

Synthesis, Characterization and Evaluation of Lectin-Binding Properties of 1,2-*cis*-Glycosides

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Abstract

This dissertation presents a state-of-the-art research of 1,2-*cis*-glycosides, including synthetic methodology development, combinatorial total synthesis of galactoside ligands, construction of glycomicroarray surfaces, and quantitative evaluation of carbohydrate–lectin binding properties.

In Chapter 1, as a general introduction, various strategies that have been developed for the stereoselective construction of 1,2-*cis*-*O*-glycosidic bond are reviewed. 1,2-*cis*-*O*-Glycoside structural units are ubiquitous in nature, and they are extensively involved in numerous biological activities. The systematic identification and evaluation of the roles of carbohydrates usually relies on practical synthetic approaches to afford pure carbohydrates in considerable quantities. Further development of general and efficient approaches to achieve the intrinsic organizational diversity of carbohydrates is still urged in order to meet the substantial carbohydrates in demand for biological, medicinal, and therapeutic studies.

In Chapter 2, I report a 1,2-*cis*-glycosidation protocol that makes use of unprotected phenyl 1-thioglycosyl donors. Glycosylation of various functionalized alcohols was accomplished in moderate to high yield and selectivity to give the 1,2-*cis*-glycosides. In order to quickly optimize glycosylation conditions, a flow injection analysis method was established that enabled rapid and quantitative evaluation of yield on small scale. This methodology, together with nuclear magnetic resonance spectroscopy, allowed for rapid evaluation of the overall reactions.

In Chapter 3, I describe the synthesis of alpha-linked propargyl terminated galactosides with various spatial presentations, which are set to be applied in the

construction of synthetic carbohydrate microarrays to mimic featured carbohydrate presentations on a cell surface. Through the routes, glycosyl acceptors were obtained via flexible and efficient regioselective protection strategies, and they were glycosylated with an alpha-directing glycosyl donor to have the alpha-linked galactosides in exclusive stereoselective and satisfactory yield.

In Chapter 4, the establishment of a synthetic carbohydrate microarray is described. The carbohydrate surface was interrogated by a fluorescence-labeled lectin to quantitatively analyze the carbohydrate-binding affinities and dissociation constants. This study will add new dimensions to our understanding of the effects of spatial arrangement of carbohydrate ligands in carbohydrate–lectin binding, and shed light on elucidating the structure–affinity relationship of carbohydrate recognition with receptors.

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Abbreviations

Ac	Acetyl
AcOH	Acetic Acid
AgOTf	Silver trifluoromethanesulfonate
All	Allyl
Bn	Benzyl
Bz	Benzoyl
COSY	Correlated spectroscopy
DMAP	4-(Dimethylamino)pyridine
DMF	<i>N,N</i> -Dimethylformamide
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
TBAF	Tetrabutylammonium fluoride

Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS	Trimethylsilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOF	Time of flight

Chapter 1 1,2-*cis*-Glycosylation in Carbohydrate Chemistry:
An Overview

Abstract

Oligosaccharides and glycoconjugates containing 1,2-*cis*-glycoside structural units are ubiquitous in nature with numerous biological roles. An increasing understanding of the biological roles has led to a significant demand for these carbohydrates for biological, medicinal, and therapeutic studies. Therefore, considerable effort has been devoted to develop efficient and general strategies for the synthesis of glycosides with 1,2-*cis*-stereocontrol. The scope of this overview is on the recent advances that have been developed to address the challenge of stereoselective 1,2-*cis*-*O*-glycosylation. Glycosyl donors with diverse leaving groups, protecting groups, and their corresponding activation conditions are presented, and the subtle influences of these factors on glycosylation outcomes are also compiled and discussed.

1.1 Background

As one of the essential components in living systems, carbohydrates, including natural glycosides, oligosaccharides, polysaccharides and glycocalyx, are ubiquitous in nature. Tons of carbohydrates are produced by plants and microorganisms on a yearly basis through the photosynthesis process. Cellulose, which is a structural polysaccharide with β -1,4-linkage, is the most abundant chemical substance on the planet. Carbohydrates perform numerous biological roles and sustain the metabolism in living systems. In the past, the functionalities ascribed to carbohydrates were limited to energy storage and skeletal components. However, with the first isolation and identification of a carbohydrate-binding protein, their diverse roles in biological systems sparked great interest and a multitude of efforts, which resulted in the emerging of ‘Glycobiology’ as a science. Nowadays, people realize that carbohydrates are heavily involved in the cell–cell recognition and cell–external agent interactions. Since cell membranes are covered with a thick layer of glycoconjugates, they form the basis for molecular interactions at the cell surface. These interactions mediate cell adhesion, fertilization, cell growth and differentiation, immunological processes, blood clotting, and host–pathogen interactions, all of which are critical for proper development and higher order function in living systems.^{1, 2}

Biologically significant carbohydrates usually exist as complicated oligomers or polymers, or in conjugation with other biomolecules to form bioconjugates such as glycolipids, glycoproteins, or glycosylphosphatidylinositol (GPI) anchors.³ In order to systematically identify and evaluate the roles of carbohydrates, glycobiologists need access to particular target carbohydrates in sufficient quantities, as isolating these

molecules from nature in high purity is still difficult due to their low abundance and heterogeneity. Therefore, developing practical synthetic approaches is an important method to afford pure carbohydrates in considerable quantities.

The synthesis of glycosides always includes the regioselective and stereoselective construction of glycosidic bonds between glycosyl donors and acceptors, a process called glycosylation. Although considerable effort has been expended and progress has been made in developing efficient glycosylation approaches,⁴⁻⁹ the rapid construction of complex oligosaccharides or glycoconjugates with specific structures still remains challenging. Carbohydrate building blocks are present in more than one form (pyranose and furanose forms), and the variations of their size, configuration, connectivity, and spatial arrangement leads to diverse structures ranging from monosaccharides to oligosaccharides to highly branched polysaccharides and glycodendrimers. Each sugar unit is a unique stereogenic center, and the stereochemical control of the glycosidic connections requires precise design and prudent manipulation. Furthermore, from the mechanistic viewpoint, the process also lacks generality and consistency in rationalization of the glycosylation reactions.¹⁰ The glycosylation process usually initiates from the activation of a donor and via glycosyl oxacarbenium ion formation, which is reacted or coordinated with a counterion to form a covalent intermediate, or an equilibrating close-contact and loosely bound ion pair. These covalent intermediates or ion-pairs react with an acceptor in either S_N1-like or S_N2-like pathways to afford the corresponding products. During this process, many factors including donor type, promoter, protecting group, solvent, concentration, temperature, *etc.*, are capable of affecting the glycosylation outcomes, and sometimes the combined efforts of several

factors will dominate the outcomes.^{11, 12} Therefore, proposing a comprehensive glycosylation method is a challenging task.

Among the various glycosidic linkages, the *O*-glycosidic linkage is one of the representative linkage types based on its high generality and abundance in nature. Typically, the *O*-glycosides can be classified into either 1,2-*cis*- and 1,2-*trans*-*O*-glycosides or α - and β -*O*-glycosides. 1,2-*cis*-*O*-Glycosides are generally α -*O*-glycosides, but there are exceptions such as α -*O*-mannosides and α -*O*-arabinosides, which belong to 1,2-*trans*-*O*-glycosides. The α or β designation is based on the stereochemical relationship between the anomeric center (C-1) and the configuration of the highest numbered stereogenic center at the hemiacetal stage. If the hydroxyl groups bound to this center are *cis* to the substituent at the highest-numbered stereocenter, this anomer is called the α -anomer, otherwise the one with a *trans* relationship is called the β -anomer.¹ Several typical glycoside configurations are shown in Figure 1.1. Sometimes if the 2-hydroxyl group is replaced by hydrogen, such as in 2-deoxyglycosides and sialosides, we no longer define them as 1,2-*cis* or 1,2-*trans* glycosides, and they are referred to as α - and β -glycosides. Both 1,2-*cis* and 1,2-*trans* glycosidic linkages are essential and indispensable in natural compounds, but the extremely difficult syntheses of 1,2-*cis* glycosidic linkages are barriers and could be encountered in many carbohydrate syntheses. The management of these difficulties is the focus of this review.¹³

1,2-*cis*-*O*-Glycosidic linkages can be found in many oligosaccharides or glycoconjugates of high biological importance and therapeutic potential. Several typical naturally occurring oligosaccharides and glycolipids comprising 1,2-*cis*-*O*-glycosidic bonds are displayed in Figure 1.2. For example, glycosphingolipids (GSLs) of the *globo*

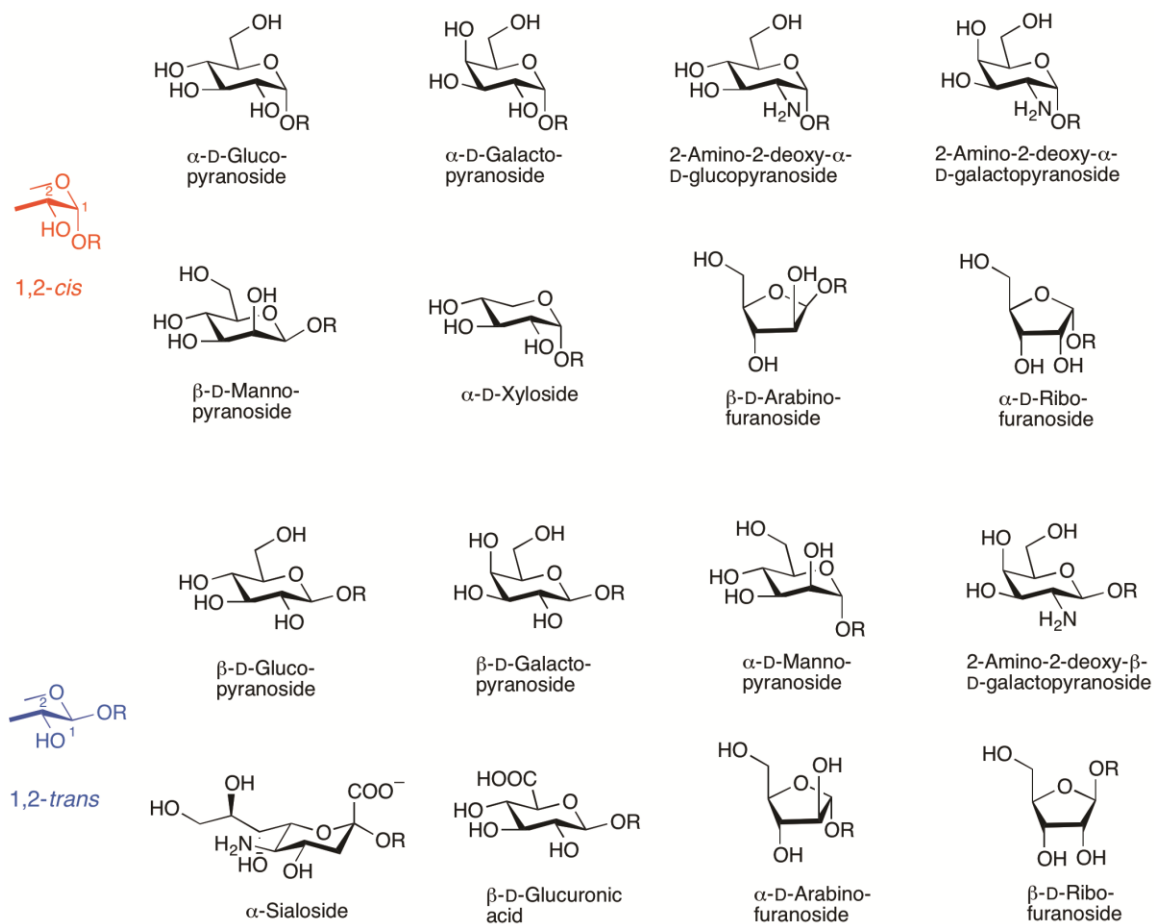


Figure 1.1 Typical glycoside configurations found in nature.

series, which is a current target for tumor-specific antigen, have the α -linked galactosyl residue.¹⁴ Simplexide, another kind of glycolipid of marine origin with immunological potential, also consists of an α -D-glucosyl-(1 $\rightarrow$ 4)- β -D-(1 $\rightarrow$ 6)-galactosyl disaccharide residue.¹⁵ The bacterium *Azospirillum brasilense* SR80 consists of a lipopolysaccharide with an O-tetrasaccharide repeating unit, which contains a 2-O-(α -L-fucopyranosyl)- α -D-galactosyl-(1 $\rightarrow$ 3)- β -D-galactosyl residue.¹⁶ The capsular polysaccharide A of *Bacteroides fragilis* NCTC 9343, which has been shown in mediating the formation of intra-abdominal abscesses in experimental animal models, is composed of a 1,2-*cis*-glycosaminoglycan motif.¹⁷ *Clostridium difficile*, which triggers nosocomial infections, has been found to express glycans containing 1,2-*cis*-linkage on the bacterial cell surface.¹⁸ Several asymmetrically branched N-glycans bearing 1,2-*cis*- β -D-mannoside residues are important synthetic targets to represent glycan structures of those found on human and animal airway epithelia. These are involved in the recognition of influenza-virus hemagglutinins.¹⁹

There are several methods that can be applied to govern the stereochemistry of glycosylation reaction. The most general method is to put specific protecting groups on glycosyl donors. Various protecting groups have been developed and applied to afford desired stereochemical glycosides. Generally, they can participate in the formation of the oxacarbenium ions by either forming a covalent acetal bond, or by molecular interaction such as hydrogen bonding, or by steric effects to steer a specific nucleophilic attack pathway. For example, an acyl group on C-2 usually forms an acyloxonium ion with the anomeric center to reliably achieve 1,2-*trans* glycosides, which is called neighboring-group participation (NGP). The NGP concept is also applied to the formation of 1,2-*cis*

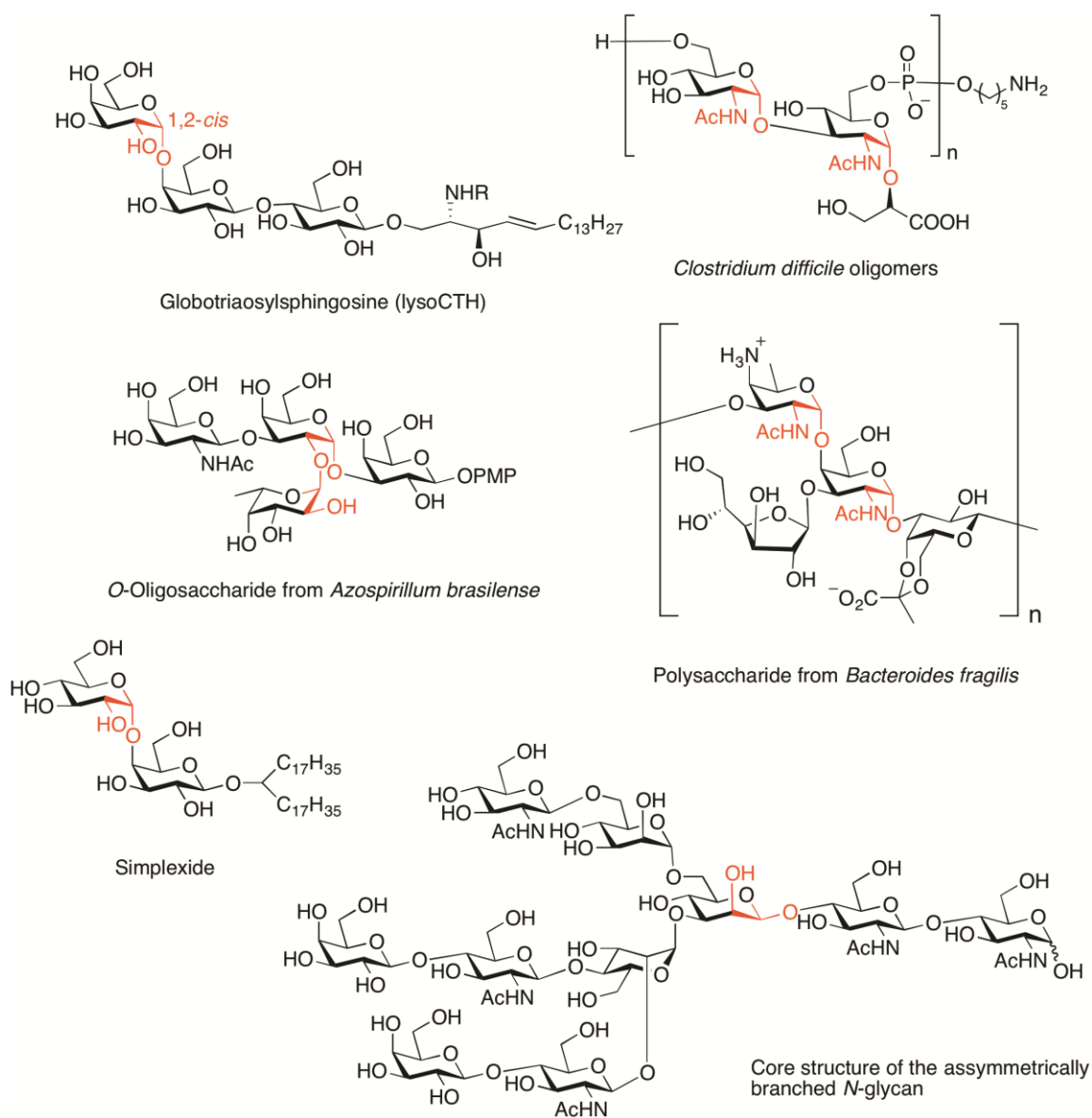


Figure 1.2 Representative naturally occurring oligosaccharides and glycolipids comprising 1,2-*cis*-O-glycosidic bonds (specified in tangerine color).

glycosidic bonds. Some kind of remote protecting groups, such as picolinoyl and 2-quinolinecarbonyl groups,^{20, 21} have been demonstrated to guide the 1,2-*cis* stereoselectivity of glycosylation via hydrogen-bonding effects. Moreover, several bulky cyclic protecting groups, including di-*tert*-butylsilylene (DTBS)²² and benzylidene,²³ are powerful 1,2-*cis*-directing groups, which have been extensively applied in the construction of the α -linkage in galactosides and the β -linkage in mannosides. Besides using an effective protecting group, the application of nucleophilic ether solvents, such as diethyl ether, dioxane, and tetrahydrofuran, are also highly regarded to facilitate the 1,2-*cis*-stereoselectivity of glycosylation under different glycosylation conditions.^{24, 25} Recently, I reported a straightforward 1,2-*cis*-alkyl glycosylation strategy by utilizing unprotected thiophenol glycosyl donors, which were glycosylated with a variety of reactive acceptors to make the corresponding 1,2-*cis*-*O*-glycosides in moderate to high yield and selectivity (see Chapter 2).²⁶

This overview focuses on the advances that have been developed to rise to the challenge of stereoselective glycosylation, especially of the 1,2-*cis*-*O*-glycosylation. The aim of this perspective is to overview (i) the application of diverse glycosyl donors and activation conditions on 1,2-*cis*-*O*-glycosylation, (ii) the influence of various protecting groups on 1,2-*cis*-*O*-glycosylation, and (iii) outlook of the 1,2-*cis*-*O*-glycosylation methods.

1.2. Glycosyl Donors and Their Applications in 1,2-*cis*-Glycosylations

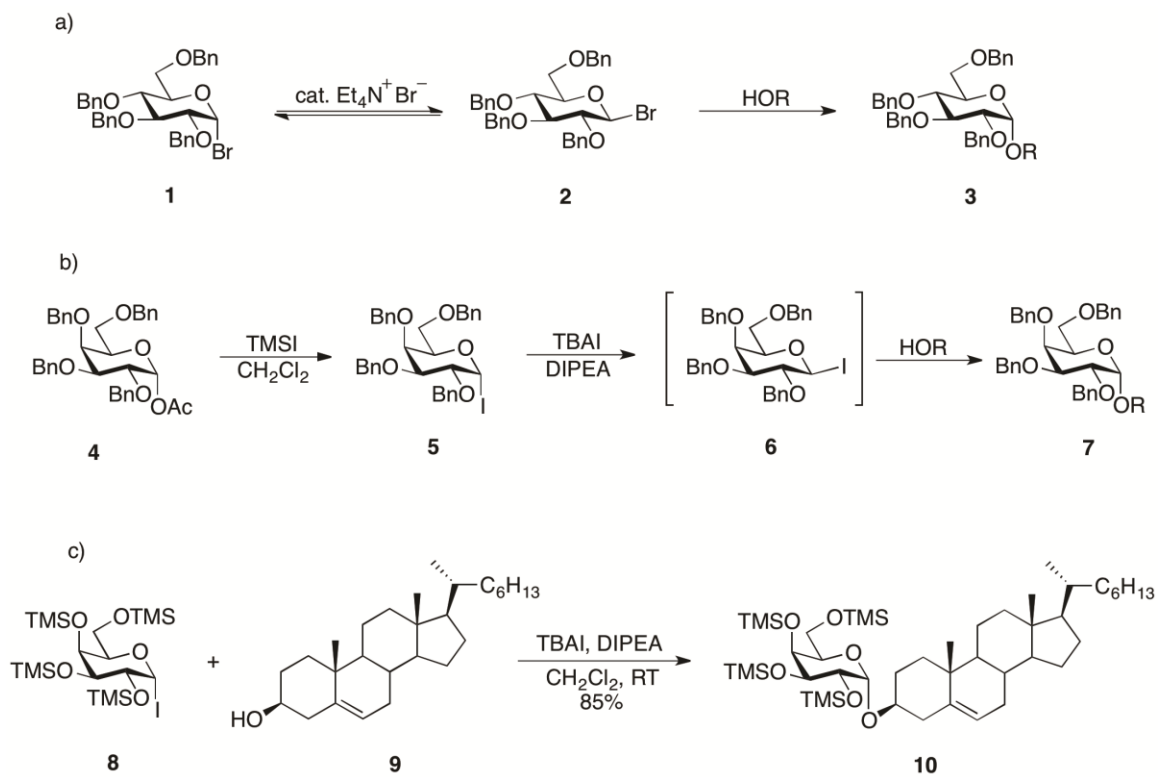
1.2.1 Glycosyl halides

In 1901, glycosyl chlorides and glycosyl bromides were first introduced as glycosylation agents by Wilhelm Koenigs and Eduard Knorr,²⁷ and this classical method

is still widely used nowadays in the stereospecific synthesis of glycosides. Heavy metal salts, such as Ag_2O , Ag_2CO_3 , AgOTf , AgClO_4 , HgBr_2 , $\text{Hg}(\text{CN})_2$, HgO or CdCO_3 usually serve as promoters in this reaction.¹ Another benefit of using metal carbonates and oxides as activators is they are also acid scavengers, which can neutralize the acid released in the glycosylation reaction. Sometimes organic bases, for example *s*-collidine, lutidine, tetramethylurea, and the sterically hindered 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) also perform the same function.²

Besides a leaving group, the halide ion is also used as a stereodirecting agent. Lemieux introduced halide-ion catalyzed glycosylation (Scheme 1.1a), which is to combine promoters such as Et_4NBr or Bu_4NBr with Hünig's base to yield high stereoselectivity of 1,2-*cis* glycosides.^{28, 29} During this process, the generated bromide ion (Br^-) performs as a nucleophile to convert α -glucosyl bromide **1** into β form **2**, which was glycosylated with an acceptor to give 1,2-*cis*- α -glucoside **3**. However, the Lemieux glycosylation often takes long reaction times (>2 days), and only applies to reactive glycosyl halides and acceptors. Fortunately, these drawbacks can be avoided by using alternate glycosyl donors, to generate glycosyl halides *in situ*, such as to activate thioglycoside donors by $\text{CuBr}_2\text{--Bu}_4\text{NBr}$ ³⁰ or $\text{DMTST--Bu}_4\text{NBr}$,³¹ to progress through glycosyl halide intermediates.

Although these were the only donor choices for a long period of time, glycosyl halides are now losing their prevalence in glycosylation reactions. They suffer from two weaknesses: first, equimolar amount of poisonous, costly and often moisture- and light-sensitive heavy metal reagents are utilized as promoters; second, the lability of glycosyl halides obstructs chemical manipulation on the other functional without impairing the



Scheme 1.1 Stereoselective glycosylation by glycosyl halide donors.

activity of the very sensitive anomeric halides. These drawbacks can be circumvented by either applying benzyl or silyl protecting groups on glycosyl halide donors, or by using more stable donors such as thioglycosides as effective stereospecific glycosylation methods that have been developed.

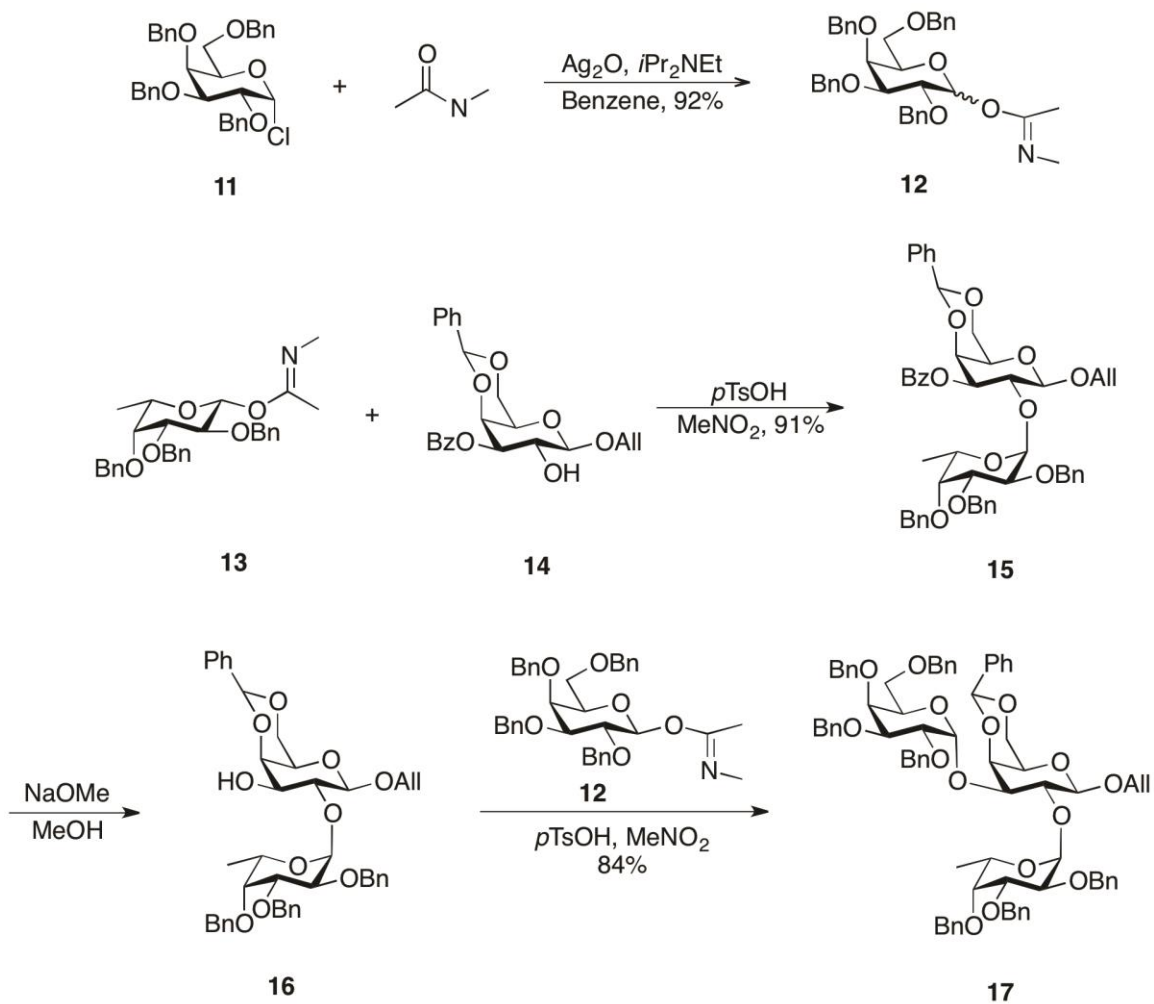
Although glycosyl iodides were first prepared many years ago,³² their utility as glycosyl donors has not been fully explored until recent decades, which is due to their instability and high reactivity. The halide ion-catalyzed glycosylation method paves way for their *in situ* preparation, and they have showed delightful properties in reaction rate, efficiency, and stereoselectivity.^{33–35} Most reported glycosylation reactions by glycosyl iodide donors are equipped with arming (activating) protecting groups, usually *O*-benzyl, *O*-trimethylsilyl, or other electron-donating groups.³⁶ The direct nucleophilic displacement of an anomeric iodide by an acceptor via an S_N2 process could achieve a straightforward synthesis of a 1,2-*cis* glycosyl linkage, which avoids the introduction of stereodirecting groups that require further removal. For example, benzylated glycosyl acetate **4** can be activated by iodotrimethylsilane (TMSI) to yield the α -glycosyl iodide **5**. Without isolation, α -glycosyl iodide is activated by TBAI and Hünig's base to produce β -glycosyl iodide **6** *in situ*, and subsequent axial attack on the anomeric center affords the α -glycoside **7** in high stereoselectivity (Scheme 1.1b).^{37, 38} Besides the benzyl protecting group, the trimethyl silylated glycosyl iodide **8** can also be synthesized and applied in constructing the biologically active α -D-cholesteryl glycoside **10** in high 1,2-*cis* selectivity (Scheme 1.1c).³⁹ Because of their high reactivity and instability, methods are needed to generate glycosyl iodides *in situ*, and further improvements are expected to increase their stability and develop other activation conditions for a broader usage.

1.2.2 Glycosyl imidates

In glycosyl imidates, the anomeric oxygen atom is derivatized to a labile leaving group, which can be readily removed by Lewis acid activators such as TMSOTf or $\text{BF}_3 \cdot \text{OEt}_2$. Sinaÿ prepared the first β -*O*-glycosyl acetimidates by treating benzylated glycosyl chlorides **11** with *N*-methyleacetamide,⁴⁰ and applied them in the construction of the blood group B determinant. The acetimidates **12** and **13** were each glycosylated with glycosyl acceptors **16** and **14**, respectively, by the promotion of *p*TsOH, and 1,2-*cis* glycoside **17** was thus synthesized in excellent stereoselectivity (Scheme 1.2).^{41, 42}

In the 1980s, Schmidt reported *O*-glycosyl trichloroacetimidates and *O*-glycosyl trifluoroacetimidates as advanced imide donors with better general applicability.⁴³ After that, a series of *N*-substituted *O*-glycosyl trihaloacetimidates was synthesized from the corresponding glycosyl hemiacetals and trihaloacetonitrile or *N*-substituted trihaloacetimidoyl halides in a basic medium.⁴⁴ Generally, trichloroacetimidate donors are more competent than trifluoroacetimidate donors with respect to reactivity and reaction yield, while in some specific cases^{45, 46} *O*-glycosyl *N*-phenyl-trifluoroacetimidates are more reactive. There are several factors including the existence of a substituent, the basicity and/or the size of the leaving group, and its conformational change synergistically determining the donor efficiency.⁴⁷

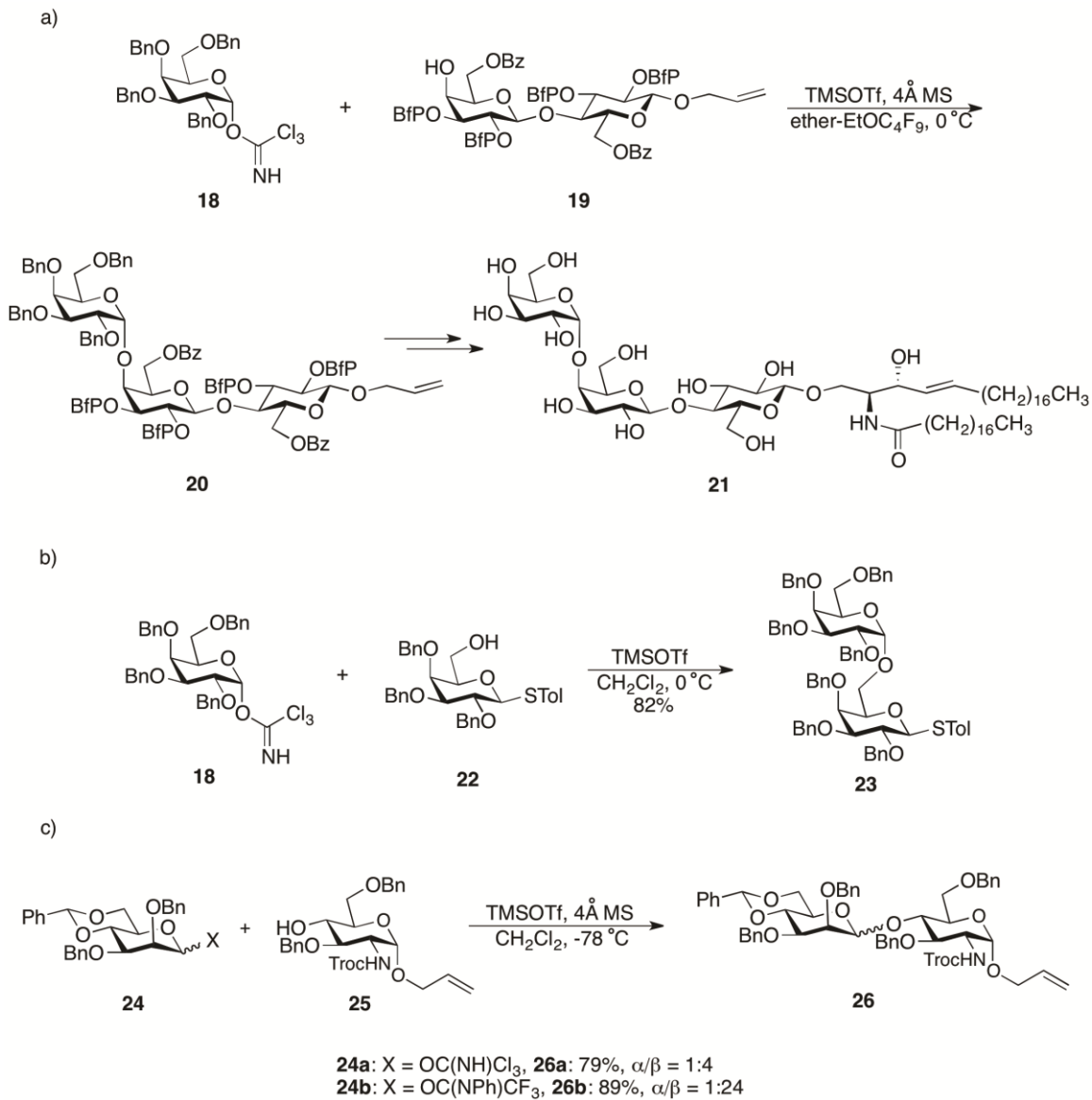
The glycosylations with trichloroacetimidates are usually carried out under acidic conditions, and various Lewis acids have been tested and demonstrated, ranging from typically used TMSOTf and $\text{BF}_3 \cdot \text{OEt}_2$,⁴³ to $\text{Sm}(\text{OTf})_3$ ⁴⁸ and $\text{Yb}(\text{OTf})_3$ ⁴⁹ for the specific activation of armed and disarmed *O*-glycosyl donors, respectively. The activation of *N*-aryl-trifluoroacetimidates needs more forceful conditions, and successful activation



Scheme 1.2 Application of glycosyl acetimidate donors in the synthesis of blood group B determinant.

systems include $\text{Bi}(\text{OTf})_3$, $\text{I}_2/\text{Et}_3\text{SiH}$, and $\text{TMSB}(\text{C}_6\text{F}_5)_4$.^{46, 50} For the purpose of reaching desired 1,2-*cis*-stereoselectivity, the glycosylation conditions need to be precisely controlled, usually assisted by the use of non-participating protecting groups (benzyl group), nonpolar solvents, mild Lewis acid catalysts ($\text{BF}_3 \cdot \text{OEt}_2$), and low reaction temperatures. Such conditions favor the $\text{S}_{\text{N}}2$ processes, and β -trichloroacetimidate will preferentially provide α -glycosides. If more powerful conditions were applied, such as using strong acid catalysts (TMSOTf , TfOH), higher temperature and more polar solvents, both α - and β -trichloroacetimidates would generate the thermodynamically stable products, which are the α -glycosides in general. Besides the glycosylation conditions, the nature of the counter anion of the Lewis acid also has an influence on the stereoselectivity. For example, in some specific cases HClO_4 favors the formation of α -glycosides, while $\text{HB}(\text{C}_6\text{F}_5)_4$ does not; yet the reason behind these observations has not been explained.⁵¹

The *O*-glycosyl trihaloacetimidate donors have been extensively employed in the synthesis of 1,2-*cis*-*O*-glycosides. Benzylated *O*-galactosyl trichloroacetimidate **18** was glycosylated with the acceptor **19** to exclusively furnish the α -(1 $\rightarrow$ 4)-linked trisaccharide **20**, for the synthesis of oligosaccharide moieties **21** of a globotriaosylceramide (Scheme 1.3a).⁵² They were also used in the stereoselective construction of α -(1 $\rightarrow$ 6)-linked saccharides such as verbascose, raffinose or sucrose (Scheme 1.3b).⁵³ Benzylated *O*-glucosyl trichloroacetimidates also displayed comparable properties. The glycosylations can be successfully realized with either 2-, 3-, 4- or 6-OH on glycosyl acceptors in the presence of TMSOTf at low reaction temperature, in order to obtain predominantly α -linked oligosaccharides.⁵⁴ Besides, it has also been reported that *N*-phenyl-



Scheme 1.3 Synthesis of 1,2-*cis*-O-glycosides with glycosyl trihaloacetimidate donors.

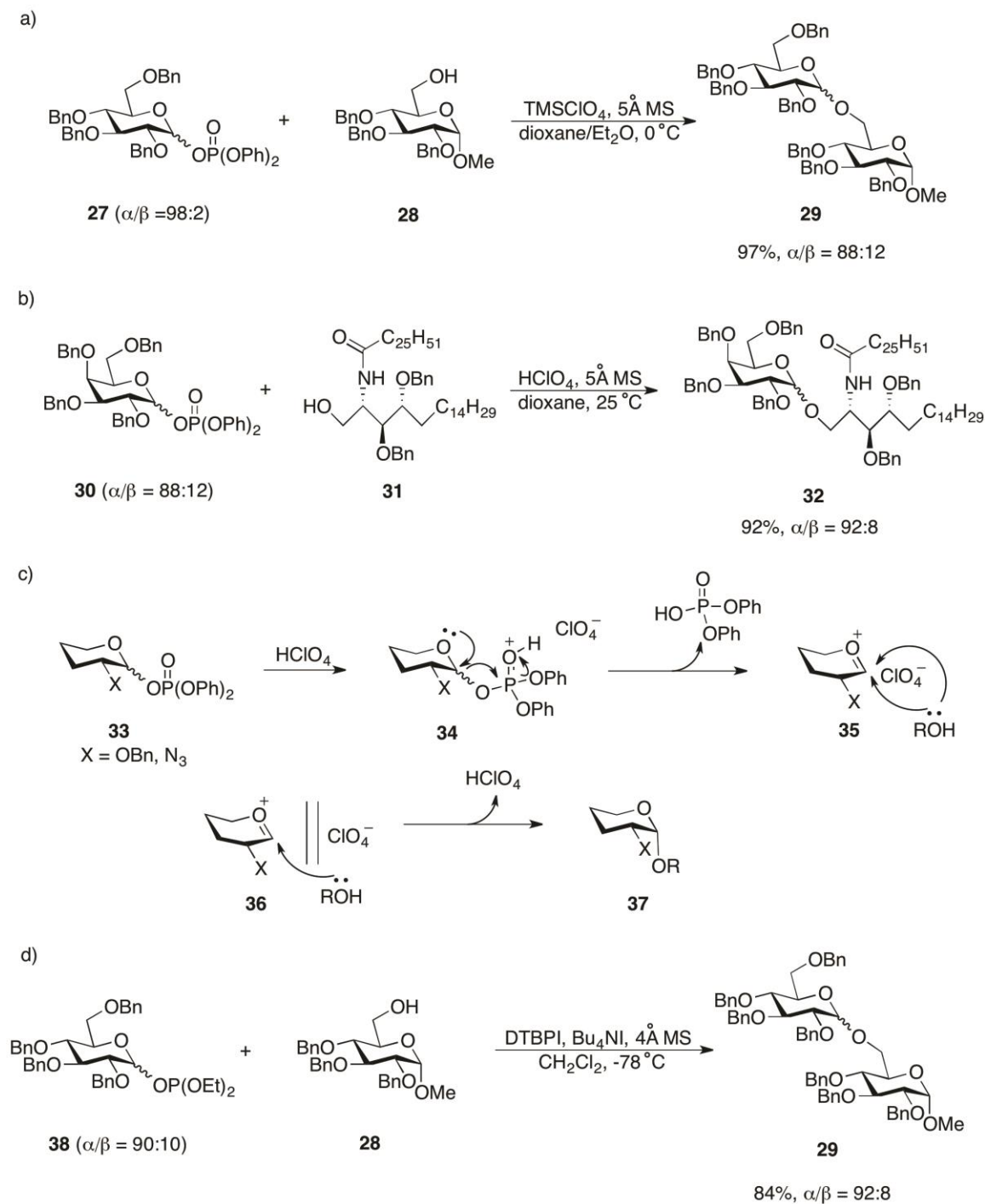
trifluoroacetimidate **24b**, in some specific cases, is superior to trichloroacetimidate **24a**, such as achieving higher yield and better stereoselectivity in the β -mannosylation reactions (Scheme 1.3c).⁵⁵

The disadvantages of this method still exist: the high reactivity and lability of glycosyl trichloroacetimidates can give rise to side reactions during glycosylations; the donor may decompose during long-time storage; the rearrangement of the donor may occur to produce the non-reactive glycosyl trichloroacetamide. All these will lead to low glycosylation yields, side products, and challenges in purification. Moreover, the structural characterization of donors could be complicated by their invertomers, and further aggravated by the splitting of the carbon signals by adjacent fluorine.⁴⁻⁹

1.2.3 Glycosyl phosphates and phosphites

The first synthesis of glycosyl diphenyl phosphates as effective glycosyl donors was reported in 1989.⁵⁶ After that, many glycosyl donors bearing phosphorus leaving groups were developed by adopting different substituents on the phosphorus atom.^{57, 58} In most circumstances, both α - and β -glycosyl phosphate donors are easy to prepare, store, and manipulate; yet the glycosylation properties were found inferior to the corresponding *O*-glycosyl trichloroacetimidates. The traditional reaction method was to use TMSOTf as the promoter in at least a stoichiometric amount, which is due to the formation of an equal amount of silyl phosphate to be the driving force of glycosylation. Recently, perchloric acid was employed in only a catalytic amount as an efficient activator, and enhanced α -selectivity was obtained.⁵⁹

Likewise, the glycosyl phosphate and phosphite donors are capable of forming 1,2-*cis*- α -*O*-glycosidic linkages. For instance, benzylated glucosyl diphenyl phosphates



Scheme 1.4 Glycoside syntheses with glycosyl phosphates (a, b) and glycosyl phosphite (d) as donors, and rationalization of the high α -selectivity upon the activation of HClO_4 (c).

27 (mixed isomers with high α) was reported as the donor to glycosylate with glycosyl acceptor **28** to provide glycoside **29** in good yields and α -selectivity upon activation with catalytic amount of TMSClO_4 or HClO_4 in a dioxane/ Et_2O mixed solvent (Scheme 1.4a).⁶⁰ This strategy has also been practiced in the preparation of α -galactosylceramide KRN 7000, a ligand for natural killer T cells activation on antigen-presenting cells. Benzylated galactosyl diphenyl phosphate **30** was coupled with the ceramide acceptor **31** under a catalytic amount of HClO_4 in dioxane, and the glycoside **32** was formed in respectable yield and α -selectivity (Scheme 1.4b).⁵⁹ It is believed that during the glycosylation process a contact ion pair (CIP) **35** is formed with an oxocarbenium ion and a perchlorate counter ion after the cleavage of the phosphate group, and ethereal solvent facilitates the formation of solvent-separated ion pair (SSIP) **36**, which favors the axial addition of acceptors from the α -face due to the kinetic anomeric effect (Scheme 1.4c).^{61, 62}

Glycosyl phosphite exhibits the preference in α -glycosylations as well. Glycosyl diethyl phosphite **38** was reacted with the acceptor **28** to predominantly furnish α -glycoside **29** under the activation of 2,6-di-*tert*-butylpyridinium iodide (DTBPI) and TBAI. The glycosyl iodides were supposed to be generated as intermediates during the *in situ* anomerization process (Scheme 1.4d).⁶³

1.2.4 Thioglycosides

Thioglycosides are amongst the most popular types of glycosyl donors in oligosaccharide synthesis from their first preparation over a century ago.⁶⁴ Thioglycosides have many benefits as glycosyl donors, which include long shelf life for storage, easy accessibility, great stability under various reaction conditions for protecting-

group operation, favorable state as crystalline, and most importantly, amenability to desired chemoselective transformations when necessary. Therefore, thioglycosides satisfy most requirements as ideal glycosyl donors.^{2, 4-9}

Accordingly, a great variety of strategies have been developed for the synthesis of thioglycosides. The most popular method for thioglycoside preparation is to treat per-*O*-acetylated carbohydrates with thiols such as thioethanol or thiophenol in the presence of Lewis acids, usually $\text{BF}_3 \cdot \text{OEt}_2$, to predominantly furnish 1,2-*trans* thioglycosyl donors.⁶⁵ They have also been prepared by a two-step procedure that involves a nucleophilic displacement of acetylated glycosyl halides to give the corresponding glycosyl thioacetates, followed by alkylation/arylation of the *in situ* generated thiols by selective *S*-deacetylation.⁶⁶ This protocol is quite versatile when different alkyl/aryl thiol glycosides or *S*-linked disaccharides need to be synthesized.⁶⁷ The acetylated thioglycosides thus prepared can survive in deacylation and many other protecting group manipulations, whereas glycosyl bromides and glycosyl trichloroacetimidates cannot.

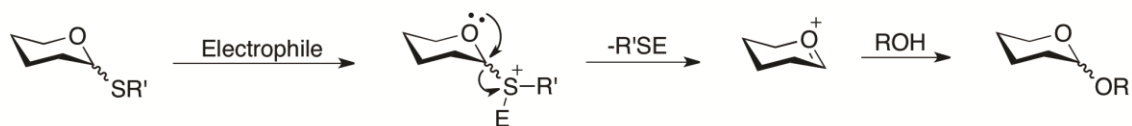
A wide range of activation systems for thioglycoside donors has evolved as well. The use of iodonium ions generated from *N*-iodosuccinimide (NIS) with a catalytic dose of triflic acid (TfOH), which was first reported in 1990, has been regarded as the preferred method to activate thioglycosides.⁶⁸ Since then, the activation ability of iodonium ions on thioglycosides has been demonstrated by many successful applications with variations, and besides, many other thiophilic promoters have been developed. For example, the employment of NIS with other types of Lewis acids, including AgOTf ,⁶⁹ $\text{TrB}(\text{C}_6\text{F}_5)_4$,⁷⁰ $\text{Bi}(\text{OTf})_3$,⁷¹ HClO_4 -silica⁷² *etc.*, as alternatives to TfOH for the activation of thioglycosides with comparable effects. Sometimes *N*-bromosuccinimide (NBS),⁷³

interhalogen compounds (ICl or IBr),⁷⁴ or iodine (I₂)⁷⁵ functions similarly as substituent halo sources. 1-Fluoropyridinium triflates were also reported⁷⁶ as versatile promoters in the transformation of thioglycosides to *O*-glycosides. All these activation systems rely on the intrinsic soft nucleophilicity and especially the halophilicity of the sulfur atom in the thioglycosides. The glycosylation process initiates from the activation with soft electrophilic reagents (halogen in such cases) and the formation of a sulfonium ion, which is a better leaving group. Cleavage of the sulfonium ion leads to the formation of an oxocarbenium ion as a common intermediate in glycosylation, and the following nucleophilic addition by the glycosyl acceptor affords the *O*-glycoside (Scheme 1.5a).⁷⁷

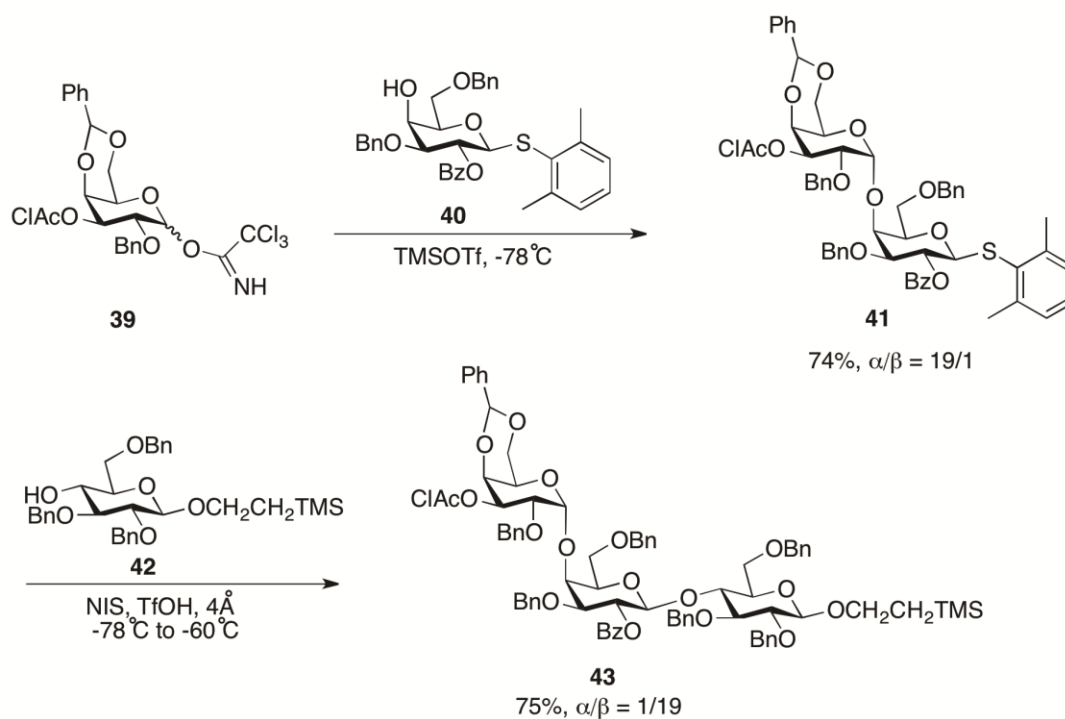
Recently another type of soft electrophile, the organo-sulfur compounds, has been evaluated as valuable promoters for the activation of thioglycosides. Representative activation systems involve sulfonium triflates such as DMTST,³¹ MeSOTf,⁷⁸ or PhSOTf,⁷⁹ and sulfinates in combination with Tf₂O such as benzenesulfinyl morpholine (BSM)/Tf₂O,⁸⁰ 1-(phenylsulfinyl)piperidine (BSP)/Tf₂O,⁸¹ diphenyl sulfoxide (Ph₂SO)/Tf₂O⁸² or Me₂S₂/Tf₂O^{ts83}. Since the sulfinyl/triflic anhydride systems are able to preactivate thioglycosides at low temperatures, the thioglycosides can be activated in the presence of another and be treated as glycosyl acceptors as well. Besides, the relative stability of thioglycosides facilitates the selective glycosylation of glycosyl imidate donors. These features have often been applied in the iterative and one-pot synthesis of complex oligosaccharides (Scheme 1.5b, Scheme 1.6a).⁴⁻⁹

The applications of thioglycosides in 1,2-*cis*-glycosylation reactions are frequently reported. For example, stereoselective construction of 1,2-*cis*- α -*O*-glycosidic linkages in some oligosaccharide syntheses was performed by benzylated thiomethyl

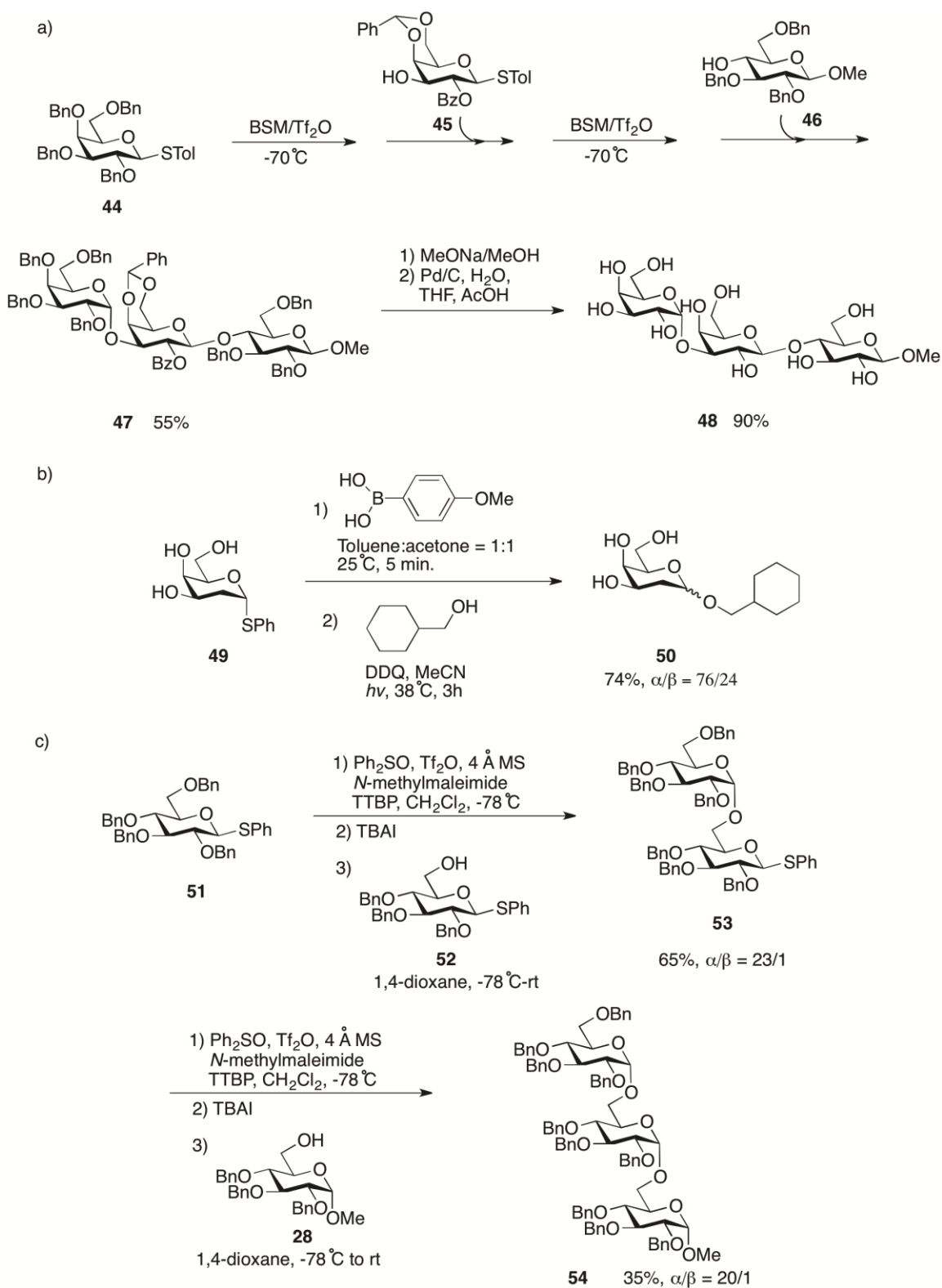
a)



b)



Scheme 1.5 Glycosylation by a thioglycoside via the activation of an electrophile (a), and selective activation of O-glycosyl trichloroacetimidates in the presence of thioglycoside donor (b).



Scheme 1.6 Oligosaccharide and alkyl glycoside syntheses with thioglycosides as donors.

glycosides in the presence of iodine as an efficient promoter.⁸⁴ Thiogalactoside was also reported to be glycosylated with acceptors by using BSM/Tf₂O in a preactive one-pot synthesis fashion, to give the trisaccharide **48** bearing a 1,2-*cis* linkage as analogue of a tumor-associated antigen IsoGb3 (Scheme 1.6a).⁸⁵ The unprotected deoxythioglycoside **49** was coupled with alcohols under the assistance of DDQ and long-wavelength UV irradiation to effectively give α -O-glycoside **50a** as the major product (Scheme 1.6b). Specifically, *p*-methoxyboronic acid was demonstrated to temporarily protect the 1,3-diol (C-4 and C-6 positions) and increase the reaction yields.⁸⁶ Very recently, a method using thioglycoside donors was developed for the highly selective *O*-glycosylation. The thioglycoside **51** was activated with Ph₂SO/Tf₂O, followed by TBAI, to possibly generate a glycosyl iodide intermediate that undergoes stereoselective 1,2-*cis*- α -glycosylation in high yield. This method permits iterative oligosaccharide synthesis (Scheme 1.6c) as well, and shows promise in synthesizing complex α -glycosides with no specialized protective groups.⁸⁷

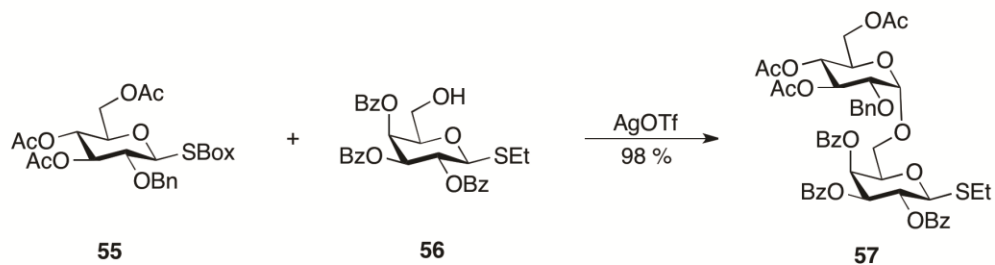
In conclusion, lots of attention and effort have been generated in the preparation and activation of thioglycosides, and many strategies have been proposed during the past decade. Since thioglycosides are representative glycosyl donors with many benefits, they were designed to play a key role in oligosaccharide synthesis. Therefore, advanced approaches, which emphasize regio- and stereoselective reactions in high yield with easy isolation of products and minimum protecting group usage and manipulation, are still desired.

1.2.5 Other glycosyl donors and activators

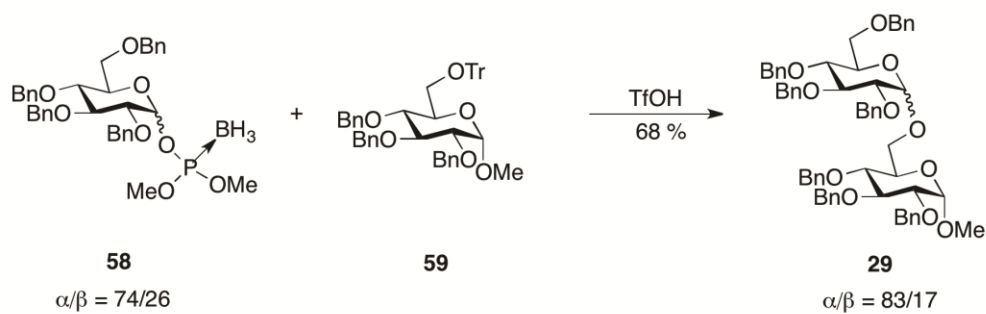
The previous sections review the glycosyl donors most typically used in oligosaccharide synthesis and especially their applications in 1,2-*cis*-*O*-glycosylations. Although these glycosylation strategies have offered significant advances for chemselective glycosidic bond formation, it is still necessary to evaluate other less commonly used glycosyl donors. Adopting dissimilar glycosyl donors alters activation patterns and glycosylation potential, which might be vital to solve a particular glycosylation problem.

Glycosyl thioimidates, which contain an $\text{SCR}^1=\text{NR}^2$ aglycone, bear properties of both a thioglycoside and a glycosyl imidate. Glycosyl thioimidates are efficient donors, and examples such as *S*-benzoxazolyl (SBox) glycoside⁸⁸ and *S*-thiazolinyl (STaz) glycoside⁸⁹ have been employed in the 1,2-*cis*-*O*-glycosylation with complete stereoselectivity (Scheme 1.7a). The high stability of glycosyl thioimidates makes them competent to tolerate harsh reaction conditions that sometimes have to be designated for the activation of other glycosyl donors, and these properties popularize them in the synthesis of complex oligosaccharide.⁹⁰ Recently, benzylated glycosyl boranophosphate **58** was developed with modified reactivity as a glycosyl phosphate analog. The displacement of the $\text{P}=\text{O}$ moiety by a $\text{P}\rightarrow\text{BH}_3$ group enables the donor to be stable in the presence of Lewis acids, while its specific activation can be easily fulfilled by the trityl cation (Tr^+). A trityl salt such as trityl *N,N*-bis(trifluoromethanesulfonic)imidate (TrNTf_2) can be used, while the reaction can also be carried out with the *O*-trityl ether **59** that serves as both the Tr^+ source and the glycosyl acceptor (Scheme 1.7b), which provides improved *O*-glycosylation yields and 1,2-*cis* stereoselectivity as well.⁹¹ Moreover, 1,2-

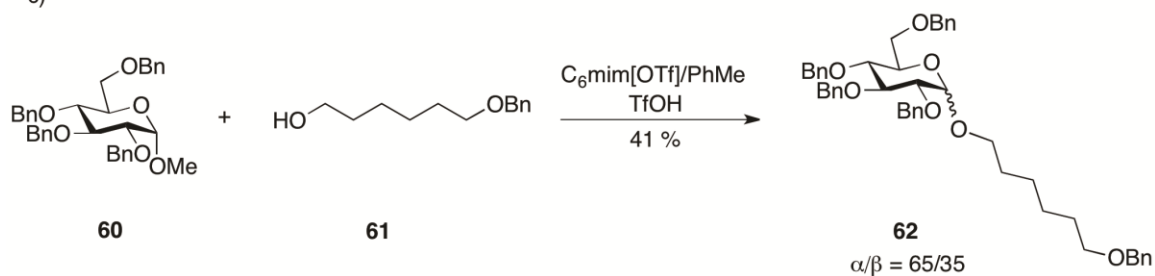
a)



b)



c)



Scheme 1.7 Stereoselective glycosylation with other typical glycosyl donors.

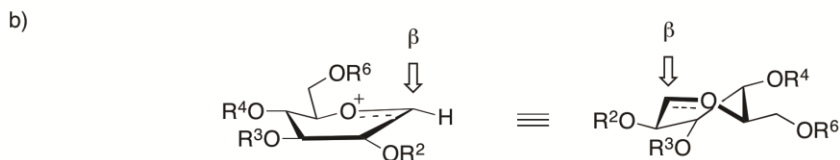
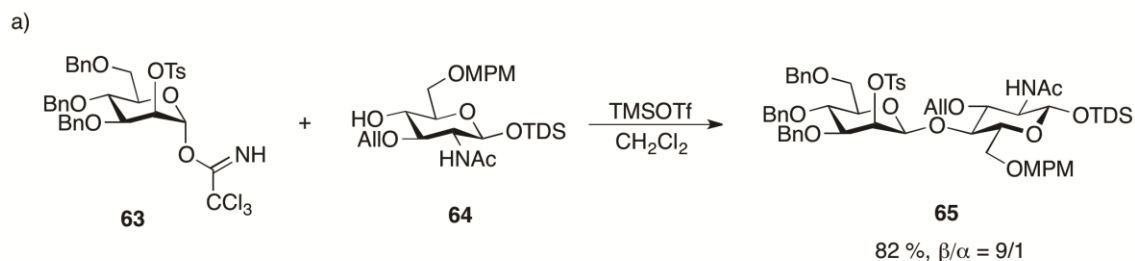
orthoesters, which are usually intermediates in glycosylation processes, can also give 1,2-*cis*-glycosides through rearrangement.^{45, 92, 93} 2-Nitroglycals are particularly valuable in the *cis*-formation of 2-acetamido galactosides.⁹⁴ Even if those inactive glycosides such as methyl glycoside **60** and 1-hydroxy sugars are now used as glycosyl donors under specific conditions with moderate to high reactivity (Scheme 1.7c).⁹⁵

1.3. 1,2-*cis*-Directing Protecting Groups

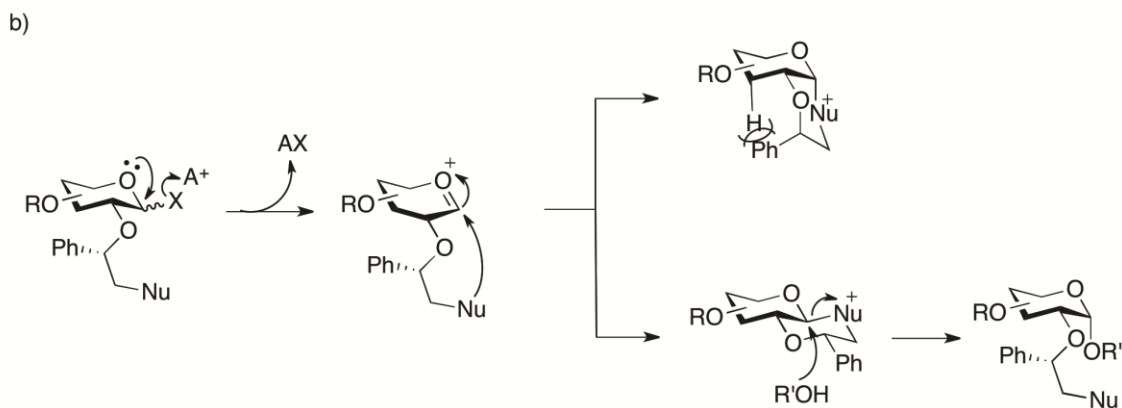
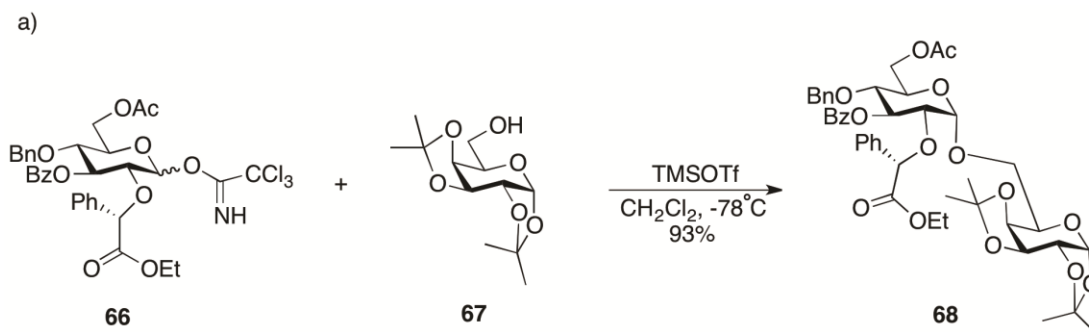
Nowadays, the role of protecting groups in carbohydrate chemistry is no longer limited to simply provide blocking of hydroxyl groups, because it has been realized that protecting groups can have a pronounced impact on the reactivities of both glycosyl donors and acceptors, as well as on the stereoselectivity of the glycosylation. This section will focus on the formation of 1,2-*cis* glycosidic bonds via installation of special protecting groups that preferentially direct the 1,2-*cis*-stereoselective. Based on the directing effects, the protecting groups are typically classified into two major categories, which are 1,2-*cis*-directing through electronic effects (Section 1.3.1) and through torsional effects (Section 1.3.2). Besides, unprotected glycosyl donors and their applications in 1,2-*cis*-glycoside synthesis have also been reported (Section 1.3.3).

1.3.1 1,2-*cis*-Directing protecting groups through electronic effects

Ether protecting groups are often used in the synthesis of 1,2-*cis* gluco- or galactosides for the neighboring hydroxyl protection. They are generally treated as nonparticipating protecting groups, and the 1,2-*cis* selectivity is achieved by the anomeric effect. Similarly, results can also be reached by aryl sulfonyl protecting groups, especially in the construction of β -mannosyl (Scheme 1.8a) and rhamnosyl glycosides. They are nonparticipating groups, but with strongly electron-withdrawing properties.



Scheme 1.8 Stereoselective synthesis through electronic effects by a neighboring protecting group (a). Flattened twist-boat intermediate (b).

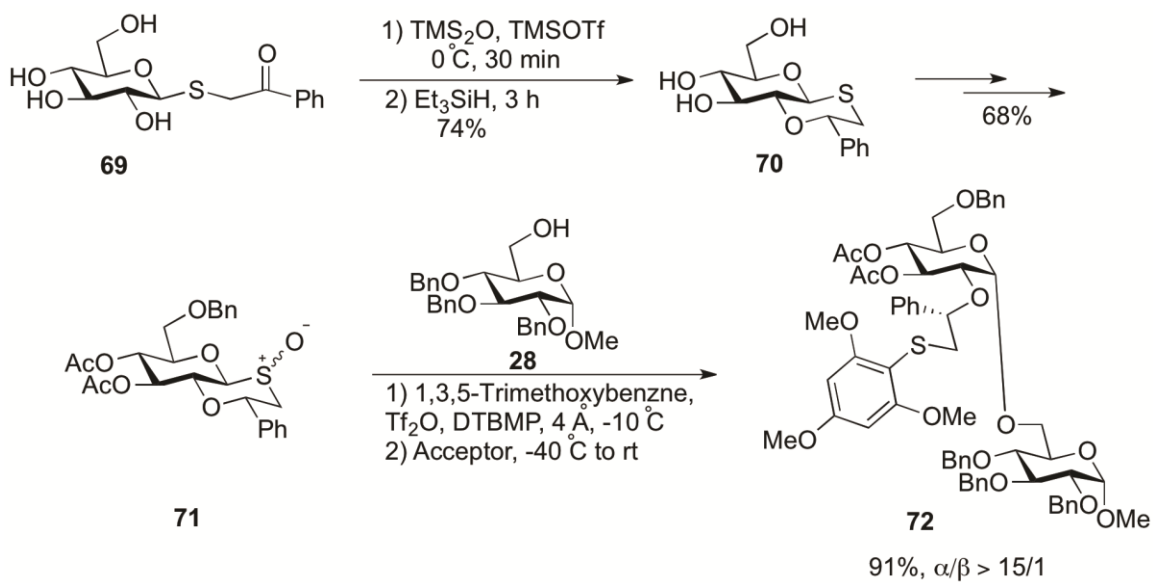


Scheme 1.9 Stereoselective glycosylation with (*S*)-(ethoxycarbonyl)benzyl-protected glycosyl donor (a), and neighboring-group participation by an (*S*) auxiliary at C-2 leading to 1,2-*cis* stereoselectivity (b).

When the donor is activated, the strong dipole effect facilitates the formation of a flattened twist-boat intermediate (Scheme 1.8b), and it is attacked favorably from the β face to give a 1,2-*cis*-glycoside.^{96, 97}

A new general strategy was developed for the formation of 1,2-*cis*-glycosidic linkages by introducing a neighboring auxiliary group at the 2-*O*-position, such as (*S*)-ethoxycarbonyl)benzyl chiral substituent depicted in Scheme 1.9a.⁹⁸ The addition of the ethoxycarbonyl group from the chiral auxiliary to the anomeric center, which is called neighboring-group participation, leads to the formation of a stable dioxolenium ion. It is expected that the (*S*)-stereochemistry of the phenyl group would occupy the equatorial face, which prevents unfavorable 1,3-diaxial steric interactions. Therefore, the attack of the dioxolenium ion by acceptors could only be feasible from the bottom face to give α -glycosides (Scheme 1.9b). Instead, β -glycosides can be readily afforded with the help of an *R*-configuration auxiliary. Moreover, the introduction of an electron-withdrawing group at the C-3 position could destabilize the oxacarbenium ion and facilitate the auxiliary group participation as well as the formation of α -glycosides. However, a drawback of this strategy is that an auxiliary group has to be installed on the glycosyl donor and cleaved after glycosylation, which requires relatively harsh conditions and tedious operations.

Recently, a simplified approach was proposed based on the virtue of a neighboring auxiliary group towards the preparation of 1,2-*cis*-glycosides. The glucosyl 1,2-oxathiane ether was made from thioglucoside **69**, and its further oxidation gave glucosyl sulfoxide donor **70** in a bicyclic anomeric sulfonium ion state. Glycosylations of



Scheme 1.10 Synthesis of 1,4-oxathiane-protected glucosyl donor and its employment in stereoselective glycosylation.

the donor with various glycosyl acceptors furnished corresponding disaccharides in high yields and 1,2-*cis* stereoselectivity (Scheme 1.10). The new donor is capable of withstanding many kinds of reaction conditions with respect to protecting group manipulations, and it has been successfully applied in the synthesis of a branched glucoside as a polysaccharide derivative isolated from a natural fungi.⁹⁹

The influence of remote substituents on configuration of the anomeric center during glycosylations has been exploited in recent years as well. Several kinds of protecting groups, ranging from commonly used acetyl¹⁰⁰ and carbonate¹⁰¹ groups to deliberately designed picolinyl (Pic)¹⁰² and picoloyl (Pico)¹⁰³ protecting groups, are carefully studied and determined to be effective in the stereo-formation of glycosidic bonds. For example, a comparative study¹⁰⁰ revealed that when trichloroacetimidate mannoside donors contain electron-withdrawing substituents such as an acetyl or benzoyl group at the C-3 position, their glycosylations with primary glycosyl acceptors could provide the disaccharides in great yields and 1,2-*cis*-selectivity. However, if the electron-withdrawing substituent at C-3 is changed to benzylsulfonyl, the stereoselectivity of the corresponding disaccharide would be inverted to 1,2-*trans*-selectivity. Recently, a parallel study¹⁰⁴ was carried out on the glycosylations of glucosides with both conformationally restricted and non-restricted glucosyl donors. The results indicated that when the glucosyl *N*-phenyltrifluoroacetimidate donors bear acetyl groups at C-3 or C-6, the glycosylations with acceptors were accomplished in high yields and 1,2-*cis* selectivity. When the acetyl group at C-6 was switched to a benzoyl group, a further selectivity increase was observed. Comparable yields and selectivity were also applied to the conformationally constrained 4,6-*O*-benzylidene-protected glucosyl donor. These

experimental results support the hypothesis that electron-withdrawing or sterically bulky groups, specifically those at C-6, favor 1,2-*cis*-glycosylation. The protecting groups electronically or sterically shield the top face of the pyranose rings, and facilitate the nucleophilic addition from the opposite face to afford 1,2-*cis*-glycosides.¹⁰⁵

The study of 2-azido-2-deoxy glycosyl donors showed a distinctive relationship between the remote protecting groups and stereoselectivity of glycosylations.¹⁰⁶ It was observed that acetyl substituents at C-3 and C-6 positions on GlcN₃ donors directed α -glycosylations, whereas acetyl group at C-4 was more β -directing.¹⁰⁷ The application of 2-azidomannouronate ester donor in 1,2-*cis*-glycosylation was investigated as well.^{108, 109} The donor could be coupled with a glycosyl acceptor with the activation of Ph₂SO and TfOH at low temperature to give predominantly 1,2-*cis*-glycosides. The proposed mechanism states that the oxacarbenium ion is formed as an intermediate in association with the triflate counterion. Meanwhile, the strong electron-withdrawing carboxylate group on C-5 dominates a pseudo-axial location and stabilizes the positive charge on the intermediate, which expedites the following nucleophile attack from the bottom face and provides 1,2-*cis* selectivity exclusively.

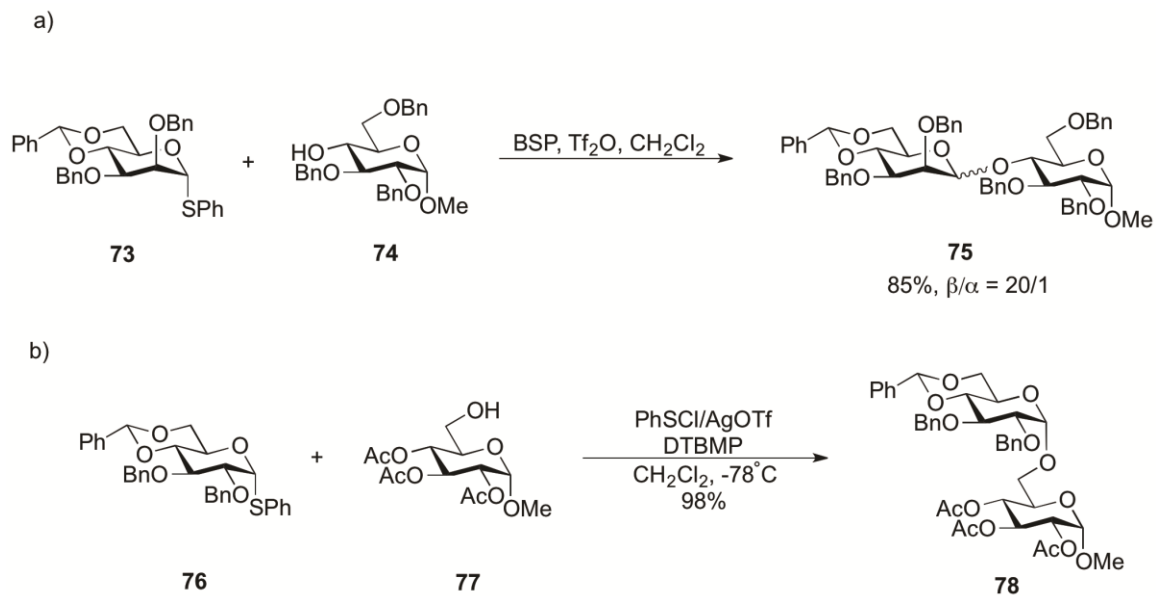
In addition, an innovative stereodirecting glycosylation strategy, which is defined as H-bond-mediated aglycone delivery (HAD), has been carefully designed and applied with the assistance of specific protecting groups such as picolinyl (Pic) and picoloyl (Pico) groups.²⁰ When incorporating these substituents at C-3 or C-4, a hydrogen bond is formed between the picolinyl nitrogen with the oxygen of the acceptor at the syn face, and a high stereoselectivity is achieved. The applicability of this strategy was demonstrated by many applications. Various glycosyl donors, including 4-*O*-picoloyl

glucosyl donor, 3-*O*-picoloyl mannosyl donor, 5-*O*-(2-quinolinecarbonyl) arabinosyl donor, as well as the 6-*O*-picoloylated 2-deoxy glycosyl donors, all successfully contributed to 1,2-*cis* glycosidic bond formation. Besides, it was observed that this strategy is particularly effective under dilute conditions (5 mM). The use of 4-*O*-picoloyl glucosyl donors provides faster and increased 1,2-*cis* selectivity compared with those acquired at higher concentration.¹⁰³

1.3.2 1,2-*cis*-Directing protecting groups through conformational constraining effects

Recently, much attention has been paid to the conformationally constrained glycosyl donors in effective glycosylations. Since the first report¹¹⁰ of the deactivation effect of a cyclic acetal group on glycoside donors, conformationally restricted protecting groups have been largely applied¹⁰¹ to modulate the reactivity of donors, especially to enhance the stereoselectivity and enable orthogonal oligosaccharide syntheses that involve considerable synthetic challenge. Conformational constraining protecting groups, which are usually cyclic bifunctional protecting groups, can confine the flexibility of the sugar ring and lock the donors to certain conformations. When the donors are activated, glycosyl oxocarbenium ions are generated with face discrimination, which makes them principally accessible from one side.

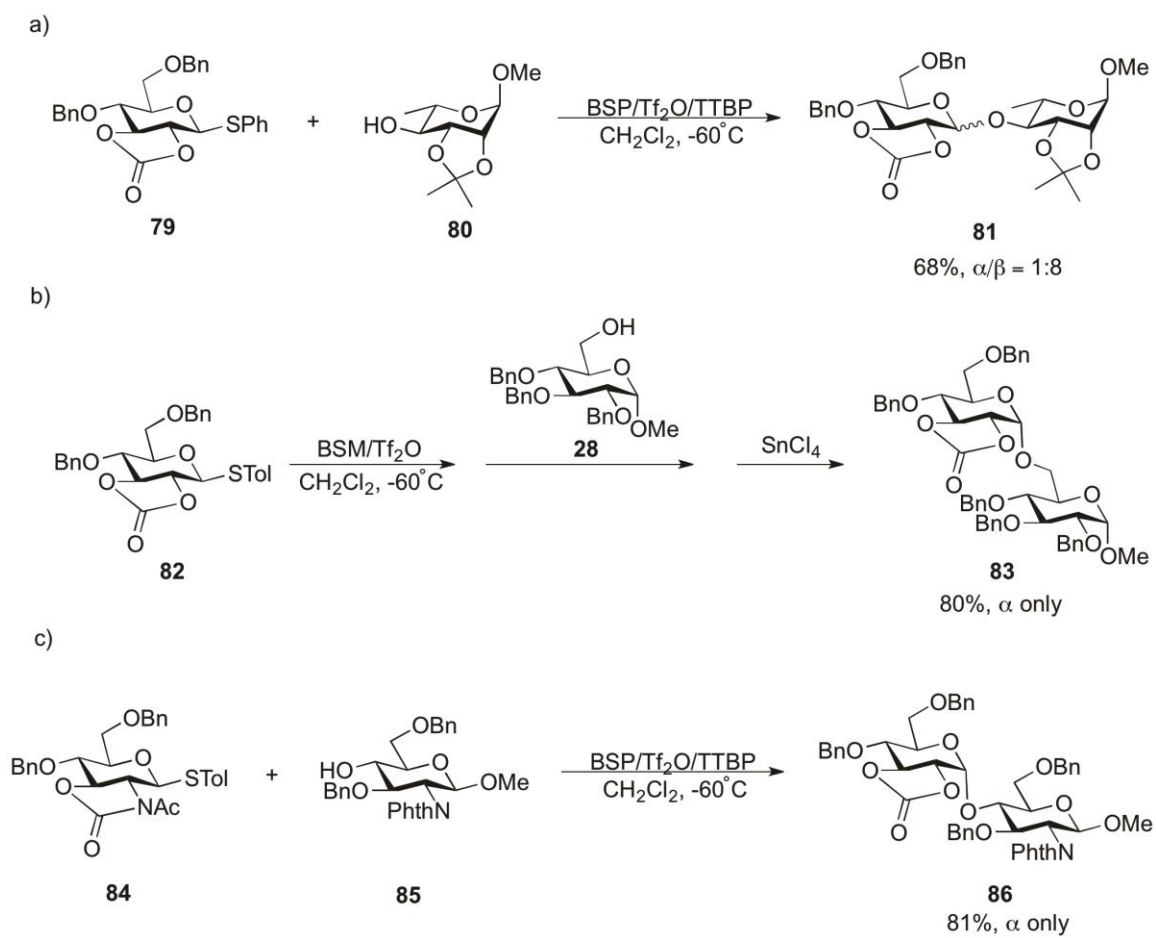
The employment of 4,6-*O*-benzylidene protecting group has been demonstrated as a successful strategy of β -selective mannosylations (Scheme 1.11a).^{111–114} Early examples involve using 4,6-*O*-benzylidene sulfoxide donors and thioglycoside donors to directly form β -mannosidic linkages. It was believed that the benzylidene protecting group stabilized the generated α -mannosyl triflate intermediates, in which the following



Scheme 1.11 Application of 4,6-*O*-benzylidene protecting group in 1,2-*cis*-glycosylation reactions.

additions had to be preferentially carried out from the β face.¹¹⁵ Subsequently, high β -selective mannosylations were practiced with other types of mannosyl donors possessing 4,6-*O*-benzylidene protections, such as TCAI, PTFA, phosphite, iodide and phthalate donors, and many of which were even activated by non-triflate promoters other than forming the mannosyl triflate intermediates.^{55, 116–119} Therefore, it has been rationalized that the high β -mannosylations are induced by a torsional effect imposed by the benzylidene group. The stereoselective glycosylation processes proceed via the formation of a conformationally constrained twist-boat structure intermediate, other than the α -mannosyl triflate intermediate. This proposal accounts for the great β -stereoselectivity of 4,6-*O*-benzylidene 2-deoxy-2-iodoglucosyl donors as well. However, it has to be added that other factors, including leaving groups, glycosyl acceptors, substituents at the 2-/3-position, and activation conditions, can all influence the stereoselectivity outcomes to different extents.^{120, 121}

Besides its applications in β -mannosylations, the 4,6-*O*-benzylidene protecting group was also used in other kinds of glycosyl donors to achieve α -glycosylations. For instance, 4,6-*O*-benzylidene-protected thioglucoside donor **78** was glycosylated with various acceptors to give α -glucosides with excellent selectivities (Scheme 1.11b).¹²² We know that during the glycosylation processes, numerous intermediates with different reactivities and stabilities are likely to exist, and many factors can determine the compositions and equilibrium proportions. In such circumstances, however, the conformational constraining effect imposed by the benzylidene group still contributes a major impact towards the glycosylation pathways, and could be responsible for the observed stereoselectivities.



Scheme 1.12 Application of carbonate and oxazolidinone protecting groups in 1,2-*cis*-glycosylation reactions.

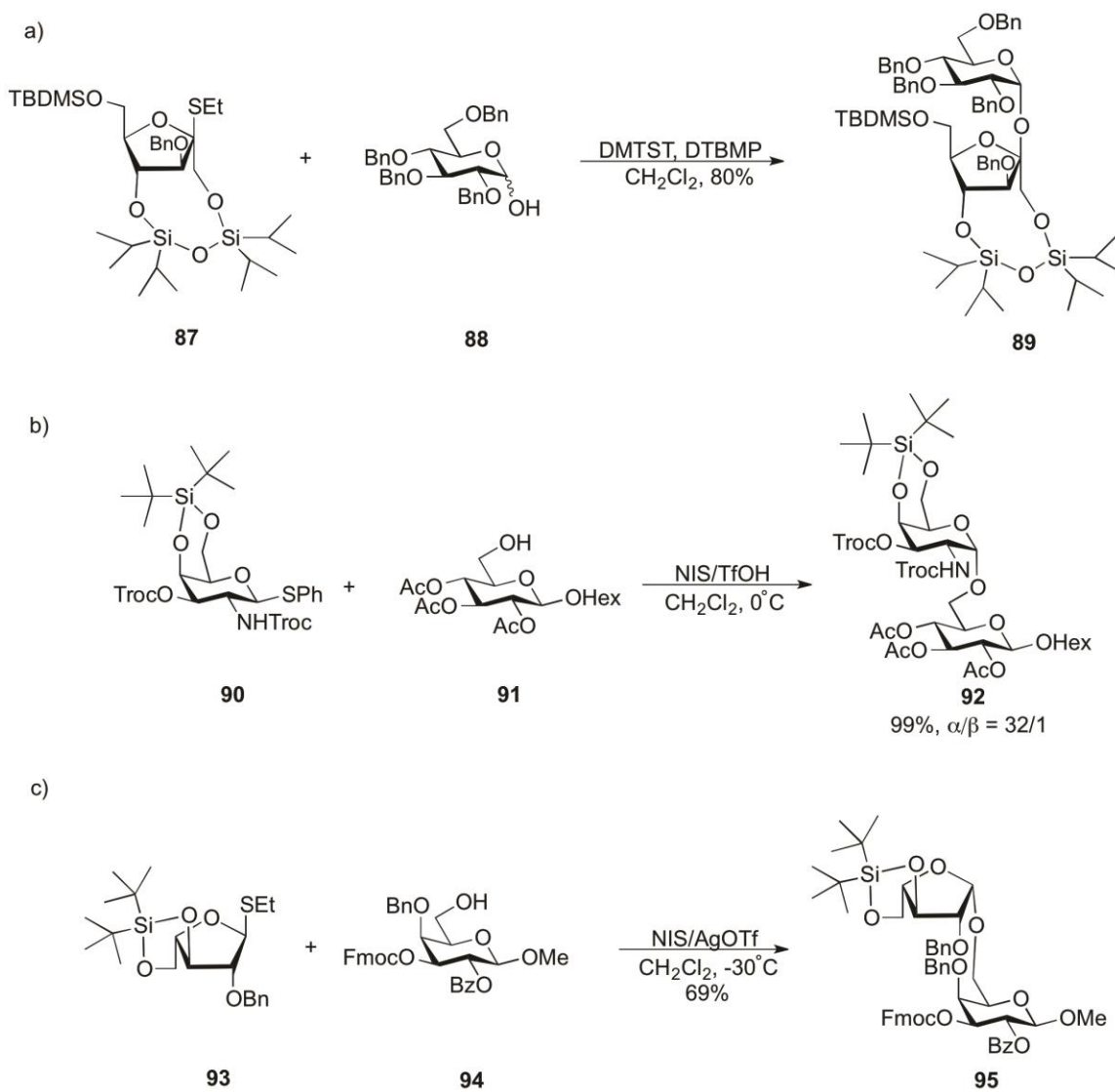
Inspired by the strong stereospecific effect of the 4,6-*O*-benzylidene group, the strategy of using a 2,3-*O*-carbonate group as a conformationally constraining protecting group has also been established.¹²³ In many cases, 2,3-*O*-carbonate thioglucosides and thiogalactosides have been shown to be good α -glycosylating agents, but these reactions always relied on specific activators and/or solvent systems.¹²⁴ Although neighboring-group participation is unavailable due to the incorporated carbonate ring, some kinds of 2,3-*O*-carbonate donors also present β -stereoselectivity and have been used in the synthesis of β -glycosides (Scheme 1.12a).¹²⁵ Fortunately, there is not a huge gap for the transition from a β - to an α -stereodirecting effect, and the corresponding switching method has been developed. With the assistance of either a catalytic or stoichiometric amount of Lewis acid, the β -stereospecificity of 4,6-di-*O*-benzyl-2,3-*O*-carbonate thioglucoside was inverted completely to α -selectivity (Scheme 1.12b).¹²⁶ Moreover, the carbonate group deactivates the glycosyl donors both electronically and conformationally. Thus, the disarming effect enables them to serve as acceptors at the same time in an orthogonal glycosylation synthesis, where chemoselective activations of other armed glycosyl donors can be performed.

The 2,3-*N,O*-oxazolidinone group has been used as an α -directing group in glycosylation reactions as well, specifically in the preparation of α -linked 2-amino-2-deoxyglycosides.¹²⁷ However, side reactions may occur sometimes, and the nitrogen atom on the oxazolidinone group has the propensity to undergo *N*-glycosylation or *N*-sulfenylation. Therefore, a revised method is to use *N*-acetylated oxazolidinone donors (Scheme 1.12c).¹²⁸ The stereospecificity of the donors in glycosylations can also be adjusted by tuning the reaction conditions such as relative reactivity, configuration of the

acceptors, and the activation systems. For example, the *N*-acetylated oxazolidinone donors were β -stereoselective when applying the NIS/AgOTf activation system; when an excess amount of AgOTf was used, *in situ* anomerization would occur to generate α products. Meanwhile, the torsional oxazolidinone ring played a key role, as an endocyclic C-1–O-5 bond cleavage pathway was found to be operative.¹²⁹ The employment of *N*-benzyl-2,3-oxazolidinone thioglycosides as glycosyl donors for α -glycosylations was reported recently, and satisfactory yields and selectivities were observed.¹³⁰

Silyl groups are among the most frequently used protecting groups in carbohydrate synthesis based on their high stability and of easy operation. However, it was until the recent applications of di-*tert*-butylsilylene (DTBS) protecting group in 1,2-*cis*-galactosylations that their stereodirecting ability had been widely recognized.^{131, 132} Since then, cyclic bifunctional silyl protecting glycosyl donors had been largely applied in the stereoselective syntheses of glycosides.¹³³ Compared with mono-substituted silyl protected donors, cyclic bifunctional silyl protecting groups exhibit stronger governance on the stereoselectivity of glycosylation through rigidifying the donor ring conformation and imposing steric hindrance during glycosylation. For example, the 1,1,3,3-tetraisopropylidisiloxane-protected thiofructofuranoside **87** was synthesized as a stereospecific donor (Scheme 1.13a).¹³⁴ The highly bulky silyl group blocks the α -face of the donor to guarantee a completely β -glycosylation with the glycosyl acceptor.

The employment of DTBS-protected donors is becoming a popular method for the construction of 1,2-*cis* glycosidic bonds, especially in the syntheses of α -galactosides. It has been demonstrated that DTBS-protected galactosyl donors are able to glycosylate various acceptors to get the corresponding α -galactosides exclusively. Even if



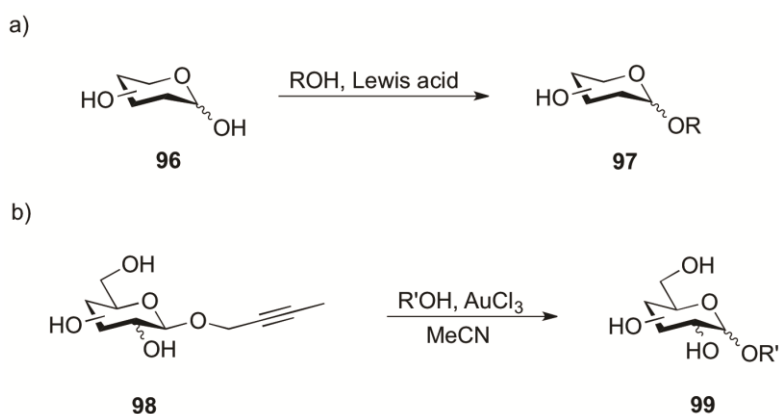
Scheme 1.13 Application of cyclic silyl protecting groups in directing 1,2-*cis*-glycosylation reactions.

neighboring participating groups such as benzoyl (Bz) or 2,2,2-trichlorethoxycarbonyl (Troc) were installed at C-2 (Scheme 1.13b), high α -selectivities were also observed, which revealed the convincing effect of DTBS group.^{22, 131, 132} A plausible mechanism has been proposed to account for the powerful α -directing effect of DTBS group. As for the galactopyranoside, it has been demonstrated that the axially oriented oxygen at C-4 contributes electrons to stabilize the generated oxocarbenium ion during the glycosylation process, which is called “through-space electron donation”.¹³⁵ This effect steadies the intermediate and prevents the neighboring groups with moderate nucleophilicity from participating. Meanwhile, the bulky *tert*-butylsilylene group entails a closer positioning to the anomeric center, and prevents the nucleophilic acceptors to add from the β -side.¹³³ Besides its applications in galactosylations, the DTBS group was also incorporated into arabinofuranoside to obtain a conformationally constrained donor to control the anomeric configuration. Glycosylations were performed to have expected β -selectivity with many acceptors, which claimed its potential in the construction of β -arabinoside domains from lipoarabinomannan and mycobacterial arabinogalactan (Scheme 1.13c).^{136, 137}

1.3.3 Protective group free glycosylations

Generally, a well-established glycosyl donor should contain the following properties, which are, (a) easy accessibility; (b) high generality for various alcohols and glycosyl acceptors; (c) applicability with alternative reaction conditions or catalysts; (d) high efficiency on both yield and stereoselectivity. Adopting unprotected glycosyl donors directly into the glycosylations is an appealing strategy. Unprotected glycosyl donors often hold higher reactivities, and their usage avoids the cumbersome protection and deprotection processes. Despite the tremendous growth of glycosylation strategies over

the past century, the original Fischer glycosidation method is still the most utilized method for glycosylations with simple alcohols. Fischer glycosidation refers to the reaction of a cyclic hemiacetal (a free sugar) with an alcohol to form a glycoside by the catalysis of an acid, which named after Emil Hermann Fischer who developed this method a century ago (Scheme 1.14a).¹³⁸ The Fischer glycosidation involves an equilibrium process, and usually it is shifted by the treatment of an excess amount of alcohol, acid catalyst, high temperature and long reaction times. This strategy often provides a mixture of stereoisomeric products, of which the alpha anomer is normally the major product, as it is the most thermodynamically stable product owing to the anomeric effect. Up to now, the traditional Fischer glycosidation has been enhanced by exploiting different kinds of acid catalysts¹³⁹ and reaction conditions, such as microwave irradiation¹⁴⁰ and ultrasonication.¹⁴¹ However, since free carbohydrates have limited solubility in alcohols, it usually requires harsh conditions (e.g., high temperature, microwave, ultrasonication) to push the reaction, which results in decomposition of the products, encroachment of side products, and tedious separation processes.



Scheme 1.14 Glycosylation by unprotected glycosides.

Some kinds of unprotected glycosyl donors, such as glycosyl trichloroacetimidates and 3-methoxy-2-pyridyloxy (MOP) glycosyl donors, were applied into α -stereodirecting glycosylations.¹⁴² However, drawbacks including low stability and limited generality, impair their versatility. Later, advanced unprotected glycosyl donors were prepared to rise to the challenges. The glycosylations using unprotected alkynyl glycosyl donors under the activation of gold(III) have been reported (Scheme 1.14b).¹⁴³ Several terminal alkynyl glycosides were glycosylated with various primary alcohols in excess amount under reflux in acetonitrile and 5% AuCl₃. The 2-butylnyl glycosides were most reactive, giving good yields with moderate to high α -selectivities. The secondary alcohols were also tested but showed reduced efficiency.

A recent study investigated the photoinduced *O*-glycosylation of unprotected deoxythioglycosides (Scheme 1.6b). It was revealed that glycosylations could be performed with alcohols in the presence of DDQ and long-wavelength UV irradiation to give respectable yields and α -selectivities. It has also been demonstrated that *p*-methoxyboronic acid could temporarily protect the 1,3-diols (C-4 and C-6 positions) to prevent self-coupling of the donor or formation of a 1,6-anhydroglycoside.⁸⁶

1.4. Conclusions and Outlook

The advances of chemical glycosylation strategies summarized herein have significantly enriched our tactics to build various glycosidic linkages and access complex oligosaccharides and glycoconjugates of great biological importance. Nevertheless, it is still too early to claim that all roughness associated with glycosidic bond formation has been smoothed. Carbohydrates are amongst the most complicated biomolecule systems in nature, and a variety of aspects, including the glycosyl donor type, protecting groups,

activator/catalyst, and reaction conditions, are influential or decisive to the glycosylation outcomes. Even for the construction of a simple oligosaccharide molecule, a carefully designed synthetic route and a meticulous experimental operation are often required to obtain the target molecule in high yield and selectivity. Therefore, development of general, efficient, and flexible strategies to achieve the intrinsic organizational diversity of carbohydrates is still imperative.

How do we reach this ultimate goal? From my perspective, a continuous screening of alternative glycosyl donors and protecting groups may not spark fundamental advancement of the current approaches for glycosylations. Instead, more concerns should be put into the discovery of glycosylation mechanistic principles and the driving forces behind these reactions. There has been proposed many sorts of glycosylation pathways and intermediates, such as generations of oxacarbenium triflate ion-pair and solvent separated ion-pair, conformation-dependent reactivity, H-bonding stereodirecting effects, and so forth, but none of these can be completely demonstrated. A comprehensive understanding of glycosylation mechanisms will be beneficial for further advances in glycosylations and to generate innovations, such as the development of “enzyme-like catalysts”. The enzyme-like catalyst will not only have comparable properties as enzymes such as transglycosidases or glycosyl transferases with high selectivities and a minimum of protections, but these reagents also exhibit superior glycosylation performance with respect to stability and efficiency, and availability in industrial production. It could specifically detect the target glycosides, and provide the platform and the activation required. It is inspiring that some preliminary attempts have been performed to obtain glycosides in great regio- and stereoselectivity.^{144, 145}

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**Chapter 2 1,2-*cis* Alkyl Glycosides: Straightforward
Glycosylation from Unprotected 1-Thioglycosyl Donors**

Abstract

A 1,2-*cis*-alkyl glycosidation protocol that makes use of unprotected phenyl 1-thioglycosyl donors is reported. Glycosylation of various functionalized alcohols was accomplished in moderate to high yield and selectivity to give the 1,2-*cis*-glycosides. In order to quickly develop optimum glycosylation conditions, an FIA (flow injection analysis)–ESI-TOF-MS method was developed that enabled rapid and quantitative evaluation of yield on small scale. This methodology, coupled with NMR spectroscopy, allowed for rapid evaluation of the overall reactions.

2.1 Background

As essential components in the cell membrane, carbohydrates and glycoconjugates serve many protective, stabilizing, organizational, barrier, and recognition functions.¹ The chemical synthesis of these glycoconjugates, including proteoglycans, glycolipids, and glycoproteins, is in great demand for biological studies of their functions as cell-wall components that are collectively termed the glycocalyx. Anomerically pure alkyl glycosides serving as fundamental building blocks are in demand to achieve the stereoselective synthesis of these cell-wall structures. Some alkyl glycosides, such as propargyl² and allyl³ glycosides are simple approaches for the construction of microarrays^{4, 5} and glycodendrimers.⁶

Generally, 1,2-*trans*-alkyl glycosidation can be reliably achieved via neighboring-group participation of a C-2 acyl group on a glycosyl donor, while stereochemical control for 1,2-*cis*-alkyl glycosidation can be challenging.⁷ The conventional Fischer glycosidation reaction, a straightforward way to afford short-chain, uncomplicated, thermodynamically favored 1,2-*cis* alkyl glycosides, has been improved by using various acid catalysts,^{8–10} microwave irradiation,¹¹ ultrasonication,¹² and ionic liquids.¹³ Since free sugars have limited solubility in longer-chain alcohols (acceptors), harsh conditions (e.g., high temperature, microwave, ultrasonication) are often required to push the reaction, which results in decomposition of the products,⁸ formation of various side products, time-consuming separation processes, and low yields and poor stereoselectivities. Ether protecting groups, most often the benzyl group, are routinely used for protecting free hydroxyl groups in the synthesis of 1,2-*cis*-glycosides,¹⁴ but benzyl deprotection by H₂/Pd will destroy a number of groups (alkene, alkyne, nitro,

halogen) on functionalized alkyl glycosides.¹⁵ The elegant intramolecular aglycon delivery (IAD) approach offers a stereospecific 1,2-*cis*-glycosidic synthesis, albeit from selectively protected intermediates.^{16–19}

In principle, many of these issues can be circumvented through conversion of an unprotected glycosyl donor directly into the desired 1,2-*cis* alkyl glycosides. Glycosylation by an unprotected sugar donor has several practical values:²⁰ the often tedious protection and deprotection process can be avoided;²¹ unprotected donors possess higher reactivity compared to *O*-acyl-protected donors, and the better solubility of unprotected donors in short-chain alcohols (acceptors) enables glycosylation at lower temperatures, which reduces the formation of by-products.

Mamidyala and Finn have reported glycosylation using unprotected alkynyl donors and AuCl₃ as an effective activator.^{22, 23} Very recently, Nitz and co-workers reported glycosidation in relatively good yields using a protecting-group-free protocol with 1-*p*-toluenesulfonyl hydrazide and glycosyl chloride donors; however, anomeric selectivities were generally lacking.²⁴ Among the various classes of glycosyl donors, phenyl 1-thioglycosyl compounds have been regarded as ideal choices for donor precursors (including precursors for light-induced glycosidation²¹) because they are stable, easily synthesized, and for the most part, crystalline.²⁵ Herein, I report a 1,2-*cis*-alkyl glycosidation protocol that makes use of unprotected phenyl 1-thioglycosyl donors.²¹

2.2 Glycosidations

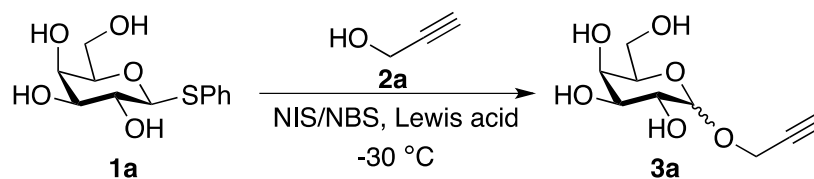
Phenyl 1-thio- β -D-galactopyranoside (**1a**)²⁶ and propargyl alcohol (**2a**) were selected as the unprotected glycosyl donor and acceptor, respectively, for the model

glycosylation reaction (Table 2.1). Initially, the reaction was carried out between **1a** and dry **2a** (40 equiv) under the activation of *N*-iodosuccinimide (NIS)/trimethylsilyl triflate (TMSOTf). The desired product was obtained in respectable yield and stereoselectivity (Table 2.1, entry 1). TLC analysis of the crude product showed only the desired α,β isomers. Further experiments revealed other Lewis acids, $\text{BF}_3\cdot\text{OEt}_2$ ⁷ and TfOH ²⁷, that used for 1,2-*cis*-glycosidations, provided similar yields and stereoselectivities, while $\text{H}_2\text{SO}_4\cdot\text{SiO}_2$ ⁹ gave a lower yield (a result also reported from another laboratory²⁸) but higher stereoselectivity (entries 2–4). I surmise that the results may be due to the heterogeneity of the $\text{H}_2\text{SO}_4\cdot\text{SiO}_2$ catalyst. The bisulfate counterion would be trapped in the silica gel matrix, leading to the formation of a loosely solvent-separated ion pair (SSIP) between the bisulfate counterion and the oxocarbenium ion, suggesting a unimolecular ($\text{S}_{\text{N}}1$) favored transition state and better α selectivity due to the anomeric effect.^{29–31} I also examined the activation by Lewis acids and *N*-bromosuccinimide (NBS). As anticipated, relatively lower yields and stereoselectivities were observed (entries 5 and 6), which could be attributed to the diminished electrophilic properties of the bromonium ion. Moreover, experimentation showed that glycosylation was most favored when the amount of alcohol was in the range of 40–60 equiv (entries 1–3 and 9 vs. entries 7–8 and 10). Experiments further demonstrated neither TMSOTf nor NIS alone was able to trigger the glycosylation reaction (entries 11 and 12).

2.3 Rapid High-Throughput Screening of Reactions

In order to rapidly evaluate and optimize glycosylation conditions for a number of reactions, I adapted the concept of high-throughput screening using mass spectrometry similar to that reported by Ito and co-workers, who employed MALDI-TOF-MS.^{32, 33}

Table 2.1 Optimization of reaction conditions^a

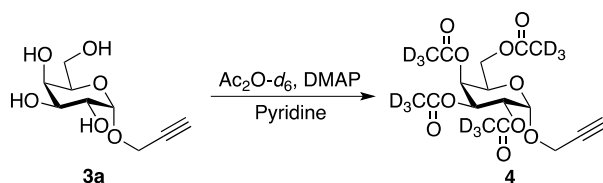


Entry	Lewis acid	Halogen source	Propargyl alcohol (mol equiv)	Yield (%) ^b	$\alpha:\beta$ ^c
1	TMSOTf	NIS	40	75	10:1
2	BF ₃ ·OEt ₂	NIS	40	74	10:1
3	TfOH	NIS	40	76	9:1
4	H ₂ SO ₄ ·SiO ₂	NIS	40	52	15:1
5	TMSOTf	NBS	40	59	5:1
6	BF ₃ ·OEt ₂	NBS	40	38	5:1
7	TMOSTf	NIS	20	50	7:1
8	TMSOTf	NIS	30	53	10:1
9	TMSOTf	NIS	60	76	10:1
10	TMSOTf	NIS	100	58	11:1
11	TMSOTf	None	40	0	-
12	None	NIS	40	0	-

^aThe reaction was conducted using 0.37 mmol of **1a**, 1.03 mmol of NIS/NBS, and 0.07 mmol of Lewis acid. ^bThe yield was determined after acetylation by FIA–ESI–TOF–MS. ^cThe anomeric ratio was determined by integration of H-1 in the ¹H NMR spectrum of the crude product.

In the reactions with small molecules, I used a coupled flow-injection system with ESI-TOF-MS (FIA–ESI-TOF-MS) that enabled quantitative evaluation of glycosylation yield with products of MW <500 amu. Furthermore, the method provided a more accurate estimation of yield in two ways: (1) An average value from a certain volume of sample (e.g., 2.5 μ L with our flow-injection equipment) was evaluated rather than a tiny spot excited by the laser on MALDI. (2) Integration of the ion intensity peaks was used to calculate yield instead of the m/z peak height as in the MALDI method.^{32, 33}

In order to provide an internal standard for the FIA–ESI-TOF-MS studies, propargyl α -D-galactopyranoside (**3a**, Scheme 2.1) was acetylated with $\text{Ac}_2\text{O}-d_6$ to afford the per-deuterated glycoside **4**. While it is known that the ionizing properties of deuterated and nondeuterated glycosides are nearly identical,³² the fact was confirmed in this study specifically for these compounds. The FIA–ESI-TOF-MS responses were found essentially the same for either the ^1H - or ^2H -labeled compounds, thus facilitating a relatively uncomplicated rapid analysis of the reactions.



Scheme 2.1 Synthesis of deuterated glycoside substrate as the internal standard.

2.3.1 Flow-injection–ESI-TOF-MS (FIA–ESI-TOF-MS) instrumentation

The QStar Elite mass spectrometer was outfitted with a flow-injection analysis (FIA) device to provide rapid and effective injection of multiple samples for ESI-TOF-MS analysis. A picture of the FIA equipment is shown in Figure 2.1.

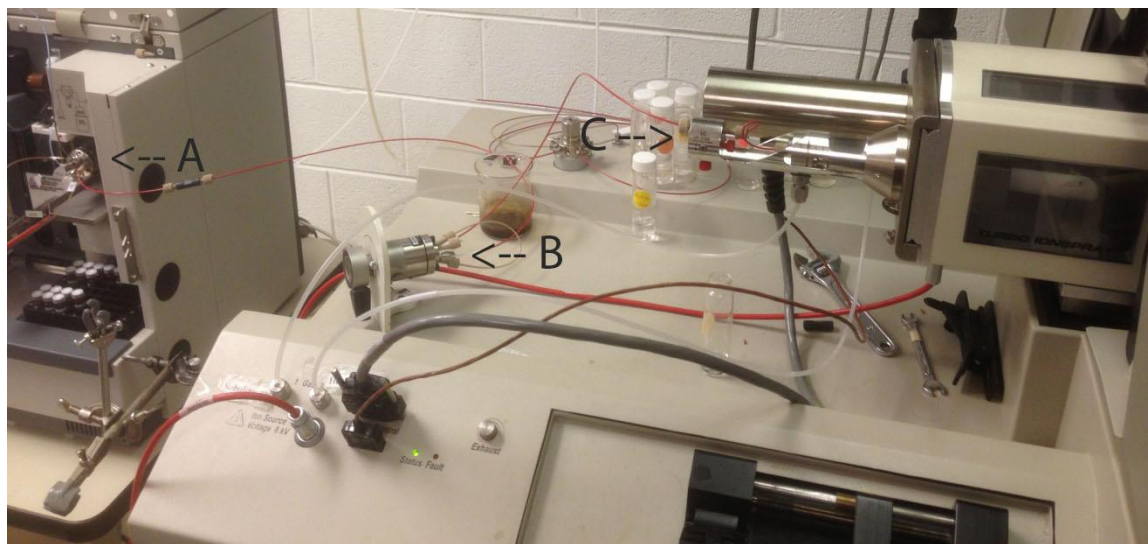


Figure 2.1 Flow-injection device outfitted on QStar Elite TOF-MS instrument. A: Dionex Ultimate fully integrated micro-, capillary-, and nano-HPLC system (Sunnyvale, CA, USA), B: 6-Port manual injection valve (Rheodyne, Rohnert Park, CA), and C: AB Sciex QSTAR Elite triple quadrupole time-of-flight (QTOF) mass spectrometer (Concord, Ontario, Canada) with an ESI ion source.

FIA–ESI-TOF-MS conditions

- FIA flow
 - Solvent: MeOH
 - Flow rate: 120 $\mu\text{L}/\text{min}$
- Manual injection
 - Sample concentration: 10 $\mu\text{g}/\text{mL}$; 25 μL diluted to 1.00 mL with MeOH for injection
 - Loop volume: 2.5 μL
 - Syringe size: 250 μL
 - Injections at 2-min intervals; 10 times each

2.3.2 High-throughput screening/reaction optimization by flow-injection analysis–ESI-TOF-MS (FIA–ESI-TOF-MS)

Before the screening for glycosylation conditions, I carefully examined whether the ionizing properties of deuterated and nondeuterated glycoside isomers are identical. To establish a quantitative calibration curve, deuterium-labeled glycoside solution [10 $\mu\text{g}/\text{mL}$, propargyl 2,3,4,6-tetra-*O*-(acetyl- d_3)- α -D-galactopyranoside (**4**)] and non-labeled glycoside solution [propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4a**)] were prepared separately, mixed in five concentration ratios, and diluted to the same volume by MeOH to afford five standards (Table 2.2). A six-port manual injection valve was installed between the LC pump and the ESI-QTOF instrument. MeOH was chosen as the FIA solvent. Each sample was injected into the six-port manual injection valve with a 2.5- μL sample loop and analyzed by ESI-QTOF-MS for 10 replicates. Non-labeled glycoside ($[\text{M}_\text{H}+\text{Na}]^+$ ion) and labeled ($[\text{M}_\text{D}+\text{Na}]^+$ ion) were observed at m/z 409 and m/z

Table 2.2 Comparison of $\frac{[C_H]}{[C_D]}$ ^a and $\frac{PA_H}{PA_D}$ ^b

entry	$\frac{[C_H]}{[C_D]}$ ^a	$\frac{[PA_H]}{[PA_D]}$ ^b	Standard deviation
1	0.2	0.2061	0.0095
2	0.4	0.3955	0.0094
3	1	0.9946	0.0158
4	2.5	2.5619	0.0354
5	5	5.2201	0.4221

^a Concentration ratios of non-labeled standards/labeled standards. ^b Peak area ratios using isotope-diluted FIA–ESI-TOF-MS.

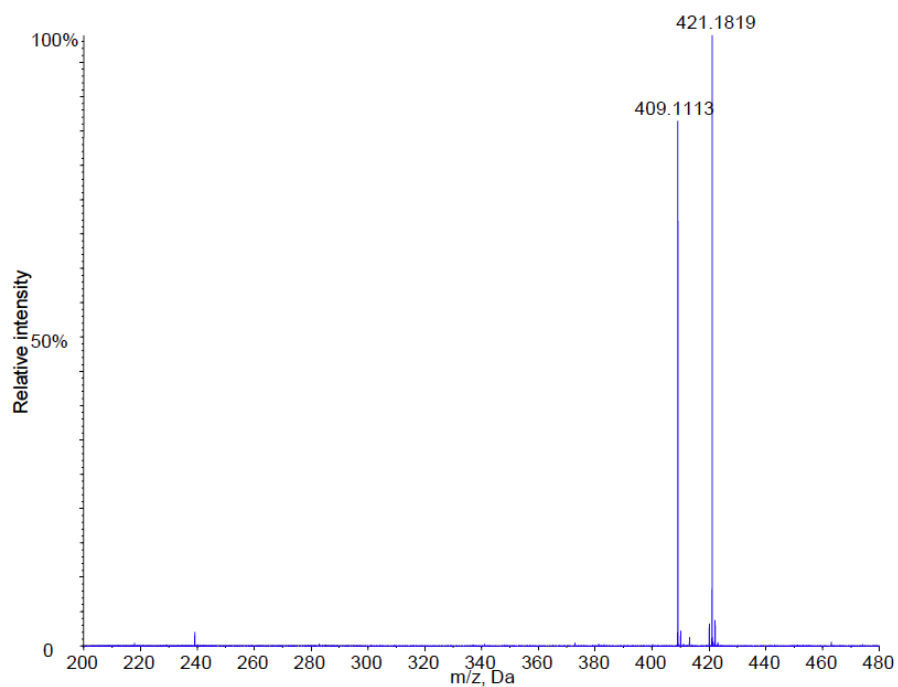


Figure 2.2 ESI-TOF-MS of product **4a** ($[M_H+Na]^+$) ion at m/z 409) and internal standard (deuterated) **4** ($[M_D+Na]^+$) ion at m/z 421).

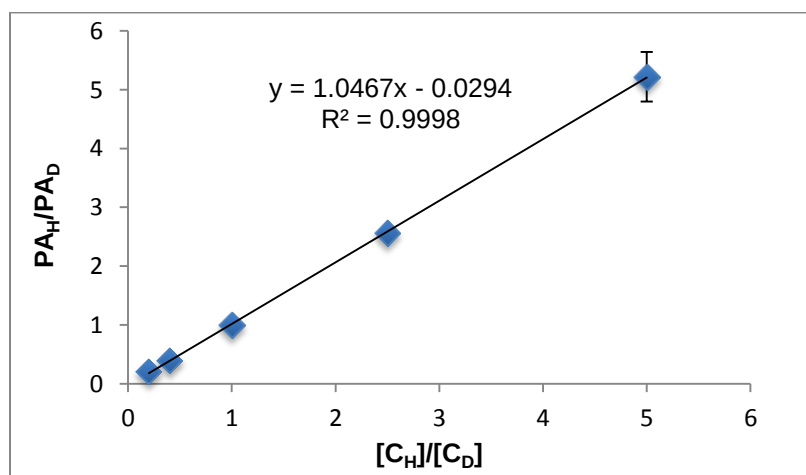


Figure 2.3 Relationship between concentration ratios of standards ($\frac{[C_H]}{[C_D]}$) and peak area ratios ($\frac{PA_H}{PA_D}$).

421, respectively (Figure 2.2). Integration of peak areas under extracted peak profiles at m/z 409 and m/z 421 were performed, and peak area ratios were calculated for each entry. The results indicated that the ionizing properties of the deuterated and nondeuterated glycoside isomers are almost identical, which enables quantification of the nondeuterated glycoside when deuterated glycoside is added as an internal standard (Figure 2.3).

For a general analytical method, the internal standard was dissolved in CH_2Cl_2 to afford a 10 $\mu\text{g/mL}$ solution. The crude product (non-labeled glycoside) was also dissolved in CH_2Cl_2 to get an estimated 10 $\mu\text{g/mL}$ solution. Samples (20 μL) of both labeled and non-labeled solutions were mixed and diluted with MeOH to get a 1 mL of solution. The solution was injected into the six-port injection valve, and the injection was repeated 10 times at 2-min intervals. The peaks of labeled ($[\text{M}_\text{D}+\text{Na}]^+$ ion at m/z 421) and non-labeled ($[\text{M}_\text{H}+\text{Na}]^+$ ion at m/z 409) glycoside were observed (Figure 2.2). After analyzing the ratio of the two peak areas, the concentration of the non-labeled glycoside solution was obtained.

2.3.3 Optimization studies and scope of the reaction

Optimization studies were conducted as in the following paragraphs in which several solvents and solvent mixtures were examined. The conditions were those of Table 2.1, with variations. The effect of *N,N*-dimethylformamide (DMF) in the solvent on stereoselective α -glycosylation has been well documented,³⁴ and I anticipated that adding a catalytic amount of DMF might promote the formation of the 1,2-*cis*-glycosidic bond. After screening with added DMF, other solvents, including CH_2Cl_2 , THF and Et_2O (0.2 equiv), were examined; however, no obvious improvement in yield or α selectivity was observed with any of these additives, and a further increase of the amount of solvent

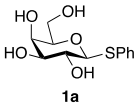
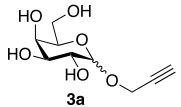
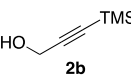
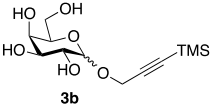
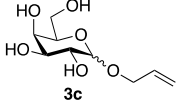
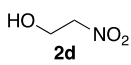
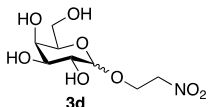
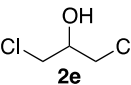
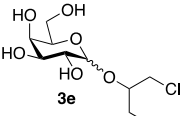
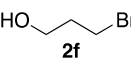
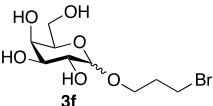
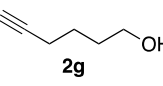
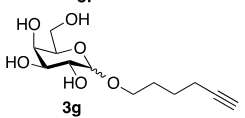
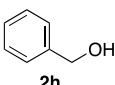
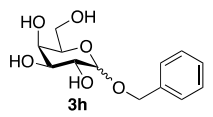
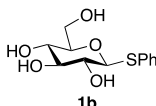
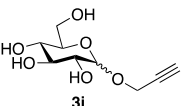
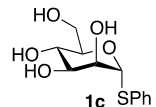
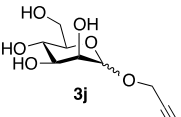
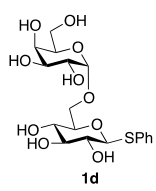
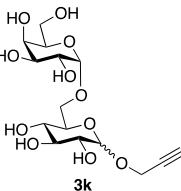
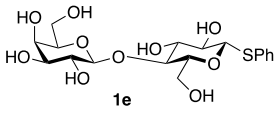
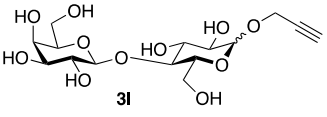
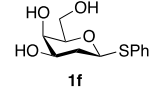
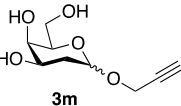
added (6 equiv) led to a decrease in stereoselectivity and yield, which indicates that a neat alcohol environment is essential for optimum glycosylation under these conditions.

With the appropriate conditions for 1,2-*cis*-alkyl glycosidation in hand, I investigated the scope of the reaction with several unprotected glycosyl donors and alcohols bearing various functional groups. (See Table 2.3.) The stereoselectivity of glycosylation with unprotected D-glucose, D-mannose and disaccharide donors and propargyl alcohol spanned from modest to high (Table 2.3, entries 1, 9–12). It is noteworthy that the major product from phenyl 1-thio- α -D-mannoside is the β (*cis*) anomer (Table 2.3, entry 10) that is formed. Glycosylation with various functionalized alcohols was accomplished without difficulty (Table 2.3, entries 2–8). A variety of groups on alcohols were tolerated to provide the corresponding 1,2-*cis*-substituted-alkyl glycosides. These results indicate the generality and applicability of the present glycosylation method.

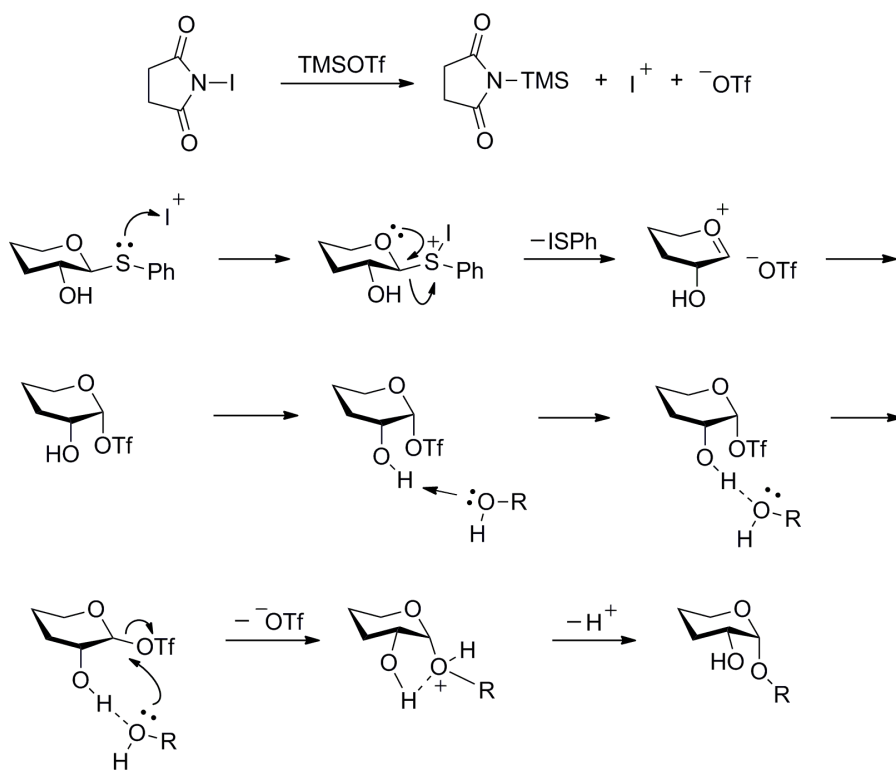
2.4 Proposed Mechanism

A mechanistic explanation of the observed results from these glycosylations is no doubt complex, as numerous alcohol–substrate–Lewis acid reagent associations (including H-bonding interactions) are possible and difficult to sort out. I presume the role of NIS/TMSOTf is critical for the activation of related systems.^{35, 36} Perhaps noteworthy is the fact (Table 2.3, entry 13) that a 2-deoxy-1-thioglycoside (**1f**), an analog of **1a**, gives a significantly diminished α selectivity of only 1.7:1, possibly indicating a special role for the 2-OH group that might coordinate with the reagent alcohol and account for the generally higher *cis*-selectivities in the other examples. The role of such H-bonding in stereoselection in glycosylation has been addressed in many articles.^{37–41}

Table 2.3 Scope of the reaction^a

Entry	Donor	Acceptor	Product	% Yield ^b	$\alpha:\beta^c$
1	 1a	 2a	 3a	75	10:1
2 ^d	1a	 2b	 3b	62	10:1
3	1a	 2c	 3c	71	7:1
4	1a	 2d	 3d	93 ^e	5:1 ^f
5 ^g	1a	 2e	 3e	85	5:1
6 ^d	1a	 2f	 3f	56	3:1
7 ^d	1a	 2g	 3g	42	> 20:1
8 ^d	1a	 2h	 3h	72	3:1
9	 1b	2a	 3i	79	7:1
10	 1c	2a	 3j	81	1:2
11	 1d	2a	 3k	69	8:1
12	 1e	2a	 3l	57	12:1
13	 1f	2a	 3m	67	1.7:1

^a For details of the synthetic procedures, see the Experimental Section. ^b Isolated yield after acetylation of the products to give **4a–4m** except for **3d**. ^c The anomeric ratio was determined by integrating the ¹H NMR spectrum of the crude product. ^d The reaction was performed at –10 °C. ^e Isolated yield without acetylation. ^f The anomeric ratio was determined from isolated products. ^g The reaction was performed at 0 °C.



Scheme 2.2 Plausible mechanism.

A mechanism for the 1,2-*cis*-glycosylation (Scheme 2.2) is proposed based on the analysis above. Upon the activation of the iodonium ion²⁸ generated by NIS/TMSOTf, the phenyl 1-thioglycoside is converted to an oxocarbenium ion, which traps the triflate counterion to form an oxocarbenium–triflate ion pair.²⁹ The generally good solubility of the unprotected donors in alcohols (acceptors) indicates H-bond formation, one of which will coordinate the 2-OH with the oxygen of the alcohol, increasing its nucleophilicity³⁰ and steering the nucleophilic attack for *cis* selectivity via a five-membered ring transition state. A final deprotonation step achieves the 1,2-*cis* glycosides, which is in accordance with the fact that only catalytic amount of Lewis acid is needed.

2.5 Experimental Section

2.5.1 General methods

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Alcohols that were opened and stored for a period of time were pre-dried by Drierite[®] (anhyd calcium sulfate). Reagent grade dichloromethane (CH₂Cl₂), diethyl ether (Et₂O), tetrahydrofuran (THF), methanol (MeOH), *N,N*-dimethylformamide (DMF) and toluene were obtained from the Pure-Solv (Innovation Technologies) solvent system that uses alumina columns except for DMF, which was dried over a column of 5 Å molecular sieves. Pyridine was distilled over CaH₂ prior to use. All reactions were performed under anhydrous conditions unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on silica gel precoated aluminum plates. Zones were detected by UV irradiation using a 254 nm lamp and/or by heat/charring with *p*-anisaldehyde–sulfuric acid development reagent.⁴² Column chromatography was performed on silica gel (40–63 μm). Optical rotation values were

obtained at the sodium D line using a Perkin–Elmer 241 polarimeter. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature with a Varian Inova 500 MHz instrument. Chemical shifts are reported in δ -units (ppm) relative to the residual ^1H CDCl_3 at δ 7.26 ppm and ^{13}C at δ 77.16 ppm. All two-dimensional experiments (gCOSY, gHSQC and gHMBC) were recorded on the same instrument using Varian protocols. Mass spectrometric analysis was performed on a QSTAR Elite quadrupole time-of-flight (QTOF) mass spectrometer with an ESI source.

2.5.2 General synthetic procedures

(1). Synthesis of the phenyl thioglycoside donors **1a–1f**²⁶

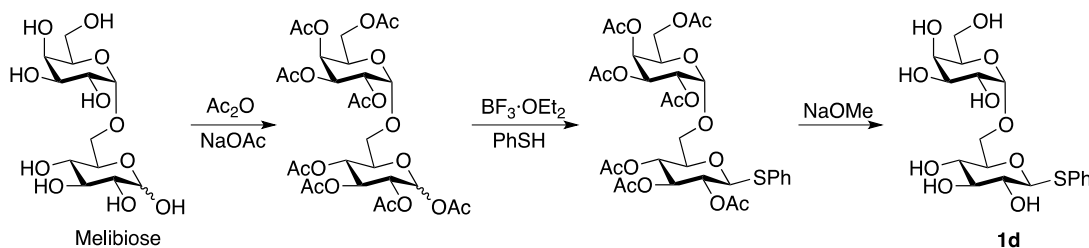
The selected free sugar (5.0 g, 27.8 mmol for D-galactose, 1.0 g, 5.6 mmol for D-glucose, D-mannose and 2-deoxy-D-galactose, and 1.0 g, 2.9 mmol for disaccharide) was suspended in a mixture of NaOAc (2.50 g, 30.5 mmol for D-galactose, 0.50 g, 6.1 mmol for other sugars) and Ac_2O (25 mL, 262.3 mmol for D-galactose, 5 mL, 52.5 mmol for other sugars), and the mixture was heated under an N_2 atmosphere to 70 °C. After 24 h, the yellow solution was cooled to room temperature, poured onto ice and quenched with satd aq NaHCO_3 . The aqueous phase was extracted with CH_2Cl_2 (3 $\times$ 50 mL). The organic extract was washed successively with water and brine, dried over anhyd Na_2SO_4 , and concentrated to afford the per-acetylated sugar as a solid that was used directly without further purification.

Thiophenol (3.60 mL, 35.2 mmol for the per-acetylated D-galactose; amounts for the other sugars were adjusted correspondingly) was then added to a solution of per-acetylated sugar (10.6 g, 27.1 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, and the mixture were stirred for 30 min. Then $\text{BF}_3\cdot\text{Et}_2\text{O}$ (10.3 mL, 81.3 mmol) was slowly injected into

the mixture, which was allowed to warm to room temperature. After 5 h, the mixture was diluted by CH₂Cl₂, washed with satd aq NaHCO₃ and brine, dried over anhyd Na₂SO₄, concentrated in vacuo, and purified by column chromatography (hexanes–EtOAc 5:1, hexanes–EtOAc 2.5:1 for the disaccharides) to afford the per-acetylated phenyl thioglycoside as a colorless syrup.

The per-acetylated phenyl thioglycoside (22.5 mmol for per-acetylated phenyl 1-thio-D-galactoside, amounts for other sugars were reduced correspondingly) was then dissolved in dry MeOH (20 mL), followed by the addition of a small amount of NaOMe to afford pH 9. After 2 h the solution was quenched by the addition of Amberlite[®] IR 120 (H⁺) resin. The resin was filtered off, and the solvent was removed in vacuo to afford the unprotected phenyl thioglycoside donor as a white powdery solid.

Literature reports for the phenyl thioglycosides **1a–c** and **1e** are as follows: phenyl 1-thio-β-D-galactopyranoside (**1a**),²⁶ phenyl 1-thio-β-D-glucopyranoside (**1b**),⁴³ phenyl 1-thio-α-D-mannopyranoside (**1c**),⁴⁴ and phenyl β-D-galactopyranosyl-(1,4)-1-thio-β-D-glucopyranoside (**1e**).⁴⁵ Phenyl α-D-galactopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (**1dβ**), a new compound, was prepared as in the foregoing paragraphs and is characterized NMR spectroscopy and high-resolution MS in the following paragraph.



Scheme 2.3 Synthesis of phenyl α-D-galactopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (**1dβ**).

^1H NMR (500 MHz, CD_3OD): δ 7.56–7.54 (m, 2H), 7.36–7.33 (m, 2H), 7.29–7.26 (m, 1H), 4.89 (d, J = 3.5 Hz, 1H, H-1'), 4.70 (d, J = 9.8 Hz, 1H, H-1), 3.92 (dd, J = 10.8, 6.1 Hz, 1H), 3.88 (ddd, J = 6.6, 5.6, 1.4 Hz, 1H), 3.83 (m, 1H), 3.80–3.73 (m, 3H), 3.70 (m, 2H), 3.59–3.56 (m, 1H), 3.43 (t, J = 8.8 Hz, 1H), 3.37 (m, 1H), 3.27 (dd, J = 9.8, 8.6 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 135.29, 132.15, 130.01, 128.26, 100.10, 89.04, 80.29, 79.65, 73.92, 72.11, 71.53, 71.49, 71.12, 70.38, 67.94, 62.84. HRESIMS: (m/z) ($\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{18}\text{Na}^+$ 457.1144; found 457.1146.

(2). Glycosylation to give glycosides 3a–3m.

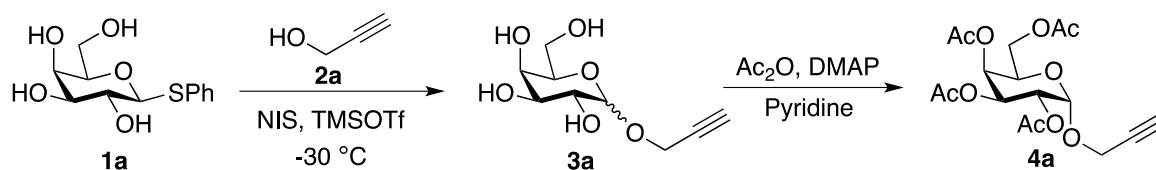
Phenyl 1-thiogalactoside donor **1a** (100 mg, 0.37 mmol) was dissolved in propargyl alcohol (**2a**, 0.87 mL, 0.82 g, 14.71 mmol), followed by the addition of pre-activated powdered 4 Å molecular sieves (150 mg), and stirring was continued for 1 h under nitrogen at room temperature. Then the mixture was cooled to $-30\text{ }^\circ\text{C}$, and NIS (232 mg, 1.03 mmol) and TMSOTf (13.3 μL , 0.074 mmol) were added, which made a black (maroon with most other alcohols) solution. After 2 h, satd aq $\text{Na}_2\text{S}_2\text{O}_3$ was added to quench the reaction, and the dark color faded. The mixture was then filtered through Celite[®], and the solution was concentrated in vacuo to give crude **3a**. Other alcohols were reacted in a similar manner to give glycosides **3b–3m**.

(3). Acetylation of alkyl glycosides to give per-acetylated glycosides 4a–4c and 4e–4m

The residue from the foregoing step (**3a**) was dissolved in dry pyridine (10 mL), and 4-(dimethylamino)pyridine (DMAP, catalytic amt.) and Ac_2O (1 mL, 10.6 mmol) were added with stirring overnight at room temperature. After concentrating the mixture, the residue was partitioned between EtOAc and water, and the organic layer was washed

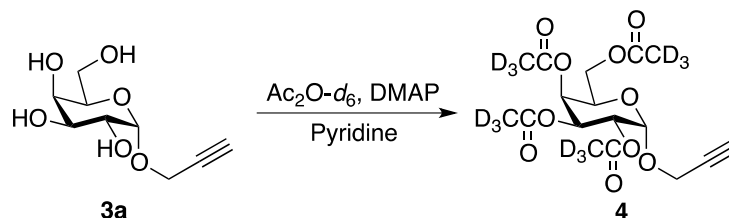
with satd aq NaHCO₃ and brine, dried over anhyd Na₂SO₄, and concentrated in vacuo to afford the crude product **4a**. In a similar manner compounds **4b–4c**, **4e–4m** were prepared.

2.5.3 Experimental data for compounds 3d, 4, 4a–4c and 4e–4m



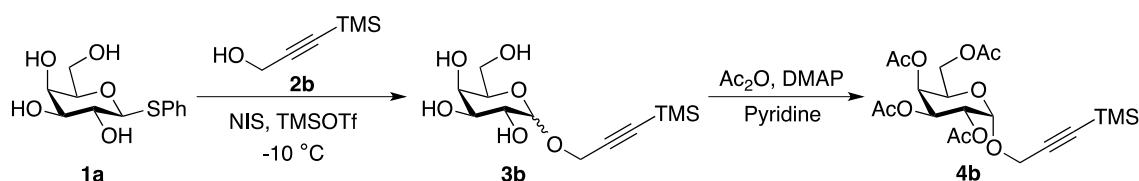
Scheme 2.4 Synthesis of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4a**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4:1, hexanes–EtOAc) to give **4a** (106.7 mg, 75.1%, $\alpha/\beta = 10:1$) as a mixture of isomers. **4aa**: R_f 0.23 (2.5:1, hexanes–EtOAc). $[\alpha]_D^{20} +148.5$ (c 1.00, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.46 (1H, dd, $J = 3.4, 1.4$ Hz, H-4), 5.36 (1H, dd, $J = 10.9, 3.4$ Hz, H-3), 5.32 (1H, d, $J = 3.6$ Hz, H-1), 5.17 (1H, dd, $J = 10.9, 3.7$ Hz, H-2), 4.27 (2H, dd, $J = 2.4, 1.0$ Hz, CH₂–C $\equiv$ CH), 4.25 (1H, m, H-5), 4.11–4.09 (2H, m, H-6^a, H-6^b), 2.45 (1H, t, $J = 2.4$ Hz, CH₂–C $\equiv$ CH), 2.14, 2.08, 2.04, 1.98 (12H, 4s, 4 $\times$ COCH₃). ¹³C NMR (125 MHz, CDCl₃): δ : 170.50, 170.48, 170.29, 170.05 (4 $\times$ COCH₃) 95.08 (C-1), 78.39 (CH₂–C $\equiv$ CH), 75.32 (CH₂–C $\equiv$ CH), 68.11 (C-4), 67.88 (C-2), 67.53 (C-3), 66.94 (C-5), 61.63 (C-6), 55.43 (CH₂–C $\equiv$ CH), 20.91, 20.83, 20.78, 20.77 (4 $\times$ COCH₃). HRESIMS: (m/z) calcd for C₁₇H₂₂O₁₀Na⁺ (M+Na)⁺ 409.1111; found 409.1114. Compound **4a** has been reported (NMR spectral data match those above) from the silica gel/H₂SO₄ glycosidation of the free sugar, a process I was unable to duplicate in yield and purity.⁹ A similar problem has been reported by at least one other laboratory.²⁸



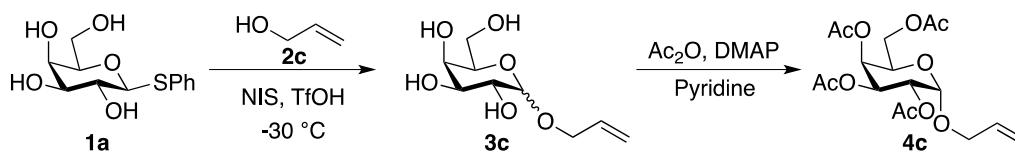
Scheme 2.5 Synthesis of propargyl 2,3,4,6-tetra-*O*-(acetyl-*d*₃)- α -D-galactopyranoside (**4**).

Compound **3a** (64.3 mg, 0.295 mmol) was dissolved in dry pyridine (10 mL), and DMAP (catalytic amt.) and Ac₂O-*d*₆ (0.56 mL, 5.92 mmol) was added with stirring overnight at room temperature. After concentration, the residue was partitioned between CH₂Cl₂/water, and the organic layer was washed with satd aq NaHCO₃ and brine, dried over anhyd Na₂SO₄ and concentrated in vacuo. The crude product was purified by silica gel chromatography (4:1 hexanes–EtOAc) to give **4** (106.1 mg, 90.4%) as a colorless syrup. *R*_f 0.23 (2.5:1, hexanes–EtOAc). [α]_D²¹ +148.2 (*c* 1.00, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.46 (1H, dd, *J* = 3.4, 1.4 Hz, H-4), 5.36 (1H, dd, *J* = 10.9, 3.3 Hz, H-3), 5.31 (1H, d, *J* = 3.8 Hz, H-1), 5.16 (1H, dd, *J* = 10.9, 3.7 Hz, H-2), 4.26 (2H, dd, *J* = 2.4, 1.1 Hz, CH₂–C $\equiv$ CH), 4.25 (1H, m, H-5), 4.10 (2H, m, H-6^a, H-6^b), 2.45 (1H, t, *J* = 2.4 Hz, CH₂–C $\equiv$ CH). ¹³C NMR (125 MHz, CDCl₃): δ 170.49 ($\times 2$), 170.30, 170.05 (4 $\times$ COCH₃) 95.06 (C-1), 78.38 (CH₂–C $\equiv$ CH), 75.31 (CH₂–C $\equiv$ CH), 68.06 (C-4), 67.83 (C-2), 67.48 (C-3), 66.93 (C-5), 61.58 (C-6), 55.42 (CH₂–C $\equiv$ CH). HRESIMS: (*m/z*) calcd for C₁₇H₁₀D₁₂O₁₀Na⁺ (*M*+Na)⁺ 421.2050; found 421.2048.



Scheme 2.6 Synthesis of 3-(trimethylsilyl) propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4b**).

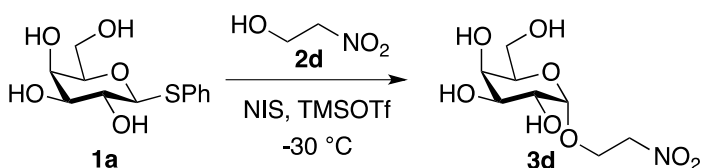
The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4.5:1, hexanes–EtOAc) to give **4b** (104.9 mg, 62.2%, $\alpha/\beta = 10:1$) as a mixture of anomers. **4ba**: R_f 0.37 (2.5:1, hexanes–EtOAc). $[\alpha]_D^{20}$ 144.4 (c 1.00, CHCl_3). ^1H NMR (500, CDCl_3 MHz): δ 5.46 (1H, dd, $J = 3.4, 1.3$ Hz, H-4), 5.38 (1H, dd, $J = 10.9, 3.4$ Hz, H-3), 5.34 (1H, d, $J = 3.7$ Hz, H-1), 5.16 (1H, dd, $J = 10.9, 3.7$ Hz, H-2), 4.26 (3H, m, $\text{CH}_2\text{--C}\equiv\text{CH}$, H-5), 4.14–4.05 (2H, m, H-6^a, H-6^b), 2.14, 2.09, 2.04, 1.99 (12H, 4s, 4 $\times$ COCH₃), 0.17 (9H, s, $\text{--Si}(\text{CH}_3)_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 170.51, 170.36, 170.34, 170.11 (4 $\times$ COCH₃), 99.92 ($\text{CH}_2\text{--C}\equiv\text{C--TMS}$), 94.71 (C-1), 92.52 ($\text{CH}_2\text{--C}\equiv\text{C--TMS}$), 68.11 (C-4), 67.93 (C-2), 67.56 (C-3), 66.84 (C-5), 61.58 (C-6), 56.05 ($\text{CH}_2\text{--C}\equiv\text{C--TMS}$), 20.93, 20.83, 20.81, 20.79 (4 $\times$ COCH₃), -0.16 ($\text{--Si}(\text{CH}_3)_3$). HRESIMS: (m/z) calcd for $\text{C}_{20}\text{H}_{30}\text{O}_{10}\text{SiNa}^+$ ($\text{M}+\text{Na}$)⁺ 481.1506; found 481.1507.



Scheme 2.7 Synthesis of allyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4c**).

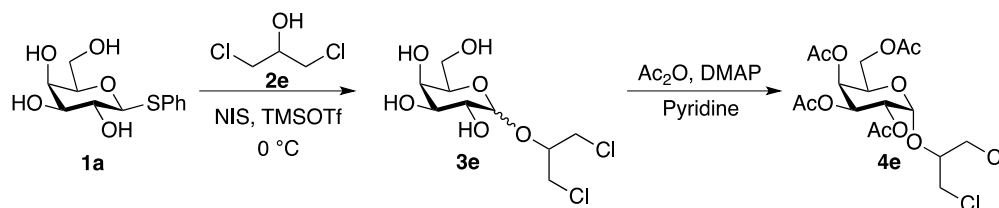
The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4:1, hexanes–EtOAc) to give **4c** (101. 2 mg, 70.9%, $\alpha/\beta = 7:1$) as a mixture of anomers. **4ca**: R_f 0.29 (2.5:1, hexanes–EtOAc). $[\alpha]_D^{20}$ +163.0 (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.87 (1H, dddd, $J = 17.2, 10.4, 6.1, 5.2$ Hz, $\text{CH}_2\text{CH=CH}_2$) 5.45 (1H, dd, $J = 3.4, 1.3$ Hz, H-4), 5.38 (1H, dd, $J = 12.0, 3.4$ Hz, H-3), 5.31 (1H, dq, $J = 17.2, 1.6$ Hz, $\text{CH}_2\text{CH=CH}_2$), 5.22 (1H, dq, $J = 10.4, 1.3$ Hz, $\text{CH}_2\text{CH=CH}_2$), 5.15 (1H, d, $J = 3.8$ Hz, H-1), 5.12 (1H, m, H-2), 4.24 (1H, m, H-5), 4.18

(1H, ddt, 13.1, 5.2, 1.4 Hz, H-6^a/H-6^b), 4.09 (2H, m, CH₂CH=CH₂), 4.02 (1H, ddt, 13.1, 6.1, 1.4 Hz, H-6^a/H-6^b), 2.13, 2.07, 2.04, 1.97 (12H, 4s, 4×COCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 170.50, 170.48, 170.33, 170.09 (4×COCH₃), 133.34 (CH₂CH=CH₂), 118.12 (CH₂CH=CH₂), 95.47 (C-1), 68.90 (C-6), 68.25 (C-4), 68.21 (C-2), 67.73 (C-3), 66.49 (C-5), 61.86 (CH₂CH=CH₂), 20.91, 20.81, 20.78, 20.77 (4×COCH₃). HRESIMS: (*m/z*) calcd for C₁₇H₂₄O₁₀Na⁺ (M+Na)⁺ 411.1267; found 411.1267. The β anomer of compound **4c** has been characterized.⁴⁶



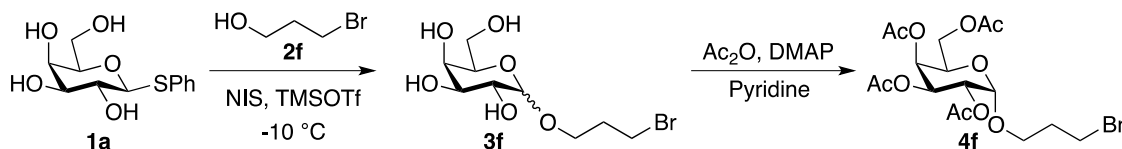
Scheme 2.8 Synthesis of 2-nitroethyl α-D-galactopyranoside (**3d**).

The compound was synthesized according to the general glycosylation procedures B.2 above (The compound partially decomposed when subjected to the acetylation conditions). The crude product was purified by silica gel chromatography (15:1 to 7:1, EtOAc–MeOH) to give **3d** (86.6 mg, 93.0%, α/β = 5:1) as a mixture of anomers. **3da**: *R_f* 0.43 (3:1, EtOAc–MeOH). [α]_D²⁰ +24.7 (*c* 1.00, CH₃OH). ¹H NMR (500 MHz, CD₃OD): δ 4.94 (1H, d, *J* = 3.9 Hz, H-1), 4.78 (2H, m, OCH₂CH₂NO₂), 4.33 (1H, ddd, *J* = 11.9, 5.8, 4.6 Hz, OCH₂CH₂NO₂), 4.04 (1H, ddd, *J* = 11.9, 5.8, 4.6 Hz, OCH₂CH₂NO₂), 3.96 (1H, dd, *J* = 3.3, 1.2 Hz, H-4), 3.85 (1H, m, H-5), 3.82 (1H, m, H-2), 3.79–3.72 (3H, m, H-3, H-6^a, H-6^b). ¹³C NMR (125 MHz, CD₃OD): δ 100.91 (C-1), 75.87 (OCH₂CH₂NO₂), 72.60 (C-5), 71.24 (C-3), 71.05 (C-4), 69.90 (C-2), 65.06 (OCH₂CH₂NO₂), 62.69 (C-6). HRESIMS: (*m/z*) calcd for C₈H₁₅O₈NNa⁺ (M+Na)⁺ 276.0695; found 276.0694. Compound **3d** has been reported.⁴⁷ Partial characterization was by ¹³C NMR spectroscopy (reported only seven peaks) in DMSO-*d*₆.



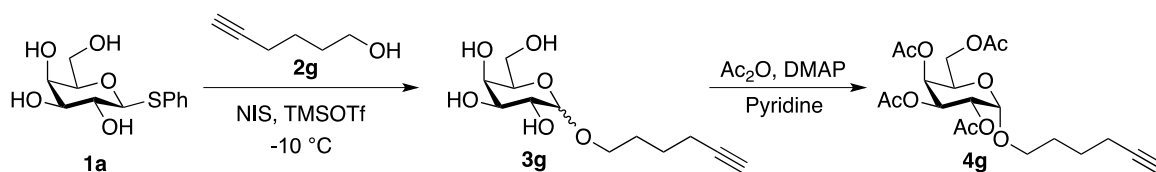
Scheme 2.9 Synthesis of 1,3-dichloro-2-propyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4e**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4:1, hexanes–EtOAc) to give **4e** (143.1 mg, 84.9%, $\alpha/\beta = 5:1$) as a mixture of anomers. **4e** α : R_f 0.38 (2:1, hexanes–EtOAc). $[\alpha]_D^{22} +154.3$ (c 1.00, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.48 (1H, dd, $J = 3.4, 1.3$ Hz, H-4), 5.36 (1H, d, $J = 3.9$ Hz, H-1), 5.34 (1H, dd, $J = 11.0, 3.4$ Hz, H-3), 5.08 (1H, dd, $J = 11.0, 3.9$ Hz, H-2), 4.45 (1H, ddd, $J = 6.9, 5.6, 1.1$ Hz, H-5), 4.10 (2H, m, H-6^a, H-6^b), 4.00 (1H, m, CH(CH₂Cl)₂), 3.74 (2H, d, $J = 5.1$ Hz, CH(CH₂Cl)₂), 3.66 (2H, m, CH(CH₂Cl)₂), 2.14, 2.08, 2.05, 2.00 (12H, 4s, 4×COCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 170.77, 170.49, 170.26, 170.07 (4×COCH₃), 96.87 (C-1), 78.97 (CH(CH₂Cl)₂), 68.14 (C-2, C-4), 67.44 (C-3), 67.34 (C-5), 62.14 (C-6), 44.16, 43.59 (CH(CH₂Cl)₂), 20.93, 20.80, 20.79, 20.75 (4×COCH₃). HRESIMS: (m/z) calcd for C₁₇H₂₄O₁₀Cl₂Na⁺ (M+Na)⁺ 481.0644; found 481.0645, 483.0619, 485.0626 (ratio of molecular ion isotopic peak heights $\approx$ 9:6:1).



Scheme 2.10 Synthesis of 3-bromopropyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4f**).

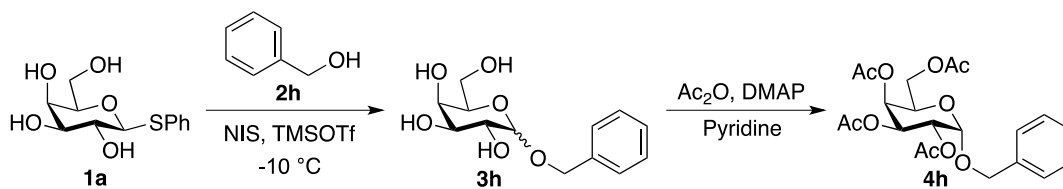
The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4.5:1, hexanes–EtOAc) to give **4f** (96.5 mg, 56.0%, $\alpha/\beta = 3:1$) as a mixture of anomers. **4fa**: R_f 0.37 (2:1, hexanes–EtOAc). $[\alpha]_D^{20} +98.6$ (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.45 (1H, dd, $J = 3.4, 1.4$ Hz, H-4), 5.34–5.31 (1H, m, H-3), 5.13 (1H, m, H-2), 5.12 (1H, d, $J = 3.7$ Hz, H-1), 4.23 (1H, m, H-5), 4.10 (2H, m, H-6^a, H-6^b), 3.87 (1H, ddd, $J = 9.9, 6.0, 5.0$ Hz, one of $\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 3.58–3.49 (3H, m, the other $\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$ and two $\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 2.17–2.10 (5H, m, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$, COCH_3), 2.08, 2.04, 1.98 (9H, 3s, $3 \times \text{COCH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 170.56, 170.49, 170.35, 170.20 ($4 \times \text{COCH}_3$), 96.63 (C-1), 68.26 (C-2), 68.19 (C-4), 67.69 (C-3), 66.56 (C-5), 65.96 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 61.91 (C-6), 32.15 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 30.17 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 20.93, 20.86, 20.81, 20.78 ($4 \times \text{COCH}_3$). HRESIMS: (m/z) calcd for $\text{C}_{17}\text{H}_{25}\text{O}_{10}\text{BrNa}^+$ ($\text{M}+\text{Na}$) $^+$ 491.0529, 493.0511; found 491.0526, 493.0502 (ratio of molecular ion isotopic peak heights $\approx 1:1$). Compound **4f** has been reported.⁴⁸ NMR spectral data match those above.



Scheme 2.11 Synthesis of 5-hexynyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4g**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4.5:1, hexanes–EtOAc) to give **4g** (66.3 mg, 42.1%, $\alpha/\beta > 20:1$) as a colorless syrup. **4ga**: R_f 0.38 (2:1, hexanes–EtOAc). $[\alpha]_D^{20} +139.1$ (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.45 (1H, dd, $J = 3.4, 1.3$ Hz, H-4), 5.36–5.32 (1H, m, H-

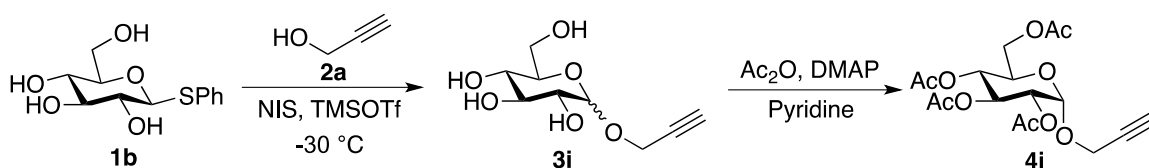
3), 5.12 (1H, m, H-2), 5.10 (1H, d, $J = 3.4$ Hz, H-1), 4.21 (1H, m, H-5), 4.10 (2H, m, H-6^a, H-6^b), 3.73 (1H, dt, $J = 9.9, 6.1$ Hz, one of $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 3.45 (1H, dt, $J = 9.9, 6.3$ Hz, the other one of $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 2.23 (2H, tdd, $J = 6.9, 2.7, 0.7$ Hz, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 2.14, 2.07, 2.04, 1.98 (12H, 4s, $4\times\text{COCH}_3$), 1.95 (1H, t, $J = 2.6$ Hz, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 1.75 – 1.69 (2H, m, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 1.64–1.59 (2H, m, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$). ^{13}C NMR (125 MHz, CDCl_3): δ 170.56, 170.54, 170.37, 170.17 ($4\times\text{COCH}_3$), 96.29 (C-1), 84.10 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 68.85 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 68.36 (C-2), 68.26 (C-4), 68.16 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 67.80 (C-3), 66.39 (C-5), 61.96 (C-6), 28.45 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 25.21 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$), 20.93, 20.84, 20.81, 20.79 ($4\times\text{COCH}_3$), 18.23 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-C}\equiv\text{CH}$). HRESIMS: (m/z) calcd for $\text{C}_{20}\text{H}_{28}\text{O}_{10}\text{Na}^+$ ($\text{M}+\text{Na}$)⁺ 451.1580; found 451.1580.



Scheme 2.12 Synthesis of benzyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4h**).

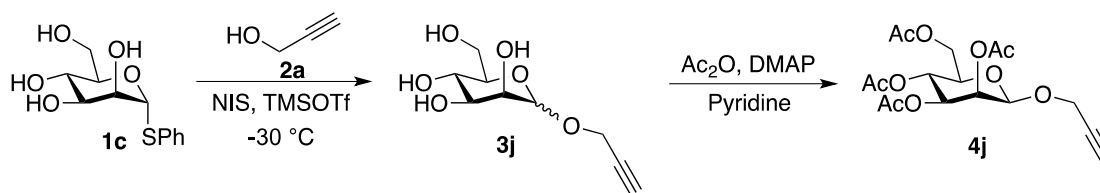
The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4.5:1, hexanes–EtOAc) to give **4h** (116.5 mg, 72.3%, $\alpha/\beta = 3:1$) as a mixture of anomers. **4ha**: R_f 0.40 (2:1, hexanes–EtOAc). $[\alpha]_D^{21} +126.8$ (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 7.37–7.31 (5H, m, H_{arom}), 5.46 (1H, dd, $J = 3.5, 1.3$ Hz, H-4), 5.40 (1H, dd, $J = 10.7, 3.4$ Hz, H-3), 5.18 (1H, d, $J = 3.7$ Hz, H-1), 5.14 (1H, dd, $J = 10.7, 3.7$ Hz, H-2), 4.74–4.53 (2H, dd, $J = 96.5, 12.1$ Hz, CH_2Ph), 4.27 (1H,

td, $J = 6.7, 1.4$ Hz, H-5), 4.08 (2H, qd, $J = 11.2, 6.6$ Hz, H-6^a, H-6^b), 2.13, 2.05, 2.03, 1.98 (12H, 4s, 4×COCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 170.50, 170.40, 170.33, 170.11 (4×COCH₃), 136.90, 128.65, 128.24, 128.01 (C_{arom}), 95.52 (C-1), 70.09 (CH₂Ph), 68.23 (C-4), 68.20 (C-2), 67.78 (C-3), 66.63 (C-5), 61.81 (C-6), 20.86, 20.84, 20.79, 20.77 (4×COCH₃). HRESIMS: (m/z) calcd for C₂₁H₂₆O₁₀Na⁺ (M+Na)⁺ 461.1424; found 461.1426. Compound **4h** has been used in experiments apparently without characterization.⁴⁹ The β anomer is characterized in another paper.⁵⁰



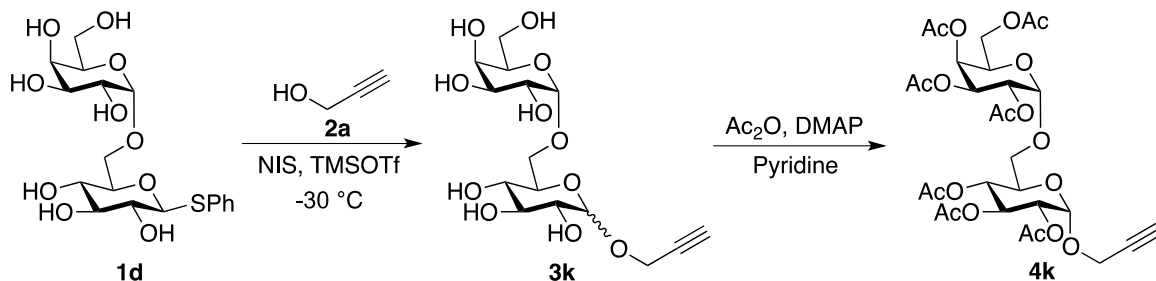
Scheme 2.13 Synthesis of propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside (**4i**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (5:1 to 4:1, hexanes–EtOAc) to give **4i** (112.8 mg, 79.4%, $\alpha/\beta = 7:1$) as a mixture of anomers. **4ia**: R_f 0.35 (2:1, hexanes–EtOAc). $[\alpha]_D^{21} +163.6$ (c 1.00, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.48 (1H, m, H-3), 5.28 (1H, d, $J = 3.8$ Hz, H-1), 5.08 (1H, dd, $J = 10.1, 9.4$ Hz, H-4), 4.91 (1H, dd, $J = 10.3, 3.8$ Hz, H-2), 4.27 (2H, d, $J = 2.4$ Hz, CH₂–C $\equiv$ CH), 4.25–4.10 (2H, m, H-6^a, H-6^b), 4.04 (1H, m, H-5), 2.44 (1H, t, $J = 2.4$ Hz, CH₂–C $\equiv$ CH), 2.08, 2.07, 2.02, 2.00 (12H, 4s, 4×COCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 170.75, 170.22, 170.14, 169.66 (4×COCH₃) 94.69 (C-1), 78.28 (CH₂–C $\equiv$ CH), 75.41 (CH₂–C $\equiv$ CH), 70.56 (C-2), 70.04 (C-3), 68.52 (C-4), 67.94 (C-5), 61.82 (C-6), 55.52 (CH₂–C $\equiv$ CH), 20.84, 20.79, 20.78, 20.72 (4×COCH₃). HRESIMS: (m/z) calcd for C₁₇H₂₂O₁₂Na⁺ (M+Na)⁺ 409.1111; found 409.1112. Compound **4i** has been reported.⁴⁶ The NMR data match those reported above.



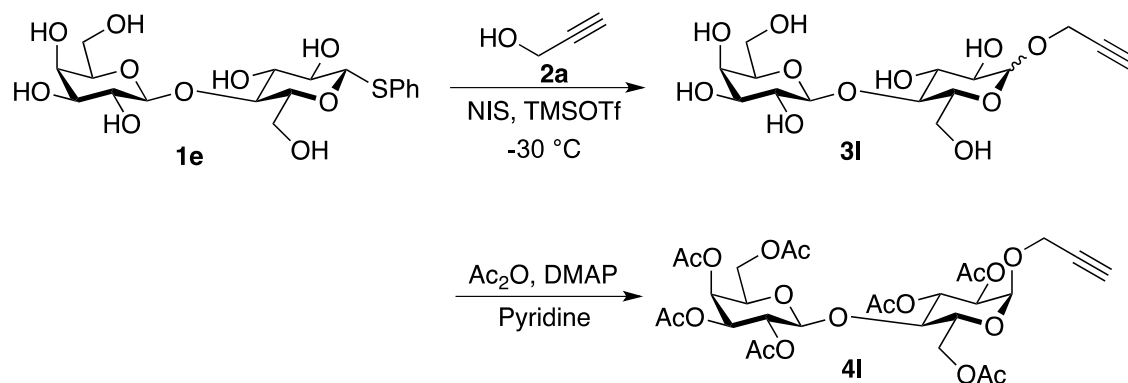
Scheme 2.14 Synthesis of propargyl 2,3,4,6-tetra-*O*-acetyl- β -D-mannopyranoside (**4j**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (4:1 to 2.5:1, hexanes–EtOAc) to give **4j** (115.6 mg, 81.4%, $\alpha/\beta = 1:2$) as a mixture of anomers. **4j β** : R_f 0.18 (2:1, hexanes–EtOAc). $[\alpha]_D^{20} -82.8$ (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.48 (1H, dd, $J = 3.3, 1.1$ Hz, H-2), 5.26 (1H, t, $J = 9.9$ Hz, H-4), 5.09 (1H, dd, $J = 10.0, 3.3$ Hz, H-3), 4.94 (1H, d, $J = 1.1$ Hz, H-1), 4.38 (2H, m, $\text{CH}_2\text{--C}\equiv\text{CH}$), 4.31 (1H, dd, $J = 12.3, 5.3$ Hz, H-6^a), 4.16 (1H, dd, $J = 12.3, 2.5$ Hz, H-6^b), 3.69 (1H, ddd, $J = 9.9, 5.3, 2.6$ Hz, H-5), 2.48 (1H, t, $J = 2.4$ Hz, $\text{CH}_2\text{--C}\equiv\text{CH}$), 2.17, 2.08, 2.03, 1.98 (12H, 4s, $4\times\text{COCH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 170.79, 170.36, 170.10, 169.68 ($4\times\text{COCH}_3$), 95.76 (C-1, $J_{\text{C1-H1}} = 158.76$ Hz), 77.94 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 76.09 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 72.66 (C-5), 71.21 (C-3), 68.85 (C-2), 66.08 (C-4), 62.45 (C-6), 55.91 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 20.95, 20.88, 20.81, 20.69 ($4\times\text{COCH}_3$). HRESIMS: (m/z) calcd for $\text{C}_{17}\text{H}_{22}\text{O}_{12}\text{Na}^+$ ($\text{M}+\text{Na}$)⁺ 409.1111; found 409.1111. The $J_{\text{C1-H1}}$ cited above is in line with that generally expected for a β -D-mannopyranoside.⁵¹ A recent example is that of Demchenko and co-workers.⁵²



Scheme 2.15 Synthesis of propargyl 6-O-(2,3,4,6-tetra-O-acetyl- α -D-galactopyranosyl)-2,3,4-tri-O-acetyl- α -D-glucopyranoside (**4k**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (2:1, hexanes–EtOAc) to give **4k** (172.1 mg, 69.4%, α/β = 8:1) as a mixture of anomers. **4k** α : R_f 0.29 (1:1, hexanes–EtOAc). $[\alpha]_D^{20}$ +166.2 (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.48 (1H, dd, J = 10.3, 9.3 Hz, H-3), 5.45 (1H, dd, J = 3.4, 1.3 Hz, H-4'), 5.34 (1H, dd, J = 10.8, 3.3 Hz, H-3'), 5.24 (1H, d, J = 3.7 Hz, H-1), 5.16 (1H, d, J = 3.7 Hz, H-1'), 5.11 (1H, dd, J = 10.8, 3.6 Hz, H-2'), 5.05 (1H, dd, J = 10.2, 9.3 Hz, H-4), 4.85 (1H, dd, J = 10.3, 3.8 Hz, H-2), 4.28 (2H, dd, J = 2.5, 0.7 Hz, $\text{CH}_2\text{-C}\equiv\text{CH}$), 4.25 (1H, m, H-5'), 4.07 (2H, m, H-6'^{a,b}), 4.02 (1H, m, H-5), 3.72 (1H, dd, J = 11.3, 5.4 Hz, H-6^a), 3.55 (1H, dd, J = 11.3, 2.4 Hz, H-6^b), 2.49 (1H, t, J = 2.4 Hz, $\text{CH}_2\text{-C}\equiv\text{CH}$), 2.13, 2.11, 2.07, 2.04, 2.03, 2.00, 1.97 (21H, 7s, 7 $\times$ COCH₃). ^{13}C NMR (125 MHz, CDCl_3): δ 170.66, 170.52, 170.32, 170.27, 170.19, 170.00, 169.64 (7 $\times$ COCH₃), 96.39 (C-1'), 94.49 (C-1), 78.31 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 75.57 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 70.63 (C-2), 70.09 (C-3), 69.16 (C-4), 68.93 (C-5), 68.27 (C-4'), 68.25 (C-2'), 67.60 (C-3'), 66.58 (C-5'), 66.31 (C-6), 61.90 (C-6'), 55.51 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 20.94, 20.86, 20.82, 20.81 ($\times 2$), 20.79, 20.78 (7 $\times$ COCH₃). HRESIMS: (m/z) calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{18}\text{Na}^+$ ($\text{M}+\text{Na}$)⁺ 697.1956; found 697.1956.

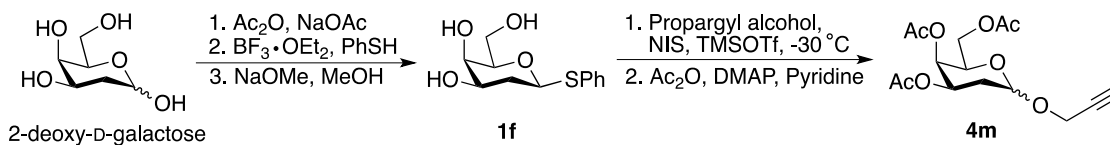


Scheme 2.16 Synthesis of propargyl 4-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2,3,6-tri-O-acetyl- α -D-glucopyranoside (**4I**).

The compound was synthesized according to the general glycosylation and acetylation procedures B.2 and B.3, above. The crude product was purified by silica gel chromatography (2:1 to 1.5:1, hexanes–EtOAc) to give **4I** (140.6 mg, 56.7%, $\alpha/\beta = 12:1$) as a mixture of anomers. **4Ia**: R_f 0.22 (1:1, hexanes–EtOAc). $[\alpha]_D^{22} +59.7$ (c 1.00, CHCl_3). ^1H NMR (500 MHz, CDCl_3): δ 5.47 (1H, dd, $J = 10.3, 9.2$ Hz, H-3), 5.34 (1H, dd, $J = 3.6, 1.2$ Hz, H-4'), 5.20 (1H, d, $J = 3.8$ Hz, H-1), 5.10 (1H, dd, $J = 10.4, 7.9$ Hz, H-2'), 4.95 (1H, dd, $J = 10.4, 3.5$ Hz, H-3'), 4.83 (1H, dd, $J = 10.3, 3.8$ Hz, H-2), 4.48 (1H, d, $J = 7.9$ Hz, H-1'), 4.45 (1H, dd, $J = 12.0, 2.1$ Hz, H-6^a), 4.25 (2H, dd, $J = 3.3, 2.4$ Hz, $\text{CH}_2\text{--C}\equiv\text{CH}$), 4.14 (2H, m, H-6^b, H-6'^a), 4.07 (1H, dd, $J = 11.1, 7.5$ Hz, H-6'^b), 3.96 (1H, m, H-5), 3.86 (1H, ddd, $J = 7.5, 6.3, 1.2$ Hz, H-5'), 3.76 (1H, dd, $J = 10.1, 9.2$ Hz, H-4), 2.43 (1H, t, $J = 2.4$ Hz, $\text{CH}_2\text{--C}\equiv\text{CH}$), 2.14, 2.12, 2.06, 2.05, 2.04, 2.04, 1.95 (21H, 7s, $7\times\text{COCH}_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 170.50, 170.48, 170.47, 170.30, 170.21, 169.59, 169.13 ($7\times\text{COCH}_3$), 101.16 (C-1'), 94.41 (C-1), 78.30 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 76.42 (C-4), 75.37 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 71.19 (C-3'), 70.79 (C-2), 70.77 (C-5'), 69.76 (C-3), 69.27 (C-2'), 68.85 (C-5), 66.74 (C-4'), 61.86 (C-6), 60.94 (C-6'), 55.30 ($\text{CH}_2\text{--C}\equiv\text{CH}$), 21.00 ($\times 2$), 20.84, 20.78 ($\times 2$), 20.77, 20.64 ($7\times\text{COCH}_3$). HRESIMS: (m/z) calcd for

$C_{29}H_{38}O_{18}Na^+ (M+Na)^+$ 697.1956; found 697.1956. Compound **4l** has been reported.¹²

However, the NMR data differ from those I report and assign above. Our assignments are based on 2D NMR data. See the NMR spectra in the Supplementary Information section.



Scheme 2.17 Synthesis of propargyl 3,4,6-tri-O-acetyl-2-deoxy- α,β -D-lyxo-hexopyranoside (**4m**).

In addition, the thiophenyl glycoside (donor) of 2-deoxy-D-lyxo-hexopyranose (phenyl 2-deoxy-1-thio- β -D-lyxo-hexopyranoside, **1f**) was also synthesized and applied in this glycosylation reaction to afford propargyl 3,4,6-tri-O-acetyl-2-deoxy- α,β -D-lyxo-hexopyranoside (alias: “propargyl 3,4,6-tri-O-acetyl-2-deoxy- α,β -D-galactopyranoside,” **4m**).⁵³ However, a moderate yield (67%) and low stereoselectivity ($\alpha:\beta = 1.7:1$) were achieved. The acetylated mixture **4m** was not further separated into its anomers.

2.6 Conclusions

In summary, a facile and general strategy for the direct construction of 1,2-*cis* alkyl glycosides has been developed. Glycosylations between several unprotected phenyl 1-thioglycosyl donors and alcohols bearing various functional groups proceed smoothly to give satisfying yields and 1,2-*cis* selectivity. Use of an FIA-ESI-TOF-MS/NMR protocol facilitated rapid and efficient optimization of conditions. I anticipate that the synthetic procedures described herein will find application in a number of areas where 1,2-*cis* selectivity in glycosylated products is required.

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Chapter 3 Convergent Synthesis of Diversely Presented Galactoside Ligands

Abstract

In order to construct a synthetic carbohydrate microarray to imitate featured carbohydrate presentations on cell surface, I have developed straightforward synthetic routes for the preparation of α -linked propargyl terminated galactosides with various spatial presentations. Through the routes, glycosyl acceptors were obtained via flexible and efficient regioselective protection strategies, which were followed by glycosylations with 4,6-di-*tert*-butylsilylene protected thiogalactoside donor to get the α -linked galactosides in exclusive stereoselective and satisfied yield. These galactoside ligands could be readily immobilized on the azide-functionalized surface.

3.1 Background

Cell membranes are extensively covered with oligosaccharides and glycoconjugates, which have been determined to be crucial in mediating many biological processes, such as cell adhesion, cell recognition, ion conductivity and cell signaling.¹ The carbohydrate landscape provides an ideal basis for these biological functions, which are based on their highly branched structures and varied linkage types. Moreover, carbohydrates can modify the intrinsic properties of proteins to which they are attached by altering the stability, protease resistance, quaternary structure, and dynamics involved.² However, explaining the mechanisms involved is still challenging. Fortunately, the accumulation of experience in both carbohydrate synthesis^{3, 4} and glycomicroarray construction^{5, 6} enables us to explore the mechanisms systematically.

Our goal is to emulate this cell membrane landscape by construction of microarrays with a collection of diversely presented carbohydrate ligands. Glycoclusters with capped-galactose will be assembled in a modular fashion and immobilized on glass surfaces at defined densities to control molecular presentation. The compounds synthesized will be new glycoclusters of α -galactose units that mimic features of carbohydrates on cell surfaces. The significance of α -galactosides has been well documented, as they are extensively involved in many biological recognition processes, such as being ABO histo-blood antigens,^{7, 8} Chagas disease antigens,⁹ and precursors of Fabry disease symptoms pathogenesis.¹⁰ Recently, Commins and co-workers reported the anaphylaxis to mammalian red meat that is caused by the serum anti- α -Gal IgE antibodies to the oligosaccharides with the Gal- α -(1,3)-Gal structure.¹¹ They demonstrated that IgE antibodies were produced in patients after tick bites (such as by the tick *Amblyomma*

americanum). These patients are then experienced severe hypersensitivity reactions after taking mammalian red meat or an mAb cetuximab cancer drug, which contain Gal- α -(1,3)-Gal oligosaccharides.¹²

As the type of linkage from a sugar cap on an oligosaccharide to the neighboring sugar residue is a major distinguishing feature among natural galactans, I want to systematically investigate the influence of linkage type and size of α -linked galactosides on their binding with receptors, and get more insights in elucidating the recognition mechanism and developing novel diagnostic methods and therapies. This chapter will primarily focus on the synthesis of α -linked galactoside ligands. Six propargyl-terminated galactose derivatives will be synthesized with totally 1,2-*cis*- α -O-glycosidic linkages. I synthesized a collection of galactoside ligands (α -linked 1,2-, 1,3-, 1,4- and 1,6- galactose disaccharides, as well as mono- and α -linked trisaccharide, as displayed in Figure 3.1, compounds **1–6**). The installed propargyl terminus of galactosides enables rapid and efficient incorporation with an azide-terminated cluster via “click chemistry”,¹³ which allows a quantitative introduction of glycosyl groups onto carbohydrate microarray surface. Furthermore, a previous study conducted in our lab revealed that the α -linked mannose disaccharide presented highest binding affinity with Con A lectin compared with mono- and trisaccharide.¹⁴ Therefore, I intentionally designed the following galactoside candidates.

Herein, I will take advantage of combinatorial synthesis for the construction of the propargyl galactoside ligands. Combinatorial synthesis is an efficient means of making several compounds simultaneously from different combinations of modular building blocks.^{15, 16} It is specifically favorable in the preparation of a series of

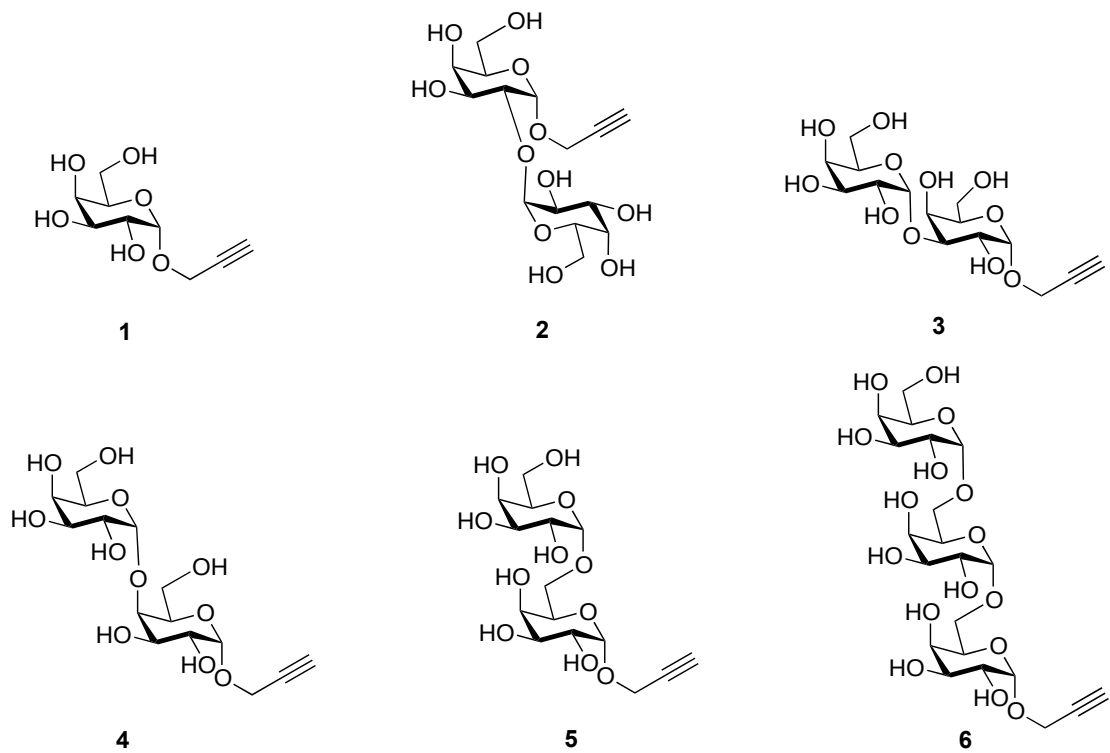


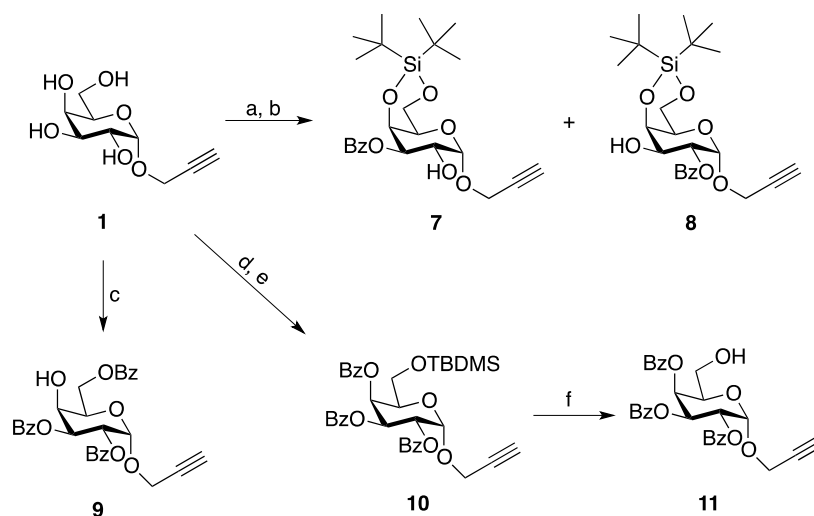
Figure 3.1 Structures of defined α -linked galactosides with propargyl terminus to be incorporated into carbohydrate microarrays.

oligosaccharides, which can be efficiently achieved through the combinations of different glycosyl donors and acceptors. In most cases, the benzyl ether protecting group is a popular choice in 1,2-*cis*-glycosylation reactions based on the virtue of non-participation during glycosylations, but debenzylation always needs H₂/Pd system that would destroy the propargyl terminus. Therefore, the selection of a protecting group that favors 1,2-*cis*-glycosylation and tolerates propargyl group as well is a main challenge.

In 2003, Kiso *et al.* first reported¹⁷ the employment of di-*tert*-butylsilylene (DTBS) protecting group in presence of C-2 benzoyl participating groups in α -selective galactosylation. The cyclic bifunctional silyl protecting groups present strong power in controlling the α -stereoselectivity of glycosylation. Accordingly, the 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl protected galactosyl donors have been prepared and used in many α -galactosylations.¹⁸ The highly bulky silyl group blocks the β -face of the donor to guarantee a completely α -glycosylation with the glycosyl acceptor.¹⁹ This type of donor shows great accessibility, compatibility, and most importantly, high tolerance with sensitive groups such as alkene, alkyne, nitro and halogen. The deprotection of silyl and benzoyl group can be performed at mild conditions without impairing the anomeric functional groups, which satisfies our requirements in the synthesis of α -linked propargyl galactoside ligands.

Combinatorial synthesis was performed to afford the α -linked galactose ligands **1–6** from different combinations of glycosyl donor and acceptors. Propargyl α -D-galactoside **1** was synthesized according to the method described in Chapter 2, through the glycosylation of unprotected 1-thiogalactosyl donor with propargyl alcohol.²⁰ It was then regioselectively protected by different strategies^{21–23} with modifications to afford

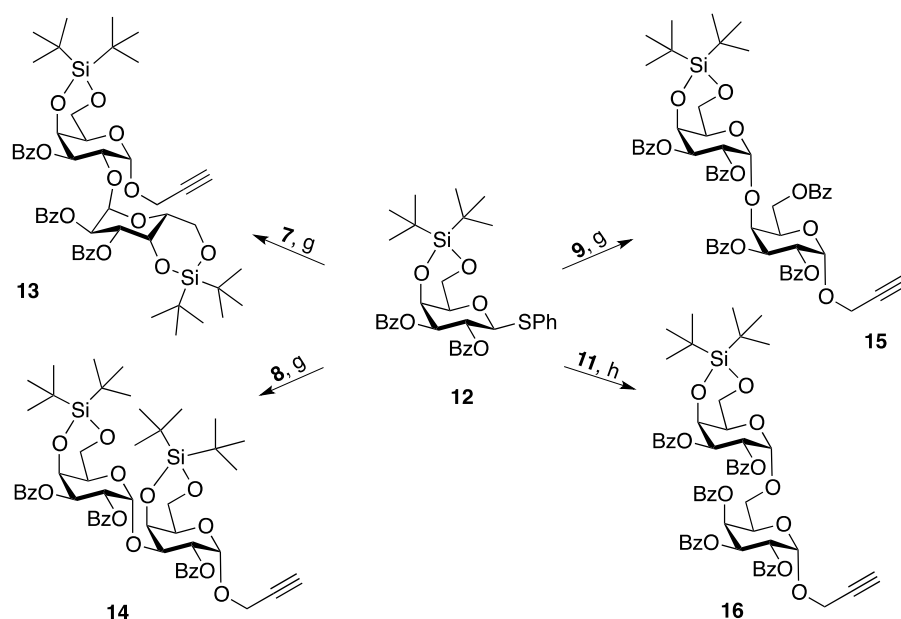
four galactose acceptors **7–9** and **11** with propargyl terminus, which are 2, 3, 4, 6 –OH free respectively. For the synthesis of the acceptors **7** and **8**, I developed a concise method. The galactoside **1** was protected with the bifunctional silyl-protecting group to have the compound with the 4 and 6 –OH blocked, and it was treated with an equimolar benzoyl chloride. As the reactivities of 2 and 3 –OH of galactose are similar, it gave both acceptors **7** and **8** at the same time. To the best of our knowledge, these synthetic approaches used minimal steps and protecting group manipulations (Scheme 3.1).



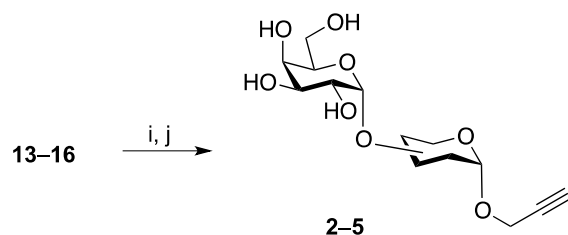
Scheme 3.1 Synthesis of the glycosylation acceptors **7–9** and **11**. *Reagents and conditions:* (a) DTBS(OTf)₂, DMF, –40 °C, 83%; (b) BzCl, Pyridine, 47% for **7**, 30% for **8**; (c) BzCl, Pyridine, –30 °C, 48%; (d) TBDMSCl, Imidazole, Pyridine, 65%; (e) BzCl, Pyridine, DMAP, 63%; (f) TBAF, AcOH, THF, 84%.

The synthesized glycosyl acceptors were glycosylated with the α -directing glycosyl donor **12**¹⁷ upon the activation with trifluoromethanesulfonic acid (TfOH) and *N*-iodosuccinimide (NIS). Expected α -configurations were achieved in moderate to high yield (Scheme 3.2). It is noteworthy that acceptors **7–9** and **11**, yet having similar structures, possess different reactivity towards the glycosylation with donor **7**.

Accordingly, besides the conventional glycosylation procedure (with acceptor **11**), an inverse-addition (I-A) procedure²⁴ (with acceptors **7–9**) was also applied to achieve more effective glycosylation and to optimize the yield. I speculate that the I-A procedure favors the formation of oxocarbenium ion due to kinetic anomeric effect and through-space electron donation effect,¹⁹ which enables weakly nucleophilic acceptors (such as these secondary alcohols) to attack it through S_N1 process. Removal of the silyl and benzoyl groups furnished the α -linked disaccharides **2-5** (Scheme 3.3).

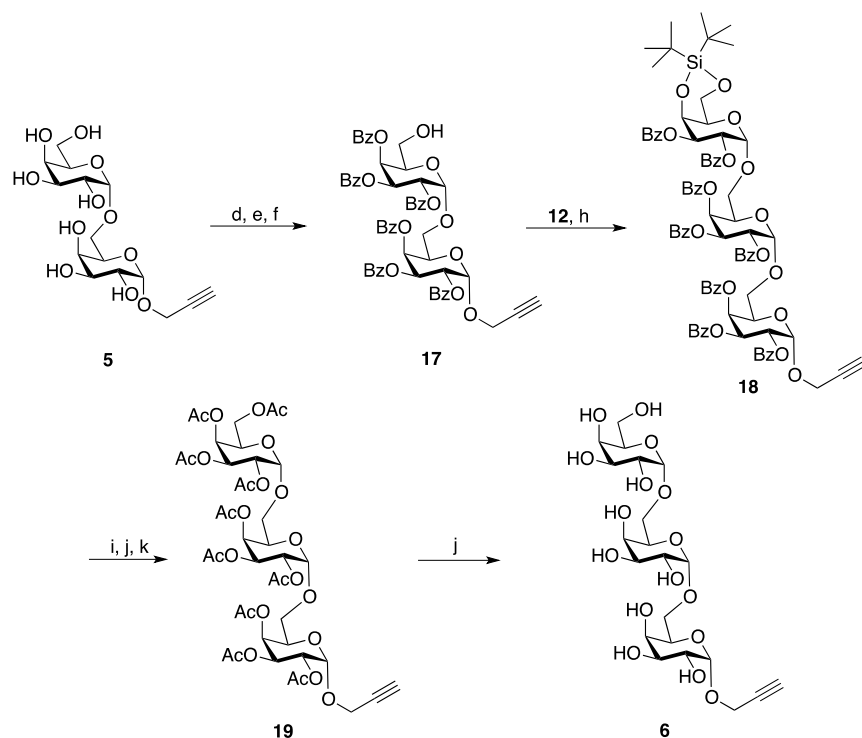


Scheme 3.2 Synthesis of disaccharides **13–16**. *Reagents and conditions:* (g) NIS, TfOH, CH₂Cl₂, –40 °C to rt, I-A procedure, 53–80%; (h) NIS, TfOH, CH₂Cl₂, 0 °C, 64%.



Scheme 3.3 Deprotection of disaccharides **13–16** to afford **2–5**. *Reagents and conditions:*

(i) TBAF, AcOH; (j) NaOMe, MeOH, 72–84% over two steps.



Scheme 3.4 Synthesis of trisaccharide **6**. *Reagents and conditions:* (d) TBDMSCl, Imidazole, Pyridine; (e) BzCl, Pyridine, DMAP; (f) TBAF, AcOH, THF; (h) NIS, TfOH, CH₂Cl₂, 0 °C; (i) TBAF, AcOH; (j) NaOMe, MeOH; (k) Ac₂O, DMAP, Pyridine, 11% over 8 steps.

The synthesis of trisaccharide **6** was in a similar fashion with disaccharide **5**, as they shared the same Gal- α -(1,6)-Gal structure. Regioselective protection of disaccharide **5** gave the acceptor **17**, which was then glycosylated with donor **12** to construct **18** with

the α -1-6-linkage at the top. Deprotection of **18** afforded the trisaccharide **6**, which was acetylated again for a further purification and better characterization. Final deprotection by sodium methoxide gave defined trisaccharide **6** in high purity (Scheme 3.4).

3.2 Experimental Section

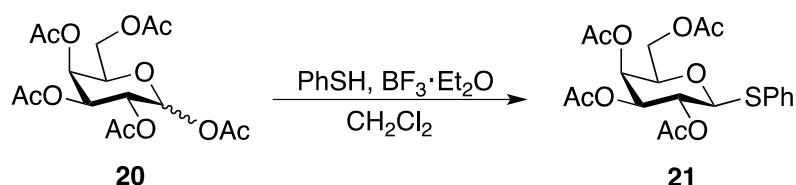
3.2.1 General methods

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Reagent grade dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), tetrahydrofuran (THF), methanol (MeOH), *N,N*-dimethylformamide (DMF) and toluene were obtained from the Pure-Solv (Innovation Technologies) solvent system that uses alumina columns except for DMF, which was dried over a column of 5 Å molecular sieves. Pyridine was distilled over CaH_2 prior to use. All reactions were performed under anhydrous conditions unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on silica gel precoated aluminum plates. Zones were detected by UV irradiation using a 254 nm lamp and/or by heat/charring with *p*-anisaldehyde–sulfuric acid development reagent. Column chromatography was performed on silica gel (40–63 μm). Optical rotation values were obtained at the sodium D line using a Perkin–Elmer 241 polarimeter. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature with a Varian Inova 500 MHz instrument. Chemical shifts are reported in δ -units (ppm) relative to the residual ^1H CDCl_3 at δ 7.26 ppm and ^{13}C at δ 77.16 ppm. All two-dimensional experiments (gCOSY, gHSQC and gHMBC) were recorded on the same instrument using Varian protocols. Mass spectrometric analysis was performed on a QSTAR Elite quadrupole time-of-flight (QTOF) mass

spectrometer with an ESI source. MALDI-TOF mass spectra were obtained on a Voyager-DE PRO BioSpectrometry workstation.

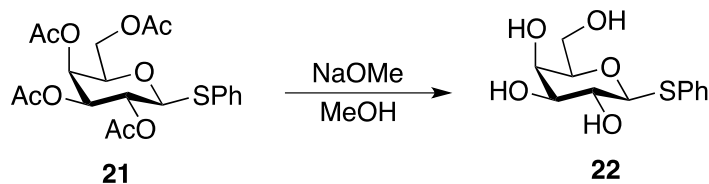
3.2.2. Synthesis of carbohydrate ligands

(1) Synthesis of the phenyl thioglycoside donor.



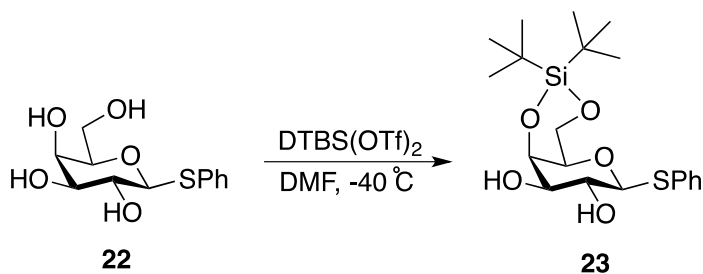
Scheme 3.5 Synthesis of phenyl 2,3,4,6-tetra-O-acetyl-1-thio- β -D-galactopyranoside **21**.

Thiophenol (2.78 mL, 27.2 mmol) was added to a solution of compound **20** (8.15 g, 20.9 mmol) in dry CH_2Cl_2 at 0 °C and the mixture was stirred for 30 min. Then $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (7.95 mL, 62.7 mmol) was slowly injected to the mixture, which was allowed to warm to room temperature. After 5 hours, the mixture was diluted by CH_2Cl_2 , washed with sat NaHCO_3 and brine, dried over Na_2SO_4 , concentrated in vacuo, and purified by column chromatography (Hexane–EtOAc 6:1) to afford compound **21** as colorless syrup (7.56 g, 82.2%). $R_f = 0.39$ (2.5:1, hexane–EtOAc). $[\alpha]_D^{23} = + 3.6$ ($c = 1.00$, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 7.50 (m, 2H, Ar-H), 7.30 (m, 3H, Ar-H), 5.41 (dd, $J = 3.4, 1.1$ Hz, 1H, H-4), 5.23 (m, 1H, H-2), 5.06 (dd, $J = 10.0, 3.4$ Hz, 1H, H-3), 4.73 (d, $J = 10.0$ Hz, 1H, H-1), 4.18 (m, 1H, H-6_a), 4.11 (m, 1H, H-6_b), 3.94 (ddd, $J = 7.1, 6.2, 1.1$ Hz, 1H, H-5), 2.10, 2.08, 2.03, 1.96 (4s, 12H, $4 \times \text{COCH}_3$). ^{13}C NMR (126 MHz, Chloroform- d) δ 170.35, 170.18, 170.02, 169.42, 132.58, 132.49, 128.91, 128.17, 86.56, 74.45, 72.03, 67.31, 67.28, 61.68, 20.86, 20.68, 20.65, 20.60.



Scheme 3.6 Synthesis of phenyl 1-thio-β-D-galactopyranoside **22**.

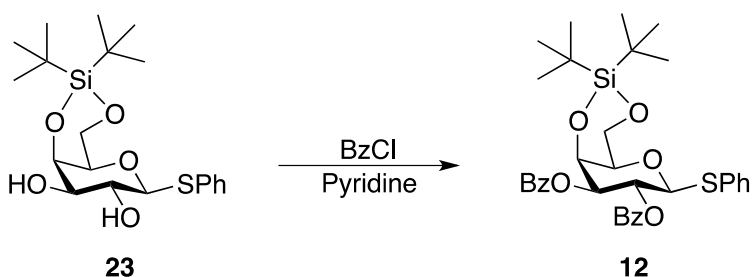
Compound **21** (5.85 g, 13.3 mmol) was dissolved in dry MeOH (50 mL), followed by adding NaOMe in small amount to afford pH 9. After 2 h the solution was quenched by Amberlite® IR 120 (H⁺). The resin was filtered off and the solvent was removed in vacuo to afford a white powdery solid **22** (3.53 g, 97.6%). $R_f = 0.53$ (4:1, EtOAc–MeOH). m.p. 106 – 108 °C. $[\alpha]_D^{21} = -48.4$ ($c = 0.93$, CH₃OH). ¹H NMR (500 MHz, Methanol-d) δ 7.58 (m, 2H, Ar-H), 7.31 (m, 2H, Ar-H), 7.25 (m, 1H, Ar-H), 4.63 (d, $J = 9.7$ Hz, 1H, H-1), 3.93 (dd, $J = 3.4, 1.0$ Hz, 1H), 3.80 (m, 1H), 3.74 (m, 1H), 3.64 (t, $J = 9.4$ Hz, 1H), 3.59 (ddd, $J = 6.6, 5.3, 1.1$ Hz, 1H), 3.54 (dd, $J = 9.2, 3.3$ Hz, 1H). ¹³C NMR (126 MHz, Methanol-d) δ 136.00, 132.05, 129.82, 127.97, 90.22, 80.55, 76.29, 70.98, 70.38, 62.59.



Scheme 3.7 Synthesis of phenyl 4,6-O-di-tert-butylsilanediyl-1-thio-β-D-galactopyranoside **23**.

Compound **22** (3.53 g, 13.0 mmol) was dissolved in dry DMF (40 mL) and cooled to -40 °C. Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (4.02 mL, 12.3 mmol) was added dropwise to the mixture, which was stirred for 30 min, followed by addition of

pyridine (3.2 mL). The reaction mixture was stirred for another 15 min, then diluted with diethyl ether, washed by water (200 mL) and brine (100 mL) and dried over anhydrous Na₂SO₄. The organic layer was concentrated in vacuo and purified by column chromatography (DCM/Acetone 15:1 to 10:1) to afford compound **23** (4.83 g, 90.2%) as colorless syrup. $R_f = 0.56$ (10:1, DCM–Acetone). $[\alpha]_D^{22} = -78.0$ ($c = 1.00$, CHCl₃). ¹H NMR (500 MHz, Chloroform-d) δ 7.57 (m, 2H, Ar-H), 7.30 (m, 3H, Ar-H), 4.57 (d, $J = 9.8$, 1H, H-1), 4.45 (dd, $J = 3.5, 1.1$ Hz 1H), 4.27 (m, 2H), 3.76 (td, $J = 9.4, 2.5$ Hz, 1H), 3.55 (ddd, $J = 10.5, 8.9, 3.5$ Hz, 1H), 3.48 (m, 1H), 2.81 – 2.70 (br, 2s, 2H, OH), 1.06 (s, 9H), 1.05 (s, 9H). ¹³C NMR (126 MHz, Chloroform-d) δ 133.27, 132.77, 129.07, 128.02 (Ar-C), 89.21 (C-1), 75.37, 75.27, 72.67, 70.83, 67.22, 27.69, 27.55, 23.47, 20.79.

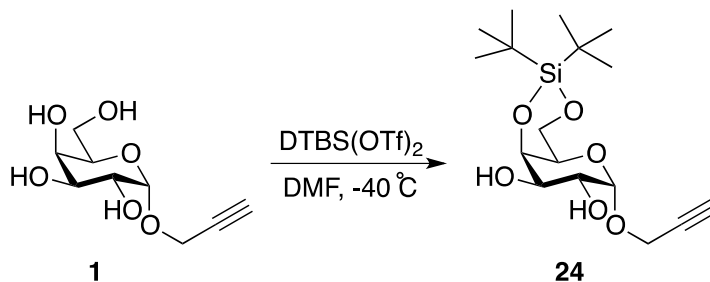


Scheme 3.8 Synthesis of phenyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl-1-thio- β -D-galactopyranoside **12**.

Compound **23** (4.28 g, 10.39 mmol) was dissolved in dry pyridine (30 mL), followed by adding benzoyl chloride (2.90 mL, 24.96 mmol) at room temperature. The reaction mixture was stirring for 3 hours, which was then quenched by EtOH (5 mL) and concentrated in high vacuo. The residue was diluted by EtOAc (60 mL), washed with 1N HCl (50 mL), sat. aq. NaHCO₃ (60 mL) and brine (50 mL). The aqueous layer was extracted with EtOAc (60 mL), and the combined organics were dried over anhydrous Na₂SO₄. After concentration, the crude product was purified by silica gel

chromatography (10:1, hexane–EtOAc) to give **12** (5.69 g, 88.3%) as a white solid. R_f = 0.32 (6:1, hexane–EtOAc). m.p. 72 – 74 °C. $[\alpha]_D^{22}$ = +136.9 (c = 1.03, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 7.99 (ddd, J = 8.5, 5.0, 1.3 Hz, 4H, Ar-H), 7.49 (m, 4H, Ar-H), 7.38 (m, 4H, Ar-H), 7.26 (m, 3H, Ar-H), 5.93 (t, J = 10.0 Hz, 1H, H-2), 5.22 (dd, J = 9.8, 3.1 Hz, 1H, H-3), 4.95 (d, J = 10.1 Hz, 1H, H-1), 4.89 (dd, J = 3.1, 0.9 Hz, 1H, H-4), 4.33 (m, 2H, H-6_{a,b}), 3.65 (q, J = 1.3 Hz, 1H, H-5), 1.16 (s, 9H, CH_3 -*t*Bu-Si), 0.96 (s, 9H, CH_3 -*t*Bu-Si). ^{13}C NMR (126 MHz, Chloroform- d) δ 166.27, 165.58, 133.94, 133.37, 133.29, 132.60, 129.95, 129.93, 129.75, 129.59, 129.04, 128.53, 128.49, 127.94, 87.67, 75.61, 75.16, 70.61, 68.26, 67.27, 27.65, 27.60, 23.40, 20.86. MALDI-TOFMS: calcd for $\text{C}_{34}\text{H}_{40}\text{O}_7\text{SSi}\cdot\text{Na}^+$: 643.2156; found: m/z 643.2146.

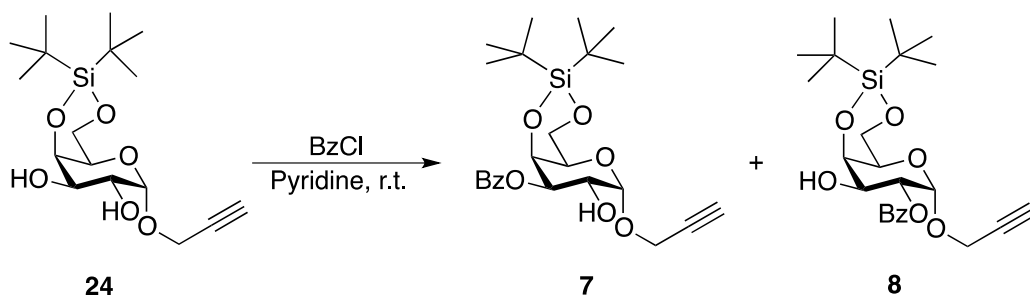
(2) Synthesis of galactosyl acceptors



Scheme 3.9 Synthesis of propargyl 4,6-*O*-di-*tert*-butylsilanediyl- α -D-galactopyranoside **24**.

Compound **1** (1.91 g, 8.76 mmol) was dissolved in dry DMF (20 mL) and cooled to -40 °C. Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (2.70 mL, 8.32 mmol) was added dropwise to the mixture, which was stirred for 30 min, followed by addition of pyridine (2.1 mL). The reaction mixture was stirred for another 15 min, then diluted with diethyl ether, washed by water (100 mL) and brine (100 mL) and dried over anhydrous Na_2SO_4 . The organic layer was concentrated in vacuo and purified by column

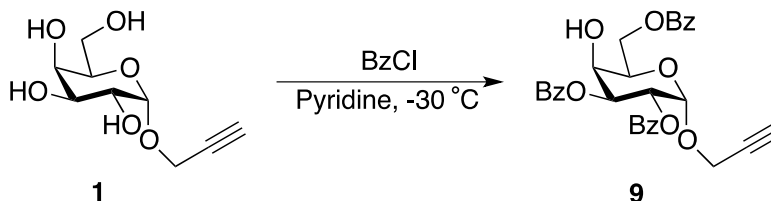
chromatography (DCM/Acetone 40:1 to 20:1) to afford compound **24** (2.59 g, 82.6%) as an amorphous white foam. $R_f = 0.41$ (9:1, DCM–Acetone). $[\alpha]_D^{22} = 138.6$ ($c = 1.00$, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 5.13 (d, $J = 3.9$, 1H, H-1), 4.46 (dd, $J = 3.5$, 1.2 Hz, 1H), 4.30 – 4.27 (m, 3H), 4.18 (dd, $J = 12.6$, 1.7 Hz, 1H), 3.88 (dd, $J = 9.8$, 3.9 Hz, 1H), 3.80 (m, 1H), 3.72 (dd, $J = 9.8$, 3.4 Hz, 1H), 2.45 (t, $J = 2.4$ Hz, 1H), 1.05 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, Chloroform- d) δ 98.18, 78.93, 75.12, 73.32, 71.29, 69.82, 67.95, 66.87, 55.50, 27.72, 27.34, 23.51, 20.84. HRMS ESI (m/z) ($\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{17}\text{H}_{30}\text{O}_6\text{SiNa}^+$ 381.1709, found 381.1709.



Scheme 3.10 Synthesis of propargyl 3-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl- α -D-galactopyranoside **7** and propargyl 2-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl- α -D-galactopyranoside **8**.

Compound **24** (1.69 g, 4.72 mmol) was dissolved in dry pyridine (20 mL), followed by adding benzoyl chloride (0.58 mL, 4.96 mmol) at room temperature. The reaction mixture was stirring for 1 hour, which was then quenched by EtOH (2 mL) and concentrated in high vacuo. The residue was diluted by EtOAc (60 mL), washed with 1N HCl (50 mL), sat. aq. NaHCO_3 (60 mL) and brine (50 mL). The aqueous layer was extracted with EtOAc (60 mL), and the combined organics were dried over anhydrous Na_2SO_4 . After concentration, the crude products were purified by silica gel chromatography (10:1, hexane–EtOAc) to give **7** (1.02 g, 46.8%) and **8** (0.66 g, 30.3%)

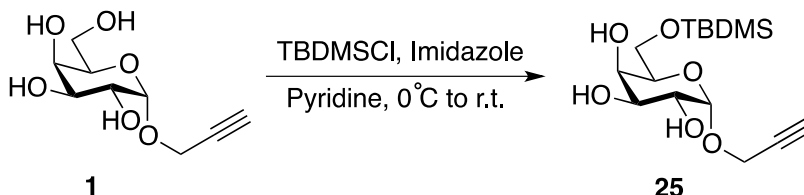
as amorphous white foam. **8**: $R_f = 0.47$ (3:1, hexane–EtOAc). $[\alpha]_D^{20} = +97.2$ ($c = 1.00$, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 8.12 (m, 2H, Ar-H), 7.56 (m, 1H, Ar-H), 7.44 (m, 2H, Ar-H), 5.39 (dd, $J = 10.2, 3.7$ Hz, 1H), 5.30 (d, $J = 3.8$ Hz, 1H, H-1), 4.54 (dd, $J = 3.5, 1.3$ Hz, 1H), 4.33 (dd, $J = 12.7, 2.2$ Hz, 1H), 4.26 (dd, $J = 3.1, 2.4$ Hz, 2H), 4.22 (dd, $J = 12.6, 1.6$ Hz, 1H), 4.11 (m, 1H), 3.90 (m, 1H), 2.58 (m, 1H), 2.33 (t, $J = 2.4$ Hz, 1H), 1.11 (s, 9H, $\text{CH}_3\text{-tBu-Si}$), 1.07 (s, 9H, $\text{CH}_3\text{-tBu-Si}$). ^{13}C NMR (126 MHz, Chloroform- d) δ 166.86, 133.27, 130.06, 129.99, 128.46, 96.45, 79.02, 74.82, 73.78, 71.34, 68.52, 67.81, 66.80, 55.64, 27.68, 27.41, 23.52, 20.90. MALDI-TOFMS: calcd for $\text{C}_{24}\text{H}_{34}\text{O}_7\text{Si}\cdot\text{Na}^+$: 485.1972; found: m/z 485.1972. **7**: $R_f = 0.42$ (3:1, hexane–EtOAc). $[\alpha]_D^{20} = +182.6$ ($c = 1.00$, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 8.11 (m, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 7.45 (m, 2H, Ar-H), 5.23 (dd, $J = 10.4, 3.2$ Hz, 1H), 5.19 (d, $J = 3.9$ Hz, 1H, H-1), 4.72 (m, 1H), 4.34 (m, 3H), 4.30 (dd, $J = 12.6, 2.2$ Hz, 1H), 4.21 (dd, $J = 12.6, 1.7$ Hz, 1H), 3.87 (m, 1H), 2.47 (t, $J = 2.4$ Hz, 1H), 2.04 (m, 1H), 1.07 (s, 9H, $\text{CH}_3\text{-tBu-Si}$), 0.98 (s, 9H, $\text{CH}_3\text{-tBu-Si}$). ^{13}C NMR (126 MHz, Chloroform- d) δ 166.91, 133.25, 130.29, 129.95, 128.50, 98.37, 78.85, 75.24, 73.83, 71.49, 68.00, 66.97, 66.85, 55.50, 27.69, 27.35, 23.43, 20.84. MALDI-TOFMS: calcd for $\text{C}_{24}\text{H}_{34}\text{O}_7\text{Si}\cdot\text{Na}^+$: 485.1972; found: m/z 485.1972.



Scheme 3.11 Synthesis of propargyl 2,3,6-tri-*O*-benzoyl- α -D-galactopyranoside **9**.

Benzoyl chloride (1.15 mL, 9.89 mmol) was added dropwise to a solution of **1** (0.52 g, 2.39 mmol) in pyridine (20 mL) at $-30\text{ }^\circ\text{C}$. After stirring for 2 hrs, the reaction

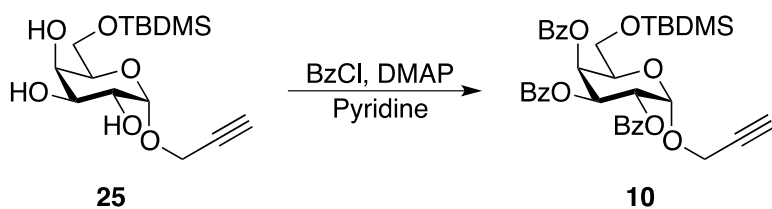
mixture was quenched by satd NaHCO₃, extracted by DCM (100 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by chromatography column (20:15:3, Hex-DCM–EtOAc) to give **9** (0.61 g, 48.2%) as a glassy solid. m.p. 138 – 140 °C. $[\alpha]_D^{21} = +120.4$ ($c = 1.00$, CHCl₃). $R_f = 0.30$ (3:1, hexane–EtOAc). ¹H NMR (CDCl₃, 500 MHz) δ 8.12 – 7.98 (m, 5H), 7.63 – 7.35 (m, 10H), 5.76 (m, 2H), 5.52 (d, $J = 3.6$ Hz, 1H), 4.69 (dd, $J = 11.4, 6.2$ Hz, 1H), 4.57 (dd, $J = 11.4, 6.7$ Hz, 1H), 4.44 (m, 2H), 4.34 (d, $J = 2.4$ Hz, 2H), 2.35 (t, $J = 2.4$ Hz, 1H). ¹³C NMR (CDCl₃, 125 MHz) δ 166.62, 166.20, 165.88, 133.84, 133.55, 133.44, 133.38, 130.32, 129.99, 129.93, 129.86, 129.70, 129.48, 129.38, 128.60, 128.49, 95.70, 78.60, 75.23, 70.83, 68.54 ($\times 2$), 68.18, 63.22, 55.50. HRMS ESI (m/z) ($M+Na$)⁺ calcd for C₃₀H₂₆O₉Na⁺ 553.1475, found 553.1475.



Scheme 3.12 Synthesis of propargyl 6-*O*-(*tert*-butyldimethylsilyl)- α -D-galactopyranoside **25**.

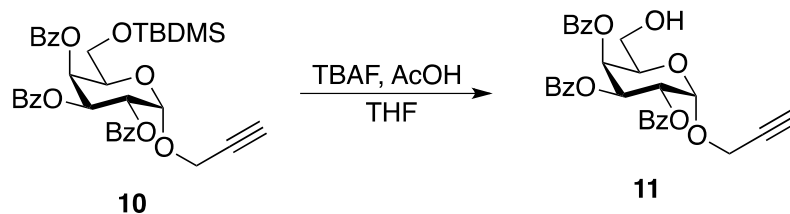
To a cold solution of **1** (3.84 g, 17.61 mmol) in anhydrous pyridine (30 mL), imidazole (2.40 g, 35.29 mmol) was added and reaction was stirred until all was dissolved. Then, *tert*-butylchlorodimethylsilane (3.18 g, 21.10 mmol) was added, and the mixture was stirred at room temperature for 24 h under nitrogen gas protection. The mixture was poured into ice/water. The crystalline precipitate was thoroughly washed with cold water and hexane to obtain the product **25** (3.78 g, 64.6%). ¹³C NMR (CD₃OD, 75 MHz): δ : 97.13 (C-1), 78.53 (CH₂–C $\equiv$ CH), 74.65 (CH₂–C $\equiv$ CH), 71.39, 70.10, 69.30,

68.54 (C-2 to C-5), 62.23 (C-6), 53.60 (CH₂-C≡CH), 24.92 (C(CH₃)₃), 17.69 (-C(CH₃)₃), -6.59 (2) (-Si(CH₃)₂).



Scheme 3.13 Synthesis of propargyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldimethylsilyl)- α -D-galactopyranoside **10**.

To a solution of **25** (3.21 g, 9.67 mmol) in dry pyridine (40 mL), benzoyl chloride (6.74 mL, 58.02 mmol) and a catalytic amount of DMAP was added. The mixture was stirred at room temperature for overnight. EtOH (10 mL) was added to quench the reaction and the solution was concentrated on high vacuum and diluted by DCM. It was washed by 1M HCl (2 x 50 mL) and brine (2 x 60 mL), dried over anhydrous Na₂SO₄ to get the organic layer, which was purified by silica gel chromatography (10:1, hexane–EtOAc) to give **10** (3.93 g, 63.1%) as colorless syrup. R_f = 0.21 (10:1, hexane–EtOAc) ¹H NMR (CDCl₃, 500 MHz): δ 8.09 (m, 2H), 8.00 (m, 2H), 7.81 (m, 2H), 7.61 (m, 1H), 7.50 (m, 3H), 7.42 (m, 1H), 7.37 (m, 2H), 7.24 (m, 2H), 6.00 (m, 2H), 5.69 (ddd, J = 11.8, 3.8, 1.6 Hz, 1H), 5.62 (d, J = 3.7 Hz, 1H, H-1), 4.37 (m, 3H), 3.78 (m, 2H), 2.37 (t, J = 2.4 Hz, 1H, CH₂-C≡CH), 0.86 (s, 9H, -C(CH₃)₃), 0.01, -0.03 (2s, 2×3H, -Si(CH₃)₂). ¹³C NMR (CDCl₃, 125 MHz): δ 166.20, 165.63, 165.53 (3×COPh), 133.42, 133.38, 133.12, 129.97, 129.92, 129.83, 129.75, 129.46, 129.42, 128.69, 128.48, 128.30, 95.30 (C-1), 78.54 (CH₂-C≡CH), 75.19 (CH₂-C≡CH), 70.19, 69.33, 69.04, 68.70 (C-2 to C-5), 61.28 (C-6), 55.30 (CH₂-C≡CH), 25.87 (-C(CH₃)₃), 18.26 (-C(CH₃)₃), -5.46, -5.51 (-Si(CH₃)₂). MALDI-TOFMS: calcd for C₃₆H₄₀O₉Si·Na⁺: 667.2334; found: m/z 667.2339.



Scheme 3.14 Synthesis of propargyl 2,3,4-tri-*O*-benzoyl- α -D-galactopyranoside **11**.

A solution of **10** (3.78 g, 5.87 mmol) and glacial acetic acid (1.00 mL, 17.48 mmol) in dry THF (20 mL) was treated with 1 M TBAF (11.74 mL, 11.74 mmol) in THF at room temperature for overnight. After concentration, the reaction mixture was quenched by water, extracted by DCM (300 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by chromatography column (30:1, DCM–EtOAc) to give **11** (2.61 g, 83.8%) as an amorphous white foam. m.p. 59 – 61 °C. $[\alpha]_D^{21} = +264.3$ ($c = 1.00$, CHCl₃). $R_f = 0.27$ (2:1, hexane–EtOAc). ¹H NMR (CDCl₃, 500 MHz) δ 8.03 (m, 2H), 7.92 (m, 2H), 7.73 (m, 2H), 7.55 (ddt, $J = 8.8, 7.2, 1.3$ Hz, 1H), 7.42 (m, 3H), 7.32 (m, 2H), 7.17 (m, 2H), 5.90 (dd, $J = 10.8, 3.4$ Hz, 1H), 5.81 (dd, $J = 3.5, 1.1$ Hz, 1H), 5.70 (dd, $J = 10.7, 3.8$ Hz, 1H), 5.53 (d, $J = 3.7$ Hz, 1H), 4.34 (m, 1H), 4.28 (dd, $J = 2.4, 1.3$ Hz, 2H), 3.70 (dt, $J = 11.8, 6.9$ Hz, 1H), 3.57 (dt, $J = 11.8, 6.8$ Hz, 1H), 2.45 (t, $J = 7.0$ Hz, 1H), 2.31 (t, $J = 2.4$ Hz, 1H). ¹³C NMR (CDCl₃, 125 MHz) δ 166.87, 166.25, 165.57, 133.91, 133.50, 133.34, 130.16, 130.00, 129.81, 129.34, 129.24, 129.04, 128.82, 128.55, 128.42, 95.67, 78.56, 75.40, 70.10, 70.00, 69.22, 68.48, 60.88, 55.77.

(3) General glycosylation procedure.

Method A: The galactosyl donor **12** (1.2 equiv) and acceptor (1.0 equiv) were dissolved in dry CH₂Cl₂ (25 mL), followed by adding pre-activated 4 Å molecular sieves powder and stirring for 1 h under nitrogen at room temperature. Then the mixture was cooled to 0

°C and added NIS (2.0 equiv) and TfOH (0.2 equiv) which made a purple solution. After the acceptor was completely consumed on TLC analysis, the mixture was filtered through Celite, washed with CH₂Cl₂ for twice, and the combined filtrate was washed with satd NaHCO₃ aq., satd Na₂S₂O₃ aq. and brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo.

Method B: A mixture of galactosyl acceptor (1.0 equiv) and activated 4Å molecular sieves in CH₂Cl₂ (0.05 M) was stirred for 1 h under nitrogen at room temperature, followed by cooled to -20 °C and the addition of NIS (2.0 equiv) and TfOH (0.2 equiv). A solution of thiophenol donor **12** (1.2 equiv) in CH₂Cl₂ (0.25 M) was added dropwise to the reaction mixture. After stirring for 30 min, the reaction mixture was warmed to room temperature. When the acceptor was completely consumed on TLC analysis, the mixture was filtered through Celite, washed with CH₂Cl₂ for twice, and the combined filtrate was washed with satd NaHCO₃ aq., satd Na₂S₂O₃ aq. and brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo.

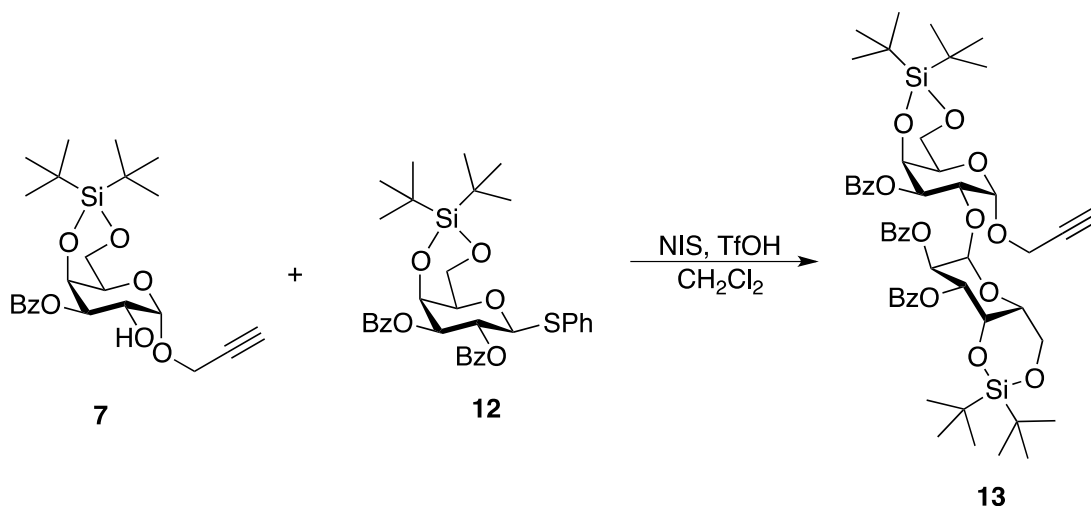
(4) General procedure for di-*tert*-butylsilylidene group removal.

A solution of saccharide (1 equiv) and glacial acetic acid (7 equiv) in dry THF (10.0 mL) was treated with 1 M tetrabutylammonium fluoride (TBAF) (10 equiv) in THF at room temperature for overnight. After concentration, the reaction mixture was quenched by water, extracted by DCM (100 mL), dried over anhydrous Na₂SO₄ and concentrated.

(5) General procedure for debenzoylation.

Benzoylated saccharide was dissolved in dry MeOH (20 mL), followed by adding NaOMe in small portion to afford pH 9. After overnight stirring, the solution was

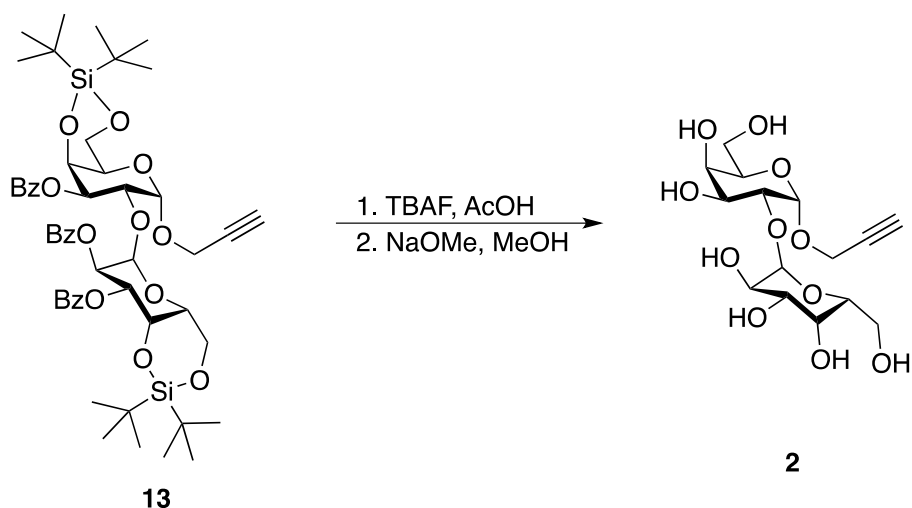
quenched by Amberlite[®] IR 120 (H⁺). The resin was filtered off and the solvent was removed in vacuo.



Scheme 3.15 Synthesis of propargyl 3-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl-2-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside **13**.

Compound **13** was synthesized according to the general glycosylation method B above. The crude product was purified by silica gel chromatography (20:3:1, hexane–CH₂Cl₂–EtOAc) to give **13** (238 mg, 58.3 %) as a white amorphous foam. R_f = 0.42 (10:3:1, hexane–CH₂Cl₂–EtOAc). $[\alpha]_D^{21}$ = +190.9 (c = 1.00, CHCl₃). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.06 (m, 4H, Ar-H), 7.96 (m, 2H, Ar-H), 7.59 (m, 1H, Ar-H), 7.54 – 7.47 (m, 4H, Ar-H), 7.42 – 7.34 (m, 4H, Ar-H), 5.65 (dd, J = 10.5, 3.6 Hz, 1H, H-2'), 5.50 (d, J = 3.8 Hz, 1H, H-1'), 5.48 (m, 1H, H-3'), 5.42 (dd, J = 10.3, 3.2 Hz, 1H, H-3), 5.12 (d, J = 3.6 Hz, 1H, H-1), 4.76 (dd, J = 3.3, 1.1 Hz, 1H, H-4), 4.64 (dd, J = 3.1, 1.1 Hz, 1H, H-4'), 4.43 (dd, J = 10.3, 3.6 Hz, 1H, H-2), 4.25 (dd, J = 12.7, 2.2 Hz, 1H, H-6_{a/b}), 4.14 (dd, J = 12.7, 1.7 Hz, 1H, H-6_{a/b}'), 4.05 (dd, J = 12.5, 1.5 Hz, 1H, H-6'_{a/b}), 3.96 (q, J = 1.5 Hz, 1H, H-5'), 3.95 – 3.87 (m, 4H, H-5, H-6'_{a/b}, CH₂–C $\equiv$ CH), 2.28 (t, J = 2.4

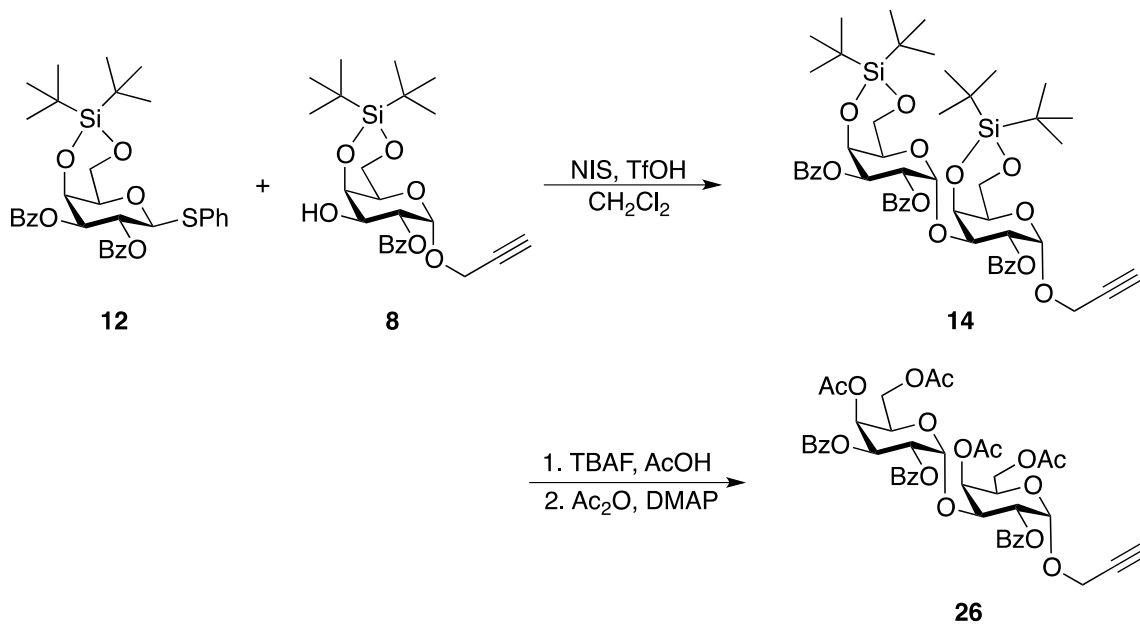
Hz, 1H, CH₂-C≡CH), 1.04, 1.02, 0.92, 0.87 (4s, 36H, 4 CH₃-tBu-Si). ¹³C NMR (151 MHz, Chloroform-d) δ 166.42, 166.22, 166.06 (3 C=O_{Bz}), 133.42, 133.30, 133.13, 130.54, 130.07, 129.78, 129.64, 129.62, 128.66, 128.57, 128.44, (Ar-C), 96.15 (C-1), 95.78 (C-1'), 78.88 (CH₂-C≡CH), 74.79 (CH₂-C≡CH), 72.02 (C-3), 71.50 (C-2), 71.05 (C-4), 71.01 (C-3'), 70.92 (C-4'), 69.04 (C-2'), 67.40 (C-5), 67.27 (C-5'), 66.96 (C-6'), 66.82 (C-6), 55.32 (CH₂-C≡CH), 27.59, 27.56, 27.35, 27.34 (4 CH₃-tBu-Si), 23.36, 23.31, 20.80, 20.79 (4 C_q-tBu-Si). MALDI-TOFMS: calcd for C₅₂H₆₈O₁₄Si₂·Na⁺: 995.4040; found: *m/z* 995.4038.



Scheme 3.16 Synthesis of propargyl 2-O-(α-D-galactopyranosyl)-α-D-galactopyranoside **2**.

The crude product was purified by silica gel chromatography (4:1, EtOAc–MeOH) to give **2** as a white powdery solid (49 mg, 84.2 %). *R*_f = 0.44 (1:1, EtOAc–MeOH). [α]_D²⁰ = 84.0 (*c* = 1.00, CH₃OH). ¹H NMR (600 MHz, Methanol-d₄) δ 5.31 (d, *J* = 3.7 Hz, 1H, H-1), 5.11 (d, *J* = 3.8 Hz, 1H, H-1'), 4.37 (t, *J* = 2.2 Hz, 2H, CH₂-C≡CH), 4.18 (m, 1H, H-5'), 4.07 (dd, *J* = 10.1, 3.7 Hz, 1H, H-2), 4.00 (m, 1H, H-4), 3.96 (dd, *J* = 3.3, 1.2 Hz, 1H, H-4'), 3.93 (dd, *J* = 10.1, 3.4 Hz, 1H, H-3), 3.89 – 3.86 (m, 2H, H-5, H-

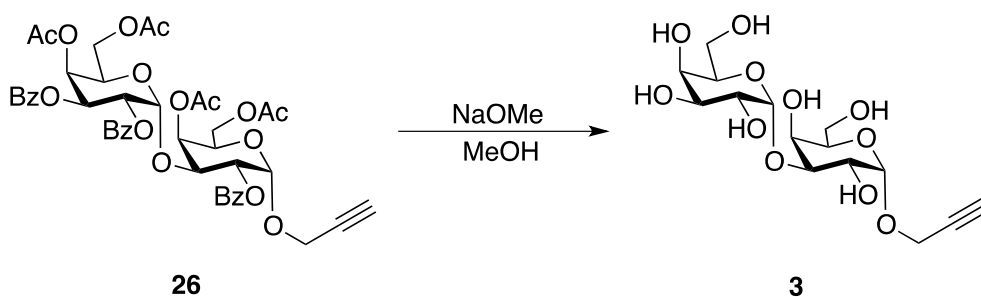
3'), 3.83 – 3.74 (m, 5H, H-6_{a,b}, H-2', H-6'_{a,b}) 2.92 (t, $J = 2.4$ Hz, 1H, CH₂-C≡CH). ¹³C NMR (151 MHz, Methanol-d₄) δ 98.08 (C-1'), 96.13 (C-1), 80.00 (CH₂-C≡CH), 76.28 (CH₂-C≡CH), 74.25 (C-2), 72.76 (C-5), 72.53 (C-5'), 71.47 (C-3'), 71.17 (C-4'), 71.13 (C-4), 70.33 (C-2'), 69.76 (C-3), 62.90 (C-6'), 62.61 (C-6), 55.34 (CH₂-C≡CH). HRMS ESI (m/z) (M+Na)⁺ calcd for C₁₅H₂₄O₁₁Na⁺ 403.1216, found 403.1218.



Scheme 3.17 Synthesis of propargyl 2-*O*-benzoyl-4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside **26**.

Compound **14** was synthesized according to the general glycosylation method B above. After removal the di-*tert*-butylsilylidene group, the reaction mixture was dissolved in dry pyridine (10 mL), followed by adding acetic anhydride (6 equiv) and a catalytic amount of DMAP. The mixture was stirred at room temperature for overnight. Toluene (3×25 mL) was added and the solution was concentrated, and it was extracted with CH₂Cl₂ (150 mL) and satd NaHCO₃ aq. The organic layer was then separated and washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude product

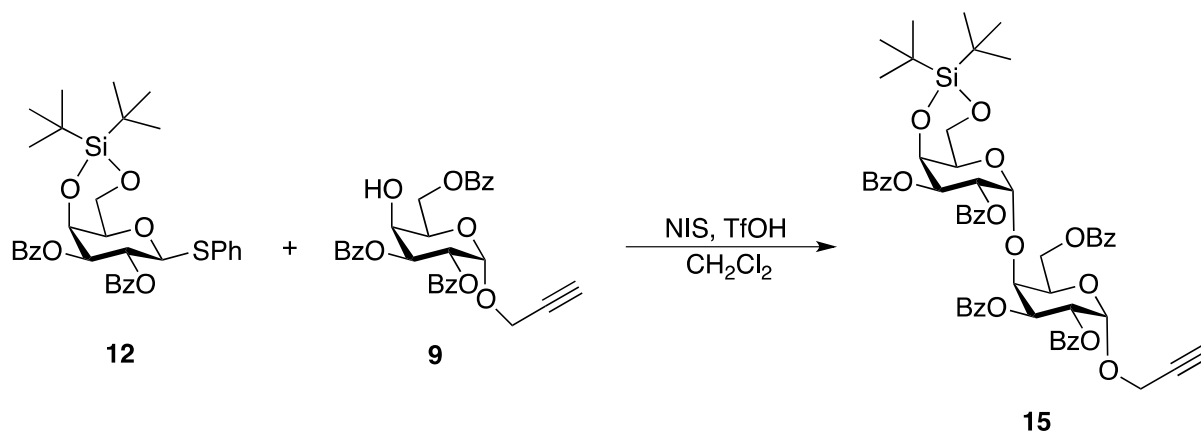
was purified by silica gel chromatography (3:1, hexane–EtOAc) to give **26** (621 mg, 79.4 %) as a white crystal. m.p. 148 – 150 °C. R_f = 0.36 (1.5:1, hexane–EtOAc). $[\alpha]_D^{20}$ = +234.6 (c = 1.00, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 8.12 (m, 2H), 7.97 (m, 2H), 7.78 (m, 2H), 7.62 (m, 1H), 7.51 – 7.27 (m, 8H), 5.61 (dd, J = 10.9, 3.5 Hz, 1H, H-2'), 5.55 – 5.47 (m, 4H, H-2, H-4, H-1', H-3'), 5.39 (d, J = 3.8 Hz, 1H, H-1), 5.24 (dd, J = 3.4, 1.3 Hz, 1H, H-4'), 4.55 (dd, J = 10.7, 3.1 Hz, 1H, H-3), 4.44 (ddd, J = 7.7, 4.9, 1.4 Hz, 1H, H-5'), 4.25 (d, J = 2.5 Hz, 2H, $\text{CH}_2\text{-C}\equiv\text{CH}$), 4.23 (m, 1H, H-5), 4.13 – 4.01 (m, 4H, H-6_{a,b}, H-6'_{a,b}), 2.32 (t, J = 2.3 Hz, 1H, $\text{CH}_2\text{-C}\equiv\text{CH}$), 2.11, 2.08, 2.02, 1.36 (4s, 12H, 4 $\times\text{COCH}_3$). ^{13}C NMR (126 MHz, Chloroform- d) δ 170.46, 170.45, 169.94, 169.92 (4 C=O_{Ac}), 166.02, 165.91, 165.12 (3 C=O_{Bz}), 133.74, 133.33, 133.27, 130.05, 129.77, 129.64, 129.61, 129.52, 129.23, 128.77, 128.45, 128.44 (Ar-C), 95.68 (C-1), 92.57 (C-1'), 78.47 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 75.26 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 69.15 (C-2), 68.63 (C-3), 68.36 (C-4'), 67.86 (C-2'), 67.72 (C-3'), 67.23 (C-5), 67.20 (C-5'), 65.42 (C-4), 62.23 (C-6'), 61.69 (C-6), 55.67 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 20.87, 20.83, 20.66, 19.64 (4 $\times\text{COCH}_3$). MALDI-TOFMS: calcd for $\text{C}_{44}\text{H}_{44}\text{O}_{18}\cdot\text{Na}^+$: 883.2420; found: m/z 883.2423.



Scheme 3.18 Synthesis of propargyl 3-O-(α -D-galactopyranosyl)- α -D-galactopyranoside **3**.

The crude product was purified by silica gel chromatography (4:1, EtOAc–MeOH) to give **3** as a white powdery solid (154 mg, 76.6 %). R_f = 0.48 (1:1, EtOAc–

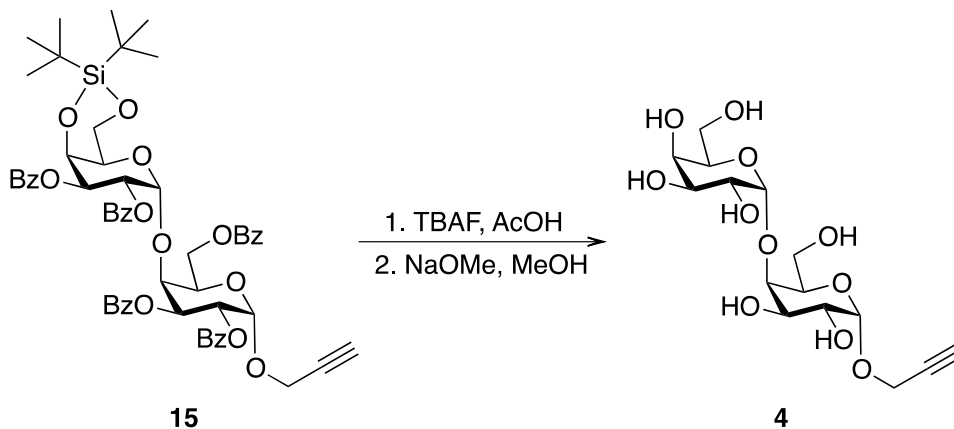
MeOH). $[\alpha]_D^{24} = 265.8$ ($c = 1.00$, CH₃OH). ¹H NMR (600 MHz, Deuterium oxide) δ 5.18 (d, $J = 3.6$ Hz, 1H, H-1), 5.17 (d, $J = 3.9$ Hz, 1H, H-1'), 4.37 (m, 2H, CH₂-C $\equiv$ CH), 4.27 (m, 1H, H-4), 4.19 (m, 1H, H-5'), 4.04 – 3.95 (m, 5H, H-2, H-3, H-5, H-3', H-4'), 3.88 (dd, $J = 10.4, 3.9$ Hz, 1H, H-2'), 3.79 – 3.75 (m, 4H, H-6_{a,b}, H-6'_{a,b}), 2.92 (t, $J = 2.4$ Hz, 1H, CH₂-C $\equiv$ CH). ¹³C NMR (126 MHz, Deuterium oxide) δ 97.16 (C-1), 94.94 (C-1'), 78.90 (CH₂-C $\equiv$ CH), 75.90 (CH₂-C $\equiv$ CH), 74.03 (C-2), 70.99 (C-3'), 70.82 (C-5'), 69.22 (C-3), 69.12 (C-4'), 68.13 (C-2'), 66.28 (C-5), 65.29 (C-4), 60.95 (C-6'), 60.89 (C-6), 54.71 (CH₂-C $\equiv$ CH). HRMS ESI (m/z) ($M+Na$)⁺ calcd for C₁₅H₂₄O₁₁Na⁺ 403.1216, found 403.1218.



Scheme 3.19 Synthesis of propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butyl-silanediy)- α -D-galactopyranosyl)- α -D-galactopyranoside **15**.

Compound **15** was synthesized according to the general glycosylation method B above. The crude product was purified by silica gel chromatography (10:1, hexane–EtOAc) to give **15** (693 mg, 52.9 %) as a white amorphous foam. $R_f = 0.46$ (3:1, hexane–EtOAc). $[\alpha]_D^{21} = +207.4$ ($c = 1.00$, CHCl₃). ¹H NMR (500 MHz, Chloroform-*d*) δ 8.06 – 7.98 (m, 8H), 7.87 – 7.85 (m, 2H), 7.56 – 7.49 (m, 4H), 7.42 – 7.35 (m, 9H), 7.30 (m, 2H), 5.91 (dd, $J = 10.8, 3.6$ Hz, 1H, H-2'), 5.78 (m, 2H, H-2, H-3), 5.75 (dd, $J = 10.8, 3.0$

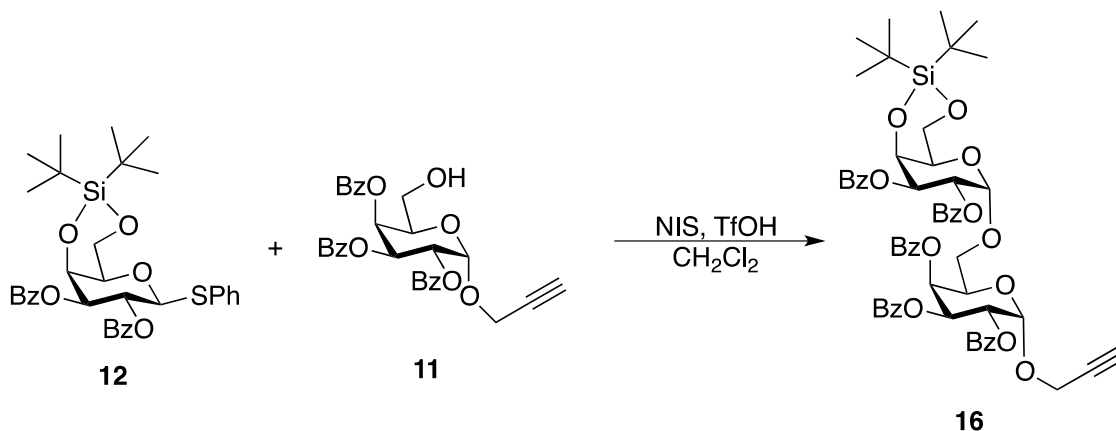
Hz, 1H, H-3'), 5.51 (d, $J = 3.7$ Hz, 1H, H-1'), 5.48 (d, $J = 3.6$ Hz, 1H, H-1), 4.94 (dd, $J = 3.1, 1.1$ Hz, 1H, H-4'), 4.68 (dd, $J = 10.7, 6.3$ Hz, 1H, H-6_a), 4.48 (m, 1H, H-4), 4.38 (m, 1H, H-5), 4.34 – 4.31 (m, 4H, H-5', H-6_b, CH₂-C≡CH), 3.84 (qd, $J = 12.9, 1.9$ Hz, 2H, H-6'_{a,b}), 2.35 (t, $J = 2.4$ Hz, 1H, CH₂-C≡CH), 1.08 (s, 9H, CH₃-*t*Bu-Si), 0.93 (s, 9H, CH₃-*t*Bu-Si). ¹³C NMR (126 MHz, Chloroform-*d*) δ 166.40, 166.27, 166.15, 166.05, 165.67 (5 C=O_{Bz}), 133.66, 133.45, 133.40, 133.25, 133.20, 130.07, 130.01, 129.99, 129.98, 129.85, 129.76, 129.52, 129.40, 129.34, 129.07, 128.75, 128.59, 128.49, 128.48 ($\times 2$) (Ar-C), 98.73 (C-1'), 95.84 (C-1), 78.58 (CH₂-C≡CH), 76.54 (C-4), 75.27 (CH₂-C≡CH), 71.37 (C-4'), 71.02 (C-3'), 70.19 (C-2), 69.11 (C-2'), 69.04 (C-5), 68.37 (C-3), 68.15 (C-5'), 66.74 (C-6'), 61.87 (C-6), 55.50 (CH₂-C≡CH), 27.60, 27.36 (2 CH₃-*t*Bu-Si), 23.35 (C_q-*t*BuSi), 20.85 (C_q-*t*BuSi). MALDI-TOFMS: calcd for C₅₈H₆₀O₁₆Si·Na⁺: 1063.3543; found: m/z 1063.3543.



Scheme 3.20 Synthesis of propargyl 4-O-(α -D-galactopyranosyl)- α -D-galactopyranoside **4**.

The crude product was purified by silica gel chromatography (4:1, EtOAc–MeOH) to give **4** as a white powdery solid (163 mg, 84.0 %). $R_f = 0.50$ (1:1, EtOAc–MeOH). $[\alpha]_D^{20} = 183.9$ ($c = 1.00$, CH₃OH). ¹H NMR (500 MHz, Methanol-*d*₄) δ 5.06 (d,

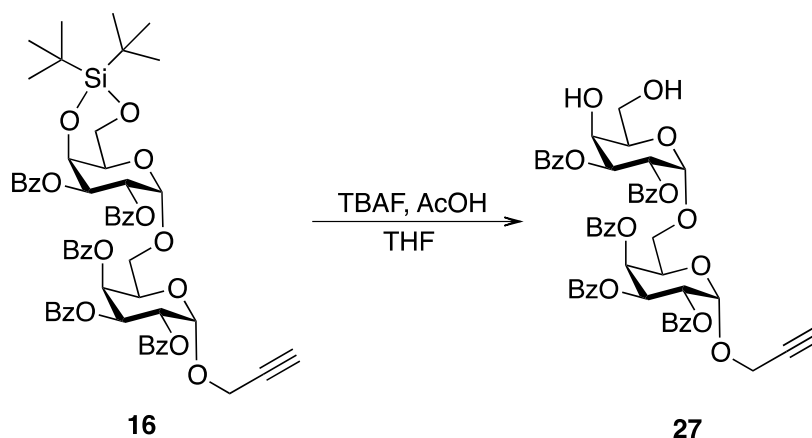
$J = 3.7$ Hz, 1H, H-1), 4.99 (d, $J = 3.8$ Hz, 1H, H-1'), 4.30 (dd, $J = 2.5, 0.9$ Hz, 2H, $\text{CH}_2\text{-C}\equiv\text{CH}$), 4.21 (ddd, $J = 6.8, 5.1, 1.3$ Hz, 1H, H-5'), 4.06 (m, 1H, H-4), 3.92 (m, 2H, H-4', H-5), 3.86 – 3.68 (m, 8H, H-2, H-3, H-6_{a,b}, H-2', H-3', H-6'_{a,b}), 2.88 (t, $J = 2.4$ Hz, 1H, $\text{CH}_2\text{-C}\equiv\text{CH}$). ^{13}C NMR (126 MHz, Methanol- d_4) δ 102.75 (C-1'), 99.16 (C-1), 81.18 (C-4), 80.15 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 76.07 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 73.01 (C-5'), 72.39 (C-5), 71.18 (C-2'), 71.12 (C-2), 70.96 (C-4'), 70.49 (C-3), 70.30 (C-3'), 62.66 (C-6'), 61.40 (C-6), 55.66 ($\text{CH}_2\text{-C}\equiv\text{CH}$). HRMS ESI (m/z) ($\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{15}\text{H}_{24}\text{O}_{11}\text{Na}^+$ 403.1216, found 403.1216.



Scheme 3.21 Synthesis of propargyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butyl-silanediy)- α -D-galactopyranosyl)- α -D-galactopyranoside **16**.

Compound **16** was synthesized according to the general glycosylation method A above. The crude product was purified by silica gel chromatography (16:3:1, hexane–DCM–EtOAc) to give **16** (2.64 g, 64.0 %) as an amorphous white foam. $R_f = 0.33$ (4:1, hexane–EtOAc). m.p. 106 – 108 °C. $[\alpha]_D^{21} = +233.6$ ($c = 1.00$, CHCl_3). ^1H NMR (500 MHz, Chloroform- d) δ 8.03 – 7.98 (m, 7H), 7.76 (dd, $J = 8.3, 1.4$ Hz, 2H), 7.60 (m, 1H), 7.52 – 7.36 (m, 13H), 7.22 (m, 2H), 5.94 (dd, $J = 10.6, 3.4$ Hz, 1H), 5.89 (dd, $J = 3.4, 1.3$ Hz, 1H), 5.78 (dd, $J = 10.6, 3.6$ Hz, 1H), 5.70 (dd, $J = 10.7, 3.7$ Hz, 1H), 5.60 (d, $J = 3.7$

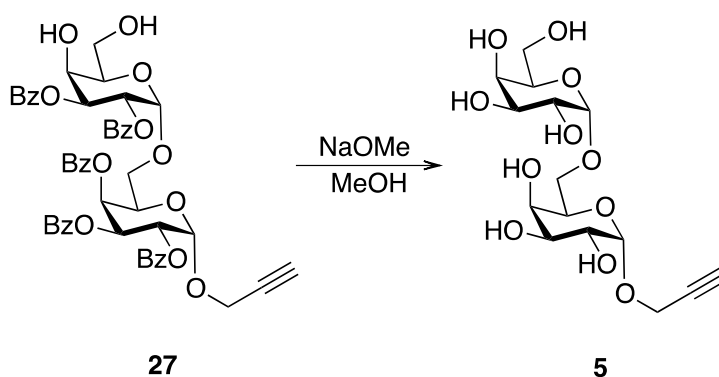
Hz, 1H), 5.58 (dd, $J = 10.6, 3.1$ Hz, 1H), 5.27 (d, $J = 3.6$ Hz, 1H), 4.90 (dd, $J = 3.1, 1.1$ Hz, 1H), 4.53 (ddd, $J = 7.3, 4.6, 1.3$ Hz, 1H), 4.39 – 4.31 (m, 3H), 4.27 (dd, $J = 12.7, 1.7$ Hz, 1H), 4.08 – 4.07 (m, 1H), 3.89 (dd, $J = 10.7, 7.5$ Hz, 1H), 3.69 (dd, $J = 10.7, 4.4$ Hz, 1H), 2.44 (t, $J = 2.4$ Hz, 1H), 1.12 (s, 9H), 0.98 (s, 9H). ^{13}C NMR (126 MHz, Chloroform- d) δ 166.31, 166.22, 166.20, 165.54, 165.53 (5 $\text{C}=\text{O}_{\text{Bz}}$), 133.66, 133.47, 133.31, 133.22, 133.19, 130.05, 130.04, 130.01, 129.94, 129.85, 129.81, 129.62, 129.38, 129.30, 129.24, 128.75, 128.54, 128.53, 128.48, 128.34 (Ar-C), 97.08, 95.32 (C-1 and C-1'), 78.47, 75.56, 71.34, 71.06, 69.63, 69.07, 68.74, 68.62, 68.49, 67.46, 67.14, 67.07, 55.52 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 27.68, 27.39 (2 $\text{CH}_3\text{-}t\text{Bu-Si}$), 23.45 ($\text{C}_{q\text{-}t\text{BuSi}}$), 20.87 ($\text{C}_{q\text{-}t\text{BuSi}}$). MALDI-TOFMS: calcd for $\text{C}_{58}\text{H}_{60}\text{O}_{16}\text{Si}\cdot\text{Na}^+$: 1063.3543; found: m/z 1063.3543.



Scheme 3.22 Synthesis of propargyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3-di-*O*-benzoyl- α -D-galactopyranosyl)- α -D-galactopyranoside **27**.

The crude product was purified by chromatography column (5:1, DCM–EtOAc) to give **27** (1.90 g, 91.5 %) as an amorphous white foam. m.p. 102 – 104 °C. $[\alpha]_{\text{D}}^{21} = +237.4$ ($c = 1.00$, CHCl_3). $R_f = 0.36$ (1:1.5, hexane–EtOAc). ^1H NMR (500 MHz, Chloroform- d) δ 8.02 – 7.94 (m, 7H), 7.75 (m, 2H), 7.59 (m, 1H), 7.52 – 7.32 (m, 13H), 7.22 (m, 2H), 5.94 (m, 2H), 5.73 – 5.65 (m, 3H), 5.60 (d, $J = 3.7$ Hz, 1H), 5.29 (d, $J = 3.5$

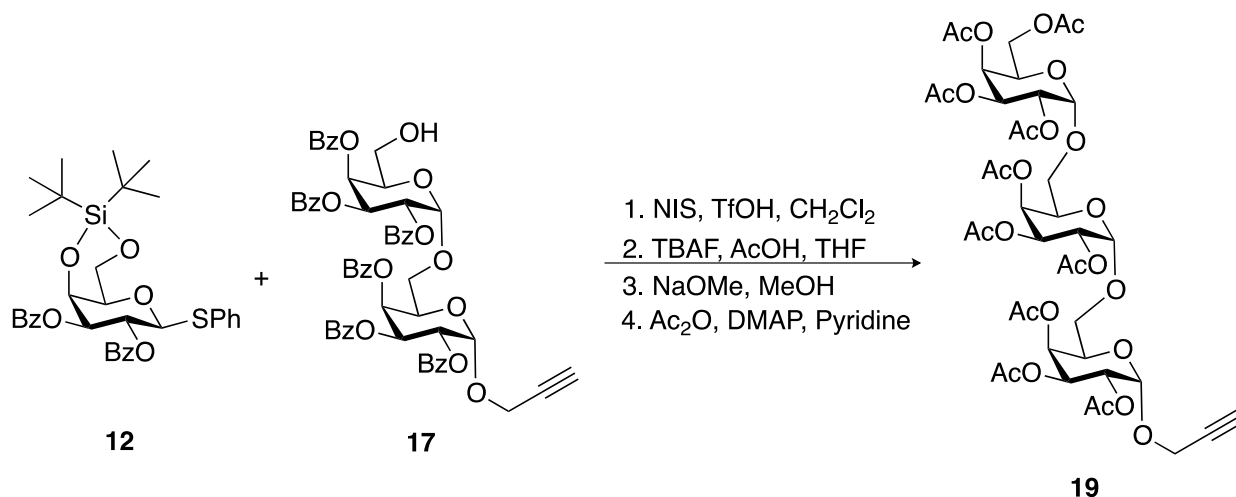
Hz, 1H), 4.58 (ddd, $J = 6.8, 5.5, 1.3$ Hz, 1H), 4.49 (td, $J = 2.7, 1.2$ Hz, 1H), 4.41 – 4.31 (qd, $J = 16.0, 2.4$ Hz, 2H), 4.21 (td, $J = 4.7, 1.4$ Hz, 1H), 4.03 – 3.95 (m, 1H), 3.89 (dd, $J = 10.7, 7.0$ Hz, 1H), 3.72 (dd, $J = 10.7, 5.4$ Hz, 1H), 3.11(d, $J = 3.0$ Hz, 1H), 2.55 (m, 1H), 2.49 (t, $J = 2.4$ Hz, 1H). ^{13}C NMR (126 MHz, Chloroform-d) δ 166.26, 166.18, 165.86, 165.65, 165.56 (5 $\text{C}=\text{O}_{\text{Bz}}$), 133.63, 133.46, 133.43, 133.31, 133.23, 130.03, 129.99, 129.94, 129.91, 129.80, 129.51, 129.44, 129.34, 129.26, 129.22, 128.74, 128.55, 128.51, 128.50, 128.32 (Ar-C), 97.31, 95.63 (C-1 and C-1'), 78.58, 77.41, 77.16, 76.91, 75.62, 71.11, 69.80, 69.79, 69.46, 69.05, 68.76, 68.61, 68.48, 67.23, 63.26, 55.77($\text{CH}_2\text{-C}\equiv\text{CH}$). MALDI-TOFMS: calcd for $\text{C}_{50}\text{H}_{44}\text{O}_{16}\cdot\text{Na}^+$: 923.2522; found: m/z 923.2526.



Scheme 3.23 Synthesis of propargyl 6-O-(α -D-galactopyranosyl)- α -D-galactopyranoside **5**.

The crude product was purified by chromatography column (4:1, EtOAc–MeOH) to give **5** (543 mg, 80.4%) as a white powdery solid. $R_f = 0.43$ (1:1, EtOAc–MeOH). $[\alpha]_{\text{D}}^{20} = +160.6$ ($c = 1.00$, CH_3OH). ^1H NMR (500 MHz, Methanol- d_4) δ 5.03 (d, $J = 3.8$ Hz, 1H, H-1), 4.87 (d, $J = 3.7$ Hz, 1H, H-1'), 4.29 (t, $J = 2.4$ Hz, 2H, $\text{CH}_2\text{-C}\equiv\text{CH}$), 4.00 (m, 1H, H-5), 3.96 (dd, $J = 3.3, 1.2$ Hz, 1H, H-4), 3.91 – 3.85 (m, 3H, H-4', H-5', H-6_a), 3.81 – 3.70 (m, 6H, H-2, H-2', H-3, H-3', H-6'_a, H-6'_b), 3.65 (dd, $J = 10.1, 6.0$ Hz, 1H,

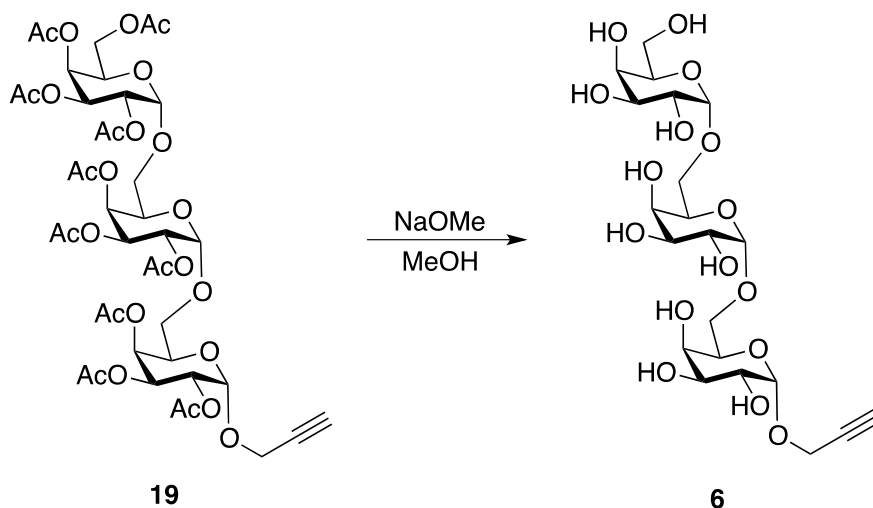
H-6_b), 2.89 (t, $J = 2.4$ Hz, 1H, $\text{CH}_2\text{-C}\equiv\text{CH}$). ^{13}C NMR (126 MHz, Methanol- d_4) δ 100.33 (C-1'), 98.89 (C-1), 80.13 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 76.17 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 72.39 (C-5'), 71.51 (C-3'), 71.25 (C-2'), 71.09 (C-4'), 70.76 (C-4), 70.66 (C-5), 70.19 (C-3), 69.98 (C-2), 67.62 (C-6), 62.71 (C-6'), 55.46 ($\text{CH}_2\text{-C}\equiv\text{CH}$). HRMS ESI (m/z) ($\text{M}+\text{Na}$) $^+$ calcd for $\text{C}_{15}\text{H}_{24}\text{O}_{11}\text{Na}^+$ 403.1216, found 403.1216.



Scheme 3.24 Synthesis of propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside **19**.²⁵

Compound **19** was synthesized according to the general glycosylation method A above. The crude product was purified by silica gel chromatography (4:1, hexane–EtOAc). It was deprotected, acetylated, and purified again by silica gel chromatography (1:1.5, hexane–EtOAc) to give **19** (153 mg, 42.8%) as colorless oil. $R_f = 0.29$ (1:1.5, hexane–EtOAc). $[\alpha]_D^{21} = 165.7$ ($c = 1.00$, CHCl_3). ^1H NMR (600 MHz, Chloroform- d) δ 5.51 – 5.45 (m, 3H, H-3, H-4'', H-4'), 5.34 (dd, $J = 10.9, 3.3$ Hz, 1H, H-3'), 5.28 (m, 1H, H-3''), 5.26 (d, $J = 3.8$ Hz, 1H, H-1), 5.16 (d, $J = 3.5$ Hz, 1H, H-1'), 5.14 – 5.12 (m, 2H, H-4, H-2''), 5.10 (dd, $J = 10.9, 3.6$ Hz, 1H, H-2'), 4.95 (d, $J = 3.5$ Hz, 1H, H-1''), 4.89

(dd, $J = 10.3, 3.7$ Hz, 1H, H-2), 4.31 – 4.25 (m, 4H, $\text{CH}_2\text{-C}\equiv\text{CH}$, H-5'', H-5'), 4.19 (dd, $J = 11.3, 5.6$ Hz, 1H, H-6''_{a/b}), 4.07 – 4.01 (m, 2H, H-5, H-6'_{a/b}), 3.75 (dd, $J = 11.5, 5.2$ Hz, 1H, H-6_{a/b}), 3.70 (dd, $J = 10.1, 6.4$ Hz, 1H, H-6'_{a/b}), 3.58 (dd, $J = 11.4, 2.3$ Hz, 1H, H-6_{a/b}), 3.40 (dd, $J = 10.1, 6.0$ Hz, 1H, H-6'_{a/b}), 2.57 (t, $J = 2.4$ Hz, 1H, $\text{CH}_2\text{-C}\equiv\text{CH}$), 2.13 ($\times 2$), 2.11, 2.07 ($\times 3$), 2.05, 2.01, 1.97($\times 2$) ($10\times\text{COCH}_3$). ^{13}C NMR (151 MHz, Chloroform-d) δ 170.70, 170.65, 170.60, 170.31, 170.25, 170.22, 170.16, 169.97, 169.93, 169.65 ($10\times\text{C=O}_{\text{Ac}}$), 96.36 (C-1'), 96.23 (C-1''), 94.42 (C-1), 78.35 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 75.76 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 70.57 (C-2), 70.18 (C-3), 69.12 (C-5), 68.97 (C-4), 68.57 (C-4'), 68.34 (C-4''), 68.23 (C-2'), 67.78 (C-3'), 67.75 (C-2''), 67.48 (C-3''), 67.32 (C-5'), 66.77 (C-5''), 66.21 (C-6), 65.88 (C-6'), 62.11 (C-6''), 55.45 ($\text{CH}_2\text{-C}\equiv\text{CH}$), 20.96, 20.86 ($\times 2$), 20.82 ($\times 3$), 20.79, 20.77, 20.76, 20.74 ($10\times\text{COCH}_3$). MALDI-TOFMS: calcd for $\text{C}_{41}\text{H}_{54}\text{O}_{26}\cdot\text{Na}^+$: 985.2796; found: m/z 985.2798.



Scheme 3.25 Synthesis of propargyl 6-O-(6-O- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside **6**.

The crude product was purified by chromatography column (1:1, EtOAc–MeOH) to give **6** (48 mg, 84.9%) as a white powdery solid. $R_f = 0.5$ (MeOH). $[\alpha]_D^{21} = 154.2$ ($c = 1.00$, CH₃OH). ¹H NMR (600 MHz, Deuterium Oxide) δ 5.13 (d, $J = 3.8$ Hz, 1H, H-1), 4.99 (2d, $J = 3.6$ Hz, 2H, H-1', H-1''), 4.37 (m, 2H, CH₂–C $\equiv$ CH), 4.19 (m, 1H, H-5'), 4.04 (m, 1H, H-4'), 4.01 – 3.98 (m, 3H, H-4, H-4'', H-6_{a/b}), 3.91 – 3.81 (m, 6H, H-3', H-3, H-6'_{a/b}, H-3'', H-2', H-2''), 3.75 – 3.67 (m, 5H, H-6''_{a,b}, H-6_{a/b}, H-6'_{a/b}, H-5), 3.62 (m, 1H, H-2), 3.53 (m, 1H, H-5''), 2.92 (m, 1H, CH₂–C $\equiv$ CH). ¹³C NMR (151 MHz, Deuterium oxide) δ 97.92 (C-1'', $J_{C1''-H1''} = 170.55$ Hz), 97.81 (C-1', $J_{C1'-H1'} = 169.56$ Hz), 97.00 (C-1, $J_{C1-H1} = 170.92$ Hz), 78.73 (CH₂–C $\equiv$ CH), 76.00 (CH₂–C $\equiv$ CH), 73.16 (C-5), 70.89 (C-4), 70.83 (C-2), 70.56 (C-3), 69.41 (C-3''), 69.38 (C-5''), 69.33 (C-3'), 69.25 (C-4'), 69.14 (C-4''), 68.68 (C-5'), 68.29 (C-2''), 68.19 (C-2'), 66.36 (C-6'), 65.26 (C-6), 61.05 (C-6''), 54.84 (CH₂–C $\equiv$ CH). HRMS ESI (m/z) ($M+Na$)⁺ calcd for C₂₁H₃₄O₁₆Na⁺ 565.1745, found 565.1748.

3.3 Conclusions

I have described straightforward syntheses of α -linked propargyl terminated galactosides with various spatial presentations. Flexible and efficient regioselective protection strategies were performed on propargyl galactoside to give the corresponding glycosyl acceptors. The high α -stereoselective glycosylations were accomplished with the usage of 4,6-di-*tert*-butylsilylene protected thiogalactoside donor. After mild deprotection sequences, the desired galactoside ligands were obtained in satisfied yield, and their structures were rigorously confirmed by using ¹H NMR, ¹³C NMR, 2D NMR, and MALDI-TOF mass spectra.

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Chapter 4 Construction of an α -Galactosides Featured Microarray and Evaluation of Its Lectin-Binding Properties

Abstract

A synthetic carbohydrate microarray with the immobilization of a collection of diversely presented galactoside clusters was constructed to imitate featured carbohydrate presentations on natural cell surface. The synthetic surface was interrogated by a fluorescence-labeled lectin to quantitatively analyze their binding affinities and dissociation constants. The lectin showed stronger binding affinities with disaccharides that possess the non-reducing terminal Gal- α -(1,3)-Gal, Gal- α -(1,4)-Gal and Gal- α -(1,6)-Gal aglycones. This study will add new dimensions to our understanding of the effects of spatial arrangement of carbohydrate ligands in carbohydrate–lectin binding, and shed light on elucidating the structure/affinity relationship of carbohydrates recognition with receptors.

4.1 Background

The glycocalyx, which is the thick layer of glycoconjugates covering both eukaryotic and prokaryotic cell membrane surfaces, forms the basis for molecular recognitions. Its interactions with lectins have been recognized to be critical in mediating biological activities such as cell–cell communication and adhesion, host–pathogen interactions and immune defense.¹ However, understanding the mechanism of carbohydrates–lectin interactions is arduous.² A major impediment is the complex structure of carbohydrates, which makes both chemical synthesis of the oligosaccharide structures involved and biochemical analysis of their interactions with recognition proteins highly challenging. Carbohydrate structures display tremendous diversity and informational storage capacity because many permutations of size, composition, connectivity, and spatial arrangement can be generated. Moreover, the multimeric architecture of lectins allows simultaneous binding to multiple carbohydrate ligands to generate multivalent binding types, while proper presentation of the carbohydrate ligands with matched spacing and orientation is necessary.^{3, 4}

In order to systematically investigate the molecular basis of carbohydrate–lectin interactions, carbohydrate microarrays have been developed as an imitation of natural glycosylated surfaces.⁵ Synthetic carbohydrate ligands, which can be acquired in sufficient amount and high purity, are made and immobilized on solid supports with a linker incorporated.⁶ Several carbohydrate microarrays have been constructed by immobilizing homo or hetero glycoclusters,⁷ multiplexed glycoconjugates,⁸ sequence–defined heteromultivalent glycooligomers,⁹ and glycoclusters with different valency,¹⁰ to generate multivalent ligand landscapes that can create adequately affinity with lectins;¹¹

yet, less emphasis has been put on the integration of spatial and chemical patterning of the synthetic glycan surface.¹²

Herein, I report a modular approach to constructing carbohydrate microarray by immobilizing various α -linked galactoside clusters to investigate the influence of spatial organization and site-specific ligand modifications on the recognition and binding with lectin. The synthesized α -linked galactoside ligands **1–6** (Figure 3.1) were coupled with the azido-PEG-biotin linker by click chemistry to afford the glycoconjugates **G1–G6**, respectively, which were immobilized to an avidin-coated glass slide to afford the carbohydrate microarray at defined densities. The synthetic carbohydrate landscape was interrogated by using fluorescence imaging technique to assess the recognition with *Griffonia Simplicifolia* Lectin I-isolectin B₄ (GSL I-B₄), a C-type lectin that shows high affinity to α -D-galactosyl end groups.^{13–15} C-Type lectins are Ca²⁺-dependent glycan-binding proteins, and the binding occurs primarily through the coordination of the calcium ion with the specific amino acid residues and the 3- and 4-hydroxyl groups on the sugar.¹⁶ 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.^{17, 18} Thus, how strongly a particular biological surface is recognized depends heavily on presentation, the subtleties of which the present work specifically seeks to explore.

4.2 Construction of Carbohydrate Microarray

The synthesized α -galactoside ligands **1–6** were used directly for the construction of microarray. The choice of the linker structure is governed by a combination of factors,

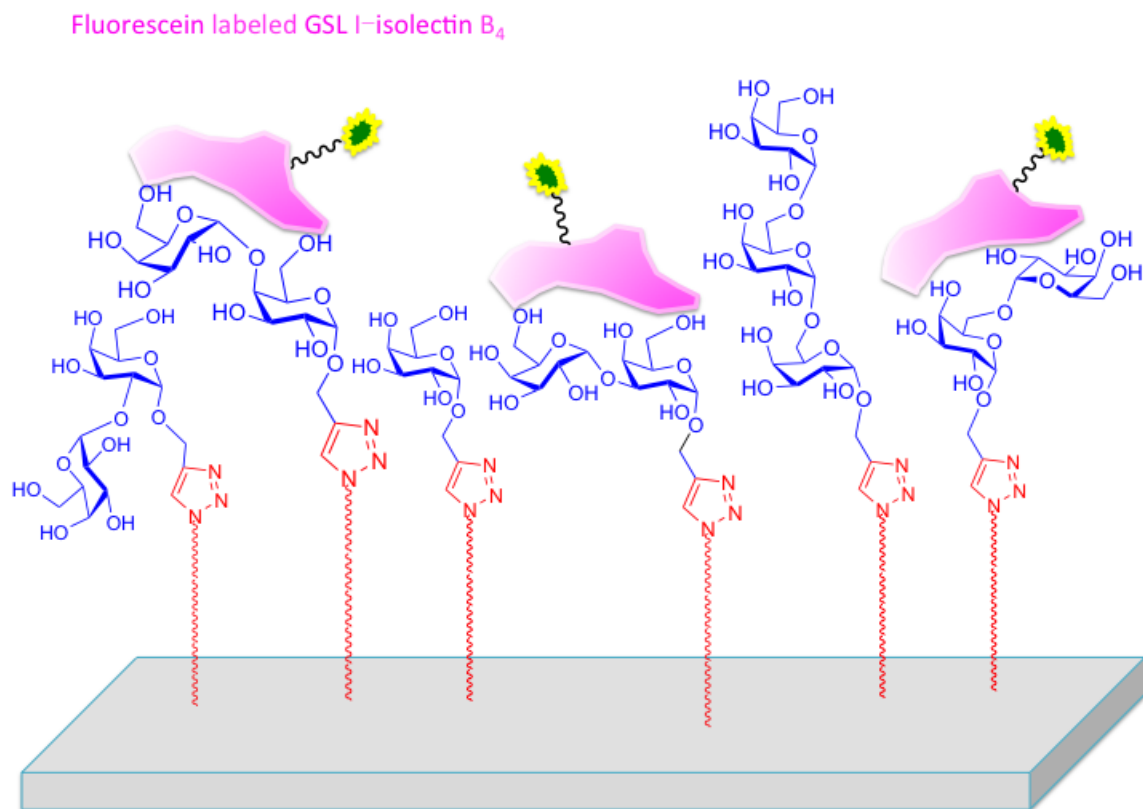
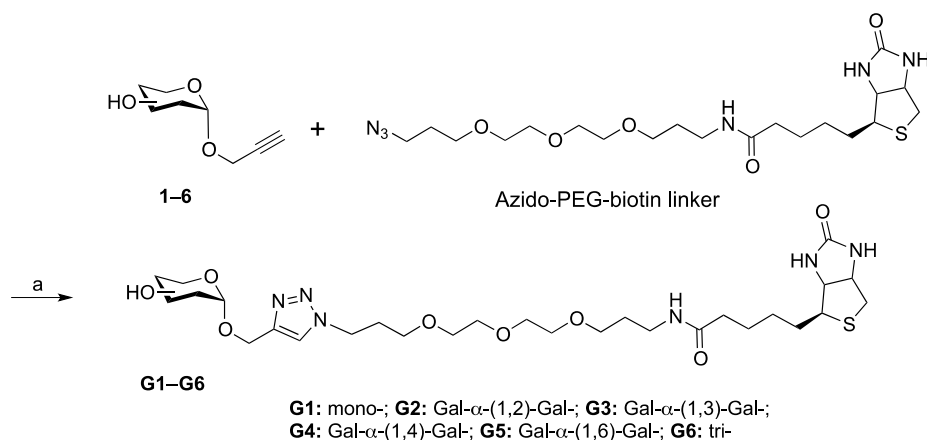


Figure 4.1 Landscape of synthetic α -linked galactosides microarray and its carbohydrate–lectin binding evaluations.

primarily the geometry of the lectin multimers, the need for structures that have good water solubility and resistance to non-specific protein adsorption, and the desire to use readily available azide derivatives that can be incorporated readily into the modular architecture. It is expected that substantially long length of linkers may be needed, as the linear path between binding sites is not physically possible with a short linker, and some added flexibility will be helpful in facilitating proper orientation of the terminal sugar residues. Therefore, azido-PEG-biotin linker, derived from polyethylene glycol (PEG), was synthesized¹⁹ and used, which is hydrophilic and highly flexible. It was coupled with propargyl α -D-galactosides **1–6** by Cu-catalyzed azide–alkyne 1,3-dipolar cycloaddition (CuAAC) “click chemistry”²⁰ to synthesize the glycoconjugates **G1–G6** (Scheme 4.1).

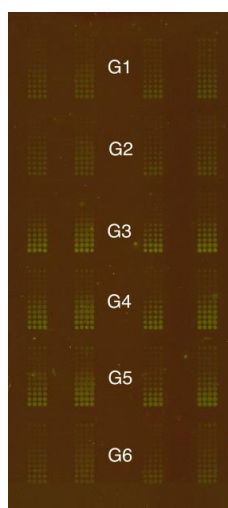


Scheme 4.1 Conjugation of propargyl terminated galactosides with azido-PEG-biotin linker yielded glycoconjugates **G1–G6** primed with a biotin group for immobilization on avidin-coated slides. *Reagents and conditions:* (a) CuSO₄, Sodium ascorbate, THPTA.

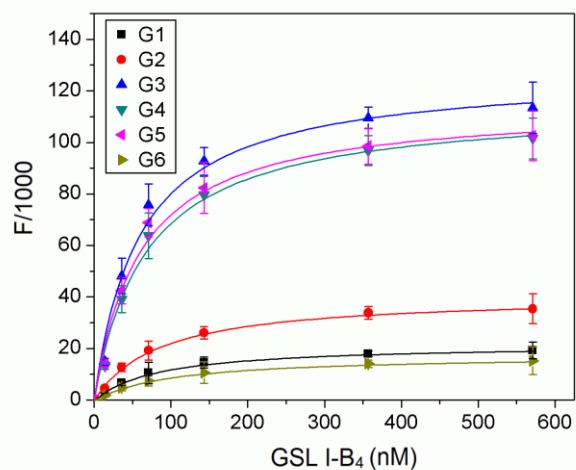
The resulting glycoconjugates were printed at 12 concentrations (1 μ M – 100 μ M, by glycan) on avidin-coated glass slides using a robotic spotter to afford microarrays with increasing glycoconjugates densities. Each concentration was spotted as four replicate spots, and four replicate grids were printed for each glycocluster. The printing of multiple

grids allowed for the systematic modification of both sugar and protein concentrations. The microarray slides were incubated overnight in a humid chamber at 55 °C to allow biotin–avidin binding to proceed. After washing to remove unbound glycans, the microarray slides were incubated with DyLight 594-labeled GSL I-B₄ lectin, and visualized using a fluorescence scanner (Figure 4.2a).

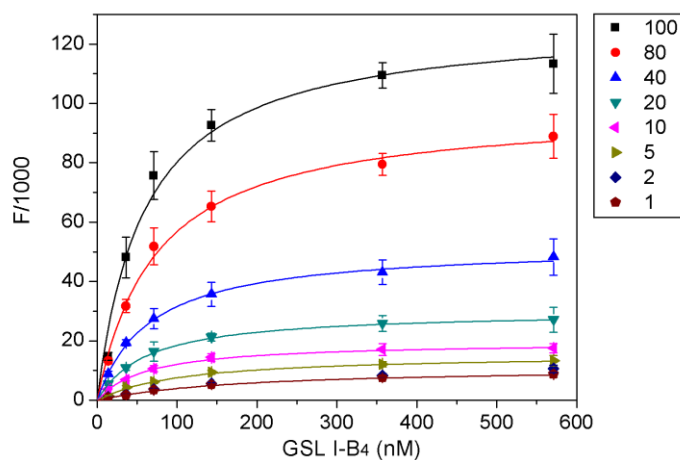
The binding curves (Figure 4.2b) were plotted from the surface bound glycoconjugates **G1–G6**, showing the fluorescence resulting from the bound lectin as a function of the concentration of GSL I-B₄ lectin that was incubated with the slide.²¹ It shows that all of the arrayed glycoconjugates **G1–G6** were recognized by the GSL I-B₄ lectin, while their affinities varied depending on the glycoconjugate structure. **G3–G5** showed stronger bindings than **G1**, **G2**, and **G6**. The dissociation constants (K_d) of each glycoconjugate were also determined to add more quantitative insights (Table 4.1). **G3** and **G5** exhibited lower K_d values (58.9 nM and 59.5 nM, respectively) and higher binding affinities, which is followed by **G4** (67.2 nM); while **G1**, **G2** and **G6** displayed higher K_d values (82.5 nM, 83.6 nM and 100.3 nM, respectively) and lower binding affinities. It indicated that the disaccharides showed generally better binding affinities than the monosaccharide and the trisaccharide. I surmise that it may be due to the less binding sites (hydroxyl groups) of monosaccharide than disaccharide, and the bulky size of trisaccharide that limits lectin's accessibility. **G2** possessing an terminus Gal- α -(1,2)-Gal disaccharide, unexpectedly, showed low binding affinity. A mechanistic explanation of it is no doubt complicated, as many factors are possible and difficult to sort out. Perhaps noteworthy is the downward orientation fact of the terminal α -Gal, which is to some extent, concealing itself among the carbohydrate arrays.



(a)



(b)



(c)

Figure 4.2 (a) Fluorescence scanning of carbohydrate microarray after DyLight 594-labeled GSL I-B₄ lectin incubation ($C_{\text{glycan}} = 1 \mu\text{M}$ to $100 \mu\text{M}$); (b) Binding curves obtained from the surface bound glycoconjugates **G1–G6** as a function of GSL I-B₄ lectin concentration; (c) Binding curves for the glycocluster **G3** printed at different concentrations.

Table 4.1 Dissociation constants of **G1–G6** determined by GSL I-B₄ binding curves

$$F = \frac{F_{max}[L]}{[L] + K_d}$$

Glycoconjugates	K_d (nM)	Error (%)	Glycoconjugates	K_d (nM)	Error (%)
G1	82.5	6.6	G4	67.2	8.2
G2	83.6	5.9	G5	59.5	8.9
G3	58.9	9.1	G6	100.3	3.5

Table 4.2 Relationship of different printing concentrations of **G3** and the dissociation constants (K_d), and the errors

Ligands Printing concentration (μM)	K_d (nM)	Error (%)	Ligands Printing concentration (μM)	K_d (nM)	Error (%)
100	58.9	9.1	10	61.6	4.2
80	72.9	6.5	5	104.4	7.7
40	65.0	4.1	2	217.4	18.6
20	61.7	1.6	1	173.5	10.5

The binding curves of the glycocluster **G3** at different concentrations were plotted as well to further explore the relationship between ligand concentration with lectin-binding affinity (Figure 3(c)). Eight binding curves were plotted representing eight printing concentrations of **G3** (100 μ M, 80 μ M, 40 μ M, 20 μ M, 10 μ M, 5 μ M, 2 μ M, 1 μ M, respectively). When the printing concentrations of **G3** were within 100 to 10 μ M, the K_d values obtained are narrowly distributed (mean K_d = 64 nM; SD = 5.4 nM; Table 4.2). However, when the printing concentrations were below 10 μ M, the K_d values increase and the binding affinities decrease. This is probably due to the increased distance between the ligands on the surface. If the distance between **G3** ligands on the surface is close enough, a multivalent binding type with GSL I-B₄ lectin will form. However, if the printing concentration is below 10 μ M, the average distance between immobilized **G3** ligand is too far for a multivalent interaction with the lectin.

4.3 Experimental Section

4.3.1 General procedure for glycoconjugates G1–G6 synthesis

Stock solutions were prepared as follows: six propargyl-terminated saccharides **1–6** in water (10 mM, respectively); azido-PEG-biotin linker in water (10 mM); CuSO₄ in water (50 mM); sodium ascorbate in water (250 mM); THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) in PBS buffer (1 M, pH 7, 0.5 % DMSO).

Solutions were added to six Eppendorf tubes (respectively with six glycoconjugates) in the following order (vortex after adding each solution): 850 μ L PBS, 10 μ L saccharide, 10 μ L azido-PEG-biotin linker, 10 μ L CuSO₄, 110 μ L THPTA, and 10 μ L sodium ascorbate. All tubes were shaken at room temperature for overnight. The

solutions were then diluted to 12 concentrations ranged from 1 μ M to 100 μ M, and printed on to streptavidin-coated slides.

4.3.2 Preparation of microarrays

For microarray experiments, all water used was obtained from a Milli-Q water purification system (Millipore). Six glycoclusters with biotin linkers in phosphate-buffered saline (PBS) buffer (1x PBS, pH 7.4 and 0.05% Tween-20) were immobilized at 12 concentrations (1 μ M – 100 μ M, by glycan) on streptavidin-coated glass (SuperAvidin Microarray Substrates, ArrayIt®). Each concentration was spotted as four replicate spots, and four replicate super grids were printed for each glycocluster. The printed slides were incubated overnight in a humid chamber at 55 °C. The slides were rinsed with PBS buffer (1XPBS, pH 7.4 and 0.05% Tween-20, 3 x 5 min), blocked in Superblock solution (ArrayIt®) for 1 h on shaker, washed in PBS (3 x 5 min) again, and dried over a flow of nitrogen.

4.3.3 Microarray–lectin binding assay

DyLight® 594 *Griffonia Simplicifolia* Lectin I (GSL I) – Isolectin B4 lectin (DyLight® 594 GSL I – B4 Lectin, Vector Lab) was diluted to 2, 5, 10, 20, 50, and 80 μ g/mL, respectively, in PBS (1x PBS, 0.05% Tween-20, 0.15 M NaCl), and incubated with six printed carbohydrate microarray slides, correspondingly. Slides were incubated in a humid chamber for 3 h at room temperature, rinsed with PBS (1XPBS, pH 7.4 and 0.05% Tween-20, 3 x 5 min), water (3 x 5 min), and dried over a flow of nitrogen. The slides were scanned with a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA), and images were analyzed with the GenePix Pro 7 software (Molecular Devices).

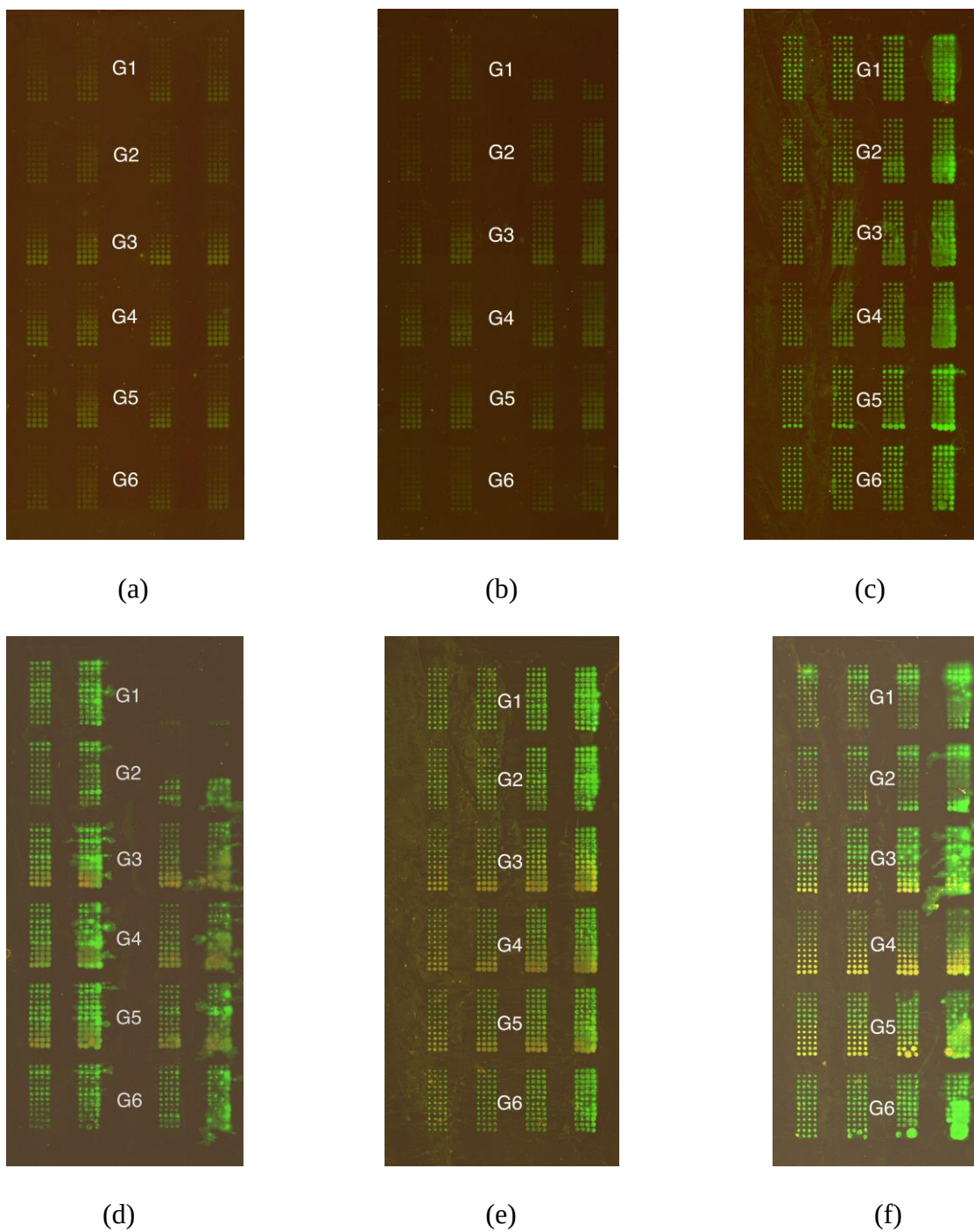


Figure 4.3 Scanned images of GSI B₄ lectin-incubated carbohydrate slides. Lectin concentrations: (a) 2 µg/mL, (b) 5 µg/mL, (c) 10 µg/mL, (d) 20 µg/mL, (e) 50 µg/mL, (f) 80 µg/mL. The two columns on right side in (d) and (f) were not included in the K_d calculation due to inconsistent printing.

4.3.4 Calculation of K_d

Slides were scanned at 532 nm then at 635 nm with the microarray scanner. Fluorescence intensities were obtained from the image analysis. K_d values were determined from the surface bound glycoconjugates **G1–G6** as a function of lectin concentration [L] by the equation below. OriginPro 8 software was used to generate the equation for nonlinear curve-fitting of each glycoconjugate and plotted binding curves.

4.4 Conclusion

I have successfully developed a microarray with controlled carbohydrate presentations by immobilizing six synthetic α -linked glycoconjugates varied in linkage and size to an avidin-coated glass slide to imitate featured carbohydrate presentations. Quantitative evaluation of their binding properties toward fluorescence-labeled GSL I-B₄ lectin was achieved by directly fluorescence scanning and determination of K_d values. The lectin displayed stronger affinity with saccharides that possess the non-reducing terminal disaccharide Gal- α -(1,3)-Gal, disaccharide Gal- α -(1,4)-Gal and Gal- α -(1,6)-Gal. The insights gained will find applications in a wealth of biological recognition activities and more importantly, it will have broad implications for the construction of analytical platforms that display the complex arrangements of chemical epitopes commonly found in natural systems.

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Chapter 5 Conclusions

The fundamental role of oligosaccharides and glycoconjugates with 1,2-*cis* glycosidic linkages in biological activities of living systems has been discussed, and various strategies developed for the stereoselective construction of 1,2-*cis*-O-glycosidic bond has been thoroughly reviewed in Chapter 1. Although considerable achievements have been accomplished to access complex oligosaccharides, the development of general, efficient, and flexible approaches to achieve the intrinsic organizational diversity of carbohydrates is still urged in order to meet the substantial carbohydrates demand for biological, medicinal, and therapeutic studies.

In this dissertation, a facile and general strategy for the direct construction of 1,2-*cis* alkyl glycosides has been developed. Glycosylations between several unprotected phenyl 1-thioglycosyl donors and various functionalized alcohols were performed under the activation of NIS/TMSOTf. The reactions proceeded smoothly with satisfying yields and 1,2-*cis*-selectivity. The optimization of the glycosylation condition was carried out efficiently by an FIA–ESI-TOF-MS/¹H NMR protocol, which enabled quantitative evaluation of reaction yield and stereoselectivity. The synthetic procedures described herein are anticipated to find application in the areas where 1,2-*cis*-linked glycosides is required.

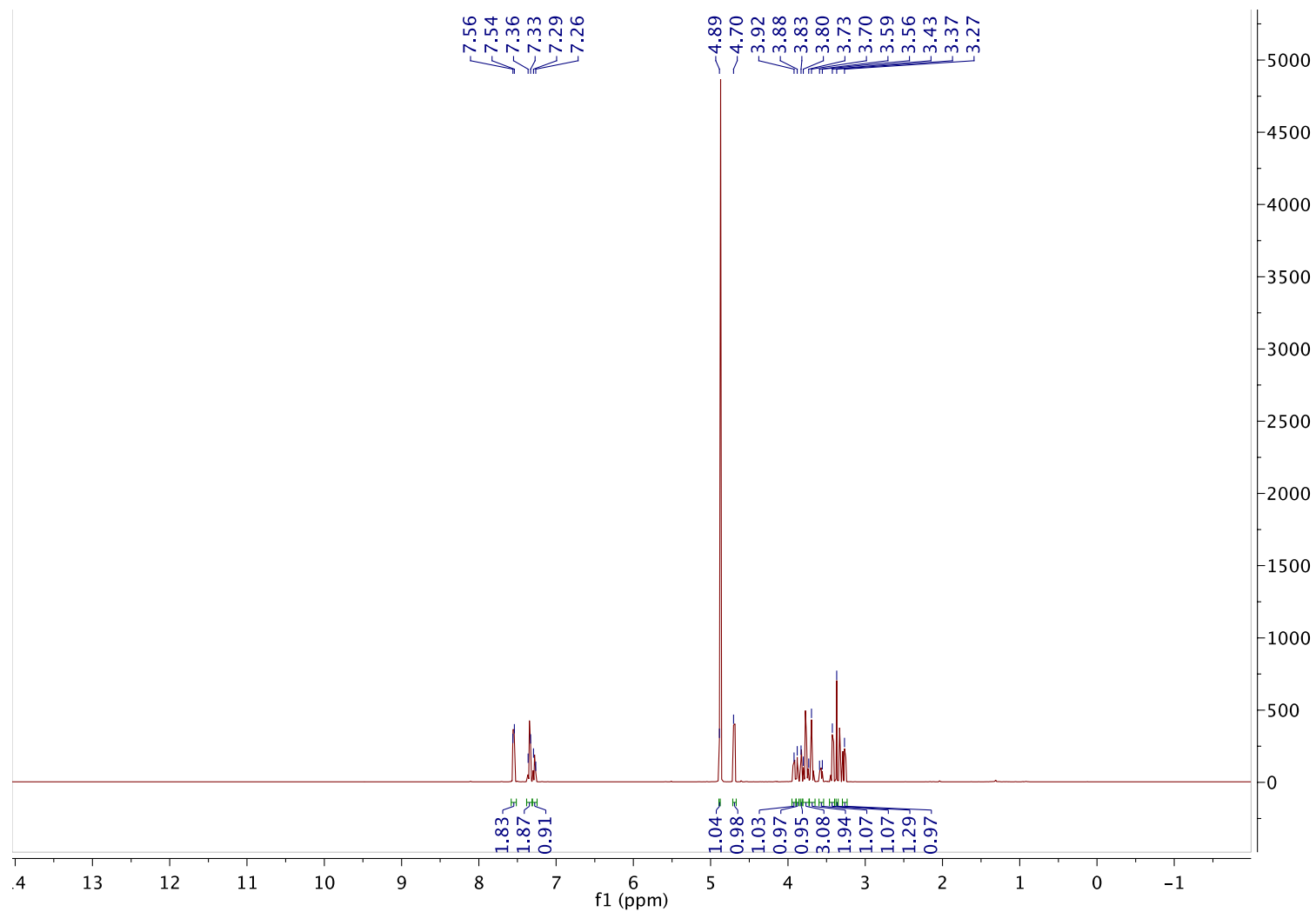
The development of synthetic glycoconjugate surfaces to imitate the natural carbohydrate landscape on the cell surface was also presented in order to facilitate the understanding of the structure–affinity relationships among carbohydrates in recognition with protein receptors. Six α -linked propargyl terminated galactosides with various spatial presentations were carefully designed for the construction of synthetic carbohydrate microarrays. Combinatorial syntheses were performed to afford these

glycan ligands from different combinations of glycosylation donor and acceptors. The glycosyl acceptors were obtained via flexible and efficient regioselective protection strategies, and they were glycosylated with 4,6-di-*tert*-butylsilylene protected thiogalactoside donor to get the α -linked galactosides in exclusive stereoselective and satisfying yield. A monosaccharide, α -linked 1 $\rightarrow$ 2, 1 $\rightarrow$ 3, 1 $\rightarrow$ 4 and 1 $\rightarrow$ 6 disaccharides, and an α -linked galactose trisaccharide with propargyl terminus were synthesized and coupled with the azido-PEG-biotin linker by click chemistry. The prepared glycoconjugates were immobilized to an avidin-coated glass slide to afford the carbohydrate microarrays. A fluorescent-labeled lectin, DyLight 594-labeled GSL I-B₄ lectin, was employed with varied concentrations to determine the glycoarray recognitions and analyze their binding affinities and dissociation constants. The lectin showed stronger binding affinities with disaccharides that possess the non-reducing terminal Gal- α -(1,3)-Gal, Gal- α -(1,4)-Gal and Gal- α -(1,6)-Gal aglycones. This study could add new dimensions to our understanding of the effects of carbohydrate ligands density, avidity, and spatial arrangement in carbohydrate–lectin binding process.

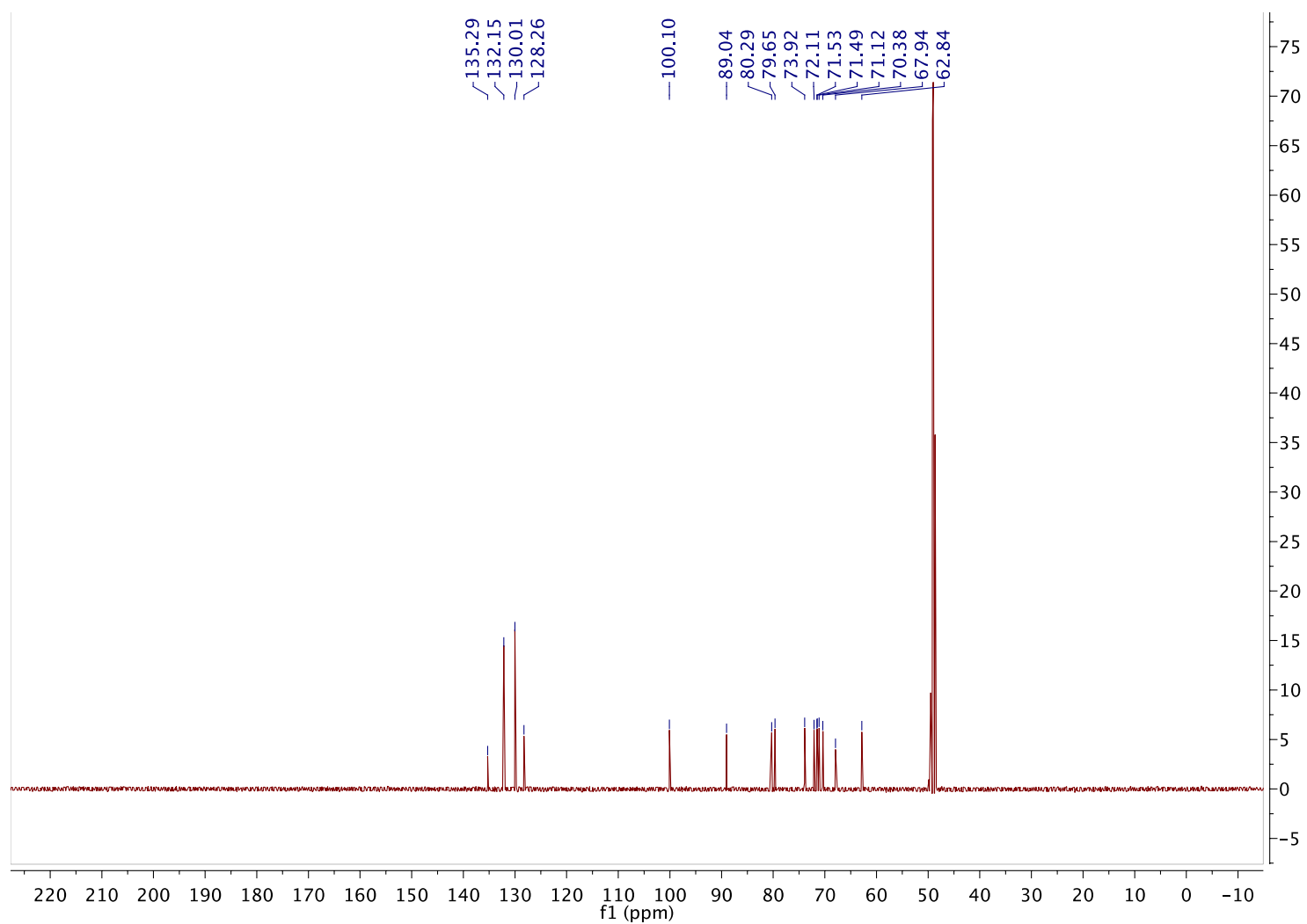
Appendix

^1H NMR, ^{13}C NMR, gCOSY NMR, gHSQC NMR and gHMBC

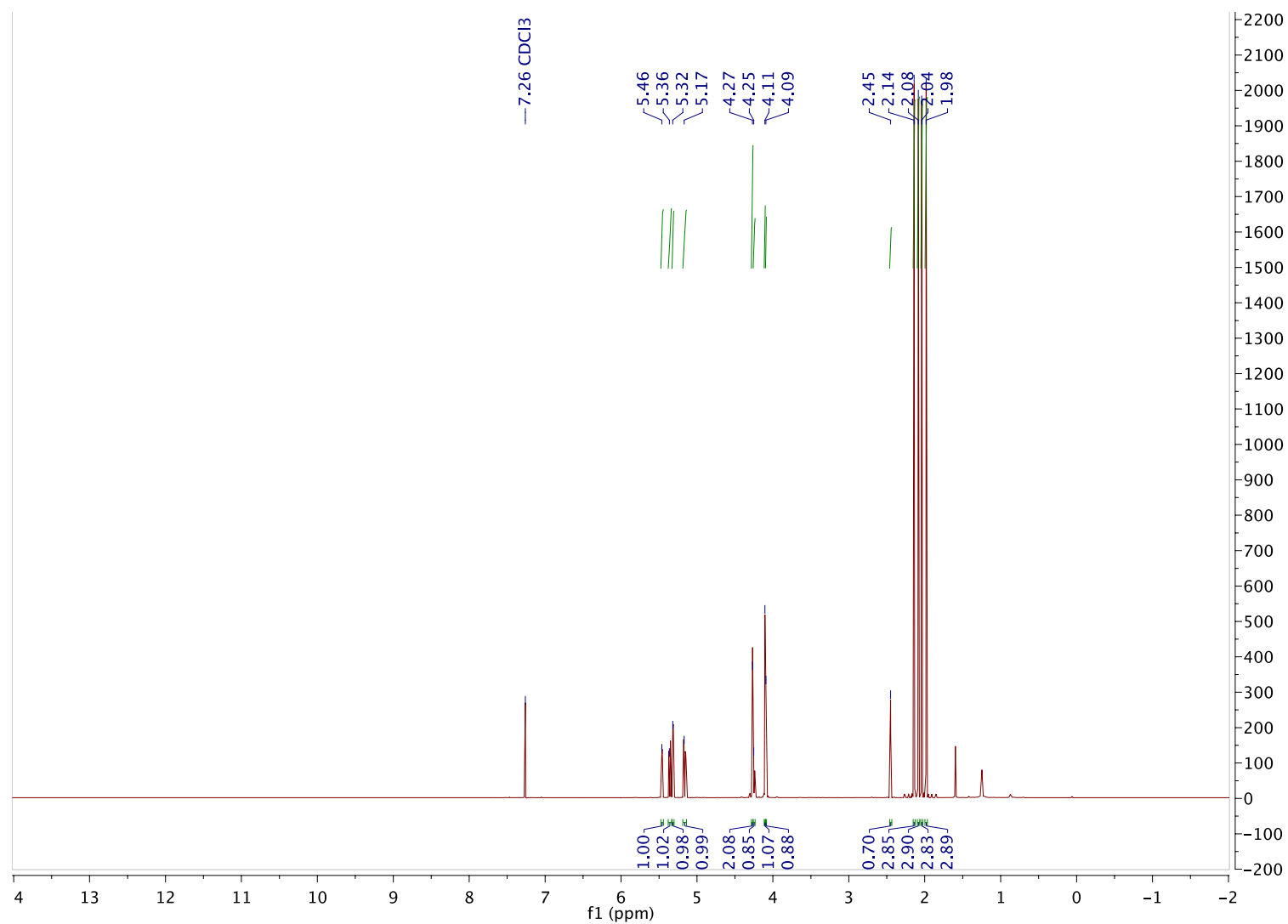
NMR Spectra of Synthesized Carbohydrates



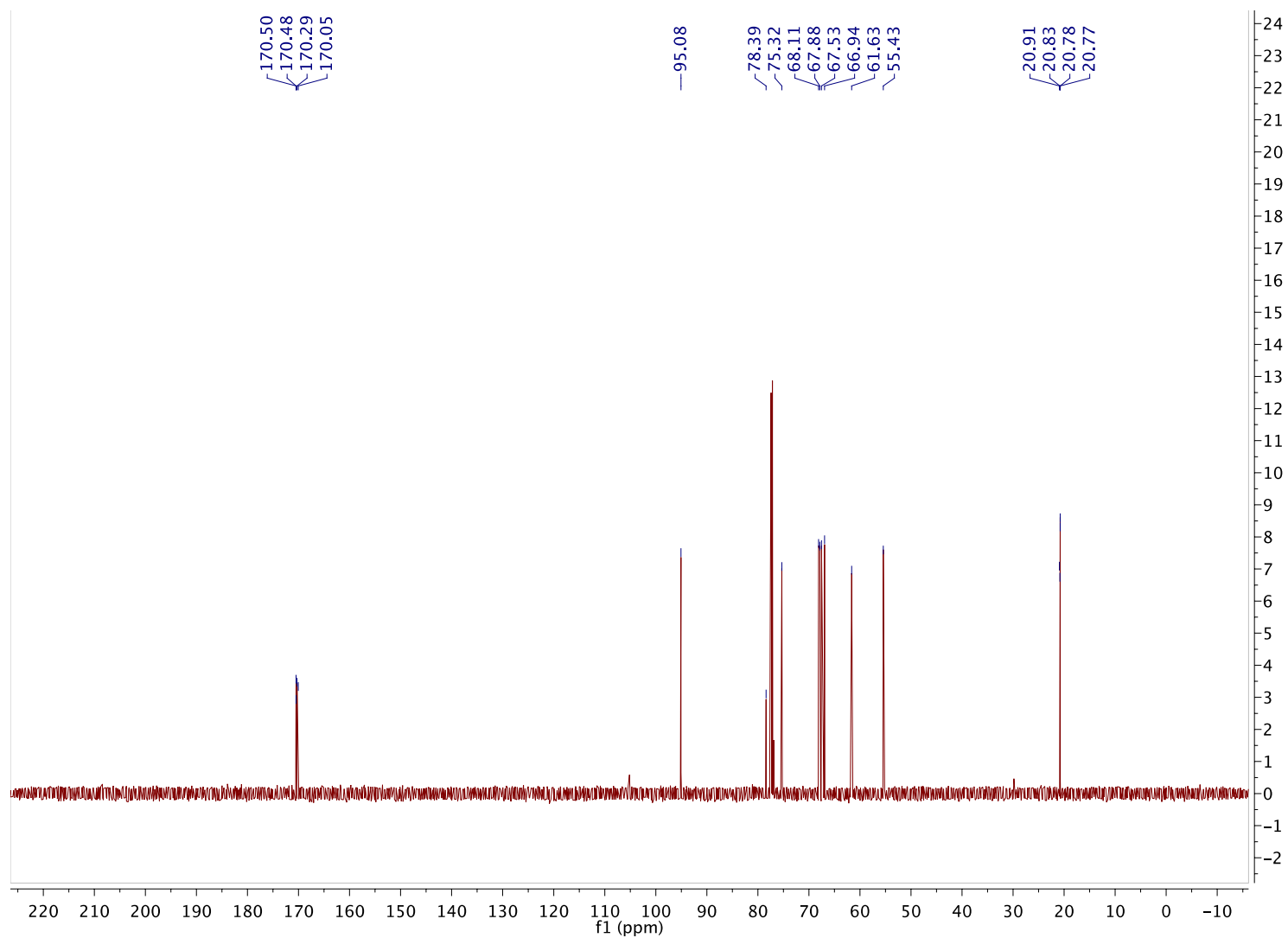
¹H NMR (500 MHz, CDCl₃): Phenyl α-D-galactopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (**1dβ**)



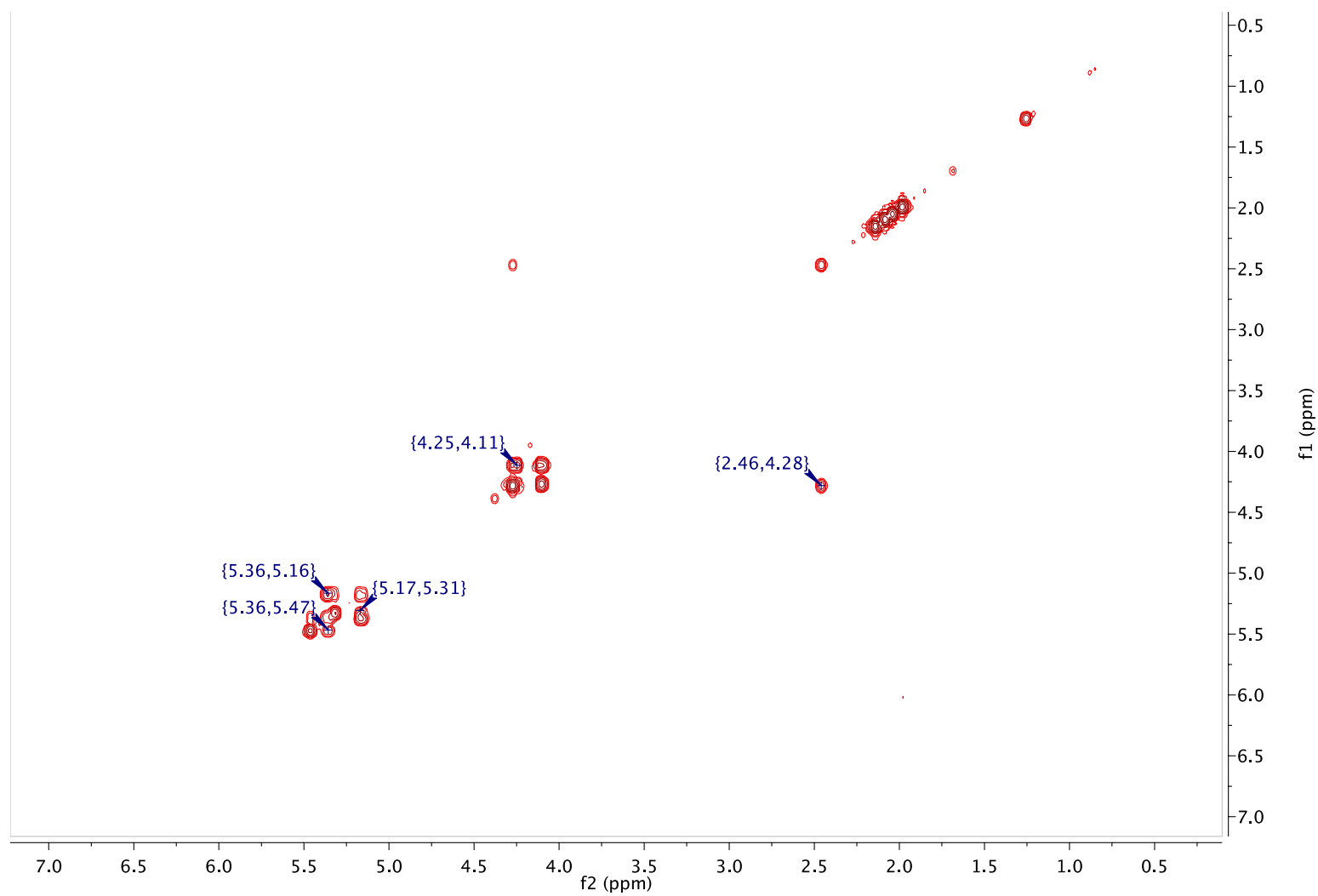
¹³C NMR (125 MHz, CDCl₃): Phenyl α-D-galactopyranosyl-(1,6)-1-thio-β-D-glucopyranoside (**1dβ**)



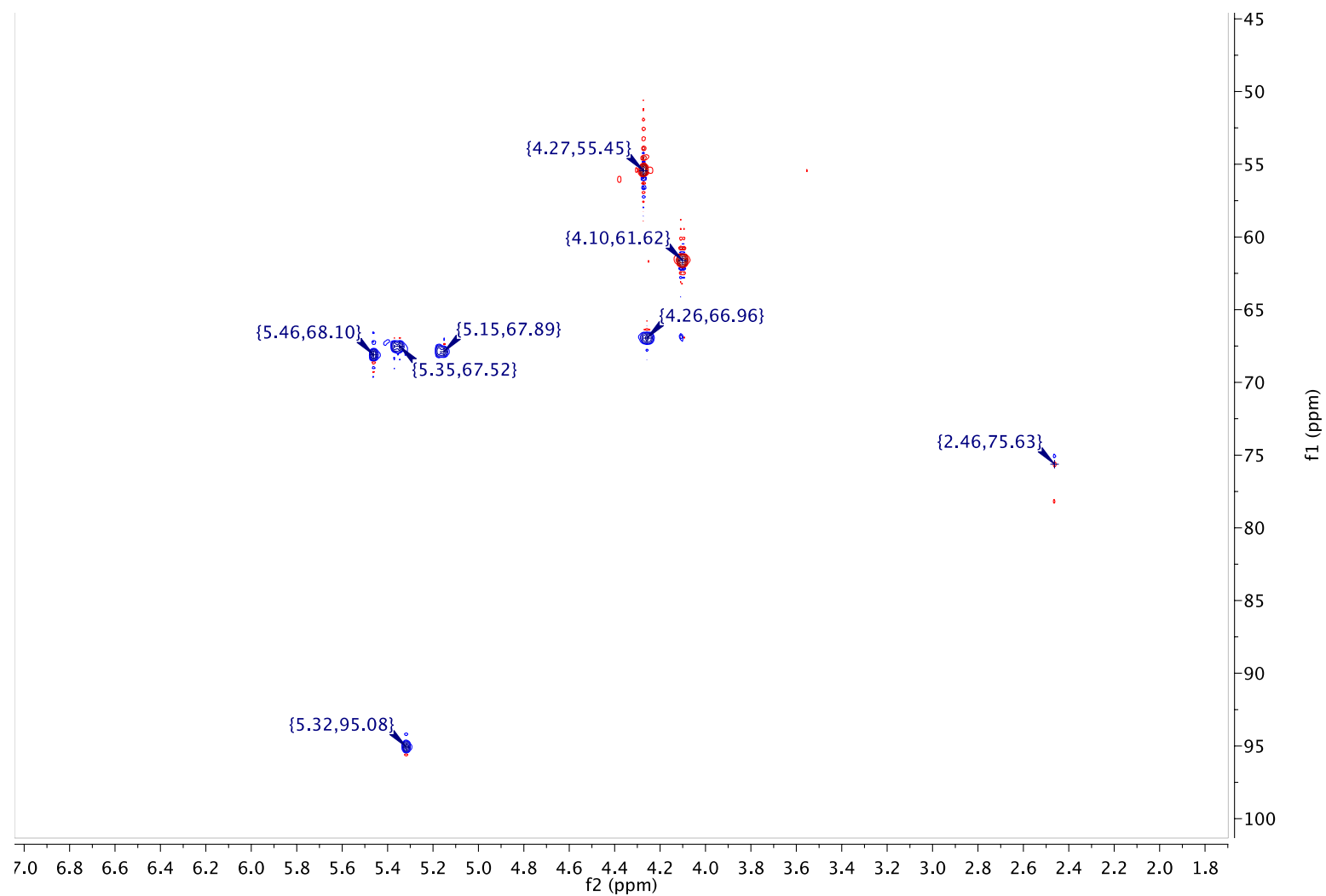
¹H NMR (500 MHz, CDCl₃): Propargyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (4a)



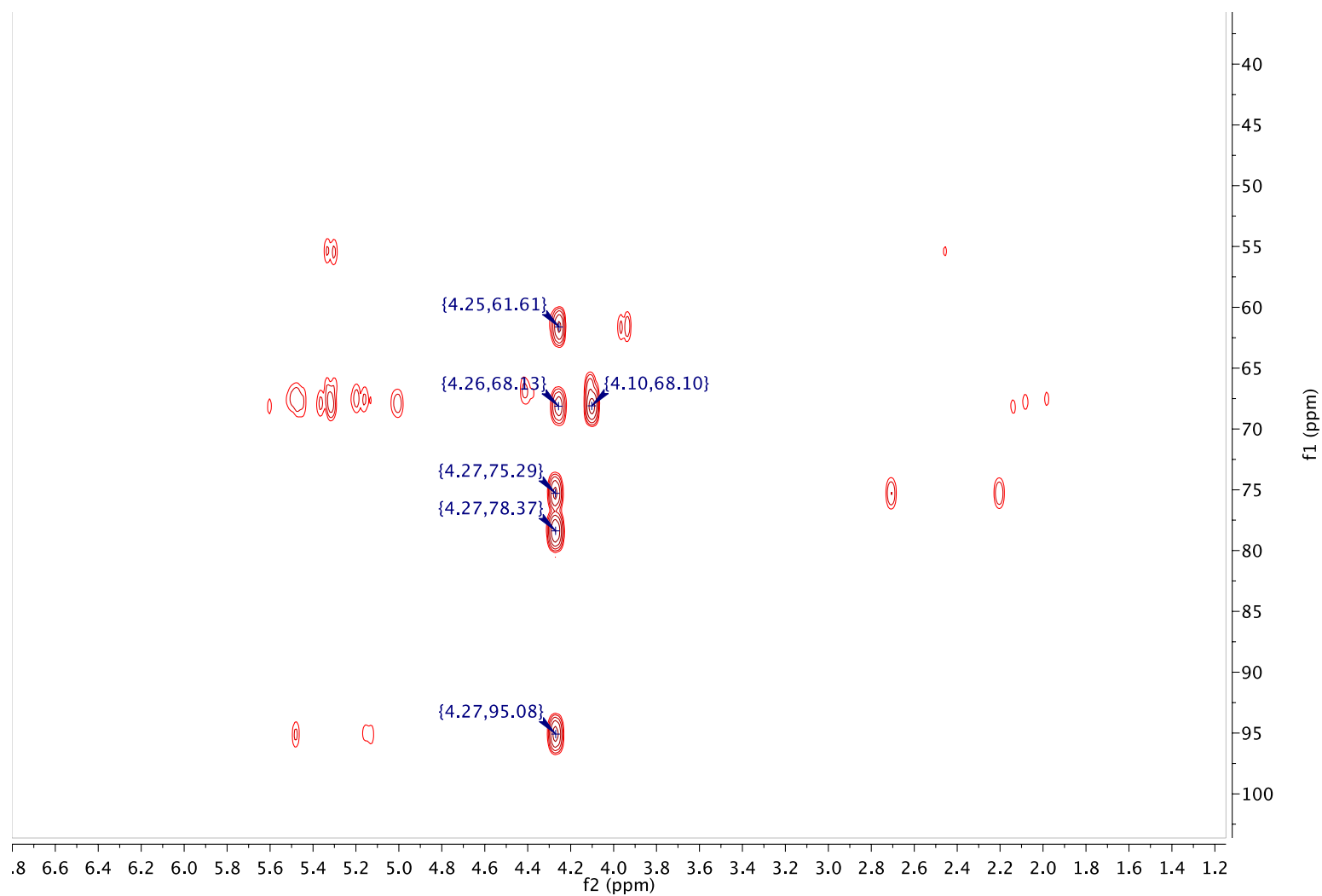
^{13}C NMR (125 MHz, CDCl_3): Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4a**)

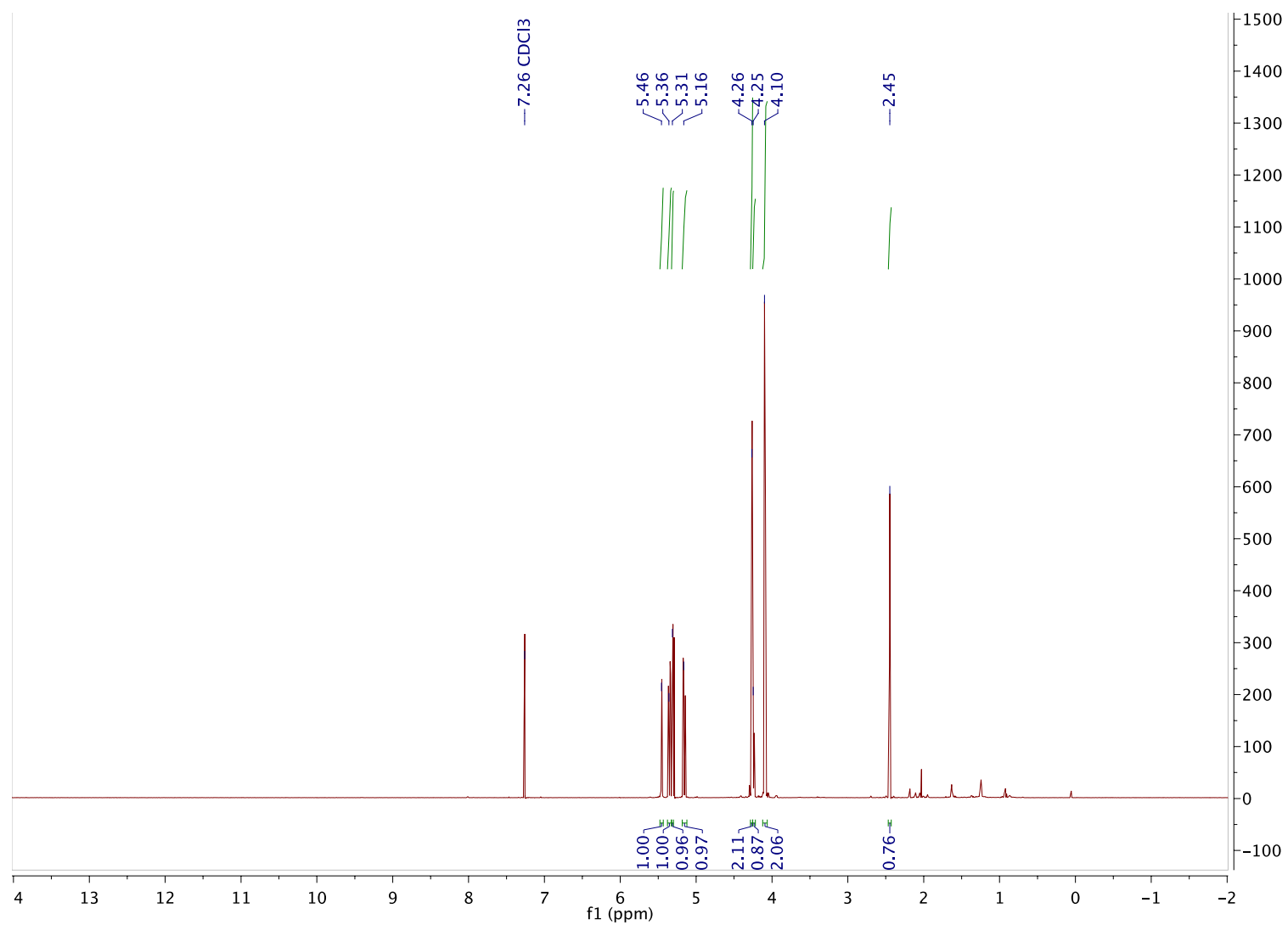


gCOSY NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (4a**)**

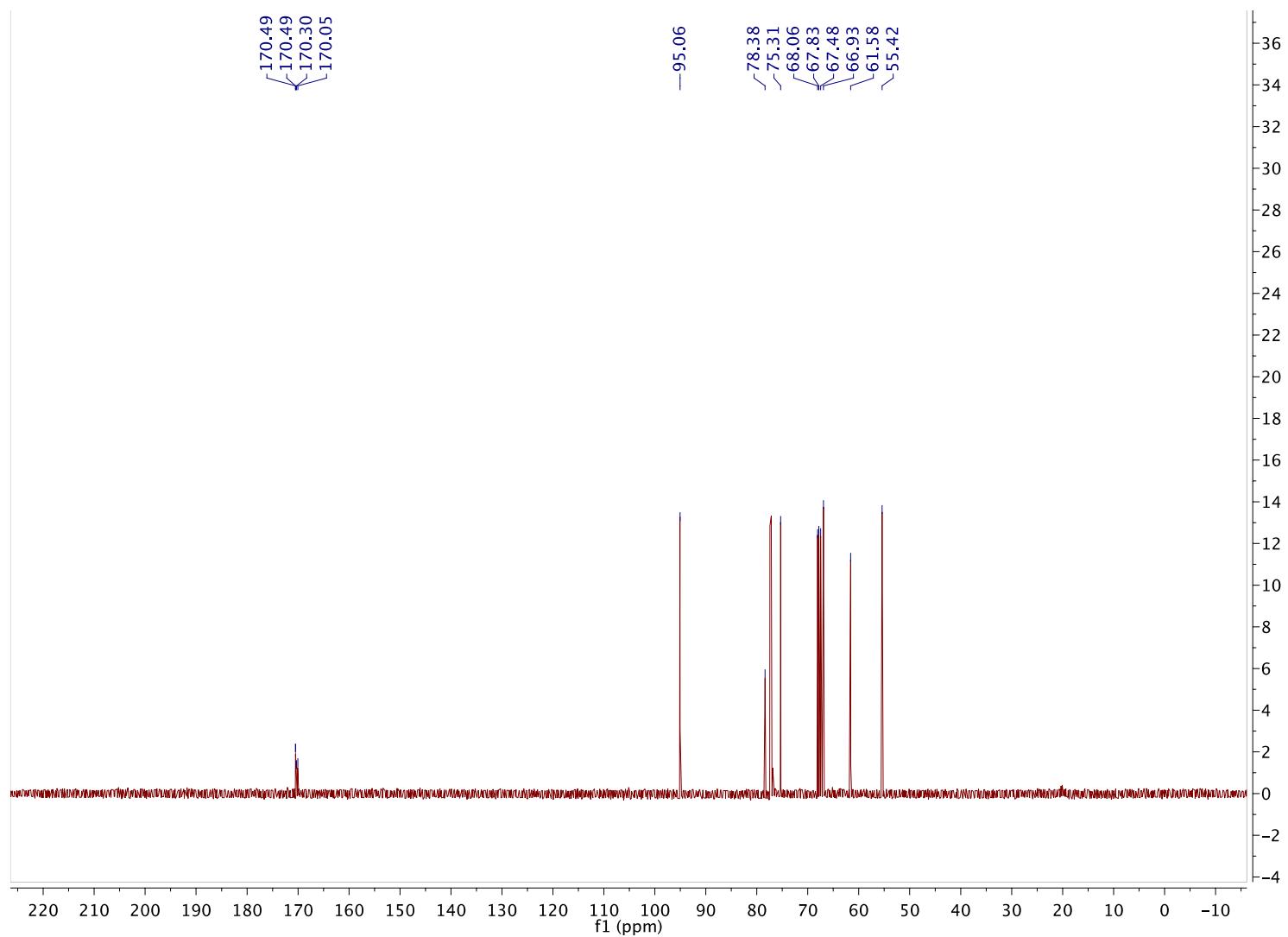


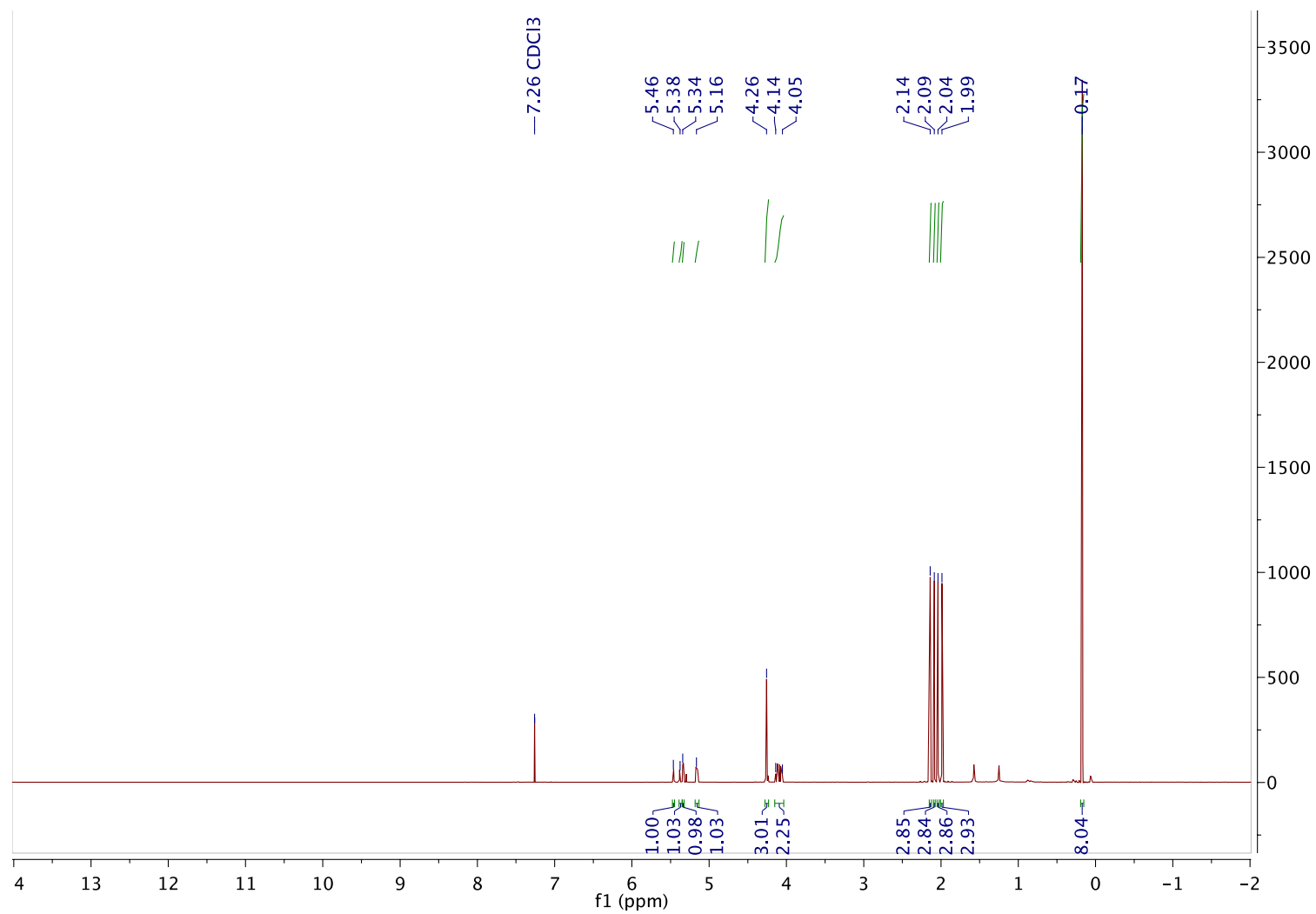
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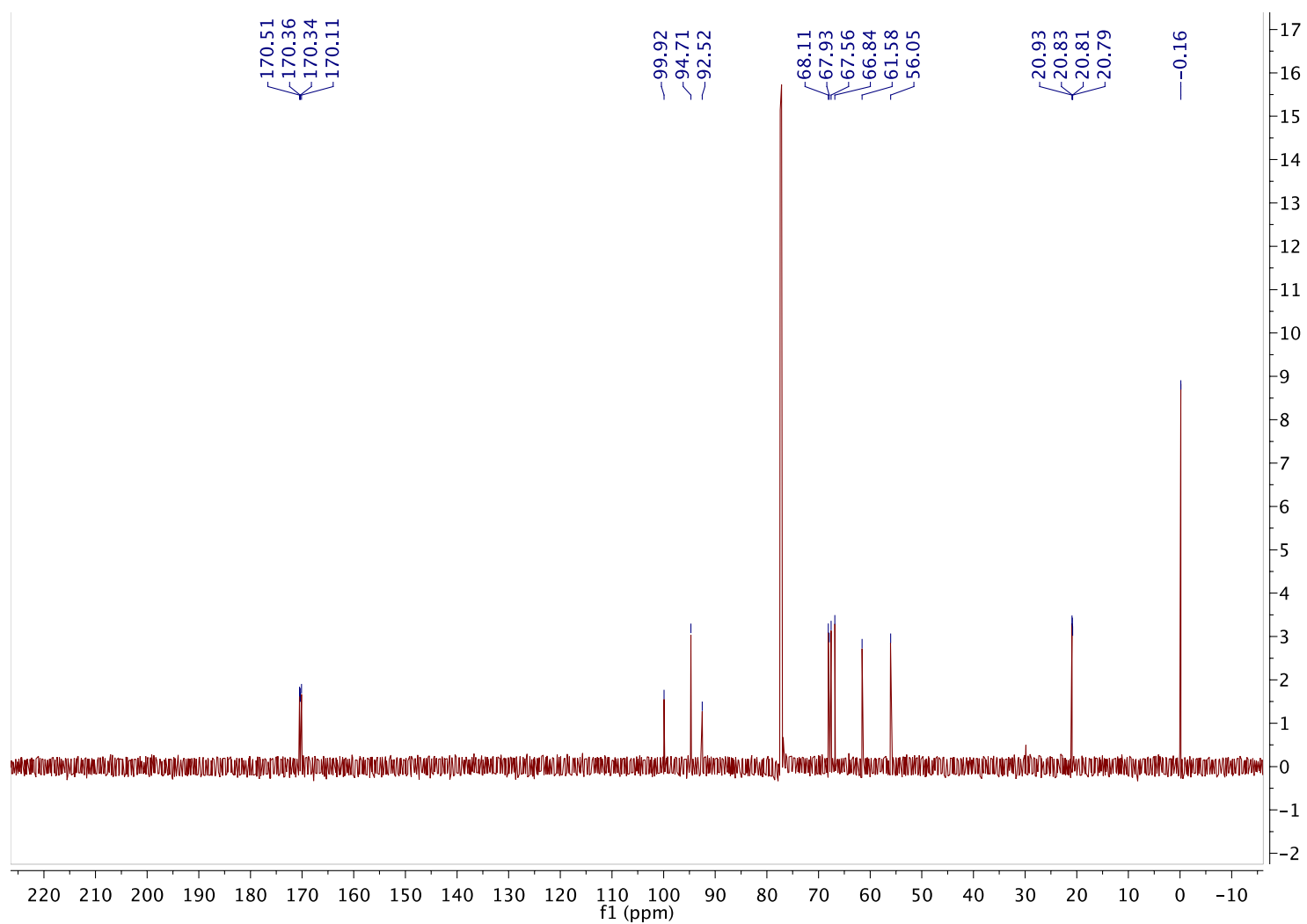


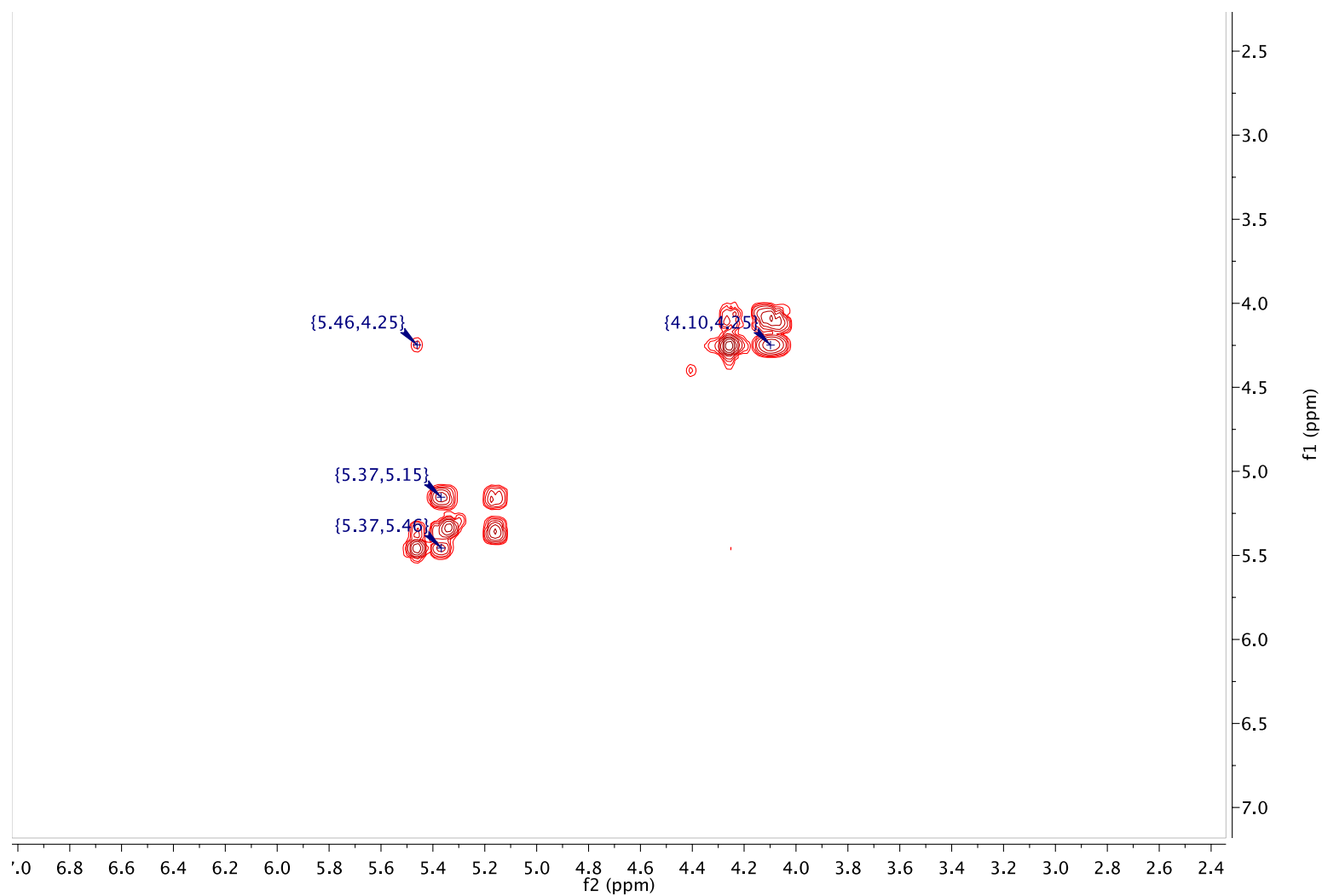
¹H NMR (500 MHz, CDCl₃): Propargyl 2,3,4,6-tetra-O-(acetyl-d₃)-α-D-galactopyranoside (4)



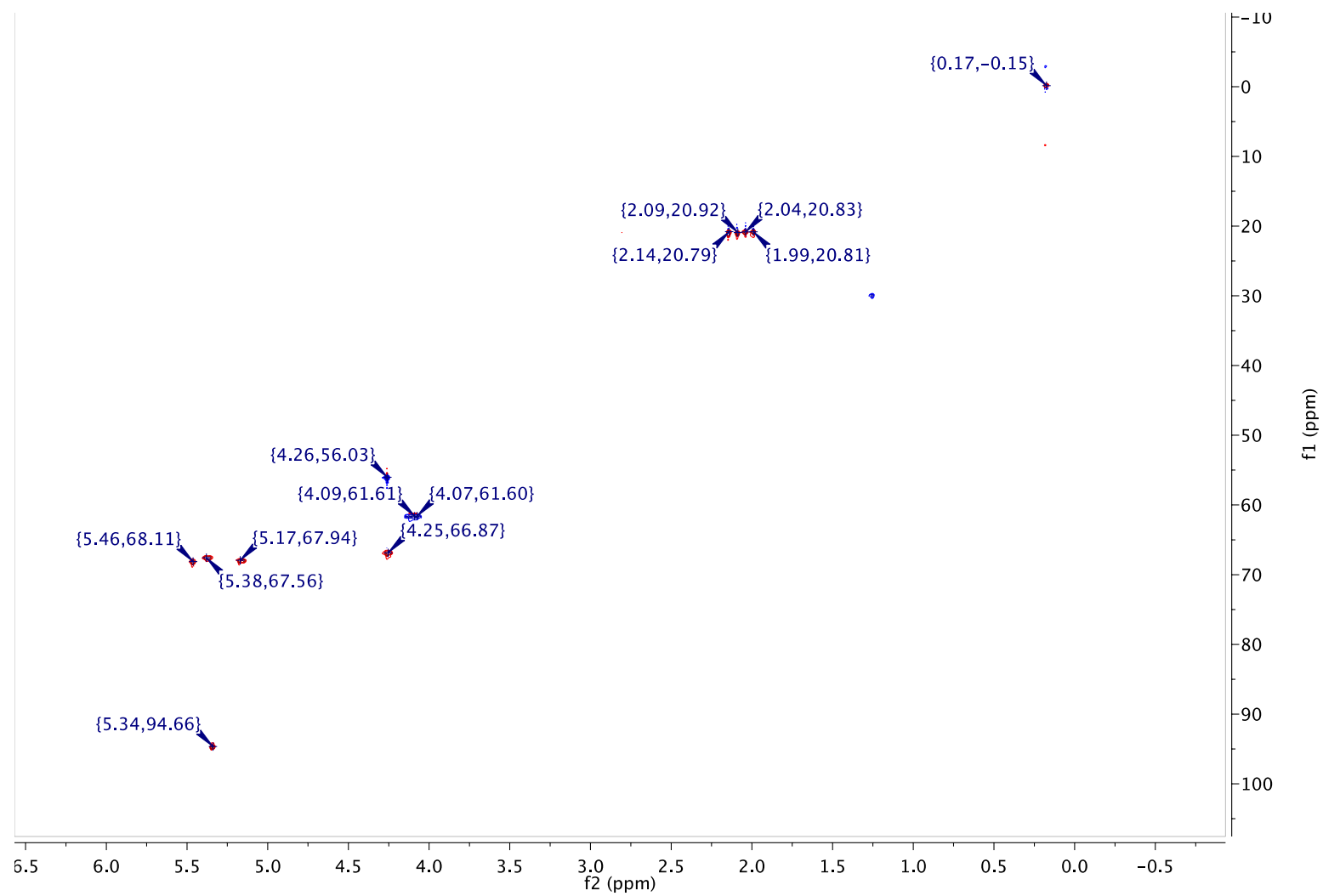


¹H NMR (500 MHz, CDCl₃): 3-(Trimethylsilyl)propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**4b**)

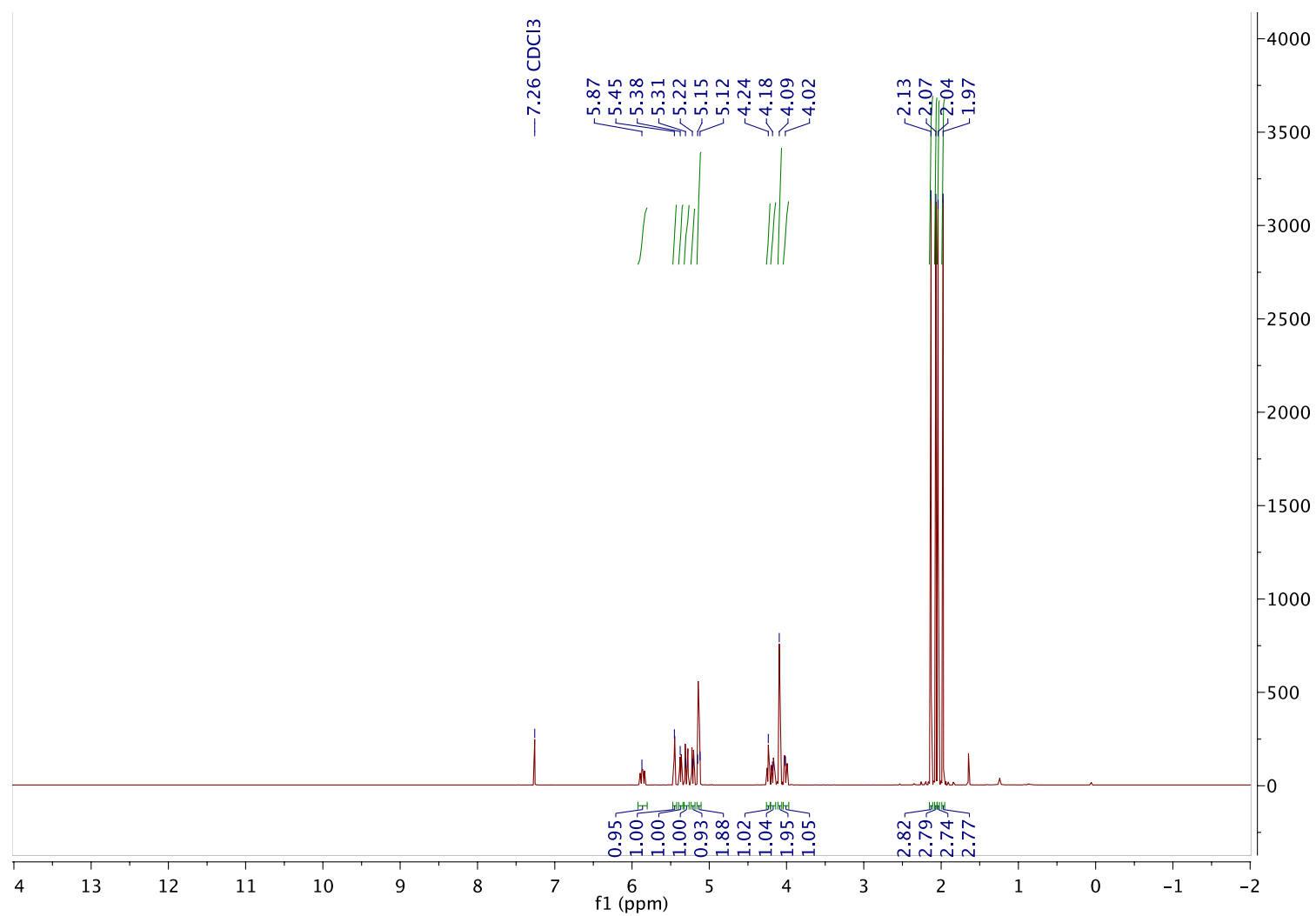




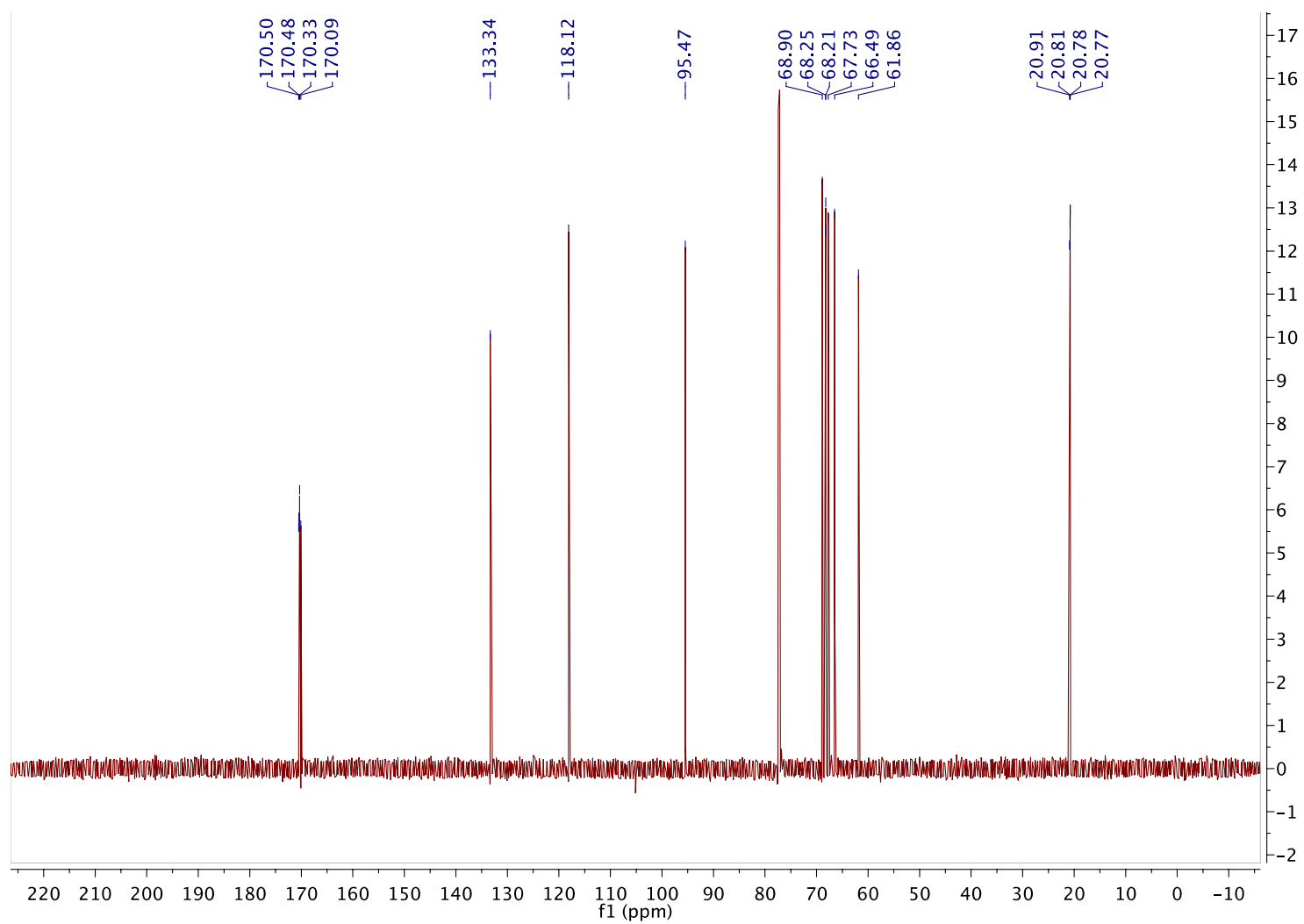
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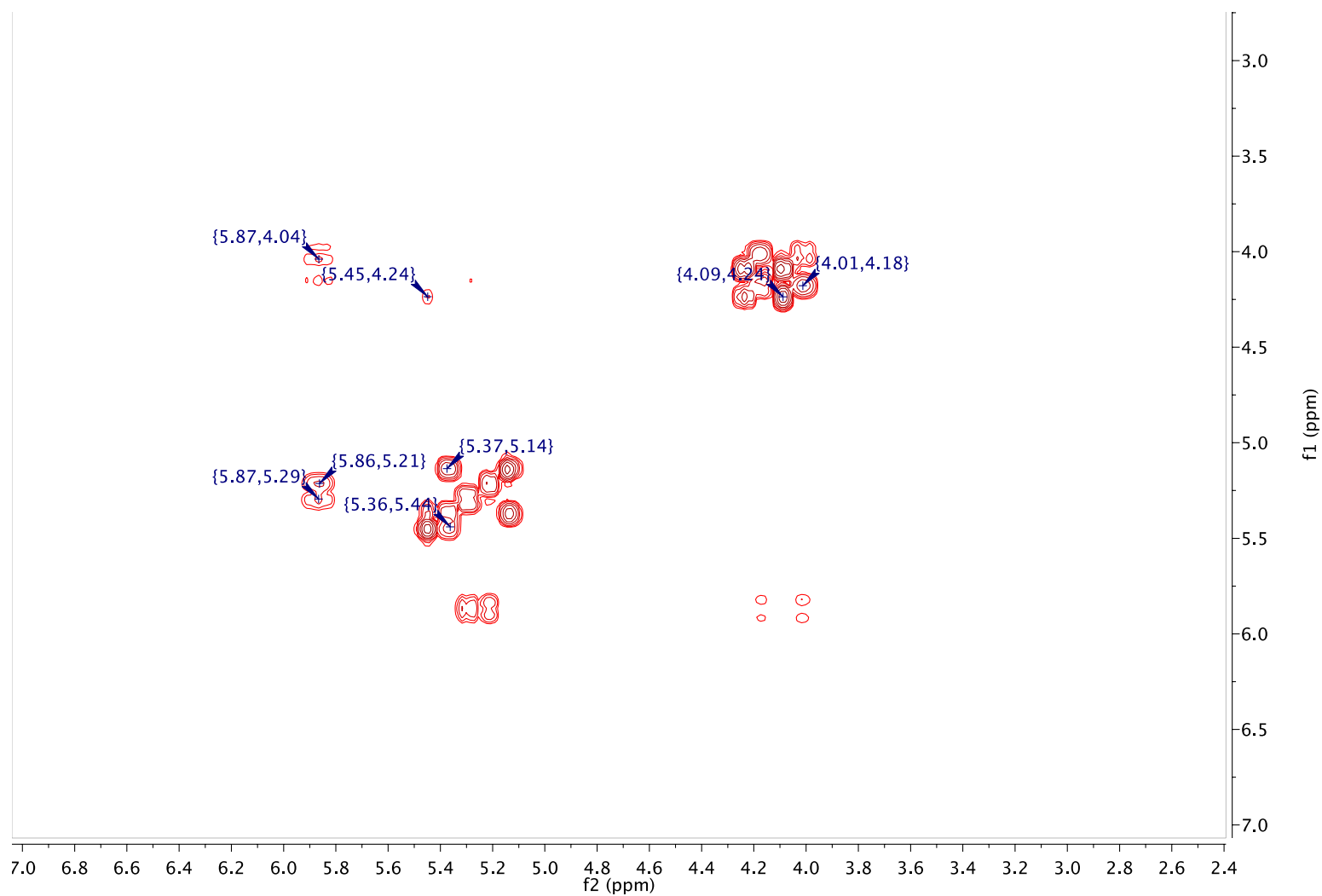


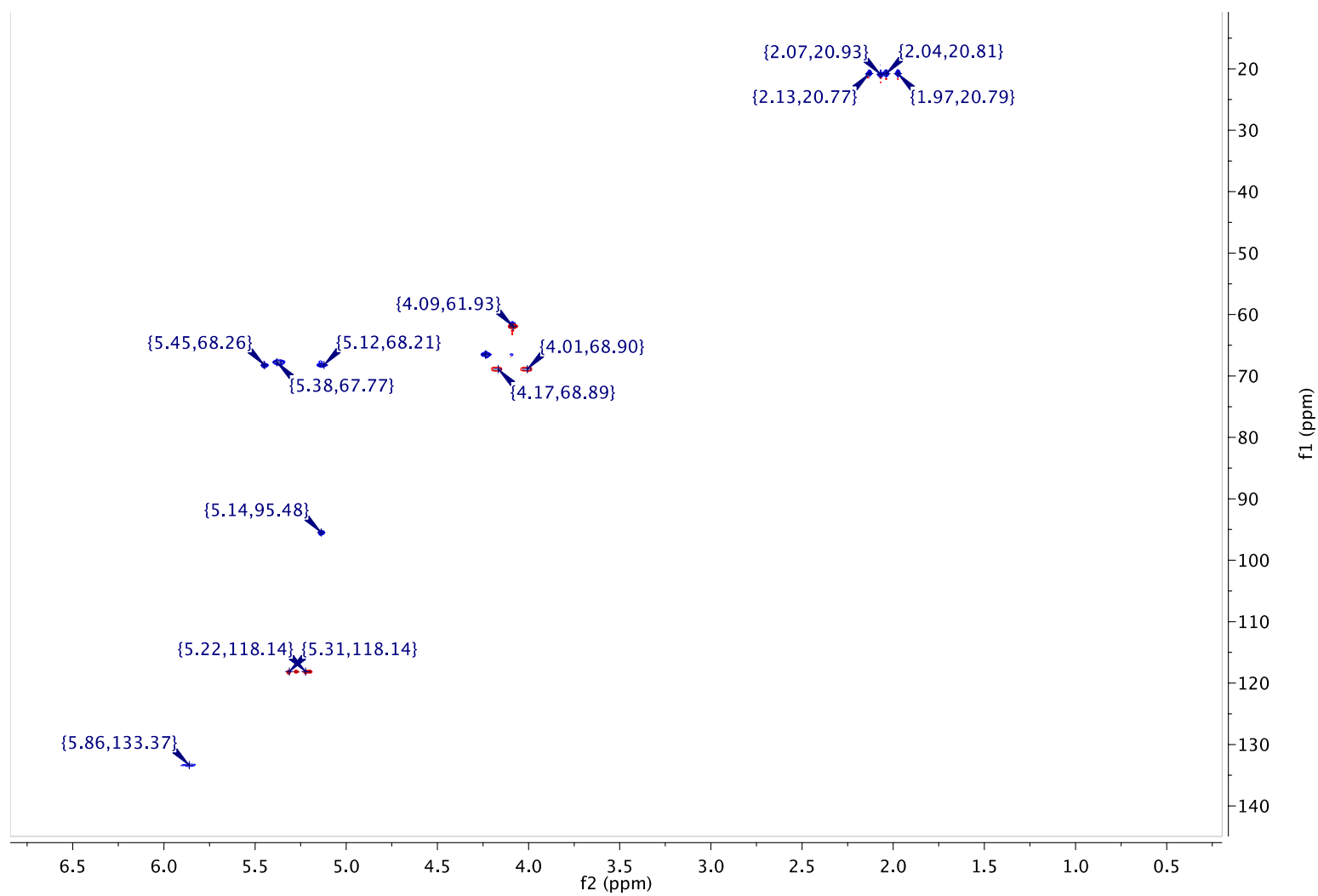
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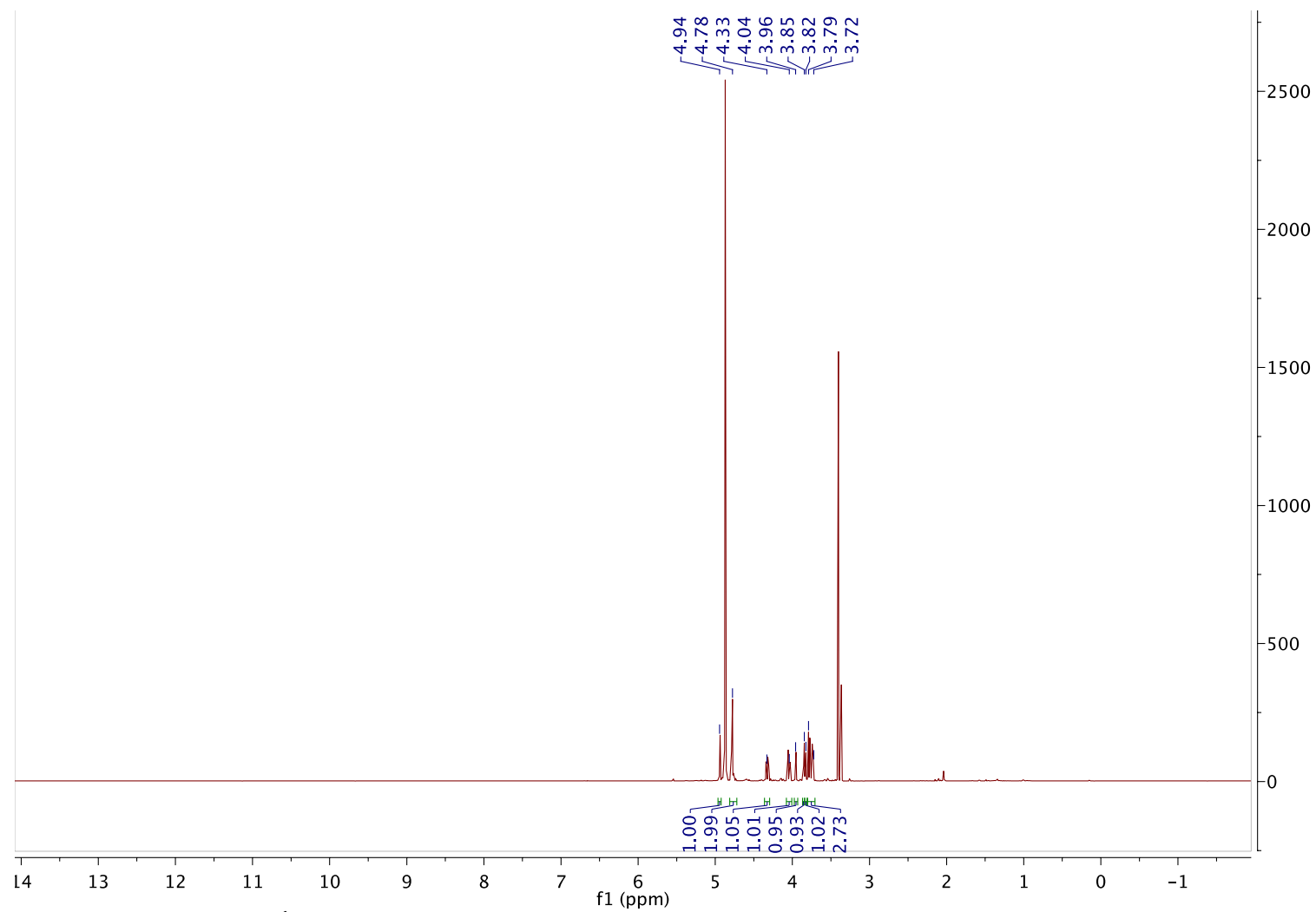
¹H NMR (500 MHz, CDCl₃): Allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (**4c**)



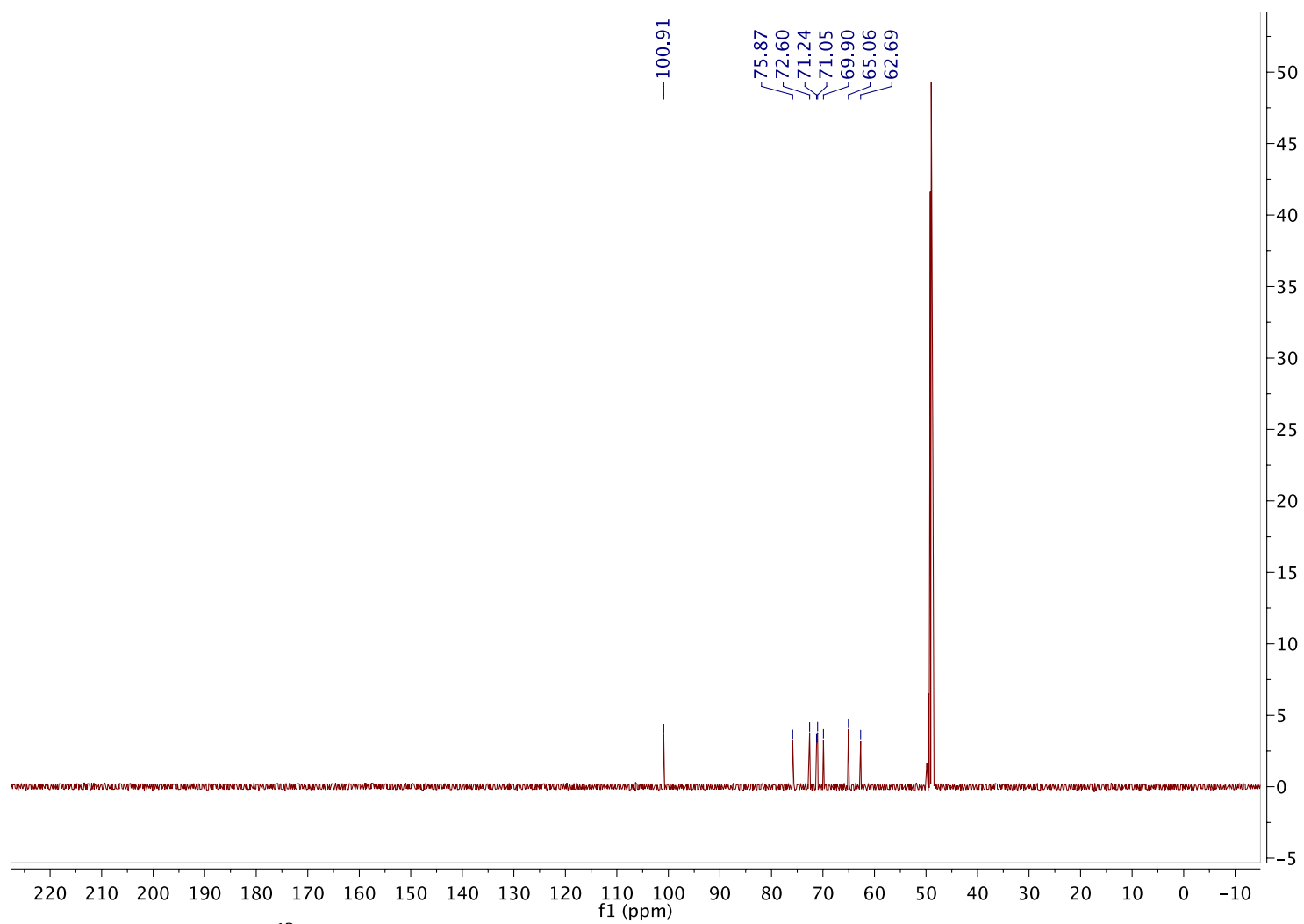


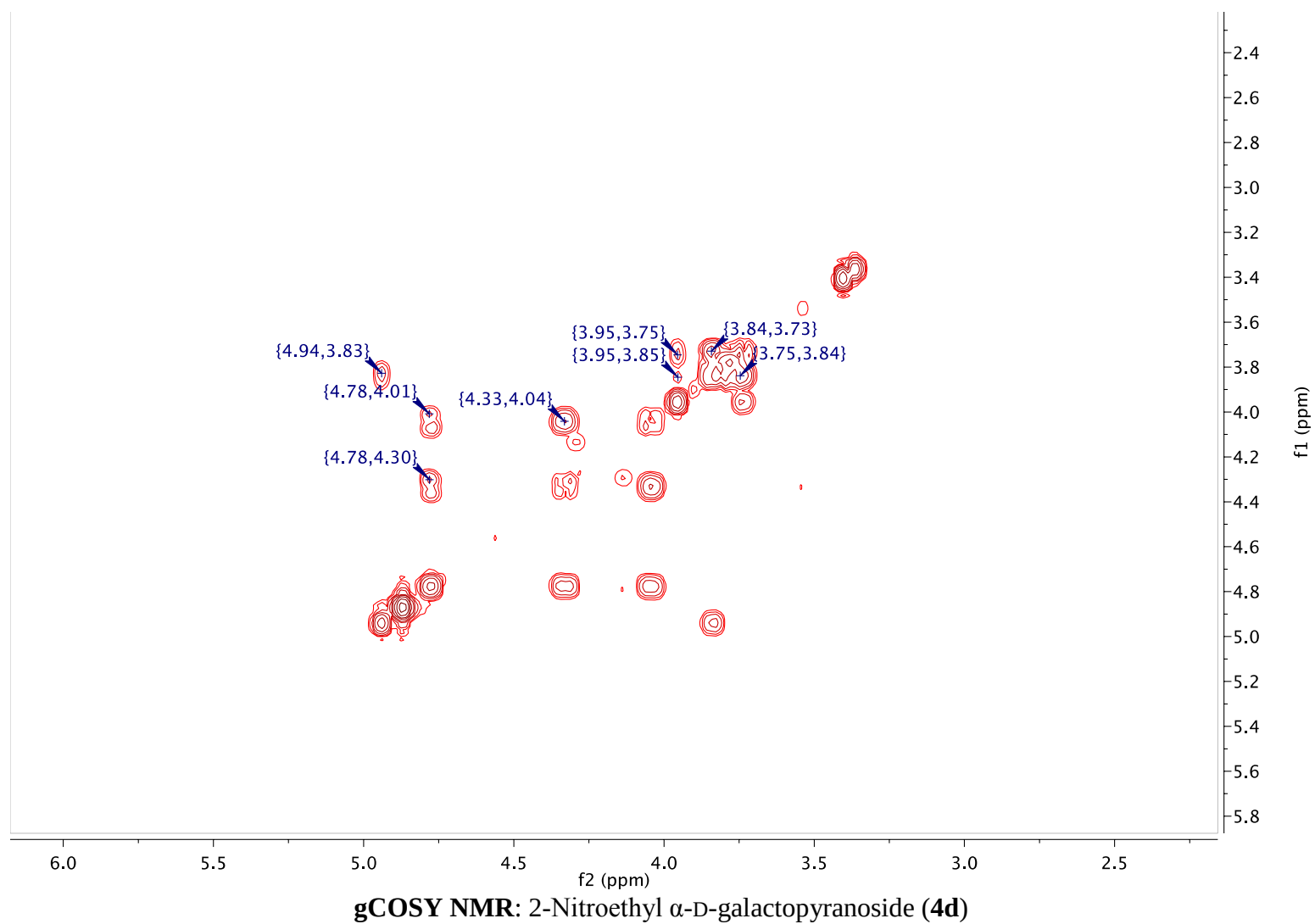


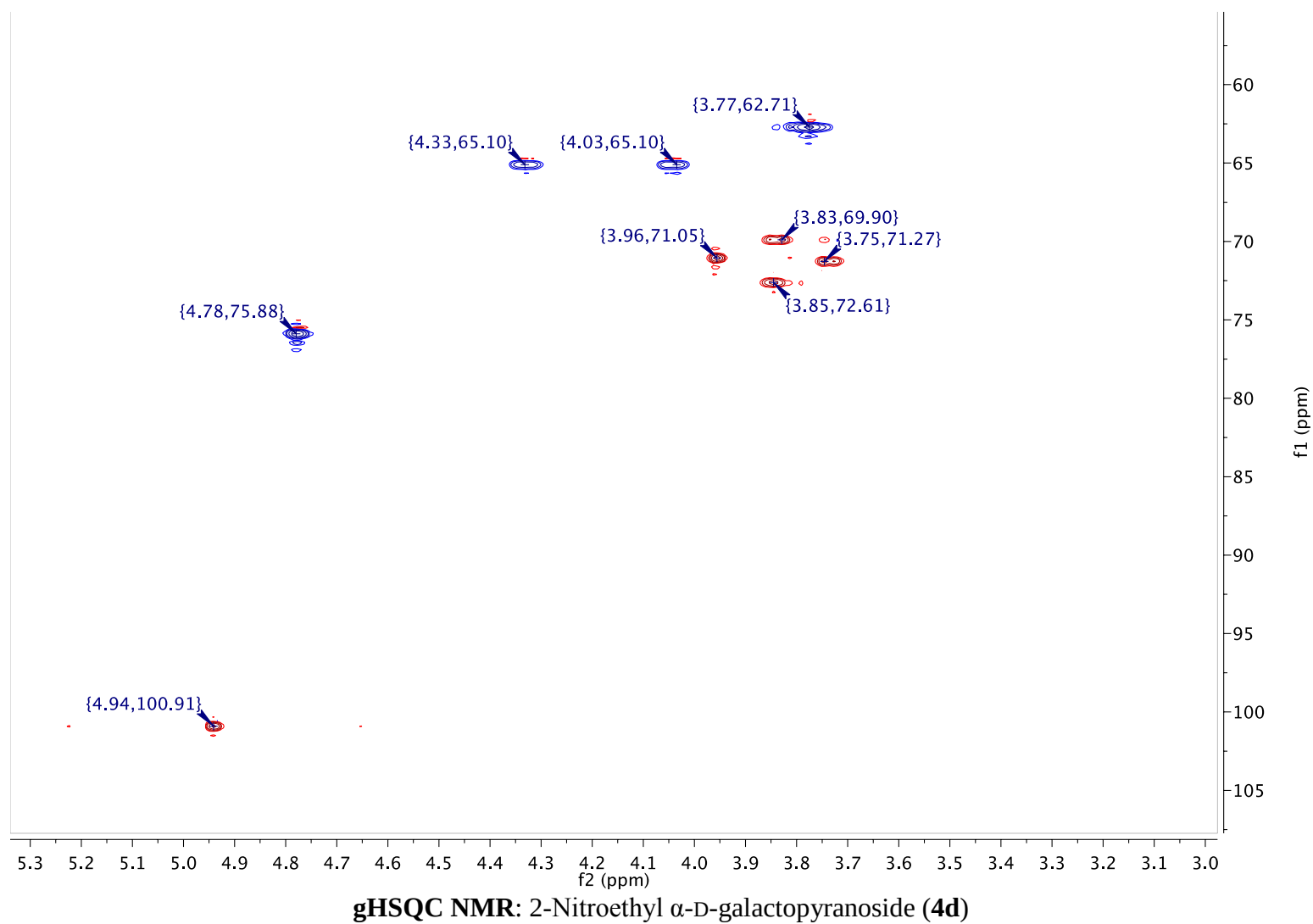
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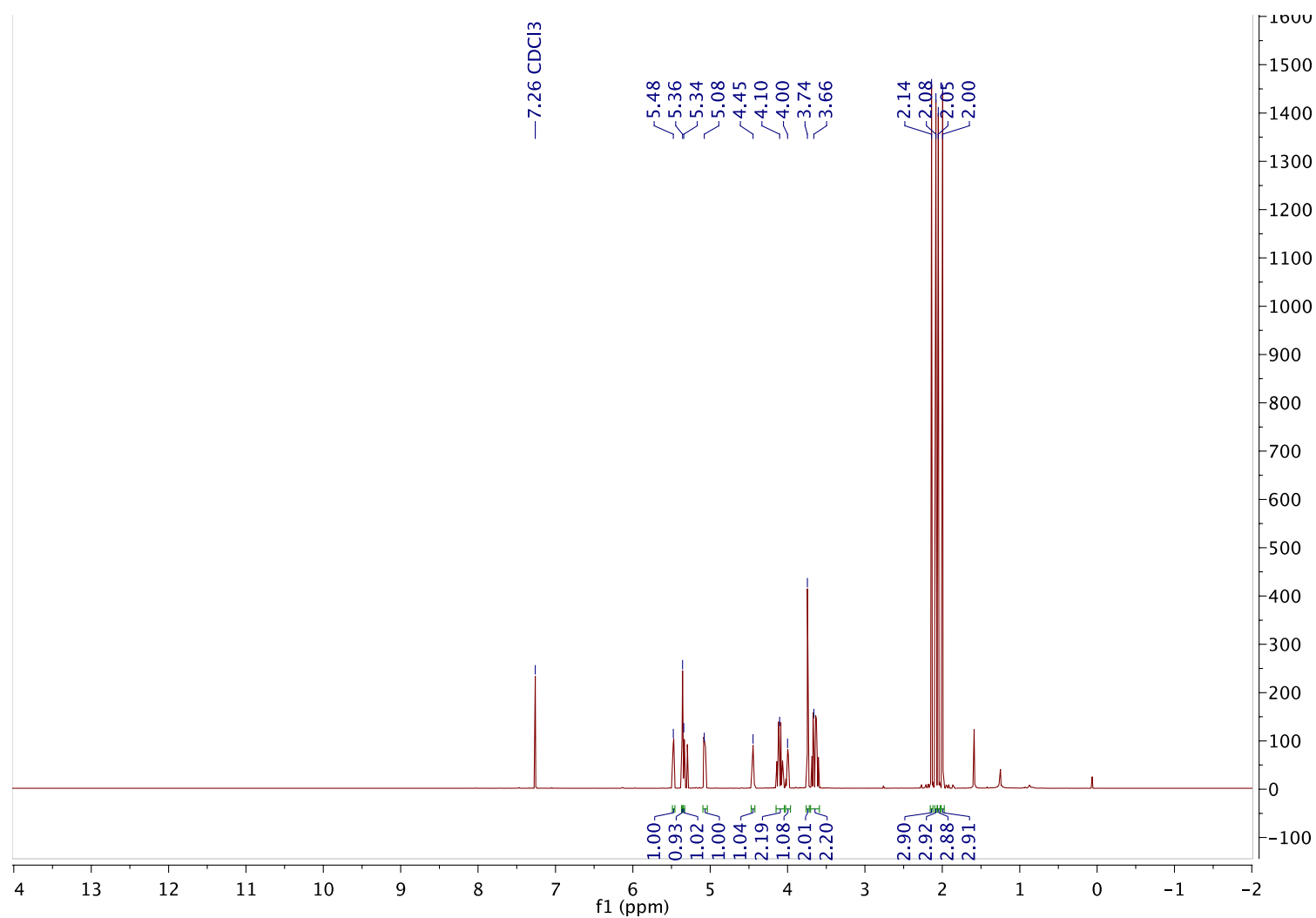


¹H NMR (500 MHz, CD₃OD): 2-Nitroethyl α-D-galactopyranoside (**4d**)

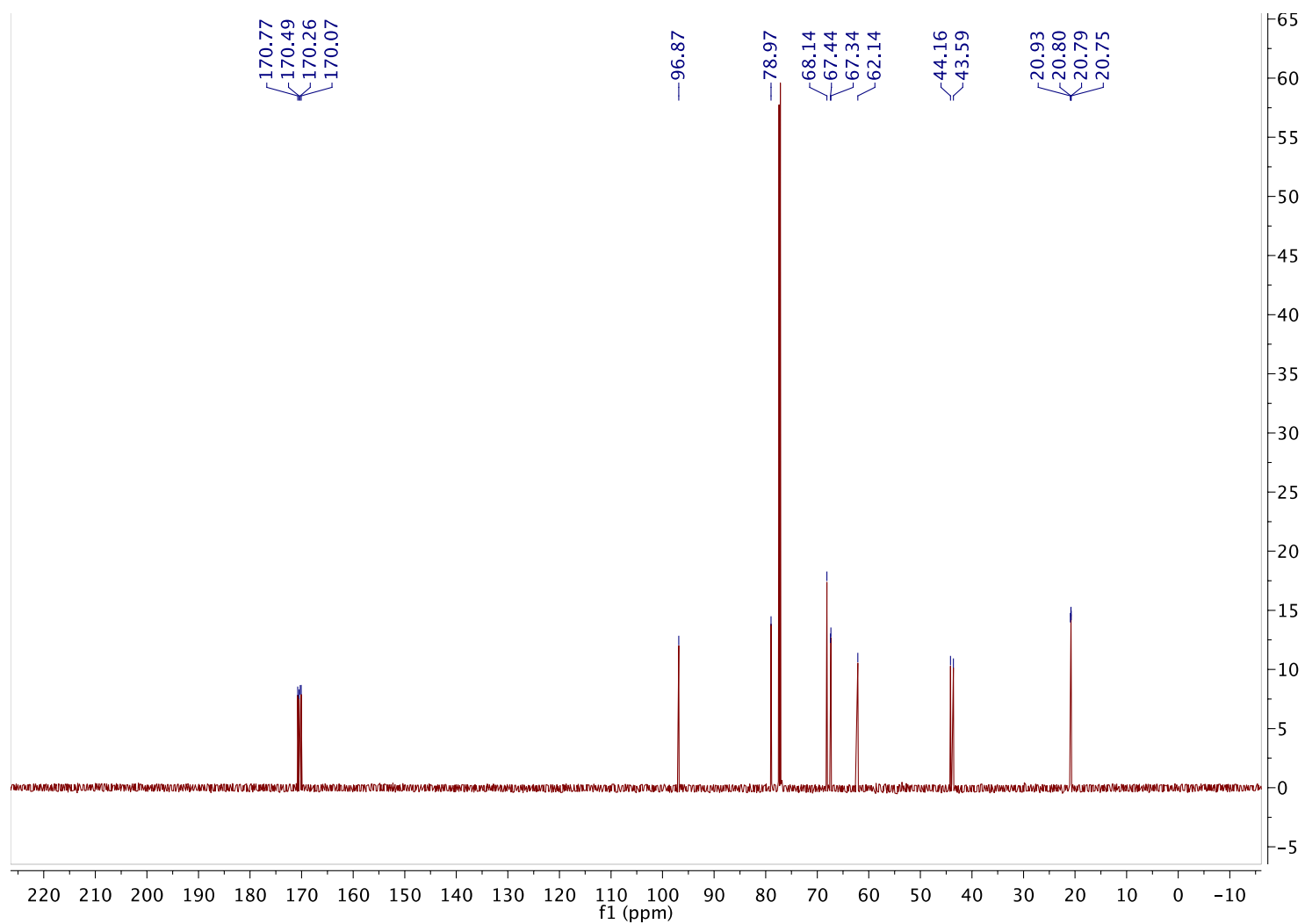


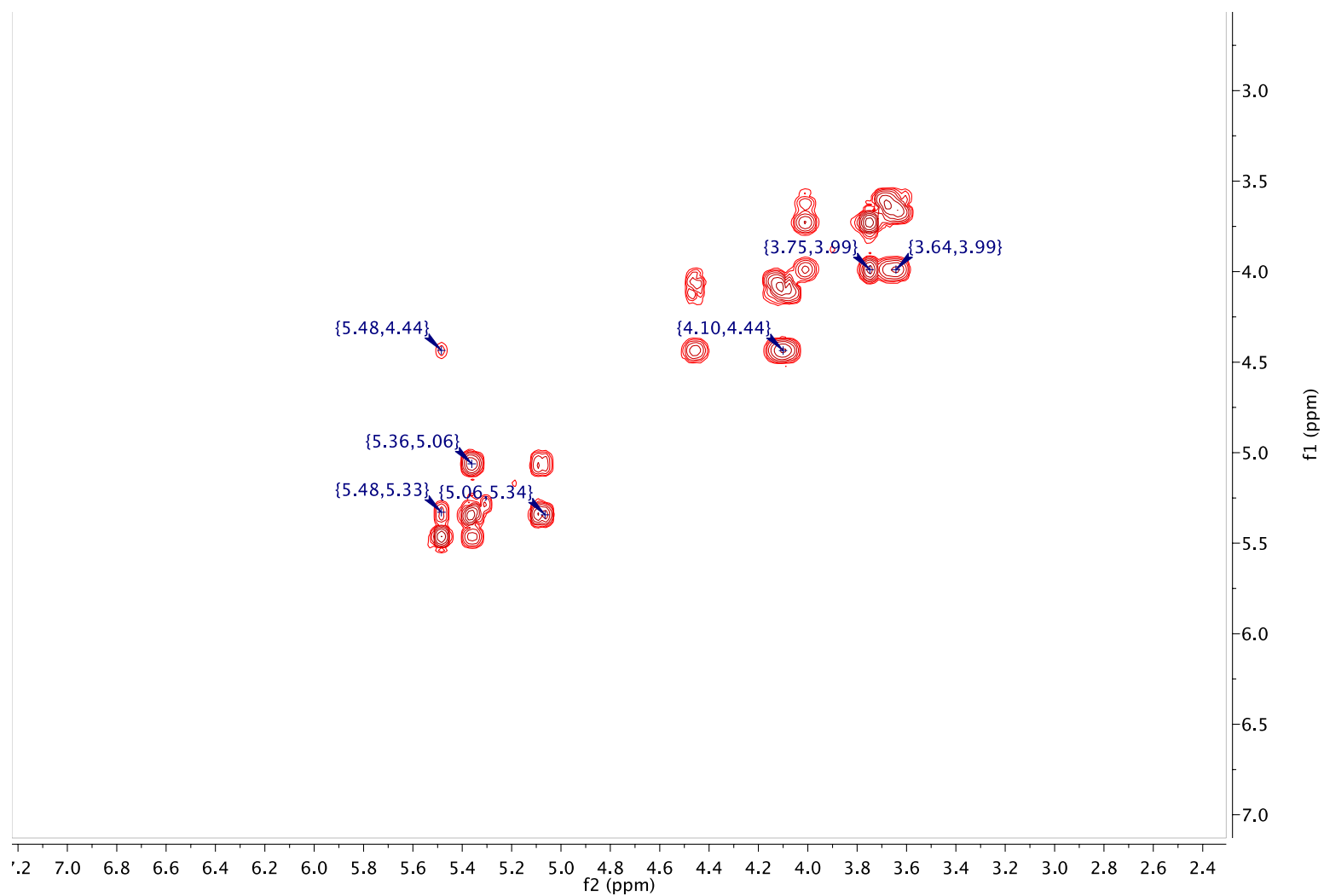




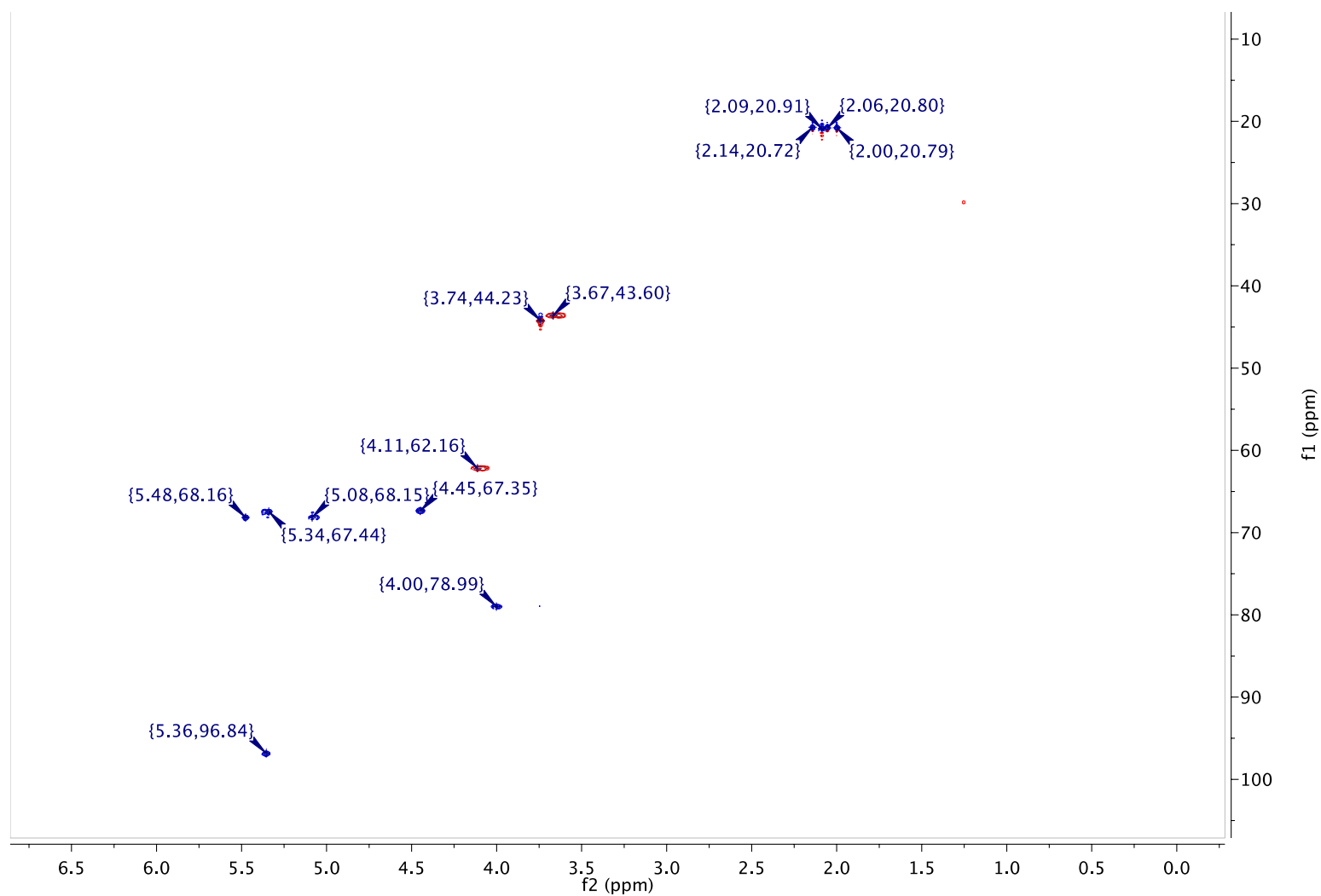


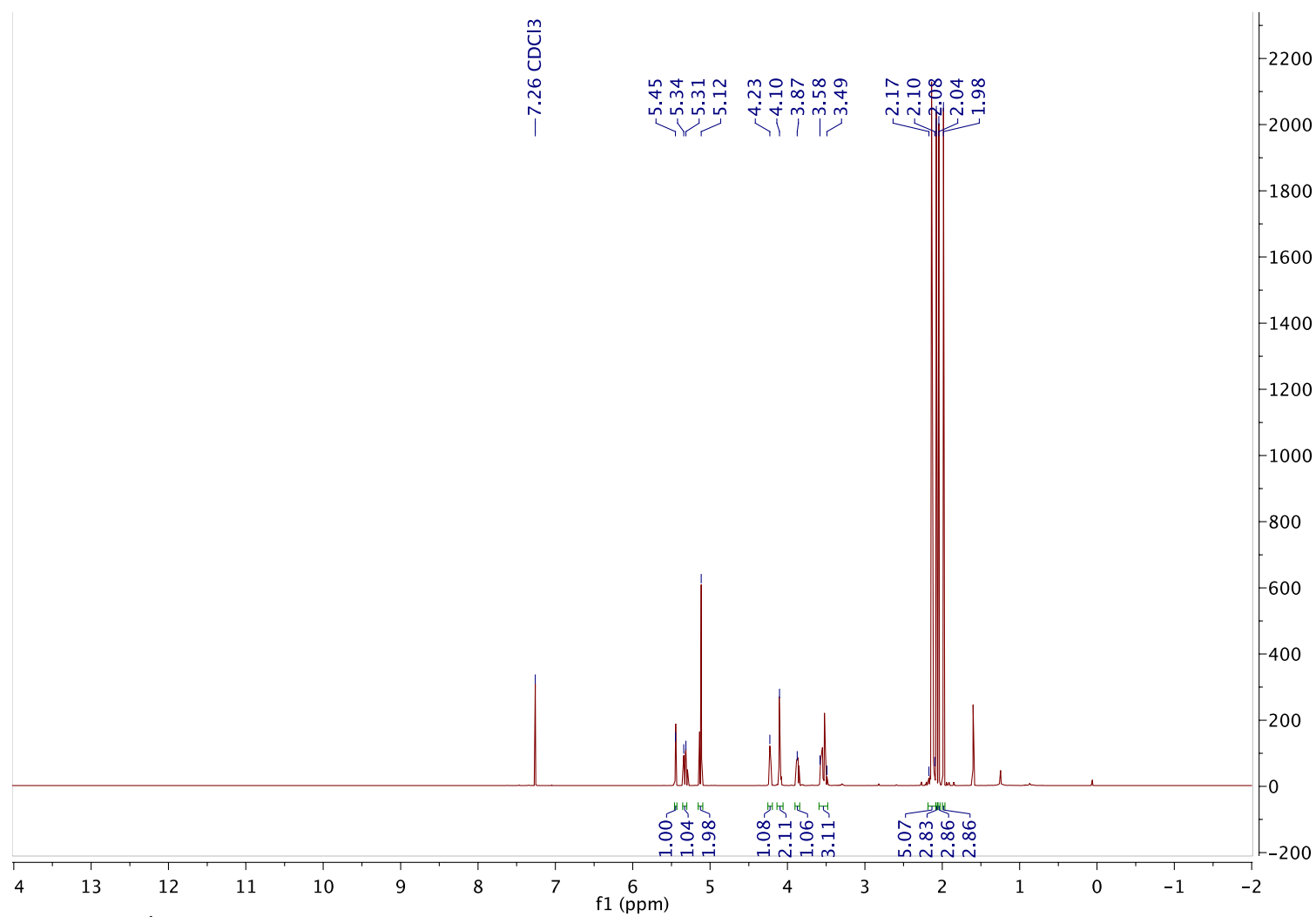
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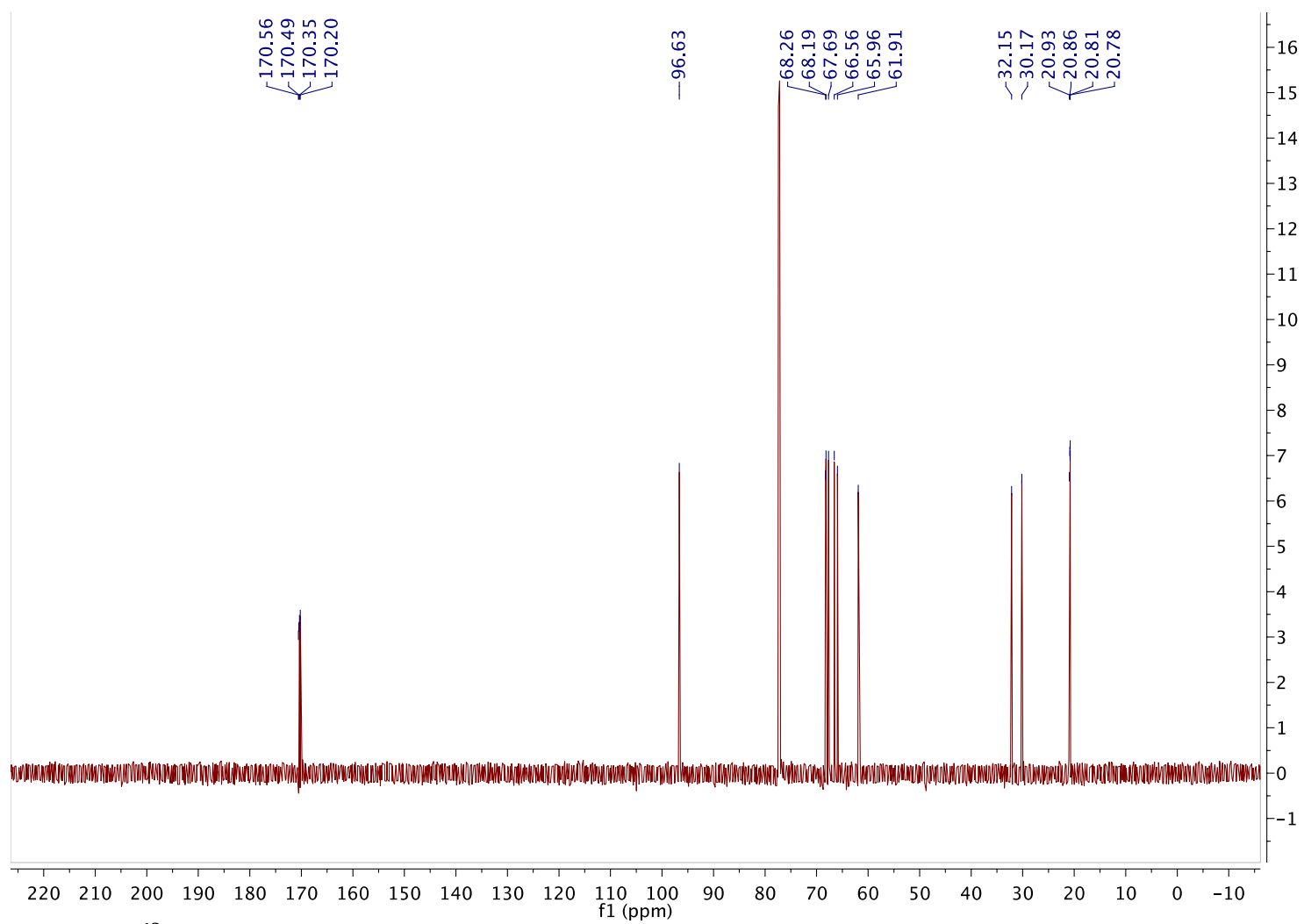


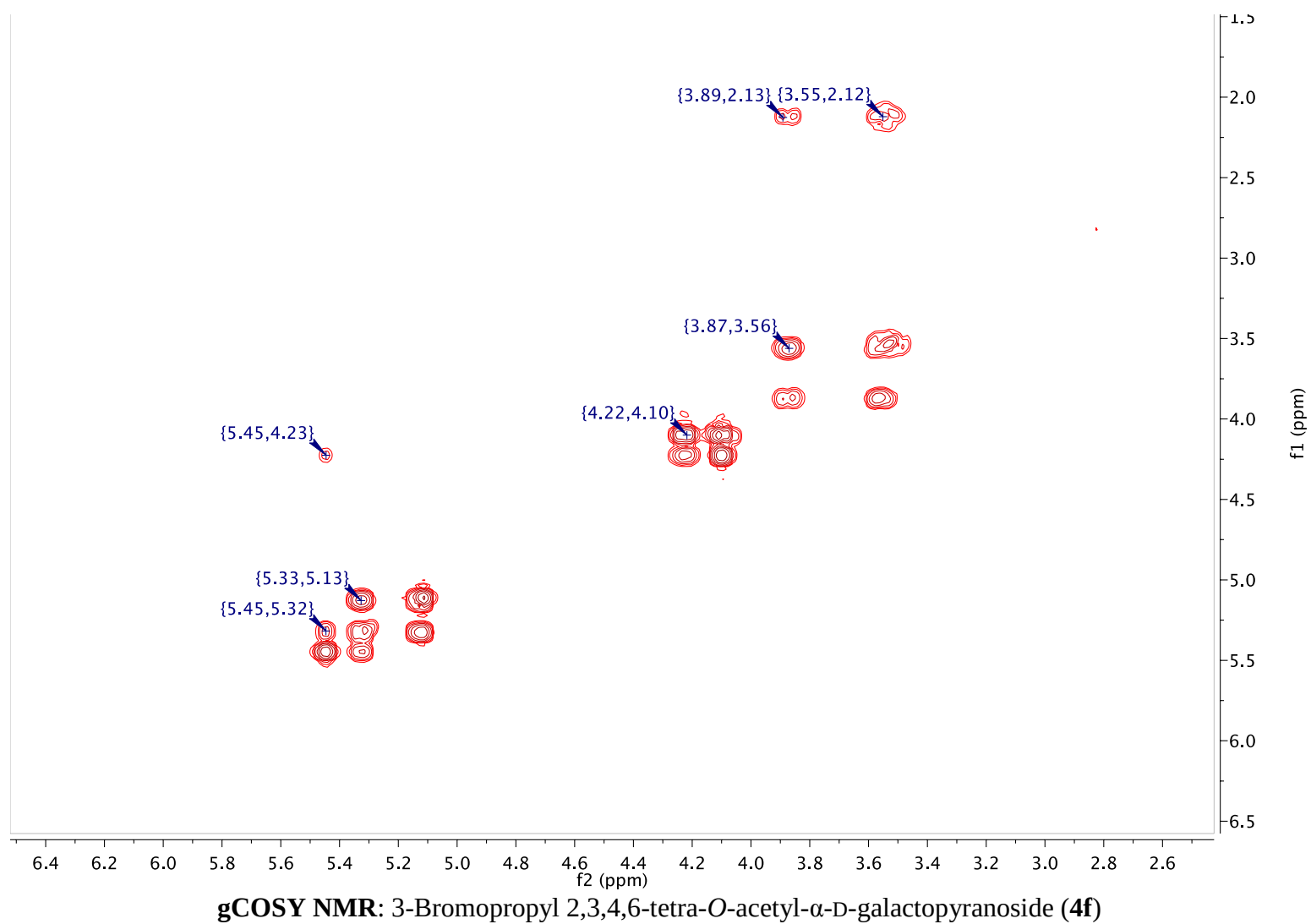


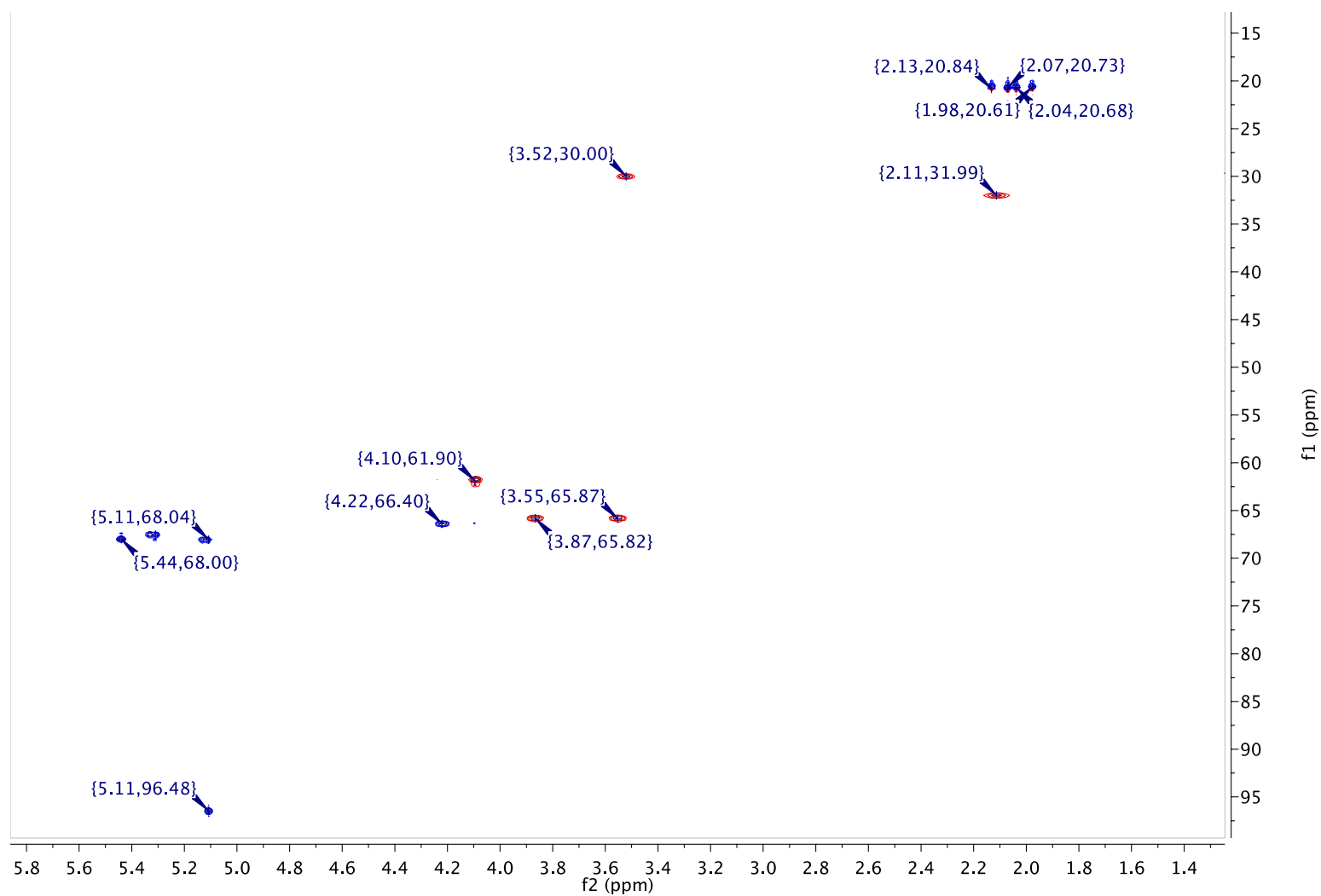
gCOSY NMR: 1,3-Dichloro-2-propyl 2,3,4,6-tetra-O-acetyl- α -D-galactopyranoside (4e**)**



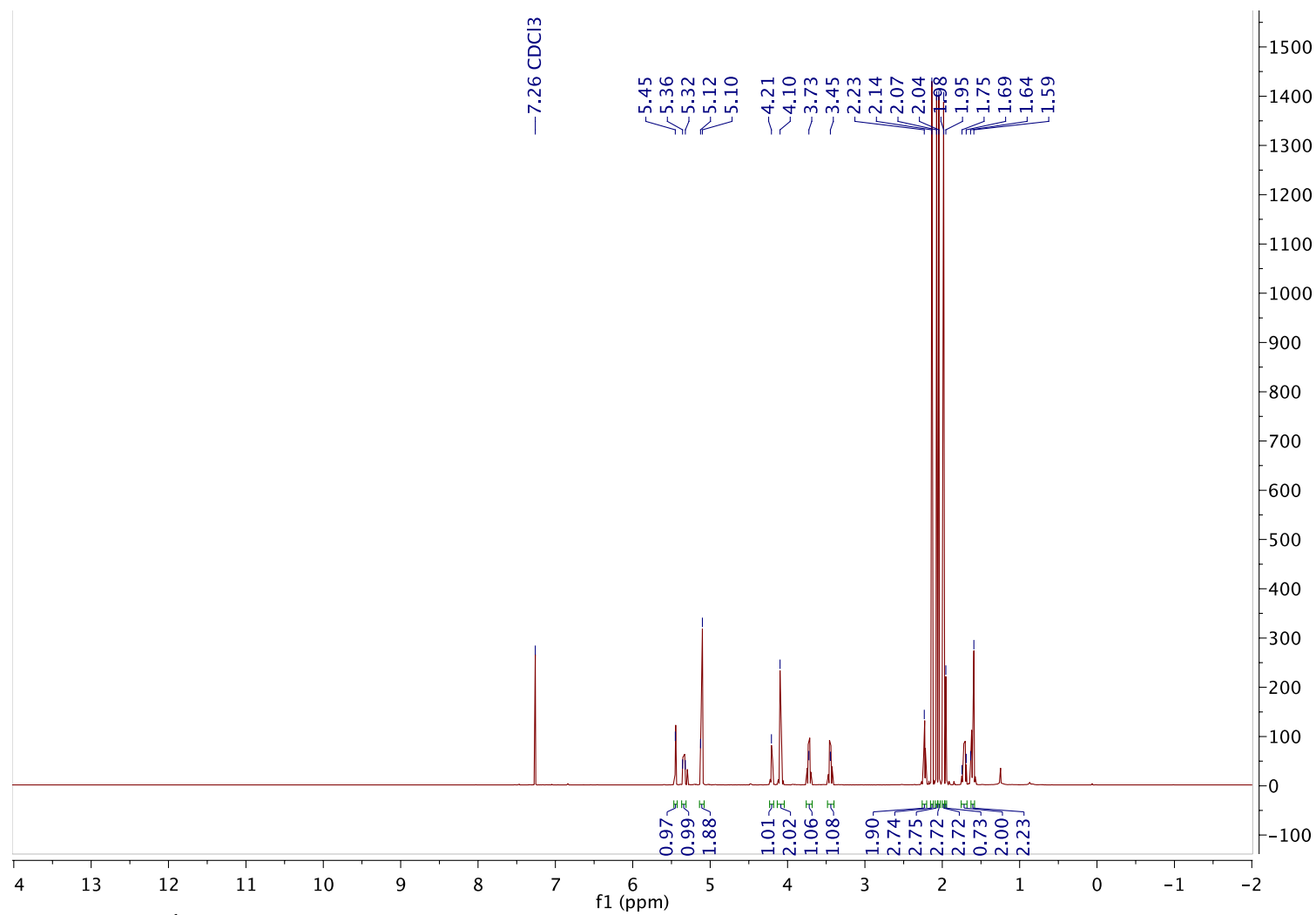




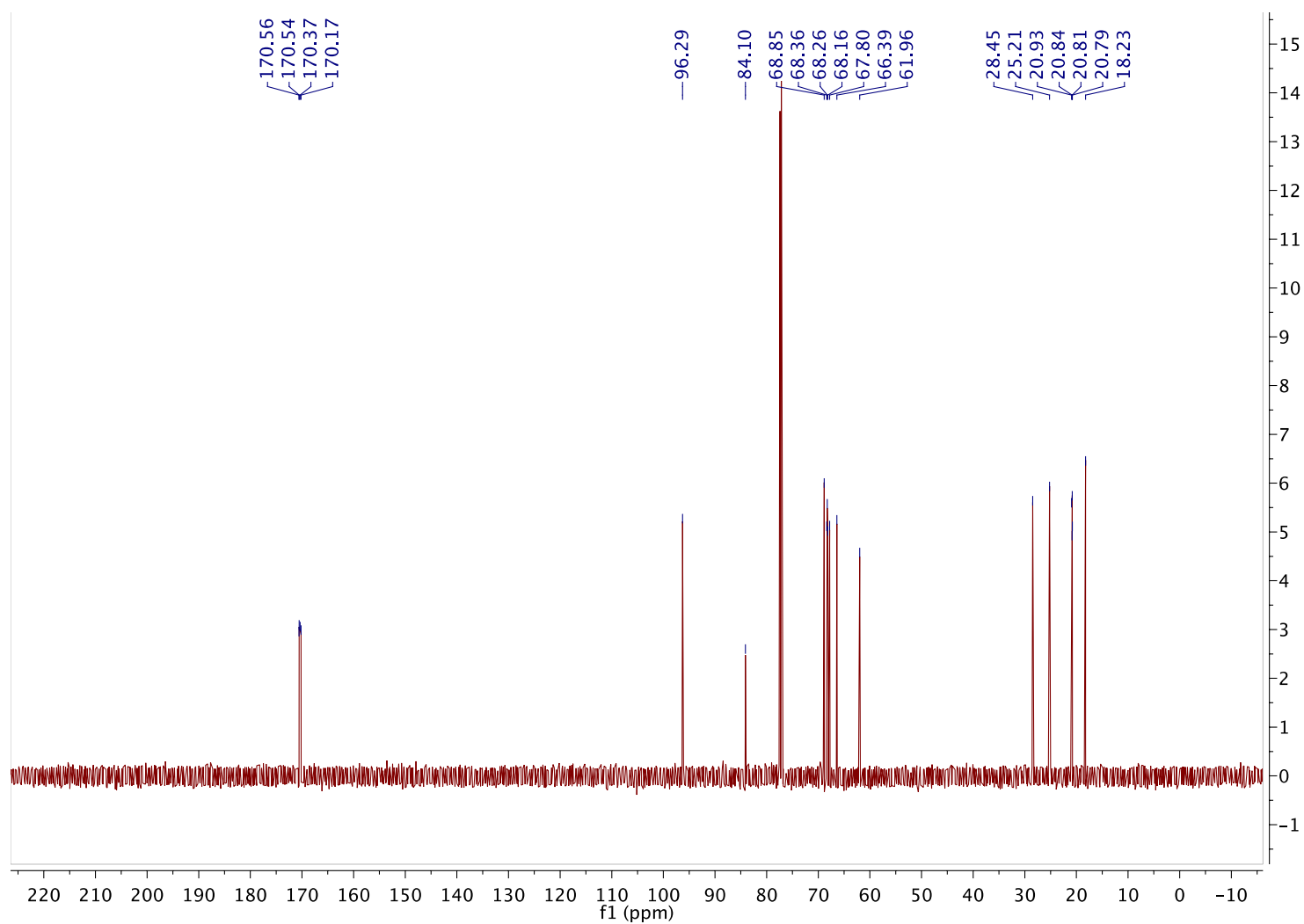


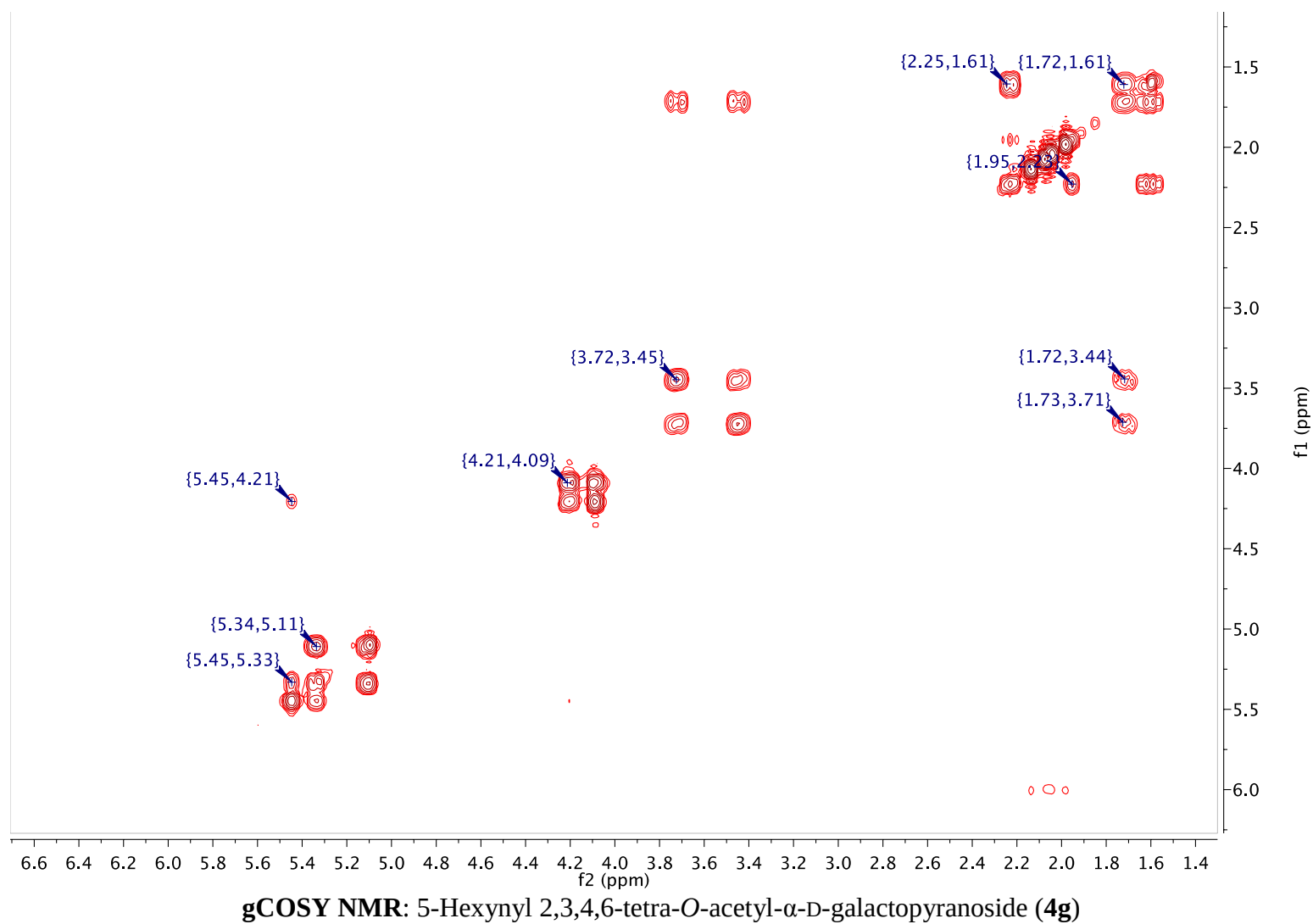


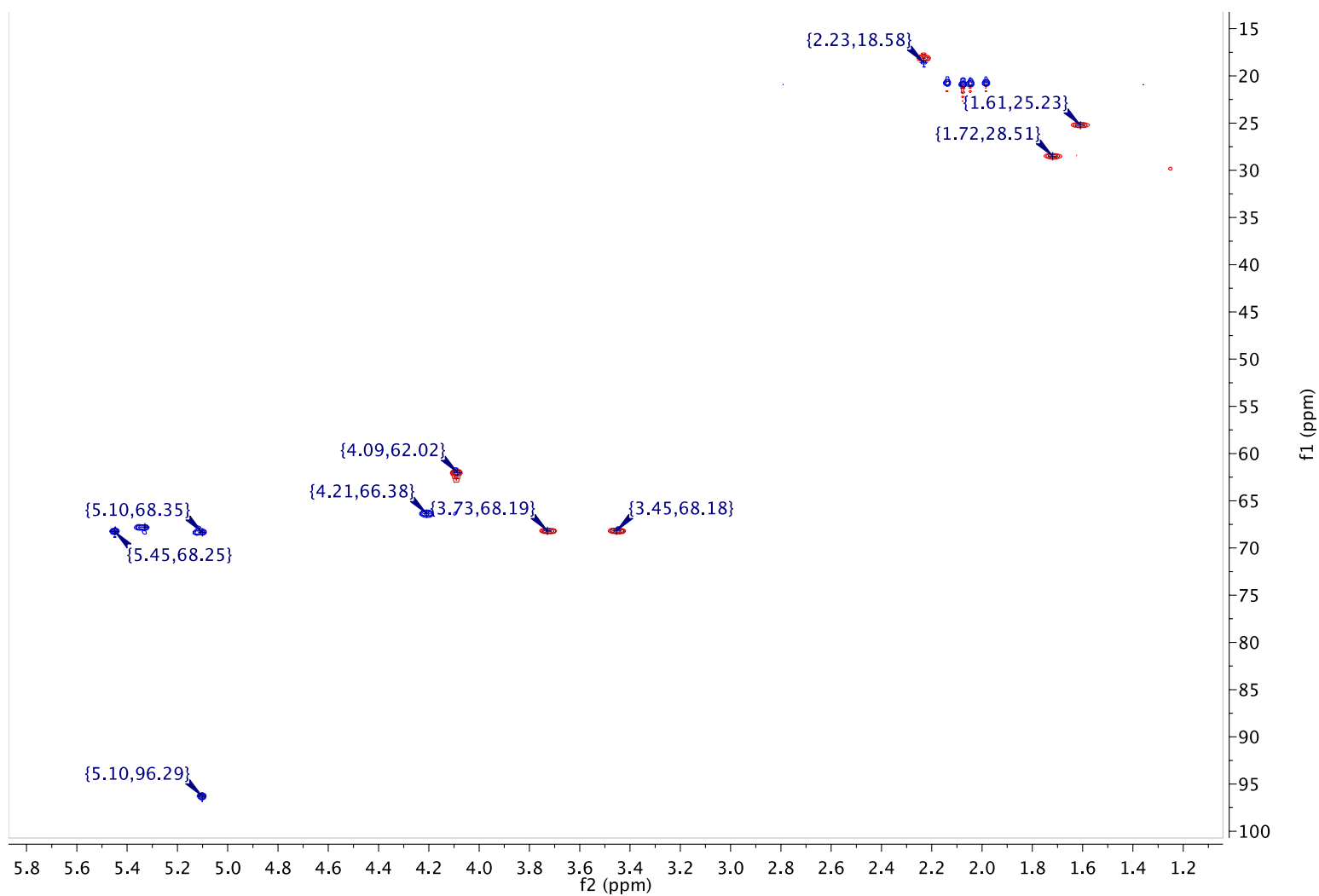
gHSQC NMR: 3-Bromopropyl 2,3,4,6-tetra-O-acetyl- α -D-galactopyranoside (4f)



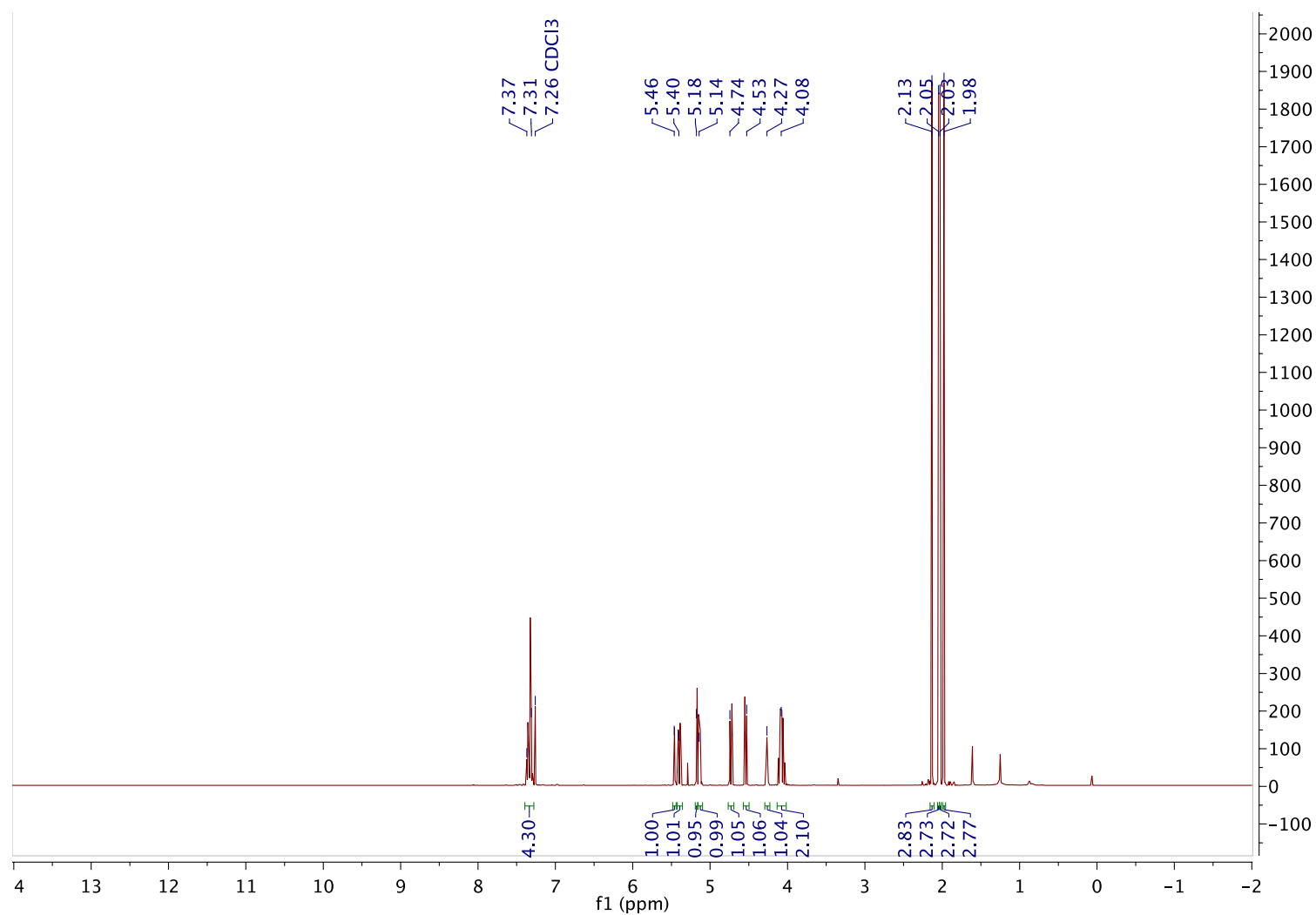
¹H NMR (500 MHz, CDCl₃): 5-Hexynyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (**4g**)



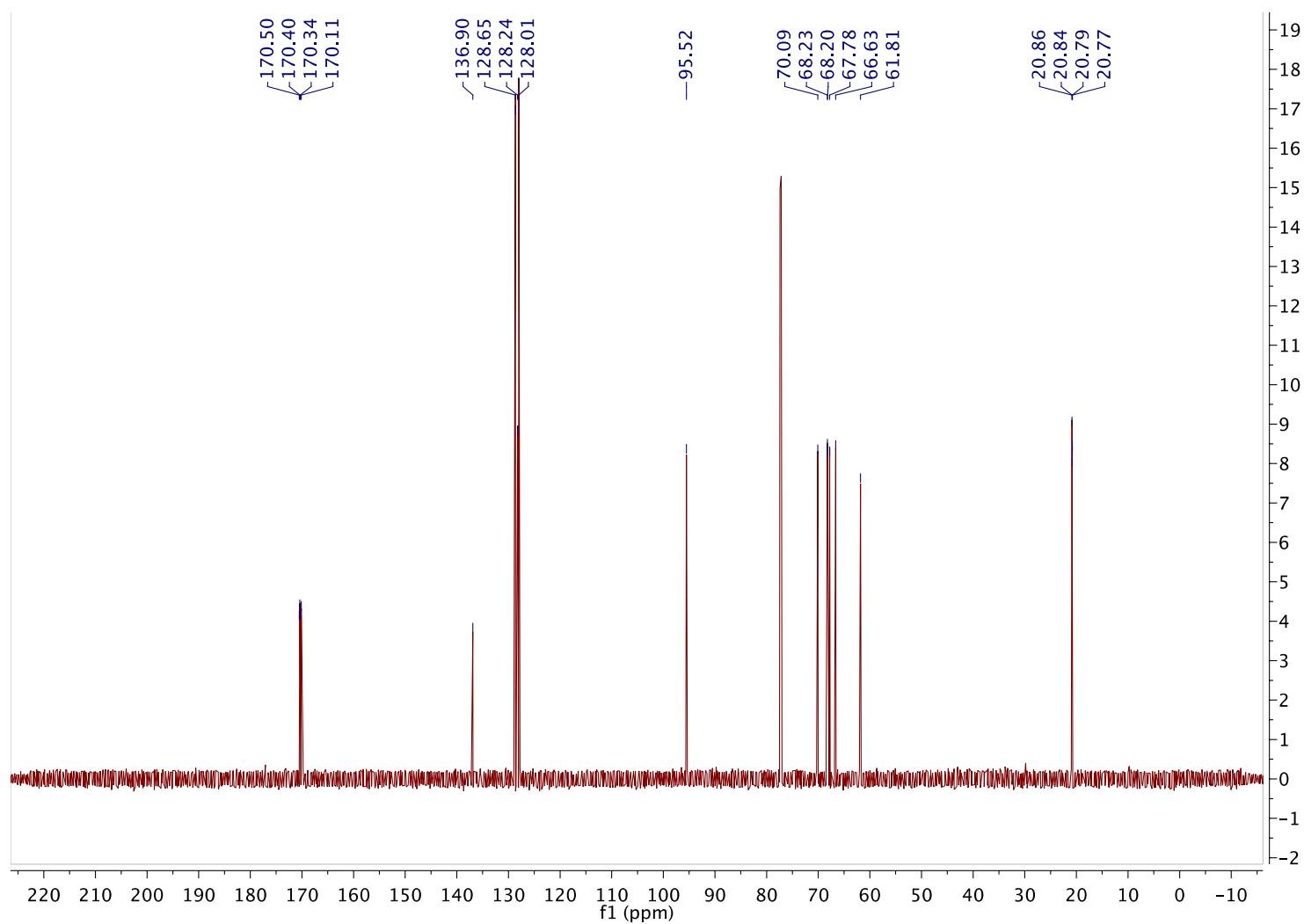




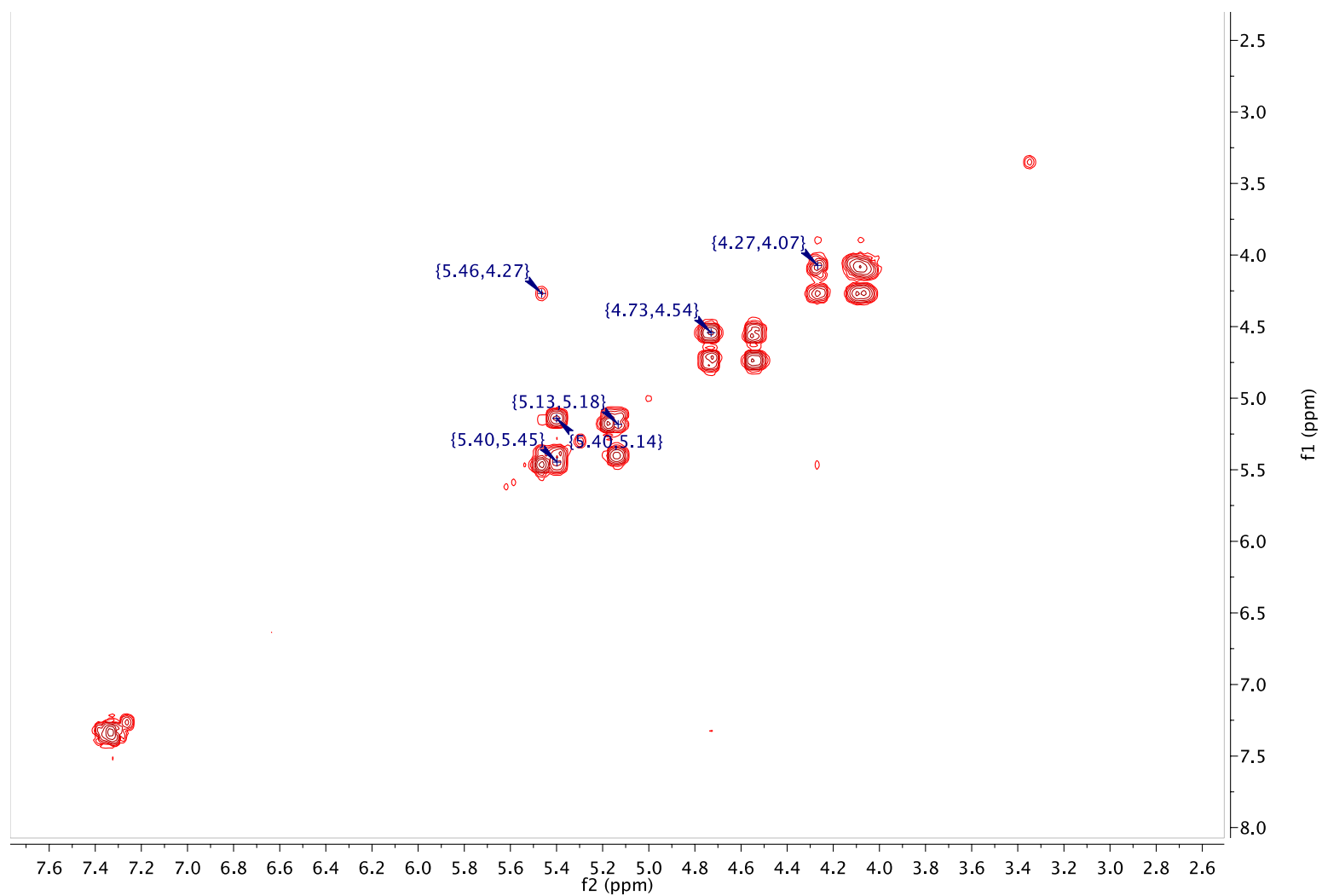
gHSQC NMR: 5-Hexynyl 2,3,4,6-tetra-O-acetyl- α -D-galactopyranoside (4g)



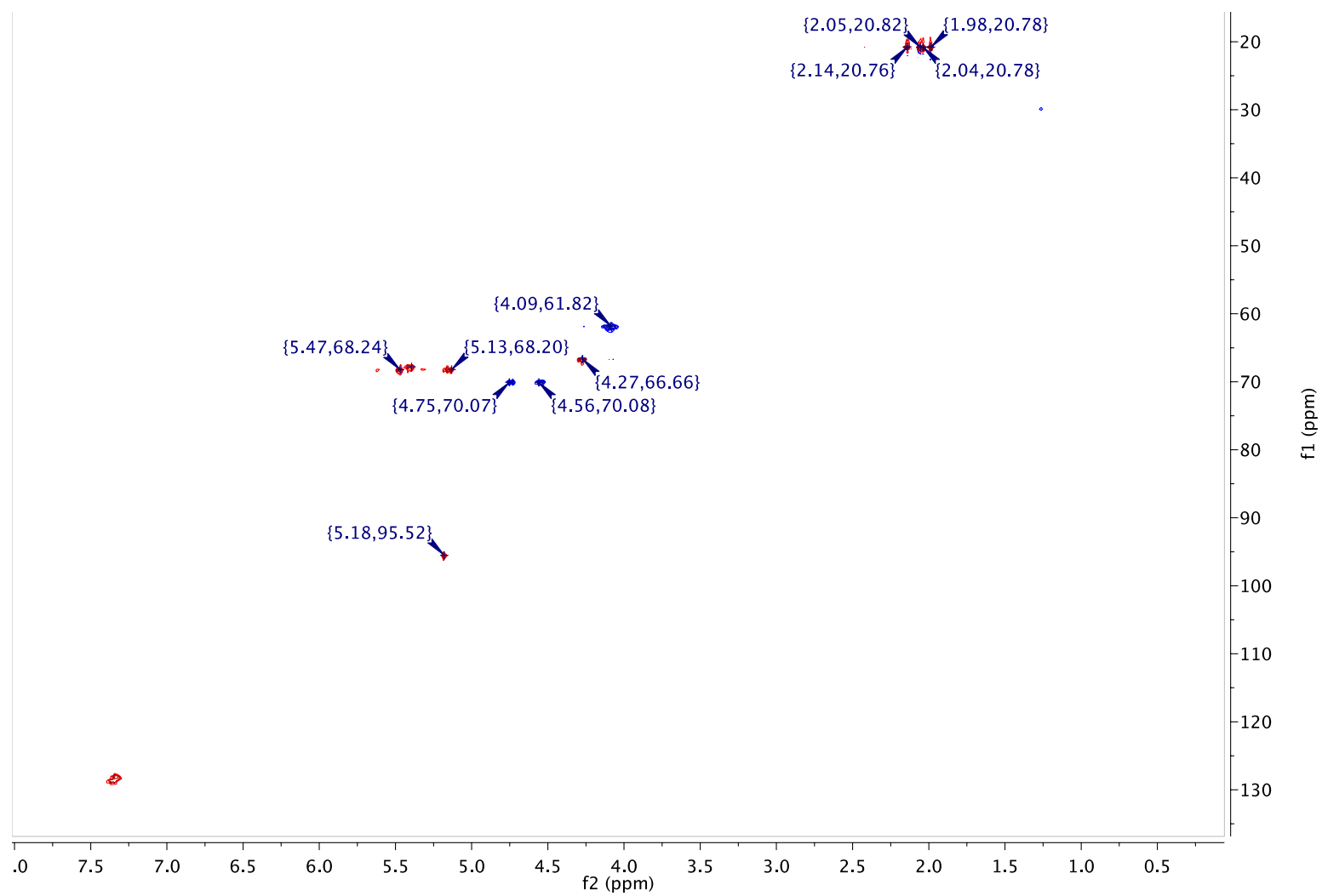
¹H NMR (500 MHz, CDCl₃): Benzyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (**4h**)



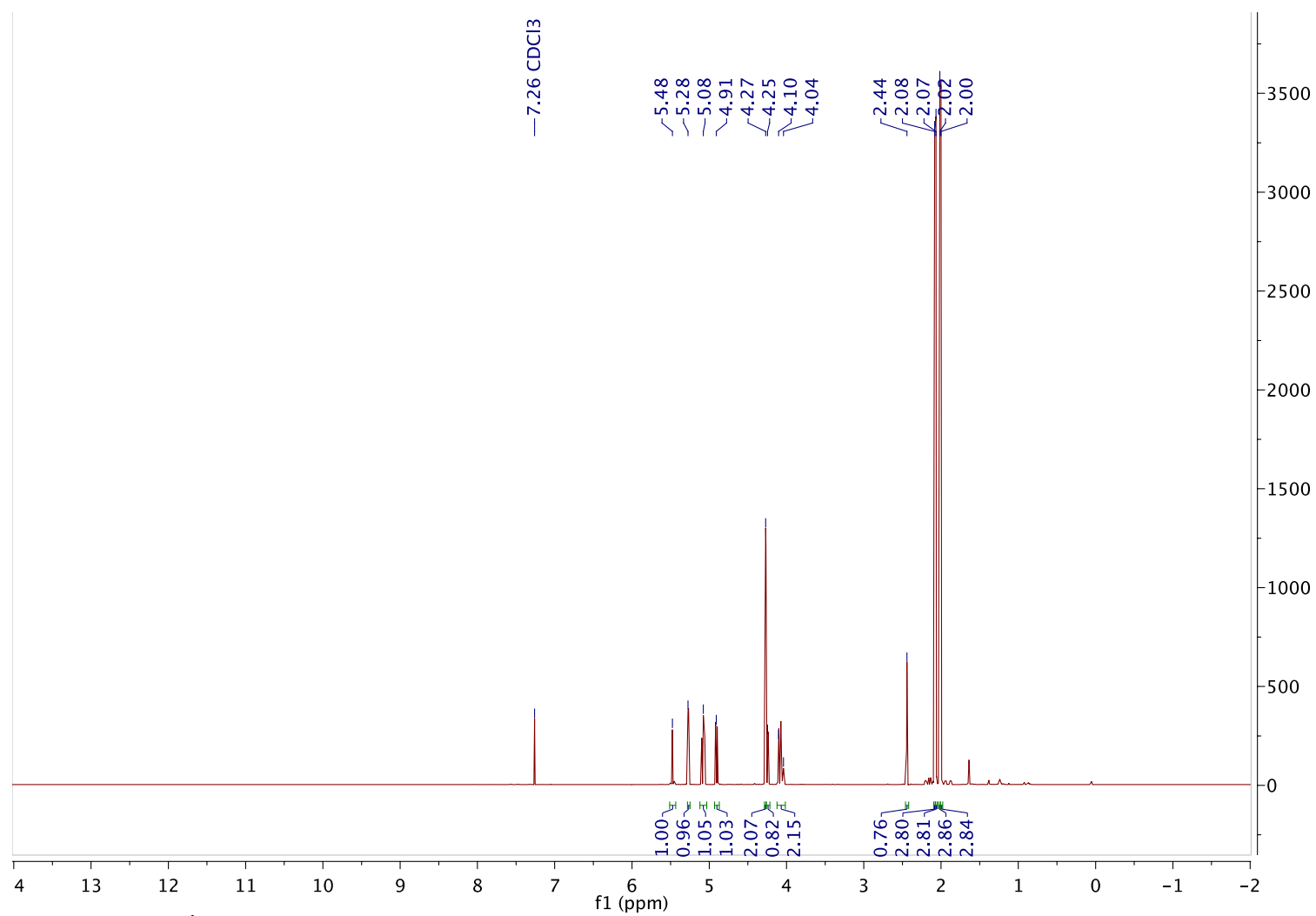
^{13}C NMR (125 MHz, CDCl_3): Benzyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (**4h**)



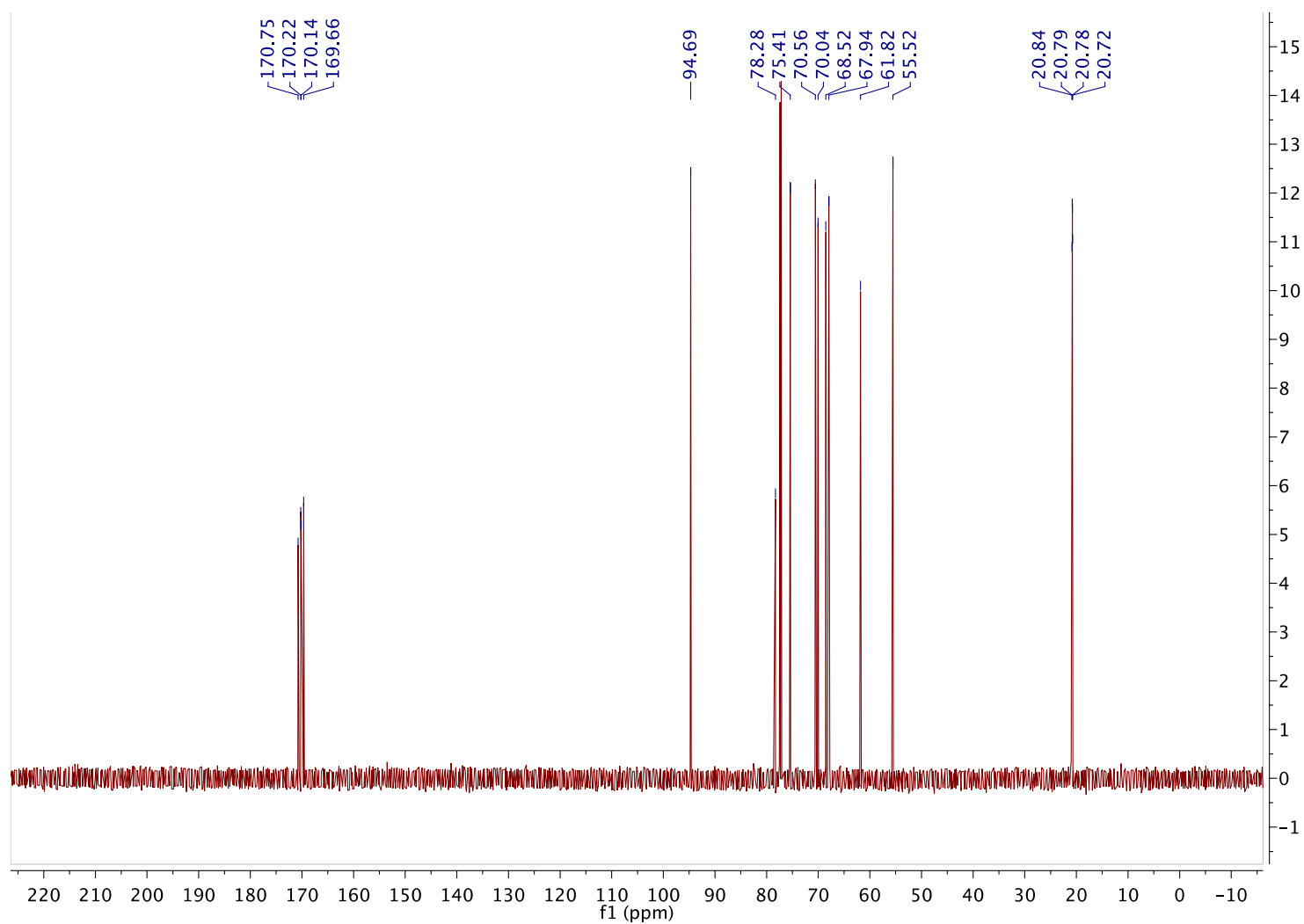
gCOSY NMR: Benzyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (4h)



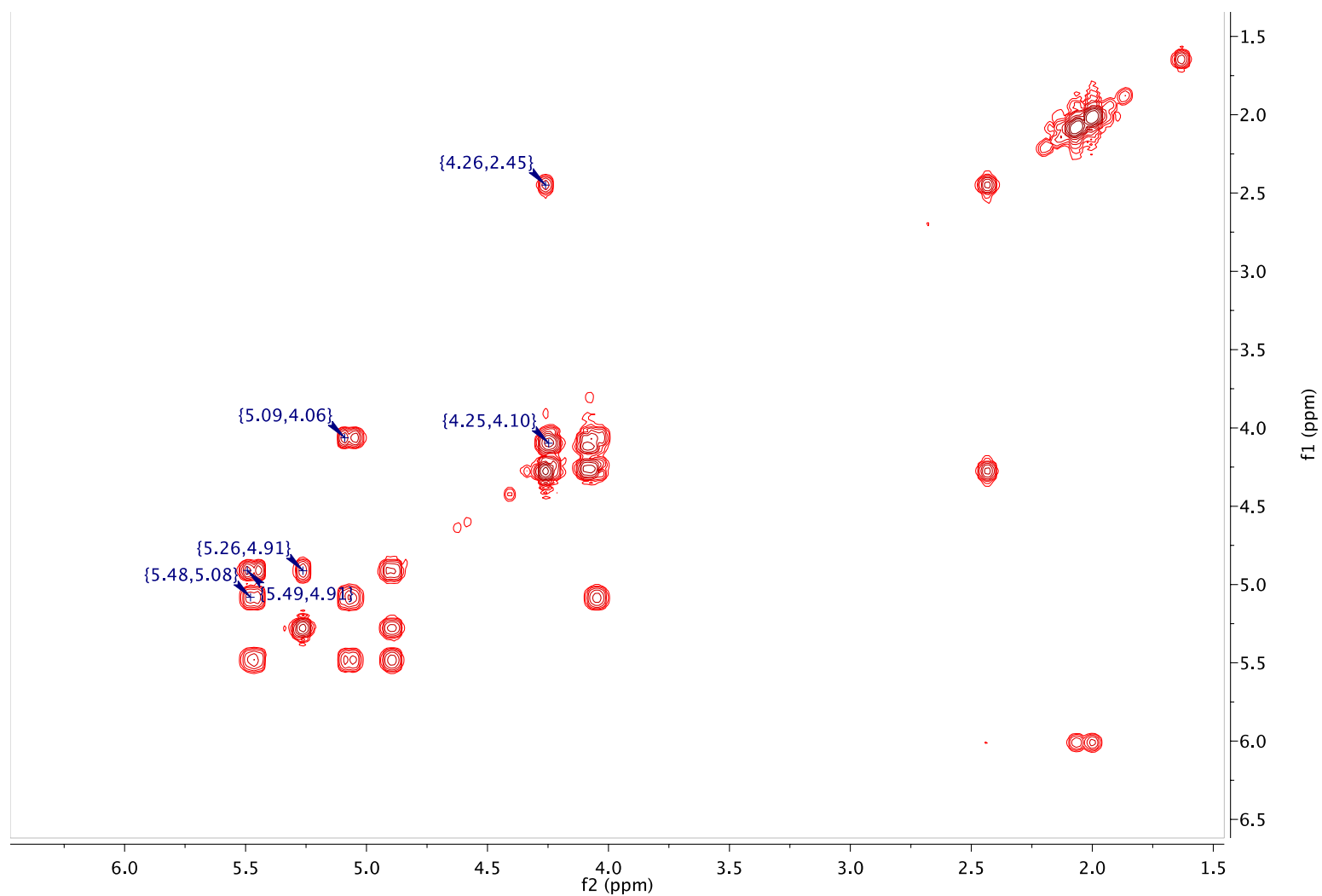
gHSQC NMR: Benzyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (4h)



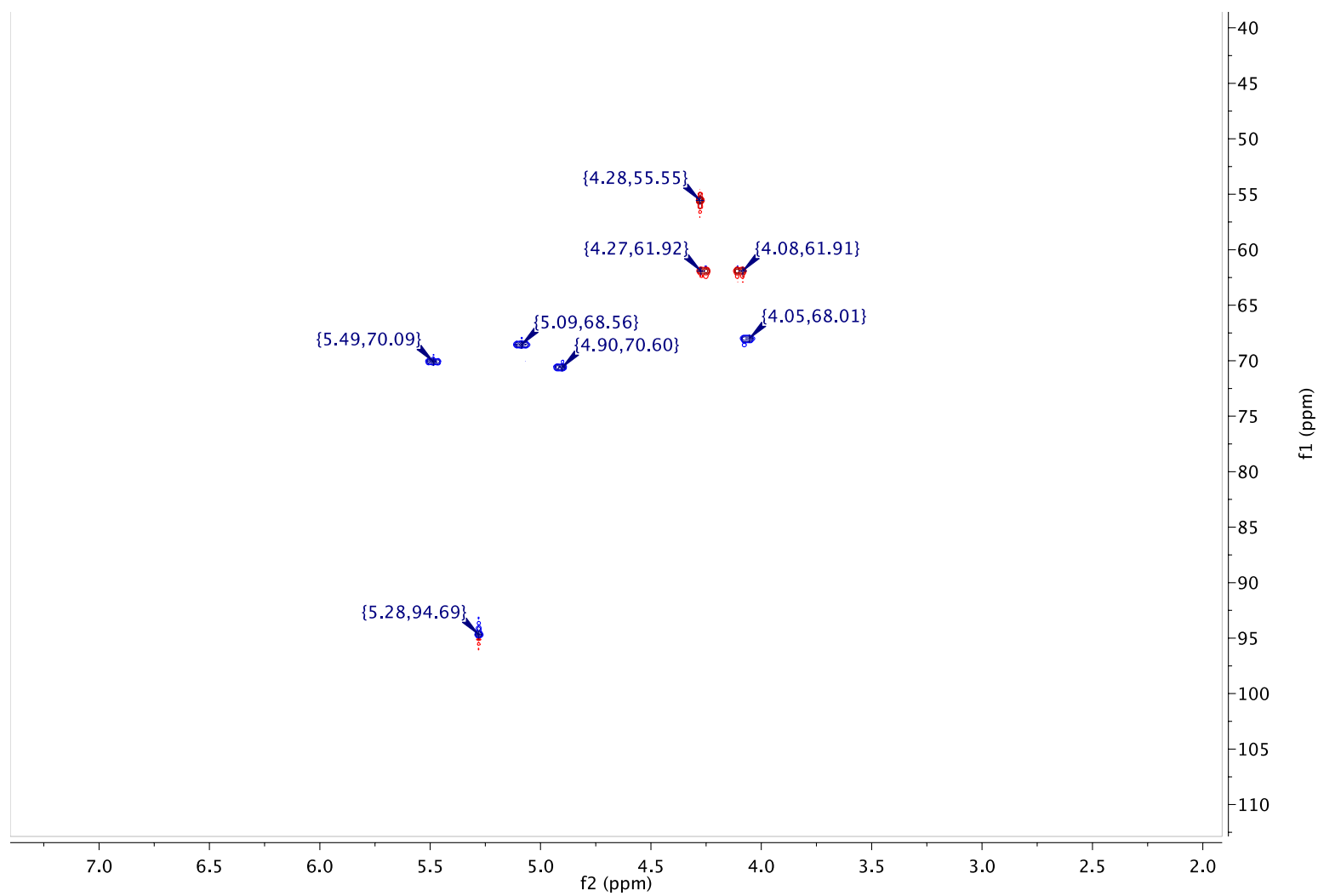
¹H NMR (500 MHz, CDCl₃): Propargyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**4i**)



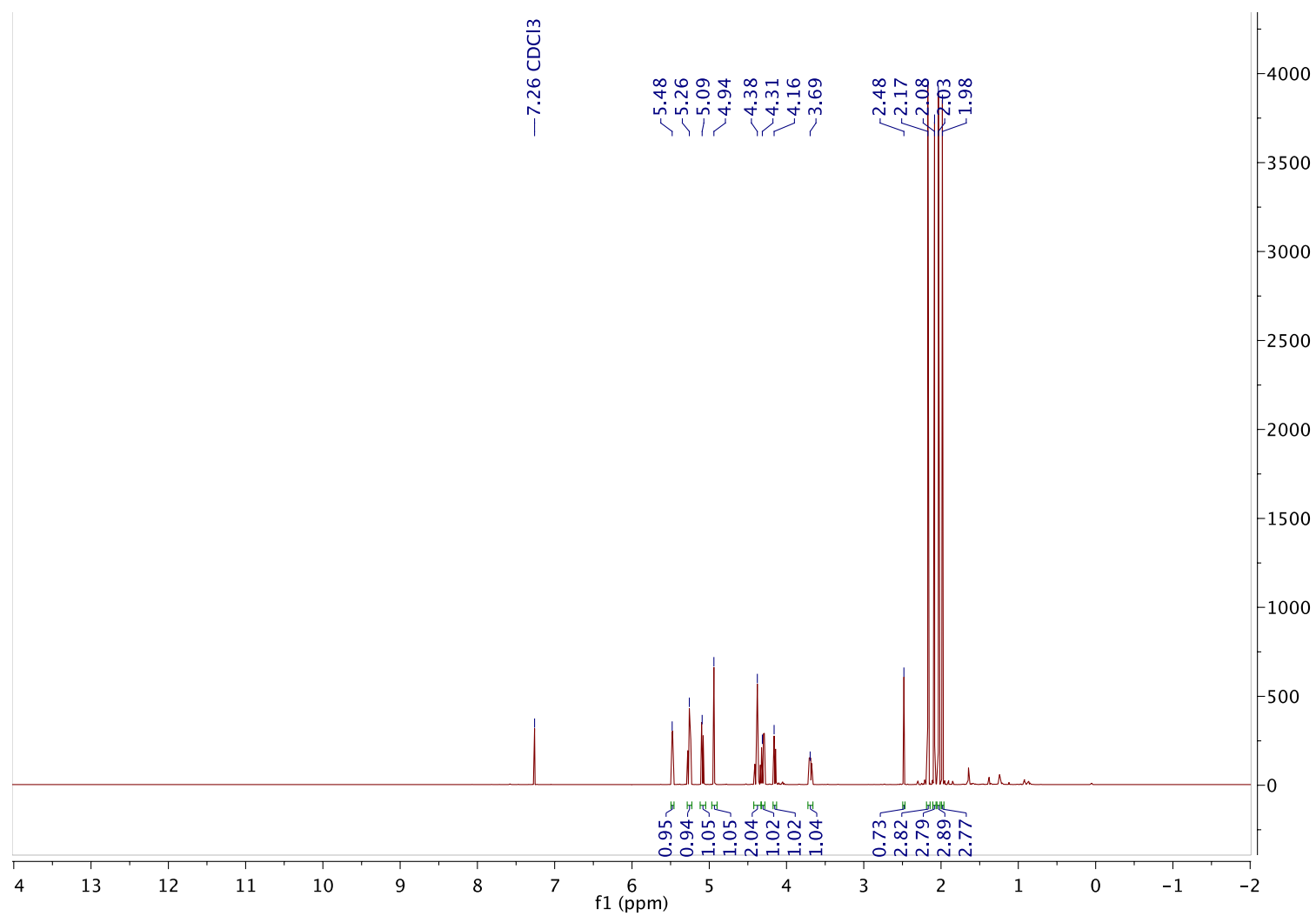
¹³C NMR (125 MHz, CDCl₃): Propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4i**)



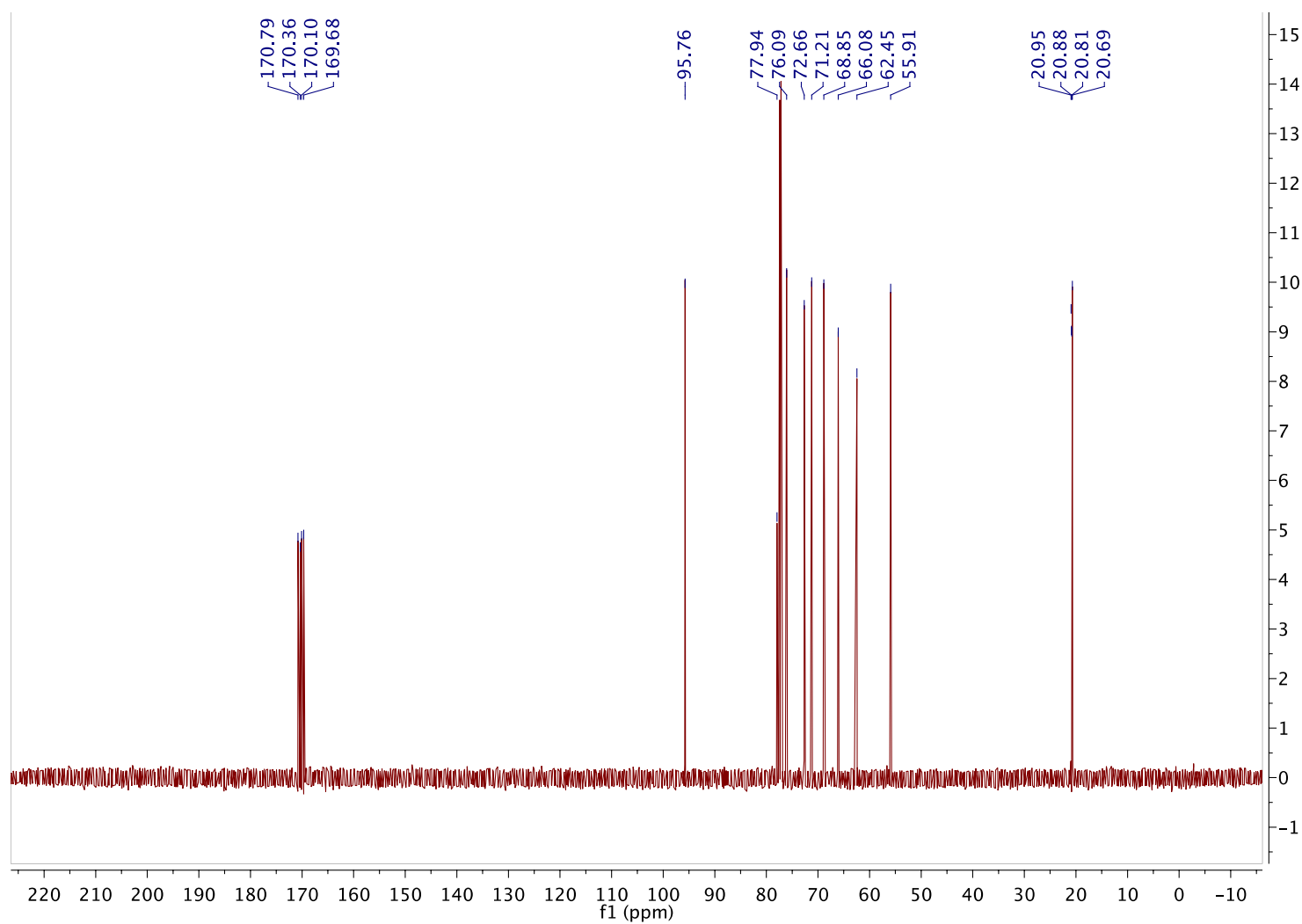
gCOSY NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside (4i**)**

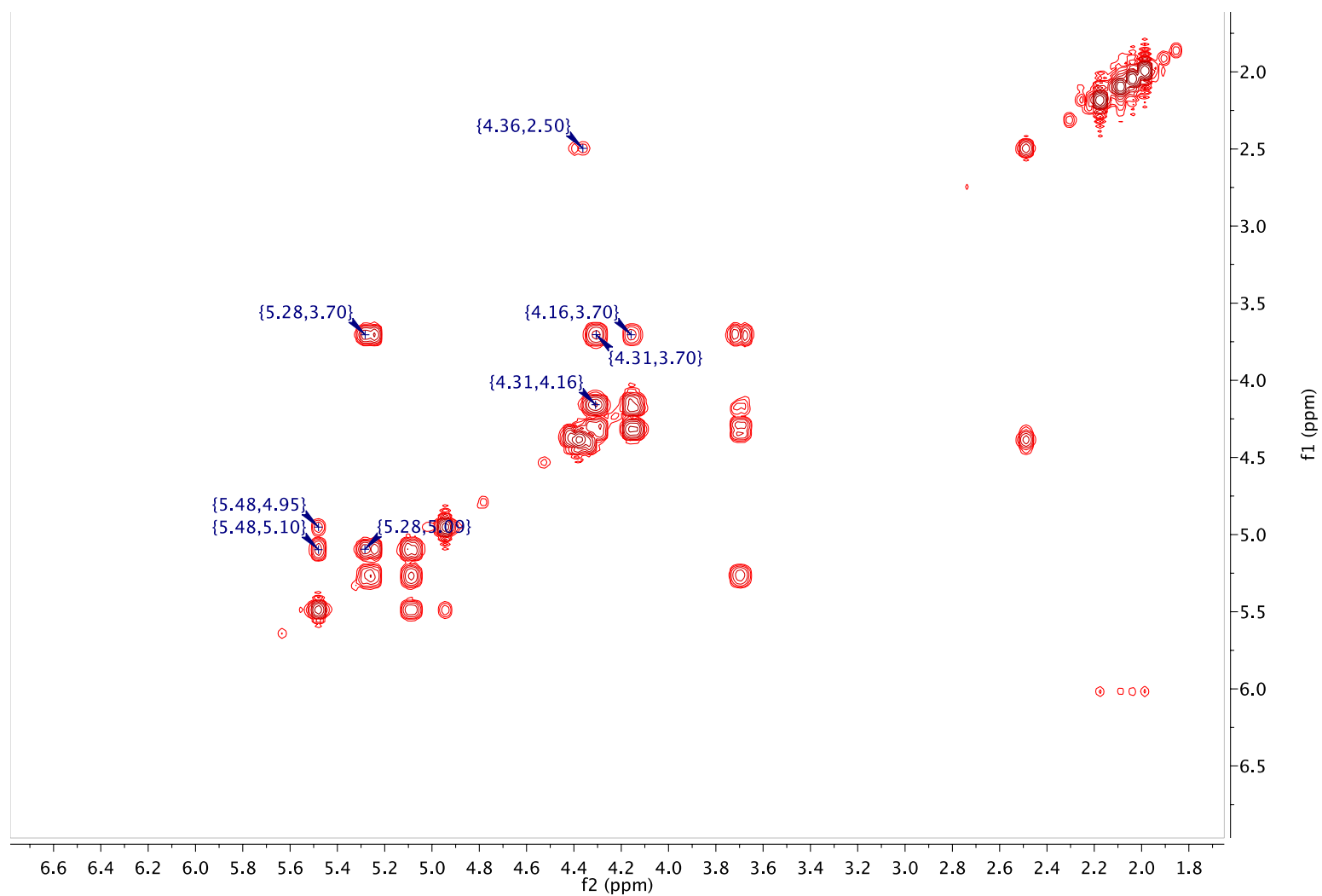


gHSQC NMR: Propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside (4i)

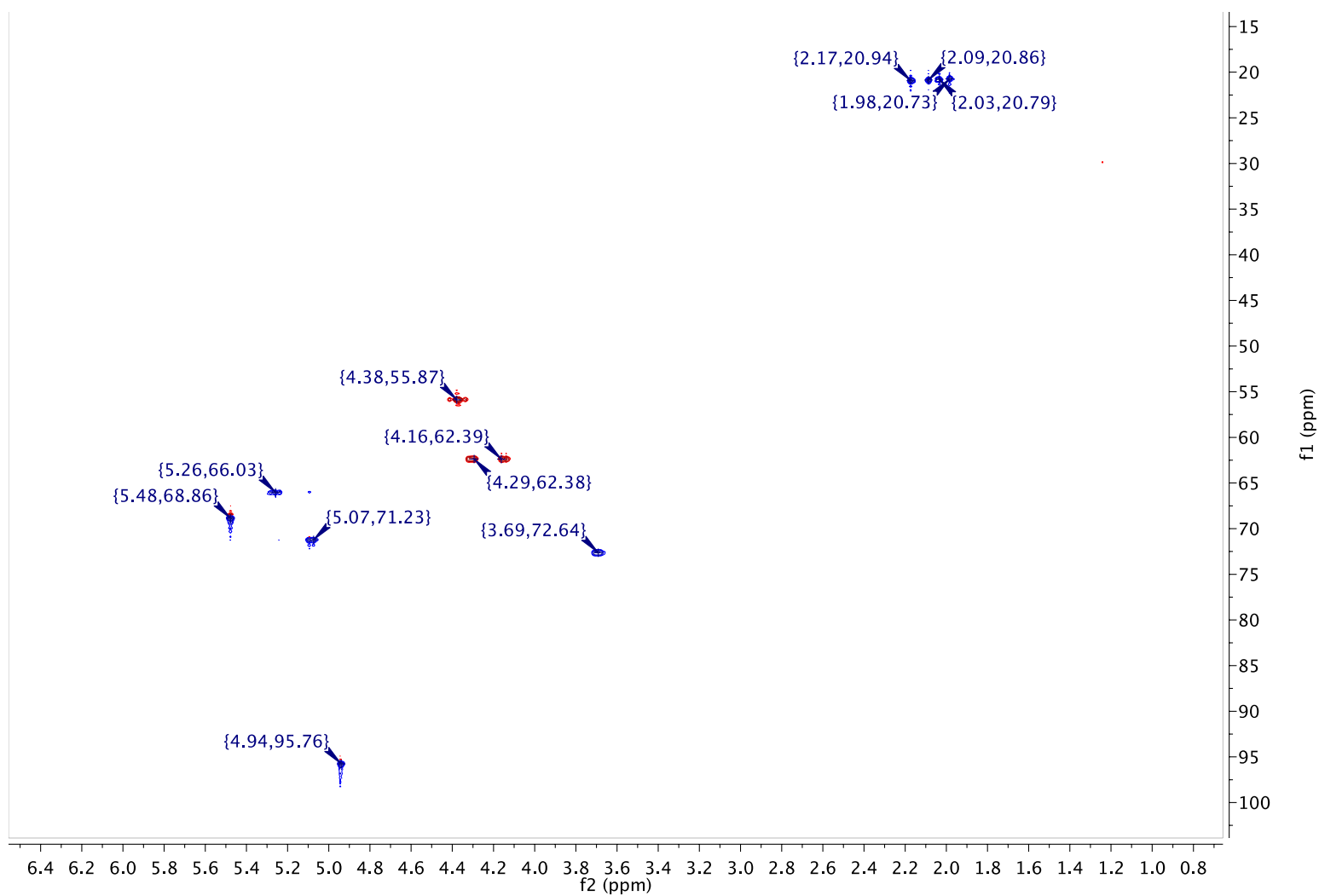


¹H NMR (500 MHz, CDCl₃): Propargyl 2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside (4j**)**

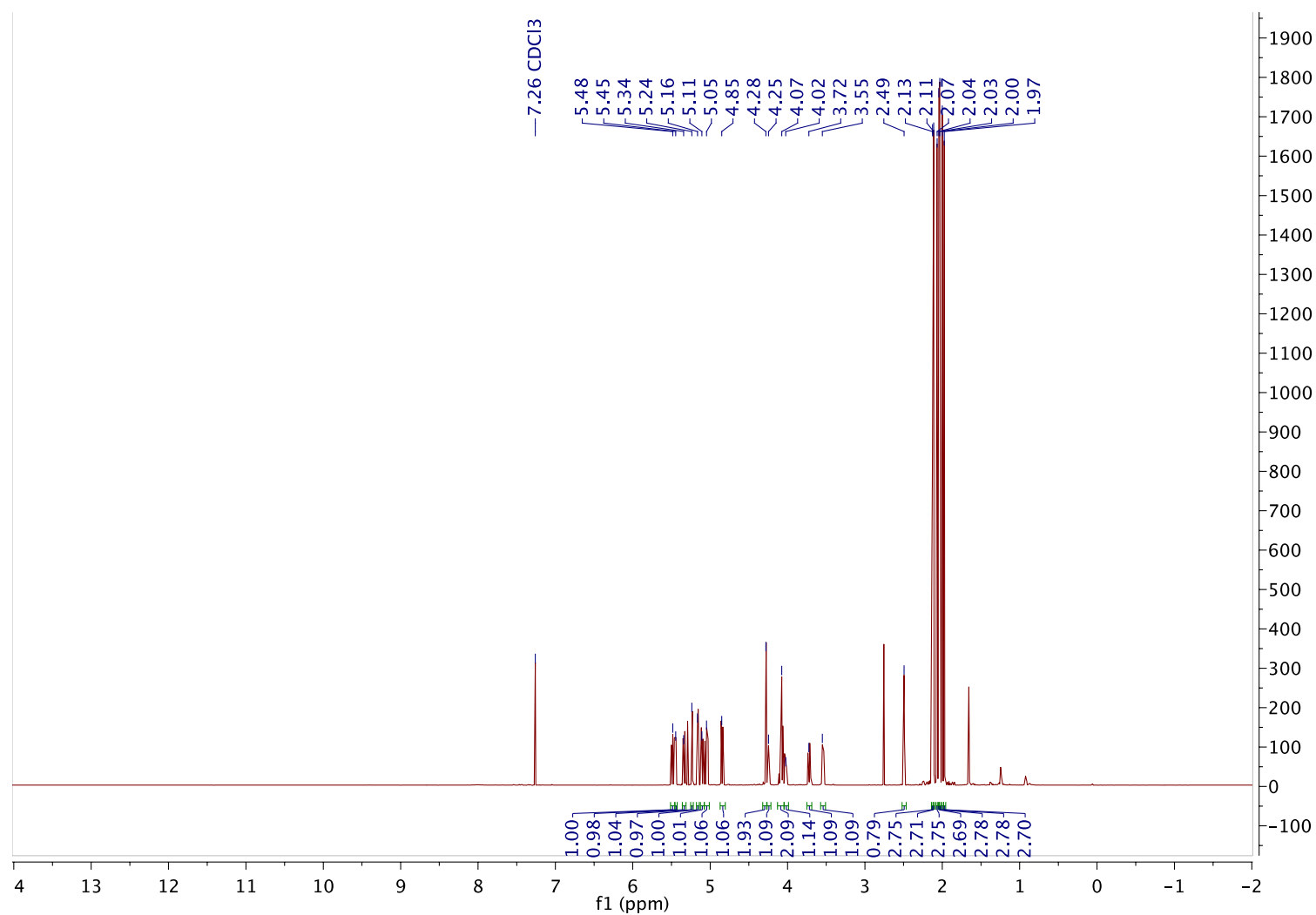




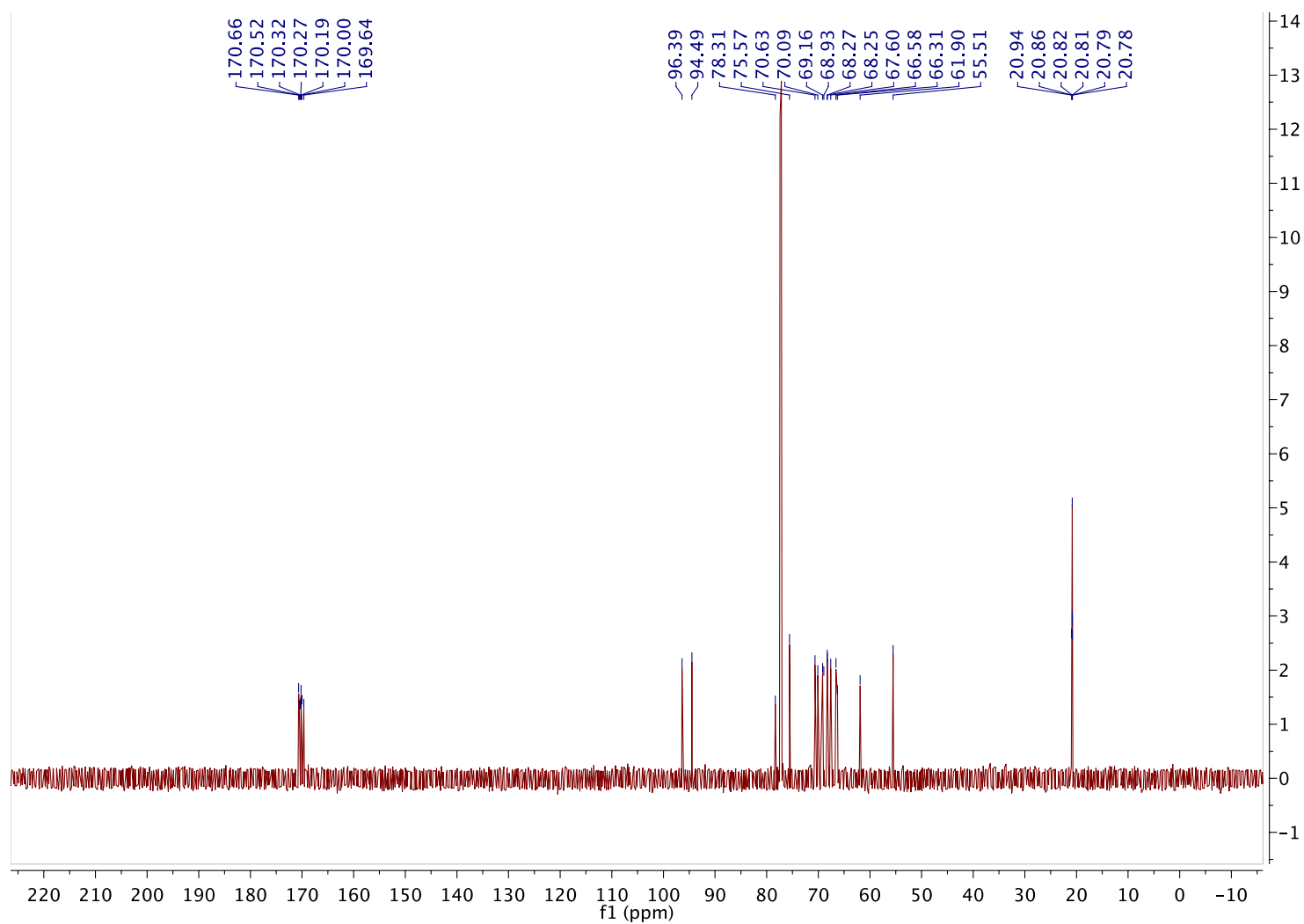
gCOSY NMR: Propargyl 2,3,4,6-tetra-O-acetyl- β -D-mannopyranoside (4j**)**



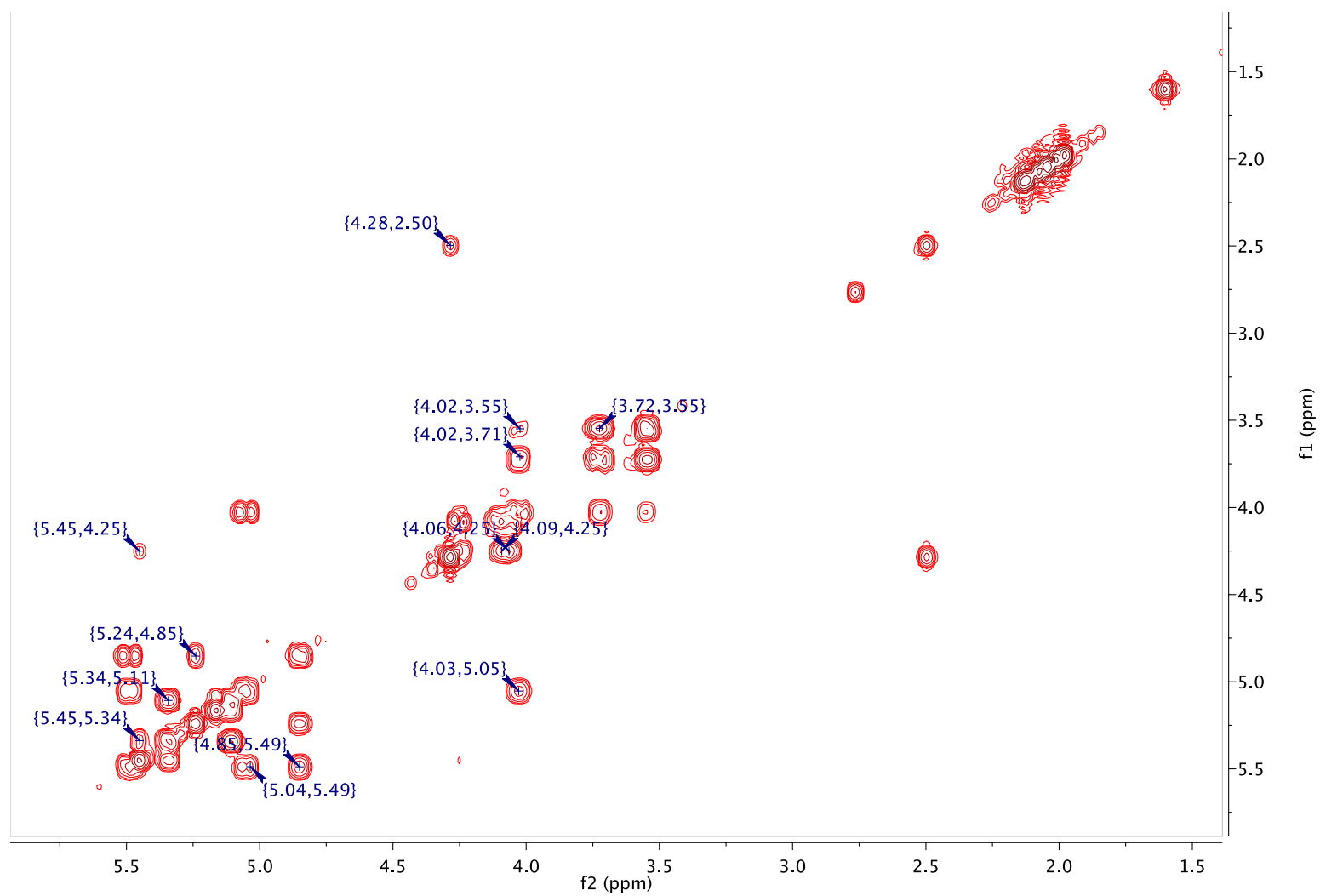
gHSQC NMR: Propargyl 2,3,4,6-tetra-O-acetyl- β -D-mannopyranoside (4j)



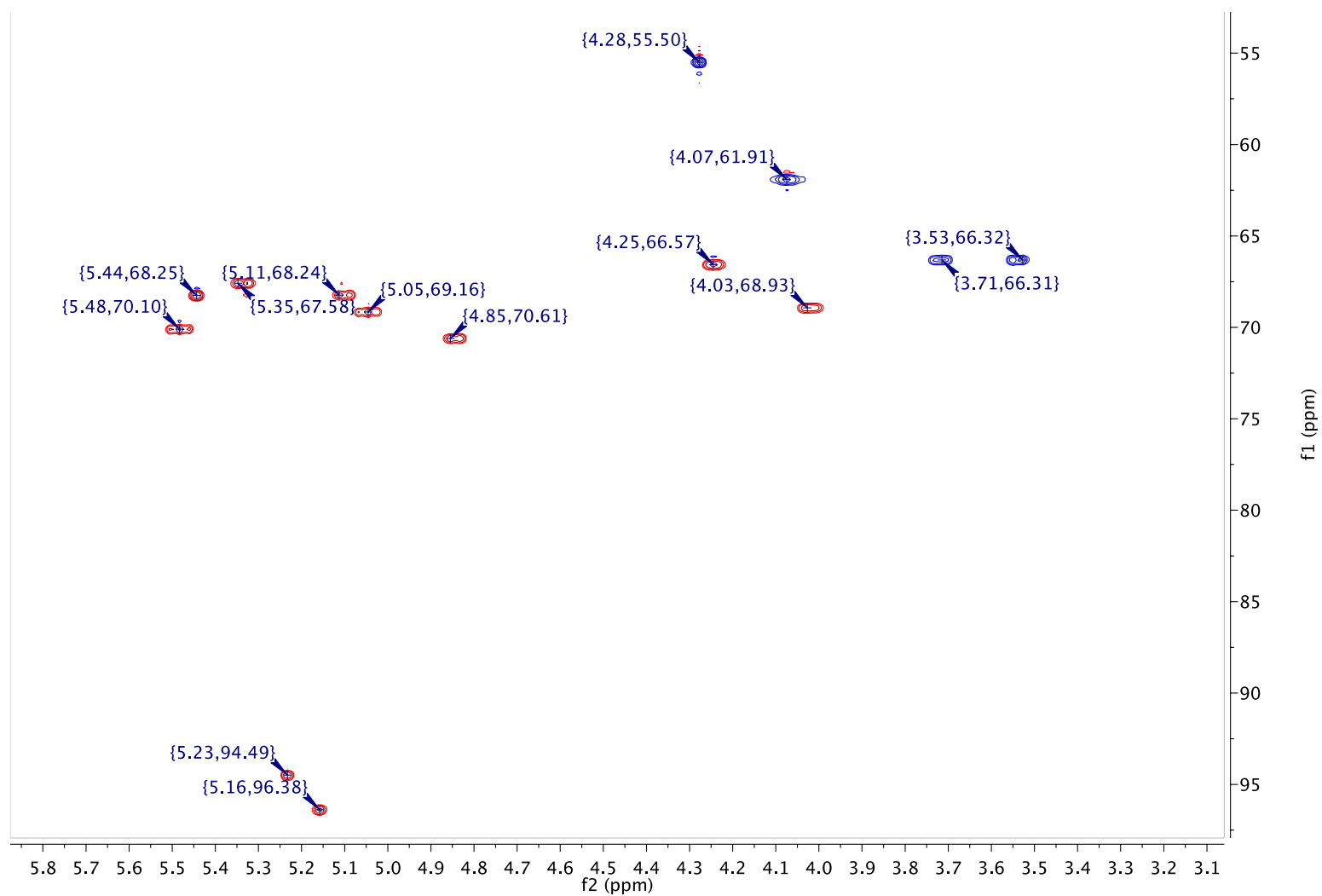
¹H NMR (500 MHz, CDCl₃): Propargyl 6-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2,3,4-tri-O-acetyl-α-D-glucopyranoside (4k)



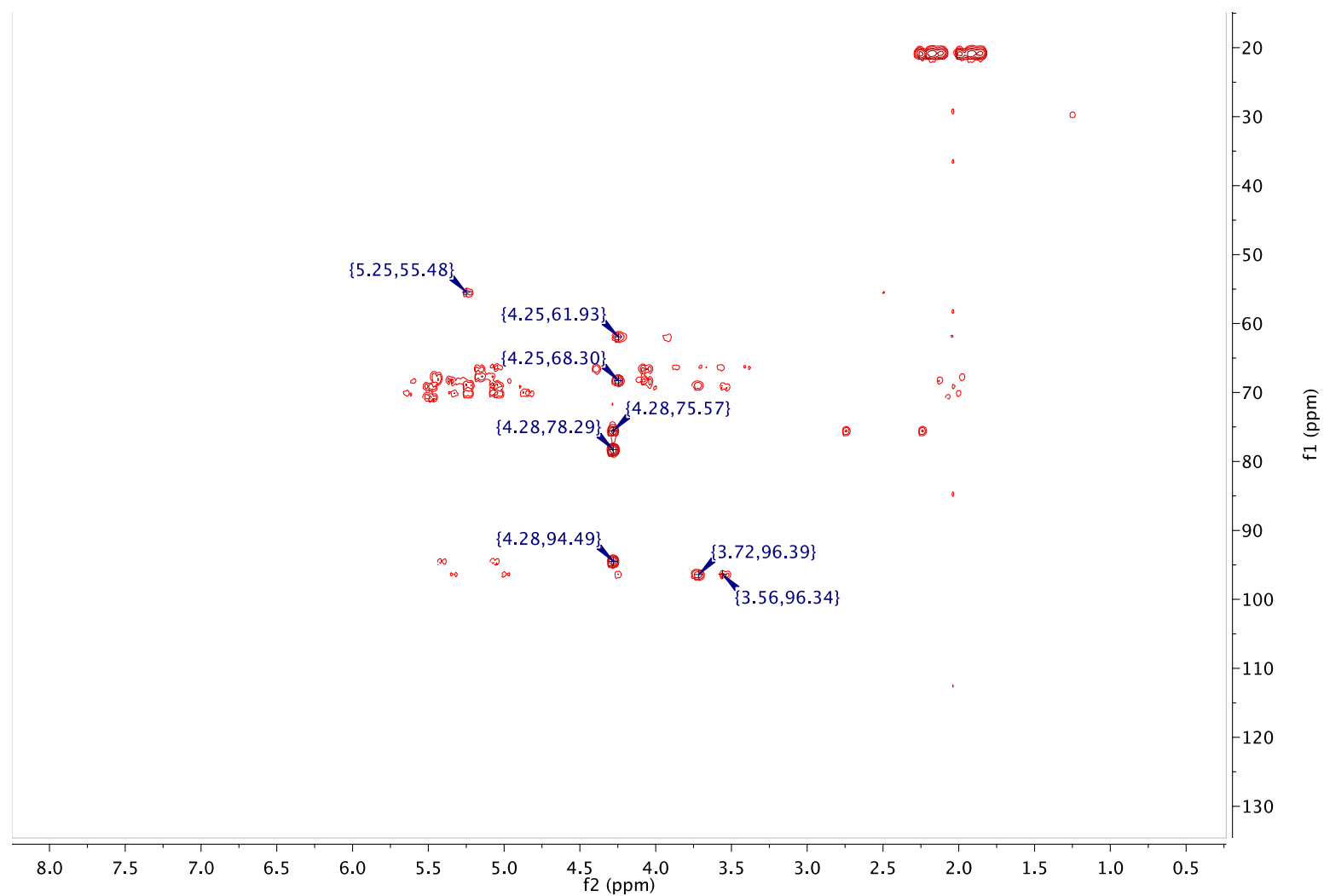
^{13}C NMR (125 MHz, CDCl_3): Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-2,3,4-tri-*O*-acetyl- α -D-glucopyranoside (**4k**)



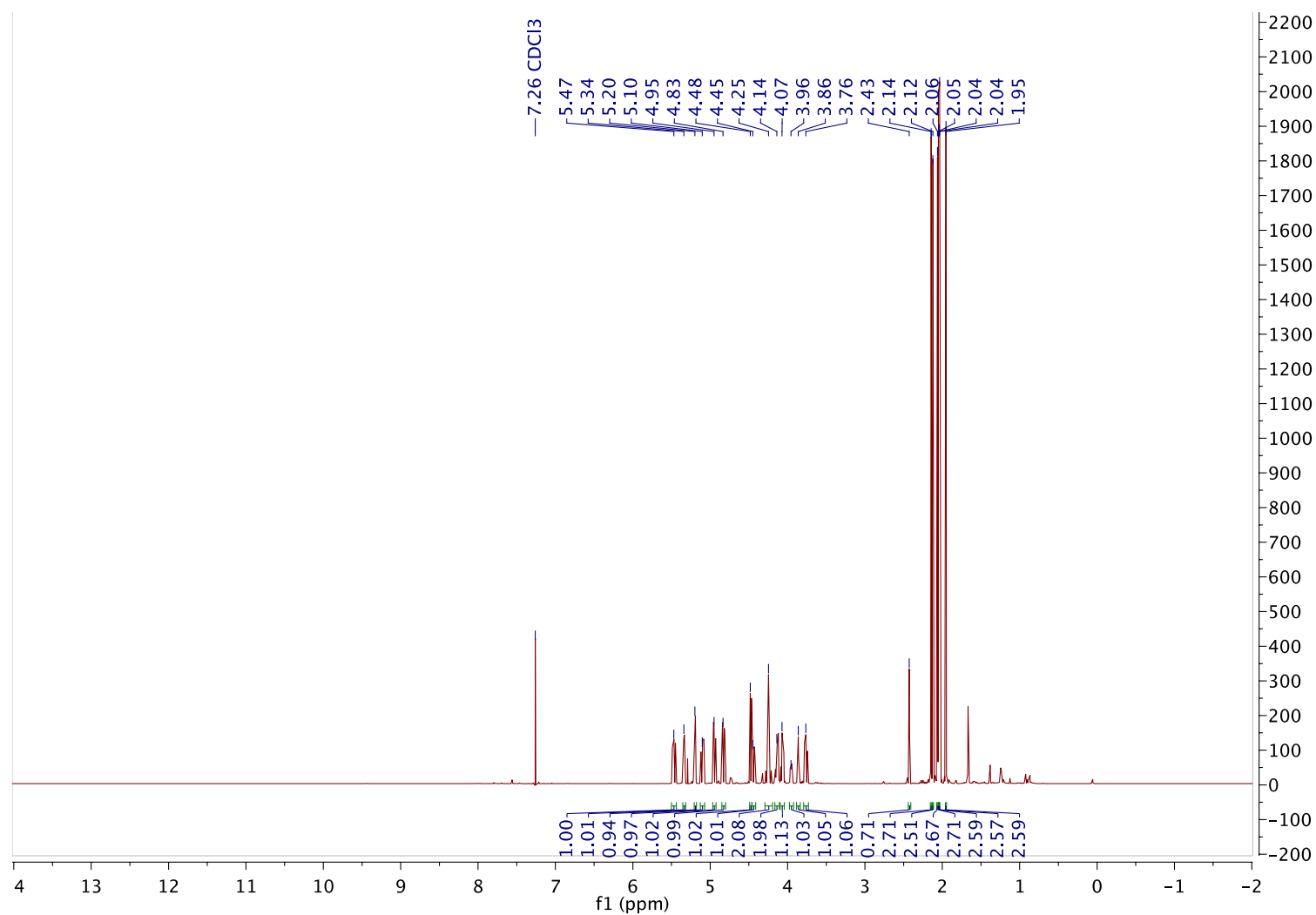
gCOSY NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-2,3,4-tri-*O*-acetyl- α -D-glucopyranoside (4k**)**



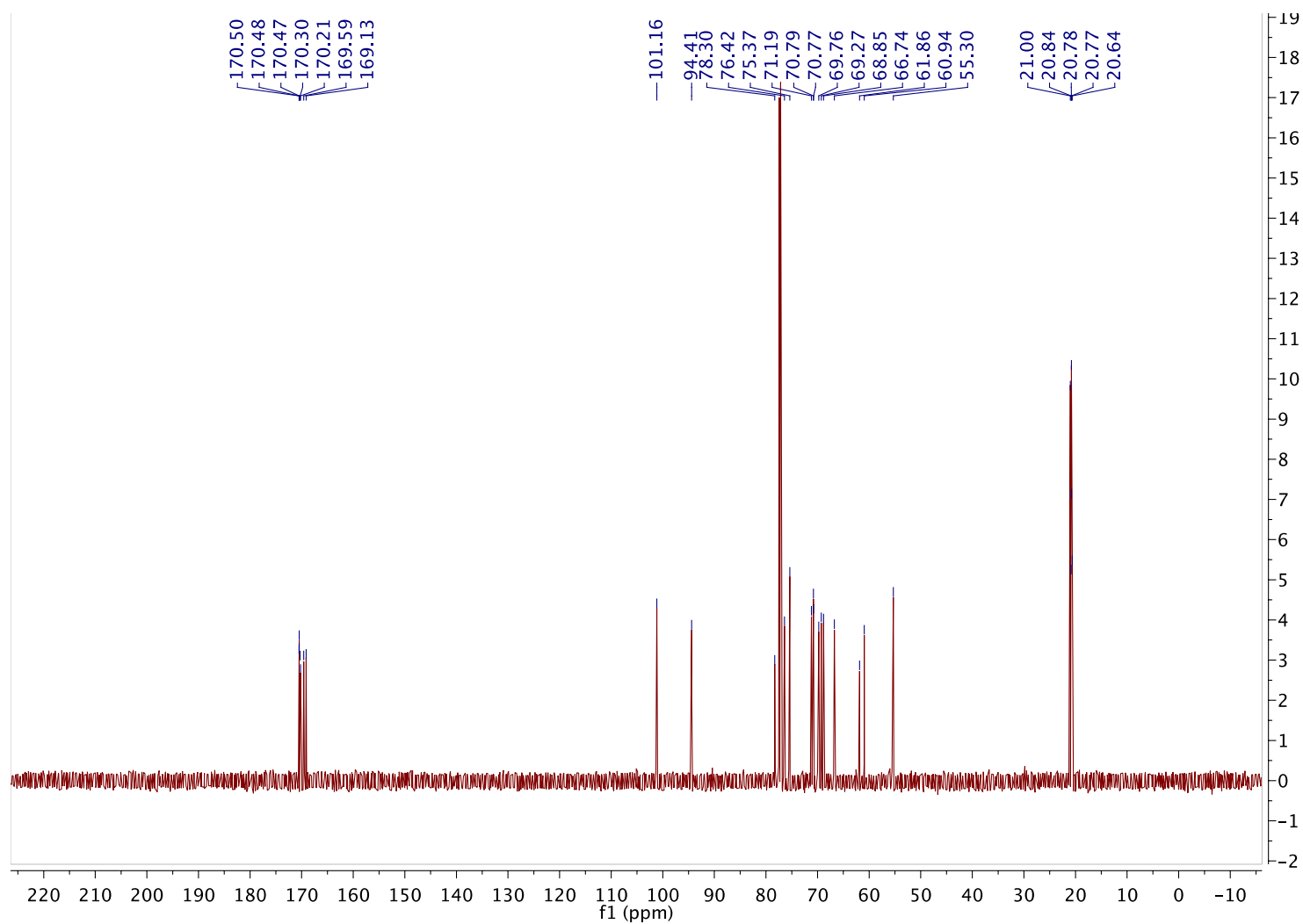
gHSQC NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-2,3,4-tri-*O*-acetyl- α -D-glucopyranoside (**4k**)



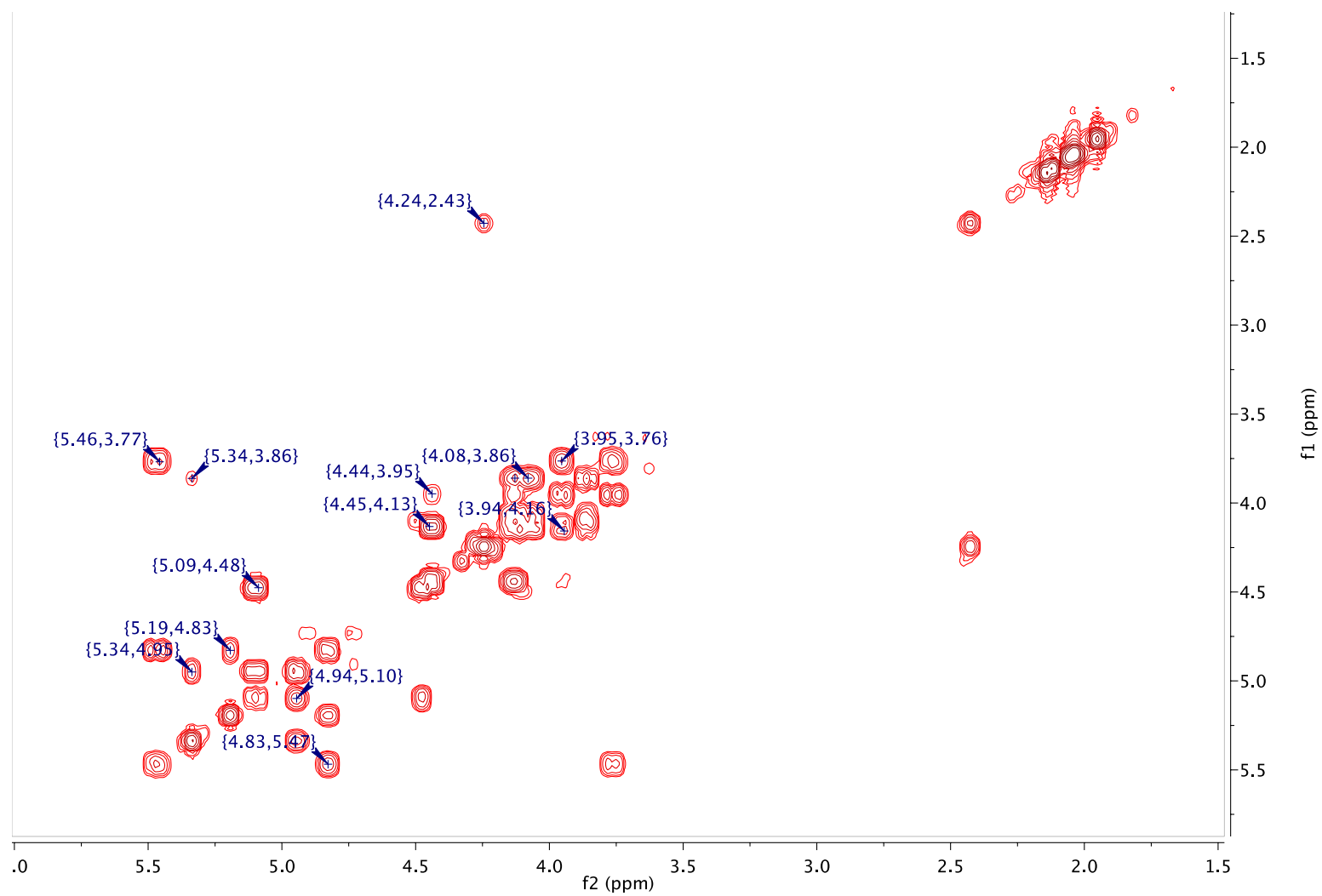
gHMBC NMR: Propargyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-2,3,4-tri-*O*-acetyl- α -D-glucopyranoside (**4k**)



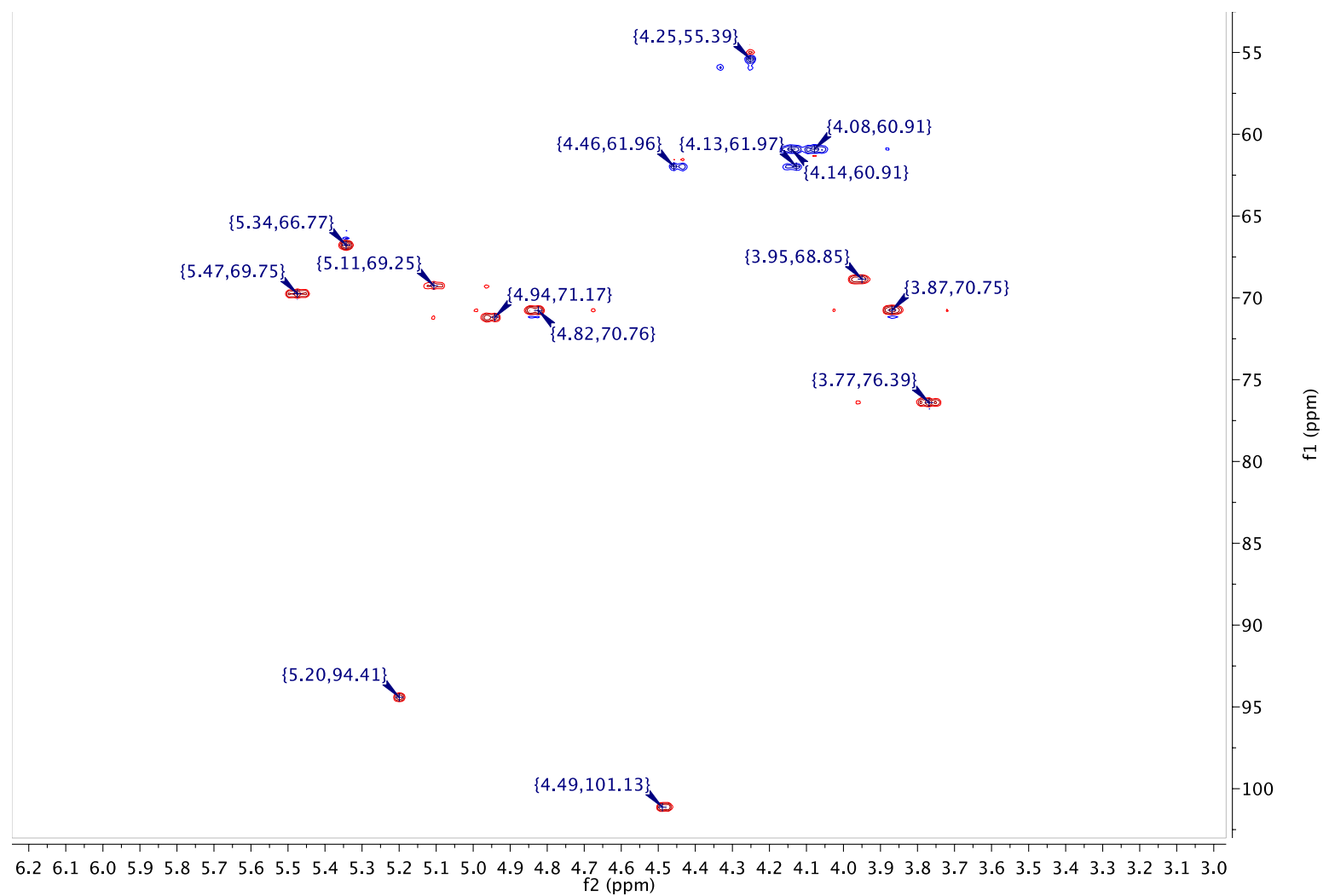
¹H NMR (500 MHz, CDCl₃): Propargyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-α-D-glucopyranoside (4I)



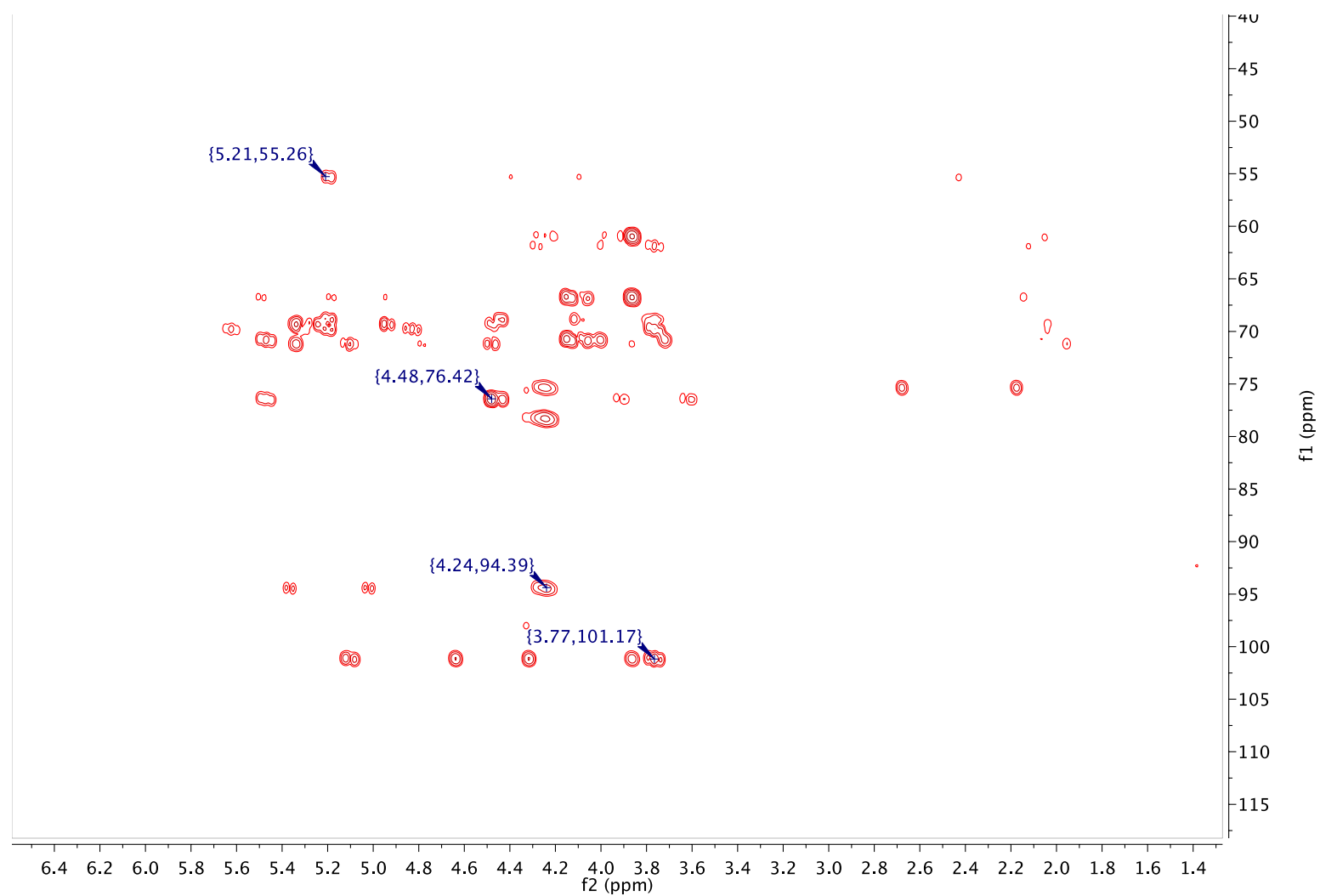
^{13}C NMR (125 MHz, CDCl_3): Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (**4I**)



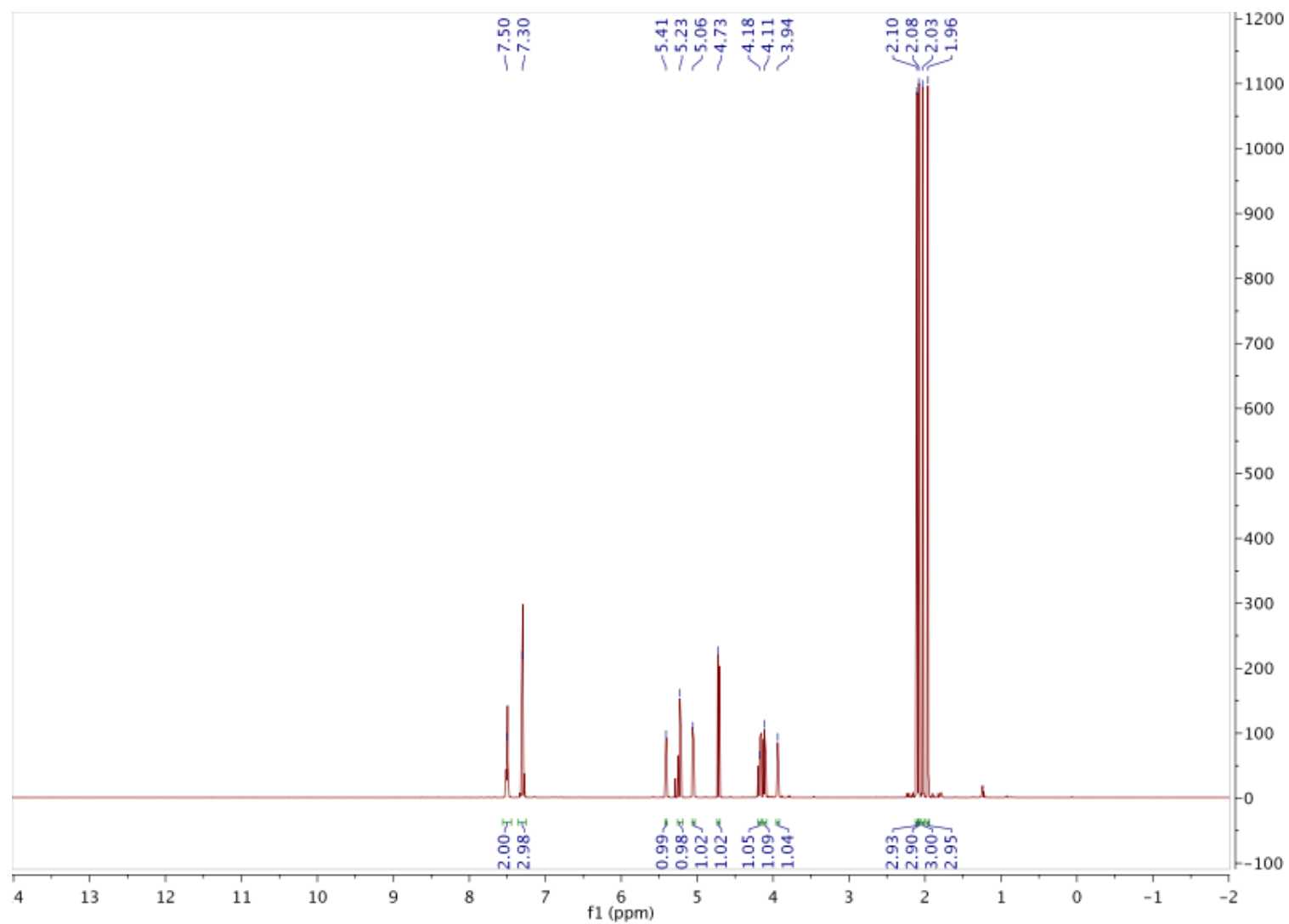
gCOSY NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (**4l**)



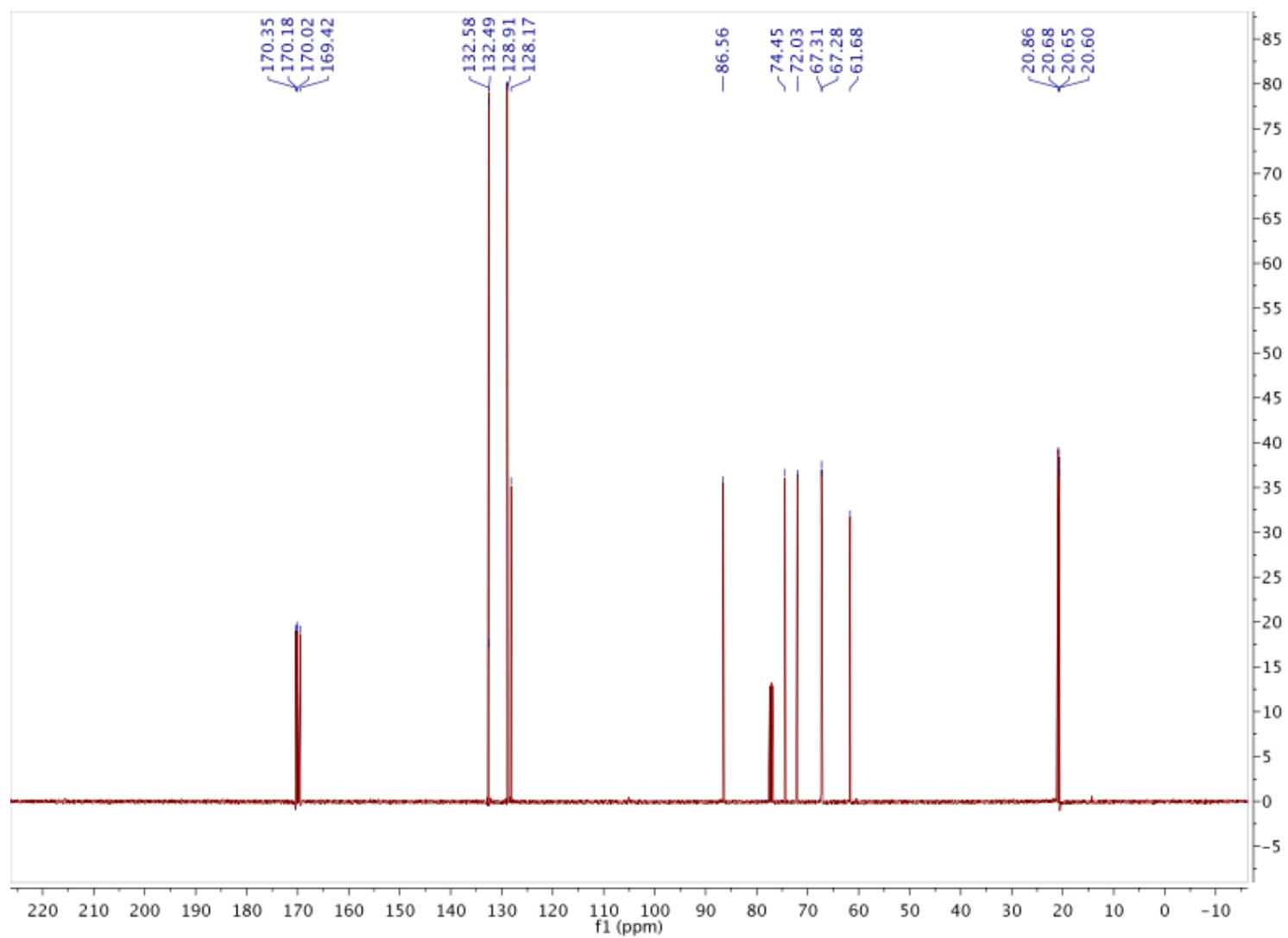
gHSQC NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (**4l**)



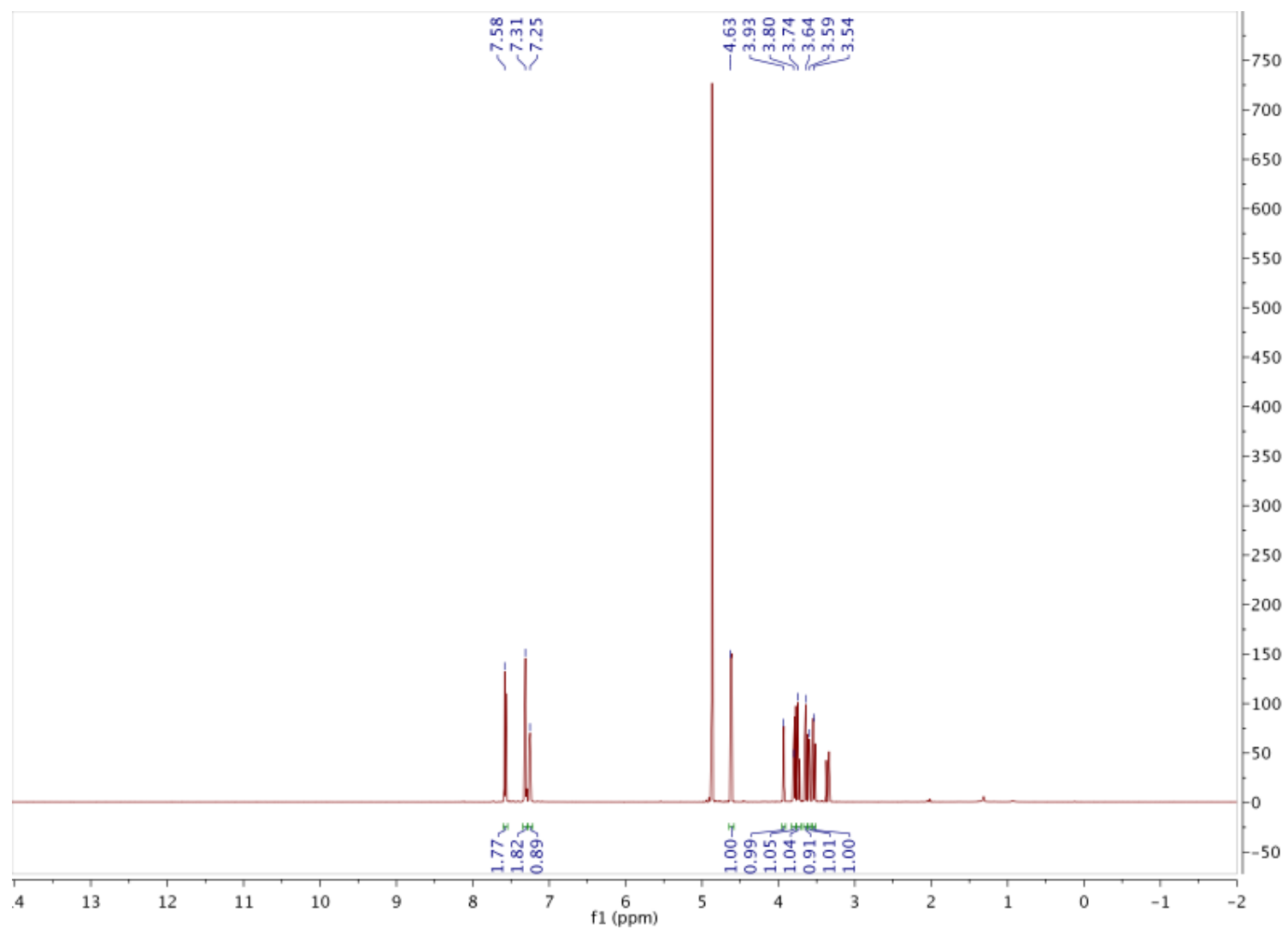
gHMBC NMR: Propargyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (**4l**)



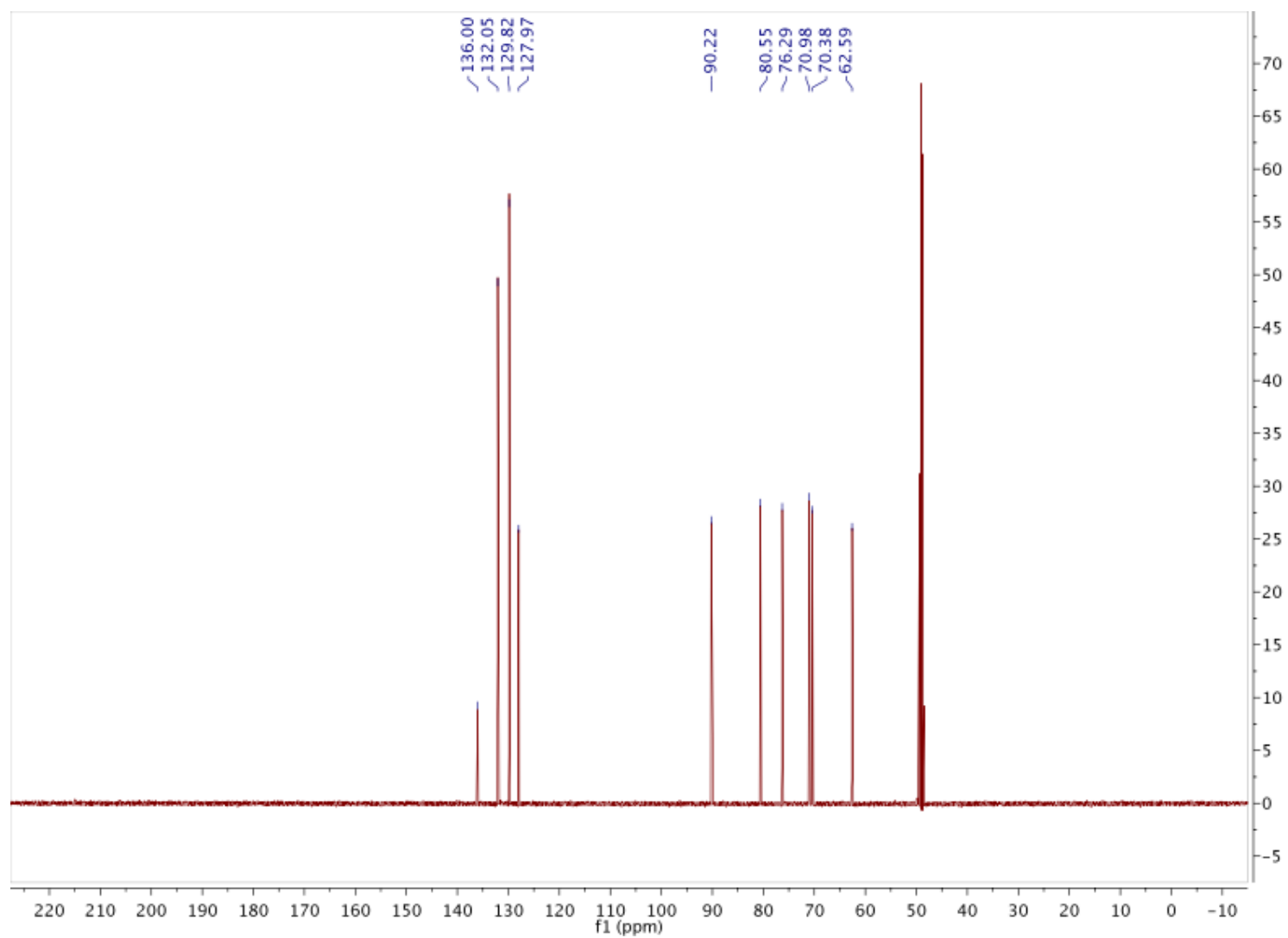
^1H NMR Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio- β -D-galactopyranoside (21)



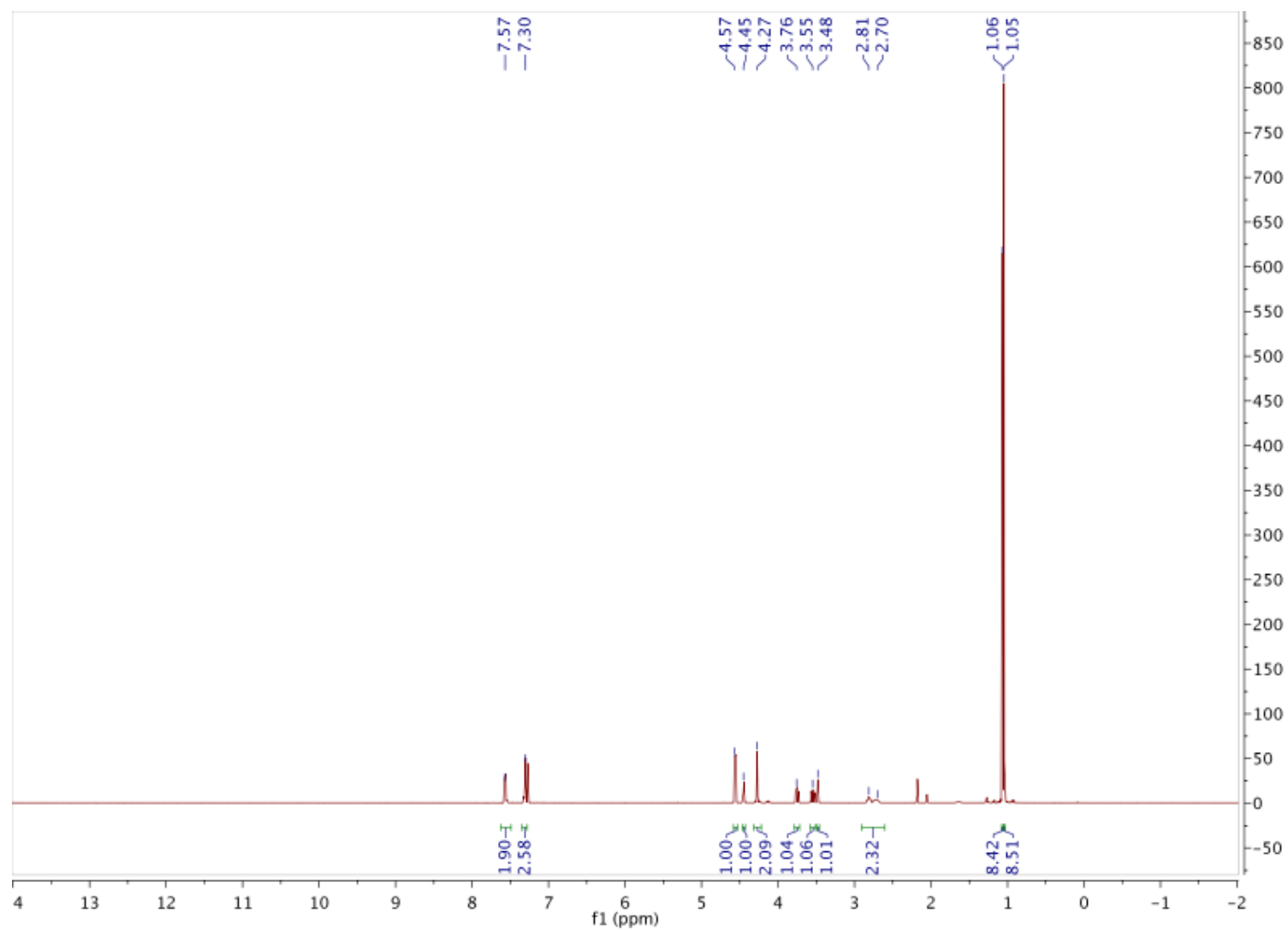
¹³C NMR Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (21)



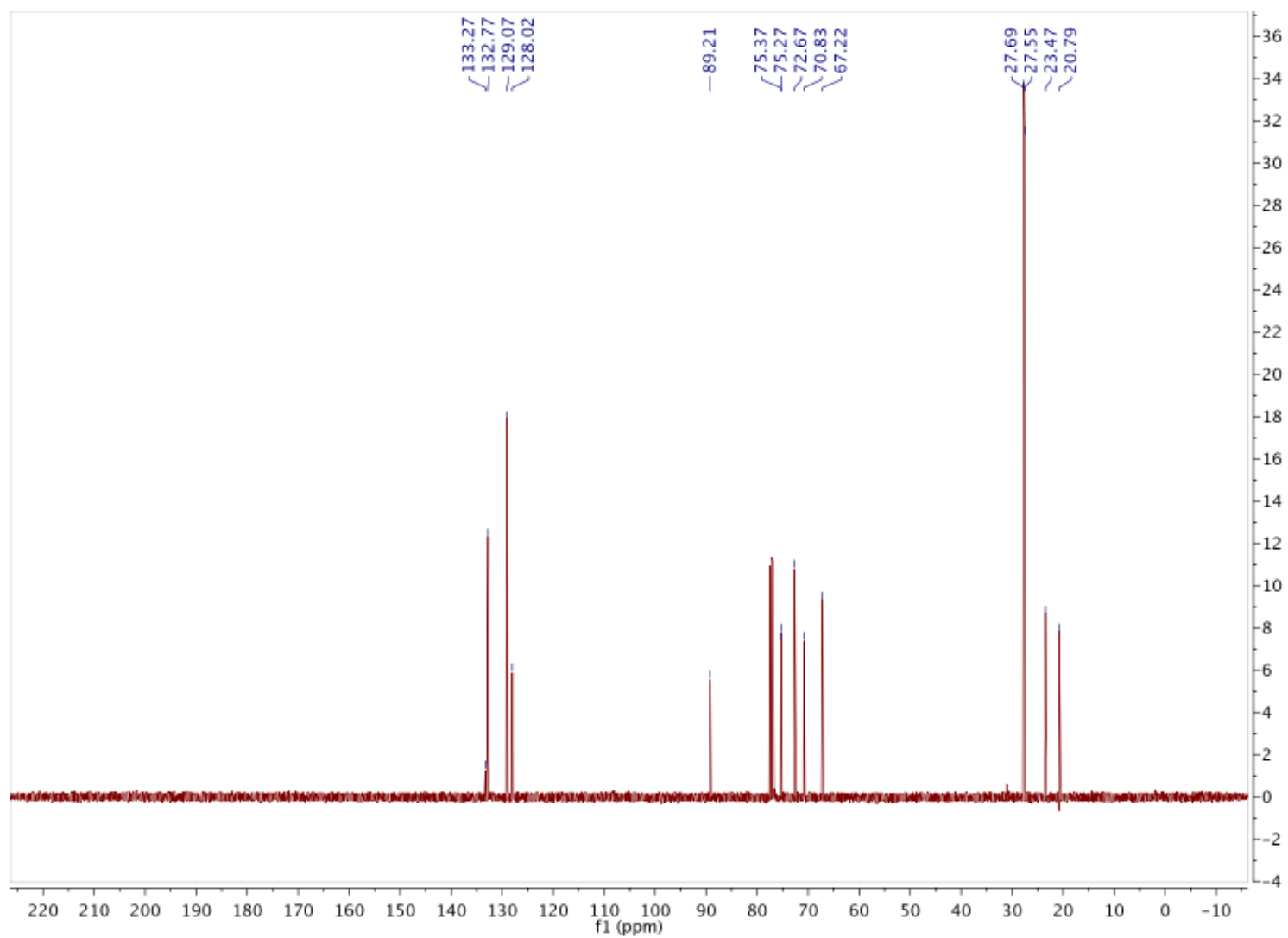
¹H NMR Phenyl 1-thio-β-D-galactopyranoside (22)



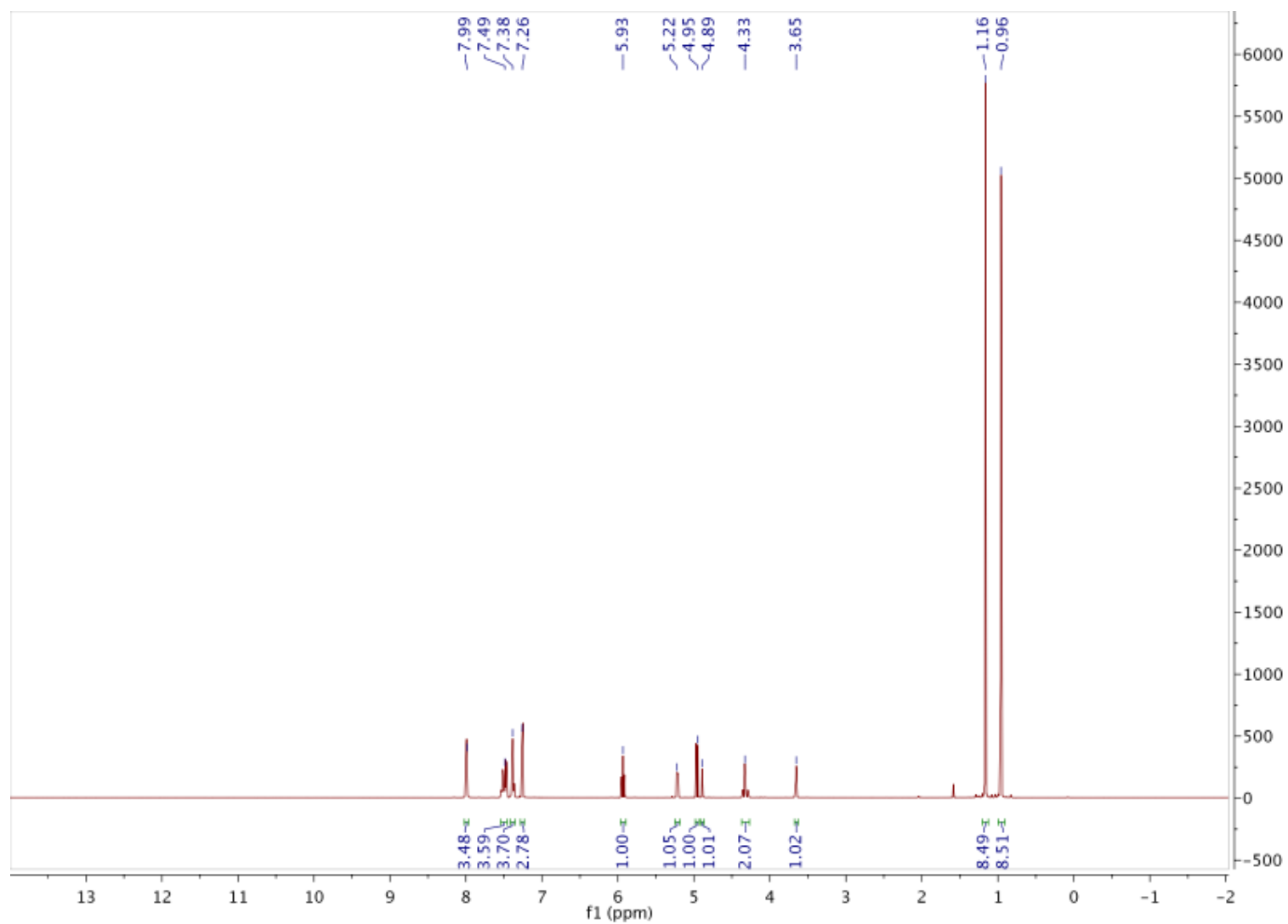
¹³C NMR Phenyl 1-thio-β-D-galactopyranoside (22)



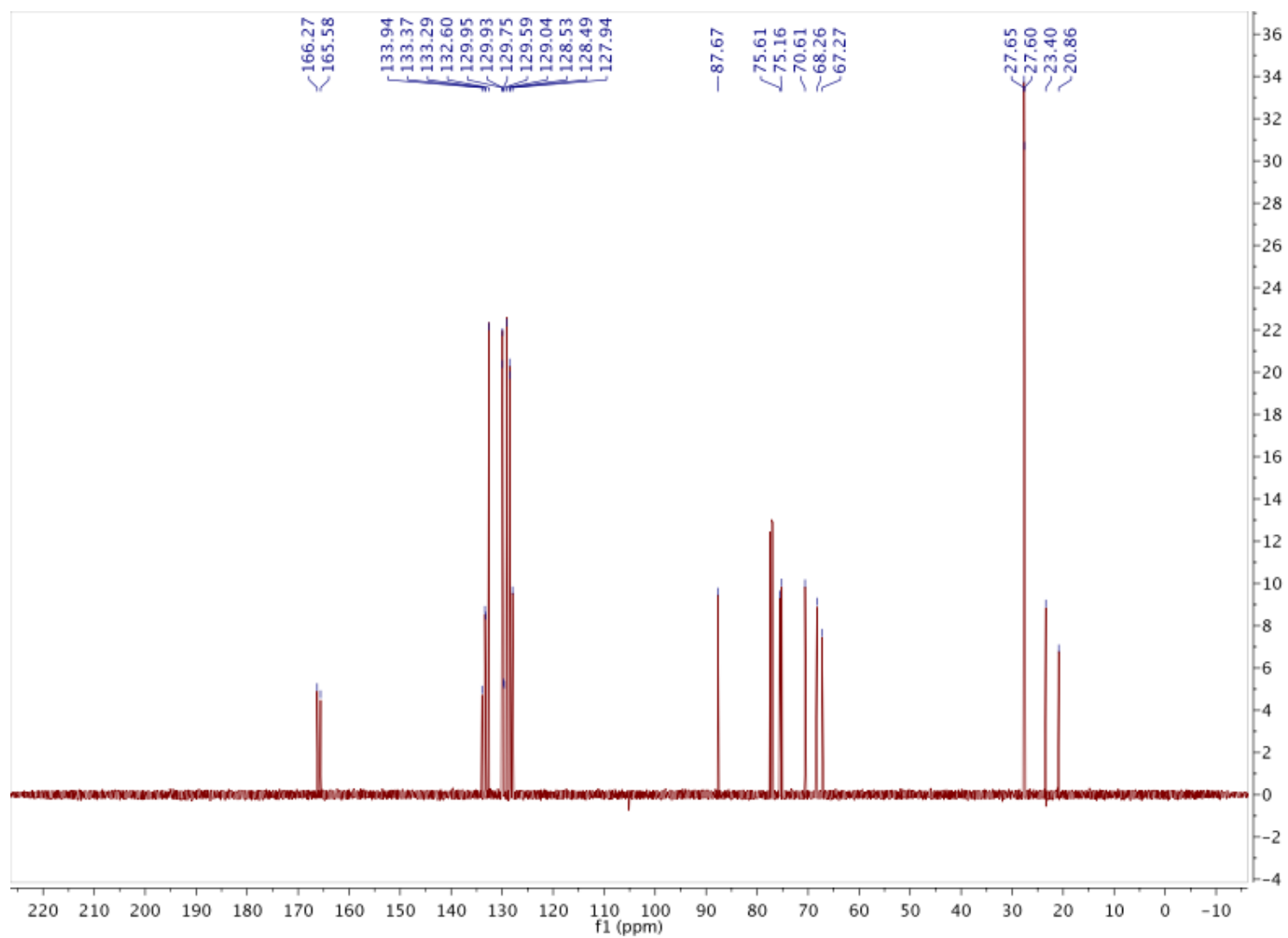
¹H NMR Phenyl 4,6-O-Di-*tert*-butylsilanediyl-1-thio-β-D-galactopyranoside (23)



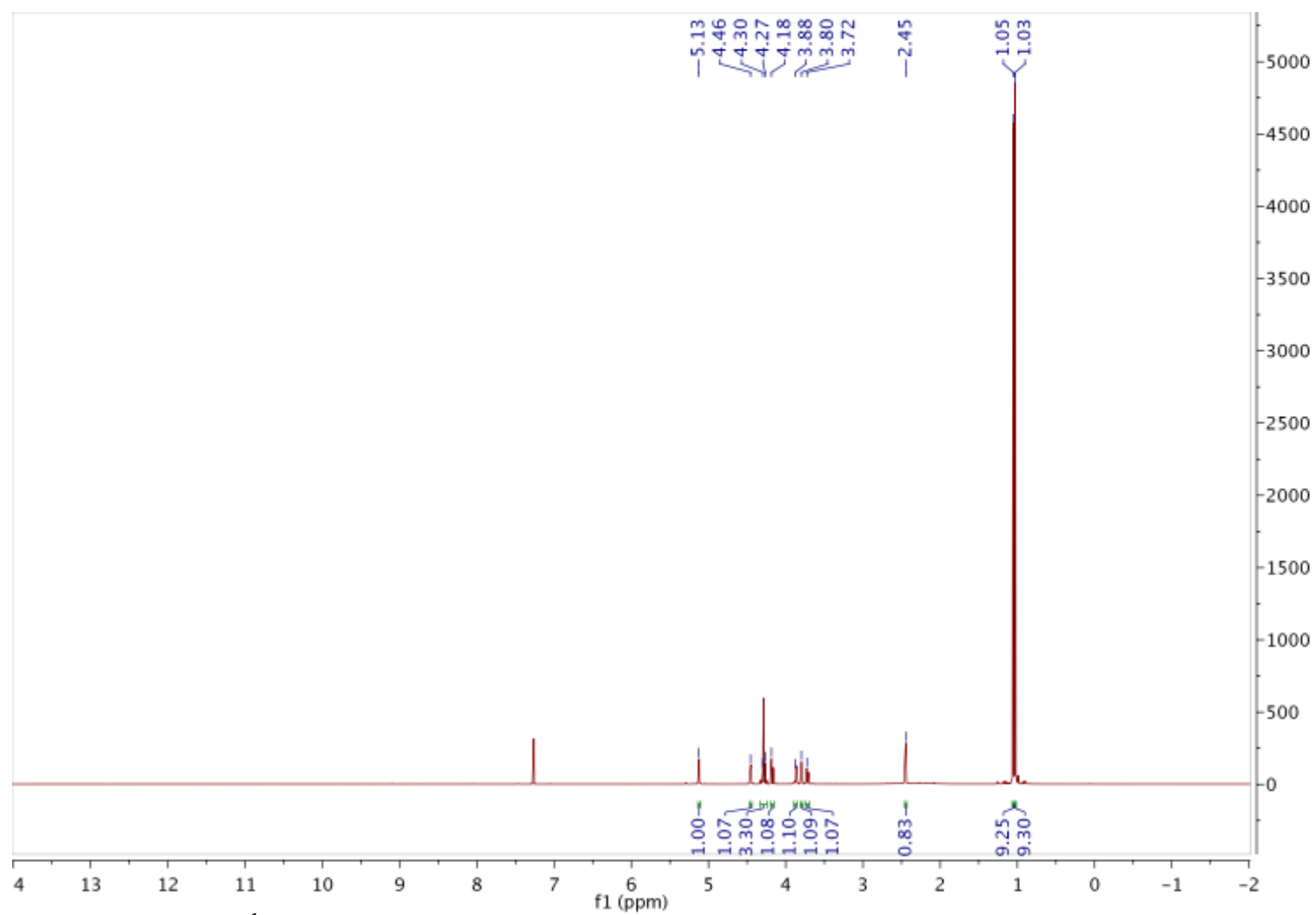
¹³C NMR Phenyl 4,6-*O*-Di-*tert*-butylsilanediyl-1-thio-β-D-galactopyranoside (23)



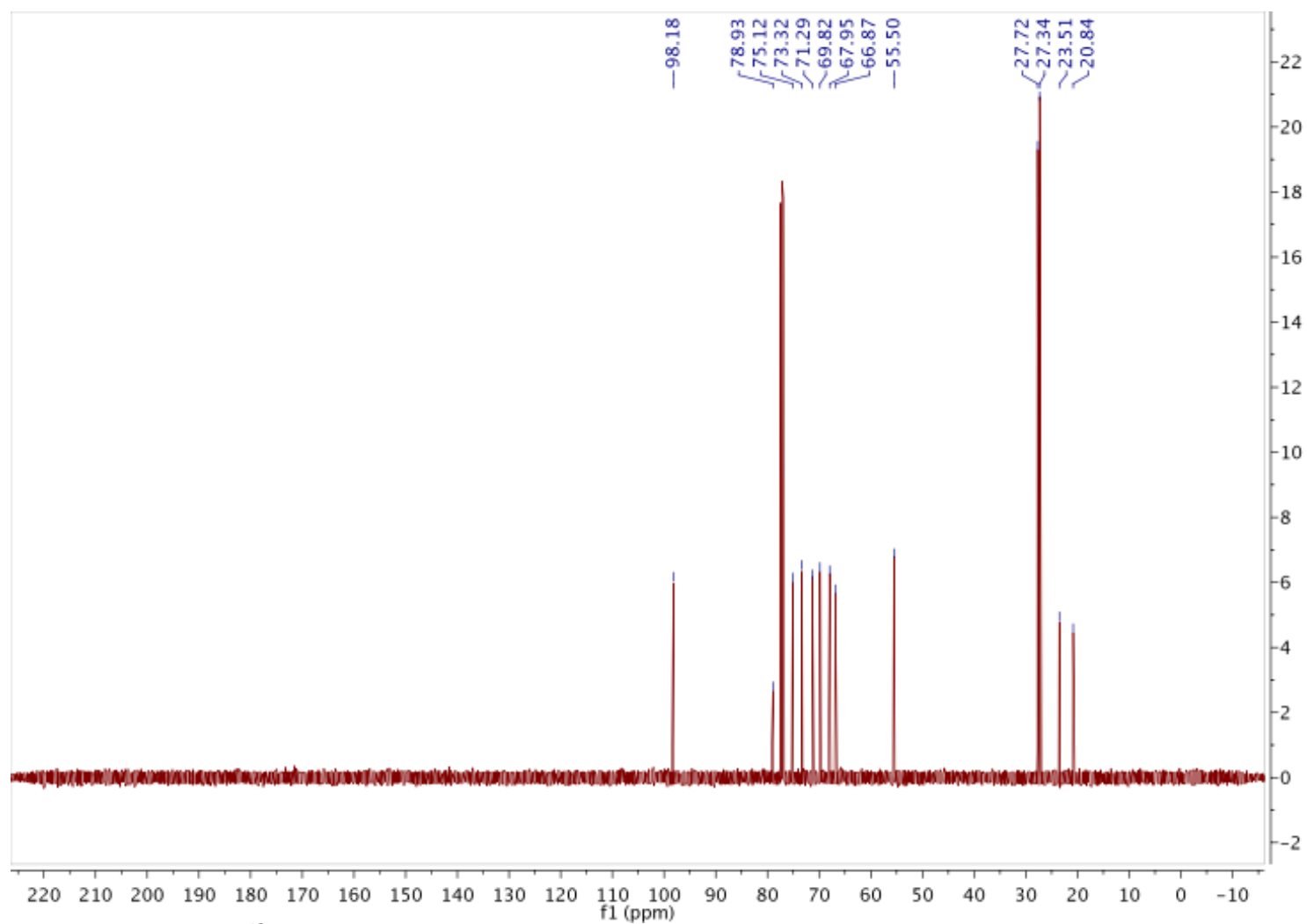
¹H NMR Phenyl 2,3-Di-O-benzoyl-4,6-O-di-*tert*-butylsilanediyl-1-thio-β-D-galactopyranoside (**12**)



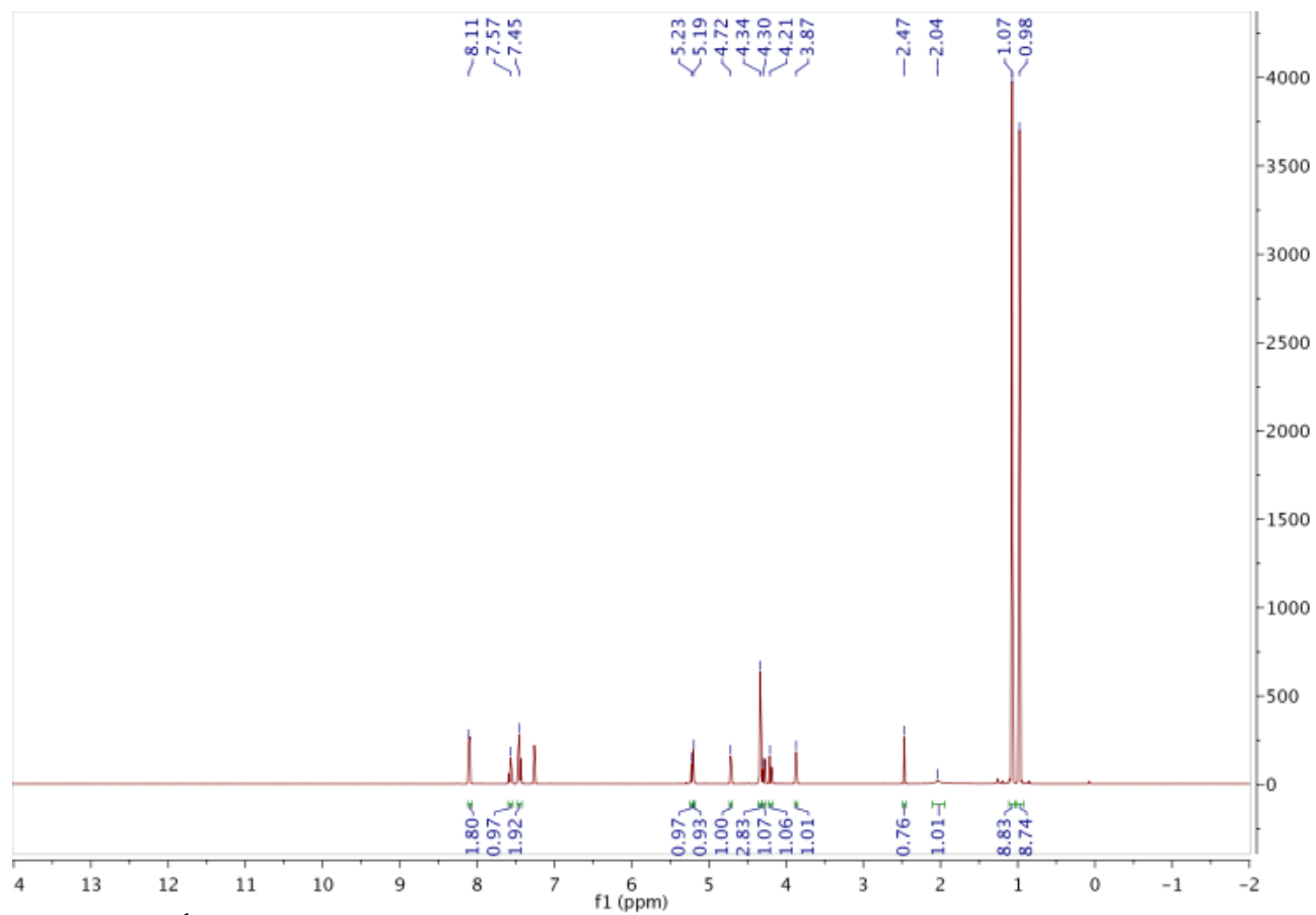
^{13}C NMR Phenyl 2,3-Di-O-benzoyl-4,6-O-di-*tert*-butylsilanediyl-1-thio- β -D-galactopyranoside (**12**)



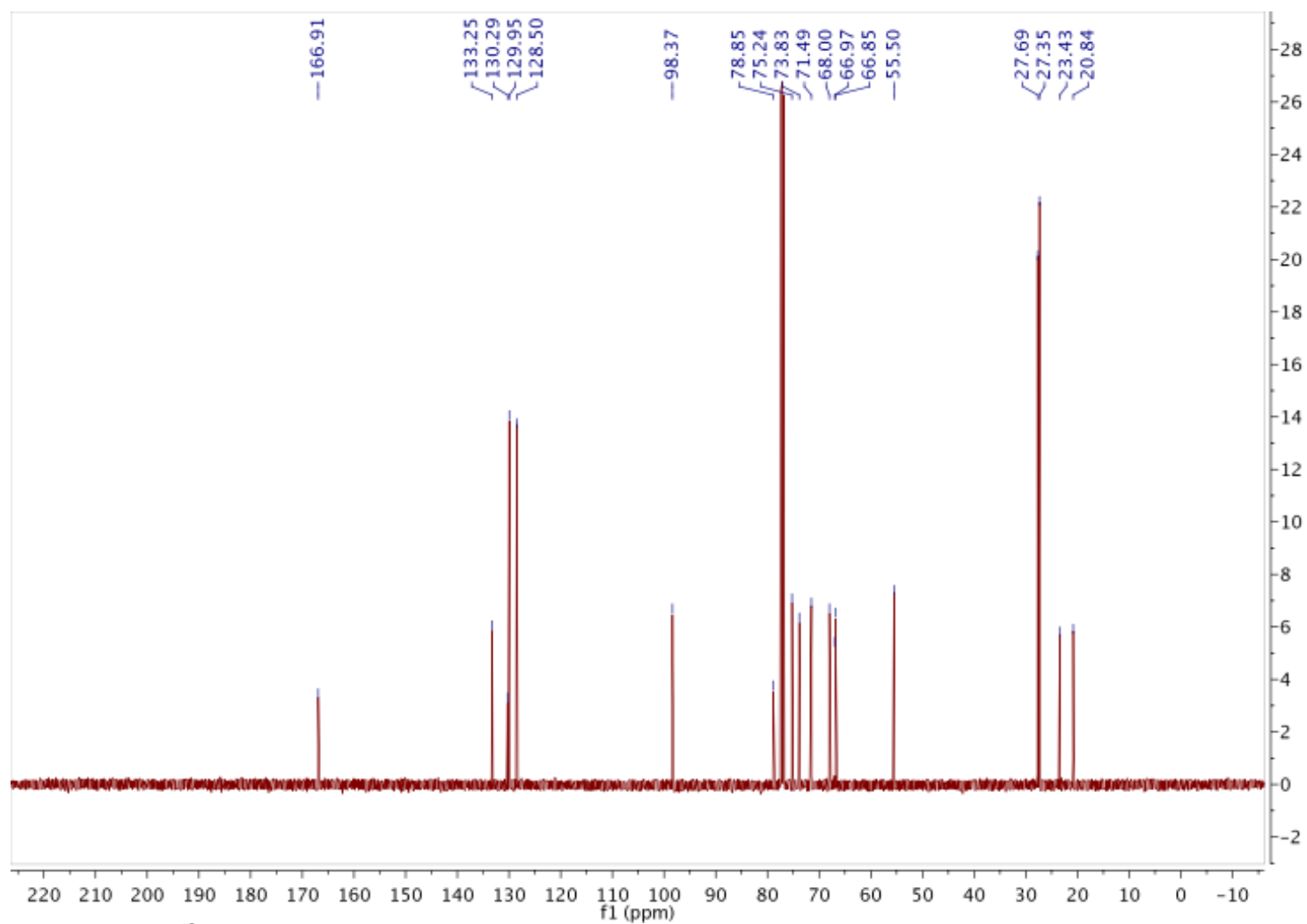
¹H NMR Propargyl 4,6-O-di-*tert*-butylsilanediyl- α -D-galactopyranoside (24)



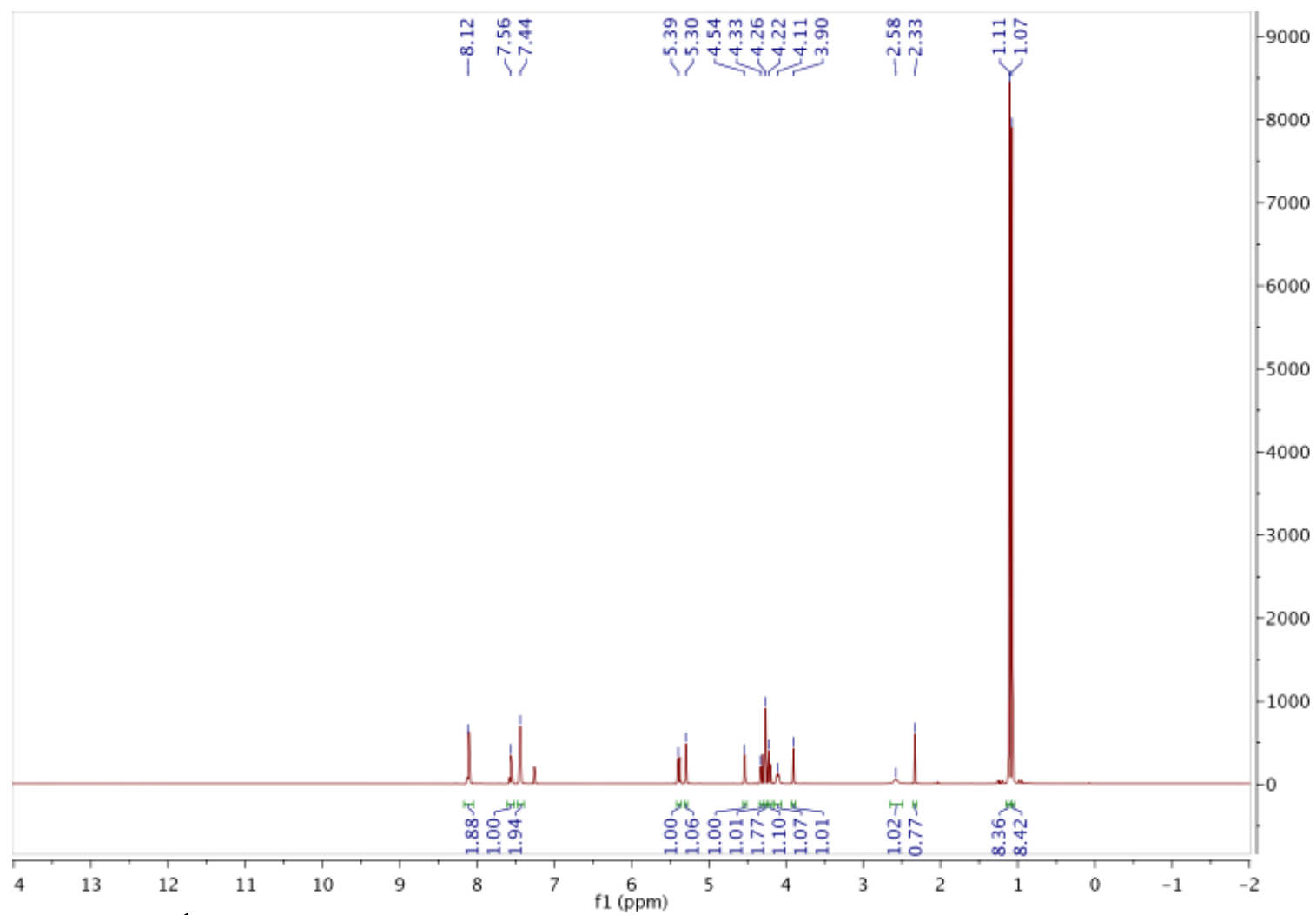
¹³C NMR Propargyl 4,6-*O*-di-*tert*-butylsilanediyl-α-D-galactopyranoside (24)



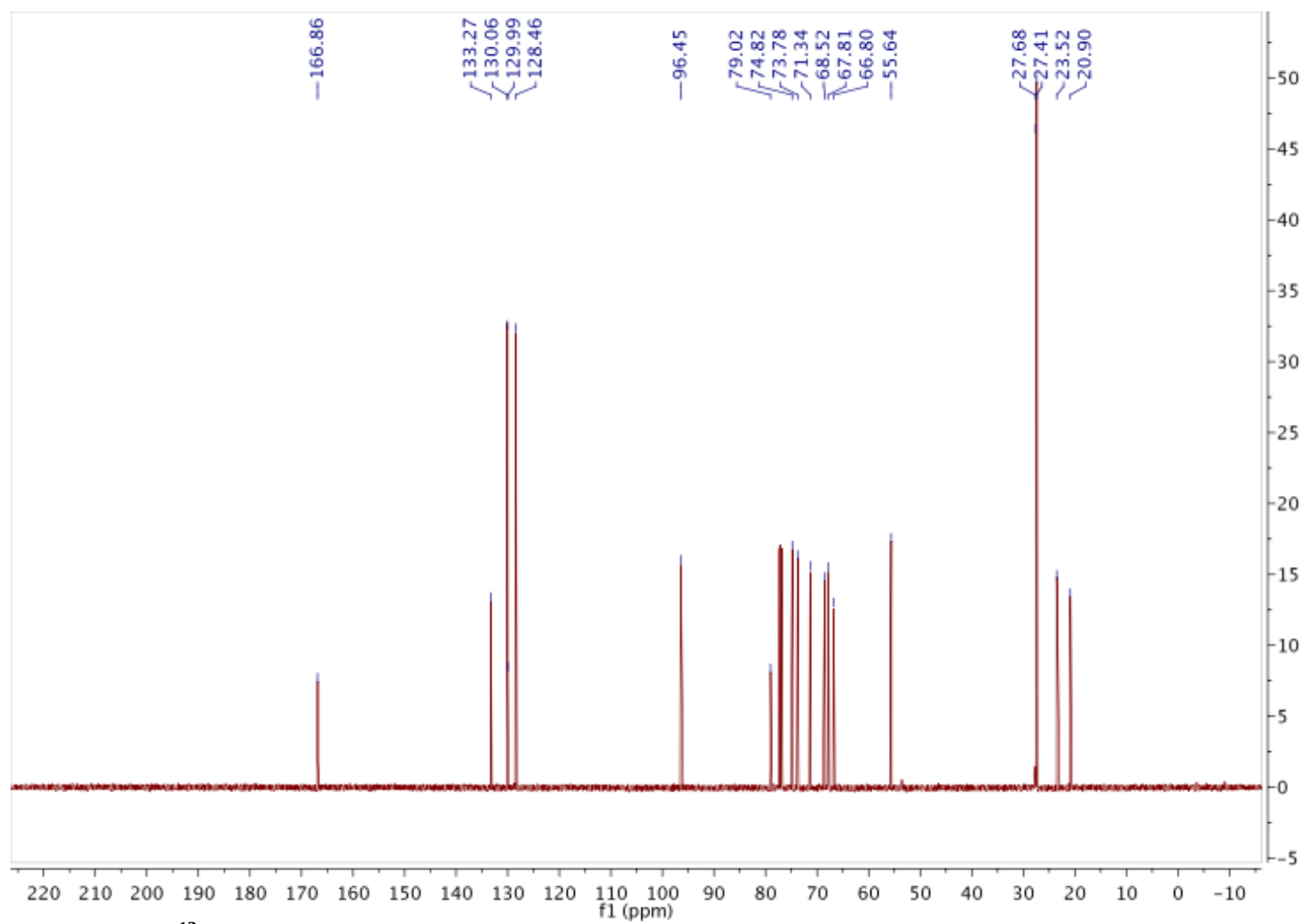
¹H NMR Propargyl 3-O-benzoyl-4,6-O-di-tert-butylsilanediyl-α-D-galactopyranoside (7)



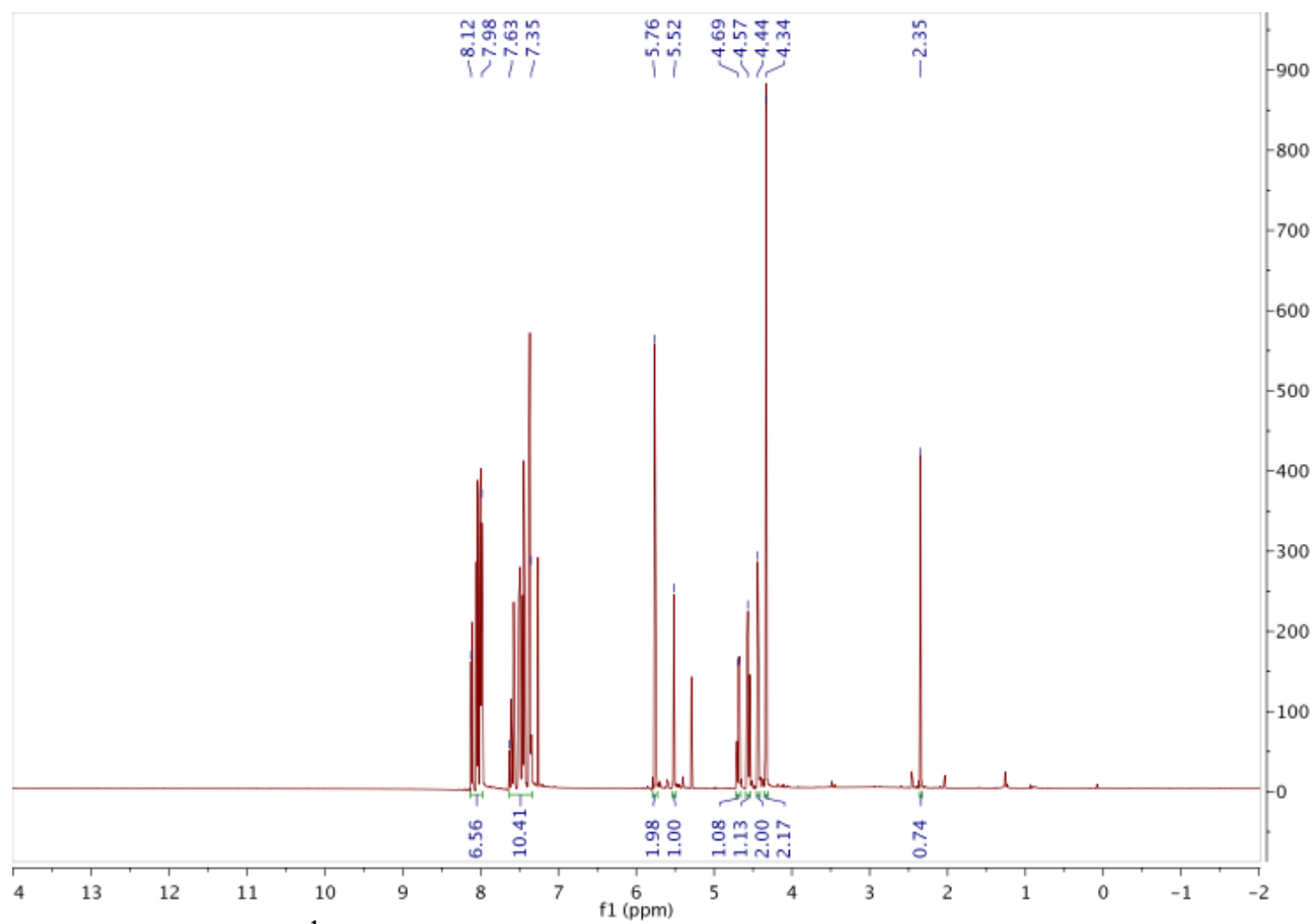
¹³C NMR Propargyl 3-O-benzoyl-4,6-O-di-*tert*-butylsilanediyl- α -D-galactopyranoside (7)



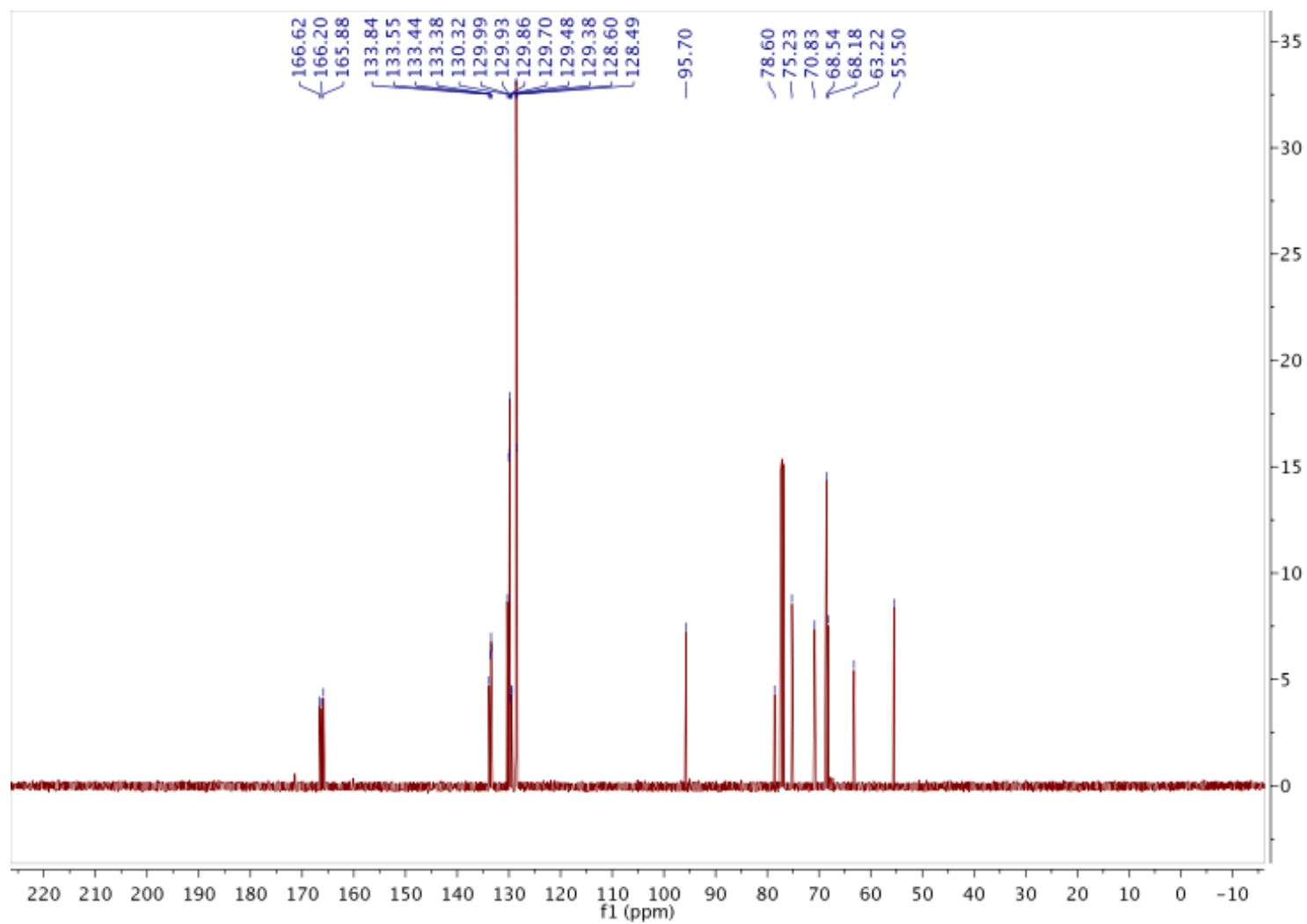
¹H NMR Propargyl 2-O-benzoyl-4,6-O-di-*tert*-butylsilanediyl-α-D-galactopyranoside (**8**)



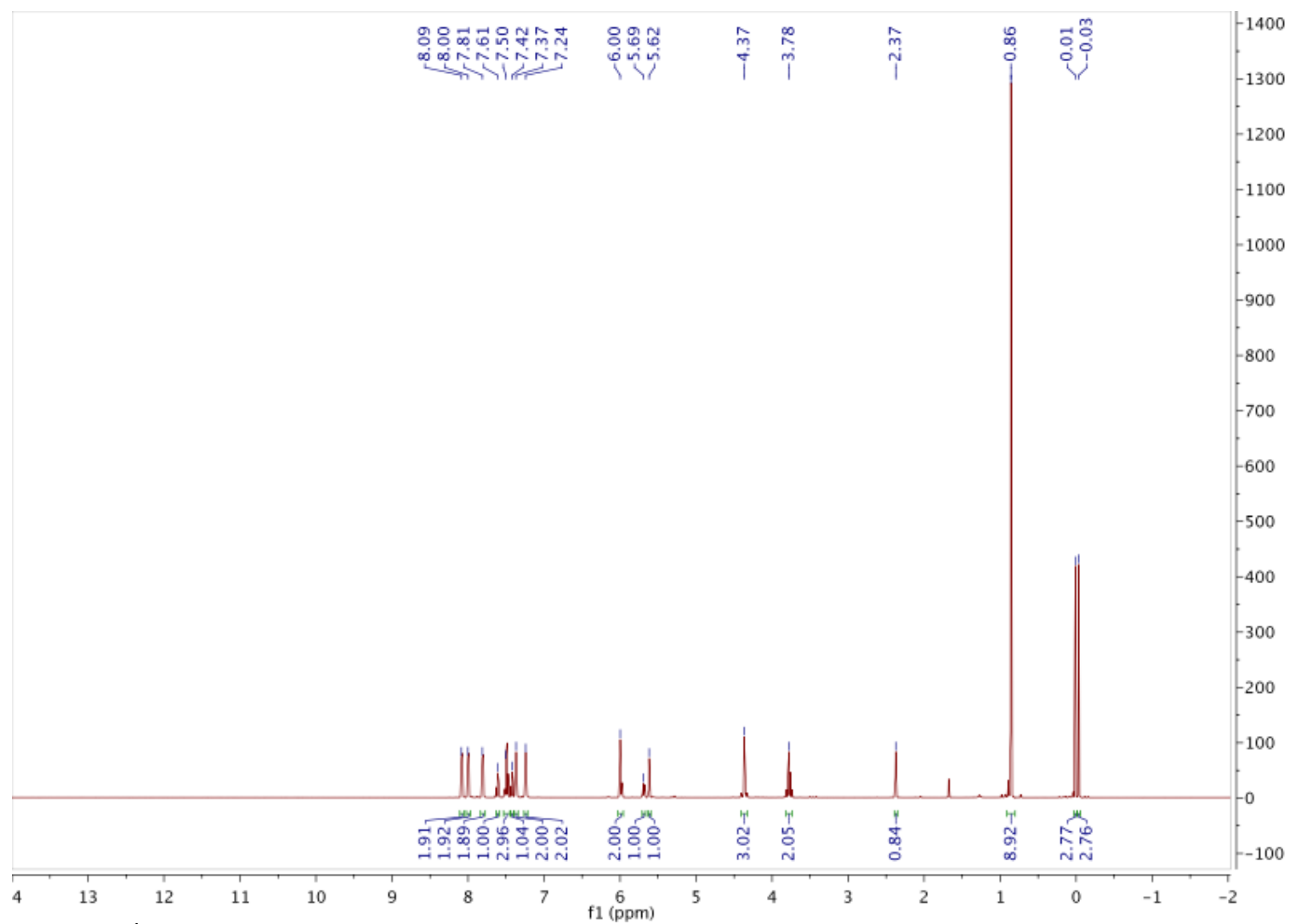
¹³C NMR Propargyl 2-O-benzoyl-4,6-O-di-*tert*-butylsilanediyl- α -D-galactopyranoside (**8**)



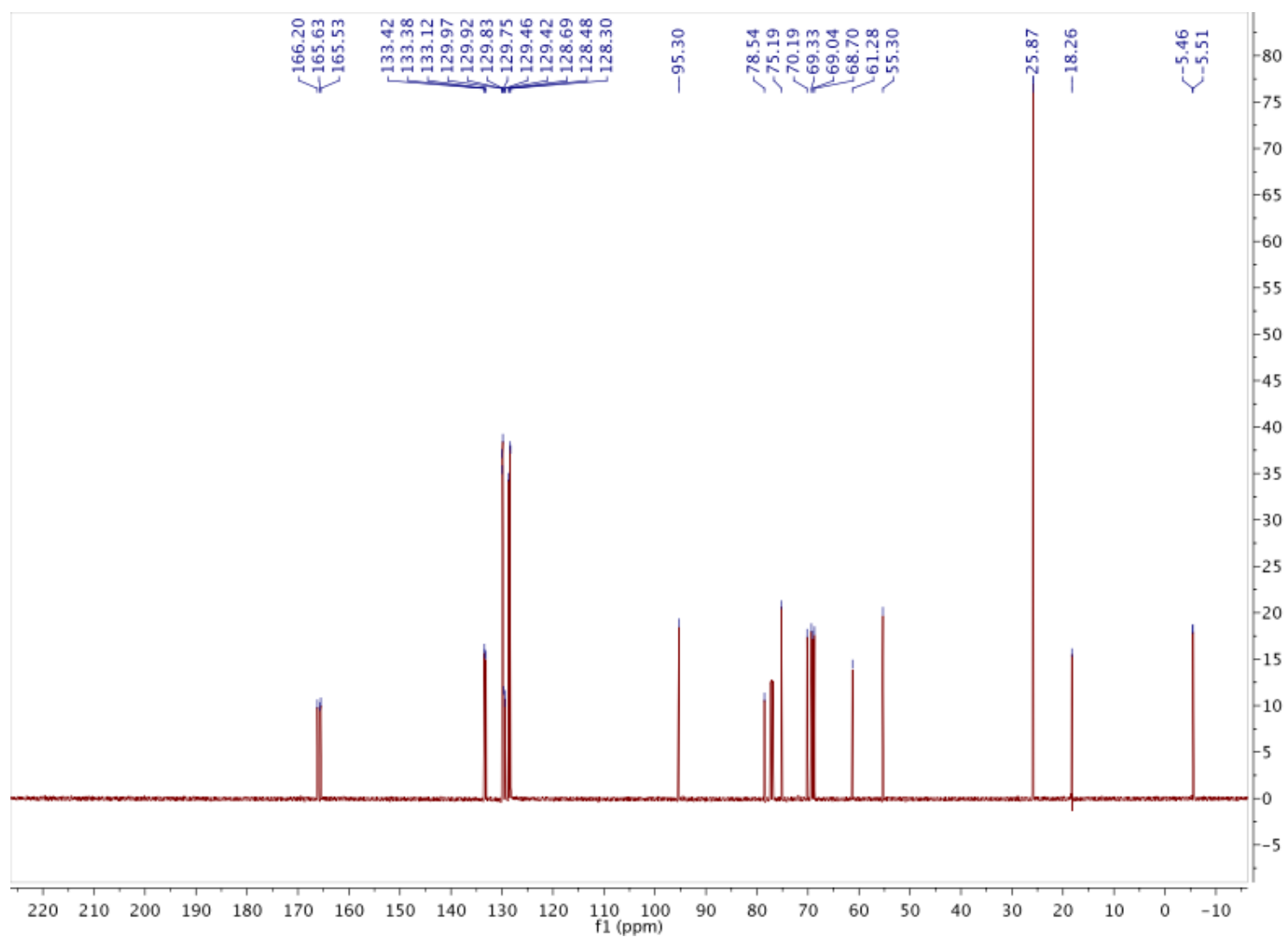
¹H NMR Propargyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (9)



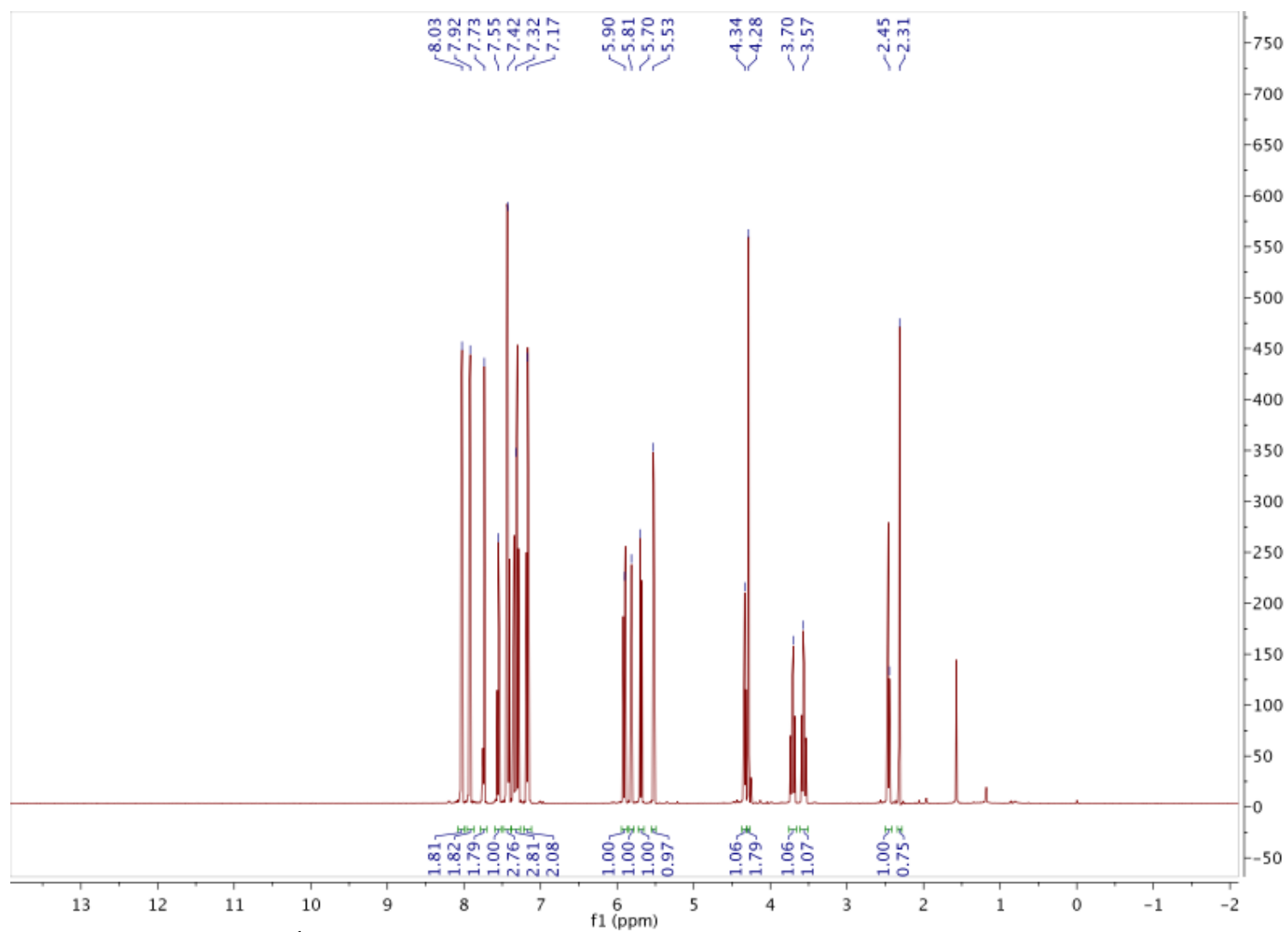
^{13}C NMR Propargyl 2,3,6-tri-*O*-benzoyl- α -D-galactopyranoside (**9**)



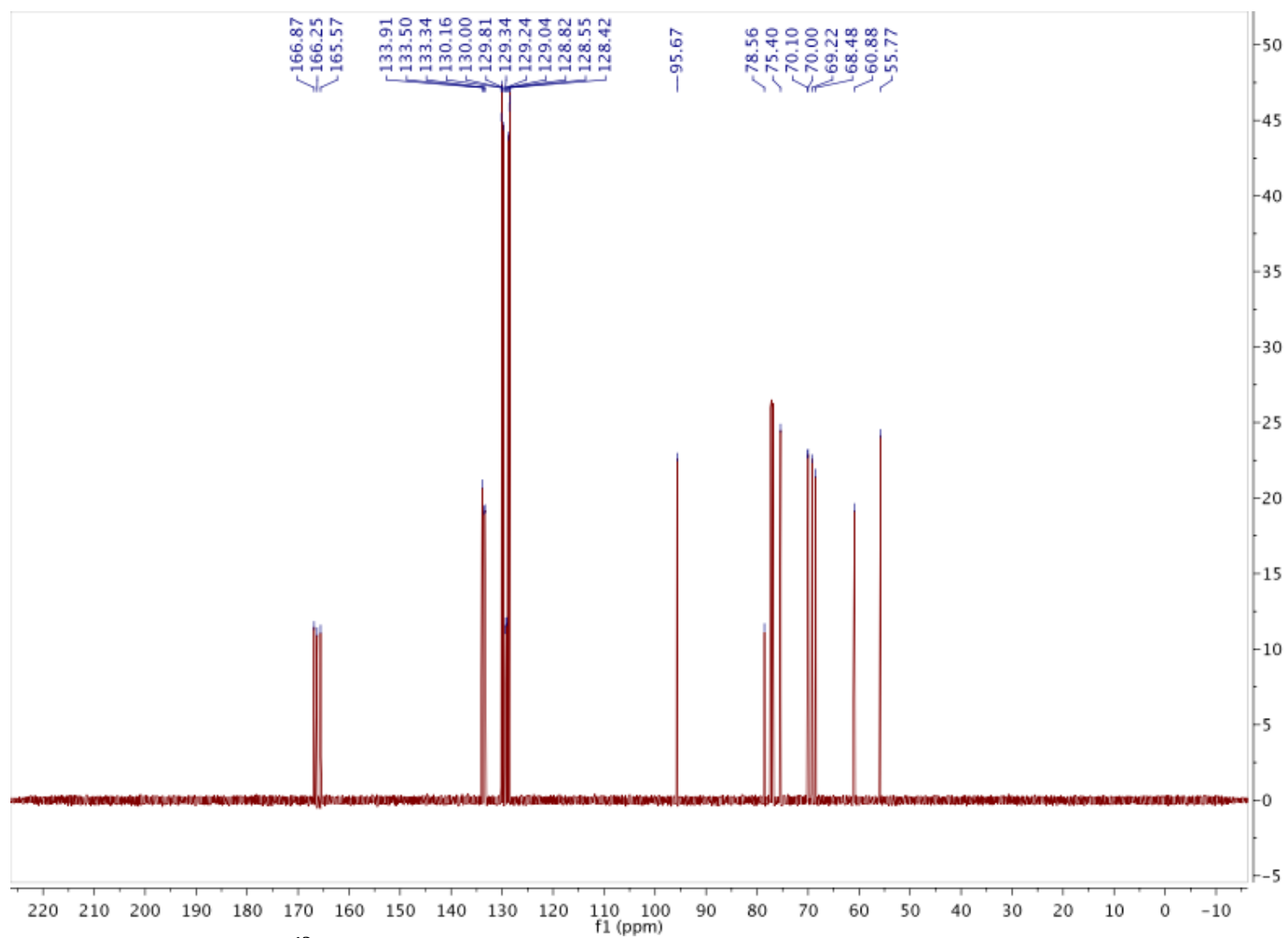
^1H NMR Propargyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldimethylsilyl)- α -D-galactopyranoside (**10**)



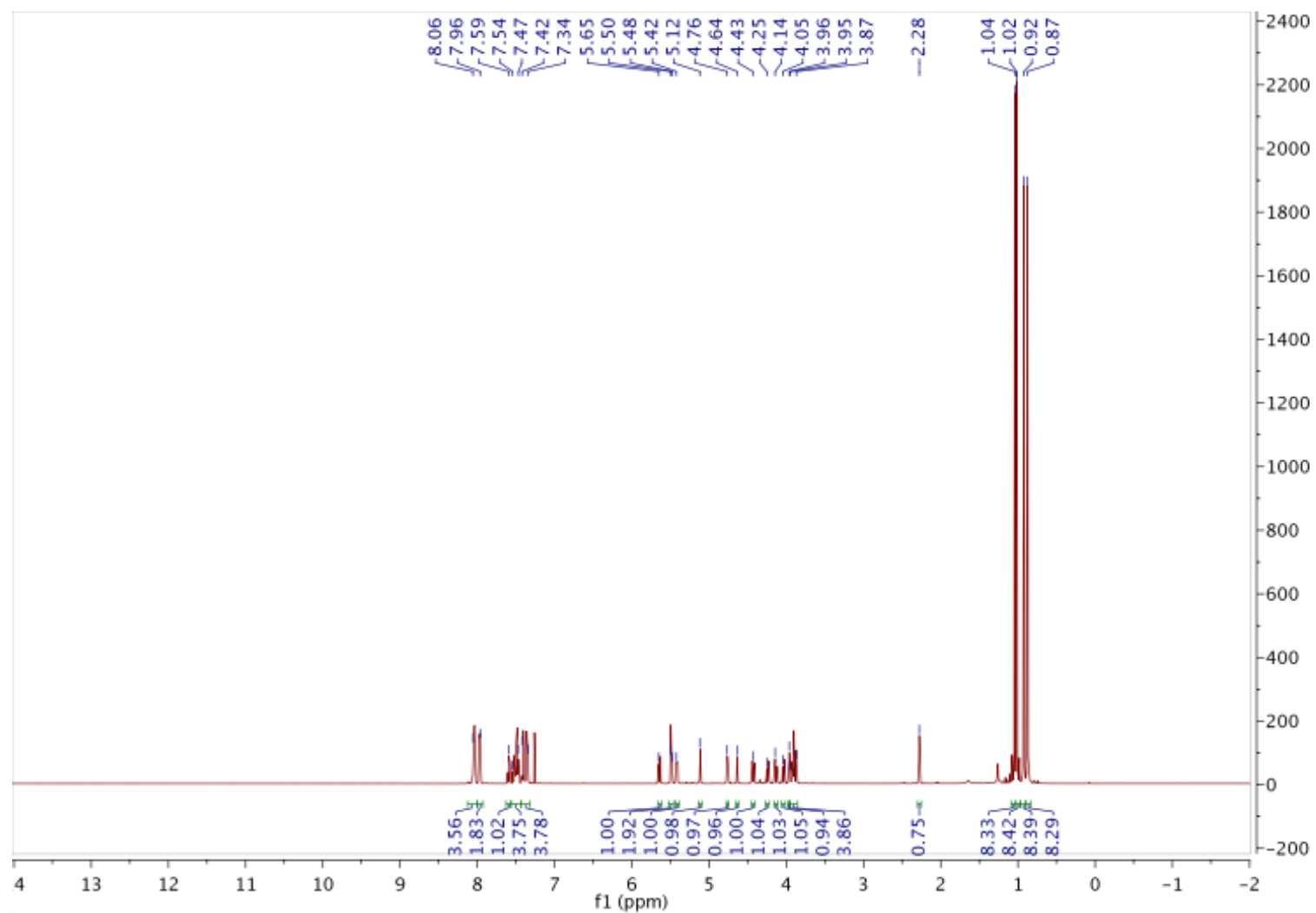
¹³C NMR Propargyl 2,3,4-tri-O-benzoyl-6-O-(*tert*-butyldimethylsilyl)-α-D-galactopyranoside (**10**)



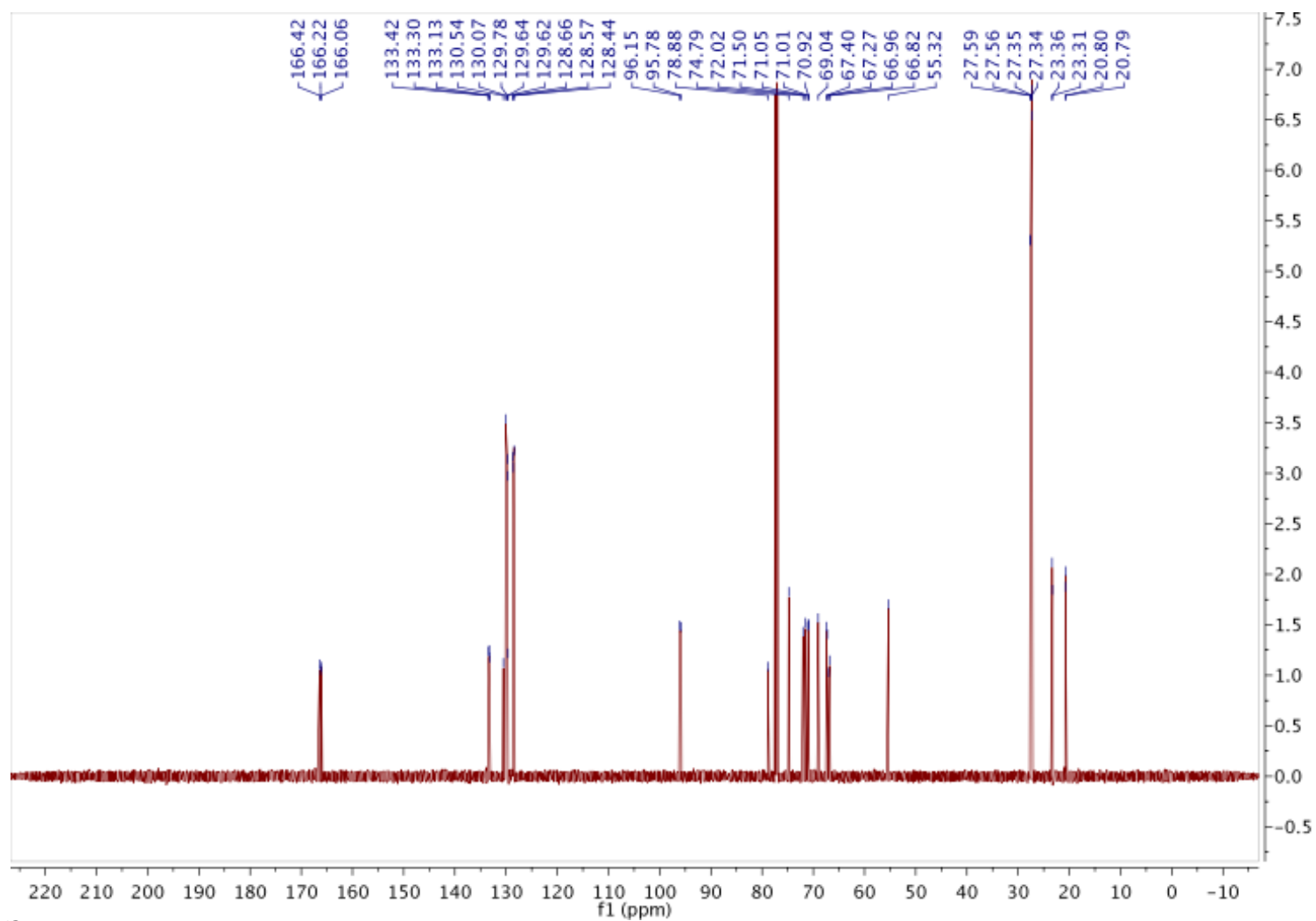
¹H NMR Propargyl 2,3,4-tri-O-benzoyl-α-D-galactopyranoside (**11**)



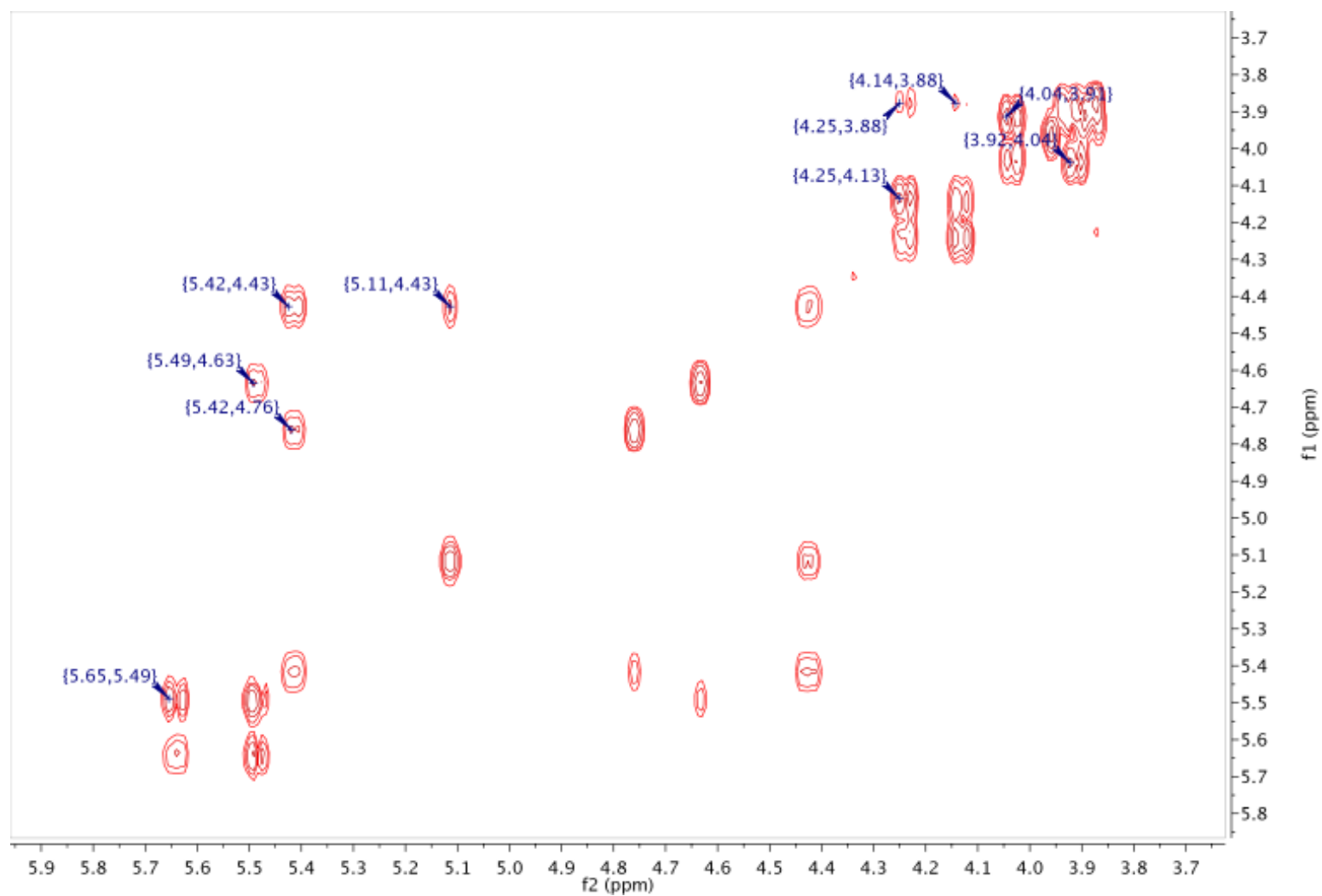
¹³C NMR Propargyl 2,3,4-tri-O-benzoyl- α -D-galactopyranoside (11)



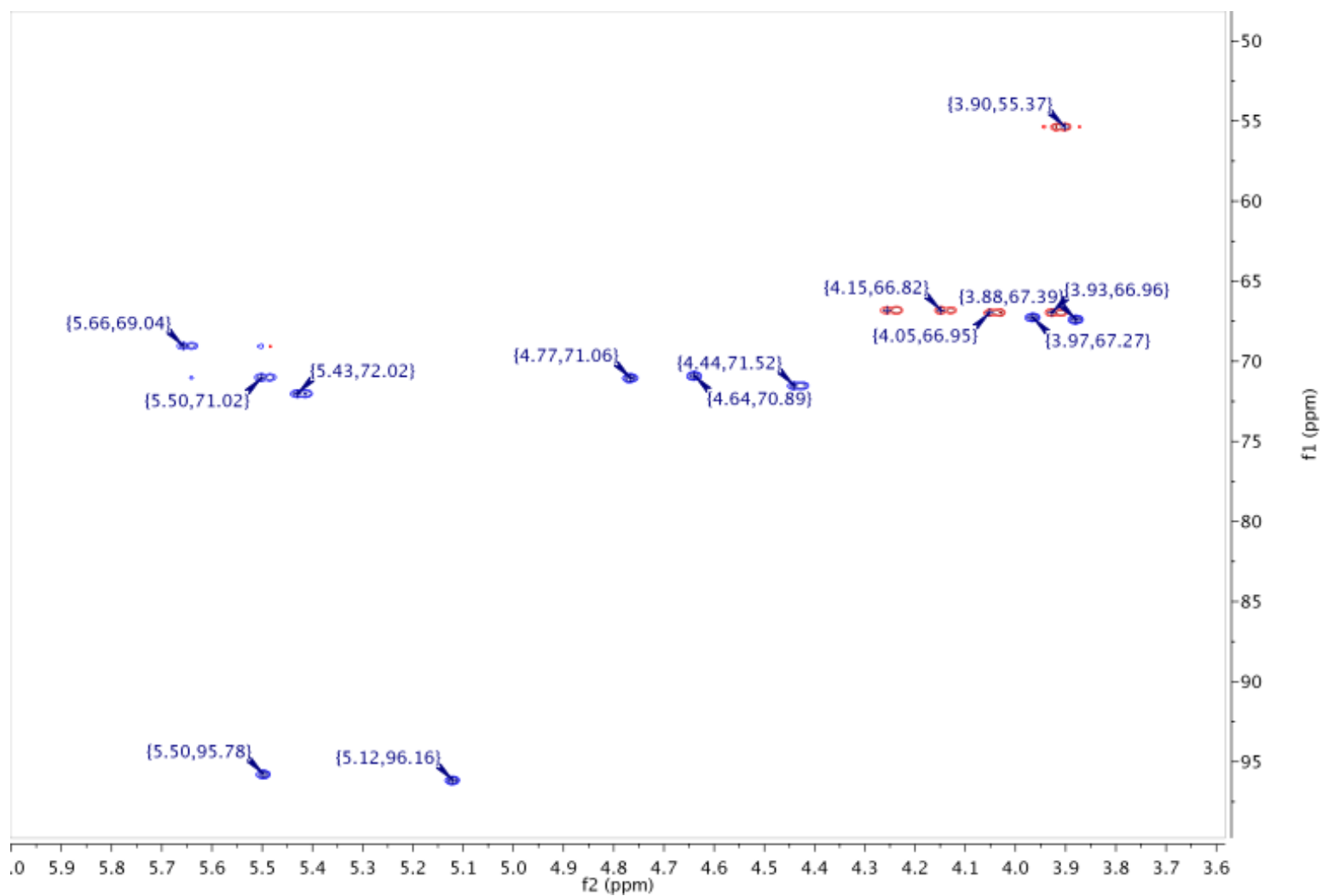
^1H NMR Propargyl 3-O-benzoyl-4,6-di-O-*tert*-butylsilanediyl-2-O-(2,3-di-O-benzoyl-4,6-di-O-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**13**)



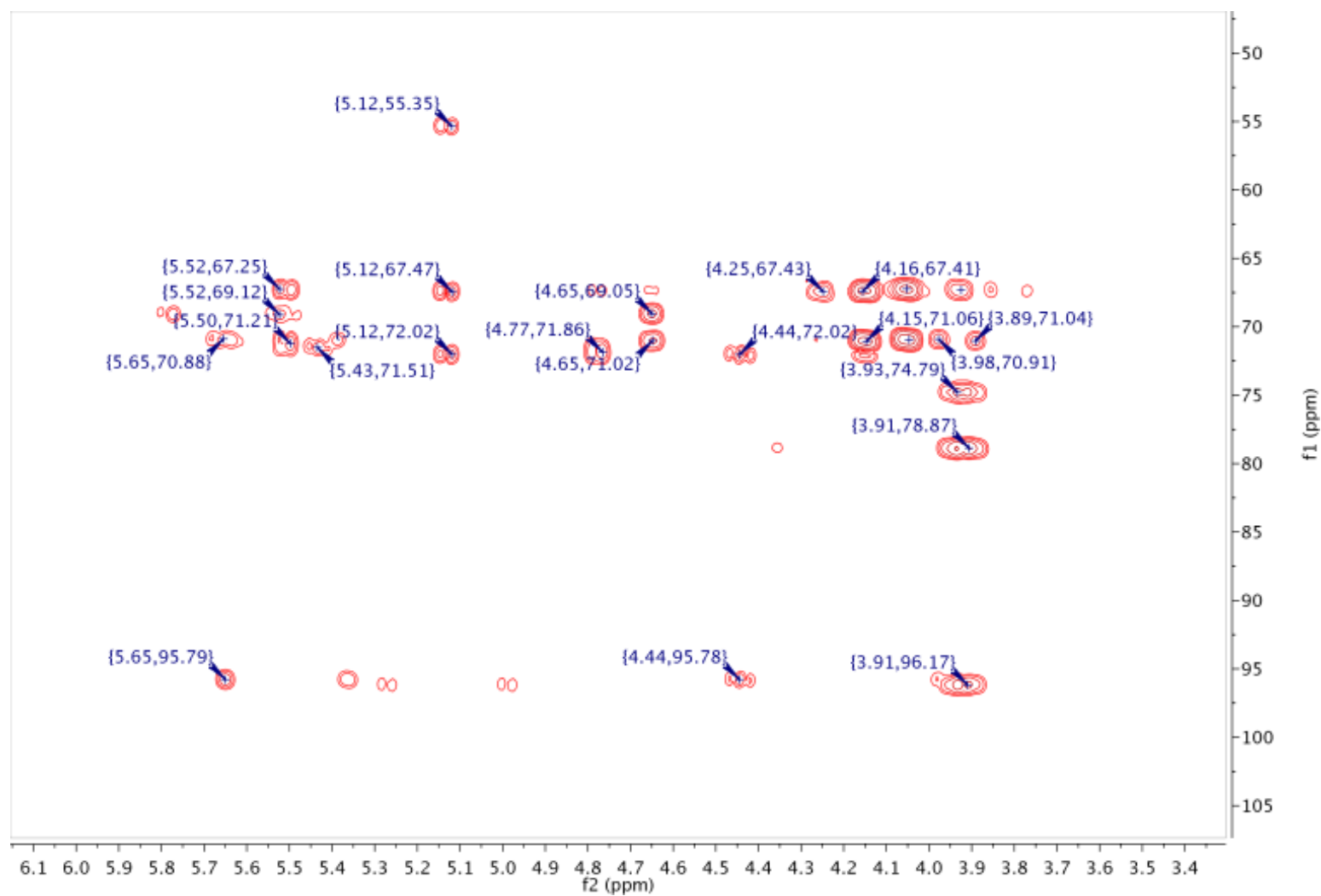
^{13}C NMR Propargyl 3-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl-2-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**13**)



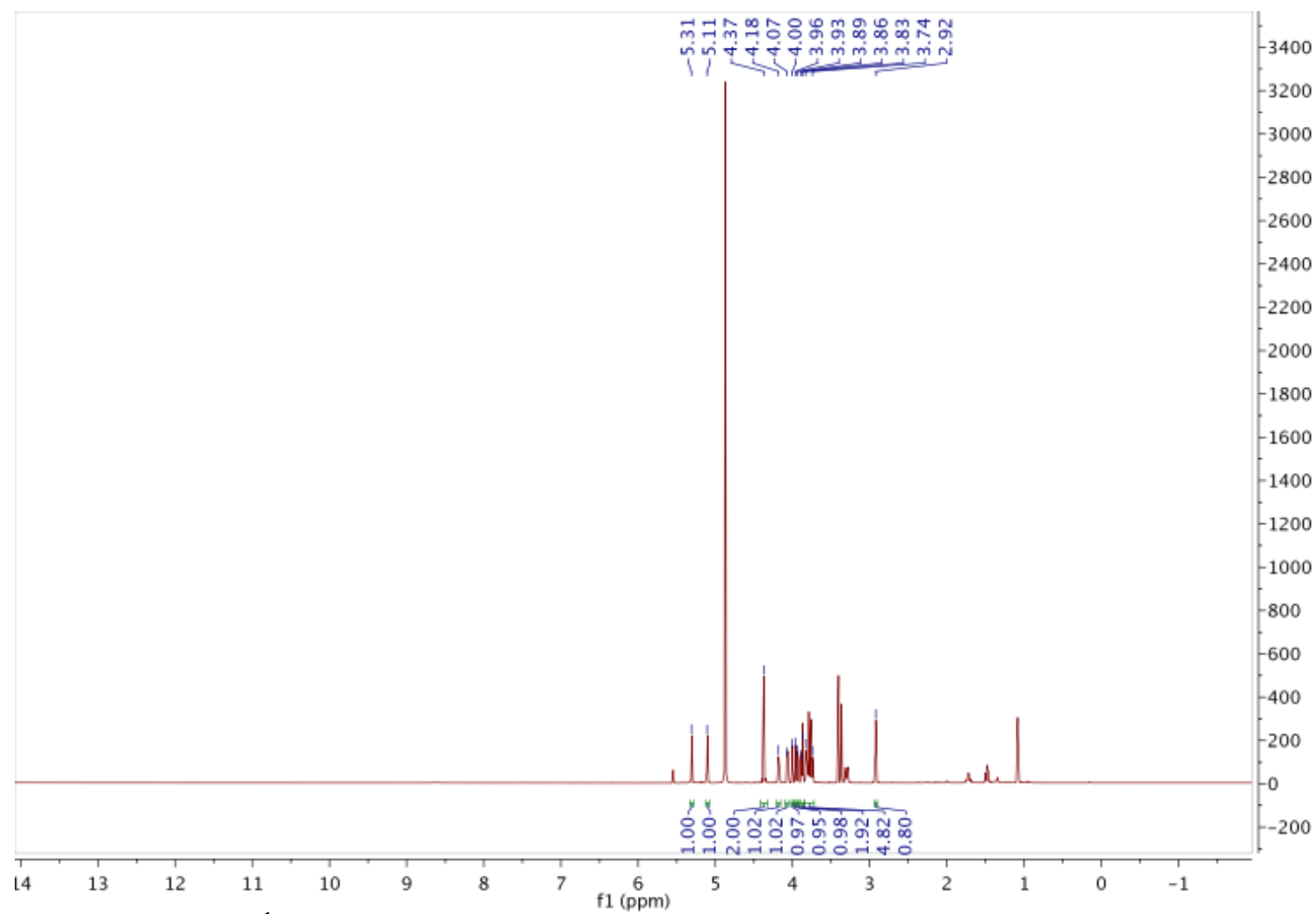
gCOSY NMR Propargyl 3-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl-2-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**13**)



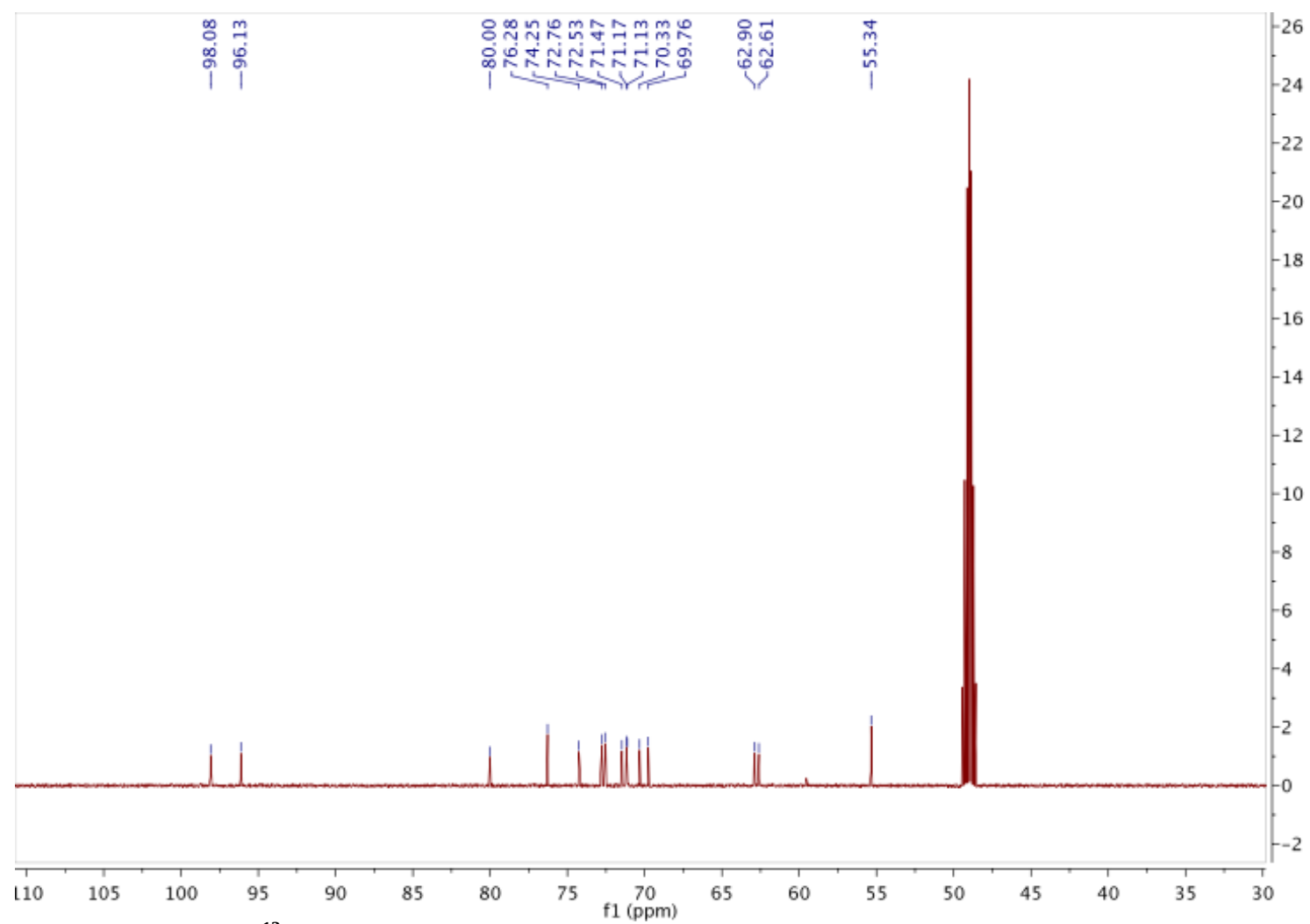
gHSQC NMR Propargyl 3-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl-2-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**13**)



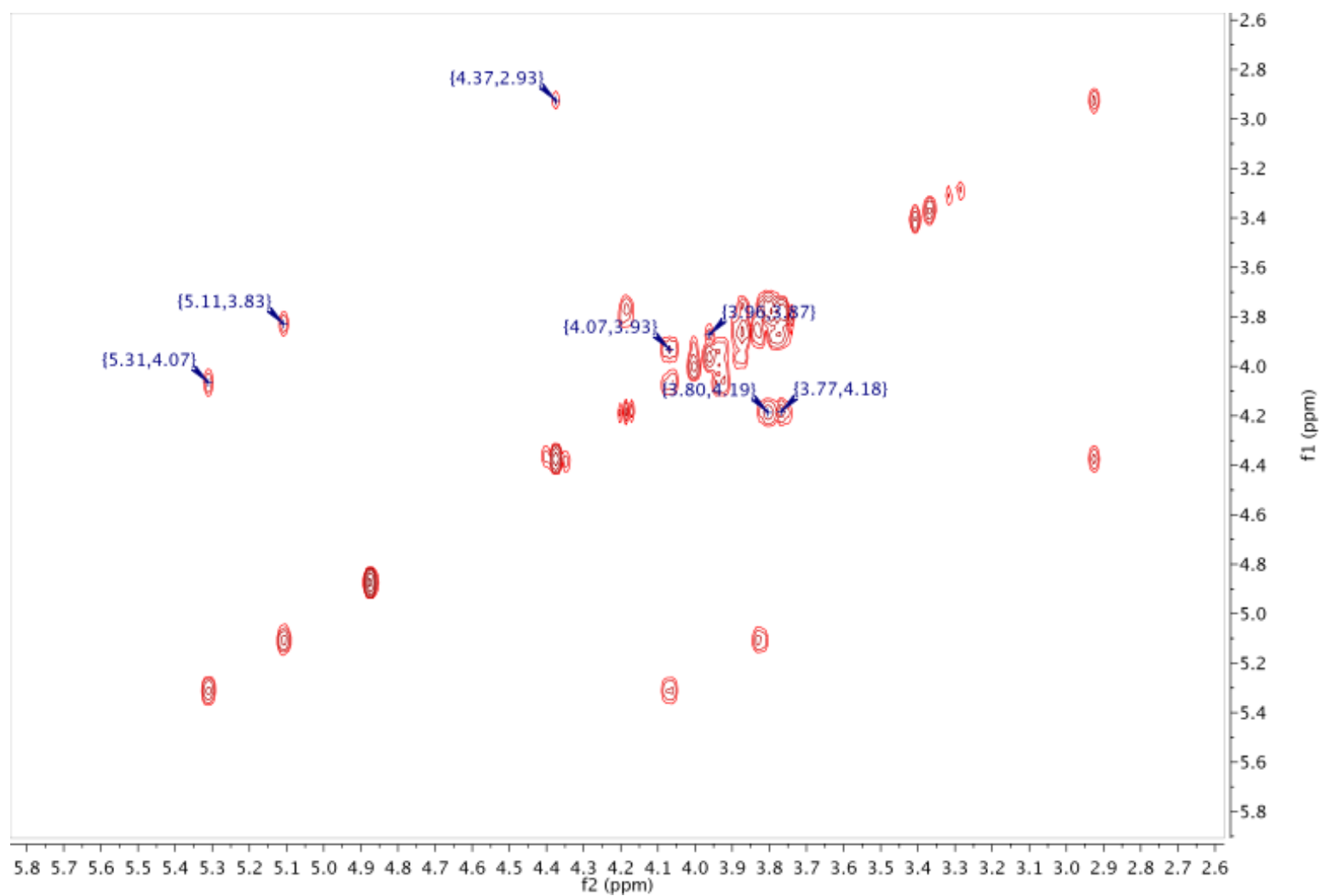
gHMBC NMR Propargyl 3-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl-2-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-*tert*-butylsilanediyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**13**)



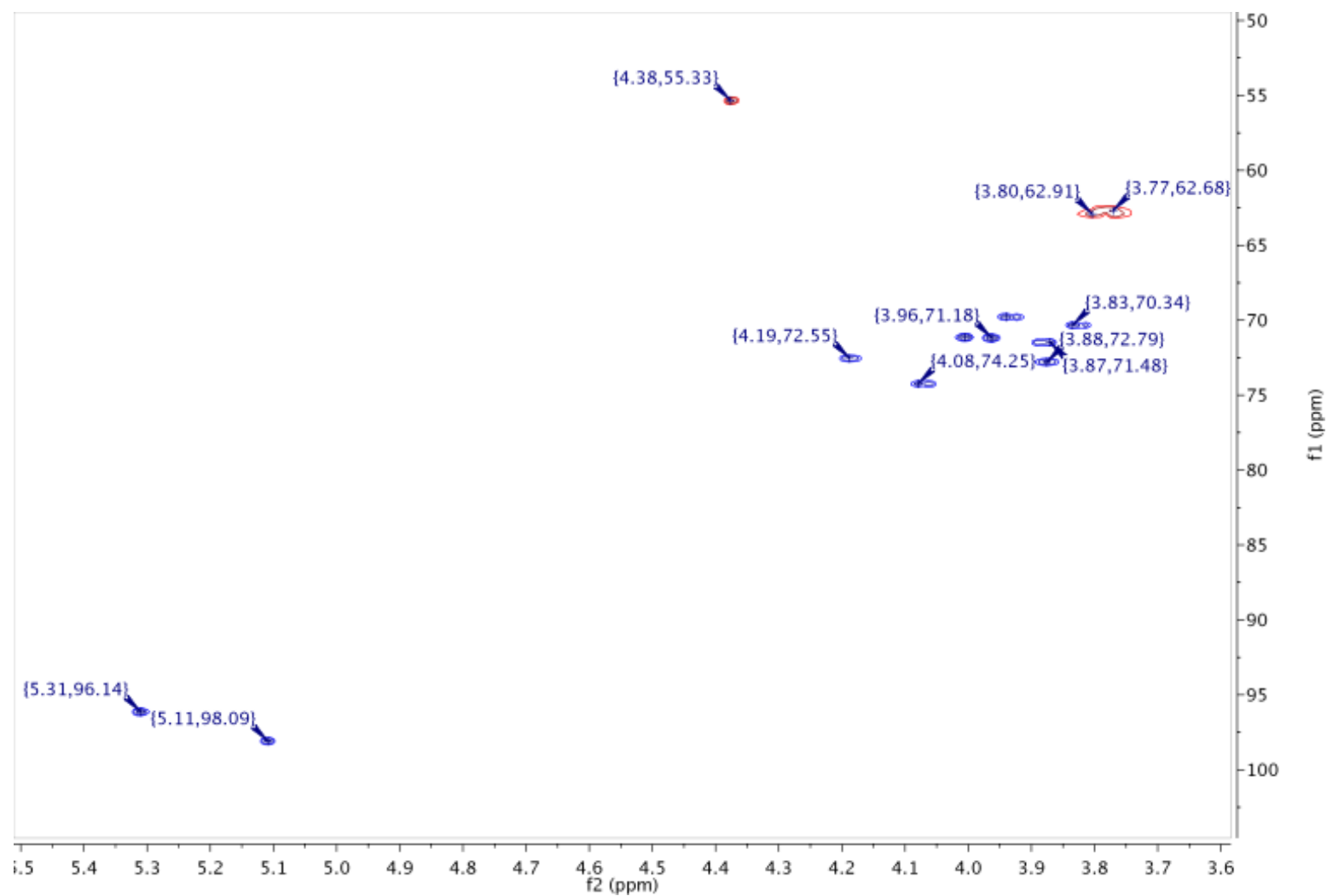
¹H NMR Propargyl 2-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (2)



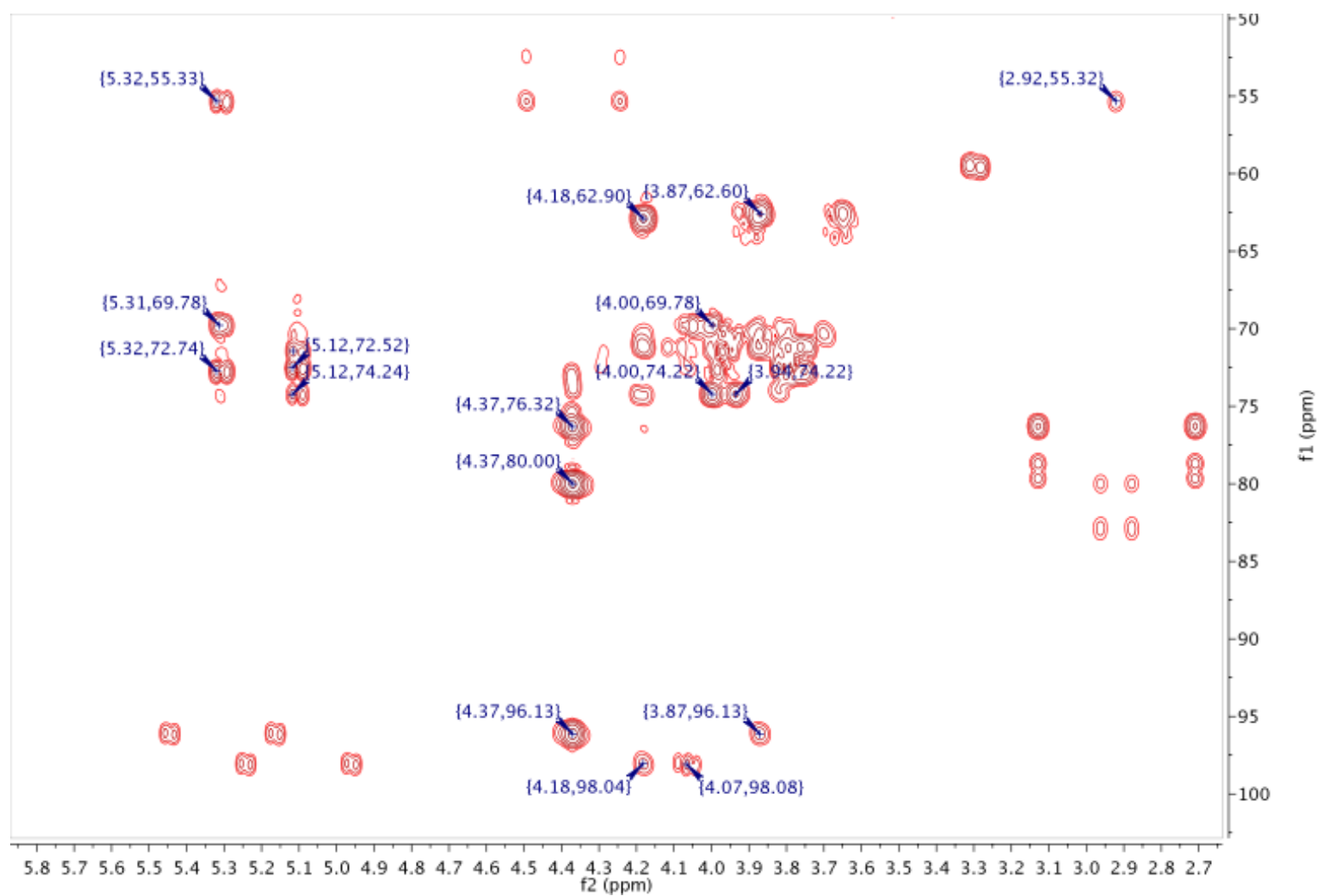
¹³C NMR Propargyl 2-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (2)



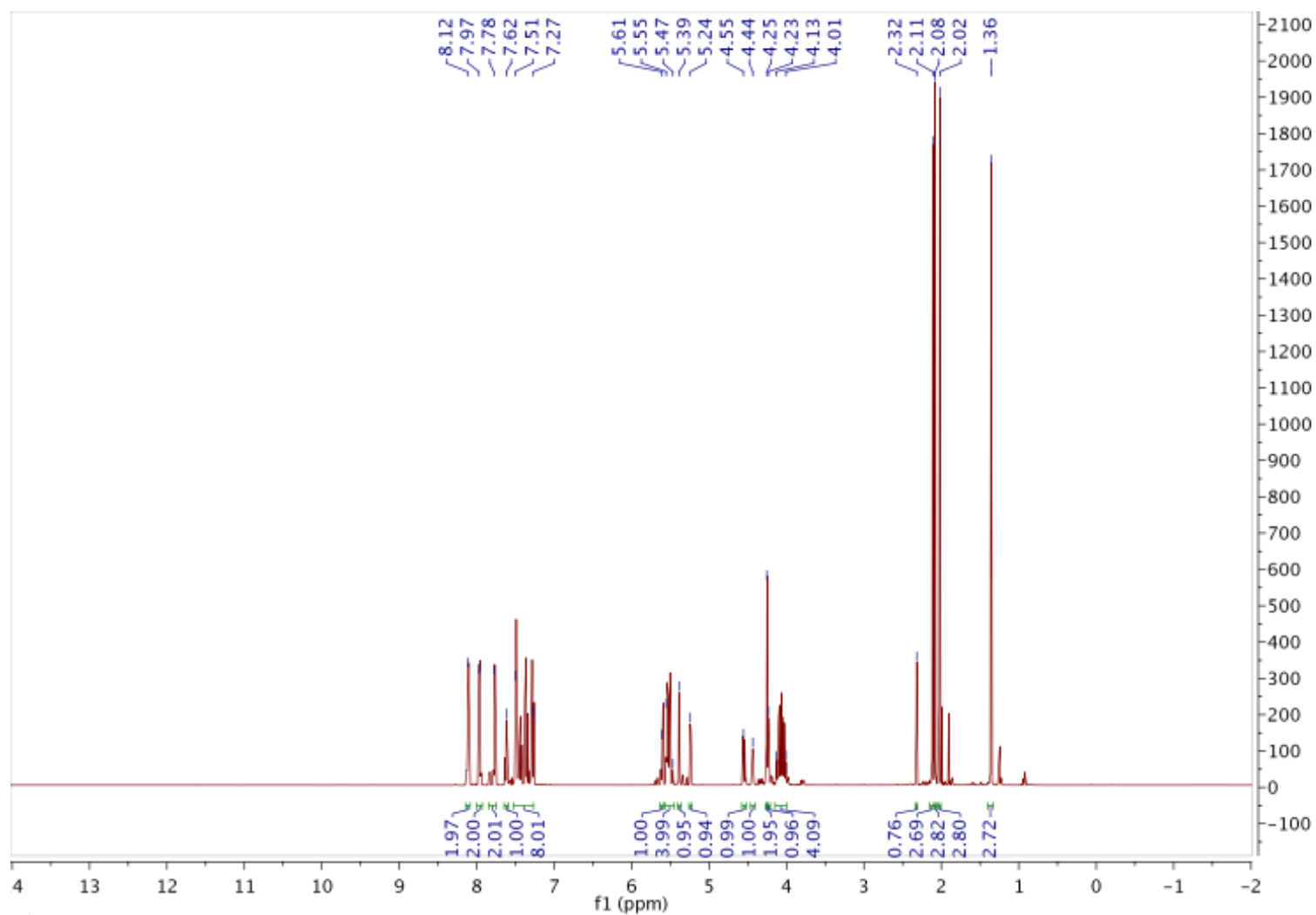
gCOSY NMR Propargyl 2-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (2)



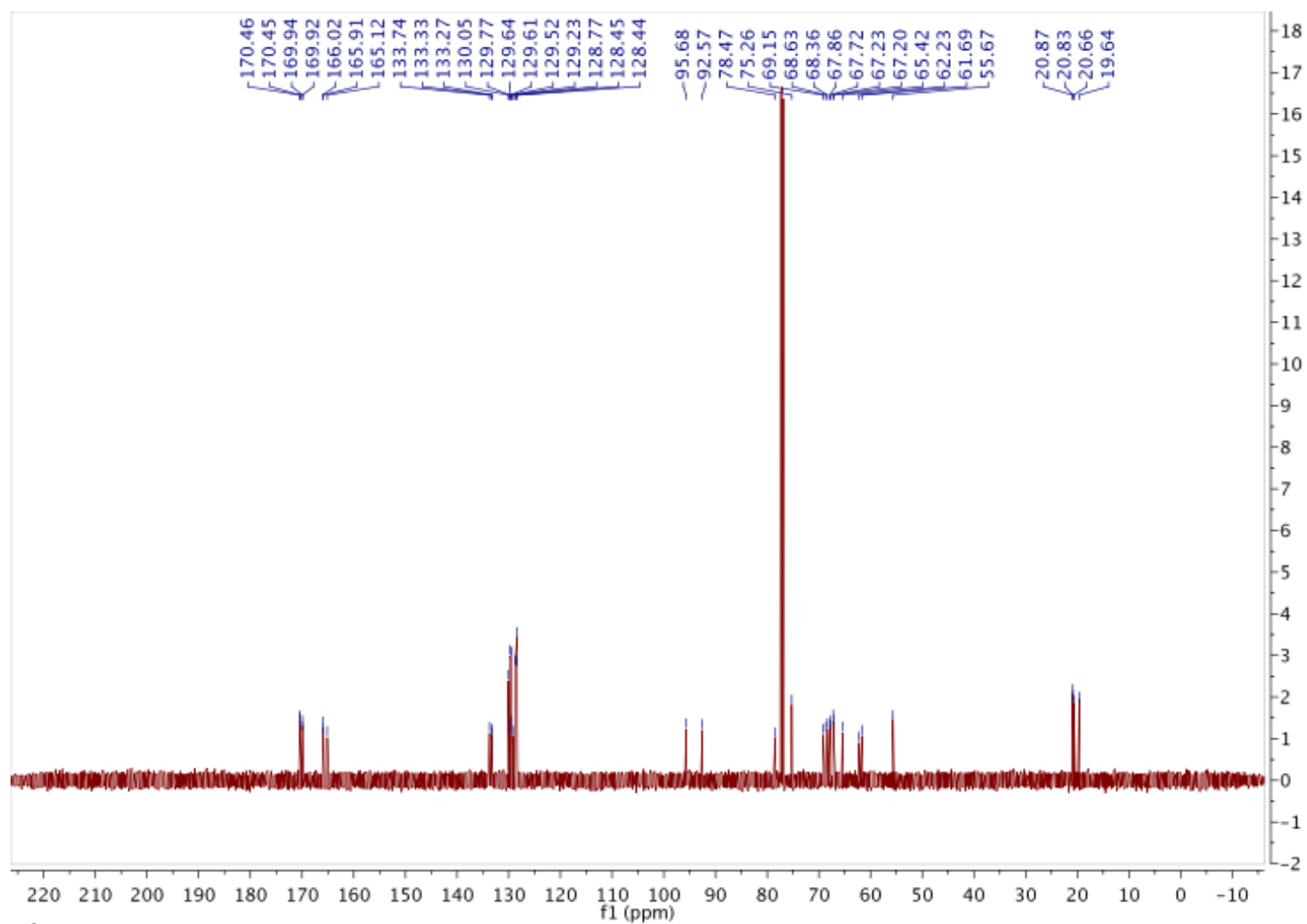
gHSQC NMR Propargyl 2-*O*-(α -D-galactopyranosyl)- α -D-galactopyranoside (2)



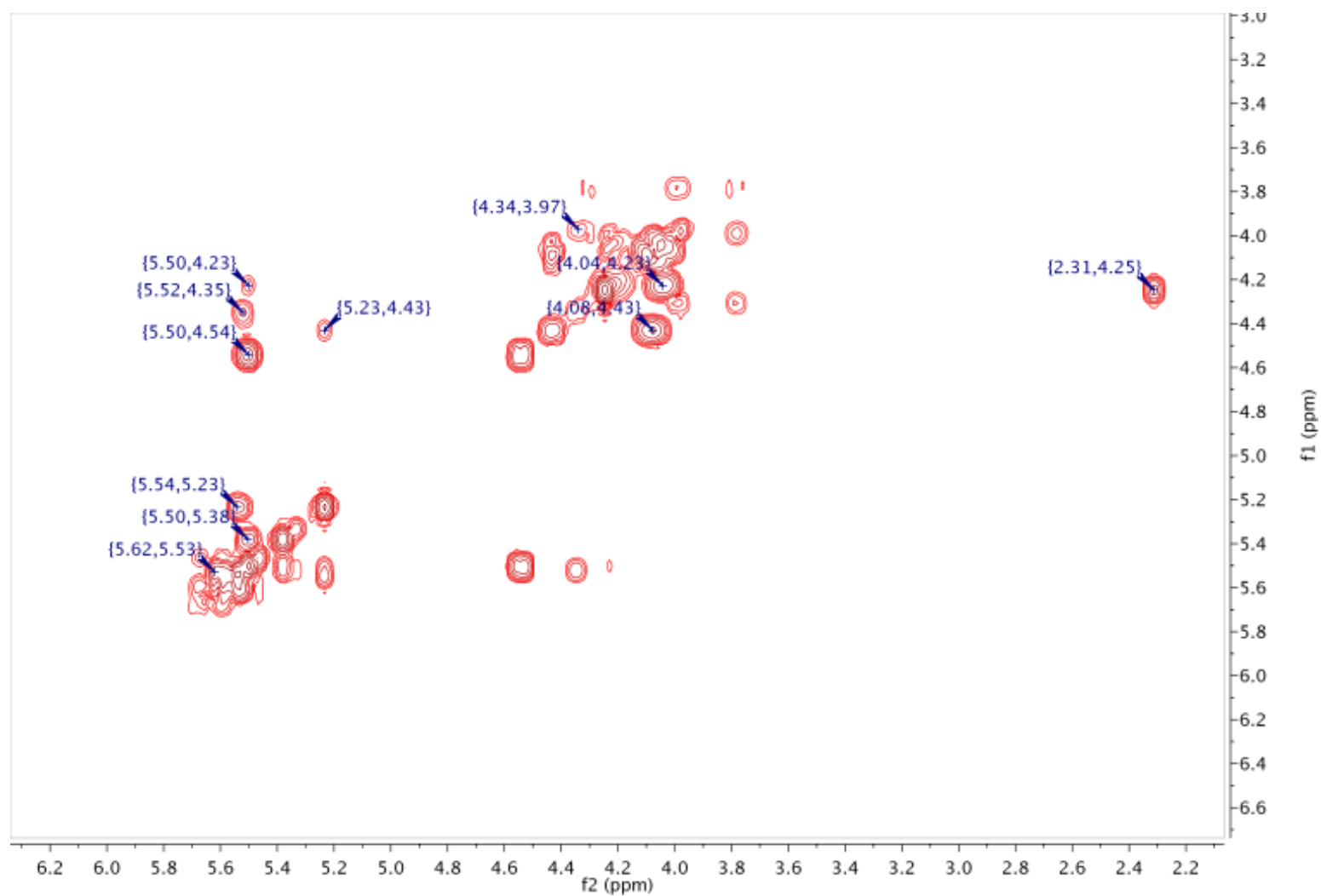
gHMBC NMR Propargyl 2-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (2)



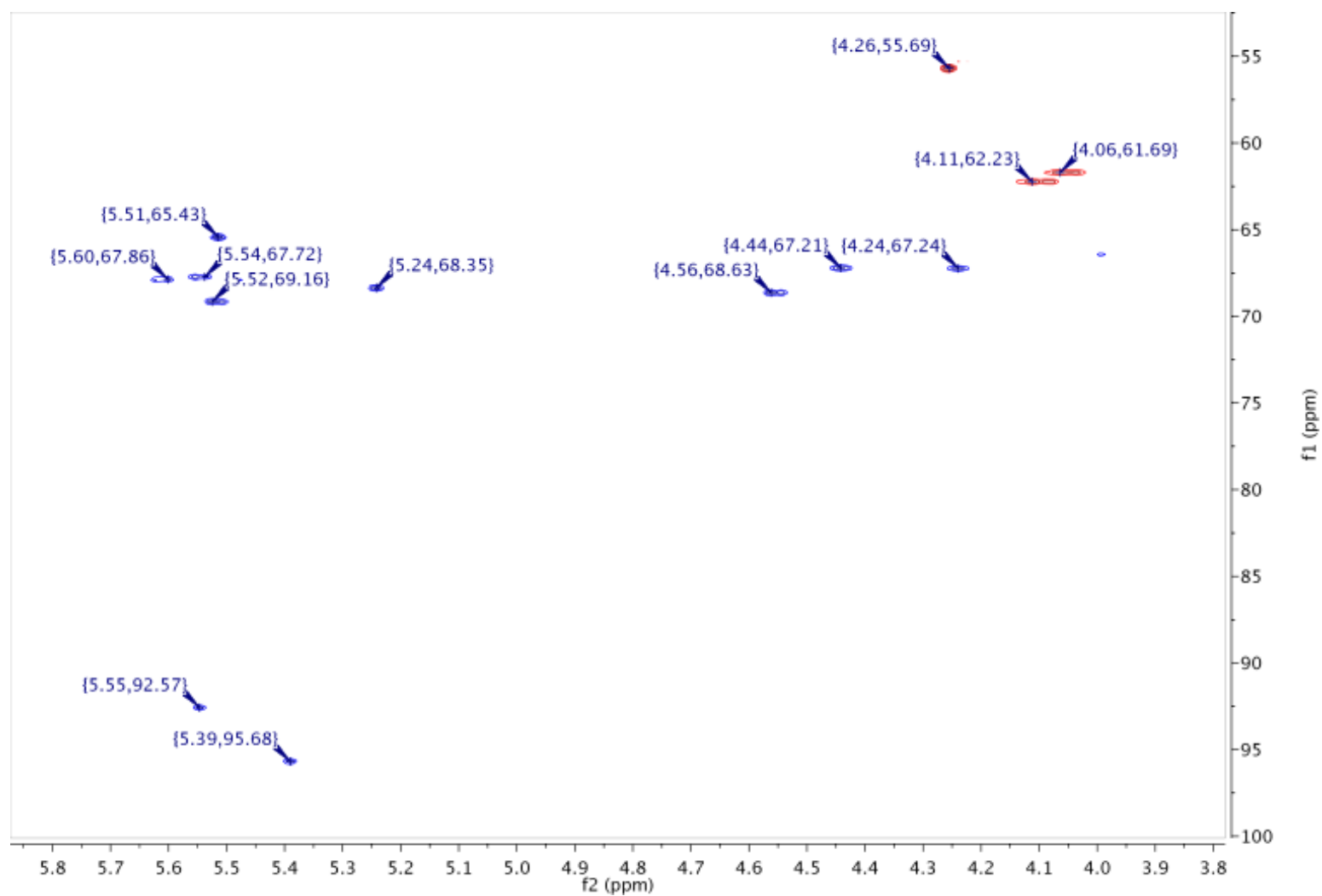
^1H NMR Propargyl 2-O-benzoyl-4,6-di-O-acetyl-3-O-(2,3-di-O-benzoyl-4,6-di-O-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**26**)



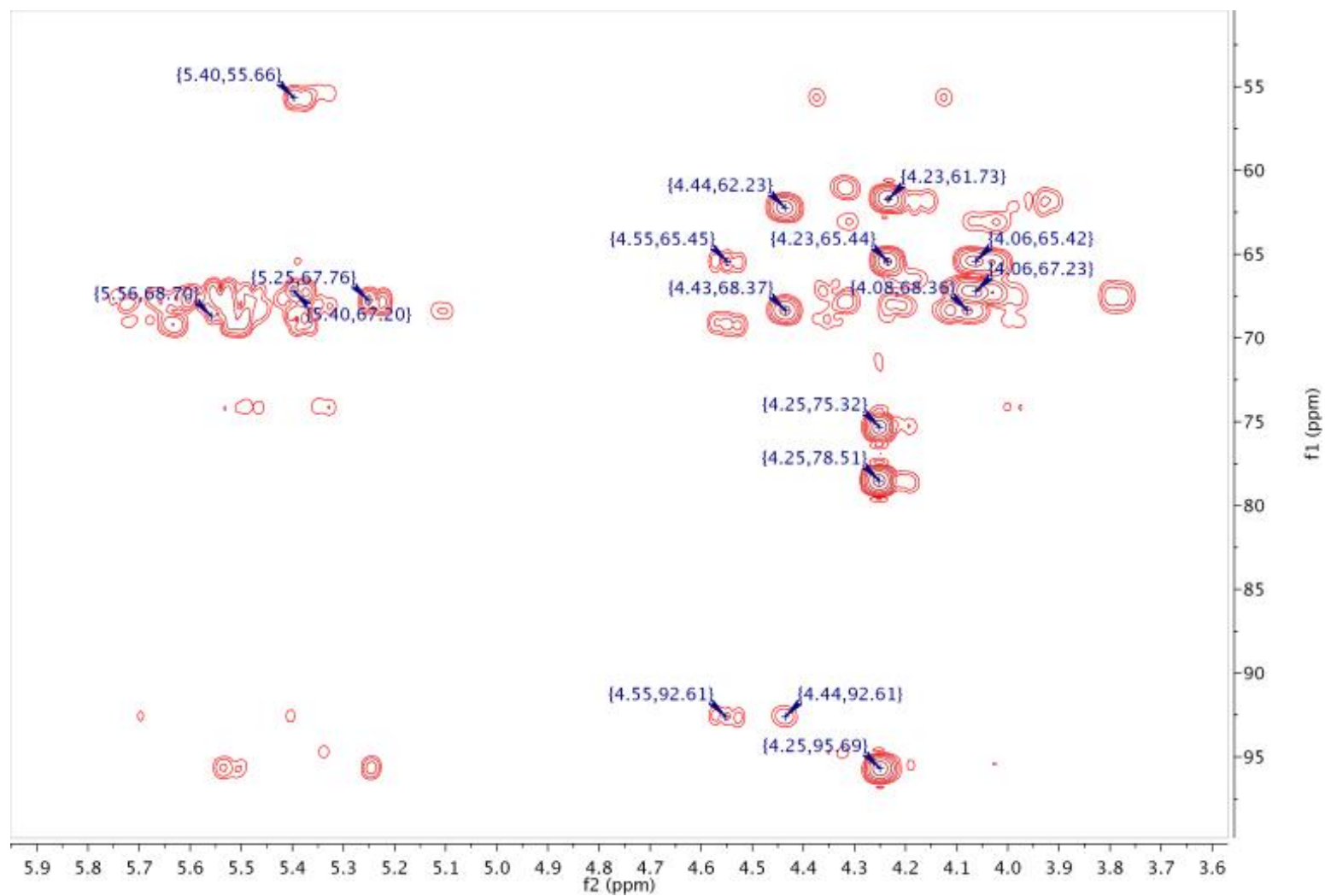
^{13}C NMR Propargyl 2-O-benzoyl-4,6-di-O-acetyl-3-O-(2,3-di-O-benzoyl-4,6-di-O-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**26**)



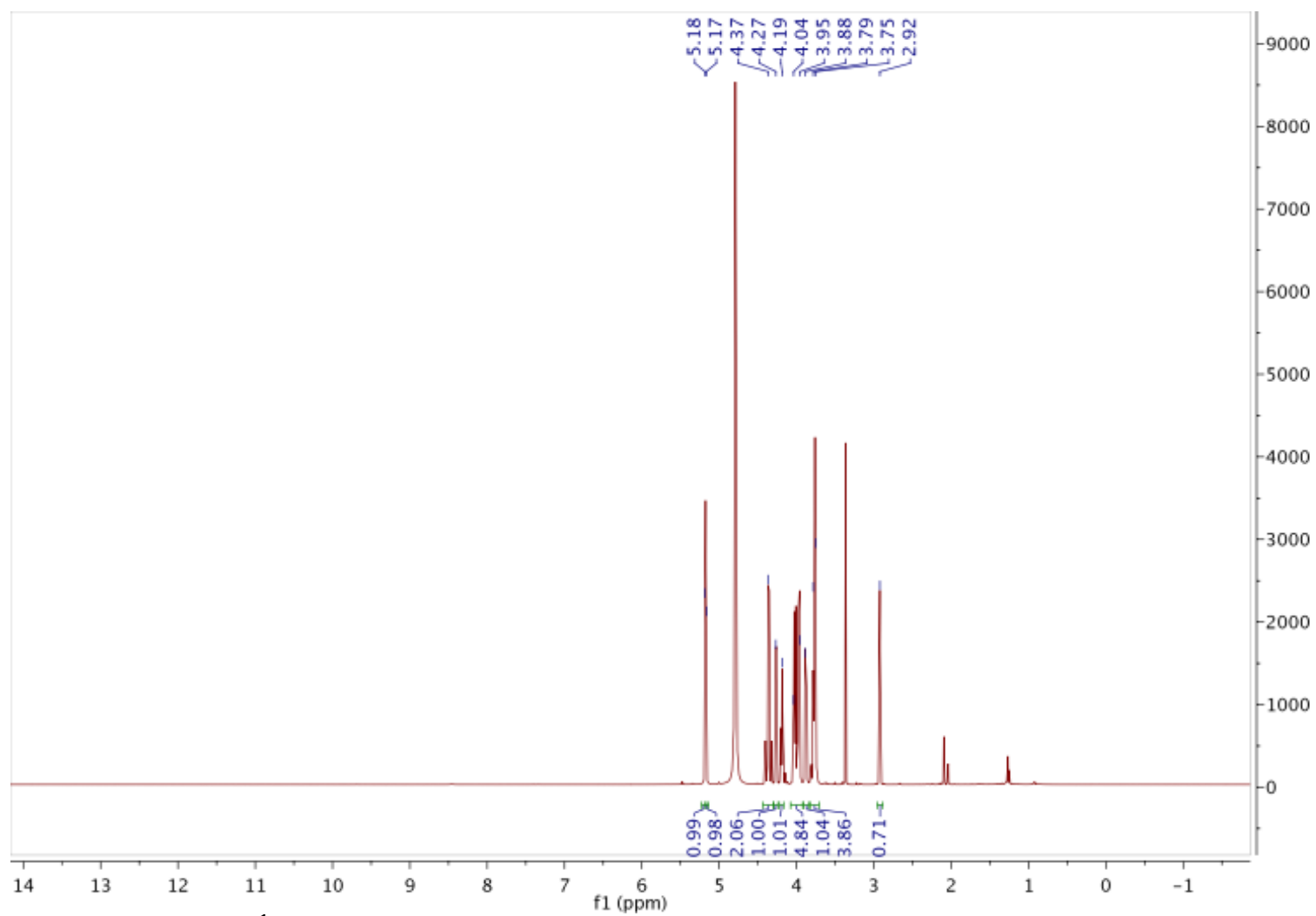
gCOSY NMR Propargyl 2-*O*-benzoyl-4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**26**)



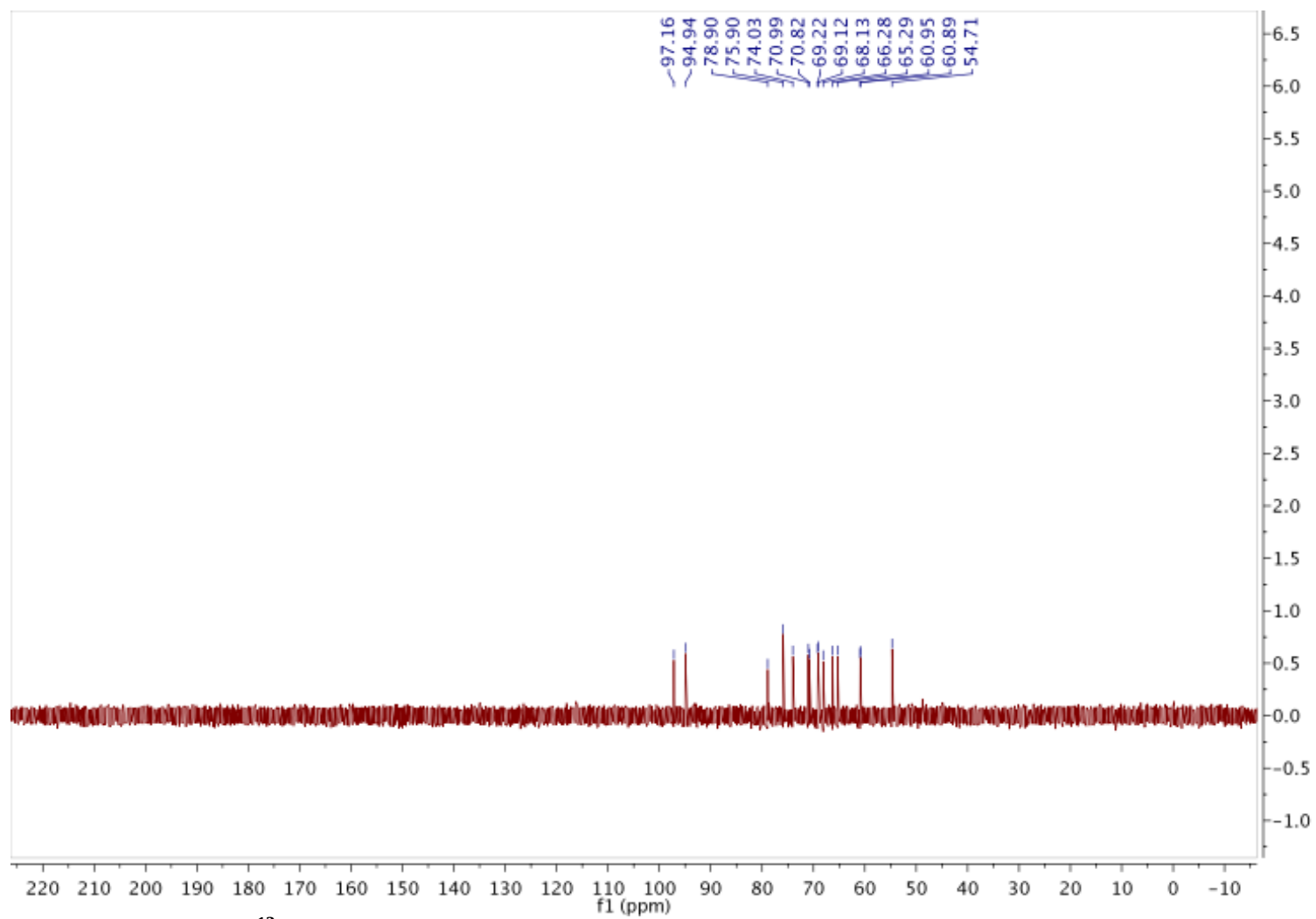
gHSQC NMR Propargyl 2-*O*-benzoyl-4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**26**)



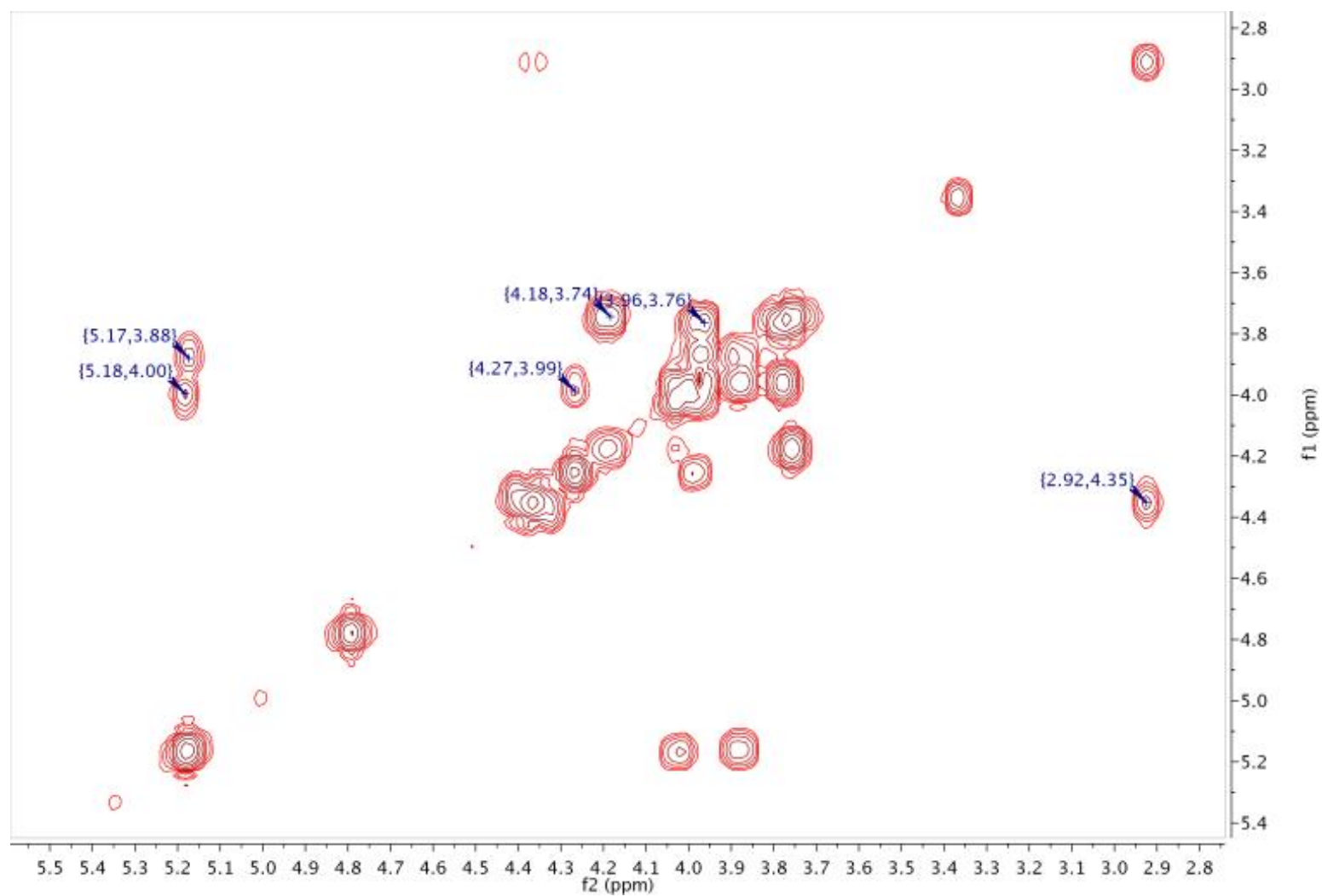
gHMBC NMR Propargyl 2-*O*-benzoyl-4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-acetyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**26**)



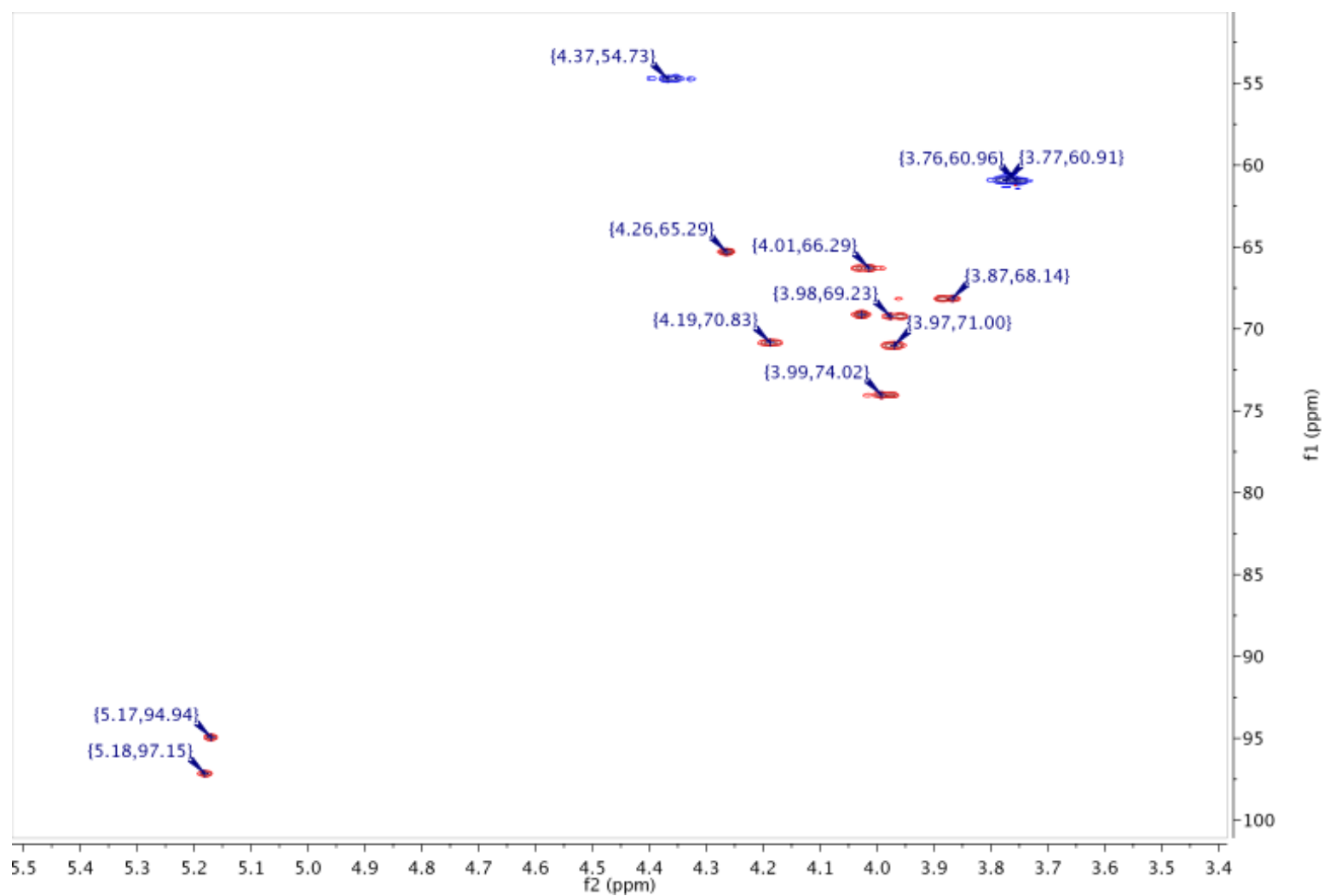
¹H NMR Propargyl 3-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (3)



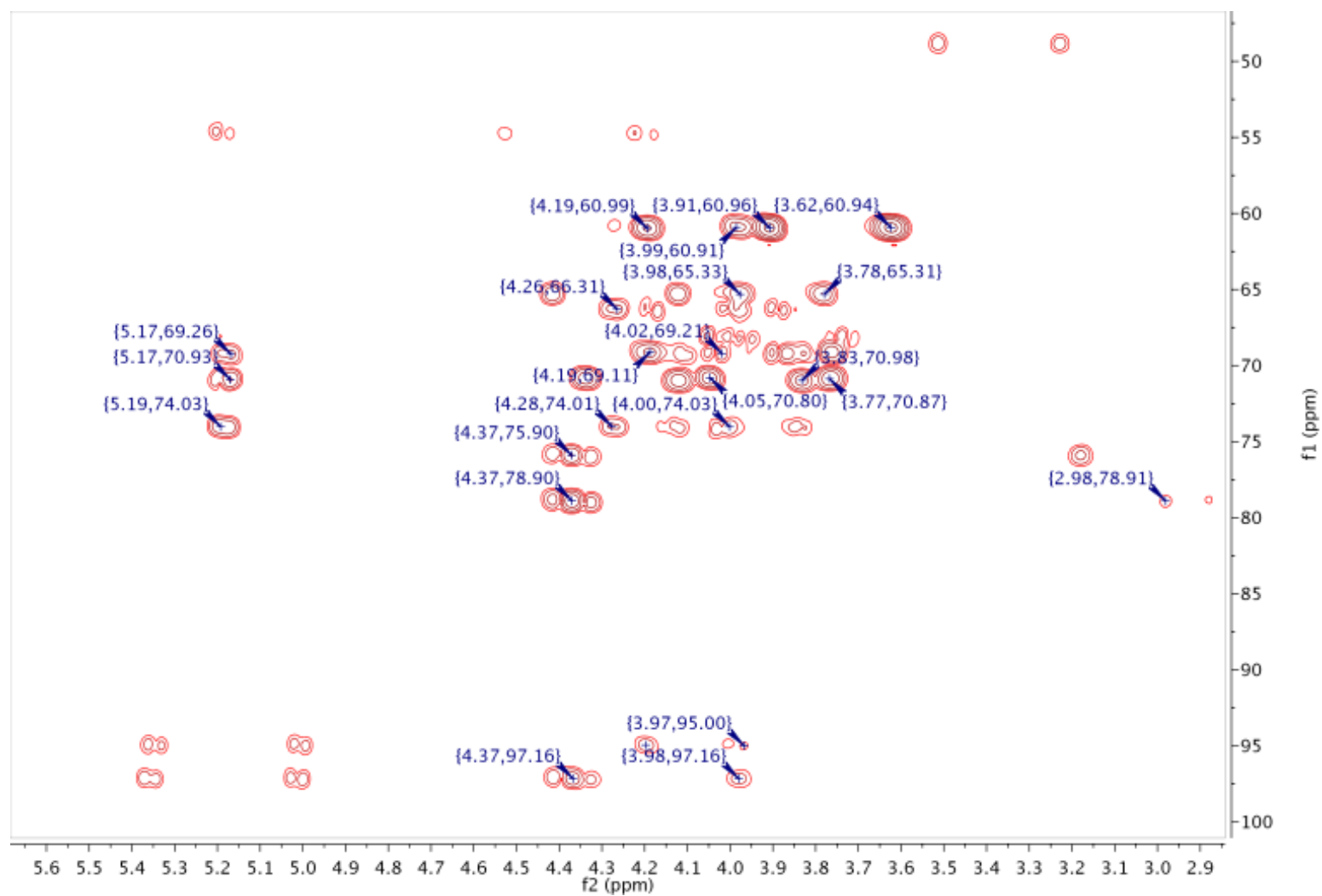
¹³C NMR Propargyl 3-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (3)



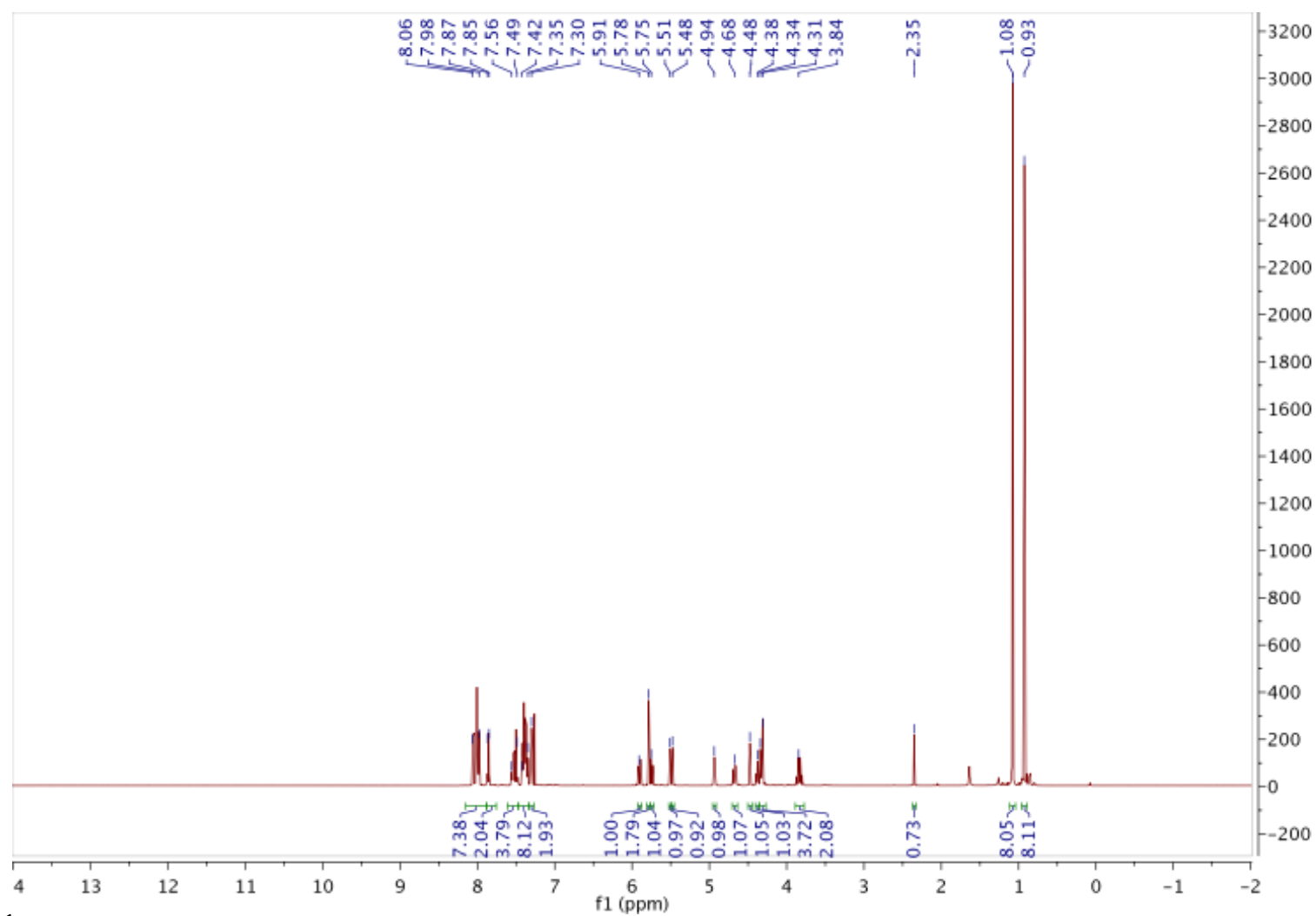
gCOSY NMR Propargyl 3-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (3)



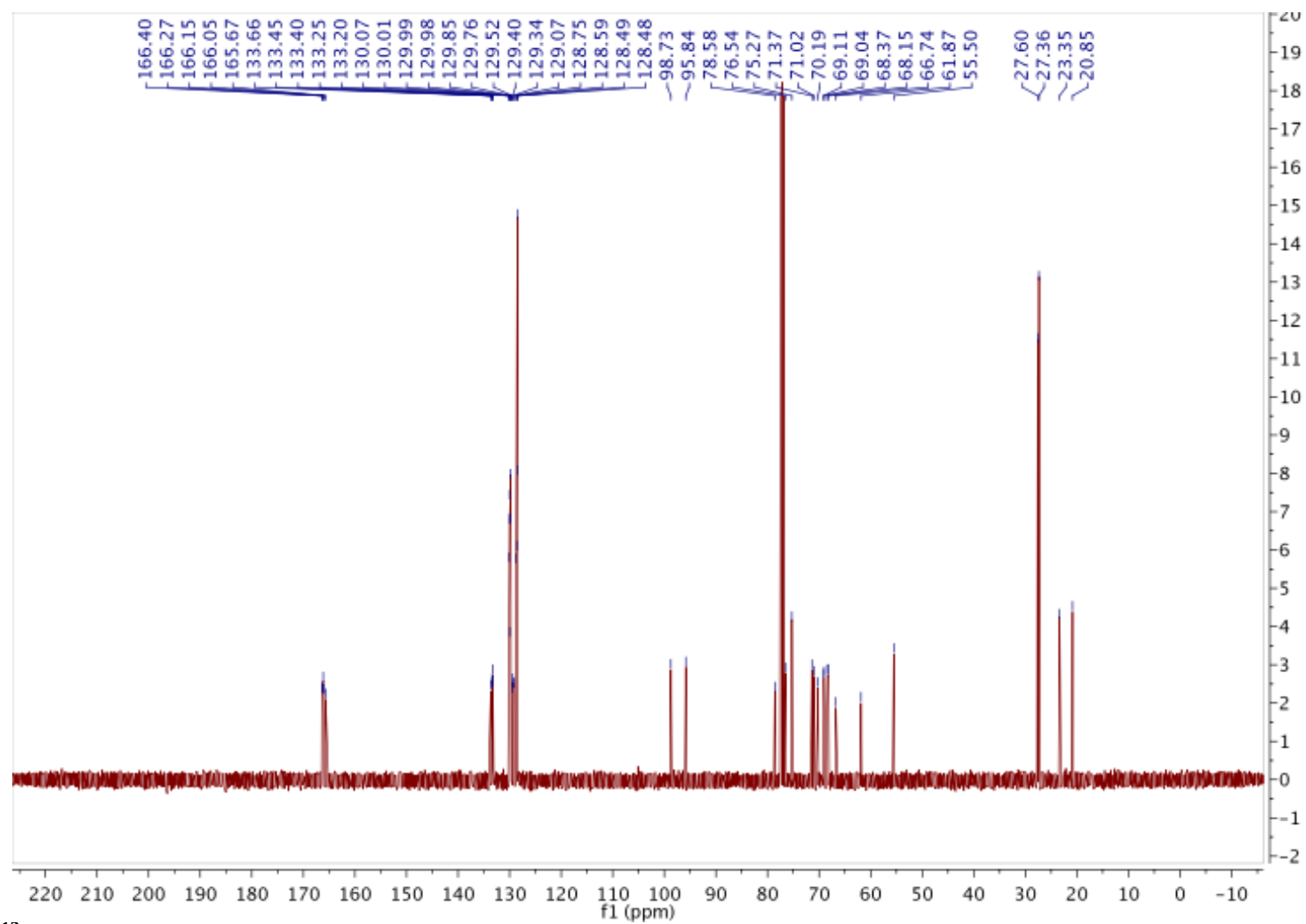
gHSQC NMR Propargyl 3-*O*-(α -D-galactopyranosyl)- α -D-galactopyranoside (**3**)



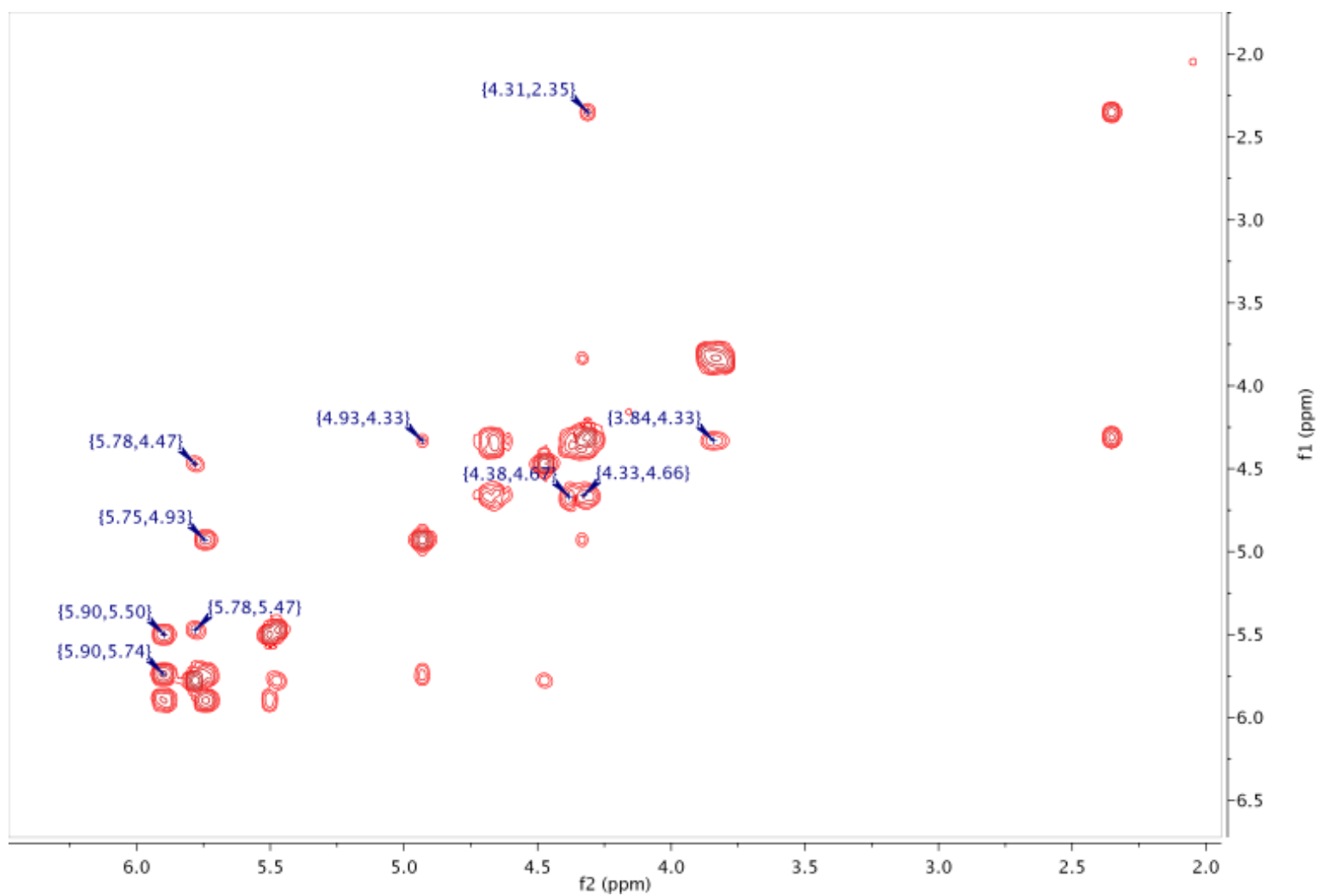
gHMBC NMR Propargyl 3-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (**3**)



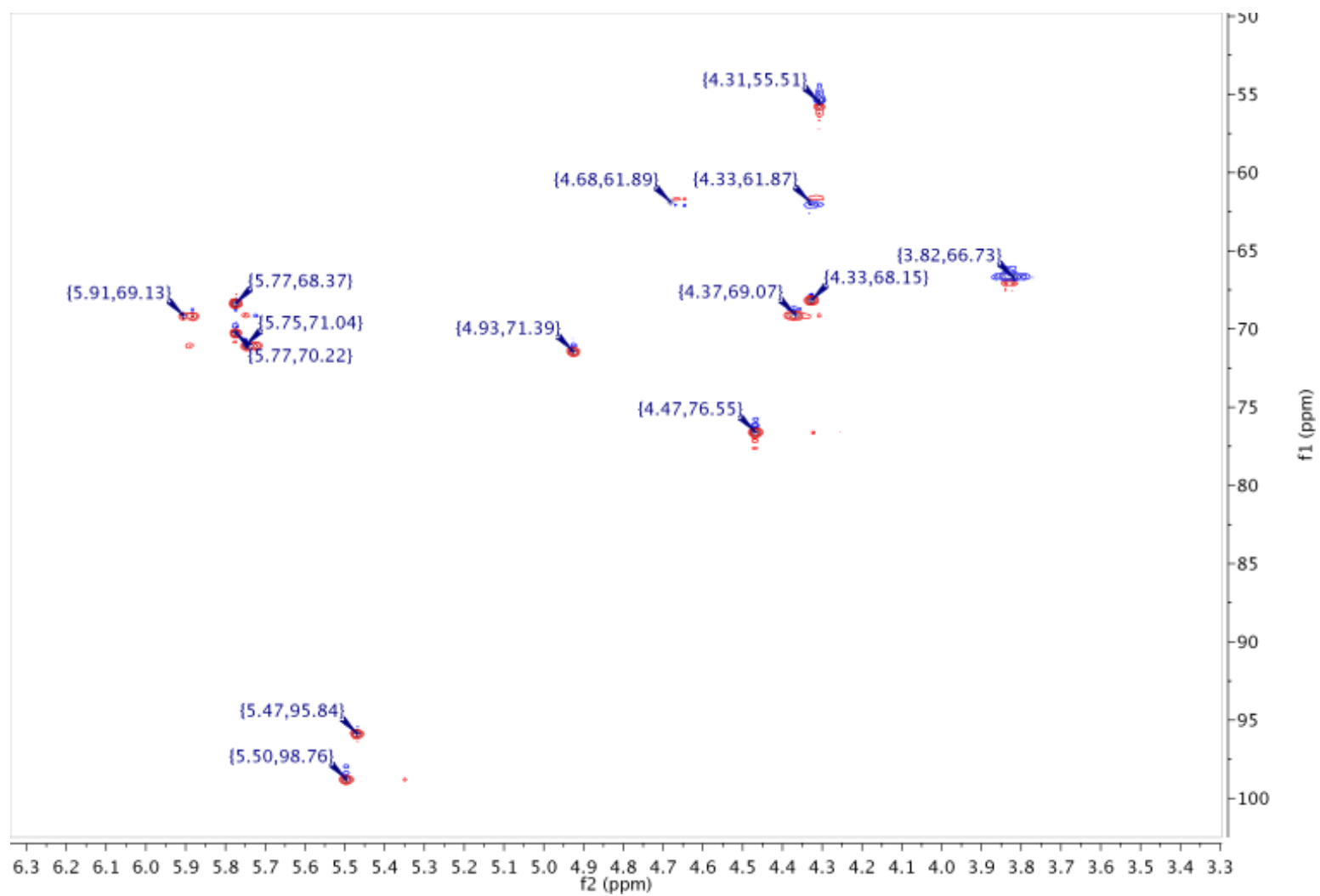
^1H NMR Propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**15**)



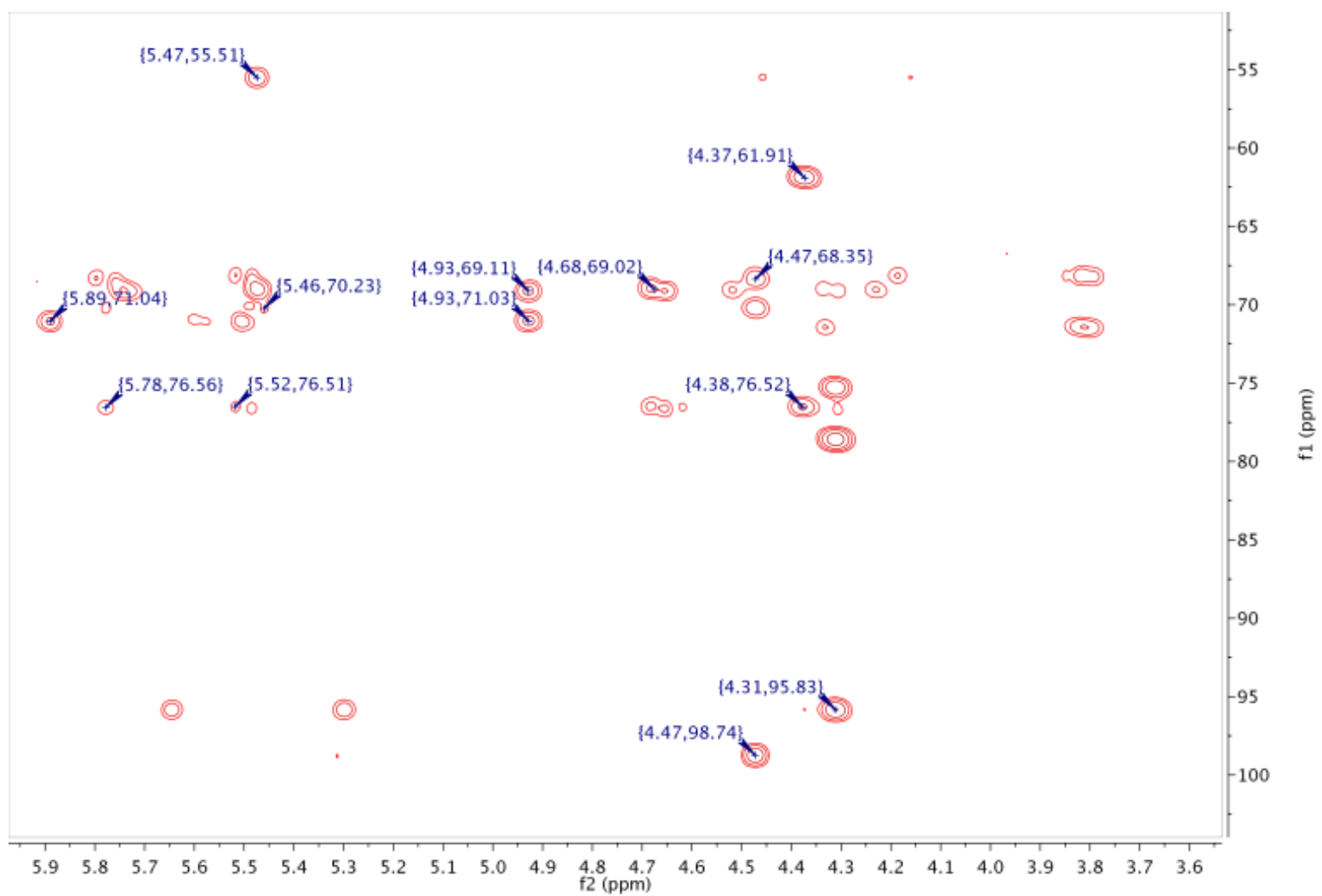
^{13}C NMR Propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**15**)



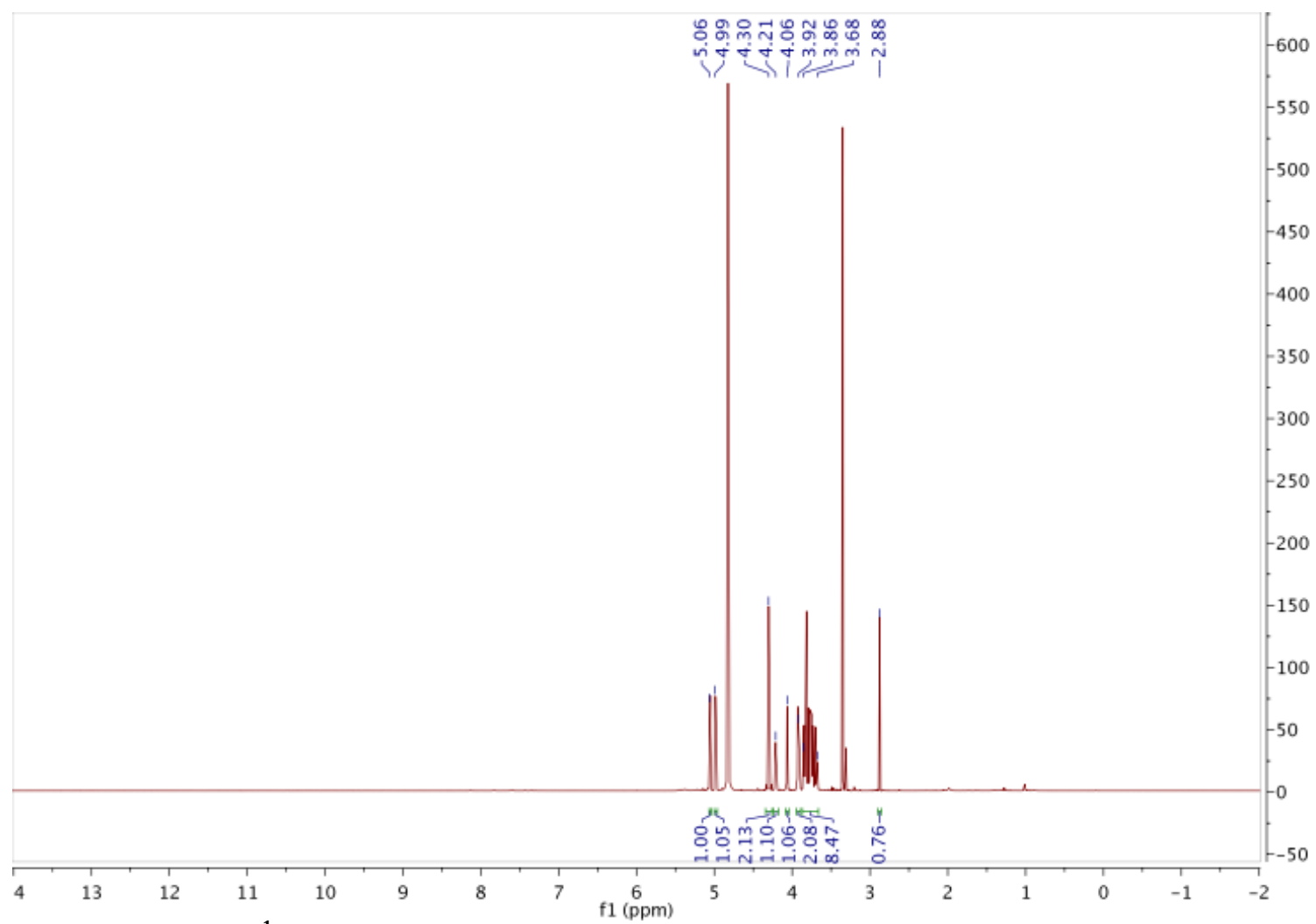
gCOSY Propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**15**)



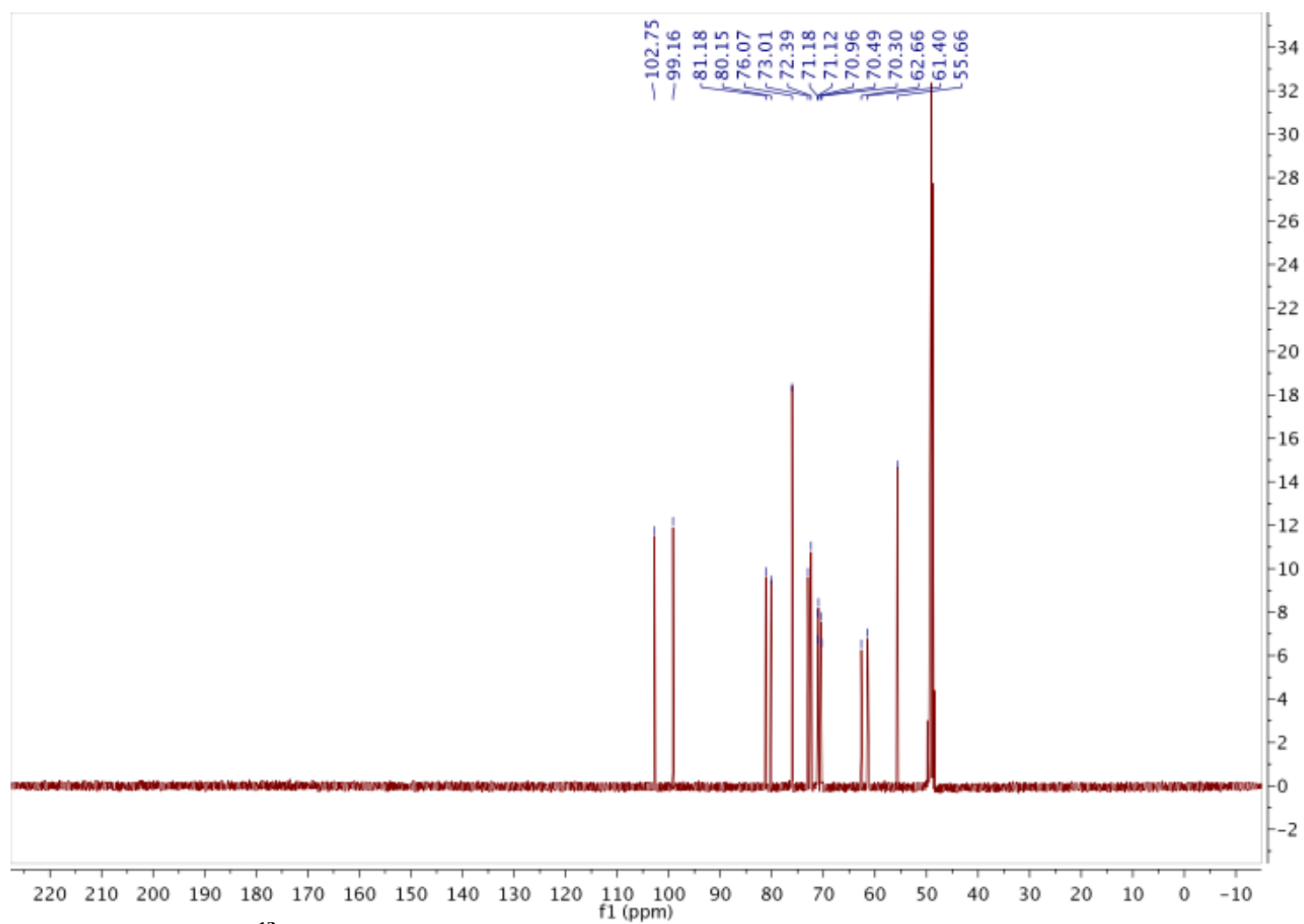
gHSQC Propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**15**)



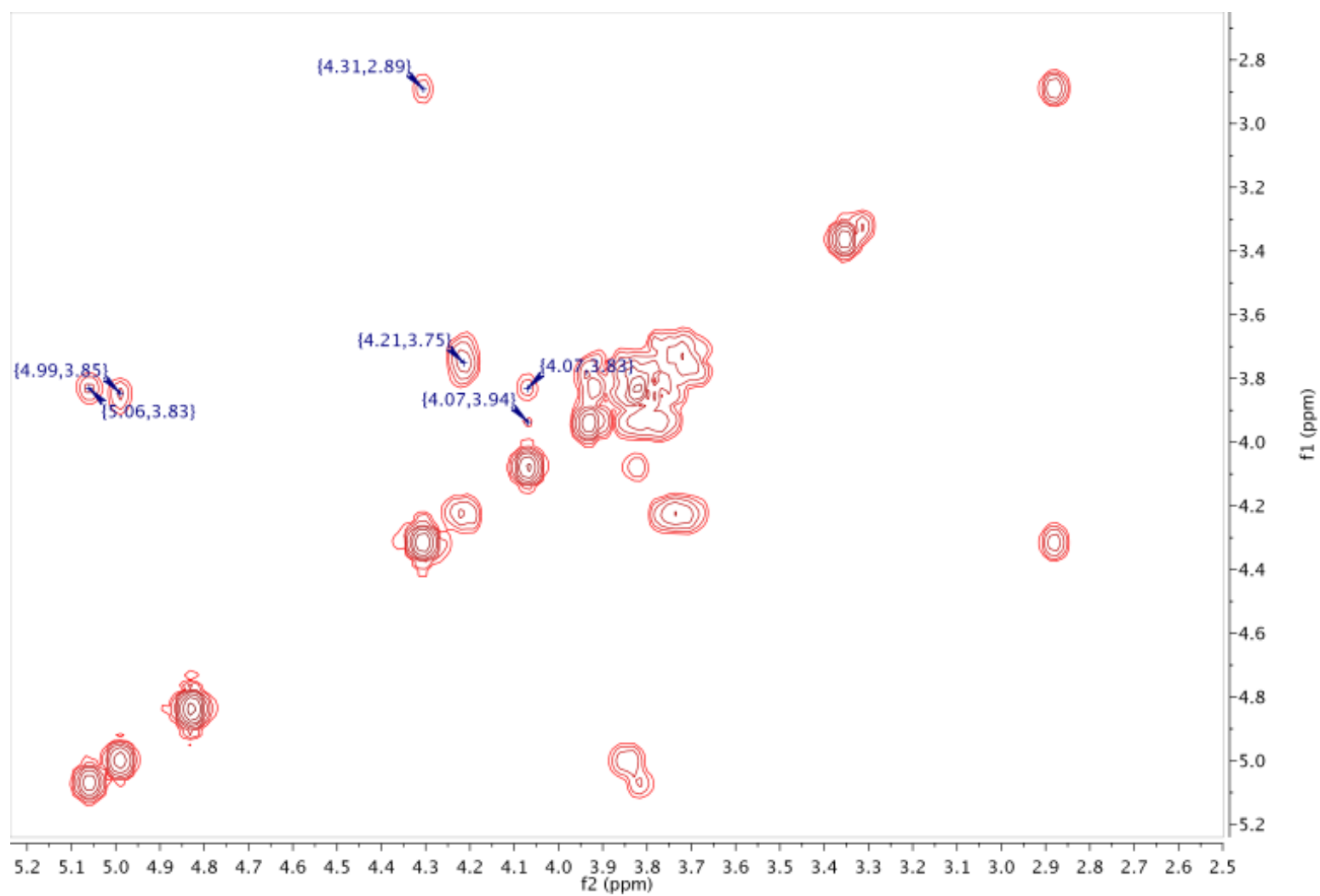
gHMBC Propargyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl)- α -D-galactopyranosyl)- α -D-galactopyranoside (**15**)



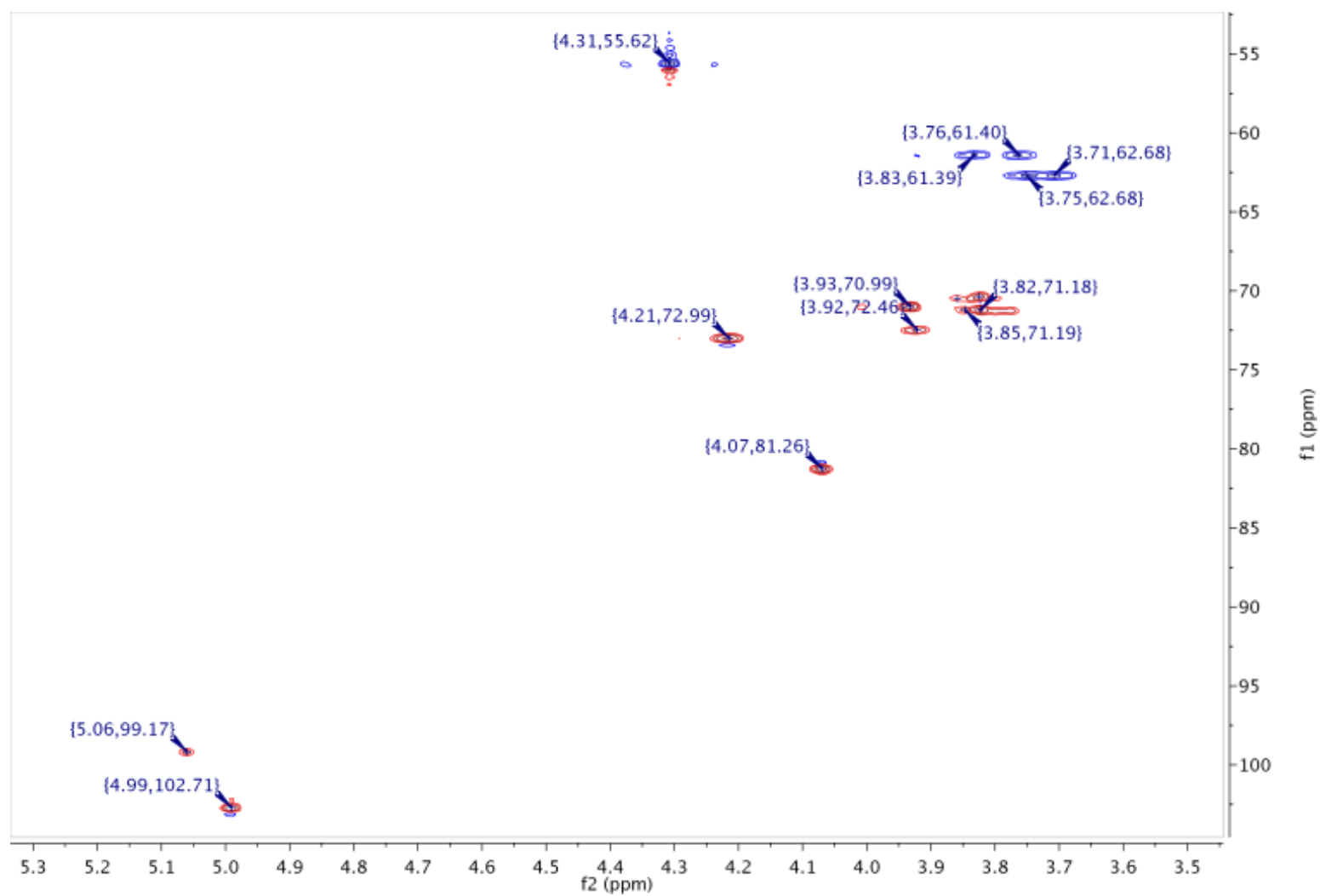
¹H NMR Propargyl 4-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (4)



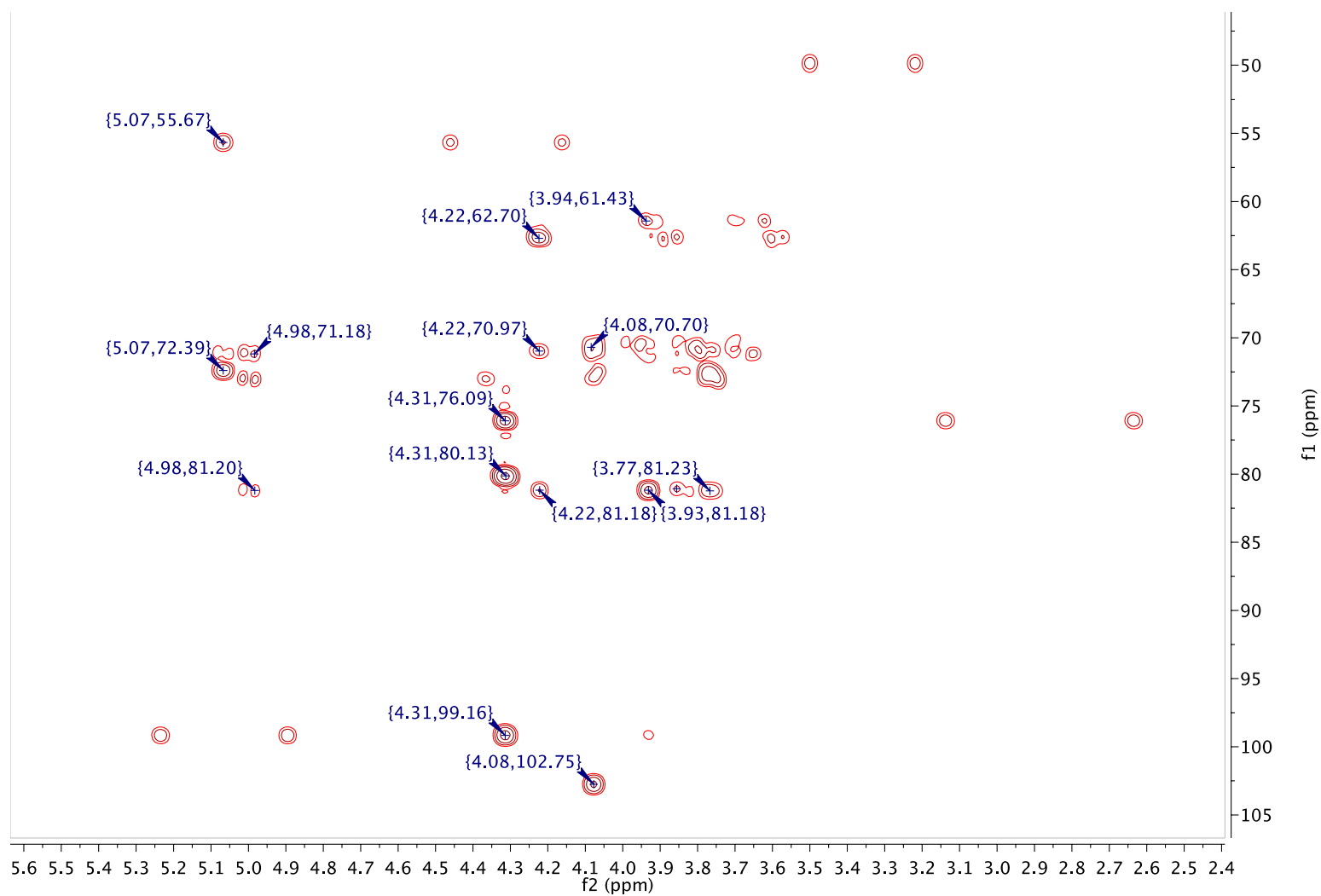
¹³C NMR Propargyl 4-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (4)



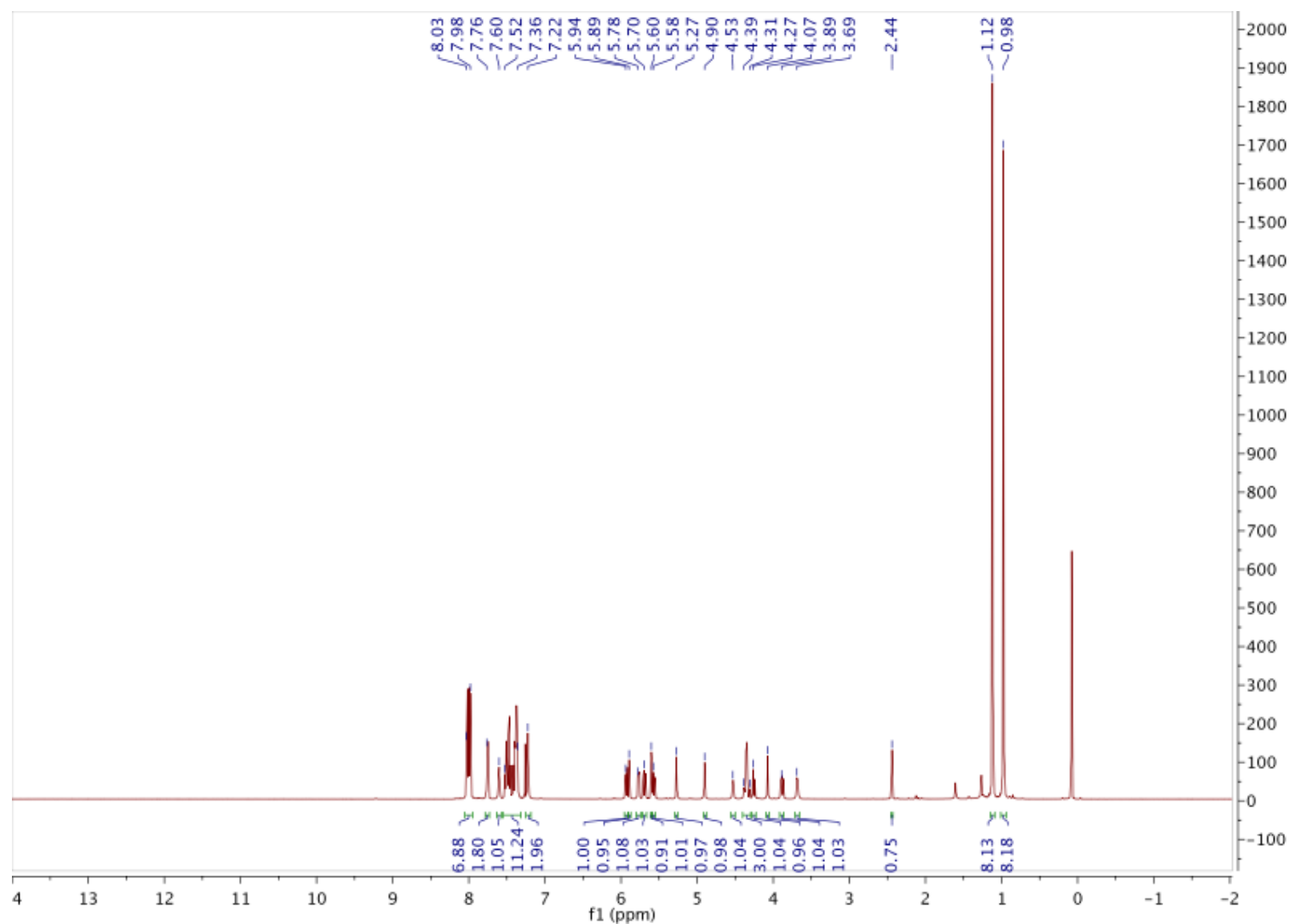
gCOSY NMR Propargyl 4-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (4)



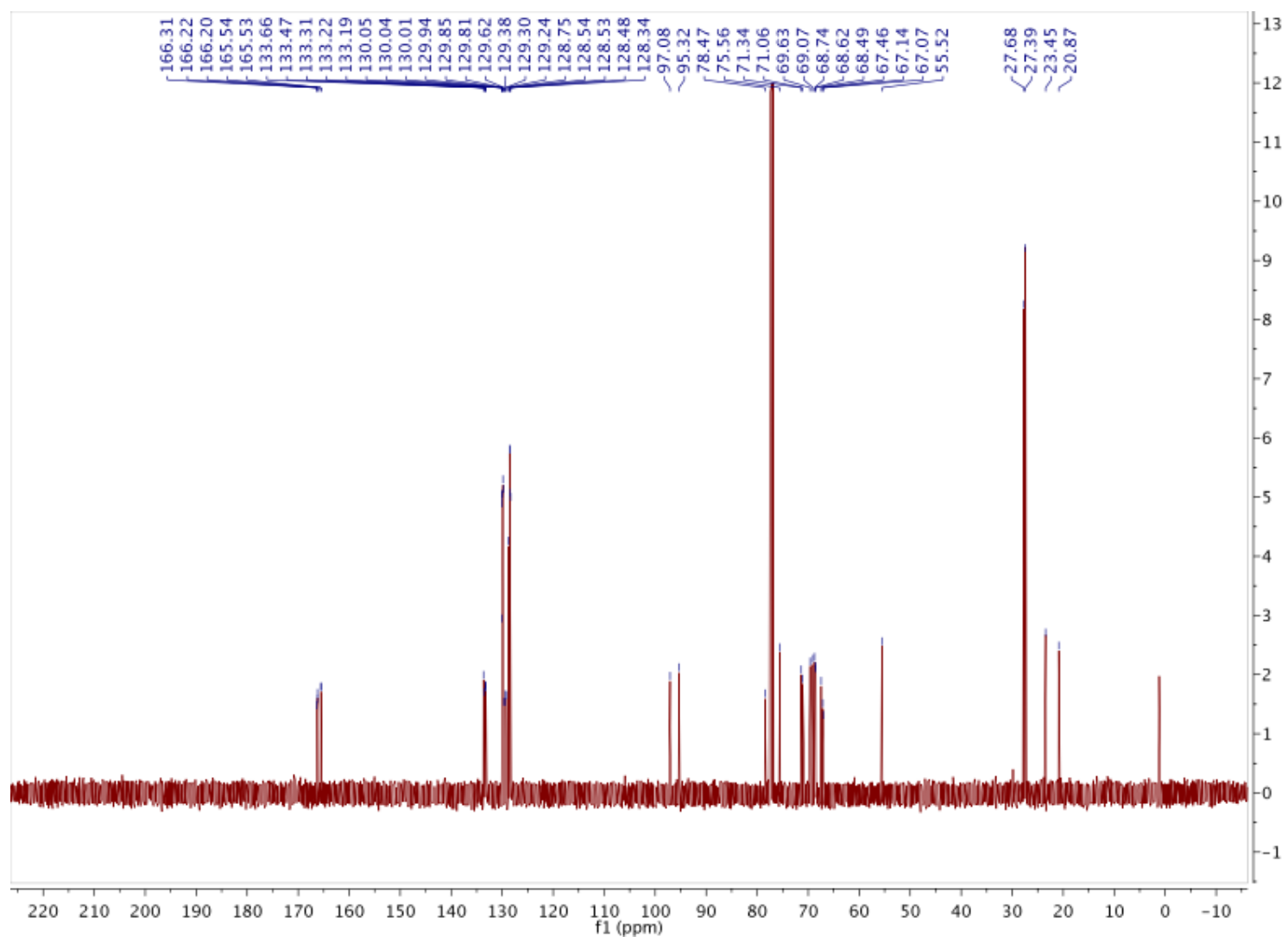
gHSQC NMR Propargyl 4-*O*-(α -D-galactopyranosyl)- α -D-galactopyranoside (**4**)



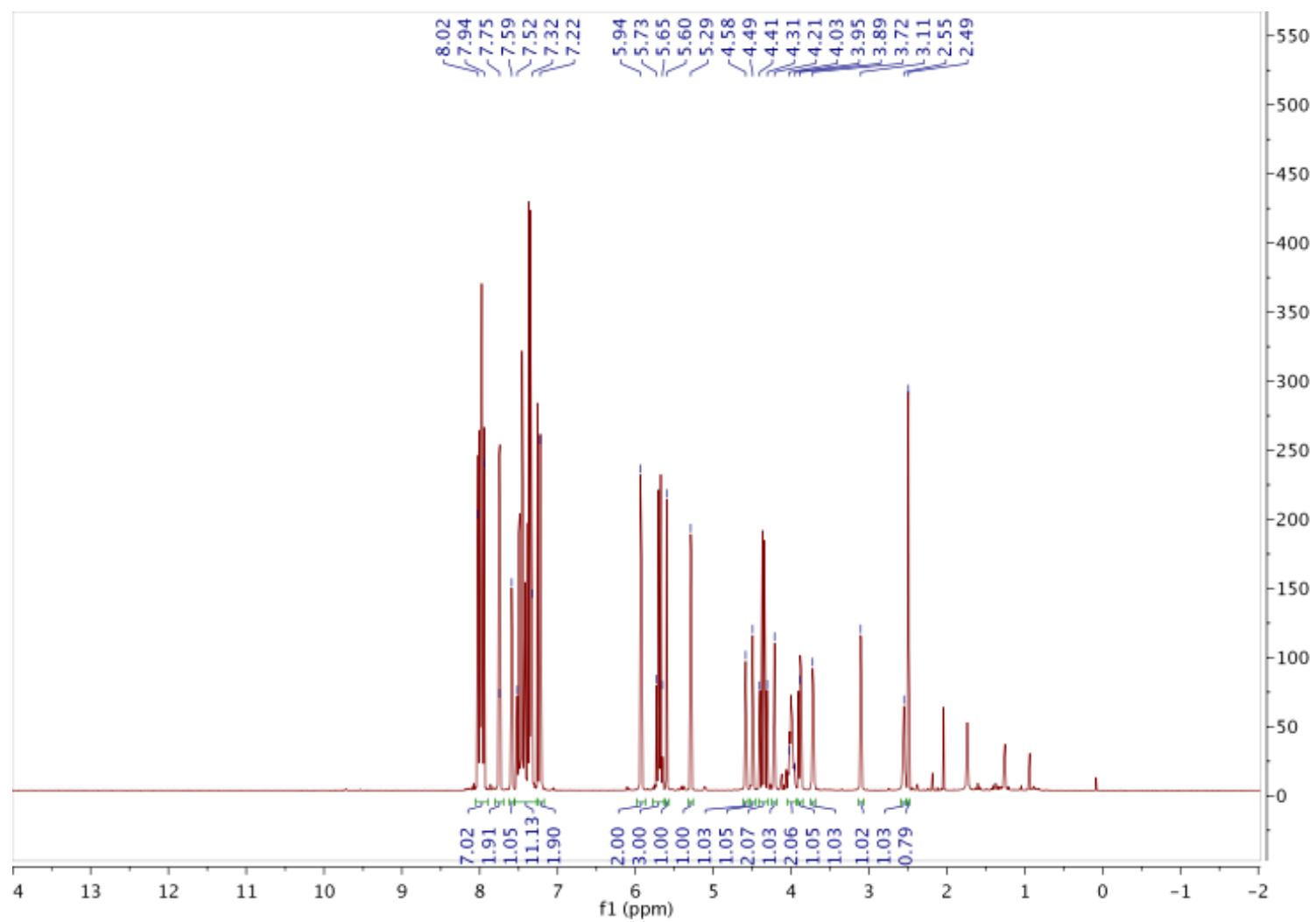
gHMBC NMR Propargyl 4-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (4)



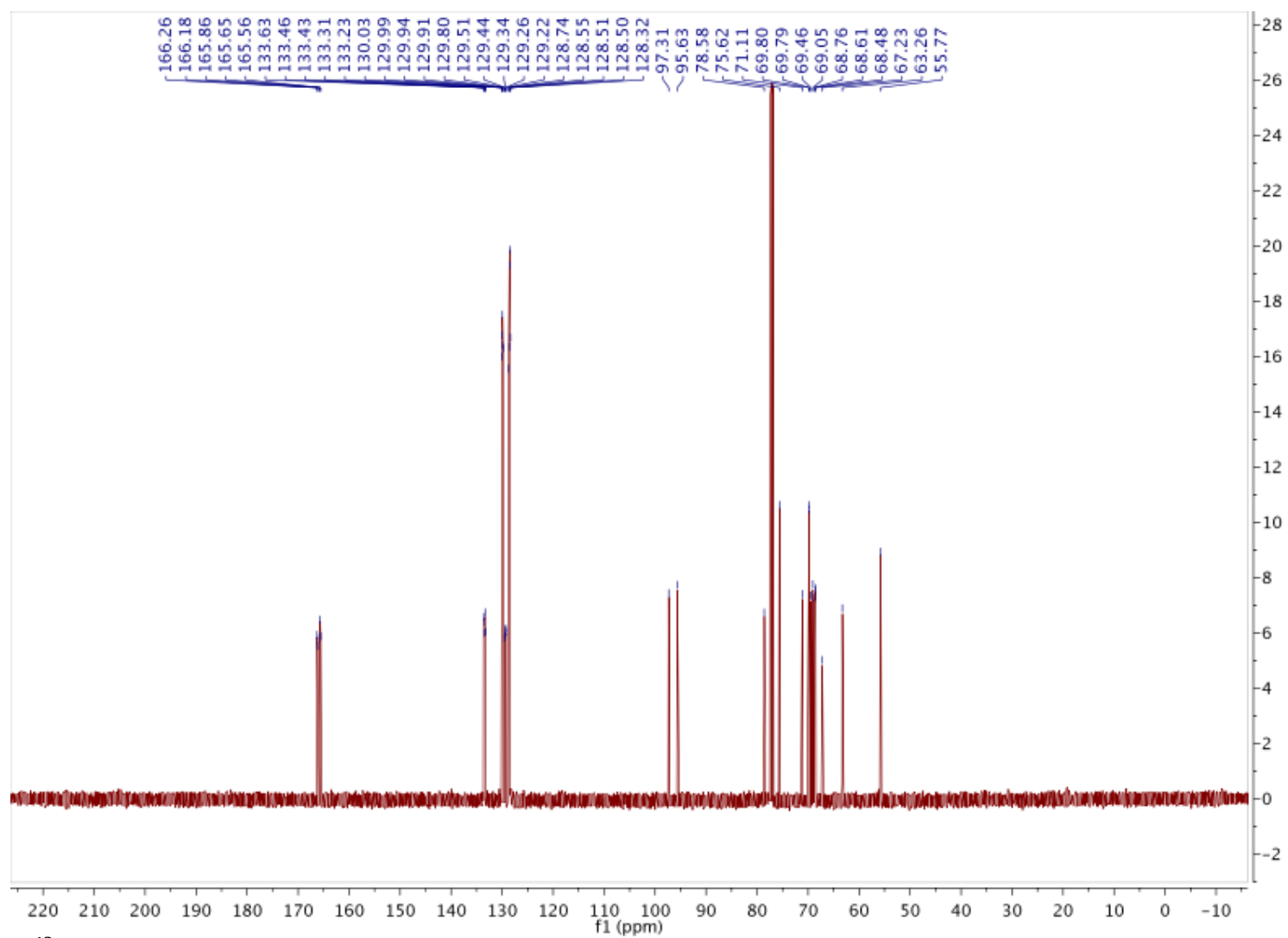
^1H NMR Propargyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilanediyl- α -D-galactopyranosyl)- α -D-galactopyranoside (**16**)



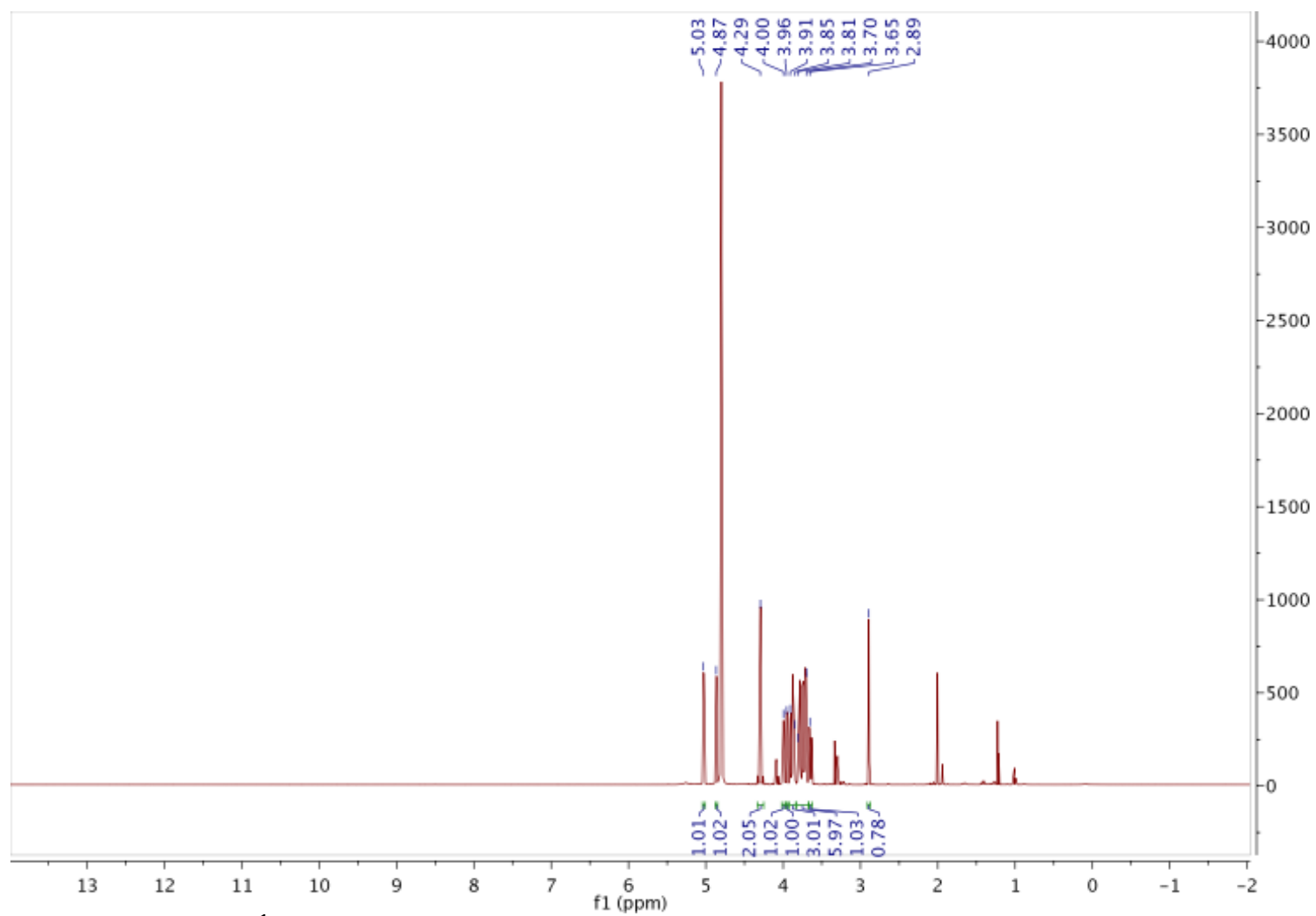
¹³C NMR Propargyl 2,3,4-tri-O-benzoyl-6-O-(2,3-di-O-benzoyl-4,6-O-di-tert-butylsilanediyl)-α-D-galactopyranosyl)-α-D-galactopyranoside (16)



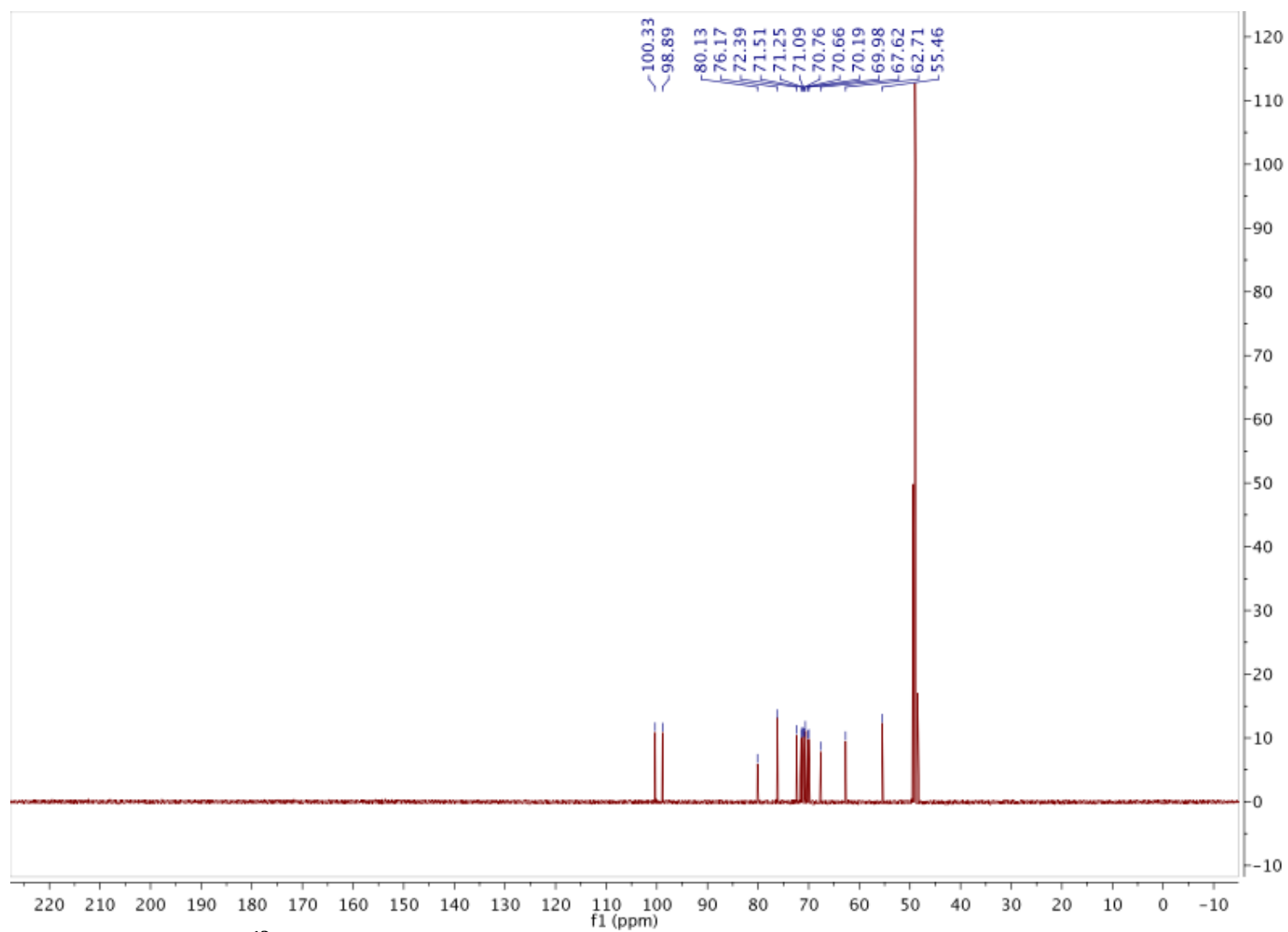
¹H NMR Propargyl 2,3,4-tri-O-benzoyl-6-O-(2,3-di-O-benzoyl-α-D-galactopyranosyl)-α-D-galactopyranoside (27)



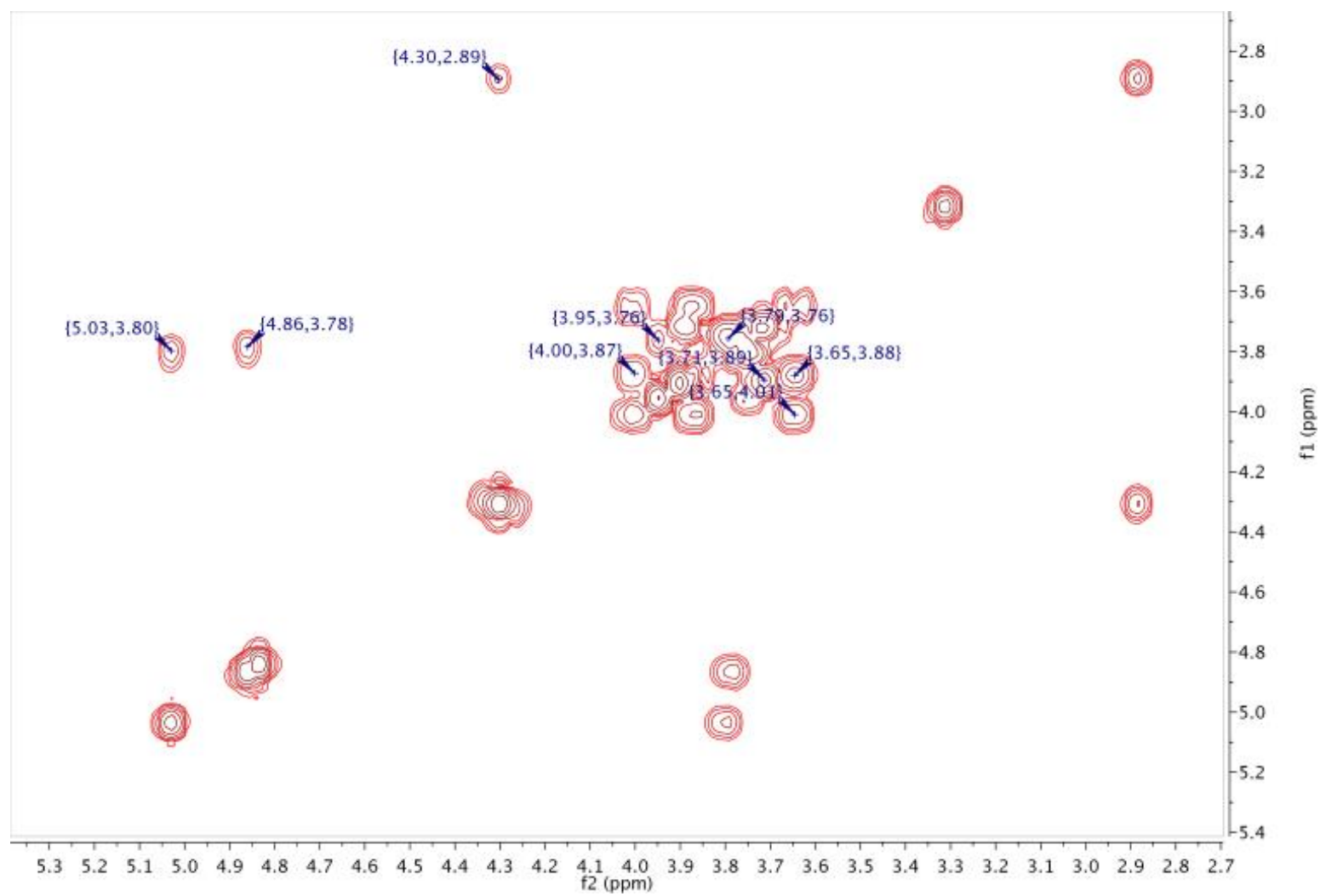
¹³C NMR Propargyl 2,3,4-tri-O-benzoyl-6-O-(2,3-di-O-benzoyl- α -D-galactopyranosyl)- α -D-galactopyranoside (27)



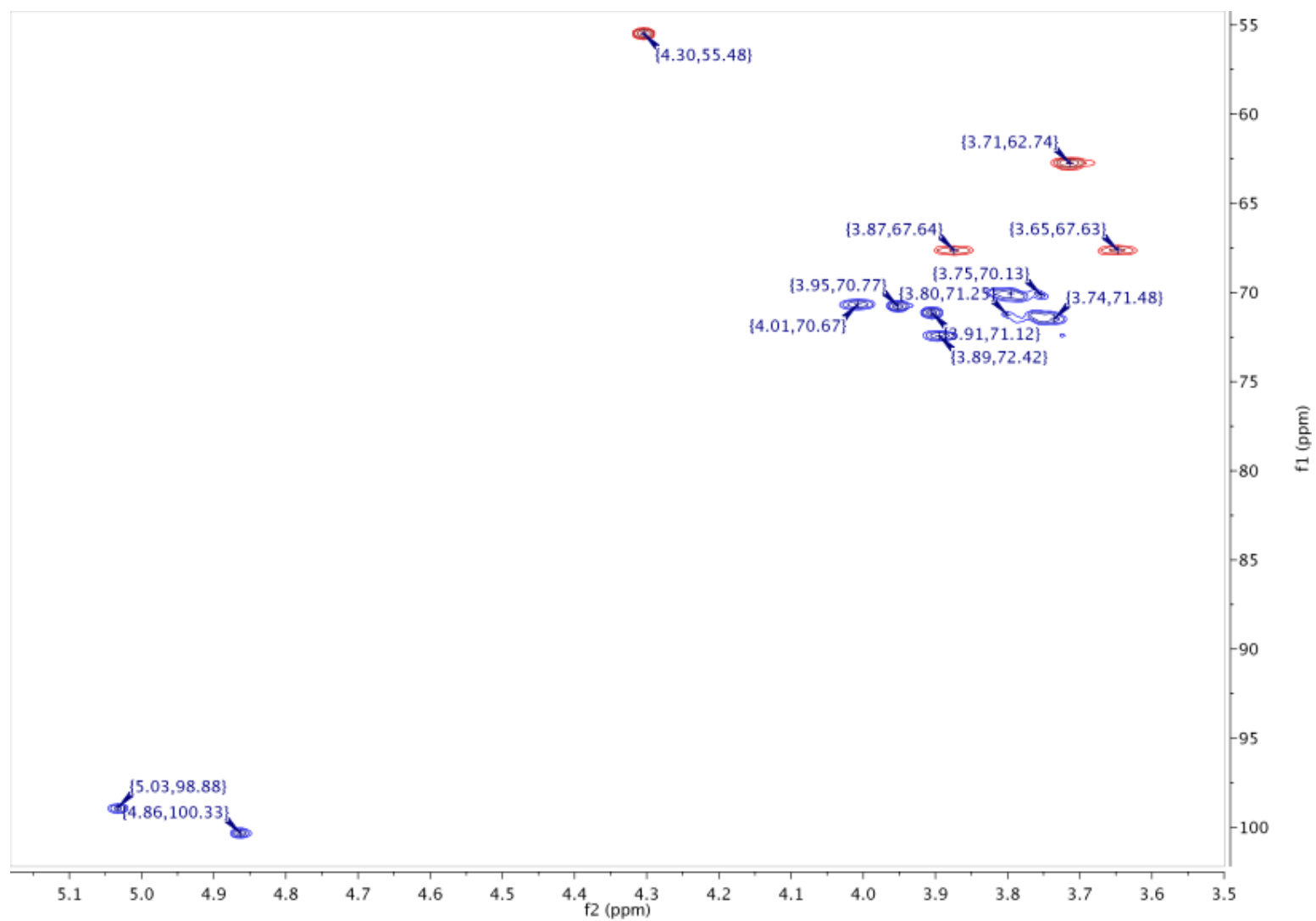
¹H NMR Propargyl 6-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (5)



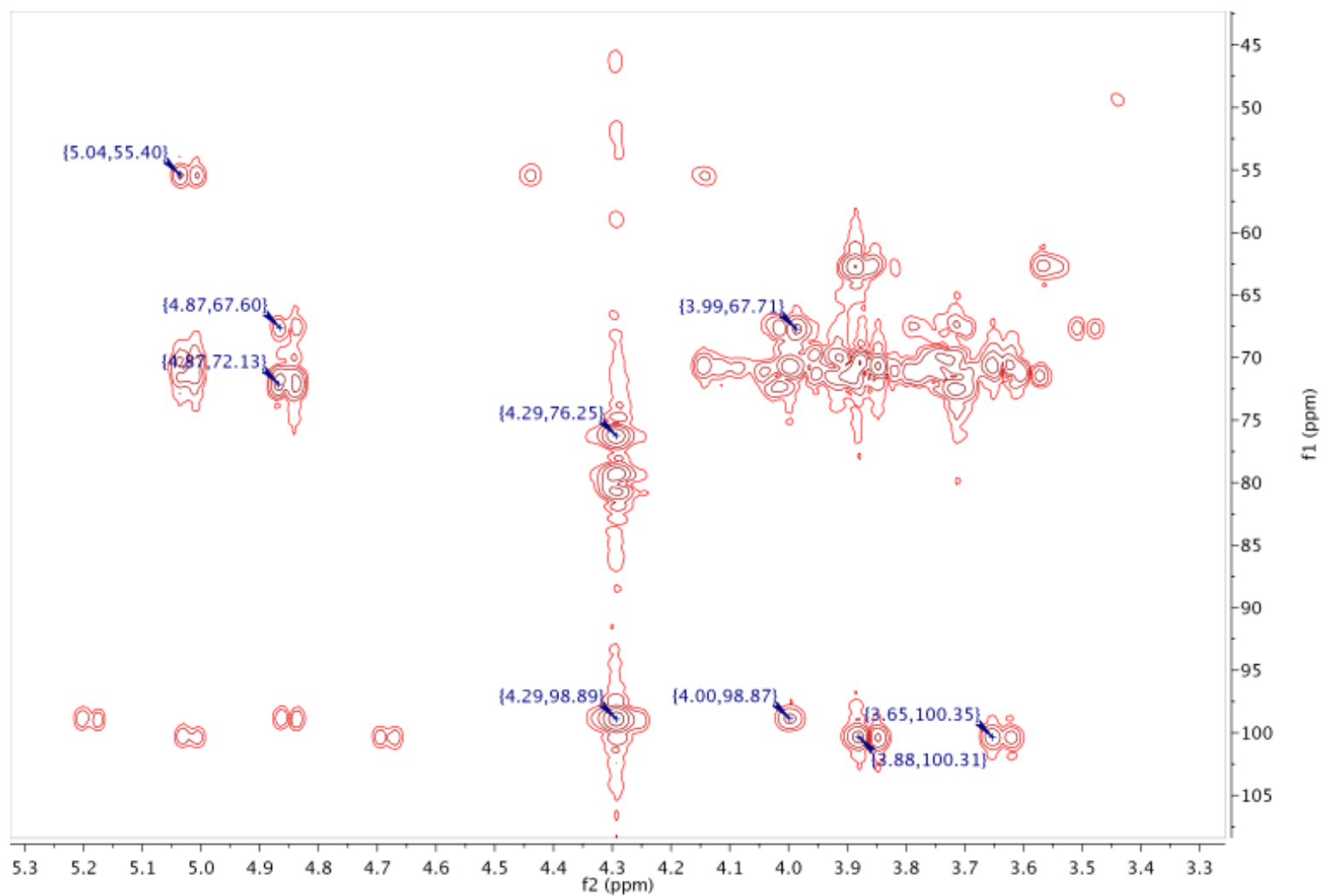
¹³C NMR Propargyl 6-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (5)



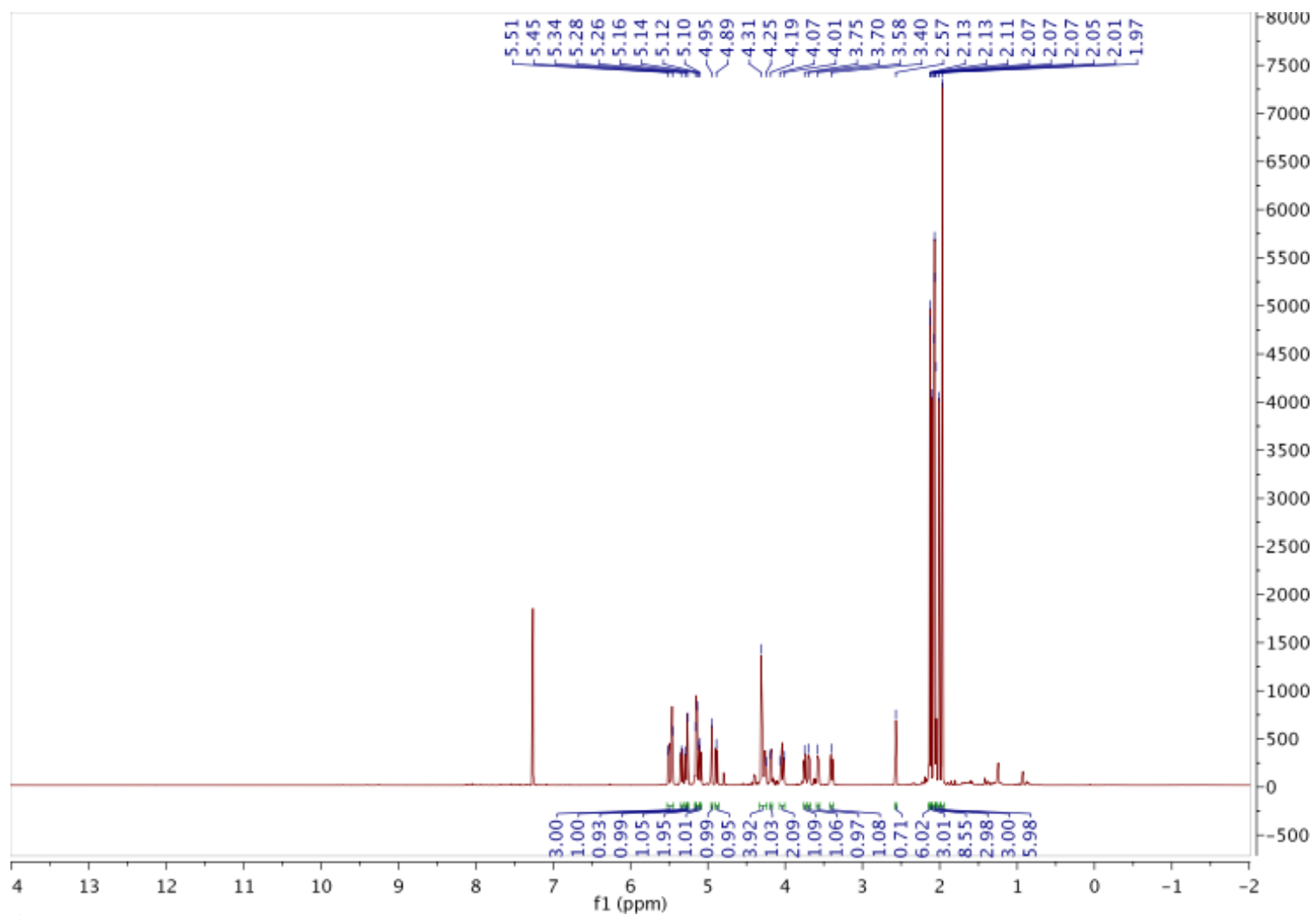
gCOSY NMR Propargyl 6-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (5)



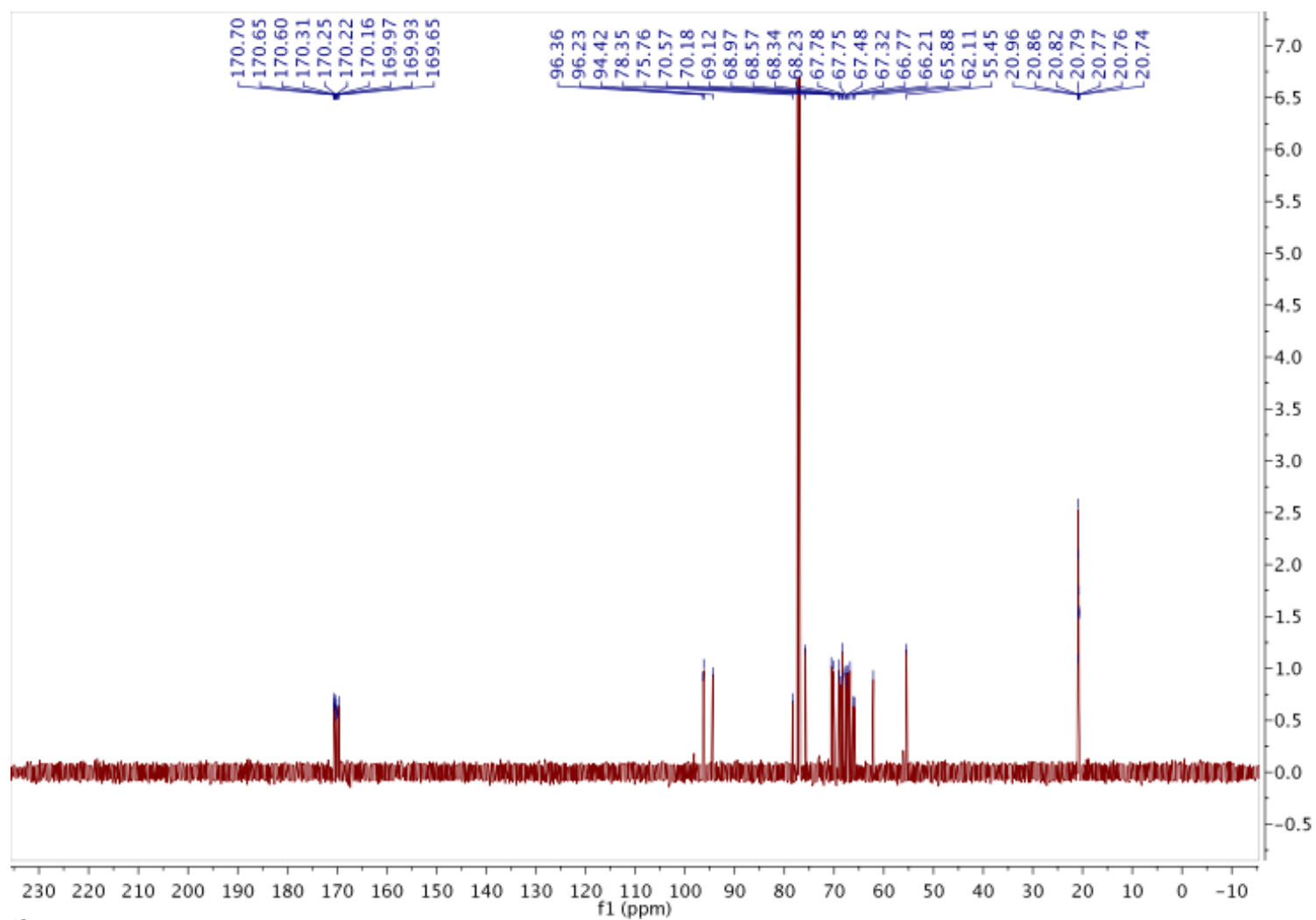
gHSQC NMR Propargyl 6-*O*-(α -D-galactopyranosyl)- α -D-galactopyranoside (**5**)



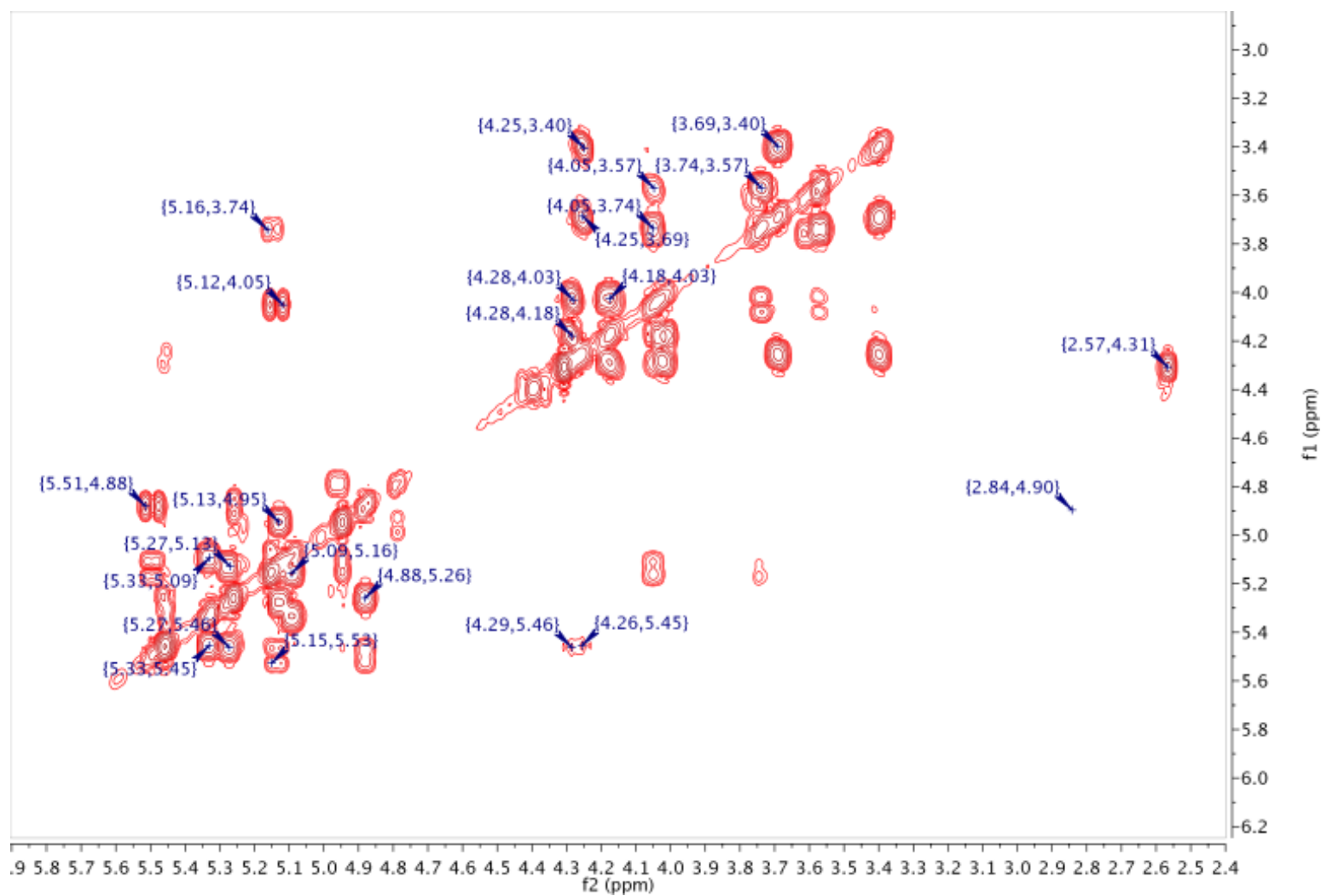
gHMBC NMR Propargyl 6-O-(α -D-galactopyranosyl)- α -D-galactopyranoside (5)



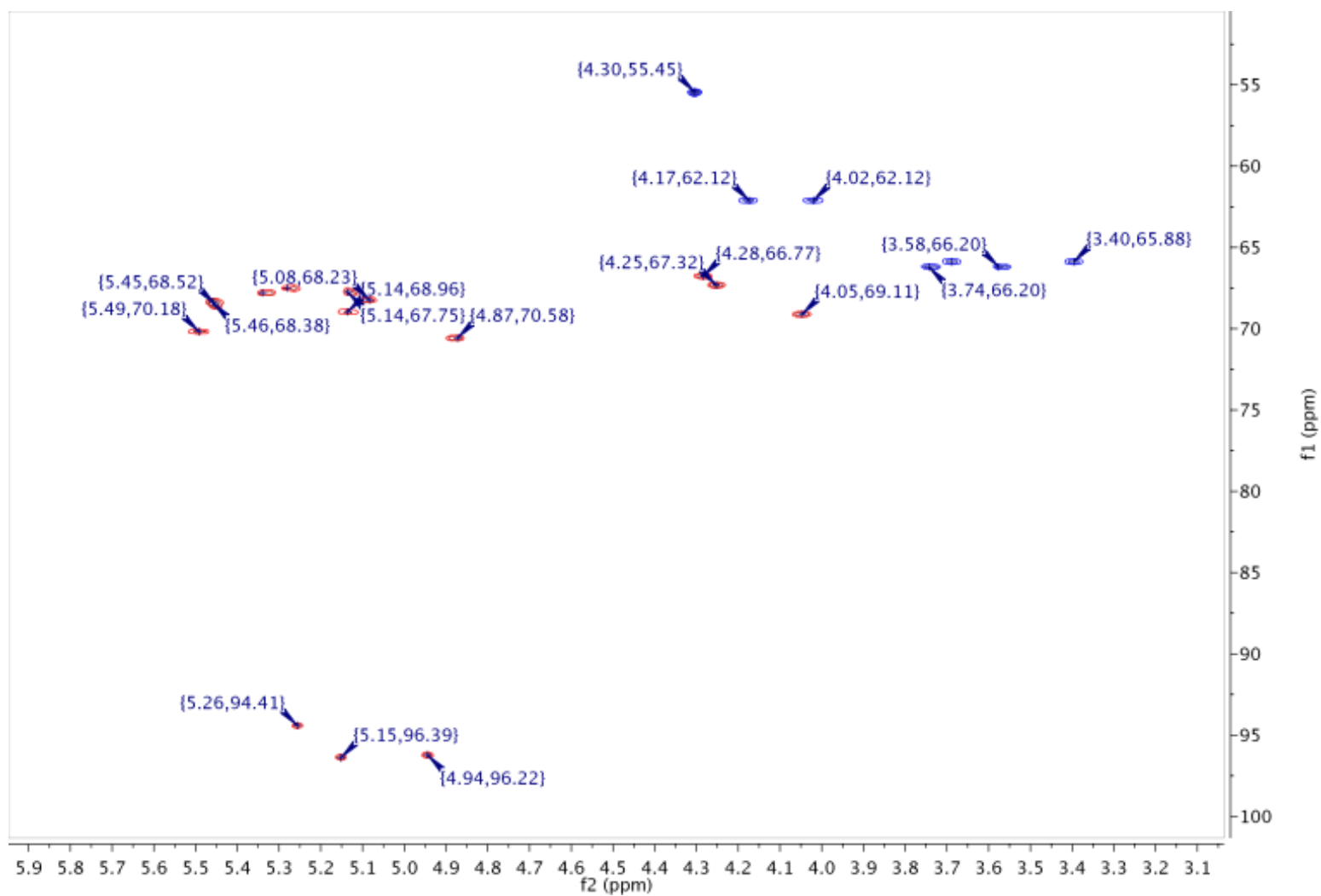
^1H NMR Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside (**19**)



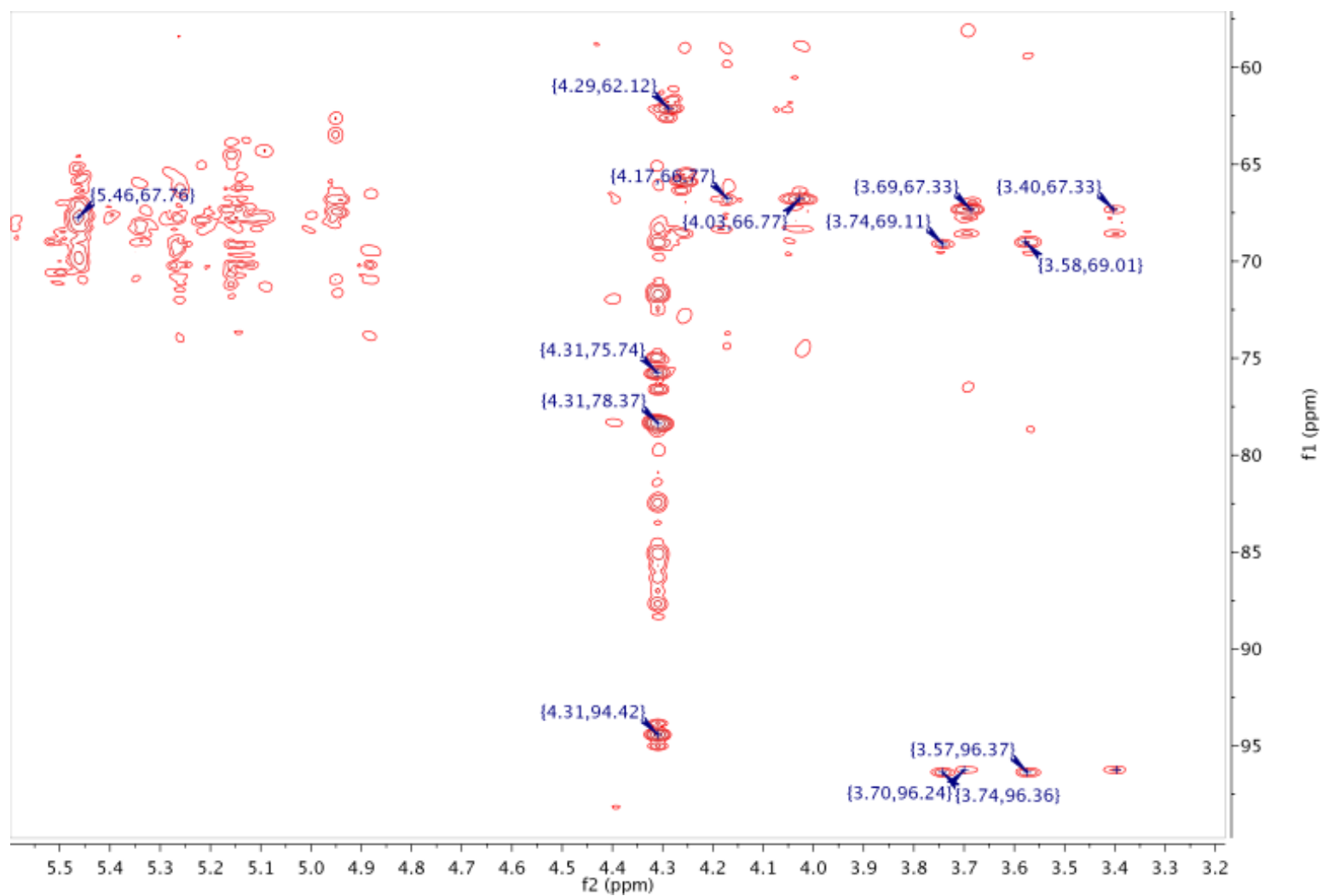
^{13}C NMR Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside (**19**)



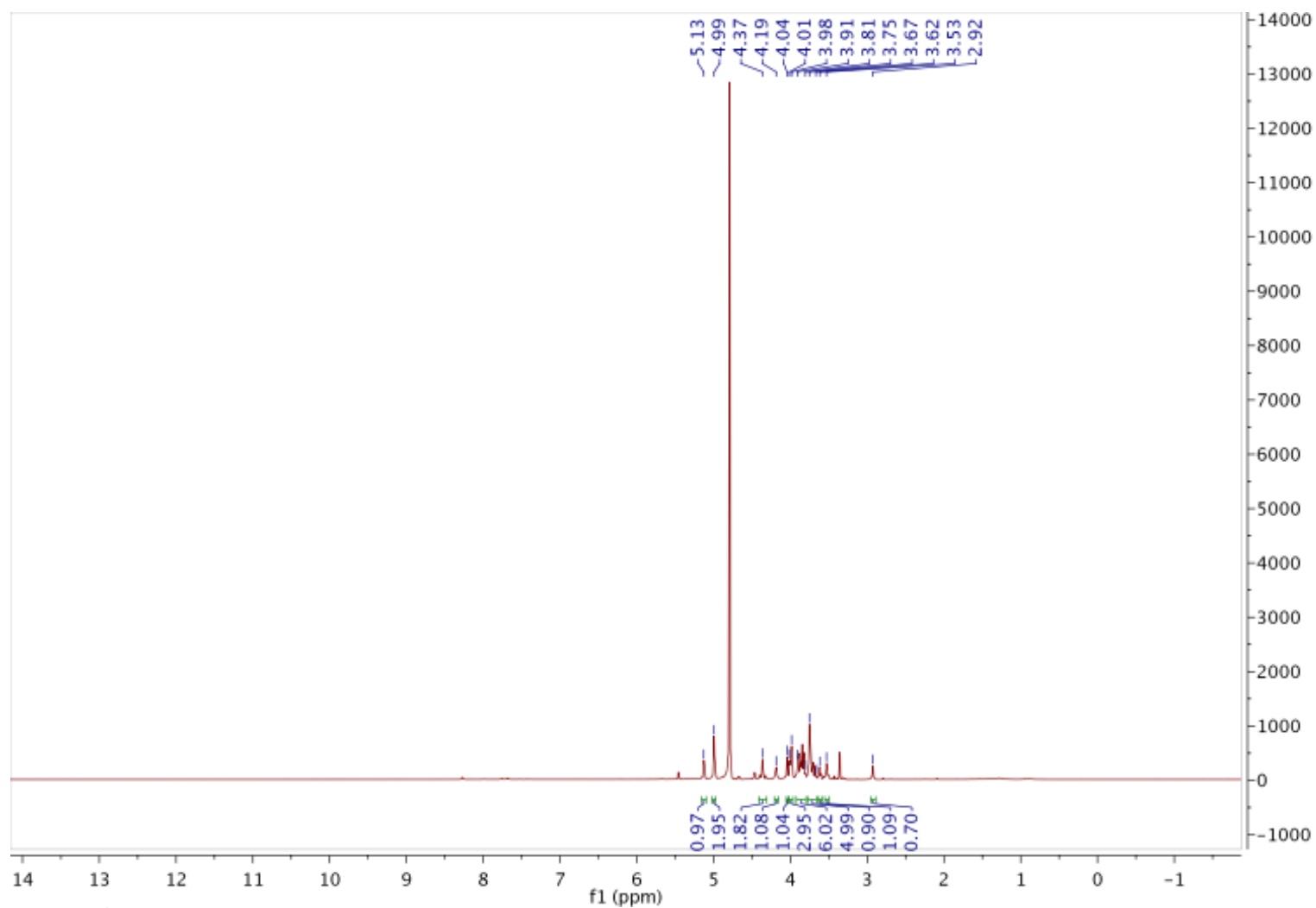
gCOSY NMR Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside (**19**)



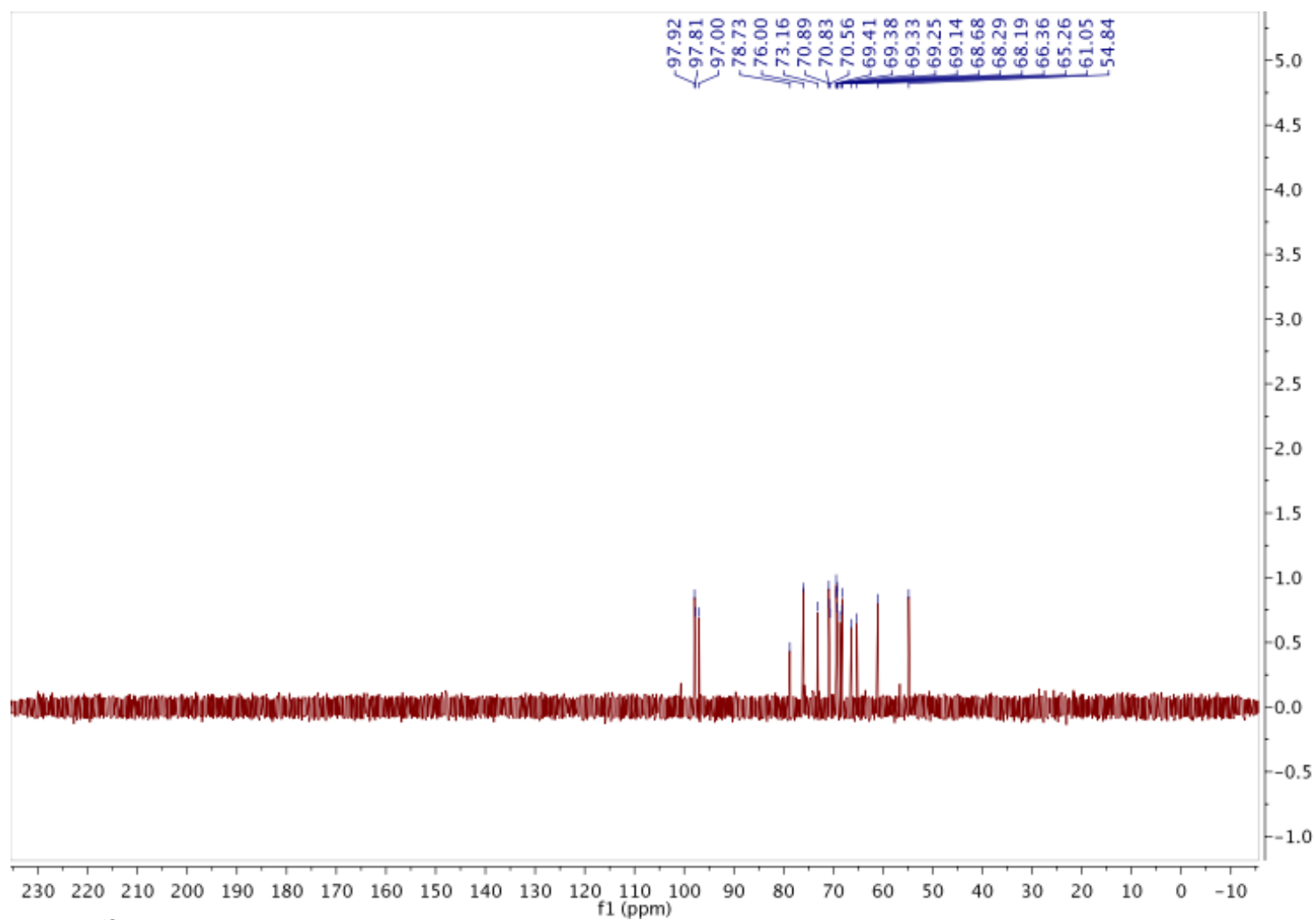
gHSQC NMR Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside (**19**)



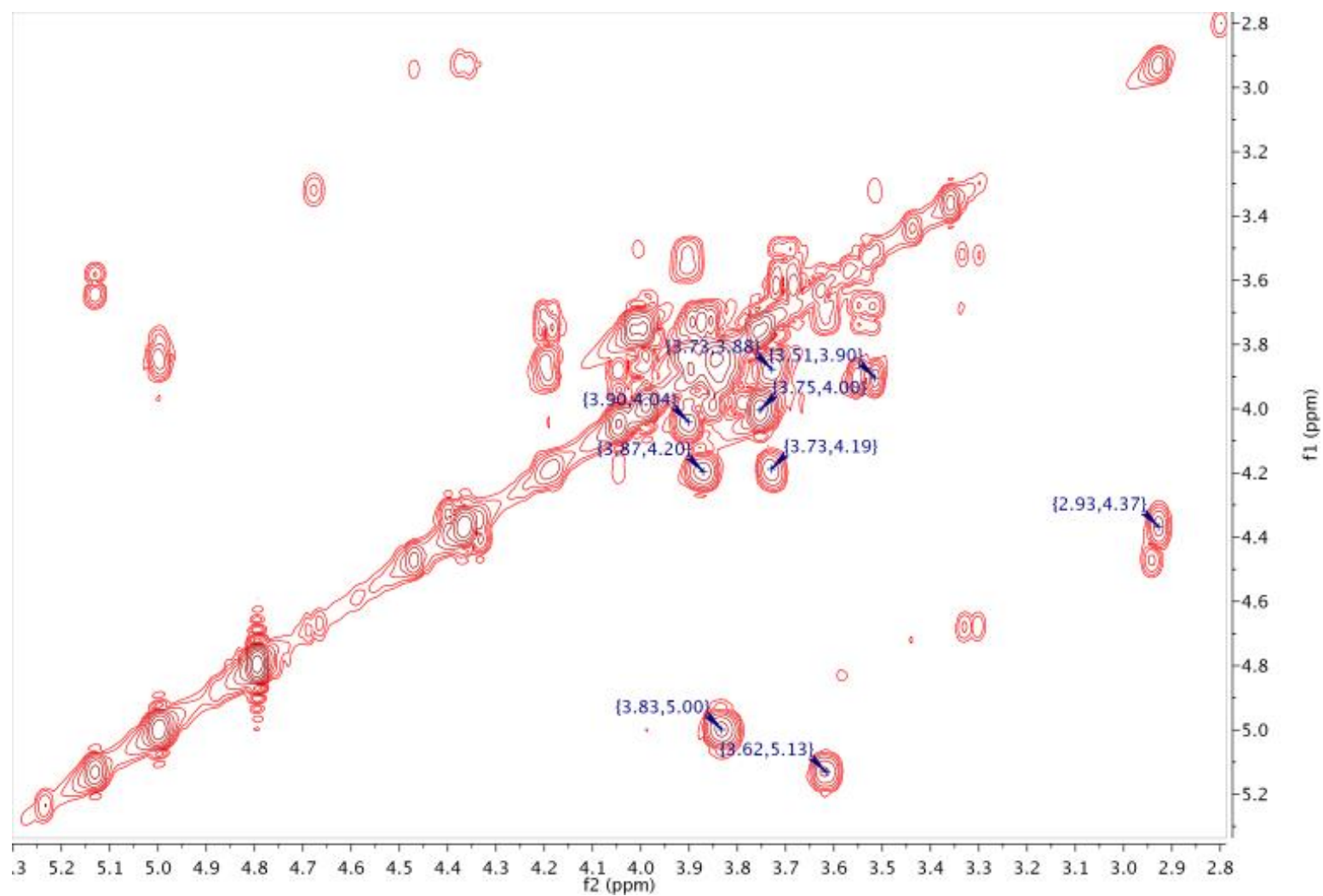
gHMBC NMR Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside)- α -D-galactopyranosyl)- α -D-galactopyranoside (**19**)



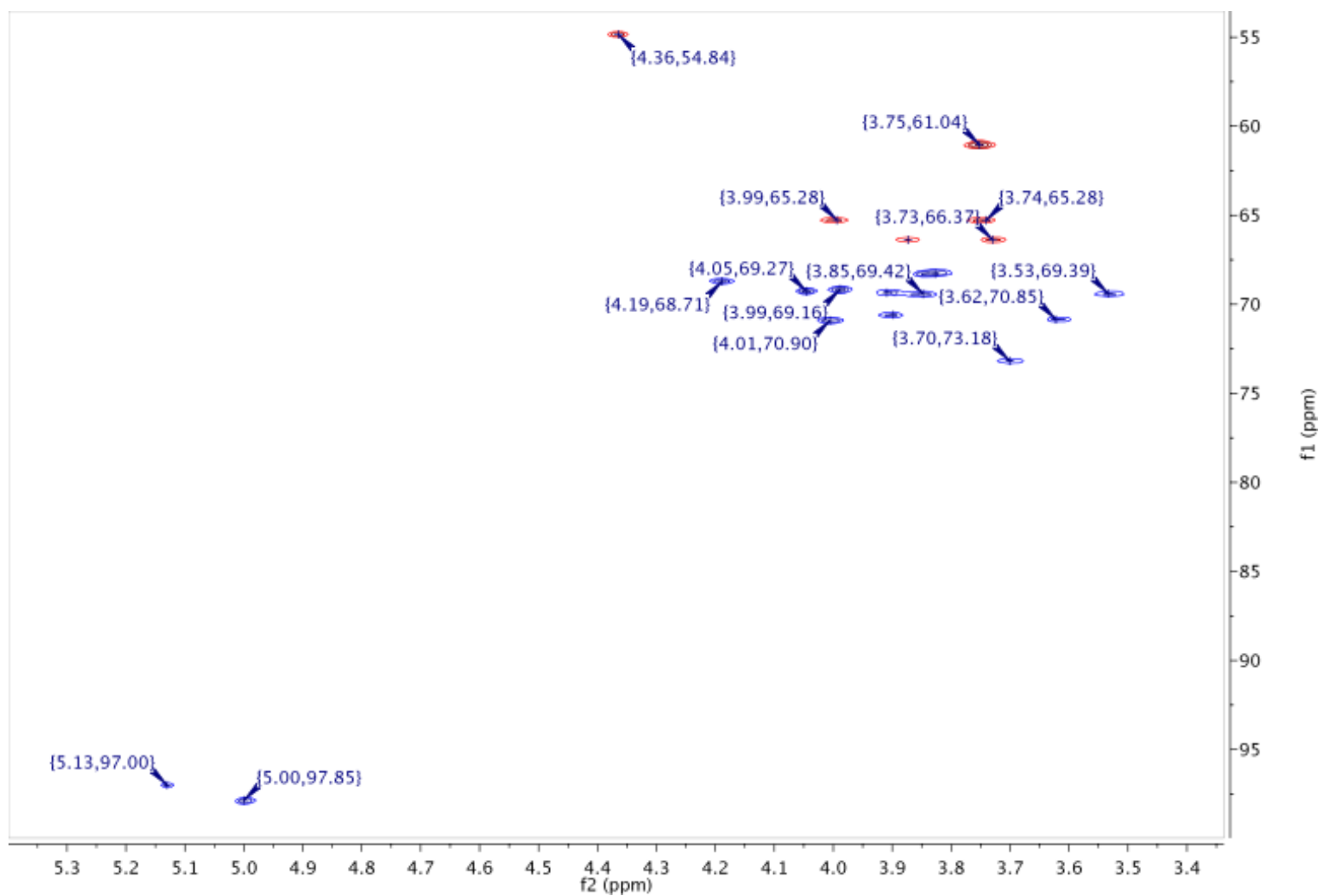
¹H NMR Propargyl 6-O-(6-O- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside (**6**)



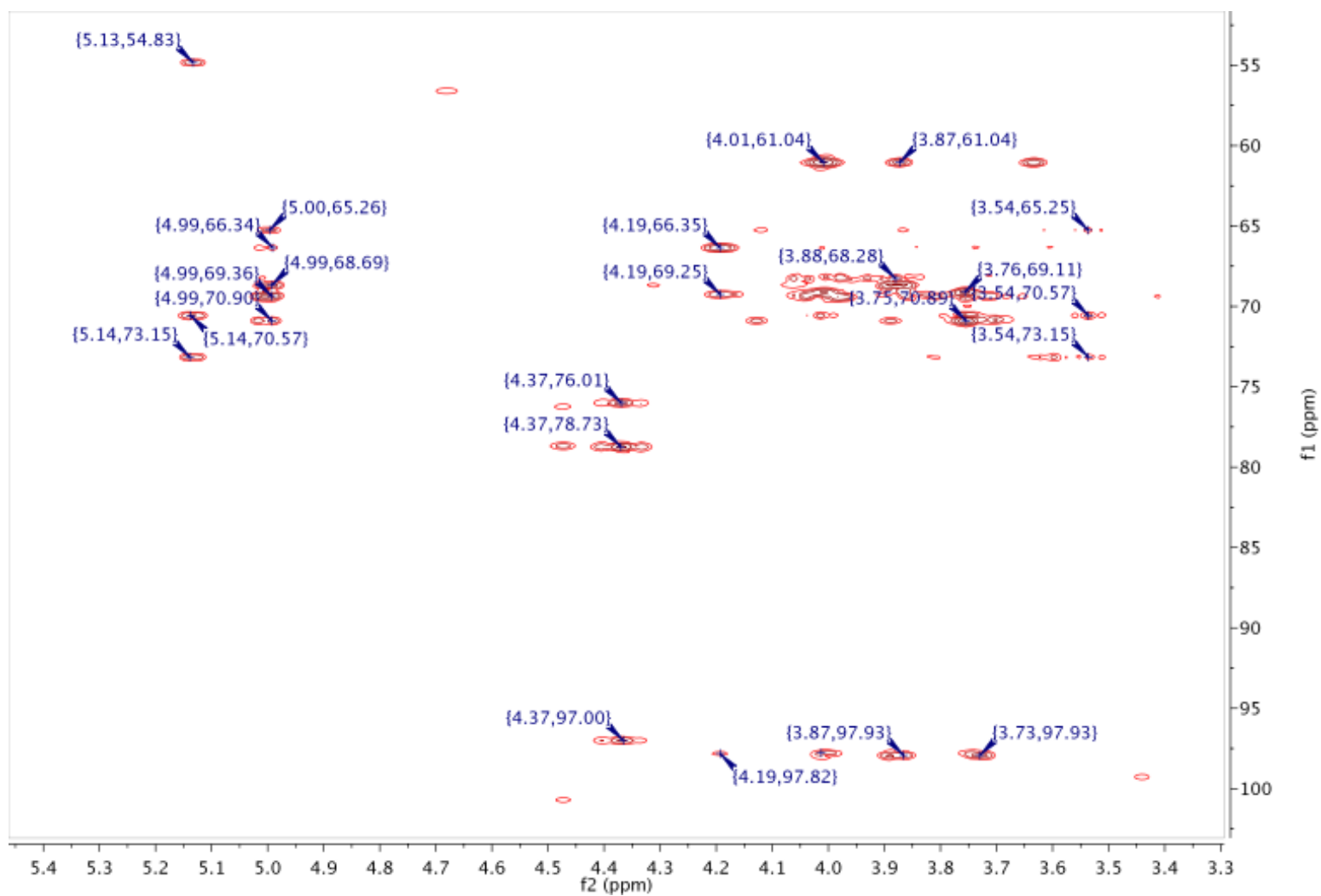
^{13}C NMR Propargyl 6-*O*-(6-*O*- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside (**6**)



gCOSY NMR Propargyl 6-*O*-(6-*O*- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside (**6**)



gHSQC NMR Propargyl 6-*O*-(6-*O*- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside (**6**)



gHMBC NMR Propargyl 6-*O*-(6-*O*- α -D-galactopyranoside- α -D-galactopyranosyl)- α -D-galactopyranoside (**6**)

Vita

Bo Meng was born in Taiyuan, China. After graduation from the Affiliated High School of Shanxi University, he attended Beijing Institute of Technology in 2006, where he received his B.E. degree in Chemical Engineering & Technology. He was enrolled as a graduate student in Department of Chemistry at the University of Tennessee, Knoxville in 2010, and joined Professor David. C. Baker's group. His PhD work mainly focused on the development of synthetic methodologies of 1,2-*cis*-glycosides and quantitative evaluation of carbohydrate–lectin binding properties. Bo Meng received a Doctor of Philosophy Degree in Organic Chemistry from the University of Tennessee in August 2015.