mannopyranoside (**6**, 10.0 g, 51.5 mmol), benzaldehyde dimethyl acetal (17.60 mL, 103.1 mmol), and *p*-toluenesulfonic acid monohydrate (0.09 g, 0.51 mmol) in anhyd DMF (50 mL) was heated at $50\text{ }^\circ\text{C}$ on a rotary evaporator under water aspirator pressure ($\sim 22\text{ mm Hg}$) for 2 h. The temperature was then increased to $65\text{ }^\circ\text{C}$, and the mixture was concentrated in volume to 20 mL. This remaining solution was poured into stirred slurry of ice (25 g), satd aq NaHCO_3 (50 mL), and Et_2O (50 mL). The white precipitate that formed was filtered, washed with hexanes (50 mL $\times$ 3) and then water (50 mL $\times$ 2) to give a white solid in 84% yield. $[\alpha]_{\text{D}} = +69.7^\circ$ ($c = 1.0$, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 7.43 (m, 5H, Ar), 5.89 (m, 1H $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.53 (s, 1H, ArCH), 5.29 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2\text{a}$), 5.22 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2\text{b}$), 4.81 (d, $J_{1,2} = 1.5\text{ Hz}$, 1H, H-1), 4.23 (m, 1H, H-6a), 4.15 (ddd, 1H, $\text{OCH}_2\text{aCH}=\text{CH}_2$), 4.02 (dd, $J = 9.64, 3.42\text{ Hz}$, 1H, H-3), 3.92 (m, 3H, H-2, H-4, $\text{OCH}_2\text{bCH}=\text{CH}_2$), 3.81 (m, 2H, H-5, H-6b), 3.54 (bs, 2H, 2 OH). ^{13}C NMR (125 MHz, CD_3OD): δ 137.12 (Ar), 133.41 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 129.09 (Ar), 128.18 (Ar $\times$ 2), 126.19 (Ar $\times$ 2), 117.56 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 102.03 (ArCH), 99.49 (C-1), 78.72 (C-4), 70.84 (C-2), 68.60 (C-6), 68.39 (C-3), 68.08 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 63.20 (C-5). The NMR data matched that previously reported.⁸⁴

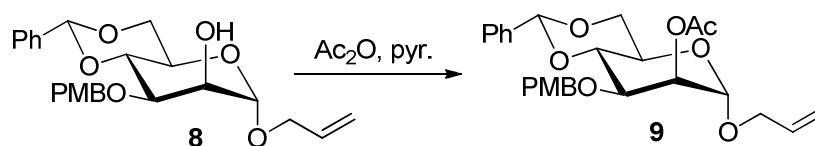
Allyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (8)⁷⁹



To a suspension of allyl 4,6-*O*-benzylidene- α -D-mannose (6.02 g, 19.6 mmol) in toluene (130 mL) was added Bu_2SnO (4.91 g, 19.6 mmol). The mixture was heated to reflux for 3 h and cooled to room temperature. Tetrabutylammonium bromide (6.72 g, 21.0 mmol), CsF (3.05 g, 20.1 mmol) and PMBCl (2.80 mL, 20.6 mmol) were added, respectively. The mixture was stirred for 48 h and then heated to reflux for 1 h, after which it was cooled to room temperature and washed with satd aq NaHCO_3 (70 mL). The water layer was then extracted with EtOAc (3 $\times$ 50 mL). The organic layers were combined and washed with water and brine, and then dried over MgSO_4 and filtered. The resulting solution was concentrated, and the crude product was purified by flash chromatography on silica gel to give a yellow oil (6.79 g, 76%). ^1H NMR (300 MHz, CDCl_3): δ 7.28–7.53 (m, 7H, Ar), 6.87 (m, 2H, Ar), 5.91 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.62 (s, 1H, ArCH), 5.28 (m, 2H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 4.89 (s, 1H, H-1), 4.80 (d, $J = 11.35$ Hz, 1H, OCH_2aAr), 4.65 (d, $J = 11.35$ Hz, 1H, OCH_2bAr), 3.87–4.29 (m, 8H, H-2, H-3, H-4, H-5, H-6a,6b, $\text{OCH}_2\text{CH}=\text{CH}_2$), 3.80 (s, 3H, OCH_3), 2.77 (s, 1H, OH). ^{13}C (75 MHz, CD_3OD): δ 159.35 (Ar), 137.52 (Ar), 133.46 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 130.06 (Ar), 129.49 (Ar $\times$ 2), 128.87 (Ar), 128.17 (Ar $\times$ 2), 126.01 (Ar $\times$ 2), 117.79 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 113.82 (Ar $\times$ 2), 101.52 (ArCH), 99.12 (C-1), 78.83 (C-4), 75.32 (C-3), 72.74 (OCH_2Ar), 69.95 (C-2), 68.81

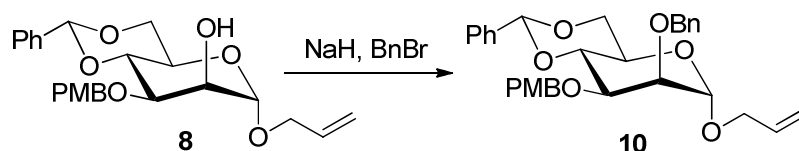
(OCH₂CH=CH₂), 68.12 (C-6), 63.35 (C-5), 55.22 (CH₃O). The spectroscopic data matched that previously reported.⁶²

Allyl 2-O-acetyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside (9**)**



A mixture of monosaccharide (**8**, 100 mg, 0.230 mmol) and Ac₂O (65 μ L, 0.69 mmol) in pyridine (10 mL) was cooled down to -10 °C and DMAP (3.0 mg, 0.023 mmol) was added. The solution was stirred overnight in an N₂ atmosphere as it slowly warmed up to room temperature. The reaction was quenched by adding ice-cold water, and the mixture was concentrated with the addition and evaporation of toluene (3 $\times$ 5 mL) from the residue. Purification by column chromatography on silica gel (2:2:1 hexane–DCM–EtOAc) afforded pure **9** as a colorless oil (95 mg, 86%). ¹H NMR (300 MHz, CDCl₃): δ 7.42 (m, 2H, Ar), 7.34–7.26 (m, 2H, Ar), 7.19 (m, 2H, Ar), 6.76 (m, 2H, Ar), 5.90–5.70 (m, 1H, OCH₂CH=CH₂), 5.55 (s, 1H, ArCH), 5.32 (m, 1H, H-2), 5.25–5.09 (m, 2H, OCH₂CH=CH₂), 4.75 (d, $J_{1,2}$ = 1.6 Hz 1H, H-1), 4.53 (q, J = 11.6 Hz, 2H, OCH₂Ar), 4.23–4.15 (m, 1H, OCH₂*a*CH=CH₂), 4.09 (m, 1H, OCH₂*b*CH=CH₂), 4.01–3.87 (m, 3H, H-3, H-4, H-5), 3.79 (dd, J = 13.2, 10.9 Hz, 2H, H-6a, H-6b), 3.71 (m, 3H, CH₃OAr), 2.07 (s, 3H, COCH₃). MALDI-TOFMS: calcd for C₂₆H₃₀O₈Na [M+Na]⁺ m/z 493.1838; found m/z 493.1789.

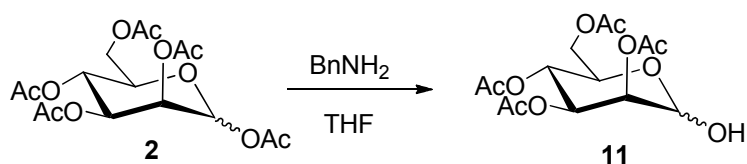
Allyl 2-O-benzyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside (10**)**



Following the procedure described in Chao Wang's thesis,⁶⁹ the foregoing compound **8** (500 mg, 1.17 mmol) dissolved in anhydr THF (60 mL) was added NaH (46 mg, 1.92 mmol) and stirred under an N₂ atmosphere for 15 min. Benzyl bromide (0.2 mL, 1.8 mmol) was added and the solution was stirred at 60 °C under an N₂ atmosphere for 18 h. The resulting yellow, cloudy solution was then cooled, MeOH (2 mL) was carefully added and the solution was stirred for 15 mins. The solvent was evaporated and the residue was taken up in CH₂Cl₂, washed with water and dried with MgSO₄. After concentration, the crude product was purified by silica gel chromatography (5:1 hexane–EtOAc) to yield pure **10** as a colorless oil (503 mg, 83%). ¹H NMR (300 MHz, CDCl₃): δ 7.53 (m, 2H, Ar), 7.46–7.24 (m, 10H, Ar), 6.91–6.83 (m, 2H, Ar), 5.92–5.76 (m, 1H, OCH₂CH=CH₂), 5.67 (s, 1H, CHAr), 5.22 (m, 2H, OCH₂CH=CH₂), 4.85 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.78 (m, 3H, OCH₂Ar, OCH₂aAr), 4.67–4.58 (m, 1H, OCH₂bAr), 4.32–4.22 (m, 2H, OCH₂aCH=CH₂, H-4), 4.16 (ddt, $J_{6a,6b}$ = 13.0 Hz, $J_{5,6a}$ = 5.1 Hz, 1H, H-6a), 4.05–3.89 (m, 3H, H-3, H-6b, OCH₂bCH=CH₂), 3.88–3.79 (m, 5H, H-5, H-2, CH₃OAr). ¹³C NMR (75 MHz, CDCl₃): δ 159.17, 138.20, 137.77 (Ar $\times$ 3), 133.55 (OCH₂CH=CH₂), 130.83 (Ar), 129.28 (Ar $\times$ 2), 128.90 (Ar), 128.47 (Ar $\times$ 2), 128.26 (Ar $\times$ 2), 128.18 (Ar $\times$ 2), 127.84 (Ar), 126.15 (Ar $\times$ 2), 117.65 (OCH₂CH=CH₂), 113.76 (Ar $\times$ 2), 101.50 (CHAr), 98.62 (C-1), 79.20 (C-4), 76.39 (C-2), 76.17 (C-3), 73.65 (CH₂Ar), 72.92

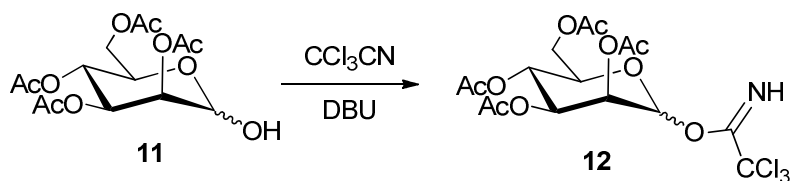
(CH₂Ar), 68.90 (OCH₂CH=CH₂), 68.01 (C-6), 64.35 (C-5), 55.34 (CH₃OAr). MALDI-TOFMS: calcd for C₃₁H₃₄O₇Na [M+Na]⁺ *m/z* 541.2202; found *m/z* 541.2301.

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranose (**11**)



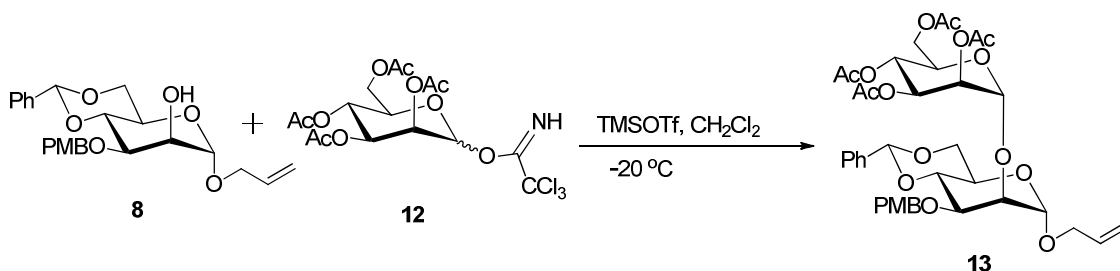
Penta-*O*-acetyl- α/β -D-mannopyranoside (**2**, 30.0 g, 76.9 mmol) and BnNH₂ (16.10 mL, 146.4 mmol) in freshly distilled THF (160 mL) were stirred for 20 h at room temperature. The reaction was quenched with dilute HCl (1 M, 10 mL), the solvent was evaporated, and the crude product was re-dissolved in CH₂Cl₂ and washed with water. The organic phase was dried with anhyd MgSO₄ and evaporated to dryness. The residue was submitted to silica gel column chromatography (2:1 hexane–EtOAc) to give 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (**11**) as a dark-brown oil (16.2 g, 60.5%). Spectroscopic data for α anomer: ¹H NMR (300 MHz, CDCl₃): δ 5.39 (dd, 1H, *J*_{2,3} = 2.7 Hz, *J*_{3,4} = 9.6 Hz, H-3), 5.27 (dd, 1H, *J*_{3,4} = *J*_{4,5} = 9.9 Hz, H-4), 5.20–5.23 (m, 2H, H-1, H-2), 4.59–5.00 (m, 1H, OH), 4.20–4.26 (m, 2H, H-5, H-6'), 4.09–4.14 (m, 1H, H-6), 2.14, 2.08, 2.04, 1.98 (4s, 12H, 4 COCH₃). ¹³C (75 MHz, CDCl₃): δ 170.9, 170.3, 170.1, 169.8 (4s, 4 COCH₃), 92.1 (C-1), 70.1 (C-2), 68.8 (C-3), 68.4 (C-5), 66.2 (C-4), 62.6 (C-6), 20.7, 20.9 (2s, 4 COCH₃). The NMR data were in agreement with those described in the literature.⁹¹

2,3,4,6-Tetra-O-acetyl-1-O-(2,2,2-trichloroethanimidoyl)-D-mannopyranose (12)



2,3,4,6-Tetra-O-acetyl- α -D-mannopyranoside (**11**, 5.10 g, 14.6 mmol), CCl_3CN (14.6 mL, 145 mmol) and activated powdered 4Å molecular sieves (700 mg) were suspended in anhyd DCM (100 mL) and left stirring at 0 °C for a period of 2 h. DBU (0.43 mL, 2.88 mmol) was added. After 2.5 h of stirring at room temperature, the solution was filtered through Celite and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (1:1, hexane–EtOAc) to afford 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl trichloroacetimidate (**12**, 6.3 g, 87.4%) as a clear oil. ^1H NMR (300 MHz, CDCl_3): δ 8.80 (s, 1H, NH); 6.29 (d, 1H, $J_{1,2} = 1.9$ Hz, H-1), 5.48 (dd, 1H, $J_{2,3} = 2.1$ Hz, H-2), 5.40–5.42 (m, 2H, H-3, H-4), 4.28 (dd, 1H, $J_{5,6a} = 4.3$ Hz, $J_{6a,6b} = 11.8$ Hz, H-6a), 4.15–4.22 (m, 2H, H-5, H-6b), 2.20, 2.07, 2.09, 2.29 (4s, 12H, 4 COCH_3). 170.5, 169.8, 169.7, 169.6, 159.7 (5s, 4 CO, C=NH, CCl_3). 90.5 (C-1), 71.2 (C-5), 67.8 (C-2), 68.8 (C-3), 65.4 (C-4), 62.0 (C-6), 21.0, 20.7, 20.65, 20.6 (4C, 4 COCH_3). The spectroscopic data matched that of the known compound.⁹¹

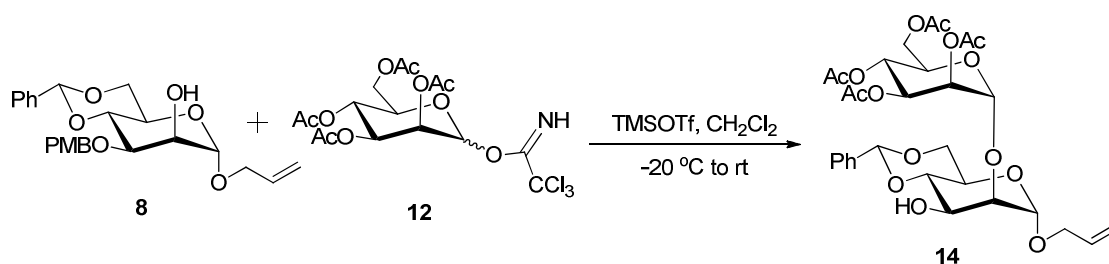
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (13**)**



Following the general procedure 4, disaccharide **13** was synthesized using 1.41 g (3.29 mmol) of acceptor **8**. Purification by column chromatography (2:1 hexane–EtOAc) gave **13** as a pale-yellow oil (1.2 g, 48%): $[\alpha]_{\text{D}} = +22.18^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (300 MHz, CDCl_3): δ 7.52–6.81 (m, 9H, Ar), 5.94–5.81 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.68 (s, 1H, CHAr), 5.31 (m, 5H, $\text{OCH}_2\text{CH}=\text{CH}_2\text{a}$, H-2^{II} , H-3^{I} , H-4^{II} , $\text{OCH}_2\text{CH}=\text{CH}_2\text{a}$), 5.11 (d, $J_{1,2} = 1.46$ Hz, 1H, H-1^{II}), 4.83 (d, $J_{1,2} = 1.57$ Hz, 1H, H-1^{I}), 4.79 (d, $J = 11.59$ Hz, 1H, OCH_2aAr), 4.54 (d, $J = 11.63$ Hz, 1H, OCH_2bAr), 4.32–3.61 (m, 14H, H-6a^{I} , H-6a^{II} , $\text{OCH}_2\text{aCH}=\text{CH}_2$, H-3^{II} , H-6b^{II} , H-5^{II} , H-2^{I} , H-4^{I} , $\text{OCH}_2\text{bCH}=\text{CH}_2$, H-5^{I} , H-6b^{I} , OCH_3), 2.05 (4 s, 12H, 4 COCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 170.60, 169.93, 169.77, 169.59 (4C, COCH_3), 159.05 (Ar), 137.51 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 133.31 (Ar), 130.52 (Ar), 129.13 (Ar $\times$ 2), 128.85 (Ar), 128.16 (Ar $\times$ 2), 126.07 (Ar $\times$ 2), 117.71 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 113.64 (Ar $\times$ 2), 101.45 (CHAr), 99.54 (C-1^{I}), 98.89 (C-1^{II}), 79.03, 76.82, 75.35 (C-2^{II} , C-2^{I} , C-4^{I}), 73.02 (OCH_2Ar), 69.17, 68.99, 68.84, 68.62, 68.05, 66.28 (6C, C-3^{II} , C-3^{I} , C-4^{II} , C-5^{II} , C-6^{I} , $\text{OCH}_2\text{CH}=\text{CH}_2$), 64.08 (C-5^{I}), 62.56 (C-6^{II}), 55.20 (OCH_3), 20.95 (COCH_3),

20.85 (COCH₃), 20.82 (2C, COCH₃). MALDI-TOFMS: calcd for C₃₈H₄₆O₁₆Na [M+Na]⁺, *m/z* 781.2684; found *m/z* 781.2699.

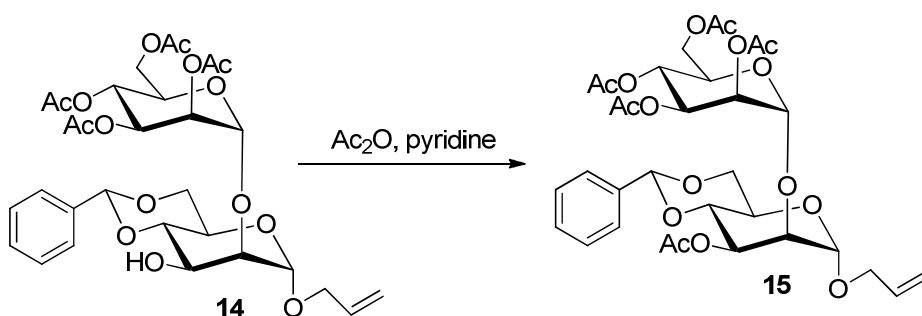
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene- α -D-mannopyranoside (14**)**



Following general procedure 5, the disaccharide **14** was synthesized on an 800 mg (1.87 mmol) acceptor scale. Purification by column chromatography (2:1 hexane–EtOAc) gave **14** as an oil (500 mg, 42%): $[\alpha]_D = +23.6^\circ$ (*c* = 1 in CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 7.46 (m, 5H, Ar), 5.90 (m, 1H, OCH₂CH=CH₂), 5.64 (s, 1H, CHAr), 5.33 (m, 5H, OCH₂CH=CH₂, H-2^{II}, H-3^I, H-4^{II}), 5.15 (s, 1H, H-1^I), 4.94 (s, 1H, H-1^{II}), 4.17 (m, 7H, H-6a^I, H-6a^{II}, OCH₂aCH=CH₂, H-6b^{II}, H-5^{II}, H-2^I), 3.90 (m, 4H, H-4^I, OCH₂bCH=CH₂, H-5^I, H-6b^I), 2.15, 2.09, 2.07, 2.01 (4 s, 12H, 4 COCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.54, 170.00, 169.81, 169.67 (4C, 4 COCH₃), 137.06 (OCH₂CH=CH₂), 133.17 (Ar), 129.11 (Ar), 128.22 (Ar $\times$ 2), 126.17 (Ar $\times$ 2), 117.67 (OCH₂CH=CH₂), 101.98 (CHAr), 99.69 (C-1^I), 98.54 (C-1^{II}), 78.81 (C-2^I), 78.39 (C-4^I), 69.02, 68.92, 68.86, 68.58, 68.50, 68.14, 66.08 (7C, C-2^{II}, C-5^{II}, C-3^{II}, C-6^I, C-3^I, OCH₂CH=CH₂, C-4^{II}), 63.55 (C-5^I), 62.51 (C-6^{II}), 20.81 (COCH₃), 20.70 (COCH₃).

20.65 (2C, 2 COCH₃). MALDI-TOFMS: calcd for C₃₀H₃₈O₁₅Na [M+Na]⁺ *m/z* 661.2108; found *m/z* 661.2087.

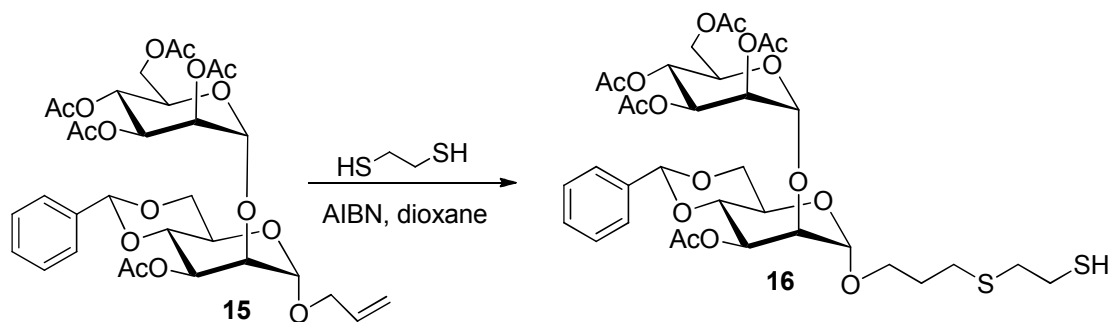
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (15)



Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**15**) was synthesized from **14** (450 mg, 0.705 mmol) following general procedure 1. Yield: 390 mg, 81%. ¹H NMR (300 MHz, CDCl₃): δ 7.44 (m, 5H, Ar), 5.90 (m, 1H, OCH₂CH=CH₂), 5.62 (s, 1H, CHAr), 5.43 (dd, *J*_{3,4} = 10.24, *J*_{2,3} = 3.10 Hz, 1H, H-3^{II}), 5.32 (m, 5H, OCH₂CH=CH₂, H-2^{II}, H-3^I, H-4^{II}), 4.91 (d, *J*_{1,2} = 1.59 Hz, 1H, H-1^I), 4.90 (d, *J*_{1,2} = 1.75 Hz, 1H, H-1^{II}), 4.26 (m, 1H, H-6a^I), 4.21 (m, 2H, H-6a^{II}, OCH₂aCH=CH₂), 4.16 (m, 1H, H-2^I), 4.08 (m, 3H, H-6b^{II}, H-5^{II}, H-4^I), 4.00 (dd, 1H, OCH₂bCH=CH₂), 3.94 (m, 1H, H-5^I), 3.88 (m, 1H, H-6b^I), 2.17 (s, 3H, COCH₃), 2.10 (s, 6H, 2 COCH₃), 2.08 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.86, 170.54, 170.31, 170.60, 169.73 (5C, 5 COCH₃), 137.66 (OCH₂CH=CH₂), 133.13 (Ar), 129.05 (Ar), 128.24 (Ar $\times$ 2), 126.19 (Ar $\times$ 2), 118.01 (OCH₂CH=CH₂),

101.79 (CHAr), 99.67 (C-1^I), 98.48 (C-1^{II}), 77.69 (C-2^I), 76.07 (C-4^I), 69.78 (C-3^I), 69.49 (C-2^{II}), 69.08 (C-5^{II}), 68.66 (C-3^{II}), 68.61 (C-6^I), 68.36 (OCH₂CH=CH₂), 66.23 (C-4^{II}), 63.92 (C-5^I), 62.51 (C-6^{II}), 20.87, 20.83, 20.73, 20.67 (5C, 5 COCH₃). MALDI-TOFMS: calcd for C₃₂H₄₀O₁₆Na [M+Na]⁺: *m/z* 703.2214; found *m/z* 703.2240.

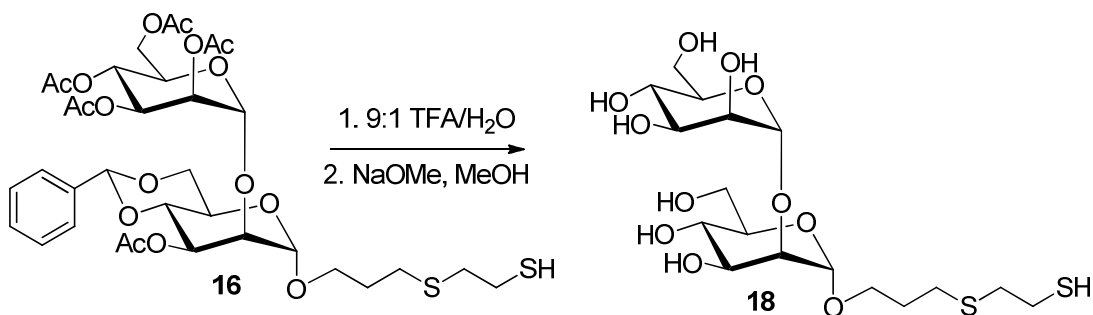
3-[(2-Sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (16**)**



Compound **16** was synthesized following general procedure 3 using 360 mg (0.529 mmol) of the foregoing disaccharide **15**. Purification on a silica gel column with 2:1 hexane–EtOAc as the eluent gave pure 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**16**) as a colorless oil (320 mg, 78%). [α]_D = +21.5° (*c* = 1 in CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 7.42 (m, 5H, Ar), 5.61 (s, 1H, CHAr), 5.41 (dd, *J*_{3,4} = 10.11, *J*_{2,3} = 3.25 Hz, 1H, H-3^{II}), 5.27 (m, 3H, H-2^{II}, H-3^I, H-4^{II}), 4.89 (1H, H-1^I), 4.87 (1H, H-1^{II}) 4.24 (m, 2H, H-6a^I, H-6a^{II}), 4.08 (m, 5H, OCH₂aCH=CH₂, H-2^I, H-6b^{II}, H-5^{II}, H-4^I), 3.84 (m, 3H, OCH₂bCH=CH₂, H-5^I, H-6b^I), 3.50 (m, 1H, H-6b^{II}), 2.73 (m, 4H, SCH₂CH₂SH),

2.64 (t, $J = 6.93$, 6.93 Hz, 2H, $\text{OSCH}_2\text{CH}_2\text{CH}_2\text{S}$), 2.16 (s, 3H, OCOCH_3), 2.09 (s, 6H, 2 OCOCH_3), 2.07 (s, 3H, OCOCH_3), 2.03 (s, 3H, OCOCH_3), 1.88 (dd, $J = 13.13$, 6.86 Hz, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 1.72 (s, 1H, SH). ^{13}C NMR (75 MHz, CDCl_3): δ 170.63, 170.49, 170.14, 170.06, 169.86 (5C, 5 COCH_3), 137.23 (Ar), 129.17 (Ar), 128.36 (Ar $\times$ 2), 126.30 (Ar $\times$ 2), 101.88 (CHAr), 99.82 (C-1^{II}), 99.45 (C-1^I), 77.95 (C-2^I), 76.12 (C-4^I), 69.90 (C-3^I), 69.60 (C-2^{II}), 69.21 (C-5^{II}), 68.75 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 66.39 (C-5^I), 66.22 (C-4^{II}), 64.07 (C-6^I), 62.74 (C-6^{II}), 36.29 ($\text{SCH}_2\text{CH}_2\text{SH}$), 29.22 ($\text{OSCH}_2\text{CH}_2\text{CH}_2\text{S}$), 28.68 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 24.77 64 ($\text{SCH}_2\text{CH}_2\text{SH}$), 21.00, 20.98, 20.92, 20.87, 20.85 (5C, COCH_3). MALDI-TOFMS: calcd for $\text{C}_{34}\text{H}_{46}\text{O}_{16}\text{S}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 797.2125; found m/z 797.2149.

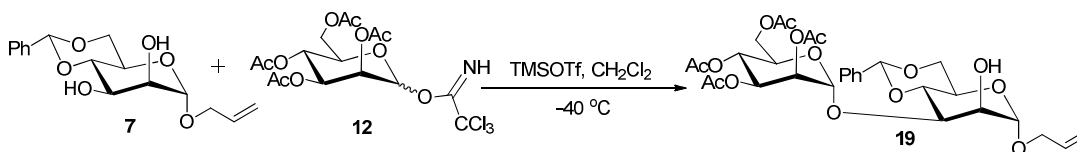
3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranoside (18**)**



The benzylidene group of foregoing compound **16** (280 mg, 0.361 mmol) was removed following general procedure 6. The resulting product was concentrated and deacetylated following general procedure 2. The residue was redissolved in water and submitted to LH-20 gel column chromatography to give **18** as colorless oil (113 mg, 66%). $[\alpha]_{\text{D}} = 90$

+8.32° (*c* = 1 in MeOH). ¹H NMR (300 MHz, CD₃OD): δ 5.07 (d, *J*_{1,2} = 1.57 Hz, 1H, H-1^{II}), H, 4.96 (d, *J*_{1,2} = 1.56 Hz, 1H, H-1^I), 3.97 (dd, *J* = 3.06, 1.67 Hz, 1H, H-2^I), 3.81 (m, 5H, H-2^{II}, H-3^I, H-4^I, H-5^I, H-6a^I), 3.59 (m, 8H, H-5^{II}, OCH₂*a*CH₂CH₂S, H-6a^{II}, H-6b^I, H-6b^{II}, H-4^{II}, H-3^{II}, OCH₂*b*CH₂CH₂S), 2.92 (ddd, *J* = 8.51, 5.30, 1.77 Hz, 2H, SCH₂CH₂SH), 2.84 (ddd, *J* = 7.94, 5.36, 1.92 Hz, 2H, OCH₂CH₂CH₂S), 2.66 (t, *J* = 7.24, 7.24 Hz, 2H, SCH₂CH₂SH), 1.87 (m, 2H, OCH₂CH₂CH₂S). ¹³C NMR (75 MHz, CD₃OD): δ 104.22 (C-1^{II}), 99.93 (C-1^I), 80.67 (C-2^I), 75.01 (C-2^{II}), 74.69 (C-5^I), 72.40 (C-5^{II}), 72.16 (C-3^I), 71.87 (C-3^{II}), 68.97 (C-4^I), 68.81 (C-4^{II}), 67.02 (OCH₂CH₂CH₂S), 63.12 (C-6^I), 63.00 (C-6^{II}), 39.58 (SCH₂CH₂SH), 32.22 (OCH₂CH₂CH₂S), 30.75 (OCH₂CH₂CH₂S), 29.57 (SCH₂CH₂SH). MALDI-TOFMS: calcd for C₁₇H₃₂O₁₁S₂Na [M+Na]⁺ *m/z* 499.1284, found *m/z* 499.1305.

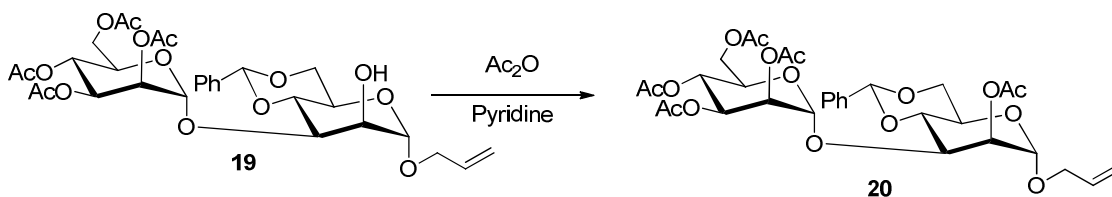
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene- α -D-mannopyranoside (19**)**



Allyl 4,6-*O*-benzylidene- α -D-mannopyranoside (**7**, 1.41 g, 4.57 mmol) was used to synthesize disaccharide **19** following general procedure 5. Purification by column chromatography (2:1 petroleum ether–EtOAc) gave **19** as a colorless oil (1.35 g, 46%). [α]_D = -2.16° (*c* = 1 in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.30 (m, 5H, Ar), 5.81 (m, 1H, OCH₂CH=CH₂), 5.53 (s, 1H, CHAr), 5.38 (dd, *J*_{2,3} = 3.22 Hz, *J*_{1,2} = 1.72 Hz, 1H, H-

2^I), 5.34 (dd, $J_{3,4} = 9.83$ Hz, $J_{2,3} = 3.48$ Hz, 1H, H-3^{II}), 5.32–5.17 (m, 5H, OCH₂CH=CH₂, H-4^{II}, H-1^{II}, H-5^{II}), 4.84 (1H, H-1^I), 4.28–4.05 (m, 7H, H-3^I, H-6a^{II}, H-6a^I, OCH₂aCH=CH₂, H-2^{II}, H-6b^I, H-4^I), 3.94 (dd, $J = 12.85, 6.34$ Hz, 1H, OCH₂bCH=CH₂), 3.79 (m, 2H, H-5^I, H-6b^{II}), 2.01 (2 s, 6H, 2 COCH₃), 1.98, 1.92 (2 s, 6H, 2 COCH₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.73, 170.13, 169.74, 169.69 (4C, 4 COCH₃), 137.22 (OCH₂CH=CH₂), 133.40 (Ar), 128.85 (Ar), 128.14 (Ar $\times$ 2), 126.01 (Ar $\times$ 2), 118.11 (OCH₂CH=CH₂), 101.42 (CHAr), 99.44 (C-1^{II}), 98.45 (C-1^I), 78.45 (C-4^I), 73.95 (C-2^I), 71.02 (C-3^I), 69.22 (C-2^{II}), 69.03 (C-3^{II}), 68.99 (C-5^{II}), 68.69 (C-6^I), 68.24 (OCH₂CH=CH₂), 66.43 (C-4^{II}), 63.67 (C-5^I), 62.73 (C-6^{II}), 20.76 (COCH₃), 20.74 (COCH₃), 20.72 (2C, 2 COCH₃). MALDI-TOFMS: calcd for C₃₀H₃₈O₁₅Na [M+Na]⁺ m/z 661.2108; found m/z 661.2137.

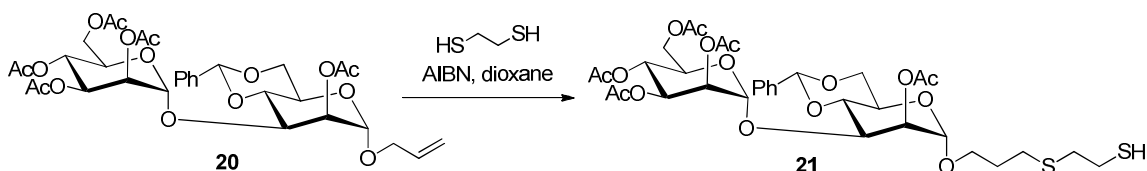
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene α -D-mannopyranoside (20**)**



Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-4,6-*O*-benzylidene- α -D-mannopyranoside (**19**, 1.35 g, 2.11 mmol) was acetylated according to general procedure 1. The residue was submitted to silica gel column chromatography (2:1 hexanes–EtOAc) to give compound **20** as a white solid (1.25 g, 89%). $[\alpha]_D = +6.5^\circ$ ($c = 1$ in CHCl₃). ¹H NMR

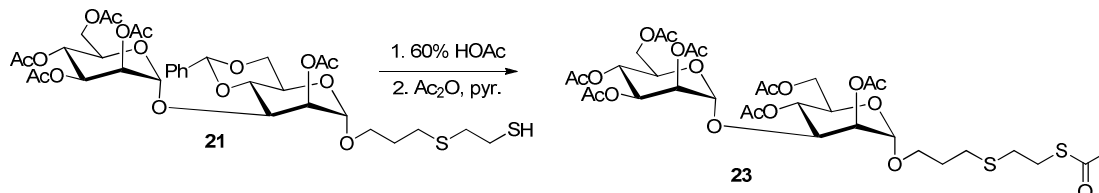
(600 MHz, CDCl₃): δ 7.37 (m, 5H, Ar), 5.86 (m, 1H, OCH₂CH=CH₂), 5.60 (s, 1H, CHAr), 5.34 (m, 2H, H-2^I, H-3^{II}), 5.29 (m, 1H, OCH₂CH=CH_{2a}), 5.23 (m, 2H, H-4^{II}, OCH₂CH=CH_{2b}), 5.19 (m, 2H, H-1^{II}, H-5^{II}), 4.81 (d, J = 1.14 Hz, 1H, H-1^I), 4.32 (dd, J = 9.89, 3.76 Hz, 1H, H-3^I), 4.27 (m, 2H, H-6a^{II}, H-6a^I), 4.17 (m, 1H, OCH_{2a}CH=CH₂), 4.09 (m, 3H, H-2^{II}, H-6b^I, H-4^I), 4.00 (dd, J = 12.76, 6.37 Hz, 1H, OCH_{2b}CH=CH₂), 3.86 (m, 2H, H-5^I, H-6b^{II}), 2.23, 2.08, 2.07, 2.06, 1.96 (5 s, 15H, 5 COCH₃). ¹³C NMR (151 MHz, CDCl₃): δ 170.78, 170.52, 170.03, 169.97, 169.85 (5C, 5 COCH₃) 137.12 (OCH₂CH=CH₂), 133.12 (Ar), 128.99 (Ar), 128.23 (Ar $\times$ 2), 126.05 (Ar $\times$ 2), 118.57 (OCH₂CH=CH₂), 101.49 (CHAr), 98.32 (C-1^{II}), 97.90 (C-1^I), 79.15 (C-4^I), 71.07 (C-2^I), 70.89 (C-3^I), 69.38 (C-2^{II}), 69.31 (C-3^{II}), 68.99 (C-5^{II}), 68.66 (C-6^I), 68.48 (OCH₂CH=CH₂), 65.81 (C-4^{II}), 63.60 (C-5^I), 62.58 (C-6^{II}), 20.93, 20.91, 20.88, 20.84, 20.78 (5C, 5 COCH₃). MALDI-TOFMS: calcd for C₃₂H₄₀O₁₆Na [M+Na]⁺ m/z 703.2214; found m/z 703.2206.

3-[(2-Sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (21)



The general procedure 3 was adopted to synthesize **21** using 1.25 g (1.84 mmol) of disaccharide **20**. Yield: 900 mg, 63%. $[\alpha]_D = +36.4^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 7.35 (m, 5H, Ar), 5.61 (s, 1H, CHAr), 5.31 (m, 3H, H-5^{II} , H-3^{I} , H-4^{II}), 5.22–5.17 (m, 2H, H-1^{II} , H-3^{II}), 4.77 (s, 1H, H-1^{I}), 4.32–4.23 (m, 3H, $\text{OCH}_2\text{aCH}_2\text{CH}_2\text{S}$, H-6a^{II} , H-2^{I}), 4.13–4.02 (m, 3H, H-4^{I} , H-2^{II} , H-6b^{II}), 3.88–3.77 (m, 3H, H-6a^{I} , $\text{OCH}_2\text{bCH}_2\text{CH}_2\text{S}$, H-5^{I}), 3.52 (td, $J_{6\text{a},6\text{b}} = 10.05$ Hz, $J_{5,6} = 6.11$ Hz, 1H, H-6b^{I}), 2.74 (m, 4H, $\text{SCH}_2\text{CH}_2\text{SH}$), 2.62 (t, $J = 7.0$ Hz, 2H, $\text{OSCH}_2\text{CH}_2\text{CH}_2\text{S}$), 2.24 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.07 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 1.97 (s, 3H, COCH_3), 1.88 (m, 2H, $\text{OSCH}_2\text{CH}_2\text{CH}_2\text{S}$), 1.72 (dd, $J = 7.87, 7.23$ Hz, 1H, SH). ^{13}C NMR (126 MHz, CDCl_3): δ 170.56, 170.40, 169.90, 169.79, 169.69 (5C, 5 COCH_3), 136.94 (Ar), 128.86 (Ar), 128.09 (Ar $\times 2$), 125.91 (Ar $\times 2$), 101.35 (CHAr), 98.72 (C-1^{II}), 98.31 (C-1^{I}), 78.91 (C-4^{I}), 71.14 (C-2^{I}), 70.89 (C-3^{I}), 69.18 (2C, C-5^{II} , C-2^{II}), 68.83 (C-3^{II}), 68.53 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 66.21 (C-6^{I}), 65.65 (C-4^{II}), 63.49 (C-5^{I}), 62.36 (C-6^{II}), 36.21 ($\text{SCH}_2\text{CH}_2\text{SH}$), 29.12 ($\text{OSCH}_2\text{CH}_2\text{CH}_2\text{S}$), 28.62 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 24.64 ($\text{SCH}_2\text{CH}_2\text{SH}$), 20.80, 20.76, 20.73, 20.70, 20.64 (5C, COCH_3). MALDI-TOFMS: calcd for $\text{C}_{34}\text{H}_{46}\text{O}_{16}\text{S}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 797.2125, found m/z 797.2164.

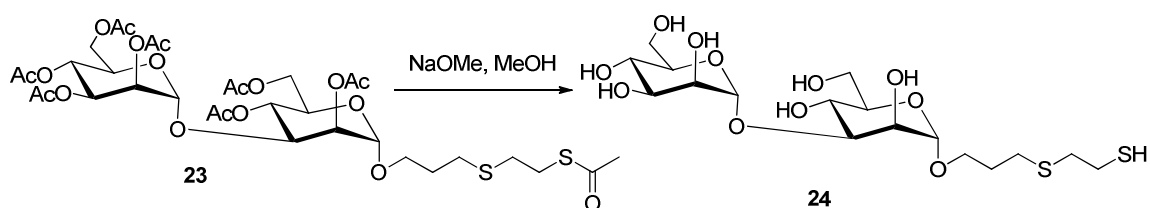
S-(2-[(3-([2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 3)-2,4,6-tri-O-acetyl- α -D-mannopyranosyl]oxy)propyl)sulfanyl]ethyl) ethanethioate (23**)**



Compound **21** (500 mg, 0.645 mmol) was treated with 60% aq HOAc (30 mL) at 70 °C for 40 min.⁶⁵ Concentration and azeotropic distillation with toluene afforded a dry residue that was acetylated following general procedure 1. After concentration, purification by column chromatography on silica gel afforded **23** (420 mg, 79%) as a colorless oil. $[\alpha]_D = +15.4^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (300 MHz, CDCl_3): δ 5.23 (m, 4H, H-3^I, H-4^I, H-2^I, H-3^{II}), 5.01 (m, 2H, H-2^{II}, H-1^{II}), 4.82 (d, $J = 1.40$ Hz, 1H, H-1^I), 4.25 (m, 4H, H-6a^I, H-6a^{II}, H-4^{II}, H-6b^I), 4.15 (m, 1H, H-5^{II}), 4.07 (m, 1H, H-6b^{II}), 3.85 (m, 2H, H-5^I, $\text{OCH}_2\text{aCH}_2\text{CH}_2\text{S}$), 3.53 (m, 1H, $\text{OCH}_2\text{bCH}_2\text{CH}_2\text{S}$), 3.06 (m, 2H, $\text{SCH}_2\text{CH}_2\text{S}$), 2.66 (m, 4H, $\text{SCH}_2\text{CH}_2\text{S}$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 2.34 (s, 3H, SCOCH_3), 2.20 (s, 3H, OCOCH_3), 2.13 (2s, 6H, 2 OCOCH_3), 2.10 (s, 6H, 2 OCOCH_3), 2.04 (s, 3H, OCOCH_3), 1.98 (s, 3H, OCOCH_3), 1.89 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$). ^{13}C NMR (75 MHz, CDCl_3): δ 195.46 (SCOCH_3), 170.83, 170.72, 170.54, 170.13, 170.02, 169.95, 169.71 (7C, 7 OCOCH_3), 99.13 (C-1^I), 97.57 (C-1^{II}), 75.33 (C-3^I), 71.07 (C-2^I), 70.09 (C-2^{II}), 69.51 (C-5^{II}), 68.94 (C-5^I), 68.44 (C-3^{II}), 67.75 (C-4^{II}), 66.53 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 66.03 (C-4^I), 62.72 (C-6^I), 62.49 (C-6^{II}), 31.89 (SCOCH_3), 30.78 ($\text{SCH}_2\text{CH}_2\text{S}$), 29.27 ($\text{SCH}_2\text{CH}_2\text{S}$), 29.18 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 28.59 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 21.10, 20.98, 20.95, 20.88, 20.82 (5C, 5

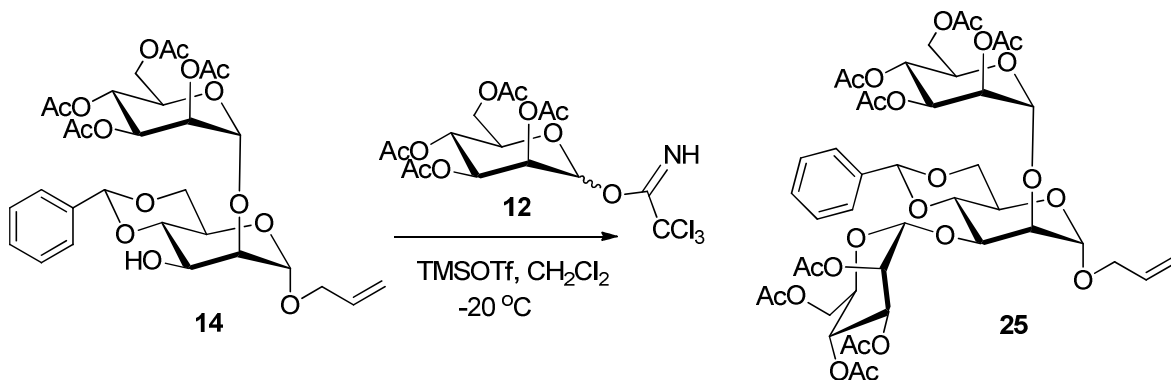
OCOCH₃), 20.78 (2C, OCOCH₃). MALDI-TOFMS: calcd for C₃₃H₄₈O₁₉S₂Na [M+Na]⁺ *m/z* 835.2129; found *m/z* 835.2156.

3-[(2-Sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside (23)



Following the general procedure 1, 3-[(2-sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside (**24**) was synthesized on a 300-mg (0.37 mmol) scale. Yield: 112 mg, 64%. $[\alpha]_D = +2.38^\circ$ (*c* = 1 in MeOH). ¹H NMR (500 MHz, CD₃OD): δ 5.08 (1H, H-1^{II}), 4.74 (1H, H-1^I), 4.03 (m, 1H, H-2^I), 3.98 (d, *J* = 1.22 Hz, 1H, H-2^{II}), 3.81 (m, 7H, H-3^I, H-4^I, H-5^I, H-6a^I, H-6a^{II}, H-5^{II}, OCH₂*a*CH₂CH₂S), 3.72 (m, 2H, H-6b^I, H-6b^{II}), 3.62 (t, *J*_{3,4} *J*_{4,5} = 9.57 Hz, 1H, H-4^{II}), 3.56 (m, 2H, H-3^{II}, OCH₂*b*CH₂CH₂S), 2.92 (m, 2H, SCH₂CH₂SH), 2.85 (dd, *J* = 8.83, 5.31 Hz, 2H, OCH₂CH₂CH₂S), 2.68 (t, *J* = 7.16, 7.16 Hz, 2H, SCH₂CH₂SH), 1.89 (m, 2H, OCH₂CH₂CH₂S). ¹³C NMR (126 MHz, CD₃OD): δ 103.85 (C-1^{II}), 101.69 (C-1^I), 80.63 (C-3^I), 74.87 (C-3^{II}), 74.86 (C-5^I), 72.47 (C-5^{II}), 72.12 (C-2^{II}), 71.48 (C-2^I), 68.77 (C-4^{II}), 67.54 (C-4^I), 67.02 (OCH₂CH₂CH₂S), 62.84 (C-6^{II}), 62.77 (C-6^I), 39.67 (SCH₂CH₂SH), 32.28 (OCH₂CH₂CH₂S), 30.65 (OCH₂CH₂CH₂S), 29.61 (SCH₂CH₂SH). MALDI-TOFMS: calcd for C₁₇H₃₂O₁₁S₂Na [M+Na]⁺ *m/z* 499.1284; found *m/z* 499.1297.

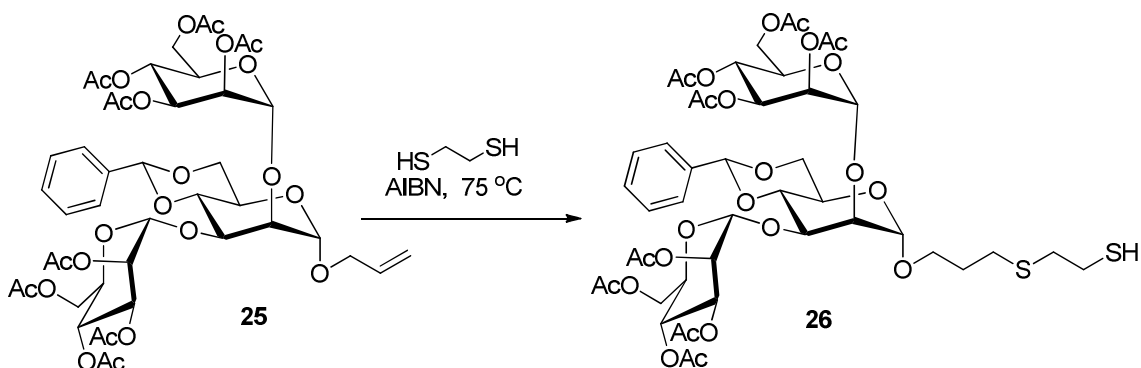
Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (25)



Following the general procedure 4, compound **25** was synthesized on a 1-g (1.57 mmol) acceptor scale. Yield: 900 mg, 59%. $[\alpha]_D = +21.3^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 7.44 (m, 2H, Ar), 7.32 (m, 3H, Ar), 5.88 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.67 (s, 1H, CHAr), 5.43 (m, 2H, H-3^{III} , H-5^{II}), 5.30 (m, 4H, H-4^{II} , H-5^{III} , H-1^{III} , $\text{OCH}_2\text{CH}=\text{CH}_2\text{a}$), 5.21 (m, 4H, $\text{OCH}_2\text{CH}=\text{CH}_2\text{b}$, H-3^{II} , H-1^{II} , H-4^{III}), 4.89 (s, 1H, H-1^{I}), 4.27 (m, 3H, H-3^{I} , H-6a^{III} , H-6a^{II}), 4.16 (m, 4H, H-6a^{I} , $\text{OCH}_2\text{aCH}=\text{CH}_2$, H-4^{I} , H-6b^{II}), 4.08 (m, 3H, H-6b^{III} , H-2^{I} , H-2^{III}), 3.99 (m, 2H, $\text{OCH}_2\text{bCH}=\text{CH}_2$, H-2^{II}), 3.89 (m, 1H, H-6^{I}), 3.82 (m, 1H, H-5^{I}), 2.16 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.06 (m, 9H, 3 COCH_3), 2.04 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 1.94 (s, 3H, COCH_3). ^{13}C NMR (126 MHz, CDCl_3): δ 170.59, 170.51, 169.86, 169.83, 169.80, 169.76, 169.45, 169.29 (8C, 8 COCH_3), 137.08 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 133.16 (Ar), 128.79 (Ar), 128.08 (Ar $\times$ 2), 126.03 (Ar $\times$ 2), 118.16 ($\text{OCH}_2\text{CH}=\text{CH}_2$), 101.30 (CHAr), 99.48 (C-1^{II}), 98.65 (C-1^{I}), 98.29 (C-1^{III}), 78.87 (C-4^{I}), 77.46 (C-2^{I}), 72.41 (C-3^{I}), 69.35 (C-4^{II}), 69.25 (C-2^{III}), 69.20 (C-5^{II}), 69.09 (C-2^{II}), 68.72

(C-3^{III}), 68.54 (C-3^{II}), 68.46 (C-6^I), 68.28 (OCH₂CH=CH₂), 66.45 (C-5^{III}), 66.40 (C-4^{III}), 64.01 (C-5^I), 62.70 (C-6^{II}), 62.60 (C-6^{III}), 20.85 (COCH₃), 20.72 (2C, 2 COCH₃), 20.71(3C, 3 COCH₃), 20.68 (COCH₃), 20.60 (COCH₃). MALDI-TOFMS: calcd for C₄₄H₅₆O₂₄Na [M+Na]⁺ *m/z* 991.3059; found *m/z* 991.3018.

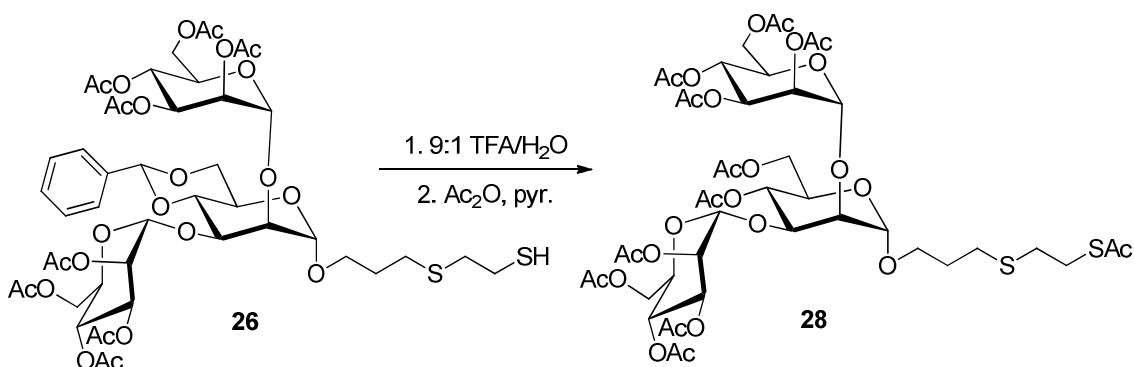
3-[(2-Sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (26)



The general procedure 3 was used to synthesize **26** on an 800-mg (0.83 mmol) scale. Yield: 690 mg, 78%. $[\alpha]_D = +37.67^\circ$ (*c* = 1 in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.44 (m, 2H, Ar), 7.32 (m, 3H, Ar), 5.67 (s, 1H, CHAr), 5.41 (m, 2H, H-3^{III}, H-5^{II}), 5.32 (m, 1H, H-4^{II}), 5.25 (m, 4H, H-5^{III}, H-1^{III}, H-3^{II}, H-4^{III}), 5.19 (s, 1H, H-1^{II}), 4.85 (s, 1H, H-1^I), 4.26 (m, 4H, H-3^I, H-6^I, H-6^{II}, H-6^{III}), 4.16 (dd, *J* = 10.98, 8.28 Hz, 2H, H-6^{II}, H-4^I), 4.06 (m, 3H, H-2^I, H-6^{III}, H-2^{III}), 3.98 (t, 1H, H-2^{II}), 3.88 (t, *J* = 10.32, 10.32 Hz, 1H, H-6^I), 3.79 (q, 2H, H-5^I, OCH₂aCH₂CH₂S), 3.53 (td, *J* = 9.73, 6.18, 6.18 Hz, 1H,

$\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 2.72 (m, 4H, $\text{SCH}_2\text{CH}_2\text{SH}$), 2.61 (t, $J = 7.00, 7.00$ Hz, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 2.15, 2.10, 2.08, 2.06, 2.05, 2.03, 2.01, 1.94 (8 s, 24H, 8 COCH_3), 1.88 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 1.71 (t, $J = 7.82, 7.82$ Hz, 1H, $\text{SCH}_2\text{CH}_2\text{SH}$). ^{13}C NMR (126 MHz, CDCl_3): δ 170.64, 170.62, 169.96, 169.93 (4C, 4 COCH_3), 169.92 (2C, 2 COCH_3), 169.58, 169.44 (2C, 2 COCH_3), 137.20 (Ar), 128.94 (Ar), 128.23 (Ar $\times$ 2), 126.18 (Ar $\times$ 2), 101.43 (CHAr), 99.63 (C-1^I), 99.62 (C-1^{II}), 98.64 (C-1^{III}), 78.82 (C-4^I), 77.84 (C-2^I), 73.09 (C-3^I), 69.49 (C-4^{II}), 69.41 (C-5^{II}), 69.38 (C-2^{III}), 69.15 (C-2^{II}), 68.81 (C-3^{II}), 68.69 (C-3^{II}), 68.63 (C-6^I), 66.66 (C-5^{III}), 66.51 (C-4^{III}), 66.34 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 64.20 (C-5^I), 62.93 (C-6^{II}), 62.69 (C-6^{III}), 36.37 ($\text{SCH}_2\text{CH}_2\text{SH}$), 29.33 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 28.81 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 24.79 ($\text{SCH}_2\text{CH}_2\text{SH}$), 20.99 (COCH_3), 20.91 (COCH_3), 20.87 (2C, 2 COCH_3), 20.86 (COCH_3), 20.85 (COCH_3), 20.81 (COCH_3), 20.74 (COCH_3). MALDI-TOFMS: calcd for $\text{C}_{46}\text{H}_{62}\text{O}_{24}\text{S}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 1085.2970; found m/z 1085.2979.

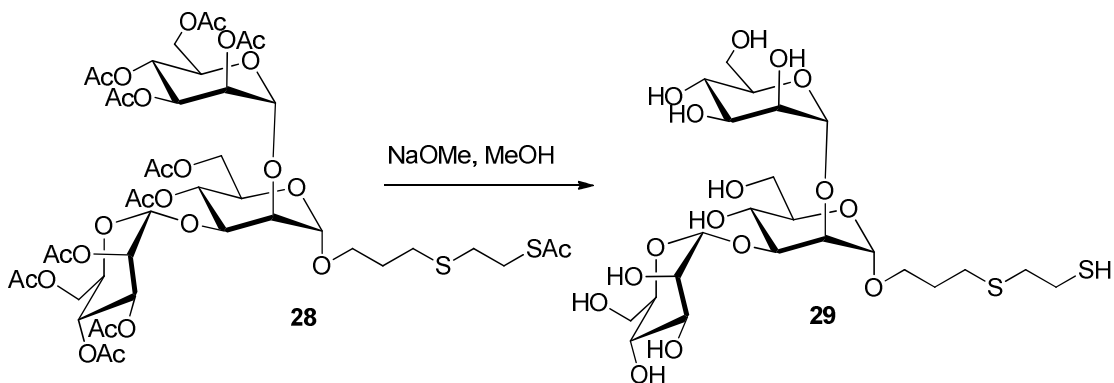
S-(2-[(3-[(2,3,4,6-Tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-O-acetyl- α -D-mannopyranosyl]oxy)propyl)-sulfanyl]ethyl) ethanethioate (28**)**



Following general procedure 6, the benzylidene group on **26** (0.60 g, 0.56 mmol) was removed and the intermediate was acetylated using general procedure 1 to afford trisaccharide **28**. Yield: 392 mg, 82% over two steps. $[\alpha]_D = +18.7^\circ$ ($c = 1$ in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.37 (m, 2H, H-3^{II}, H-5^{II}), 5.27 (m, 4H, H-2^{II}, H-4^{II}, H-3^{III}, H-3^I), 5.15 (m, 1H, H-2^{III}), 5.05 (d, $J = 1.17$ Hz, 1H, H-1^I), 5.04 (bd, 1H, H-1^{II}), 4.97 (bd, 1H, H-1^{III}), 4.28 (dd, $J = 12.14, 5.80$ Hz, 1H, H-6a^{III}), 4.20 (m, 4H, H-5^{III}, H-6a^{II}, H-6a^I, H-6b^{II}), 4.10 (m, 3H, H-6b^I, H-4^{III}, H-4^I), 4.02 (m, 2H, H-2^I, H-6b^{III}), 3.81 (m, 2H, H-5^I, OCH₂aCH₂CH₂S), 3.51 (m, 1H, OCH₂bCH₂CH₂S), 3.05 (dd, $J = 9.09, 6.59$ Hz, 2H, SCH₂CH₂S), 2.66 (m, 4H, SCH₂CH₂S, OCH₂CH₂CH₂S), 2.33 (s, 3H, SCOCH₃), 2.15 (s, 3H, OCOCH₃), 2.13 (s, 9H, 3 OCOCH₃), 2.08 (s, 3H, OCOCH₃), 2.06 (s, 3H, OCOCH₃), 2.05 (s, 3H, OCOCH₃), 2.04 (s, 3H, OCOCH₃), 1.98 (s, 3H, OCOCH₃), 1.97 (s, 3H, OCOCH₃), 1.89 (m, 2H, OCH₂CH₂CH₂S). ¹³C NMR (126 MHz, CDCl₃): δ 195.47

(SCOCH₃), 171.07, 170.58, 170.47, 170.19, 170.12, 169.93, 169.73, 169.65, 169.47, 169.33 (10C, 10 OCOCH₃), 99.66 (C-1^{III}), 99.27 (C-1^{II}), 98.11 (C-1^I), 78.91 (C-2^I), 78.06 (C-4^I), 69.77 (C-4^{II}), 69.71 (C-2^{III}), 69.42 (C-5^{III}), 69.34 (C-5^I), 69.20 (C-4^{III}), 68.88 (C-3^{III}), 68.79 (C-3^{II}), 66.62 (OCH₂CH₂CH₂S), 66.52 (C-5^{II}), 66.05 (C-2^{II}), 62.92 (C-6^{III}), 62.71 (C-6^{II}), 62.39 (C-6^I), 31.90 (OCH₂CH₂CH₂S), 30.77 (SCOCH₃), 29.26, 29.23, 28.61, 20.98 (4C, 4 OCOCH₃), 20.90 (2C, 2 OCOCH₃), 20.85 (2C, 2 OCOCH₃), 20.84, 20.80, 20.77 (3C, 3 OCOCH₃), 20.74 (2C, 2 OCOCH₃). MALDI-TOFMS: calcd for C₄₅H₆₄O₂₇S₂Na [M+Na]⁺ *m/z* 1123.2974; *m/z* found 1123.3026.

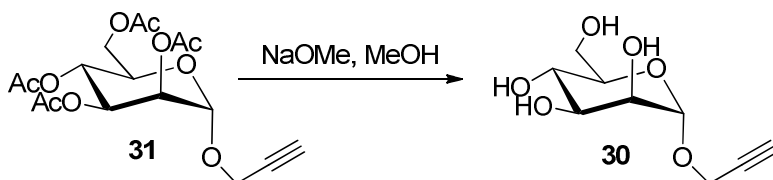
3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (29)



Acetylated trisaccharide **28** was fully deprotected following general procedure 2 on a 300-mg (0.27 mmol) scale. Yield: 133 mg, 76%. [α]_D = +47.5° (*c* = 1 in MeOH). ¹H NMR (500 MHz, CD₃OD): δ 5.10 (d, *J*_{1,2} = 1.58 Hz, 1H, H-1^{III}), 5.08 (d, *J*_{1,2} = 1.56 Hz, 1H, H-1^{II}), 5.04 (d, *J*_{1,2} = 1.56 Hz, 1H, H-1^I), 4.03 (dd, *J*_{2,3} = 2.85 Hz, *J*_{1,2} = 1.59 Hz, 1H, H-2^I), 3.97 (dd, *J*_{2,3} = 3.17 Hz, *J*_{1,2} = 1.56 Hz, 1H, H-2^{III}), 3.94 (dd, *J*_{3,4} = 9.59 Hz, *J*_{2,3} =

3.06 Hz, 1H, H-3^I), 3.88 (dd, $J_{2,3} = 3.20$ Hz, $J_{1,2} = 1.60$ Hz, 1H, H-2^{II}), 3.86 (m, 1H, H-6a^{III}), 3.82 (m, 3H, H-6a^{II}, H-6a^I, OCH₂aCH₂CH₂S), 3.69 (m, 9H, H-4^I, H-6b^I, H-6b^{II}, H-3^{III}, H-3^{II}, H-6b^{III}, H-5^{III}, H-5^{II}, H-4^{III}), 3.56 (m, 3H, H-4^{II}, H-5^I, OCH₂bCH₂CH₂S), 2.93 (m, 2H, SCH₂CH₂SH), 2.85 (m, 2H, SCH₂CH₂SH), 2.67 (t, $J = 7.20$, 7.20 Hz, 2H, OCH₂CH₂CH₂S), 1.89 (m, 2H, (OCH₂CH₂CH₂S). ¹³C NMR (126 MHz, CD₃OD): δ 104.03 (C-1^{III}), 103.45 (C-1^{II}), 100.01 (C-1^I), 79.56 (C-2^I), 79.45 (C-3^I), 75.24 (C-5^{II}), 75.10 (C-3^{II}), 74.95 (C-5^I), 72.55 (C-5^{III}), 72.37 (C-3^{III}), 72.10 (C-2^{II}), 72.05 (C-2^{III}), 68.79 (C-4^{II}), 68.73 (C-4^{III}), 68.40 (C-4^I), 67.16 (OCH₂CH₂CH₂S), 63.15 (C-6^{III}), 63.00 (C-6^{II}), 62.88 (C-6^I), 39.65 (SCH₂CH₂SH), 32.29 (SCH₂CH₂SH), 30.70 (OCH₂CH₂CH₂S), 29.64 (OCH₂CH₂CH₂S). MALDI-TOFMS: calcd for C₂₃H₄₂O₁₆S₂Na [M+Na]⁺ m/z 661.1812; found m/z 661.1789.⁹³

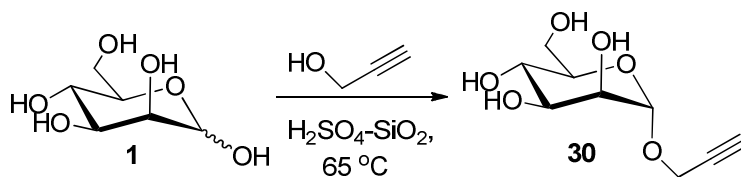
Propargyl α -D-mannopyranoside (**30**)



Following the general procedure 2 for deacetylation, compound **30** was synthesized on a 12 g (31 mmol) scale. Yield: 6.02 g (88%). ¹H NMR (300 MHz, CD₃OD): δ 4.96 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.29–4.23 (m, 2H, OCH₂CCH), 3.82 (m, 2H), 3.74–3.56 (m, 3H), 3.54–3.46 (m, 1H), 2.87–2.82 (m, 1H, OCH₂C $\equiv$ CH). ¹³C NMR (75 MHz, CD₃OD): δ

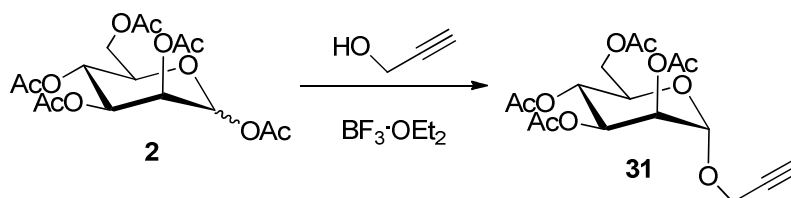
99.79 (C-1), 80.00, 75.98, 75.05, 72.45, 71.98, 68.42, 62.79, 54.82 (C-6). The spectroscopic data matched that in literature.⁹⁴

Propargyl α -D-mannopyranoside (30)⁹⁵



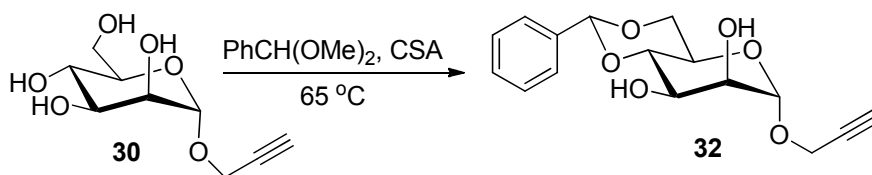
To a slurry of silica gel (10 g 200–400 mesh) in dry Et₂O (50 mL) was added commercially available concentrated H₂SO₄ (3 mL) with shaking for 5 min. The solvent was evaporated under reduced pressure resulting in free flowing H₂SO₄-silica which was then dried at 110 °C for 3 h. A suspension of D-mannose (1.8 g, 10 mmol) in propargyl alcohol (2.9 mL, 50 mmol) was heated to 65 °C while stirring. H₂SO₄-silica (50 mg) was added and stirring was continued until all the solids had dissolved (2.5 h). At this point, TLC (5:1 CH₂Cl₂-MeOH) showed complete conversion of the starting D-mannose to a faster running component. After cooling to room temperature, the reaction mixture was transferred to a short silica gel column and the excess propargyl alcohol was eluted with DCM followed by elution of the product with CH₂Cl₂-MeOH (15:1) to afford the desired propargyl glycoside (30) in 65% yield. Spectroscopic data matched that in literature.⁹⁴

Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (31**)**⁸³



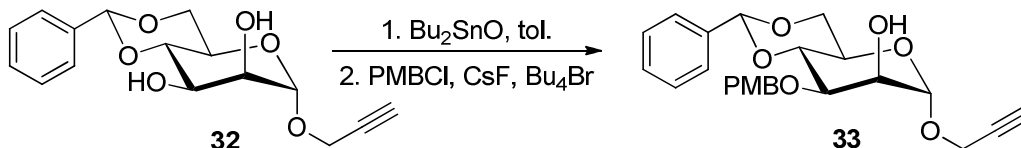
α,β -D-Mannopyranose pentaacetate (3.22 g, 8.24 mmol) and propargyl alcohol (0.77 mL, 13 mmol) were dissolved in 40 mL of dry dichloromethane (DCM). The mixture was cooled to 0 °C and boron trifluoride etherate (15 mmol, 2.13 g) was added in two portions. After removal of the cooling bath the reaction was stirred for 24 h. Subsequently, the mixture was quenched with saturated NaHCO₃ solution; the organic phase was dried over MgSO₄ and concentrated. The residue was purified by column chromatography (2:1 hexane–EtOAc) to give 2.7 g (84 %) of **31** as a colorless solid. ¹H NMR (300 MHz, CDCl₃): δ 5.31–5.18 (m, 3H, H-3, H-2, H-4), 4.98 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.27–4.16 (m, 3H, OCH₂C $\equiv$ CH, H-6a), 4.05 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-6b), 4.00–3.92 (m, 1H, H-5), 2.46 (t, J = 2.4 Hz, 1H, OCH₂C $\equiv$ CH), 2.12 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 1.99 (s, 3H, COCH₃), 1.95 (s, 3H, COCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.61, 169.92, 169.82, 169.68 (4C, 4 COCH₃), 96.22 (C-1), 77.95 (OCH₂C $\equiv$ CH), 75.69 (OCH₂C $\equiv$ CH), 69.33 (C-5), 68.97 (C-3), 68.92 (C-2), 65.98 (C-4), 62.30 (C-6), 54.94 (OCH₂C $\equiv$ CH), 20.87, 20.75, 20.69, 20.66 (4C, 4 COCH₃). The spectroscopic data matched that previously reported.⁹⁵

Propargyl 4,6-*O*-benzylidene- α -D-mannopyranoside (32)



Propargyl 4,6-*O*-benzylidene- α -D-mannopyranoside (**32**) was synthesized following similar procedure used for the synthesis of allyl 4,6-*O*-benzylidene- α -D-mannopyranoside (**7**) on a 6.02-g scale (27.6 mmol). Yield: 6.4 g (76%). ^1H NMR (300 MHz, CDCl_3): δ 7.55–7.31 (m, 5H, Ar), 5.56 (s, 1H, *CHAR*), 5.06 (s, 1H, H-1), 4.32 – 4.21 (m, 3H), 4.07 (d, J = 10.8 Hz, 2H), 3.98–3.76 (m, 3H), 2.75 (s, 2H, 2 OH), 2.48 (t, J = 2.3 Hz, 1H, $\text{OCH}_2\text{C}\equiv\text{CH}$). ^{13}C NMR (75 MHz, CDCl_3): δ 137.26, 129.47, 128.52 (Ar $\times$ 2), 126.40 (Ar $\times$ 2), 102.44 (*CHAR*), 98.96 (C-1), 78.85, 78.68, 75.20, 70.88, 68.79, 68.70, 63.60, 54.78 ($\text{OCH}_2\text{C}\equiv\text{CH}$). HRDARTMS: calcd for $\text{C}_{16}\text{H}_{18}\text{O}_6$ $[\text{M}]^+$ m/z 306.1103, calcd for $[\text{M}+\text{H}]^+$ m/z 307.1182; found m/z 307.1108.

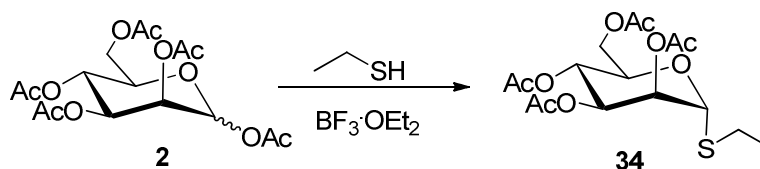
Propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (33)



Propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**33**) was synthesized following similar procedure used for the synthesis of compound **8** on a 3.5-g (11.4 mmol) scale. Yield: 3.21 g (65%). ^1H NMR (300 MHz, CDCl_3): δ 7.50 (m, 2H, Ar), 7.45–7.34 (m, 3H, Ar), 7.28 (m, 2H, Ar), 6.91–6.83 (m, 2H, Ar), 5.62 (s, 1H, *CHAR*),

5.06 (d, $J_{1,2} = 1.4$ Hz, 1H, H-1), 4.78 (d, $J = 11.4$ Hz, 1H, $\text{OCH}_2a\text{C}\equiv\text{CH}$), 4.64 (d, $J = 11.4$ Hz, 1H, $\text{OCH}_2a\text{C}\equiv\text{CH}$), 4.26 (m, 3H, OCH_2aAr , H-6a), 4.11 (m, 1H, H-5), 4.02 (m, 1H, H-2), 3.91 (dd, $J_{3,4} = 9.5$ Hz, $J_{2,3} = 3.5$ Hz 1H, H-3), 3.88–3.82 (m, 2H, OCH_2bAr , H-4), 3.80 (s, 3H, OCH_3), 2.85 (bs, 1H, OH), 2.47 (t, $J = 2.4$ Hz, 1H, $\text{OCH}_2\text{C}\equiv\text{CH}$). ^{13}C NMR (75 MHz, CDCl_3): δ 159.50, 137.61, 130.10 (Ar $\times$ 3), 129.65 (2 $\times$ Ar), 129.03 (Ar), 128.30 (2 $\times$ Ar), 126.16 (2 $\times$ Ar), 113.97 (2 $\times$ Ar), 101.69 (CHAr), 98.84 (C-1), 78.76 (C-5), 78.70 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 75.23 (C-3), 75.13 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 72.87 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 69.85 (C-2), 68.78 (CH_2Ar), 63.87 (C-4), 55.36 (ArOCH_3), 54.58 (C-6). HRDARTMS: calcd for $\text{C}_{24}\text{H}_{26}\text{O}_7$ $[\text{M}]^+$ m/z 426.1679; found m/z 426.1572.

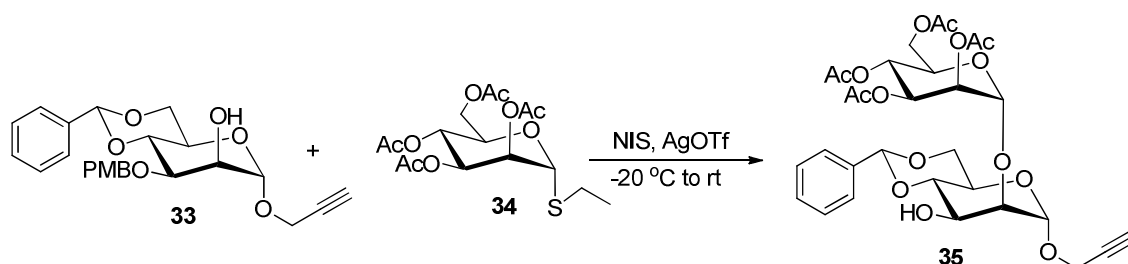
Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio- α -D-mannopyranoside (**34**)⁸⁵



Anhydrous DCM (100 mL) was added to D-mannose pentaacetate (**2**, 10.5 g, 26.9 mmol) along with ethanethiol (6.0 mL, 81 mmol). $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (10 mL, 79 mmol) was added and stirred under an argon atmosphere for 2 h at 0 °C, followed by 16 h at rt. Saturated NaHCO_3 was added and the solution was stirred for 2 h. The organic layer was dried over Mg_2SO_4 and concentrated. The residue was purified by silica gel chromatography using 3:1 toluene– EtOAc as the eluent to afford a white solid (**34**, 8.1 g, 76%). ^1H NMR (300 MHz, CDCl_3): δ 5.34–5.25 (m, 4H, H-1, H-2, H-3, H-4), 4.38 (ddd, 1H, $J_{4,5} = 9.9$ Hz, $J_{5,6a}$

= 5.3 Hz, $J_{5,6b}$ = 2.3 Hz, H-5), 4.29 (dd, 1H, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.08 (dd, 1H, $J_{5,6b}$ = 2.3 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b), 2.60–2.66 (m, 2H, SCH₂CH₃), 2.16 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 2.02 (s, 3H, COCH₃), 1.97 (s, 3H, COCH₃), 1.28 (t, 3H, SCH₂CH₃). Spectroscopic data matched that in the literature.⁸⁵

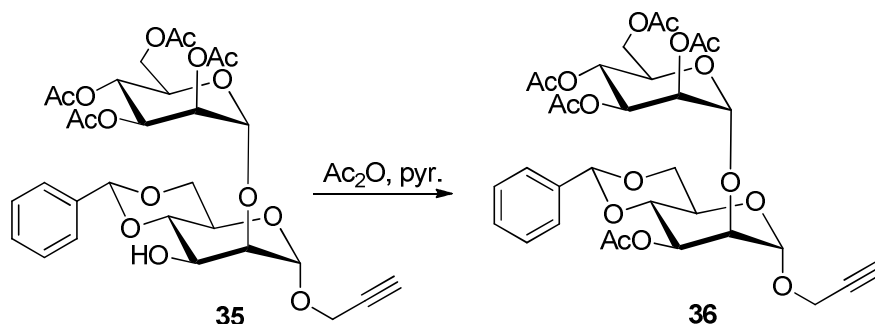
Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene- α -D-mannopyranoside (35)



Following general procedure 6 for glycosylation, the disaccharide **12** was synthesized on a 1.20-g (2.81 mmol) acceptor scale. Purification by column chromatography (2:1 hexane–EtOAc) gave **35** as a colorless oil (1.12 g, 61%). $[\alpha]_D = +31.07^\circ$ ($c = 1$ in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.54–7.45 (m, 2H, Ar), 7.40–7.30 (m, 3H, Ar), 5.64 (s, 1H, CHAr), 5.44 (dd, $J_{2,3} = 3.4$ Hz, $J_{1,2} = 1.8$ Hz, 1H, H-2^{II}), 5.38 (dd, $J_{3,4} = 10.0$ Hz, $J_{2,3} = 3.4$ Hz, 1H, H-3^{II}), 5.29 (t, $J_{3,4} = J_{4,5} = 10.0$ Hz, 1H, H-4^{II}), 5.15 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1^{II}), 5.10 (d, $J_{1,2} = 1.4$ Hz, 1H, H-1^I), 4.28–4.22 (m, 4H, OCH₂C $\equiv$ CH, H-6a^I, H-6a^{II}), 4.18–4.13 (m, 2H, H-6b^{II}, H-3^I), 4.11–4.08 (m, 1H, H-5^{II}), 4.07 (dd, $J_{2,3} = 3.4$ Hz, $J_{1,2} = 1.6$ Hz, 1H, H-2^I), 3.93 (t, $J_{3,4} = J_{4,5} = 9.4$ Hz, 1H, H-4^I), 3.89–3.79 (m, 2H, H-6b^I, H-5^I), 2.50 (t, $J = 2.4$ Hz, 1H, OCH₂C $\equiv$ CH), 2.15 (s, 3H, COCH₃), 2.11 (s, 3H, COCH₃), 2.07

(s, 3H, COCH₃), 2.01 (s, 3H, COCH₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.79, 170.20, 170.00, 169.85 (4C, 4 COCH₃), 137.27 (Ar), 129.37 (Ar), 128.47 (Ar × 2), 126.42 (Ar × 2), 102.29 (CHAr), 99.97 (C-1^{II}), 98.27 (C-1^I), 78.90 (C-4^I), 78.47 (OCH₂C≡CH), 78.19 (C-2^I), 75.44 (OCH₂C≡CH), 69.30 (C-2^{II}), 69.28 (C-5^{II}), 69.20 (C-3^{II}), 68.84 (C-3^I), 68.62 (C-6^I), 66.36 (C-4^{II}), 64.19 (C-5^I), 62.64 (C-6^{II}), 54.87 (OCH₂C≡CH), 21.01, 20.93, 20.86, 20.85 (4C, 4 COCH₃). MALDI-TOFMS: calcd for C₃₀H₃₆O₁₅Na [M+Na]⁺ *m/z* 659.1952; found *m/z* 659.1980.

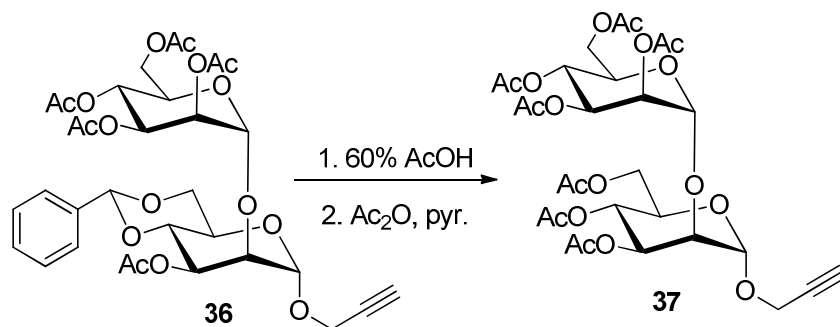
Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (36)



Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**36**) was synthesized from compound **35** (1.03 g, 1.62 mmol) following general procedure 1. Yield: 951 mg, 86%. [α]_D = +26.80° (*c* = 1 in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.49–7.43 (m, 2H, Ar), 7.38–7.32 (m, 3H, Ar), 5.61 (s, 1H, CHAr), 5.41 (dd, *J*_{3,4} = 10.0 Hz, *J*_{2,3} = 3.4 Hz, 1H, H-3^{II}), 5.35–5.25 (m, 3H, H-4^{II}, H-3^I, H-2^{II}), 5.09 (d, *J*_{1,2} = 1.5 Hz, 1H, H-1^{II}), 4.90 (d, *J*_{1,2} = 1.6 Hz, 1H, H-1^I),

4.29– 4.20 (m, 4H, $\text{OCH}_2\text{C}\equiv\text{CH}$, H-6a^I, H-6a^{II}), 4.15 (dt, $J = 5.4, 2.7$ Hz, 1H), 4.14–4.06 (m, 3H), 3.97–3.90 (m, 1H), 3.87 (dd, $J = 12.5, 7.7$ Hz, 1H), 2.49 (t, $J = 2.4$ Hz, 1H, $\text{OCH}_2\text{C}\equiv\text{CH}$), 2.15 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 2.08 (s, 3H, COCH_3), 2.06 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 170.67, 170.34, 170.08, 169.99, 169.77 (5C, 5 COCH_3), 137.23 (Ar), 129.15 (Ar), 128.32 (Ar $\times$ 2), 126.31 (Ar $\times$ 2), 101.93 (CHAr), 99.81 (C-1^{II}), 97.96 (C-1^I), 78.28 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 77.53, 76.01, 75.56, 69.72, 69.59, 69.30, 68.81, 68.56, 66.31, 64.46, 62.46, 54.84 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 20.94, 20.88, 20.86, 20.81, 20.79 (5C, 5 COCH_3). MALDI-TOFMS: calcd for $\text{C}_{32}\text{H}_{38}\text{O}_{16}\text{Na}$ $[\text{M}+\text{Na}]^+$: m/z 701.2058; found m/z 701.2123.

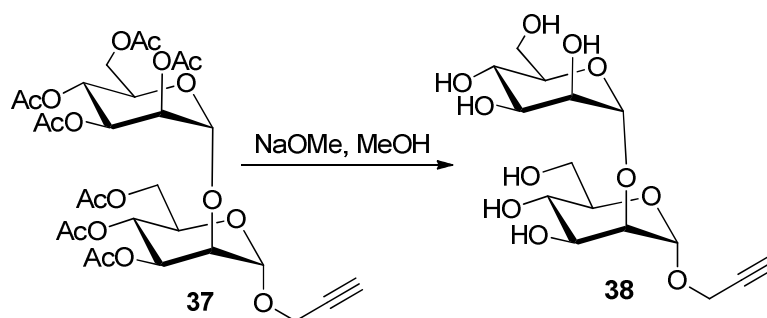
Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-3,4,6-tri-*O*-acetyl- α -D-mannopyranoside (37)



The benzylidene group of foregoing compound **36** (880 mg, 1.25 mmol) was removed following general procedure 6. The resulting product was concentrated and acetylated following general procedure 1 for easier purification and characterization. After concentration, purification by column chromatography (2:1 hexane–EtOAc) on silica gel

afforded propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-3,4,6-tri-*O*-acetyl- α -D-mannopyranoside (**37**, 620 mg, 73%) as a colorless oil. $[\alpha]_D = +37.06^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (300 MHz, CDCl_3): δ 5.4–5.33 (m, 2H), 5.34–5.24 (m, 4H), 5.15 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.93 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.28–4.05 (m, 7H), 3.95 (ddd, $J_{4,5} = 9.5$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.4$ Hz, 1H, H-5), 2.48 (t, $J = 2.4$ Hz, 1H, $\text{OCH}_2\text{C}\equiv\text{CH}$), 2.15 (s, 3H, COCH_3), 2.14 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 2.07 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 171.02, 170.70, 170.48, 169.99, 169.85, 169.60, 169.48 (7C, 7 COCH_3), 99.31 (C-1), 97.01 (C-1), 78.21 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 76.87, 75.68 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 70.16, 69.86, 69.34, 69.19, 68.51, 66.40, 66.17, 62.45, 62.13, 55.12 ($\text{OCH}_2\text{C}\equiv\text{CH}$), 21.02, 20.88, 20.85, 20.81, 20.80 (5C, 5 COCH_3), 20.79 (2C, 2 COCH_3). MALDI-TOFMS: calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{18}\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 697.1956; found m/z 697.2001.

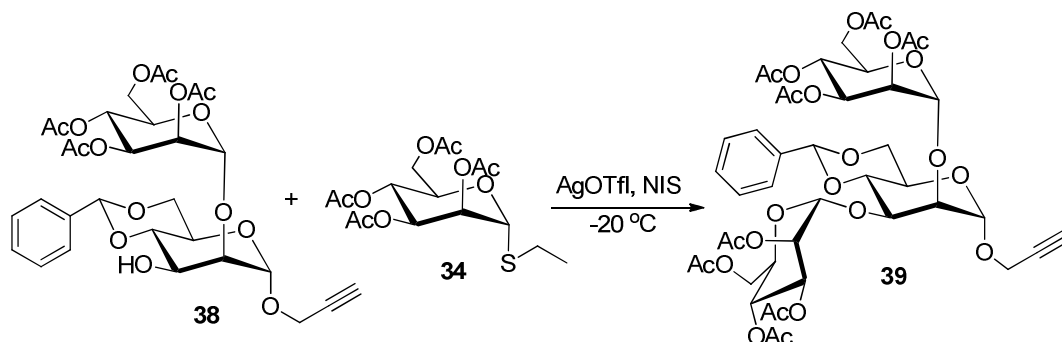
Propargyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranoside (38**)**



Following the general procedure 1, propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-3,4,6-tri-*O*-acetyl- α -D-mannopyranoside (**38**) was synthesized

on a 600-mg (0.889 mmol) scale. Yield: 201 mg, 59%. $[\alpha]_D = +50.4^\circ$ ($c = 1$ in MeOH). ^1H NMR (500 MHz, CD_3OD): δ 5.20 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.98 (d, $J_{1,2} = 1.8$ Hz, 1H, H-1), 4.31–4.20 (m, 2H, $\text{OCH}_2\text{C}\equiv\text{CH}$), 3.98 (dd, $J_{2,3} = 3.2$ Hz, $J_{1,2} = 1.8$ Hz, 1H, H-2), 3.86–3.77 (m, 4H), 3.74–3.59 (m, 6H), 3.53–3.48 (m, 1H), 2.84 (t, $J = 2.4$ Hz, 1H, $\text{OCH}_2\text{C}\equiv\text{CH}$). ^{13}C NMR (126 MHz, CDCl_3): δ 104.27 (C-1), 98.71 (C-1), 80.29, 80.12, 75.96, 75.12, 74.92, 72.44, 72.05, 71.88, 68.84, 68.56, 62.89 (C-6), 62.85 (C-6), 55.16 ($\text{OCH}_2\text{C}\equiv\text{CH}$). MALDI-TOFMS: calcd for $\text{C}_{15}\text{H}_{24}\text{O}_{11}\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 403.1216; found m/z 403.1287.

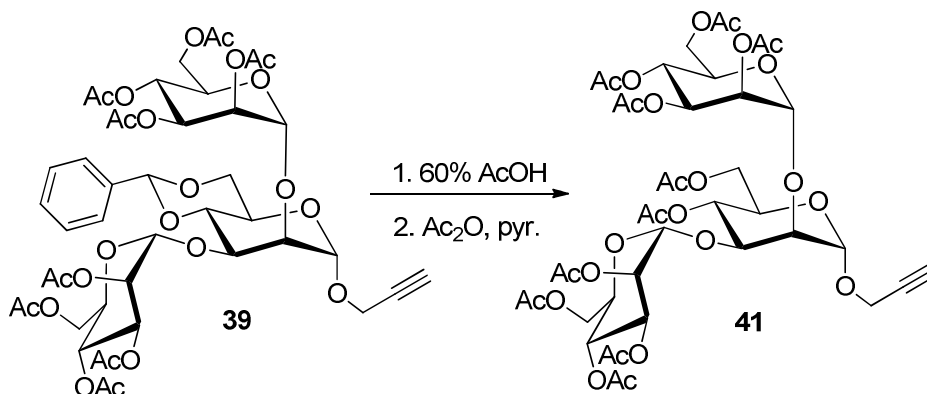
Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (39**)**



Following the general procedure 6, compound **39** was synthesized on a 1.03-g (1.62 mmol) acceptor scale. Yield: 761 mg, 49%. $[\alpha]_D = +28.23^\circ$ ($c = 1$ in CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 7.41 (m, 2.9 Hz, 2H, Ar), 7.34–7.28 (m, 3H, Ar), 5.65 (s, 1H, CHAr), 5.44–5.39 (m, 2H, H-5^{II}, H-4^{III}), 5.33–5.27 (m, 2H, H-2^{II}, H-3^{III}), 5.26 (d, $J_{1,2} = 1.7$ Hz, 1H, H-1^{III}), 5.22–5.14 (m, 3H, H-2^{III}, H-1^{II}, H-2^I), 5.04 (d, $J_{1,2} = 1.5$ Hz, 1H, H-

1^I), 4.30–4.21 (m, 6H, H-4^{II}, H-6a,b^{II}, H-6a^I, OCH₂C≡CH), 4.20–4.12 (m, 2H, H-4^I, H-6a^{III}), 4.10–4.03 (m, 3H, H-6b^{III}, H-3^{II}, H-5^I), 3.95 (ddd, $J_{4,5} = 8.8$ Hz, $J_{5,6a} = 6.3$ Hz, $J_{5,6b} = 2.2$ Hz, 1H, H-5^{III}), 3.87 (m, 1H, H-6b^I), 3.81 (dd, $J_{3,4} = 9.2$ Hz, $J_{2,3} = 4.5$ Hz, 1H, H-3^I), 2.48 (t, $J = 2.4$ Hz, 1H, OCH₂C≡CH), 2.14 (s, 3H, COCH₃), 2.10 (s, 3H, COCH₃), 2.07 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 2.02 (s, 3H, COCH₃), 2.00 (s, 3H, COCH₃), 1.92 (s, 3H, COCH₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.66, 170.65, 169.94, 169.90, 169.87, 169.82, 169.52, 169.36 (8C, 8 COCH₃), 137.11 (Ar), 128.89 (Ar), 128.16 (Ar × 2), 126.11 (Ar × 2), 101.40 (CHAr), 99.60 (C-1^{II}), 98.39 (C-1^{III}), 98.13 (C-1^I), 78.72 (C-4^I), 78.23 (OCH₂C≡CH), 77.25 (C-3^{III}), 75.53 (OCH₂C≡CH), 72.31 (C-4^{II}), 69.40 (C-2^{II}), 69.26 (2C, C-4^{III}, C-5^I), 69.23 (C-5^{III}), 68.80 (C-5^{II}), 68.62 (C-2^{III}), 68.38 (C-6^I), 66.45 (C-3^{III}), 64.49 (C-3^I), 62.66 (C-6^{II}), 62.60 (C-6^{III}), 54.67 (OCH₂C≡CH), 20.92 (COCH₃), 20.84 (COCH₃), 20.80 (2C, 2 COCH₃), 20.78 (2C, 2 COCH₃), 20.75 (COCH₃), 20.67 (COCH₃). MALDI-TOFMS: calcd for C₄₄H₅₆O₂₄Na [M+Na]⁺ m/z 989.2903; found m/z 989.2720.

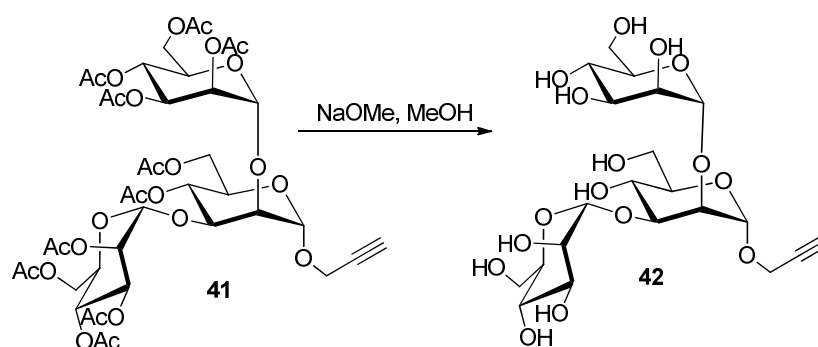
Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (39**)**



Following general procedure 7, the benzylidene group on compound **39** (612 mg, 0.632 mmol) was removed to afford an intermediate diol that was acetylated using general procedure 1 to afford trisaccharide **41**. Yield: 433 mg, 71% over two steps. $[\alpha]_D = +33.33^\circ$ ($c = 1$ in CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.42–5.33 (m, 2H), 5.32–5.18 (m, 5H), 5.12 (m, 1H, H-2), 5.08 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.96 (d, $J_{1,2} = 1.4$ Hz, 1H, H-1), 4.30–4.14 (m, 7H), 4.13–4.00 (m, 5H), 3.84 (ddd, $J = 10.01, 3.87, 2.45$ Hz, 1H), 2.47 (t, $J = 2.4$ Hz, 1H, OCH₂C $\equiv$ CH), 2.14 (s, 3H, COCH₃), 2.12 (s, 3H, COCH₃), 2.11 (s, 3H, COCH₃), 2.10 (s, 3H, COCH₃), 2.08 (s, 3H, COCH₃), 2.06 (s, 2H, COCH₃), 2.04 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 1.98 (s, 3H, COCH₃), 1.96 (s, 3H, COCH₃). ¹³C NMR (126 MHz, CDCl₃): δ 171.03, 170.61, 170.50, 170.15, 170.10, 169.87, 169.70, 169.55, 169.39, 169.31 (10C, COCH₃), 99.47 (C-1), 99.34 (C-1), 96.78 (C-1), 78.48, 78.23 (OCH₂C $\equiv$ CH), 77.36, 75.71 (OCH₂C $\equiv$ CH), 69.76 (2C), 69.63, 69.46, 69.35, 68.73, 68.71, 66.69, 66.46, 66.21, 62.95, 62.50, 62.22, 55.07 (OCH₂C $\equiv$ CH), 20.95, 20.90, 20.85,

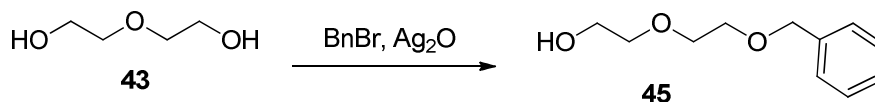
20.85, 20.82, 20.80, 20.78, 20.74, 20.72, 20.69 (10C, 10 COCH₃). MALDI-TOFMS: calcd for C₄₁H₅₄O₂₆Na [M+Na]⁺ *m/z* 985.2801; *m/z* found 985.2695.

Propargyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (42**)**



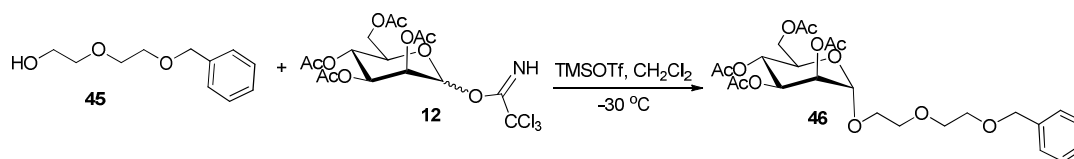
Global deprotection of acetylated trisaccharide **41** was achieved following the general procedure 2 on a 316 mg (0.328 mmol) scale. Yield: 143 mg, 80%. [α]_D = +57.52° (*c* = 1 in MeOH). ¹H NMR (500 MHz, CD₃OD): δ 5.19 (d, *J*_{1,2} = 1.7 Hz, 1H, H-1), 5.09 (m, 2H, H-1, H-1), 4.32–4.22 (m, 2H, OCH₂C $\equiv$ CH), 4.05 (dd, *J*_{2,3} = 3.1 Hz, *J*_{1,2} = 1.8 Hz, 1H, H-2), 3.97 (dd, *J*_{2,3} = 3.2 Hz, *J*_{1,2} = 1.8 Hz, 1H, H-2), 3.93 (dd, *J*_{3,4} = 9.6 Hz, *J*_{2,3} = 3.1 Hz, 1H, H-3), 3.89 (dd, *J*_{2,3} = 3.2 Hz, *J*_{1,2} = 1.8 Hz, 1H, H-3), 3.87–3.80 (m, 3H), 3.78–3.62 (m, 10H), 3.57–3.52 (m, 1H), 2.85 (t, *J* = 2.4 Hz, 1H, OCH₂C $\equiv$ CH). ¹³C NMR (126 MHz, CD₃OD): δ 104.10 (C-1), 103.52 (C-1), 98.71 (C-1), 80.03, 79.46, 79.19, 76.12, 75.35, 75.30, 75.03, 72.52, 72.37, 72.07, 72.01, 68.73, 68.55, 68.25, 62.97, 62.89, 62.72, 55.18 (OCH₂C $\equiv$ CH). MALDI-TOFMS: calcd for C₂₁H₃₄O₁₆Na [M+Na]⁺ *m/z* 565.1745; found *m/z* 565.1801.

2-[2-(Benzyloxy)ethoxy]ethanol (**45**)⁸⁸



To a mixture of diethylene glycol (**43**, 4.50 mL, 47.1 mmol) in CH₂Cl₂ (10 mL) was added Ag₂O (16.3 g, 70.7 mmol) and BnBr (6.15 mL, 51.7 mmol). The solution was stirred at room temperature for 14 h and filtered through a silica gel pad. The mixture was concentrated and flash column chromatography (1:1 hexane–EtOAc) gave pure **45** as a colorless oil (7.65 g, 83%). ¹H NMR (300 MHz, CDCl₃): δ 7.39–7.23 (m, 5H, Ar), 4.55 (s, 2H, CH₂Ar), 3.73–3.64 (m, 4H, OCH₂CH₂O), 3.63–3.55 (m, 4H, OCH₂CH₂O), 3.04 (t, *J* = 6.0 Hz, 1H, OH). ¹³C NMR (75 MHz, CDCl₃): δ 137.81 (Ar), 128.29 (Ar × 2), 127.69 (Ar × 2), 127.59 (Ar), 73.11 (CH₂Ar), 72.46 (OCH₂CH₂OBn), 70.21 (HOCH₂CH₂O), 69.27 (OCH₂CH₂OBn), 61.46 (HOCH₂CH₂O). Spectroscopic data matched that previously reported.⁸⁸

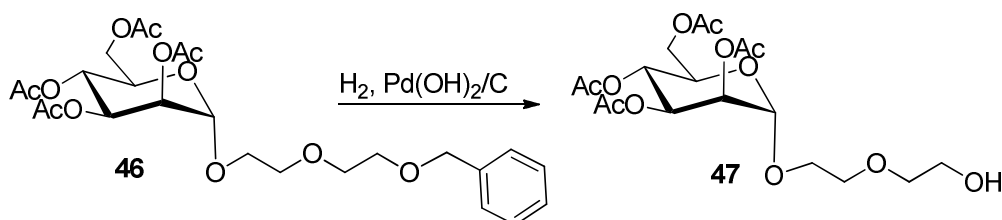
2-[2-(Benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**46**)



To a mixture of compound **45** (1.0 g, 5.1 mmol) and trichloroacetimidate donor **12** (3.7 g, 7.6 mmol) in CH₂Cl₂ (10 mL) was added powdered 4-Å molecular sieves (300 mg), and the mixture stirred at room temperature for 30 min under an N₂ atmosphere. The solution was cooled to -30 °C and TMSOTf (92 μL, 0.51 mmol) was added. After TLC showed

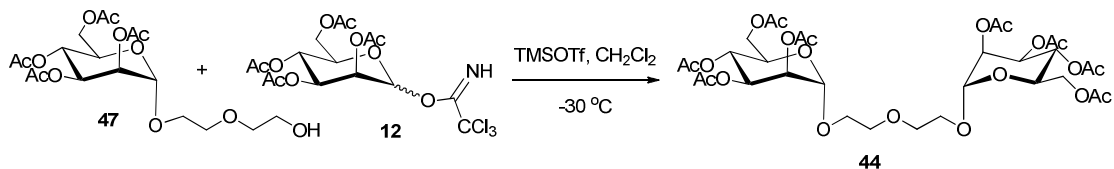
total consumption of the acceptor, Et₃N (1 mL) was added to quench the reaction. The mixture was diluted with CH₂Cl₂, filtered over a pad of Celite and concentrated. Column chromatography on silica gel (1:1 hexane–EtOAc) afforded pure **46** as a slightly brown oil (1.05 g, 39%). [α]_D = +33.53 ° (*c* = 1.0, CHCl₃). ¹H NMR (300 MHz, CDCl₃): δ 7.42–7.28 (m, 5H, Ar), 5.37 (m, 1H, H-3), 5.29 (m, 2H, H-4, H-2), 4.89 (d, *J* = 1.7 Hz, 1H, H-1), 4.56 (m, 2H, CH₂Ar), 4.31 (dd, *J*_{6a,6b} = 12.8 Hz, *J*_{5,6} = 5.4 Hz, 1H, H-6a), 4.15–4.05 (m, 2H, H-5, H-6b), 3.91–3.77 (m, 1H, CHOCH_{2a}CH₂O), 3.72–3.66 (m, 5H, CHOCH_{2b}CH₂O, OCH₂CH₂OBn, CHOCH₂CH₂O), 3.65–3.60 (m, 2H, OCH₂CH₂OBn), 2.15 (s, 3H, COCH₃), 2.11 (s, 3H, COCH₃), 2.01 (s, 3H, COCH₃), 2.00 (s, 3H, COCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.80, 170.14, 169.99, 169.83 (4C, 4 COCH₃), 138.24 (Ar), 128.46 (Ar × 2), 127.81 (Ar × 2), 127.69 (Ar), 97.78 (C-1), 73.32 (CH₂Ar), 70.85 (OCH₂CH₂OBn), 70.10 (CHOCH₂CH₂O), 69.61 (C-2), 69.48 (OCH₂CH₂OBn), 69.15 (C-3), 68.41 (C-5), 67.48 (CHOCH₂CH₂O), 66.12 (C-4), 62.44 (C-6), 21.02, 20.88, 20.81, 20.79 (4C, 4 COCH₃). MALDI-TOFMS: calcd for C₂₅H₃₄O₁₂Na [M+Na]⁺ *m/z* 549.1948; found *m/z* 549.1961.

2-(2-Hydroxyethoxy)ethyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**47**)⁸⁹



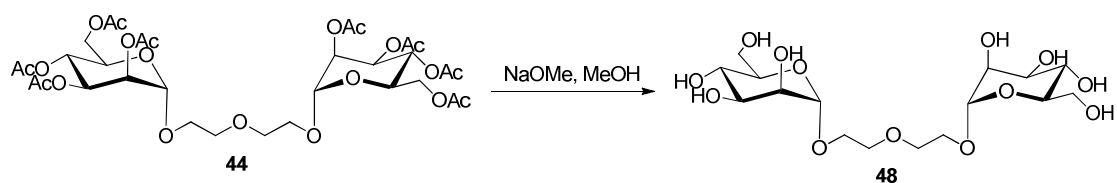
The monosaccharide **46** (800 mg, 1.52 mmol) was dissolved in 1:1 EtOAc–EtOH (120 mL) and 20% $\text{Pd}(\text{OH})_2$ (80 mg) was added. The mixture was stirred for 8 h under an H_2 atmosphere. Upon completion of reaction, the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and purified by column chromatography on silica gel (2:2:1 hexane–EtOAc–DCM) to give **47** as a colorless oil (530 mg, 80%). $[\alpha]_{\text{D}} = +5.33^\circ$ ($c = 1.0$, CHCl_3). ^1H NMR (300 MHz, CDCl_3): δ 5.31 (dd, $J_{3,4} = 10.0$ Hz, $J_{2,3} = 3.4$ Hz, 1H, H-3), 5.27–5.16 (m, 2H, H-4, H-2), 4.86 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 4.21 (dd, $J_{6a,6b} = 12.3$ Hz, $J_{5,6a} = 5.5$ Hz, 1H, H-6a), 4.10–3.98 (m, 2H, H-5, H-6b), 3.79–3.57 (m, 7H, $\text{CHOCH}_2\text{CH}_2\text{O}$, $\text{CHOCH}_2\text{CH}_2\text{O}$, $\text{OCH}_2\text{CH}_2\text{OH}$, $\text{OCH}_2\text{CH}_2\text{OH}$), 3.56–3.50 (m, 2H, $\text{OCH}_2\text{CH}_2\text{OH}$), 2.10 (s, 3H, COCH_3), 2.04 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.94 (s, 3H, COCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 170.95, 170.38, 170.12, 169.95 (4C, 4 COCH_3), 97.67 (C-1), 72.80 ($\text{CHOCH}_2\text{CH}_2\text{O}$), 70.25 ($\text{OCH}_2\text{CH}_2\text{OH}$), 69.82 (C-2), 69.06 (C-3), 68.50 (C-5), 67.39 ($\text{CHOCH}_2\text{CH}_2\text{O}$), 66.38 (C-4), 62.74 (C-6), 61.98 ($\text{OCH}_2\text{CH}_2\text{OH}$), 21.09, 20.93, 20.88, 20.87 (4C, 4 COCH_3). HRDARTMS: calcd for $\text{C}_{18}\text{H}_{28}\text{O}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 459.1478, found m/z 459.1520.

2-(2-[(2,3,4,6-Tetra-O-acetyl- α -D-mannopyranosyl)oxy]ethoxy)ethyl 2,3,4,6-tetra-O-acetyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (44**)**



To a mixture of compound **47** (500 mg, 1.15 mmol) and donor **12** (1.13 g, 2.30 mmol) in CH_2Cl_2 (13 mL) was added powdered 4-Å molecular sieves (500 mg), and the mixture was stirred at room temperature for 30 min under an N_2 atmosphere. The solution was cooled to $-30\text{ }^\circ\text{C}$ and TMSOTf (20 μL , 0.11 mmol) was added. After stirring for 2 h, Et_3N (0.3 mL) was added to quench the reaction. The mixture was diluted with CH_2Cl_2 , filtered over a pad of Celite and concentrated. Column chromatography on silica gel (1:2 hexane– EtOAc) afforded pure **44** as a clear oil (358 mg, 41%). $[\alpha]_{\text{D}} = +20.57^\circ$ ($c = 1.0$, CHCl_3). ^1H NMR (600 MHz, CDCl_3): δ 5.32 (dd, $J_{3,4} = 10.0$ Hz, $J_{2,3} = 3.4$ Hz, 1H, H-3), 5.29–5.22 (m, 2H, H-4, H-2), 4.85 (d, $J_{1,2} = 1.56$ Hz, 1H, H-1), 4.26 (dd, $J_{6a,6b} = 12.3$ Hz, $J_{5,6} = 5.1$ Hz, 1H, H-6a), 4.11–4.05 (m, 1H, H-6b), 4.04–3.97 (m, 1H, H-5), 3.82–3.75 (m, 1H, $\text{CHOCH}_2a\text{CH}_2\text{O}$), 3.69–3.61 (m, 3H, $\text{CHOCH}_2b\text{CH}_2\text{O}$, $\text{CHOCH}_2\text{CH}_2\text{O}$), 2.14, 2.07, 2.01, 1.97 (4s, 12H, 4 COCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 170.77, 170.10, 169.99, 169.84 (4C, 4 COCH_3), 97.86 (C-1), 70.24 ($\text{CHOCH}_2\text{CH}_2\text{O}$), 69.57 (C-2), 69.11 (C-3), 68.50 (C-5), 67.52 ($\text{CHOCH}_2\text{CH}_2\text{O}$), 66.17 (C-4), 62.46 (C-6), 21.00, 20.87, 20.81, 20.80 (4C, 4 COCH_3). MALDI-TOFMS: calcd for $\text{C}_{32}\text{H}_{46}\text{O}_{21}\text{Na}$ $[\text{M}+\text{Na}]^+$ m/z 789.2493; found m/z 789.2521.

2-[2-(α -D-Mannopyranosyloxy)ethoxy]ethyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (48**)**



Following the general procedure 1, the deacetylated disaccharide **48** was synthesized on a 300 mg (0.39 mmol) scale. Purification on LH-20 Sephadex column with H₂O and MeOH as eluents gave 124 mg (74%) of **48** as a colorless oil. $[\alpha]_D = +26.57^\circ$ ($c = 1.0$, CHCl₃). ¹H NMR (300 MHz, CD₃OD): δ 4.77 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1), 3.84–3.75 (m, 3H, H-3, H-4, H-2), 3.71–3.59 (m, 4H, H-6a, H-6b, CHOCH₂CH₂O), 3.57–3.51 (m, 2H, CHOCH₂CH₂O). ¹³C NMR (75 MHz, CD₃OD): δ 101.71 (C-1), 74.64 (C-5), 72.47 (C-3), 72.09 (C-2), 71.42 (CHOCH₂CH₂O), 68.60 (C-4), 67.67 (CHOCH₂CH₂O), 62.92 (C-6).

PART TWO

PRELIMINARY MICROARRAY ANALYSIS OF INTERACTIONS BETWEEN

MANNOSE DERIVATIVES AND CONCAVALIN A

VI. Carbohydrate–Protein Binding Studies

1. Characterization of carbohydrate–protein interactions

Carbohydrate–protein interactions play a central role in a wide range of biological processes such as cell–cell recognition, viral and bacterial pathogenesis, and inflammation.⁹⁶ An understanding of carbohydrate–protein interactions at the molecular level would lead to better insight into biological processes of living systems and assist in the development of therapeutic and diagnostic strategies.⁵⁹ Despite the ubiquity and importance of carbohydrates in biology, difficulties in the study of carbohydrate–protein interactions have hindered the development of a mechanistic understanding of carbohydrate structure and function.⁵⁹

Investigation of carbohydrate–protein interactions can be approached from two angles. One is from the side of the proteins, for which currently available molecular biological approaches are extremely useful. For instance, a carbohydrate–binding protein can be cloned and overexpressed, so that it can be crystallized for X-ray crystallographic studies. Site-directed mutagenesis can provide mutants, which are valuable for understanding the mode of interaction between carbohydrates and lectins.⁹⁷ In the other approach, which mostly depends on synthetic organic chemistry, carbohydrates are manipulated to bring about structural changes that facilitate the understanding of binding specificity and other fundamental properties of carbohydrate–protein interactions.⁹⁸

However, the structural complexity of carbohydrates is a major obstacle, and the binding affinities are typically weak. Different techniques have been used to characterize

carbohydrate–protein interactions. One common method involves measuring the binding of lectins to cells, tissues and glycoproteins.³² This approach frequently uncovers interesting and useful binding properties; however, cells, tissues, and glycoproteins display complex mixtures of carbohydrate epitopes. Therefore, it is exceedingly difficult to determine the specific carbohydrate structures being recognized by a particular lectin. An alternative approach involves measuring binding to structurally defined carbohydrate epitopes through techniques such as isothermal titration calorimetry (ITC), mono- and oligosaccharide inhibition studies, enzyme-linked lectin assays (ELLA) and surface plasmon resonance assays (SPR). Unfortunately, these methods can be labor intensive, require large amounts of carbohydrates, and/or can be difficult to perform in a high-throughput fashion.⁹⁶ Moreover, these studies have typically been limited to the small number of carbohydrate epitopes that were readily available. Another disadvantage with these methods is that they do not use platforms that can mimic the cell surface, and therefore do not take advantage of multivalency.

For the enthalpy gain during receptor–ligand complexation, a number of criteria must be taken into account, such as dipole–dipole interactions, dispersive forces (London forces), and specific forces such as hydrogen bonding. In aqueous solution, however, the loss of favorable interactions of both ligand and receptor with solvent has to be overcompensated in the binding event because the binding of two solutes necessarily involves the loss of favorable interactions with the solvent. Consequently, binding is largely driven by hydrophobic effects. This and the quite shallow binding sites of lectins

account for the weak binding affinities found in carbohydrate recognition. The presentation of carbohydrates in an array provides a method to simultaneously monitor multiple binding events and the effects of multivalency (discussed in part one) which increases binding affinities.

1.1. Carbohydrate Microarrays

Microarrays are miniaturized assemblies of molecules organized across a planar surface.⁹⁹ Microarray technologies are novel tools emerging at the frontier of glycomics that are revolutionizing studies of carbohydrate–protein interactions and the elucidation of carbohydrate ligands involved not only in endogenous receptor systems but also pathogen–host interactions.¹⁰⁰ Array technology is particularly important in glycomics because it can dramatically increase the output of biochemical data, and glycoarrays (arrays displaying carbohydrates) have found several applications (Figure 19).¹⁰¹

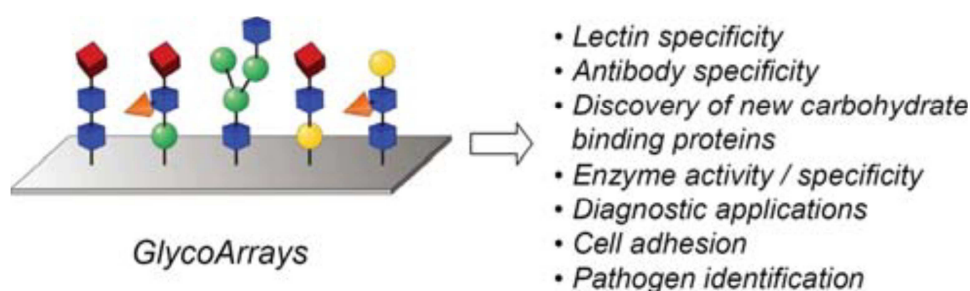


Figure 19. Current applications for glycoarrays.¹⁰¹

Analogous to DNA and protein arrays, carbohydrate microarrays contain numerous carbohydrate epitopes immobilized on a solid support in a miniaturized

fashion. Glycans attached to surfaces resemble those on cell surfaces, and consequently, they may interact with proteins in a similar way to that when they are located on cell surfaces.¹⁰² The microarray format allows one to rapidly evaluate many potential interactions with a minimum amount of sample.⁹⁶ Not only do arrays shorten the time for biochemical measurements, but glycoarrays also have the advantage of using less precious carbohydrate material because of the option of miniaturization that is not possible in solution studies.¹⁰¹ Another advantage of glycan microarray analysis is the high detection sensitivity. This is attributed to the fact that the binding of a molecule in the solution phase to an immobilized microspot of ligand in the solid phase has minimal reduction of the molar concentration of the molecule in solution.¹⁰³ Therefore, in a microarray assay it is much easier to have a binding equilibrium take place and result in a high sensitivity.¹

Generally, carbohydrate microarray methods fall into two categories: polysaccharide and oligosaccharide microarrays. Polysaccharides from natural sources can be readily and randomly immobilized on solid matrices based on hydrophobic physical absorption^{104,105} or charge-based interaction¹⁰⁶ to generate polysaccharide microarrays that are valuable for comparable antigenicity analyses.¹⁰⁰ On the other hand, oligosaccharide microarrays provide detailed information on structure–activity relationships in carbohydrate recognition events. The immobilization of oligosaccharides is, however, more challenging due to their hydrophilic nature, and chemical derivatization procedures are normally required before arraying.

Various immobilization strategies have been developed to fabricate carbohydrate microarrays. These can be divided into two main classes, depending on the type of sugar immobilization: by covalent attachment or by physical adsorption. Stable carbohydrate microarrays should be fabricated by covalent immobilization, which can be achieved by Michael addition,¹⁰⁷ Diels–Alder reaction,¹⁰⁸ Staudinger ligation,¹⁰⁹ click chemistry,¹¹⁰ Schiff base formation,^{111,112} and amide bond formation.¹¹³ Although covalent immobilization offers very stable arrays, the main drawback of this strategy is the need for the chemical modification of the sugar to introduce a linker, thus allowing its reaction with the surface.¹⁰¹ This modification is usually done by chemical manipulation, requiring complex multistep protection–glycosylation–deprotection sequences, with the need for often difficult purification. While this might not appear to be a major limitation, in the case of “simple” sugars (monosaccharides), it is a major challenge for glycoarrays of complex natural oligosaccharides as these are often available in tiny quantities (see Part One of this dissertation for the synthesis of sugars).^{100,101}

Our approach for this project will utilize covalent immobilization of thiol-terminated sugars onto epoxide-functionalized glass slides. This will afford very stable glycan microarrays for interrogation with proteins.

2. Results and Discussion

Previously, Medhanit Bahta of our group, in collaboration with Dr. Robert Standaert of the Oak Ridge National Laboratory, assessed the binding activities of self-assembled

monolayers of some carbohydrates with human serum mannose-binding lectins.⁷⁵ The carbohydrates used included a thiol-terminated monosaccharide (previously synthesized by Brian Sanders) and a Kamerling-derivatized 1,3- α -D-mannobiose. The approach used was similar to that developed by Mrksich and co-workers, whereby maleimide-functionalized glass surfaces were arrayed with thiol-terminated mannose derivatives and incubated with mannose-binding lectins.¹⁰⁸ Although the overall avidities of mannose-binding lectins were determined, the binding constants were not.

Herein, we describe the utility of microarray analysis for characterizing carbohydrate–protein interactions and the determination of dissociation constants. Microarray detection is invaluable as it allows for the simultaneous analysis of binding under numerous conditions on a single slide. Synthesized mannose derivatives were printed on epoxide-functionalized glass slides. On a single glass slide, 12 different concentrations (0, 25, 50, 100, 150, 200, 250, 300, 400, 500, 750, 1000 μ M) of each sugar were probed with 6 different concentrations of protein Con A (2, 5, 10, 20, 30, 50 μ g/mL, which corresponds to 19, 48, 96, 192, 288 and 481 nM, respectively). The sugars selected for the preliminary studies included the thiol-terminated mannose derivatives shown in Figure 20. (The synthesis of these compounds can be found in Part One of this dissertation.)

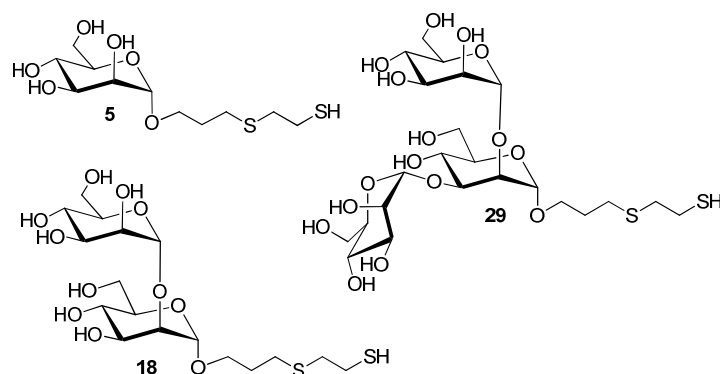


Figure 20. Thiol-terminated mannose derivatives 5, 18, and 29 used in binding studies.

These sugars were selected since they have been derivatized with thiols and will readily react with epoxide-functionalized glass slides to form covalent bonds when immobilized. Also, these sugars have hydrophobic aglycons that have been reported to promote the recognition of sugars by lectins.¹¹⁴ These hydrophobic aglycons may be a reason for the favorable change in entropy or favorable interactions within the binding site of Con A. The thiol-terminated sugars are deprotonated in a basic print buffer (PBS, pH 8.5), and the resultant anion cleaves open the epoxides on the glass surface, hence forming covalent bonds (Figure 21).

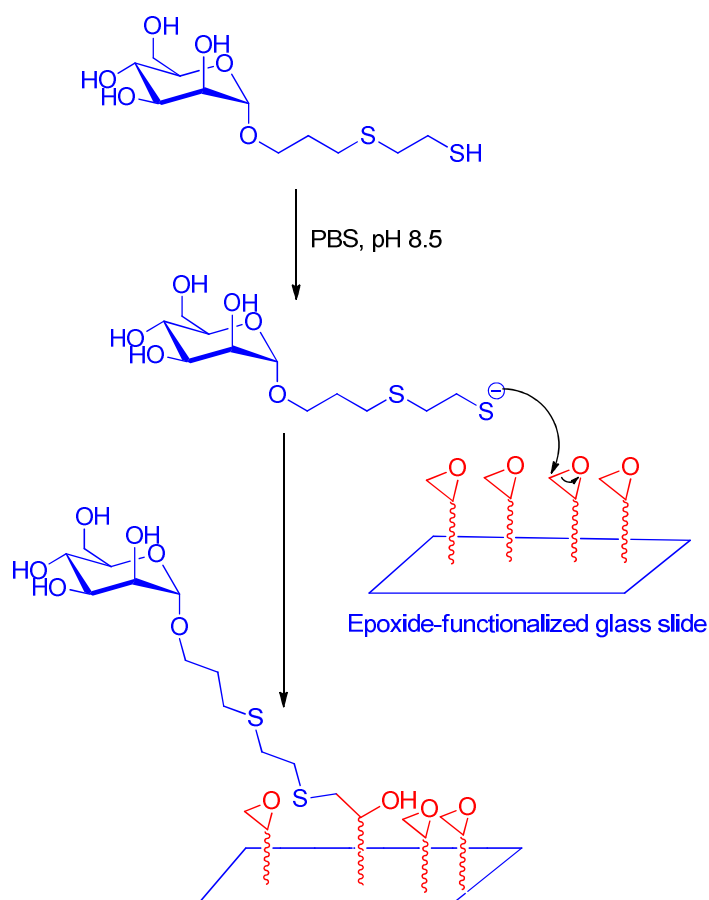


Figure 21. Covalent bond formation between a tethered sugar and the glass surface.

Using robotic printing technology, thiol-terminated mannose derivatives **5**, **18** and **29** were printed onto epoxide-functionalized glass slides (Figure 22) to achieve covalent immobilization. The pin on the robotic printer has a 0.25 μL sample channel and a tip size of 75 μm . It prints sugar spots of ~ 100 μm in diameter. Initial immobilization of thiol-terminated sugars was not very successful based on results obtained after protein

incubation. This could be attributed to the oxidation of thiol terminals of the sugars when exposed to air.

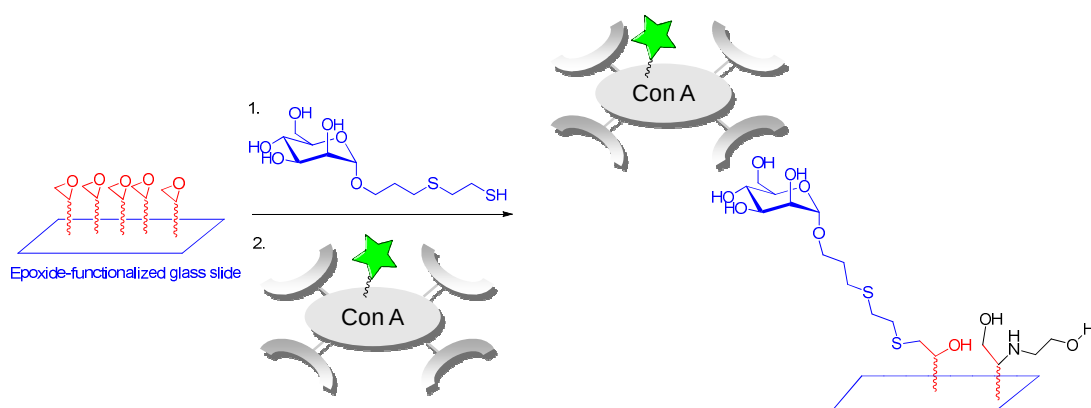
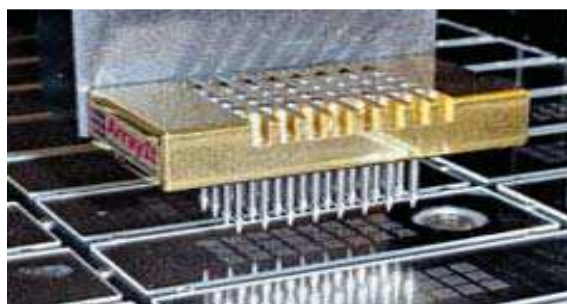
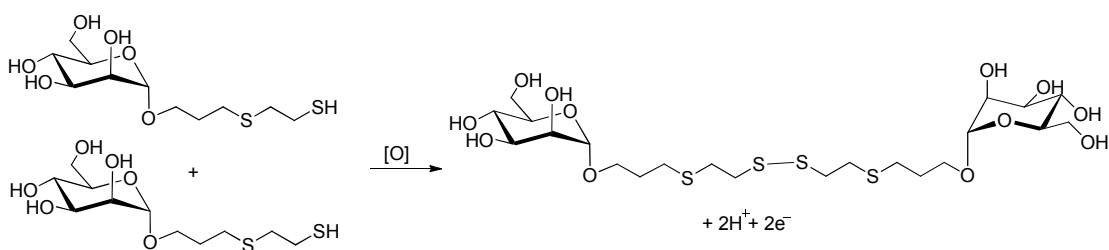


Figure 22. Top: Picture of a pin-printer. Bottom: Carbohydrate microarray fabrication and analysis on glass slides

Thiols have a tendency to oxidize and form disulfide bonds when exposed to air (Scheme 35). It was observed that formation of covalent bonds between sugars and the epoxide-derivatized surface was minimal when the sugars oxidized and became linked by disulfide bonds. Therefore, the reducing agent *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) was added to sugar solutions to cleave the disulfide bonds to



Scheme 35. Disulfide bond formation.

thiols before printing. The results showed better immobilization of sugars onto the glass surface with the addition of TCEP. After overnight incubation, the microarrays were thoroughly washed with wash buffer (PBS, pH 7.4 containing 0.05% Tween-20) to remove unbound carbohydrate. To prevent a non-specific reaction between the glass surface and protein, it was necessary to block the unreacted epoxide functional groups on the glass surface. Initial blocking with Superblock[®] solution was not very successful, as this showed too much background fluorescence as a result of the reaction between the protein and the epoxides. Ethanolamine block buffer showed better results and was used for all subsequent assays. After blocking for 1 h, the slides were washed and dried, and the supergrids were mapped out using a permanent-ink marker. Carbohydrate microarrays were incubated with fluorescent-tagged lectin (lissamine rhodamine-tagged Concanavalin A) for 1 h at room temperature. After protein incubation, the slides were washed to remove unbound protein, followed by detection of bound protein through fluorescence slide scanning.

A representative example of a fluorescence scan resulting from our microarray studies is depicted in Figure 22. In this analysis, sugars **5**, **18** and **29** were printed at 12

different concentrations ranging from 0–1000 μM (right to left) in 12×10 print patterns, which resulted in three separate grids that make up a supergrid. Each supergrid allows for the detection of protein binding as a function of the concentration of carbohydrate printed on the surface. In order to further study the effects of changing protein concentration, six supergrids were printed and subjected to six different concentrations of Con A. As depicted in the slide scan in Figure 21, each of the six supergrids (A, B, C, D, E, and F) was probed using a different concentration of lissamine rhodamine-tagged Con A ranging from 2–50 $\mu\text{g/mL}$. After incubation and thorough washing, the glass slides were scanned to obtain fluorescence intensities (Figure 21).

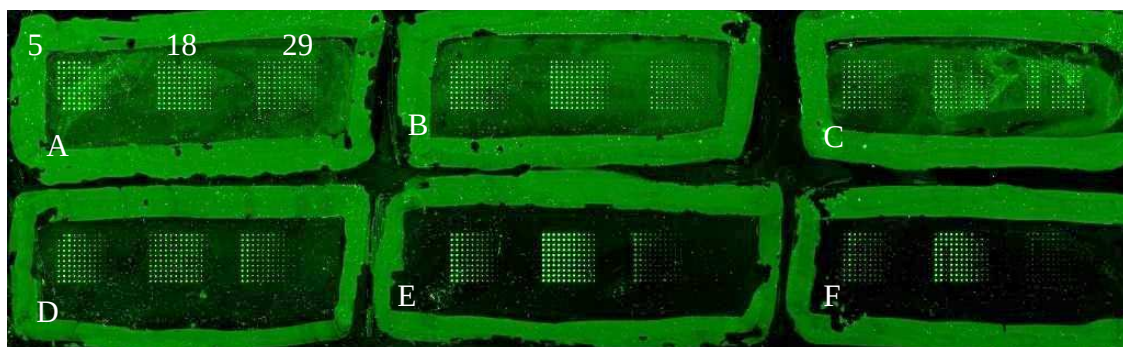


Figure 23. Scanned image of glycan microarray with Con A. Monosaccharide 5, disaccharide 18 and trisaccharide 29. Protein concentrations: 50 $\mu\text{g/mL}$ (A), 30 $\mu\text{g/mL}$ (B), 20 $\mu\text{g/mL}$ (C), 10 $\mu\text{g/mL}$ (D), 5 $\mu\text{g/mL}$ (E) and 2 $\mu\text{g/mL}$ (F).

To evaluate protein–surface binding, plots of fluorescence intensity versus both carbohydrate and protein concentration were constructed. The curves were treated as Langmuir isotherms assuming equilibrium was reached during incubation. The Langmuir isotherm relates the adsorption of proteins to the immobilized sugars on surface of glass

slide to the concentration of the protein solution (see Langmuir equation in section 4.2.3). In these studies, (1→2)-linked disaccharide **18** reproducibly showed the greatest fluorescence intensities, indicating enhanced binding with Con A (Figure 24). The trisaccharide **29** showed the least fluorescence intensities and hence was the weakest ligand for Con A. These results have important implications for understanding complex cell-surface glycan recognition by validating our hypothesis that sugars are recognized based on specific patterns of geometric presentation. The steric bulk of the trisaccharide **29** might have contributed to the poor binding to Con A that has a shallow binding pocket.

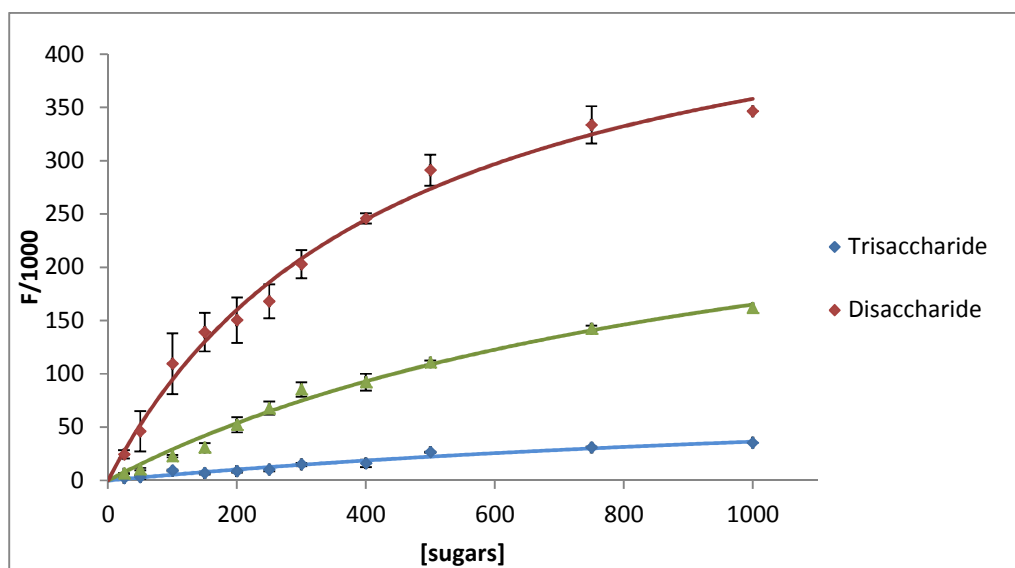


Figure 24. Binding curves for three sugars 5, 18 and 29 with Con A at a concentration of 50 $\mu\text{g/mL}$.

Although the printed concentrations of the disaccharide **18** varied by up to 40-fold from 25 to 1000 μM , the dissociation constants ($K_{\text{D,surf}}$) were narrowly distributed with an average of 61 nM (SD = 7 nM) from nine replicate experiments. The differences in dissociation constants seem to be greater at higher concentrations above 400 μM . The monosaccharide **5** had an average surface dissociation constant of 76 nM (SD = 5 nM), which is close to that reported in the literature (83 nM for the interaction of Con A and 100 μM mannose monosaccharide).⁵⁹ The trisaccharide **29** showed less binding than the other sugars **5** and **18** with a dissociation constant of 91 nM (SD = 4 nM). Generally, as the concentration of the sugars increased the fluorescence intensities observed increased, indicating that more protein was adsorbed to the surface. However, this was not necessarily the case when the protein concentrations were increased (Figure 25).

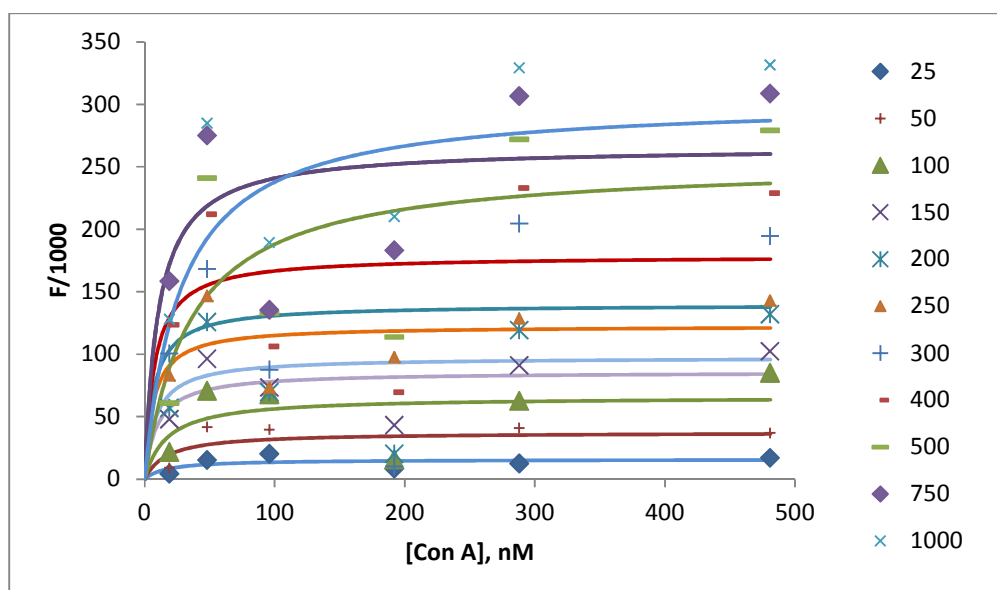


Figure 25. Binding curve for disaccharide 18 and Con A using different concentrations as shown. Errors not shown. Average deviation is ± 28 to that of Figure 24.

Binding curves for monosaccharide **5** and trisaccharide **29** also showed the same irregular change in fluorescence as the protein concentrations were increased (Figure 26). For the monosaccharide **5**, there was increased binding as the protein concentration increased from 2 to 10 $\mu\text{g/mL}$, but a decrease was observed at a protein concentration of 20 $\mu\text{g/mL}$. There was an increase in binding observed again at protein concentration of 30 $\mu\text{g/mL}$ and a small decrease at 50 $\mu\text{g/mL}$. For the trisaccharide **29**, there was a steady increase in binding from a protein concentration of 2–30 $\mu\text{g/mL}$. However, there was a small decrease in binding at 40 $\mu\text{g/mL}$ and no noticeable increase at 50 $\mu\text{g/mL}$. This might be attributed to the difference in surface coverage of each spot on the glass slide by sugars. However, in a previous study of by Wong and co-workers, it was shown that the

surface was saturated by the monosaccharide at concentrations above 100 μM .⁵⁹ Since most of our sugar concentrations were above 100 μM (except for 25 μM and 50 μM), we assumed a full coverage of surface for the monosaccharide. There was no way of measuring how much carbohydrate was attached on the surface of the slide for the different sugar concentrations. An accurate measure of surface coverage of carbohydrates on each spot is not available by the current techniques.

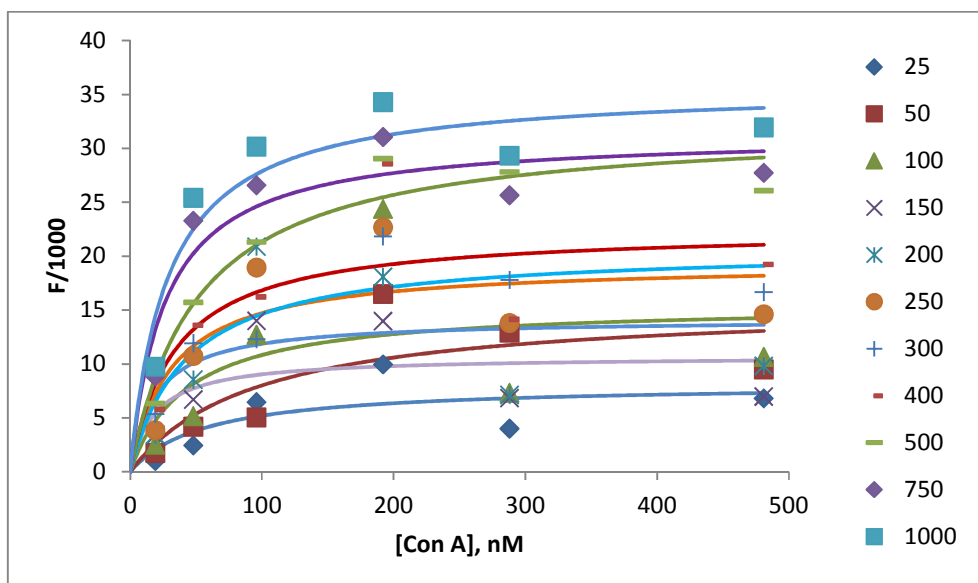
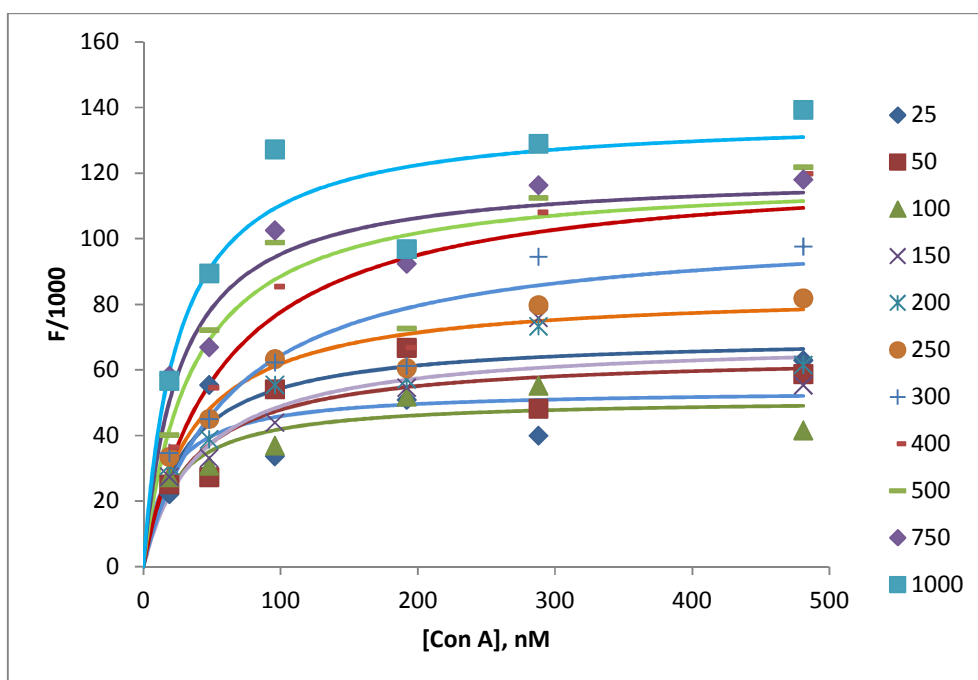


Figure 26. Top: Binding curves for monosaccharide 5. Errors not shown. Average deviation is ± 3.4 to that shown in Figure 24. Bottom: Binding curves for trisaccharide 29. Errors not shown. Average deviation is ± 8 to that shown in Figure 24.

To probe into this problem, we ran multiple binding studies of sugars (disaccharide **15** was chosen for further studies) on the same slide instead of comparing results from different slides. So, for each sugar concentration, 30 spots were generated and tested with the same concentration of protein. Figure 27 below shows the scanned results of the disaccharide investigated with six different concentrations of Con A. There was a gradual increase in fluorescence intensity between disaccharide **18** and Con A as the concentration of Con A increased.

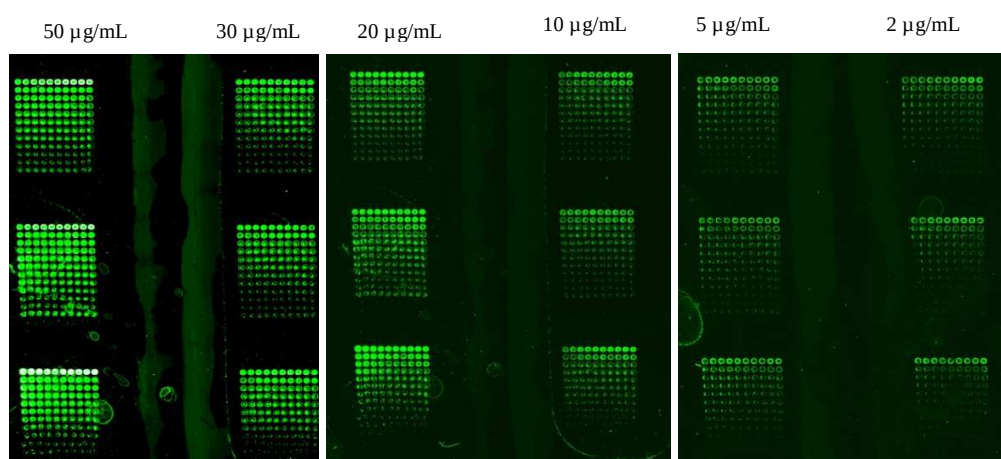


Figure 27. Scanned image of binding interaction between disaccharide **18 and six concentrations of Con A.**

The plotted values showed that there was an increase in fluorescence as protein concentration was increased (Figure 28). However, there was a slight decrease in binding at higher sugar concentrations of 750 μM and 1000 μM with a protein concentration at 30 $\mu\text{g/mL}$. Generally these results were much better than those previously obtained (Figure 25). The average binding constant (K_D) from these experiments for the disaccharide **15** was 58 nM (SD = 3.6 nM), which was not very different from 61 nM obtained previously.

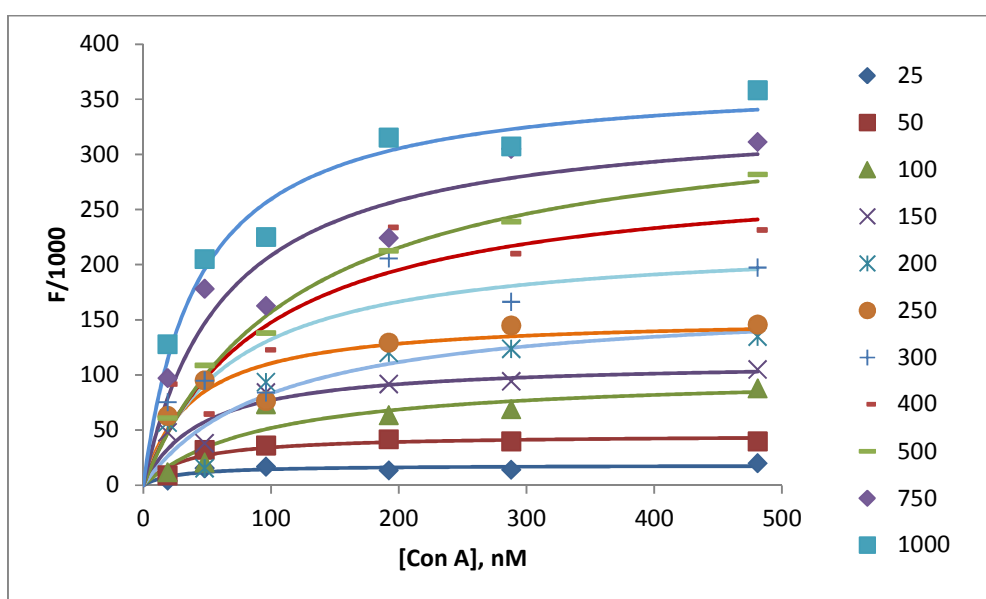


Figure 28. Binding curve for disaccharide 18 and Con A printed at different concentrations as shown. Errors not shown. Average deviation of ± 17 from that shown in Figure 24.

It was intriguing to us that the disaccharide showed better binding than the trisaccharide. This might be attributed to the trisaccharide being bulkier than the disaccharide and also due to the fact that the carbohydrate binding site of Con A is very shallow. Since the disaccharide **18** was (1→2)-linked and the trisaccharide **29** was (1→2)- and (1→3)-linked, we wanted to investigate if the (1→3)-linkage in the trisaccharide was decreasing the binding effect with protein. Therefore, the (1→3)-linked disaccharide **24** was synthesized, (For the synthesis of **24**, see Part One of this dissertation.) and its binding properties with Con A were determined by microarray analysis (Figure 29).

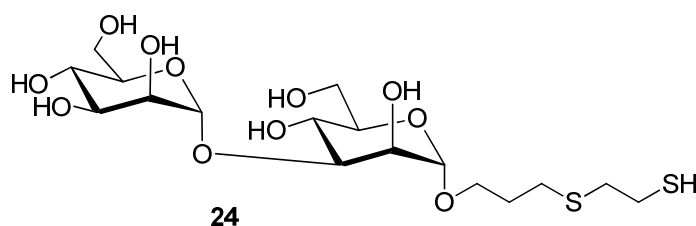


Figure 29. (1→3)-Linked thiol-terminated disaccharide 24.

The result was that the (1→3)-linked disaccharide **24** showed less binding with protein ($K_D = 68$ nM) than the (1→2)-linked disaccharide **18** (58 nM). This shows the importance of the point of connection between sugars (i.e., the sugar linkages). The (1→2)-linkage for the disaccharide construction showed enhanced binding over that of a (1→3)-linkage. The 2-position on mannose is axial, while the 3-position is equatorial; therefore, the orientations of the disaccharides **15** and **24** are very different (Figure 30).

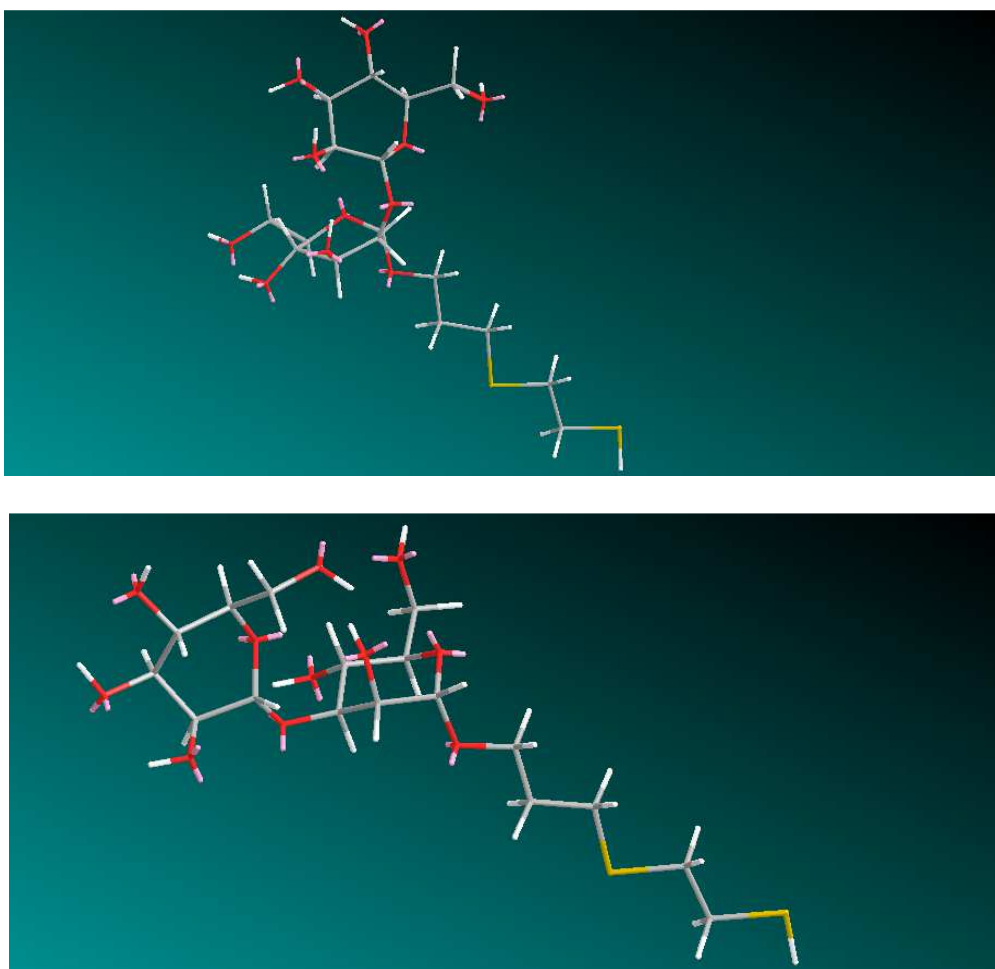


Figure 30. Three-dimensional models of disaccharides; top: (1→2)-linked disaccharide **15; bottom: (1→3)-linked disaccharide **24**. (Structures were calculated using Chem3D Pro version 12.0.2.1076).**

The 3-OH and 4-OH required for binding with Con A are well exposed in both the reducing and non-reducing sugar units of the (1→2)-linked disaccharide **15** which might have led to multivalent binding. Contrarily, only the 3-OH and 4-OH of the non-reducing sugar unit of the (1→3)-linked disaccharide **24** is exposed for binding with Con A. It is noteworthy that the (1→3)-linked disaccharide **24** also showed better binding

with Con A than the trisaccharide **29**. It has been shown before that when lectins bind disaccharides, the non-reducing residue occupies the carbohydrate binding site.⁴³ Since the trisaccharide **29** is more sterically hindered than the disaccharide, it is therefore reasonable that both the (1→2)-linked disaccharide **18** and the (1→3)-linked disaccharide **24** showed better binding to Con A. Figure 31 shows the scanned image of the binding experiment between disaccharide **24** and Con A at 30 $\mu\text{g/mL}$.

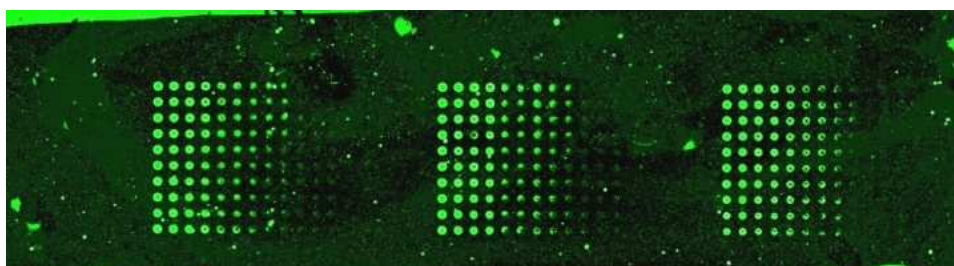


Figure 31. Scanned image of binding between (1→3)-linked disaccharide **24 and 30 $\mu\text{g/mL}$ of Con A.**

The binding was much lower at sugar concentrations below 150 μM as evident in the scanned image (Figure 31). However, from a sugar concentration of 150 μM , there was a linear increase in the binding with Con A as the sugar concentration increased. The highest binding was observed at a sugar concentration of 1000 μM and a Con A concentration of 50 $\mu\text{g/mL}$ as expected. The binding between disaccharide **24** and Con A were quite similar at high sugar concentration of 750 μM and 1000 μM when Con A was used at concentrations of 20 and 30 $\mu\text{g/mL}$.

3. Conclusions and future work

We successfully used microarray techniques to immobilize four synthesized thiol-terminated derivatives on epoxide-functionalized glass slides. Reduction of background signal from non-specific binding between protein and glass slide was the most challenging step in the microarray analysis. Using ethanolamine buffer proved to be the best block solution. The sugars used included monosaccharide **5**, (1→2)-linked disaccharide **18**, (1→3)-linked disaccharide **24** and trisaccharide **29**. Glycan arrays were probed with Con A at six different concentrations (2, 5, 10, 20, 30 and 50 µg/mL). The disaccharides **18** and **24** showed better binding with Con A with dissociation constants of 58 nM and 68 nM, respectively. This was attributed to the availability of two binding sites on the (1→2)-linked disaccharide **18** as opposed to only one binding site on the (1→3)-linked disaccharide **24**. The (1→2)-linkage is necessary for increased binding with Con A. Coverage of the surface by sugars has a direct effect on the binding with Con A. However, the current technique does not give a measure of how much sugar is immobilized on each spot. Therefore, future work will include controlling the binding site density of the ligand arrays using synthesized nodes for direct attachment onto glass surfaces. These nodes will be synthesized to have one, two, and three branches that can react with sugars (Figure 33). Higher multiplicities will be generated by linking base nodes together with linkers of varying lengths.

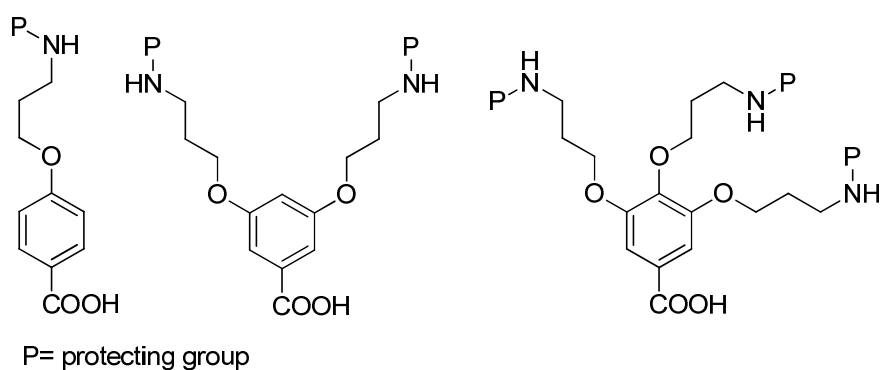


Figure 32. Chemical structures and symbolic representation of node structures.

In addition, alkyne-derivatized galactosides are currently being synthesized in the Baker Laboratory by Bo Meng and Andrew Moss (Figure 34). Glycan microarrays will be constructed with these galactose derivatives and their binding properties with mannose-binding lectins (control) and galactose-binding protein (GBP) will be determined.

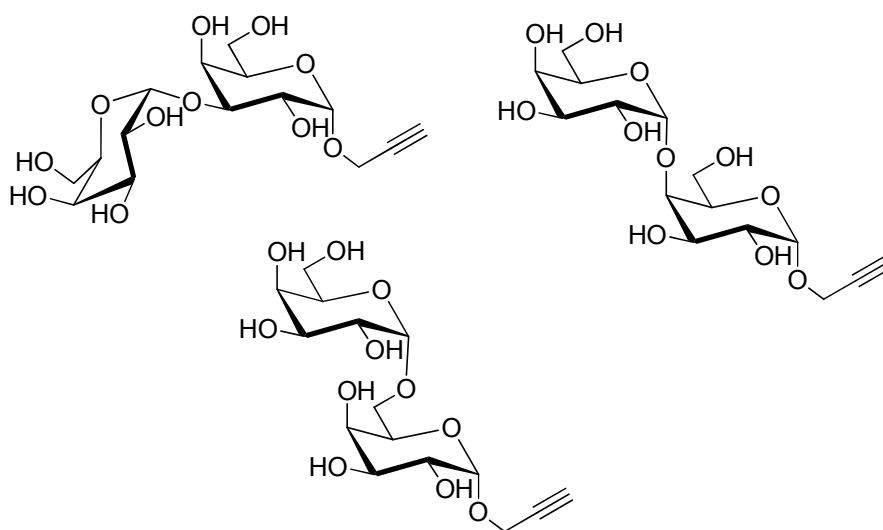


Figure 33. Alkyne-derivatized galactosides.

4. Experimental

4.1. Materials

Epoxide-derivatized glass slides (Nexterion slide E, SCHOTT North America), Rhodamine labeled Concanavalin A (Vector Labs), thiol-terminated mannose derivatives (all synthesized as reported in part one); all other standard chemicals were purchased from commercial suppliers and used as received. Glass slides were stored in a desiccator to keep them dry.

4.2. Methods

4.2.1. Microarray fabrication

Printing sugar solutions were made up of sugar (1 mg/mL stock solutions in H₂O), PBST (300 mM phosphate-buffered saline and 0.05% Tween-20) of pH 8.9 and TCEP (5 mM stock solution in H₂O). The final TCEP concentration in each prepared sugar solution was 2 mM. Eleven different concentrations (1, 2.5, 5, 10, 20, 30, 40, 50, 60, 80 and 100 mM) of each sugar were prepared in a 96-well plate. A control of 0 µg/mL of sugar was also included. Using the standard robotic pin printer (Virtek ChipwriterTM Professional Arrayer, Figure 20), the different solutions of sugars were printed on epoxide-functionalized glass slides. Six identical supergrids were printed; each super grid had 3 grids, and each grid was printed in a 12 × 10 pattern. After an overnight incubation at room temperature under 60% humidity, the slide surface was divided by drawing with permanent-ink marker to avoid contamination during protein incubation. Slides were washed by submerging them in petri dishes filled with wash buffer (PBS, pH 7.4

containing 0.05% Tween-20) and gently shaking on a waver for 30 min. To block unreacted epoxides, slides were submerged in petri dishes filled with a block solution (50 mM ethanolamine in 100 mM borate, pH 9.2) and gently shaken for 1 h on a waver. After blocking, the slides were washed three times by immersing them in centrifuge tubes filled with PBS buffer (pH 7.4) and dried by gentle purging with N₂ air. Slides were immediately used for incubation with protein.

4.2.2 Binding assay

Rhodamine-labeled Con A (5 mg/mL) was diluted in phosphate-BSA buffer (Con A buffer: 50 mM, pH 6.5; 1 mM CaCl₂, 1mM MnCl₂, 0.9% NaCl (w/v), 1% BSA (w/v), 0.05% Tween-20). Six different concentrations of Con A were obtained: 2, 5, 10, 20, 30 and 50 µg/mL. An incubation chamber was fabricated by placing a damp, warm paper towel in a covered plastic box. For incubations, 40–50 µL of protein solution was applied to each supergrid and slides were placed in the incubation chamber and placed on the shaker for 1 h at room temperature. After incubation, slides were washed three times in petri dishes filled with incubation buffer (Con A buffer), three times with PBST buffer (0.05% Tween-20), three times with distilled water, and then dried by gentle purging with N₂ air to ensure complete dryness. Each wash was approximately 5 min. The dried plates were scanned immediately to obtain results.

4.2.3. Data analysis

Fluorescence intensities of microspots were quantified, and the data was extracted by using a microarray scanner (GenePix[®] 4000B, Axon Instruments). The images were read using a 532 nm wavelength laser with the array reader. The local background was subtracted from the signal at each spot. The spots with obvious defects, no detectable signal or fluorescence lower than the background or higher were removed from the analysis. The medians of ratios from replicate spots were averaged in the same array.

To determine the $K_{D,surf}$ value, the data was plotted using SigmaPlot version 11.0. Two equations were used for best-fit curves where applicable: the ligand and the sigmoidal binding equations. Ligand binding was applied for most binding curves. The advantage to using ligand-fit binding equations was that the K_D values were automatically calculated by the SigmaPlot program. In order to calculate the binding constants, curves were analyzed as Langmuir isotherms assuming equilibrium was reached during incubation. The following equation was used to calculate the binding constants as previously reported by Wong and co-workers. The calculated values matched those generated by SigmaPlot according to the following equation:

$$K_{D,surf} = \frac{[P](F_{max} - F)}{F}$$

Where, [P] = concentration of protein,

Fmax = maximum fluorescence intensity at protein concentration, a value that is generated by SigmaPlot, and

F = Fluorescence intensity

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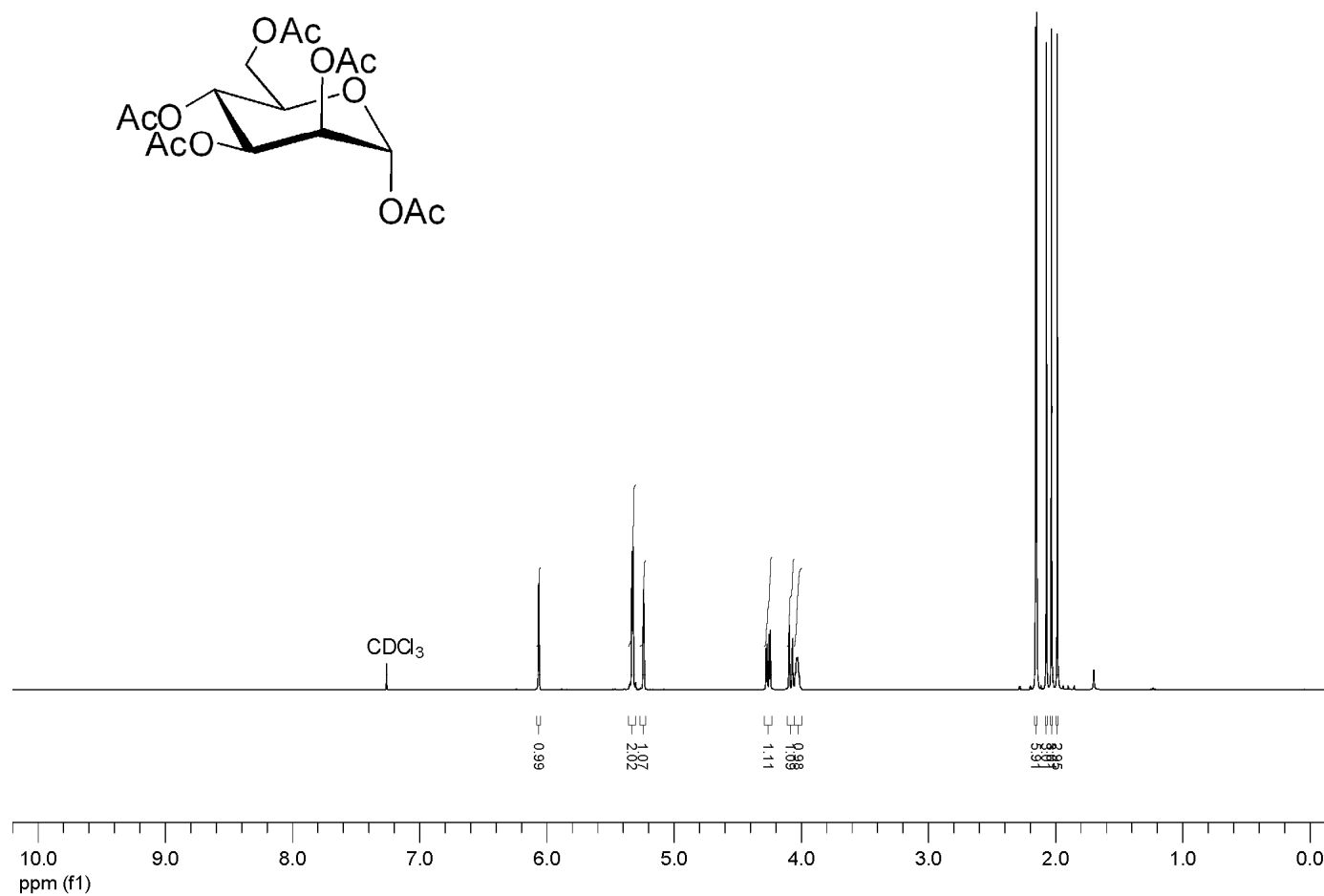
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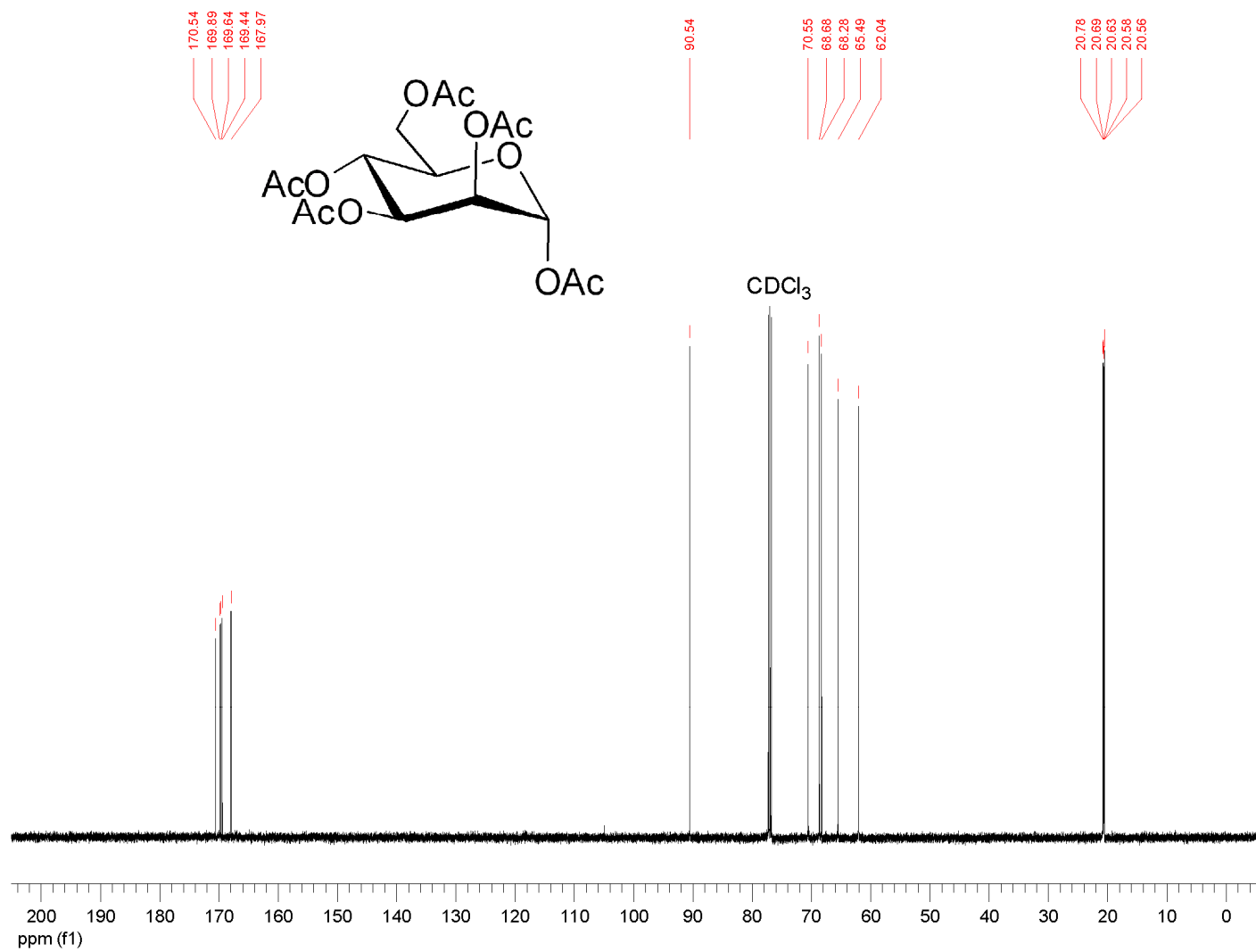
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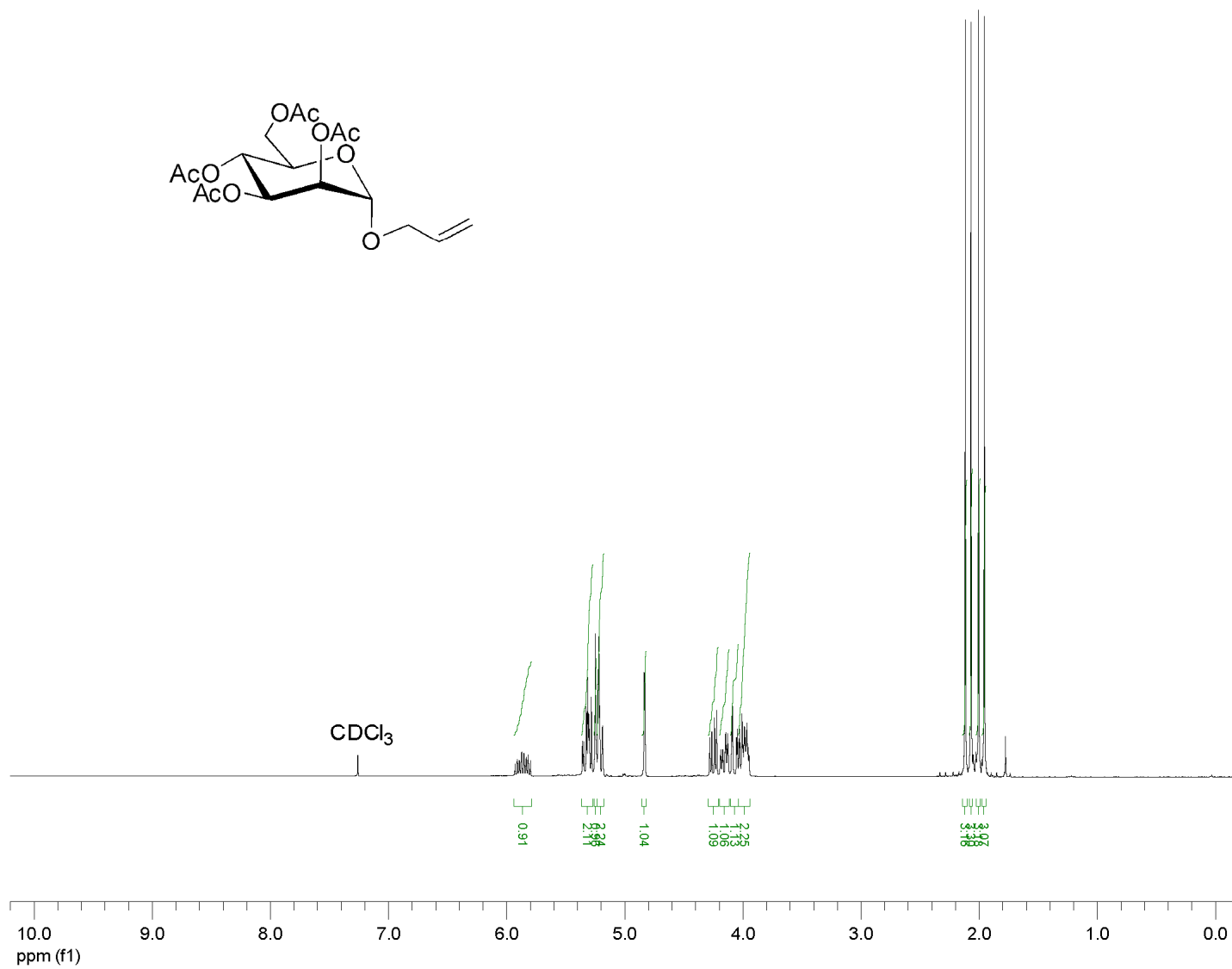
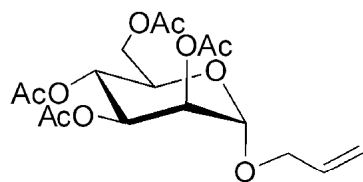
Appendix



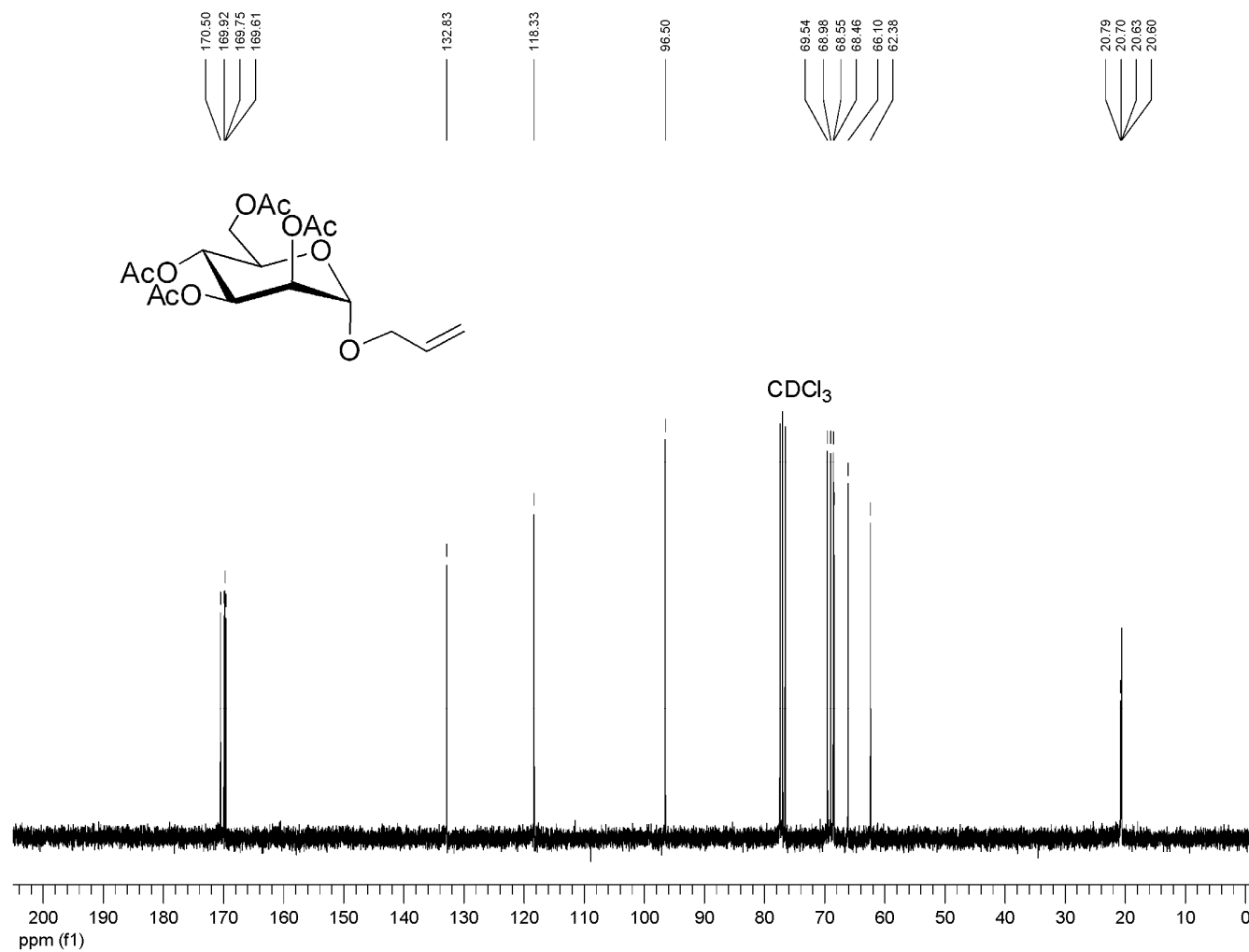
^1H NMR spectrum (300 MHz, CDCl_3) of α -anomer of 1,2,3,4,6-Penta-O-acetyl-D-mannopyranose (2).



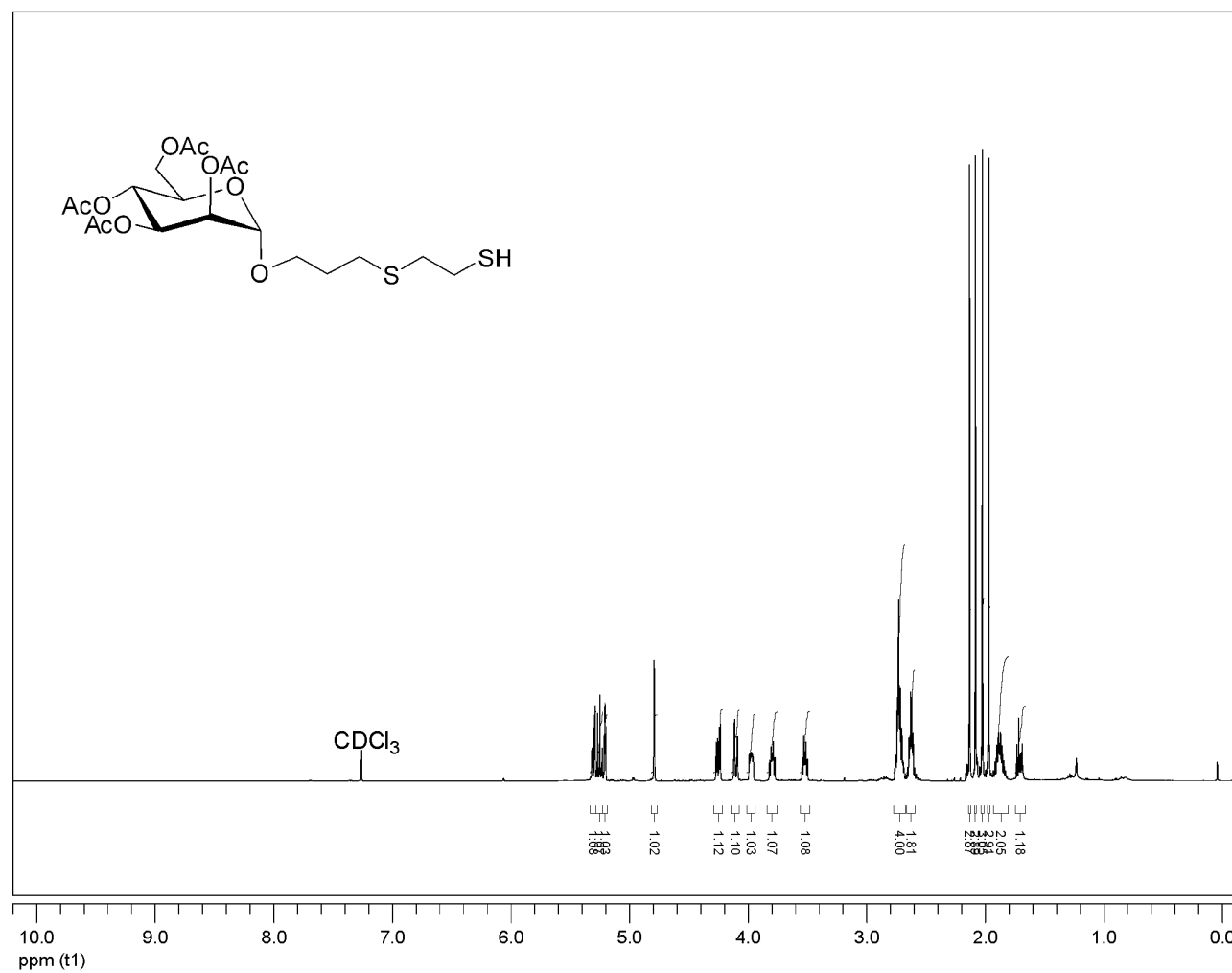
^{13}C NMR spectrum (75 MHz, CDCl_3) of α -anomer of 1,2,3,4,6-Penta-O-acetyl-D-mannopyranose (2).



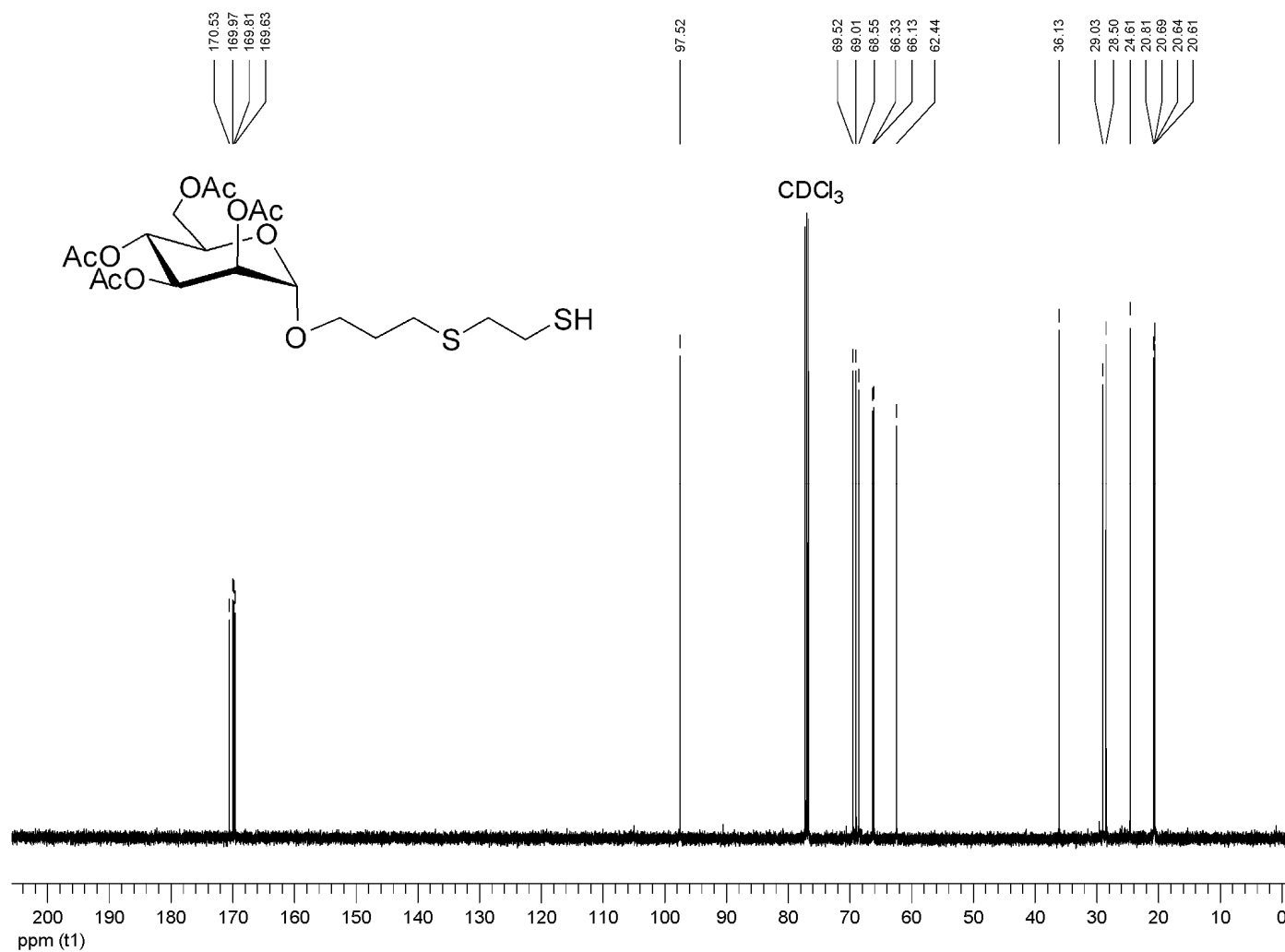
¹H NMR spectrum (300 MHz, CDCl₃) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**3**).



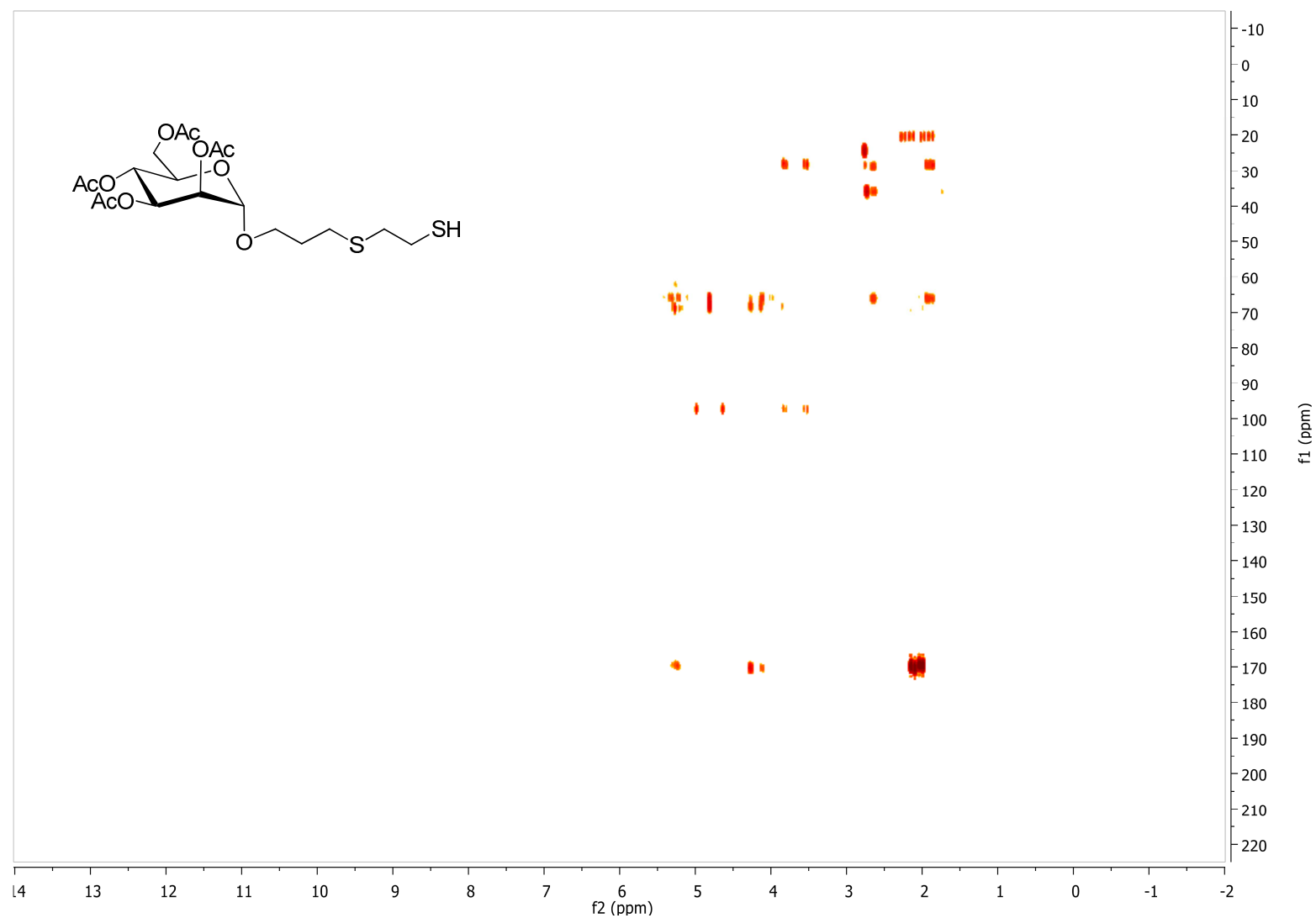
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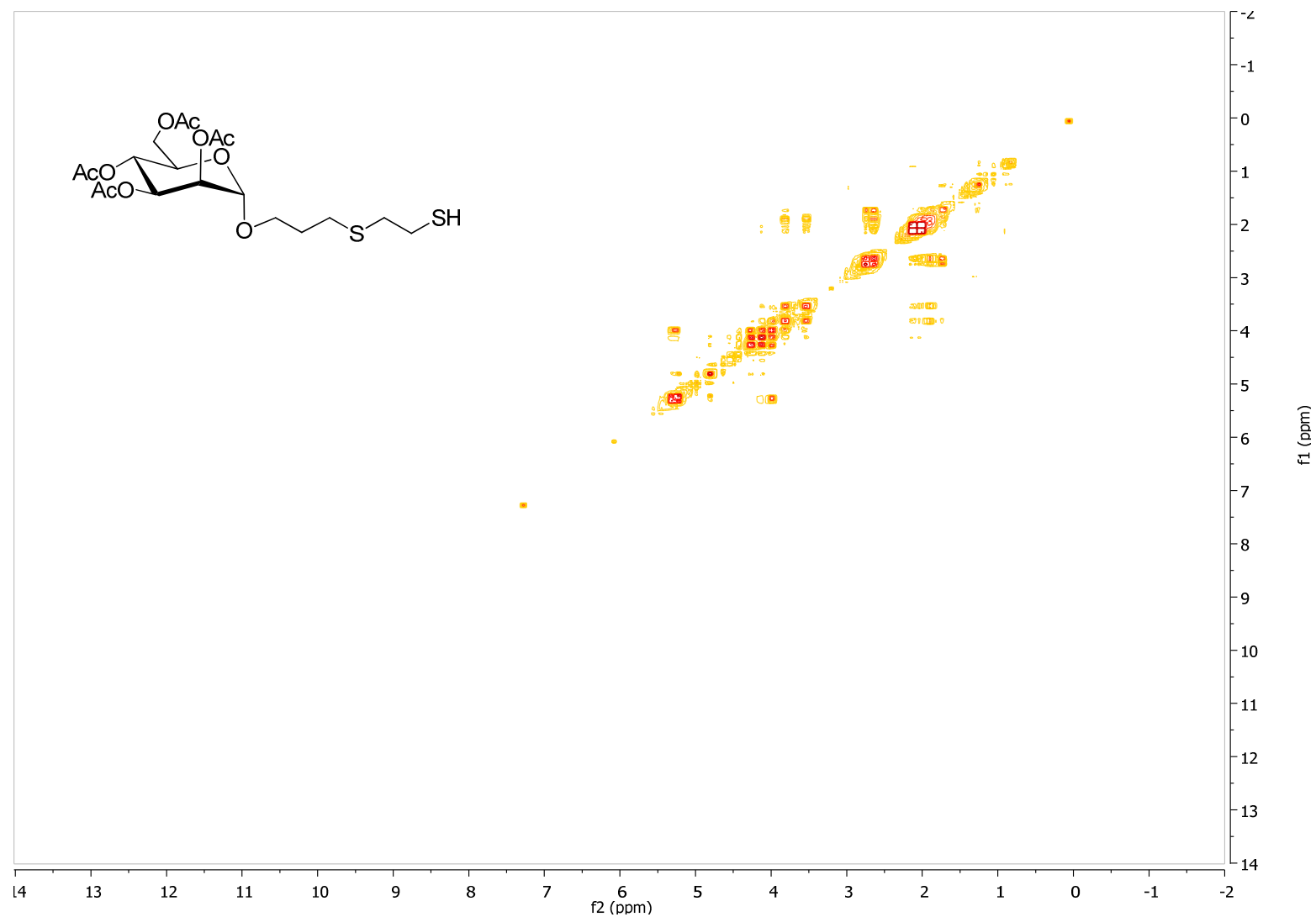
¹H NMR spectrum (300 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (4).



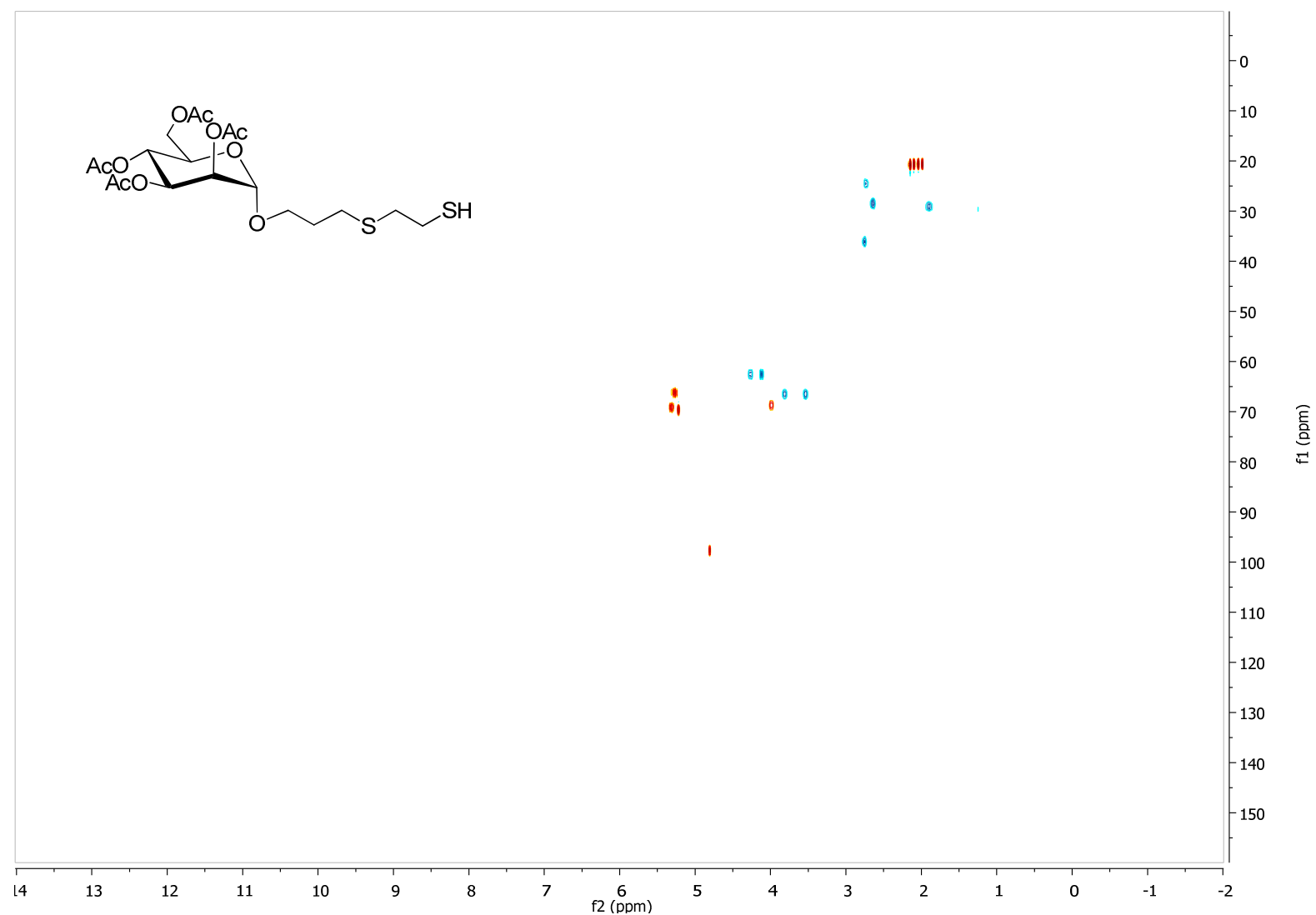
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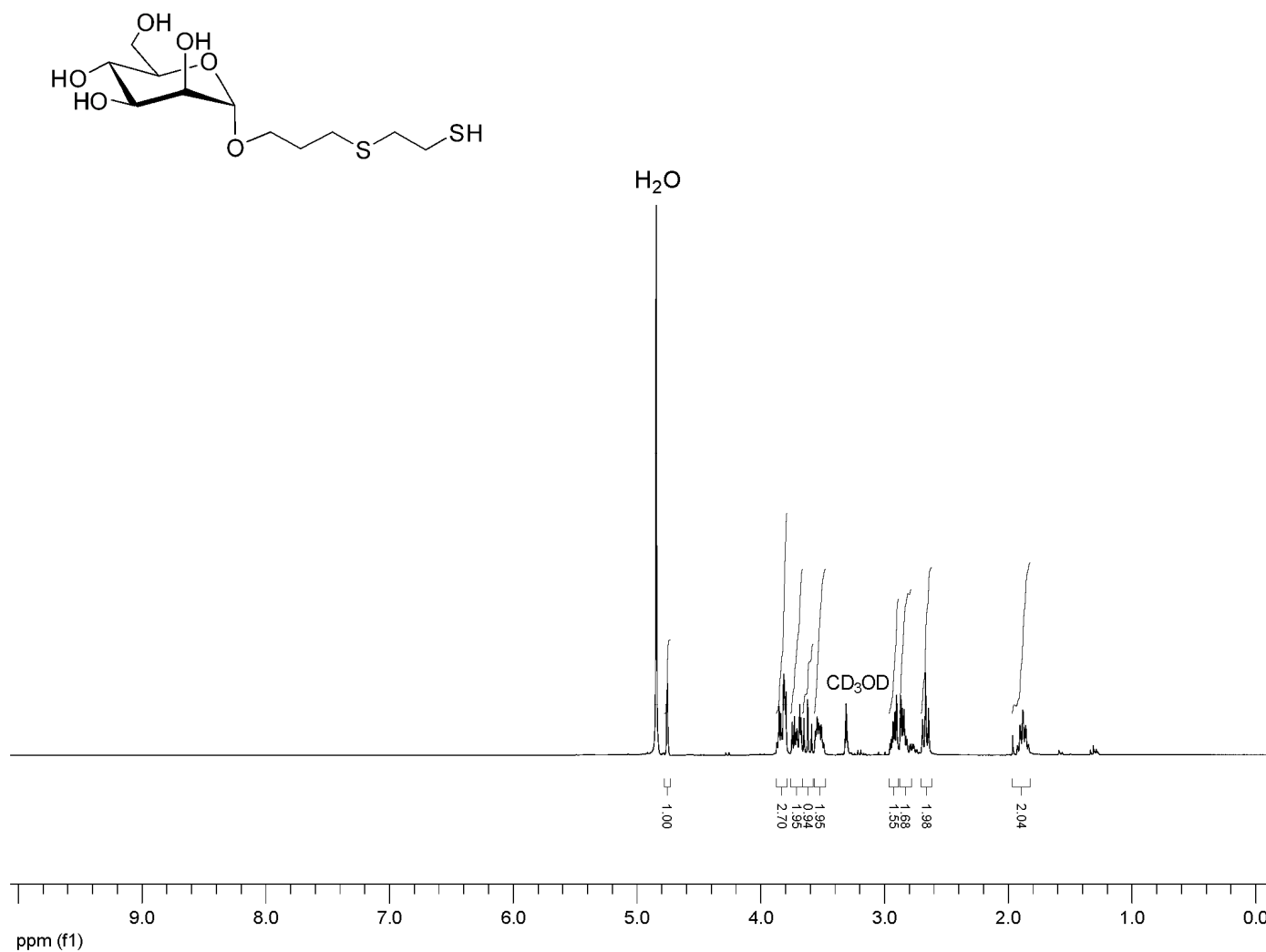
gHMBC NMR spectrum (500 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**4**).



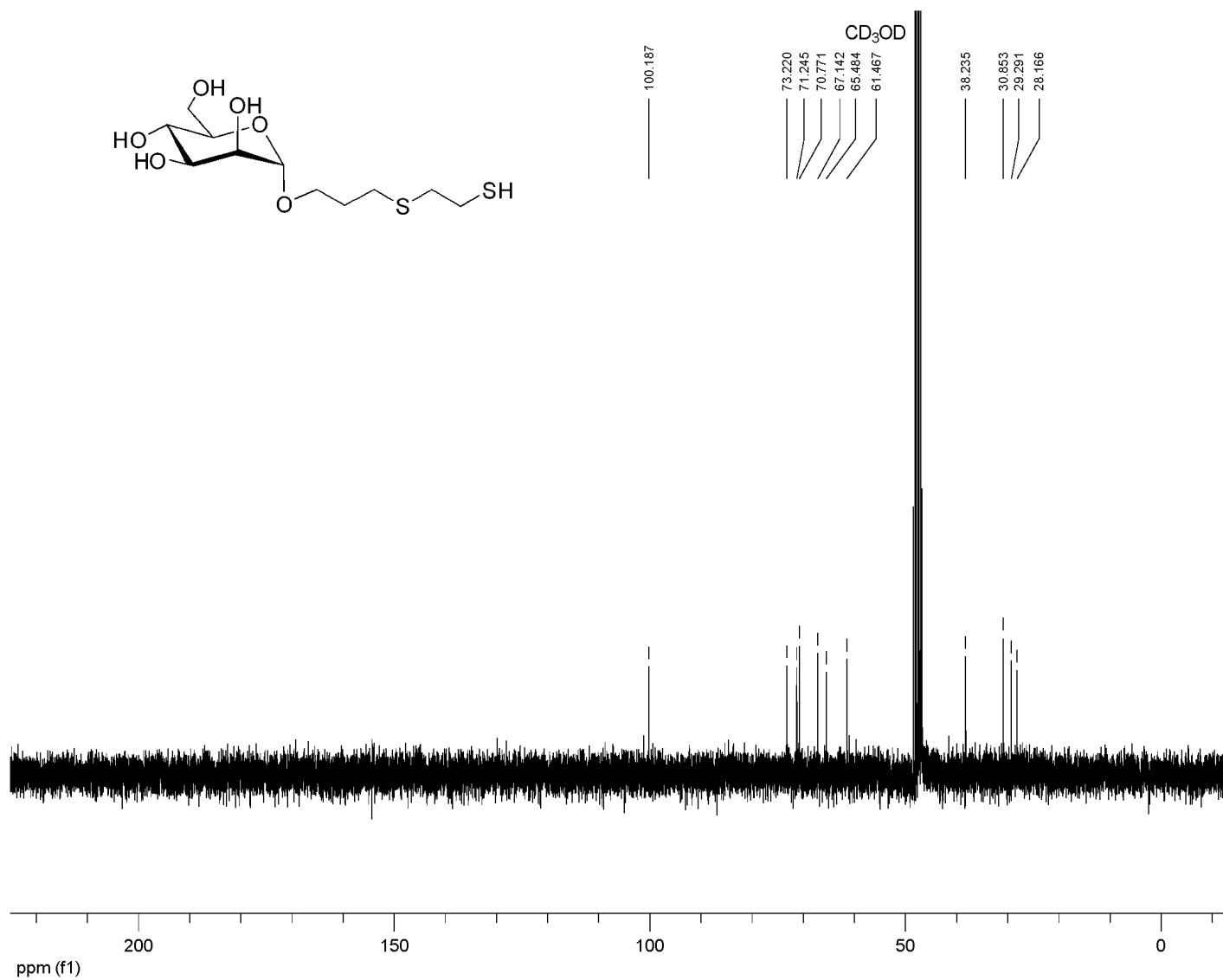
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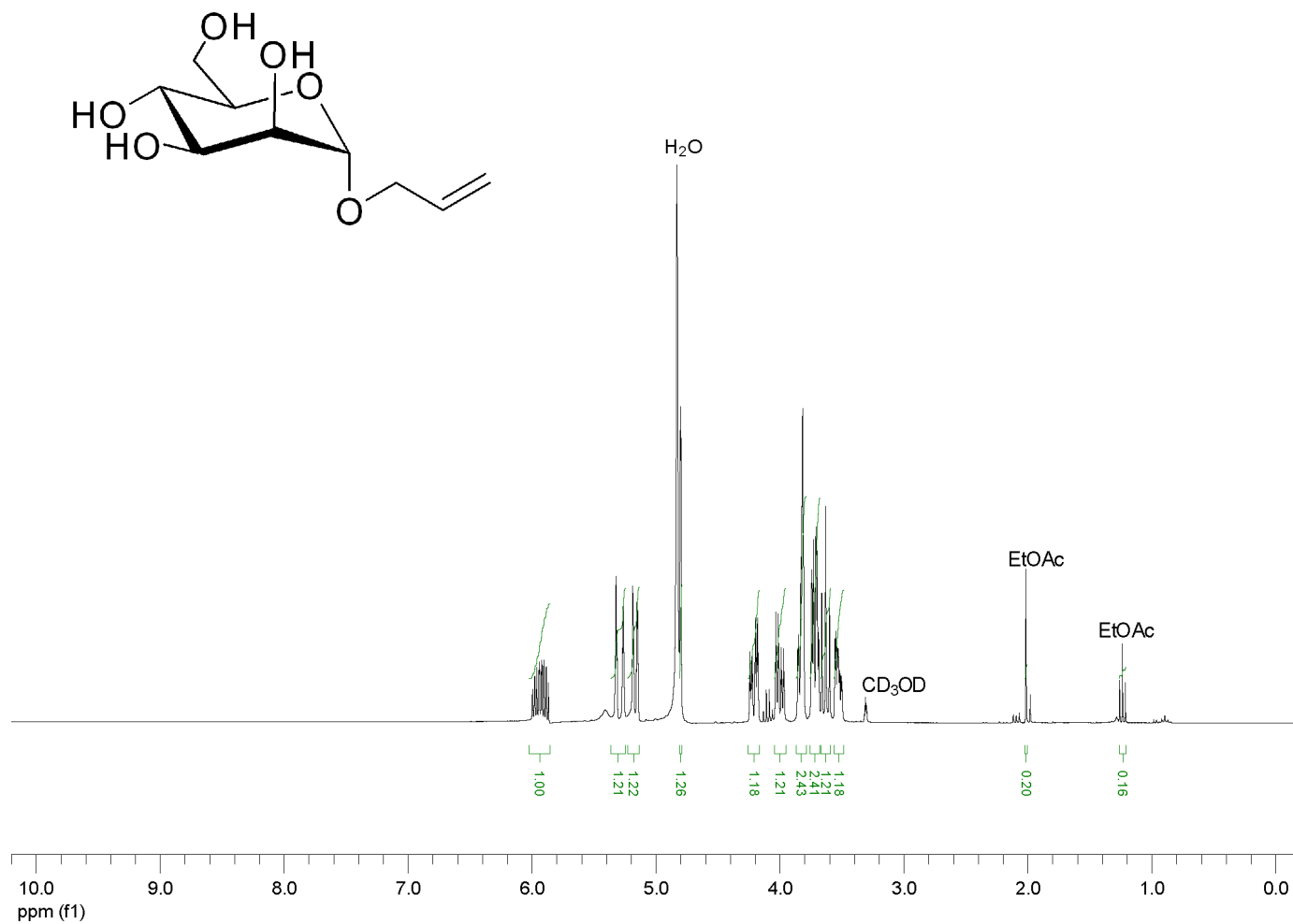
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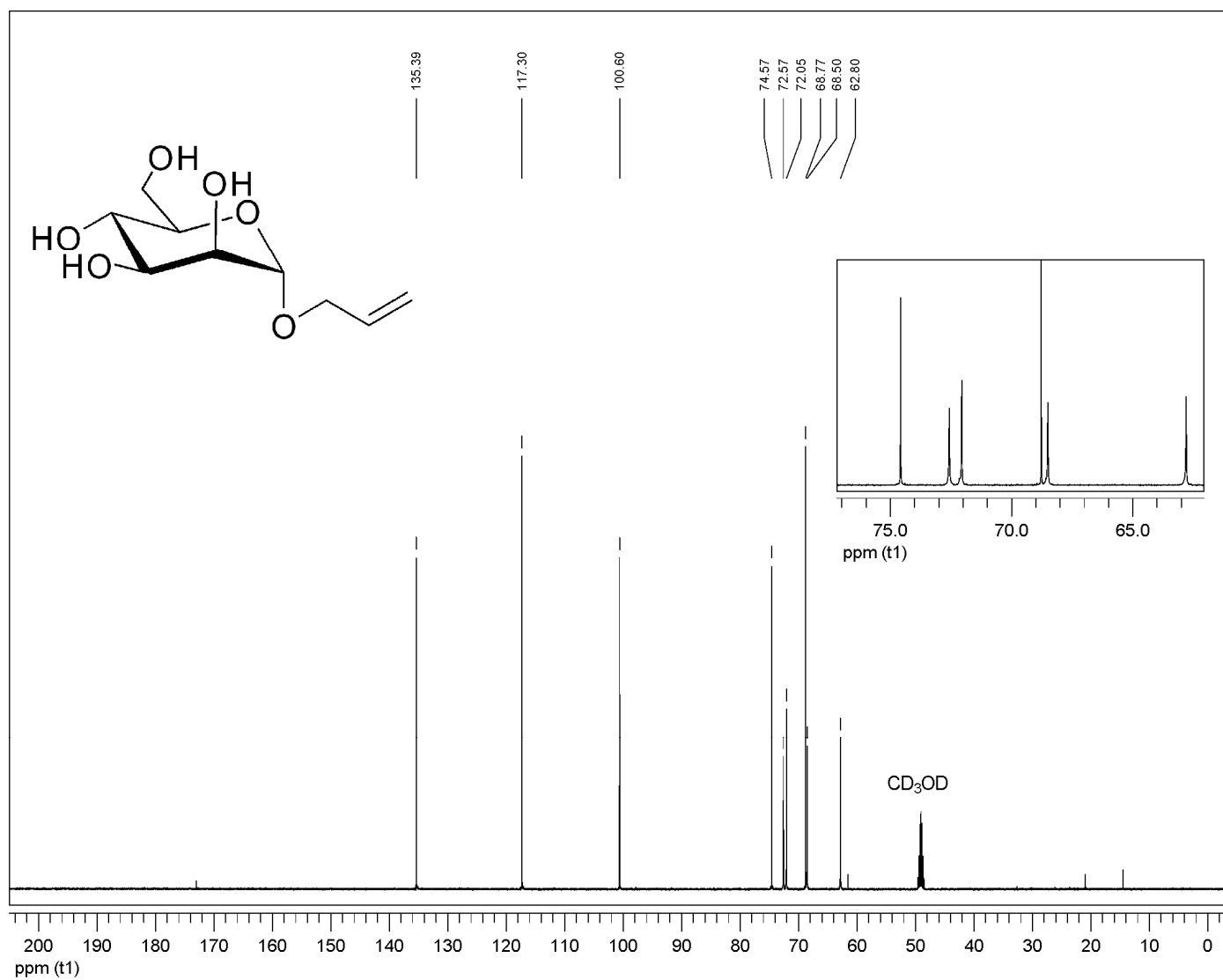
^1H NMR spectrum (300 MHz, CDCl_3) of 3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranoside (5).



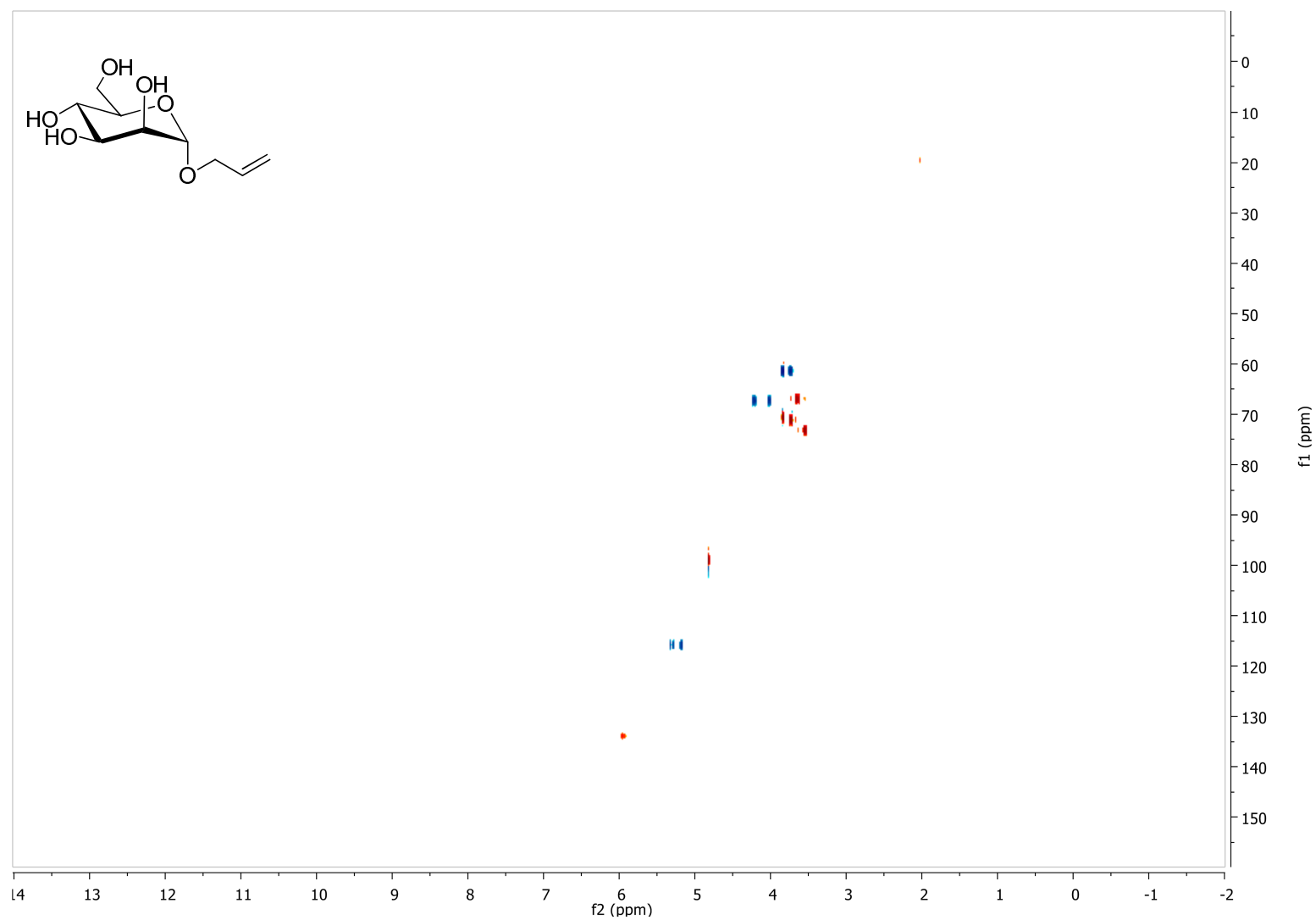
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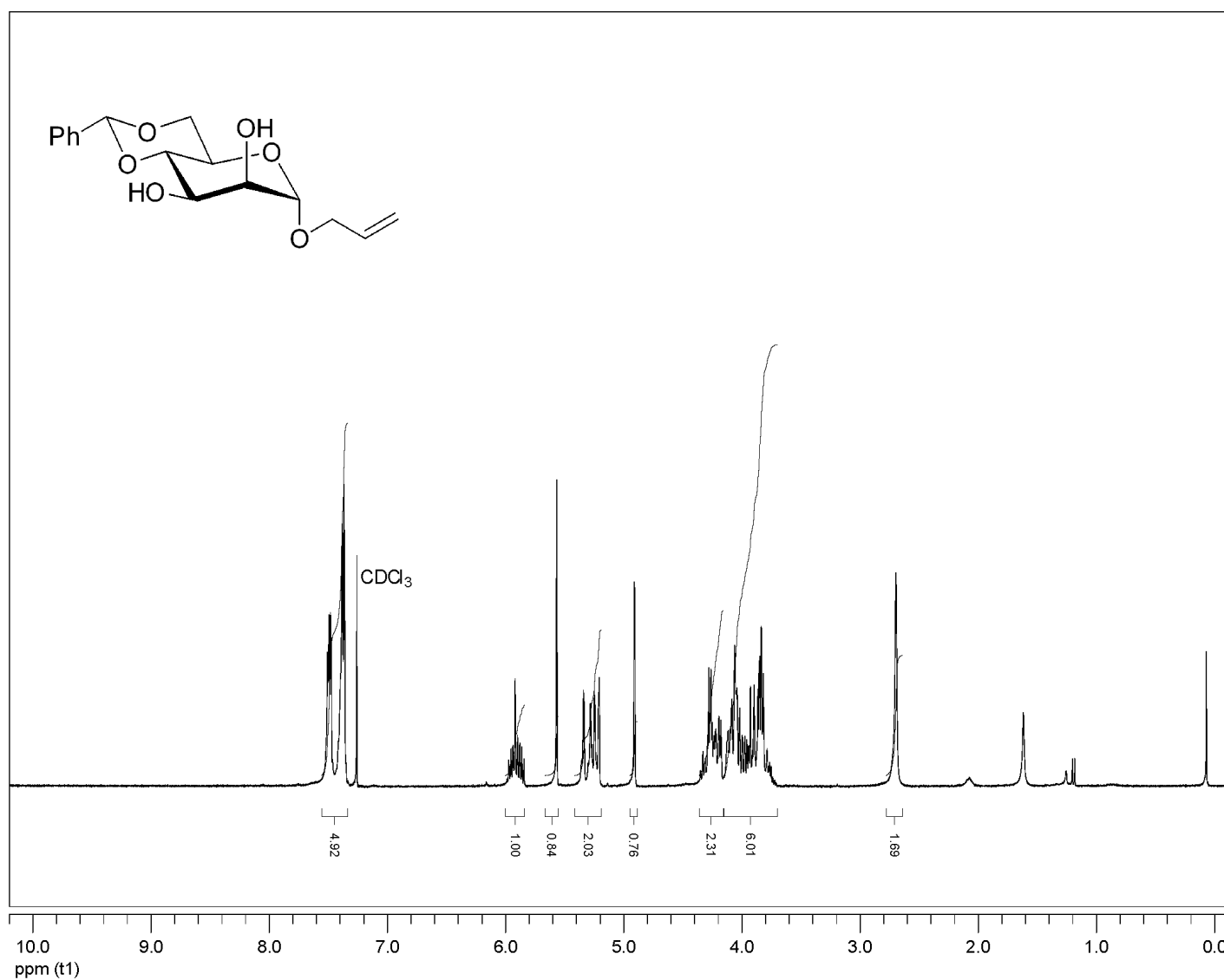


^1H NMR spectrum (300 MHz, CD_3OD) of allyl α -D-mannopyranoside (6).

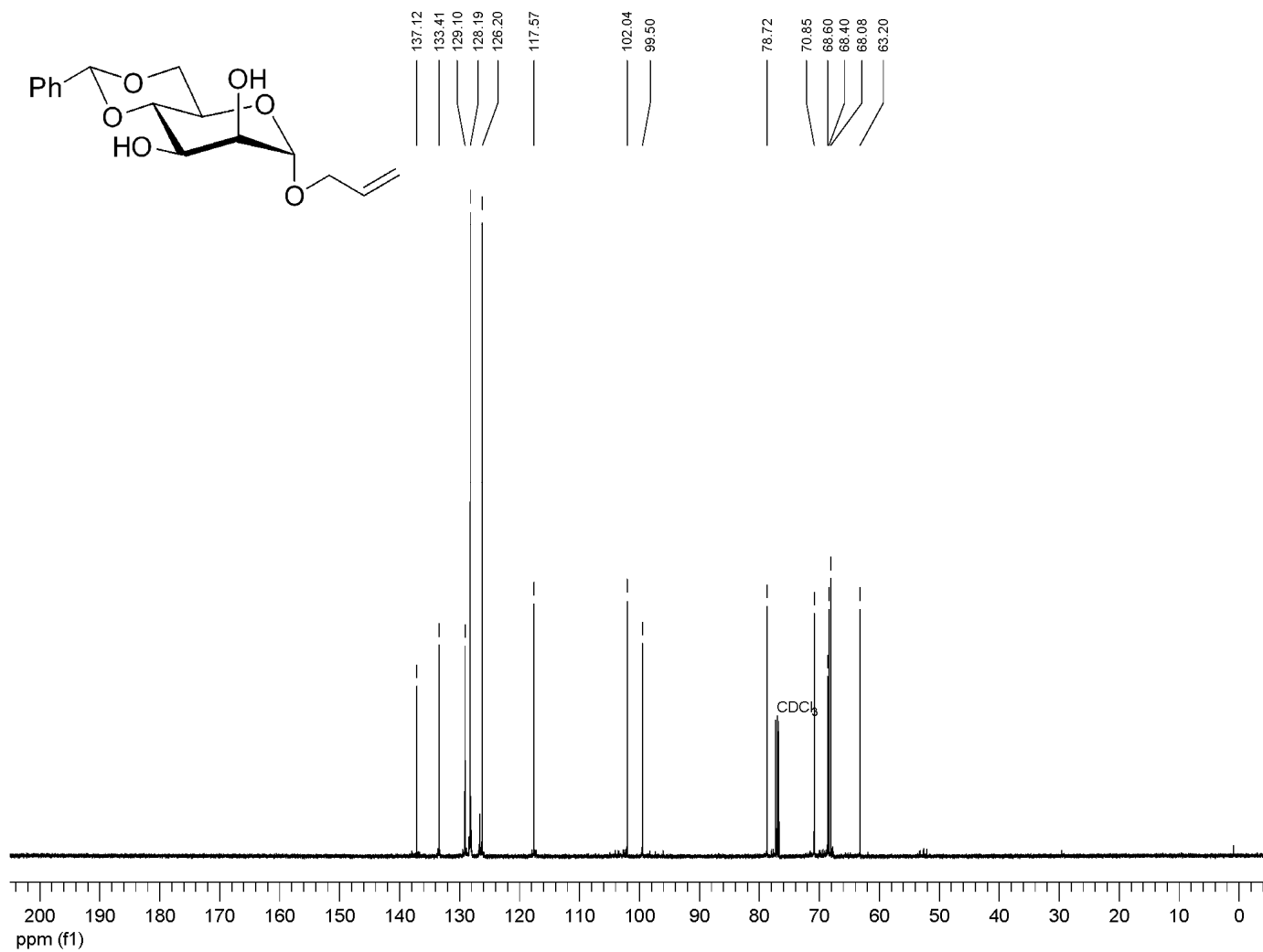


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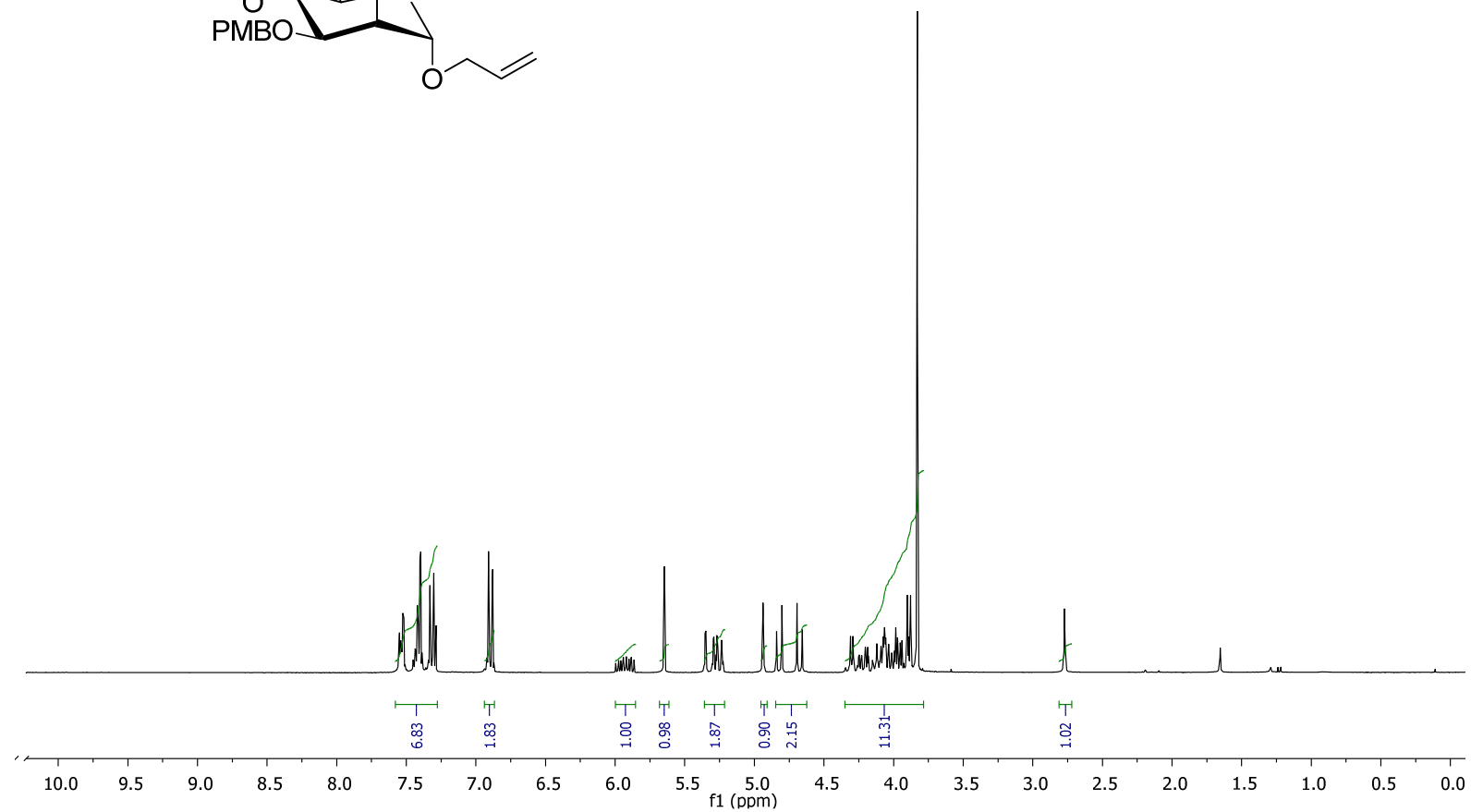
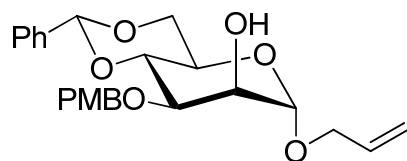




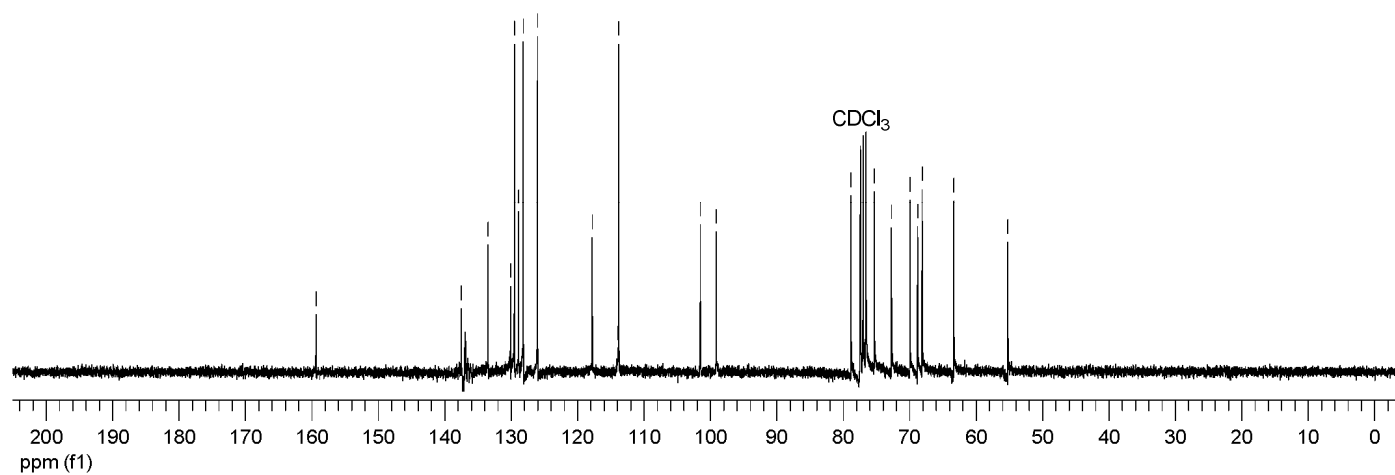
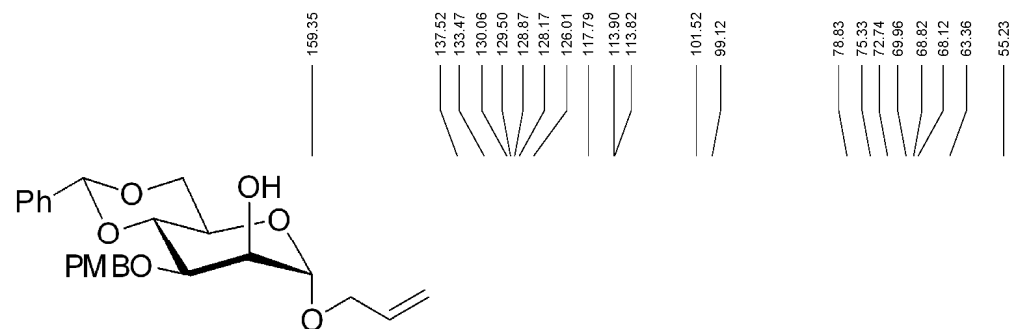
^1H NMR spectrum (300 MHz, CDCl_3) of allyl 4,6-O-benzylidene- α -D-mannopyranoside (7).



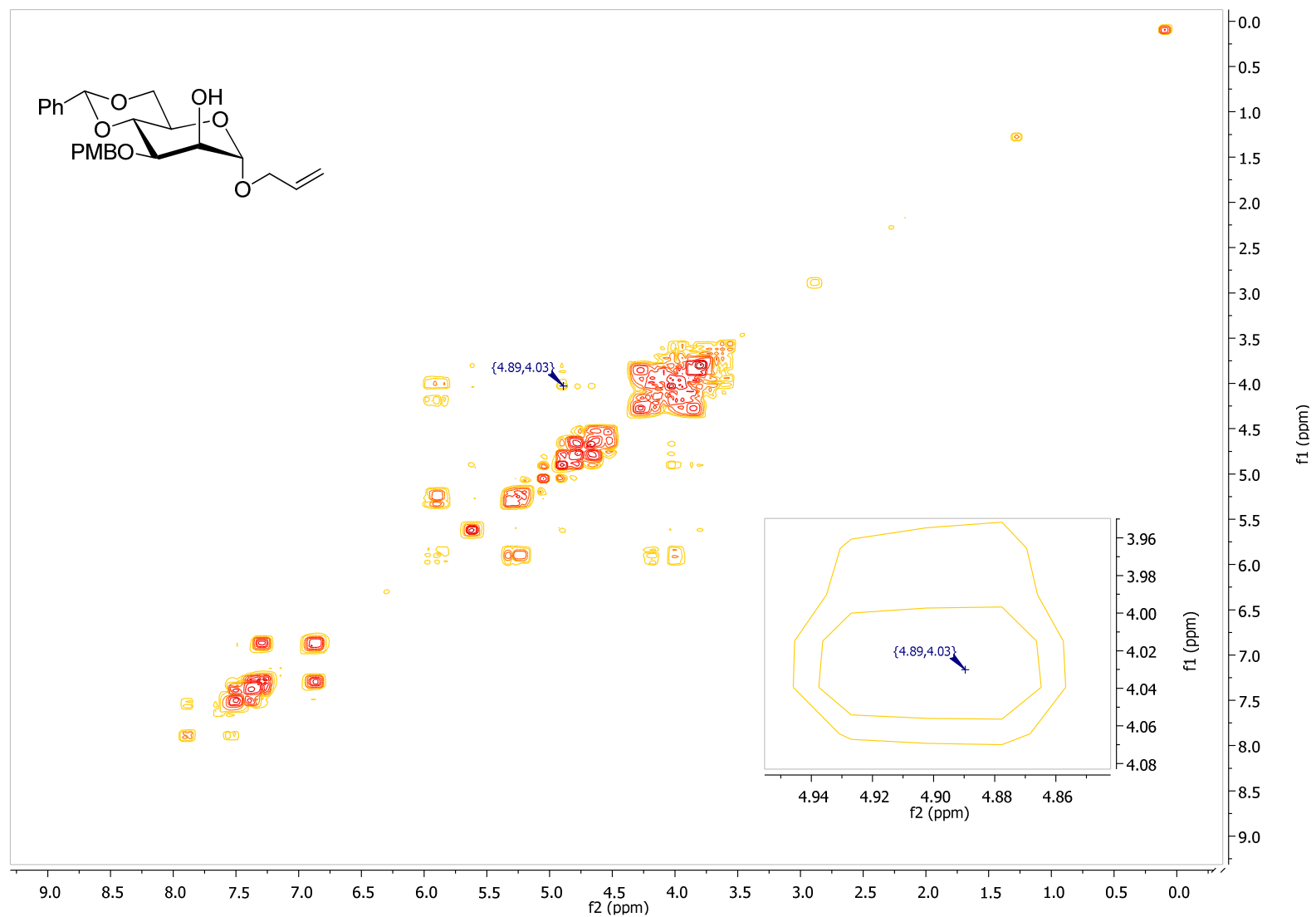
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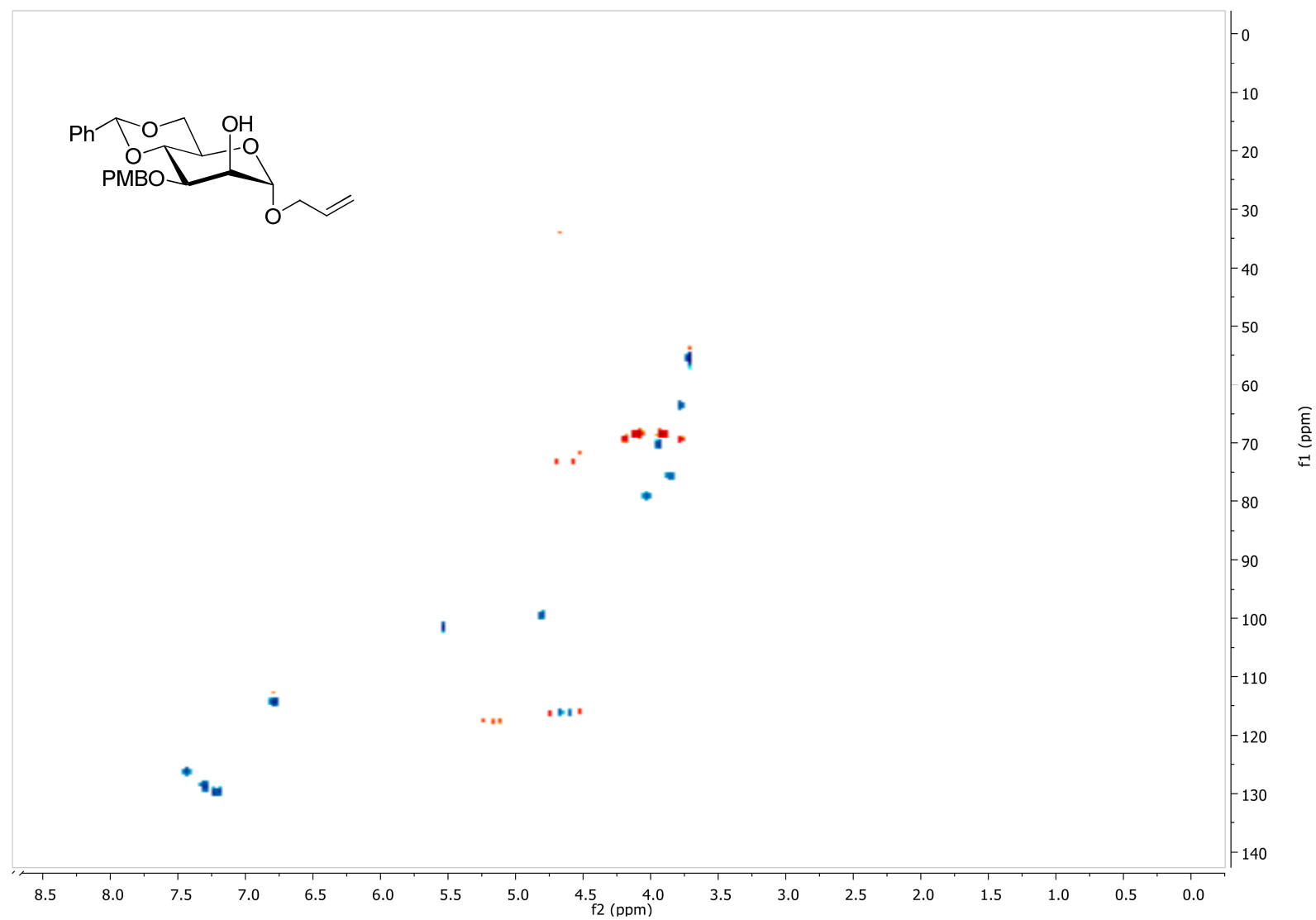
^1H NMR spectrum (300 MHz, CDCl_3) of allyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**8**).



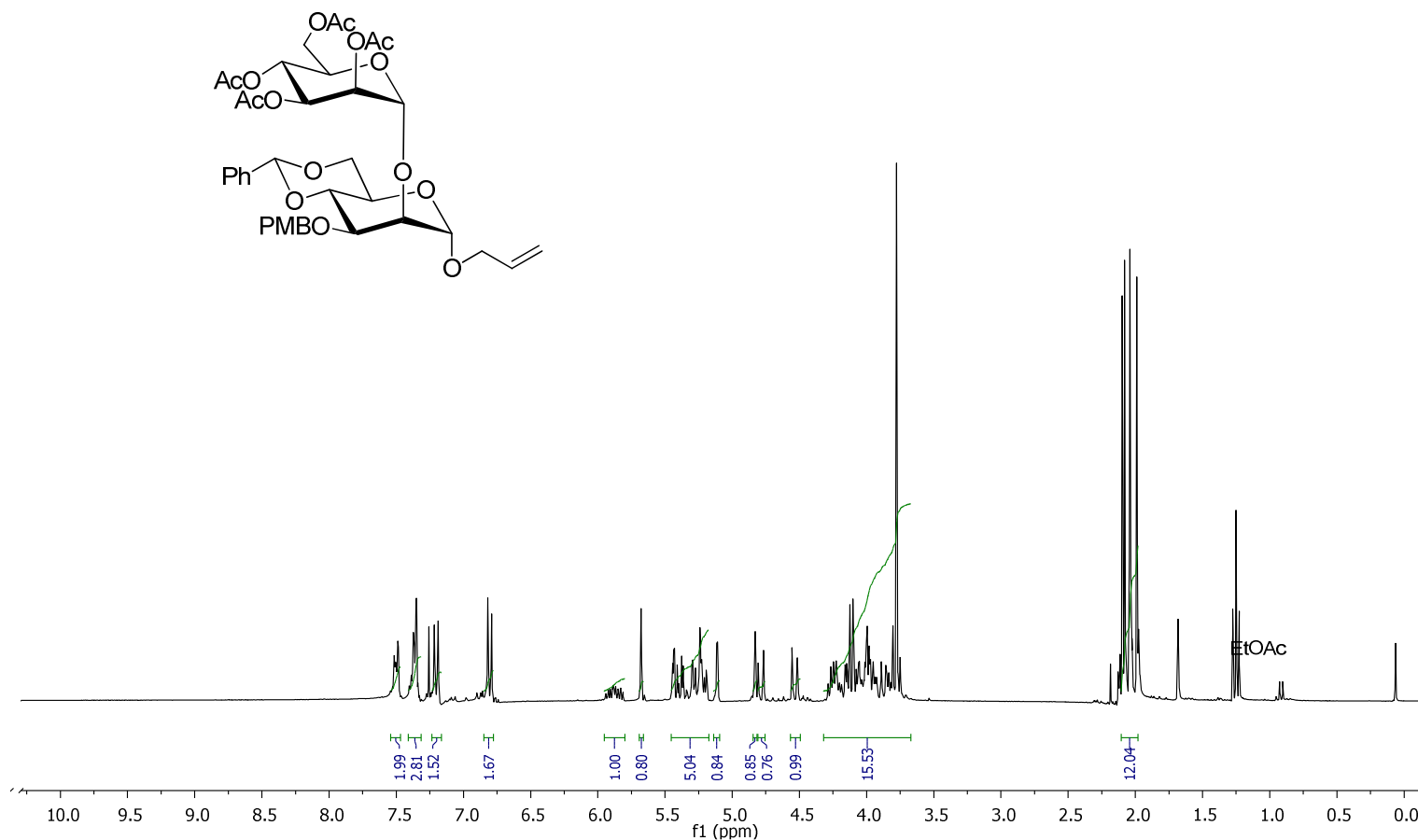
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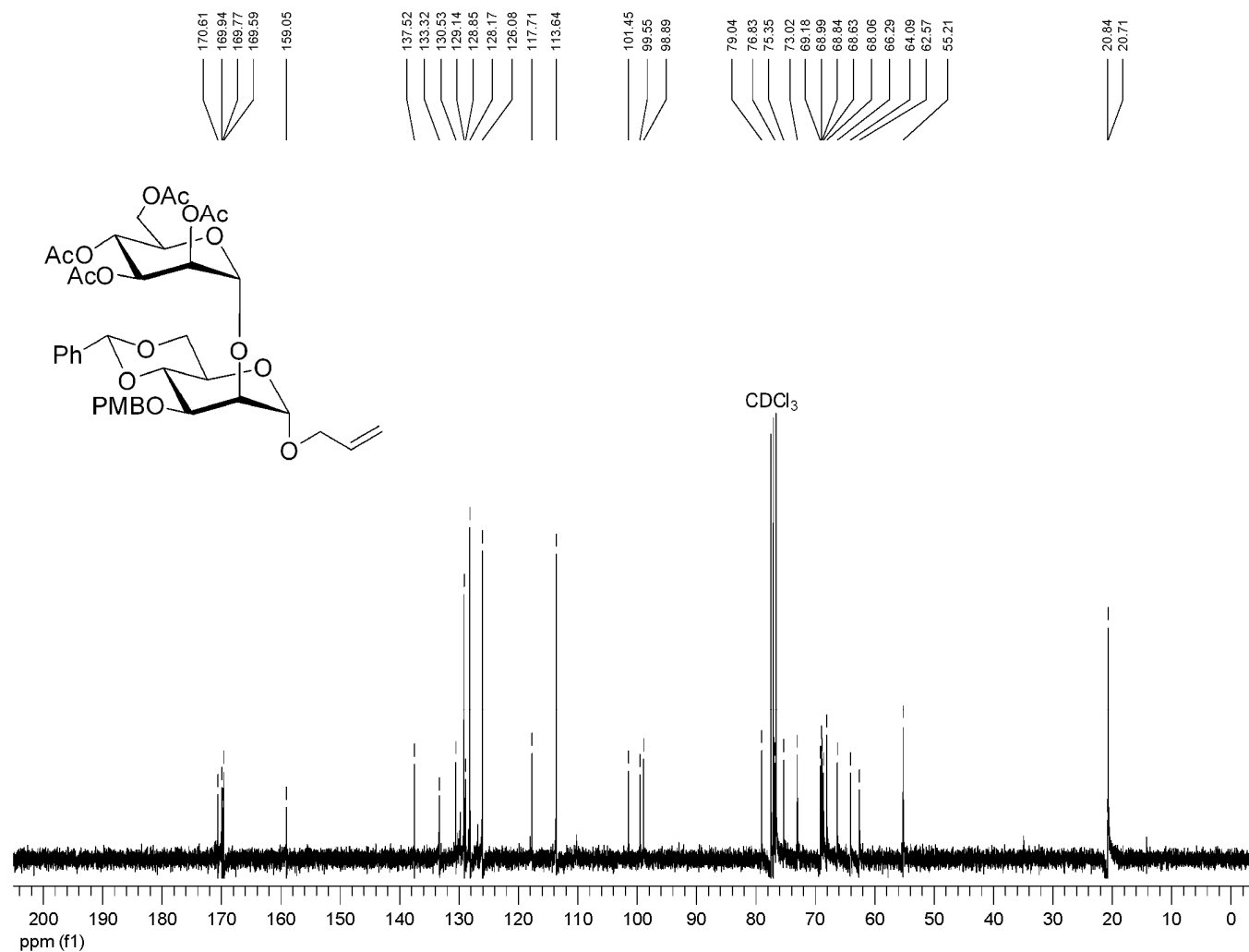
gCOSY NMR spectrum (300 MHz, CDCl₃) of Allyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**8**).



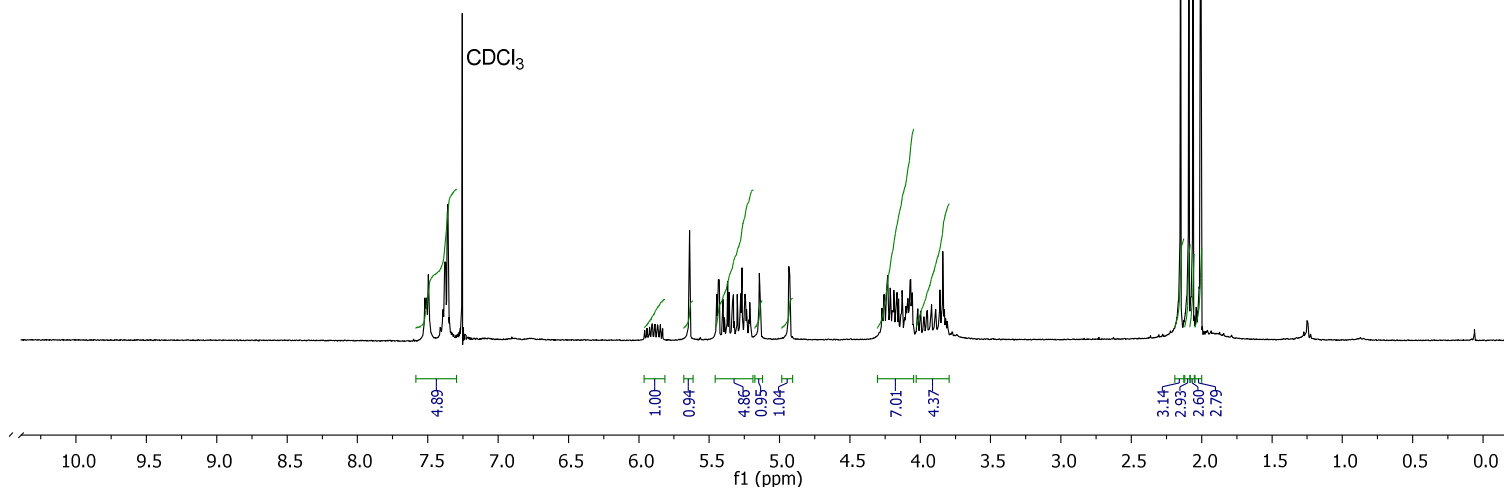
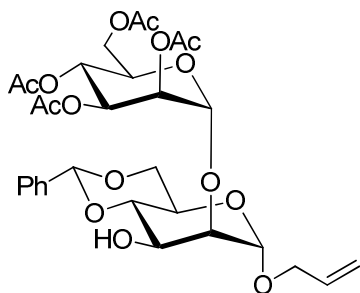
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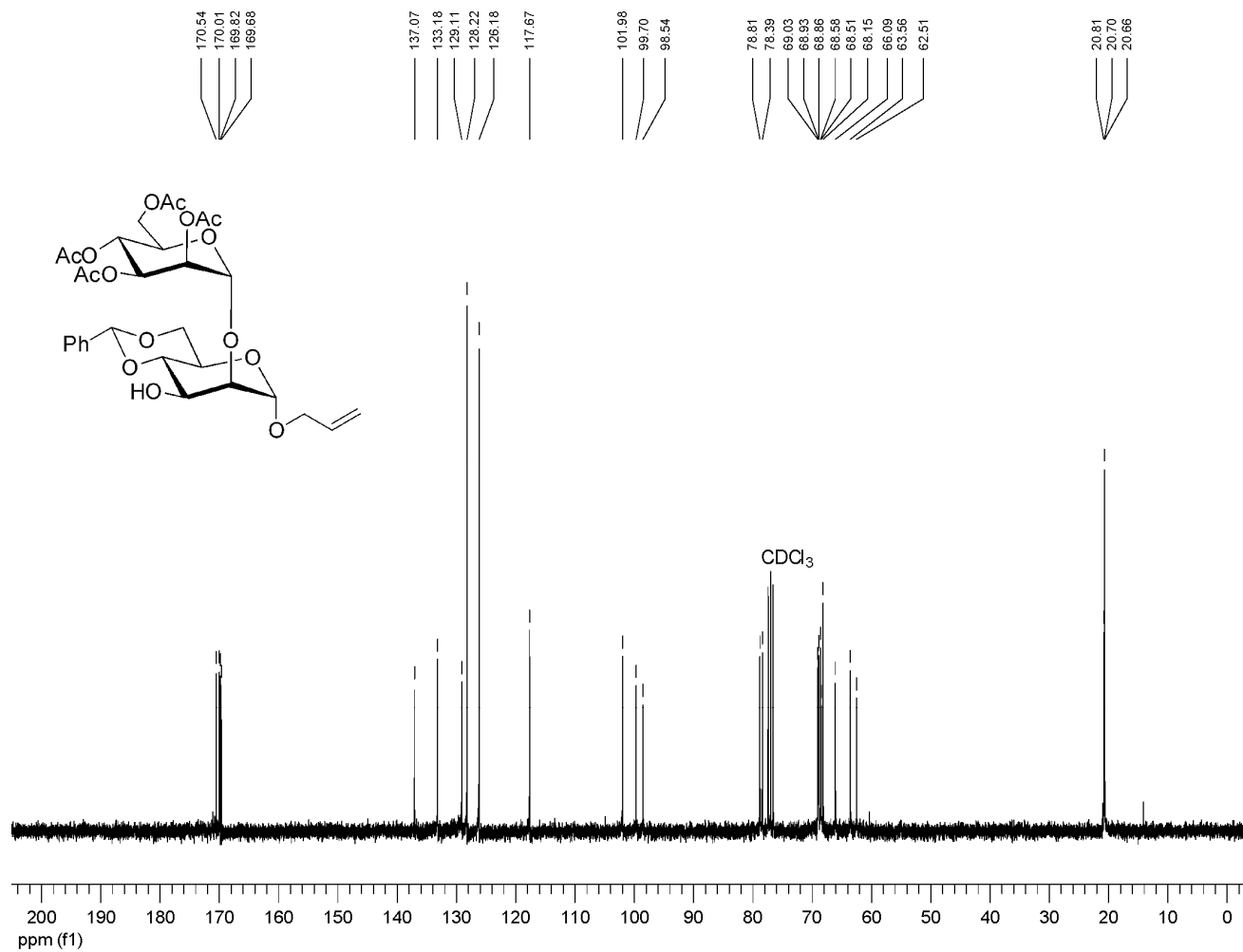
^1H NMR spectrum (300 MHz, CDCl_3) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**13**).



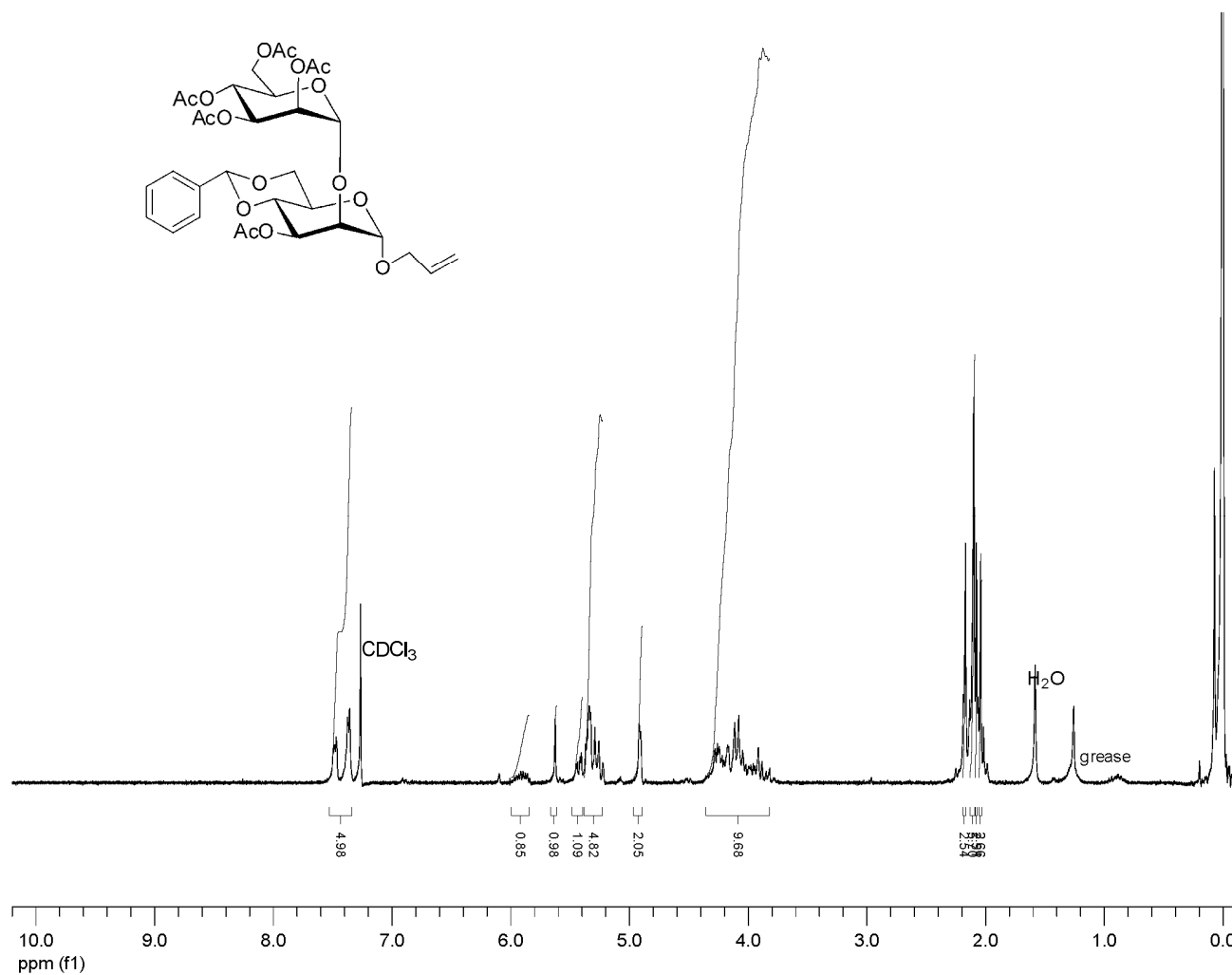
^{13}C NMR spectrum (75 MHz, CDCl_3) of allyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside (**13**).



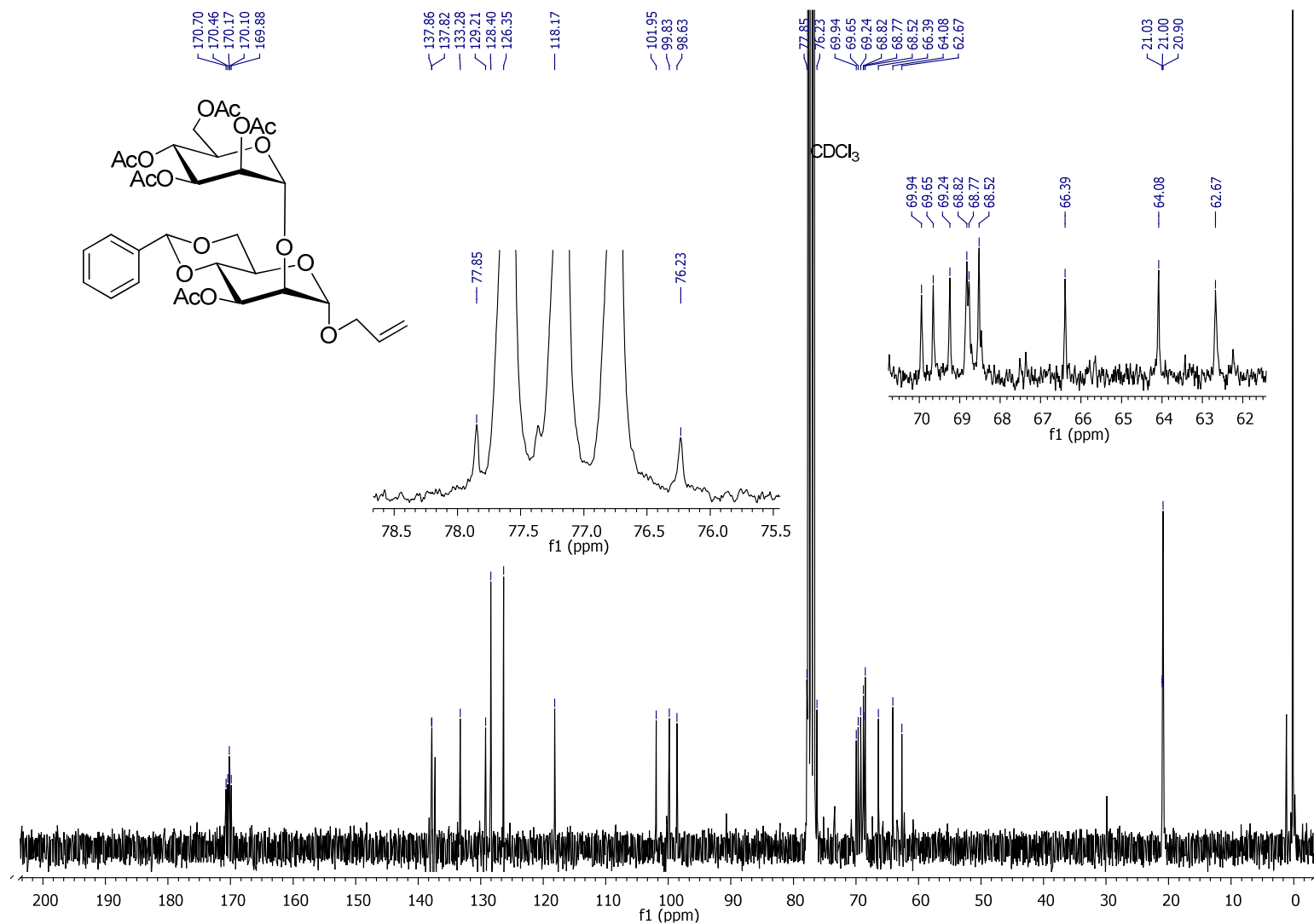
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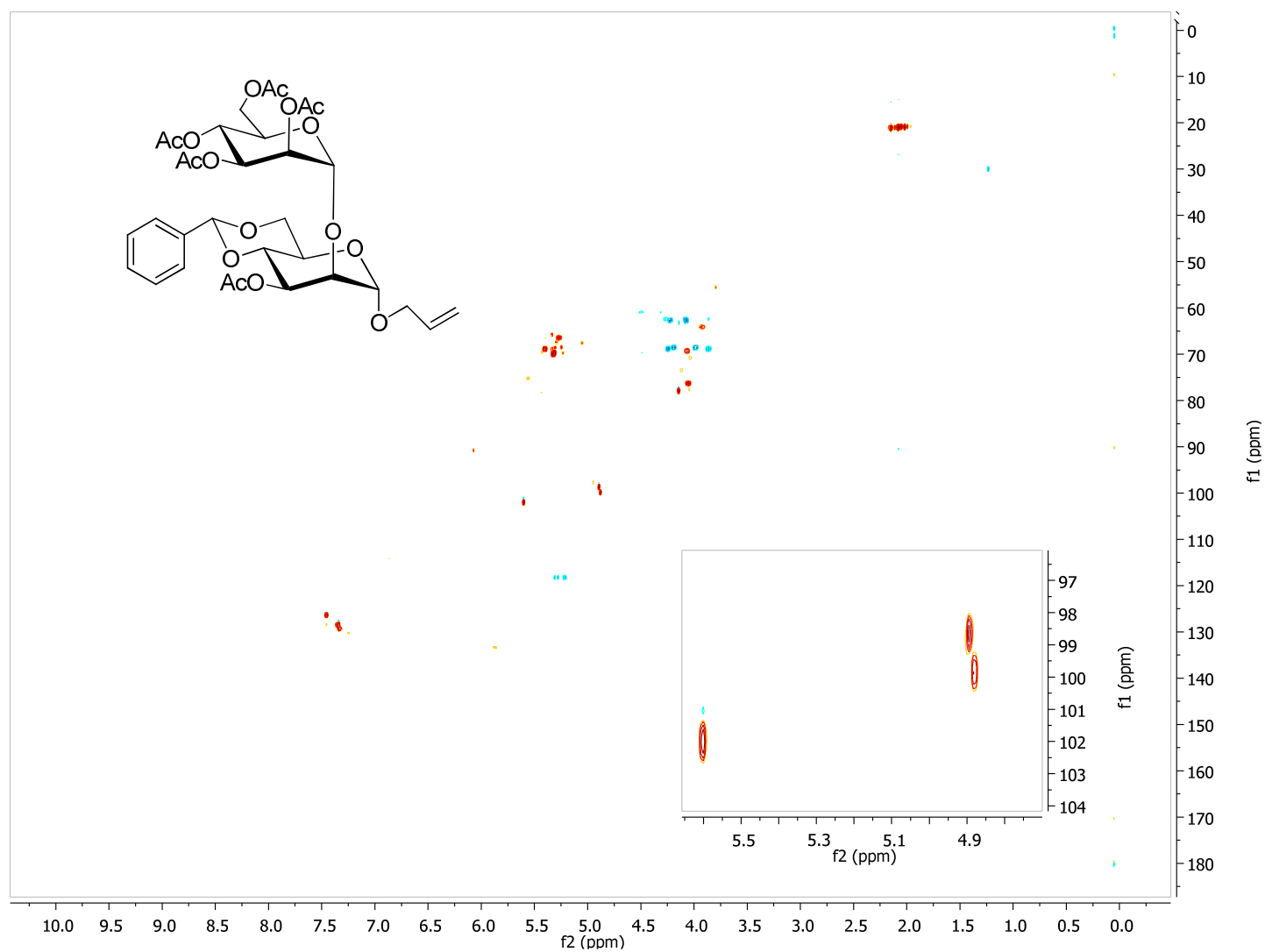
^{13}C NMR spectrum (75 MHz, CDCl_3) of a allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl- (1 $\rightarrow$ 2)- 4,6-*O*-benzylidene- α -D-mannopyranoside (**14**).



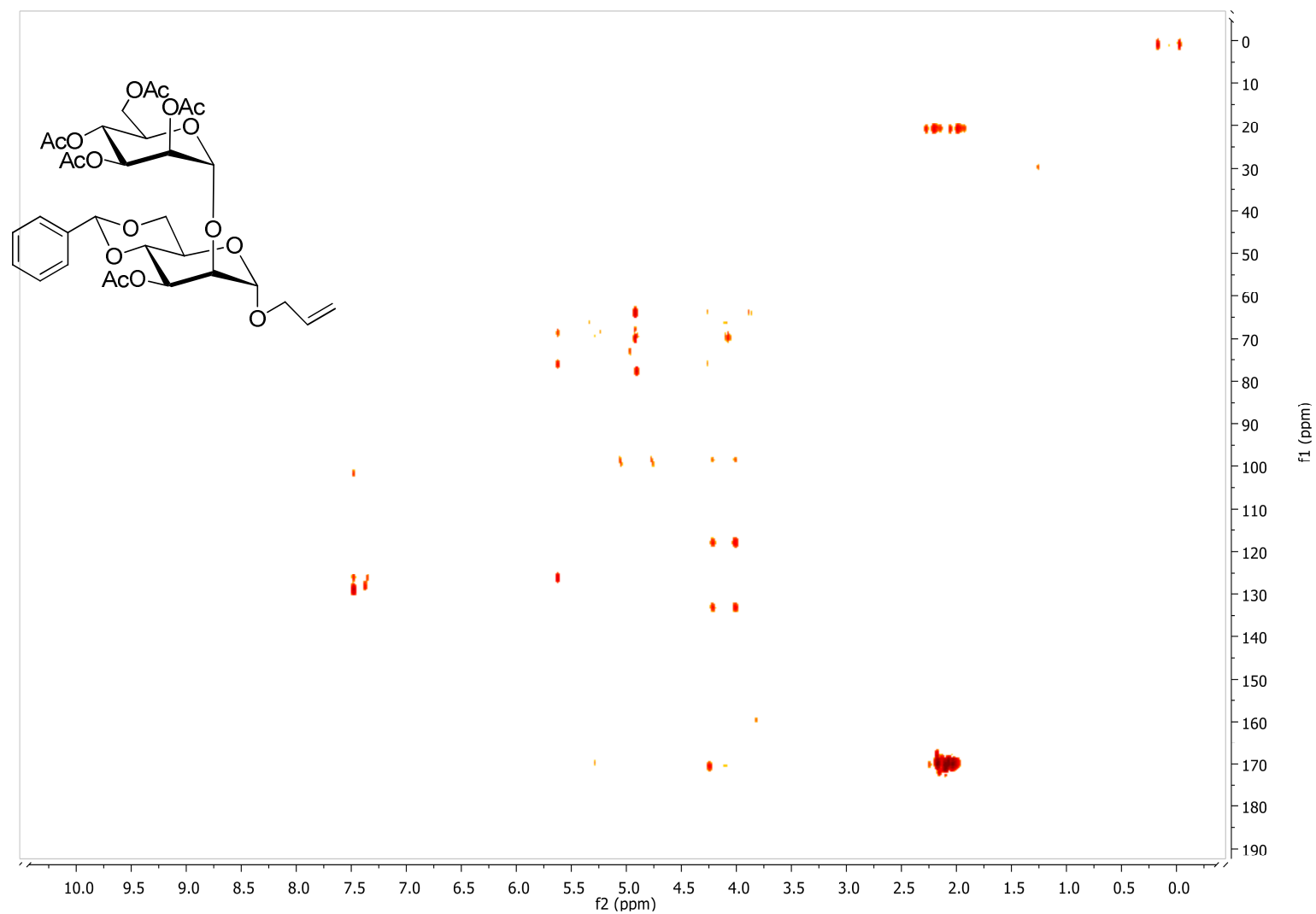
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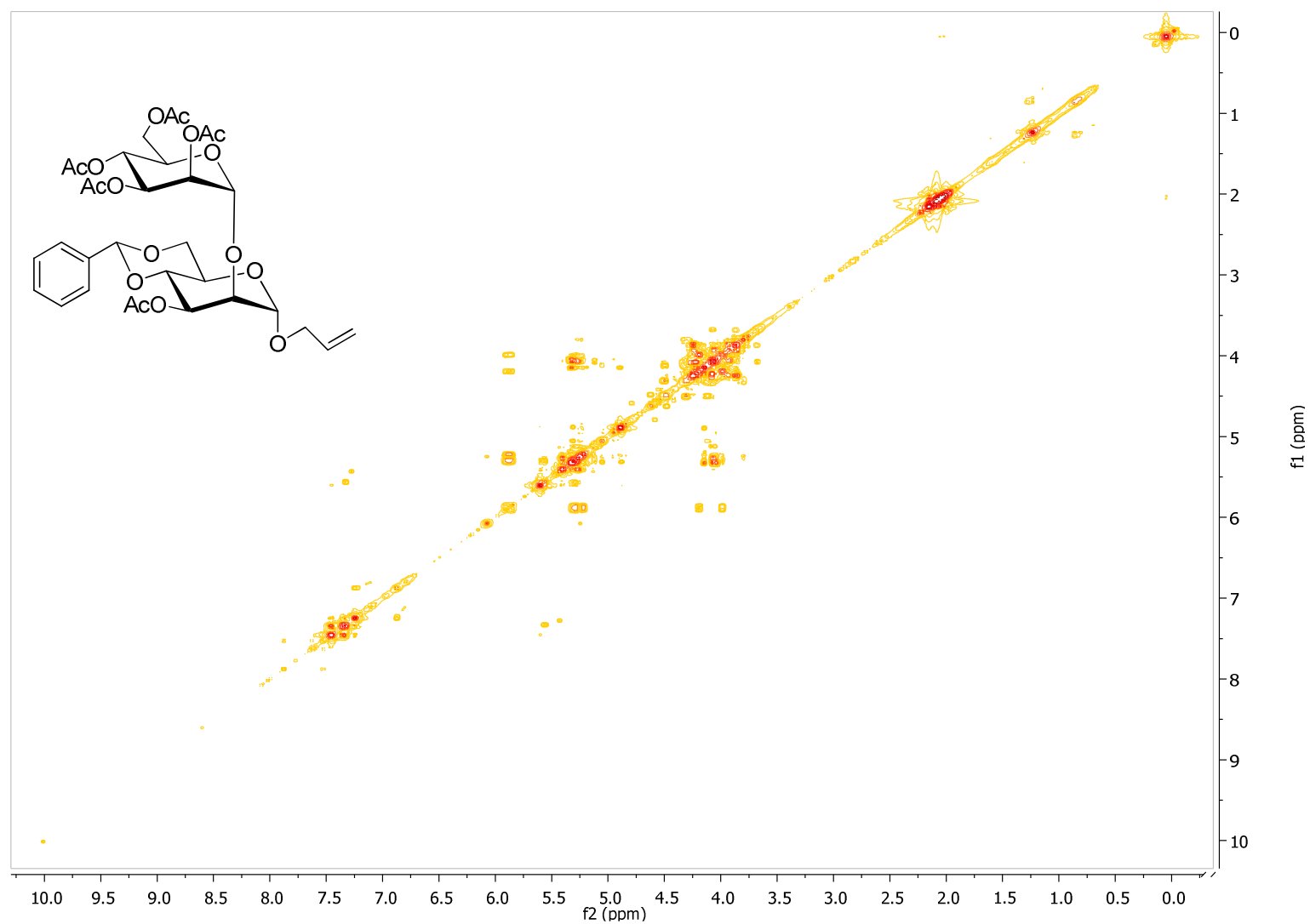


^{13}C NMR spectrum (75 MHz, CDCl₃) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**15**).

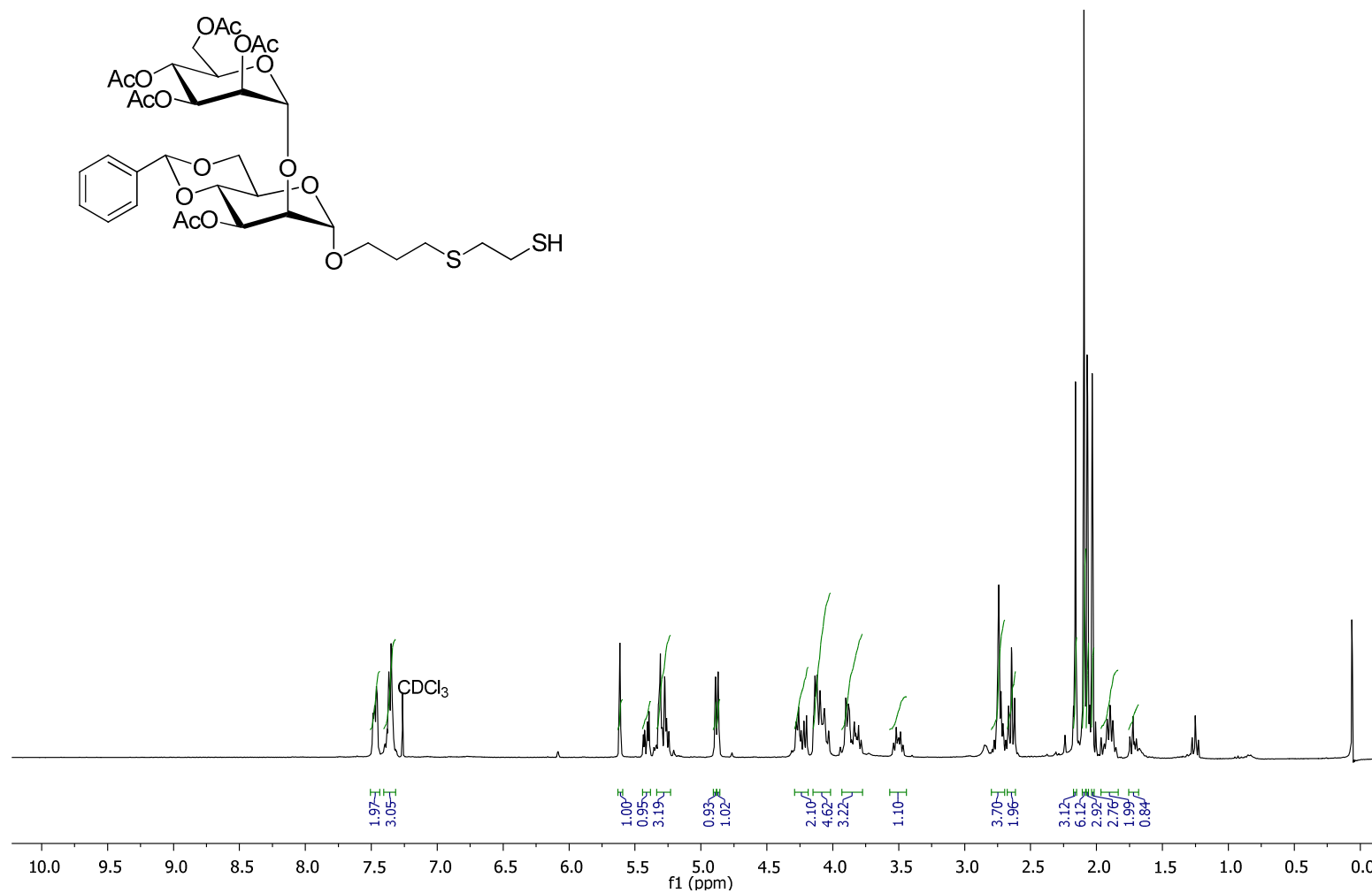


gHSQC NMR spectrum (500 MHz, CDCl₃) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**15**).

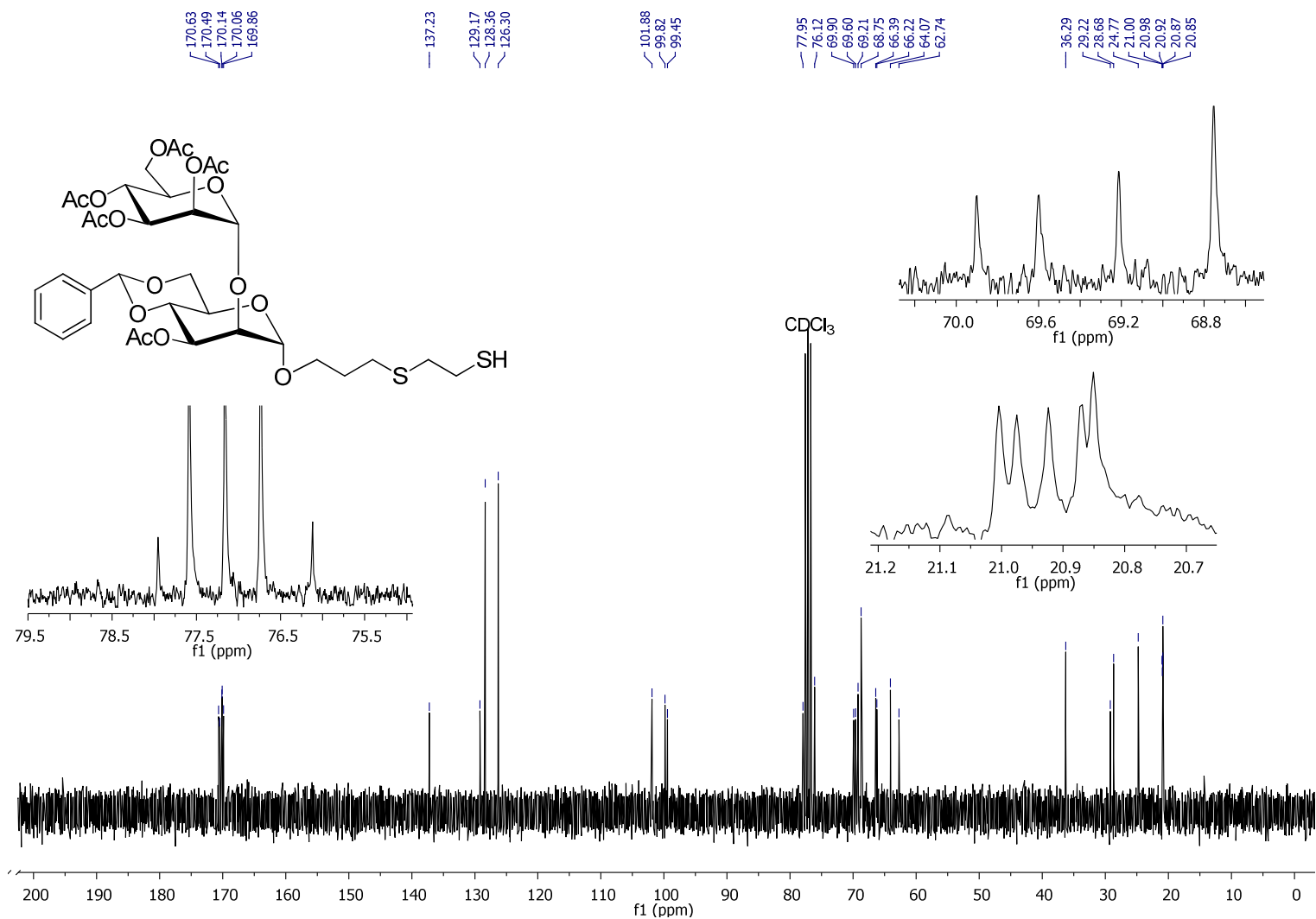




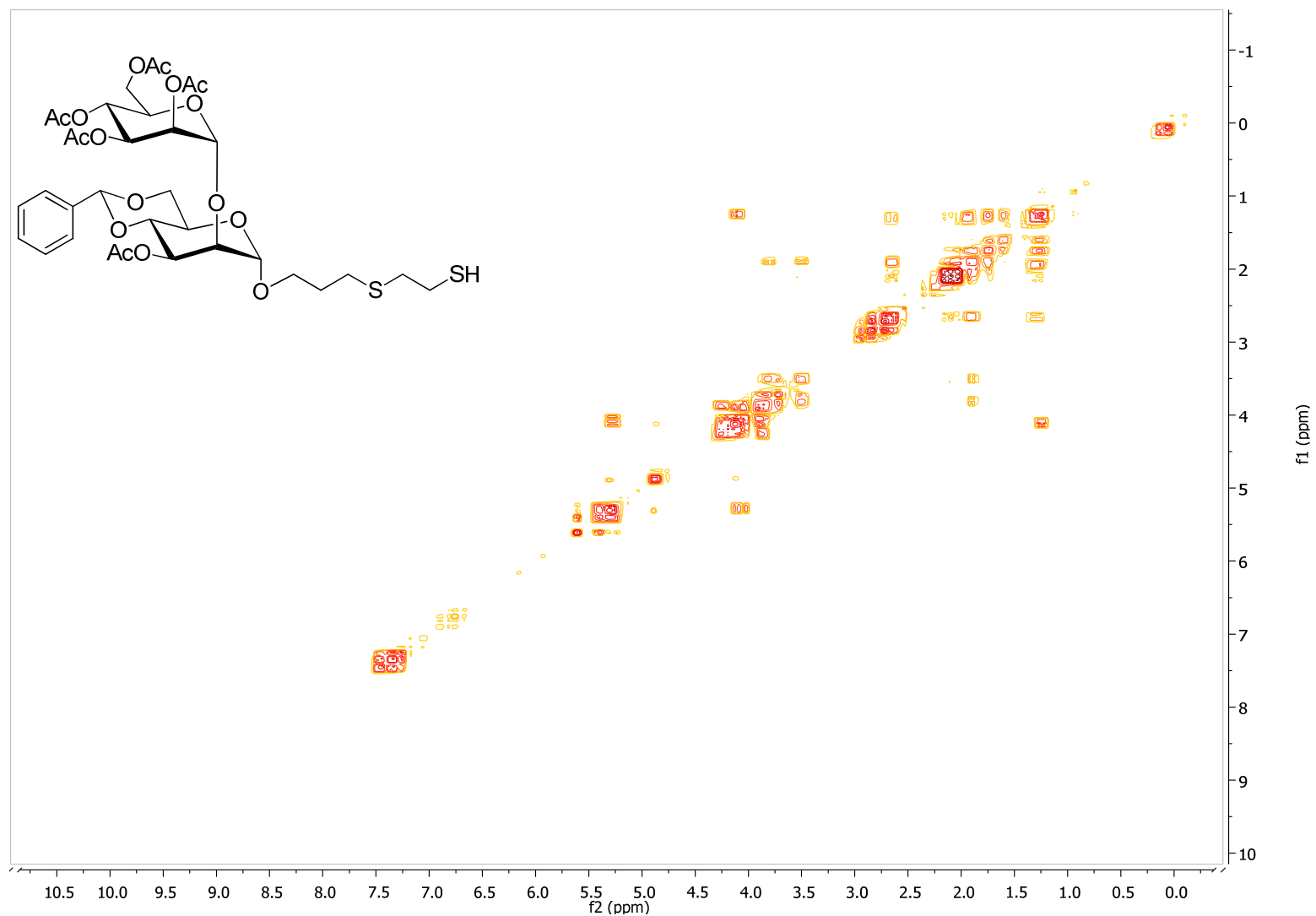
gCOSY NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**15**).



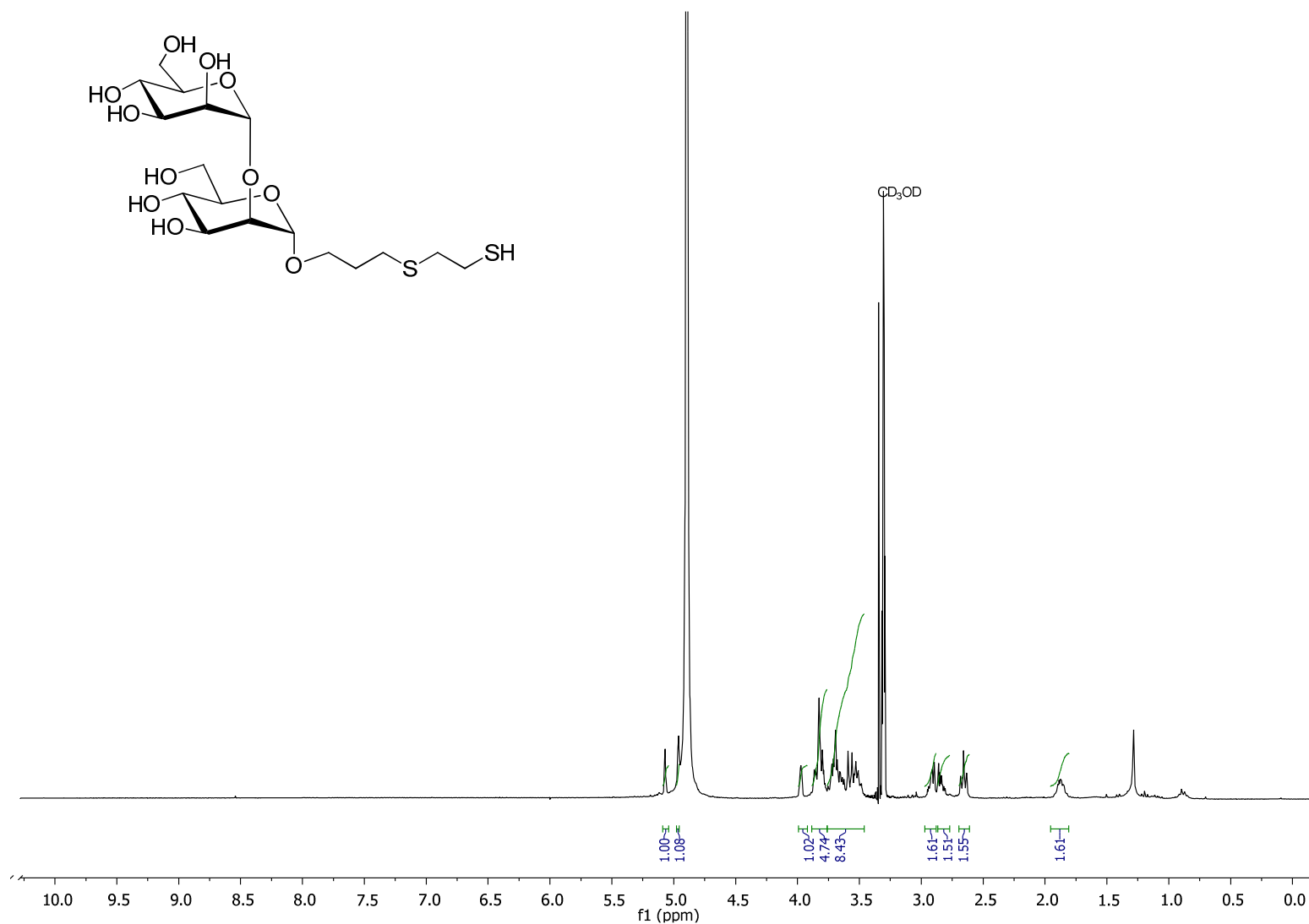
^1H NMR spectrum (300 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-O-acetyl-4,6-O-benzylidene α -D-mannopyranoside (**16**).



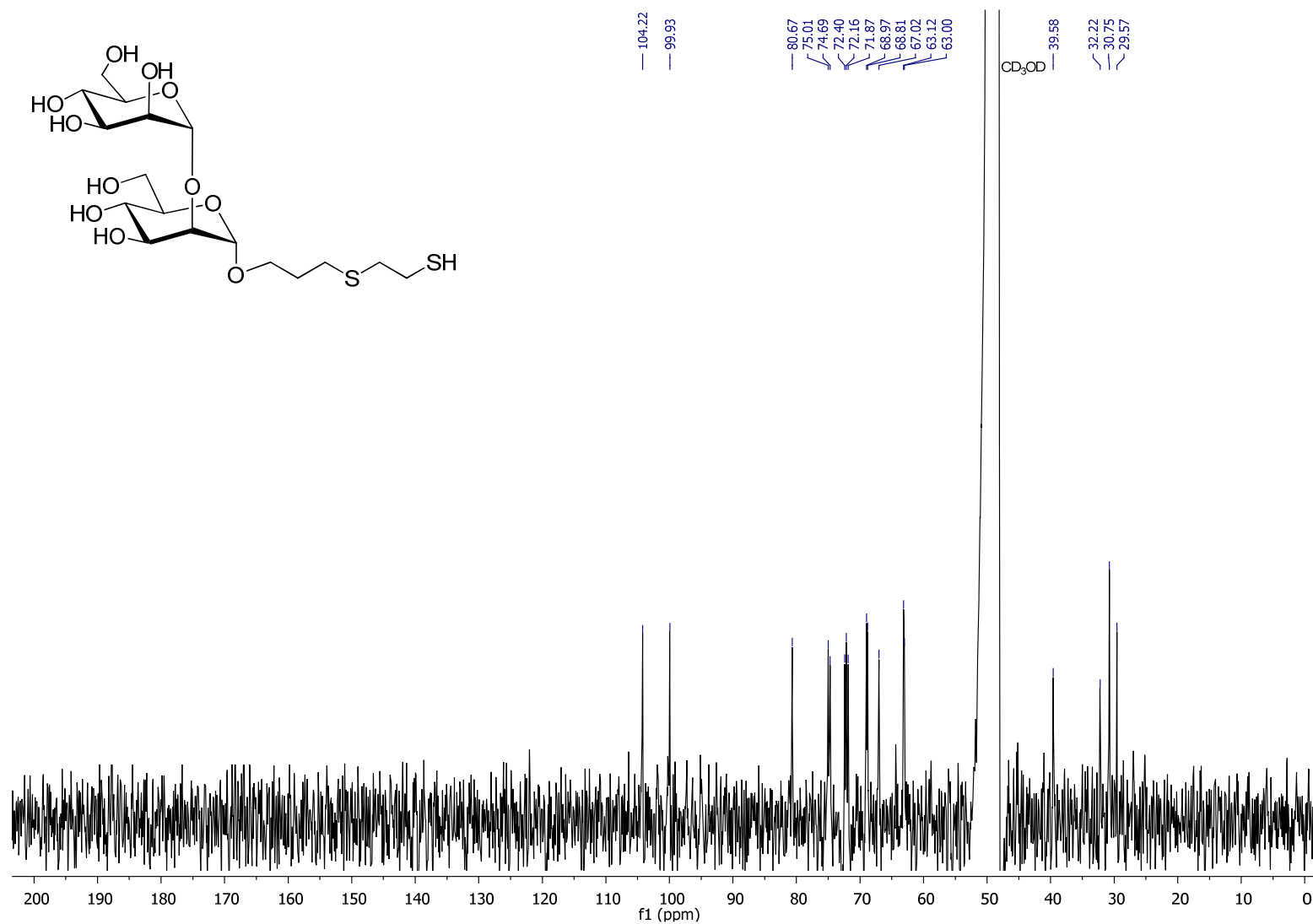
¹³C NMR spectrum (75 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)- 3-*O*-acetyl-4,6-*O*-benzylidene α -D-mannopyranoside (**16**).



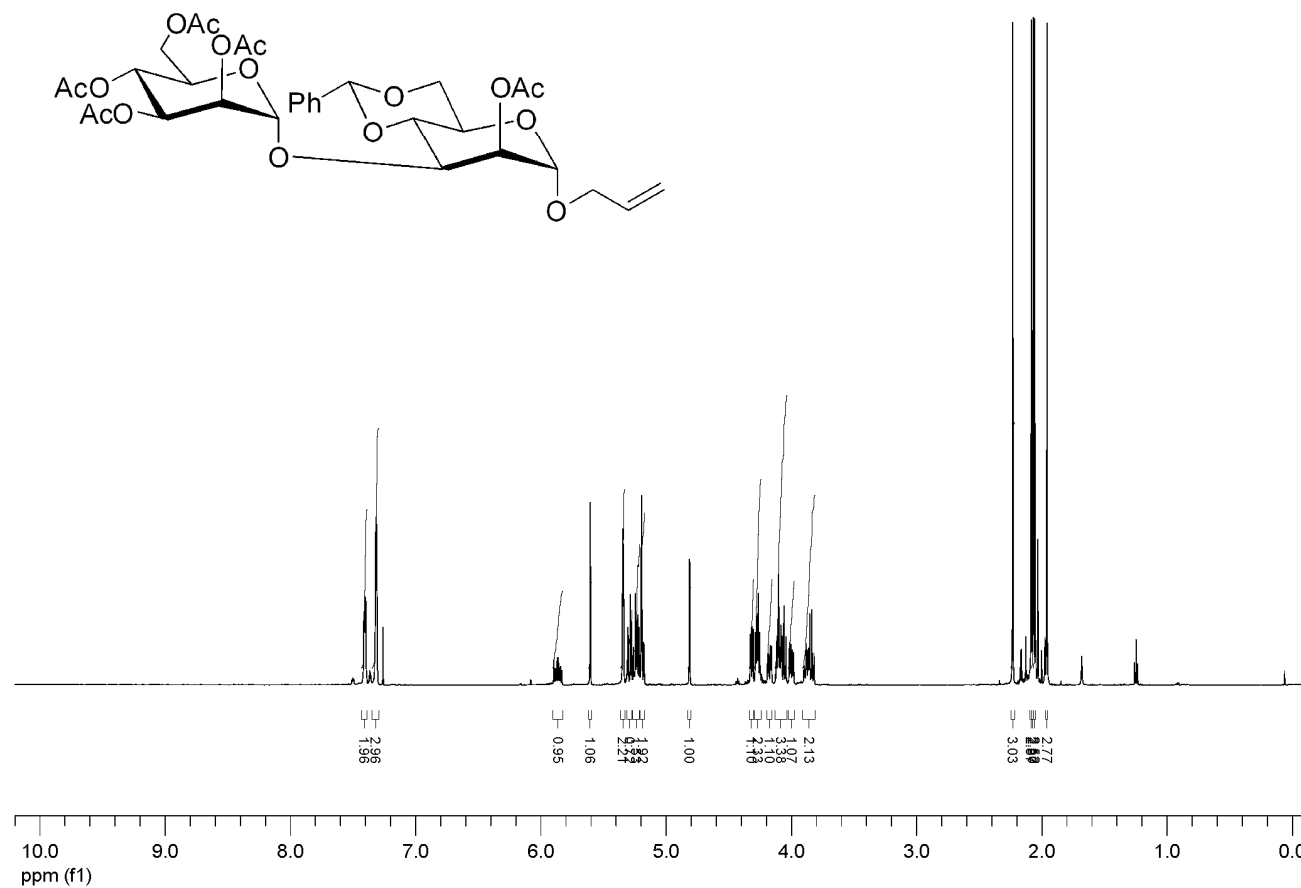
gCOSY NMR spectrum (300 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene α -D-mannopyranoside (**16**).



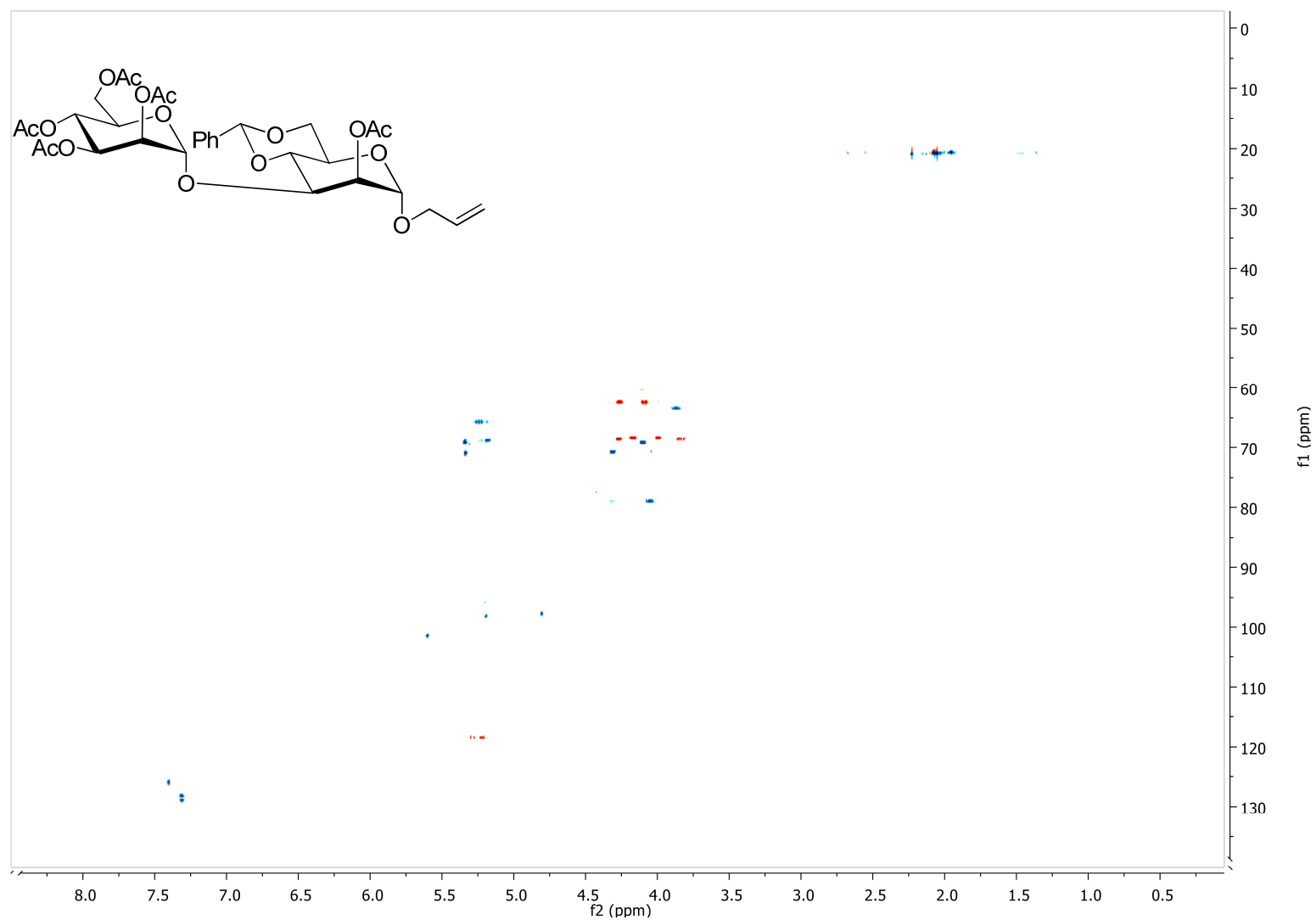
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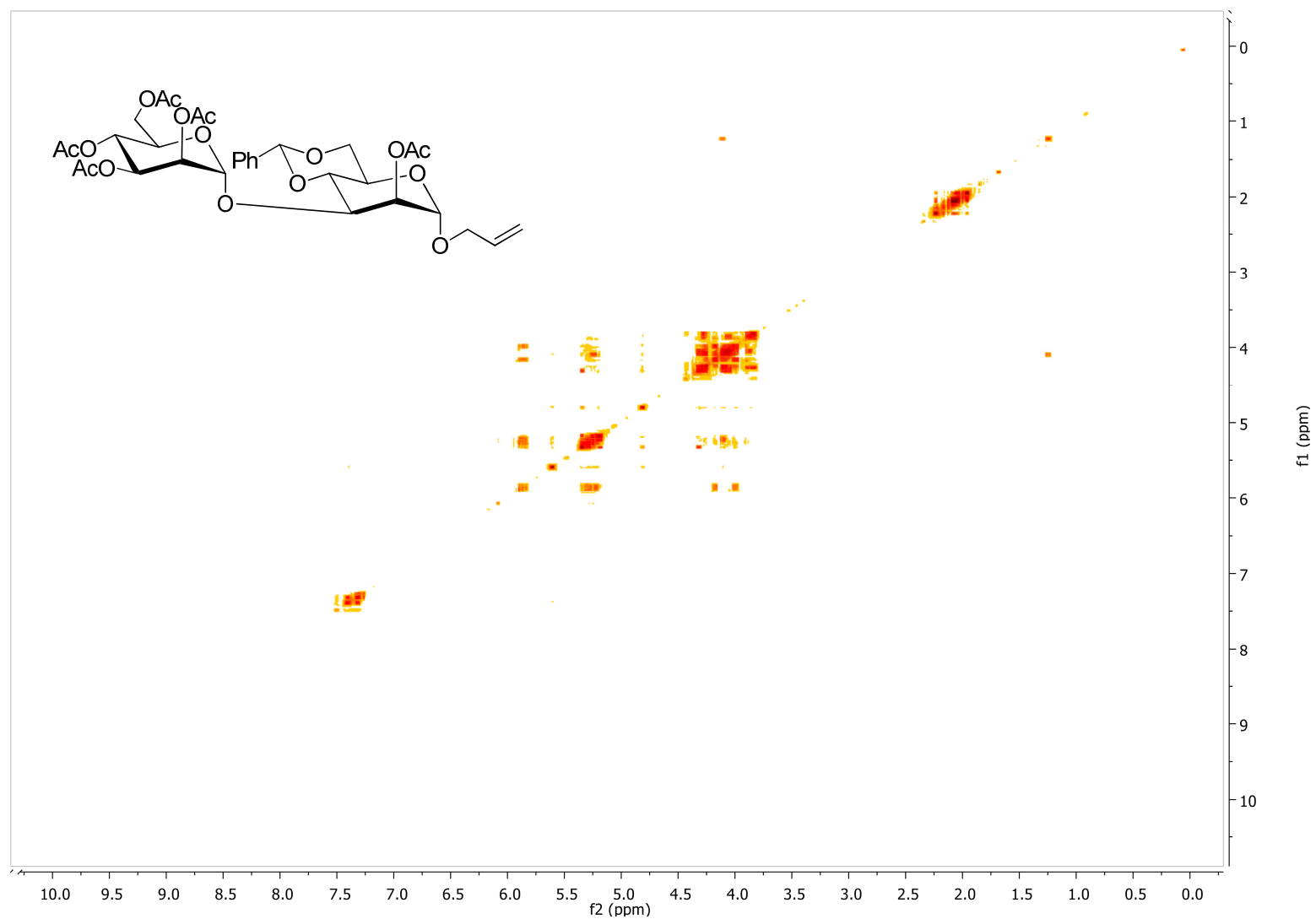


¹³C NMR spectrum (75 MHz, CD₃OD) of 3-[(2-sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (18).

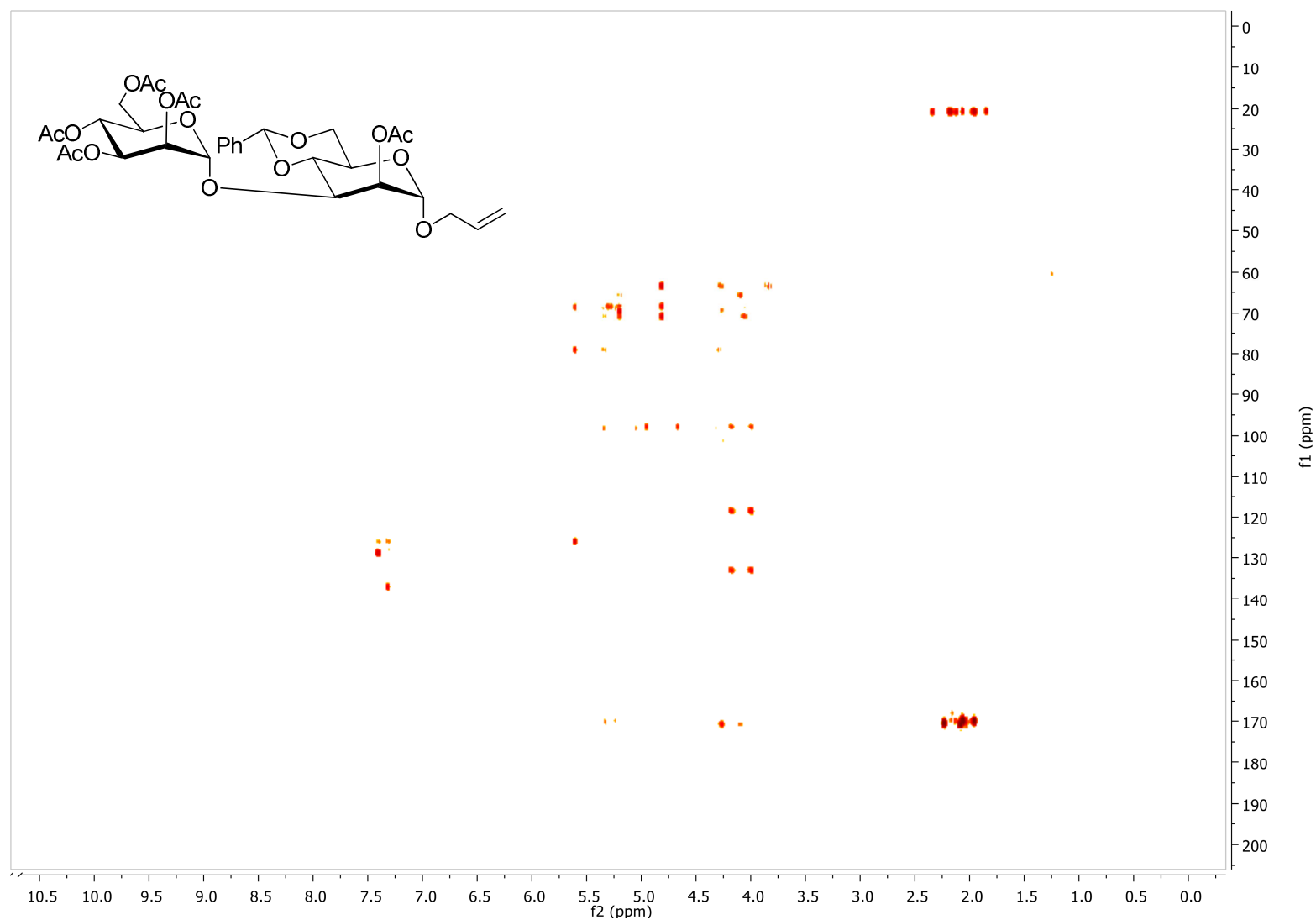


^1H NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-O-acetyl-4,6-O-benzylidene α -D-mannopyranoside (**20**).

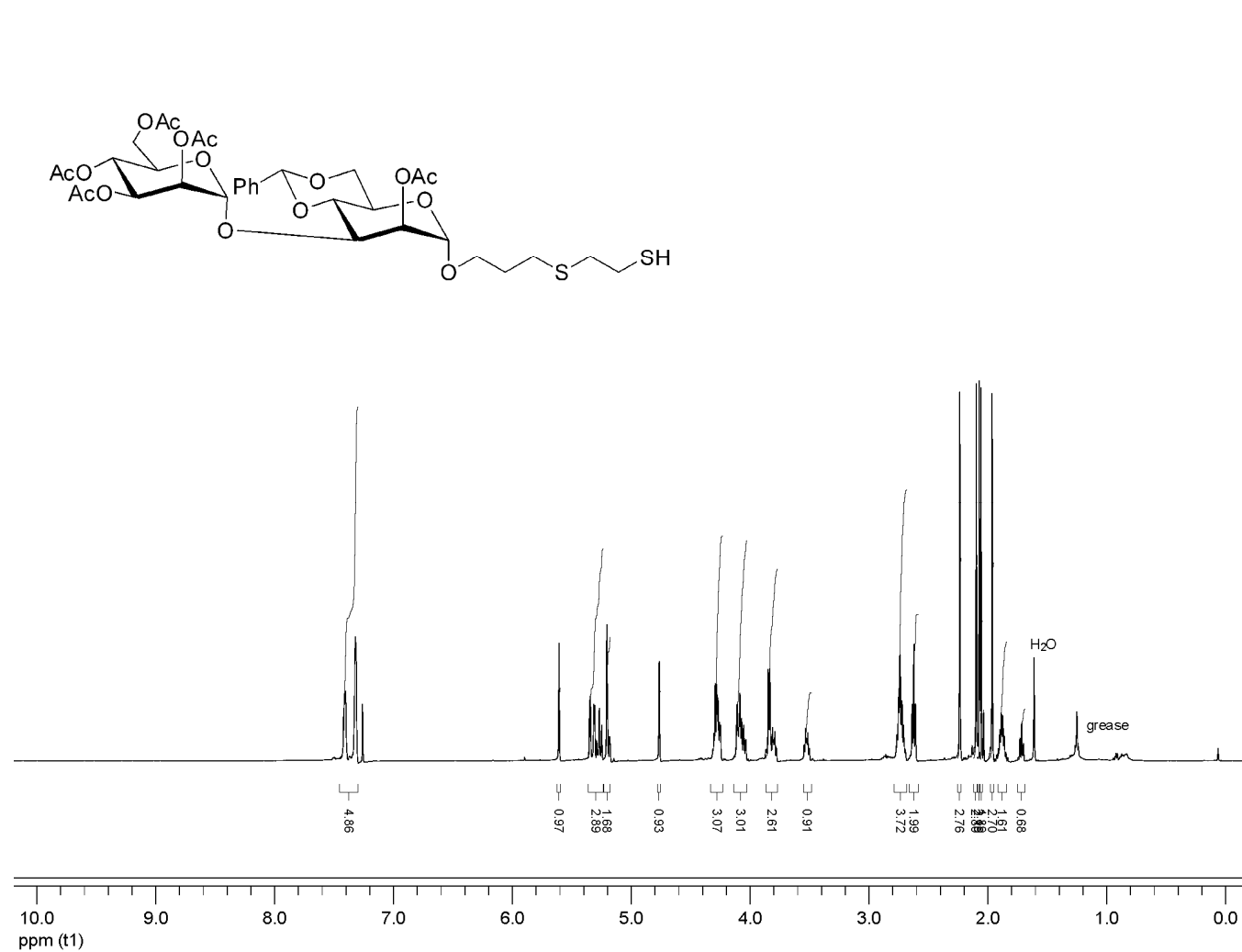




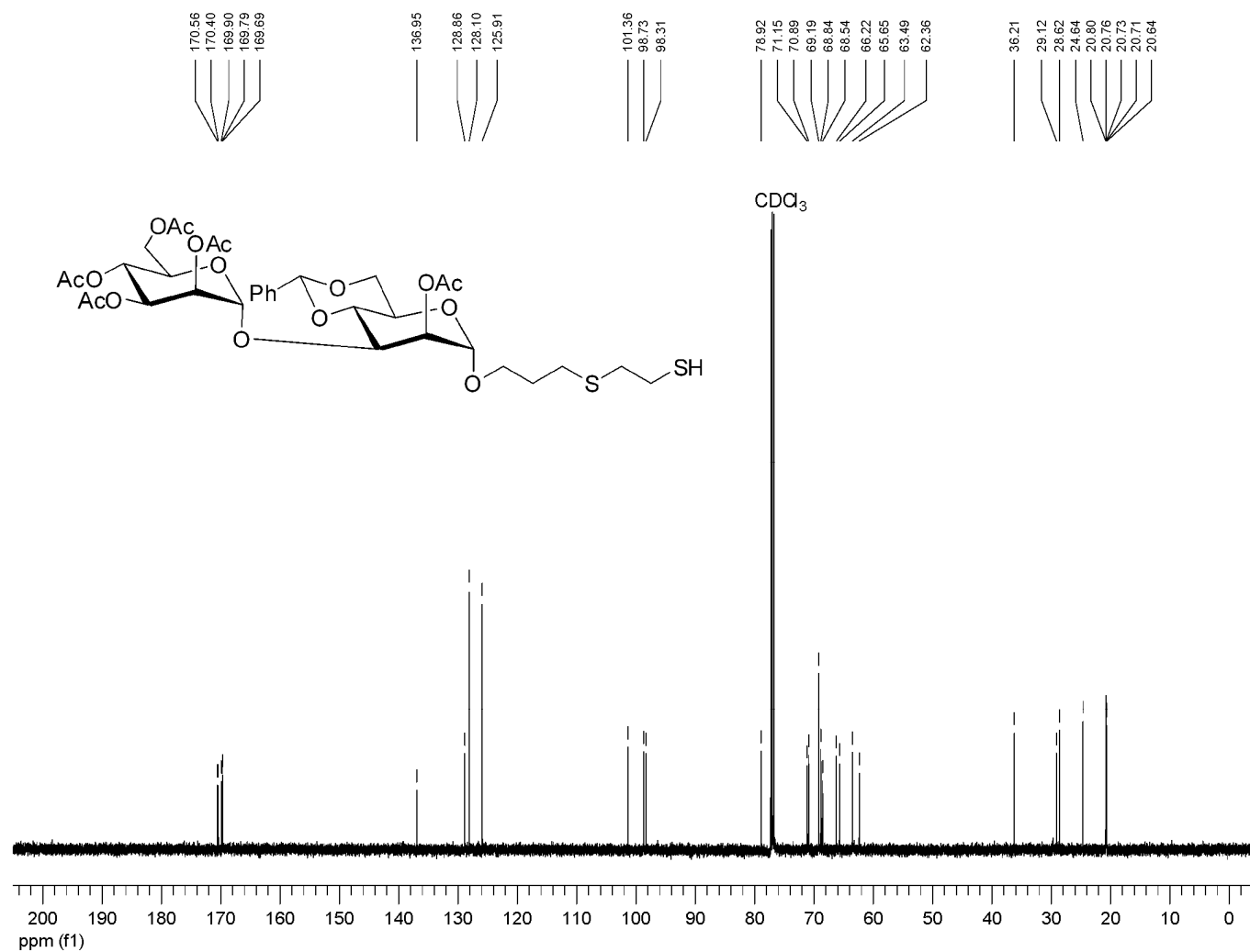
gCOSY NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene α -D-mannopyranoside (**20**).



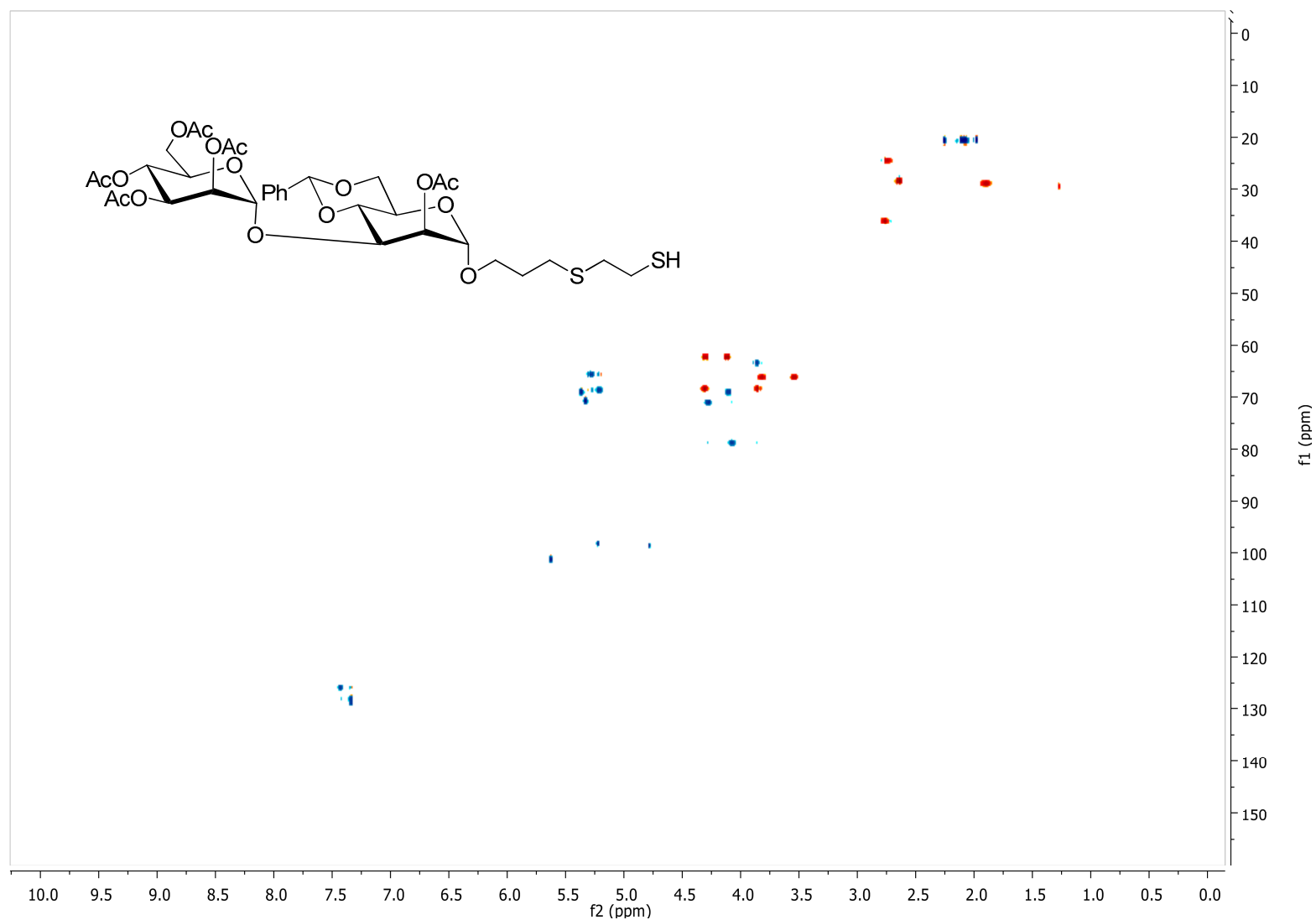
gHMBC NMR spectrum (500 MHz, CDCl_3) of Allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene α -D-mannopyranoside (**20**).

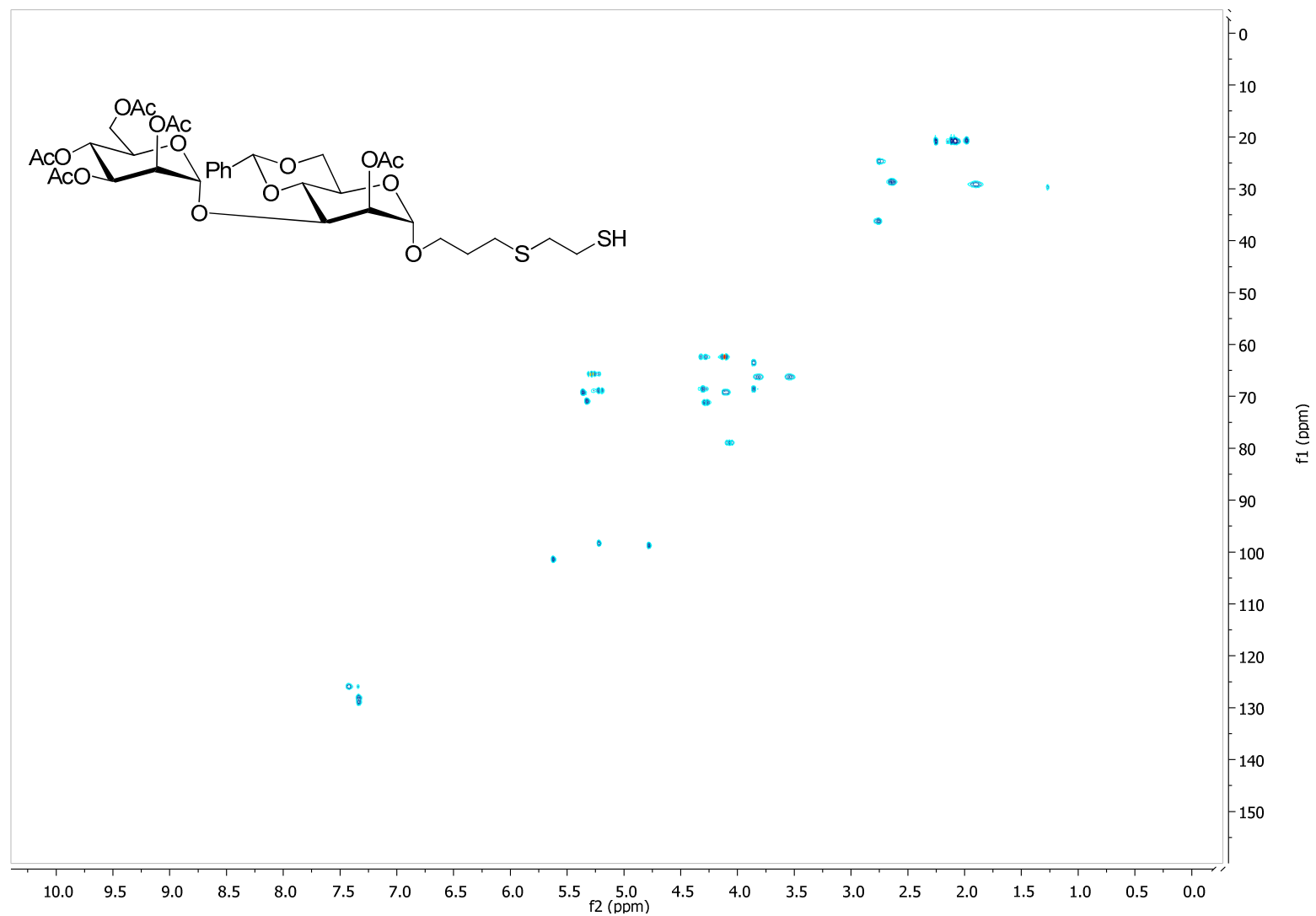


^1H NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)- 2-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**21**).

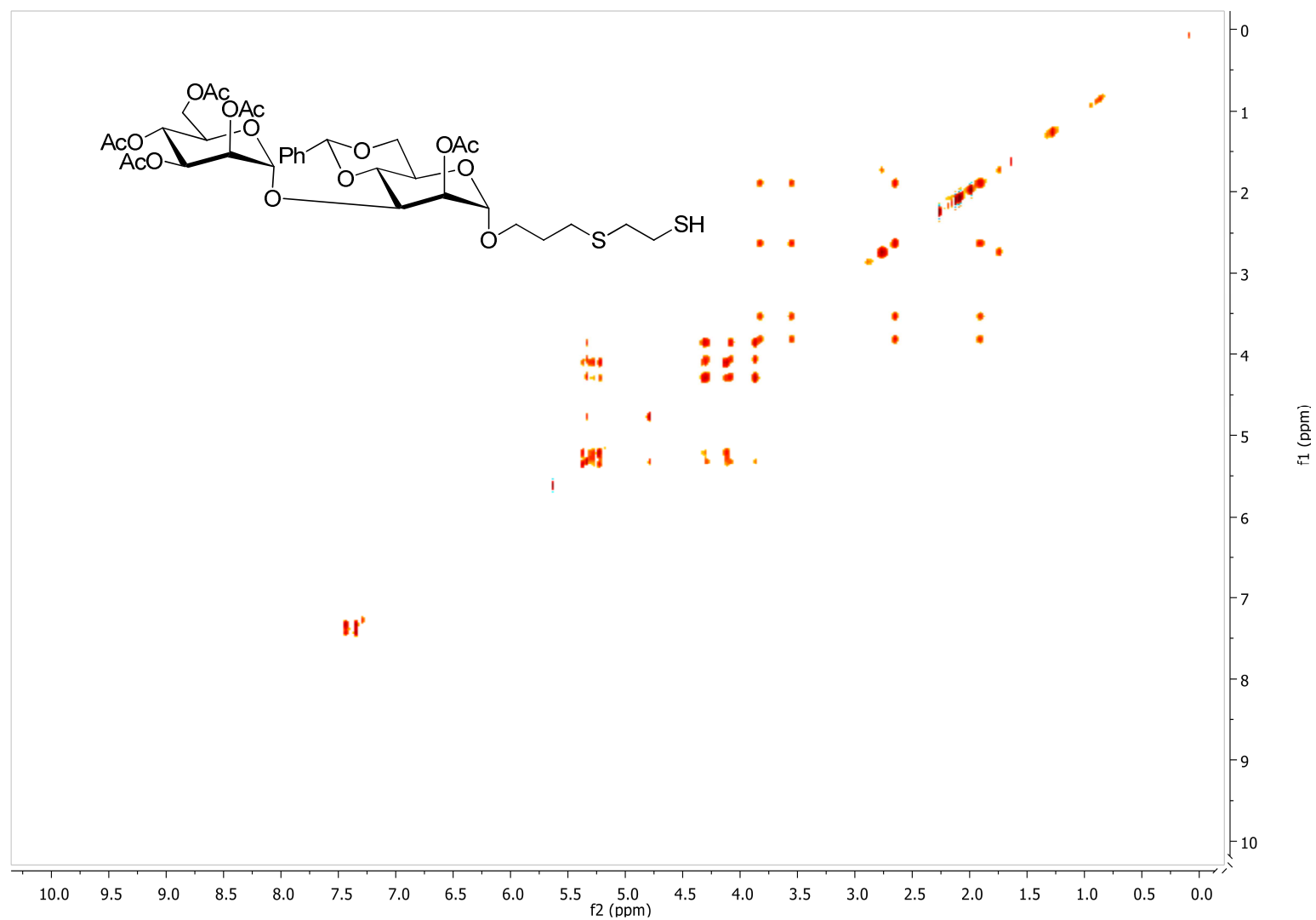


^{13}C NMR spectrum (125 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)- 2-O-acetyl-4,6-O-benzylidene- α -D-mannopyranoside (**21**).

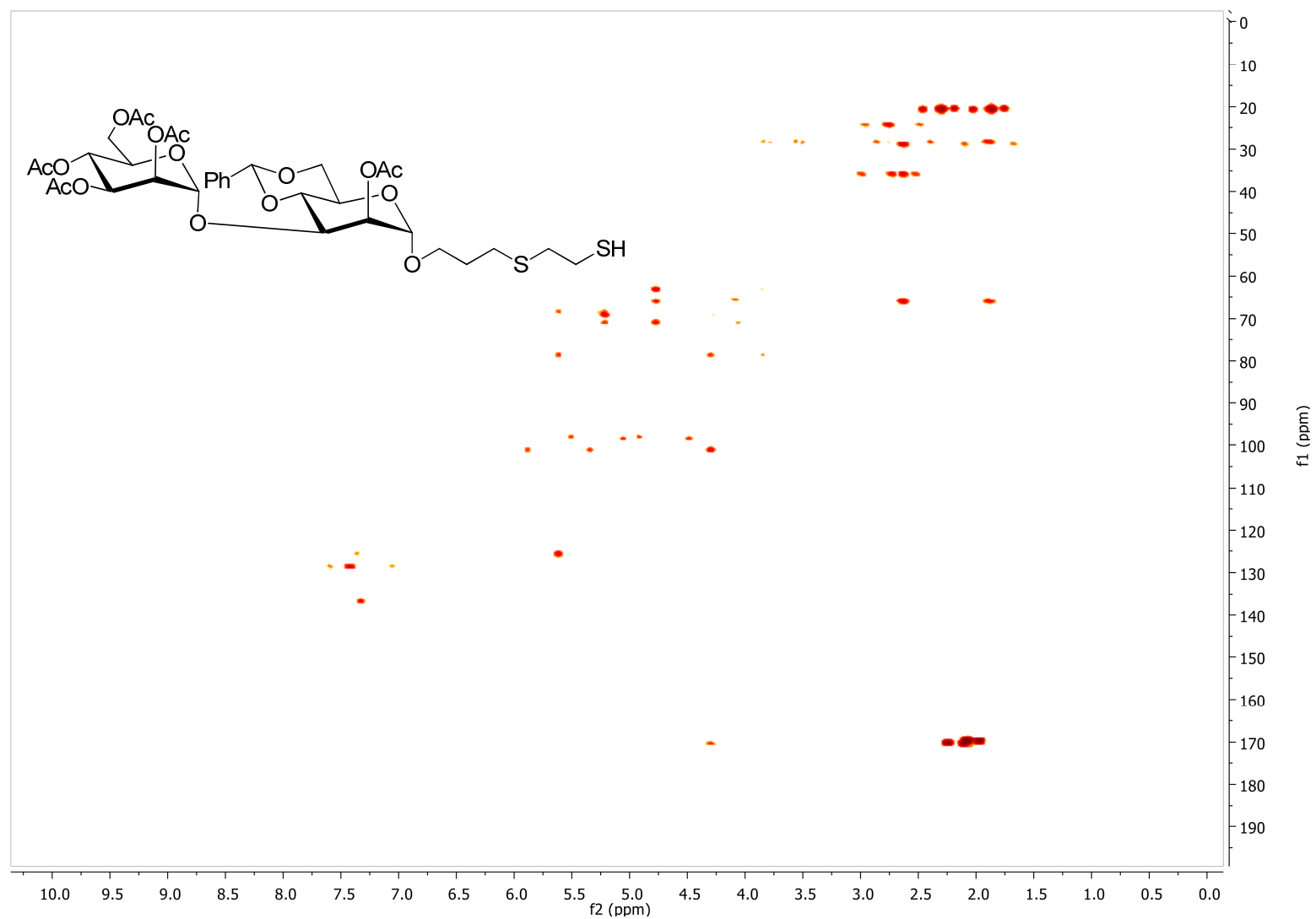




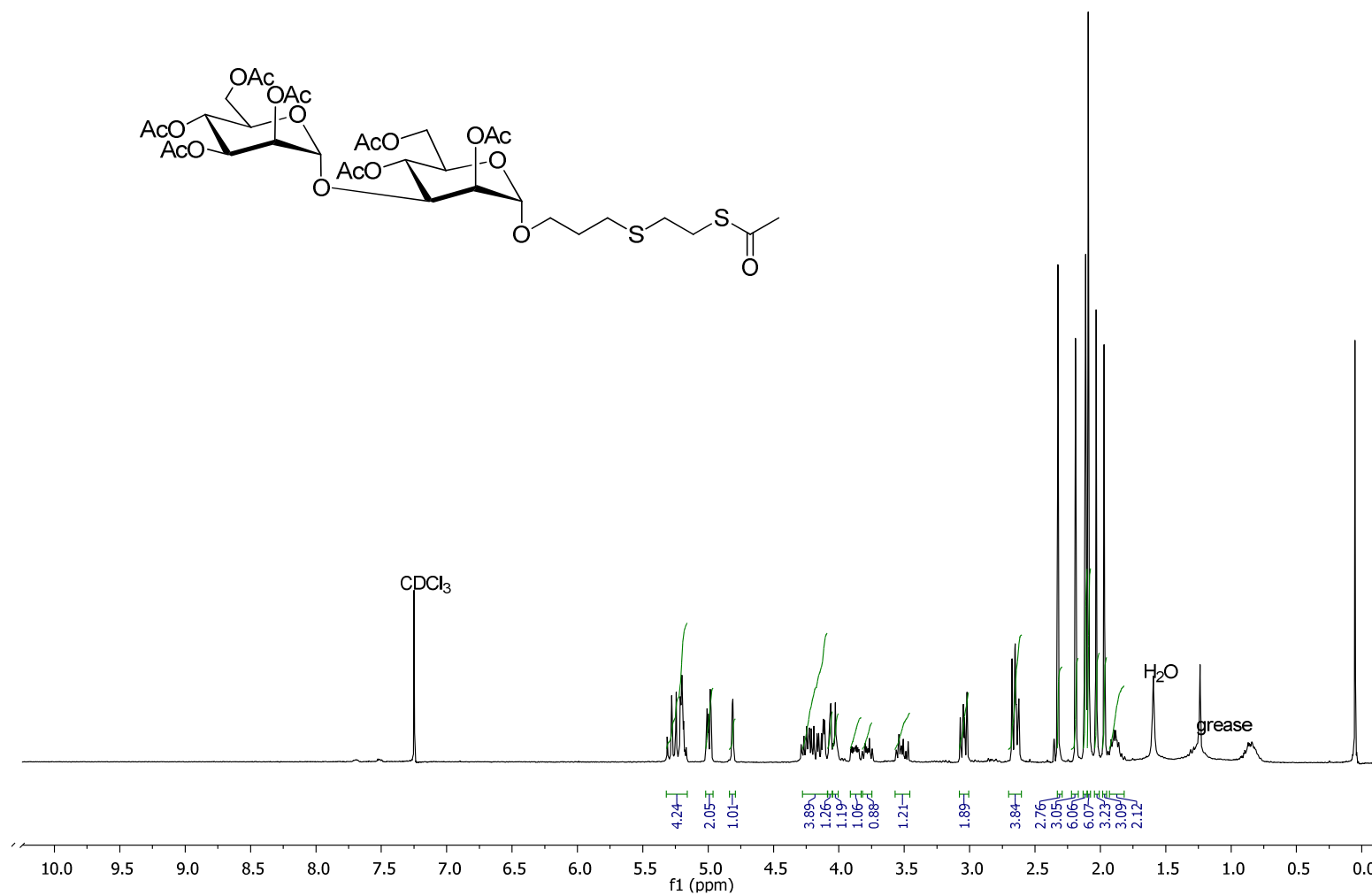
gHMQC NMR spectrum (500 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**21**).



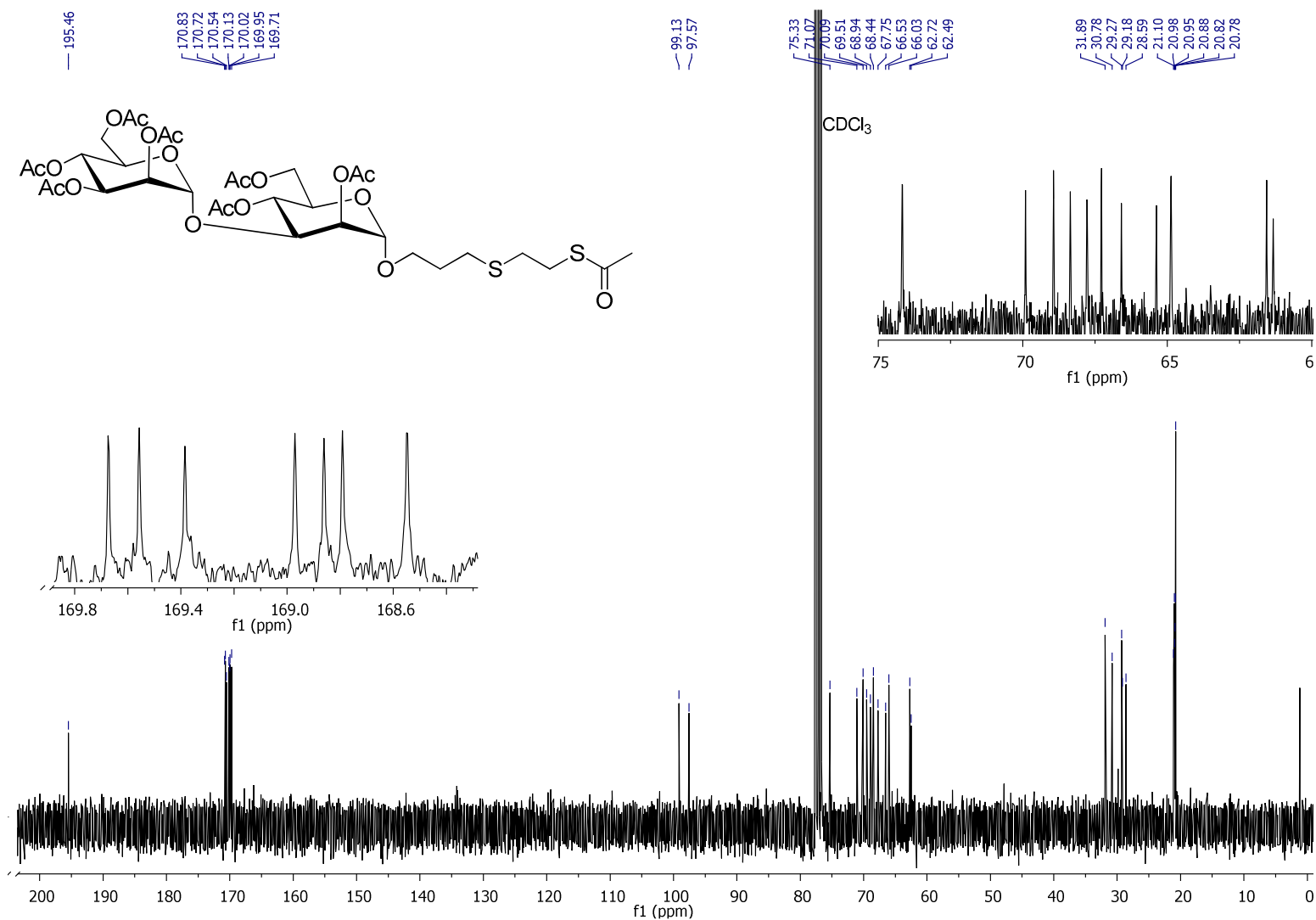
TOCSY NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)- 2-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**21**).



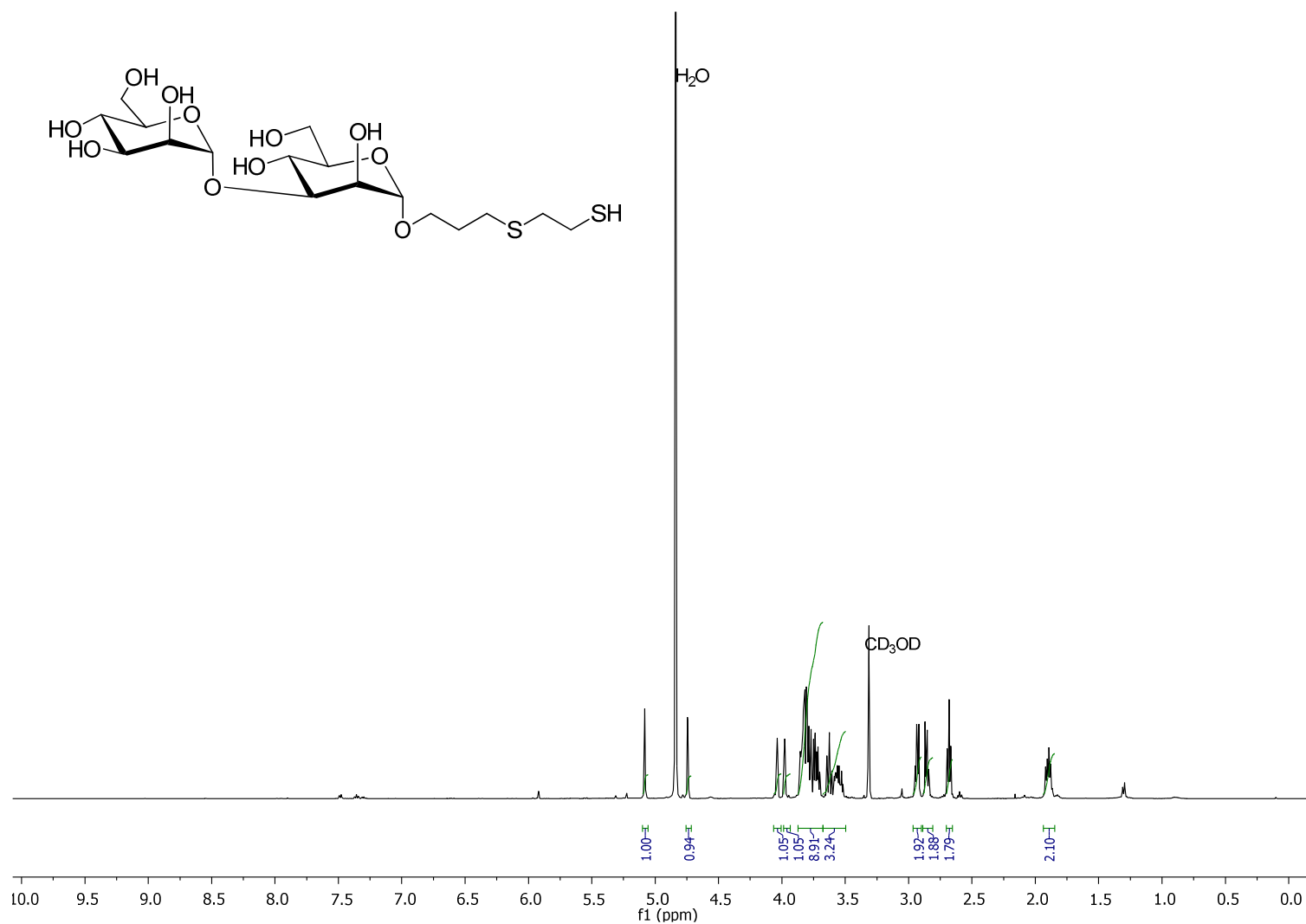
gHMBC NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)- 2-O-acetyl-4,6-O-benzylidene- α -D-mannopyranoside (**21**).



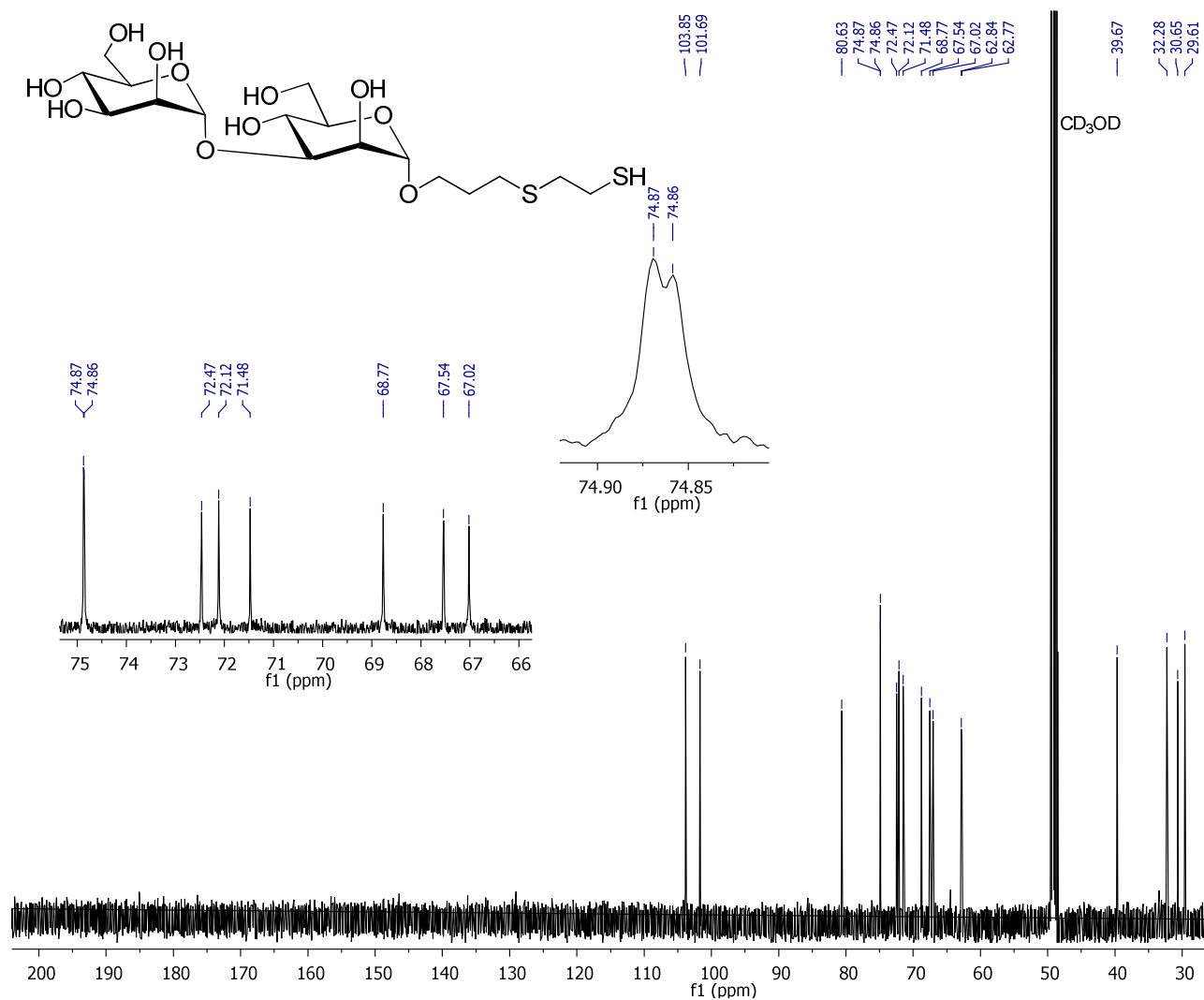
¹H NMR spectrum (500 MHz, CDCl₃) of *S*-[2-[(3-{[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 3)-2,4,6-tri-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**23**).



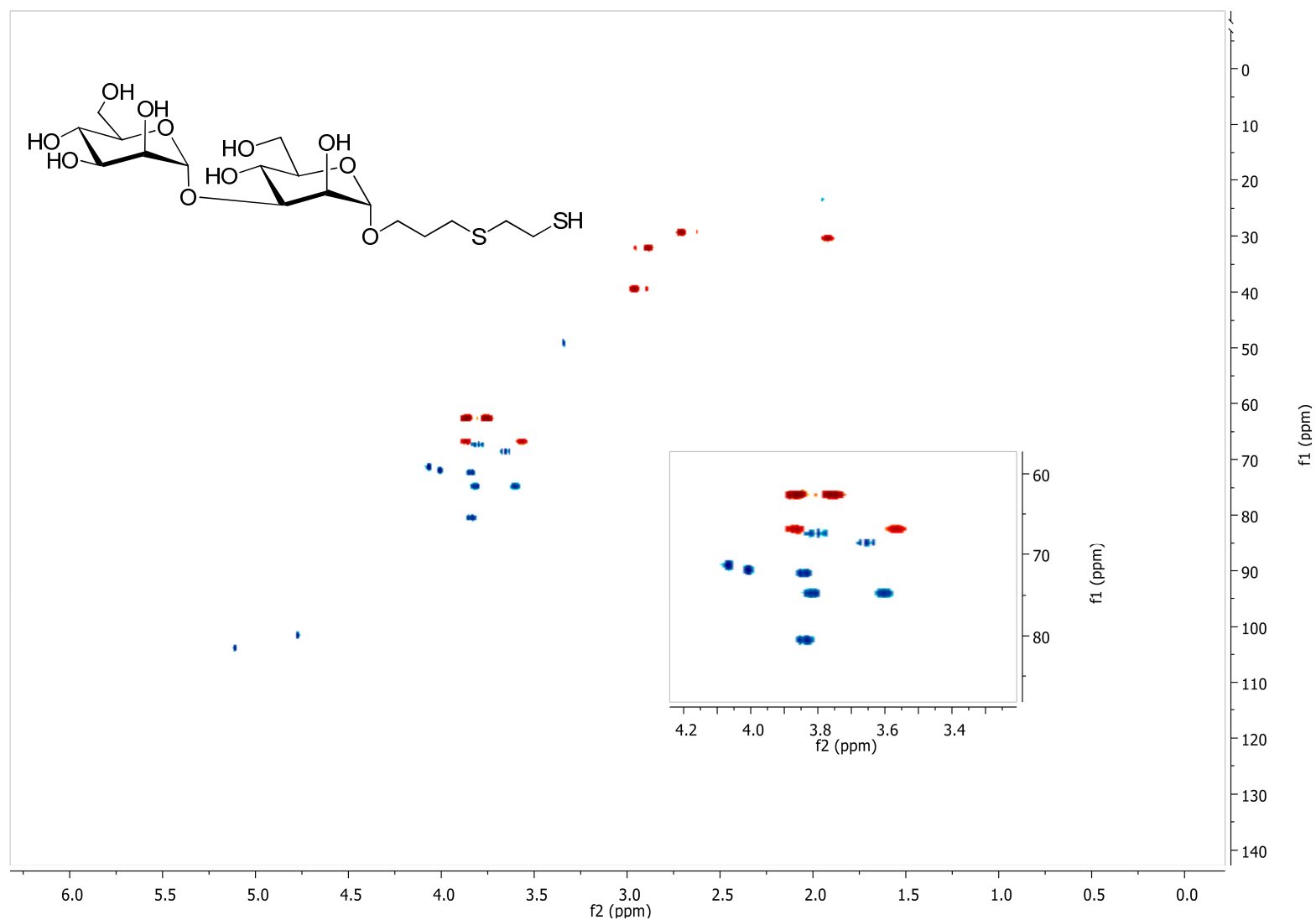
¹³C NMR spectrum (125 MHz, CDCl₃) of *S*-[2-[(3-[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 3)-2,4,6-tri-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**23**).



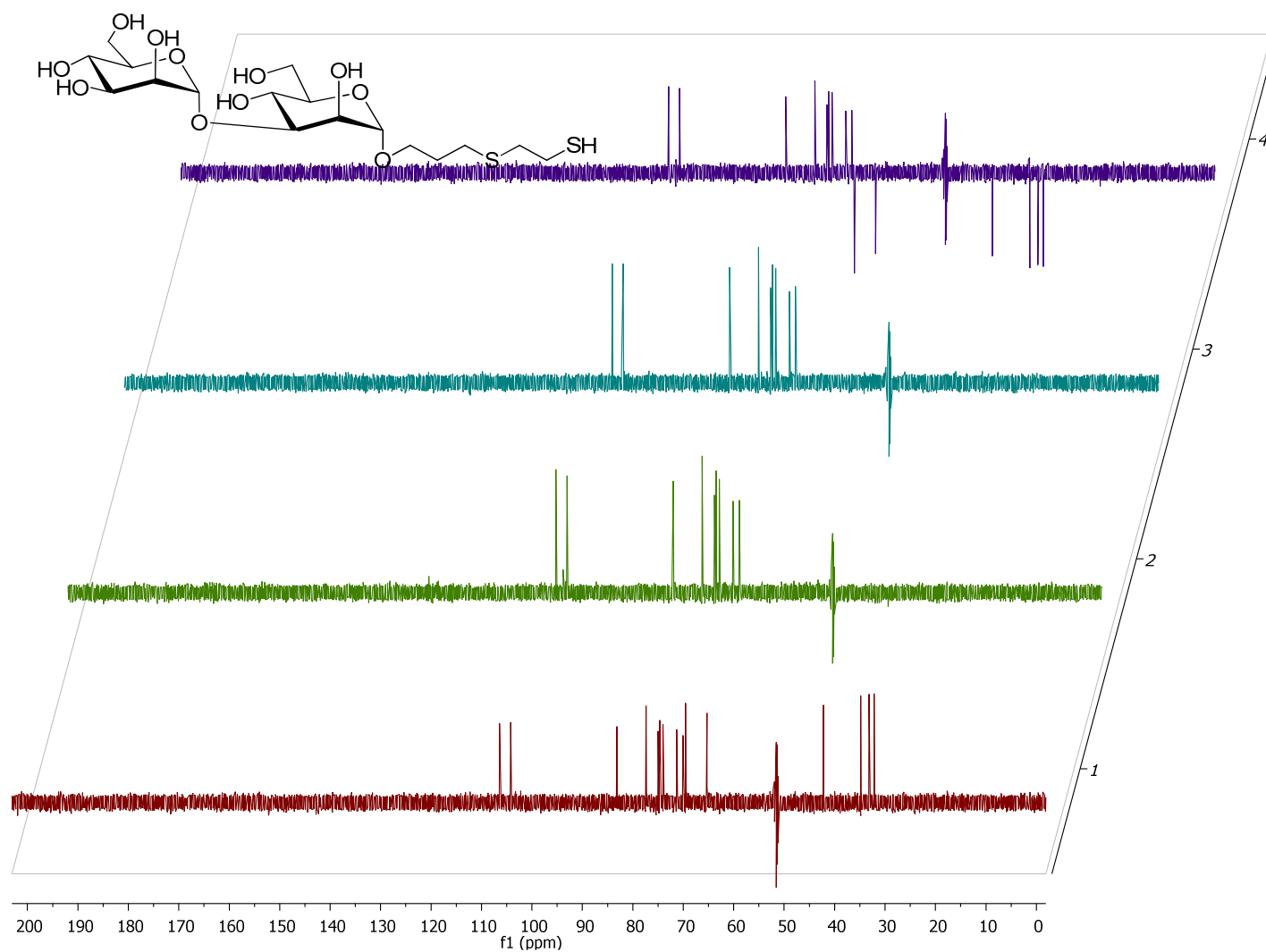
¹H NMR spectrum (500 MHz, CD₃OD) of 3-[(2-sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside (24).



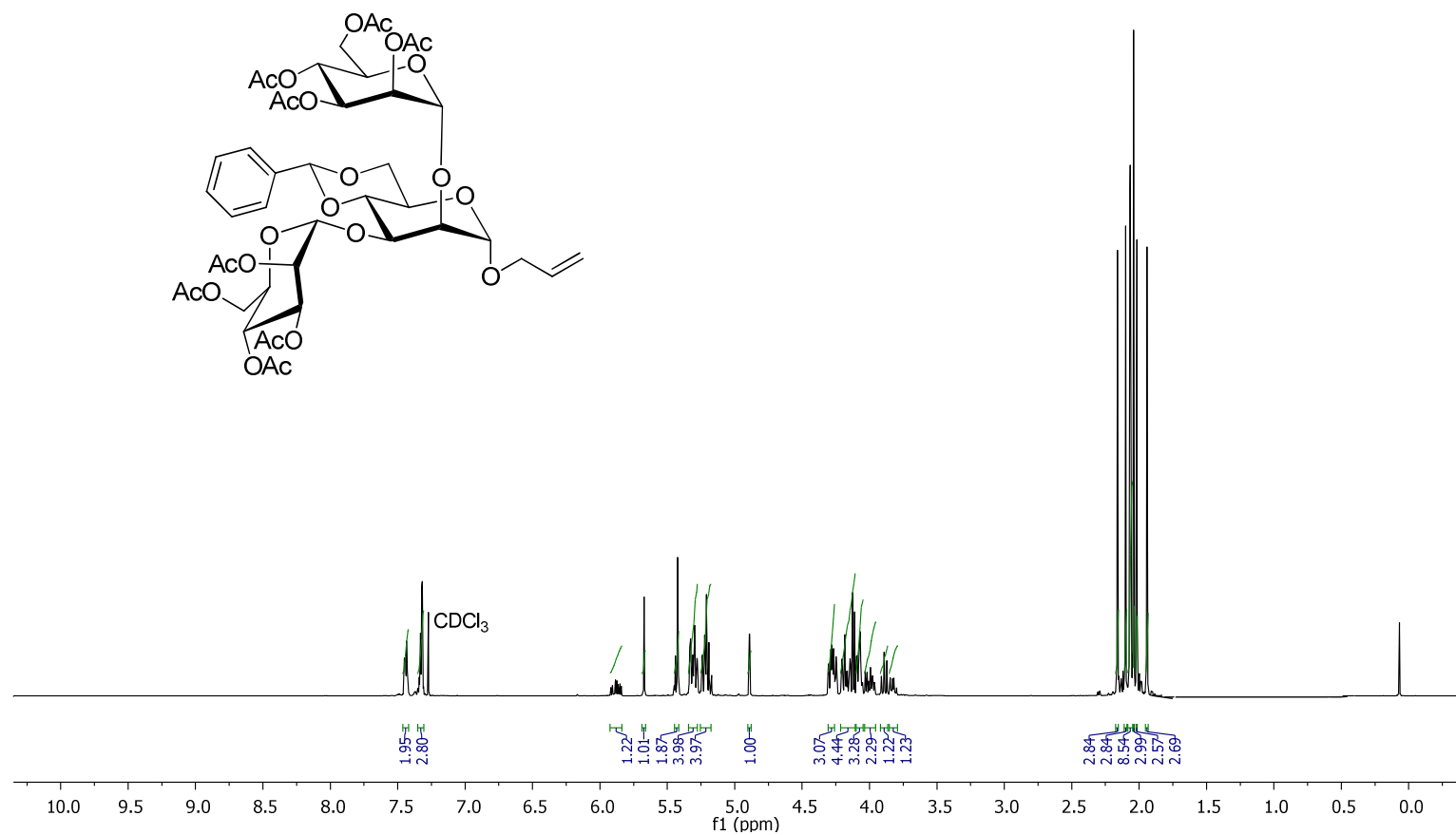
^{13}C NMR spectrum (125 MHz, CD_3OD) of 3-[(2-sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1→3)- α -D-mannopyranoside (24).



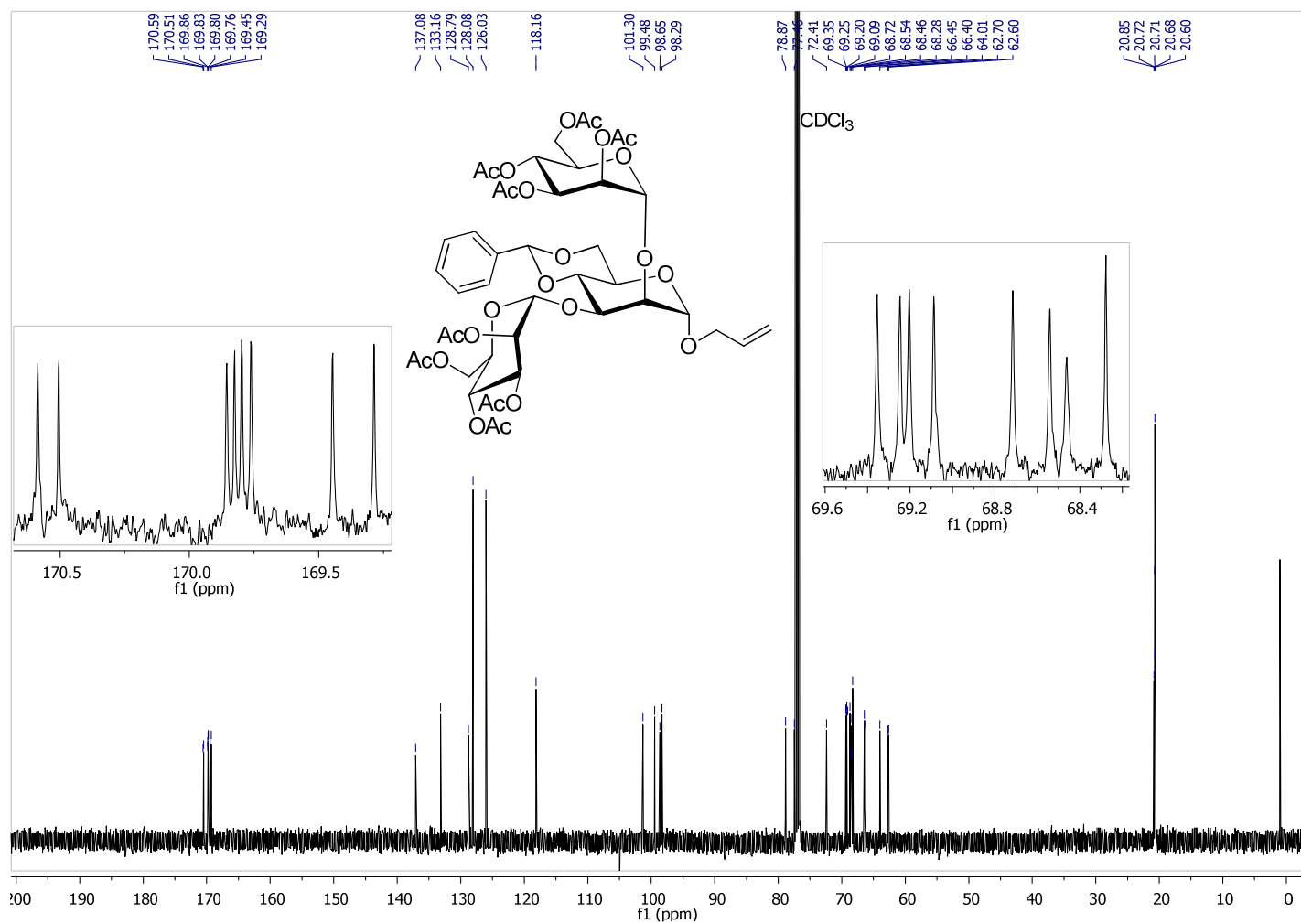
gHSQC NMR spectrum (500 MHz, CD₃OD) of 3-[(2-sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside (**24**).



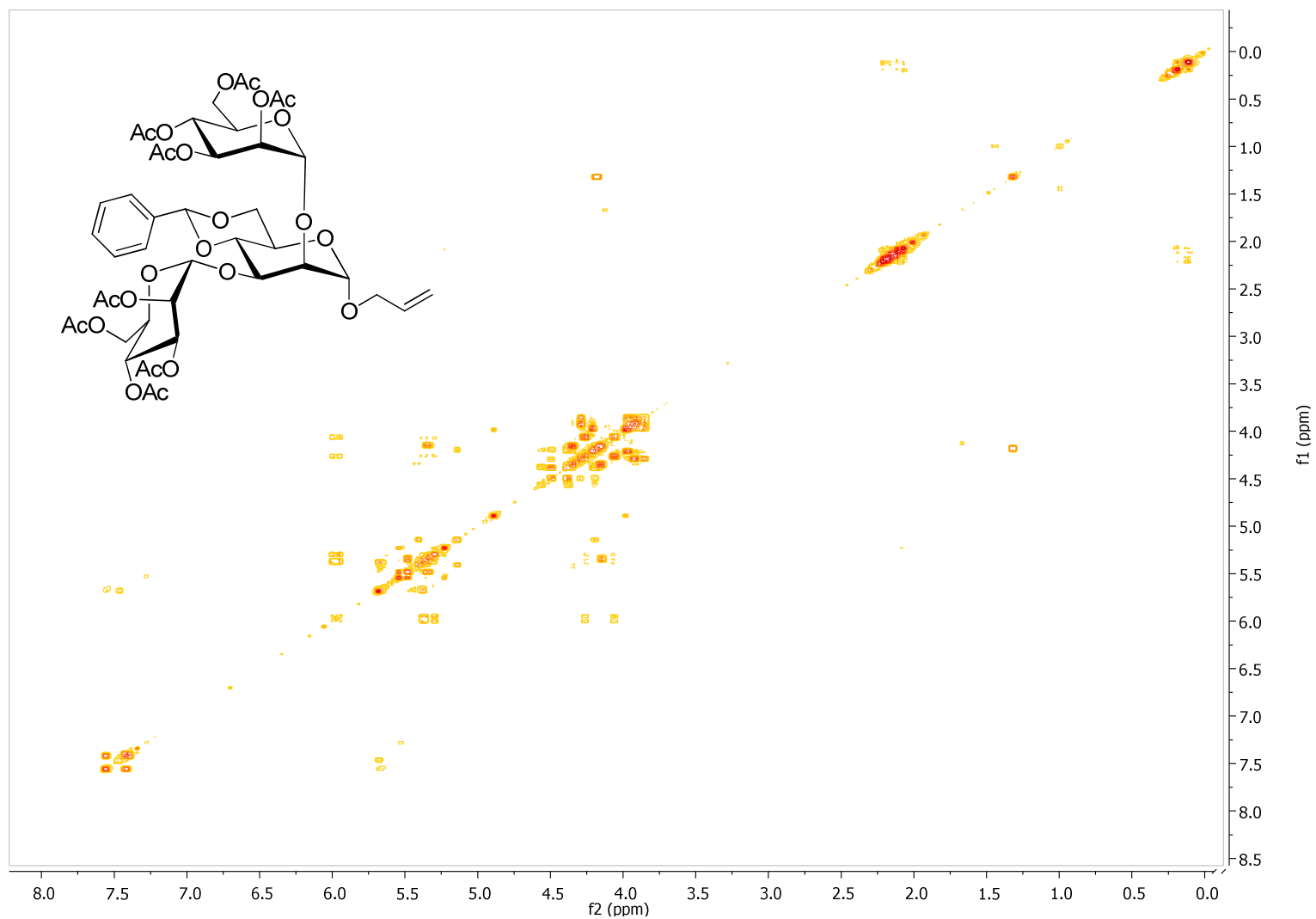
DEPT NMR spectrum (500 MHz, CD₃OD) of 3-[(2-sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside (24).



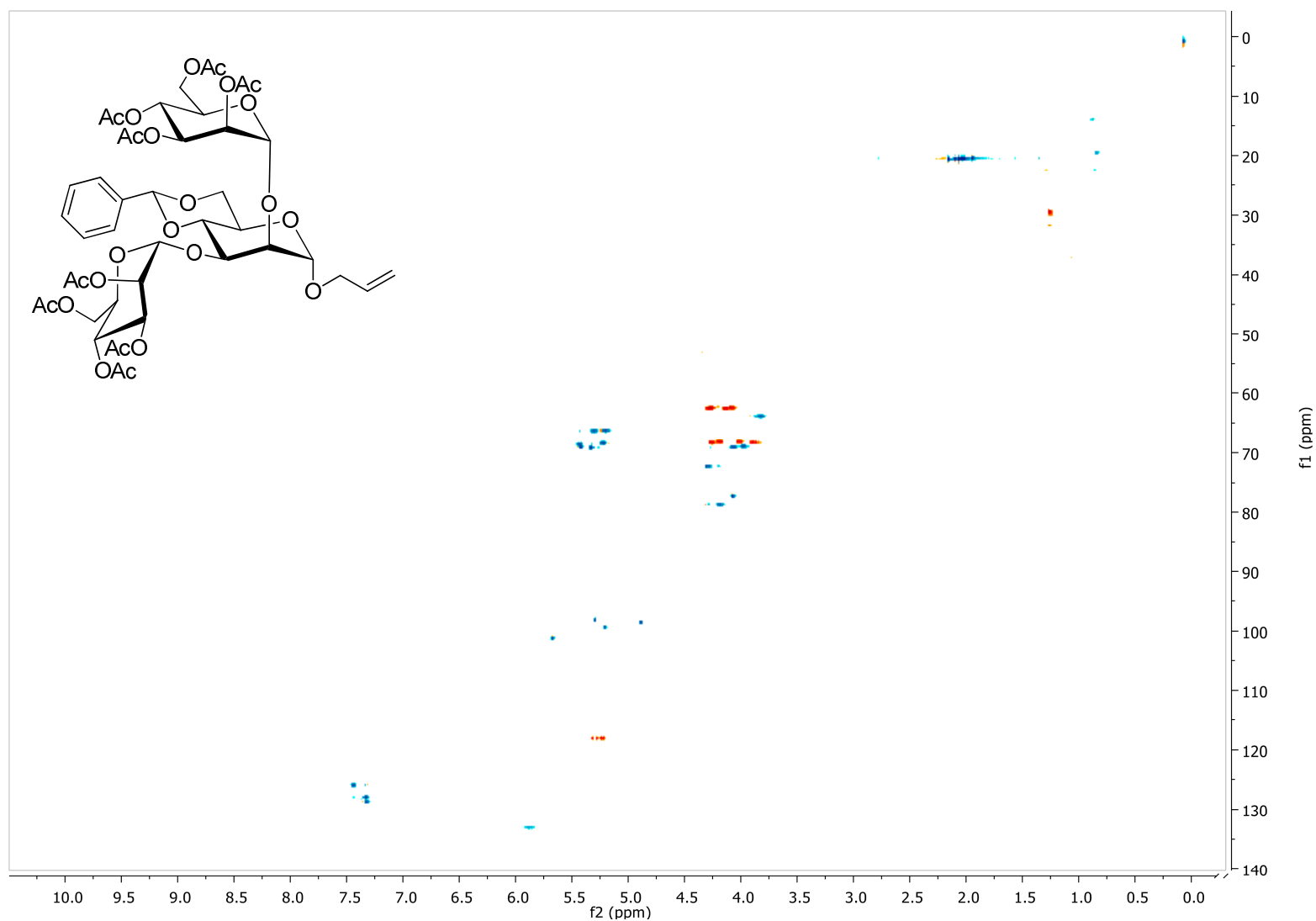
^1H NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (25).



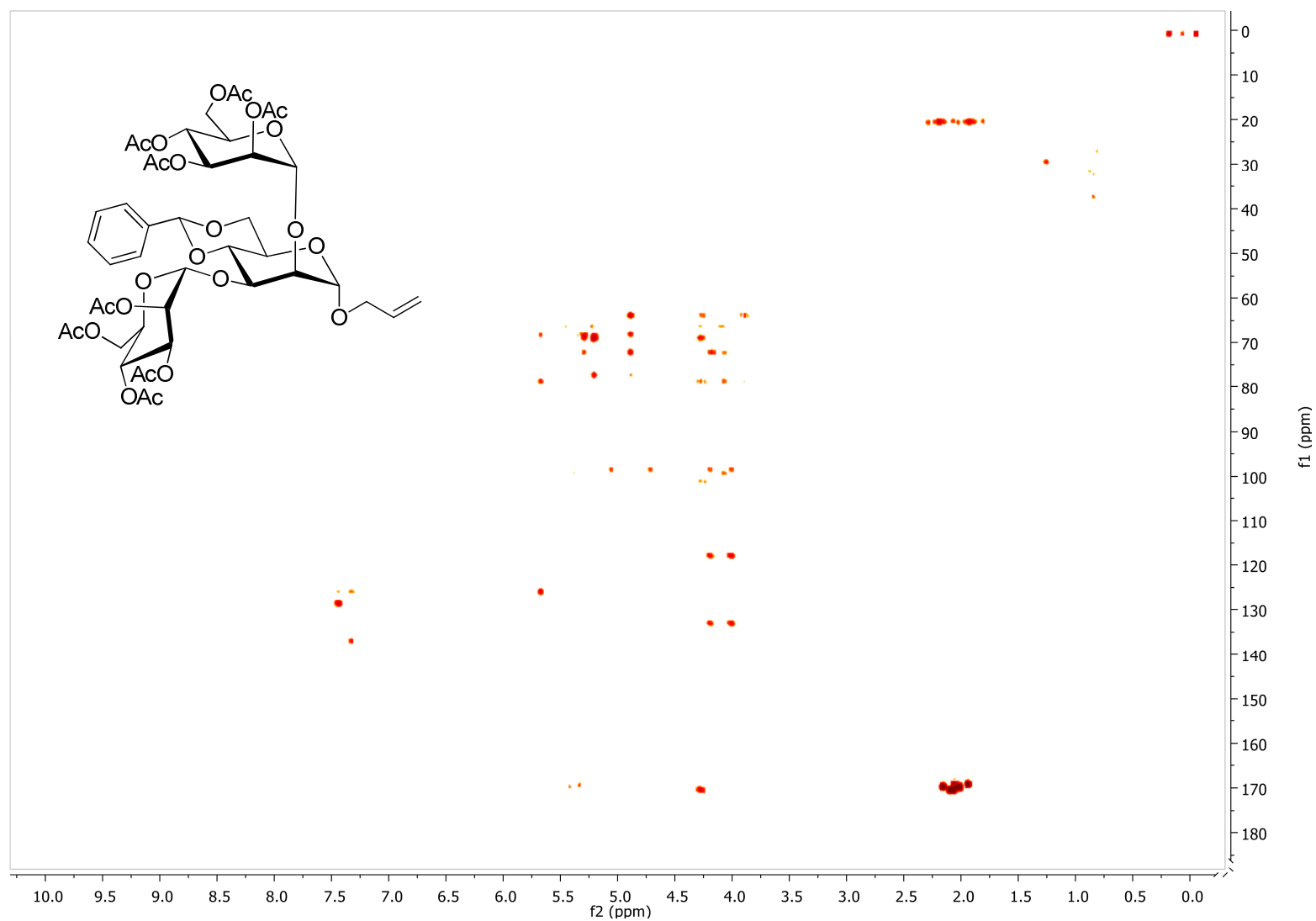
¹³C NMR spectrum (125 MHz, CDCl₃) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (25).



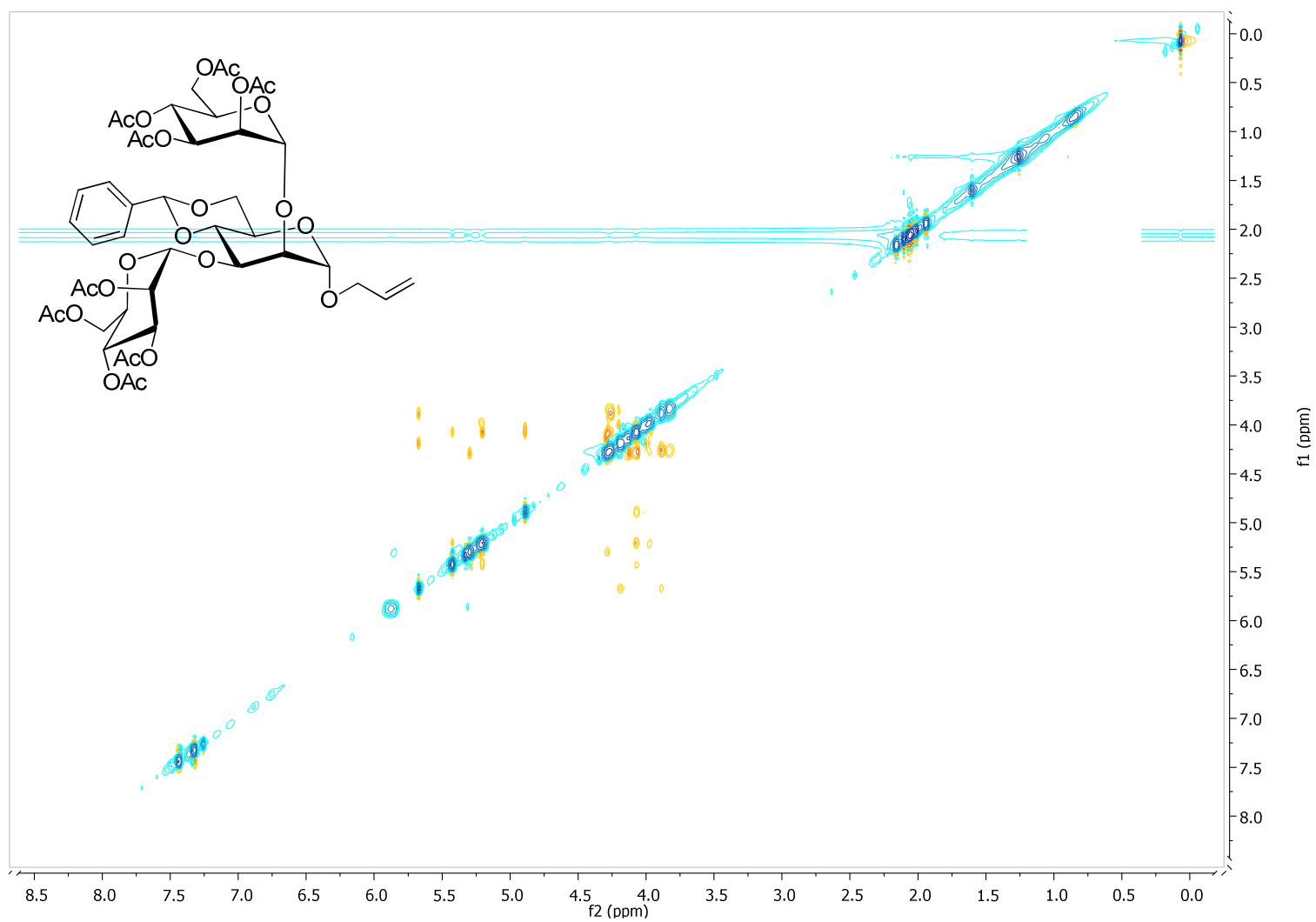
gCOSY NMR spectrum (500 MHz, CDCl₃) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (25).



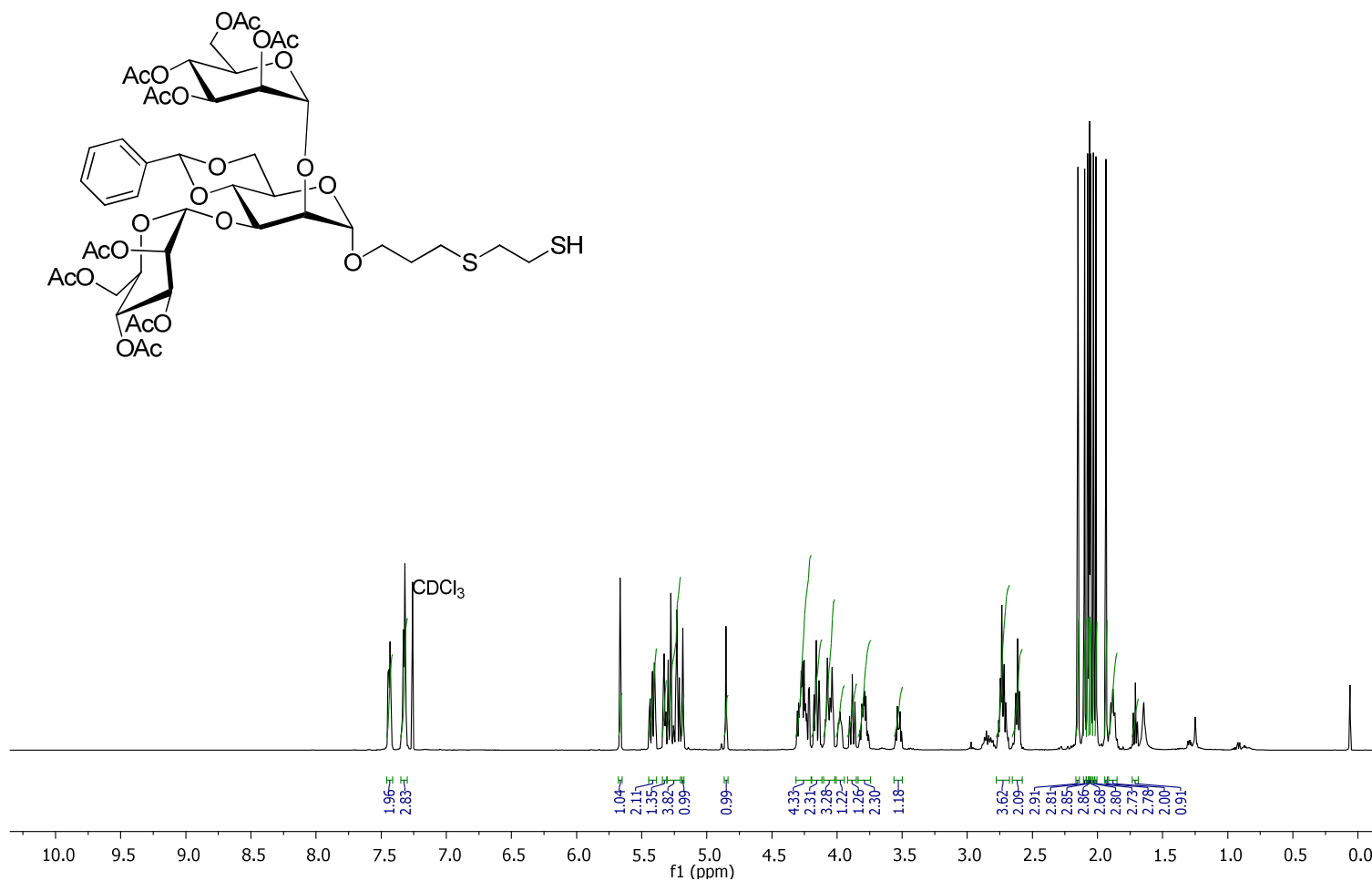
gHSQC NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (25).



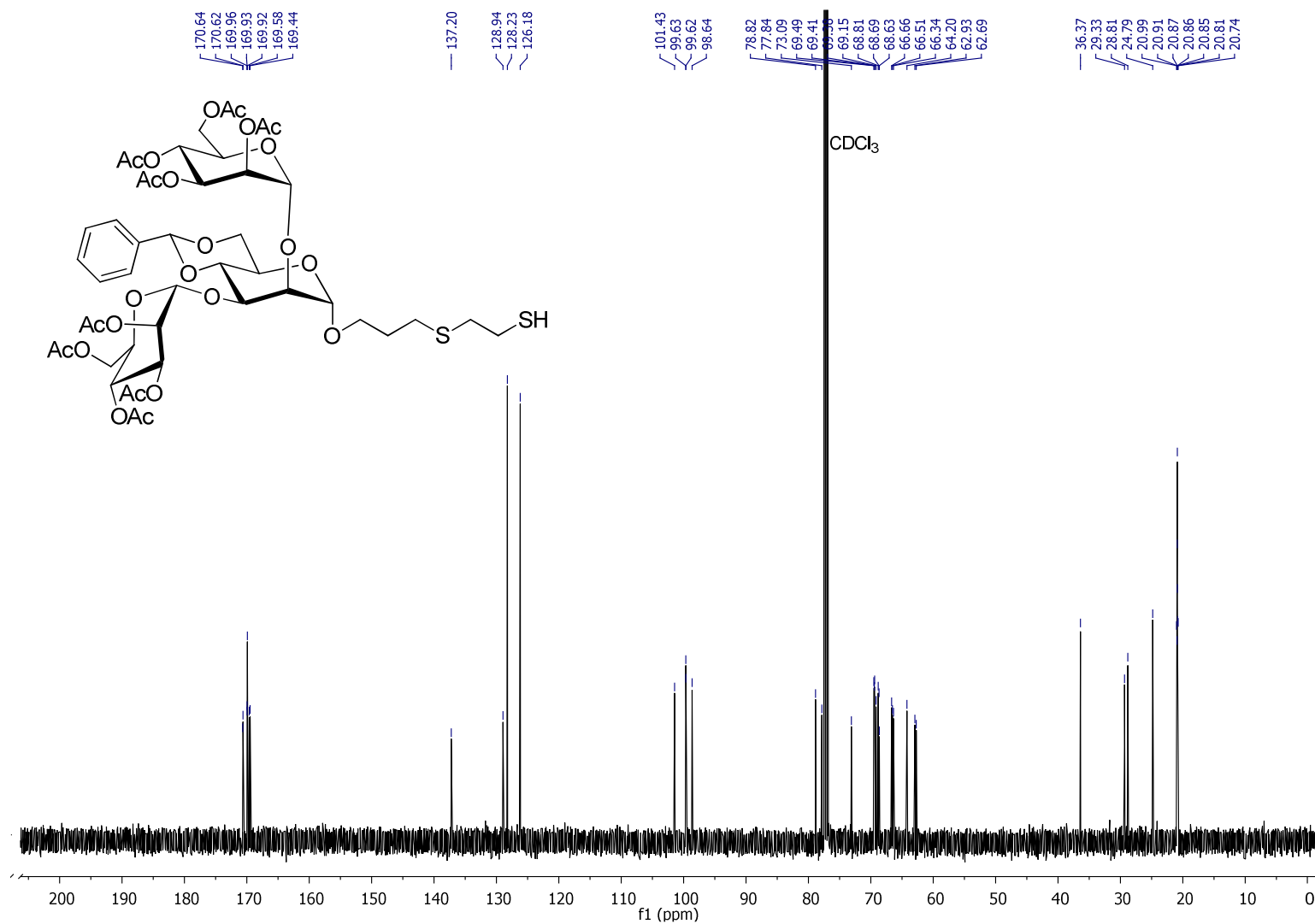
gHMBC NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (25).



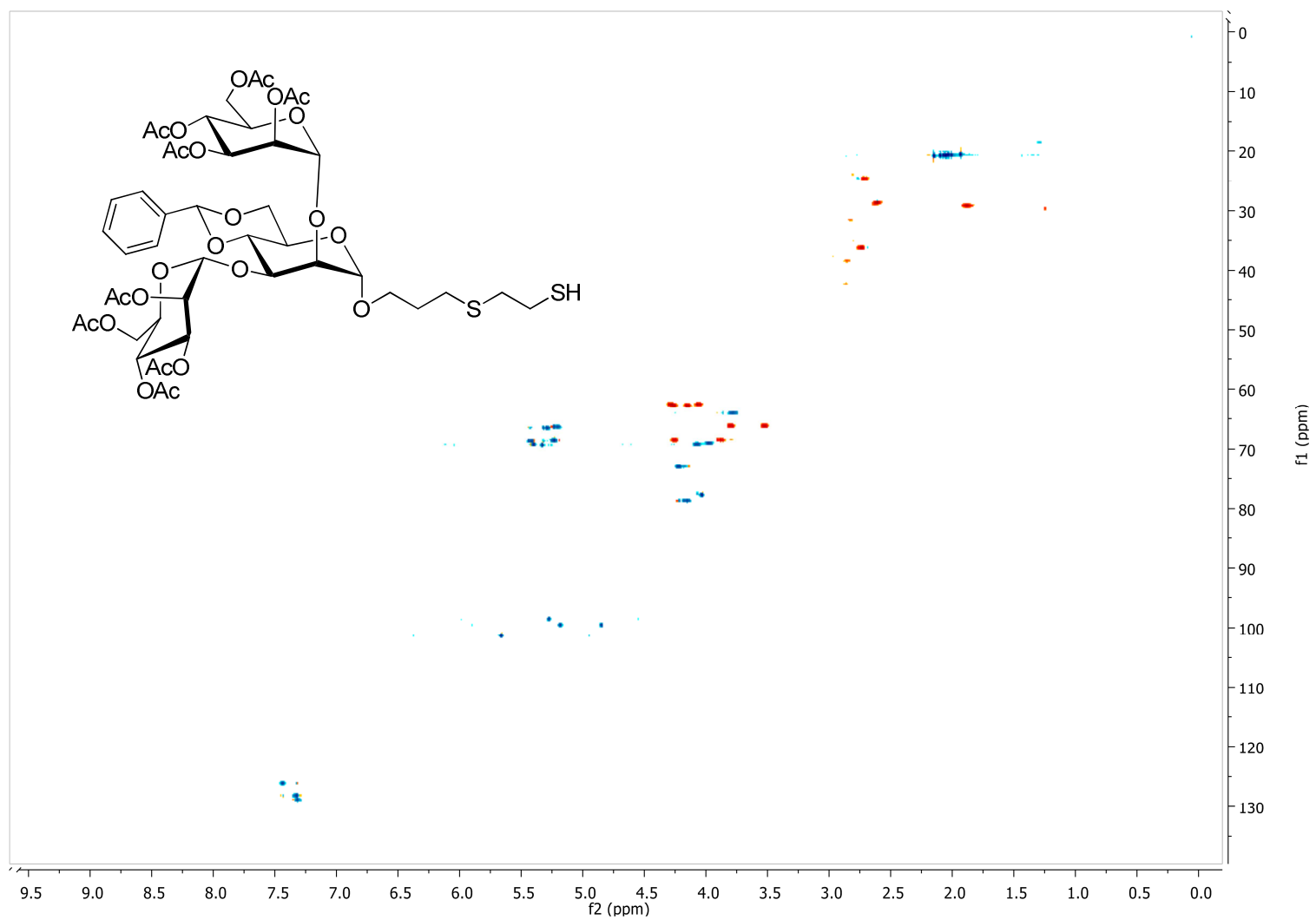
NOESY NMR spectrum (500 MHz, CDCl_3) of allyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (25).



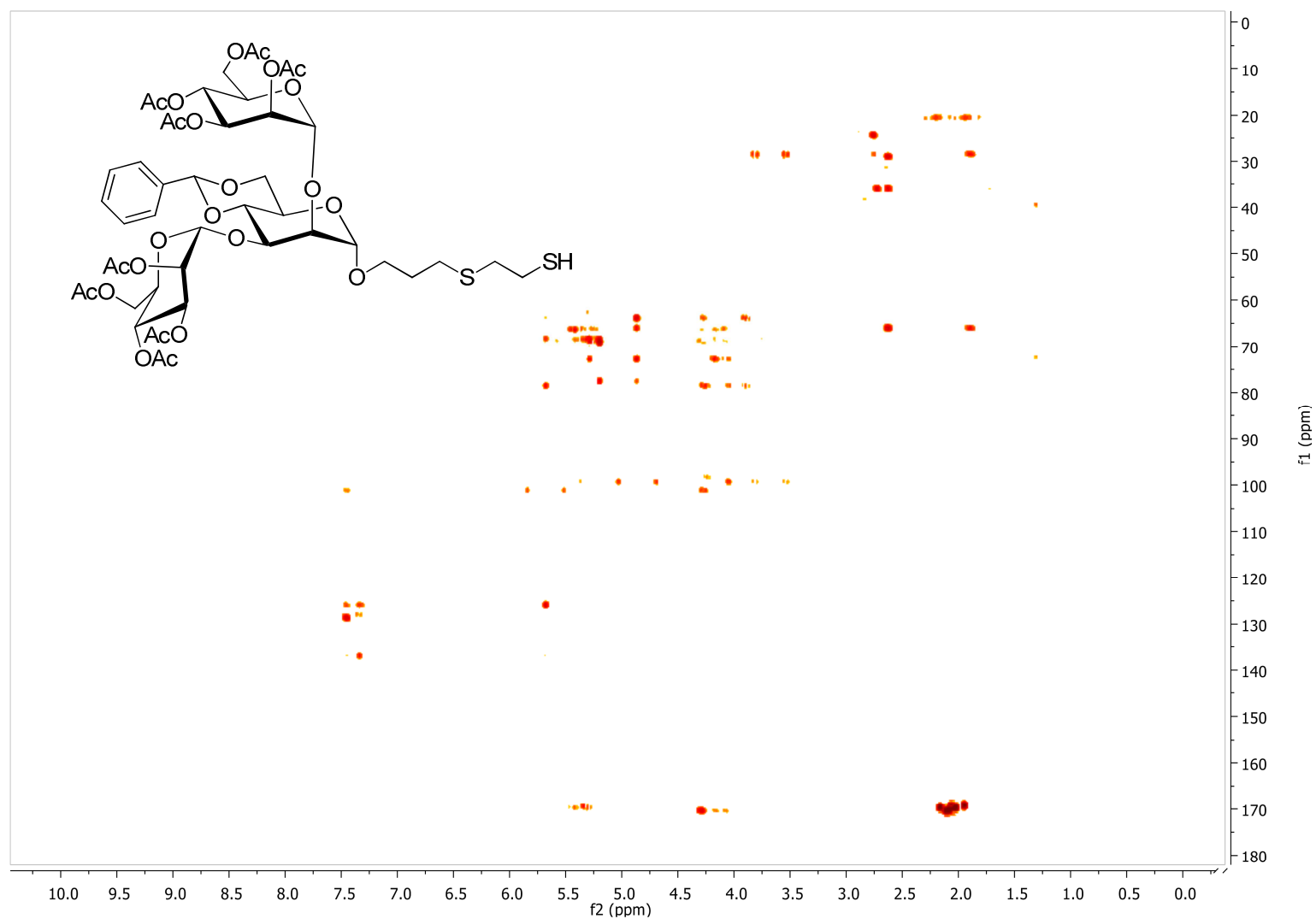
^1H NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**26**).



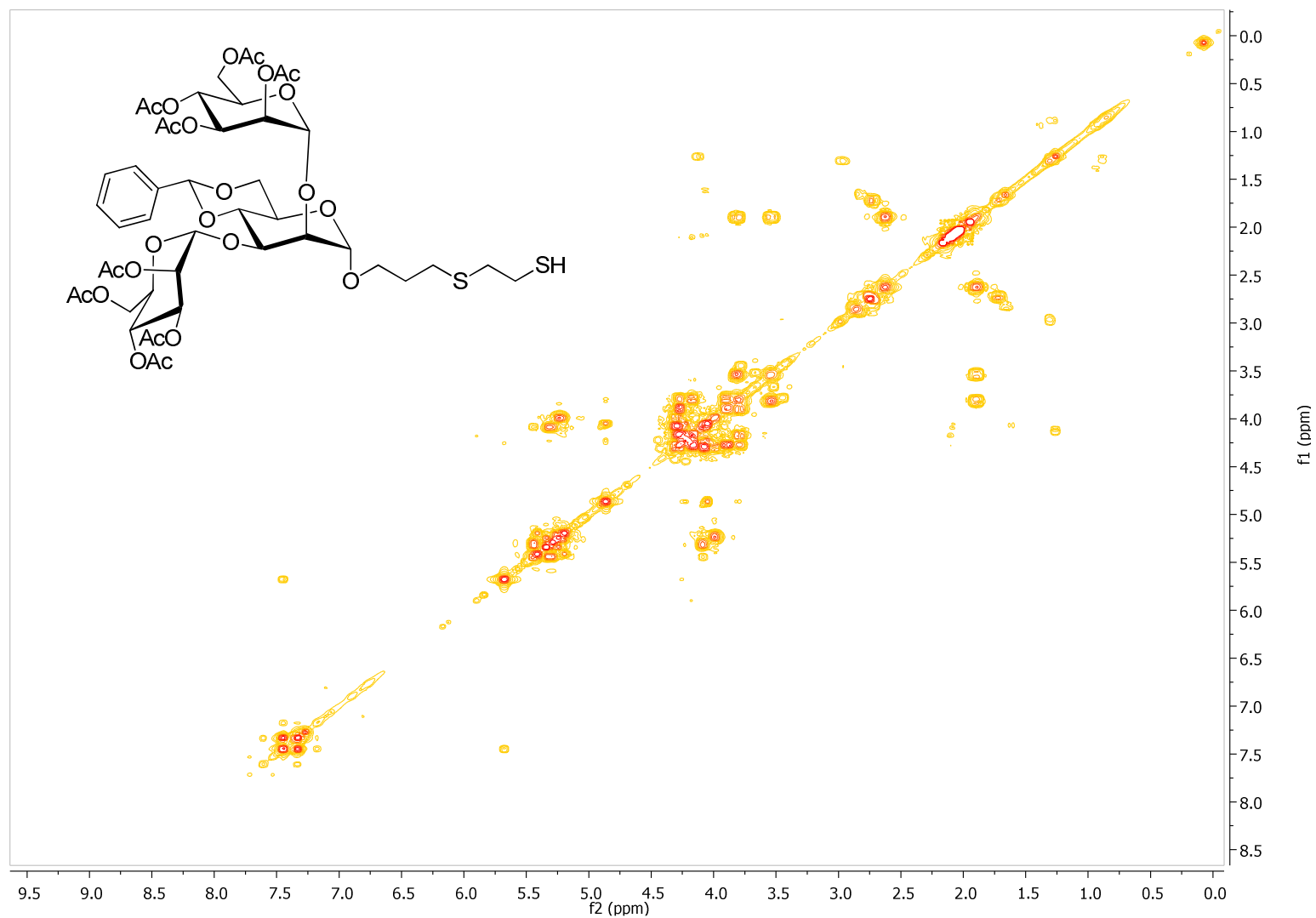
¹³C NMR spectrum (125 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (25).



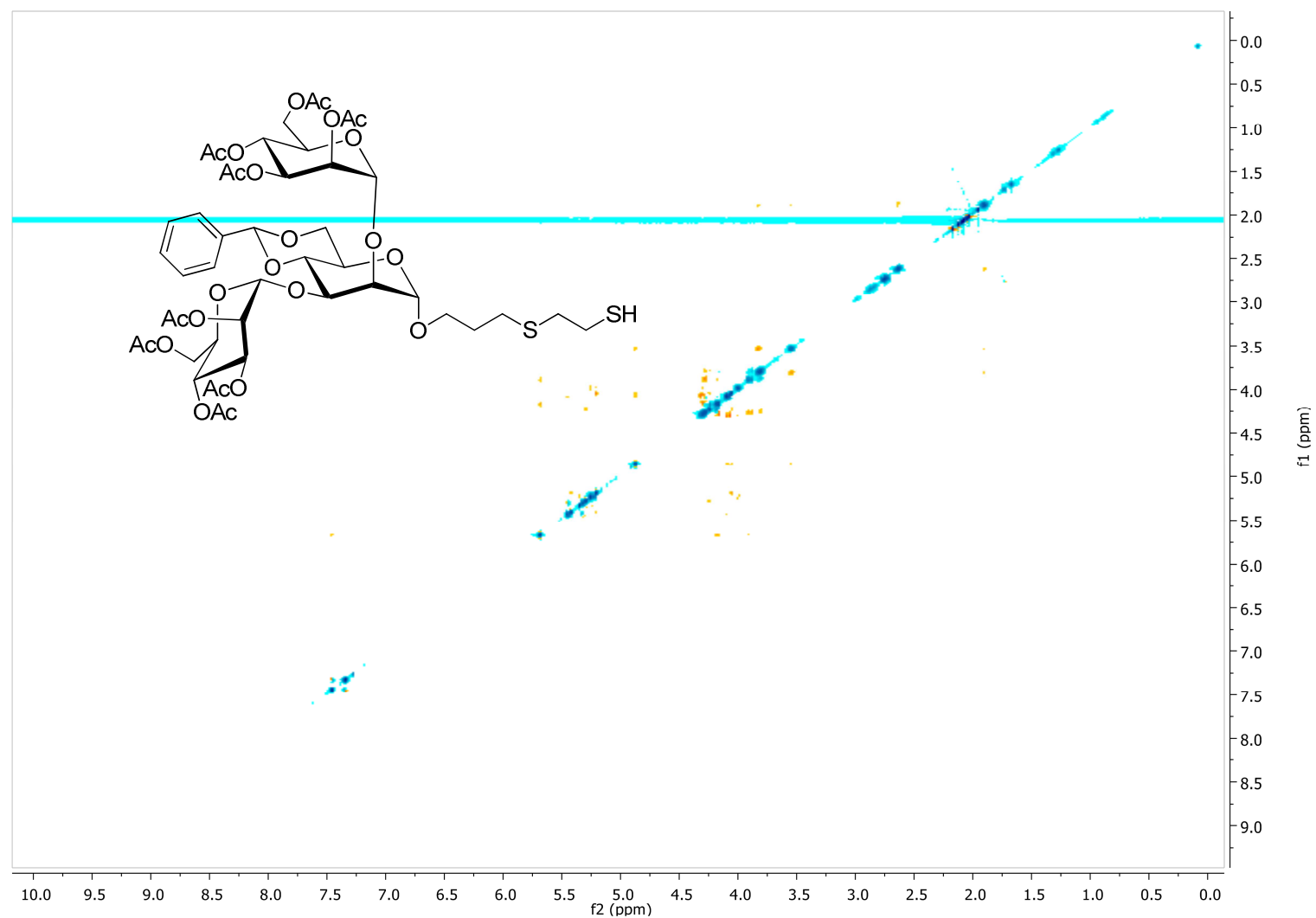
gHSQC NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (26).



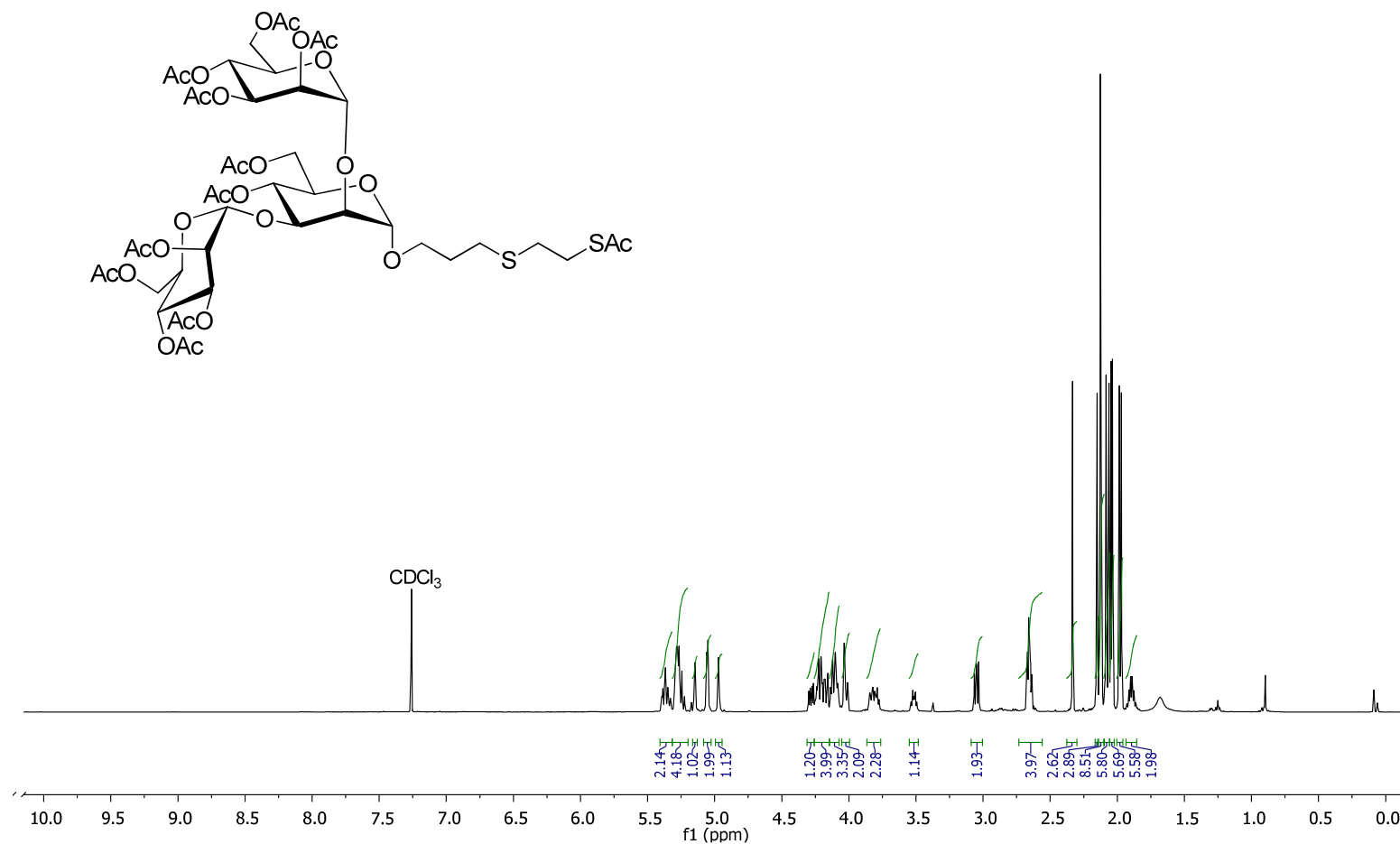
gHMBC NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**26**).



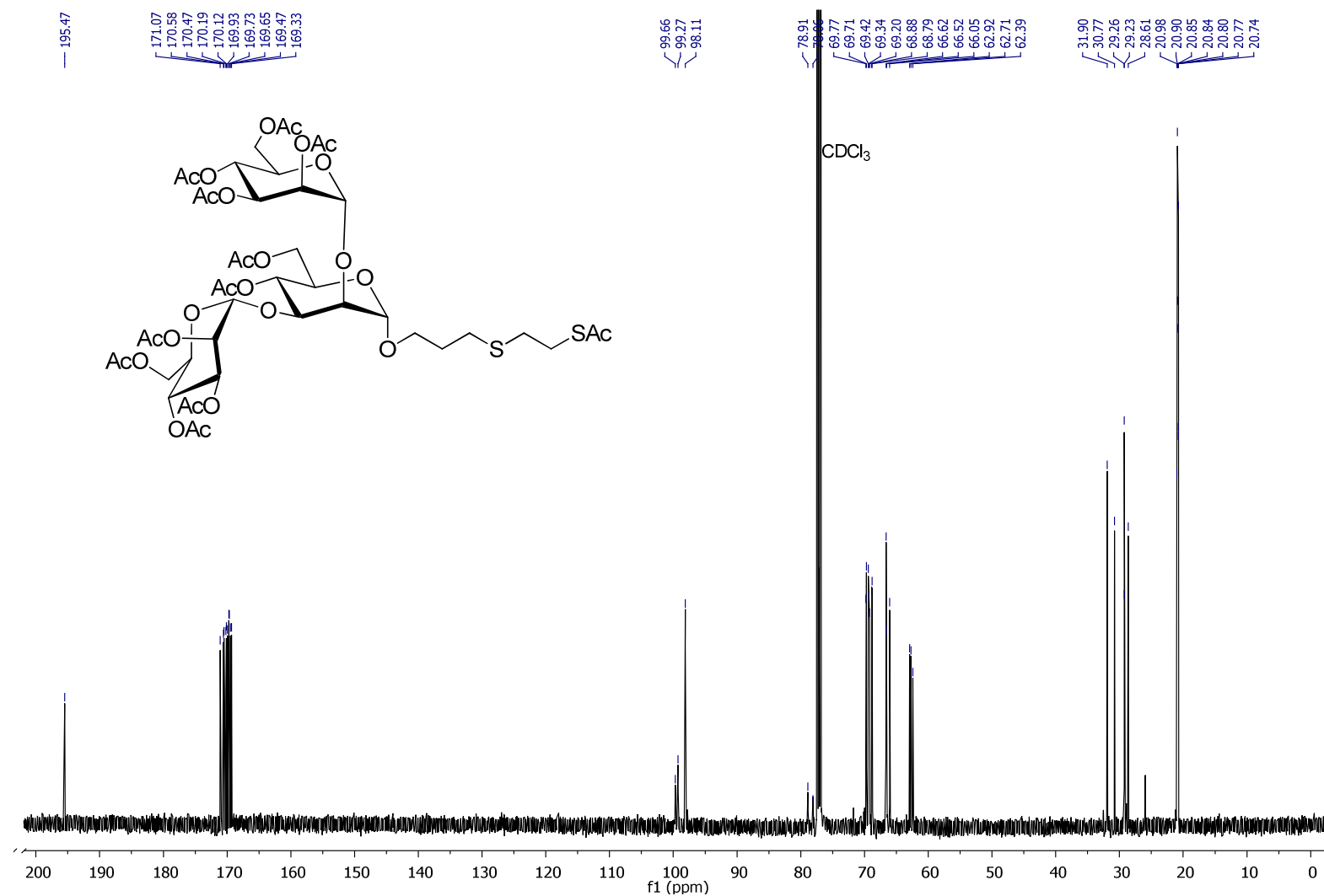
gCOSY NMR spectrum (500 MHz, CDCl_3) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**26**).



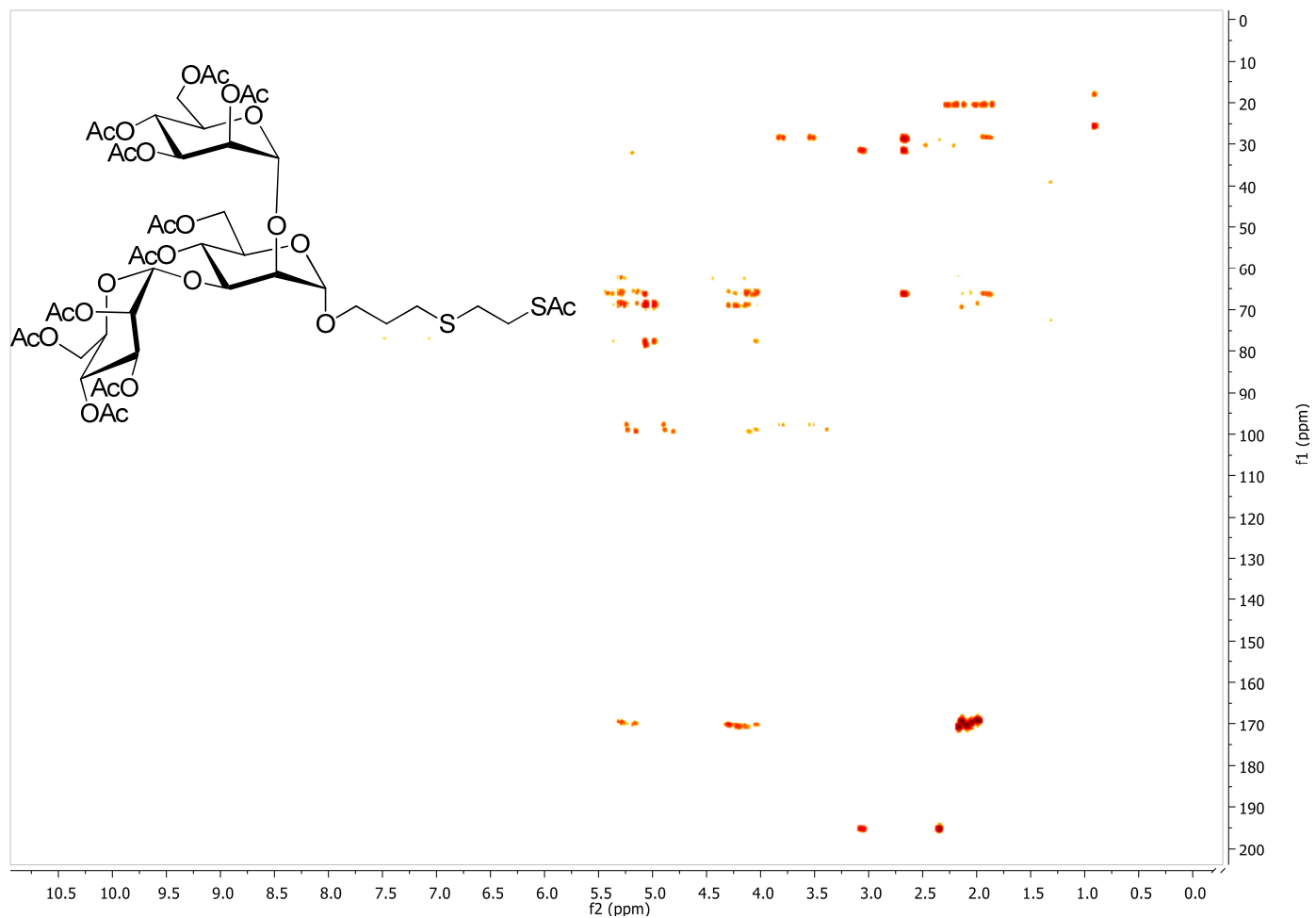
NOESY NMR spectrum (500 MHz, CDCl₃) of 3-[(2-sulfanylethyl)sulfanyl]propyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**26**).



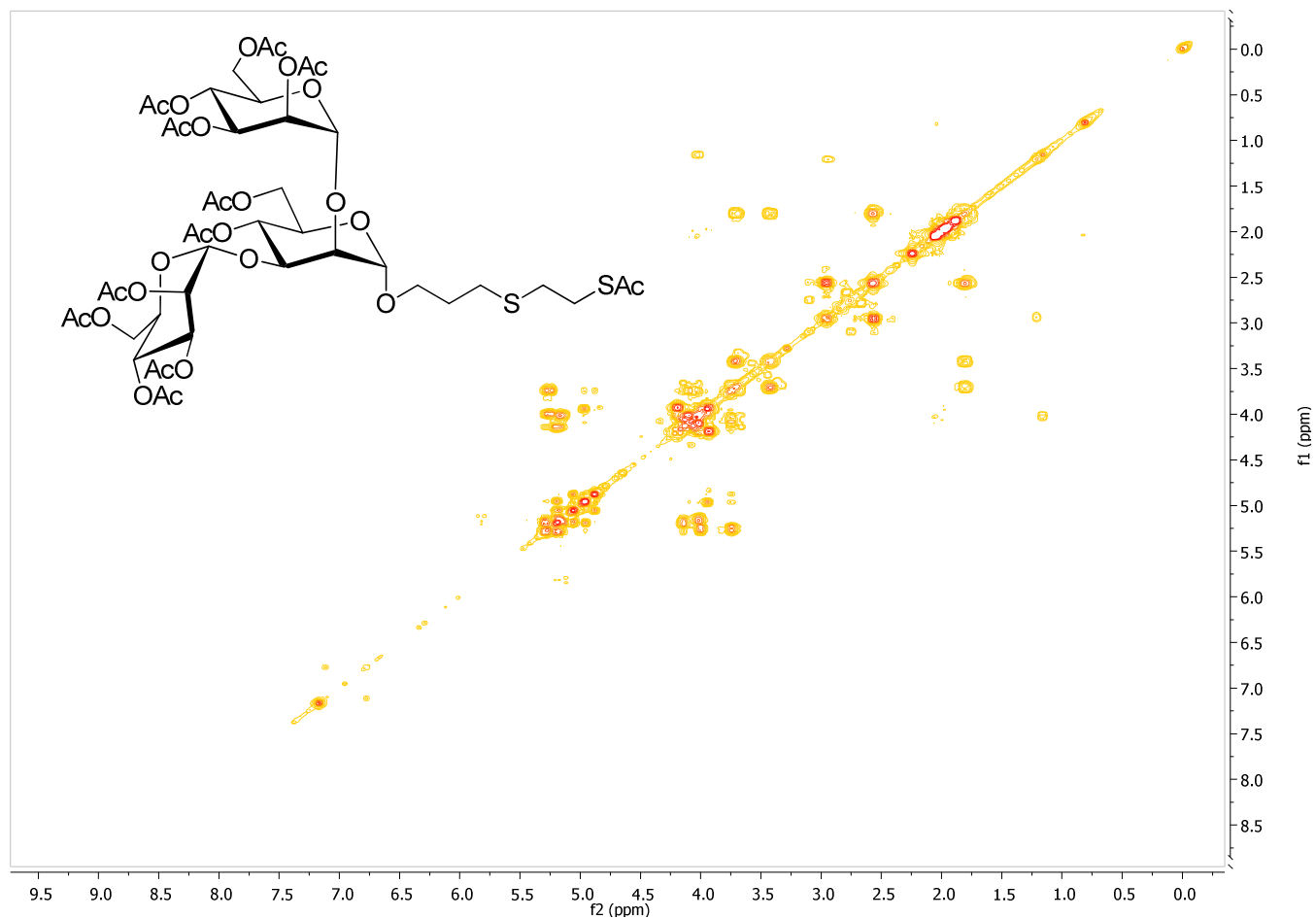
^1H NMR spectrum (500 MHz, CDCl_3) of *S*-[2-[(3-[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)]-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**28**).



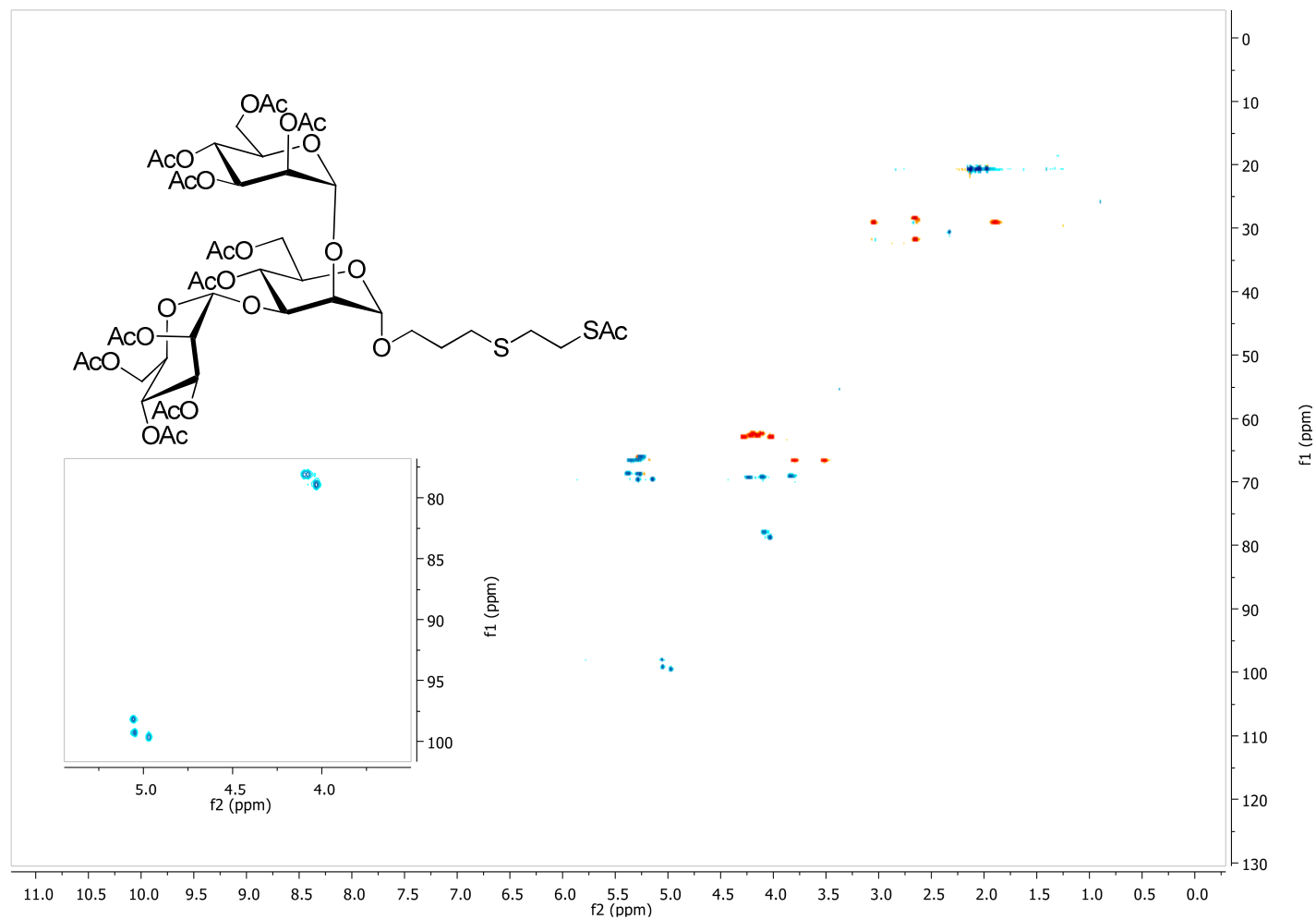
^{13}C NMR spectrum (125 MHz, CDCl_3) of *S*-[2-[(3-[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**26**).



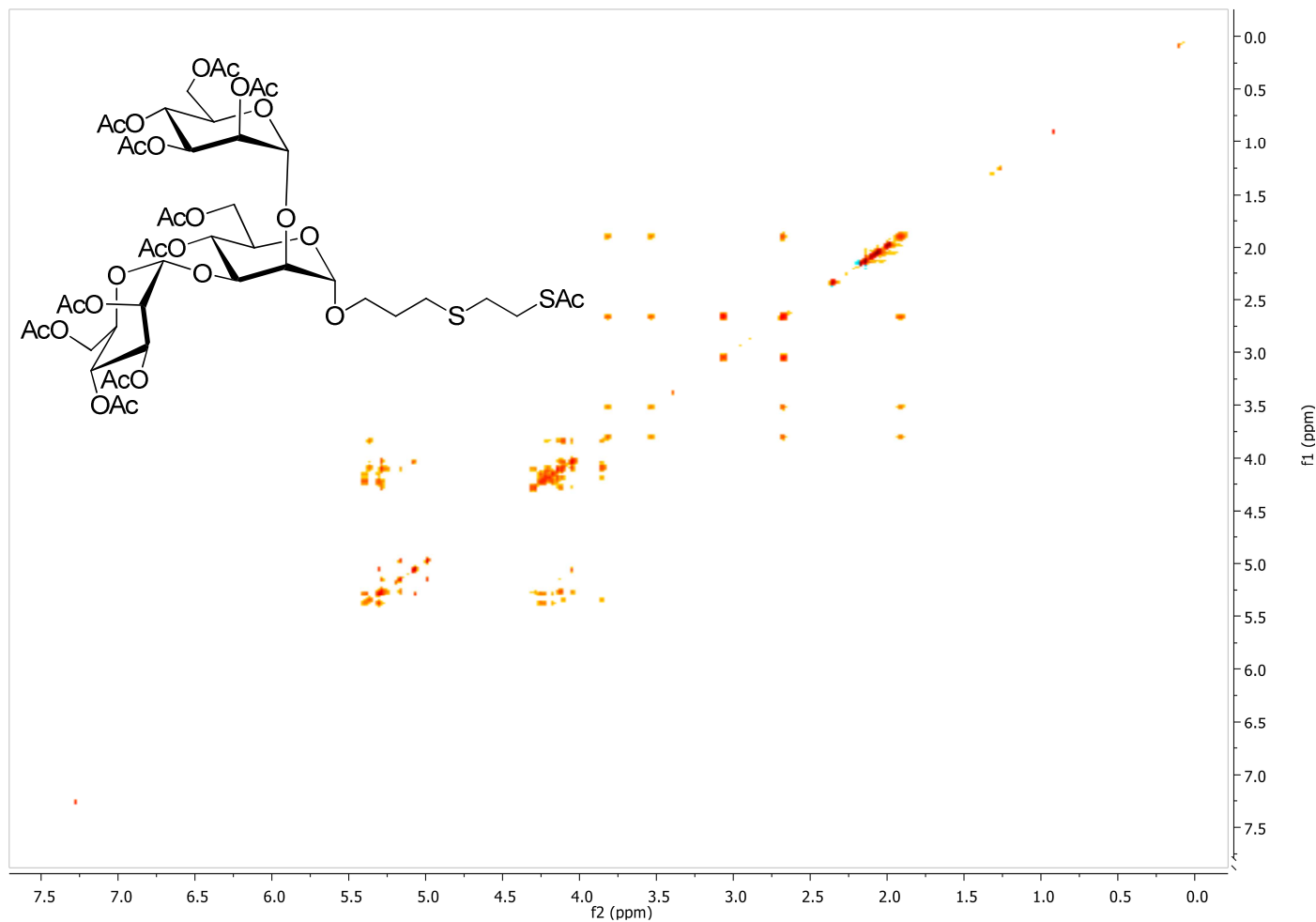
gHMBC NMR spectrum (500 MHz, CDCl_3) of *S*-[2-[(3-[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**28**).



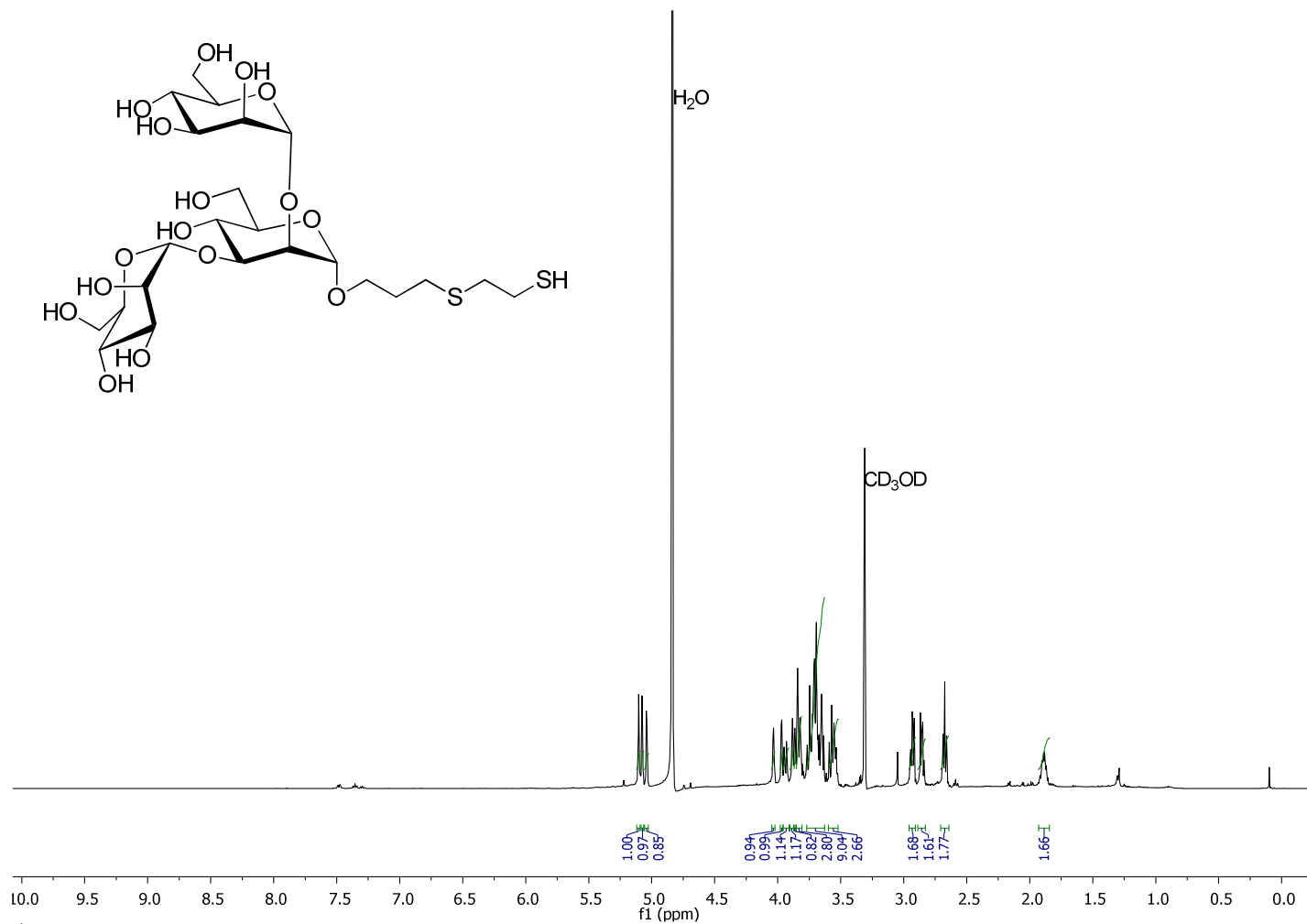
gCOSY NMR spectrum (500 MHz, CDCl_3) of S-[2-[(3-[[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-O-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**28**).



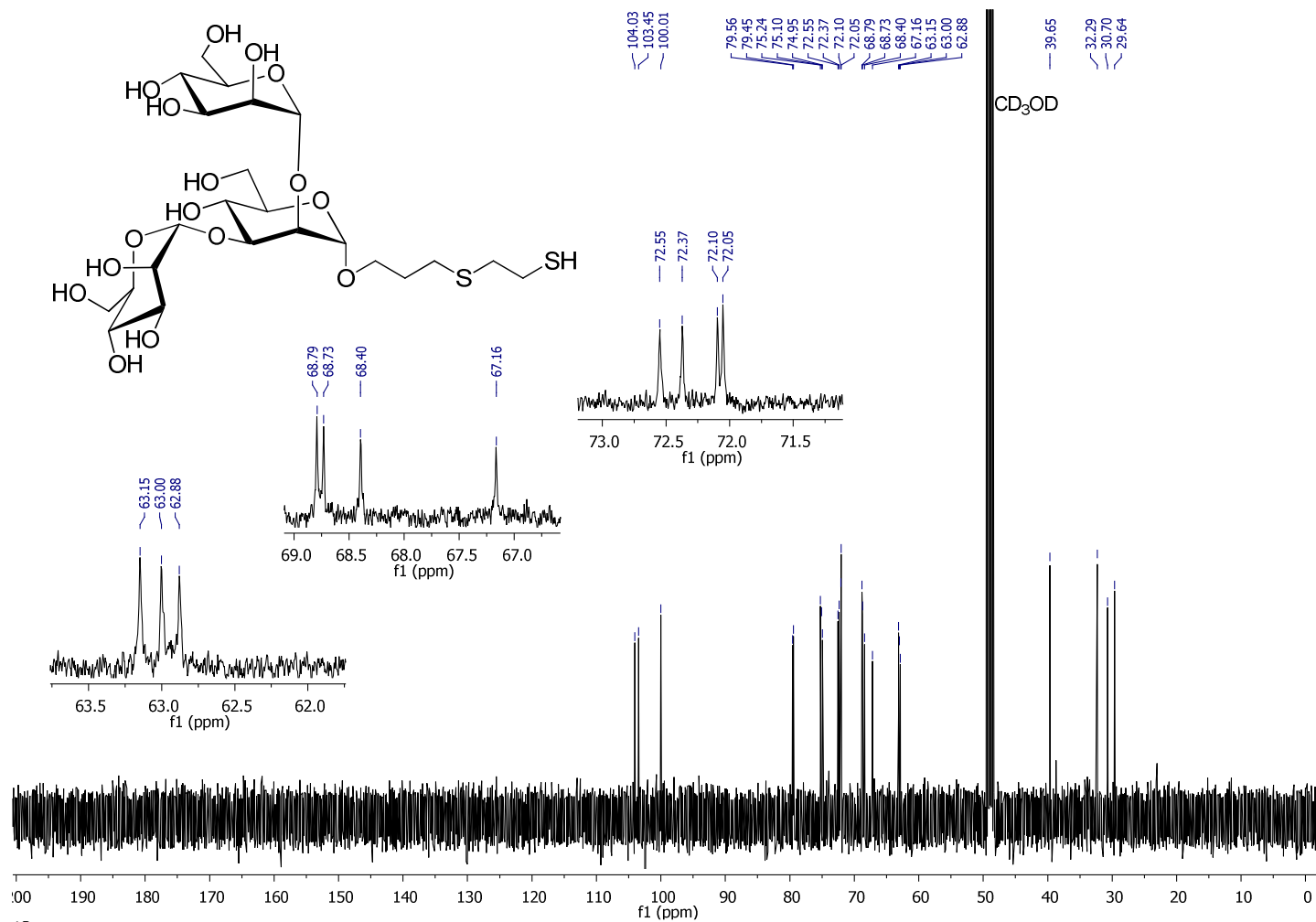
gHSQC NMR spectrum (500 MHz, CDCl₃) of S-[2-[(3-[[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-O-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**28**).



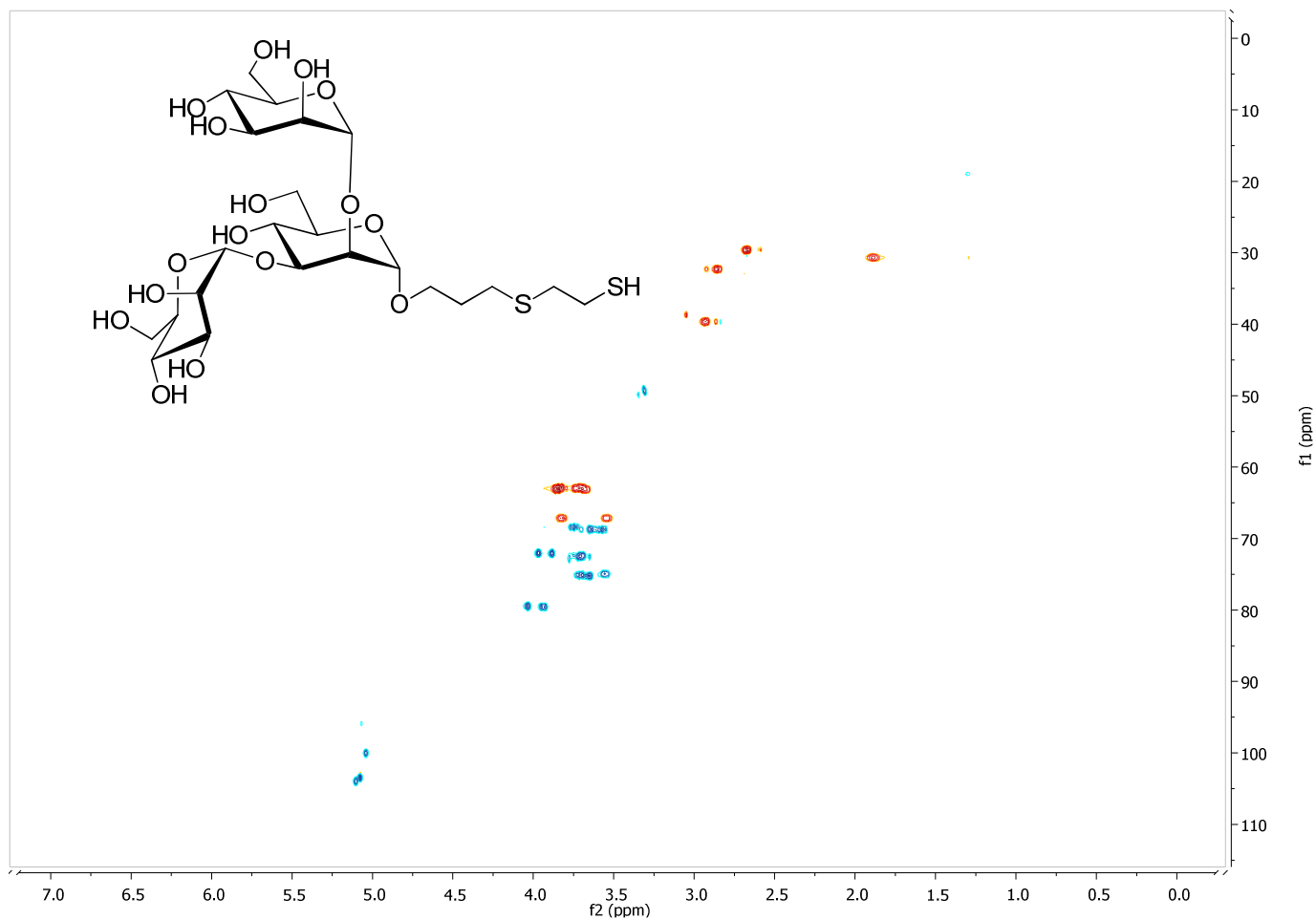
TOCSY NMR spectrum (500 MHz, CDCl_3) of *S*-[2-[(3-[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-di-*O*-acetyl- α -D-mannopyranosyl]oxy]propyl)sulfanyl]ethyl] ethanethioate (**28**).



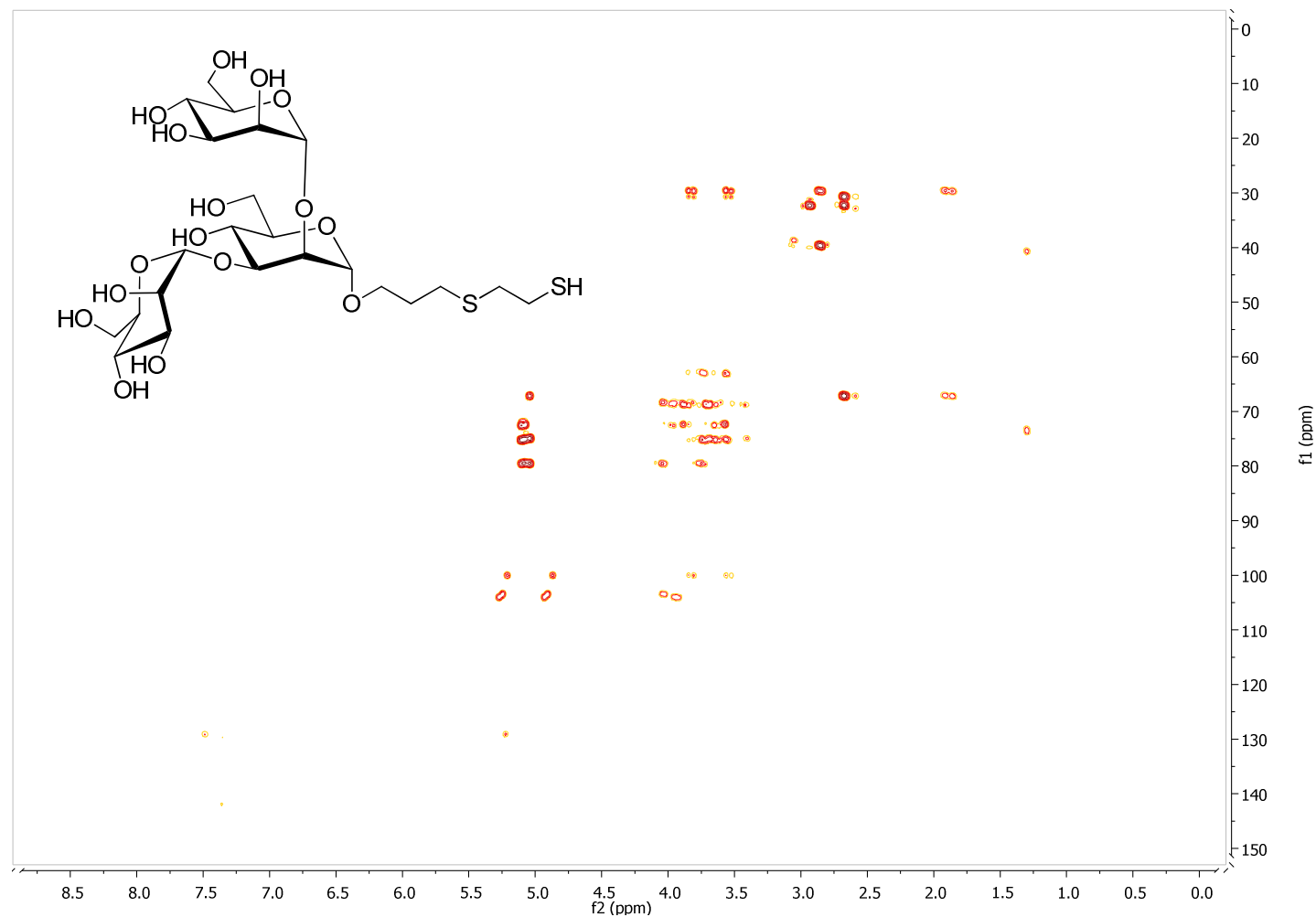
^1H NMR spectrum (500 MHz, CD_3OD) of 3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (**29**).



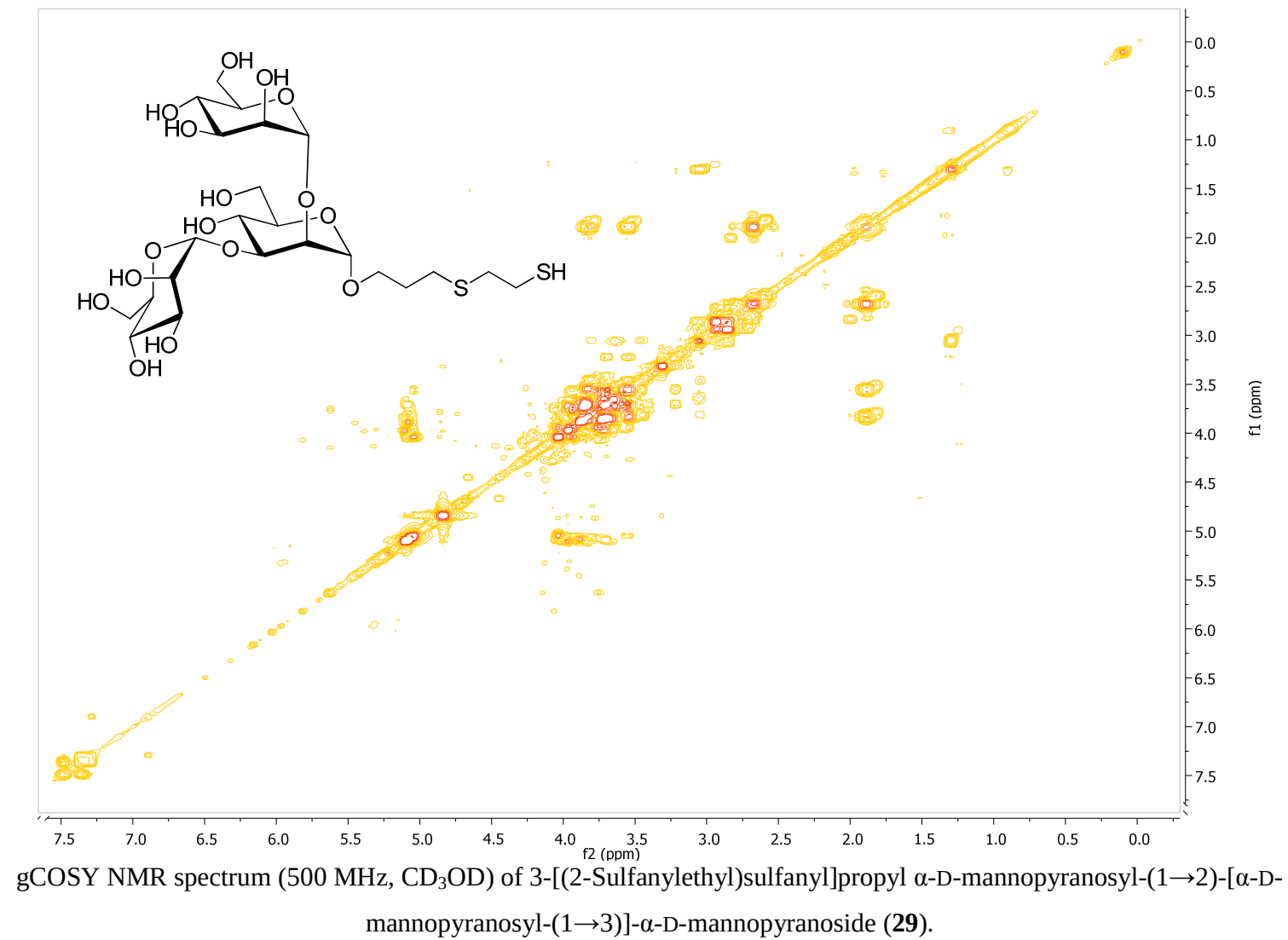
^{13}C NMR spectrum (125 MHz, CD_3OD) of 3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (**29**).

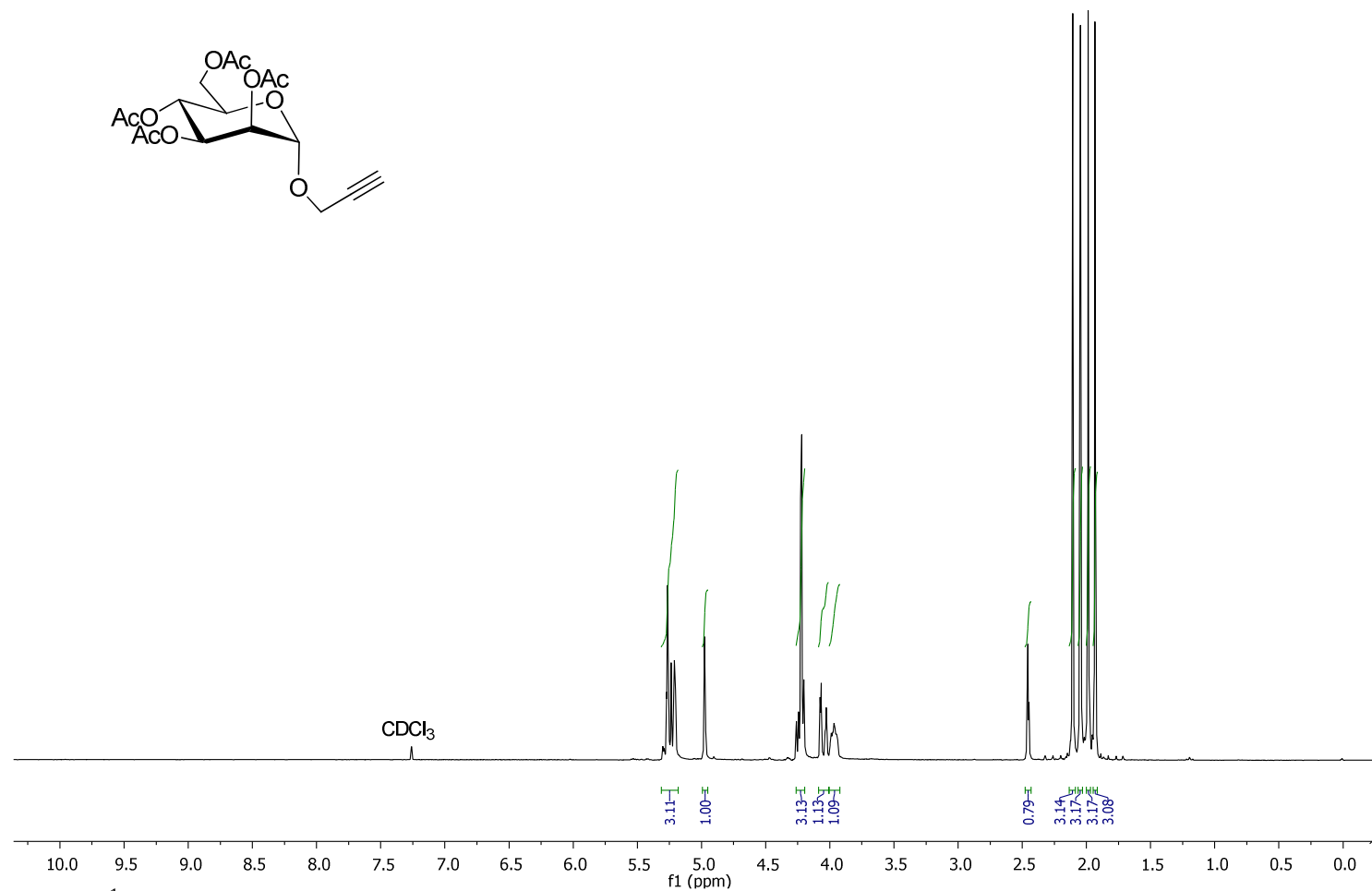
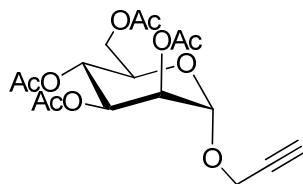


gHSQC NMR spectrum (500 MHz, CD_3OD) of 3-[(2-Sulfanylethyl)sulfanyl]propyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (**29**).

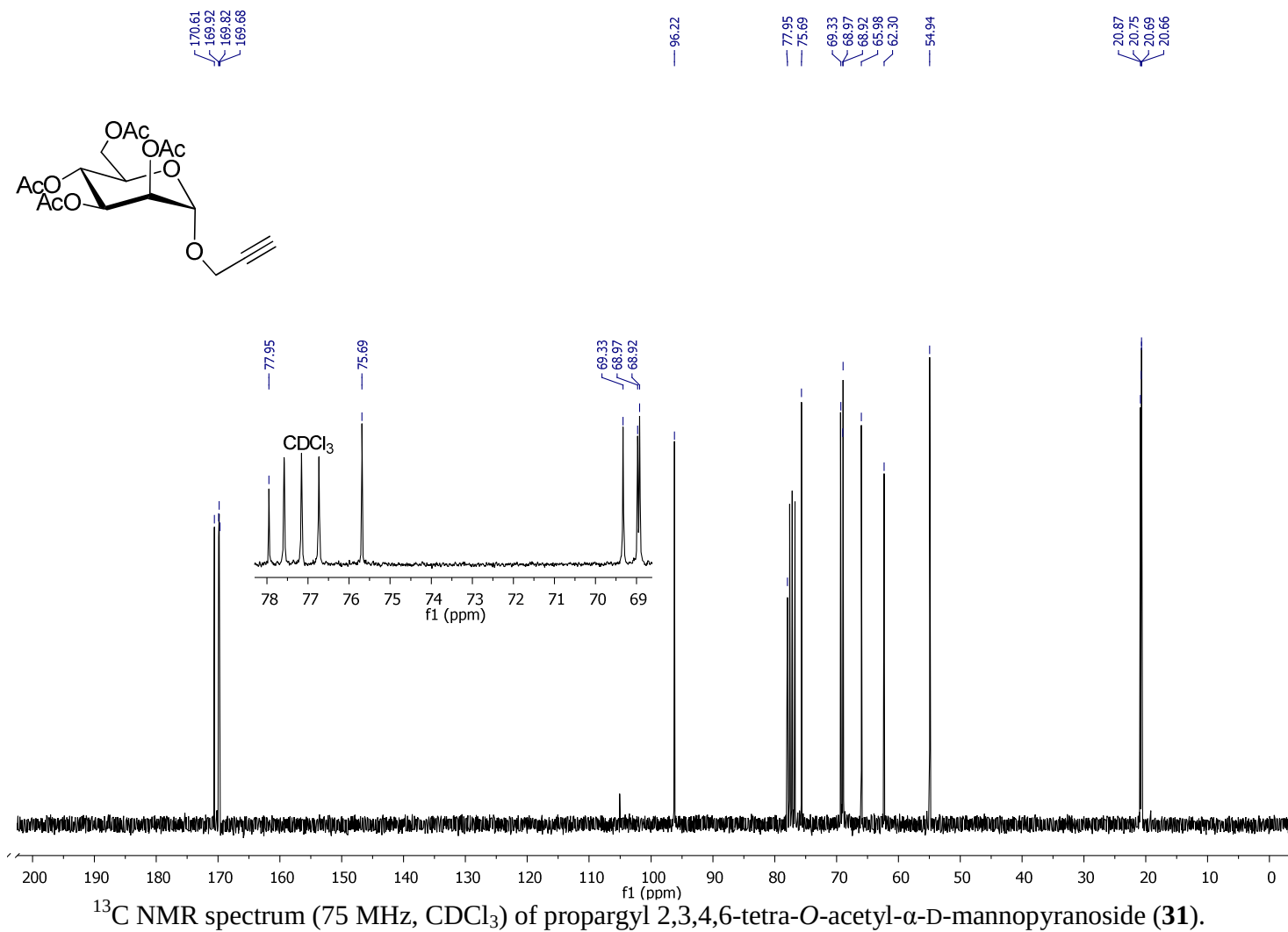


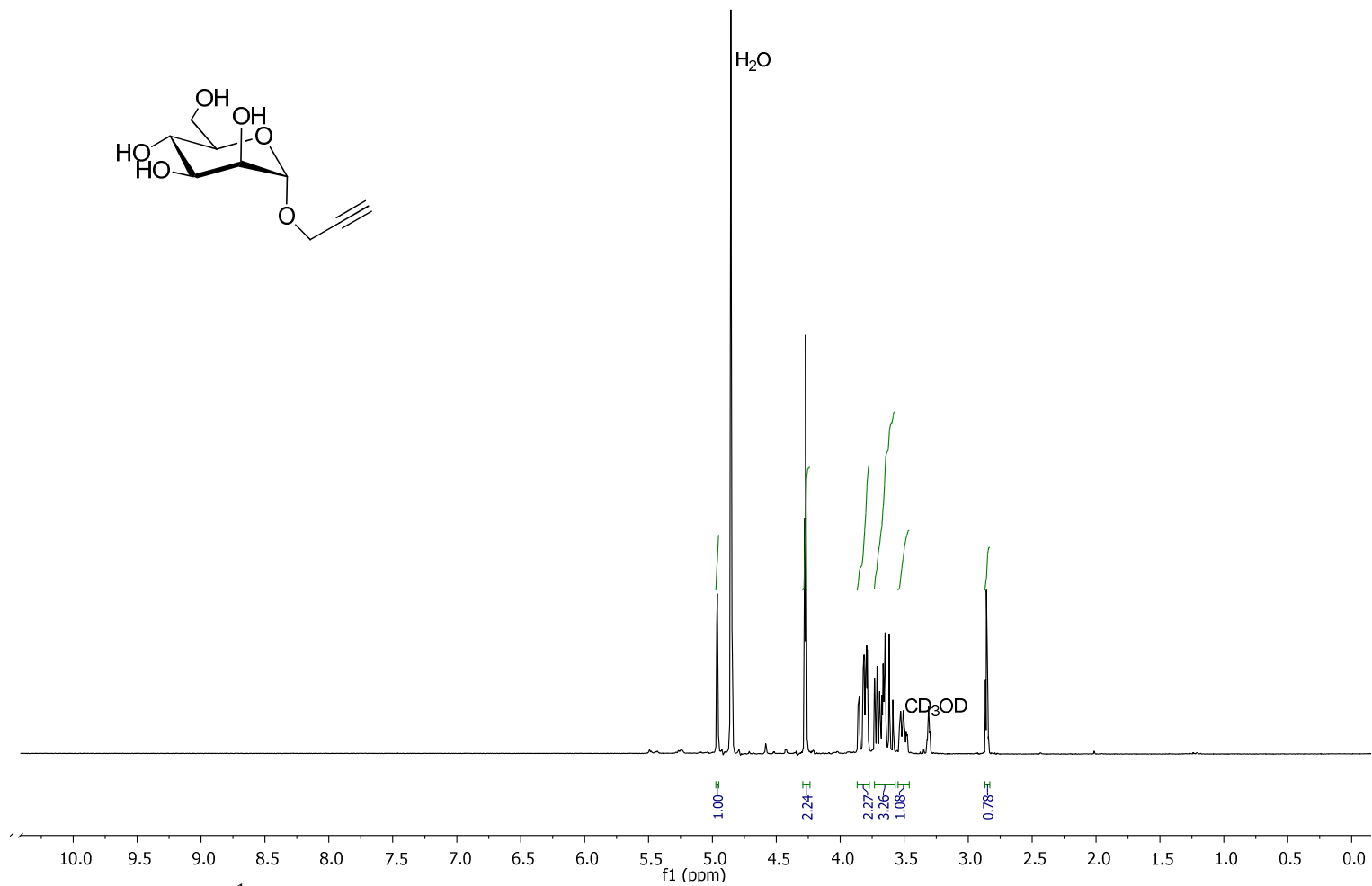
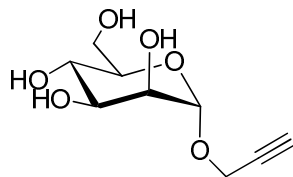
gHMBC NMR spectrum (500 MHz, CD₃OD) of 3-[(2-Sulfanylethyl)sulfanyl]propyl α-D-mannopyranosyl-(1→2)-[α-D-mannopyranosyl-(1→3)]-α-D-mannopyranoside (**29**).



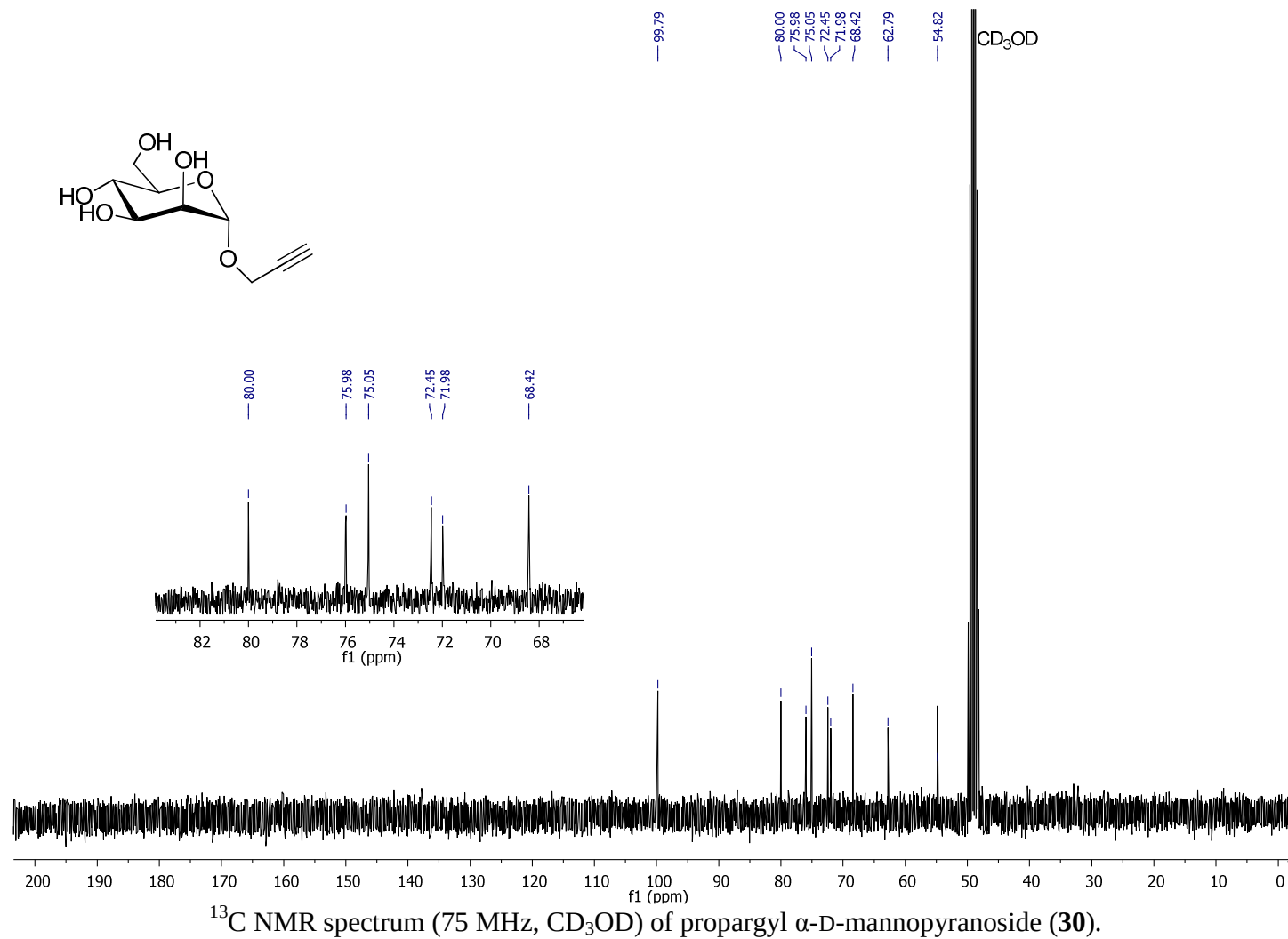


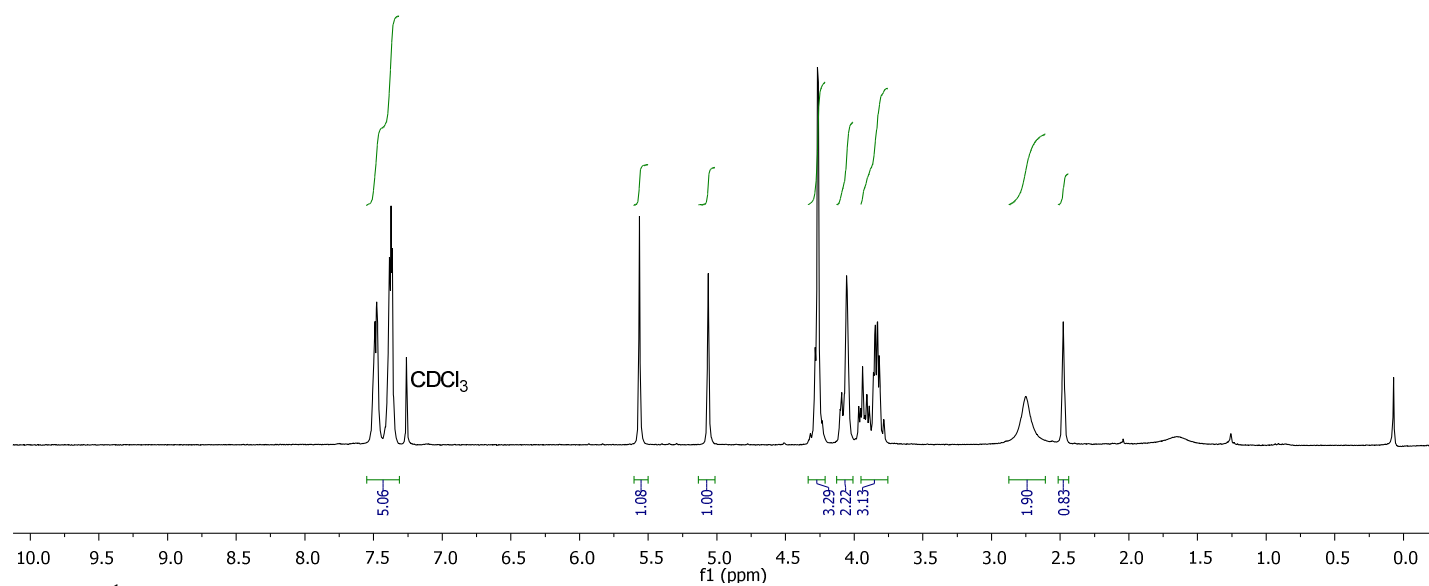
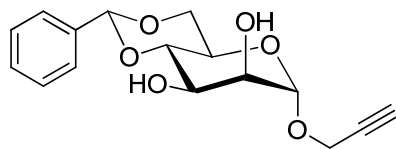
¹H NMR spectrum (300 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**31**).



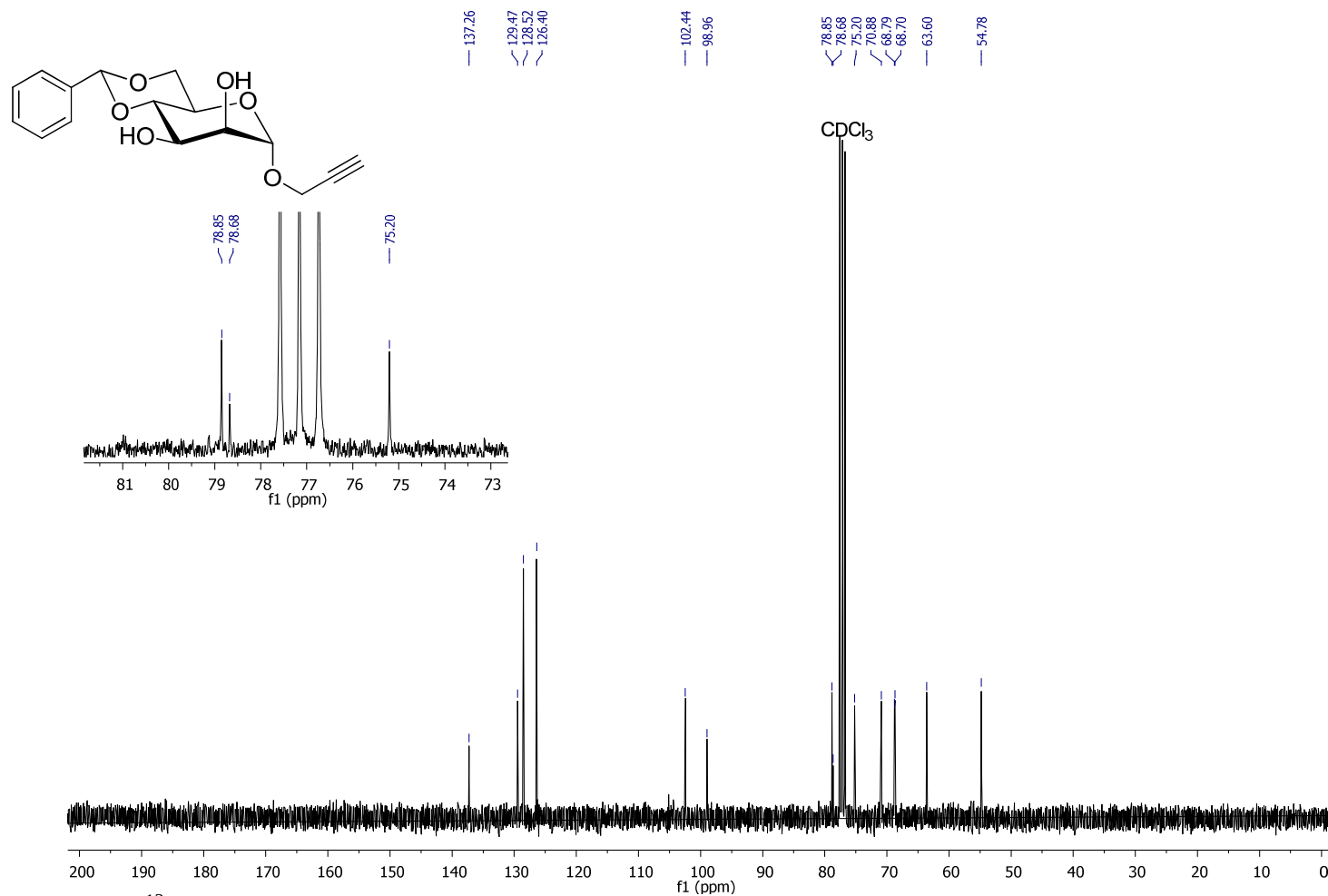


¹H NMR spectrum (300 MHz, CD₃OD) of propargyl α-D-mannopyranoside (**30**).

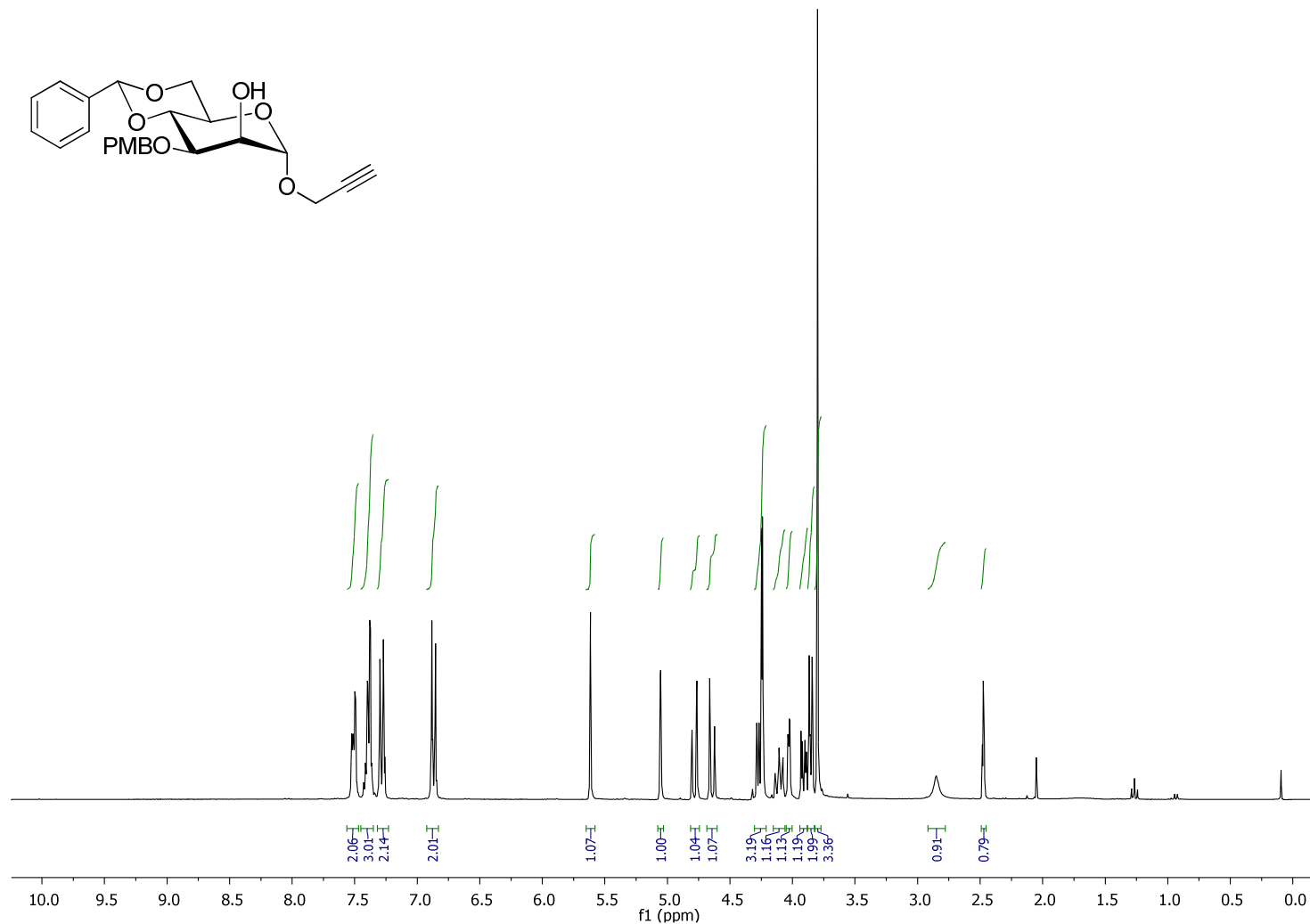
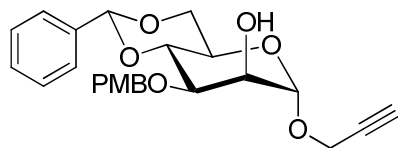




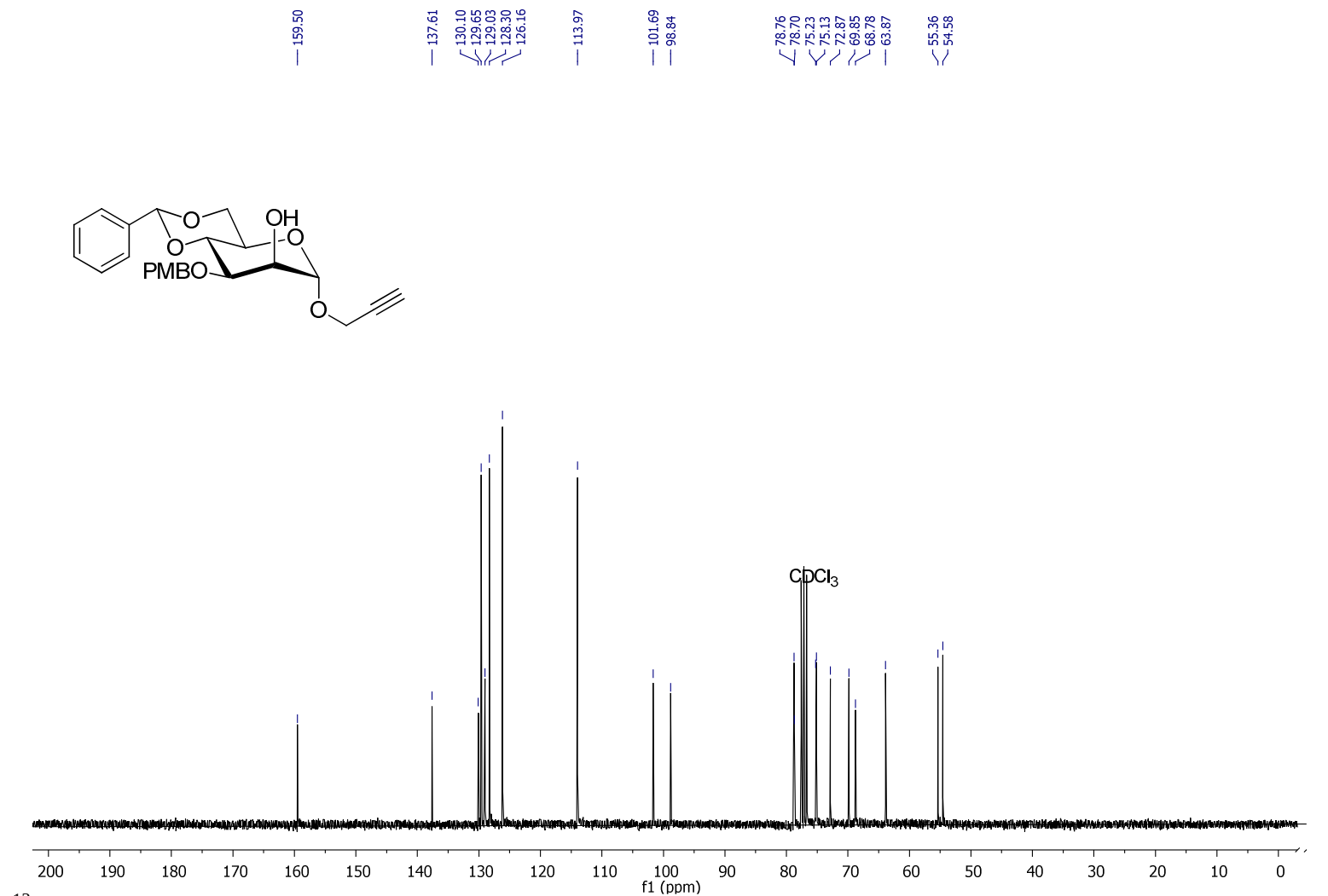
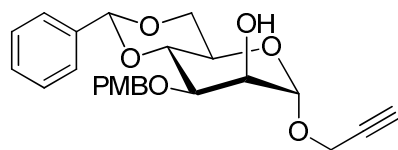
¹H NMR spectrum (300 MHz, CDCl₃) of propargyl 4,6-O-benzylidene- α -D-mannopyranoside (**32**).



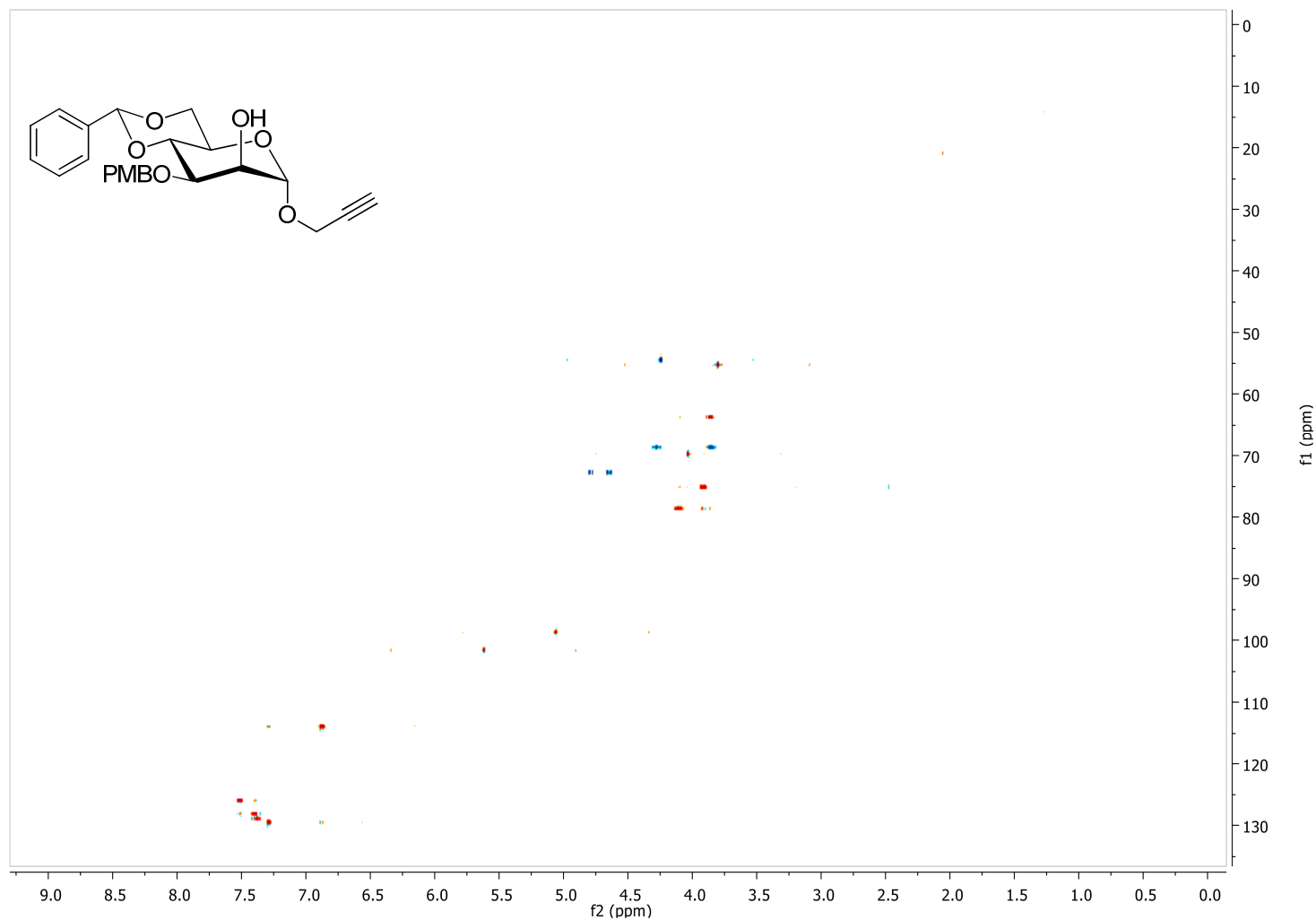
^{13}C NMR spectrum (75 MHz, CDCl_3) of propargyl 4,6-O-benzylidene- α -D-mannopyranoside (32).



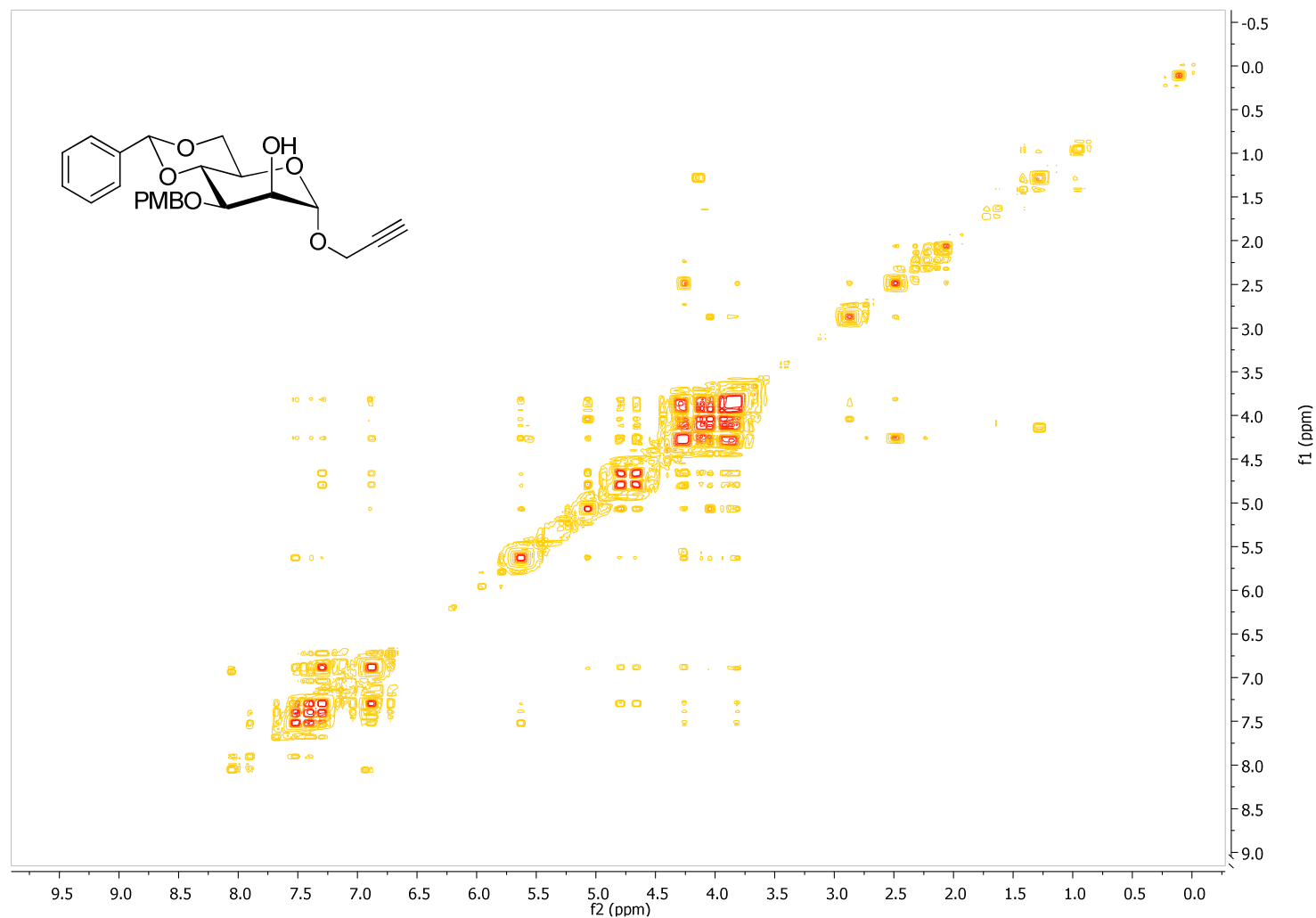
^1H NMR spectrum (300 MHz, CDCl_3) of propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**33**).



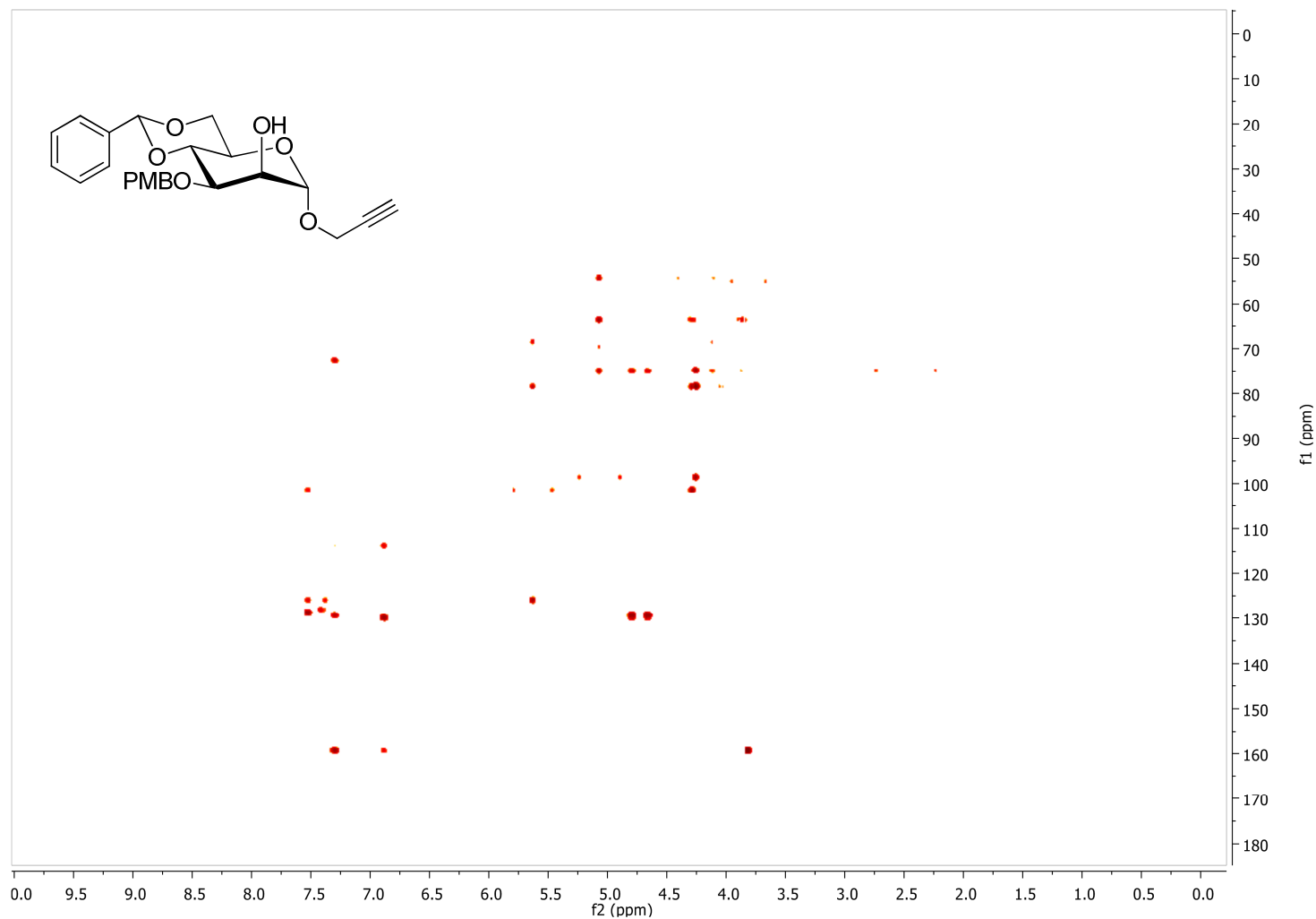
^{13}C NMR spectrum (75 MHz, CDCl_3) of propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**33**).



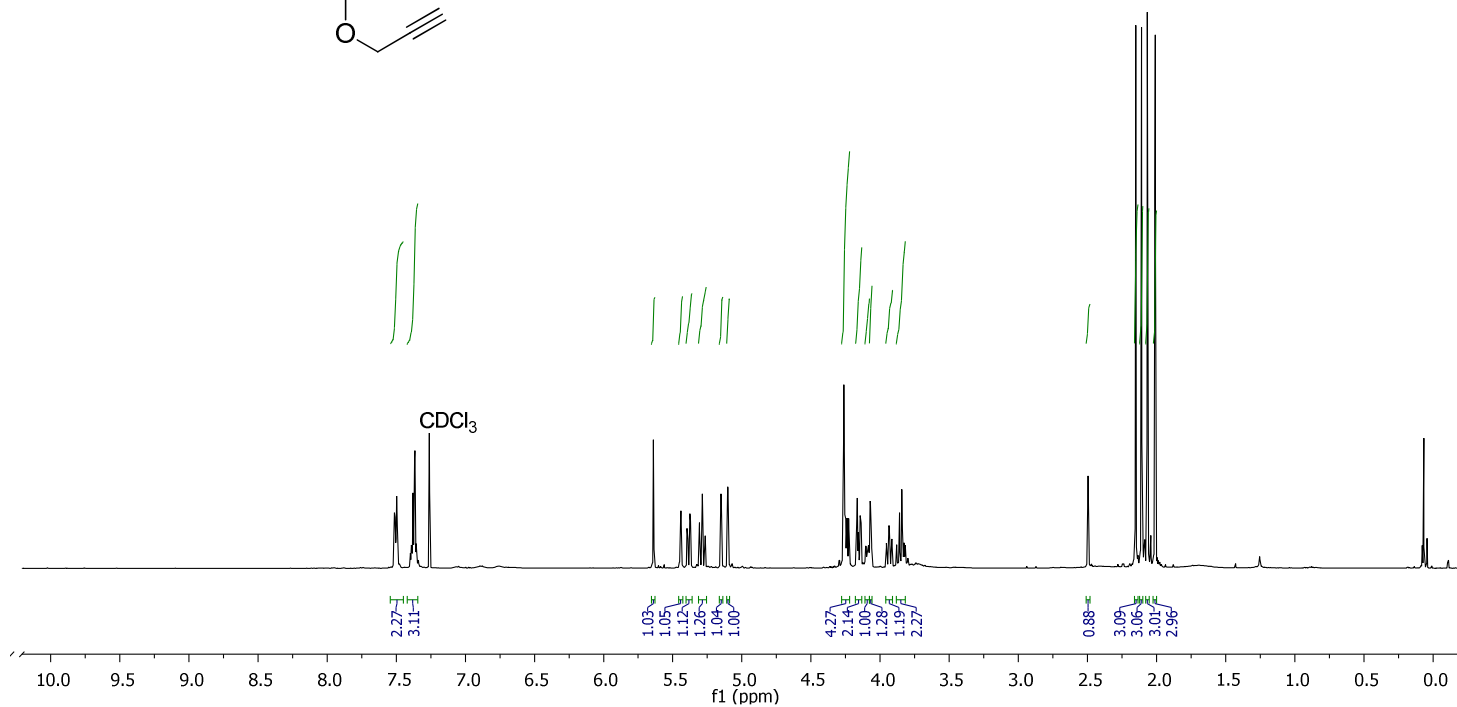
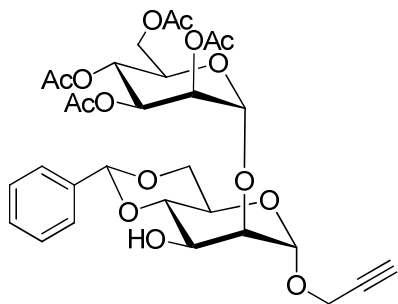
gHSQC NMR spectrum (500 MHz, CDCl₃) of propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**33**).



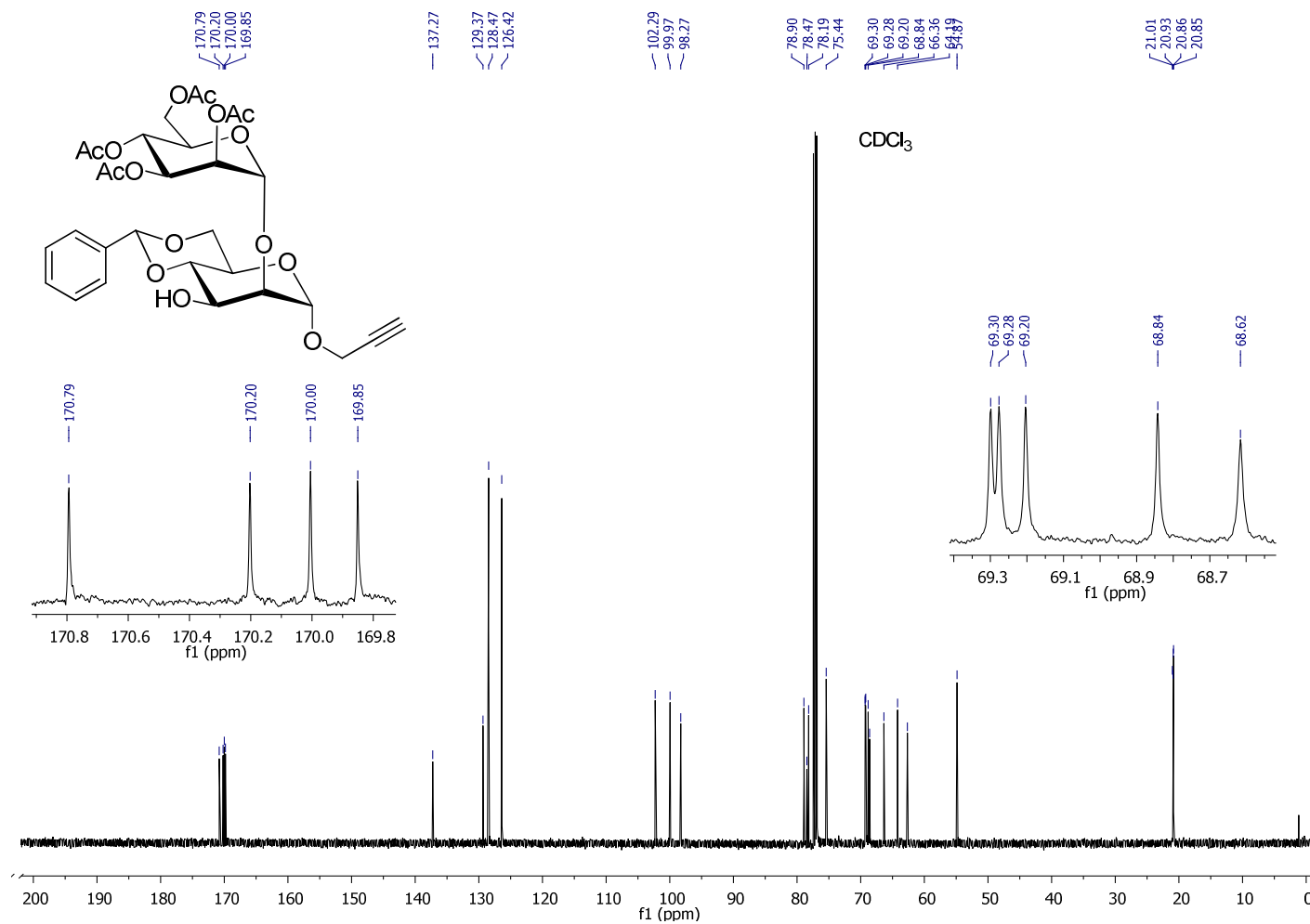
gCOSY NMR spectrum (500 MHz, CDCl_3) of propargyl 4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside (33).



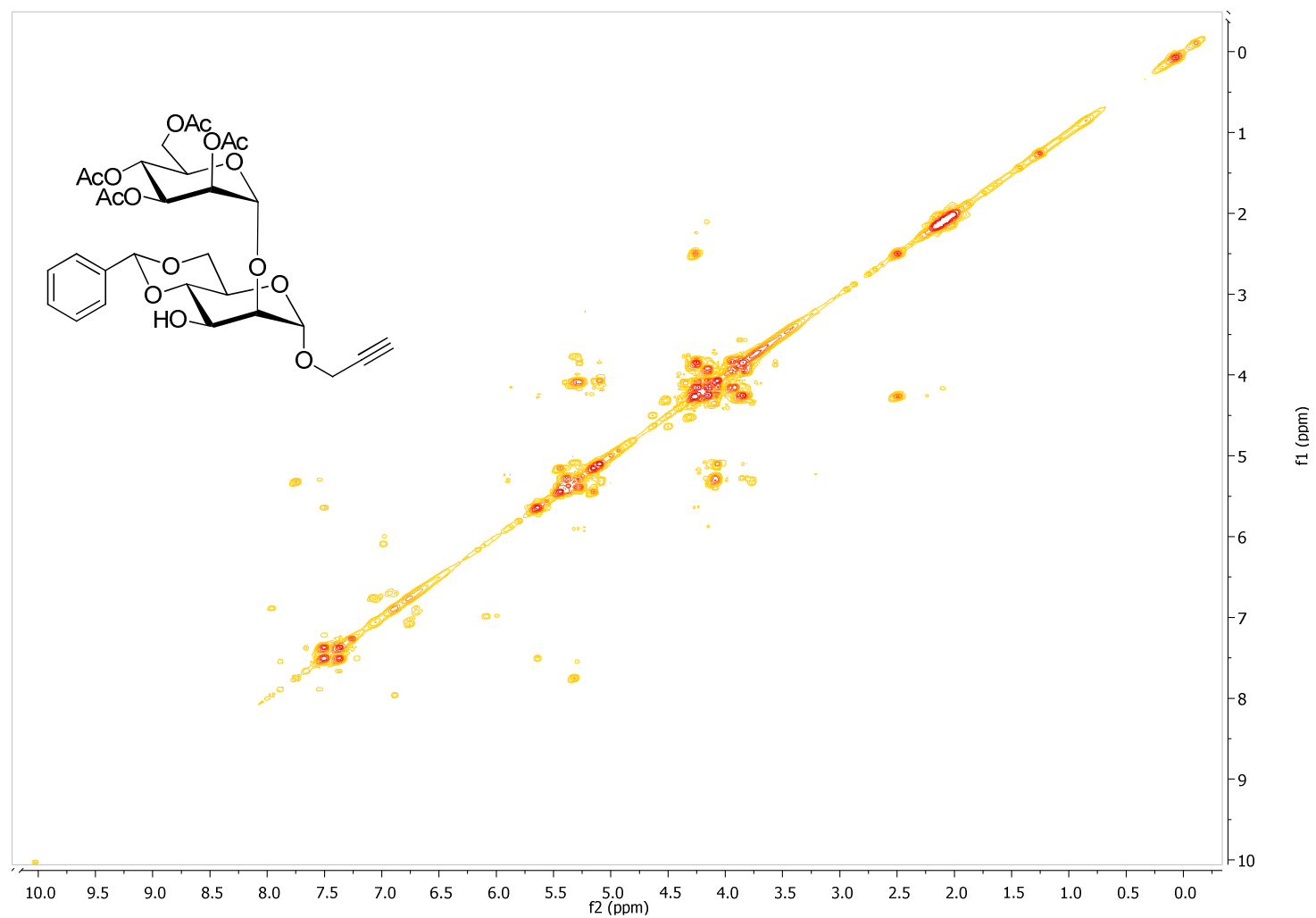
gHMBC NMR spectrum (500 MHz, CDCl_3) of propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**33**).

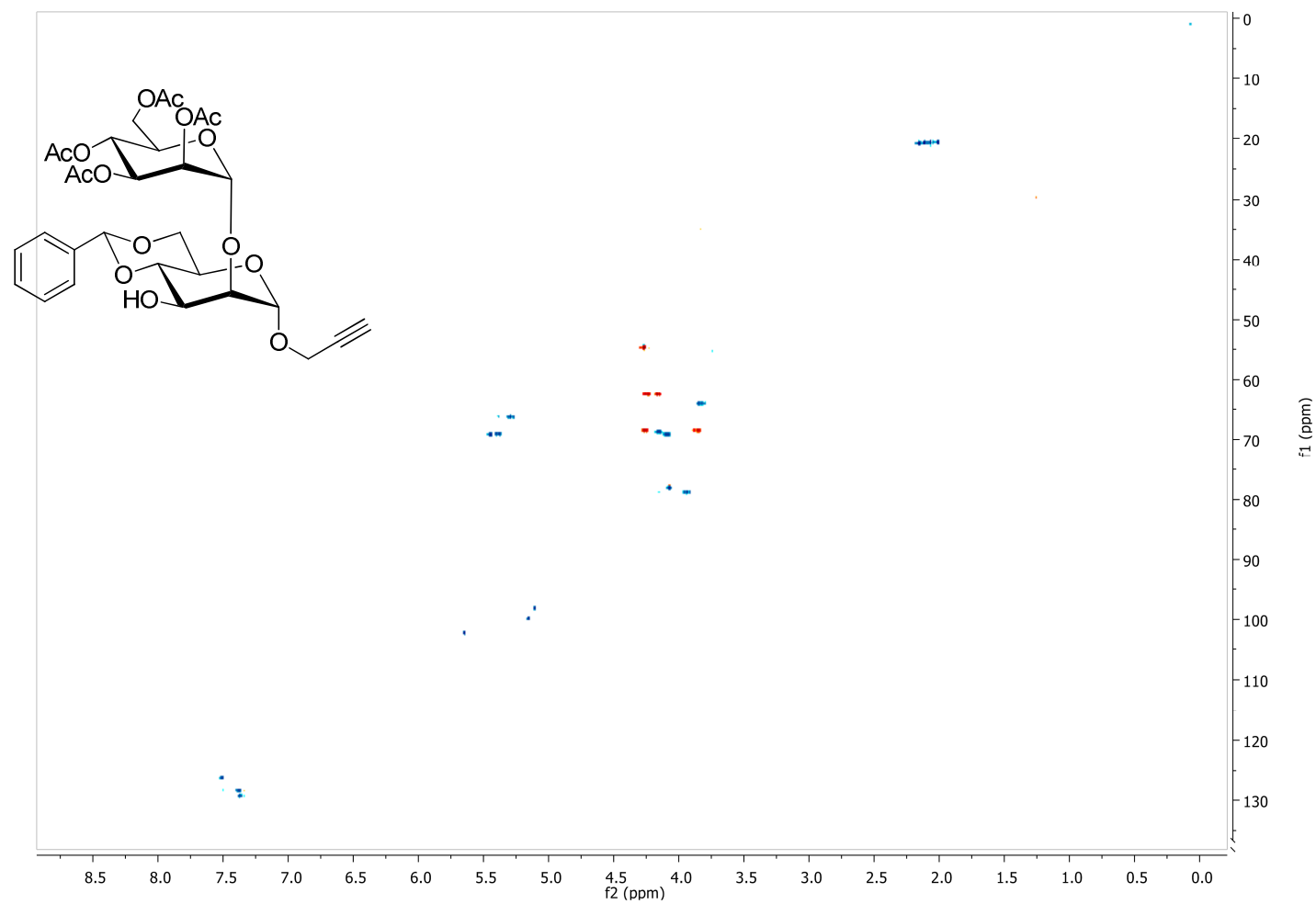


¹H NMR spectrum (500 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene- α -D-mannopyranoside (**35**).

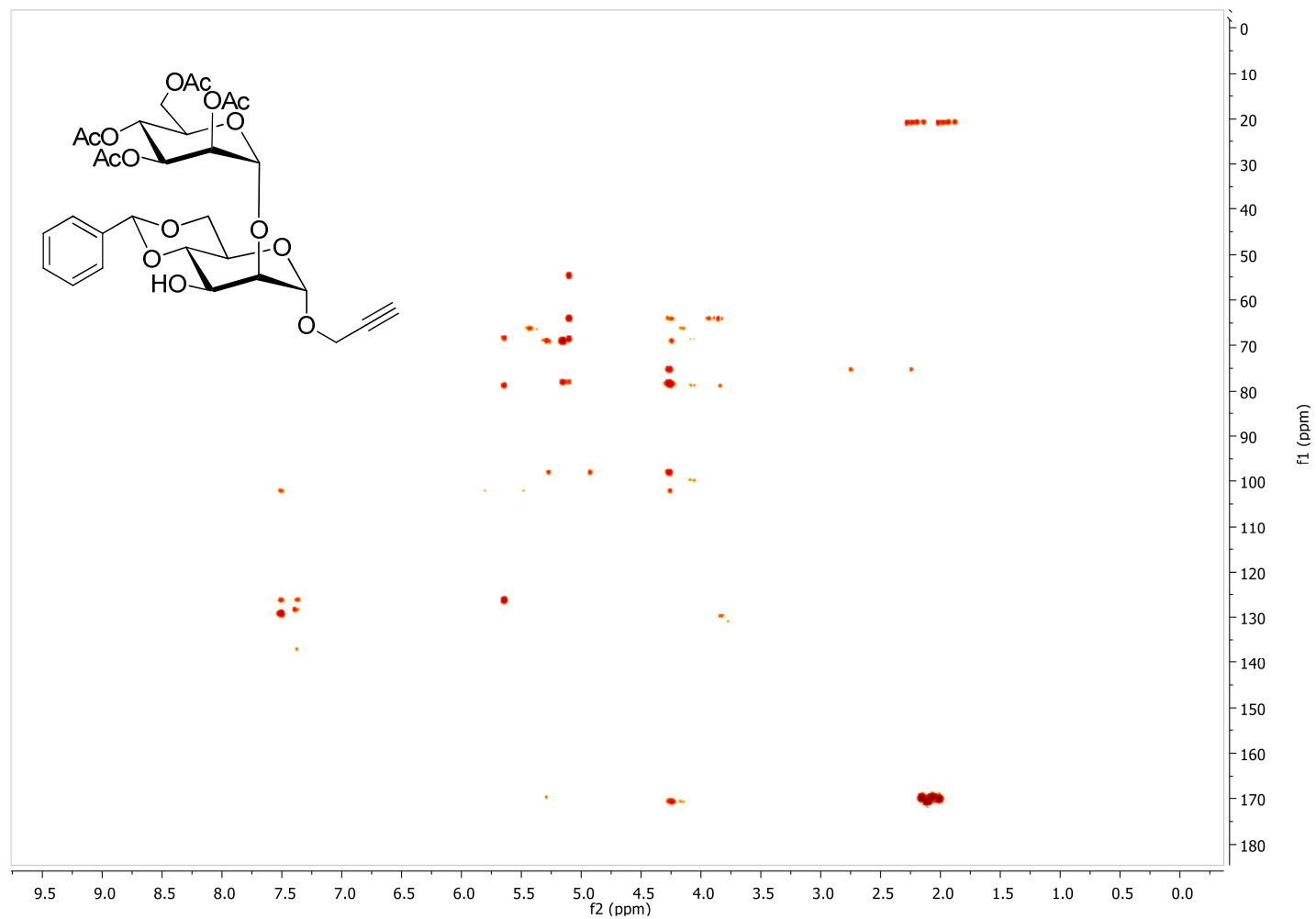


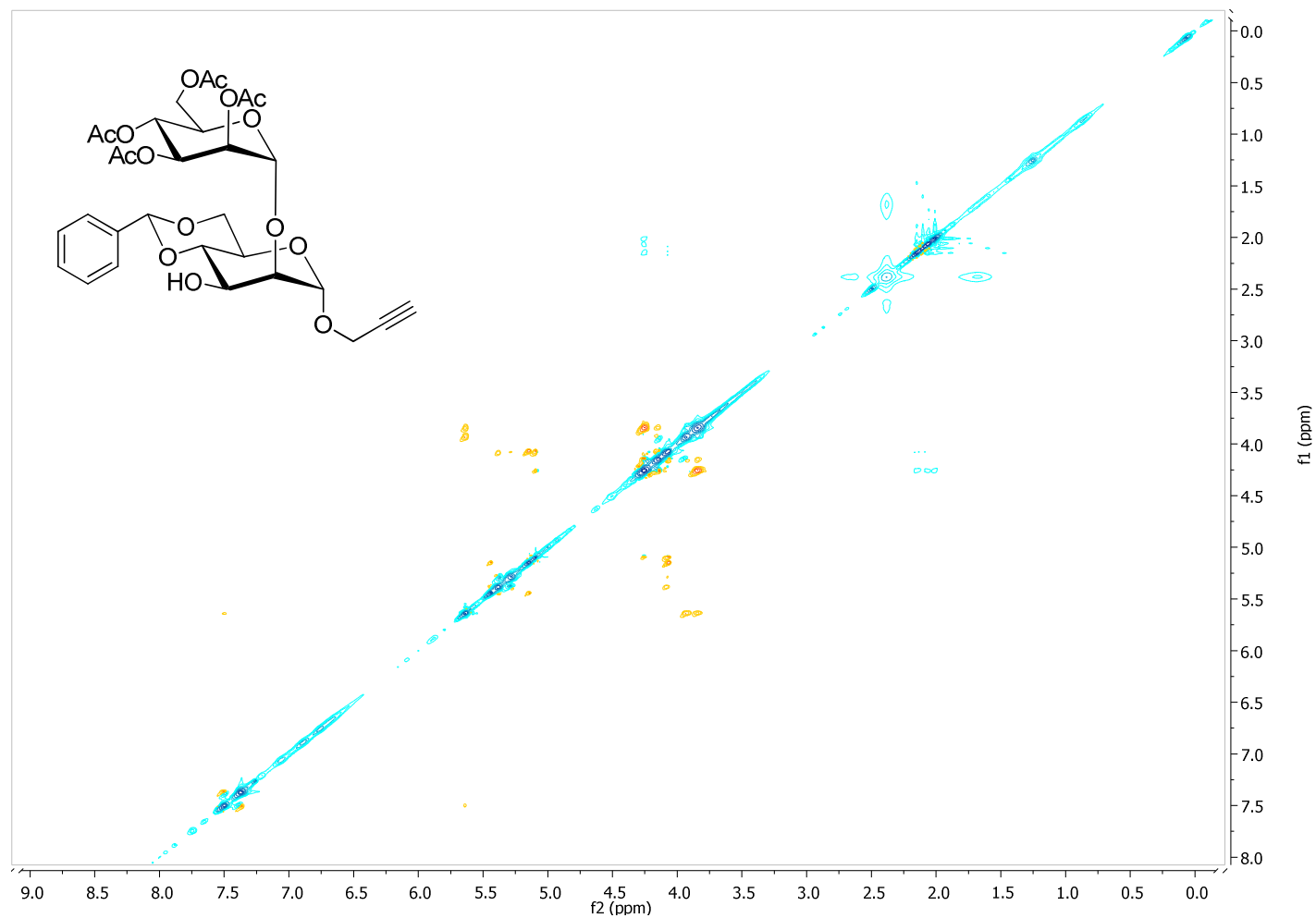
^{13}C NMR spectrum (125 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-4,6-O-benzylidene- α -D-mannopyranoside (35).



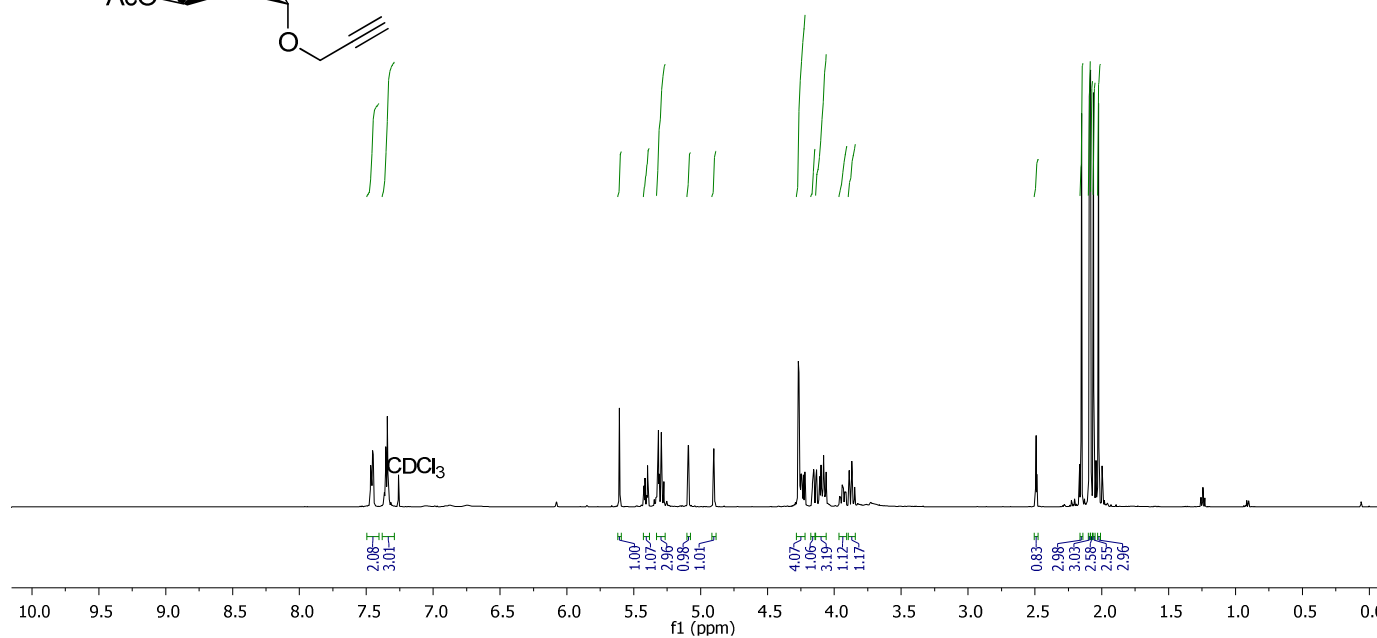
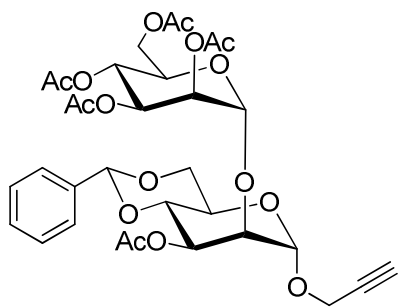


gHSQC NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-4,6-O-benzylidene- α -D-mannopyranoside (35).

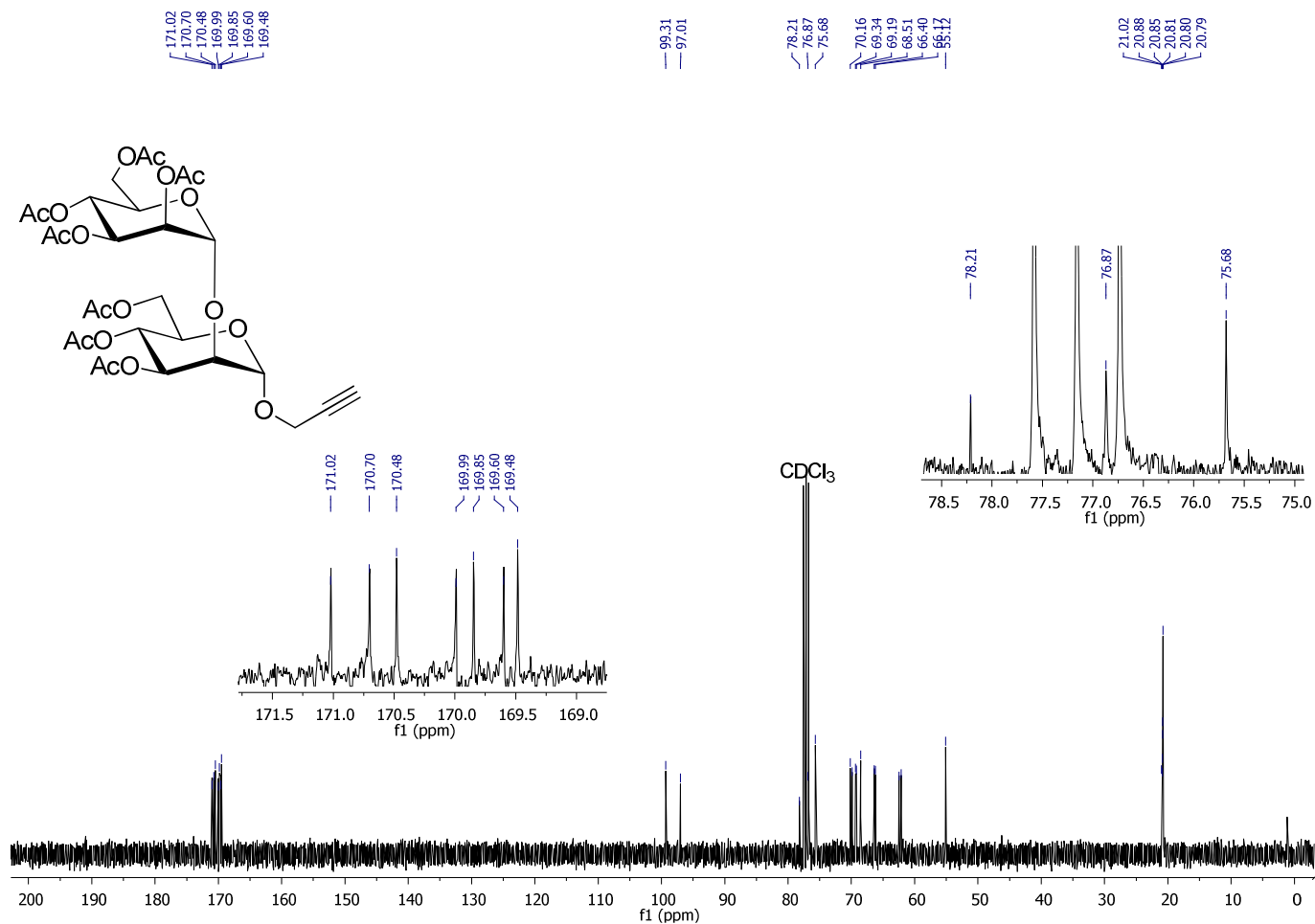




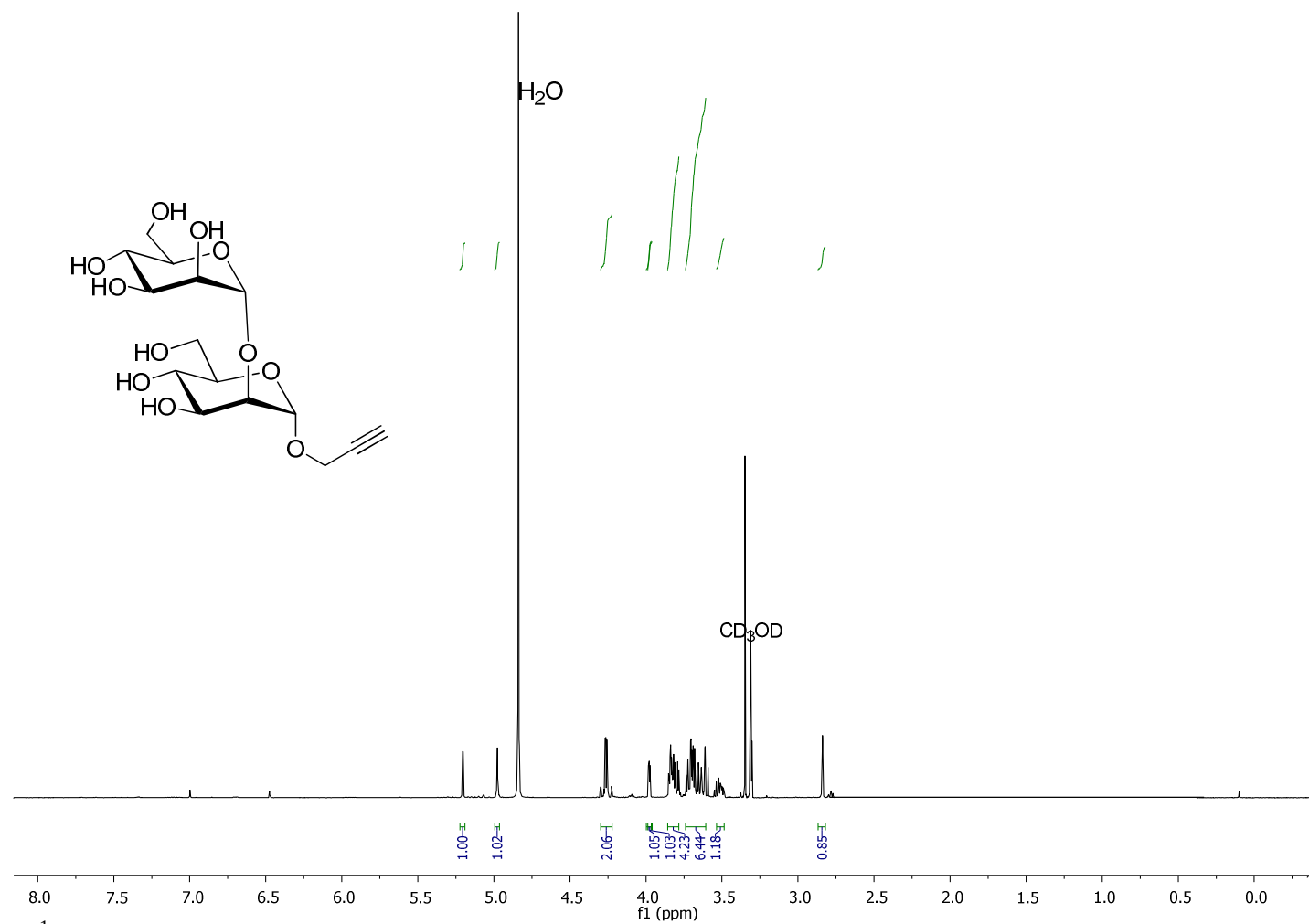
gNOESY NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene- α -D-mannopyranoside (**35**).



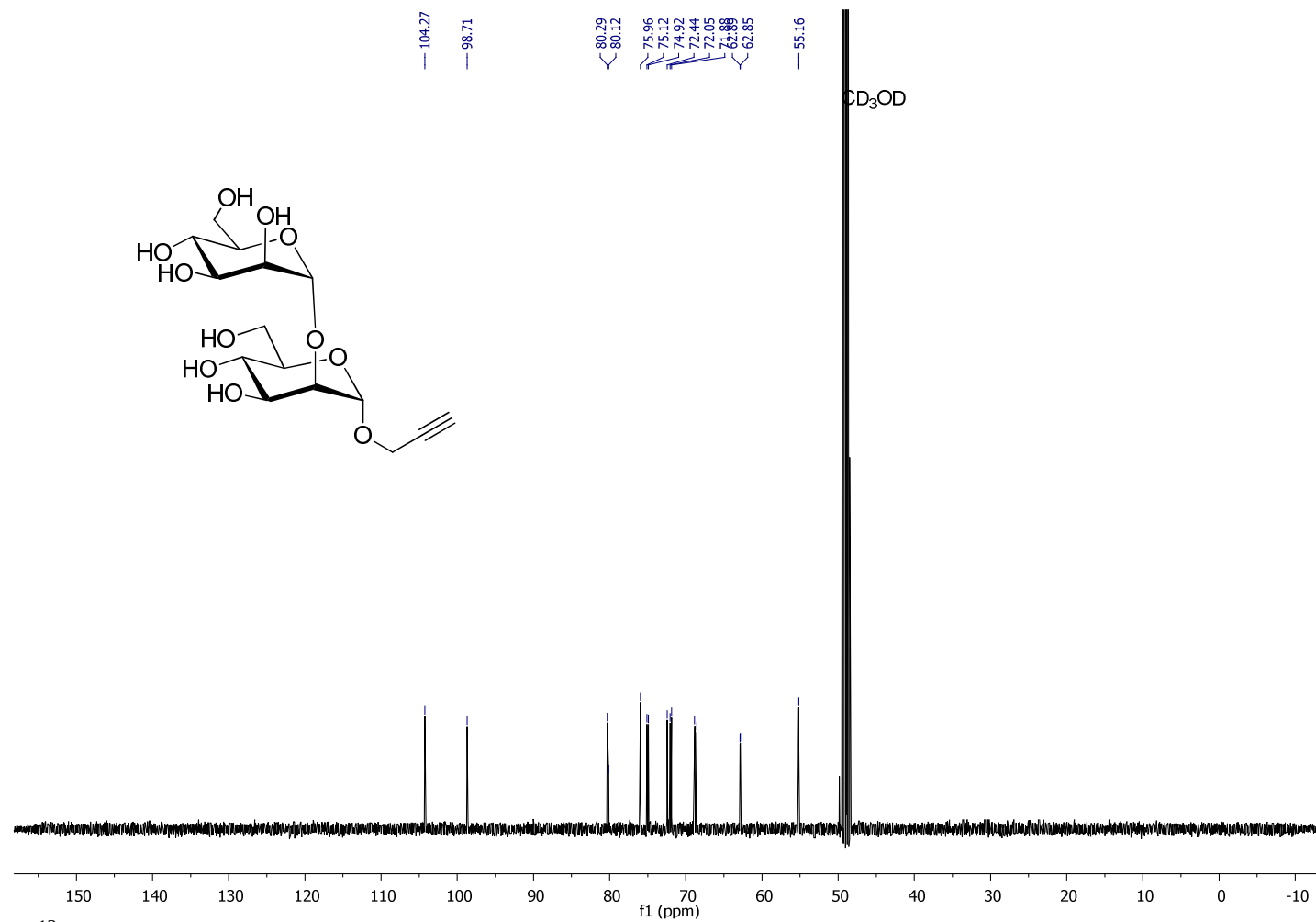
^1H NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 2)-3-*O*-acetyl-4,6-*O*-benzylidene- α -D-mannopyranoside (**36**).



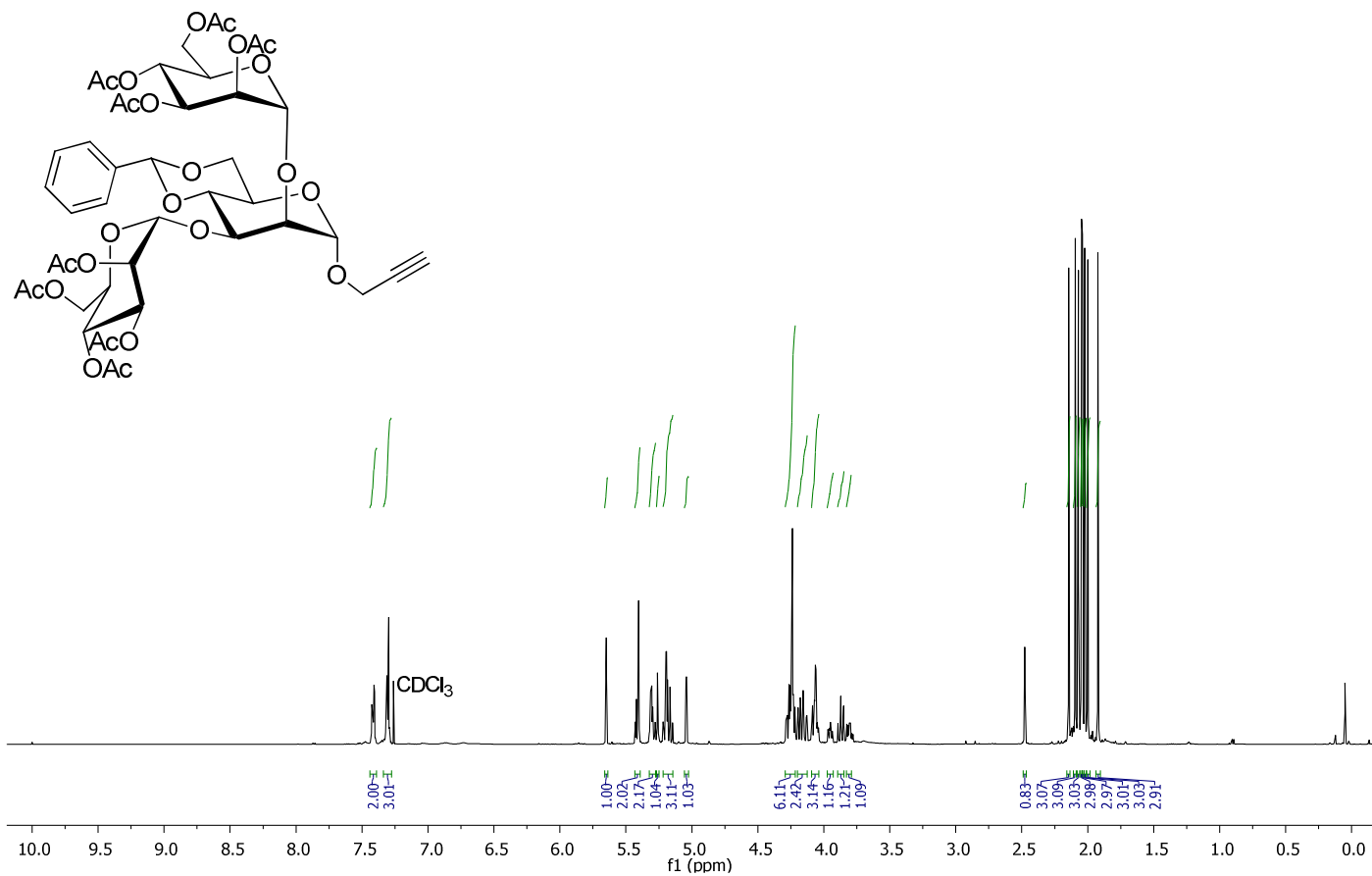
^{13}C NMR spectrum (75 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-*O*-acetyl- α -D-mannopyranoside (37).



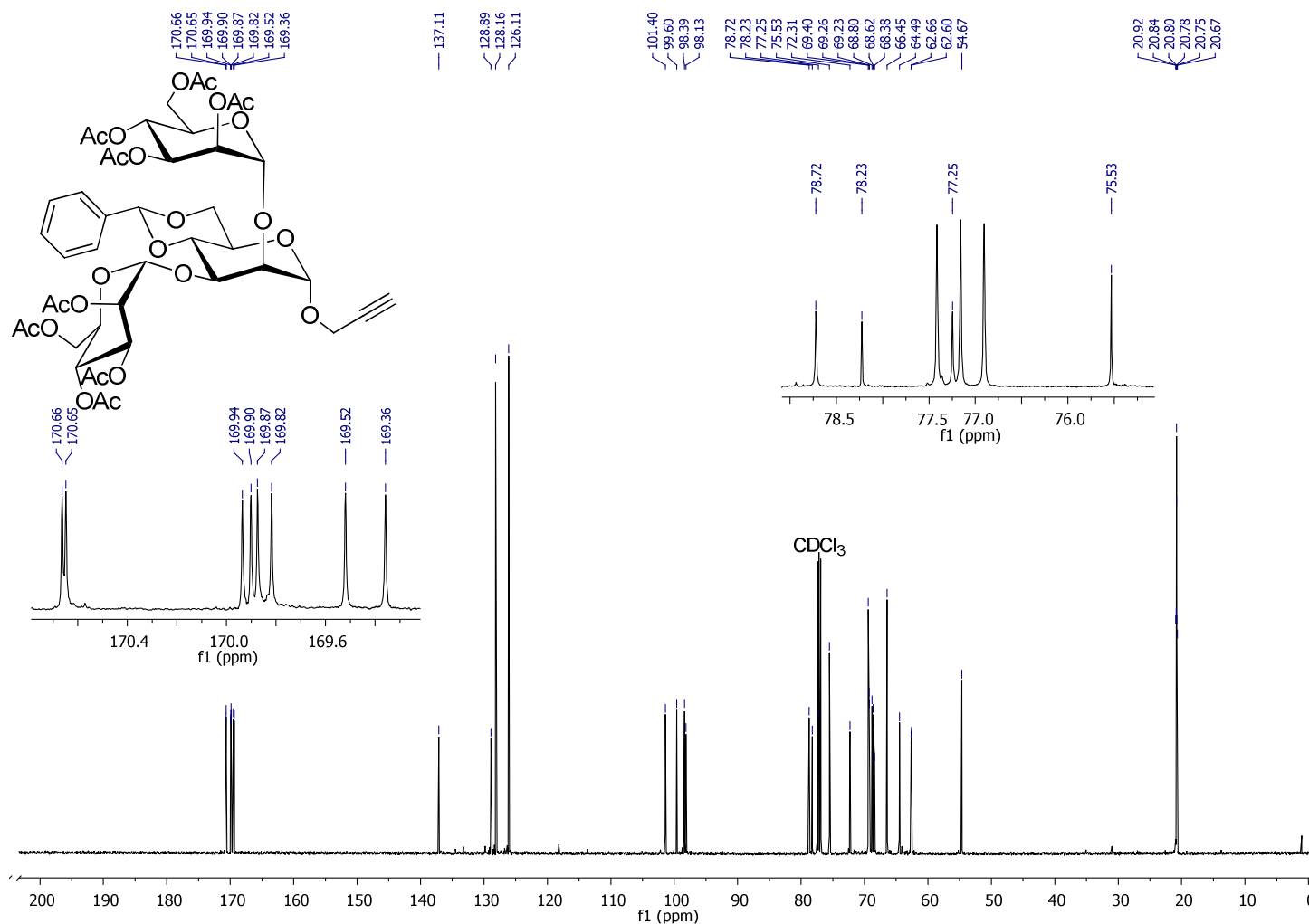
^1H NMR spectrum (500 MHz, CDCl_3) of propargyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranoside (**38**).



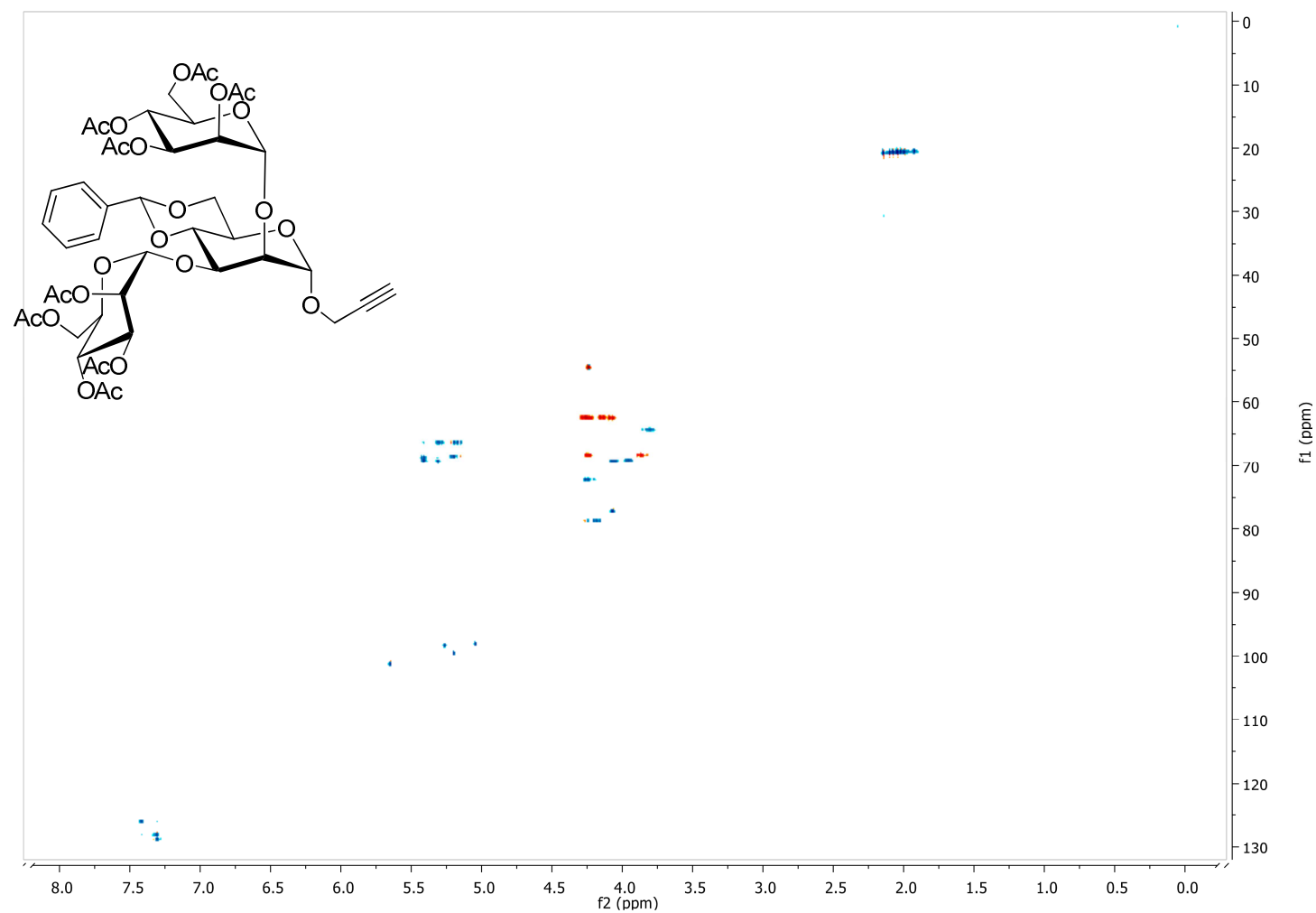
^{13}C NMR spectrum (125 MHz, CDCl_3) of propargyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranoside (38).



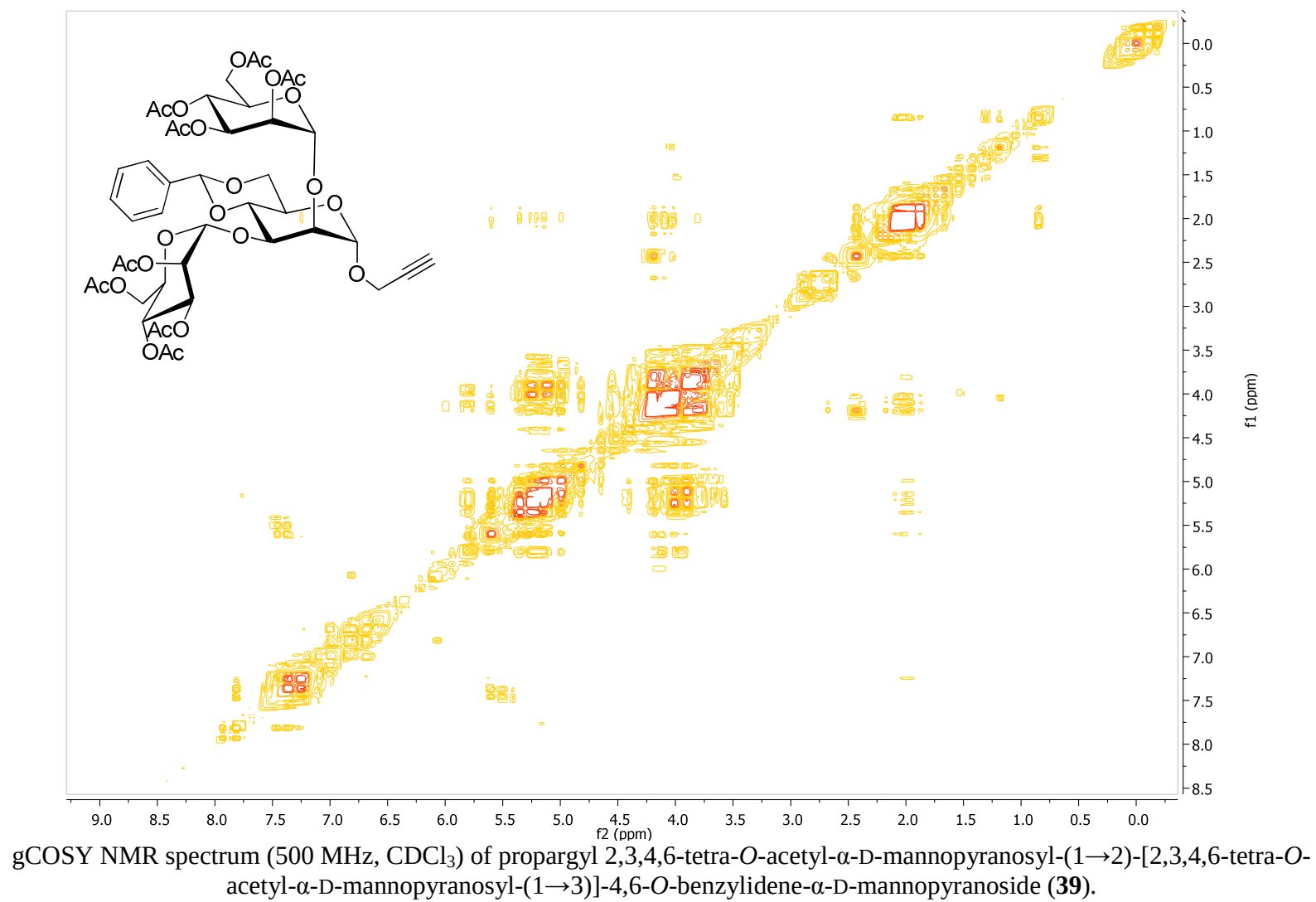
^1H NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (**39**).

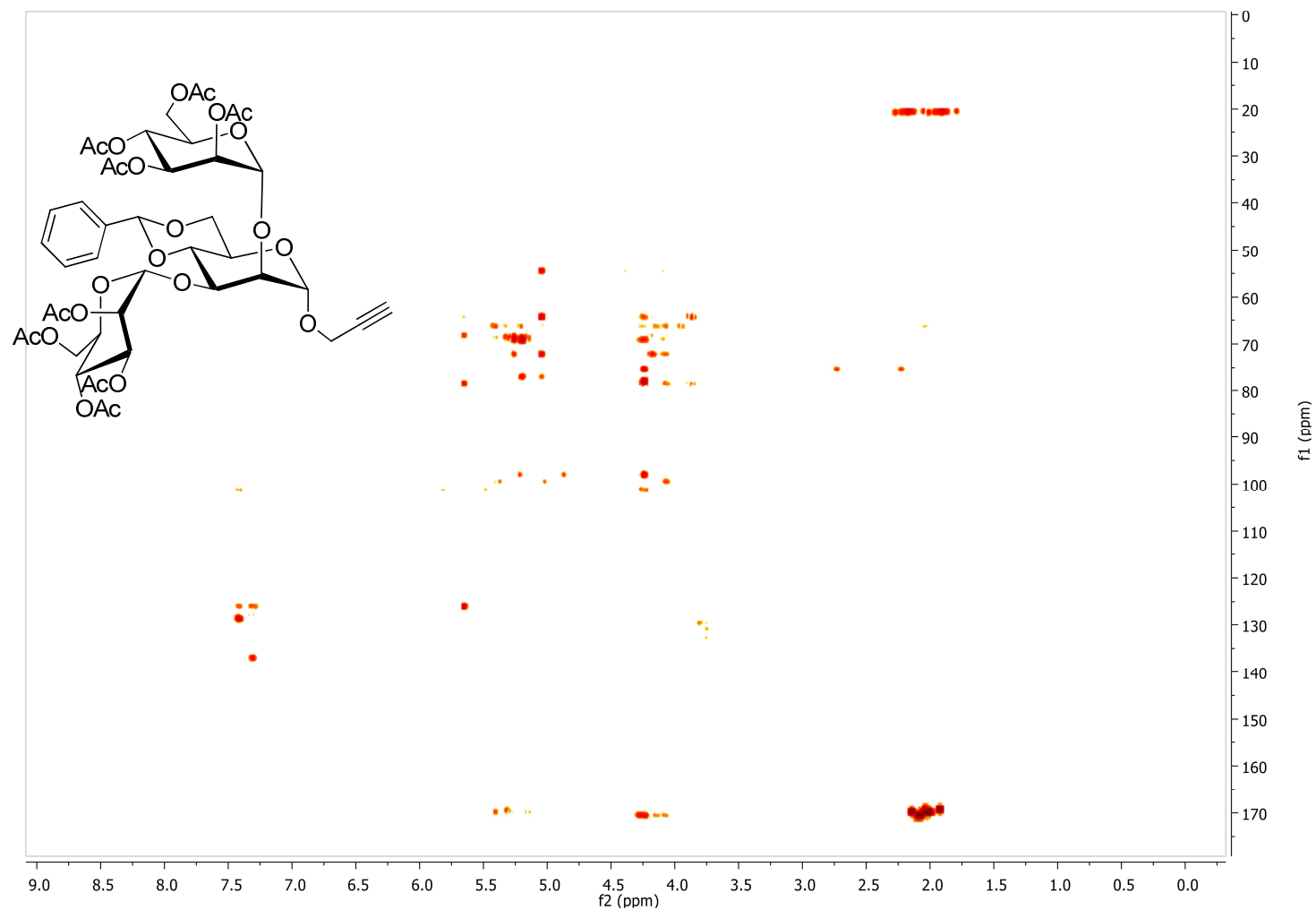


¹³C NMR spectrum (125 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**39**).

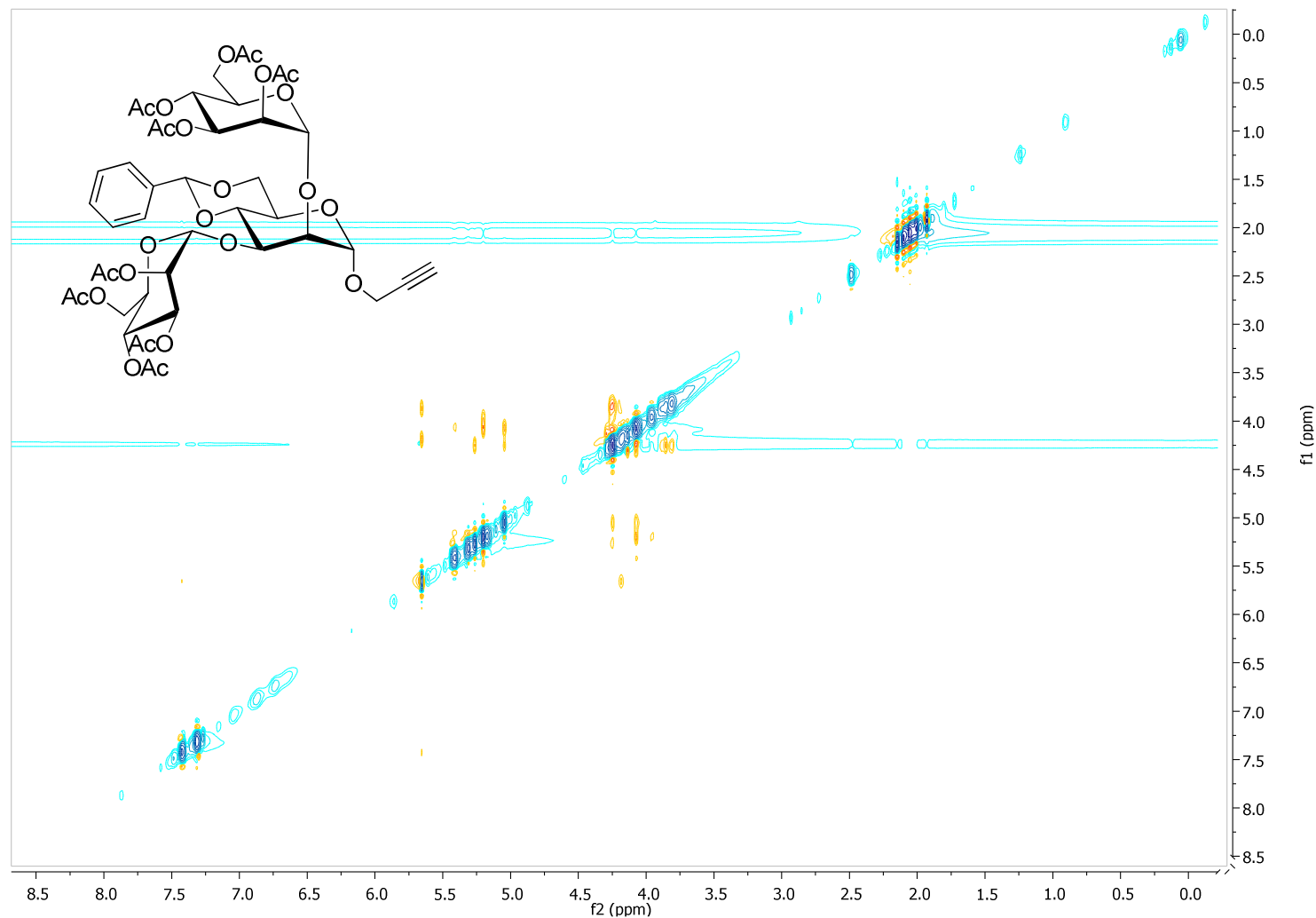


gHSQC NMR spectrum (500 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**39**).

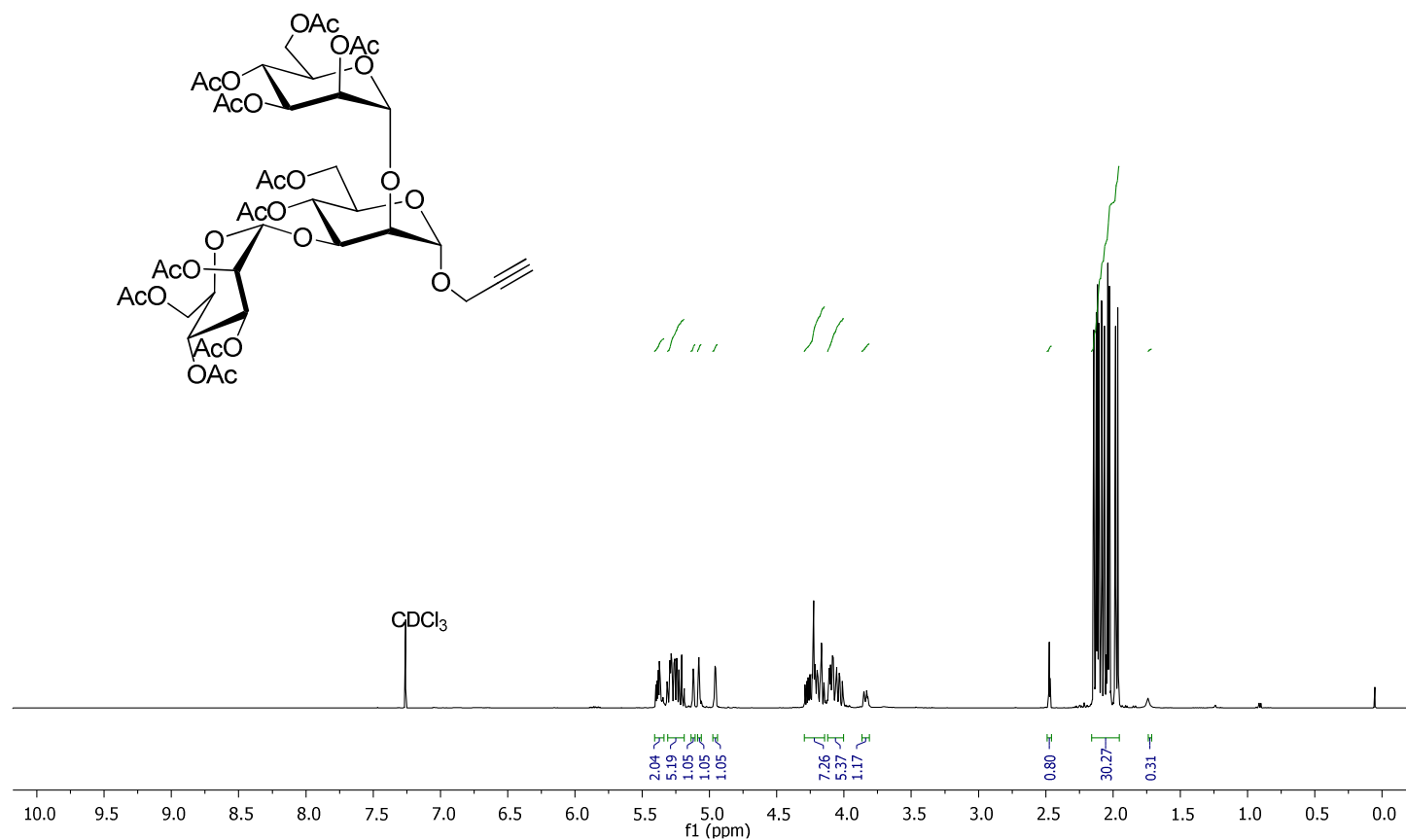




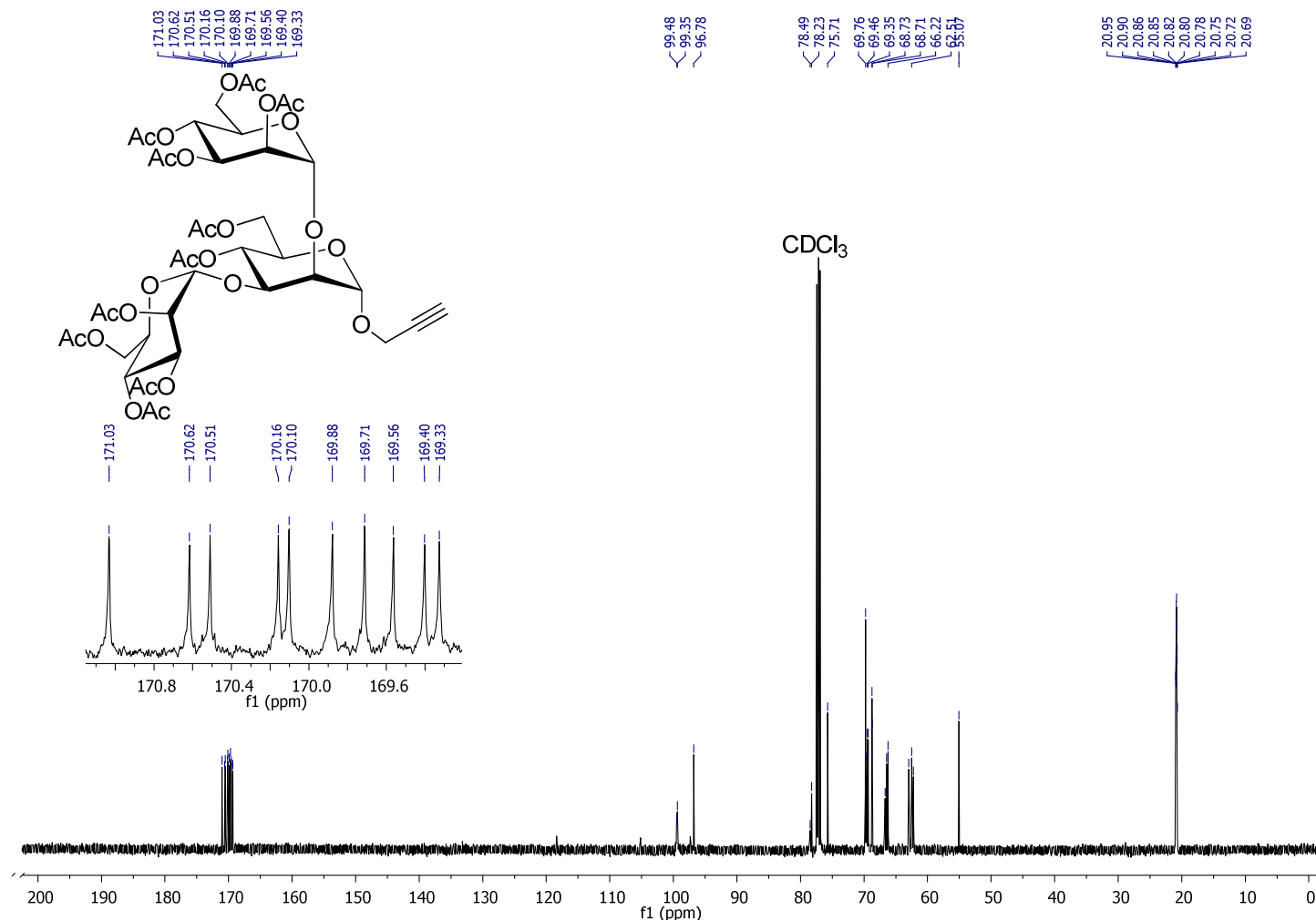
gHMBC NMR spectrum (500 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**39**).



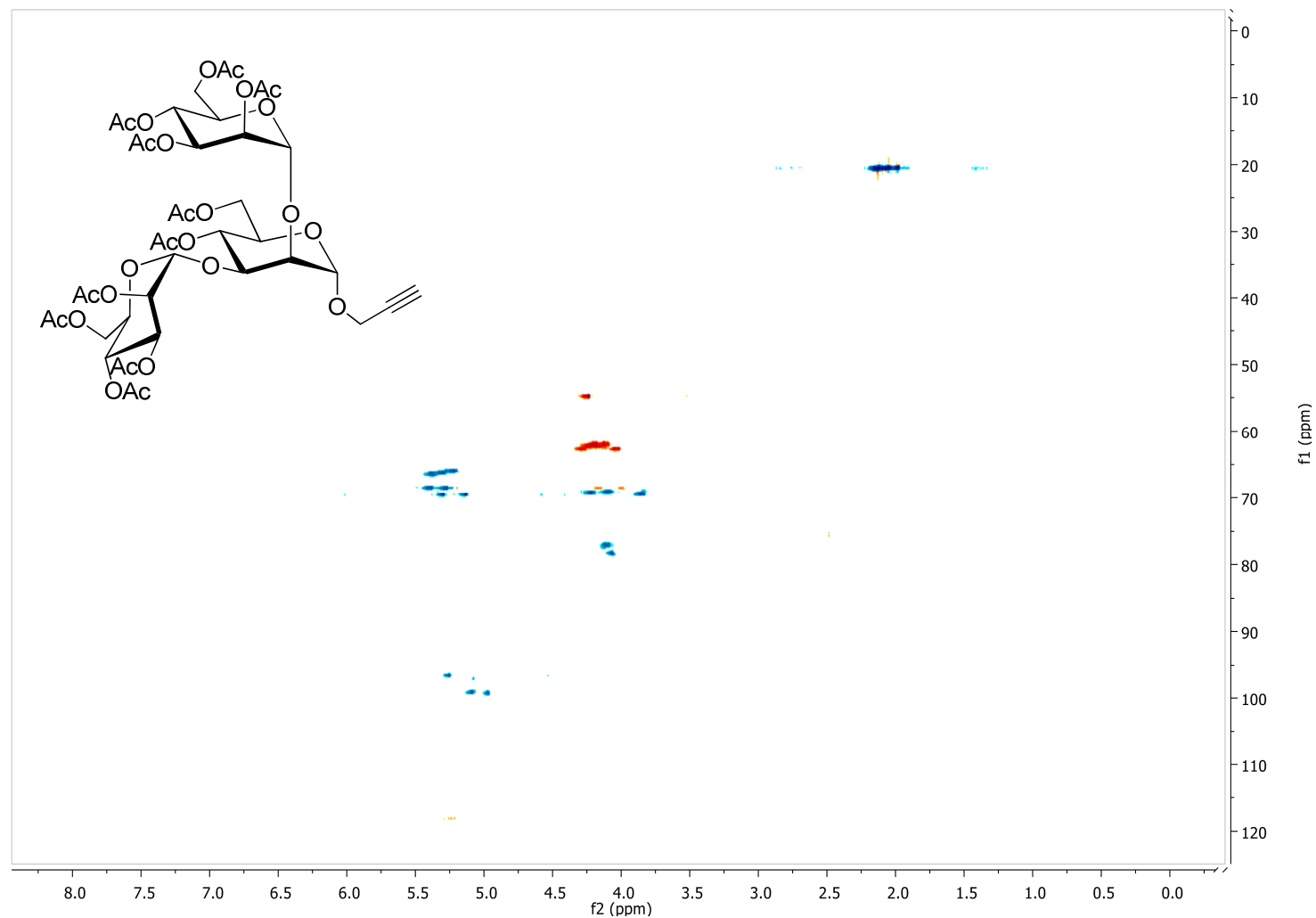
NOESY NMR spectrum (500 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**39**).



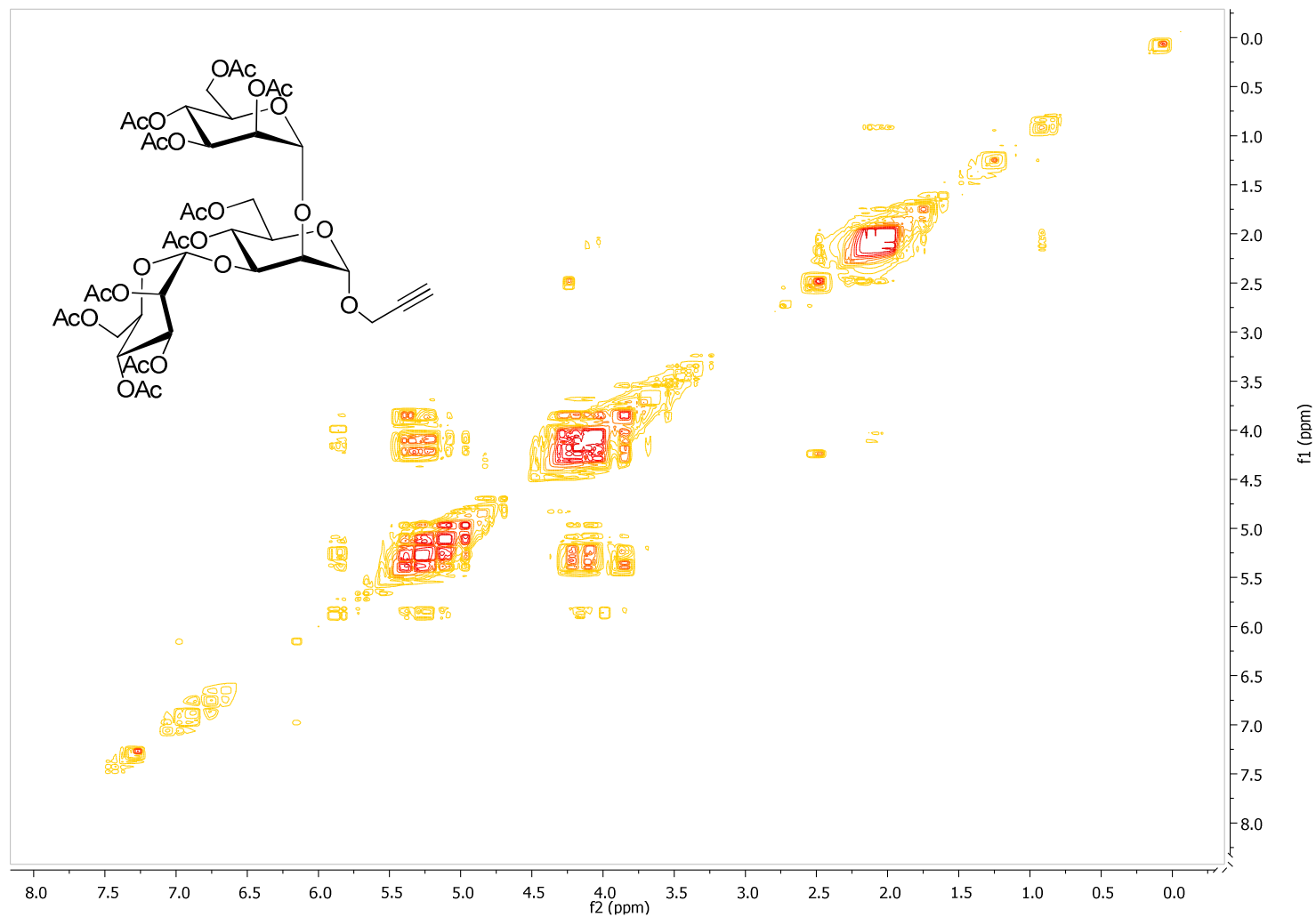
^1H NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



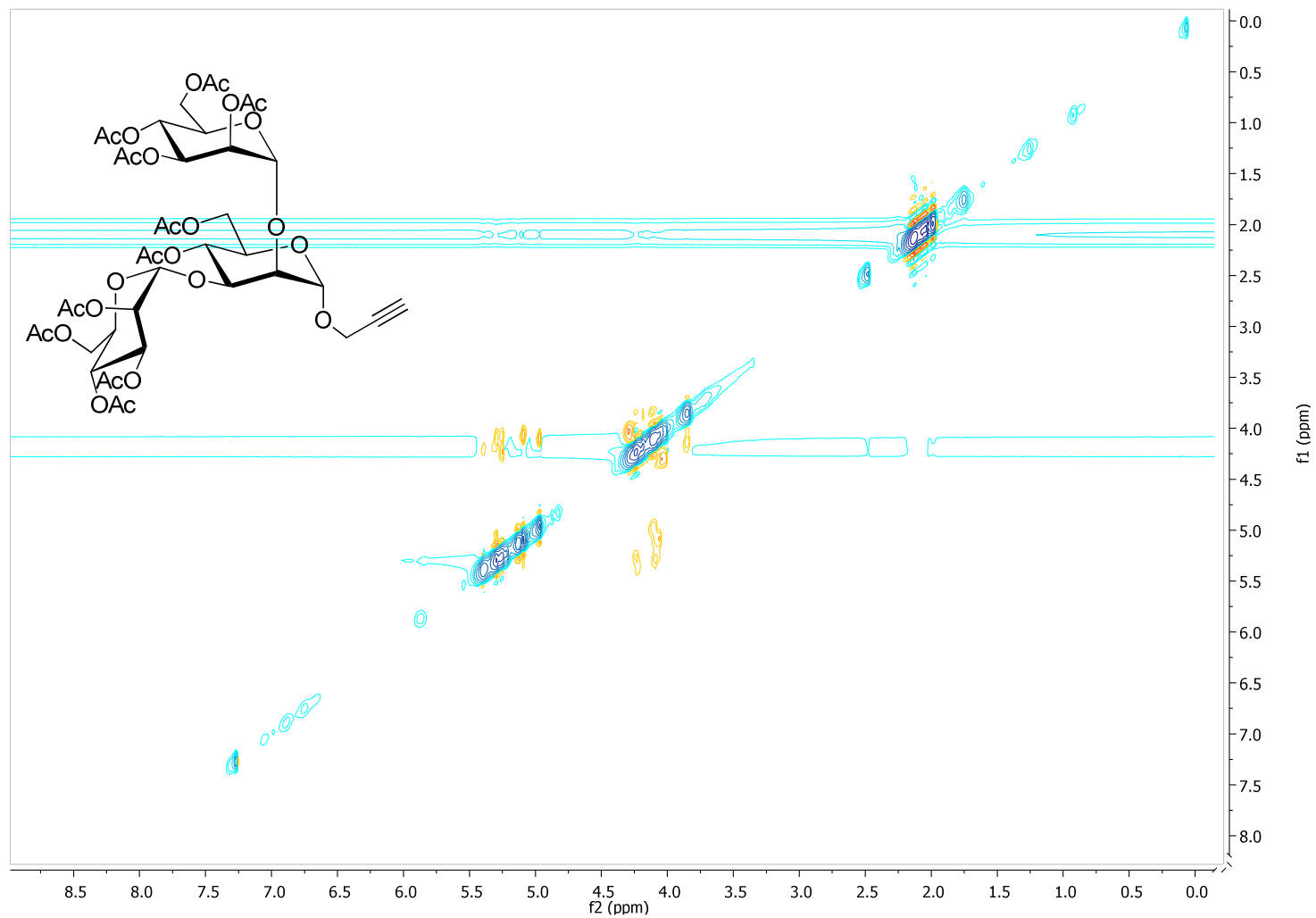
^{13}C NMR spectrum (125 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



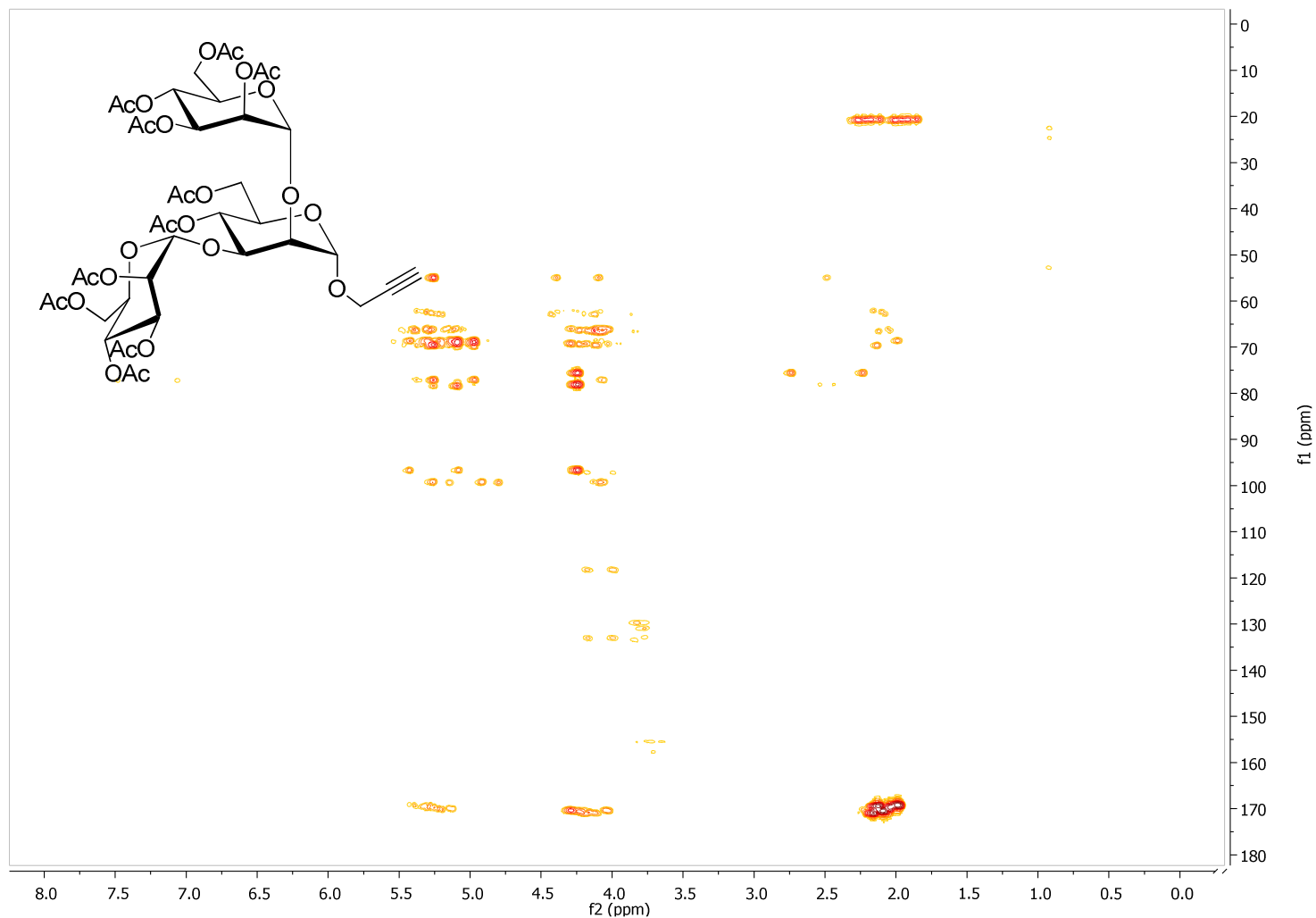
gHSQC NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



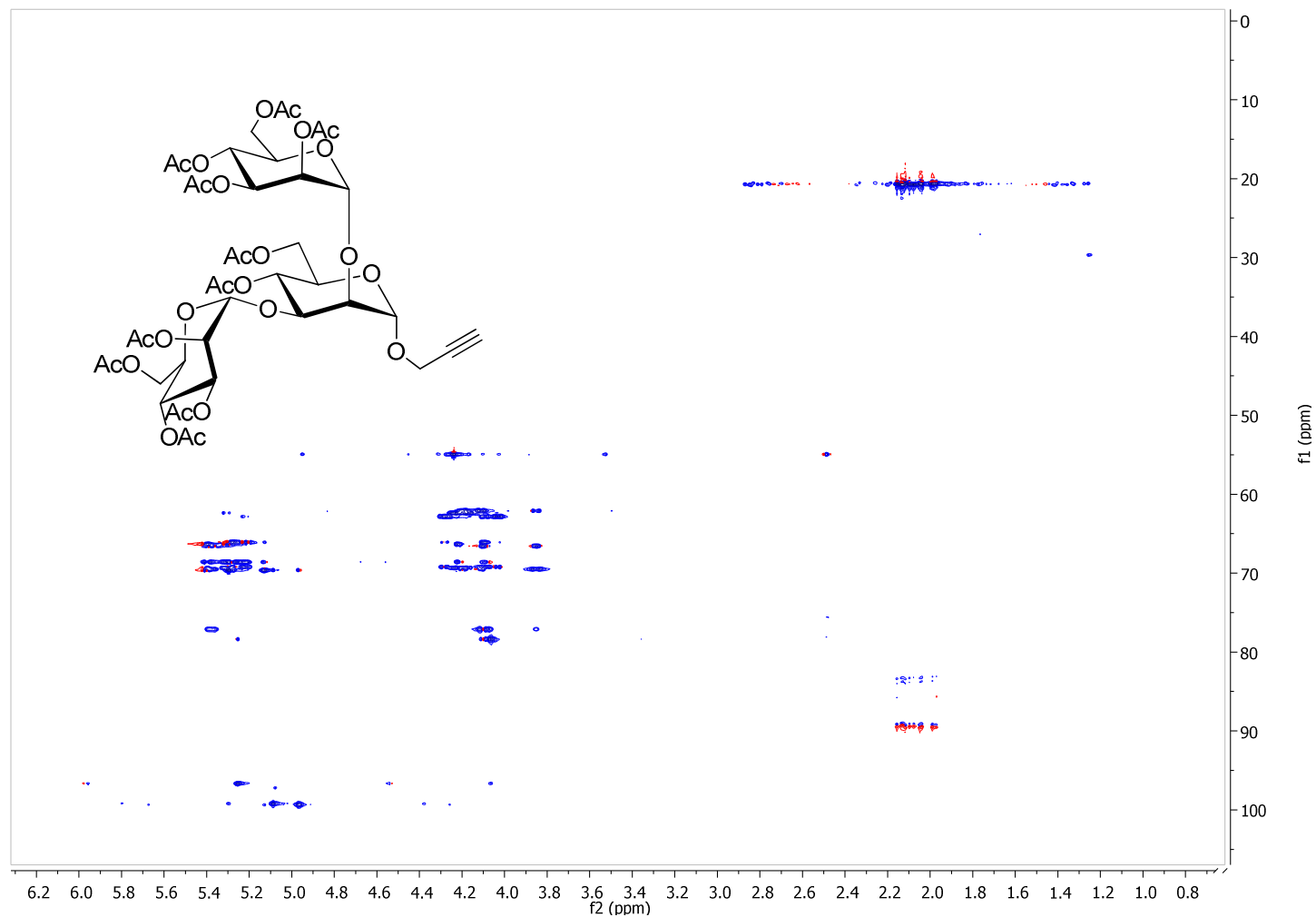
gCOSY NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



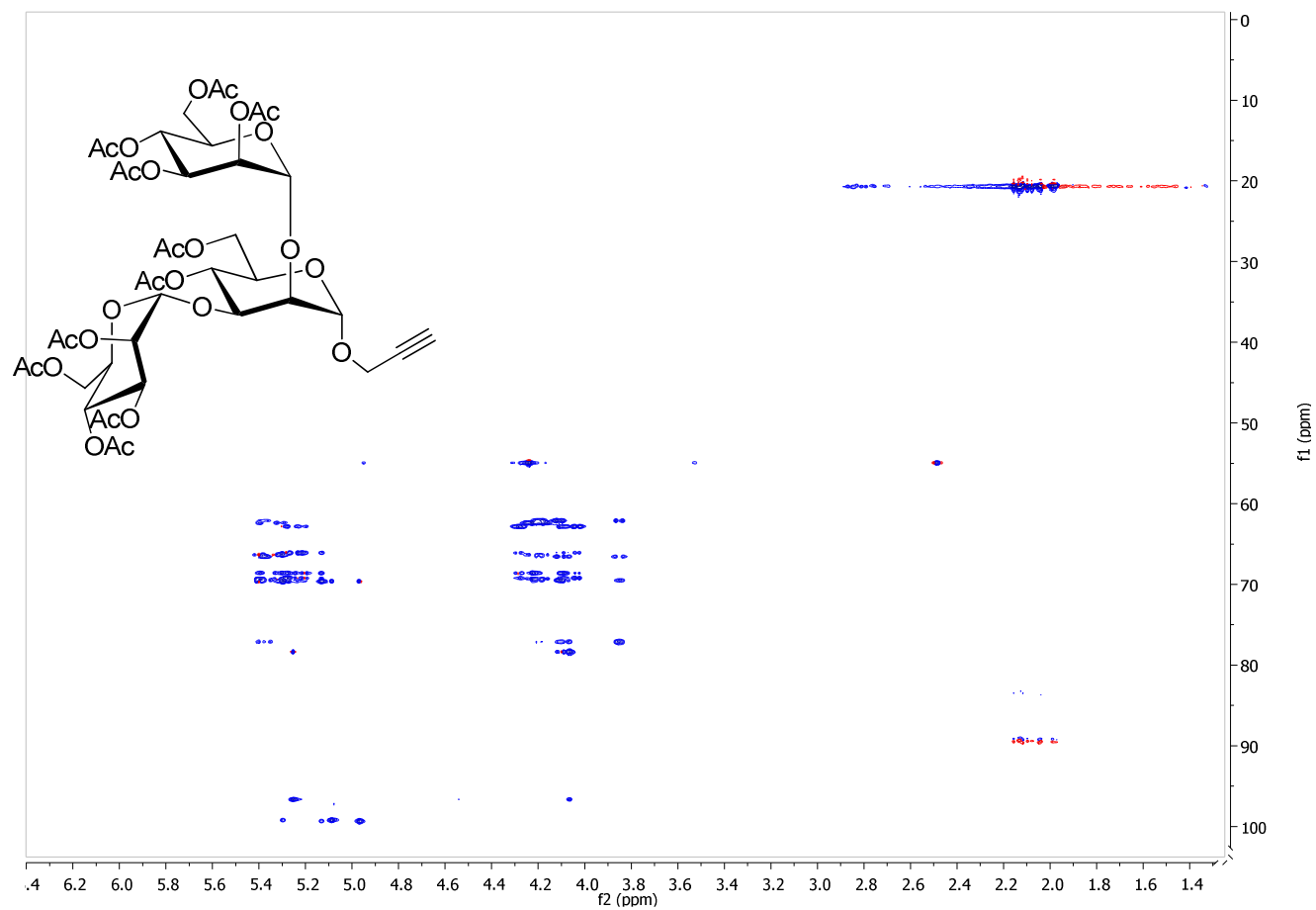
NOESY NMR spectrum (500 MHz, CDCl_3) of propargyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-O-benzylidene- α -D-mannopyranoside (**41**).



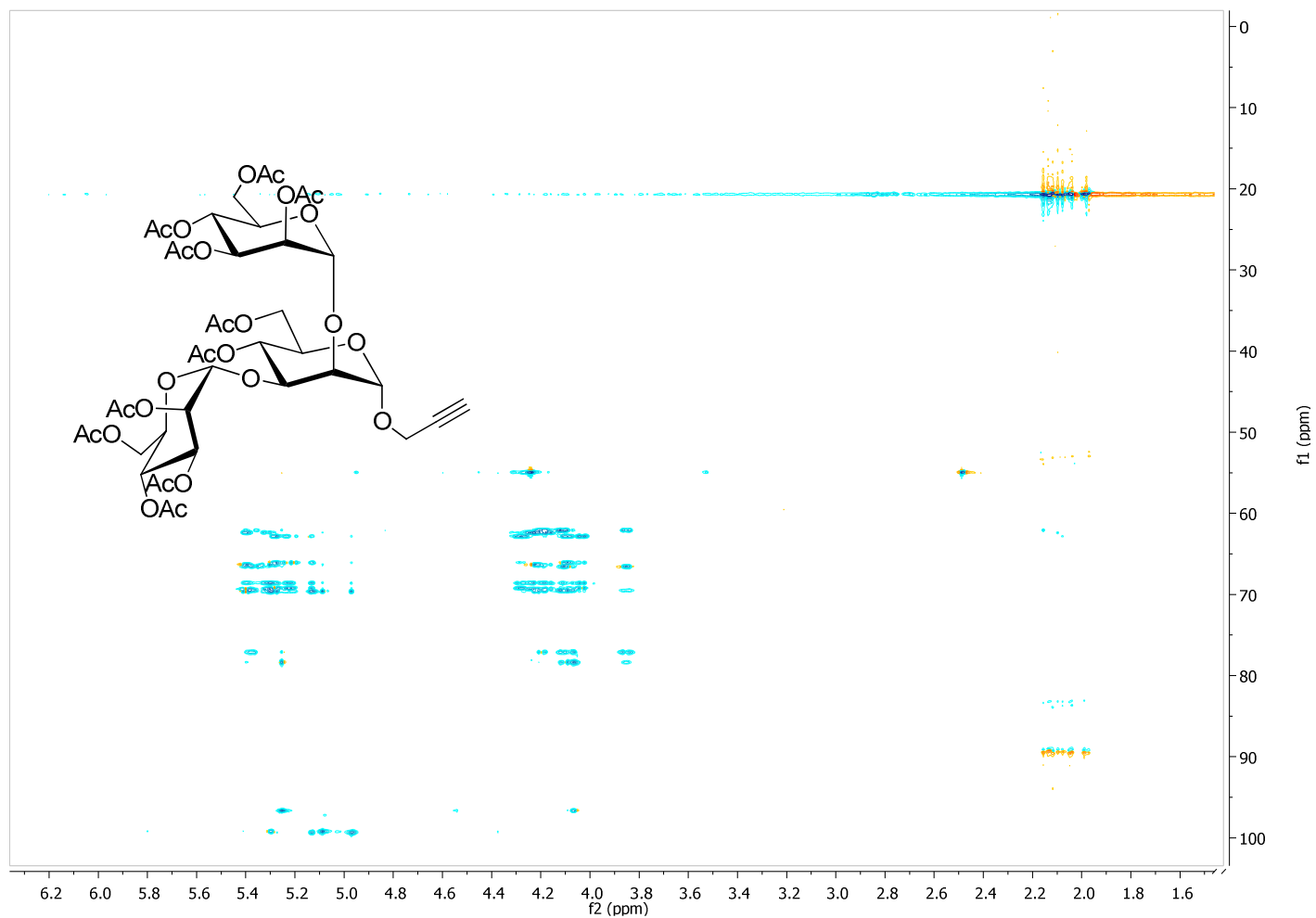
gHMBC NMR spectrum (500 MHz, CDCl₃) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



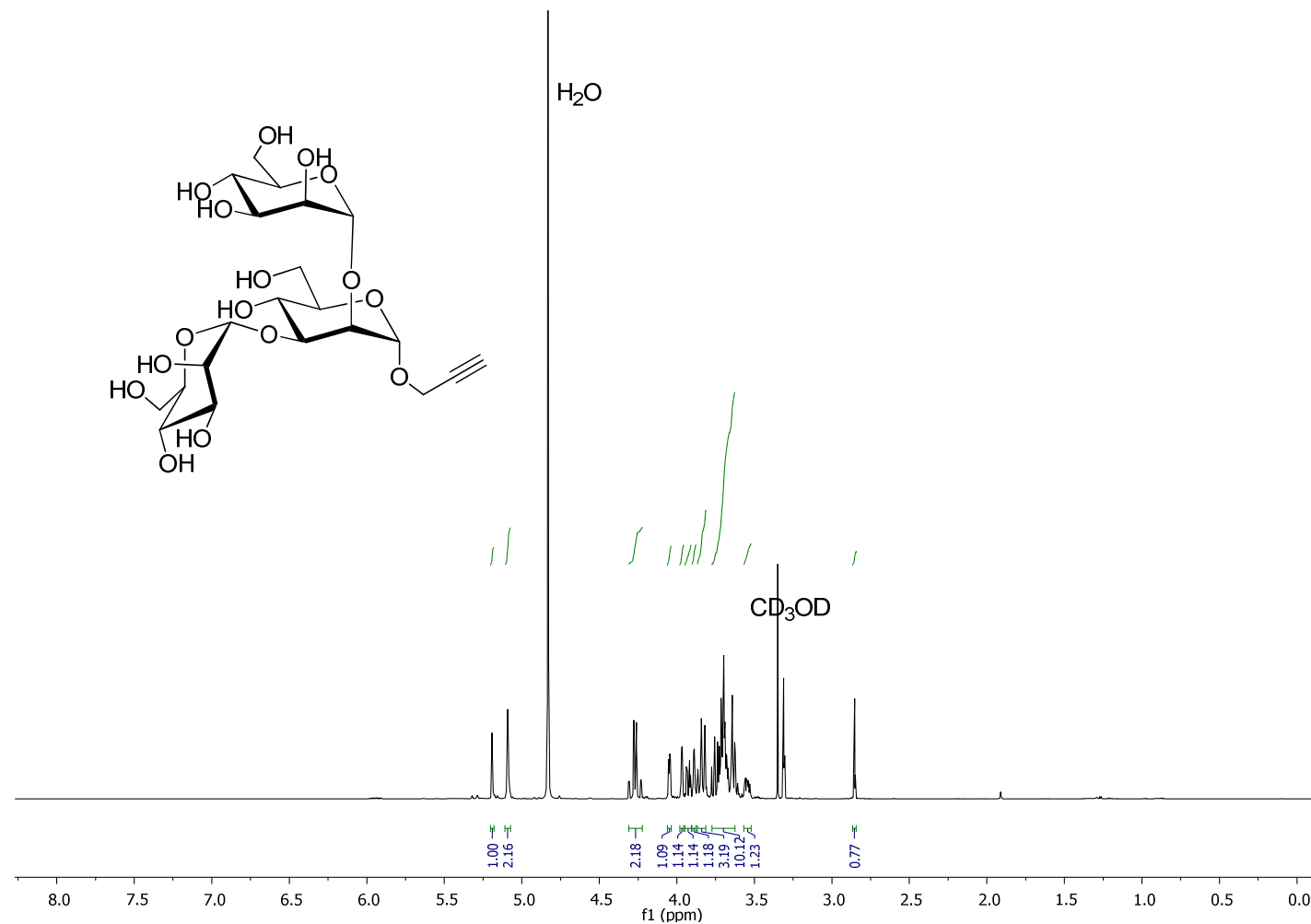
HSQCTOCSY NMR spectrum (500 MHz, CDCl₃, 24 ms) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).



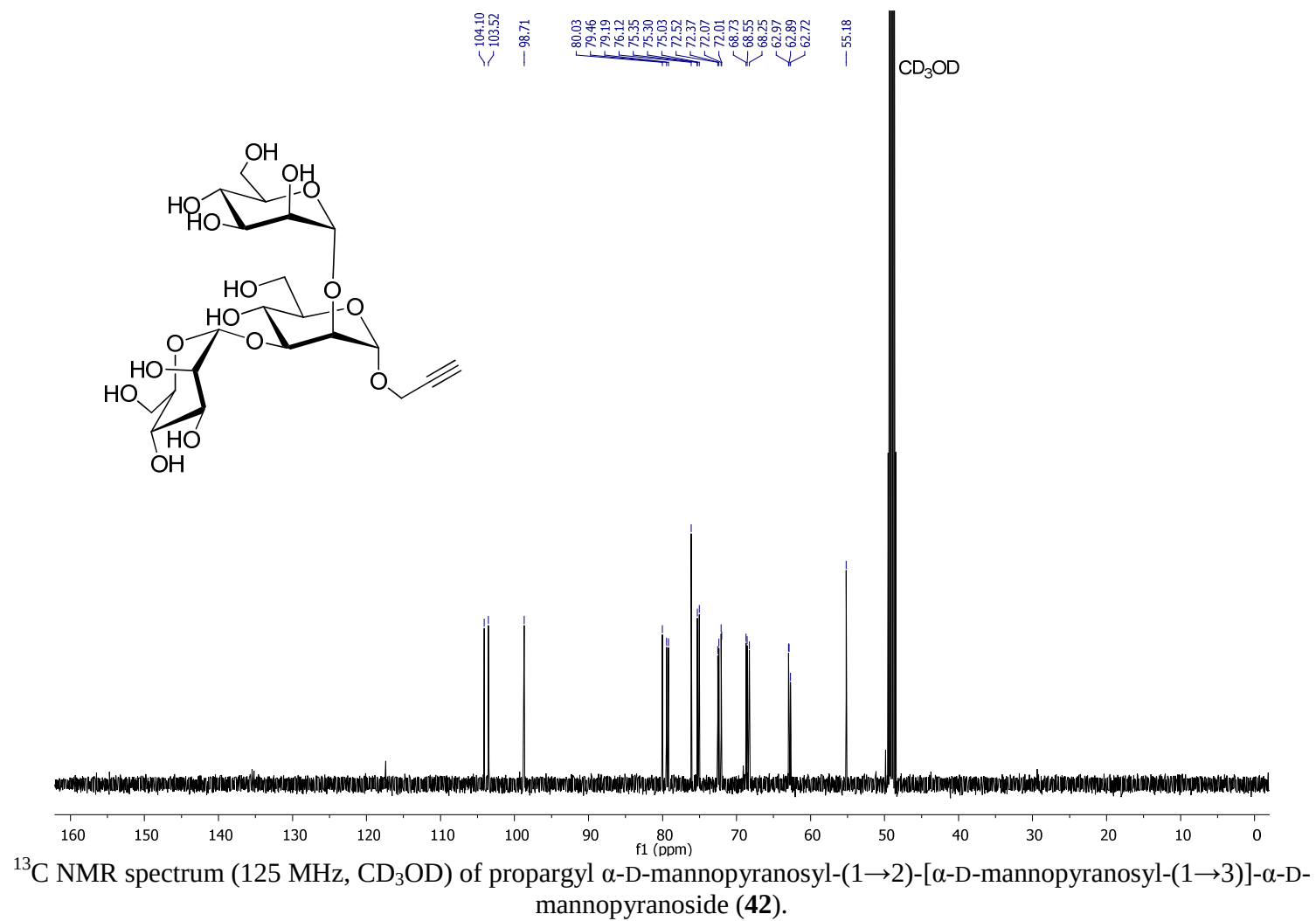
HSQCTOCSY NMR spectrum (500 MHz, CDCl_3 , 50 ms) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).

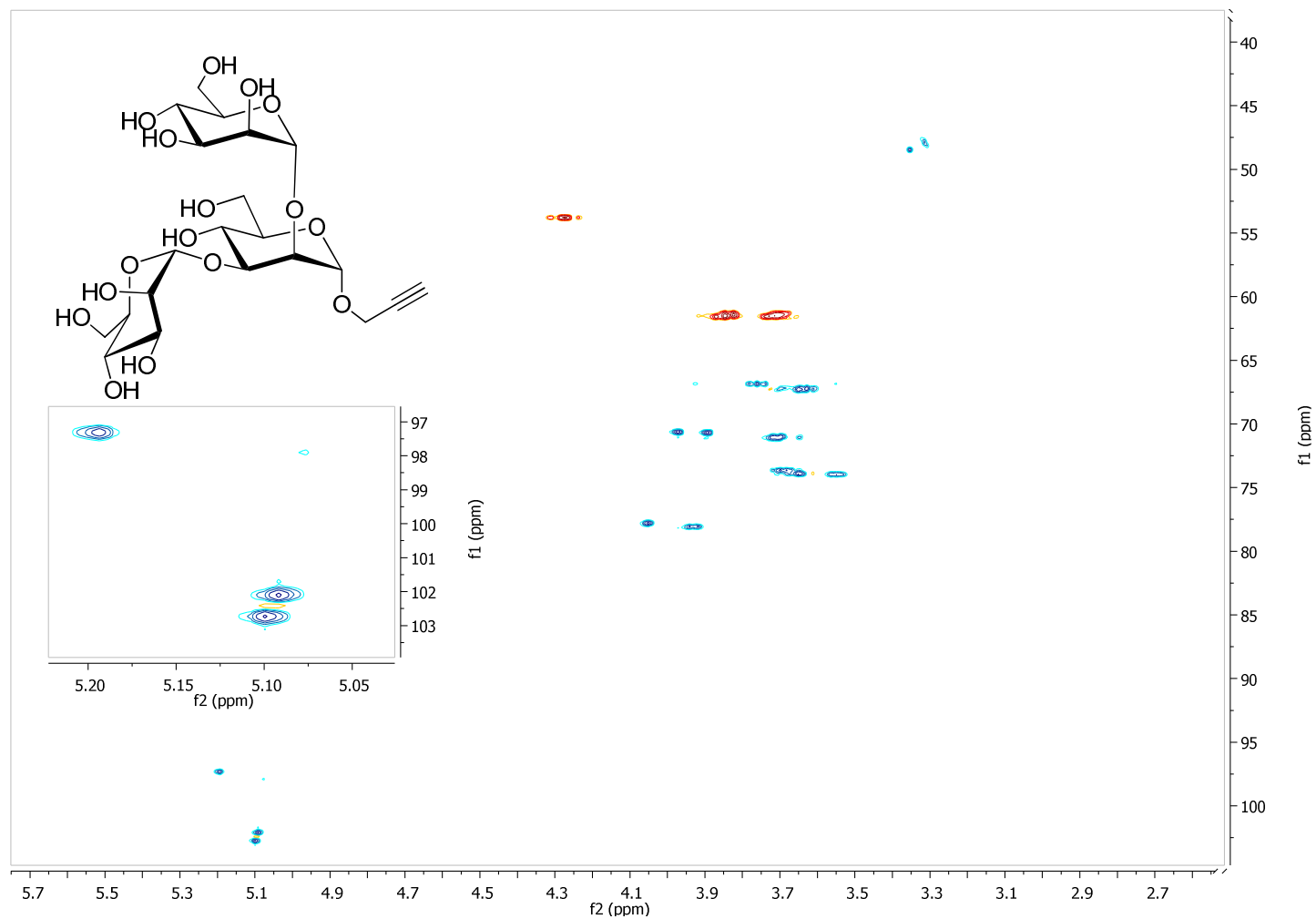


HSQCTOCSY NMR spectrum (500 MHz, CDCl₃, 80 ms) of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl-(1→3)]-4,6-*O*-benzylidene- α -D-mannopyranoside (**41**).

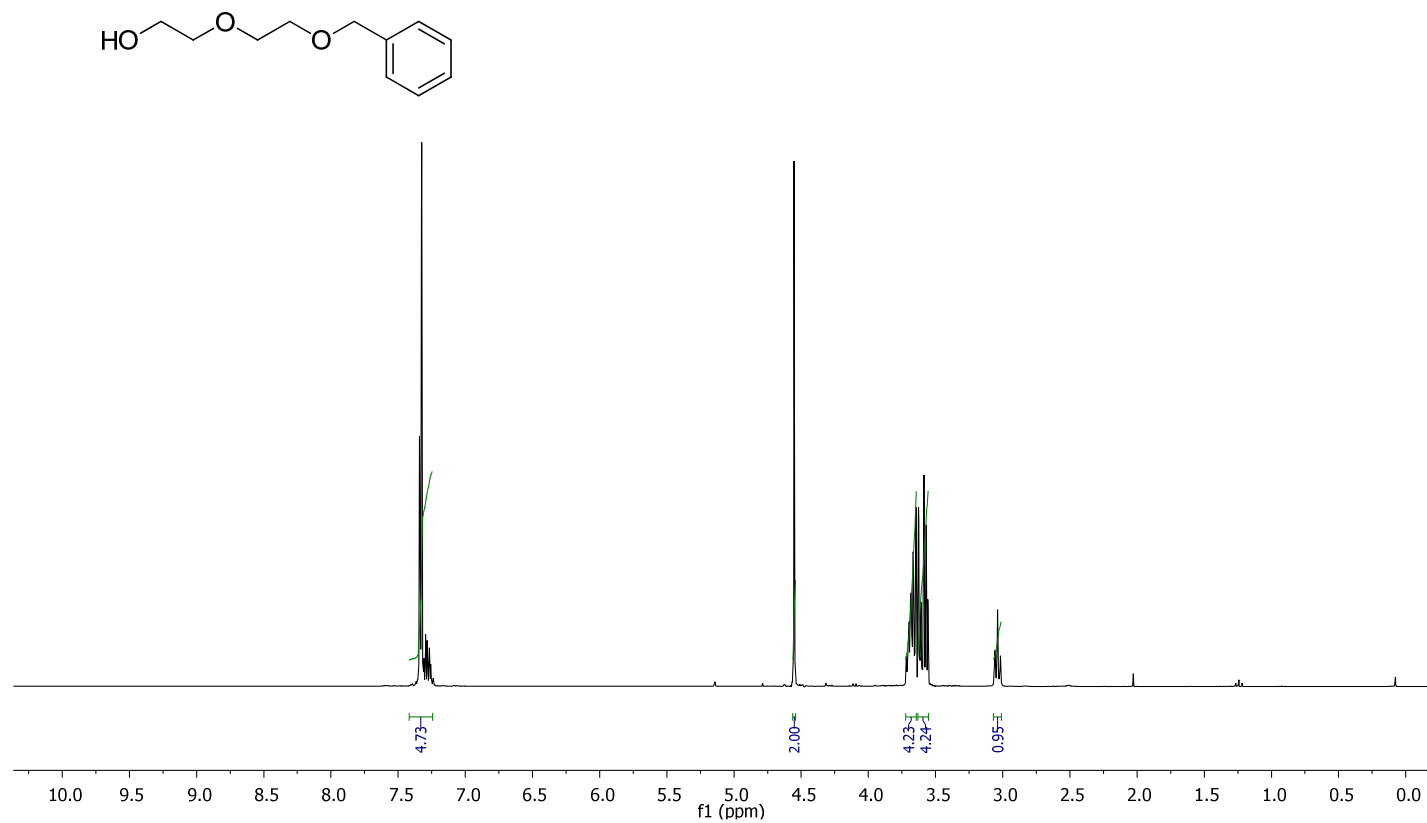


^1H NMR spectrum (500 MHz, CD_3OD) of propargyl α -D-mannopyranosyl-(1 $\rightarrow$ 2)-[α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- α -D-mannopyranoside (42).

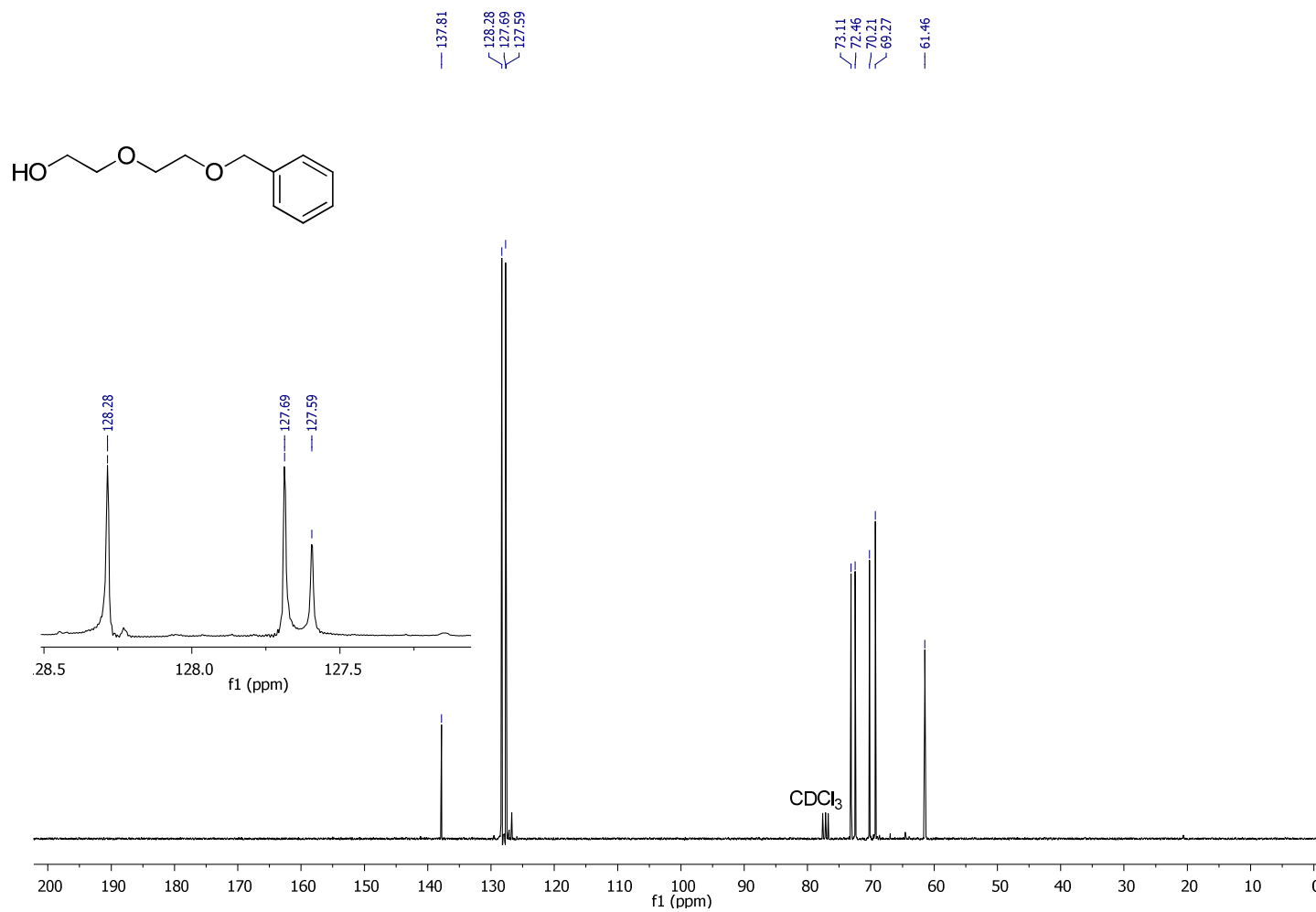




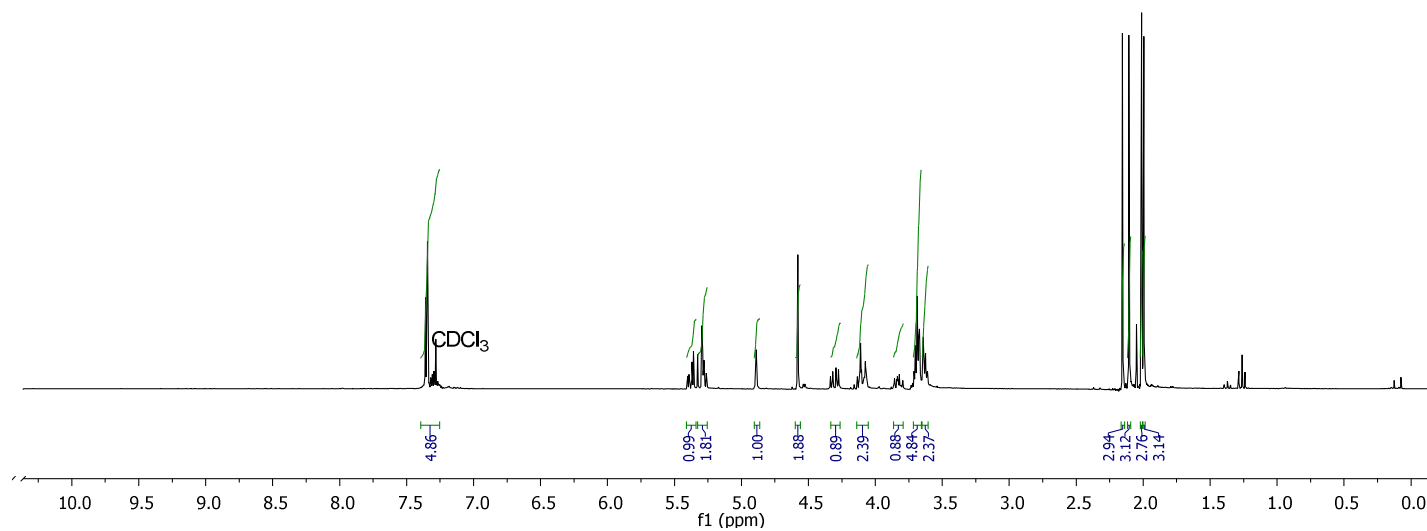
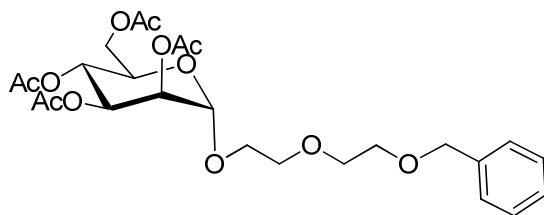
gHSQC NMR spectrum (500 MHz, CD₃OD) of propargyl α-D-mannopyranosyl-(1→2)-[α-D-mannopyranosyl-(1→3)]-α-D-mannopyranoside (**42**).



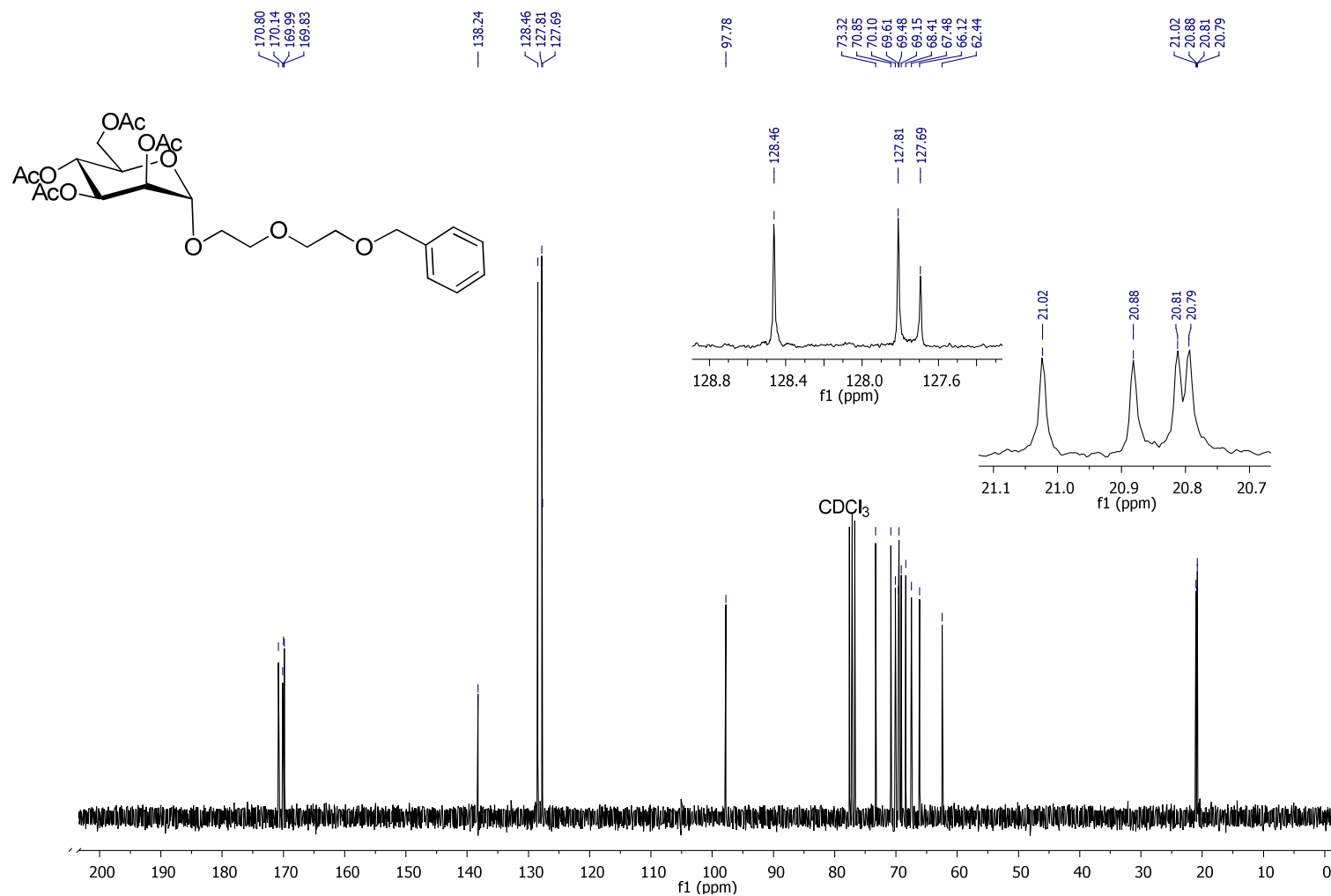
^1H NMR spectrum (300 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethanol (45).



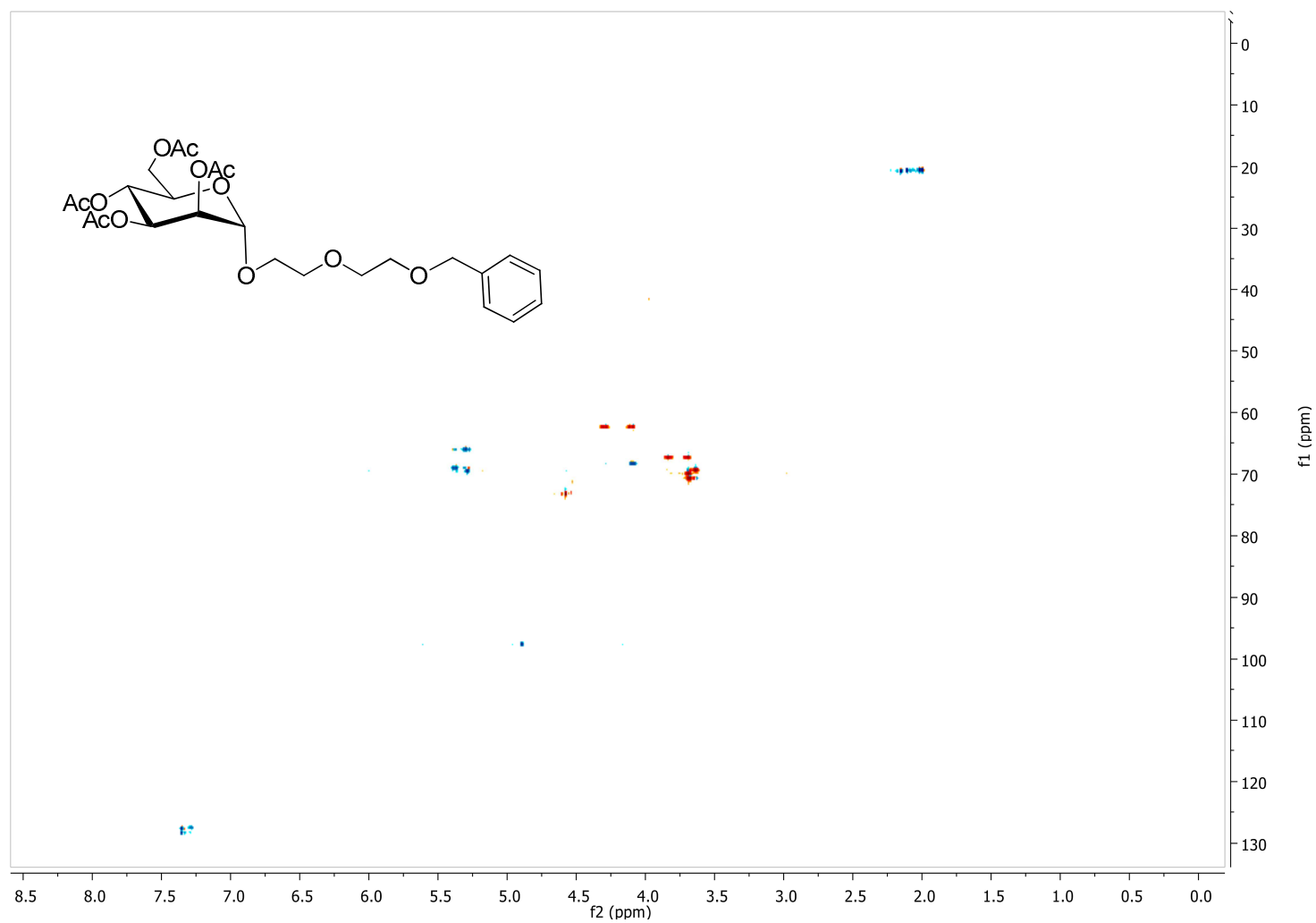
^{13}C NMR spectrum (75 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethanol (45).



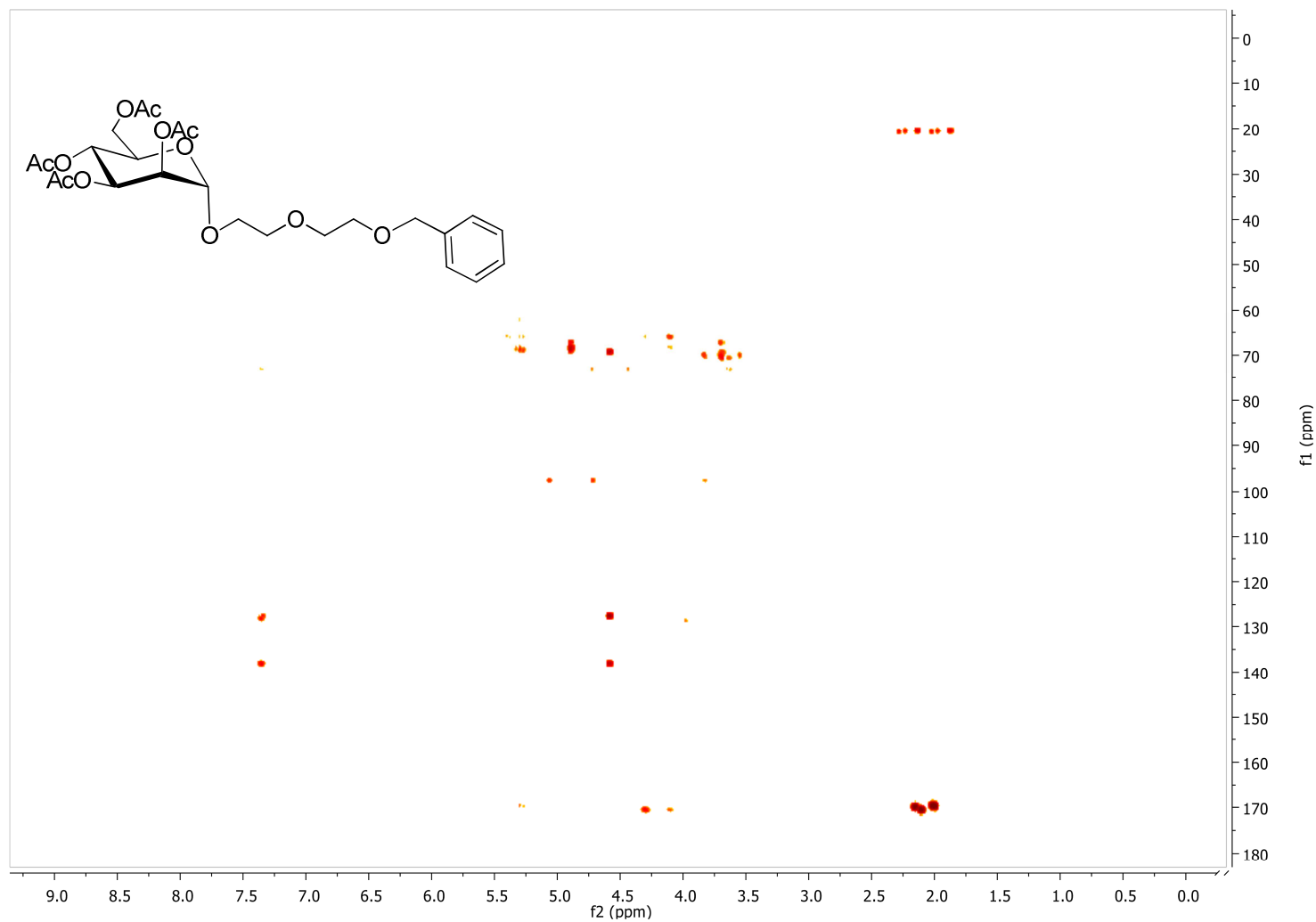
^1H NMR spectrum (300 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**46**).



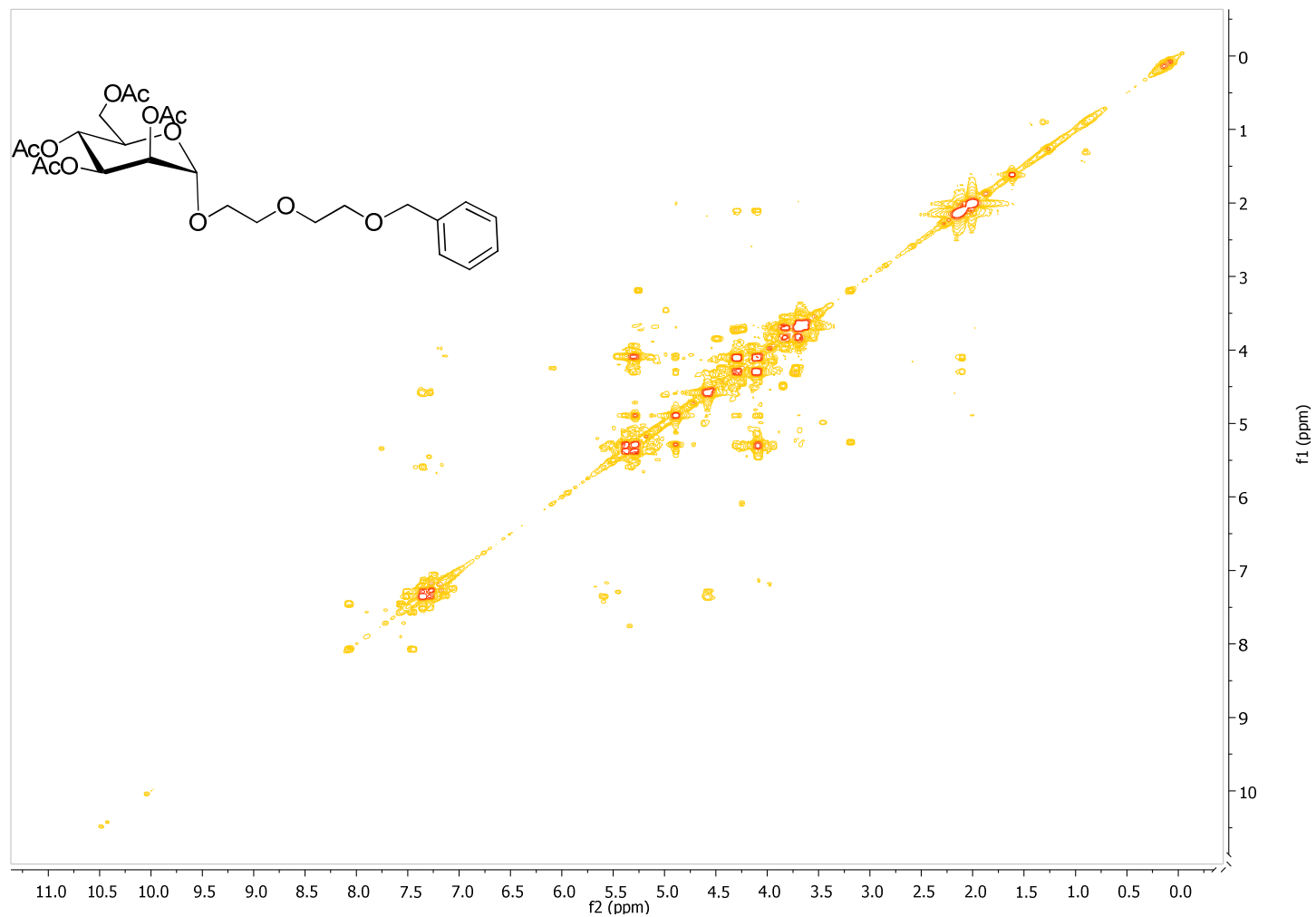
^{13}C NMR spectrum (75 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (46).



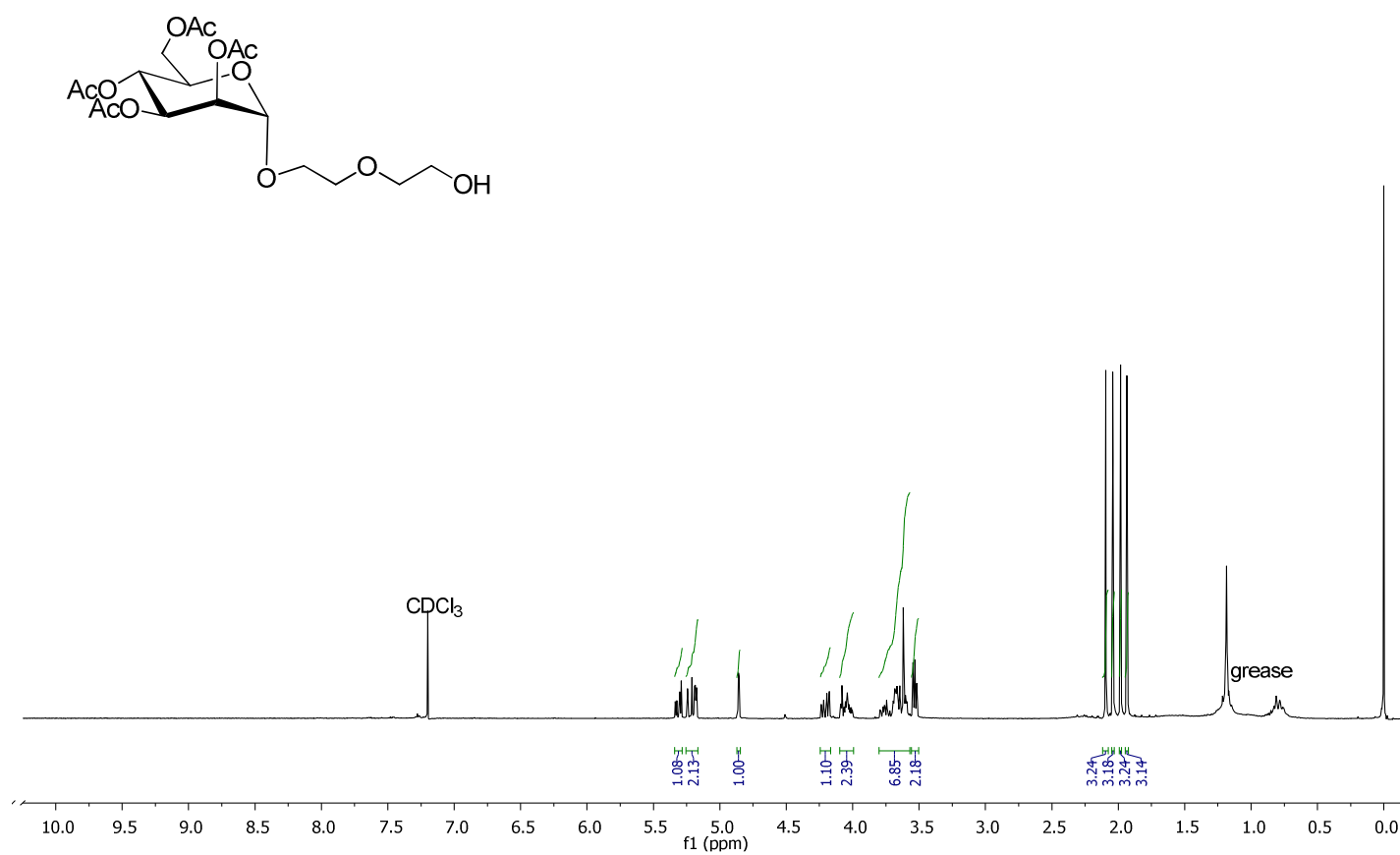
gHSQC NMR spectrum (300 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**46**).



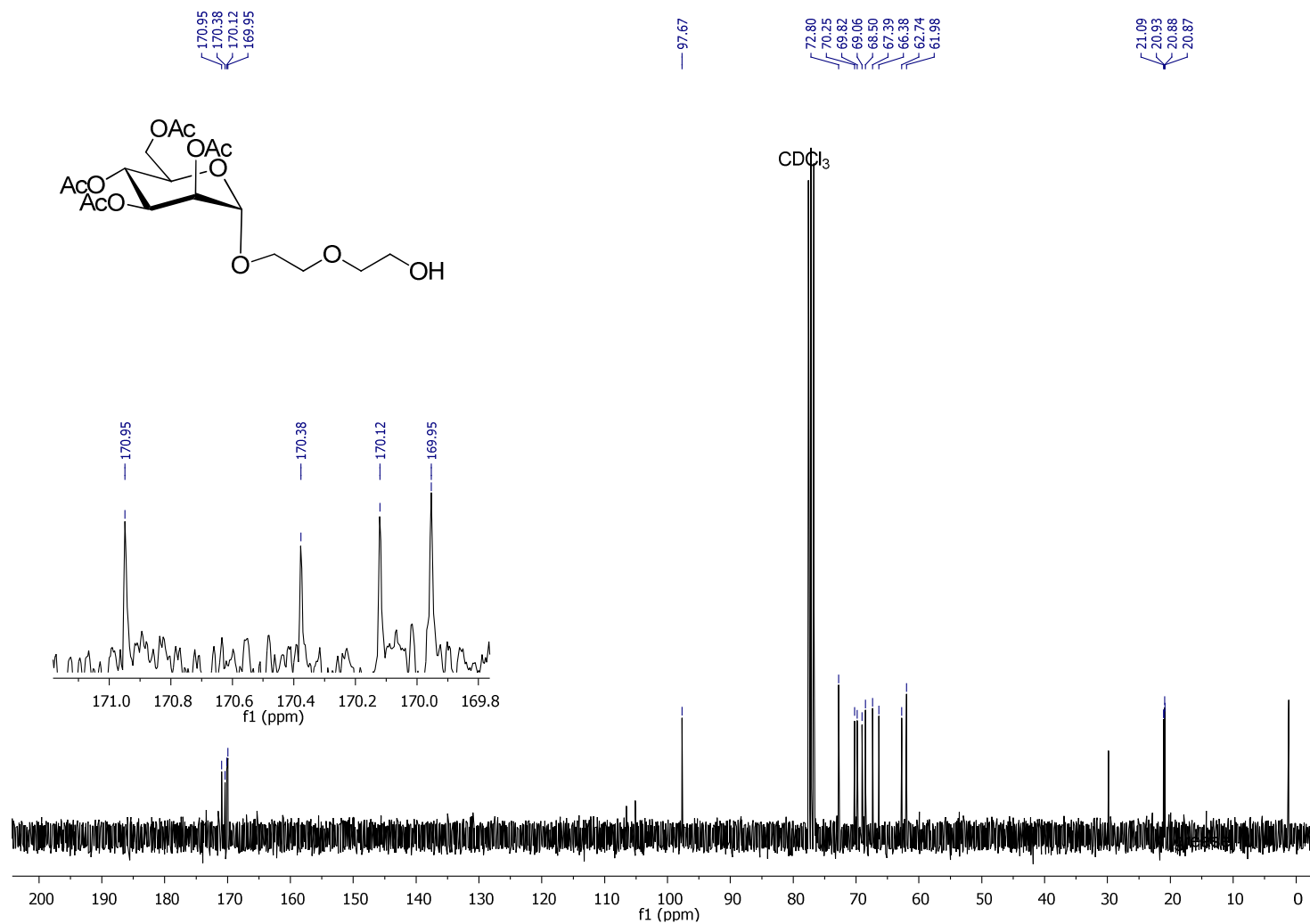
gHMBC NMR spectrum (300 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**46**).



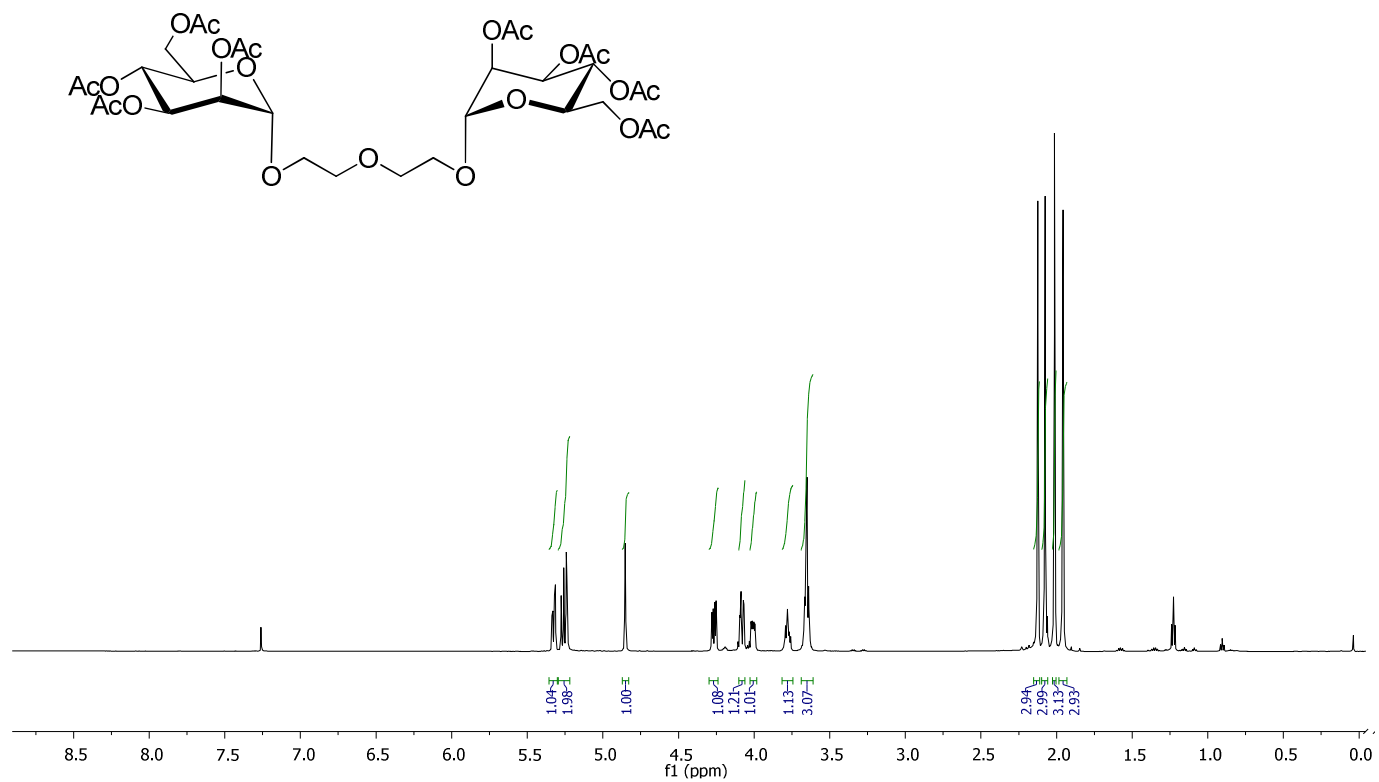
gCOSY NMR spectrum (300 MHz, CDCl_3) of 2-[2-(benzyloxy)ethoxy]ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**46**).



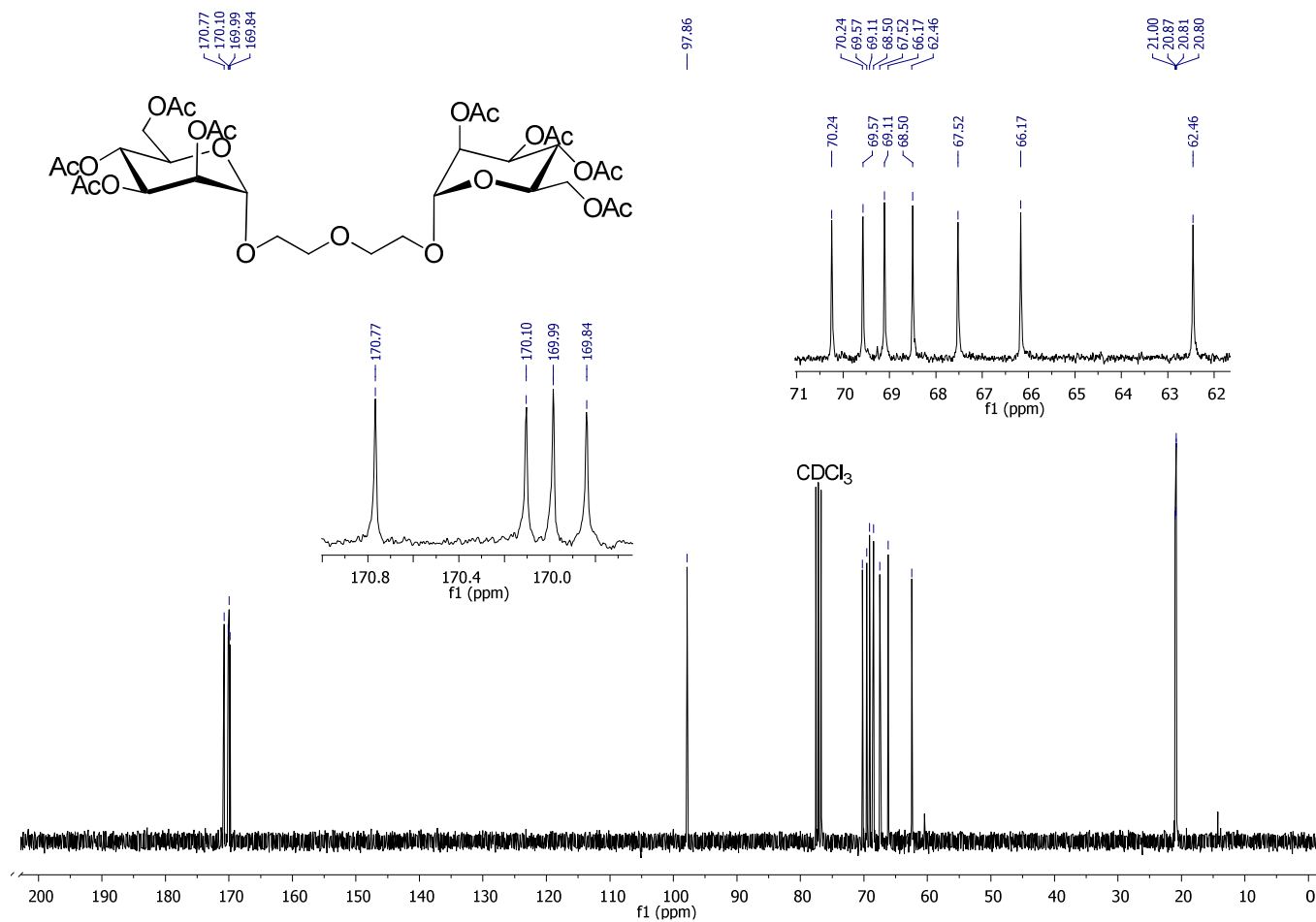
^1H NMR spectrum (300 MHz, CDCl_3) of 2-(2-hydroxyethoxy)ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**47**).



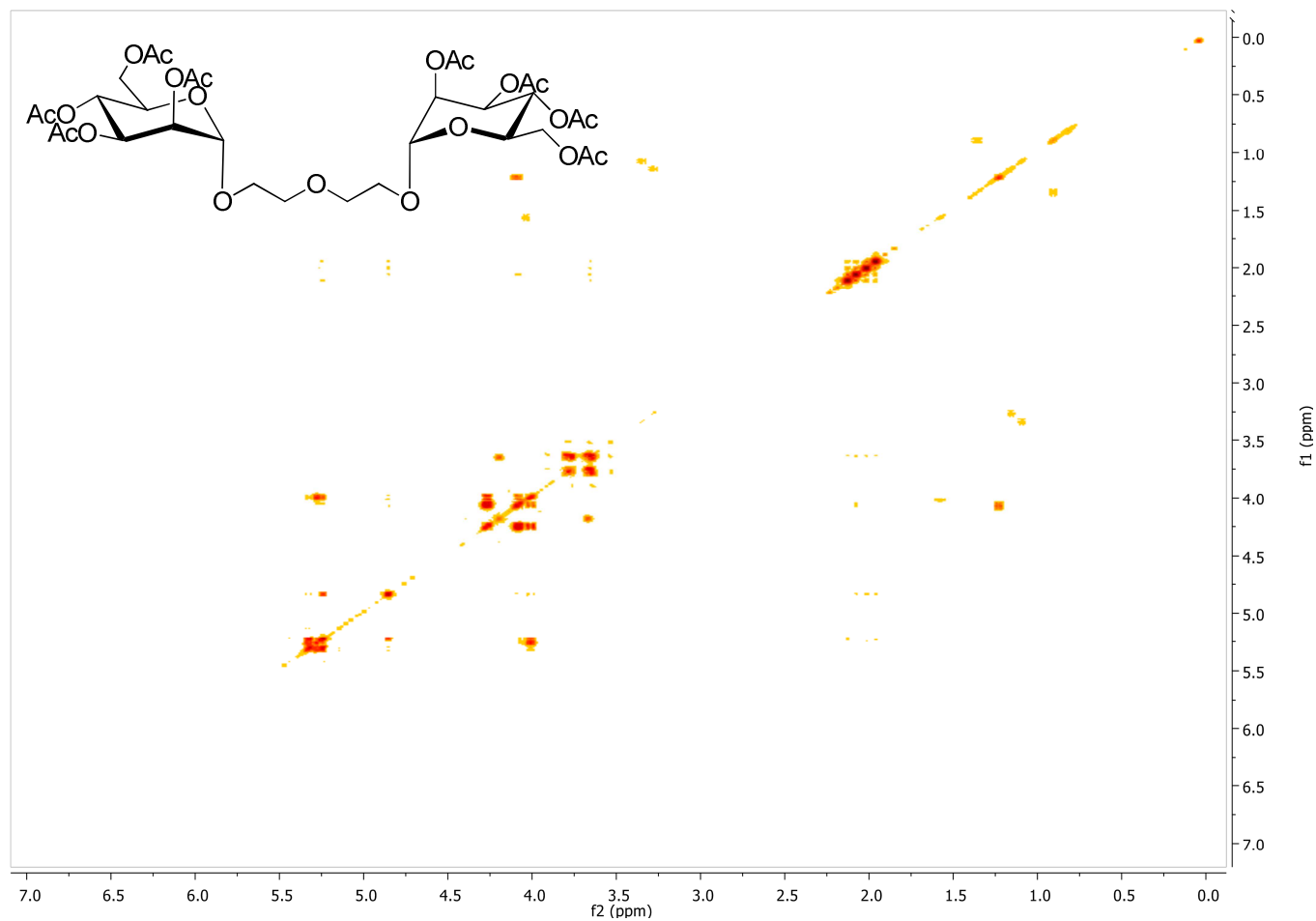
^{13}C NMR spectrum (75 MHz, CDCl_3) of 2-(2-hydroxyethoxy)ethyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**47**).
283



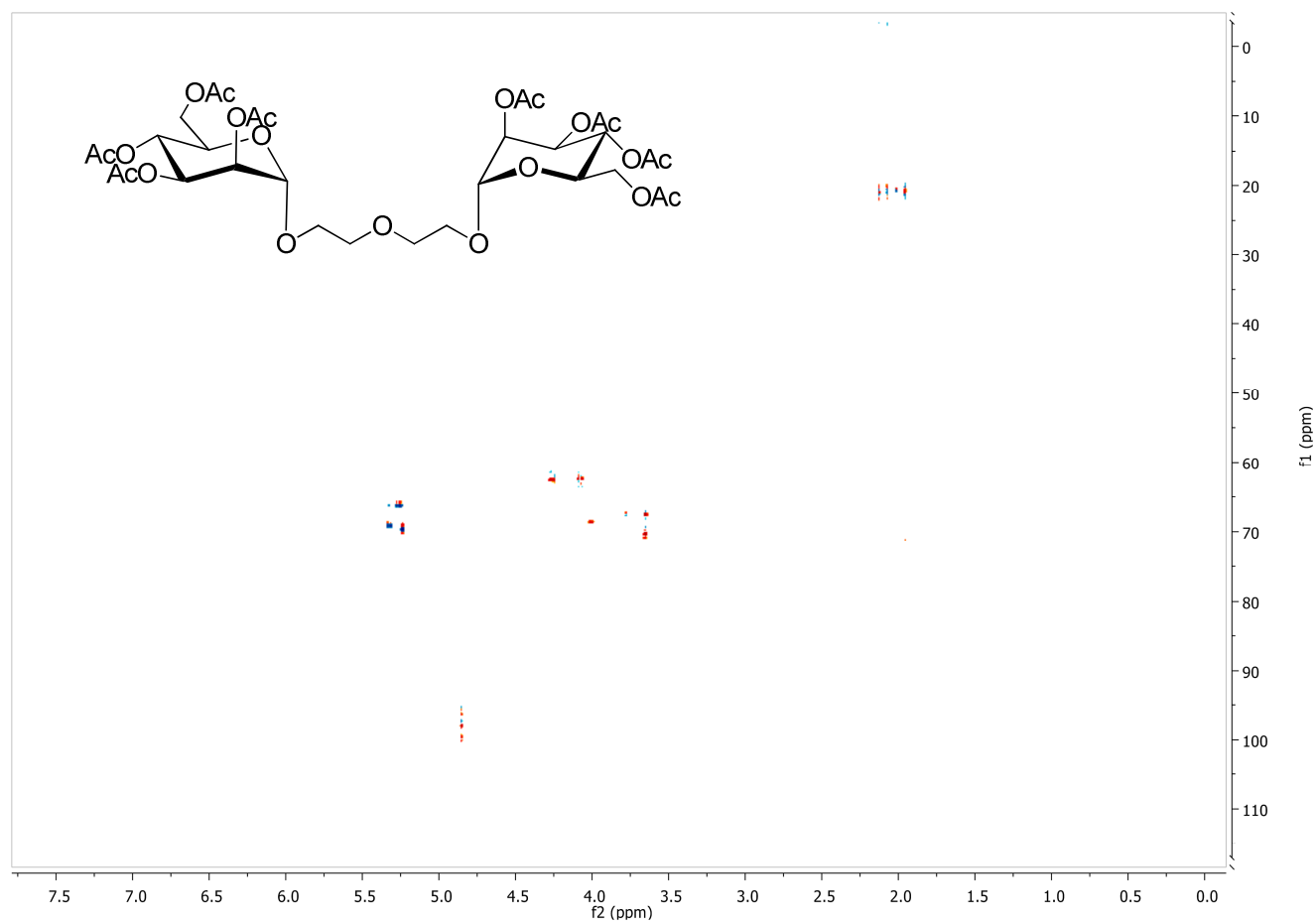
^1H NMR spectrum (300 MHz, CDCl_3) of 2-[2-[(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)oxy]ethoxy]ethyl 2,3,4,6-tetra-O-acetyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**44**).



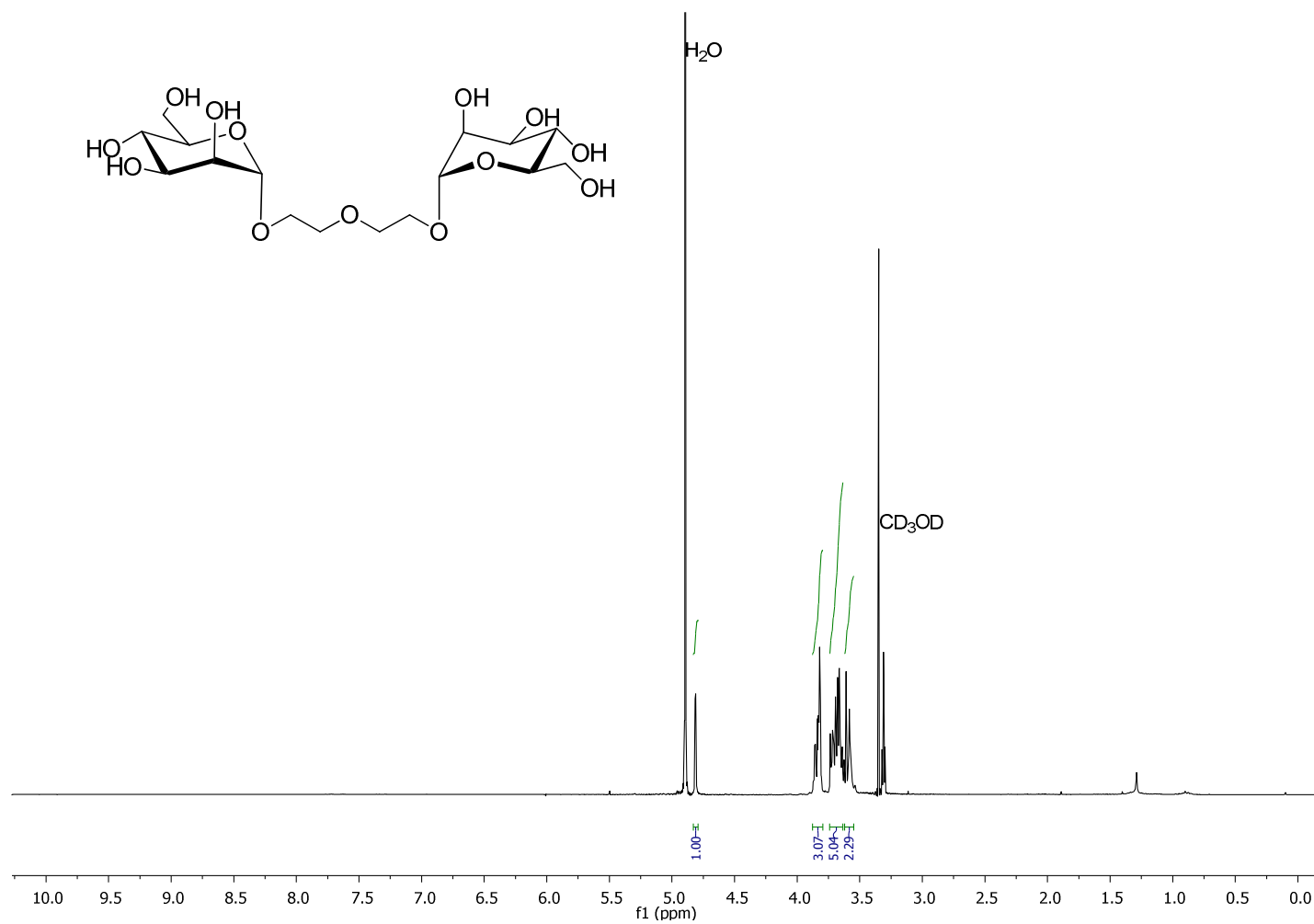
^{13}C NMR spectrum (75 MHz, CDCl_3) of 2-[2-[(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)oxy]ethoxy]ethyl 2,3,4,6-tetra-O-acetyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**44**).



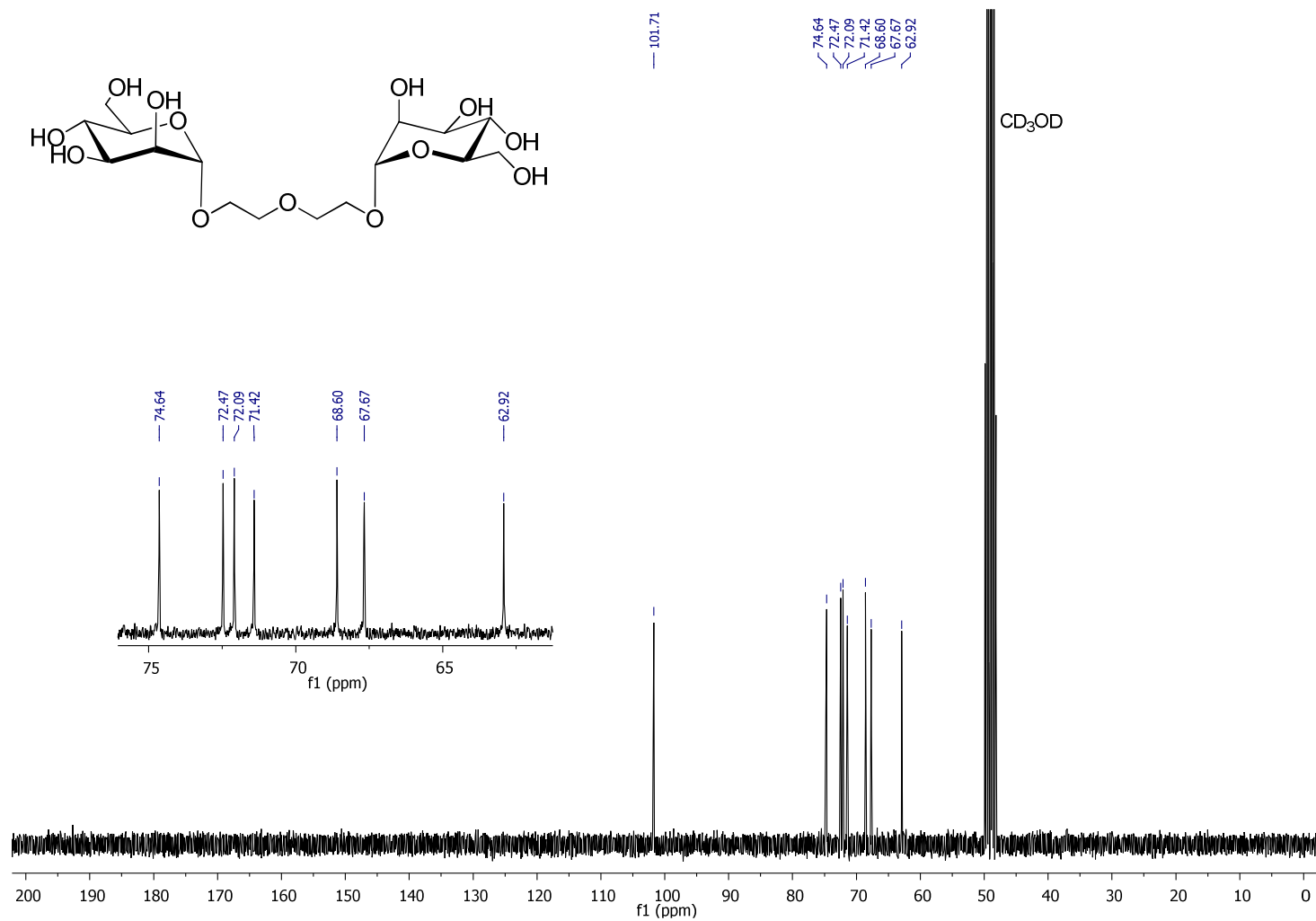
gCOSY NMR spectrum (300 MHz, CDCl_3) of 2-[2-[(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)oxy]ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**44**).



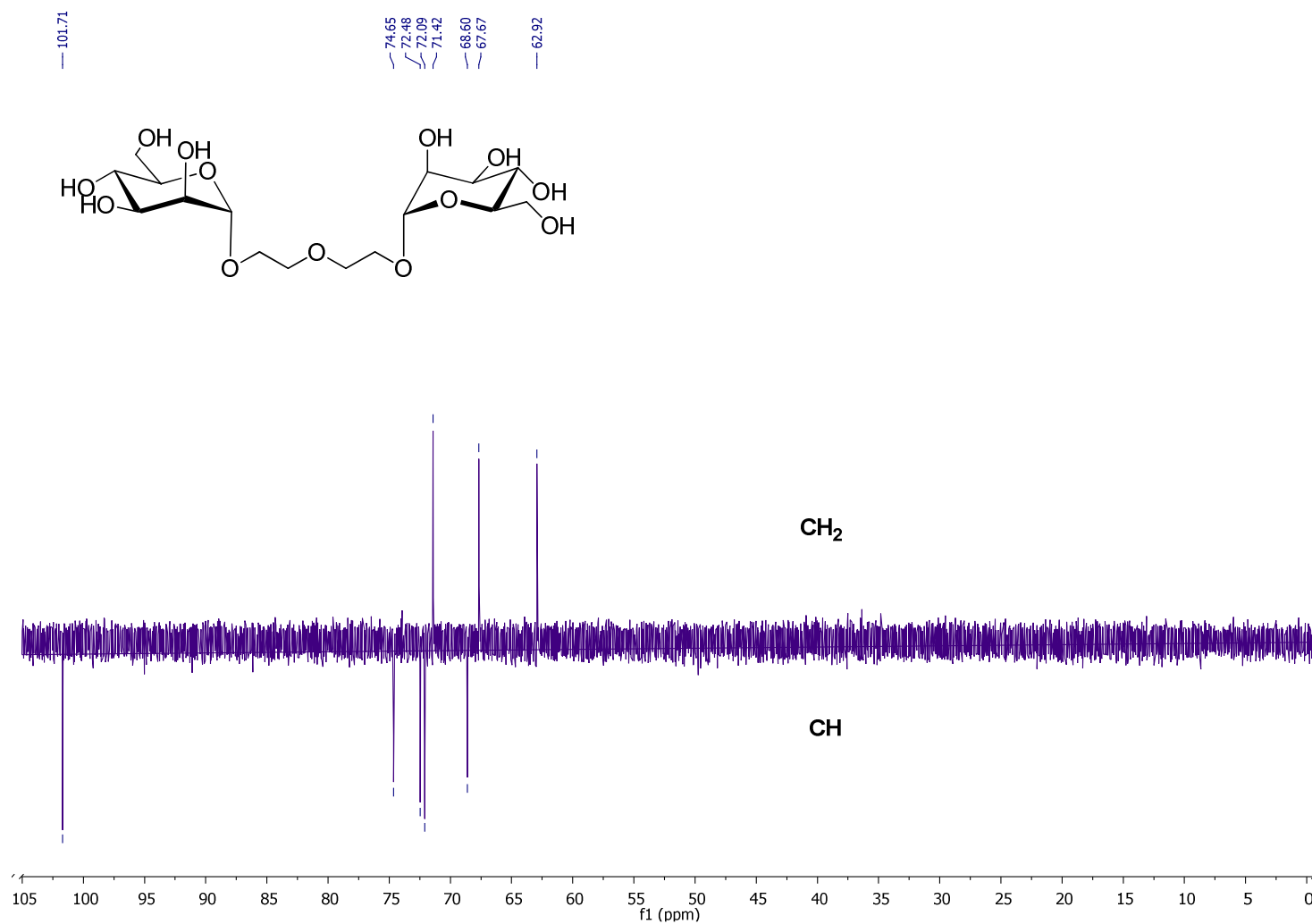
¹H NMR spectrum (300 MHz, CDCl₃) of 2-[2-[(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)oxy]ethoxy]ethyl 2,3,4,6-tetra-O-acetyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**44**).



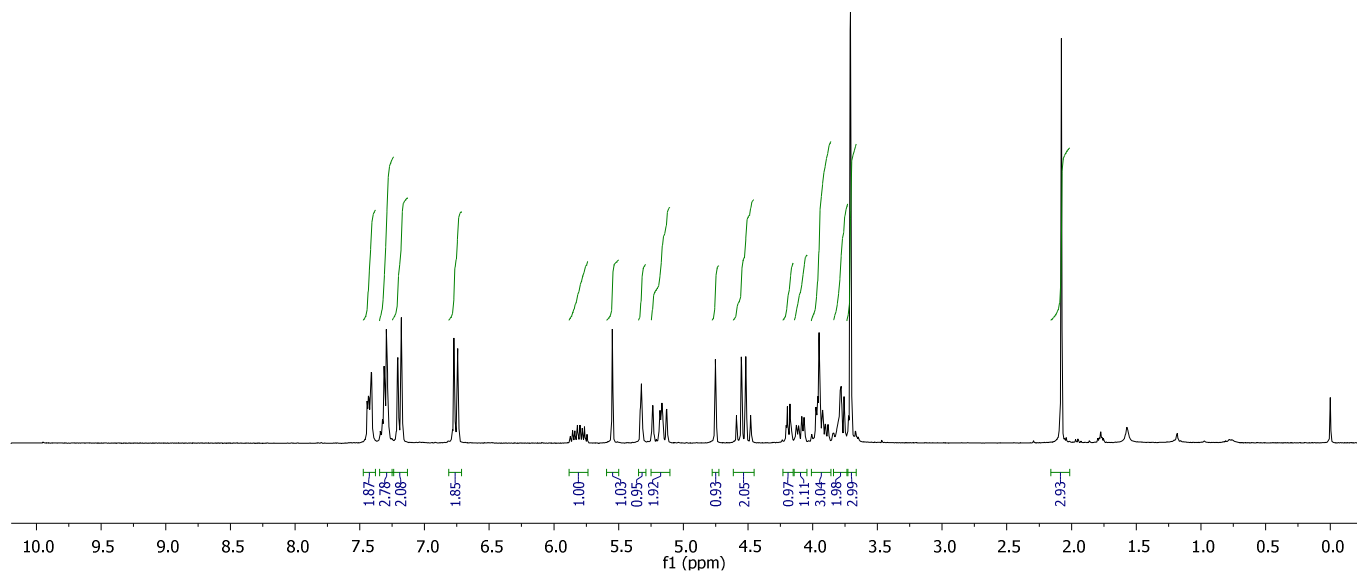
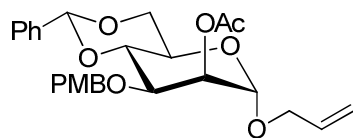
^1H NMR spectrum (300 MHz, CD_3OD) of 2-[2-(α -D-mannopyranosyloxy)ethoxy]ethyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**48**).



^{13}C NMR spectrum (300 MHz, CD_3OD) of 2-[2-(α -D-mannopyranosyloxy)ethoxy]ethyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (**48**).

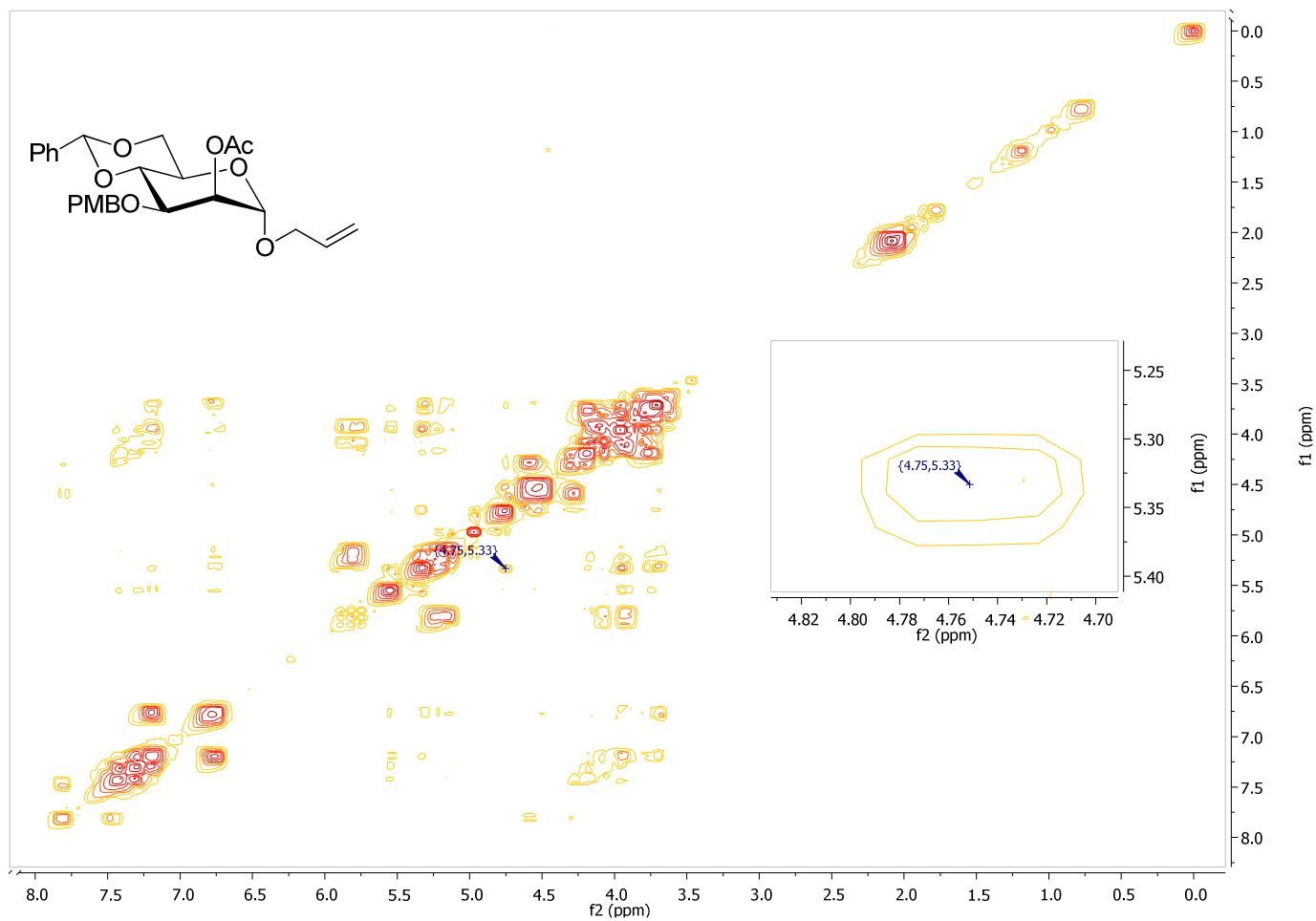


DEPT NMR spectrum (75 MHz, CD₃OD) of 2-[2-(α -D-mannopyranosyloxy)ethoxy]ethyl-(1 $\rightarrow$ 1)- α -D-mannopyranoside (49).

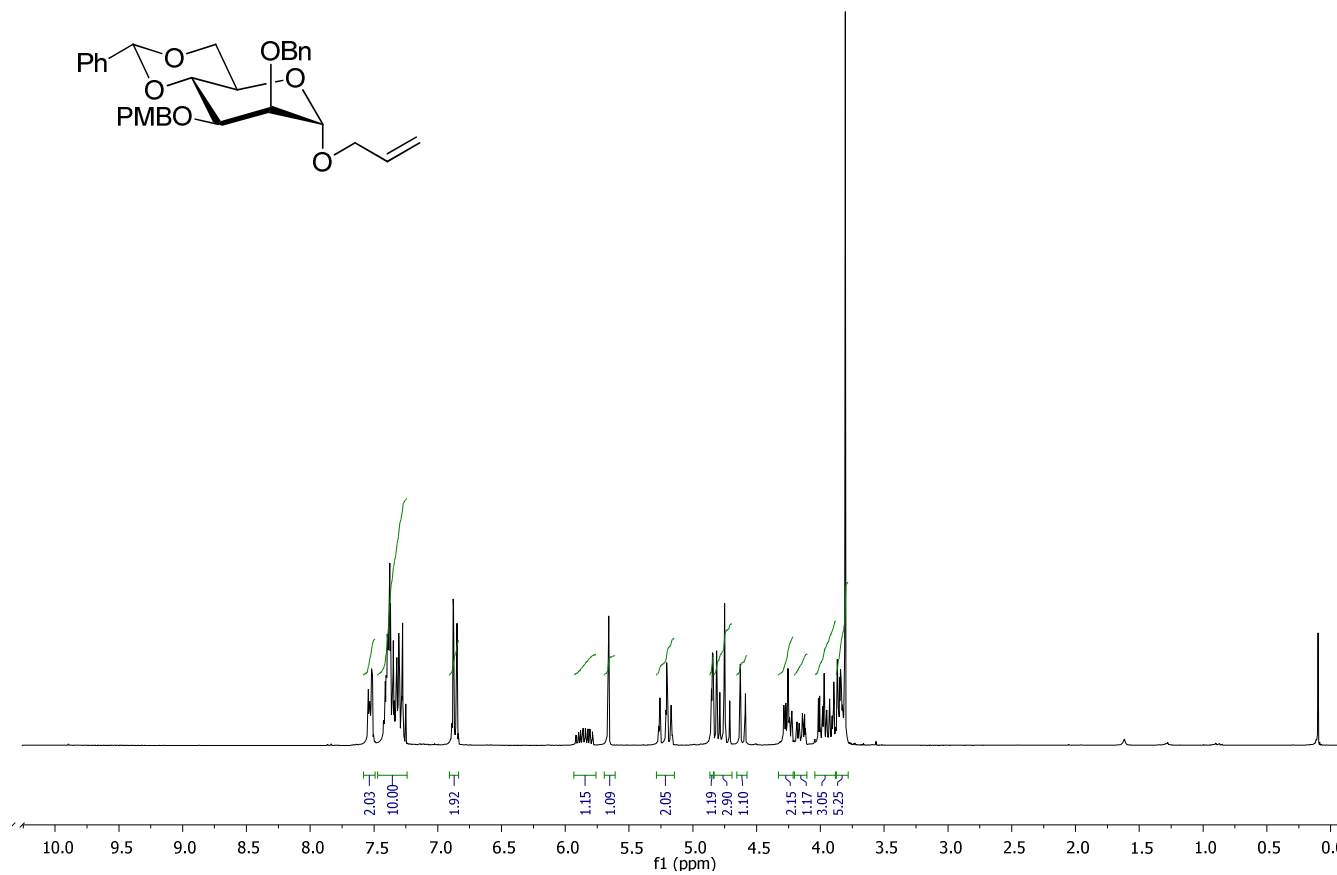


^1H NMR spectrum (300 MHz, CDCl_3) of allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside

(9).
291



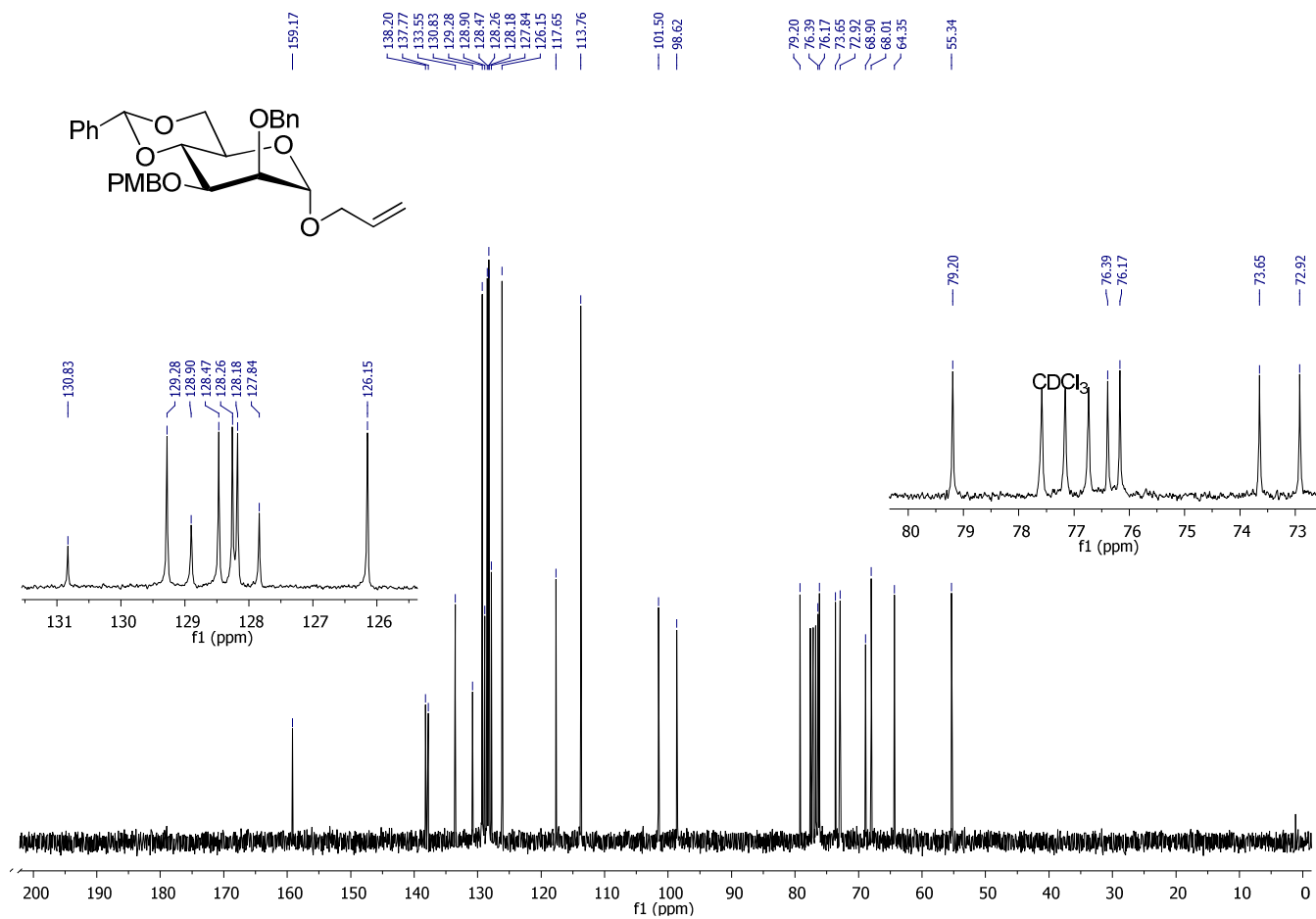
gCOSY NMR spectrum (300 MHz, CDCl₃) of allyl 2-O-acetyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside (**9**).



^1H NMR spectrum (300 MHz, CDCl_3) of allyl 2-O-benzyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside

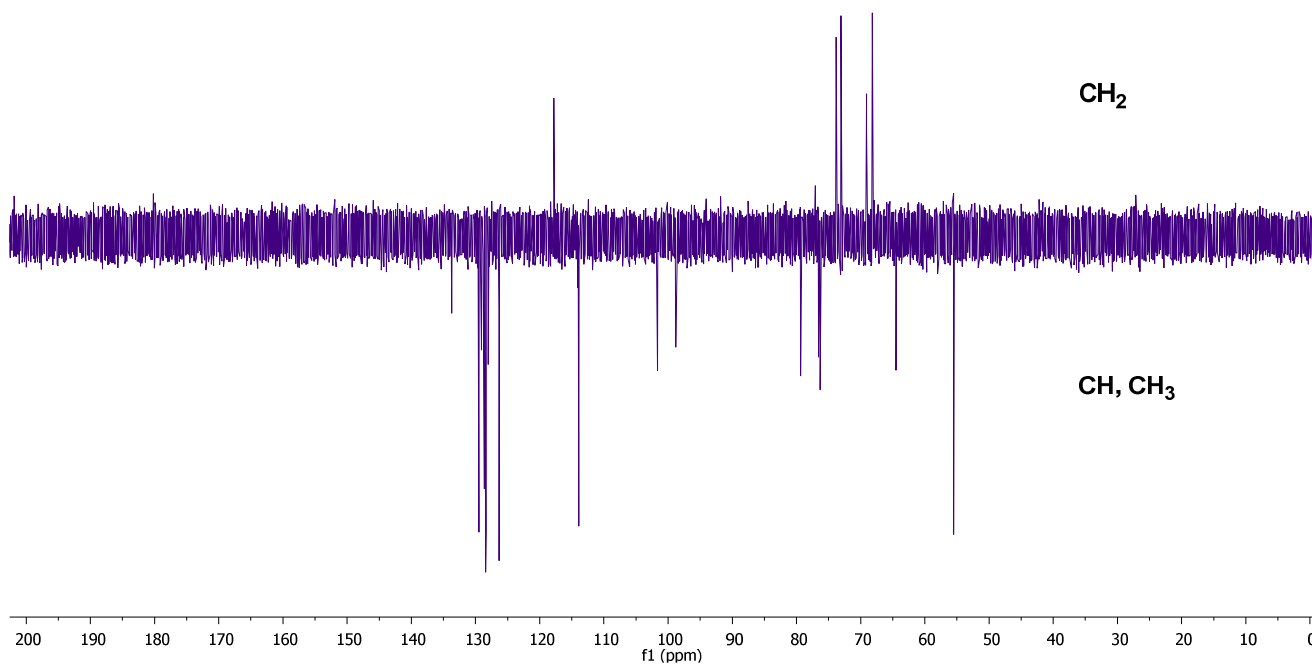
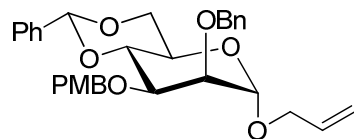
(10).

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^{13}C NMR spectrum (75 MHz, CDCl_3) of allyl 2-O-benzyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)- α -D-mannopyranoside

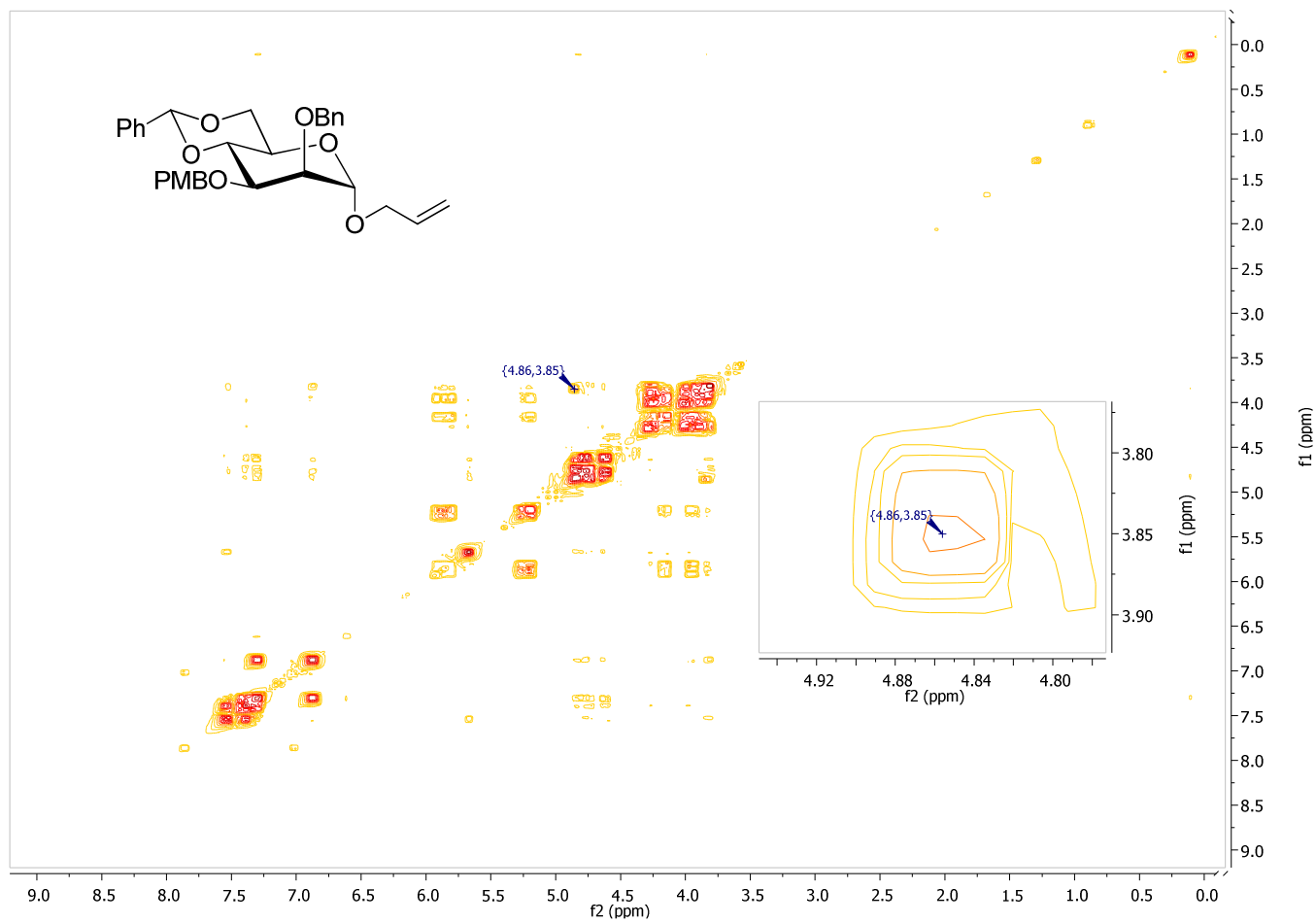
(10).



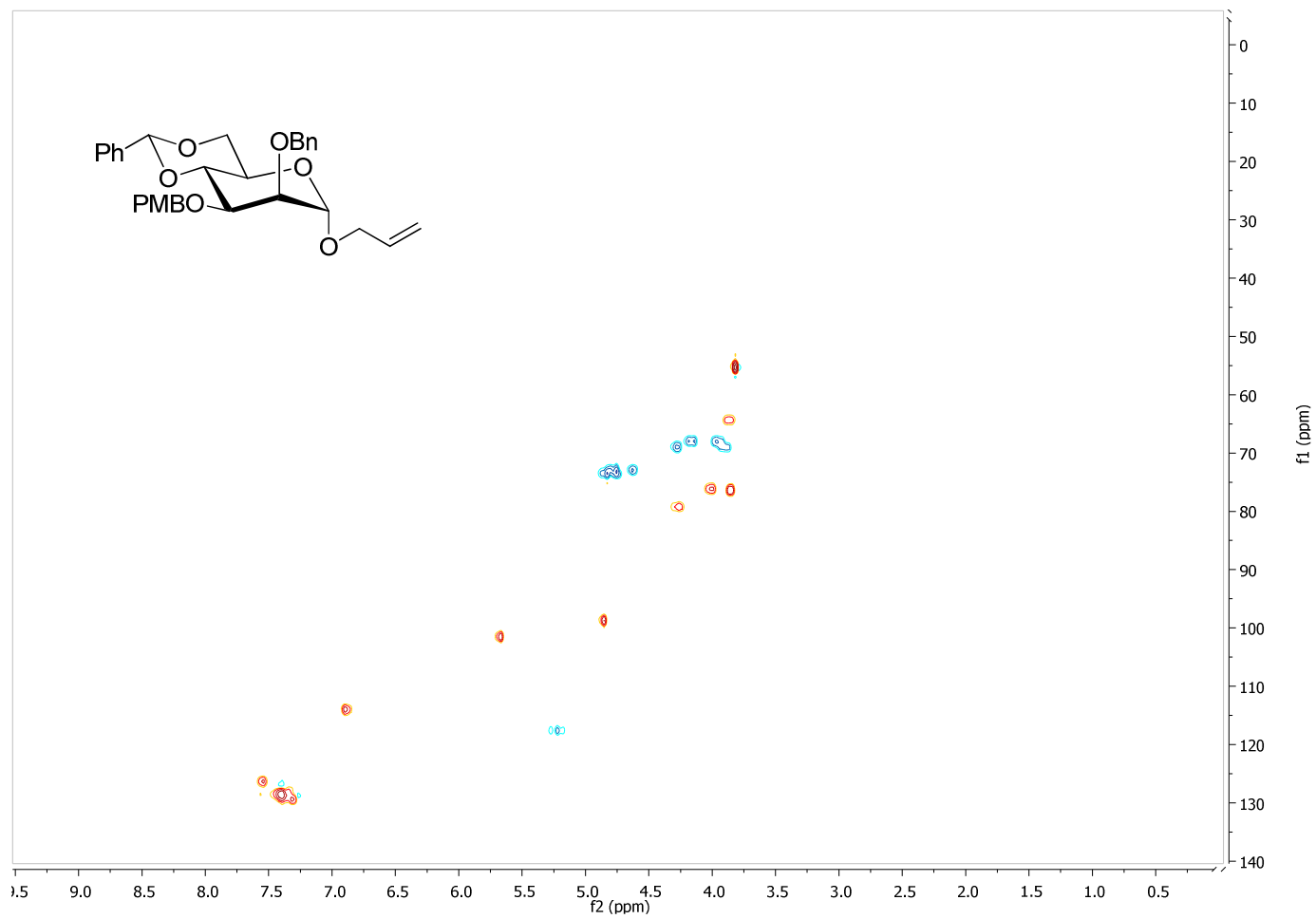
DEPT NMR spectrum (75 MHz, CDCl_3) of allyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside

(10).

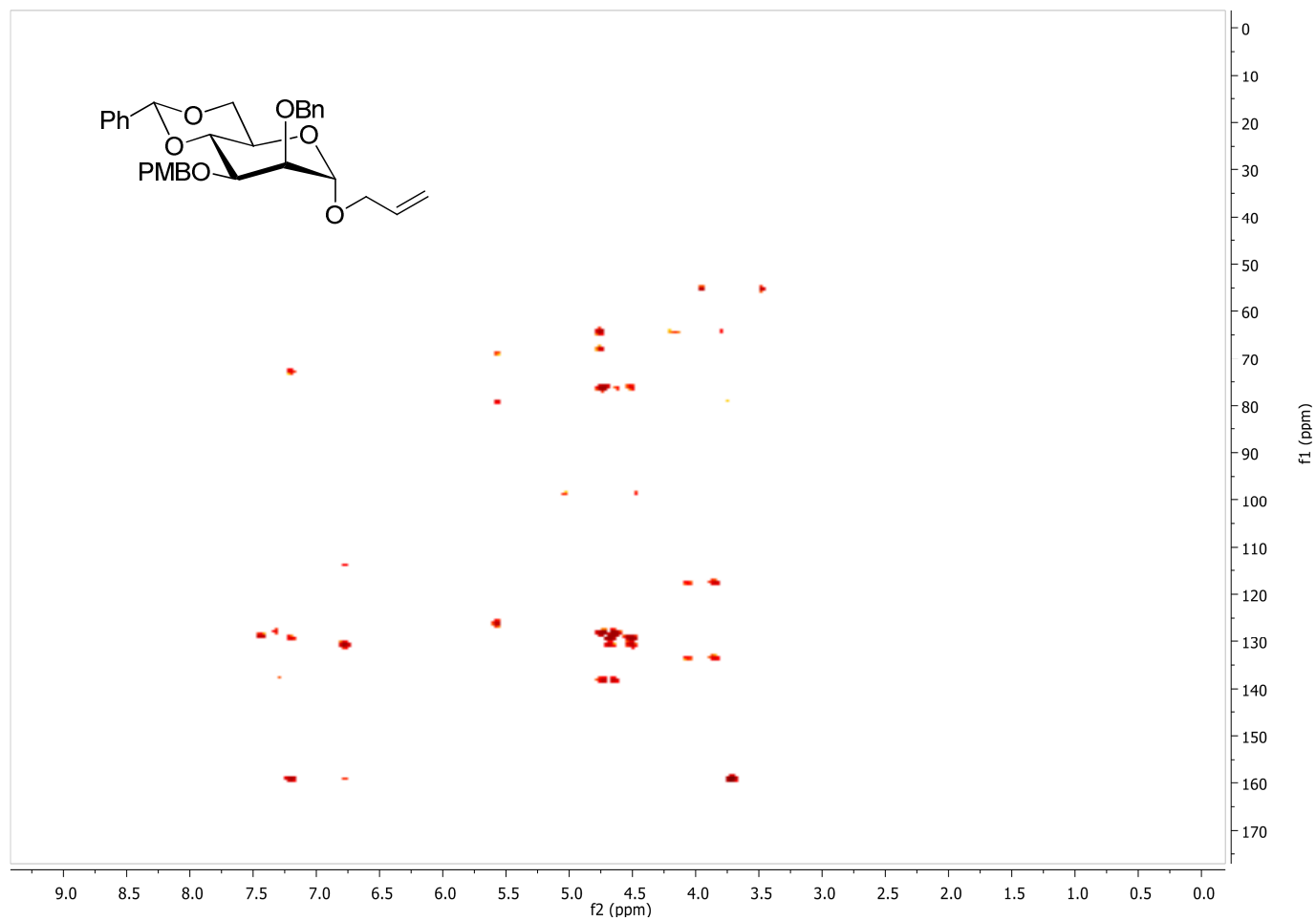
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gCOSY NMR spectrum (300 MHz, CDCl₃) of allyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**10**).



gHSQC NMR spectrum (300 MHz, CDCl_3) of allyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**10**).



gHMBC NMR spectrum (300 MHz, CDCl_3) of allyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)- α -D-mannopyranoside (**10**).

Vita

Irene Abia was born and raised in the small town Buea in Cameroon. She obtained a Bachelor's degree in Chemistry from the University of Buea, Cameroon in 2001.

Irene moved to South Africa in 2003 where she enrolled into the Medical University of Southern Africa, South Africa. There, she obtained a Bachelor Honors degree in Chemistry. From 2003 to 2006, she went on to study at the University of Pretoria, South Africa where she conducted research with Professor Jacobus Eloff earning a Master's degree in Natural Products Chemistry.

In 2006, Irene moved to USA and joined the research group of Professor David C. Baker at the University of Tennessee, Knoxville. There, she conducted research in the synthesis of carbohydrates and studied carbohydrate-protein binding interactions. Irene had never really been a fan of 'sugars' until she conducted this research and is now the self proclaimed 'queen of sugars'.