STRATEGIES FOR THE SYNTHESIS OF NOVEL GLYCOCONJUGATES

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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BY

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Dedicated to…..

My Parents

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CERTIFICATE

Certified that, the work incorporated in the thesis entitled, "***Strategies for the Synthesis of Novel Glycoconjugates***" submitted by Venkateswararao Boddu was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institution.

Date: 15th, July 2016
Pune (MH), India.

Dr. Srinivas Hotha

DECLARATION

I declare that this written submission represents my ideas in my own words and where others‟ ideas have been included; I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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General Remarks

- ^{1}H NMR spectra were recorded on AV-200 MHz, AV-400 MHz, JEOL ECX 400 MHz and DRX-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C NMR spectra were recorded on AV-50 MHz, AV-100 MHz, JEOL ECX 100 MHz and DRX-125 MHz spectrometer.
- High resolution mass spectroscopy (HRMS) was performed on Waters Synapt G2 and Maldi-TOF.
- Low resolution mass spectroscopy (LRMS) was performed on Waters Acquity UPLC-MS (H Class).
- Infrared spectra were scanned on Perkin-Elmer 1310 and Perkin-Elmer 1600 FT-IR spectrometers with sodium chloride optics and are measured in cm^{-1}.
- Optical rotations were measured with a JASCO P-2000 digital polarimeter.
- All reactions are monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV light, I_2, and anisaldehyde in ethanol as developing agents.
- All reactions were carried out under nitrogen or argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise specified.
- All evaporations were carried out under reduced pressure on Büchi and Heildoph rotary evaporators below 45 °C unless otherwise specified.
- All gold and transition metal salts were purchased from multinational commercial vendors.
- Materials were obtained from commercial suppliers and were used without further purification.
- Silica gel (60–120), (100-200), and (230-400) mesh were used for column chromatography.
- Scheme, Figure and Compound numbers in abstract and individual chapters are different.

Ac	Acetyl
Ac_2O	Acetic anhydride
AcOH	Acetic acid
aq	Aqueous
Boc	*tert*-Butoxycarbonyl
Bn	Benzyl
BnBr	Benzyl bromide
BnCl	Benzyl chloride
BSTFA	*N,O*-Bistrifluoroacetamide
Bz	Benzoyl
BzCl	Benzoyl chloride
Cat	Catalytic
Conc	Concentrated
CuAAC	Copper catalyzed Alkyne Azide Cycloaddition
Cbz	Carbobenzoloxy
DBU	1,8-Diazabicycloundec-7-ene
DIPEA	N,N-Diisopropylethylamine
DMAP	N,N-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl (methylthio) sulfonium triflate
DEPT	Distortionless Enhancement by Polarization Transfer
eq	Equivalents
Fmoc	9-Flourenylmethoxy Carbonyl
g	Gram
H	Hour
Hz	Hertz
iEDDA	Inverse electron demand Diels–Alder

J	Coupling constant
NIS	*N*-Iodosuccinimide
mL	Millilitre
mol	Mole
mmol	Millimole
Me	Methyl
MeOH	Methanol
4ÅMS	4Å Molecualar sieves
MeOTf	Methyl triflate
mg	Milligram
min	Minutes
NMR	Nuclear Magnetic Resonance
PTSA, TsOH	*para*-Tolune sulphonic acid
rt	Room temperature
TBAI	Tetra-*n*-butylammonium iodide
TESOTf	Triethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
TfOH	Trifluoromethane sulphonic acid
TLC	Thin Layer Chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Ts	Tosyl

Synopsis

The thesis entitled, "***Strategies for the Synthesis of Novel Glycoconjugates***" is divided into three chapters. Chapter one gives introduction to the available bioconjugation methods in the literature. Chapter two describes the synthesis of various *pseudo*-saccharide conjugates such as pyrimidine nucleosides, thiosaccharides and thiotrehaloses utilizing concepts from gold-catalyzed glycosylation repertoire on designer mono-saccharide building blocks. Chapter two is further divided into two sections. Section A describes the stereoselective synthesis of pyrimidine nucleosides and section B describes the stereoselective synthesis of thioglycosides and 1-thiotrehaloses. In chapter three, a tetrazine templated method for the synthesis of ternary conjugates under mild conditions is delineated.

Chapter 1: Introduction to bioconjugation methods

A chemical reaction wherein two individual molecules are coupled together covalently in the presence or absence of reagents is known as a conjugation reaction and if the reacting partners are biomolecules then it is known as bioconjugation. If the two reacting partners are saccharide and nucleobase, then, the conjugate is called as nucleoside and if a conjugate is prepared from two saccharides then, it can be termed as disaccharide. Conjugation of molecules is important for studying various cellular events such as interactions between them, imaging of cells, for the measurement of distances between epitopes, for modification of materials, etc. Amidation, Native Chemical Ligation, Staudinger ligation, azide-alkyne involved [3+2] cycloaddition ('click' reaction), olefin metathesis, imine formation, tetrazine-based ligation, Michael addition are some of the bioconjugation methods; among these, Staudinger ligation, click reaction and tetrazine-ligations are the most popular and bioorthogonal.

Chapter 2: Stereoselective *N*- and *S*- glycoconjugates by gold catalysis

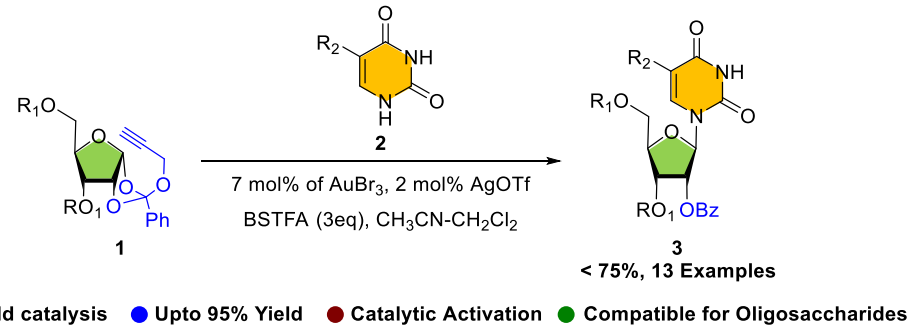
Glycoconjugates are essential for all living organisms. They regulate cell/cell and cell/invader interactions such as inflammation, tissue engineering, viral and bacterial infections, cell-growth control, wound healing, blood coagulation, tumour metastasis, and disease of nervous system; and many carbohydrate analogues have been identified and entered into the clinical trials. Stereoselective synthesis of glycoconjugates is difficult and access to chirally homogenous glycoconjugates is required for the glycochemistry world.

In this premise, recently identified propargyl 1,2-*O*-orthoesters are interesting for the synthesis of stereoselective glycoconjugates and salient feature of gold catalyzed activation of 1,2-O-orthoesters procedure are, (i) stable glycosyl donor; (ii) catalytic activation; (iii) easily accessible; (iv) high yielding; (v) leaving group traceless after the glycosylation.

Section A: Synthesis of Pyrimidine Nucleosides

Nucleosides are the basic building blocks for the biopolymers DNA and RNA, which contain the genetic information that is passed from one generation to the next. The sugar linked purine and pyrimidine nucleosides were held together by phosphodiester linkages to form the backbone of the DNA and RNA whereas the different nucleobases interact with one another *via* hydrogen bond to form the double helix structure of the DNA. In recent years, modified nucleosides have attracted more attention because of the wide spread applications including anti-HIV, anticancer and antiviral activity. Novel protocols for the chemical synthesis of such modified nucleosides are worthy due to their significance in drug development.

Pyrimidine nucleosides are synthesized by using propargyl 1,2-orthoesters and catalytic amount of $AuBr_3$ (Scheme 1). Strategically positioned 1,2-orthoesters are found to yield only 1,2-*trans* nucleosides and enable the preparation of 2′-OH containing pyrimidine nucleosides. The glycosyl donor employed in this study is stable and easily accessible. The identified high-yielding protocol is mild, diastereoselective, and catalytic.



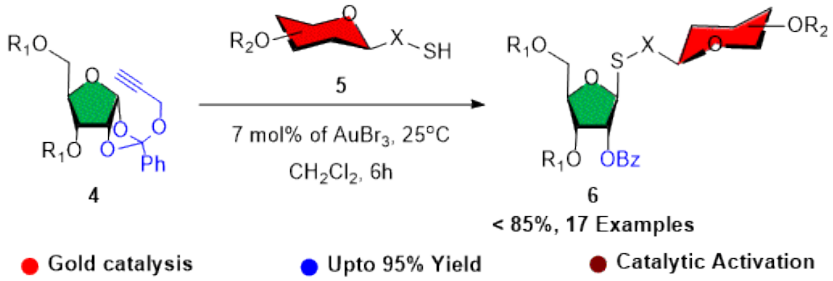
Scheme 1. Gold-catalyzed synthesis of pyrmidine nucleosides

Section B: Synthesis of Thioglycosides and 1-Thiotrehaloses

Carbohydrates with any of the hydroxyl groups being substituted by sulfur atom are not common in Nature, though sulfur is an essential element present ubiquitously in various living systems. Thioglycosides and thiotrehaloses are more resistant to the enzymatic cleavage compared to their natural *O*-analogues and hence, became promising targets for

the development of carbohydrate-based therapeutics. A few methods are available to synthesize 1,2-*trans* thioglycosides and trehaloses in a stereochemical fashion by exploiting the neighbouring group participation or steric hindrance or the combination of both of them. In this context, propargyl 1,2-orthoesters for constructing the thioglycosidic linkage in a diastereoselective fashion to achieve various thioglycosides and thiotrehaloses was hypothesized.

Various thiosaccharides and 1-thiotrehaloses were synthesized exploiting the salient features of gold-catalyzed glycosidation repertoire using alkynyl 1,2-orthoesters as glycosyl donors. The reaction was found to be general, high yielding, diastereoselective, fast, mild and catalytic (Scheme 2).



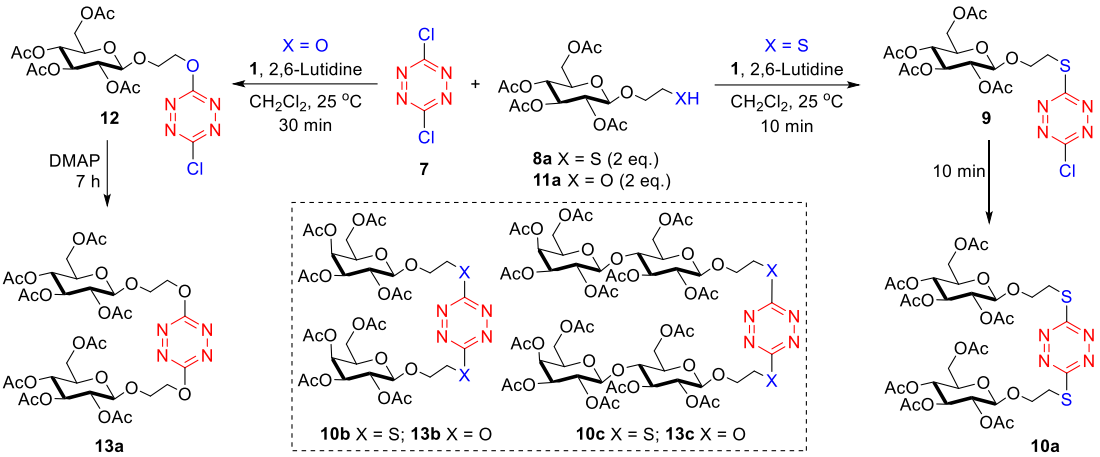
Scheme 2. Gold-catalysed synthesis of thioglycosides and 1-thiotrehaloses

Chapter 3: A Tetrazine templated method for the synthesis of ternary conjugates

Most of the methods and the one that is delineated in chapter 2 are useful to study binary complexes. In contrast, methods for multi- and poly- valent display of single molecule exploiting dendrimers, nanoparticles, calixarenes *etc*. as templates are known. However, stepwise conjugation of molecules to get ternary complexes in a modular fashion is still a challenge. In this chapter, we have successfully utilized dichlorotetrazine as a template for the synthesis of ternary conjugates in a modular fashion exploiting S_NAr and Inverse Electron Demand Diels-Alder reactions.

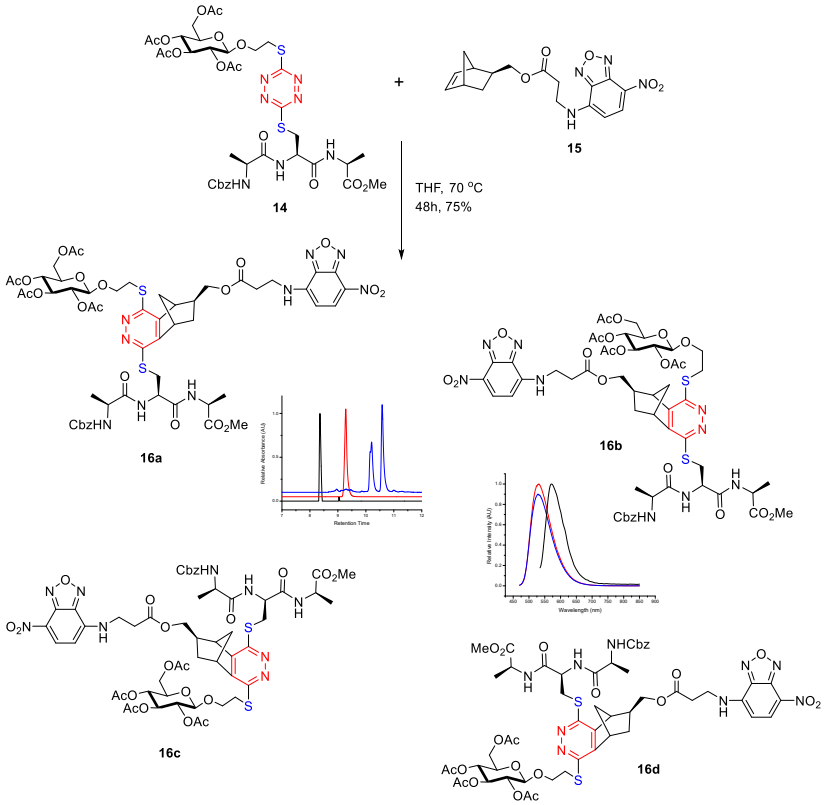
To begin our investigation, 2-thio ethyl glucopyranoside (**8a**) was treated with dichlorotetrazine (**7**) in the presence of hindered organic base in CH_2Cl_2 at 25 ^{0}C for 10min to observe formation of the mono- addition product (**9**) that got converted to the binary glycoconjugate (**10a**) in 97% yield in 20min (Scheme 3). Furthermore, glucoside (**11a)** was observed to give the mono addition product (**12**) in 30 min in the presence of 2,6-lutidine which did not get convert to the binary conjugate. Addition of one molar equivalent of more nucleophilic DMAP was observed to drive the reaction to offer binary

conjugate (**13a**) in 7 h in 95% yield (Scheme 3). Using this protocol six homodimeric and four heterodimeric glycol conjugates were synthesized.



Scheme 3

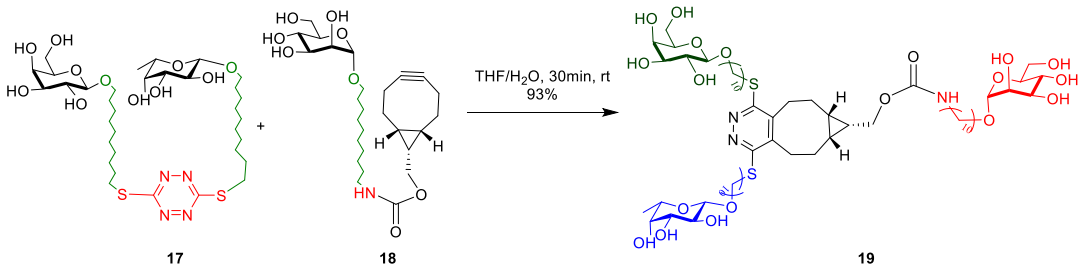
A model sugar-peptide tetrazine conjugate (**14**, SPC) was prepared from a tripeptide Cbz.Ala.Cys.Ala.OMe. Further SPC could serve as a model substrate for the IEDDA reaction to perform ternary conjugation. Among the suite of molecules available for IEDDA reaction, fluorescently labelled alkene (**15**) was considered as the resulting ternary conjugate can also be identified by the fluorescence spectroscopy.



Scheme 4. Synthesis of ternary conjugate

Accordingly, binary conjugate (**14**) and alkene (**15**) in refluxing THF underwent IEDDA reaction for 48h in 75% yield (Scheme 4). The Diels-Alder reaction between (**14**) and (**15**) can happen in an exo- or endo- manner and subsequently, undergo retro-Diels Alder reaction to release nitrogen and then gets oxidized to give aromatic nucleus. Thus, four ternary conjugates (**16a-d**) would be possible since we started with an enantiomeric mixture of alkenes **15** (Scheme 4). The outcome of the ternary conjugation reaction is confirmed by HRMS and NMR experiments.

Selectively thiol over hydroxyl functionality was utilized to prepare mono and binary conjugates in the aqueous medium. Symmetrical alkyne (**17**) was chosen to minimise the number of possible isomers during ternary conjugation. The model ternary conjugation reaction was performed in the THF/H_2O system (Scheme 5). The galactosyl-tetrazine-fucosyl binary conjugate (**17**) was subjected to ternary conjugation with cyclooctyne containing mannosyl compound (**18**) in the H_2O/THF at room temperature to form a ternary conjugate (**19**) that has Fuc*p*-, Man*p*- and Gal*p*- residues (Scheme 5). The method is modular and thus a suite of molecules can participate in ternary conjugation.



Scheme 5. Synthesis of *pseudo*-trisaccharide

In summary, salient features of tetrazine molecules were explored for the synthesis of ternary conjugates. Binary homo- and hetero- dimeric glycoconjugates are prepared by the reaction of mercaptoethyl or hydroxyethyl saccharides. The ternary conjugation was performed under inverse electron demand Diels-Alder reaction on the tetrazine nucleus. Mild ternary conjugation protocol that can be performed even in aqueous environment was identified. Selectively thiol over hydroxyl functionality was utilized to prepare mono and binary conjugates in the aqueous medium. The model ternary conjugation reaction was performed in the THF/H_2O system. The method is modular and thus a collection of molecules can participate in ternary conjugation.

*****

CHAPTER 1

Introduction

1.1 Introduction

A chemical reaction wherein two molecules are joined together covalently in the presence or absence of a reagent is known as a conjugation reaction and if one or more of the reacting partners is a biomolecule then, the process is called as bio-conjugation figure 1.1).[1]



Figure 1.1 Cartoon representation of a conjugation reaction

Bioconjugation encompasses a broad arena of science at the interface between molecular biology and chemistry. Bioconjugation techniques generally involve the covalent attachment of synthetic labels to a biomolecular framework. Examples include the modification of proteins and nucleic acids by the incorporation of fluorophores,[2] ligands, chelates,[3] radioisotopes[4] and affinity tags;[5] or modifications such as fusing two or more proteins together[6] or linking a complex carbohydrate with a peptide.[7] Bioconjugation of molecules is critical for studying various cellular events such as protein-protein interactions, imaging of cells,[8] for the measurement of distances between epitopes,[9] etc. The power of bioconjugation extends to the labelling of biomolecules *in vivo* as well.[10]

One of the toughest challenges in synthetic chemistry is the ability to have precise control over chemical reactivity and selectivity. These demands are exponentially amplified when it is necessary to perform selective reactions in chemically complex environments. Any conjugation method is called as bioorthogonal when conditions employed do not interfere with the functional groups that are commonly noticed in the highly heterogeneous biological systems (figure 1.2).[11]

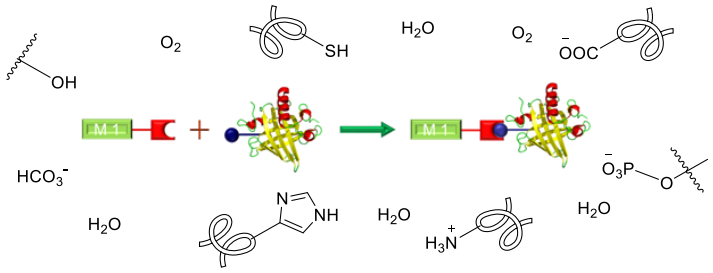


Figure 1.2 Bioorthogonal conjugation reaction

Such bioorthogonal reactions are significant for a wide range of applications comprising cell surface modification,[12] protein engineering,[13] and immunoassay development.[14] In the past decade, bioorthogonal reactions have become highly powerful tools for the

selective modification of biomolecules in various living systems.[15] Such reactions typically require the participant components to be mutually reactive and resistive to surrounding components within the cellular environment. Most of the chemical reactions are not able to meet these requirements simultaneously, rendering the development of bioorthogonal reactions an extremely challenging task. However, this area is rapidly growing and many exciting applications have been reported in recent years owing to the huge impact that might be generated by an effective bioorthogonal reaction on the study of diverse biological processes.[16]

1.2 Available methods for bioconjugation (Figure 1.3)

Amidation,[17] Native Chemical Ligation (NCL),[18] Staudinger ligation,[19] azide-alkyne involved [3+2] cycloaddition ('click' reaction),[15a,20] olefin metathesis,[21] imine formation,[22] tetrazine-based ligation,[8a,23] Michael addition[24] are some of the bioconjugation methods among which, Staudinger ligation,[19,25] 'click' reaction[20] have attracted more attention among myriad of reported bioorthogonal reactions. Most of these methods are useful to study binary complexes or two-partner conjugates. In Contrast, many examples such as dendrimers,[26] nanoparticles,[27] calixarenes[28] etc. are well known for multi- and poly- valent display of molecules is possible.

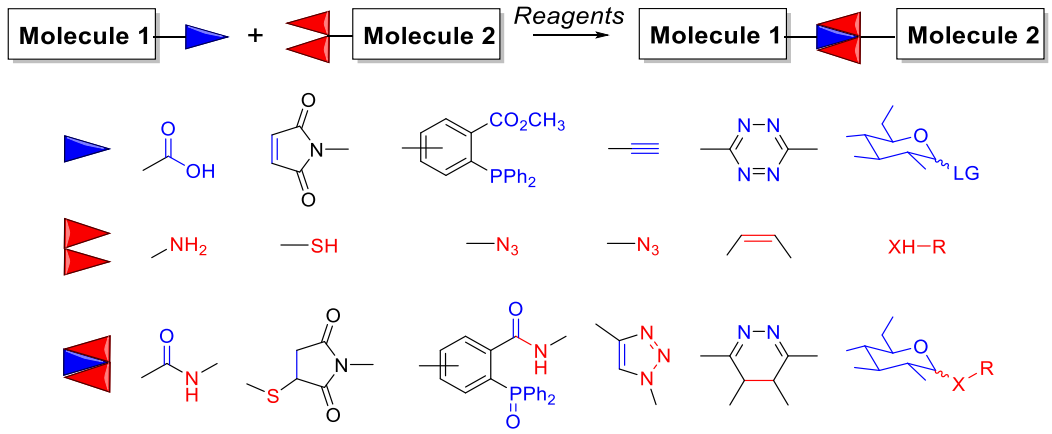


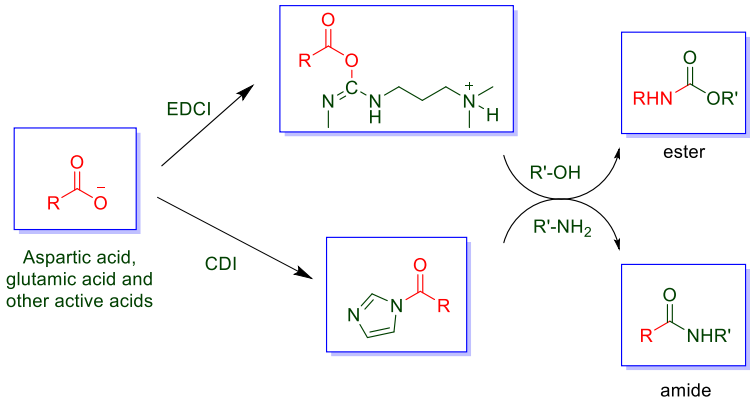
Figure 1.3 Bioorthogonal Conjugation Reaction

1.2.1 Conjugation *via* ester and amide Formation

Conjugation of proteins can be achieved by the utilization of carboxylate and amine functional groups. In proteins, the source of carboxylate and amine functionality is glutamate, aspartate and lysine respectively. Amide and ester bond formations are commonly used in the synthesis of functional nanomaterials and dendrimers.

1.2.1.1 Conjugation with carboxylic acids

Carboxylic acids are found on the *C*-terminus of proteins; and on glutamate and aspartate amino acid side chains. Carboxylic acids are strong organic acids which form the carboxylate anion on reacting with a mild base or a nucleophile. The resultant carboxylate anion is resistant to further nucleophilic addition. Thus the conjugation through carboxylate group *via* nucleophilic addition is a difficult process. Generally, harsh conditions, acid catalysis, or special activating reagents are necessary to promote these reactions. However, some carboxylate-mediated chemical reactions have been achieved with diazoalkanes[29] and diazoacetyl derivatives[30]. Further, activating agents such as carbodiimides and carbonyldiimidazole to derivatize carboxylic acids are reported to avoid harsh reaction conditions (Scheme 1.1).[31] All of these reactions generate stable covalent linkages namely esters and amides.

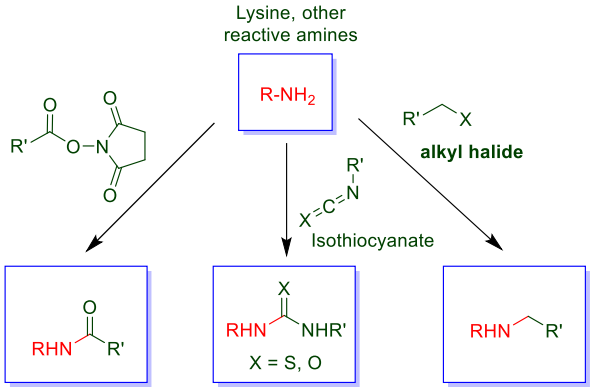


Scheme 1.1 Conjugation via coupling reagents

1.2.1.2 Conjugation with Amines

Amines are organic compounds which possess a basic nitrogen atom with a lone pair. The governing reactivity of amines is their nucleophilicity and thus most of their reactions involve nucleophilic-to-electrophilic attacks. Amine bioconjugation strategies have been largely used to modify proteins, peptides, oligosaccharides, and several other biomolecules. The two common reactions of amines are *alkylation* and *acylation* with alkyl halides and active esters respectively (Scheme 1.2). Lysine, arginine, and histidine are the sources for the free amines in the proteins. At moderately basic pH of 8–10, these amines remain unprotonated and act as good nucleophiles. Amines react with functional groups containing electrophilic carbon atoms such as isocyanate (−N=C=O) and isothiocyanate (−N=C=S) to form urea and isothiourea linkages respectively.

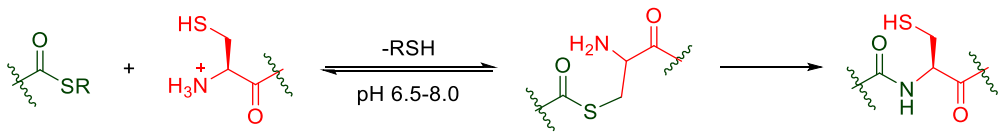
Commercially available fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) are widely used to prepare various fluorescent bioconjugates.[32]



Scheme 1.2 Conjugation with active amine

1.2.2 Native Chemical Ligation

In 1994, Native Chemical Ligation (NCL) was introduced by Kent as a method for polypeptide synthesis.[18b] NCL is a chemoselective reaction which involves the formation of an amide bond between a C-terminal thioester peptide and an N-terminal cysteine-containing peptide.[18b]



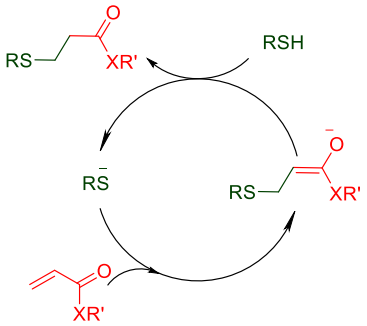
Scheme 1.3 Native Chemical Ligation[18a]

The main aspects of this reaction include that it occurs in water, using unprotected peptides at neutral pH and room temperature. An early illustration of the utility of NCL was demonstrated with the synthesis of a 72 amino acid chemokine protein [Ala33]IL-8 which was prepared from two unprotected peptides.[18] The first step of NCL is transthioesterification and the second step is an intermolecular S- to N- acyl transfer (Scheme 1.3). An NCL reaction between an intein-generated protein thioester with a synthetic cysteinyl peptide to synthesize larger proteins was termed as Expressed Protein Ligation (EPL) by Muir *et al.*.[33]

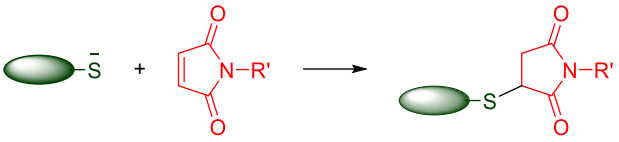
1.2.3 Michael addition

Michael addition reaction is a facile reaction between a nucleophilic species and an activated olefin under basic conditions (Scheme 1.4).[34] Important features of the Michael addition reaction are mild reaction conditions, minimal by-product formation, high functional group tolerance and high yields. Various amines, enolates and thiols can react

with acrylates, acrylamides, maleimides, acrylonitriles and cyanoacrylates, which are generally called as Michael donors and Michael acceptors, respectively.[24b,35]



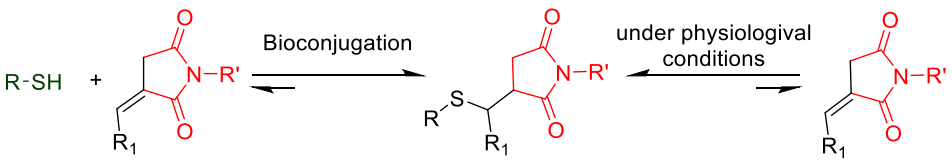
Scheme 1.4 Michal addition reaction



Scheme 1.5 Michal addition of thiolate to maleimide

Maleimides are more thiol-selective than amines.[24b,35] The conjugated double bond of maleimide easily undergoes an alkylation reaction with thiolates to generate a stable succinimidyl thioether bond (Scheme 1.5). The maleimide-thiol reaction proceeds rapidly in neutral aqueous solutions at room temperature, making it ideal for biological applications.[36] Excess maleimides can be easily removed from reaction mixture at the end of the reaction by quenching with free thiols.

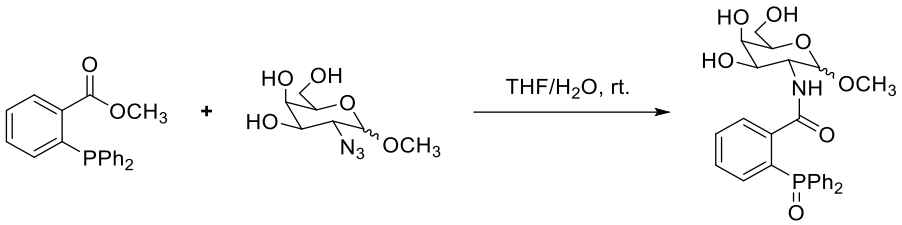
The spontaneous hydrolysis of maleimide moiety in basic aqueous media (pH > 8) gives rise to the formation of an *N*-acyl derivative that can no longer react with thiols.[5b,37] Even the thiol adduct is not stable under physiological conditions and undergoes thiol exchange or hydrolyse to give undesired products, which eventually alter the efficiency of bioconjugation.[38] Recently Kalia *et al.*[39] addressed these issues by developing an exocyclic olefinic maleimide as Micheal acceptor for the bioconjugation reaction. Under the physiological conditions thiols react with exocyclic olefinic maleimide to form a stable linkage which is resistant to thiol-exchange and stable to hydrolysis than endocyclic adducts (Scheme 1.6).[39]



Scheme 1.6 Michal addition using exocyclic olefinic maleimide

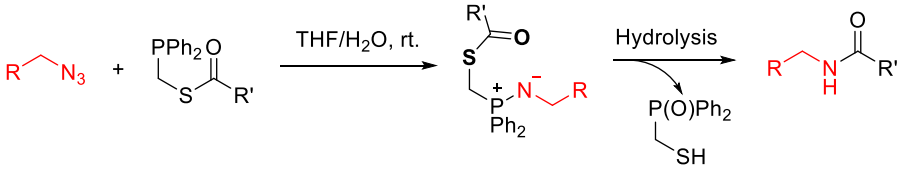
1.2.4 Staudinger ligation

The reaction of an azide with triphenylphosphine results in an aza-ylide with the loss of nitrogen, thus formed aza-ylide can undergo a numerous synthetically important reactions with a variety of electrophiles, such as ketones and aldehydes. But in the aqueous medium, the aza-ylide undergoes hydrolysis and produce amine and triphenylphosphine oxide. This reaction was reported by Staudinger and is known as Staudinger reaction. Saxon and Bertozzi showed that an ester functionalized phosphine derivative can function as an acyl donor when reacted with a glycosyl azide (Scheme 1.7).[25]



Scheme 1.7 Staudinger ligation

However, the byproduct phosphine oxide could not be separated from the ligated product. Therefore, a traceless version of the Staudinger ligation was developed for the bioconjugation in 2000 by Bertozzi[25,40] and Raines[41] groups independently based on the similar working principle. Raines[41] utilized diphenylphosphinemethanethiol, so that the auxiliary phosphine reagent can be cleaved off from the product after the ligation is completed leaving a native amide bond (Scheme 1.8).

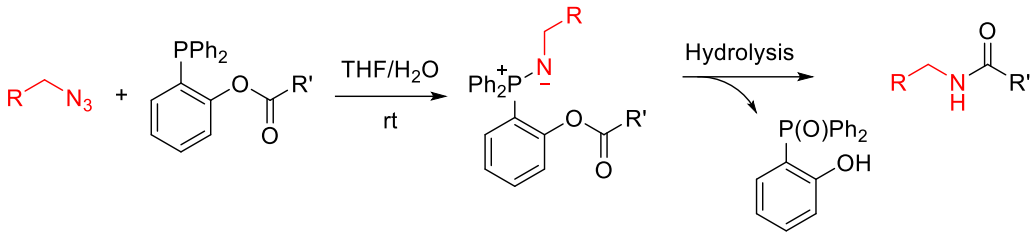


Scheme 1.8 Traceless Staudinger ligation of peptides by Raines group

Firstly, the diphenylphosphinemethanethiol reacts with azide to produce iminophosphorane which attacks the thioester to form an acyclic amidophosphonium salt to form an amide bond between the two reacting partners generating diphenylphosphinemethanethiol oxide as a sole by-product (Scheme 1.8).[41]

Bertozzi[42] developed phenol ester phosphine mediated reaction for the formation of native amide bond. Nucleophilic attack of azide on phenol ester phosphine results iminophosphorane that attacks the carbonyl centre of the phenol ester followed by

hydrolysis furnishing the desired amide along with phosphine oxide as sole by-product.[40,42]

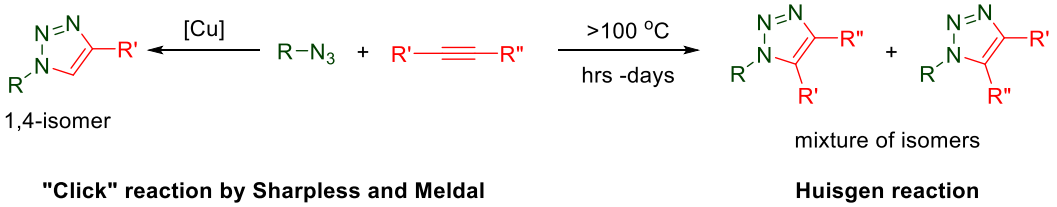


Scheme 1.9 Traceless Staudinger ligation by Bertozzi group

The Staudinger ligation has been shown to occur *in vitro* and *in vivo*; Bertozzi and co-workers successfully demonstrated that the reaction occurs in living animals with no physiological harm.[15b,43] However, many issues such as slow reaction rate, competing phosphine oxidation and limited water solubility made Staudinger ligation unsuitable to certain biological applications.

1.2.5 Azide-alkyne cycloaddition ('click' reaction)

Copper(I) catalyzed Huisgen 1,3-dipolar cycloaddition is an important chemical reaction developed during the last 30 years. Rolf Huisgen pioneered the dipolar cycloaddition of azides and alkynes in 1967,[44] it wasn't truly accessible until independent reports by Barry Sharpless[45] and Morten Meldal[46] that the dipolar cycloaddition was dramatically accelerated by factors up to 10^7 with enhanced regioselectivity using Cu(I) as a catalyst.



Scheme 1.10 1,3-dipolar azide-alkyne cycloaddition reactions

Endeavoring to create a facile modular strategy for the synthesis of medicinally relevant molecules from simple organic building blocks, Kolb, Finn and Sharpless developed a new synthetic methodology based on highly selective, reliable reactions and coined the approach "click chemistry."[47] Sharpless *et al.* compiled a set of criteria that must be met for a given reaction to fall under the umbrella term "click." The following rules must must be fulfilled by any reaction in order to fit into the ambit of 'click' chemistry:modular,wide in scope, high yielding, stereospecific, efficient under simple conditions (ideally insensitive to water and oxygen), selective, generating no or only inoffensive by-products

and the products must be: easily separable from the reaction mixture, easy to isolate, reasonably stable under physiological conditions.

While numerous reactions satisfy the criteria of click chemistry, the Huisgen Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) gained popularity, and it is simply referred to as "the click reaction." The enormous scope of the CuAAC reaction is not only due to its versatility in different reaction conditions and solvents, but also from its sustainable stability of reacting substrates. Though Cu(I)-catalyzed click chemistry has been widely used in many different biological studies, for example, to label azides in functionalized viruses, to tag azides in lipids, to modify proteins *in vitro* and *in vivo*, the toxicity of copper was a major problem which was addressed by Bertozzi *et al.*.

1.2.6 Copper-free Strain-promoted Alkyne-Azide Cycloaddition

The strain promoted 1,3-dipolar [3+2] cycloaddition of azide and cyclooctyne (SPAAC) was developed by Bertozzi and co-workers, as a modified Huisgen and Sharpless cycloaddition.[15a] In 2004, Bertozzi *et al.* initially employed this reaction towards in-vitro labelling of biomolecules under physiological conditions.[11a,15a] The simple reaction conditions and purification procedures made copper-free click chemistry to get extended into variety of disciplines including surface immobilization, synthesis of gels, oligonucleotide functionalization, dendrimer modification, and the decoration of nanostructures.[48] This reaction is selective and mild, but its relatively slow rate remained a liability for many applications. So, enhancing the rate of SPAAC reaction by invoking the principles of classical physical organic chemistry was indeed important to mention.[49] Two rate-enhancement strategies emerged experimentally, i) the addition of electron-withdrawing groups and ii) the augmentation of strain energy.[49,50] The bond angle of cyclooctyne is ~160°, which is structurally similar to the transition state of the cycloaddition reaction, resulting in a dramatic rate acceleration (figure 1.5).[50]

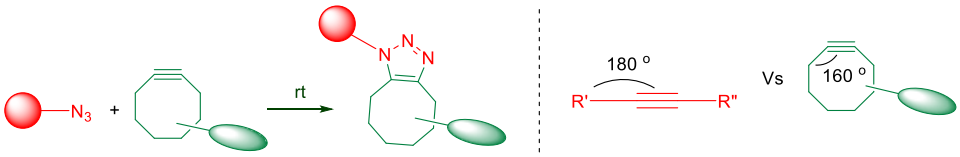


Figure 1.5 The strain promoted 1,3-dipolar [3+2] cycloaddition

The first cyclooctyne (OCT) that could react with azides at room temperature was developed by Bertozzi group. But it lacked water solubility which made it inapplicable to biosystems.[15a] Further the aryl-less octyne (ALO) was developed which had an

appreciable amount of water solubility but the reactions with azide partners were sluggish in nature.

To increase the rate of reaction through electron modulation, Florine as an electron withdrawing group was added to cyclooctyne and monofluorinated cyclooctyne (MOFO) and difluorinated cyclooctyne (DIFO) were developed (figure 1.6).[51]

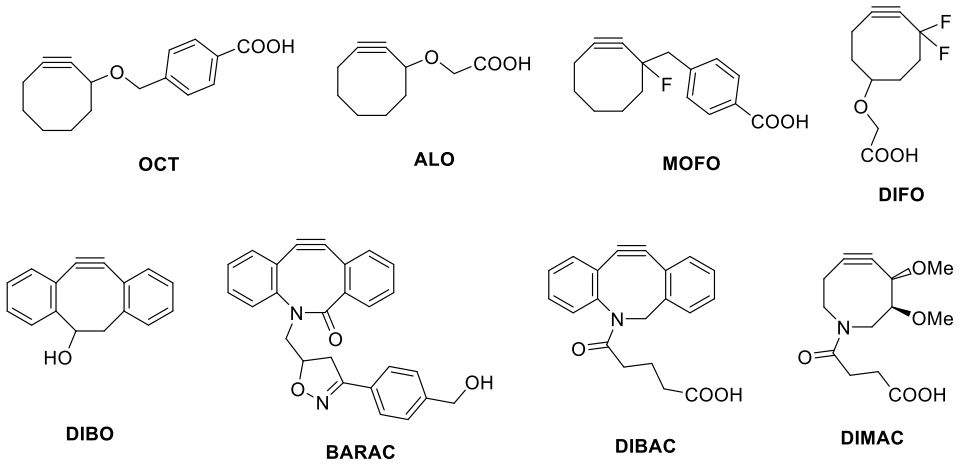


Figure 1.6 Representative Cyclooctynes

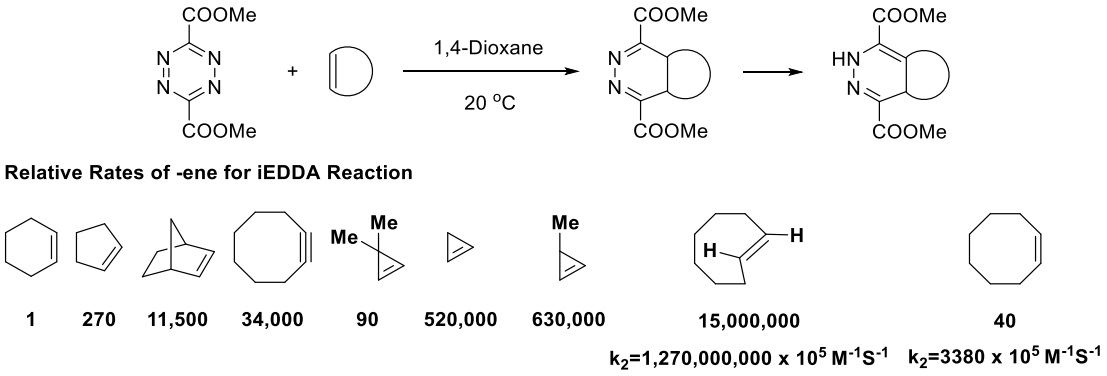
Bibenzylcyclooctyne (DIBO) was achieved by fusion of two aryl rings into a simple cyclooctyne which resulted high strain in the ring.[50] Biarylazacyclooctynone (BARAC), an analogue of DIBO with an amide bond in the ring with a sp^2 centre, further increased the reaction rate due to the ring distortion. An adjustment from the structure of BARAC to DIBAC, by reducing the steric bulk around the alkyne centre, enhanced the reactivity of the alkyne.[52] The dimethoxyazacyclooctyne (DIMAC) was also developed along with DIFO which are extremely reactive in labelling live cells.[51] The two methoxy groups α- and β- to the alkyne in DIMAC improved the water solubility, polarity and its pharmokinetics.[53]

1.2.7 Tetrazine-based ligation

Reactions that exhibit speed, selectivity, and orthogonality in biological environments have a huge impact in the areas of organic synthesis, chemical biology and material science. Unfortunately issues such as toxicity, compatibility with water, and competing side reactions have created a necessity to discover new reactions that can selectively interact with biological molecules without the inherent problems of existing methodologies. Normal electron-demand Diels-Alder reactions have been thoroughly investigated as a useful method for bioconjugation.[54] Most recently inverse electron demand Diels-Alder (iEDDA) chemistry has been extensively studied and is applied in

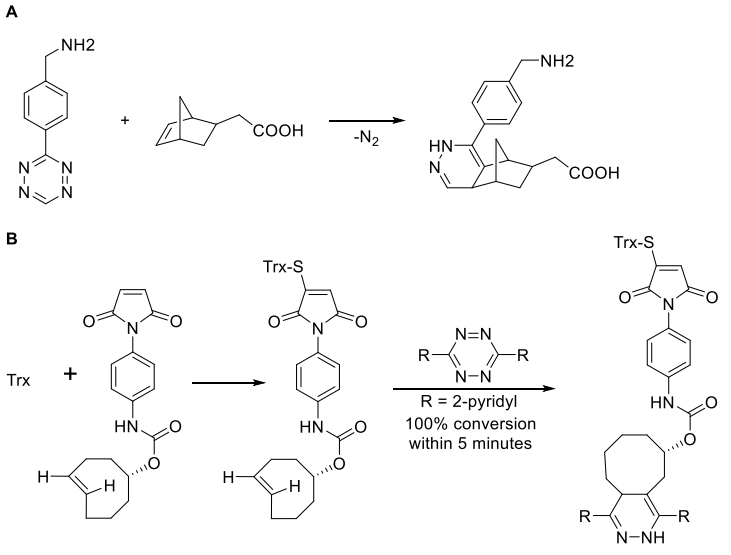
bioconjugation. It has also been established that 1,2,4,5-tetrazines are powerful dienes in iEDDA reactions of alkenes and alkynes.[23a,55]

In 1990, Sauer reported reaction kinetics of the reaction of an electron-deficient tetrazine with dienophiles and observed that the strained dienophiles display exceptional kinetics (Scheme 1.11).[56] Notably, the iEDDA reaction of transcyclooctene, cyclopropene and 3-methylcyclopropene with these tetrazines are quite rapid.[56] The highest reactivity was observed with *trans*-cyclooctene which had a high second order rate constant (k_2=1,270,000,000 x 10^5 $M^{-1}S^{-1}$).[56] Even though the iEDDA reaction of 1,2,4,5-tetrazines displays exceptional kinetics, the potential of this transformation as a novel bioconjugation method has never been explored by Sauer.



Scheme 1.11 Inverse electrondemand Diels-Alder Reaction

iEDDA was then introduced as a potential click reaction as reported almost simultaneously by two groups Fox and Hilderbrand in 2008.[23a,55] Since it has gathered a remarkable amount of attention especially in the field of life sciences, where challenging problems could be solved, it became an established method in the field of bioorthogonal and metal-free click chemistry (Scheme 1.12).[23b]



Scheme 1.12 A) Hilderbrand's reaction; **B)** Joseph fox's reaction

The first demonstration of this method involved modification of a *trans*-cyclooctene functionalised protein in cell media with a fluorescent tetrazine moiety, and this was extended to fluorescent labelling of proteins inside a cell and also on the cell surface.[55]

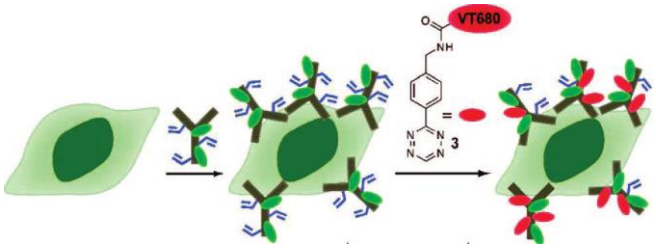


Figure 1.7 SKBR3 cells with norbornene and tetramethylrhodamine co-labelled tratsuzumab, followed by tagging the live cells with tetrazine-VT680 [55]

1.2.8 Glycoconjugates

Amongst the four key classes of biological macromolecules *viz.* proteins, DNA, lipids and carbohydrates, carbohydrates show a larger degree of structural diversity and form branched polymers and oligomers unlike proteins and DNA which form linear chains. When carbohydrates are conjugated to lipids, peptides (proteins) and steroids then the resulting conjugates are called as glycolipids, glycopeptides (glycoproteins) and saponins respectively. Conjugates of carbohydrates (Rib*f*-) with nucleobases are called as nucleosides. Further phosphorylation of these nucleosides results in nucleotides which on polymerization give DNA or RNA.

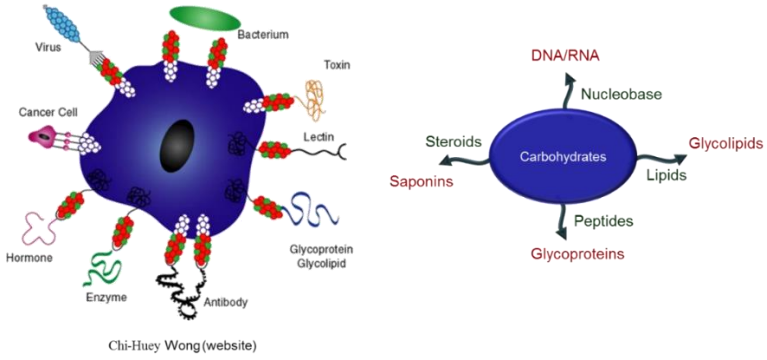


Figure 1.8 Conjugation Reaction

Carbohydrates are present in the cells in the form of oligosaccharides attached to lipids or proteins. Proteins are *O*-, *S*- or *N*-glycosylated either through serine/threonine, cysteine or asparagine amino acid residues respectively. The carbohydrate moieties of these glycoproteins play an important role in many biological processes, such as cell growth,

cell-cell adhesion and signaling, fertilization, immune defense, neuronal development and inflammation.

The foregoing discussion signifies the importance of broad theme of bioconjugation in various fields. Glycobiology is still lagging behind other biomolecule congeners probably due to the lack of proper tools to conjugation. For example, tools that exist for proteins such as expression vectors, cloning and recombinant technology or genomic modification to incorporate an unnatural amino acid in the protein or site selective mutagenesis etc. are not yet known for glycol-world. Unbelievable impact of the discovery of tools such as polymerase chain reaction, DNA polymerase etc in genomic field or discovery of green fluorescent protein in proteomics is still not seen in the glycobiology. In the absence of such exotic tools, studies on the glycan-protein or glycan-lectin and glycan-glycan interactions are extremely difficult though results from such studies pave way towards novel therapeutic agents and candidate vaccines.

In this premise, an expedient synthesis of glycoconjugates has been investigated in this thesis. A section of the thesis deals with the attachment of a nucleobase to a glycan moiety whereas the other section details our efforts in the synthesis of thioglycosides and 1-thiotrehaloses using the second generation gold-catalysed glycosidation repertoire. Importantly, we have synthesized many *pseudo*-glycan structures that are of immense significance to glycobiologists. Also, we used the *s*-tetrazine as a template for the ligation of saccharides for the first time to synthesize many *pseudo*-disaccharides and *pseudo*-tetrasaccharides in a very facile manner. Finally, inverse electron demand Diels-Alder chemistry has been applied on the tetrazine template to realize ternary-glycoconjugates. Ternary conjugation was demonstrated by synthesizing a ternary conjugate containing a glycan, a peptide and fluorescent molecule or three saccharides on the same platform using the tetrazine chemistry.

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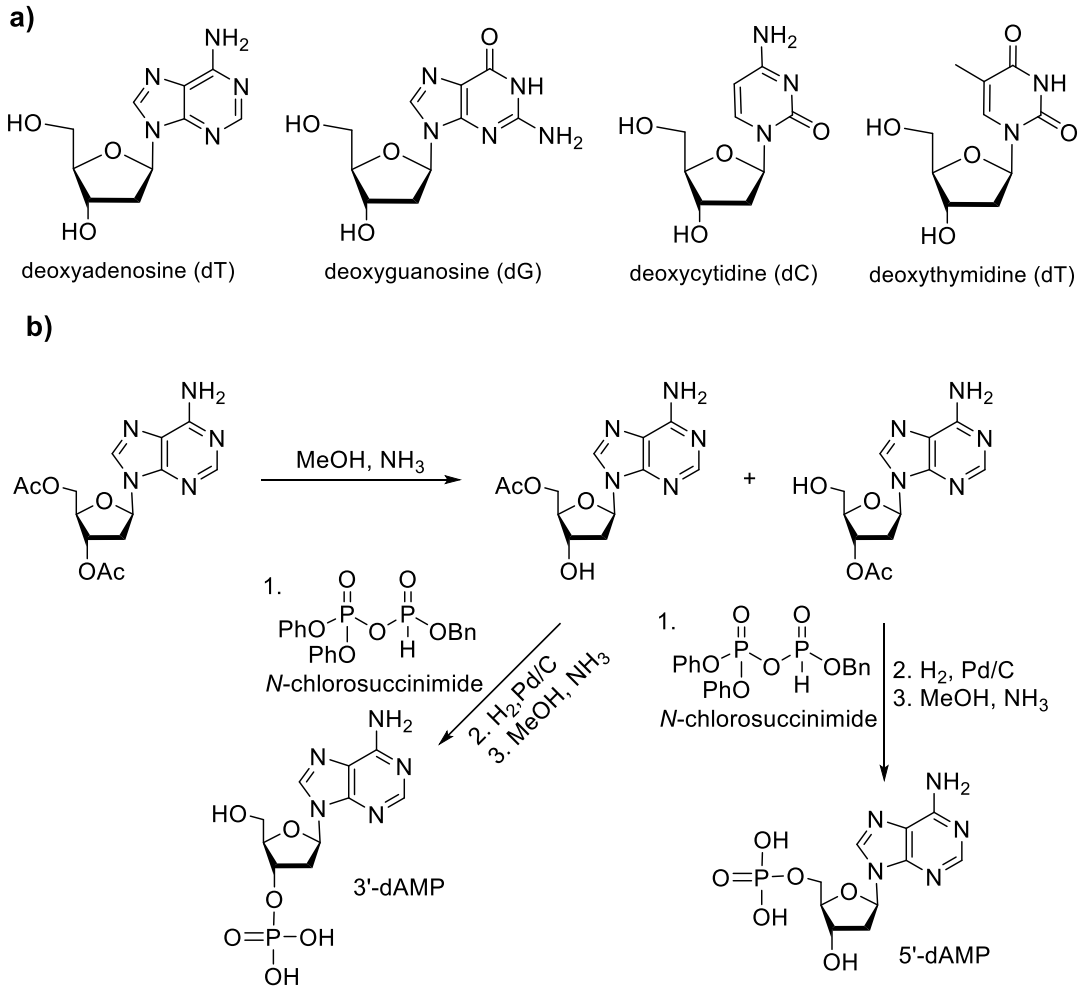
CHAPTER 2

Stereoselective N- and S- glycoconjugates by gold catalysis

2A. Synthesis of Pyrimidine Nucleosides

2A.1 Introduction to the Discovery of DNA

The story of DNA began with its discovery by Friedrich Miescher more than a century ago with the isolation of hereditary material (DNA/nuclein) and discovery that it contains phosphorus and nitrogeneous substances which were commonly observed in most cell types; named as "nuclein".[1] Richard Altmann renamed nuclein as nucleic acid and Levene identified main building blocks of DNA as deoxyadenine (dA), deoxycytosine (dC), deoxyguanine (dG), and deoxythymine (dT). Klein and Thannhauser enzymatically hydrolyzed these large and complex biopolymers (DNA) down to the monomer level and facilitated determination of the structural information.[1] Todd synthesized and confirmed four deoxynucleoside monomers, established the configuration, and β-glycosidic linkage (Scheme 2A.1).[2]



Scheme 2A.1 a) Nucleobases isolated from DNA, b) Todd's synthesis of deoxyadenosine 3′- and 5′-phosphates.[2]

Years later, the presence of hydrogen bonding interactions between the purines (A, G) and pyrimidines (C, T) was established and also found that hydrogen bonds are

particularly remained with nucleotides either in adjacent chains or within a single chain. Erwin Chargaff investigated the base composition of DNA from a variety of sources using then available paper chromatography technique to separate the products of DNA hydrolysis employing a UV spectrophotometer to quantify their relative abundance. Further, Chargaff showed that the number of purines is always equal to the number of pyrimidines.[3] In 1953, while James Watson and Francis Crick were trying to construct a model of DNA structure, realized that the enols of nucleobases T and G could actually tautomerize into the keto form. The most critical information came from the crystal data provided to Wilkins and Franklin.[4] Watson and Crick thereafter created a model with the following major features: i) two polynucleotide chains wind around the central axis to form a double helix; ii) two DNA strands run in opposite directions (antiparallel), each one forming a right-handed helix; iii) the bases occupy the core of the helix, and the surface of the double helix contains two grooves with unequal width, major and minor grooves; iv) each base forms hydrogen bonds with a base in the opposite strand resulting in a planar base pair (Figure 2A.1).[5] This is the phenomenon known as complementary base pairing, resulting in the specific association of the double helix. These developments have spurred the discovery of DNA polymerase[6] and unique genetic code.[7]

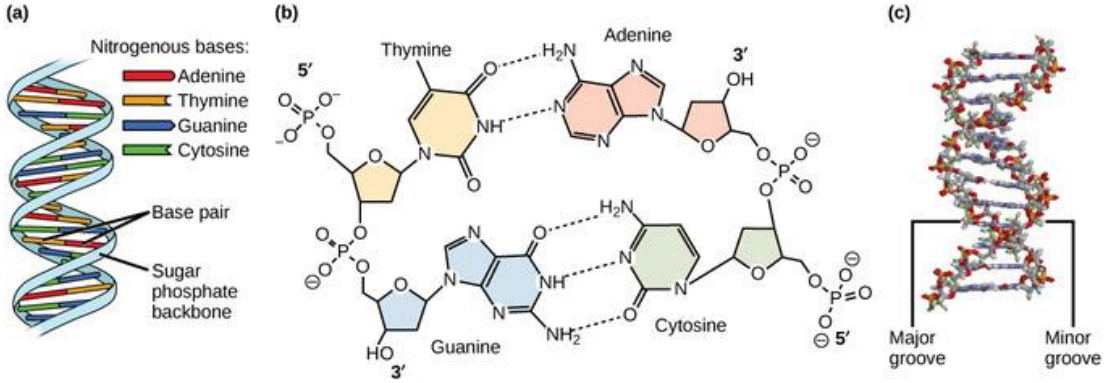


Figure 2A.1 Structure of DNA (a) a double helix structure and (b) phosphodiester bonds (c) the major and minor grooves

The decades of research on nucleic acids, revealed that the genetic information is mainly stored in the DNA through the specific sequence of the nucleoside bases that can be transformed to RNA with high accuracy by RNA polymerase enzyme through transcription process. As a consequence, introduction of unnatural nucleosides or modified nucleosides are hypothesized to alter the biological activities in search applications in biology, biotechnology or in development of various therapeutic agents.

2A.2 Modified Nucleosides

The modification of nucleosides and the nucleotides serves many purposes. Alterations can be used to create pharmaceutically active substances or to enhance our current understanding of the biological roles of the nucleic acids. In recent years, modified nucleosides have attracted more attention due to their anti-HIV, anticancer and antiviral activities.[6] Recently, modified nucleosides are utilized widely in the anti-sense oligonucleotide technology and to expand the genetic code.[7] Also modified nucleosides can serve as biological probes.[8] Modifications to the nucleosides can be conceived with reference to the sugar, the nucleobase, the phosphate backbone, or the combinations of all.

2A.2.1 Base Modifications

Base modifications have the potential to enhance stabilizing interactions between oligonucleotide strands in a significant manner (figure 2A.3). For example, expanded nucleobases featured the addition of the phenyl rings or other conjugated systems.[9] Expanded nucleobases enhance π- π stacking interactions between two adjacent base pairs which shows that the base stacking is one of the major driving forces for the double helix formation.[10]

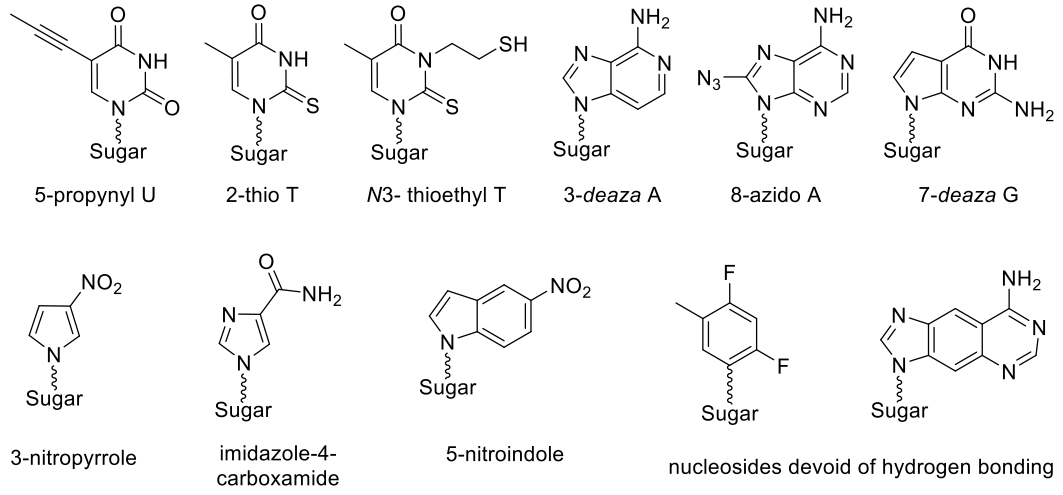


Figure 2A.2 Representative structures of base modified nucleosides

The hydrogen bonding interactions can be altered in the modified nucleobases. The most attractive sites for the modification of the nucleobases are those positions which are exposed to solvents in the major groove; 4- and 5-positions of pyrimidines and the 6- and 7-positions of purines.[11] The substitutions at these positions neither interfere with the base pairing nor induce the steric hindrance which can influence the geometry of the double

helix.[11] Alkyne and azide functional groups have been introduced for employing the 'click' or 1,3-dipolar azide-alkyne cycloaddition chemistry.[12]

2A.2.2 Sugar Modifications

Modifications to the (deoxy) ribose can be facilitated by changing the size of the ring, the stereochemistry of the sugar, or by replacing native atoms with non-traditional heteroatoms.[13] Hexose sugars can inhibit enzyme machinery due to the increased ring size.[14] One or all of the stereocenters on the sugar can be inverted, for example the enantiomeric L-*deoxy* ribose nucleosides. The atoms on the sugar itself can be altered with its substitutions such as sulfur or carbon on the *O*-4′ position.[15] The cyclic nucleoside analogues containing an extra fused ring to the sugar moiety are probed currently as SiRNA therapeutics.[16] The spironucleosides at different positions of the sugar are already synthesized and are found to affect the properties such as cytotoxicity and *C*-1' anomerization (figure 2A.3).[17]

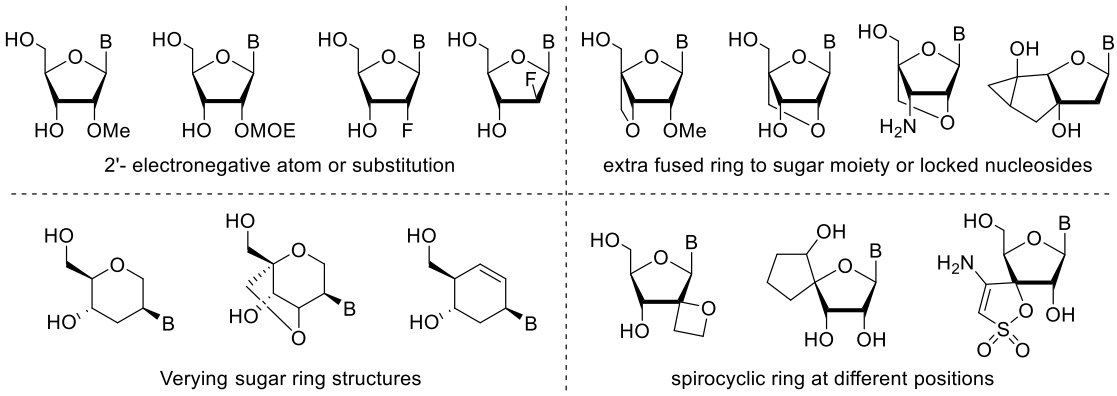


Figure 2A.3 Representative structures of sugar modified nucleosides

2A.2.3 Backbone Modifications

Backbone modifications can include the alteration of the phosphodiester linkage or the replacement of an entire backbone itself (Figure 2A.4).[18] The most famous example of a completely modified backbone system is the peptide nucleic acid or PNA.[19]

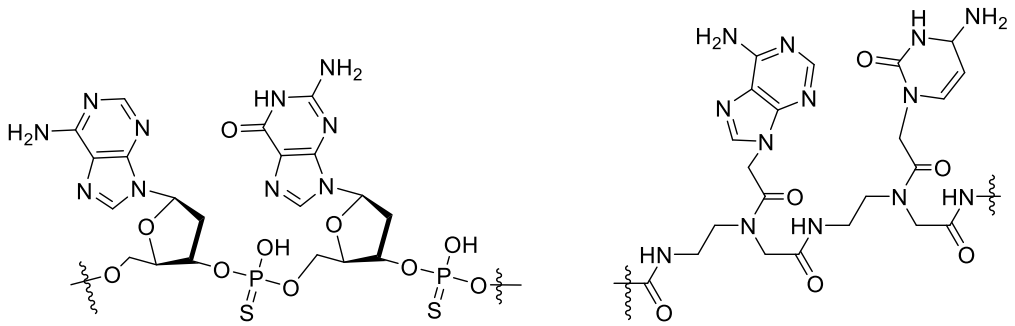
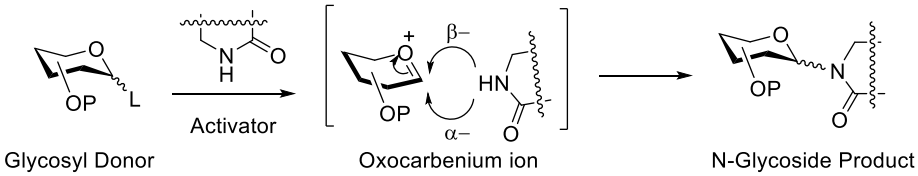


Figure 2A.4 Representative structures of backbone Modified Nucleosides.

In PNAs backbone does not contain any phosphodiester linkage rather a *pseudo*-peptide linkage of "nucleosides"; the backbone consists of repeating *N*-(2-aminoethyl)-glycine units with bases attached by a carbonyl methylene linker. PNA lacks negative charges and thus eliminates Coulombic repulsions as seen in DNA and RNA.

2A.3 Synthesis of Modified Nucleosides

Synthesis of modified nucleosides can be seen as an *N*-glycosidic coupling of sugars and nitrogen heterocycles. The chemical synthesis of *N*-glycosides usually involves the transformation of a sugar into a fully protected a form with a leaving group at its anomeric position known as the glycosyl donor and a suitable glycosyl acceptor (nucleobase or heterocycle). Usually, chemical reagents (activators) activate the leaving group forming a oxocarbenium ion which subsequently is attacked by a nitrogen nucleophile leading to the formation of *N*-glycosidic bond, and the process is called as *N*-glycosylation (Scheme 2A.2).



Scheme 2A.2 *N*- Glycosylation reaction

There are two critical issues associated with the outcome of the glycosidation reaction *viz.* (i) the regiochemistry of the resultant glycosidic linkage (more than one nitrogen atom is present in the nucleobase) and (ii) the configuration of the newly formed glycosidic bond. The type of the glycosyl donor and the substituents on the donor determine the stereochemical outcome of the glycosidation as α- or β- glycoside.

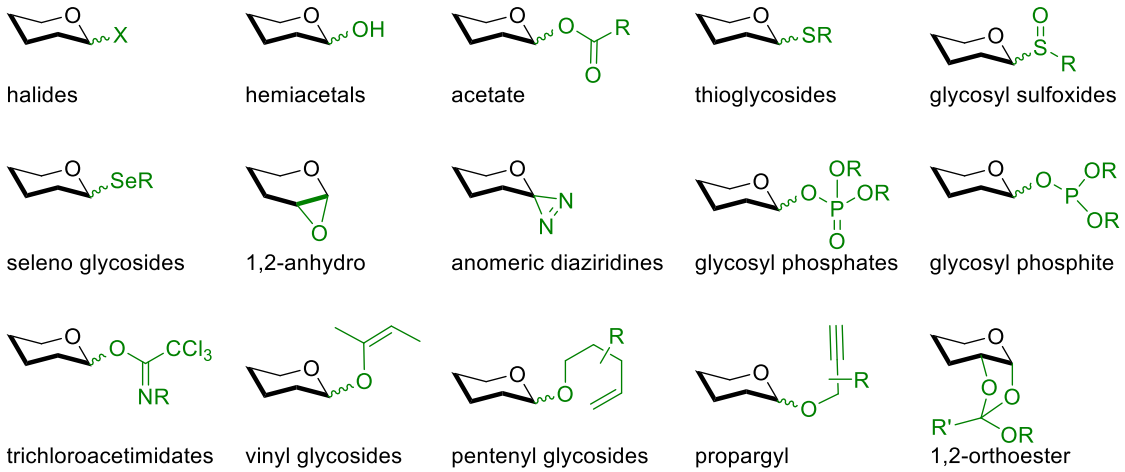
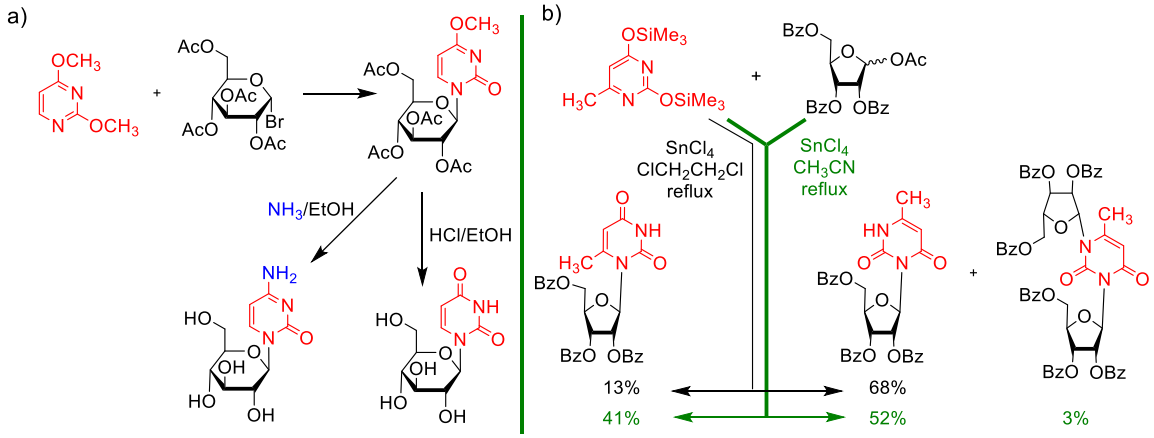


Figure 2A.4 Some of the commonly used glycosyl donors for glycosidation

Often, the presence of esters at the *C*-2 hydroxyl group of the sugar results in the formation of 1,2-*trans* stereoselective glycosides due to the neighboring group participation; whereas, the glycosylation with a ether substituents at *C*-2 position gives both 1,2-*trans* and 1,2-*cis* glycosides. From the decades of research on chemical glycosylation methods, many glycosyl donors are reported for glycosylation (Figure 2A.4); among these only a few glycosyl donors are reported for the formation of *N*-glycoside products.

In 1970, Hilbert and Johnsonn reported the first chemical synthesis of modified nucleosides through the transformation of the 2,3,4,6-tetra-*O*-acetylated-α-D-glucopyranosyl bromide with 2,4-dialkoxypyrimidine nucleobase under reflux conditions to form desired nucleoside. The resulting *N*-glycoside was further converted to sugar modified cytidine by NH_3 in ethanol treatment and subsequently converted to sugar modified thymidine by the treatment of HCl in ethanol (Scheme 2A.3).

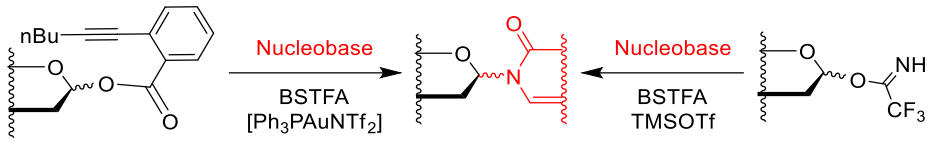


Scheme 2A.3 a) Hilbert and Johnson reaction, b) Vorbrüggen reaction

Further, Vorbrüggen reported the silyl version of Hilbert-Johnson nucleosidation wherein Lewis acids such as $SnCl_4$, TMSOTf, etc. mediated glycosylation between a labile trimethylsilyl-protected pyrimidine and protected glycosyl acetates. The presence of acyl group at *C*-2 position of ribose ensures the selective formation of the β-nucleoside due to the neighboring group participation (Scheme 2A.3).[20] The stereoselective synthesis of deoxyribonucleosides directly from deoxyribose derivatives is more difficult due to the absence of any neighboring group participation at *C*-2 position.

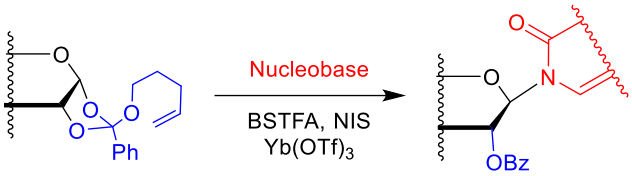
Trichloroacetimidate and more recently trifluoroacetimidate glycosyl donors also have been utilised for the synthesis of pyrimidine nucleoside.[21] In 2011, Biao Yu reported the utility of gold catalysis for the synthesis of nucleosides where *O*-alkynyl benzoate is

utilized as a leaving group that was activated by catalytic amount of [$Ph_3PAuNTf_2$] in the presence of silylating agent BSTFA in acetonitrile at room temperature (Scheme 2A.4).[22]



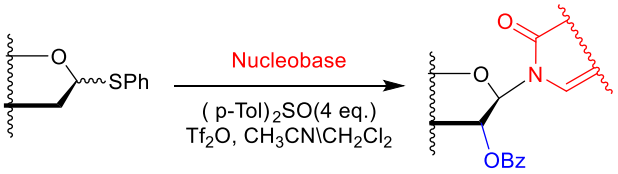
Scheme 2A.4 N-glycosylation with *O*-alkynyl benzoate and trifloroacetimidate

Subsequently, while our investigation is in progress, Fraser-Reid reported *n*-pentenyl 1,2-orthoesters[23] for nucleoside synthesis employing silylated nucleobases, NIS and $Yb(OTf)_3$ (Scheme 2A.5).[24]



Scheme 2A.5 N-glycosylation with n-pentenyl 1,2-orthoesters

More recently in 2015, while our investigation is in progress, nucleoside analogues were synthesized by the activation thio-glycosides by (*p*-Tol)$_2$SO/Tf_2O. But the stereoselectivity of resulting *N*-glycoside products depended on either the presence or absence of neighboring group at *C*-2 or temperature (Scheme 2A.6).[25]

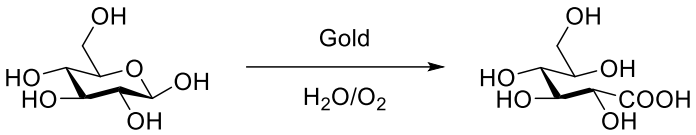


Scheme 2A.6 N-glycosylation with thio-glycoside

2A.4 Gold Catalysis in the Carbohydrate chemistry

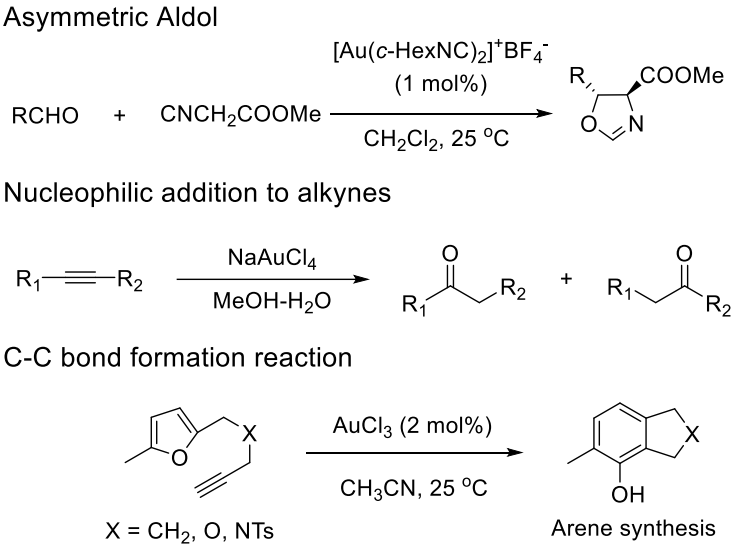
Over the last century, the interest in gold as a catalyst has increased dramatically due to its selectivity, efficiency, robustness, and versatility.[26] In earlier days, gold was considered to be a metal with low catalytic activity. There are two major areas of gold catalysis: homogeneous and heterogeneous catalysis former being is the most active area. Gold can exist in different oxidation states; the most prevalent of which are Au(0), Au(I), and Au(III). In 1973 Bond *et al.*[27] reported the hydrogenation of olefins over supported gold, which showed excellent activity of gold to drive many organic reactions over the other predominantly known catalysts.[27] More than a decade later, Haruta[28] and Hutchings[29] simultaneously and independently reported gold to be an extraordinarily excellent catalyst and ultimately demonstrated the reactivity. Haruta *et al.* investigated

the low-temperature oxidation of CO[30] and Hutchings reported the hydrochlorination of ethyne to vinyl chloride.[31] For the first time these studies showed that the gold to be an affective catalyst for organic transformations. Haruta *et al.*[32] demonstrated the high selectivity in the heterogeneous oxidation of propene to propene oxide.[32] Michele Rossi *et al.* also reported the positional-selective oxidation of different alcohols and first applied to the carbohydrates with molecular oxygen to selectively oxidize the glucose into gluconate by bubbling O_2 (Scheme 2A.7).[33]



Scheme 2A.7 Oxidation of glucose by gold catalysis

To overcome the limitations associated with the reactivity and stability of the gold catalysts, many ligands are examined to obtain more stable gold complexes and explored their utility for various organic transformations. For example, Ito *et al.* applied Au(I) catalyst and an enantiomerically pure ferrocene diphosphane ligand for the synthesis of chiral oxazolines by the addition of carbon nucleophiles to a carbonyl moiety (Scheme 2A.8).[34]

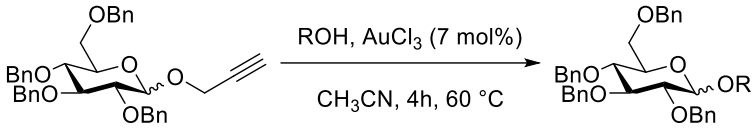


Scheme 2A.8 Homogeneous gold-catalyzed reactions

The addition of nucleophiles to alkynes was first investigated by Fukuda and Utimoto[35] for alcohols, water, and amines and demonstrated the gold cationic complexes behave as chemoselective π-acids to remove electron density and promote electrophilic character, which can then be attacked by a nucleophile easily.[35] Further, Hashmi's phenol synthesis

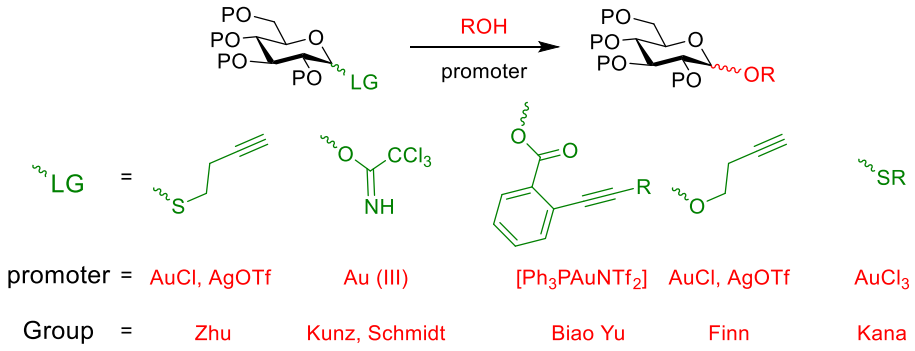
by catalytic quantity of $AuCl_3$ is the major advancement in the homogeneous gold catalysis field.[36] Subsequently Yang also investigated the intermolecular reactions of alkenes with oxygen nucleophiles with the same concept.[37]

Homogeneous gold catalysed glycosylation reactions were completely unknown, until the first report by Hotha *et al*. in 2006;[38] who showed the utility of catalytic amount of gold(III) salts for the transglycosylation reaction. This was achieved by keeping propargyloxy group at the anomeric position, and was selectively activated to generate the oxocarbenium ion intermediate. The salient features of this reaction are deployment of catalytic amount of gold, good reaction yields and mild reaction conditions, whereas the high temperature of reaction can be considered as a major drawback which was solved subsequently.[40]



Scheme 2A.9 Transglycosylation using gold (III) catalysis

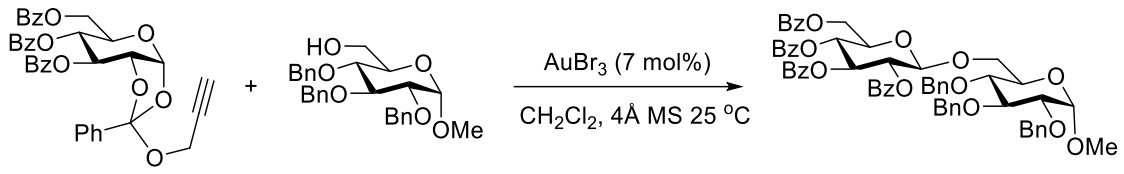
Few other groups also activated various leaving groups at anomeric center by gold catalysis. For example, *S*-but-3-ynyl thioglycoside donor was reported by Jianglong Zhu.[39] Recently trichloroacetimides are also activated with gold(III) salts by RR Schmidt.[40] *O*-Alkynyl benzoate leaving group was reported by Biao Yu[22] and homopropargyl leaving group was reported by Finn. Quite recently Sureshan reported simple alkyl and aryl thioglycosides can act as glycosyl donors under gold catalysis (Scheme 2A.10).[41]



Scheme 2A.10 Glycosylation using gold catalysis

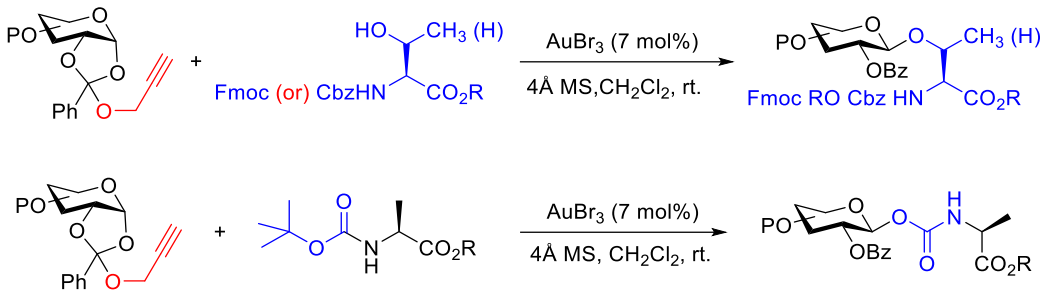
Further improvements from our group showed propargyl 1,2-orthoesters are suitable for bringing down the temperature from 60 ºC to 25 ºC, 1,2-*trans* diastereoselectivity and

increased substrate scope and yields in presence of 7 mol% of $AuBr_3$ in dichloromethane and 4Å molecular sieves powder at room temperature in good yields (Scheme 2A.11).



Scheme 2A.11 Propargyl 1,2-orthoesters as glycosyl donors

Furthermore, propargyl 1,2-orthoesters have been utilized for the synthesis of peptide glycoconjugates, glycopolymers, carbamate glycosides[42] and furanosides as well (Scheme 2A.12).[43]



Scheme 2A.12 Synthesis of glycopeptide and glycocarbamate conjugates

Addition of a silver salt as a co-catalyst is one way of improving the performance of gold catalysed transformations in organic synthesis. In gold catalysis, a silver salt with a non-coordinating counter ion is often added to abstract a halogen from the gold species, leaving the active homogenous gold catalytic system and extremely insoluble silver halides. NMR studies also revealed that the gold complexes in solution were influenced by silver, which promotes catalytic activity of gold and, suggesting a cooperative catalytic system between gold and silver salts. Based on this hypothesis a wide range of gold catalysed reactions are reported in the literature with the "silver effect" on the reactivity of the catalytic systems. Earlier studies on alkynyl glycosyl donors recommended use of silver salt as co-catalysts for improving the performance of gold catalysed glycosylation reactions.

In the era of green chemistry, processes that give higher yields by catalytic means are very important to increase the overall atom economy.[44] Our group reported[42-43,45] that propargyl 1,2-orthoesters can behave as a glycosyl donors in the presence of gold(III) salts in a diastereoselective fashion and subsequently shown to be suitable for the synthesis of glycomimetics,[45c,d] glycopolymers,[42b,45e] glycoconjugates[45f] and oligosaccharides.[43] Our continued interest in the utilization of alkynyl glycosyl donors for

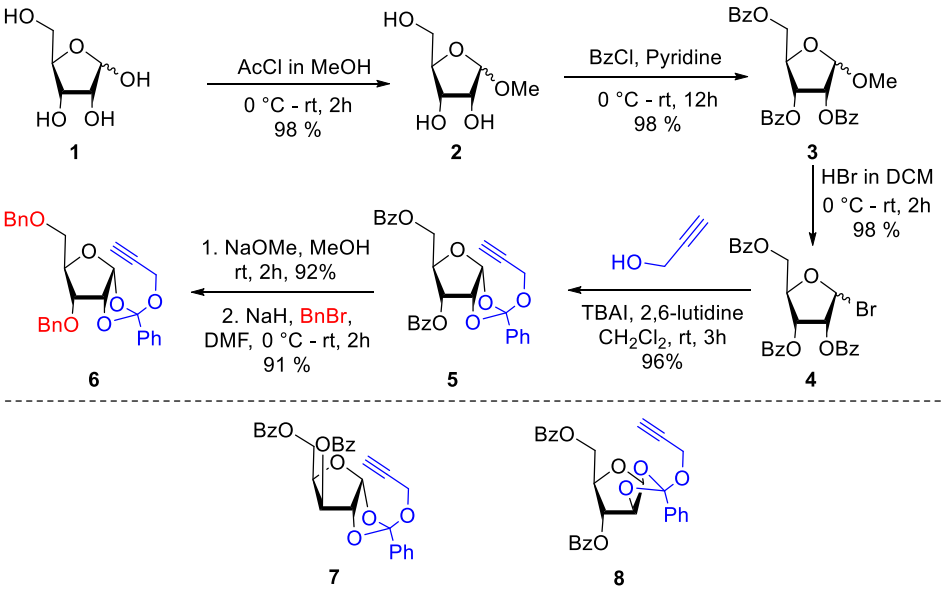
glycosides synthesis in the presence of gold salts encouraged us to explore the utility of gold-catalysed glycosidation repertoire to the synthesis of *N*-glycosides or nucleosides. Results from these studies are presented in the section of the thesis.

2A.5 Present work

Nucleosides are the basic building blocks of biopolymers DNA/RNA encoding the genetic information that is passed from one generation to the next. The sugar linked purine and pyrimidine nucleosides were held together by phosphodiester linkages to form the backbone for the DNA and RNA, whereas different nucleobases interact with one another *via* hydrogen bonds to form the double helix structure of DNA. In recent years, modified nucleosides have attracted attention because of their wide spread applications in anti-HIV, anticancer and antiviral activities.[6b] Chemical synthesis of such modified nucleosides is worthy due to their significance as therapeutic agents or as new materials. Development of an efficient glycosylation method that is reliable with diverse substrate scope would be a right step for the synthesis of nucleosides. An ideal glycosyl donor should have one or more of the following characteristics: (i) easy to prepare/access; (ii) stable to diverse chemical reactions; (iii) regio- and stereoselective; (iv) use catalytic reagents; (v) generate minimum amount of waste; and (vi) avoid use of toxic metals.

From our laboratory, stable propargyl 1,2-orthoesters were activated in the presence of catalytic amount of gold(III) salts to behave as glycosyl donors. Salient features of propargyl 1,2-orthoester based glycosylation are: (i) glycosyl donors can be prepared from easily accessible glucosylbromide; (ii) propargyl group is stable to many of the diverse chemical manipulations; (iii) can be activated by catalytic quantity of gold salt; and (iv) no offensive by-product is formed. Earlier studies from our group showed the utility of propargyl 1,2-orthoesters for the stereoselective synthesis of glycoconjugates, glycomimetics, glycopolymers, glycopolypeptides and glycans. We thought of exploring propargyl 1,2-orthoesters as glycosyl donors for the synthesis of pyrimidine nucleosides as part of our continued interest in the utilization of gold-catalysis for glycosidation. Various propargyl 1,2-orthoesters are required in order to commence our exploration. Accordingly, propargyl 1,2-orthoester glycosyl donors were prepared from simple sugars. D-Ribose (**1**) was transformed into methyl-α/β-D-ribofuranoside (**2**) in the presence of MeOH.HCl which was generated *in situ* by AcCl in MeOH. Furnoside **2** was subsequently converted to methyl 2,3,5-tri-*O*-benzoyl-α/β-D-ribofuranoside (**3**) by the treatment of benzoyl chloride in pyridine. Ribofuranoside **3** was then reacted with HBr.AcOH in dichloromethane which was generated *in situ* from acetyl bromide and methanol to afford bromide **4**. Subsequently, bromide **4** was treated with propargyl

alcohol, 2,6-lutidine and a catalytic amount of TBAI in dichloromethane at room temperature for 3h to afford 3,5-di-*O*-benzoyl-α-D-ribofuranosyl propargyl 1,2-orthoester (**5**) in very good yield (Scheme 2A.13). In the ^{1}H NMR spectrum of compound (**5**), resonances at δ_H 2.42 ppm as triplet and δ_H 4.09 ppm as a ABq clearly indicated the presence of propargyl group. Rest of resonances in the ^{1}H NMR spectrum were in complete agreement with the assigned structure (**5**). In addition, ^{13}C NMR spectrum revealed characteristic signals corresponding to the α-anomeric carbon and the quaternary carbon at δ 104.7 (C_1) and 123.6 ppm respectively while remaining resonances were in accordance with the structure of propargyl 1,2-orthoester (**5**). The assigned structure was further confirmed by mass spectroscopic analysis (*m/z* $[M + Na]^+$ calcd for $C_{29}H_{24}O_8Na^+$ 523.1363, Found: 523.1369). *N*-Glycosylation of pyrimidine nucleobase can be carried-out on orthoester (**6**); distinguishing C-2 hydroxyl group from the other two –OH groups will be cumbersome. However, the orthoester strategy enables us to obtain *C*-2 benzoates after the *N*-glycosidation is achieved. Accordingly, protection of *C*-3 and *C*-5 hydroxyl groups as their benzyl ethers would be advantageous as benzoates can be orthogonally saponified in the presence of benzyl ethers under Zemplén deacylation conditions. In addition, orthoesters are stable to basic conditions and thus NaOMe and NaH can be employed without affecting the orthoester moiety.



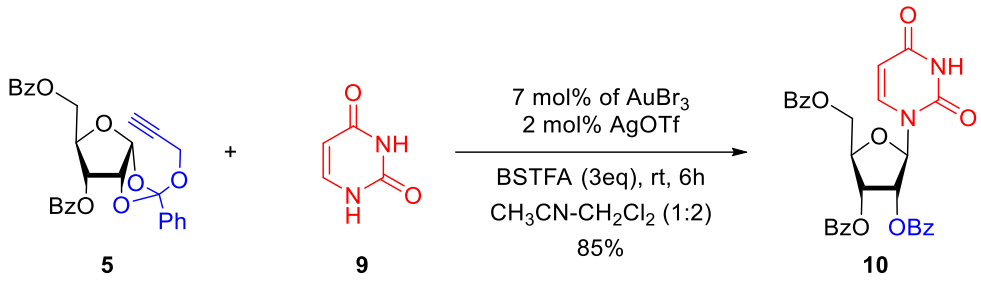
Scheme 2A.13 Synthesis of furanosyl 1,2-propargyl orthoesters

Therefore, orthoester (**5)** was saponified under Zemplén conditions to obtain a diol that was quickly treated with NaH/BnBr/DMF/TBAI/0-25 ^{0}C for 2 h to obtain the di-benzyl ether (**6)** in 91% yield. Similarly, orthoesters of D-Xylose and D-Arabinose (**7, 8**) were also prepared using the aforementioned procedure in large quantities.

To start the investigation for the synthesis of nucleosides by gold catalysis, 1 eq. of orthoester (**5**)[43] and 1.2 eq. of uracil ware taken in CH_2Cl_2 and treated with standard $AuBr_3$ (7mol%) conditions to see no formation of the desired nucleoside. The only product that was obtained in very small quantity was confirmed to be the propargyl furanoside which was earlier noticed on pyranosides as well. The resistance of the orthoester to nucleosidation has been attributed to the non-availability of nucleobase since the uracil is not soluble in dichloromethane at room temperature. However, earlier literature reports suggested addition of *N,O*-bis(trimethylsily)trifluroacetamide (BSTFA) to uracil in CH_3CN would increase the solubility of pyrimidine nucleobases by converting to the corresponding silyl enol ethers as observed by Vorbrüggen.

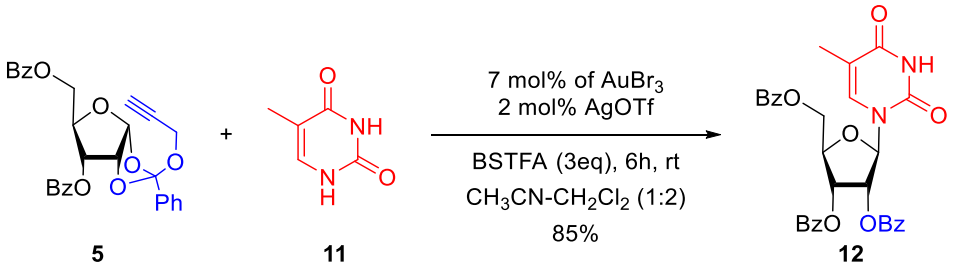
Accordingly, uracil and BSTFA were stirred in CH_3CN until the solution becomes clear (~30 min) and then a solution of orthoester **5** in CH_3CN was added dropwise. To this solution, $AuBr_3$ (7 mol%) and freshly activated 4Å MS powder was introduced and stirred for 24h to obtain the desired *N*-glycoside product (**10)** in 10% yield along with 5% of propargyl 2,3,5-tri-*O*-ribofuranoside as a by-product. Poor yield of the N-glycoside was ascribed to the poor reactivity of orthoesters in acetonitrile; it has been reported by Sureshkumar and Hotha that propargyl 1,2-orthoesters are activated better in CH_2Cl_2 over CH_3CN. In addition, our previous results showed that the addition of silver salt can increases the yield of *O*-glycosylation reactions.[45a] Inorder to examine the silver effect in the *N*-glycosidations, the reaction was performed by the addition of AgOTf as a co-catalyst to find that the yield enhancement to 35%. Propargyl orthoesters are better activated in dichloromethane than acetonitrile; but, silylation of uracil in dichloromethane requires several hours or heating is required. To avoid heating and to complete the reaction in shorter time, uracil was converted to silylated uracil by the addition of BSTFA in minimal amount of acetonitrile and then orthoester (**5**) was added in dichloromethane followed by the addition of $AuBr_3$ (7 mol%) and AgOTf (2 mol%) to observe 80% yield of the desired nucleoside **10** along with the formation of propargyl ribofuranoside.

Further, various combinations of CH_3CN and CH_2Cl_2 were tried and found that 1:2 mixture of CH_3CN:CH_2Cl_2 is best suited (85% of **10**) for the nucleosidation (Scheme 2A.14). In the ^{13}C NMR spectrum of compound **10,** characteristic resonances corresponding to *N*-glycosidic anomeric carbon at δ 88.3 ppm was noticed, all resonances due to the orthoester got disappeared confirming the formation of nucleoside **10**.



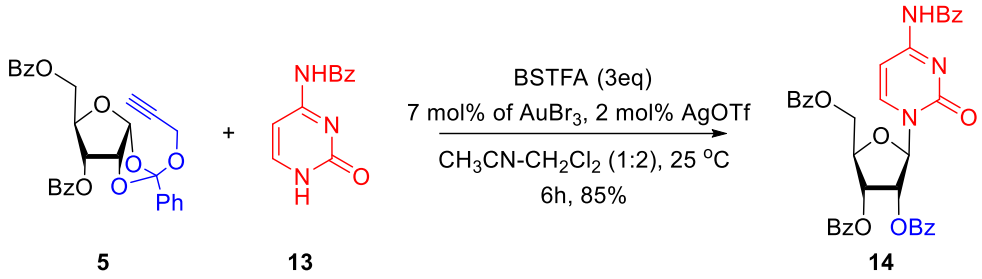
Scheme 2A.14 Gold-catalysed glycosylation for pyrimidine nucleosides

In continuation, optimized conditions were utilized for the *N*-glycoside synthesis of thymine and cytosine as well. The coupling reaction between the orthoester **5** and thymine **11** was performed under aforementioned conditions to afford the nucleoside **12**[24] in 85% yield (Scheme 2A.15). In the ^{13}C NMR spectrum of compound **12** characteristic resonances corresponding to the *N*-glycosidic anomeric carbon were noticed at δ 87.1 ppm along with other resonances in complete agreement with that of compound **12.**



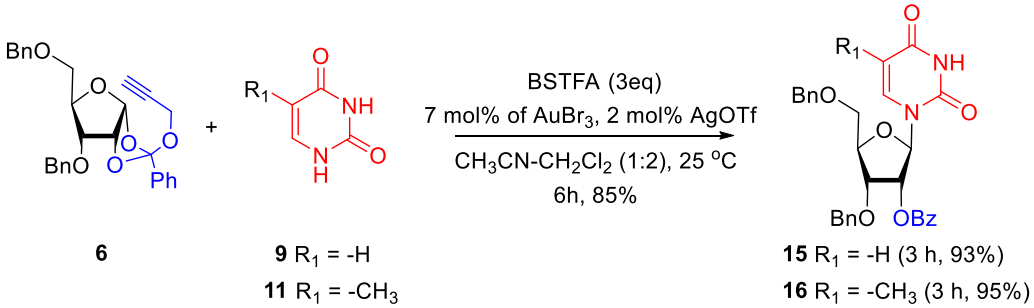
Scheme 2A.15 Gold-catalysed glycosylation for pyrimidine nucleosides

Propargyl orthoester **5** was also subjected to nucleosidation reaction with protected cytosine **13** to give *N*-benzoyl protected cytidine **14** in 85% yield (Scheme 2A.16). In the ^{13}C NMR spectrum of compound **14**, resonances due to the *N*-glycosidic anomeric carbon were identified at δ 97.3 ppm.



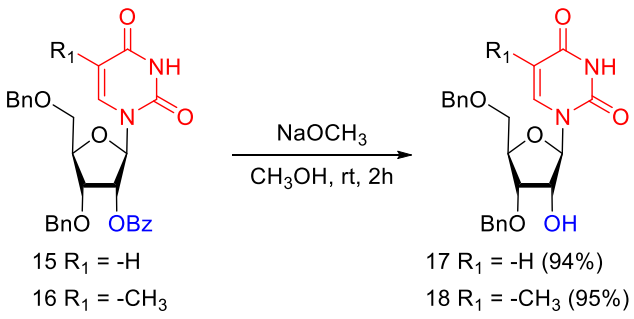
Scheme 2A.16 Gold-catalysed glycosylation for pyrimidine nucleosides

Next, di-*O*-benzyl ribofuranosyl orthoester **6** was treated with uracil **9** or thymine **11** to yield uracil nucleoside **15**[24] and thymine nucleoside **16**[46] in 93% and 95% yields respectively (Scheme 2A.17). In the ^{13}C NMR spectrum of compound **15** and **16**, the characteristic resonances corresponding to *N*-glycoside are noticed at δ 87.9 and 87.4 ppm for the anomeric carbon of compound **15** and **16** respectively.



Scheme 2A.17 Gold-catalysed glycosylation for pyrimidine nucleosides

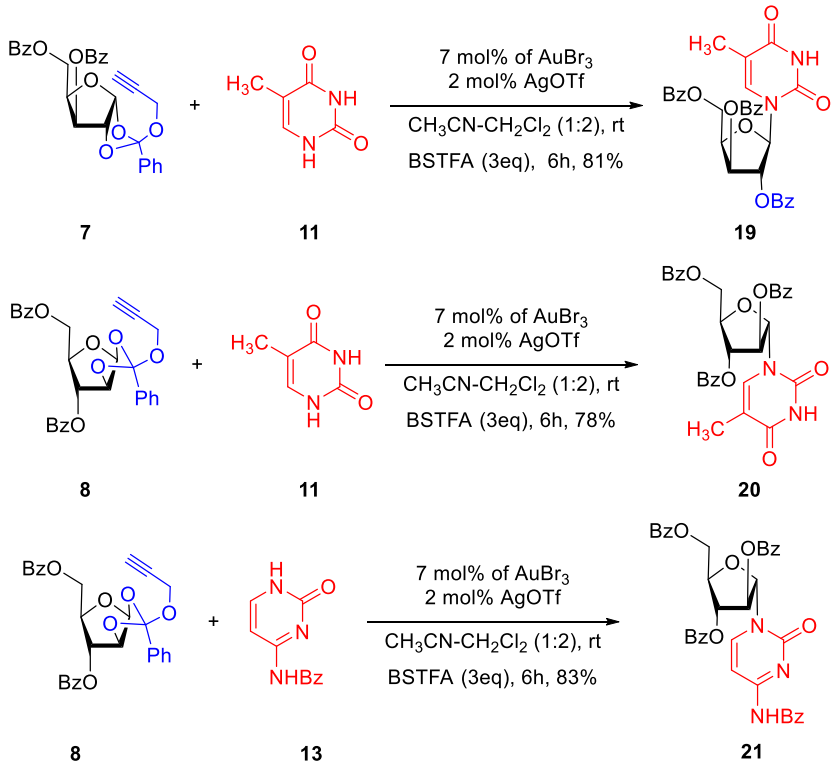
Substituents at the 2′-position of ribonucleotides are very important structurally as well as mechanistic probes for nuclease resistance and ribozyme catalysis.[47] 2′-*O*-Modified nucleosides are converted to 2′-modified nucleotides which can be incorporated into DNA by standard phosphoramidite chemistry. Many modifications such as 2′-*O*-Me, 2′-*O*-ethyl, 2′-*O*-MOM and 2′-F have been utilized in the development of antisense therapeutics;[47] such modifications can be achieved easily using *O*-benzyl orthoester **6**.



Scheme 2A.18 Synthesis of 2′-OH pyrimidine nucleosides

The presence of 2′-benzoate in nucleosides **15** and **16** is beneficial since it can be orthogonally hydrolysed in the presence of benzyl ethers. Accordingly, benzoates **15** and **16** are saponified under Zemplén conditions to obtain nucleosides containing 2′-OH (**17,18**) which can further be extrapolated to various 2′-modified oligonucleotide as antisense therapeutic agents (Scheme 2A.18).

The scope of the reaction was also investigated with other xylo- and arabino- furanosyl orthoester donors **7** and **8**. The nucleosidation reaction of donors **7**[43] and **8**[43] smoothly occurred under aforementioned conditions to obtain nucleosides **19**,[48] **20**,[49] and **21** in very high yields (81,78 and 83%) respectively and in a fully diastereoselective fashion (Scheme 2A.19). In the ^{13}C NMR spectrum of compound **19, 20** and **21,** the characteristic resonances corresponding to 1,2-*trans N*-glycosidic linkages are noticed at δ 88.3, 91.2 and 93.7 ppm for the C-1 of nucleosides **19, 20** and **21** respectively.



Scheme 2A.19 Gold-catalysed glycosylation for pyrimidine nucleosides

The occurrence of the 1,2-*trans* relationship between *C*-1 and *C*-2 was confirmed based on the ^{13}C NMR spectral signatures wherein, the anomeric carbon of the compound **19** was observed at δ 88.3 ppm whereas the anomeric carbon of the nucleoside **20** was observed at δ 91.2 ppm.[46] Compounds **19** and **20** showed 2D-NOESY correlations between H-1 and H-2,3 presumably due to the puckering of the five membered sugar

ring; however, $^{1}J_{C\text{-}H}$ values were observed to be around 171.6, 171.1 Hz respectively in the HSQC spectra, confirming the 1,2-*trans* linkage unambiguously (Figure 2A.5).[46]

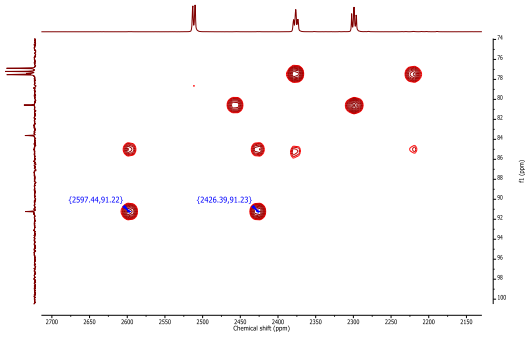
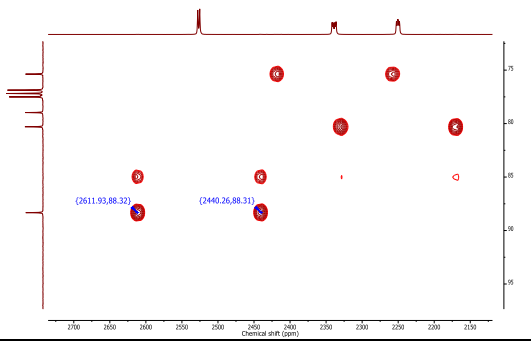
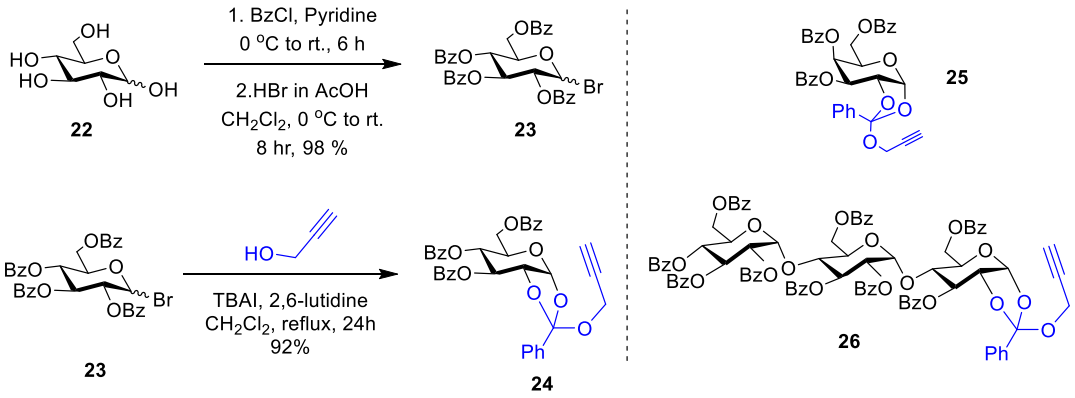
HSQC Spectrum of Compound **19**	HSQC Spectrum of Compound **20**
	

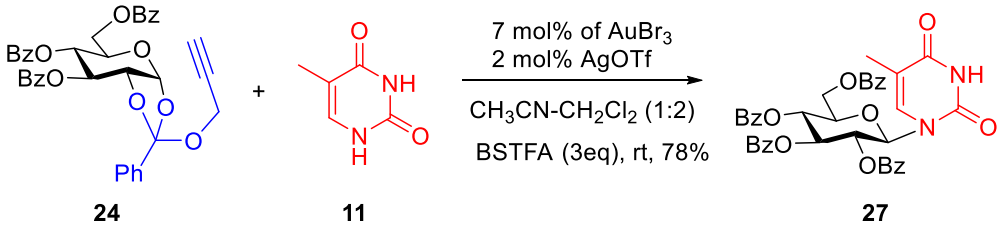
Figure 2A.5 HSQC Spectrum of Compound 19 and 20

The gold-catalyzed nucleosidation protocol for pyrimidine nucleosides was extended to afford pyranosyl nucleosides as well. Pyranose orthoesters were prepared from glucose, galactose and mannose, and maltotriose. First, D-glucose **22** was transformed to per-*O*-benzoylated glucopyranose by treating with benzoyl chloride in pyridine, which was further treated with 33% HBr in AcOH to afford glucopyranosyl bromide **23** which was then subsequently reacted with propargyl alcohol, 2,6-lutidine and a catalytic amount of TBAI in dichloromethane at reflux condition for 24 h to yield propargyl 1,2-orthoester **24** (Scheme 2A.20). In the ^{1}H NMR spectrum of compound **24**, resonances at δ_H 2.40 ppm as triplet and δ_H 3.99 ppm as a doublet clearly indicated the presence of propargyl group and a doublet at δ_H 6.11 ppm (J=5.31 Hz) which is a characteristic for β–configured anomeric proton of the orthoester was observed. Rest of the signals in the spectrum were in complete agreement with that of the assigned structure **24**



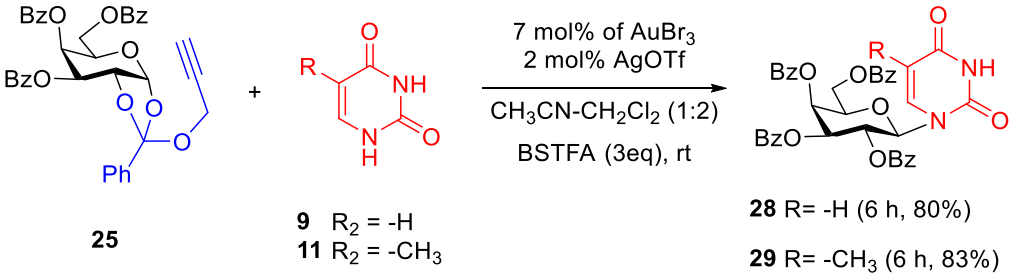
Scheme 2A.20 Synthesis of pyranose 1,2-propargyl orthoesters

In addition, ^{13}C NMR spectrum revealed characteristic signals corresponding to α-configured anomeric carbon and the quaternary carbon at δ 97.7 ppm (C1) and 121.1 ppm respectively were observed while rest of the other resonances were in accordance to the assigned structure 3,4,6-tri-*O*-benzoyl-α-D-glucopyranose-1,2(prop-2-ynyl orthobenzoate) **24**. Propargyl orthoesters of galactose **25** and maltotriose **26** were prepared by using the same procedure as elaborated for compound **24** (Scheme 2A.20). To examine the utility of the gold (III)-catalyzed nucleosidation protocol for the synthesis of pyranosyl nucleoside, glucopyranosyl orthoester **24** was coupled with thymine **11** to produce glucopyranosyl thymine nucleoside **27** using aforementioned optimized conditions (Scheme 2A.21). In the ^{13}C NMR spectrum of compound **27** the characteristic resonances corresponding to *N*-glycosidic anomeric carbon were observed at δ 80.6 ppm.



Scheme 2A.21 Gold-catalysed N-glycosylation of thymine

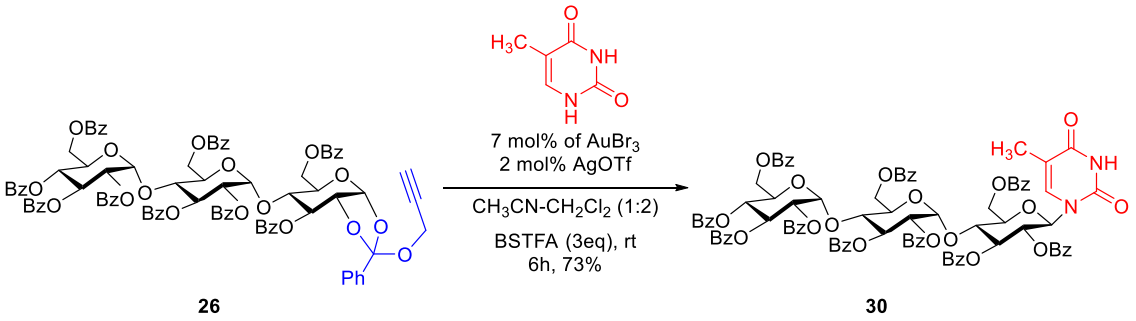
Galactose orthoester **25** was also coupled with uracil and thymine to get galactopyranosyl nucleoside **28** and **29** in 80% and 83% yields respectively (Scheme 2A.22). Characteristic resonances for thymine and uracil *N*-glycosidic carbon were observed δ 80.9 ppm for the compound **28** and **29**.



Scheme 2A.22 Gold-catalysed N-glycosylation of thymine and uracil

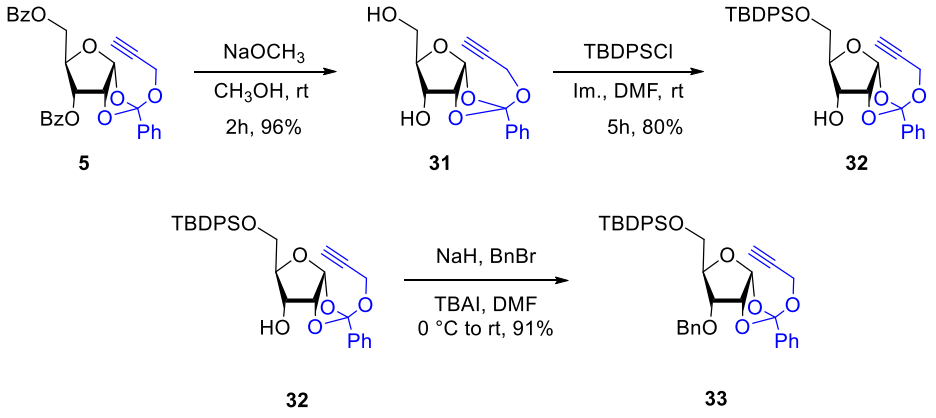
Many *N*-glycosylation procedures work well with monosaccharide-donors and may not yield fruitful results when glycosyl donors of oligosaccharides are employed. In order to check the applicability of the just identified protocol for synthesis of nucleosides for oligosaccharides, propargyl orthoester of maltotriose **26** was synthesized. Nucleosidation between orthoester **26** and the thymine occurred smoothly under aforementioned

conditions to afford nucleoside **30** in 73% yield (Scheme 2A.23). In the ^{13}C NMR spectrum of compound **30**, characteristic resonances due to *N*-glycosidic anomeric carbon at δ 80.2 ppm and two *O*-glycosidic linkages were identified at δ 96.8 and 96.9 ppm.



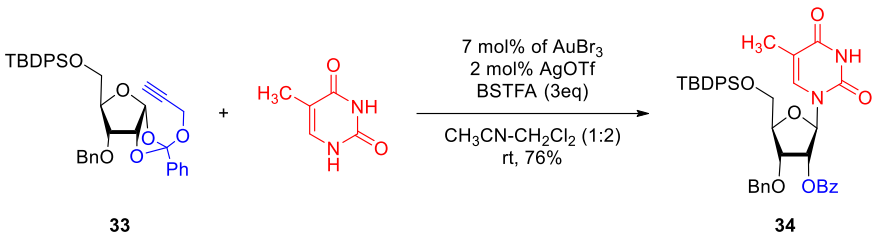
Scheme 2A.23 Gold-catalyzed glycosylation for pyrimidine nucleosides

Next, we turned our attention to the synthesis of partial deprotection of the substitutions on the sugar ring for which orthogonally cleavable protecting groups were needed. Benzyl, benzoate and silyl ethers were identified towards this objective as these protecting groups can be orthogonally installed and deprotected as well. Accordingly, compound **5** was saponified under Zemplén conditions[43] to obtain an orthoester-diol **31** that was treated with one equivalent of TBDPSCl to afford 5-*O*-TBDPS ether **32** in 80% yield which was subsequently converted into the benzyl ether **33** by the use of NaH/BnBr/TBAI/DMF in excellent yield (Scheme 2A.24).



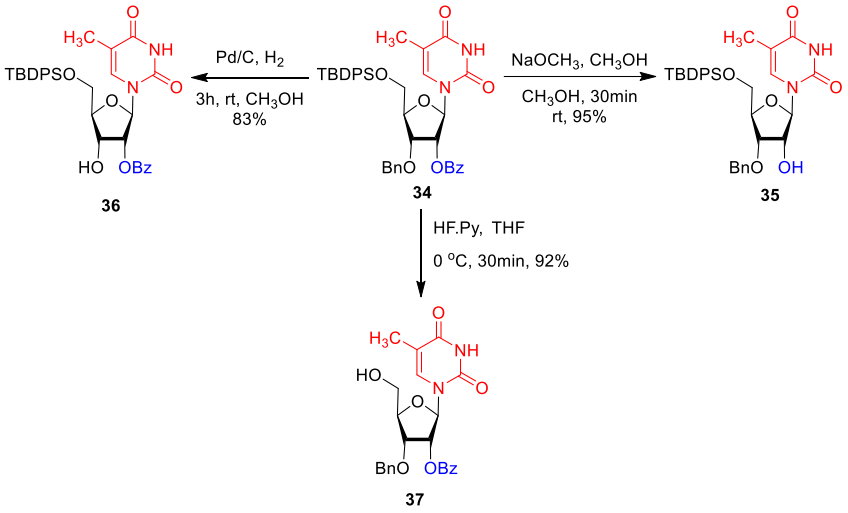
Scheme 2A.24 Preparation of orthogonally protected ribose orthoester.

Thymine nucleoside **34** was easily synthesized from the orthogonally protected orthoester under aforementioned conditions (Scheme 2A.25).



Scheme 2A.25 Preparation of orthogonally protected ribose nucleoside

The saponification of the nucleoside **34** under Zemplén conditions afforded 2′-OH containing nucleoside **35** without affecting the other two protecting group whereas the hydrogenolysis using Pd/C, H_2 in methanol at room temperature afforded the 3′-OH containing nucleoside **36** and finally, the fluoride ion mediated cleavage of the silyl ether moiety furnished the nucleoside with free 5′-OH containing **37** (Scheme 2A.26).



Scheme 2A.26 Synthesis of partial protected ribo-nucleoside

In conclusion, easily accessible propargyl 1,2-orthoesters are shown to be a good synthons for the synthesis of pyrimidine nucleosides under gold(III)-catalysis conditions. The nucleosidation reaction is catalytic, mild, high yielding and enables facile preparation of pyrimidine nucleosides with orthogonally protected 2′-hydroxyl group for further functional group interconversions. However, the procedure is not suitable for the synthesis of purine nucleosides. Thus synthesized nucleosides were deprotected to obtain either 2′, 3′ or 5′ free hydroxyl group in an orthogonal fashion. The gold(III)-catalyzed nucleosidation reaction was shown to be applicable for the synthesis of nucleoside of maltotriose as well.

2A.6 Experimental Section

General procedure for the synthesis of pyrimidine nucleosides

To a solution of nucleobase **9** (100 mg, 0.89 mmol) in acetonitrile was added BSTFA (689 mg, 709 μL, 2.68 mmol) and stirred the solution at 25 ^{0}C until it becomes clear (~30 min) solution. A solution of orthoester **5** (446 mg, 0.89 mmol) in CH_2Cl_2 was added drop wise at 25 ^{0}C, stirred for 5 min and then $AuBr_3$ (27 mg, 0.06 mmol) and AgOTf (4.5 mg, 0.02 mmol) were added and stirred at 25 ^{0}C. After the reaction is complete (adjudged by TLC-MS analysis), the reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using petroleum ether-ethyl acetate as the mobile phase to afford the nucleoside **10** (422 mg, 85%) as a colourless gum.

3,5-Di-*O*-benzoyl-α-D-ribofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**5**): This compound is prepared by employing already reported procedure starting from D-ribose (5.0 g, 33.3 mmol), Yield: (9.3 g, 56% over four steps); $[\alpha]_D^{25}$ = +122.9 (*c* 2.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 2.42 (t, *J* = 2.4 Hz, 1H), 4.09 (dABq, *J* = 3.4, 2.6 Hz, 2H), 4.22 – 4.27 (m, 1H), 4.42 (dd, *J* = 12.3, 4.8 Hz, 1H), 4.64 (dd, *J* = 12.3, 3.3 Hz, 1H), 5.10 (dd, *J* = 9.3, 5.3 Hz, 1H), 5.35 (d, *J* = 4.8 Hz, 1H), 6.27 (d, *J* = 4.2 Hz, 1H), 7.34 – 7.48 (m, 7H), 7.49 – 7.55 (m, 1H), 7.57 – 7.63 (m, 1H), 7.67 – 7.72 (m, 2H), 8.00 (tt, *J* = 8.5, 1.2 Hz, 4H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 51.5, 62.5, 72.9, 74.0, 76.3, 77.8, 79.2, 104.7, 123.6, 126.3, 126.3, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.7, 129.7, 129.7, 129.7, 129.9, 129.9, 133.2, 133.6, 135.9, 165.5, 166.0; IR ($CHCl_3$) 710, 1099, 1271, 1451, 1602, 1725, 2930, 3066, 3291 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{24}O_8Na^+$ 523.1363, Found: 523.1369.

3,5-Di-*O*-benzyl-α-D-ribofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**6**): This compound is prepared from compound **5** (4.0 g, 8 mmol) as the starting material, Yield: (3.5 g, 93%); $[\alpha]_D^{25}$ = +21.1 (*c* 0.7, $CHCl_3$); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 2.36 – 2.41 (m, 1H), 3.47 – 3.55 (m, 1H), 3.69 (d, *J* = 11.3 Hz, 1H), 3.88 – 3.95 (m, 2H), 4.01 – 4.06 (m, 2H), 4.42 – 4.57 (m, 3H), 4.72 – 4.78 (m, 1H), 4.86 – 4.93 (m, 1H), 6.09 (dd, *J* = 4.1, 1.4 Hz, 1H), 7.23 – 7.37 (m, 13H), 7.67 – 7.74 (m, 2H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 51.8, 67.4, 72.2, 73.5, 73.7, 77.1, 77.6, 78.5, 79.7, 104.8, 123.4, 126.6, 126.6, 127.7, 127.8, 127.8, 128.1, 128.1, 128.1, 128.4, 128.4, 128.4, 128.4, 128.5, 128.5, 129.7, 135.5, 137.5, 137.9; IR ($CHCl_3$) 707, 1110, 1262, 1450, 1590, 1722, 2928, 3060, 3290 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{28}NaO_6^+$ 495.1778, Found: 495.1784.

3,5-Di-*O*-benzoyl-α-D-xylofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**7**): This compound is prepared by adopting the reported procedure from D-xylose (5.0 g, 33.3 mmol) as the starting material, Yield: (9.0 g, 53% over four steps); $[\alpha]_D^{25}$ = +0.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 2.40 (t, *J* = 2.5 Hz, 1H), 4.04 – 4.07 (m, 2H), 4.44 – 4.49 (m, 1H), 4.53 – 4.59 (m, 2H), 5.04 (d, *J* = 4.1 Hz, 1H), 5.66 (d, *J* = 3.1 Hz, 1H), 6.37 (d, *J* = 4.1 Hz, 1H), 7.36 – 7.72 (m, 11H), 7.92 – 8.06 (m, 4H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 51.9, 61.7, 74.0, 76.2, 77.9, 79.3, 84.2, 105.4, 122.9, 126.3, 128.4, 128.4, 128.6, 128.6, 128.7, 128.7, 128.9, 129.5, 129.8, 129.8, 129.9, 129.9, 129.9, 130.0, 133.3, 133.9, 135.1, 165.2, 166.1; IR ($CHCl_3$) 706, 1105, 1268, 1447, 1591, 1725, 2930, 3068, 3290 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{24}O_8Na^+$ 523.1363, Found: 523.1368.

3,5-Di-*O*-benzoyl-β-D-arabinofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**8**): This compound is prepared by adopting the reported procedure from D-arabinose (5.0 g, 33.3 mmol) as the starting material, Yield: (9.6 g, 56% over four steps); $[\alpha]_D^{25}$ = -13.83 (*c* 1.00, $CHCl_3$); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 2.39 (t, *J* = 2.6 Hz, 1H), 3.98 (dd, *J* = 2.3, 0.8 Hz, 2H), 4.29 (d, *J* = 7.3 Hz, 2H), 4.66 (t, *J* = 7.2 Hz, 1H), 5.19 (d, *J* = 4.2 Hz, 1H), 5.54 (s, 1H), 6.40 (d, *J* = 4.3 Hz, 1H), 7.38 – 7.47 (m, 7H), 7.49 – 7.55 (m, 1H), 7.56 – 7.61 (m, 1H), 7.66 – 7.71 (m, 2H), 8.00 – 8.06 (m, 4H); 13C NMR (100.53 MHz, $CDCl_3$): δ 52.2, 63.9, 74.0, 77.8, 79.4, 84.6, 85.0, 106.8, 122.9, 126.6, 126.6, 128.4, 128.5, 128.6, 128.6, 128.7, 128.7, 129.0, 129.7, 129.8, 129.9, 129.9, 129.9, 130.2, 133.2, 133.8, 134.3, 165.3, 165.9; IR ($CHCl_3$) 717, 1107, 1268, 1450, 1594, 1723, 2974, 3071, 3293 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{24}O_8Na^+$ 523.1363, Found: 523.1367.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)uracil (**10**): This compound is prepared following the above mentioned general procedure using uracil **9** (0.1 g, 0.89 mmol) as the starting material, Yield: (0.42 g, 85%); $[\alpha]_D^{25}$ = -35.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 4.63 – 4.72 (m, 2H), 4.82 (dd, *J* = 11.8, 2.5 Hz, 1H), 5.60 (dd, *J* = 8.1, 1.6 Hz, 1H), 5.75 (t, *J* = 5.7 Hz, 1H), 5.86 – 5.91 (m, 1H), 6.30 (d, *J* = 5.5 Hz, 1H), 7.31 – 7.60 (m, 10H), 7.89 – 7.98 (m, 4H), 8.05 – 8.11 (m, 2H), 9.50 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 63.9, 71.2, 73.9, 80.6, 88.3, 103.5, 128.4, 128.6, 128.6, 128.6, 128.7, 128.8, 128.8, 128.9, 129.3, 129.7, 129.7, 129.9, 129.9, 130.0, 130.0, 133.7, 133.8, 133.9, 139.8, 150.3, 163.2, 165.4, 165.4, 166.2; IR($CHCl_3$): 2924, 1724, 1455, 1268, 1112, 765 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{30}H_{25}N_2O_9^+$ 557.1555, found: 557.1552.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (**12**): This compound is prepared following the above mentioned general procedure using thymine **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.39 g, 85%); $[\alpha]_D^{25}$ = -76.2 (*c* 1.00,$CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 1.58 (s, 3H), 4.62 – 4.73 (m, 2H), 4.84 – 4.91 (m, 1H), 5.79 (t, *J* = 6.1 Hz, 1H), 5.94 (dd, *J* = 6.0, 3.8 Hz, 1H), 6.45 (d, *J* = 6.3 Hz, 1H), 7.30 – 7.62 (m, 10H), 7.96 (dd, *J* = 11.9, 7.4 Hz, 4H), 8.13 (d, *J* = 7.4 Hz, 2H), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.1, 64.0, 71.5, 73.5, 80.6, 87.1, 112.2, 128.4, 128.5, 128.5, 128.6, 128.6, 128.7, 128.8, 128.9, 129.3, 129.7, 129.7, 129.8, 129.9, 129.9, 130.0, 133.7, 133.7, 133.8, 135.0, 150.6, 163.9, 165.4, 165.4, 166.0; IR($CHCl_3$): 3025, 1725, 1267, 1109, 711 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{27}N_2O_9^+$ 571.1711, found: 571.1723.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-*N*-benzoyl-cytosine (**14**): This compound is prepared following the above mentioned general procedure using cytosine **13** (0.1 g, 0.47 mmol) as the starting material, Yield: (0.25 g, 85%); $[\alpha]_D^{25}$ = -41.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 399.78 MHz): δ 4.63-4.87 (m, 3H), 5.96-6.10 (m, 2H), 6.24 (d, *J* = 2.7 Hz, 1H), 7.36-7.58 (m, 9H), 7.64 (q, *J* = 7.7, 4H), 7.91 (dd, *J* = 13.2, 7.4 Hz, 4H), 8.02 (d, *J* = 7.4 Hz, 4H), 8.33 (d, *J* = 7.3 Hz, 1H), 11.42 (s, 1H); ^{13}C NMR (DMSO-d_6, 100.53MHz): δ 64.2, 71.1, 74.4, 79.6, 92.5, 97.3, 128.9, 129.0, 129.1, 129.1, 129.1, 129.2, 129.2, 129.2, 129.2, 129.3, 129.3, 129.8, 129.8, 129.8, 129.9, 129.9, 129.9, 129.9, 129.9, 133.3, 133.5, 134.1, 134.3, 134.5, 147.9, 154.8, 164.5, 165.1, 165.2, 166.0, 167.9; IR($CHCl_3$): 3021, 1726, 1556, 1483, 1262, 750, 712 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{37}H_{29}NaN_3O_9^+$ 682.1796, found: 682.1801.

1-(2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)uracil (**15**): This compound is prepared following the above mentioned general procedure using uracil **9** (0.1 g, 0.89 mmol) as the starting material, Yield: (0.44 g, 93%); $[\alpha]_D^{25}$ = +63.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 3.59 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.87 (dd, *J* = 10.7, 2.2 Hz, 1H), 4.22 – 4.50 (m, 5H), 4.65 (d, *J* = 11.8 Hz, 1H), 5.37 (d, *J* = 8.2 Hz, 1H), 5.50 – 5.59 (m, 1H), 6.30 (d, *J* = 4.0 Hz, 1H), 7.19 – 7.46 (m, 12H), 7.56 (dd, *J* = 7.2, 1.3 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 8.08 (dt, *J* = 8.3, 1.6 Hz, 2H), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 68.7, 73.3, 73.8, 75.1, 75.8, 77.5, 82.3, 87.9, 102.5, 128.1, 128.1, 128.1, 128.2, 128.2, 128.4, 128.5, 128.5, 128.6, 128.6, 128.7, 128.8, 129.2, 130.1, 130.1, 133.6, 137.3, 140.3, 150.5, 163.8, 165.6; IR($CHCl_3$): 3201, 3062, 2921, 2866, 1721, 1454, 1381,

1266, 1122, 700 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{30}H_{29}N_2O_7^+$ 529.1969, found: 529.1974.

1-(2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)thymine (**16**): This compound is prepared following the above mentioned general procedure using thymine **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.41 g, 95%); $[\alpha]_D^{25}$ = +25.9 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78MHz): δ 1.51 (s, 3H), 3.54 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.83 (dd, *J* = 10.8, 2.0 Hz, 1H), 4.24 – 4.29 (m, 1H), 4.37 (t, *J* = 5.1 Hz, 1H), 4.41 – 4.55 (m, 3H), 4.61 (d, *J* = 11.7 Hz, 1H), 5.51 (t, *J* = 4.8 Hz, 1H), 6.29 (d, *J* = 4.5 Hz, 1H), 7.15 – 7.34 (m, 10H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.48 – 7.59 (m, 2H), 8.04 (d, *J* = 7.6 Hz, 2H), 9.04 (d, *J* = 25.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.53MHz): δ 12.1, 68.9, 73.2, 73.7, 75.0, 76.1, 82.3, 87.4, 111.3, 127.8, 127.8, 128.0, 128.0, 128.1, 128.2, 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 129.1, 130.0, 130.0, 133.6, 135.8, 137.3, 137.3, 150.4, 163.9, 165.6; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{31}N_2O_7^+$ 543.2126, found: 543.2131.

1-(3,5-Di-*O*-benzyl-β-D-ribofuranosyl)uracil (**17**): $[\alpha]_D^{25}$ = +14.8 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 3.55 (dd, *J* = 10.7, 2.0 Hz, 1H), 3.81 (dd, *J* = 10.7, 2.4 Hz, 2H), 4.07 (t, *J* = 5.1 Hz, 1H), 4.22 – 4.29 (m, 2H), 4.46 (s, 2H), 4.53 – 4.72 (m, 2H), 5.33 (d, *J* = 8.1 Hz, 1H), 5.94 (d, *J* = 4.0 Hz, 1H), 7.11 – 7.42 (m, 10H), 7.75 (d, *J* = 8.1 Hz, 1H), 9.77 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 69.1, 72.7, 73.8, 74.1, 76.7, 81.5, 90.1, 102.3, 128.0, 128.0, 128.1, 128.1, 128.3, 128.3, 128.7, 128.7, 128.7, 128.7, 137.1, 137.3, 140.4, 151.0, 163.7; IR($CHCl_3$): 3419, 3060, 2923, 2857, 1692, 1458, 1271, 1119, 699 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{23}H_{25}N_2O_6^+$ 425.1707, found: 425.1712.

1-(3,5-Di-*O*-benzyl-β-D-ribofuranosyl)thymine (**18**): $[\alpha]_D^{25}$ = -10.3 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 1.49 (s, 3H), 3.52 (dd, *J* = 10.7, 1.7 Hz, 1H), 3.79 (dd, *J* = 10.7, 2.1 Hz, 1H), 4.08 (t, *J* = 4.8 Hz, 1H), 4.21 – 4.25 (m, 1H), 4.27 (t, *J* = 4.9 Hz, 1H), 4.44 – 4.53 (m, 2H), 4.54 – 4.71 (m, 2H), 5.94 (d, *J* = 4.5 Hz, 1H), 7.18 – 7.34 (m, 11H), 7.49 (s, 1H), 9.60 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.1, 69.4, 72.7, 73.7, 74.1, 77.0, 77.4, 81.6, 89.7, 111.1, 127.7, 127.7, 128.1, 128.1, 128.2, 128.3, 128.7, 128.7, 128.7, 136.0, 137.2, 137.4, 151.0, 164.2; IR($CHCl_3$): 3421, 3032, 2924, 1694, 1469, 1118 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{24}H_{26}N_2NaO_6^+$ 461.1683, found:461.1689.

1-(2,3,5-Tri-*O*-benzoyl-β-D-xylofuranosyl)thymine (**19**): This compound is prepared following the above mentioned general procedure using nucleobase **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.37 g, 81%); $[\alpha]_D^{25}$ = 49.5 (*c* 1.00, $CHCl_3$); ^1H

NMR ($CDCl_3$, 399.78 MHz): δ 1.76 (d, *J* = 0.9 Hz, 3H), 4.68 – 4.79 (m, 2H), 4.83 (dt, *J* = 6.1, 4.1 Hz, 1H), 5.64 (dd, *J* = 2.5, 1.8 Hz, 1H), 5.86 (dd, *J* = 3.9, 1.6 Hz, 1H), 6.33 (d, *J* = 2.6 Hz, 1H), 7.39 (dt, *J* = 19.9, 7.7 Hz, 6H), 7.47 – 7.61 (m, 4H), 7.97 (d, *J* = 7.6 Hz, 4H), 8.02 – 8.06 (m, 2H), 9.75 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.5, 61.7, 75.4, 79.0, 80.3, 88.3, 111.7, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.7, 128.9, 129.3, 129.8, 129.8, 129.8, 129.9, 130.1, 130.2, 133.5, 134.0, 134.2, 135.1, 150.4, 164.0, 164.8, 164.9, 166.2; IR($CHCl_3$): 3201, 3023, 1725, 1458, 1209, 1101, 760 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{27}N_2O_9^+$ 571.1711, found: 571.1720.

1-(2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl)thymine (**20**): This compound is prepared following the above mentioned general procedure using nucleobase **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.35 g, 78%); $[\alpha]_D^{25}$ = 0.8 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 1.90 (d, *J* = 0.6 Hz, 3H), 4.63 – 4.78 (m, 2H), 4.99 (q, *J* = 5.1 Hz, 1H), 5.77 (t, *J* = 3.0 Hz, 1H), 5.96 (t, *J* = 2.9 Hz, 1H), 6.30 (d, *J* = 3.1 Hz, 1H), 7.29 – 7.32 (m, 1H), 7.41 (dt, *J* = 24.6, 7.5 Hz, 6H), 7.52 – 7.62 (m, 3H), 8.02 (td, *J* = 7.9, 1.4 Hz, 4H), 8.08 (dd, *J* = 8.7, 1.1 Hz, 2H), 9.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.7, 63.9, 77.4, 80.6, 83.6, 91.2, 111.4, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.8, 128.8, 129.5, 129.9, 129.9, 129.9, 129.9, 130.1, 130.1, 133.4, 134.0, 134.0, 136.1, 150.5, 164.2, 165.3, 165.4, 166.2; IR($CHCl_3$): 3198, 3023, 1723, 1266, 1103, 763, 711 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{27}N_2O9^+$ 571.1711, found: 571.1723.

1-(2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl)-4-*N*-benzoyl-cytosine (**21**): This compound is prepared following the above mentioned general procedure using cytosine **14** (0.1 g, 0.47 mmol) as the starting material, Yield: (0.25 g, 83%); $[\alpha]_D^{25}$ = -14.7 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 4.61–4.78 (m, 2H), 5.05–5.11 (m, 1H), 5.72 (t, *J* = 2.4 Hz, 1H), 6.04 (t, *J* = 1.6 Hz, 1H), 6.28 (d, *J* = 1.5 Hz, 1H), 7.32–7.62 (m, 13H), 7.88–7.95 (m, 5H), 8.01–8.10 (m, 4H), 9.36 (brs, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 63.9, 77.4, 80.7, 85.3, 93.7, 127.8, 128.5, 128.5, 128.5, 128.6, 128.7, 128.7, 128.7, 128.7, 129.1, 129.1, 129.5, 129.9, 129.9, 129.9, 130.0, 130.0, 130.1, 130.1, 133.0, 133.4, 133.4, 133.9, 133.9, 134.0, 134.0, 145.1, 154.8, 163.1, 165.1, 165.2, 166.2; IR($CHCl_3$): 3126, 1723, 1485, 1258, 1104, 751, 711 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{37}H_{29}NaN_3O_9^+$ 682.1796, found: 571.1798.

3,4,6-Tri-*O*-benzoyl-α-D-glucopyranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**24**): This compound is prepared from D-glucose (5.0 g, 27.7 mmol) as the starting material,

Yield: (11.8 g, 83% over three steps); $[\alpha]_D^{25}$ = -5.94 (*c* 1.00, $CHCl_3$); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 2.39 (t, *J* = 2.5 Hz, 1H), 3.89 – 4.05 (m, 2H), 4.16 (ddd, *J* = 8.1, 4.6, 3.0 Hz, 1H), 4.40 (dd, *J* = 12.1, 4.8 Hz, 1H), 4.55 (dd, *J* = 12.1, 2.9 Hz, 1H), 4.88 (ddd, *J* = 5.4, 3.0, 1.1 Hz, 1H), 5.53 (dt, *J* = 8.8, 1.1 Hz, 1H), 5.78 (dd, *J* = 3.0, 1.3 Hz, 1H), 6.11 (d, *J* = 5.3 Hz, 1H), 7.21 – 7.28 (m, 2H), 7.39 – 7.50 (m, 8H), 7.54 – 7.64 (m, 2H), 7.77 – 7.82 (m, 2H), 7.95 (ddd, *J* = 9.2, 7.4, 1.3 Hz, 4H), 8.09 (dd, *J* = 8.5, 1.2 Hz, 2H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 52.5, 64.0, 67.6, 68.5, 69.1, 72.1, 74.1, 79.3, 97.9, 121.3, 126.6, 126.6, 128.3, 128.4, 128.6, 128.6, 128.6, 128.6, 128.7, 128.7, 129.0, 129.1, 129.7, 129.7, 129.8, 130.0, 130.0, 130.1, 130.1, 130.2, 133.1, 133.7, 133.8, 134.1, 164.7, 165.3, 166.1; IR($CHCl_3$): 3743, 2339, 1719, 1249, 1092, 1019, 703 cm^{-1} HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{37}H_{30}NaO_{10}^+$ 657.1731, Found: 657.1737.

3,4,6-Tri-*O*-benzoyl-α-D-galactopyranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**25**): This compound is prepared from D-galactose (5.0 g, 27.7 mmol) as the starting material, Yield: (10.7 g, 75% over three steps); $[\alpha]_D^{25}$ = +70.53 (*c* 1.00, $CHCl_3$); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 2.41 (t, *J* = 2.3 Hz, 1H), 4.06 (d, *J* = 2.4 Hz, 2H), 4.41 (dd, *J* = 10.9, 5.1 Hz, 1H), 4.53 – 4.59 (m, 1H), 4.60 – 4.68 (m, 1H), 4.86 (t, *J* = 5.5 Hz, 1H), 5.62 (d, *J* = 5.9 Hz, 1H), 5.82 – 5.88 (m, 1H), 6.28 (d, *J* = 5.1 Hz, 1H), 7.31 – 7.43 (m, 11H), 7.47 – 7.53 (m, 3H), 7.70 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.90 – 7.98 (m, 4H); ^{13}C NMR (100.53 MHz, $CDCl_3$): 52.1, 62.3, 66.4, 69.0, 70.0, 73.5, 74.0, 79.3, 98.4, 120.2, 126.1, 126.2, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.8, 129.0, 129.4, 129.7, 129.7, 129.7, 129.7, 129.8, 129.8, 130.0, 133.2, 133.4, 133.6, 135.2, 165.2, 165.2, 165.9; IR ($CHCl_3$) 709, 1097, 1269, 1727, 2400, 2950, 3300 cm^{-1};HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{37}H_{30}NaO_{10}^+$ 657.1731, Found: 657.1739.

1-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)thymine (**27**): This compound is prepared following the above mentioned general procedure using nucleobase **9** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.44 g, 78%); $[\alpha]_D^{25}$ = +5.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$,399.78 MHz): δ 1.93 (d, *J* = 1.2 Hz, 3H), 4.39 (ddd, *J* = 10.0, 5.0, 2.7 Hz, 1H), 4.48 (dd, *J* = 12.4, 5.1 Hz, 1H), 4.67 (dd, *J* = 12.4, 2.7 Hz, 1H), 5.67 (t, *J* = 9.5 Hz, 1H), 5.78 (t, *J* = 9.8 Hz, 1H), 6.08 (t, *J* = 9.7 Hz, 1H), 6.26 (d, *J* = 9.5 Hz, 1H), 7.27 – 7.60 (m, 13H), 7.78 – 7.94 (m, 6H), 8.00 – 8.06 (m, 2H), 8.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.7, 62.7, 68.9, 70.2, 73.0, 75.4, 77.3, 80.6, 112.3, 128.0, 128.5, 128.5, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 129.4, 129.8, 129.8, 129.9, 129.9, 129.9, 130.0,

130.0, 130.1, 130.1, 133.4, 133.5, 133.8, 133.9, 134.6, 150.3, 163.1, 165.3, 165.3, 165.5, 166.1; IR($CHCl_3$): 1727, 1456, 1267, 1088, 711 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{39}H_{33}N_2O_{11}^+$ 705.2079, found:705.2091.

1-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)uracil (**28**): This compound is prepared following the above mentioned general procedure using nucleobase **9** (0.1 g, 0.89 mmol) as the starting material, Yield: (0.50 g, 80%); $[\alpha]_D^{25}$ = 123.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 4.47 (dd, *J* = 11.3, 5.5 Hz, 1H), 4.58 – 4.74 (m, 2H), 5.87 – 5.97 (m, 3H), 6.15 (s, 1H), 6.37 (p, *J* = 6.4 Hz, 1H), 7.25 (dt, *J* = 21.5, 7.8 Hz, 4H), 7.34 – 7.44 (m, 4H), 7.47 – 7.67 (m, 5H), 7.75 – 7.80 (m, 2H), 7.84 – 7.88 (m, 2H), 7.93 – 8.02 (m, 2H), 8.06 (dd, *J* = 7.8, 1.3 Hz, 2H), 9.72 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 62.2, 68.2, 68.4, 71.8, 74.5, 80.9, 104.1, 128.1, 128.5, 128.5, 128.6, 128.6, 128.6, 128.6, 128.7, 129.0, 129.0, 129.0, 129.2, 129.8, 129.8, 129.9, 129.9, 129.9, 129.9, 130.0, 130.0, 133.5, 133.6, 133.9, 134.0, 139.3, 150.6, 163.0, 165.4, 165.5, 165.6, 166.1; IR($CHCl_3$): 3436, 3067, 1728, 1455, 1270, 1102, 1025, 762 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{38}H_{31}N_2O_{11}^+$ 691.1922, found: 691.1929.

1-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)thymine (**29**): This compound is prepared following the above mentioned general procedure using nucleobase **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.47 g, 83%); $[\alpha]_D^{25}$ = 58.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 1.99 – 2.03 (m, 3H), 4.43 – 4.51 (m, 1H), 4.56 – 4.67 (m, 2H), 5.81 – 5.96 (m, 2H), 6.11 (d, *J* = 3.2 Hz, 1H), 6.31 (d, *J* = 9.1 Hz, 1H), 7.18 – 7.29 (m, 2H), 7.30 (t, *J* = 7.8 Hz, 2H), 7.33 – 7.47 (m, 5H), 7.48 – 7.56 (m, 3H), 7.66 (tt, *J* = 7.0, 1.2 Hz, 1H), 7.76 – 7.81 (m, 2H), 7.84 – 7.88 (m, 2H), 7.95 – 8.01 (m, 2H), 8.03 – 8.09 (m, 2H), 9.29 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.9, 62.2, 68.2, 68.4, 71.9, 74.4, 77.4, 80.9, 112.4, 128.2, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.9, 128.9, 129.1, 129.2, 129.8, 129.8, 129.9, 129.9, 129.9, 129.9, 130.0, 130.0, 133.4, 133.6, 133.8, 134.0, 134.7, 150.6, 163.5, 165.3, 165.4, 165.6, 166.1; IR($CHCl_3$): 3262, 3066, 1726, 1457, 1268, 1103, 763, 711 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{39}H_{33}N_2O_{11}^+$ 705.2079, found: 705.2083.

1-(2,3,6-Tri-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl α-D-glucopyranosyl)-α-D-glucopyranosyl] β-D-glucopyranosyl)thymine (**30**): This compound is prepared following the above mentioned general procedure using nucleobase **11** (0.1 g, 0.79 mmol) as the starting material, Yield: (0.92 g, 70%); $[\alpha]_D^{25}$ =

+59.2 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 1.84 (d, *J* = 0.7 Hz, 3H), 4.21 – 4.29 (m, 2H), 4.35 – 4.51 (m, 5H), 4.62 – 4.72 (m, 2H), 4.82 (dd, *J* = 12.2, 1.4 Hz, 1H), 5.02 (dd, *J* = 12.2, 1.6 Hz, 1H), 5.12 (dd, *J* = 9.9, 3.9 Hz, 1H), 5.28 (dd, *J* = 10.5, 3.9 Hz, 1H), 5.39 (t, *J* = 9.5 Hz, 1H), 5.64 (d, *J* = 3.9 Hz, 1H), 5.69 (t, *J* = 9.7 Hz, 1H), 5.79 (d, *J* = 3.9 Hz, 1H), 5.82 – 5.95 (m, 2H), 6.12 (t, *J* = 9.9 Hz, 2H), 7.07 – 7.25 (m, 12H), 7.29 – 7.67 (m, 25H), 7.67 – 7.77 (m, 6H), 7.87 (dd, *J* = 8.3, 1.1 Hz, 2H), 7.96 (dd, *J* = 8.3, 1.1 Hz, 2H), 8.03 – 8.08 (m, 2H), 8.14 – 8.20 (m, 2H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.6, 62.4, 62.8, 63.0, 69.1, 69.3, 70.0, 70.5, 70.6, 70.7, 71.0, 71.7, 73.3, 73.7, 75.1, 76.0, 77.4, 80.2, 96.8, 96.9, 112.1, 127.9, 128.1, 128.1, 128.2, 128.2, 128.2, 128.2, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 128.7, 128.7, 128.7, 128.8, 128.8, 128.9, 129.1, 129.4, 129.5, 129.6, 129.6, 129.7, 129.7, 129.7, 129.7, 129.8, 129.8, 129.8, 129.9, 129.9, 129.9, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 130.1, 130.1, 130.1, 130.2, 133.1, 133.1, 133.2, 133.3, 133.4, 133.4, 133.5, 133.6, 133.7, 133.7, 134.6, 150.2, 163.2, 164.7, 164.8, 165.2, 165.4, 165.4, 165.7, 165.7, 165.9, 166.0, 166.1; IR($CHCl_3$): 3598, 2925, 2853, 2361, 1732, 1415, 1315, 1269, 1095, 1028, 709 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{93}H_{76}N_2O_{27}Na^+$ 1675.4528, found: 1675.4519.

β-D-Ribofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**31**): This compound is prepared from orthoester **5** (5.0 g, 10.0 mmol) as the starting material, Yield: (2.8 g, 96%); $[\alpha]_D^{25}$ = +36.1 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$,399.78 MHz): δ 2.42 (t, *J* = 2.4 Hz, 1H), 3.25 (bs, 2H), 3.50 – 3.60 (m, 2H), 3.69 – 3.79 (m, 1H), 3.97 (dd, *J* = 8.3, 5.4 Hz, 1H), 4.01 (d, *J* = 2.4 Hz, 2H), 4.78 (t, *J* = 4.4, 3.9 Hz, 1H), 5.99 (d, *J* = 4.0 Hz, 1H), 7.32 – 7.40 (m, 3H), 7.64 (dd, *J* = 6.7, 2.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 51.6, 59.8, 70.3, 73.9, 79.2, 79.5, 80.5, 104.0, 122.9, 126.0, 126.1, 128.2, 128.3, 129.6, 135.0; IR($CHCl_3$): 770, 960, 1039, 1291, 1451, 1537, 1641, 2352, 2928, 3285, 3397 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{15}H_{16}NaO_6^+$ 315.0839, Found: 315.0839.

5-*O*-*tert*-Butyldiphenylsilyl-β-D-arabinofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**32**): This compound is prepared from compound **31** (2.8 g, 9.6 mmol) as the starting material, Yield: (4.1 g, 80%); $[\alpha]_D^{25}$ = +21.7 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$,399.78 MHz): δ 1.06 (d, *J* = 1.6 Hz, 9H), 2.18 (bs, 1H), 2.42 (td, *J* = 2.5, 1.0 Hz, 1H), 3.53 – 3.60 (m, 1H), 3.78 (ddd, *J* = 11.8, 3.8, 1.1 Hz, 1H), 3.88 – 3.94 (m, 1H), 4.12 (ddd, *J* = 5.9, 2.3, 0.8 Hz, 2H), 4.15 – 4.21 (m, 1H), 4.93 (t, *J* = 4.7 Hz, 1H), 6.15 (d, *J* = 4.1 Hz, 1H), 7.36 – 7.44 (m, 10H), 7.64 – 7.71 (m, 5H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 19.4, 26.9,

26.9, 26.9, 51.8, 62.0, 71.2, 73.9, 79.5, 79.7, 81.7, 104.6, 123.3, 126.3, 126.3, 127.8, 127.8, 127.8, 127.8, 128.6, 128.6, 129.8, 129.8, 129.9, 133.2, 133.3, 134.9, 135.6, 135.7, 135.7, 135.7; IR($CHCl_3$): 700, 771, 1039, 1109, 1285, 1455, 1722, 2353, 2864, 2935, 3062, 3289, 3546 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{31}H_{34}O_6NaSi^+$ 553.2017, Found: 553.2022.

2-*O*-Benzoyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranoside (prop-2-yn-1-yl)-1,2-orthobenzoate (**33**): This compound is prepared from compound **32** (4.1 g, 7.7 mmol) as the starting material, Yield: (4.4 g, 91%); $[\alpha]_D^{25}$ = +66.7 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$,399.78 MHz): δ 1.15 (s, 9H), 2.50 (t, *J* = 2.5 Hz, 1H), 3.89 (dd, *J* = 11.8, 3.0 Hz, 1H), 3.98 (dt, *J* = 8.9, 2.4 Hz, 1H), 4.05 (dd, *J* = 11.8, 1.4 Hz, 1H), 4.19 – 4.21 (m, 2H), 4.24 (dd, *J* = 8.9, 4.7 Hz, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.93 (d, *J* = 11.7 Hz, 1H), 5.07 (t, *J* = 4.4 Hz, 1H), 6.23 (d, *J* = 4.1 Hz, 1H), 7.41 – 7.52 (m, 13H), 7.74 – 7.80 (m, 5H), 7.83 – 7.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 19.4, 26.8, 26.8, 26.9, 51.6, 61.3, 72.2, 73.6, 76.6, 77.4, 77.9, 79.9, 104.8, 123.4, 126.5, 126.5, 127.7, 127.7, 127.7, 127.7, 127.9, 127.9, 128.0, 128.3, 128.5, 128.5, 129.5, 129.7, 129.7, 133.1, 133.4, 134.9, 135.5, 135.5, 135.6, 135.6, 135.8, 137.6; IR($CHCl_3$): 1046, 1108, 1290, 1455, 2334, 2359, 2856, 2927, 2959, 3036, 3067, 3291 cm^{-1}; HRMS (TOF) *m/z* $[M + Na]^+$ calcd for $C_{38}H_{40}NaO_6Si^+$ 643.2486, Found: 643.2491.

1-(2-*O*-Benzoyl-3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)thymine (**34**): This compound is prepared from compound **33** (1.0 g, 1.6 mmol) as the starting material, Yield: (0.9 g, 76%); $[\alpha]_D^{25}$ = +12.6 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 399.78 MHz): δ 1.14 (s, 9H), 1.59 (d, *J* = 0.7 Hz, 3H), 3.73 (dd, *J* = 11.7, 2.1 Hz, 1H), 4.03 (dd, *J* = 11.7, 1.9 Hz, 1H), 4.19 – 4.23 (m, 1H), 4.40 – 4.55 (m, 2H), 4.64 (d, *J* = 11.6 Hz, 1H), 5.50 (t, *J* = 5.9 Hz, 1H), 6.47 (d, *J* = 6.2 Hz, 1H), 7.16 – 7.25 (m, 5H), 7.38 – 7.49 (m, 9H), 7.56 – 7.63 (m, 1H), 7.66 – 7.73 (m, 4H), 8.03 – 8.17 (m, 2H), 9.13 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.1, 19.5, 27.2, 27.2, 27.2, 63.6, 73.4, 75.0, 76.2, 77.4, 84.0, 86.2, 111.8, 128.0, 128.1, 128.1, 128.1, 128.1, 128.2, 128.5, 128.5, 128.7, 128.7, 129.1, 130.2, 130.2, 130.3, 132.3, 132.9, 133.7, 135.2, 135.4, 135.4, 135.7, 135.7, 137.3, 150.6, 163.9, 165.8; IR($CHCl_3$):3196, 3068, 2930, 2857, 1706, 1466, 1427, 1267, 1111, 704 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{40}H_{43}N_2O_7Si^+$ 691.2834, found: 691.2844.

1-(3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)thymine (**35**): This compound is prepared from compound **34** (0.1 g, 0.14 mmol) as the starting material, Yield: (81 mg, 95%); $[\alpha]_D^{25}$ = +14.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 1.08 (s, 9H), 1.59 (d, *J* = 1.1 Hz, 3H), 3.34 (s, 1H), 3.65 (dd, *J* = 11.5, 2.0 Hz, 1H), 3.94 (dd, *J* = 11.6, 2.1 Hz, 1H), 4.10 – 4.16 (m, 2H), 4.25 (s, 1H), 4.62 (q, *J* = 11.7 Hz, 2H), 6.01 (d, *J* = 6.4 Hz, 1H), 7.28 – 7.49 (m, 12H), 7.57 – 7.67 (m, 4H), 8.86 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.1, 19.5, 27.1, 27.1, 27.2, 63.9, 73.0, 74.2, 77.4, 83.1, 88.5, 111.6, 128.1, 128.1, 128.2, 128.2, 128.3, 128.3, 128.5, 128.8, 128.8, 130.2, 130.3, 132.4, 132.9, 135.3, 135.4, 135.4, 135.6, 135.7, 136.9, 150.9, 163.7; IR($CHCl_3$): 3187, 3068, 2928, 2856, 1696, 1468, 1427, 1267, 1112, 703 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{33}H_{39}N_2O_6Si^+$ 587.2572, Found: 587.2577.

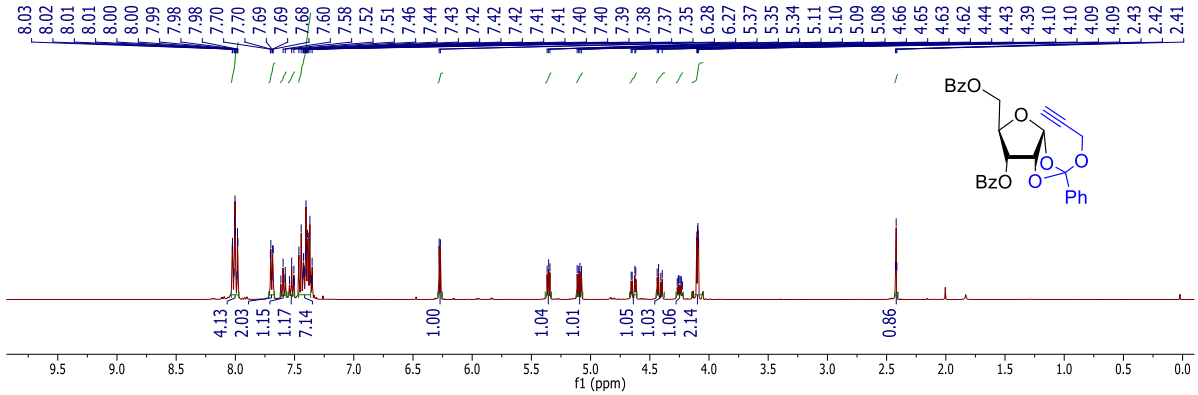
1-(2-*O*-Benzoyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl)thymine (**36**): This compound is prepared from compound **34** (0.1 g, 0.14 mmol) as the starting material, Yield: (72 mg, 83%); $[\alpha]_D^{25}$ = -19.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 1.11 (s, 9H), 1.49 (d, *J* = 0.9 Hz, 3H), 4.02 – 4.12 (m, 2H), 4.32 (d, *J* = 2.3 Hz, 1H), 4.42 (d, *J* = 9.1 Hz, 1H), 4.46 – 4.58 (m, 1H), 5.54 (dd, *J* = 5.7, 2.5 Hz, 1H), 6.33 (d, *J* = 7.1 Hz, 1H), 7.33 – 7.48 (m, 8H), 7.49 – 7.61 (m, 2H), 7.62 – 7.75 (m, 4H), 8.16 (dd, *J* = 8.2, 1.2 Hz, 2H), 9.85 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.0, 19.5, 27.1, 27.1, 27.2, 63.9, 73.4, 74.5, 77.3, 83.8, 87.6, 112.1, 128.1, 128.1, 128.2, 128.2, 128.5, 129.3, 130.2, 130.2, 130.2, 130.3, 132.0, 133.0, 133.6, 135.1, 135.3, 135.3, 135.6, 135.6, 151.4, 164.2, 166.3; IR($CHCl_3$): 3623, 2927, 2855, 2413, 2116, 1650, 1268, 1110, 772 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{33}H_{37}N_2O_7Si^+$ 601.2365, found: 601.2370.

1-(2-*O*-Benzoyl-3-*O*-benzyl-β-D-ribofuranosyl)thymine (**37**): This compound is prepared from compound **34** (0.1 g, 0.14 mmol) as the starting material, Yield: (60 mg, 92%); $[\alpha]_D^{25}$ = -15.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 399.78 MHz): δ 1.91 (d, *J* = 1.0 Hz, 3H), 2.61 (s, 1H), 3.74 (dd, *J* = 12.7, 2.5 Hz, 1H), 3.97 (dd, *J* = 12.2, 2.2 Hz, 1H), 4.23 (dt, *J* = 5.2, 2.4 Hz, 1H), 4.48 – 4.59 (m, 2H), 4.65 (d, *J* = 11.4 Hz, 1H), 5.65 (dd, *J* = 5.5, 4.6 Hz, 1H), 5.96 (d, *J* = 4.5 Hz, 1H), 7.24 (s, 5H), 7.35 (d, *J* = 1.3 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.54 – 7.65 (m, 1H), 8.07 (dd, *J* = 8.2, 1.3 Hz, 2H), 8.83 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.53 MHz): δ 12.6, 61.7, 73.5, 74.6, 75.7, 83.7, 90.9, 111.5, 128.1, 128.1, 128.2, 128.5, 128.5, 128.6, 128.6, 129.1, 130.0, 130.1, 133.7, 137.3, 137.5, 150.3,

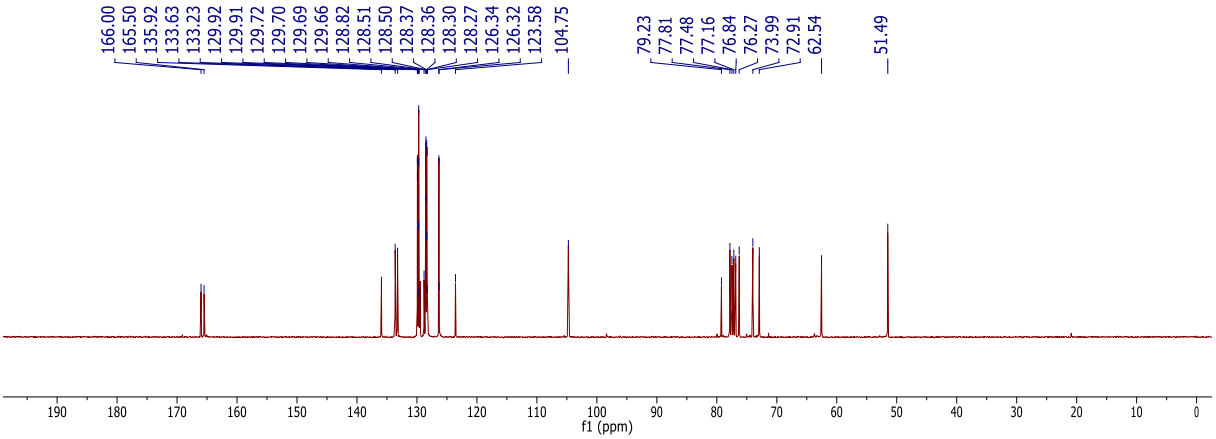
163.7, 165.9; IR($CHCl_3$): 3649, 2924, 2853, 2101, 1660, 1455, 1266, 1115, 762 cm^{-1}; HRMS (TOF) *m/z* $[M + H]^+$ calcd for $C_{24}H_{25}N_2O_7^+$ 453.1656, found: 453.1662.

2A.7 Spectral Charts

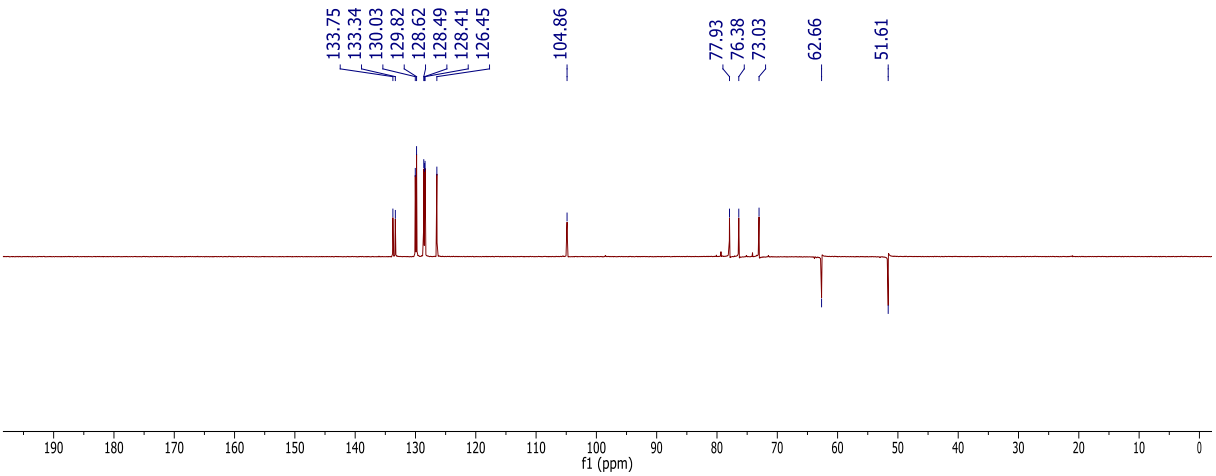
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **5**



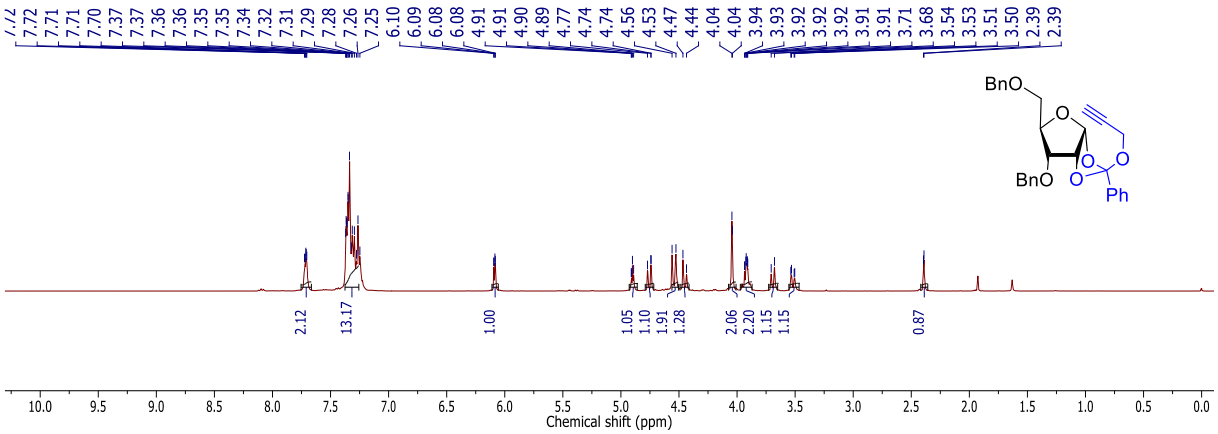
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **5**



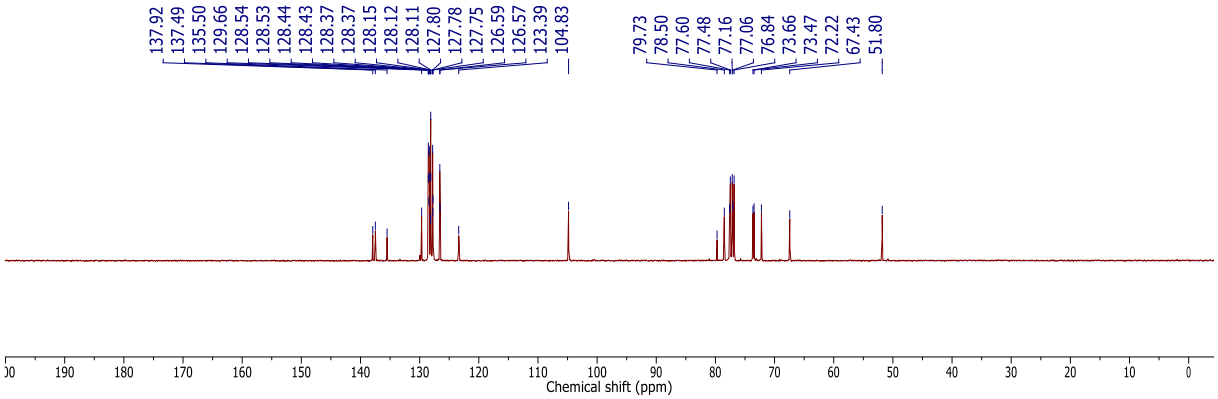
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **5**



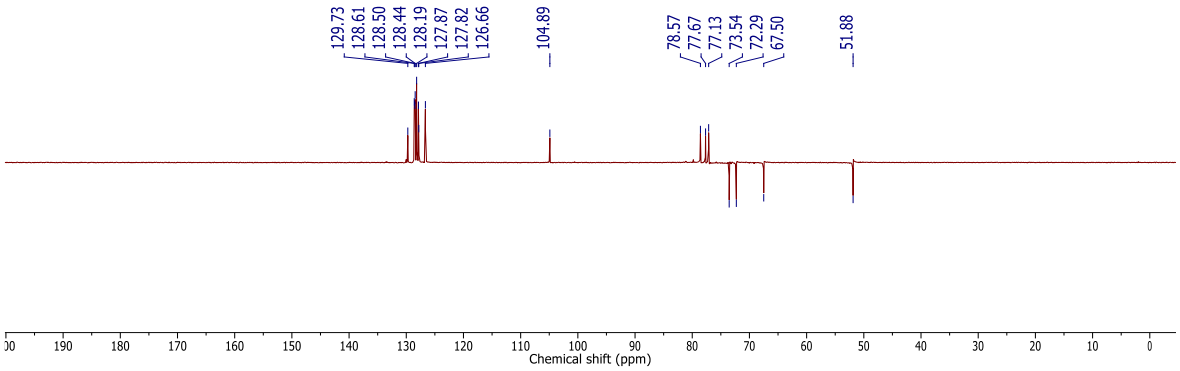
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **6**



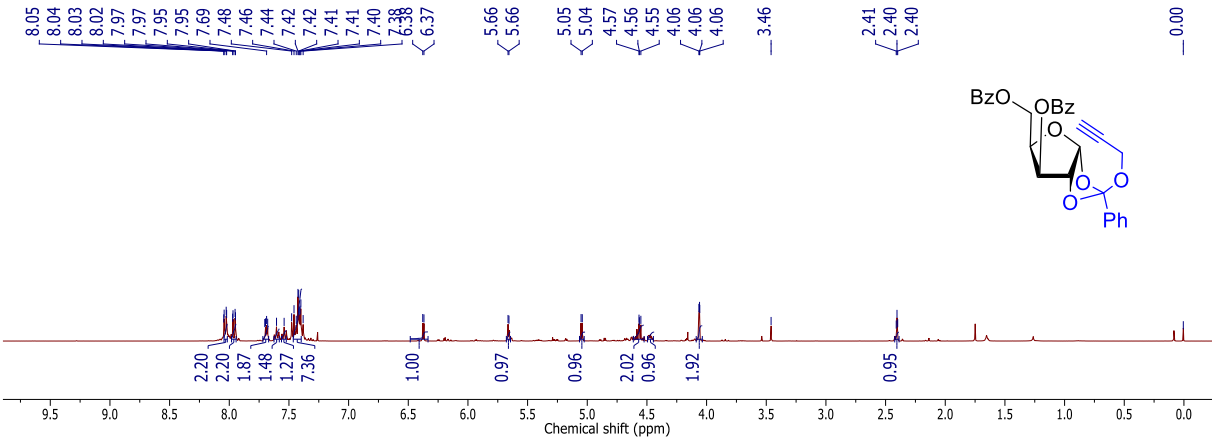
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **6**



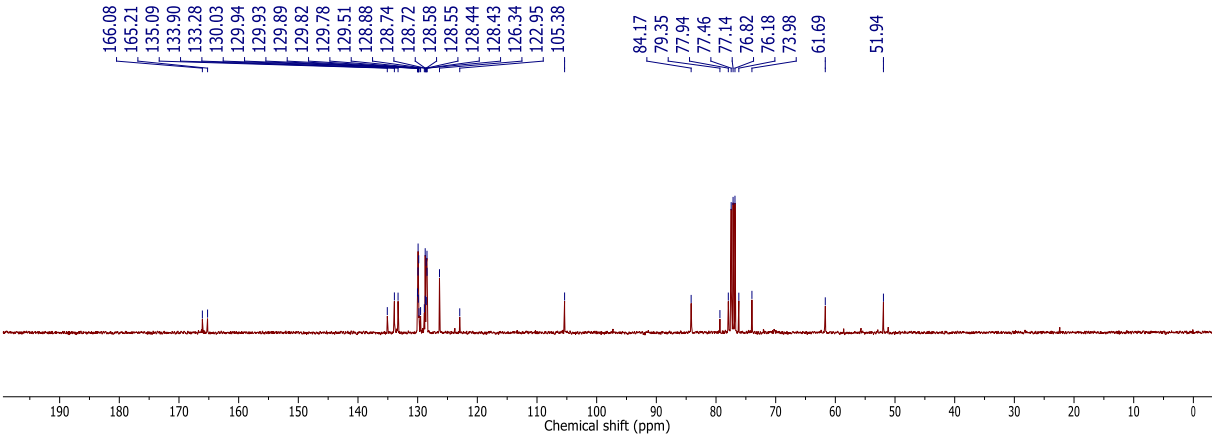
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **6**



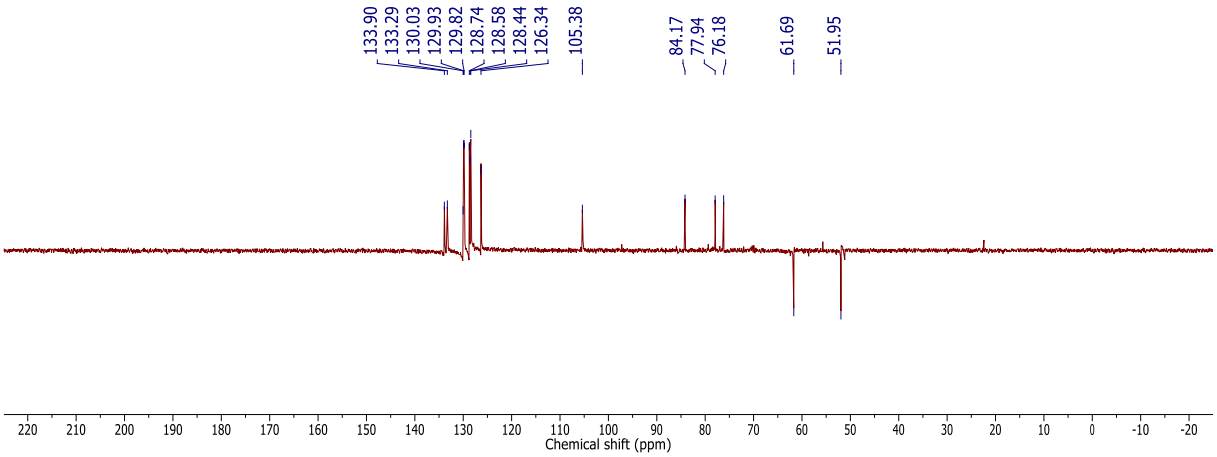
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **7**



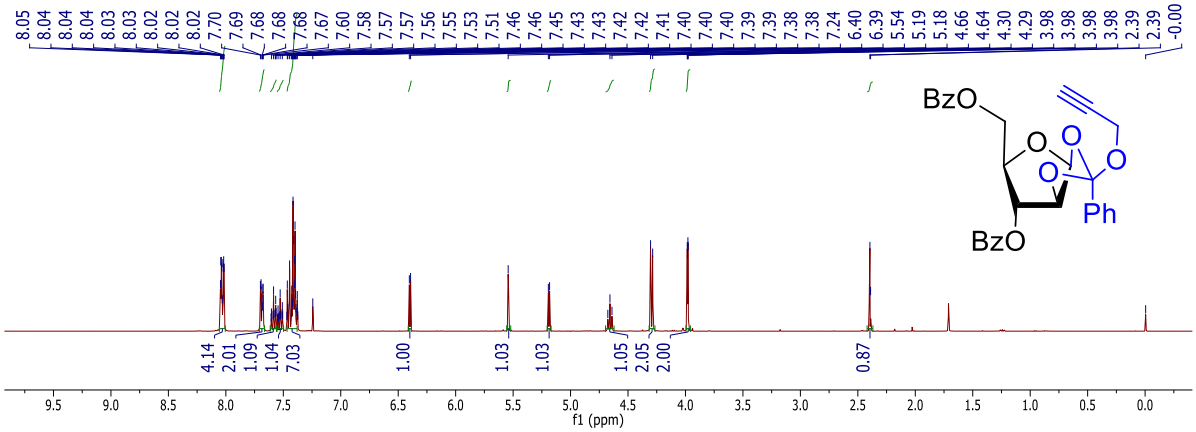
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **7**



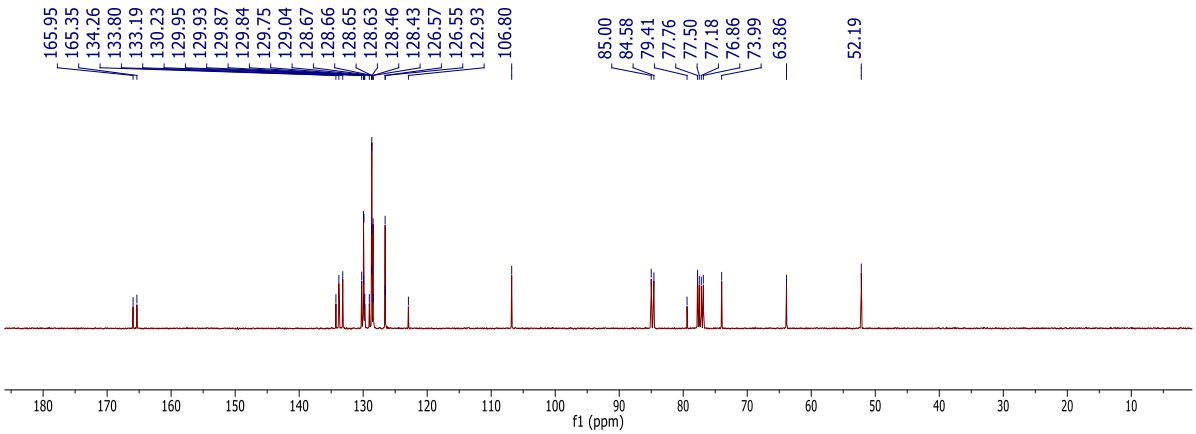
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **7**



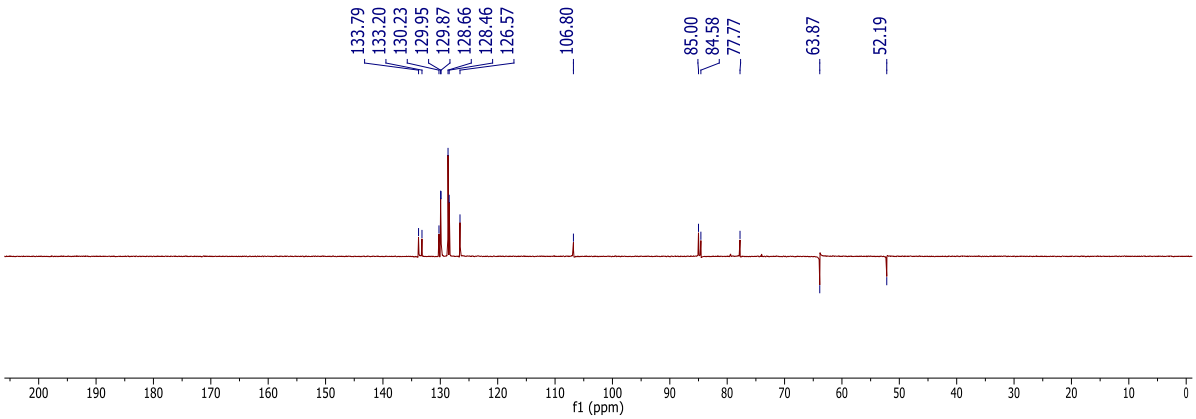
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **8**



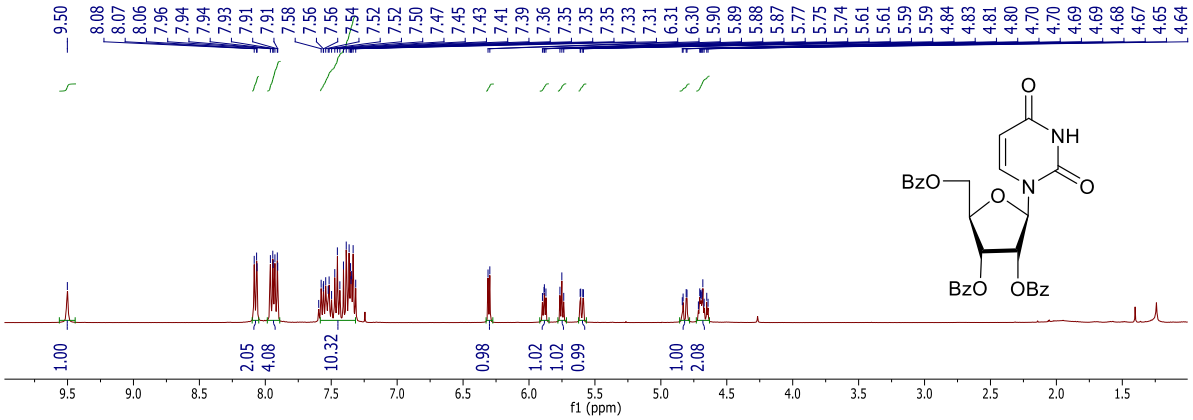
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **8**



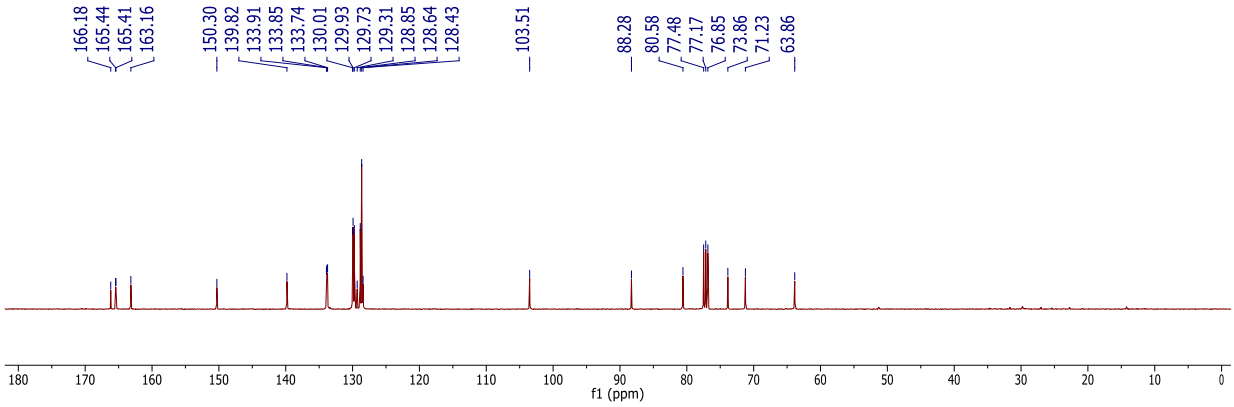
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **8**



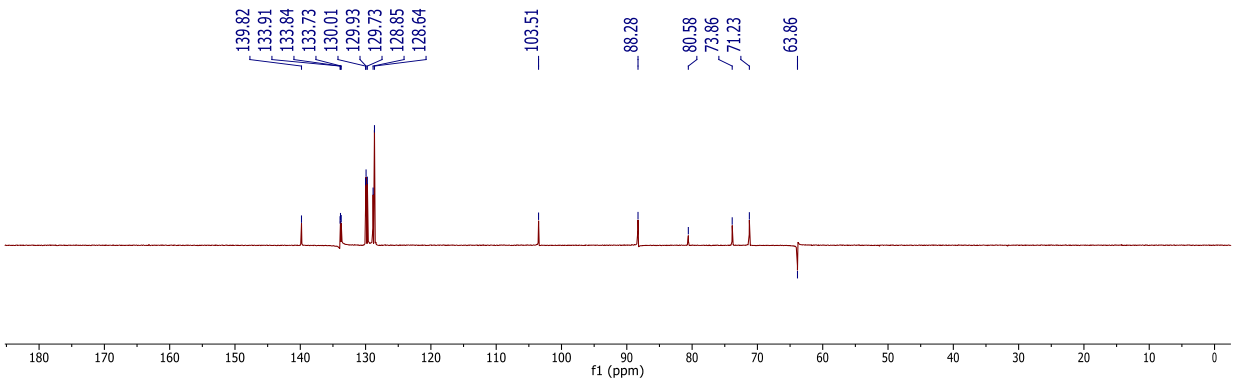
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **10**



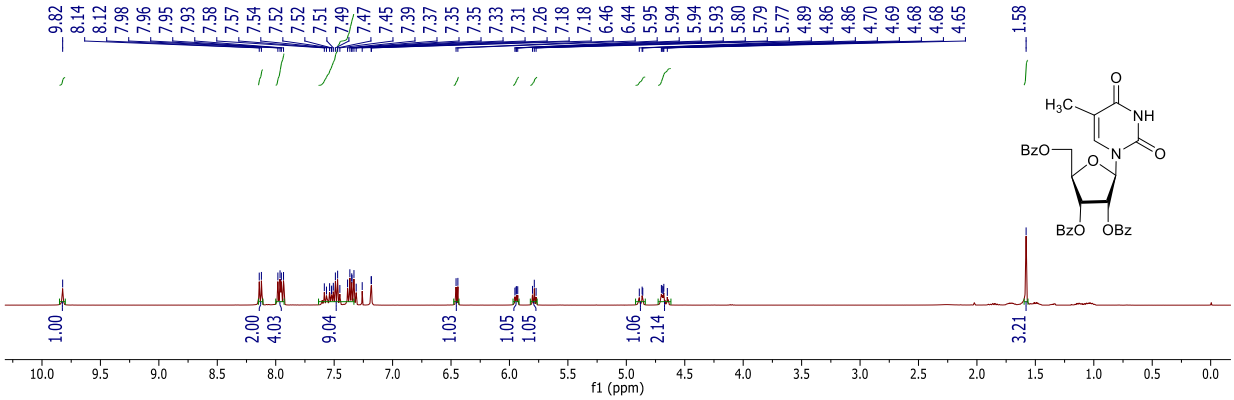
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **10**



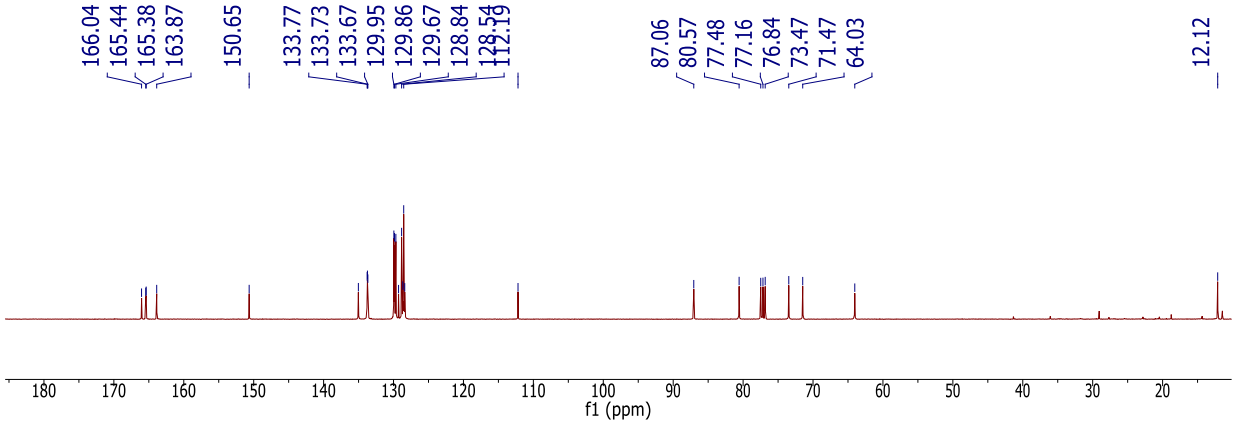
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **10**



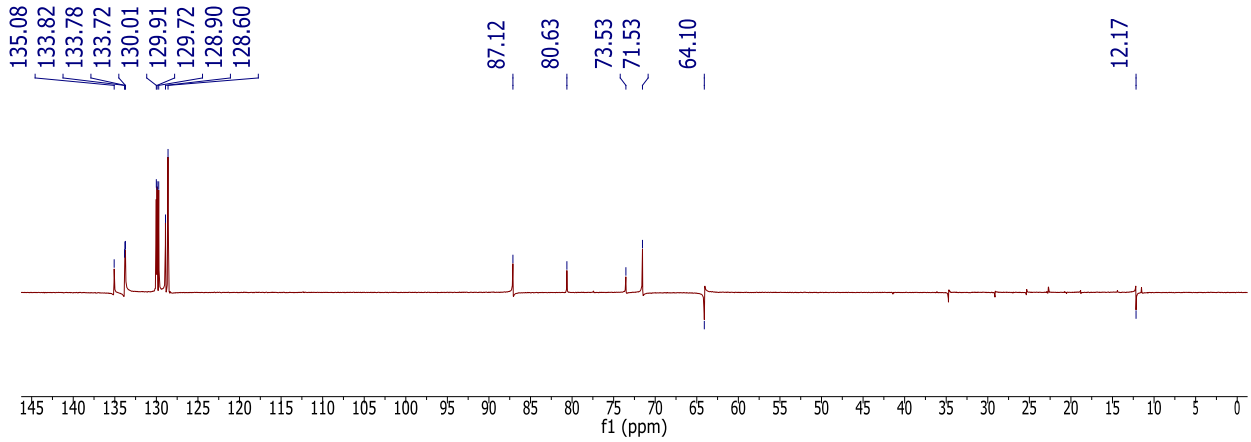
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **12**



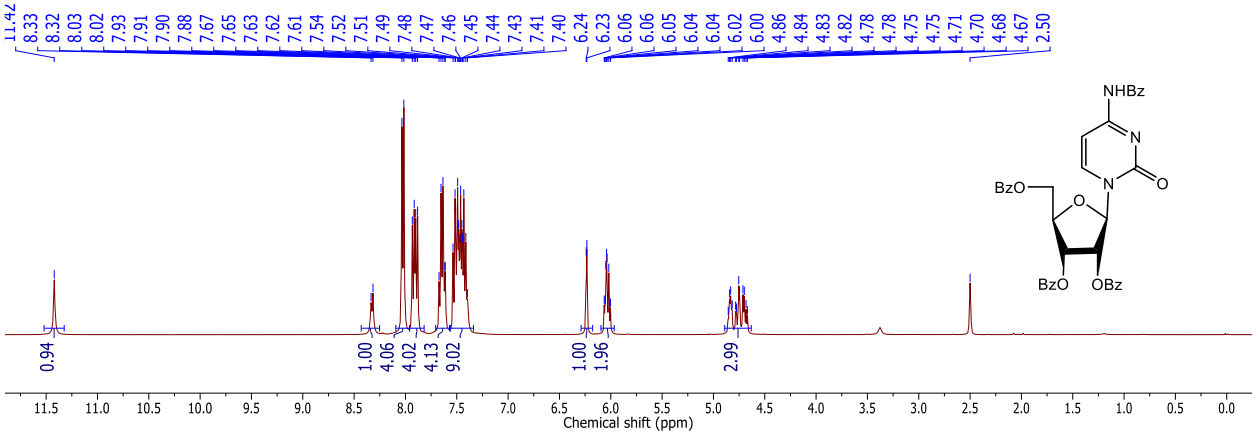
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **12**



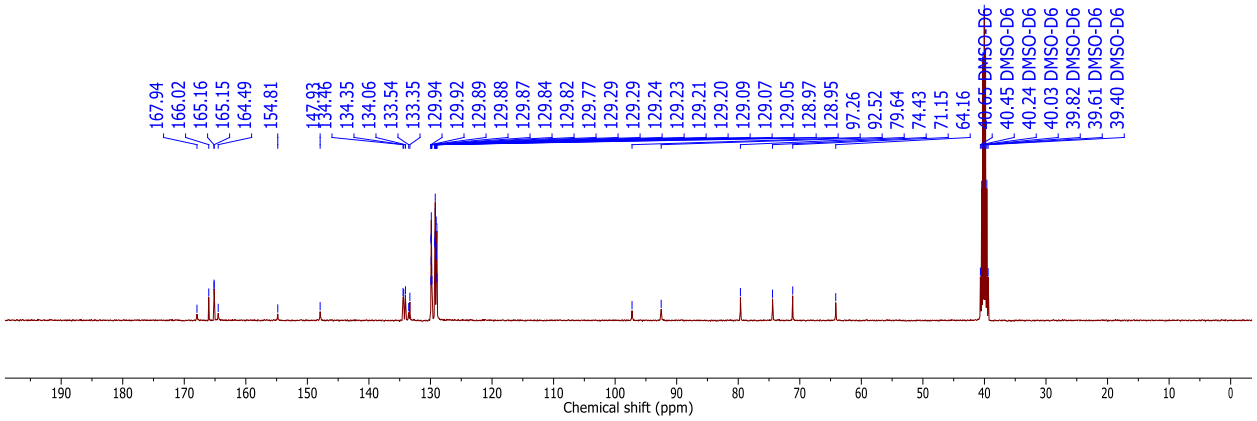
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **12**



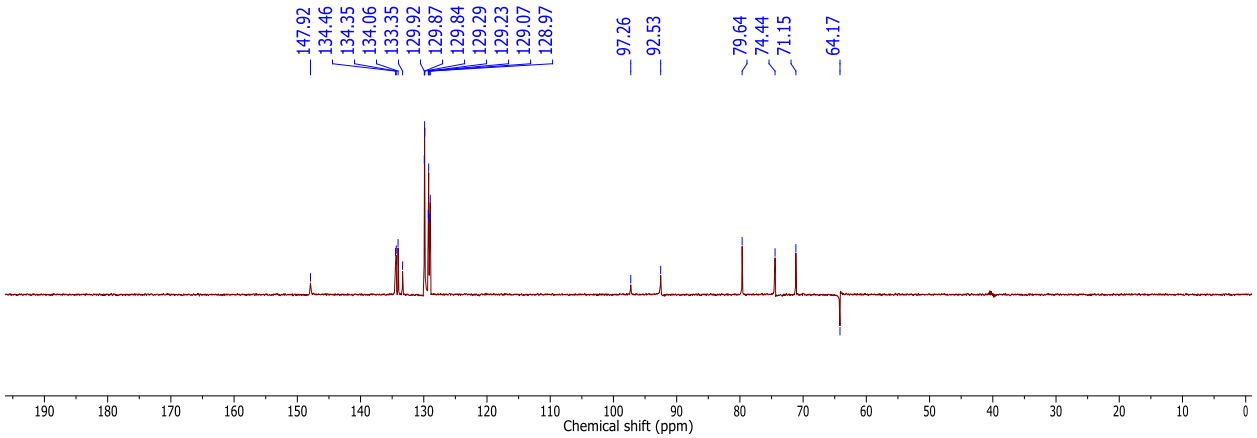
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **14**



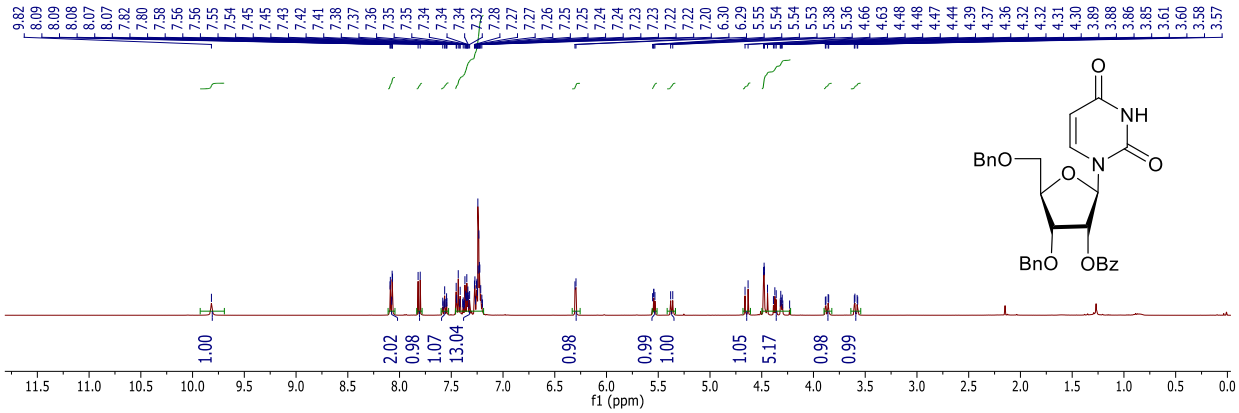
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **14**



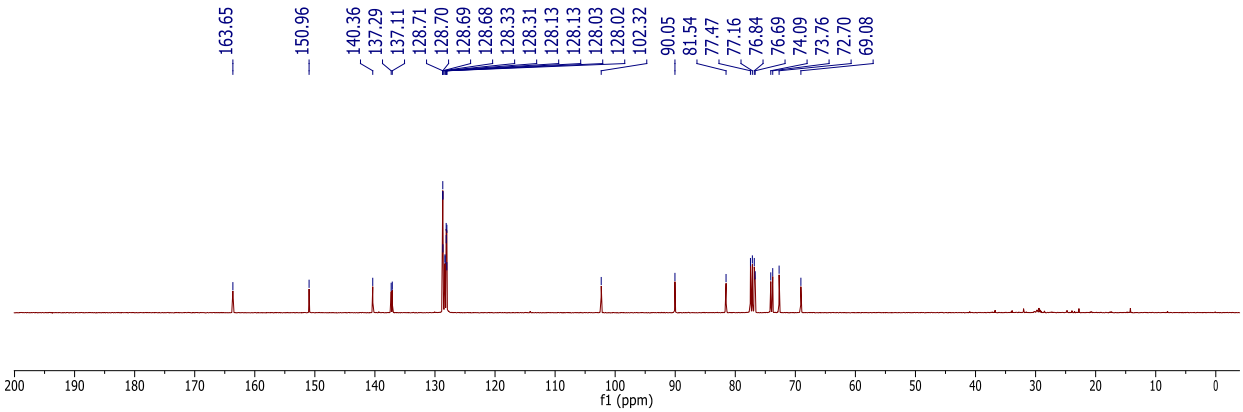
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **14**



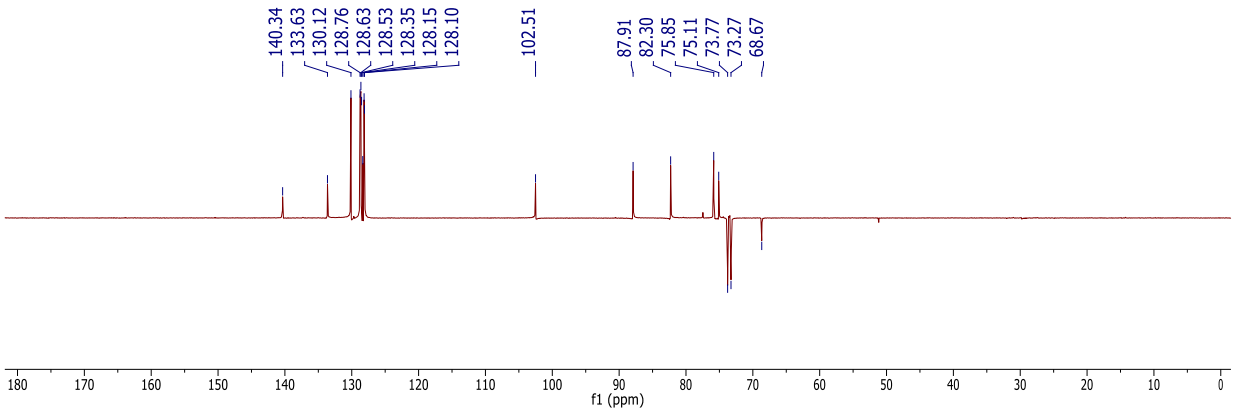
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **15**



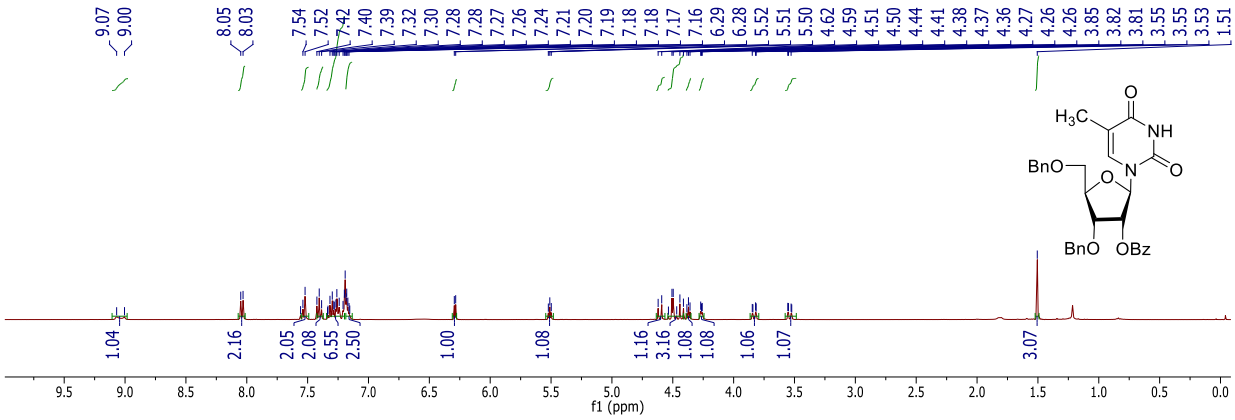
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **15**



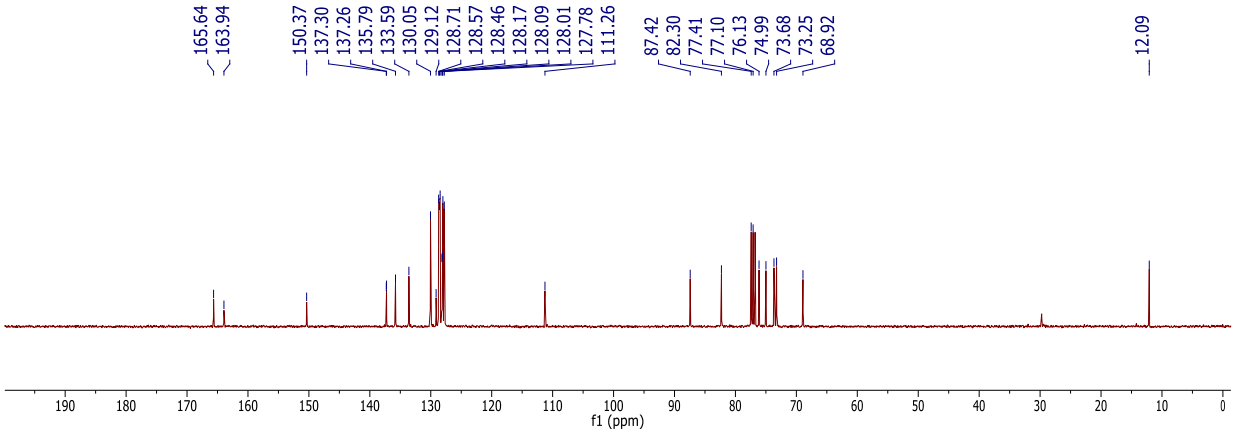
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **15**



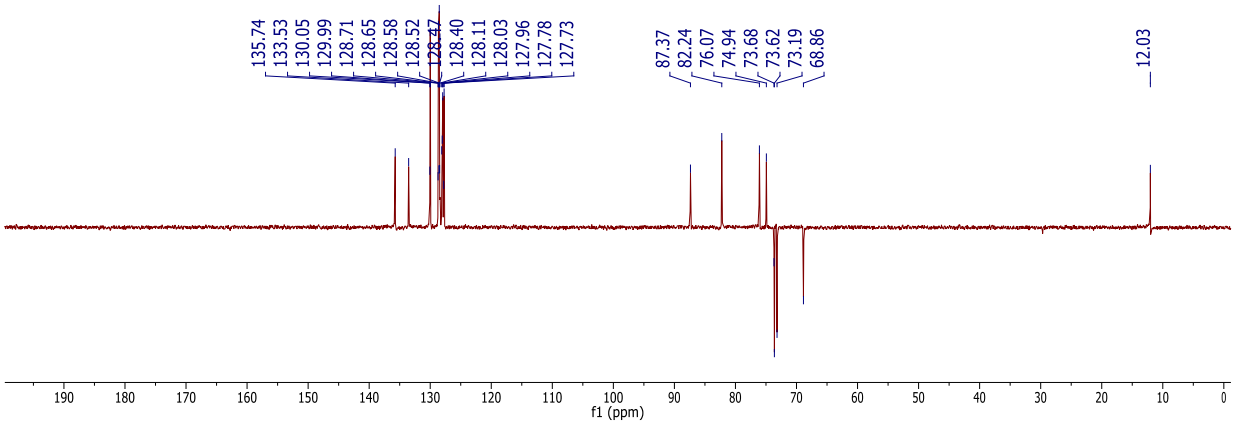
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **16**



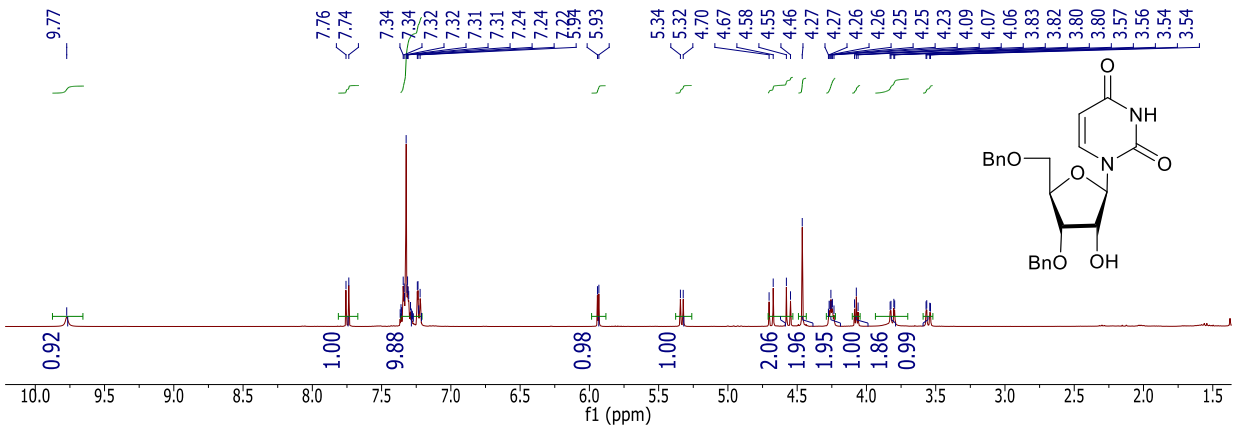
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **16**



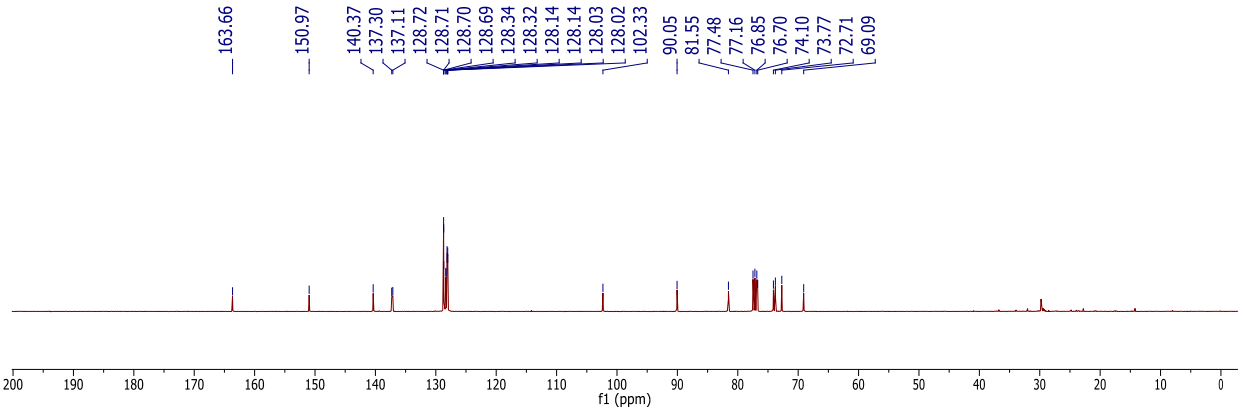
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **16**



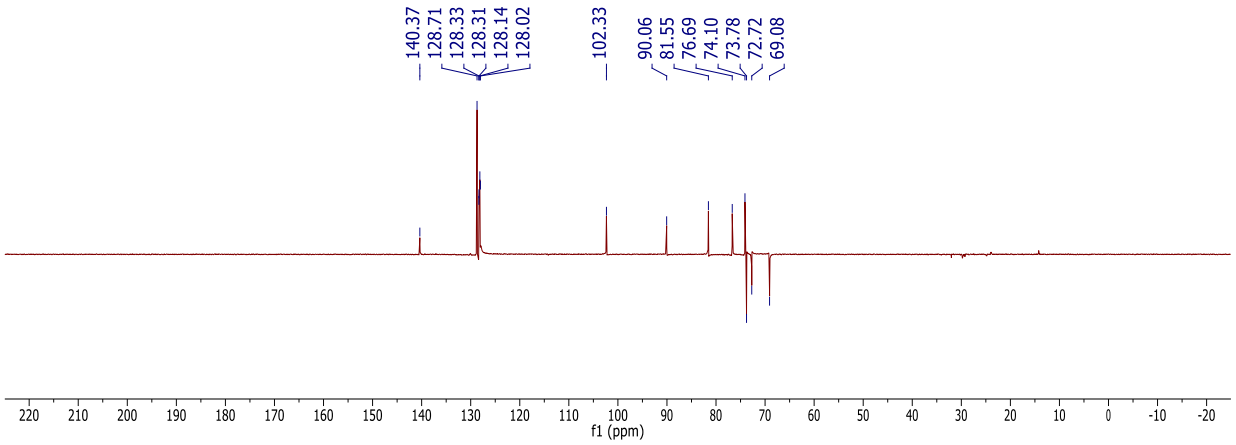
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **17**



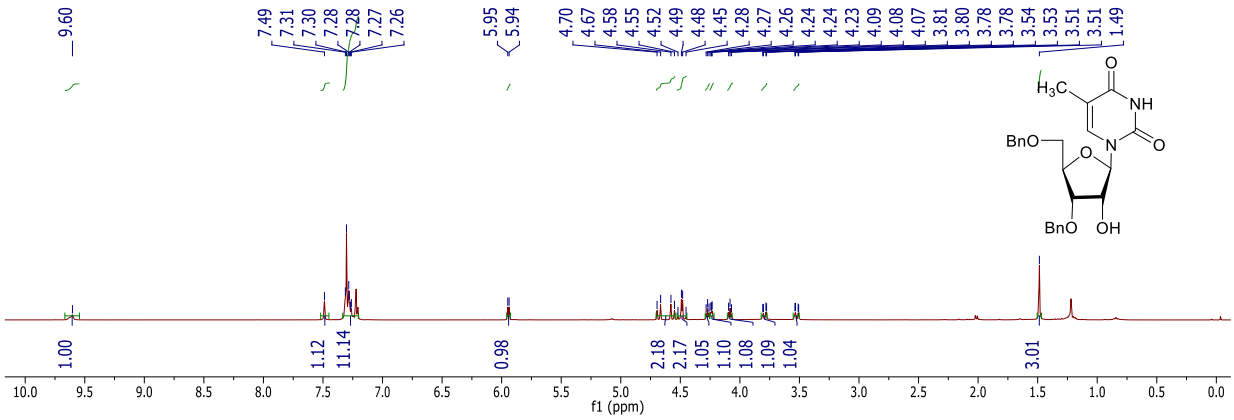
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **17**



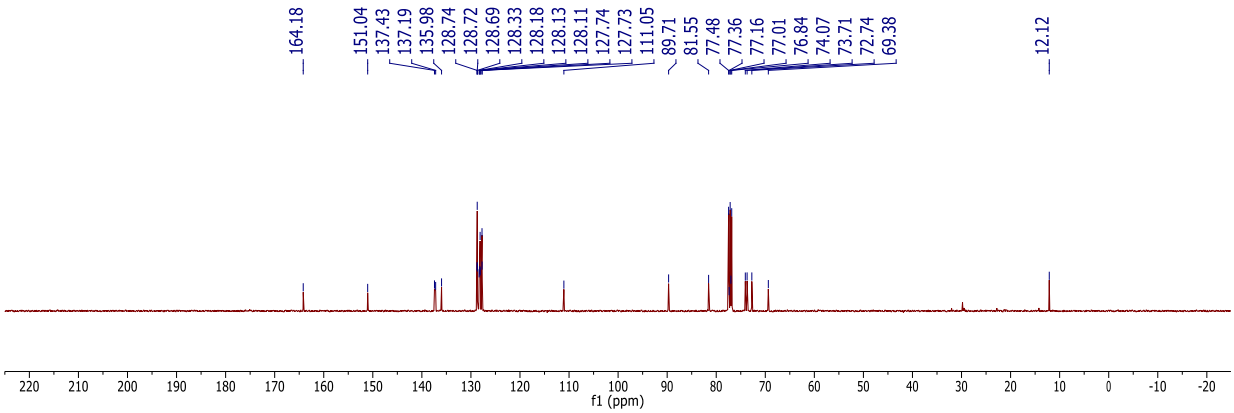
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **17**



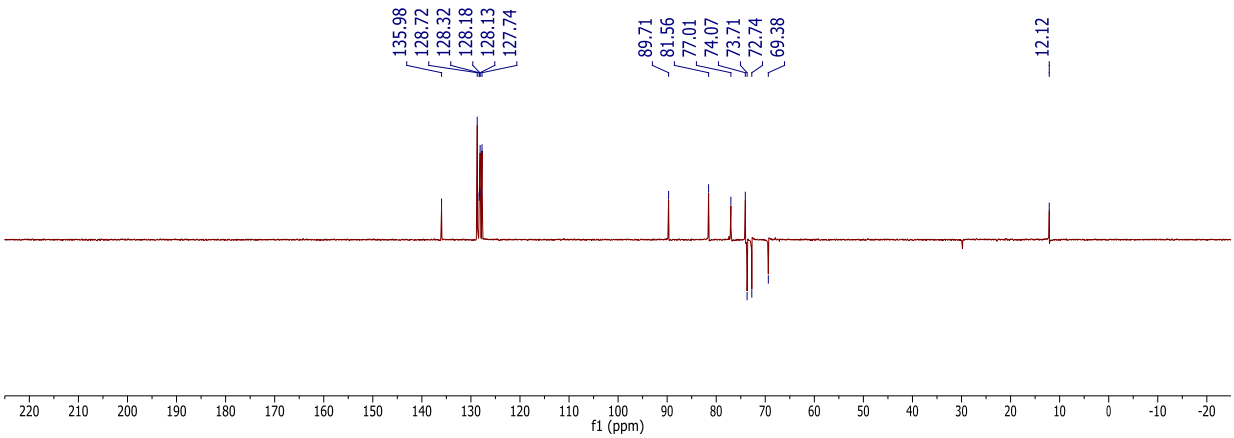
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **18**



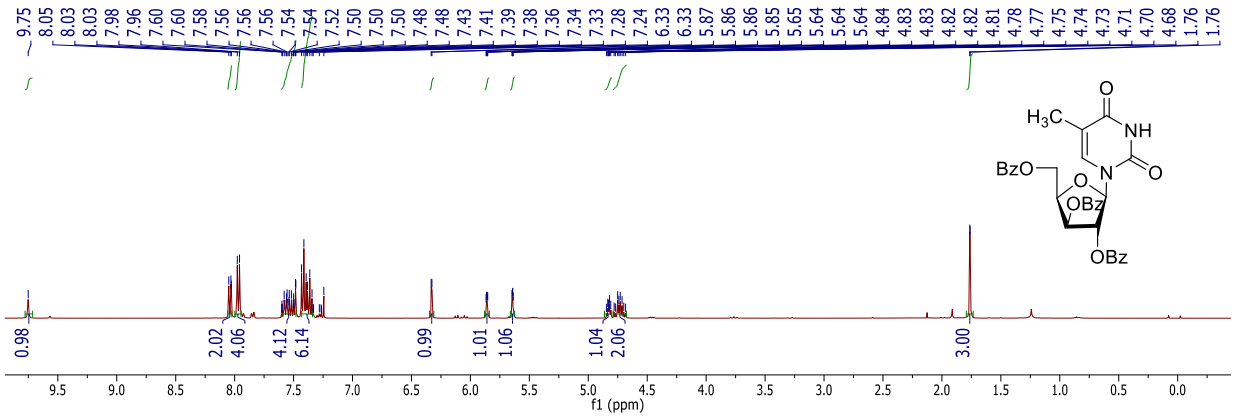
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **18**



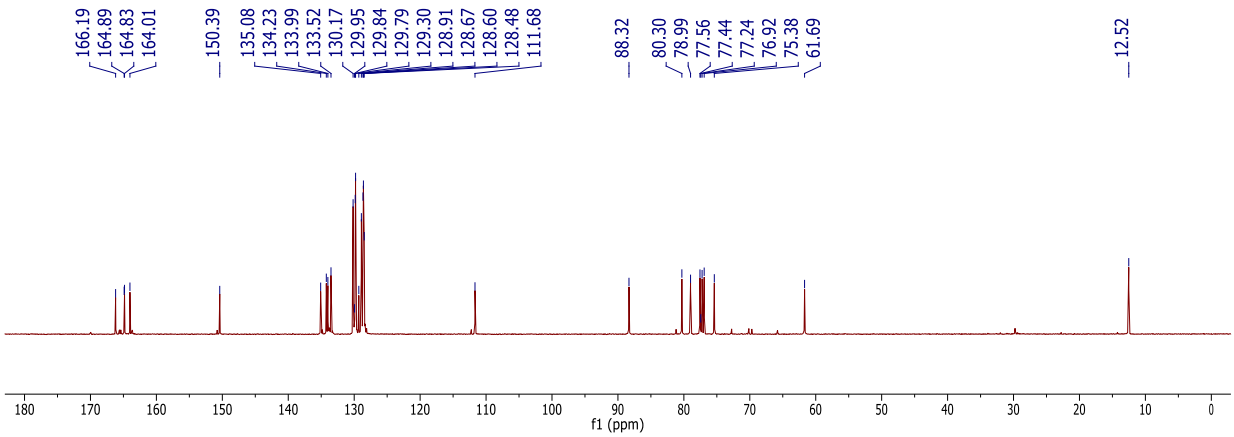
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **18**



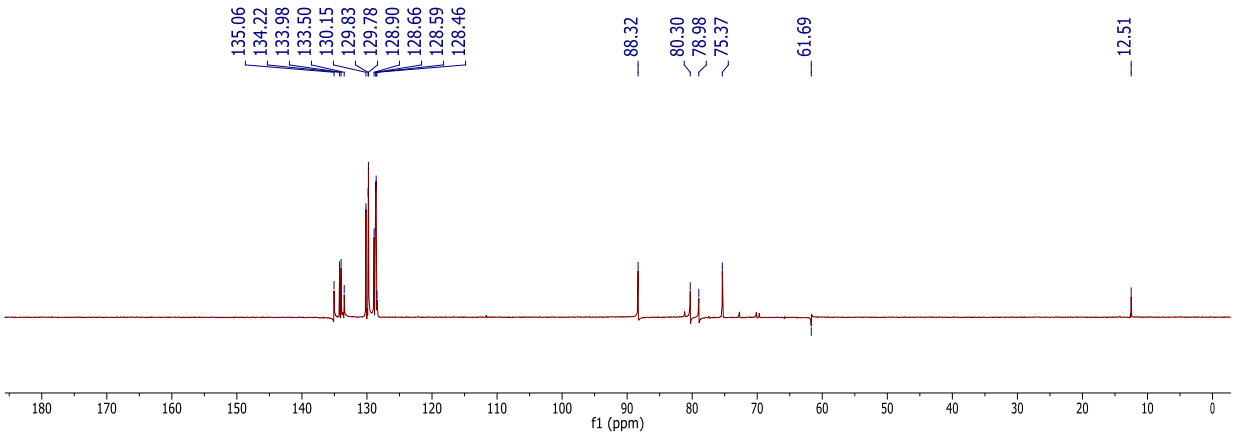
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **19**



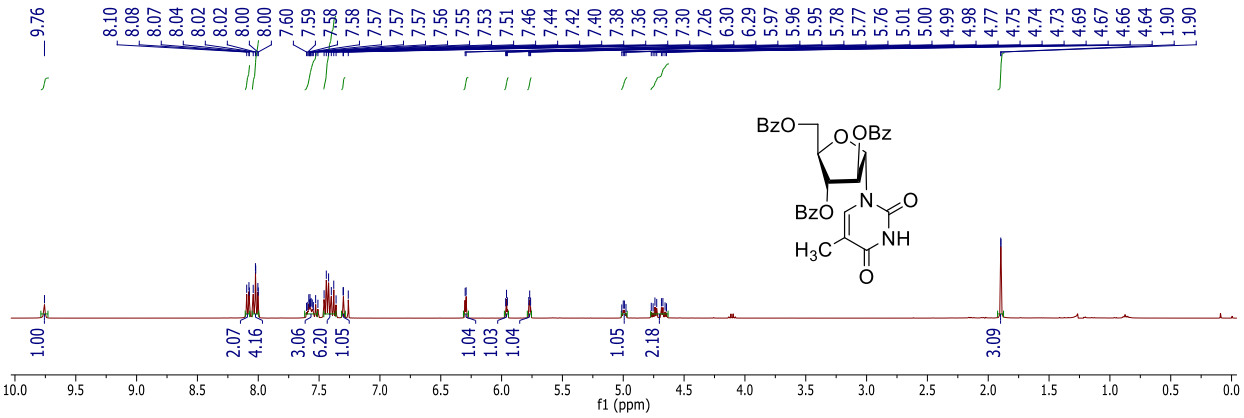
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **19**



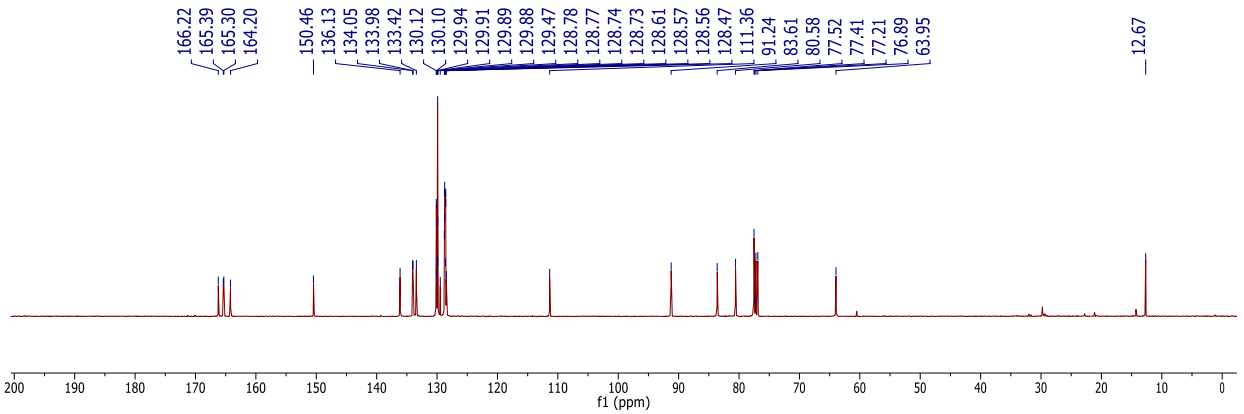
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **19**



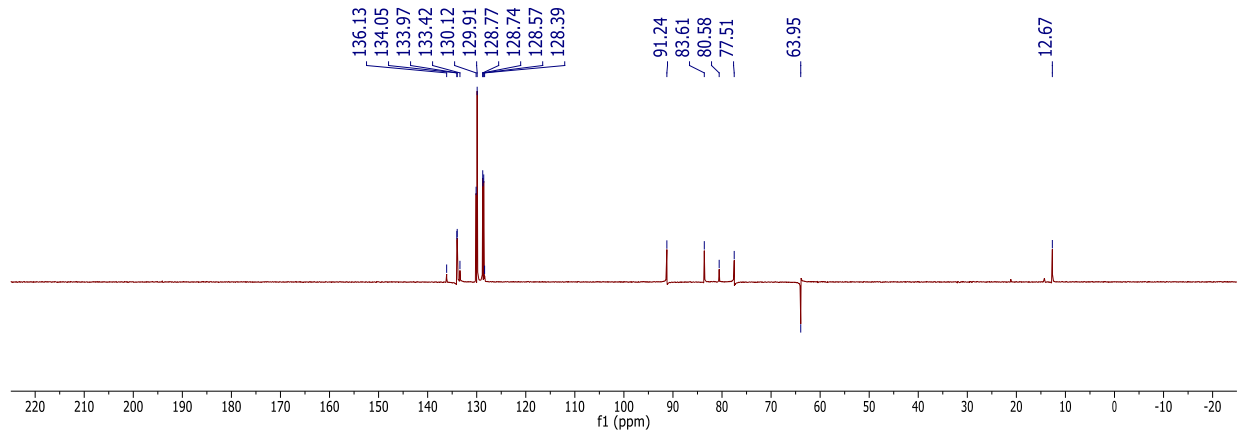
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **20**



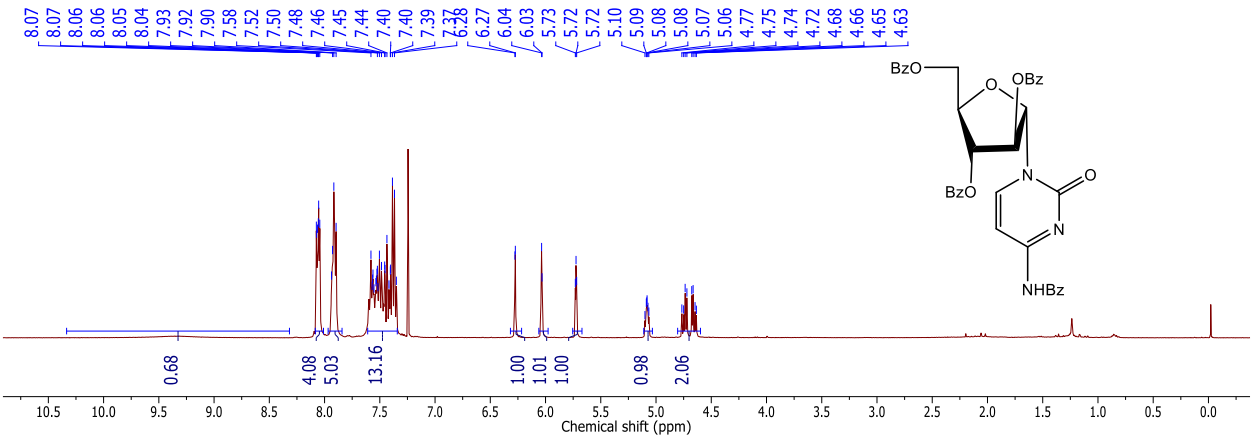
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **20**



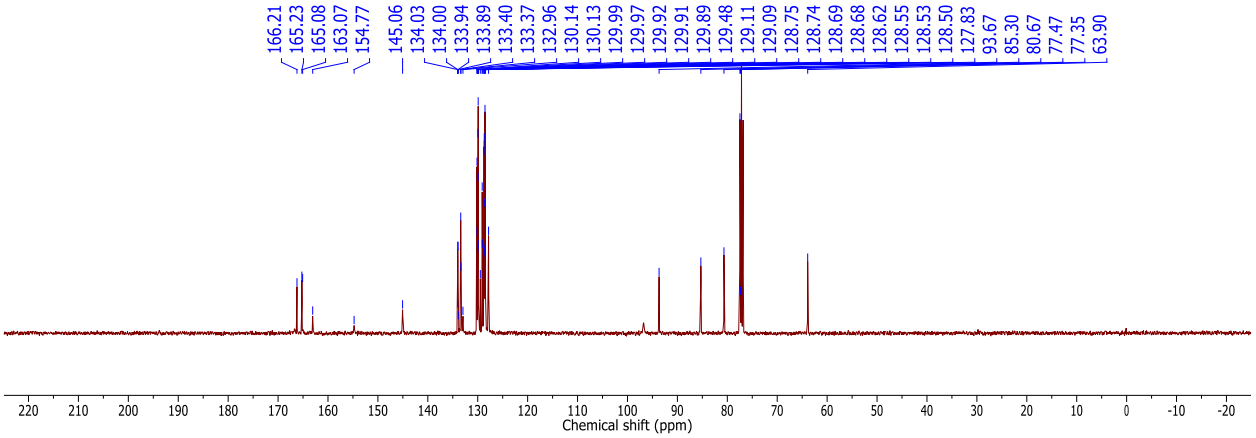
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **20**



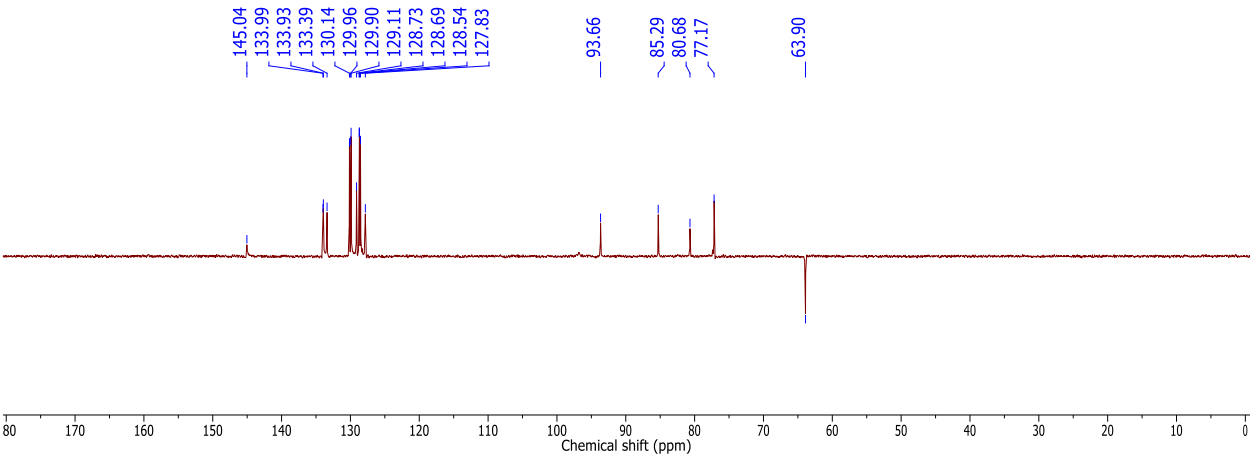
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **21**



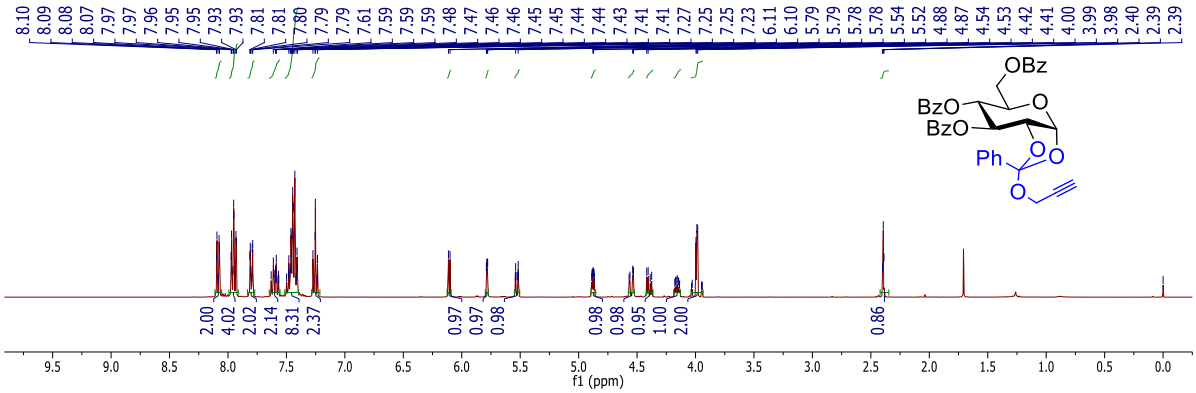
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **21**



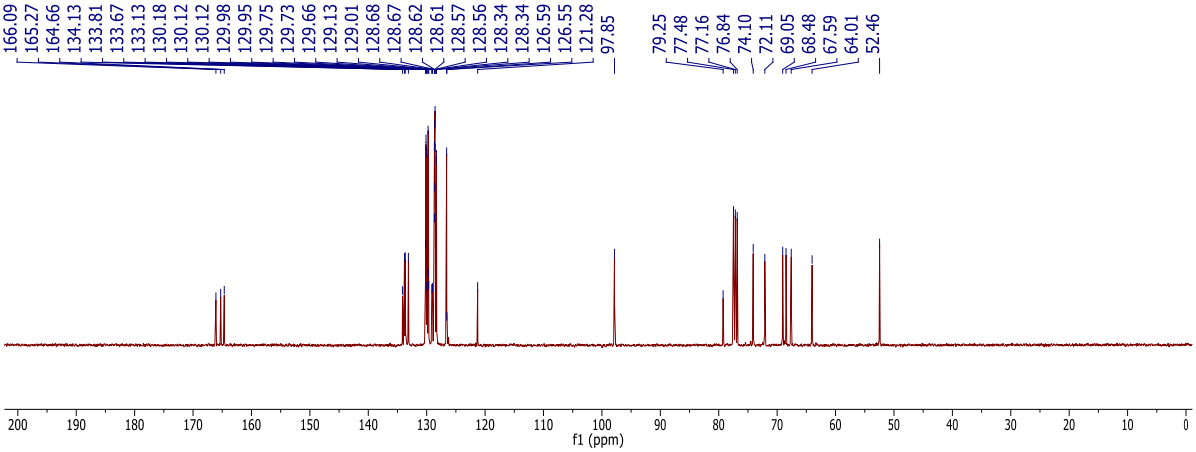
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **21**



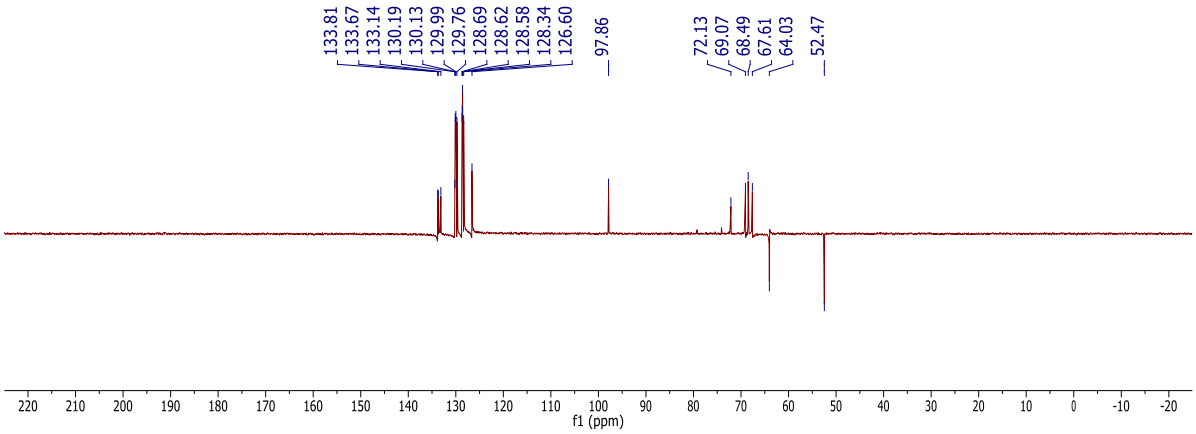
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **24**



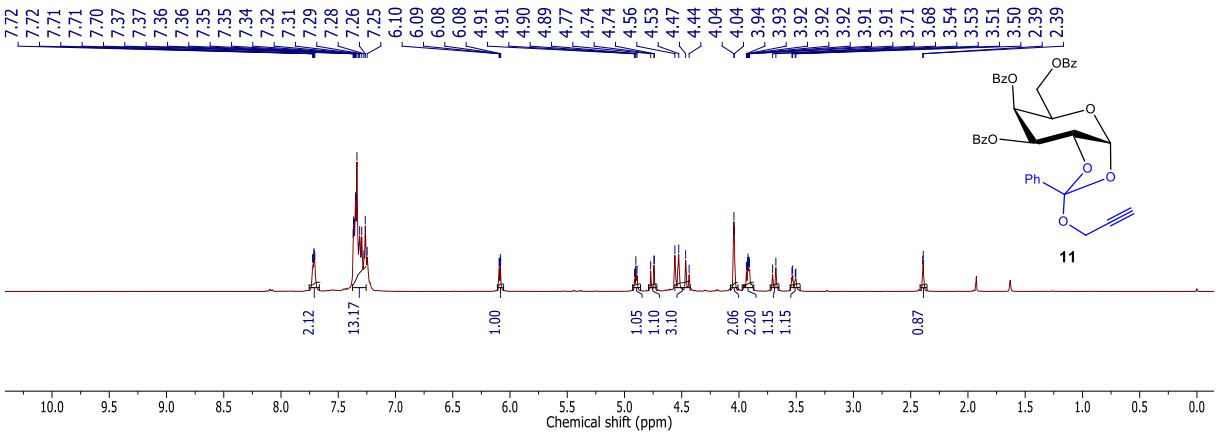
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **24**



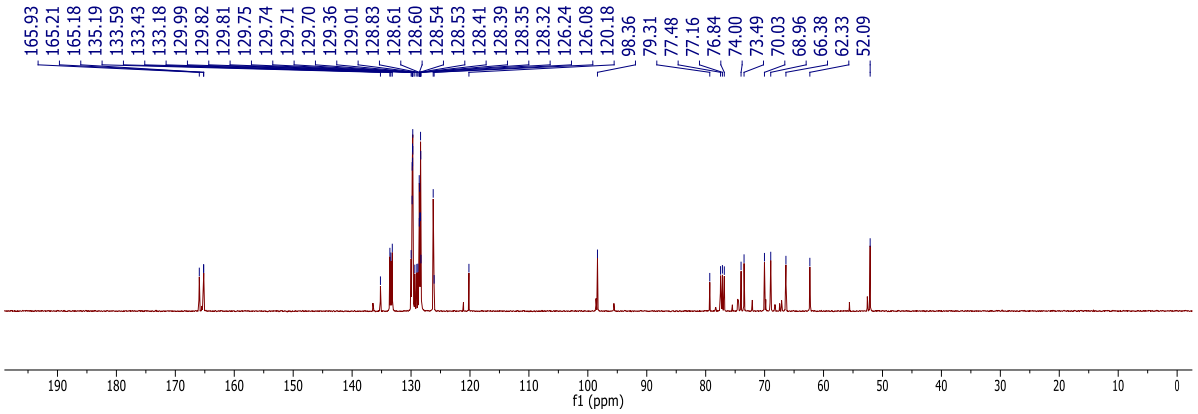
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **24**



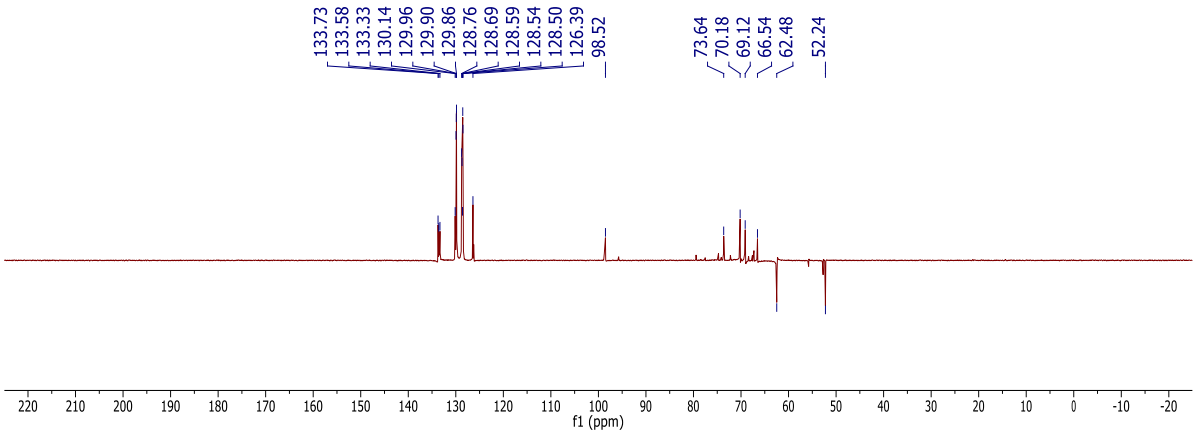
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **25**



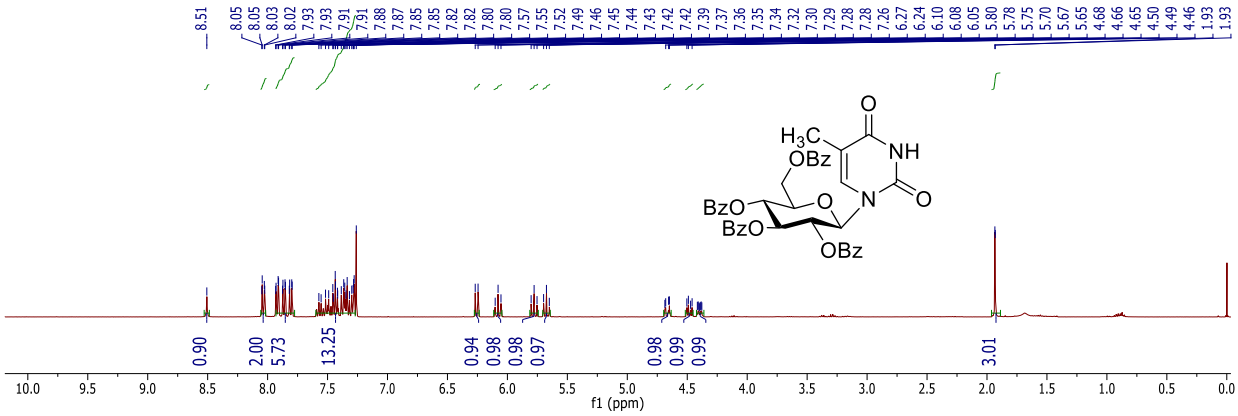
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **25**



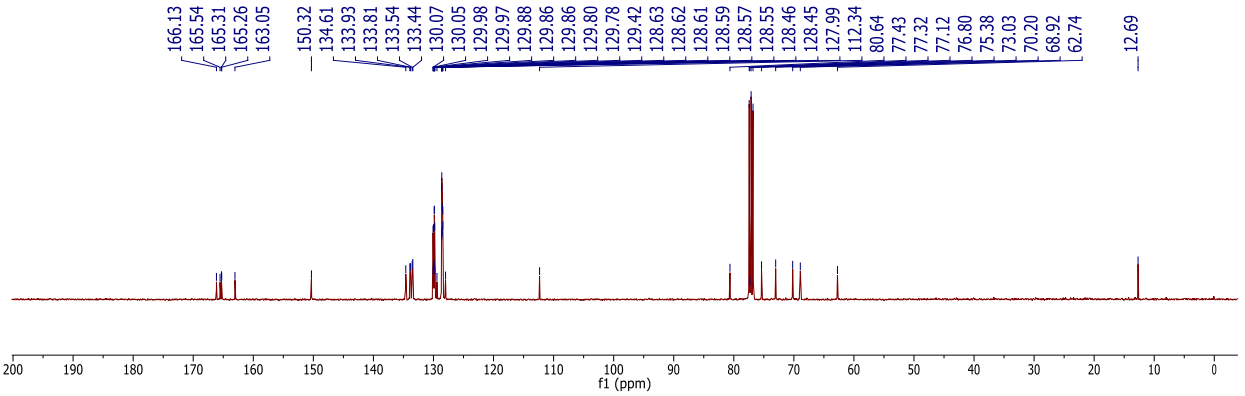
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **25**



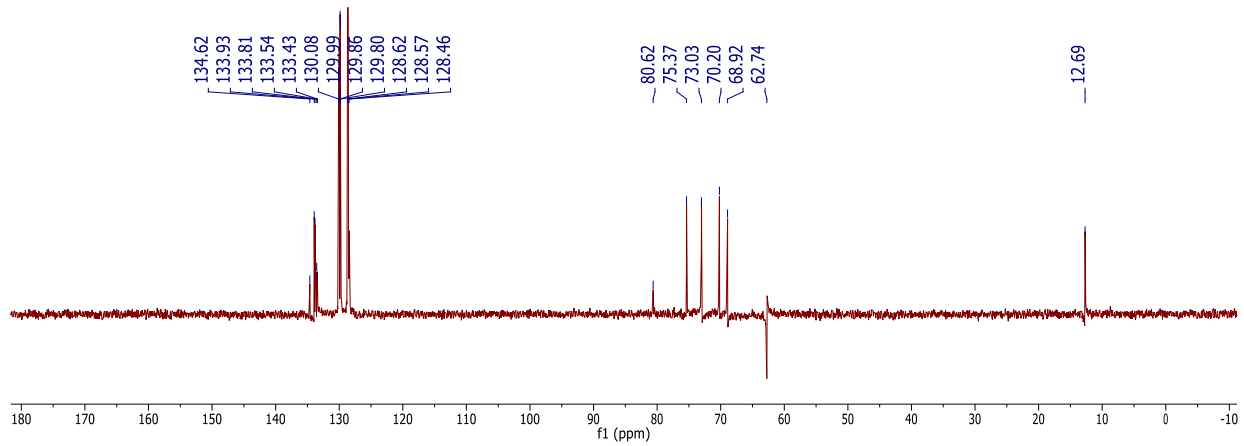
^{1}H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **27**



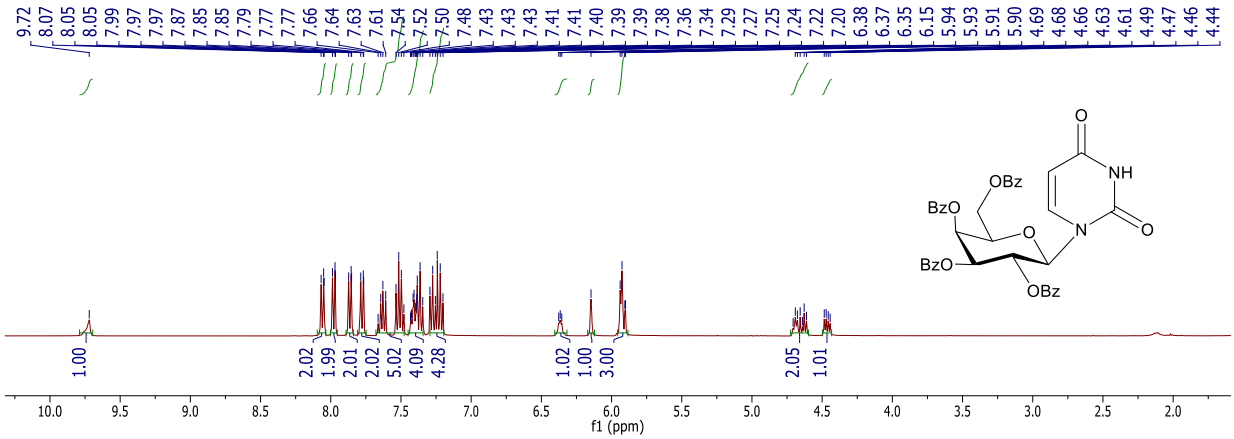
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **27**



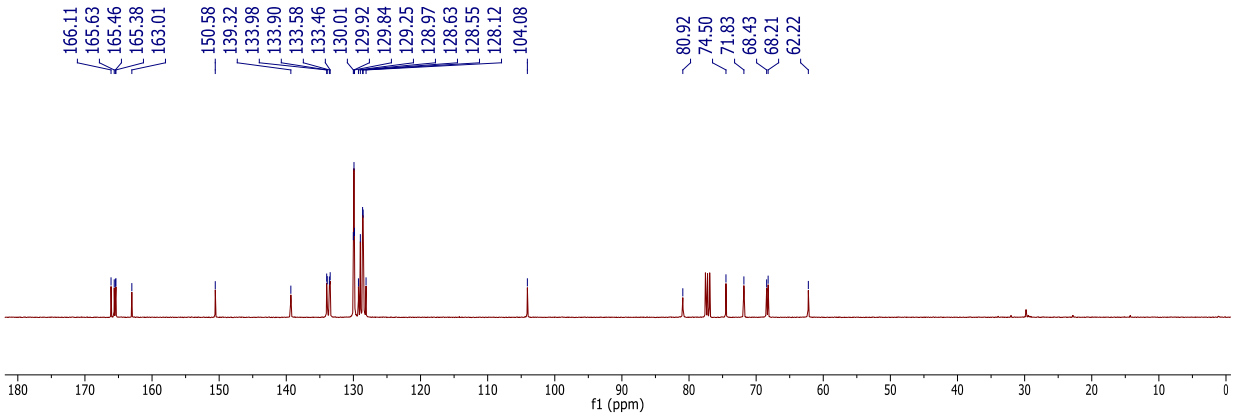
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **27**



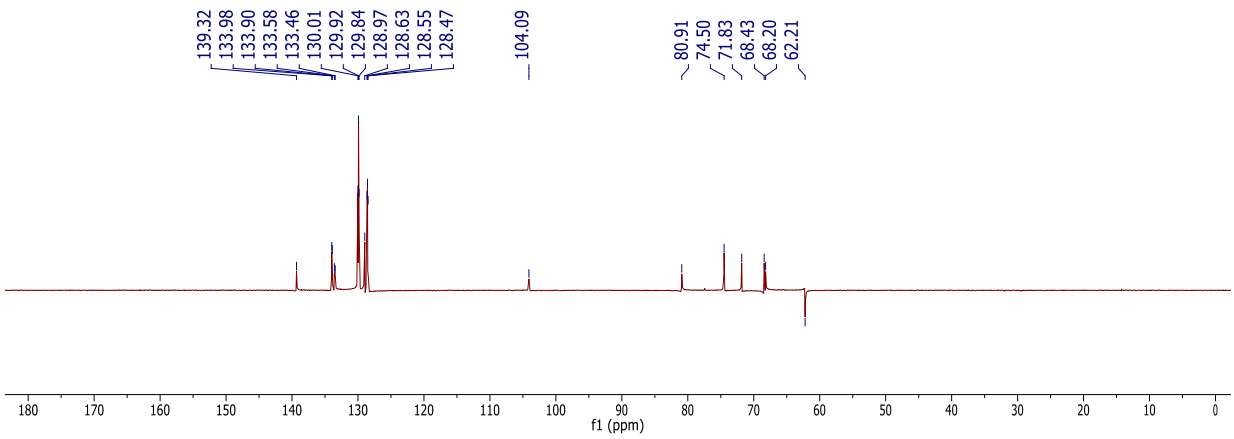
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **28**



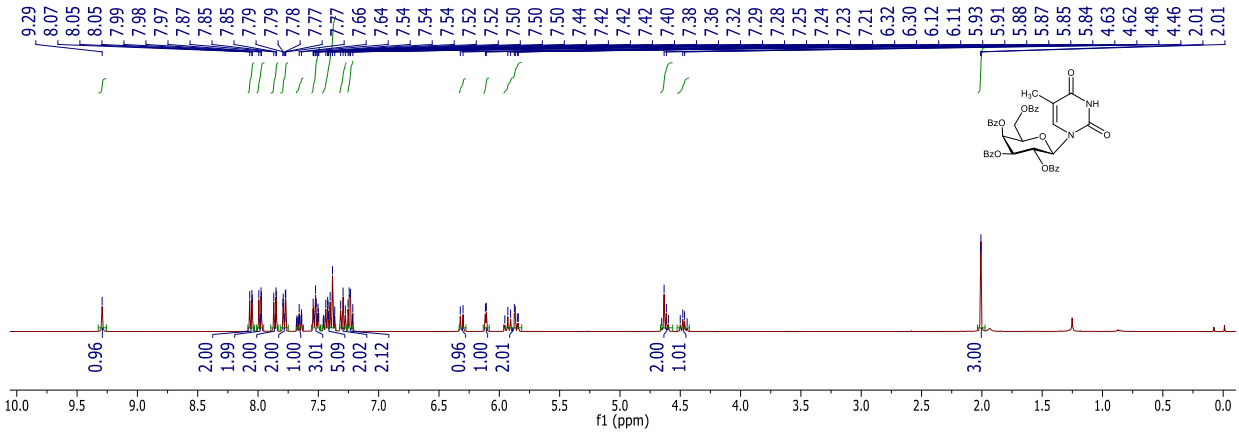
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **28**



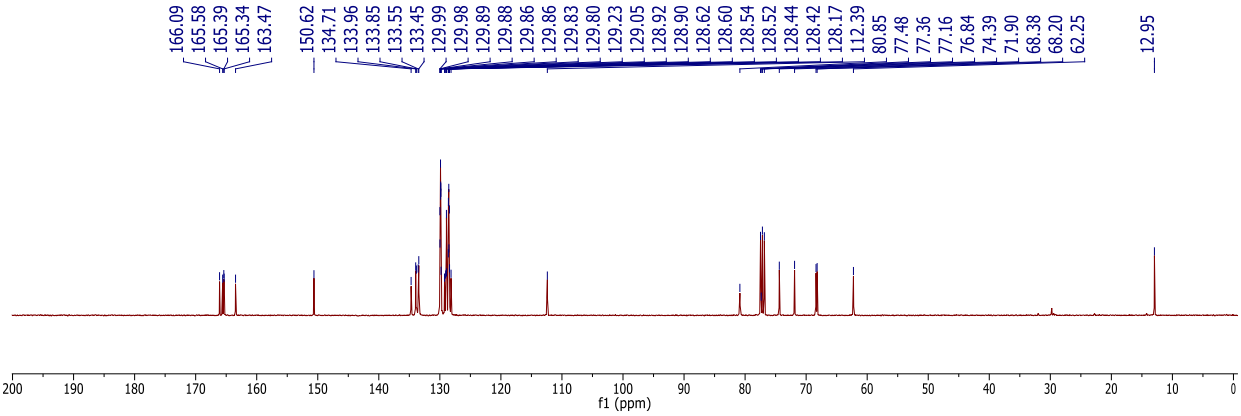
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **28**



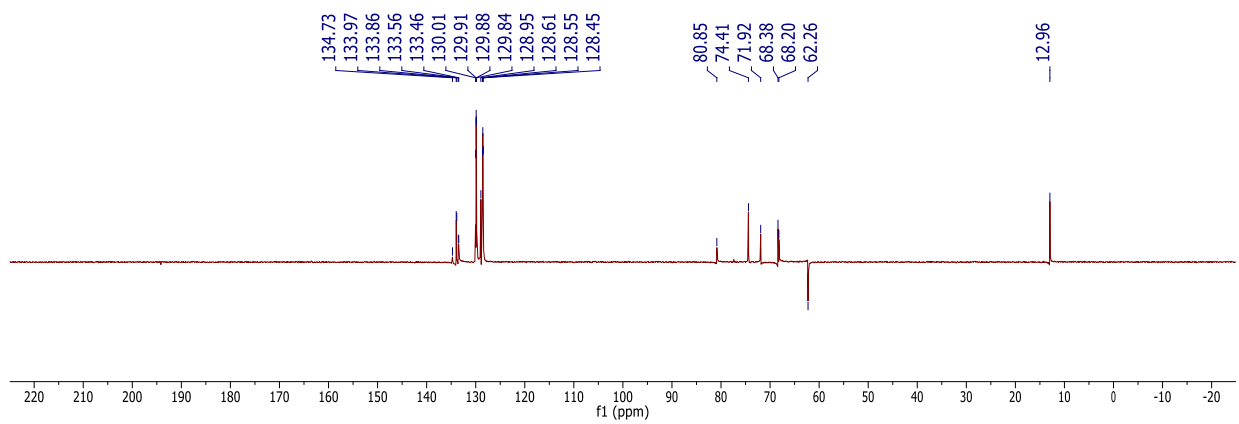
^{1}H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **29**



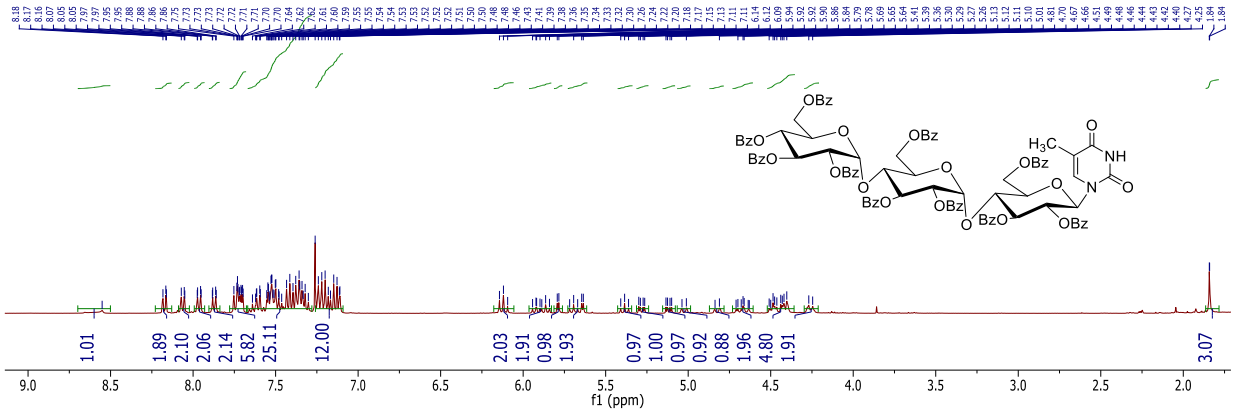
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **29**



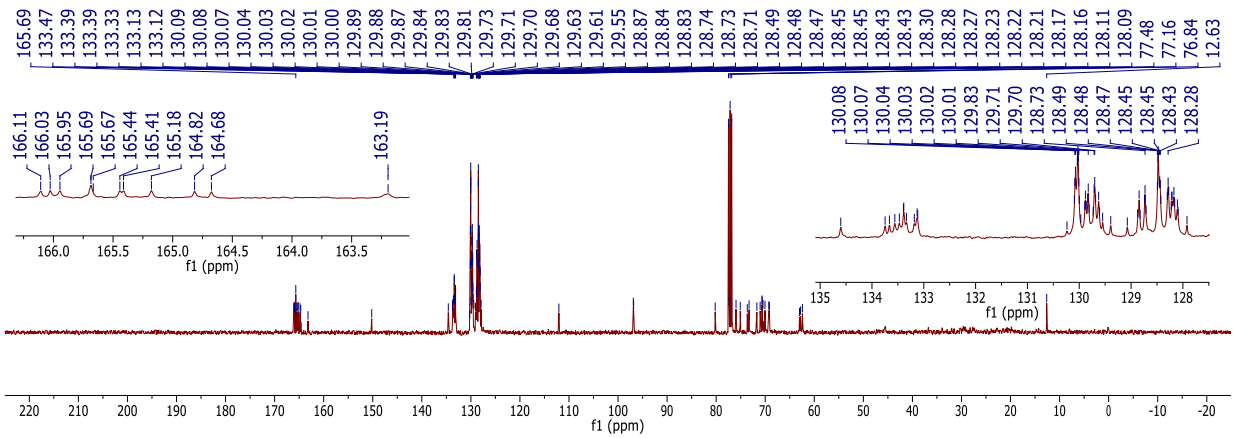
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **29**



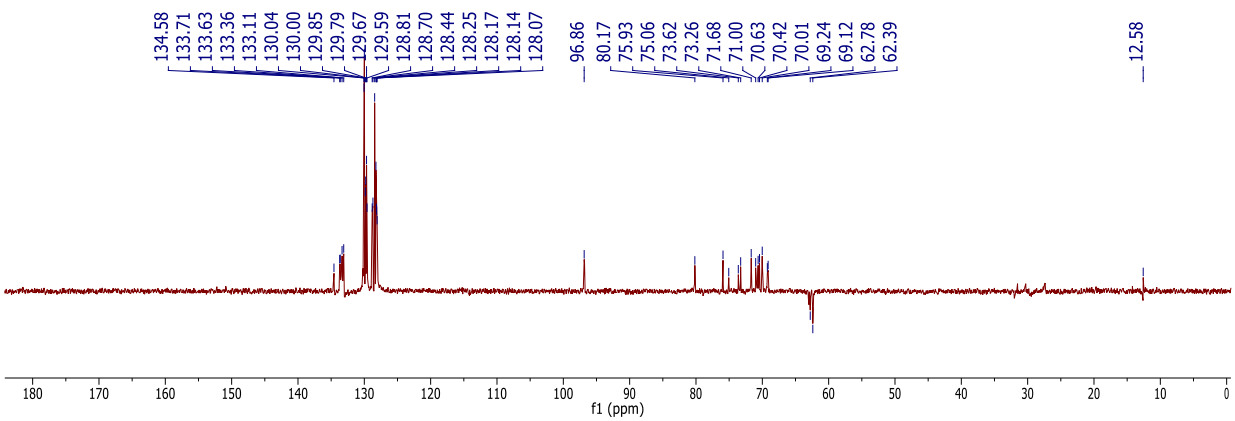
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **30**



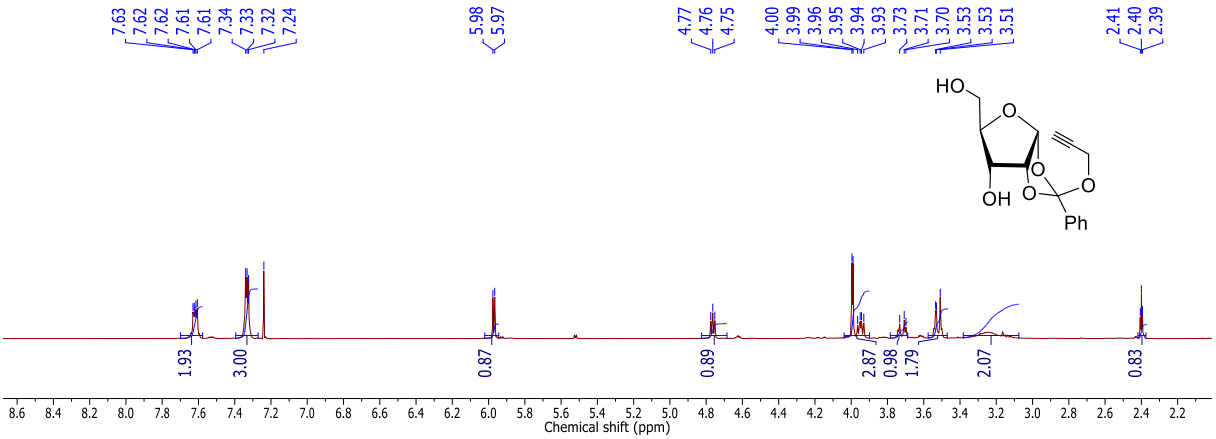
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **30**



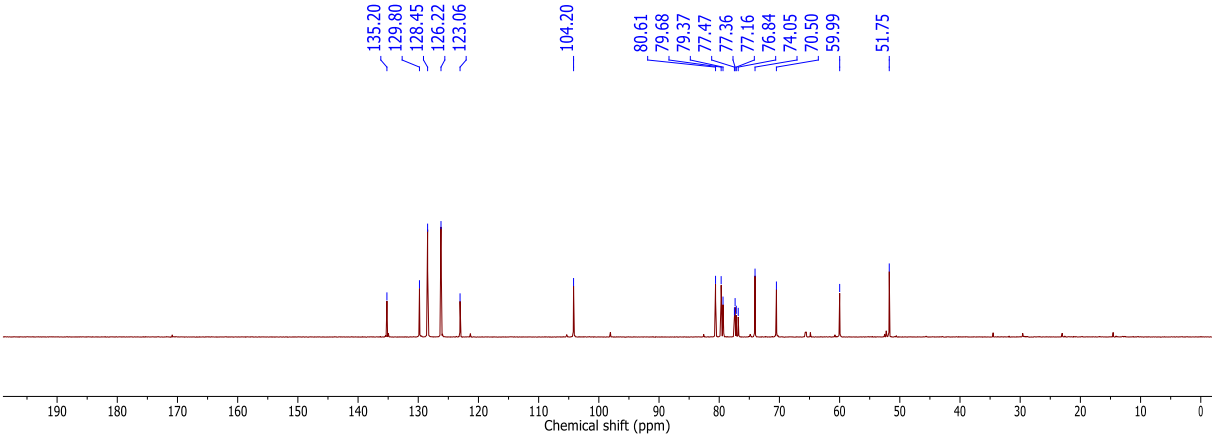
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **30**



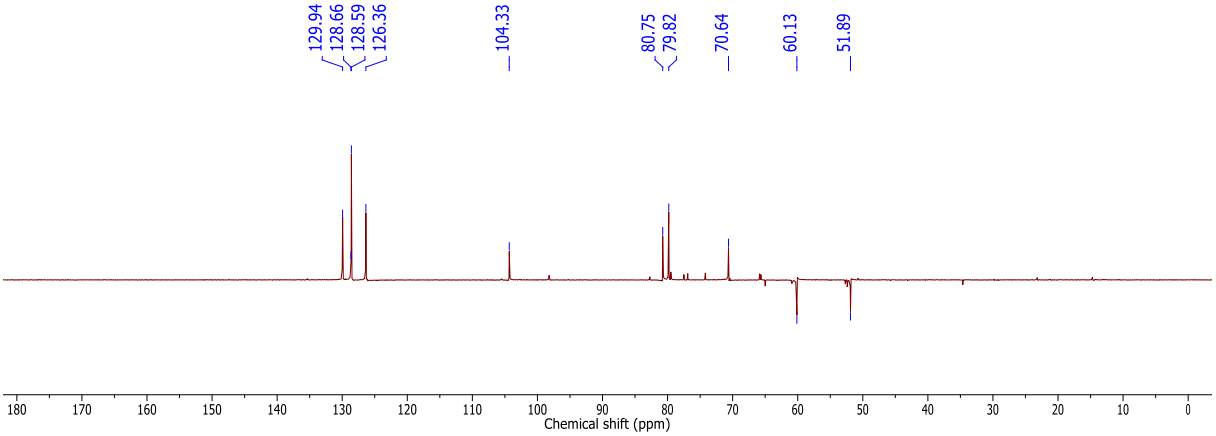
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **31**



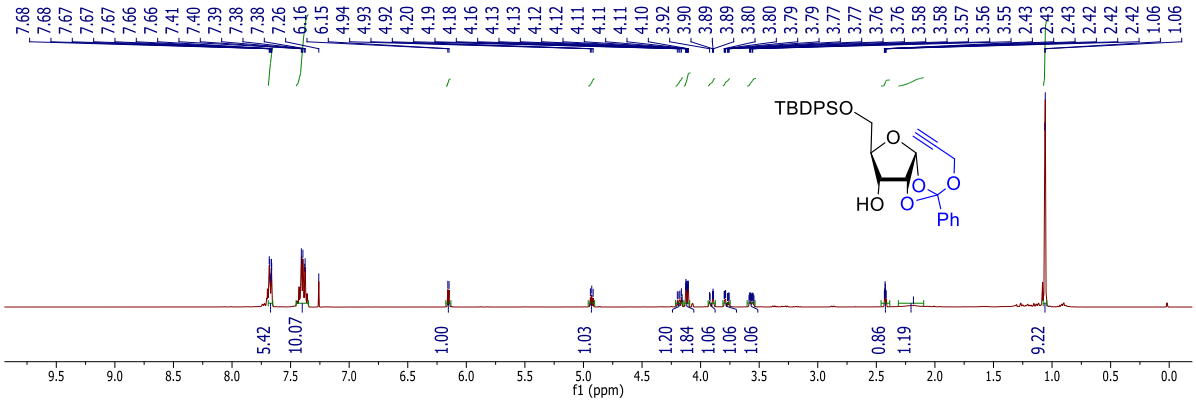
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **31**



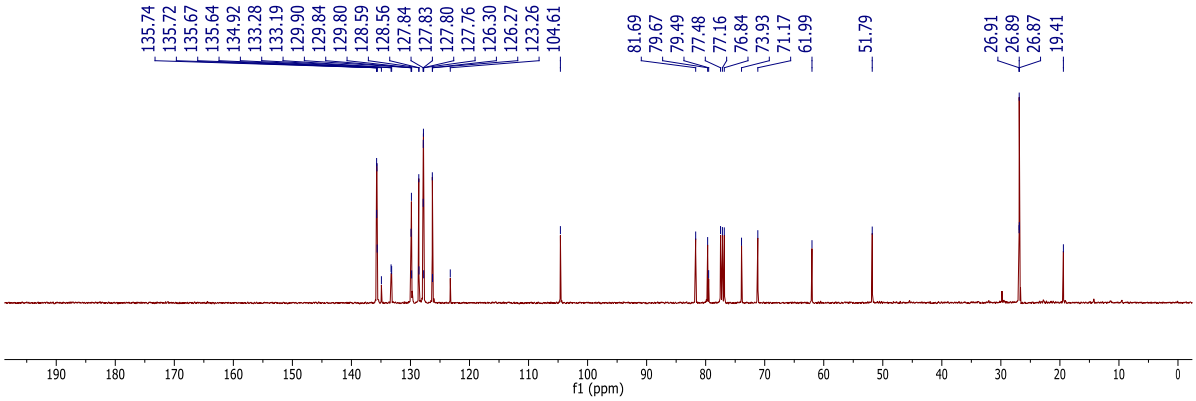
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **31**



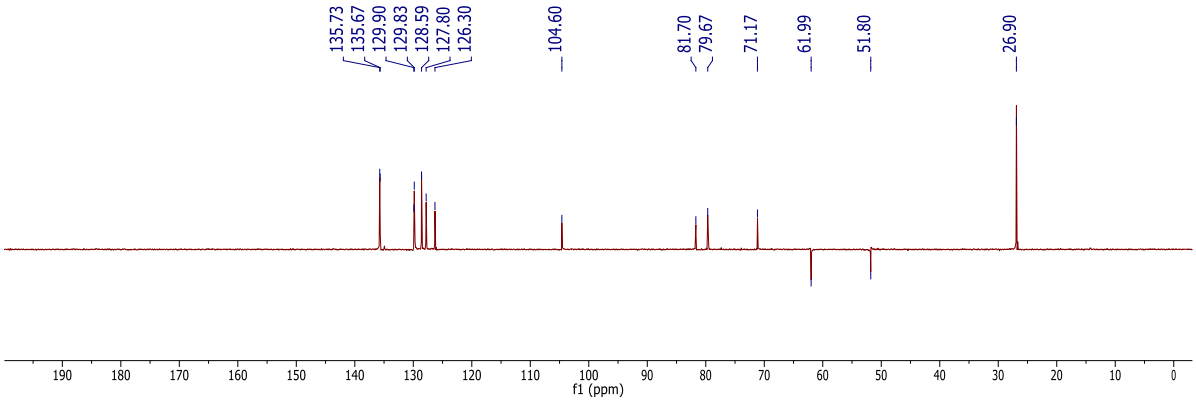
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **32**



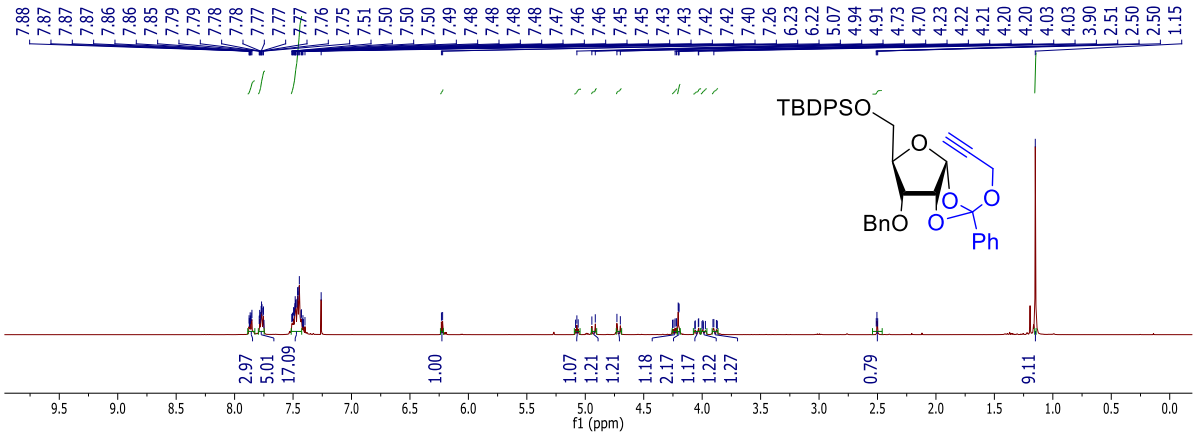
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **32**



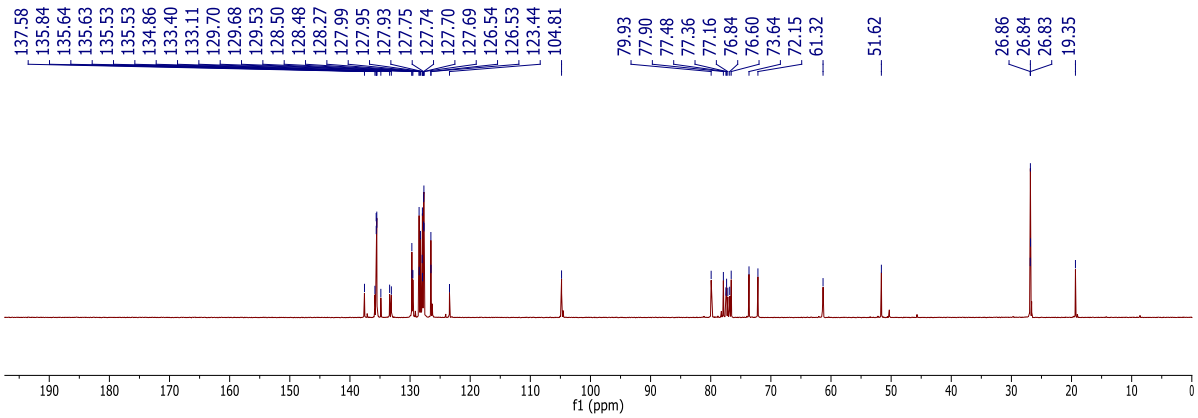
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **32**



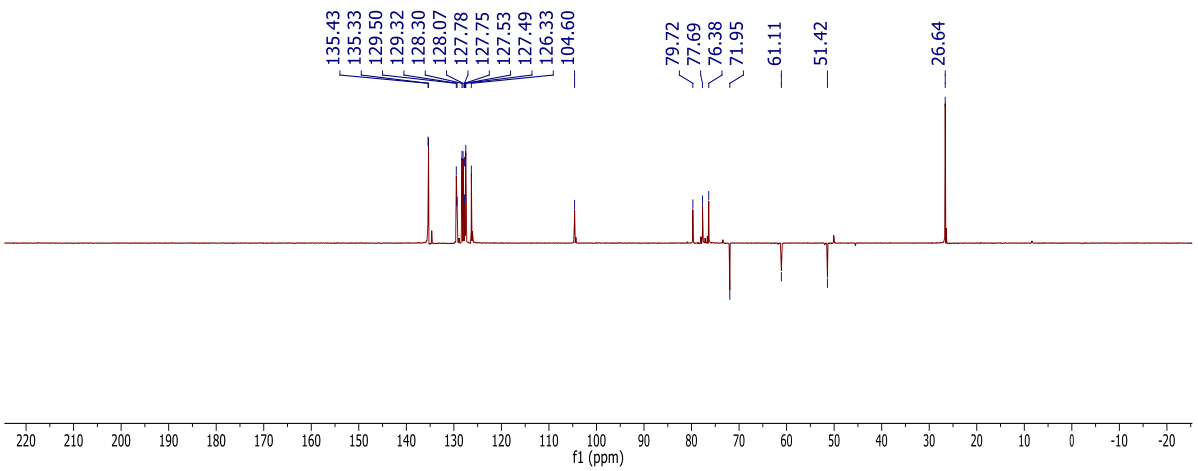
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **33**



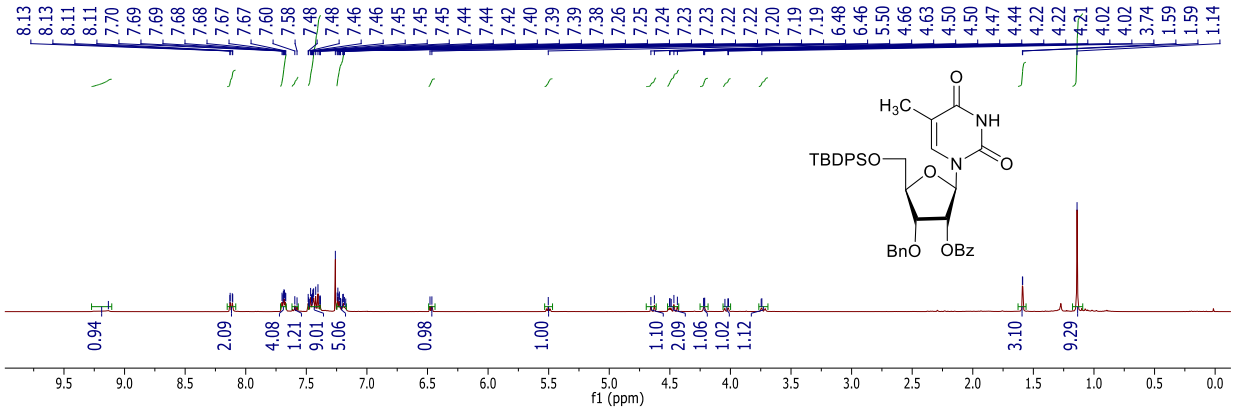
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **33**



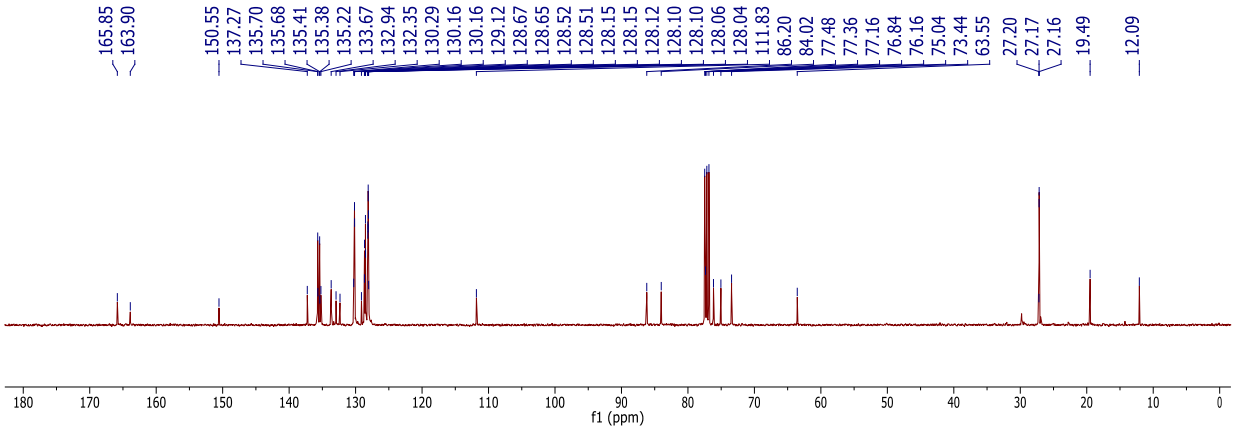
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **33**



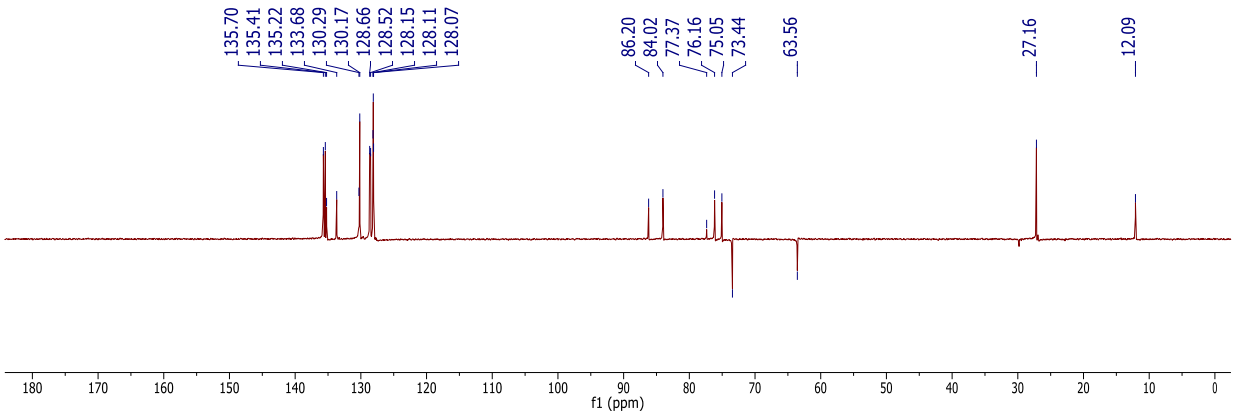
H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **34**



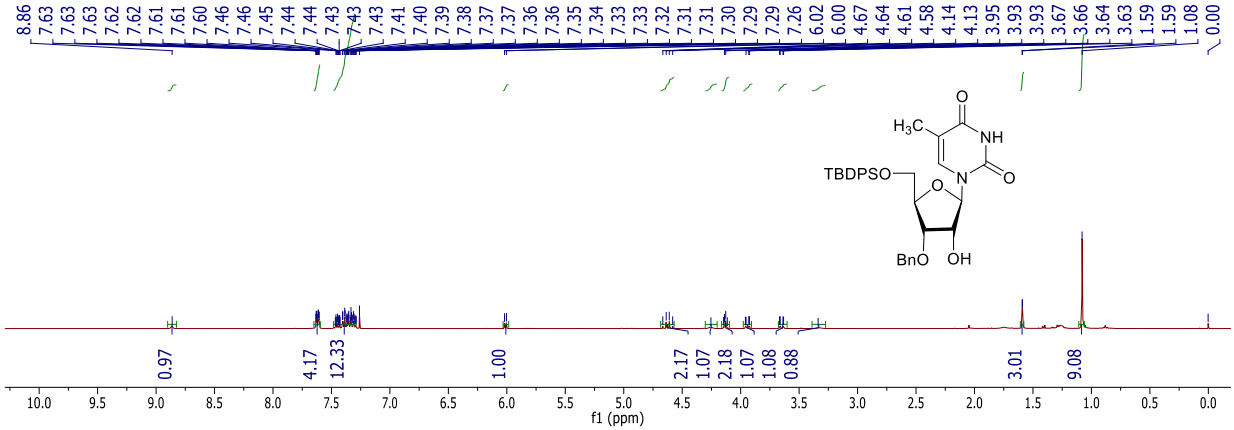
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **34**



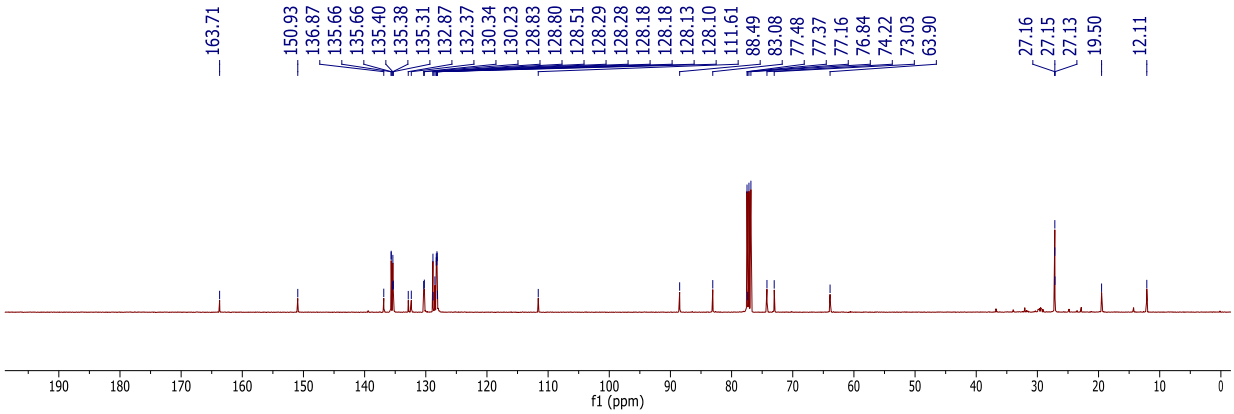
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **34**



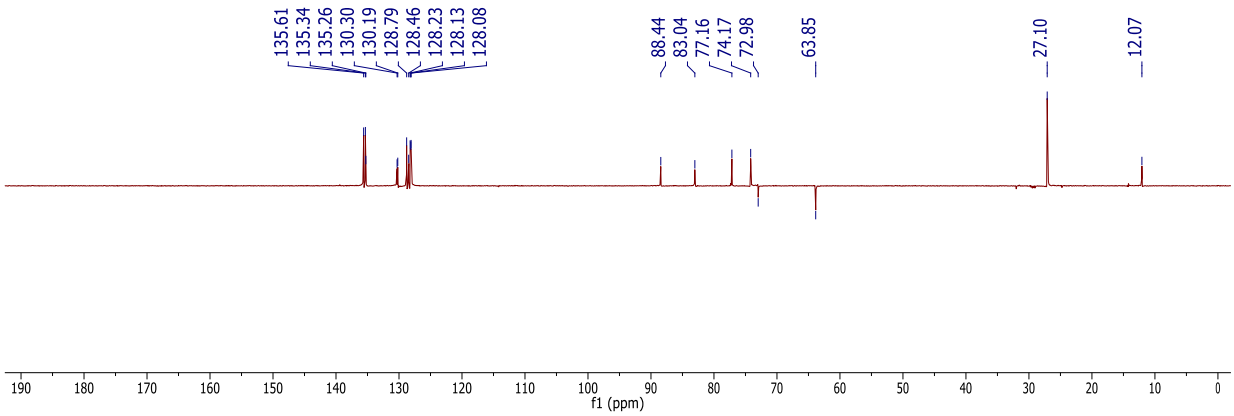
^{1}H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **35**



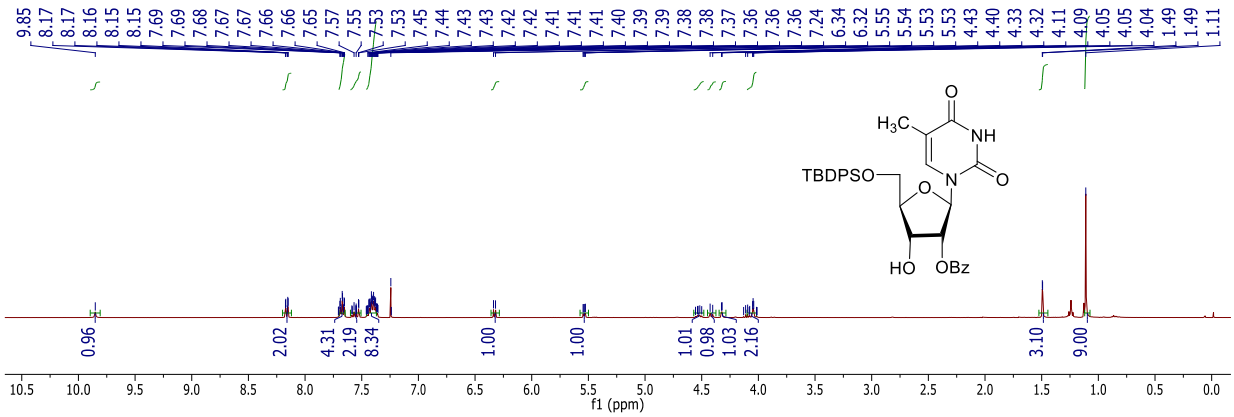
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **35**



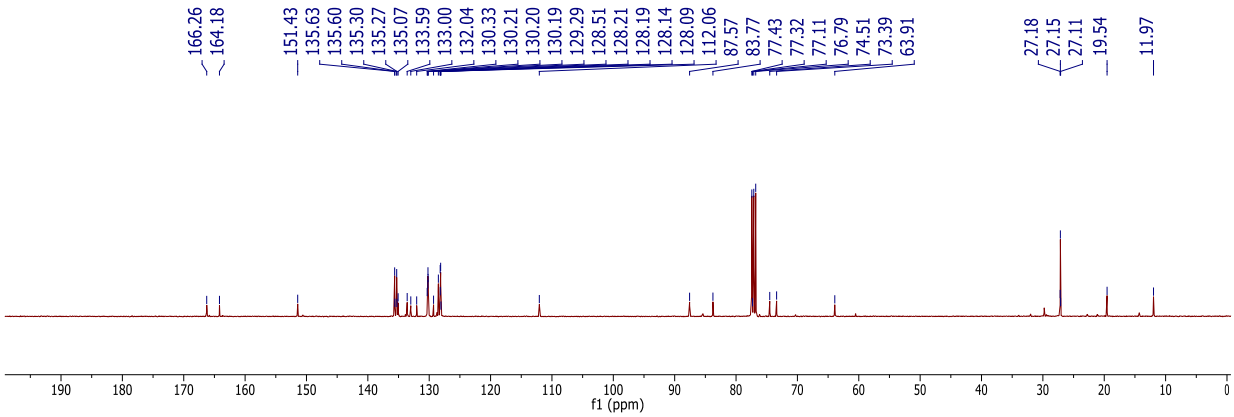
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **35**



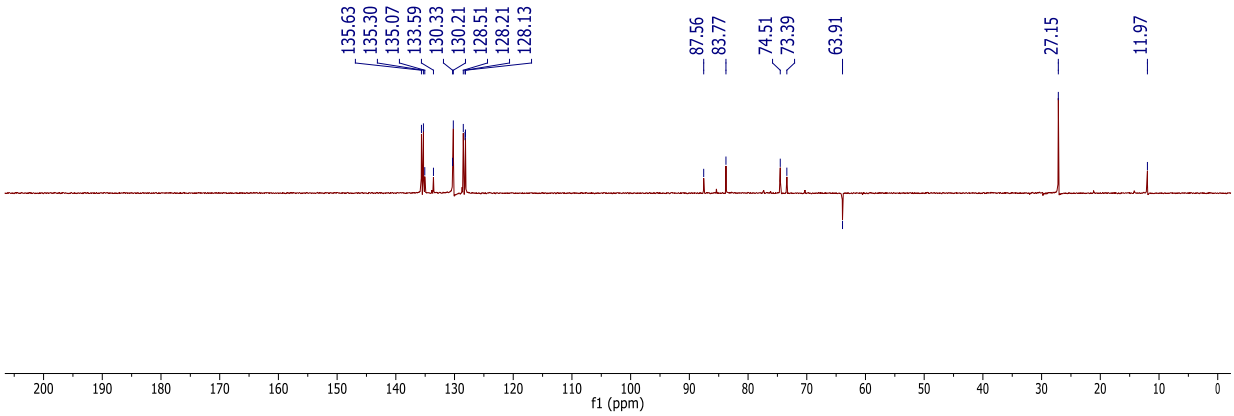
^{1}H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **36**



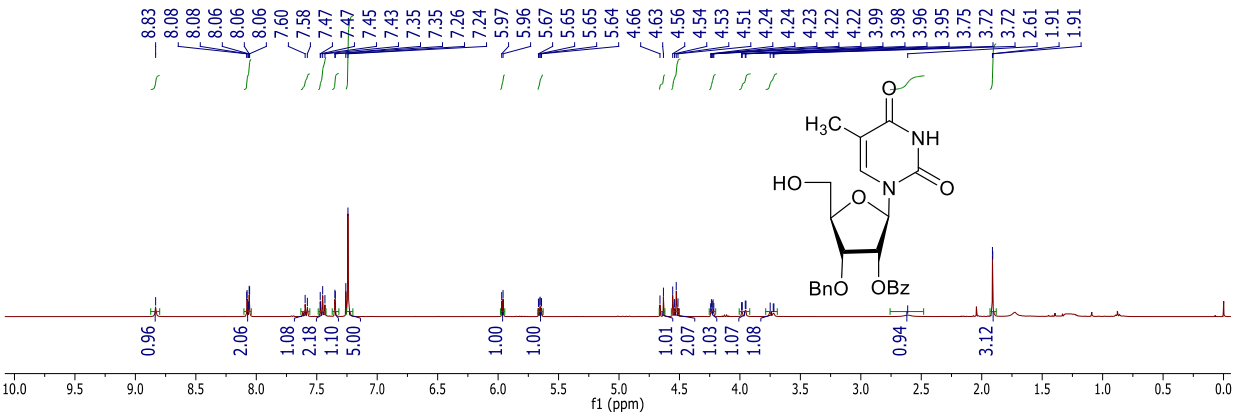
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **36**



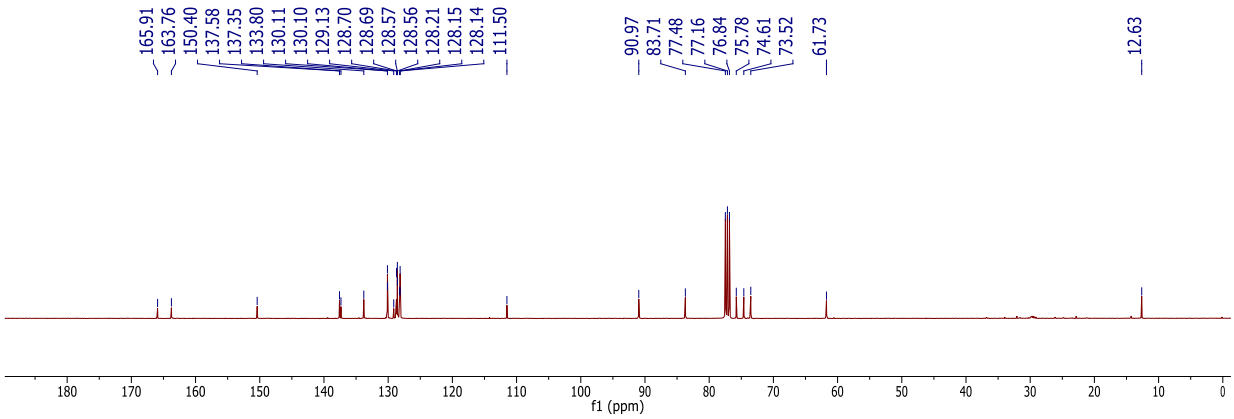
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **36**



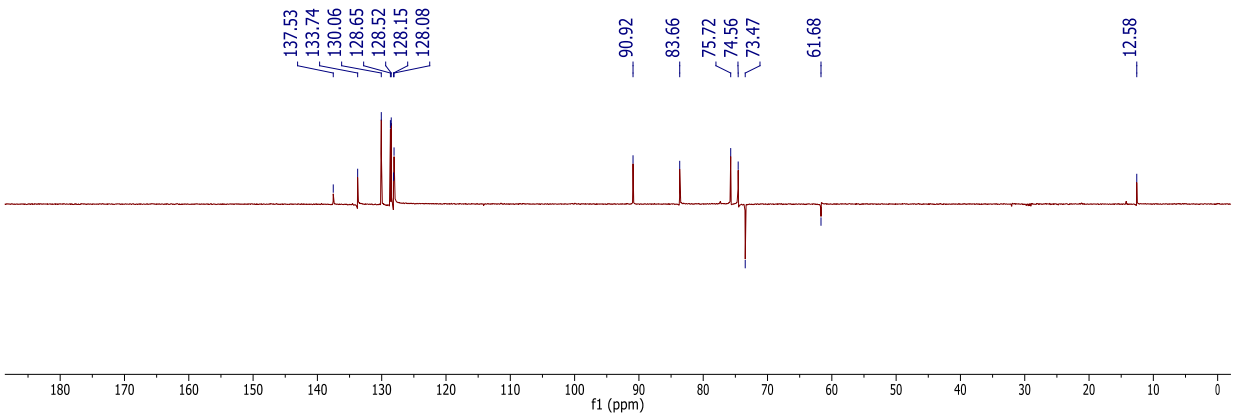
^{1}H NMR Spectrum ($CDCl_3$, 399.78MHz) of Compound **37**



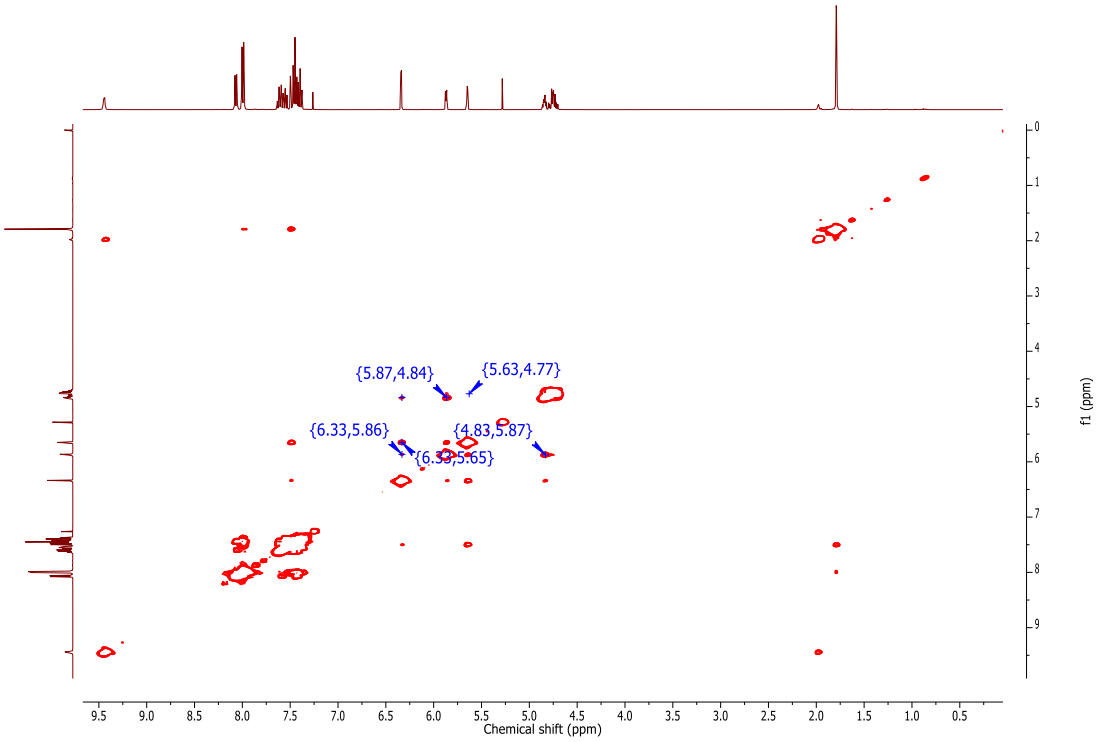
^{13}C NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **37**



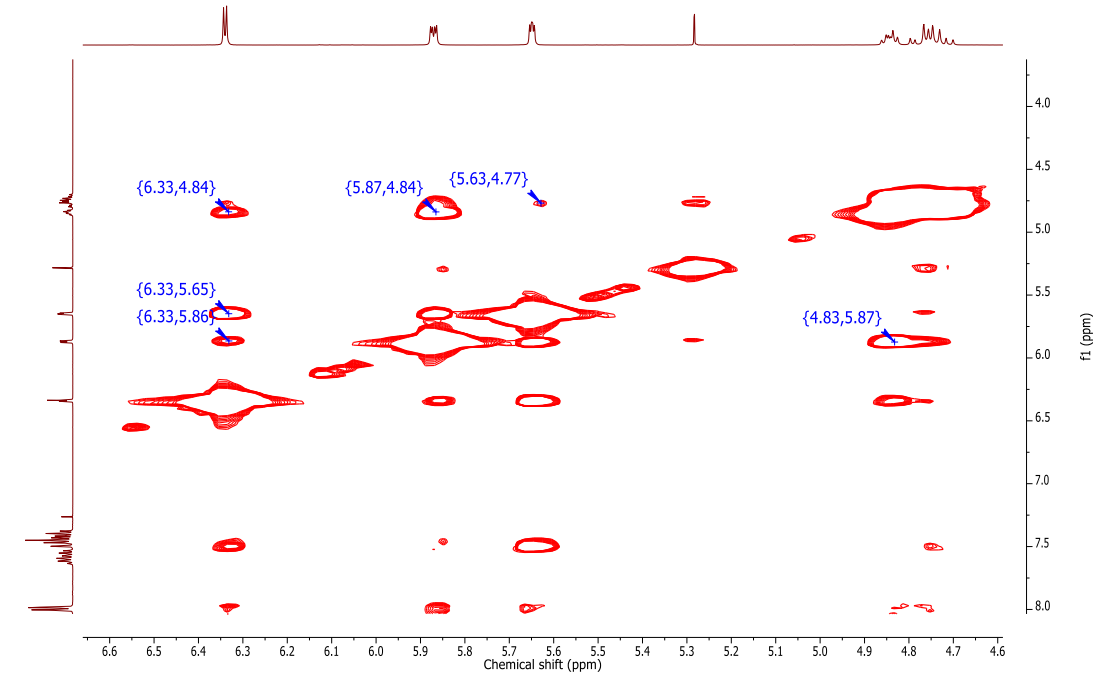
DEPT NMR Spectrum ($CDCl_3$, 100.53MHz) of Compound **37**



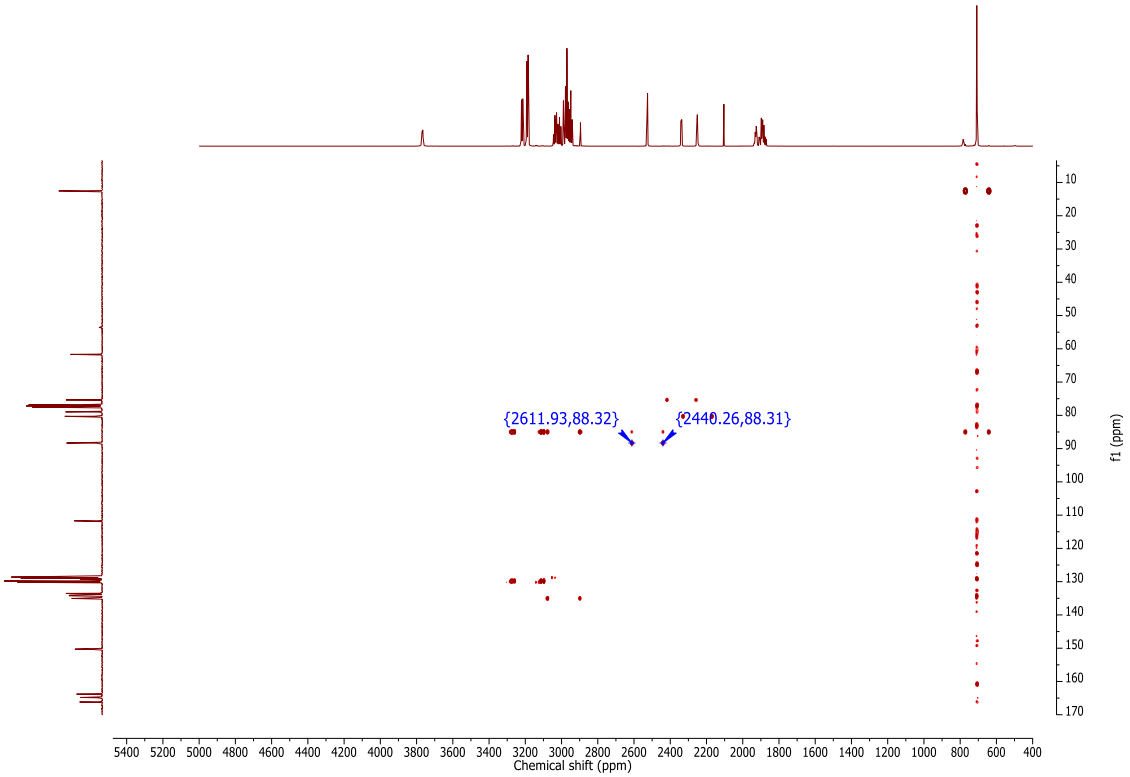
NOESY Spectrum (399.78MHz, $CDCl_3$) of Compound **19**



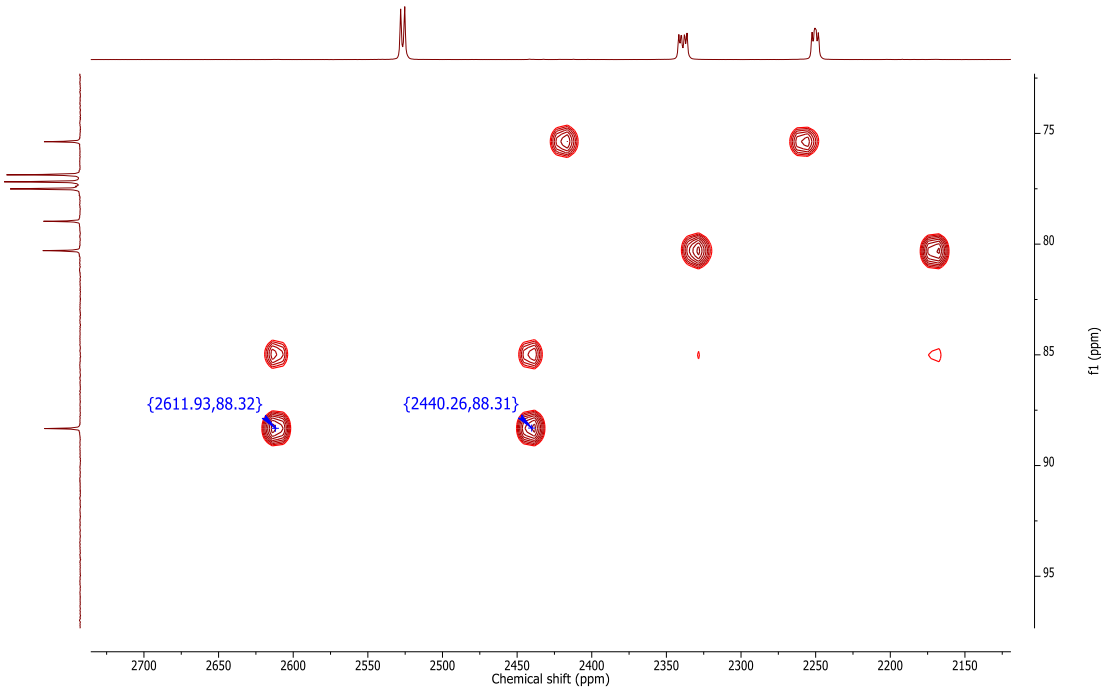
Expansion



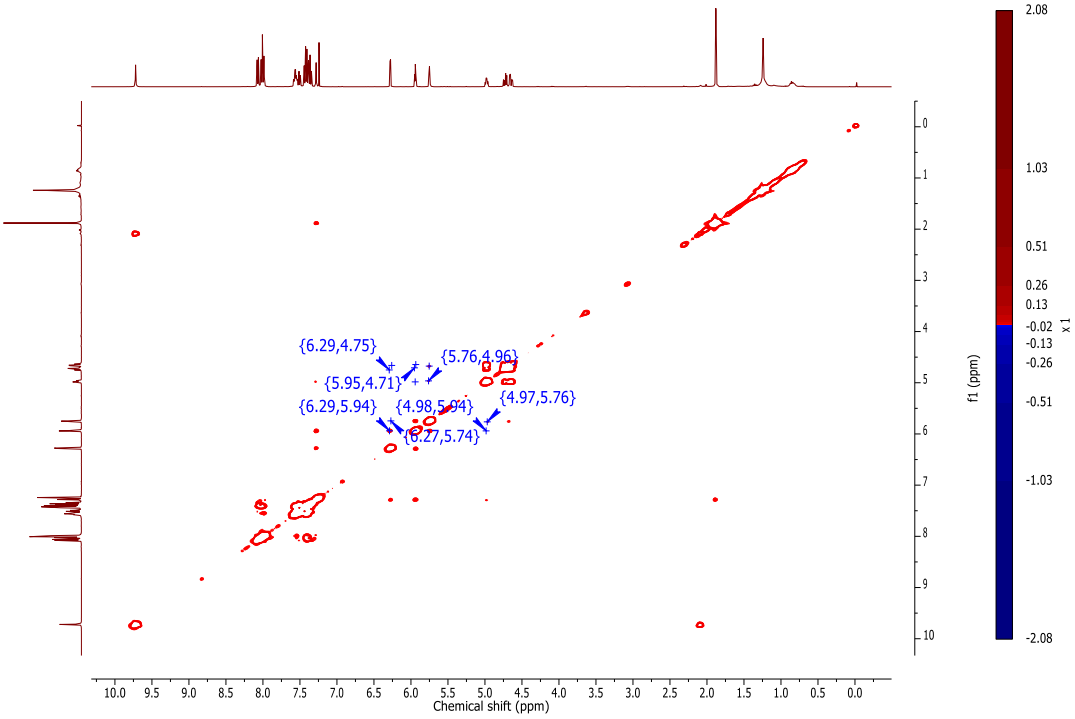
HSQC Spectrum (399.78MHz, $CDCl_3$) of Compound **19**



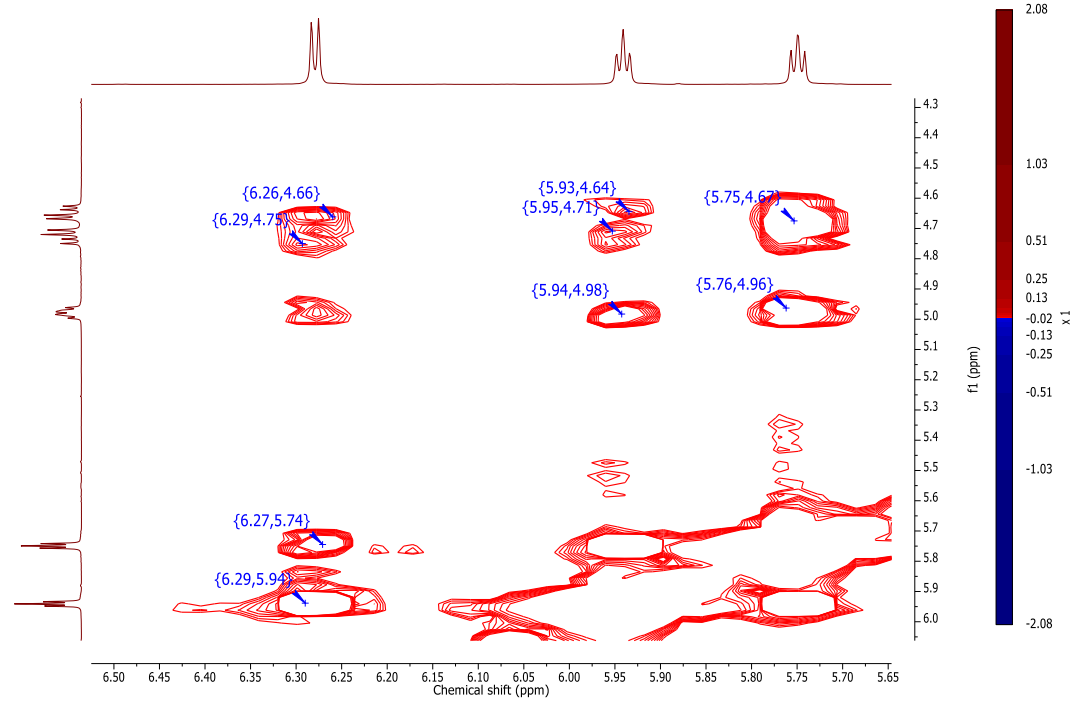
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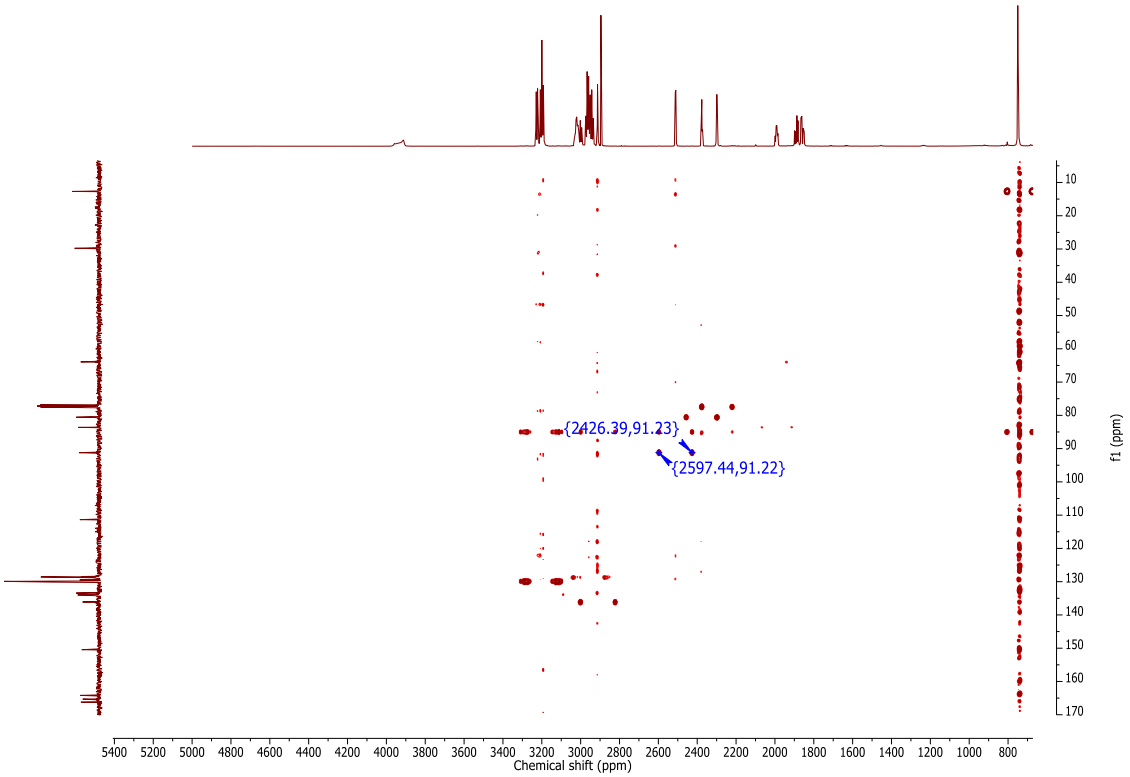
NOESY Spectrum (399.78MHz, $CDCl_3$) of Compound **20**



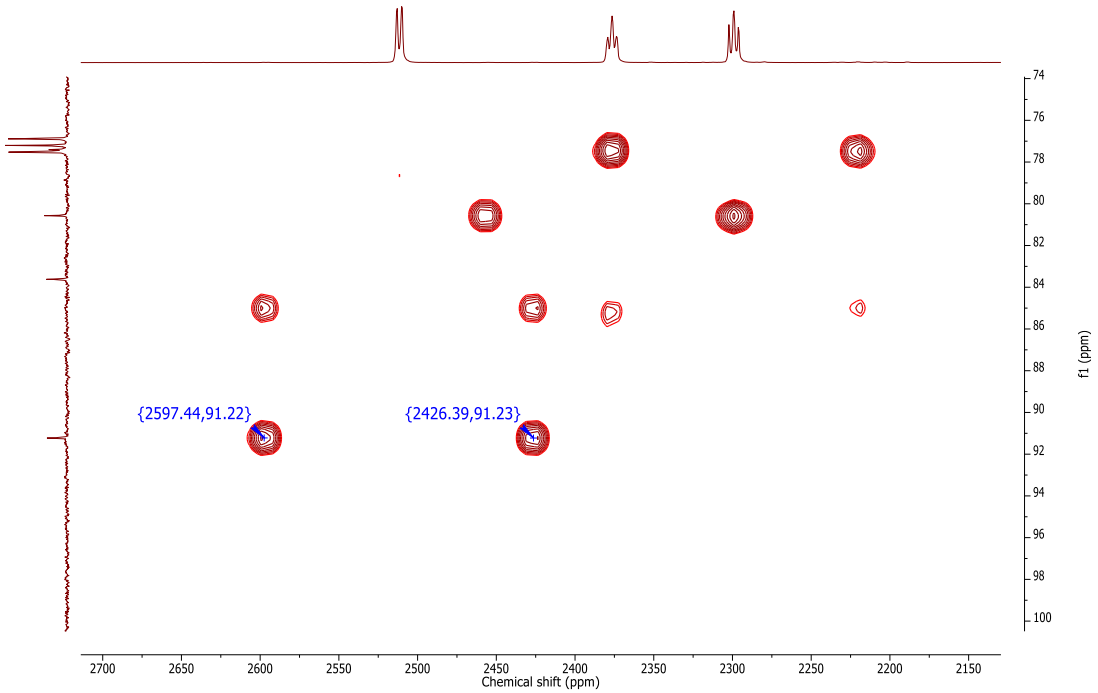
Expansion



HSQC Spectrum (399.78MHz, $CDCl_3$) of Compound **20**



Expansion



2A.8 References:

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(2) Hayes, D. H.; Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 808.

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CHAPTER 2

Stereoselective N- and S- glycoconjugates by gold catalysis

2B. Synthesis of Thioglycosides and 1-Thiotrehaloses

2B.1 Introduction

Ubiquitously present carbohydrates are present in many natural biomolecules and serve not only as energy source but also involve as structural framework in various cells and tissues. Saccharides are conjugated to different molecules to generate corresponding glycoproteins, glycolipids and steroid conjugates (saponins). These glycoconjugates play key roles in various biochemical processes such as signalling, cell-cell communication, fertilization etc. Understanding of interactions between carbohydrates and protein is desirable to develop carbohydrate-based therapeutics, drugs and vaccines as well. One of the most prominent approaches is to use modified carbohydrate analogues to elicit biological response as they can mimic the binding site of the naturally occurring saccharide antigen. Modified saccharides can be conceived as replacement of hydrolysable *O*-glycosidic linkage with other non-hydrolysable groups such as -CH_2-, -NR- or by sufur atom (-S-) among many others. Substitution of the oxygen with sulfur is one of the most suitable bioisosteric replacements in order to produce modified saccharides which are shown to be more stable than oxygen glycosides towards hydrolysis.

2B.2 Natural occurrence

The hydroxyl groups of carbohydrates being substituted by sulfur are less abundant in Nature; though, sulfur is an essential element present in many living systems.[1] Sulfur can be introduced into saccharide core structure by replacing one or more of the oxygen atom. For example, substitution of the anomeric oxygen or replacement of one of the hydroxyl groups with SH or replacement of the endocyclic oxygen with sulfur can potentially give rise to a diverse range of structures for biological exploration (Figure 2B.1).



Figure 2B.1 Possible thio-derivatives of saccharide

Thioglycosides belong to glucosinolates family which are mainly *O*-sulfated thiohydroximates of 1-thio-β-D-glucopyranosides. These important thio-glycopyranosides are largely present in four plant species; namely, *Barbarea vulgaris*, *Arabidopsis thaliana*, *Eruca sativa*, and *Isatis tinctoria*.[2] Besides, thioglycosides also exist in frostyraxthioside A from *Afrostyrax lepidophyllus*,[3] Raphanuside from the seeds of *Raphanus sativus*,[4] the clinically used antibiotic Lincomycin A[5] and Salacinol (Figure 2B.1).[6]

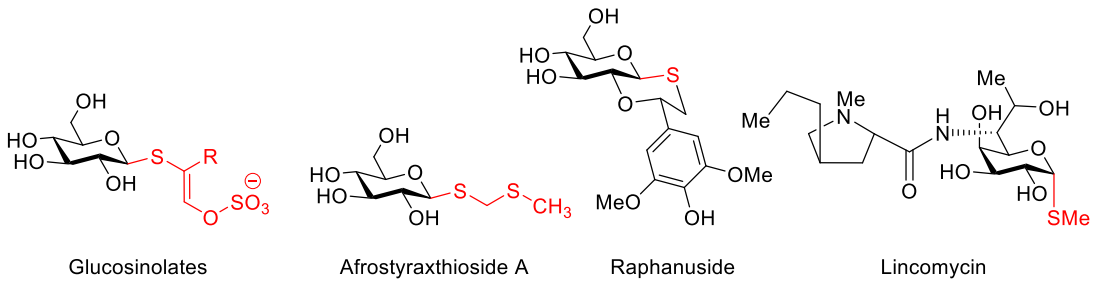
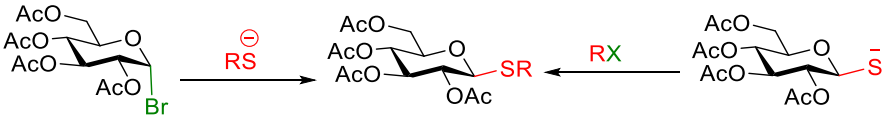


Figure 2B.2 Some of the naturally occurring thioglycosides

2B.3 Synthesis of thiosaccharides

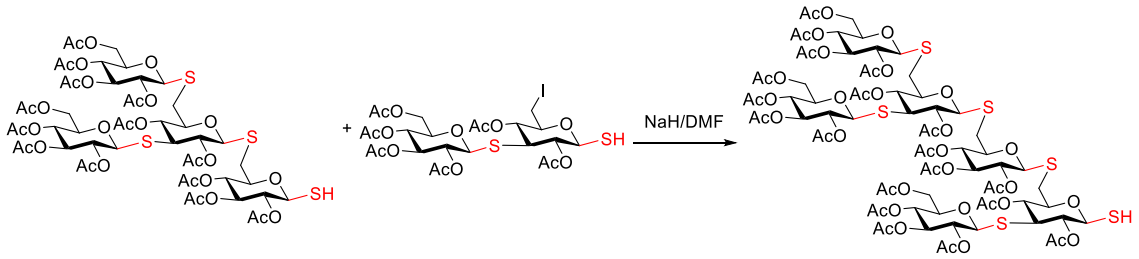
2B.3.1 Displacement method by thiolate

The first one takes advantage of the nucleophilicity of sulfur wherein the thiolate attacks the electrophilic donor to afford the thioglycoside. The electrophilic donors are generally armed with an anomeric mixture of bromide, chloride, iodide or sulfonate. The thiolates generally displace halides or sulfonates following S_N2 mechanism to afford thiosaccharides (Scheme 2B.3).[7]



Scheme 2B.3 Synthesis of thiosugars via S_N2 mechanism

In a very interesting report, *S*-linked thiomimetics of phytoalexin-elicitor-active branched oligosaccharide was synthesized to observe that thiomimietics are non-hydrolysable and they are equally potent to the naturally occurring oligosaccharides (Scheme 2B.4).[8]

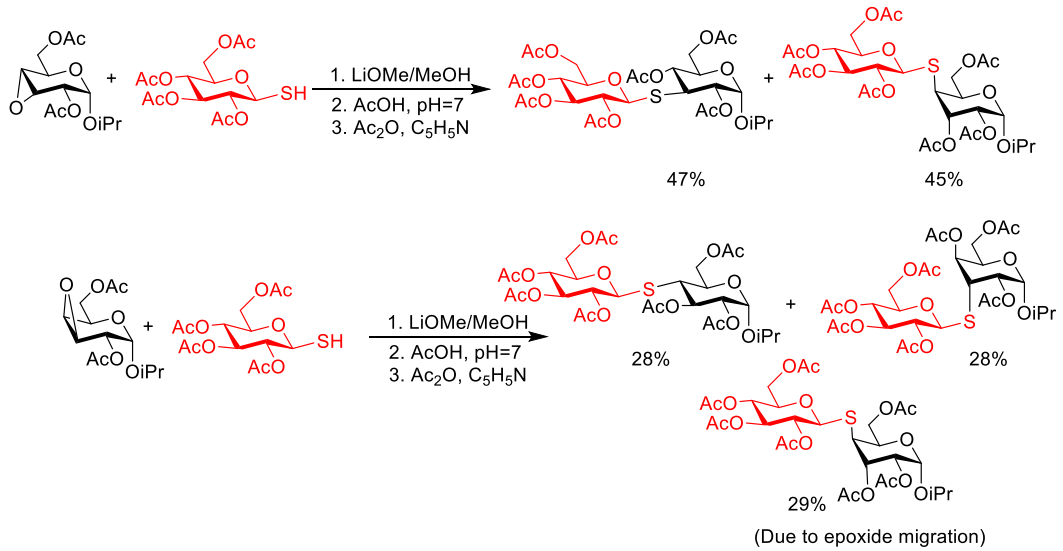


Scheme 2B.4 Synthesis of thiosaccharides[8]

2B.3.2 Synthesis of thiosaccharides under basic conditions

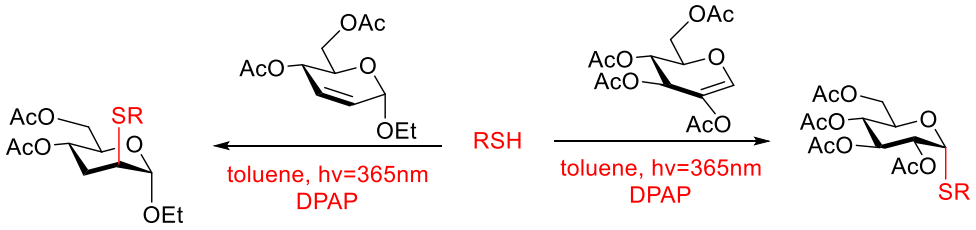
Thioglycosides or thiosugars can be synthesized by the nucleophilic addition of the thiolates to α,β-unsaturated systems,[9] epoxides,[10] 1,6-anhydride sugars and aziridines.[11] Epoxide can be opened stereospecifically by the thiolates under basic conditions to produce thioglycosides regioselectively. Varela *et al.* reported the reactivity of various sugar epoxides (anhydro sugars) with a 1-thiosugars to obtain thiosaccharides.[11]

However, the epoxide ring opening happened in a non-regiospecific manner; but partial regio- as well as stereo- selectivities can be achieved by tuning stereoelectronic properties or by invoking neighbouring group participation. Addition of the thiolate happens in a *s-trans* fashion since the nucleophilic attack happens in an anti-manner Scheme 2B.5).[11]



Scheme 2B.5 Synthesis of thiosaccharide by the addition reaction[11]

On other hand, addition of thiols to olefins generally results in the formation of both regio- and stereo- isomeric products.[9] Recently in 2012, Borbás *et al.* studied regio- and stereo-selective addition of thiols onto unsaturated sugars derivatives by photochemical means.[12] 1,2-*cis*-thioglucoside was noticed when mercaptans are treated with electron deficient 2-acetoxy glucal and manno-configured thiolate was noticed when the photochemical reaction was carried out with 2,3-di-deoxy derivative.[12]

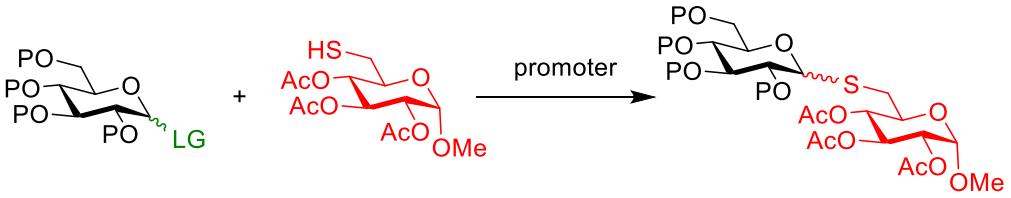


Scheme 2B.6 Photochemical addition of thiolates onto unsaturated sugar derivatives[12]

2B.3.3 Lewis acid based activation glycosylation method

Lewis acids can promote the thiolate additions on to glycosyl donors when the glycosyl acceptor has a –SH group. The stereochemical outcome of the reaction depends on the glycosyl donor and the activator used in the course of the reaction; temperature; solvent; the concentration; and/or the presence of or absence of neighbouring group participation. 1,2-*trans* stereoselectivity is observed when the *C*-2 hydroxyl group of the glycosyl donor

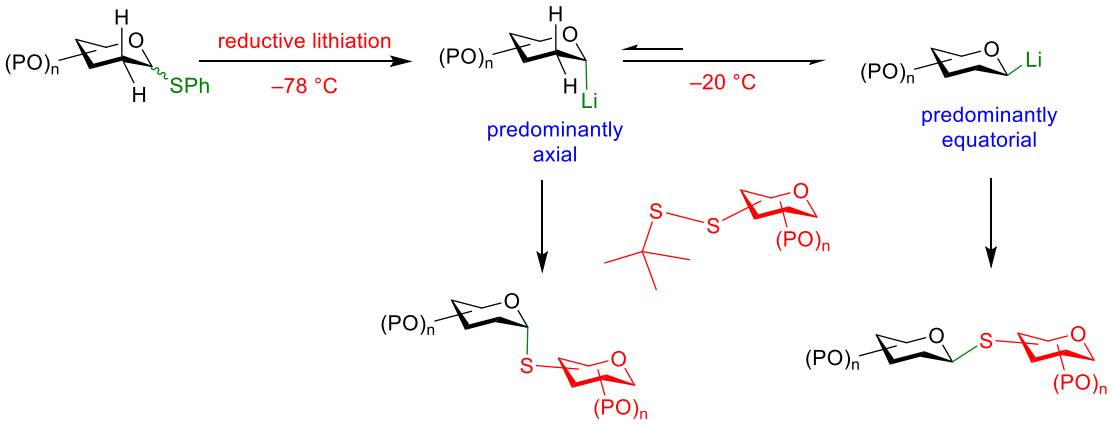
contains ester such as -OAc and -OBz whereas mixture of 1,2-*trans* and *cis* glycosides are noticed in the presence of ethers at *C*-2 position. In this connection, various glycosyl donors that are developed over the last one century or so are activated in the presence of suitable promoters to get thiosaccharides.



Scheme 2B.7 Synthesis of thiosaccharides by activation of glycosyl donors

2B.3.4 Umpolung based Method

The umpolung reactivity-based thio-glycosylation reaction was reported by Zhu *et al.*. Synthesis of the thiosaccharide can be achieved by using sulfenylation of the stereochemically defined glycosyl lithium species with an asymmetric sugar-derived disulfide. The reductive lithiation using strong lithium base such as lithium 4,4′-di-tert-butylbiphenyl leads to the elimination of the thio-phenyl group to form the lithiated donor. The anomeric selectivity was then attained by temperature control and followed by the addition of disulphide resulted the formation of thiosaccharides (Scheme 2B.6).[13]

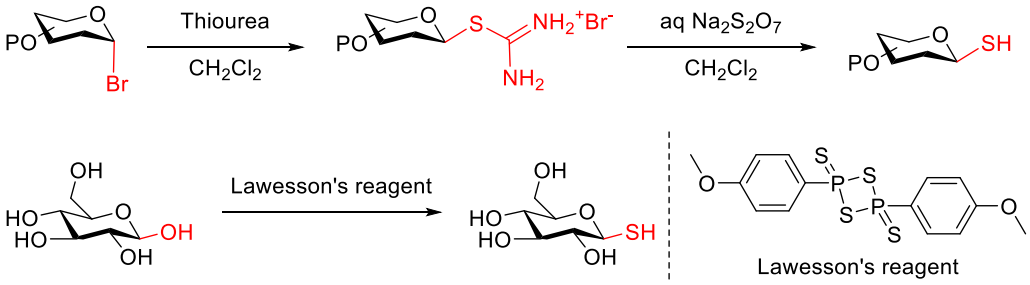


Scheme 2B.8 The umpolung based method for the synthesis of thiosaccharides[13]

2B.4 Synthesis of 1-thiosugars

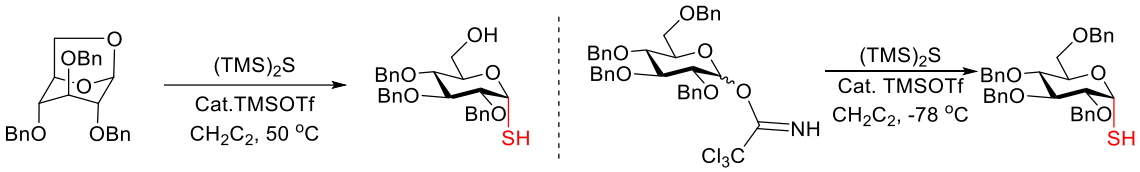
The *C*-1-thiosugars are very important synthons for the chemical synthesis of various thiooligosaccharides and many glycoconjugates due to their stability to enzymatic hydrolysis. Moreover, the thio-linked glycoconjugates are mainly considered as bioisosteric replacements in the design of various therapeutic agents. Further, the *C*-1-thiosugars are primary building blocks for the synthesis of 1,1-thiotrehaloses as well. The conventional approaches for the synthesis of 1-thiosugars include a two-step reaction

mechanism involving the S_N2 substitution reaction of a glycosyl halide with thiourea and the subsequent hydrolysis of thiourea intermediate.[14] Davis *et al.* have reported the synthesis of 1-thiosugar derivatives from sugar hemiacetals using Lawesson's reagent,[15] which were further utilized for the selenenyl-sulfide-mediated protein glycosylation.[15]



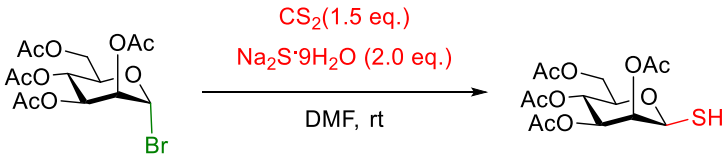
Scheme 2B.9 Synthesis of α-glycosyl thiol derivatives

Zhu *et al.* reported the exclusive preparation of α-glycosyl mercaptan derivatives from 1,6-anhydro sugars in a stereospecific manner using bis(trimethylsilyl)sulfide.[16] Schmidt developed the α-glycosyl mercaptan derivatives from glycosyl trichloroacetimidates having non-participating group at *C*-2 position using an acid-catalyzed reaction with bis(trimethylsilyl)sulfide [$(TMS)_2S$].[17]



Scheme 2B.10 Synthesis of α-glycosyl mercaptans

The preparation of 1-β-glycosyl thiol derivatives from corresponding glycosyl bromides using CS_2/Na_2S in DMF was reported by Misra *et al.* and the tetra-*O*-acetyl mannosyl bromide gave 1-β-mannosyl-thiol in good yields.[18]



Scheme 2B.11 Synthesis of β-mannosyl mercaptan

2B.5 1-Thiotrehaloses

Trehaloses are disaccharides in which two units of glucose (sugars) are directly connected to each other by oxygen atom *via* a 1,1-glycosidic bond.[19] There could be possibility of

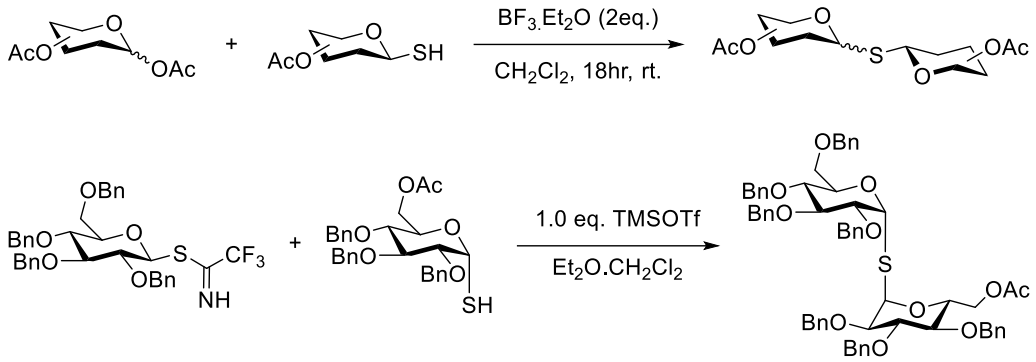
four anomeric configurations. However, the α,α-configuration is only found in animals, plants and microorganisms.[20] Physiologically, trehaloses are a quick source of energy since one mole gives two moles of glucose upon hydrolysis.[20] In addition, trehalose derived lipids are prominent components of bacterial cell walls, and are therefore necessary to cell growth.[21] Thiotrehaloses in which the glycosidic oxygen is substituted by a sulfur atom, possesses greater chemical and enzymatic stability than its corresponding *O*-linked isosteres.[22] Thiotrehaloses have attracted much attention as enzyme inhibitors; for example, the growth of *Escherichia coli* is inhibited by the trehalose analogue 1-thio-β-D-galactopyranosyl-β-D-galactopyranoside.[23] Thiotrehalose analogues have also been used for enzymatic purification and for the study of enzymatic kinetics.[24,25]



Figure 2B.12 Trehalose and 1,1-thiotrehalose

2B.5.1 Synthesis of 1,1-thiotrehaloses

Many methods are available in the literature for the synthesis of 1,1-thiodisaccharides. The condensation of per-*O*-acetylated sugar halides with potassium alkylxanthate,[26] or the reaction between a thiosugar and glycosylbromide are some of the popular ones.[7] Large number of efforts are devoted to improve reaction yields, including the mono-desulfurisation of glycosyl disulfides by tri(diethylamino)-phosphine etc.[27] Another way of synthesizing thiotrehaloses is by hydrogen fluoride-mediated reaction with D-glucose using hydrogen sulfide to form α,α-1-, α,β-1- and β,β-1-thiotrehaloses.[28] Lewis acid mediated ($BF_3.Et_2O$) glycosylation reaction of per-*O*-acetyl glycopyranosides with 1-thiosugars resulted in the formation of 1,1-thiotrehaloses presumably due to the neighbouring group participation of the *C*-2 acetate.[29] The stereoselective synthesis of α,α-1-thiotrehalose was reported recently by Zhu *et al.* by the activation of the per-*O*-benzyl glucosyl trichloroimidate donor using TMSOTf or TFA/TESH respectively with 1-thioglucose as a glycosyl acceptor.[30] However, one of the major drawbacks of these methods is that the reaction requires long time, use of toxic and malodorous nature of reagents.



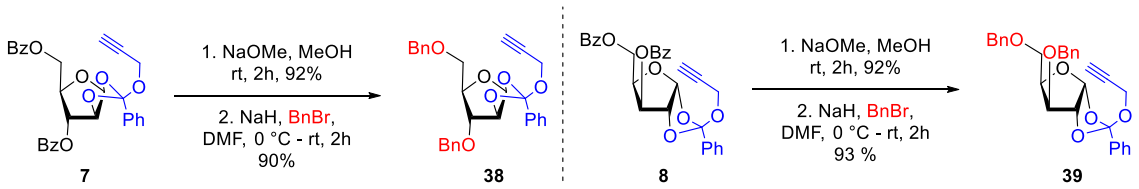
Scheme 2B.13 Synthesis of 1-thiotrehaloses[29-30]

Interestingly, thioglycosides and thiotrehaloses are resistant to the enzymatic cleavage compared to the corresponding natural *O*-analogues and thence, became a promising target for the development of carbohydrate based therapeutics.[31] Easy formation of disulfides and incompatibility with routinely used protecting groups in sugar chemistry is the major concern that is frequently encountered while synthesizing *S*-glycosides. In most of the cases, the resulting *S*-glycoside would probably be a mixture of 1,2-*trans* as well as 1,2-*cis* glycosides.[32] The possibilities would be doubled if the synthesis of 1-thiotrehaloses [(1→1′)-S-linked saccharides] is considered as an anomeric configuration of both the participating sugars. Therefore, there is indeed need to find a clean alternative for the synthesis of thiotrehaloses with full diastereoselectivity.

2B.6 Present Work

Carbohydrates with any of the hydroxyl groups being substituted by sulfur (thio-) atom are not common in Nature, though sulfur is an essential element present ubiquitously in various living systems.[1] Stable thioglycosides are investigated thoroughly for their ability to promote glycosylation reactions using a variety of thiophilic reagents.[32] Thioglycosides and thiotrehaloses are more resistant to the enzymatic cleavage compared to their natural *O*-analogues and hence, became promising targets for the development of carbohydrate based therapeutics.[31,33] A few methods are available to the synthesize 1,2-*trans* thioglycosides and trehaloses in a stereochemical fashion by exploiting the neighbouring group participation or stearic hindrance or combination of both of them. In this context, propargyl 1,2-orthoesters for constructing the thioglycosidic linkage in a diastereoselective fashion to achieve various thioglycosides and thiotrehaloses was hypothesized.

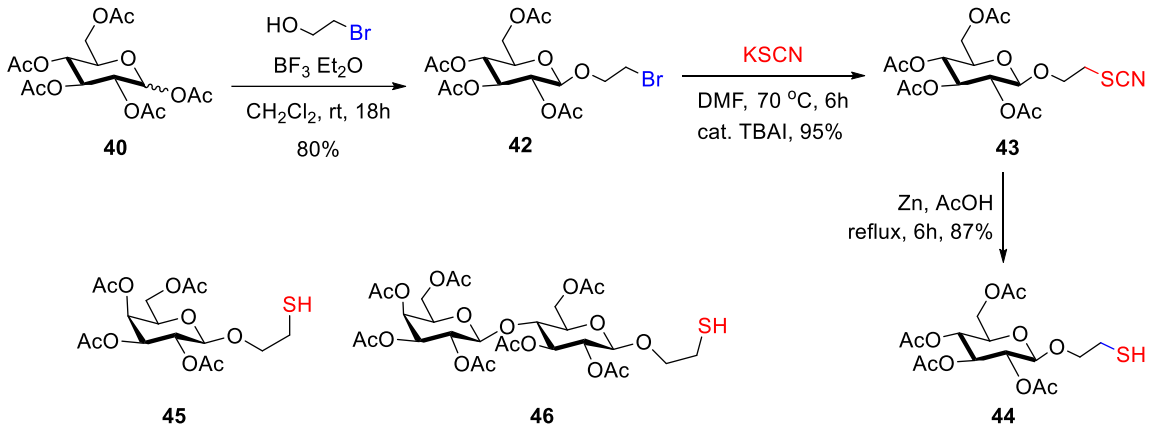
To synthesize thiosaccharides using chemical glycosylation methods, we primarily require a glycosyl donor and a glycosyl acceptor. Propargyl 1,2-*O*-orthoesters were identified as versatile glycosyl donors by Sureshkumar and Hotha. In addition, propargyl 1,2-orthoesters can be easily accessed from corresponding aldoses (See Chapter 2A). A few propargyl 1,2-orthoesters are synthesized before initiating our exploration of propargyl 1,2-orthoesters as glycosyl donors for the synthesis of thioglycosides. Propargyl orthoesters **7** and **8** prepared in the previous chapter (section 2A) were converted into corresponding benzyl ethers **38** and **39** in two steps. Initially, saponification of orthoester **7** was carried out under Zemplén deacetylation conditions followed by the treatment of NaH/BnBr/DMF/TBAI resulted in the formation of di-*O*-benzyl ether **38** in 81% yield over two steps (Scheme 2B.14).



Scheme 2B.14 Synthesis of Benzyl orthoesters

Many sugar-thiolates are required in order to explore the utility of various 1,2-orthoesters that are synthesized. If the sulfur atom (S) is present on any of the position except from *C*-1 of the sugar, then the resultant product is generally called as a thiosaccharide and if any linker is present in between, then, it is called as *pseudo*-thiosaccharide or

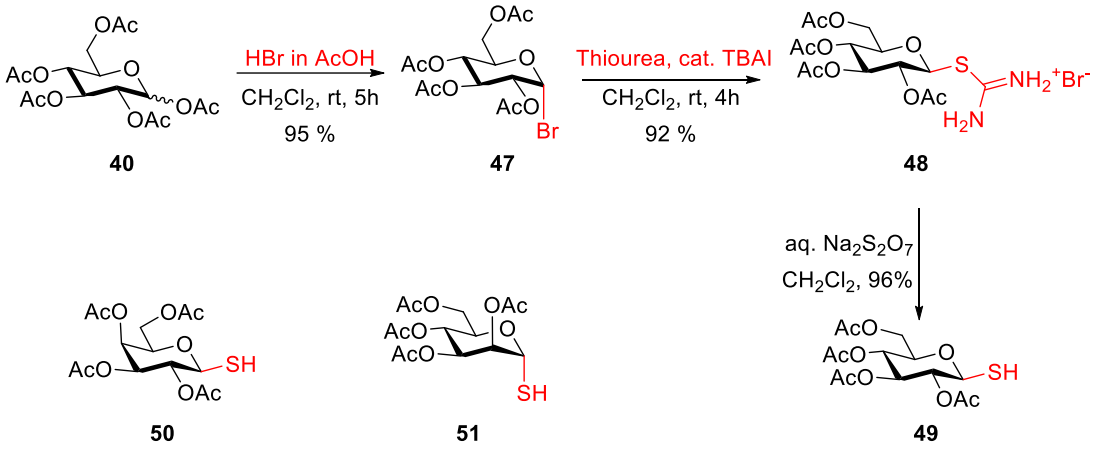
thioglycoside. If the *C*-1 thiols are directly used as glycosyl acceptors then the product is expected to be 1,1-thiotrehalose. To investigate 1,2-*O*-orthoesters as glycosyl donors, saccharides containing –SH functionality various position are synthesized. Easily available per-*O*-acetyl glucose **40** was treated with 2-bromoethanol (**41**) in the presence of $BF_3.Et_2O$ in CH_2Cl_2 for 18h to afford 2-bromoethyl glucoside **42** in 80% yield. Bromide **42** was converted into thioisocyanate **43** by reaction by displacement with KSCN, TBAI in DMF at 70 °C which was subsequently reduced under Zn/AcOH conditions to yield 2-thioethyl glucoside **44** in 87% yield. In the ^{13}C spectrum of compound **44**, the methylene (confirmed by ^{13}C-DEPT experiment as well) α- to the thiol was noticed at δ 24.2 ppm along with all other resonances in complete agreement with the assigned. The same procedure was employed for the synthesis of 2-thioethyl galactoside **45** and 2-thioethyl lactoside **46** (Scheme 2B.15).



Scheme 2B.15 Synthesis of thio sugar derivatives

In the ^1H spectrum of compound **45** and **46,** the proton resonances due to mercaptoethylene moiety are noticed at δ 1.44 ppm (2H, t, *J* = 8.3 Hz) and δ 1.50 (2H, t, J = 8.3 Hz) respectively whereas the ^{13}C-NMR spectrum resonances at δ 24.1 and 24.3 ppm are observed for the two methylenes.

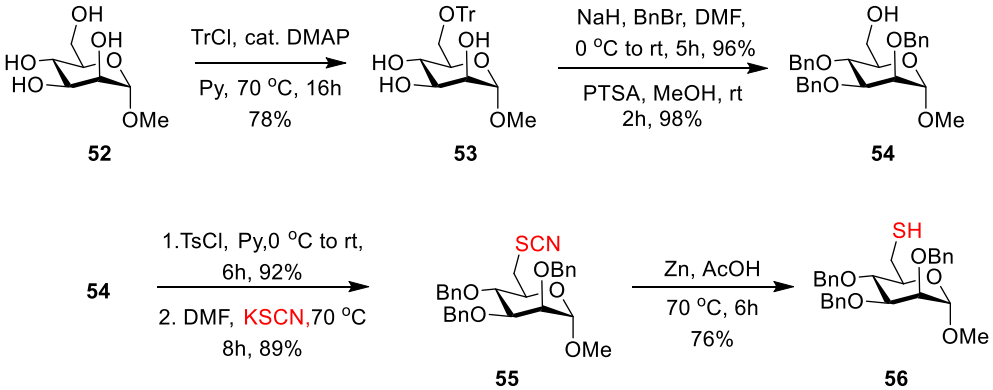
Next, our attention has turned to the synthesis of thiotrehaloses wherein a single isomer of the1-thio sugar derivatives is required. Towards this, per-*O*-acetyl glucose **40** was converted to glucosyl bromide **47** by the treatment of HBr in AcOH, which was then allowed to react with thiourea in dichloromethane to afford thiourea glucoside **48**. Further, thiourea **48** was treated with aq.$Na_2S_2O_7$ to form 1-thioglucoside **49**.[3h] Similarly 1-thiogalactoside **50** and 1-thiomannoside **51** were also prepared by using the same procedure (Scheme 2B.16).



Scheme 2B.16 Synthesis of 1-thio sugar derivatives

Another building block, 6-*deoxy*-6-thio-mannopyranoside **56** was prepared starting from mannose. Commercially available methyl mannoyranoside (**52**) was reacted with trityl chloride in pyridine to obtain trityl ether **53** which was treated with NaH BnBr/NaH/TBAI/DMF to obtain fully protected mannoside **54** in 98% yield. The trityl group of compound **54** was selectively deprotected under acidic conditions using PTSA in MeOH to afford alcohol **54**.

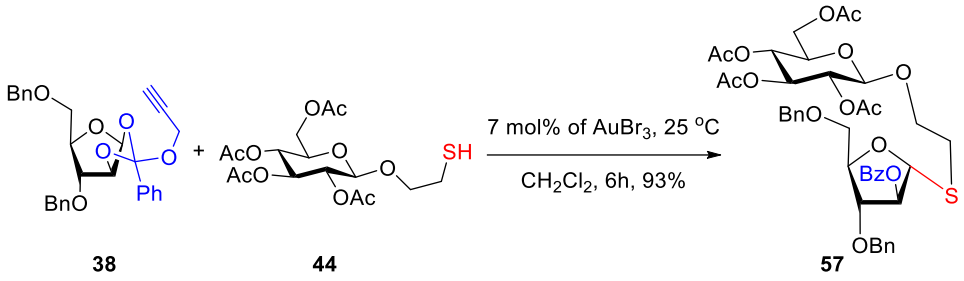
Conversion of the lone alcohol to mercaptan was achieved in three steps. Compound **54** was treated with TsCl in pyridine to get a tosylate that was subjected to nucleophilic displacement with potassium thiocyanate in DMF at 70 ^{o}C to afford thiocyanate **55** in 73% yield over two steps. The thiocyanate **55** was reduced with Zn/AcOH at 70 ^{0}C to 6-*deoxy*-6-thio mannopyranoside **56** in 76% yield. (Scheme 2B.17).



Scheme 2B.17 Synthesis of 6-*deoxy*-6-mercapto-2,3,4-tri-*O*-benzyl-mannopyranoside

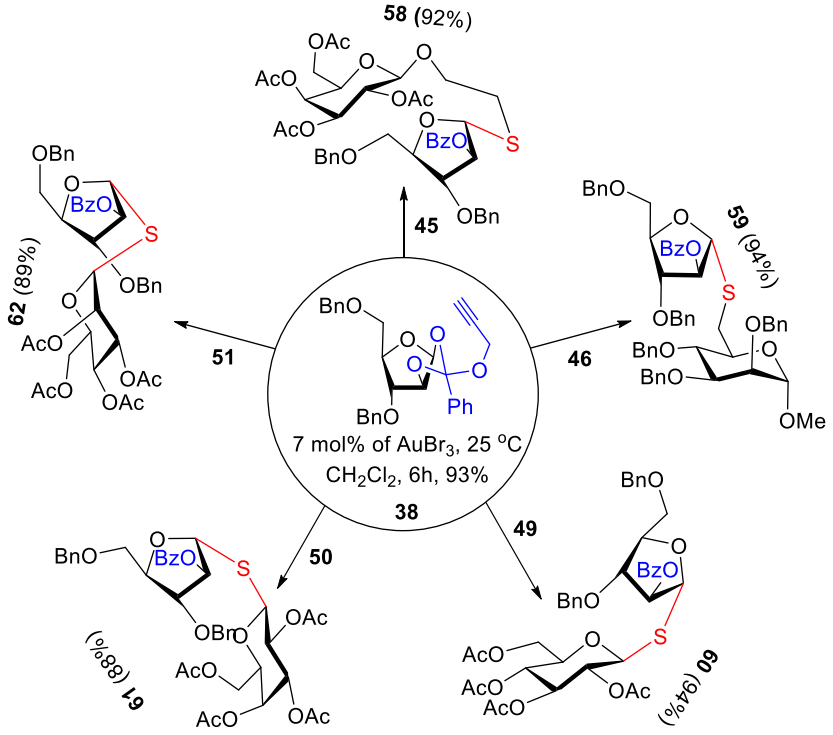
Having all the reactive building blocks necessary for exploring the utility of 1,2-orthoesters as glycosyl donors, orthoester **38** was treated with 2-thioethyl galactoside **44** and 7 mol% of $AuBr_3$ in CH_2Cl_2 at 25 °C. The progress of the reaction was monitored by conventional TLC to find that the reaction was completed in 6h to form *pseudo*-

thiodisaccharide **57** in 93% yield (Scheme 2B.18). In the ^{1}H spectrum of compound **57**, anomeric proton resonances corresponding to the thio-linked residue were noticed at δ 5.45 ppm as a singlet and the ^{13}C NMR spectrum displayed anomeric carbon linked as thio ether at δ 88.3 ppm along with all other resonances in accordance with the assigned structure.



Scheme 2B.18 Synthesis of *pseudo*-thio-disaccharide

Similarly, arabinose orthoester **38** was treated with 2-thioethyl galactoside **45** to obtain thioarabinoside **58** in 92% yield as single diastereomer. In continuation, arabinose orthoester **38** was treated with 6-*deoxy*-6-thio mannose **46** in presence of 7mol% of $AuBr_3$ to afford thio-disaccharide **59** in 94% yield as a single diastereomer (Scheme 2B.19).



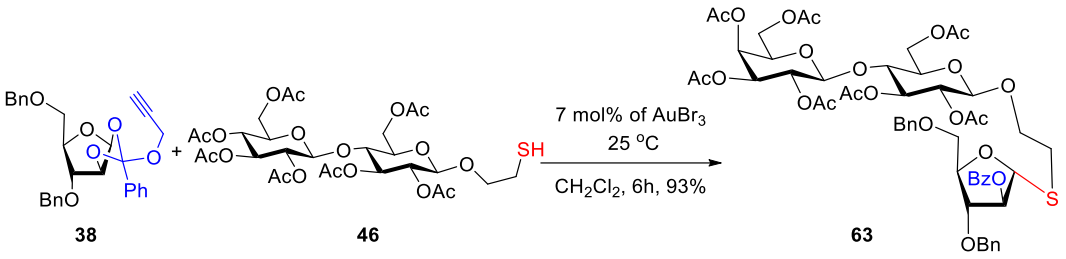
Scheme 2B.19 Synthesis of thio saccharides and thiotrehaloges of arabinose

Encouraging results allowed us to focus on the synthesis of various 1,1-thiotrehaloses as well. Accordingly, the arabinose orthoester **38** was treated with 1-thioglucose **49** in presence of 7mol% of $AuBr_3$ in CH_2Cl_2 to afford 1,1-thiotrehalose **60** in 94% yield as a

single diastereomer. The ^{13}C NMR spectrum clearly showed that the presence of one α- and one β- glycosidic linkage of thiotrehalose **60** (Scheme 2B.19).

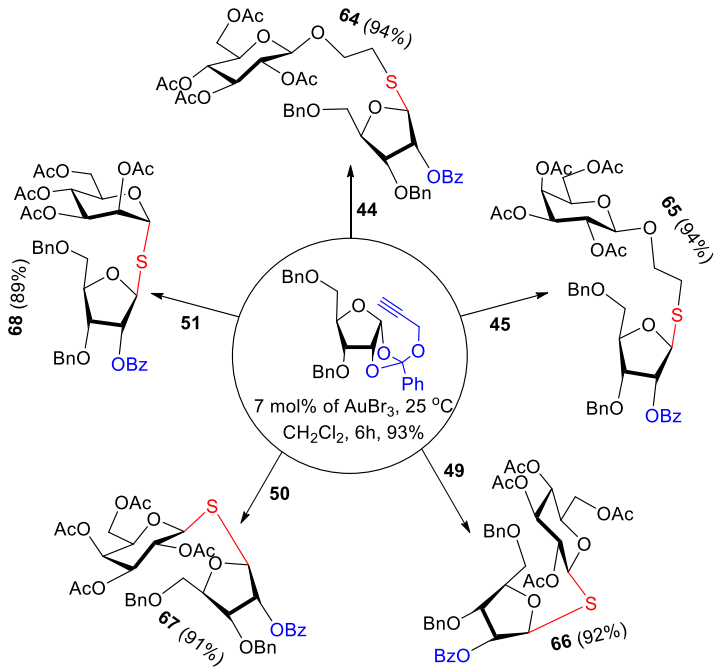
Furthermore, arabinose orthoester **38** was allowed to react with 1-thio galctose **50** and 1-thio mannose **51** under aforementioned conditions to form thiotrehaloses **61** and thiotrehalose **62** respectively (Scheme 2B.19).

2-Thioethyl lactoside **46** was also found to react with the arabinose orthoester **38** under aforementioned conditions to obtain *pseudo*-thiotrisaccharide **63** in 93% yield (Scheme 2B.20). These studies clearly showed that arabinofuranosyl donor are suitable for the synthesis of thiosaccharides and thiotrehaloses in a 1,2-*trans* diastereoselective fashion.



Scheme 2B.20 Synthesis of arabinose thiosaccharides

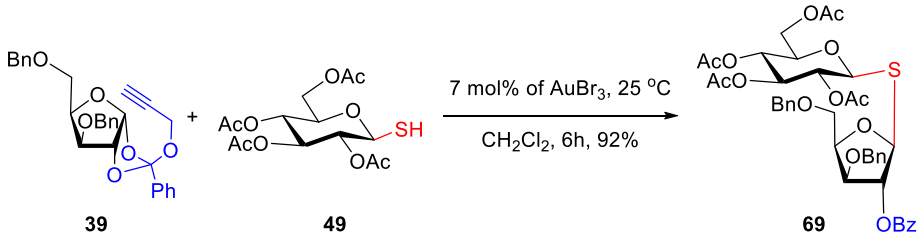
Next, we chose ribose orthoester to synthesize various β-thioglycosides. The synthesis of ribose orthoester was already discussed in the chapter 2A. The ribose orthoester **6**



Scheme 2B.21 Synthesis of thio saccharides and thiotrehaloges of ribose

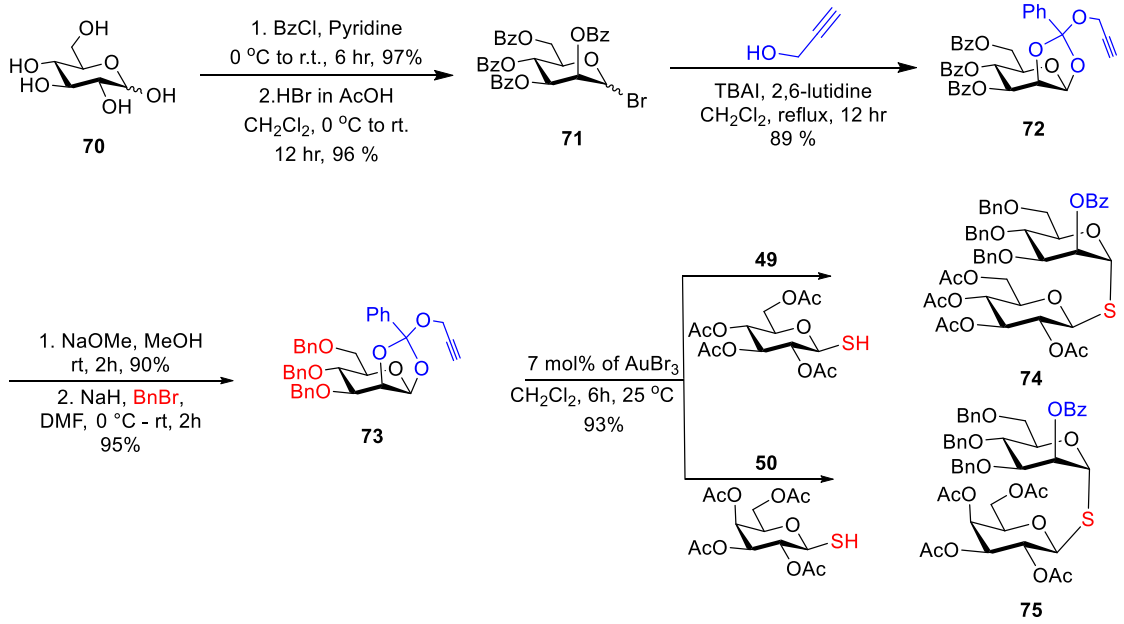
was treated with 2-thioethyl glucoside **44** and 2-thio galactoside **45** in CH_2Cl_2 in the presence of 7mol% of $AuBr_3$ to afford *pseudo*-thiodisaccharides **64** and **65** in good yield

(Scheme 2B.21). These *pseudo*-thiodisaccharides were thoroughly confirmed by NMR and mass spectral analysis. Furthermore, ribose orthoester **6** was allowed to react with 1-thioglusoce **49**, 1-thiogalactose **50** and 1-thiomannose **51** in CH_2Cl_2 in the presence of 7mol% of $AuBr_3$ to obtain ribose(1β)-glucose-(1β) thiotrehalose **66**, ribose(1β)-galactose(1β) thiotrehalose **67** and ribose(1β)-mannose(1α) thiotrehalose **68** in 92%, 91% and 89% yield respectively (Scheme 2B.21). Through NMR spectral analysis confirmed the formation of a single diastereomer during the course of the glycosidation Xylofuranosyl propargyl 1,2-orthoester **39**[34] was subjected to gold-catalysed glycosidation conditions with 1-thioglucosyl acceptor **49**[29] in the presence of 7mol% of $AuBr_3$ at 25 °C to form 1,2-*trans* thiodisaccharide **69**[35] (Scheme 2B.22).



Scheme 2B.22 Synthesis of 1,1-thiotrehaloges of xylose and glucose

The above interesting results on furanosyl donors inspired us to extend the scope of this productive methodology to pyranosyl donors as well for which a model mannopyranosyl 1,2-orthoester (**73**) was required. Synthesis started with benzoylation



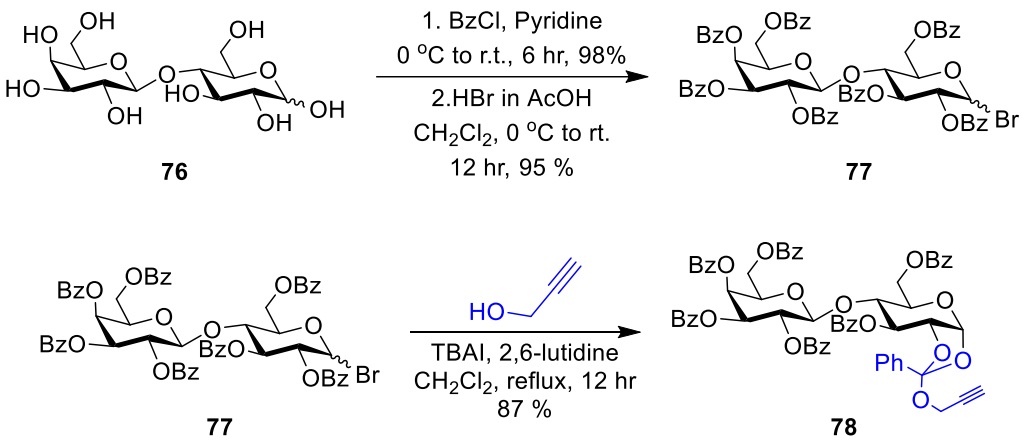
Scheme 2B.23 Synthesis of mannose orthoester and its utilization

of α-D-mannose **70** with benzoyl chloride in pyridine to form per-*O*-benzoyl mannose

which was subsequently converted to its bromoside **71** by treating with HBr in AcOH. Further, mannosyl bromoside **71** was transformed to mannose 1,2-orthoester **72** by reacting with propargyl alcohol, 2,6-lutidine and a catalytic amount of TBAI in dichloromethane at reflux conditions for 12h. Orthoester **73** was saponified under Zemplén conditions[34b] to obtain orthoester-triol that was converted into the benzyl protected orthoester **73** by the treatment of NaH/BnBr/TBAI/DMF in 93% yield (Scheme 2B.23). In continuation, propargyl 1,2-orthoester of mannose **73**[34] was treated with 1-thioglucoside **49** and 1-thiogalactoside **50** at room temperature in the presence of 7mol% of $AuBr_3$ to obtain 1,1-thiotrehaloses **74** and **75** in 93% yield respectively (Scheme 2B.23).

Similar set of reactions were performed using D-lactose (**76**) to obtain lactosyl propargyl 1,2-orthoester **78** through the per-*O*-benzoyl lactosyl bromide **77** (Scheme 2B.24).

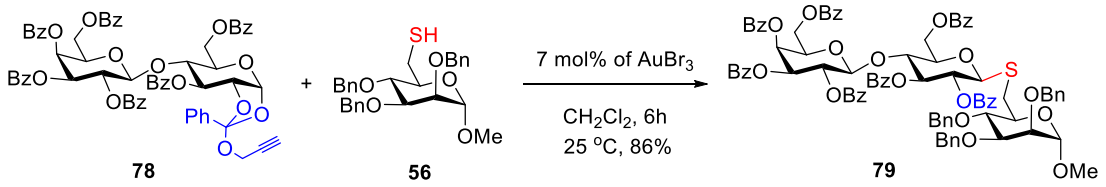
To our interest we wish to apply this methodology to the disaccharide level. To this purpose, lactosyl orthoester was also prepared from α-D-lactose to demonstrate the versatile scope of this particular protocol. Simple lactose **76** was converted to per-*O*-benzoyl lactose using benzoyal chloride and pyridine conditions. The per-*O*-benzoyl lactose transformed to lactosyl bromoside **77** by the treatment of HBr in AcOH conditions, then lactosyl bromoside **77** converted to lactose orthoester **78** by the treatment of propargyl alcohol, 2,6-lutidine and a catalytic amount of TBAI in dichloromethane at reflux condition for 12h (Scheme 2B.24). To complete the conversion of reaction the presence of catalytic amount of TBAI in reflux condition is indeed necessary.



76 **77** **77** **78**

Scheme 2B.24 Synthesis of lactose orthoester

Once the disaccharide orthoester is in our hands, the glycosylation between the lactose propargyl 1,2-orthoester **78**[36] and 6-*deoxy*-6-thio mannose **56** was carried to obtain thiolactoside **79** in excellent yield. Successful synthesis of tri-thiosaccharide **79** confirmed by mass spectrometer analysis, calculated for $C_{89}H_{80}NaO_{22}S^+$, [M+Na$^+$]: 1555.4754 and found: 1555.4761 in addition to the NMR spectroscopic analysis.



Scheme 2B.25 Synthesis of lactose thiosaccharides

In conclusion, a new synthetic methodology was developed for the facile synthesis of thioglycosides and 1-thiotrehaloses through gold-catalyzed glycosylations using stable glycosyl 1,2-orthoesters as glycosyl donros. Thioglycosides and 1-thiotrehaloses are obtained in a 1,2-*trans* diastereoselective fashion only. This simple, easy, scalable and stereoselective methodology for the synthesis of thioglycosides and thiotrehaloses would be highly useful.

2B.7 Experimental Section

General procedure for the synthesis of thioglycosides and 1-thiotrehaloses: To a solution of glycosyl donor **38** (95 mg, 0.2 mmol), glycosyl acceptor **44** (82mg, 0.2 mmol) and 4Å molecular sieves powder (50mg) in anhydrous CH_2Cl_2 (4mL) was added $AuBr_3$ (6.5mg, 0.015mmol) under argon atmosphere at room temperature. The reaction mixture was stirred at room temperature for the specified time and the completion of the reaction was judged by TLC analysis. The reaction mixture was filtered through celite and the filtrate was concentrated *in vacuo* to obtain a crude residue which was purified by conventional silica gel column chromatography using ethyl acetate-petroleum ether as mobile phase.

Compound Characterization Data:

2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylsulfane]-2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranoside **(58):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (154 mg, 93%**)**: $[\alpha]_D^{25}$ = +34.4^0 ($CHCl_3$, *c* 1.00); IR (cm^{-1}): 3024, 1751, 1449, 1371, 1225 758; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.98 – 7.93 (m, 2H), 7.61 – 7.50 (m, 1H), 7.41 – 7.36 (m, 2H), 7.28 – 7.21 (m, 10H), 5.45 (s, 1H), 5.33 (t, *J* = 1.3 Hz, 1H), 5.17 (t, *J* = 9.4 Hz, 1H), 5.04 (t, *J* = 9.7 Hz, 1H), 4.95 (dd, *J* = 9.6, 7.9 Hz, 1H), 4.74 (d, *J* = 12.1 Hz, 1H), 4.57 – 4.45 (m, 4H), 4.44 – 4.39 (m, 1H), 4.22 (dd, *J* = 12.3, 4.7 Hz, 1H), 4.10 – 4.05 (m, 2H), 4.00 (ddd, *J* = 10.3, 7.8, 5.6 Hz, 1H), 3.75 (dt, *J* = 10.3, 7.3 Hz, 1H), 3.70 – 3.58 (m, 3H), 2.92 (ddd, *J* = 13.4, 7.6, 5.7 Hz, 1H), 2.83 – 2.74 (m, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7 (2C), 20.8 (2C), 30.6, 61.9, 68.4, 68.9, 69.3, 71.2, 71.8, 72.4, 72.9, 73.5, 81.8, 82.8, 83.3, 88.6, 100.9, 127.7 (3C), 128.0, 128.0 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.3, 129.9 (2C), 133.6, 137.5, 137.9, 165.6, 169.5, 169.5, 170.3, 170.8; HRMS (TOF): calcd. for $C_{42}H_{48}NaO_{15}S^+$,[M+Na^+]: 847.2606; found: 847.2601.

2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylsulfane]-2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranoside **(59):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (152 mg, 92%**)**: $[\alpha]_D^{25}$ = +70.0^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2673, 1749, 1370, 1226, 1071, 733, 706; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.98 – 7.93 (m, 2H), 7.56 (tt, J = 7.7, 1.4 Hz, 1H), 7.39 (t, J = 7.8 Hz, 2H), 7.29 – 7.21 (m, 10H), 5.45 (s, 1H), 5.35 (d, J = 3.5 Hz, 1H), 5.33 (t, J = 1.3 Hz, 1H), 5.18 (dd, J = 10.5, 8.0 Hz, 1H), 4.98 (dd, J =

10.5, 3.5 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.59 – 4.47 (m, 4H), 4.43 (q, J = 4.8 Hz, 1H), 4.14 – 4.06 (m, 3H), 4.02 (ddd, J = 10.3, 7.9, 5.5 Hz, 1H), 3.85 (t, J = 6.7 Hz, 1H), 3.76 (dt, J = 10.3, 7.3 Hz, 1H), 3.68 (dd, J = 10.9, 3.6 Hz, 1H), 3.61 (dd, J = 10.9, 4.7 Hz, 1H), 2.93 (ddd, J = 13.3, 7.6, 5.5 Hz, 1H), 2.85 – 2.76 (m, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.8 (2C), 20.9, 30.6, 61.4, 67.1, 68.8, 68.9, 69.2, 70.7, 71.0, 72.5, 73.5, 81.8, 82.8, 83.3, 88.5, 101.4, 127.7 (3C), 128.0, 128.1 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.3, 129.9 (2C), 133.6, 137.5, 137.9, 165.6, 169.7, 170.2, 170.4, 170.5; HRMS (TOF): calcd. for $C_{42}H_{48}NaO_{15}S^+$, [M+Na^+]: 847.2606; found: 847.2599

Methyl 2,3,4-tri-*O*-benzyl-6-*deoxy*-6-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-d-arabinofuranosyl-thio)-α-D-mannopyranoside **(60):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (147 mg, 94%**)**: $[\alpha]_D^{25}$ = +117.3^0 ($CHCl_3$, c 1.00); ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 3.08 (dd, J = 13.7, 7.5 Hz, 1H), 3.38 (dd, J = 13.8, 2.3 Hz, 1H), 3.43 (s, 3H), 3.77 (qd, J = 10.9, 4.2 Hz, 2H), 3.84 – 4.03 (m, 3H), 4.09 (t, J = 9.3 Hz, 1H), 4.24 (d, J = 5.5 Hz, 1H), 4.55 – 4.73 (m, 6H), 4.76 – 4.85 (m, 4H), 4.93 (d, J = 12.1 Hz, 1H), 5.07 (d, J = 10.9 Hz, 1H), 5.61 (t, J = 1.3 Hz, 1H), 5.77 (s, 1H), 7.29 – 7.52 (m, 27H), 7.57 – 7.68 (m, 1H), 8.03 – 8.14 (m, 2H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 32.8, 54.9, 69.1, 71.6, 72.2, 72.4, 72.7, 73.5, 74.8, 75.3, 77.5, 80.4, 82.0, 82.9, 83.7, 88.5, 99.0, 127.7, 127.7, 127.7, 127.7, 127.7, 127.7, 127.8, 127.9, 127.9, 127.9, 128.0, 128.1, 128.1, 128.1, 128.1, 128.4, 128.4, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.6, 129.7, 129.9, 129.9, 133.4, 137.8, 138.1, 138.5, 138.7, 138.7, 165.4. HRMS (TOF): calcd. for $C_{54}H_{56}NaO_{10}S^+$, [M+Na^+]: 919.3486; found: 919.3491.

2-*O*-Benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **(61):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (142 mg, 91%**)**: $[\alpha]_D^{25}$ = +34.6^0 ($CHCl_3$, *c* 1.00); IR(cm^{-1}): 3024, 2366, 1753, 1371, 1221, 1105, 911, 757, 707; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.94 (dt, *J* = 7.4, 1.1 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 2H), 7.30 – 7.21 (m, 10H), 5.74 (s, 1H), 5.43 (s, 1H), 5.25 (t, *J* = 9.4 Hz, 1H), 5.14 (dt, *J* = 23.3, 9.4 Hz, 2H), 4.73 (dd, *J* = 10.9, 3.5 Hz, 2H), 4.59 – 4.44 (m, 3H), 4.41 (q, *J* = 4.2 Hz, 1H), 4.22 – 4.13 (m, 2H), 4.09 (dd, *J* = 12.4, 1.9 Hz, 1H), 3.75 – 3.68 (m, 2H), 3.63 (dd, *J* = 10.8, 4.2 Hz, 1H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.7, 20.8,

20.8, 62.0, 68.1, 68.5, 70.7, 72.4, 73.5, 74.3, 76.2, 82.2, 82.6, 83.0, 83.1, 86.9, 127.7, 127.8 (2C), 128.0 (3C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.3, 129.9 (2C), 133.6, 137.5, 138.0, 165.5, 169.3, 169.4, 170.3, 170.7. HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$,[M+Na^+]: 803.2344; found: 803.2351

2-*O*-Benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **(62):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (138 mg, 88%**)**: $[\alpha]_D^{25}$ = +41.8 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3025, 1749, 1370, 1227, 1009, 736; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.93 (dd, *J* = 8.5, 1.2 Hz, 2H), 7.60 – 7.50 (m, 1H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.30 – 7.21 (m, 10H), 5.68 (s, 1H), 5.46 (t, *J* = 1.3 Hz, 1H), 5.41 – 5.34 (m, 2H), 5.02 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.81 – 4.67 (m, 2H), 4.59 – 4.52 (m, 2H), 4.51 – 4.44 (m, 2H), 4.15 (d, *J* = 5.1 Hz, 1H), 4.09 (d, *J* = 6.7 Hz, 2H), 3.93 (t, *J* = 6.7 Hz, 1H), 3.72 – 3.60 (m, 2H), 2.08 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7 (2C), 20.8, 20.9, 61.4, 67.4, 68.0, 68.7, 72.2, 72.4, 73.5, 74.7, 77.5, 82.7, 83.1, 83.2 (2C), 87.6, 127.8 (3C), 128.0 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.3, 129.9 (2C), 133.6, 137.5, 138.0, 165.5, 169.5, 170.1, 170.4 (2C); HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$, [M+Na^+]: 803.2344; found: 803.2334

2-*O*-Benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside **(63):** This compound is prepared following the above mentioned general procedure using compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (140 mg, 89%**)**: $[\alpha]_D^{25}$ = +76.7^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3028, 2366, 1749, 1230, 1061, 706; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.93 – 7.89 (m, 2H), 7.55 (tt, J = 7.6, 1.4 Hz, 1H), 7.40 – 7.35 (m, 2H), 7.32 – 7.22 (m, 10H), 5.59 – 5.50 (m, 2H), 5.41 (dd, J = 3.0, 1.5 Hz, 1H), 5.36 – 5.25 (m, 3H), 4.73 (d, J = 12.1 Hz, 1H), 4.63 – 4.41 (m, 5H), 4.27 (dd, J = 12.3, 5.6 Hz, 1H), 4.16 (dt, J = 5.7, 1.2 Hz, 1H), 4.06 (dd, J = 12.3, 2.2 Hz, 1H), 3.66 (qd, J = 10.9, 4.2 Hz, 2H), 2.13 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 21.0, 62.5, 66.3, 68.7, 69.6, 69.7, 70.7, 72.5, 73.5, 80.5, 82.6, 82.8, 82.9, 86.2, 127.8 (3C), 128.0 (3C), 128.4 (2C), 128.6 (2C), 128.6 (2C), 129.1, 129.8 (2C), 133.7, 137.3, 137.9, 165.5, 169.8, 169.8, 169.9, 170.7; HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$, [M+Na^+]: 803.2344; found: 803.2341.

2-hydroxyethyl [2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl β-D-galactopyranosyl)-β-D-glucopyranosylsulfane]-2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranoside **(64):** This compound is prepared following the above mentioned general procedure using

compound **38** (95 mg, 0.2 mmol) as the starting material, Yield: (194 mg, 87%**)**: $[\alpha]_D^{25}$ = $+35.9^0$ ($CHCl_3$, c 1.00); IR(cm^{-1}): 2875, 2357, 1749, 1370, 1226, 1057, 755; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.95 (dt, *J* = 8.6, 1.6 Hz, 2H), 7.56 (tt, *J* = 7.2, 1.4 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.30 – 7.19 (m, 10H), 5.44 (s, 1H), 5.35 – 5.30 (m, 2H), 5.16 (t, *J* = 9.2 Hz, 1H), 5.08 (dd, *J* = 10.4, 7.9 Hz, 1H), 4.92 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.85 (dd, *J* = 9.5, 7.9 Hz, 1H), 4.74 (d, *J* = 12.1 Hz, 1H), 4.57 – 4.39 (m, 7H), 4.12 – 4.03 (m, 4H), 3.96 (ddd, *J* = 10.3, 8.0, 5.8 Hz, 1H), 3.84 (td, *J* = 7.0, 1.1 Hz, 1H), 3.77 – 3.52 (m, 5H), 2.90 (ddd, *J* = 13.6, 7.7, 5.7 Hz, 1H), 2.77 (ddd, *J* = 14.0, 7.8, 6.8 Hz, 1H), 2.13 (d, *J* = 1.6 Hz, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.6, 20.7 (3C), 20.8, 20.9, 20.9, 30.6, 60.9, 62.1, 66.7, 68.9, 69.1, 69.3, 70.7, 71.1, 71.6, 72.4, 72.6, 72.8, 73.5, 76.4, 81.8, 82.8, 83.3, 88.6, 100.7, 101.2, 127.7 (2C), 127.8, 128.0 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.3, 129.9 (2C), 133.5, 137.5, 137.9, 165.6, 169.2, 169.8, 169.9, 170.2, 170.3, 170.4 (2C), 170.5; HRMS (TOF): calcd. for $C_{54}H_{64}NaO_{23}S^+$, [M+Na^+]: 1135.3451; found: 1135.3451

2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylsulfane]-2-*O*-benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranoside **(65):** This compound is prepared following the above mentioned general procedure using compound **6** (95 mg, 0.2 mmol) as the starting material, Yield: (150 mg, 91%**)**: $[\alpha]_D^{25}$ = -9.9^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3024, 1752, 1370, 1265, 1040, 757; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.11 – 8.02 (m, 2H), 7.57 (tt, *J* = 7.0, 1.3 Hz, 1H), 7.46 – 7.41 (m, 2H), 7.35 – 7.23 (m, 5H), 7.21 – 7.14 (m, 5H), 5.42 (dd, *J* = 4.0, 3.1 Hz, 1H), 5.34 (d, *J* = 3.0 Hz, 1H), 5.16 (t, *J* = 9.5 Hz, 1H), 5.08 – 5.01 (m, 1H), 4.96 (dd, *J* = 9.6, 7.9 Hz, 1H), 4.61 – 4.42 (m, 5H), 4.31 – 4.26 (m, 2H), 4.21 (dd, *J* = 12.3, 4.7 Hz, 1H), 4.10 – 3.99 (m, 2H), 3.73 – 3.53 (m, 4H), 2.97 – 2.77 (m, 2H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 6H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.7, 20.8, 20.8, 30.3, 61.9, 68.4, 69.6, 70.5, 71.2, 71.8, 72.8, 73.1, 73.4, 75.7, 78.0, 81.8, 86.4, 100.9, 127.7 (2C), 127.8, 128.0, 128.1 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.6, 130.0 (2C), 133.5, 137.3, 138.1, 165.7, 169.4, 169.5, 170.4, 170.8; HRMS (TOF): calcd. for $C_{42}H_{48}NaO_{15}S^+$,[M+Na^+]: 847.2606; found: 847.2601.

2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylsulfane]-2-*O*-benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranoside **(66):** This compound is prepared following the above mentioned general procedure using compound **6** (95 mg, 0.2 mmol) as the starting material, Yield: (144 mg, 87%**)**: $[\alpha]_D^{25}$ = -9.3^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2873, 1749, 1225, 1071, 754; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.07 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.57

(tp, *J* = 7.7, 1.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.38 – 7.13 (m, 10H), 5.46 – 5.38 (m, 1H), 5.34 (t, *J* = 2.6 Hz, 2H), 5.17 (dd, *J* = 10.5, 8.0 Hz, 1H), 4.97 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.64 – 4.39 (m, 5H), 4.33 – 4.25 (m, 2H), 4.10 (tt, *J* = 6.0, 3.0 Hz, 2H), 4.07 – 4.01 (m, 1H), 3.86 – 3.76 (m, 1H), 3.75 – 3.61 (m, 2H), 3.63 – 3.52 (m, 1H), 2.93 (dt, *J* = 14.5, 7.4 Hz, 1H), 2.83 (ddd, *J* = 13.8, 7.0, 5.2 Hz, 1H), 2.11 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.8 (2C), 20.9, 30.3, 61.4, 67.1, 68.7, 69.6, 70.5, 70.7, 70.9, 73.1, 73.4, 75.7, 78.0, 81.8, 86.4, 101.4, 127.7 (2C), 127.8, 128.0, 128.1 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.6, 130.0 (2C), 133.5, 137.3, 138.1, 165.8, 169.6, 170.2, 170.4, 170.5; HRMS (TOF): calcd. for $C_{42}H_{48}NaO_{15}S^+$,[M+Na^+]: 847.2606; found: 847.2595.

2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **(67):** This compound is prepared following the above mentioned general procedure using compound **6** (95 mg, 0.2 mmol) as the starting material, Yield: (135 mg, 86%**)**: $[\alpha]_D^{25}$ = -19.8^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 1751, 1370, 1225, 1038, 755; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.06 (d, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.39 – 7.15 (m, 10H), 5.65 (d, *J* = 3.6 Hz, 1H), 5.53 (s, 1H), 5.24 (t, *J* = 9.3 Hz, 1H), 5.07 (dt, *J* = 29.0, 9.7 Hz, 2H), 4.85 (d, *J* = 10.3 Hz, 1H), 4.64 – 4.43 (m, 4H), 4.42 – 4.29 (m, 2H), 4.27 – 4.12 (m, 2H), 3.77 (ddd, *J* = 10.0, 4.2, 1.9 Hz, 1H), 3.67 (dd, *J* = 10.7, 2.7 Hz, 1H), 3.54 (dd, *J* = 10.7, 5.2 Hz, 1H), 2.02 (s, 3H), 1.99 (s, 6H), 1.95 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7 (3C), 20.8, 62.0, 68.2, 70.1, 70.2, 73.2, 73.5, 74.0, 75.9, 76.1, 77.4, 81.7, 82.0, 85.5, 127.7 (2C), 127.8, 128.1, 128.1 (2C), 128.5 (2C), 128.5 (2C), 128.6 (2C), 129.5, 130.0 (2C), 133.6, 137.3, 138.0, 165.7, 169.5, 169.5, 170.2, 170.7; HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$,[M+Na^+]: 803.2344; found: 803.2595.

2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **(68):** This compound is prepared following the above mentioned general procedure using compound **6** (95 mg, 0.2 mmol) as the starting material, Yield: (133 mg, 85%**)**: $[\alpha]_D^{25}$ = -1.1^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2337, 1743, 1225, 1068, 706; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.06 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.57 (tt, *J* = 7.4, 1.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.38 – 7.15 (m, 10H), 5.64 (dd, *J* = 4.3, 1.6 Hz, 1H), 5.54 (d, *J* = 1.5 Hz, 1H), 5.43 (d, *J* = 3.4 Hz, 1H), 5.23 (t, *J* = 10.1 Hz, 1H), 5.06 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.85 (d, *J* = 10.2 Hz, 1H), 4.63 – 4.43 (m, 4H), 4.41 – 4.29 (m, 2H), 4.20 – 4.06 (m, 2H), 3.98 (t, *J* = 6.7 Hz, 1H), 3.67 (dd, *J* = 10.7, 2.9 Hz, 1H), 3.54 (dd, *J* = 10.7,

5.2 Hz, 1H), 2.13 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.7, 20.8, 20.8, 61.5, 67.4 (2C), 70.3, 71.9, 73.2, 73.5, 74.5, 76.2, 77.4, 81.7, 82.4, 85.4, 127.7 (2C), 127.8, 128.1 (3C), 128.5 (2C), 128.5 (2C), 128.6 (2C), 129.5, 130.0 (2C), 133.6, 137.3, 138.0, 165.7, 169.7, 170.1, 170.4, 170.4; HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$, [M+Na^+]: 803.2344; found: 803.2338.

2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside **(69):** This compound is prepared following the above mentioned general procedure using compound **6** (95 mg, 0.2 mmol) as the starting material, Yield: (130 mg, 83%**)**: $[\alpha]_D^{25}$ = +21.9^0 ($CHCl_3$, *c* 1.00); IR(cm^{-1}): 3025, 1749, 1370, 1230, 119, 706; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.05 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.56 (tt, *J* = 7.4, 1.0 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.13 (m, 10H), 5.69 (d, *J* = 1.3 Hz, 1H), 5.61 (dd, *J* = 4.8, 2.9 Hz, 1H), 5.39 (d, *J* = 2.8 Hz, 1H), 5.36 (dd, *J* = 3.4, 1.5 Hz, 1H), 5.32 (t, *J* = 10.1 Hz, 1H), 5.19 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 2H), 4.50 – 4.38 (m, 4H), 4.31 – 4.20 (m, 2H), 4.09 (dd, *J* = 12.4, 2.2 Hz, 2H), 3.69 (dd, *J* = 10.8, 2.9 Hz, 1H), 3.53 (dd, *J* = 10.8, 4.0 Hz, 1H), 2.06 (s, 2H), 2.04 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.8, 20.8, 20.9, 62.2, 66.0, 69.1, 69.7, 69.9, 70.8, 73.1, 73.3, 76.5, 76.6, 80.8, 81.8, 86.3, 127.5 (2C), 127.7, 128.0, 128.1 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 129.5, 130.0 (2C), 133.6, 137.3, 138.0, 165.6, 169.8, 169.8, 169.9, 170.8; HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$,[M+Na^+]: 803.2344; found: 803.2346.

2-*O*-Benzoyl-3,5-di-*O*-benzyl-β-D-xylofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **(70):** This compound is prepared following the above mentioned general procedure using compound **39** (95 mg, 0.2 mmol) as the starting material, Yield: (135 mg, 88%**)**: $[\alpha]_D^{25}$ = +70.1^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2930, 1748, 1370, 1226, 1065, 759, 706; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.99 (dd, *J* = 8.2, 1.2 Hz, 2H), 7.58 (tt, *J* = 7.0, 1.2 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.35 – 7.22 (m, 10H), 5.64 (s, 1H), 5.56 (s, 1H), 5.40 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.24 (t, *J* = 10.0 Hz, 1H), 5.04 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.91 – 4.85 (m, 2H), 4.62 – 4.44 (m, 4H), 4.16 – 4.06 (m, 3H), 3.91 (td, *J* = 6.8, 0.9 Hz, 1H), 3.84 – 3.74 (m, 2H), 2.12 (s, 3H), 1.97 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7 (2C), 20.8, 20.8, 61.5, 67.4, 67.5, 68.9, 71.9, 71.9, 73.7, 74.4, 81.0 (2C), 82.3, 82.7, 85.3, 127.8 (3C), 127.9 (3C), 128.5 (4C), 128.7 (2C), 129.1, 129.8 (2C), 133.8, 137.4, 138.0, 165.3, 169.7, 170.1, 170.4 (2C); HRMS (TOF): calcd. for $C_{40}H_{44}NaO_{14}S^+$,[M+Na^+]: 803.2344; found: 803.2335.

2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylsulfane]-2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **(75):** This compound is prepared following the above mentioned general procedure using compound **74** (116 mg, 0.2 mmol) as the starting material, Yield: (174 mg, 93%**)**: $[\alpha]_D^{25}$ = +5.3^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3029, 1749, 1369, 1225, 1043, 813, 704; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.1 Hz, 2H), 7.55 (d, *J* = 6.6 Hz, 1H), 7.47 – 7.18 (m, 17H), 5.32 (s, 1H), 5.16 (d, *J* = 9.2 Hz, 1H), 5.01 (dt, *J* = 21.2, 9.3 Hz, 2H), 4.92 – 4.80 (m, 3H), 4.76 – 4.54 (m, 5H), 4.15 – 4.01 (m, 2H), 3.88 – 3.82 (m, 1H), 3.81 – 3.66 (m, 3H), 3.59 – 3.48 (m, 2H), 2.04 – 2.02 (m, 3H), 2.01 – 1.99 (m, 3H), 1.98 – 1.96 (m, 3H), 1.93 – 1.91 (m, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.6, 20.7 (2C), 20.9, 62.0, 68.2, 68.8, 70.1, 72.7, 73.5, 74.1, 75.2, 75.5, 75.9, 77.9, 79.7, 80.5 (2C), 84.4, 127.8, 127.9 (2C), 128.0, 128.1 (4C), 128.4 (2C), 128.5, 128.6 (3C), 128.6 (3C), 129.6, 130.0 (2C), 133.4, 137.7, 137.9, 138.1, 165.3, 169.4 (2C), 170.3, 170.7; HRMS (TOF): calcd. for $C_{48}H_{52}NaO_{15}S^+$,[M+Na^+]: 923.2919; found: 923.2899.

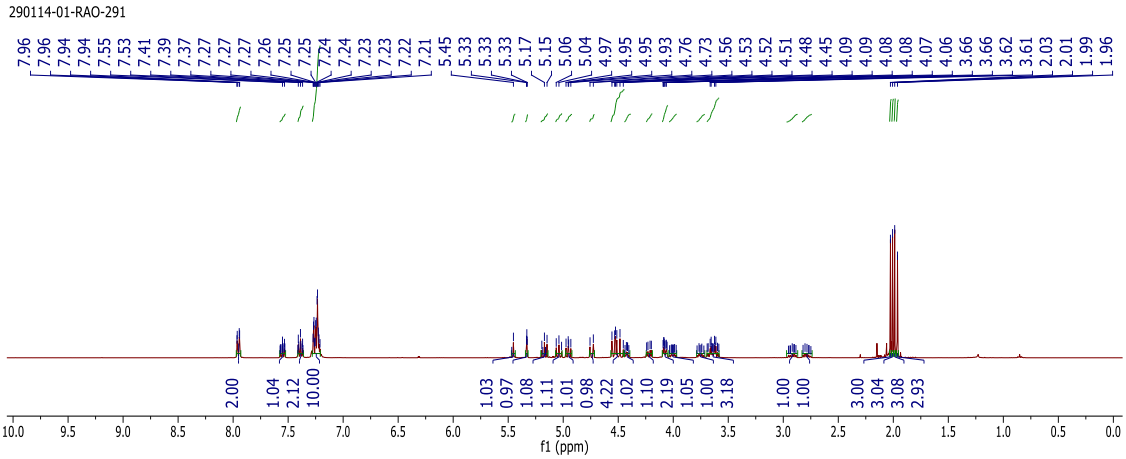
2-hydroxyethyl [2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylsulfane]-2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **(76):** This compound is prepared following the above mentioned general procedure using compound **74** (116 mg, 0.2 mmol) as the starting material, Yield: (172 mg, 92%**)**: $[\alpha]_D^{25}$ = +15.1^0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2358, 1746, 1225, 1366, 1059, 748, 703; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.01 (dd, *J* = 8.6, 1.2 Hz, 2H), 7.56 (tt, *J* = 7.4, 1.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.39 – 7.28 (m, 8H), 7.23 – 7.18 (m, 2H), 7.15 – 7.07 (m, 7H), 5.35 – 5.27 (m, 2H), 5.18 (t, *J* = 10.0 Hz, 1H), 5.00 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.91 – 4.80 (m, 3H), 4.77 – 4.64 (m, 2H), 4.63 – 4.53 (m, 3H), 4.04 (d, *J* = 6.7 Hz, 2H), 3.86 (t, *J* = 8.9 Hz, 1H), 3.81 – 3.67 (m, 4H), 3.59 – 3.53 (m, 1H), 2.07 (s, 1H), 2.02 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 20.7, 20.8 (2C), 20.8, 61.4, 67.3 (2C), 68.8, 72.0, 72.7, 73.5, 74.5, 75.2, 75.5, 77.4, 78.0, 79.7, 80.7, 81.1, 84.4, 127.8, 128.0 (3C), 128.0, 128.1 (4C), 128.4 (2C), 128.5, 128.6 (2C), 128.6 (2C), 129.6, 130.1 (2C), 133.4, 137.7, 137.9, 138.1, 165.2, 169.6, 170.1, 170.4 (2C); HRMS (TOF): calcd. for $C_{48}H_{52}NaO_{15}S^+$,[M+Na^+]: 923.2919; found: 923.2919.

(2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl β-D-galactopyranosyl) β-D-glucoopyranosyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **(80):** This compound is prepared following the above mentioned general procedure using compound **79** (198 mg, 0.2 mmol) as the starting material, Yield: (243 mg, 86%); $[\alpha]_D^{25}$ = +37.3 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2358, 1731, 1452, 1269, 1069, 708; ^{1}H NMR (399.78 MHz,

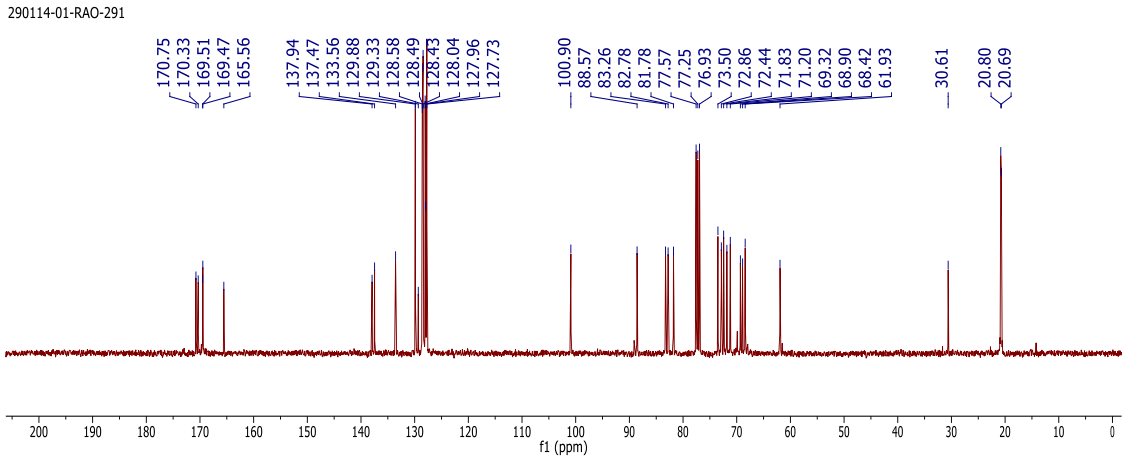
$CDCl_3$): δ 2.84 (dd, J = 14.1, 9.4 Hz, 1H), 3.04 (s, 4H), 3.58 – 3.95 (m, 8H), 4.27 (t, J = 9.5 Hz, 1H), 4.44 – 4.73 (m, 8H), 4.81 – 4.93 (m, 2H), 5.09 (d, J = 10.1 Hz, 1H), 5.34 – 5.50 (m, 2H), 5.71 – 5.79 (m, 2H), 5.84 (t, J = 9.3 Hz, 1H), 7.15 – 7.42 (m, 28H), 7.45 – 7.53 (m, 6H), 7.56 – 7.65 (m, 2H), 7.72 – 7.76 (m, 2H), 7.87 – 7.94 (m, 4H), 7.98 – 8.05 (m, 8H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 31.9, 54.6, 61.2, 62.8, 67.6, 70.0, 71.3, 71.5, 71.9, 72.1, 72.9, 73.8, 74.2, 74.6, 75.3, 76.0, 76.8, 76.9, 77.2, 77.4, 77.5, 78.3, 80.2, 84.9, 98.8, 101.1, 127.7, 127.7, 127.7, 127.7, 127.7, 127.9, 127.9, 128.0, 128.4, 128.4, 128.4, 128.4, 128.4, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.5, 128.7, 128.7, 128.7, 128.7, 128.7, 128.7, 128.7, 128.8, 128.8, 129.0, 129.3, 129.5, 129.6, 129.7, 129.7, 129.7, 129.8, 129.8, 129.8, 129.9, 129.9, 129.9, 129.9, 130.1, 130.1, 133.3, 133.4, 133.5, 133.6, 133.6, 133.7, 138.3, 138.4, 138.5, 165.0, 165.3, 165.4, 165.5, 165.6, 165.7, 166.0; HRMS (TOF): calcd. for $C_{89}H_{80}NaO_{22}S^+$, $[M+Na^+]$: 1555.4754; found: 1555.4761.

2B.8 Spectral Charts

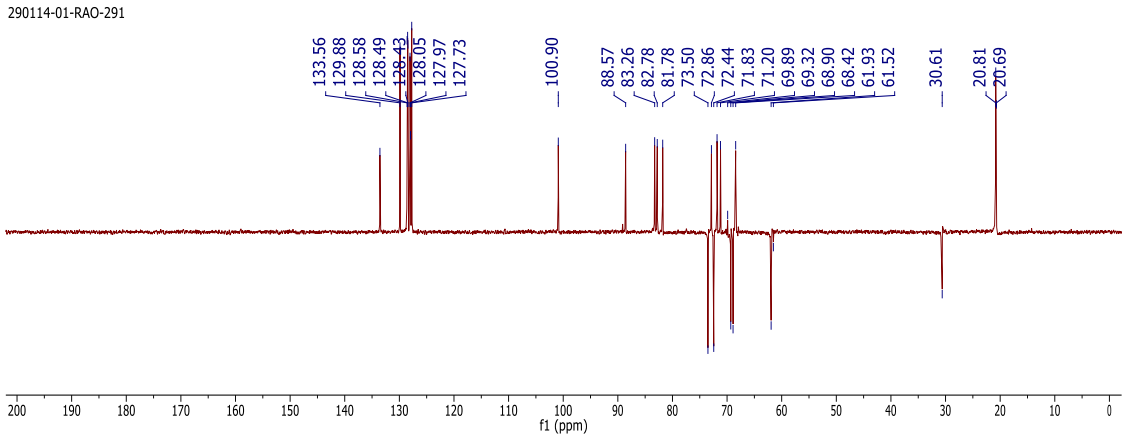
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(58)**



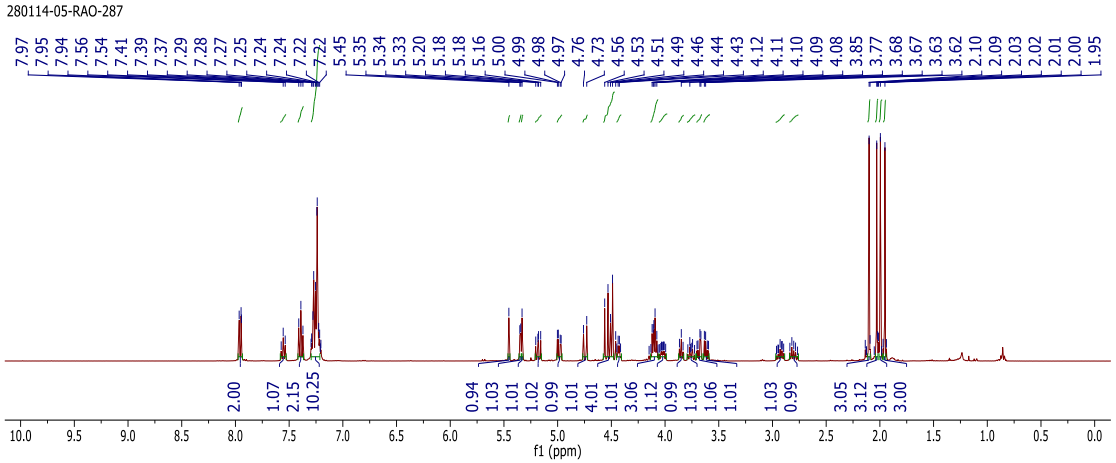
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(58)**



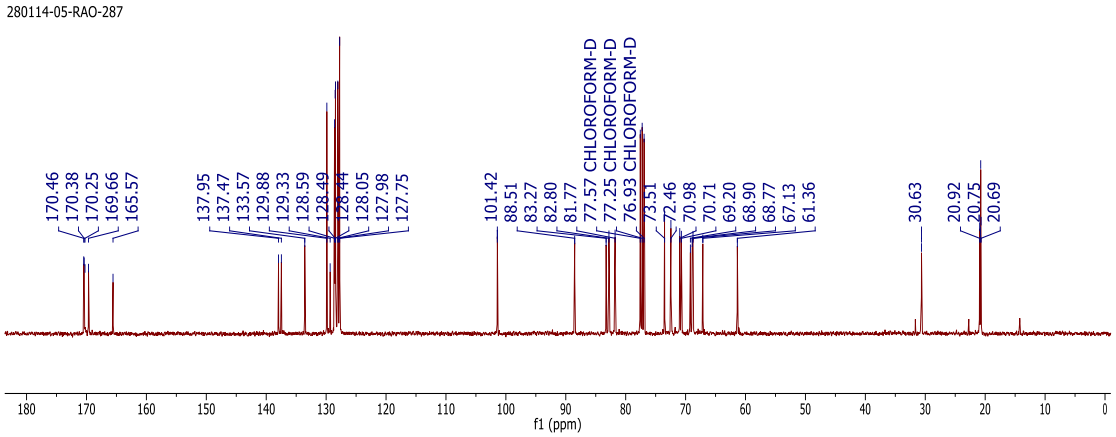
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(58)**



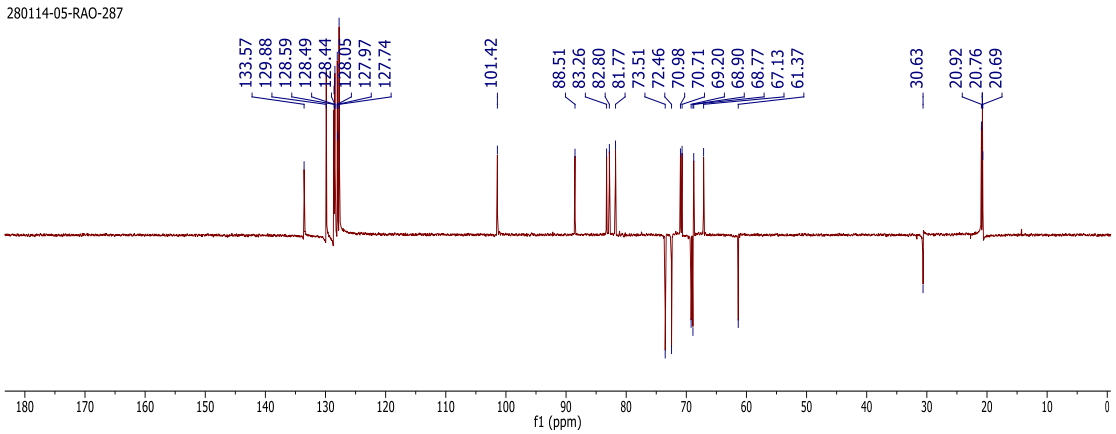
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(59)**



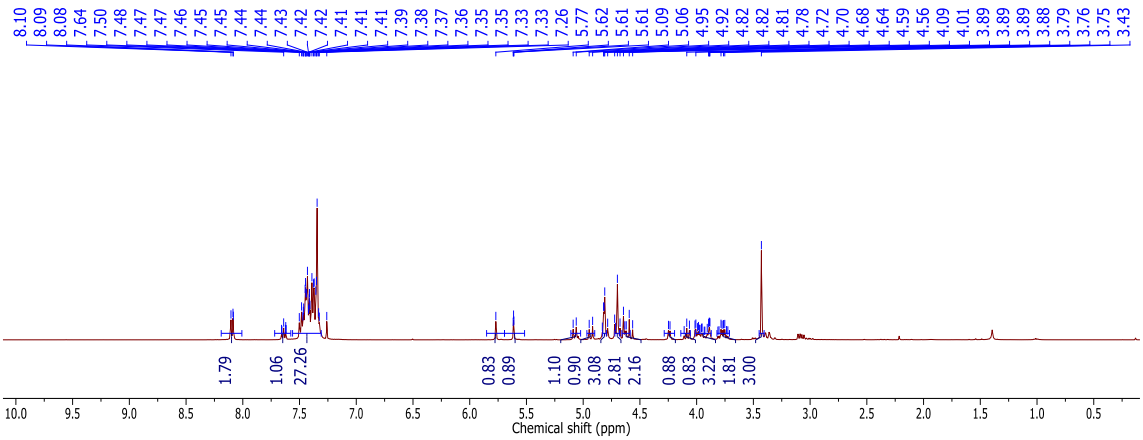
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(59)**



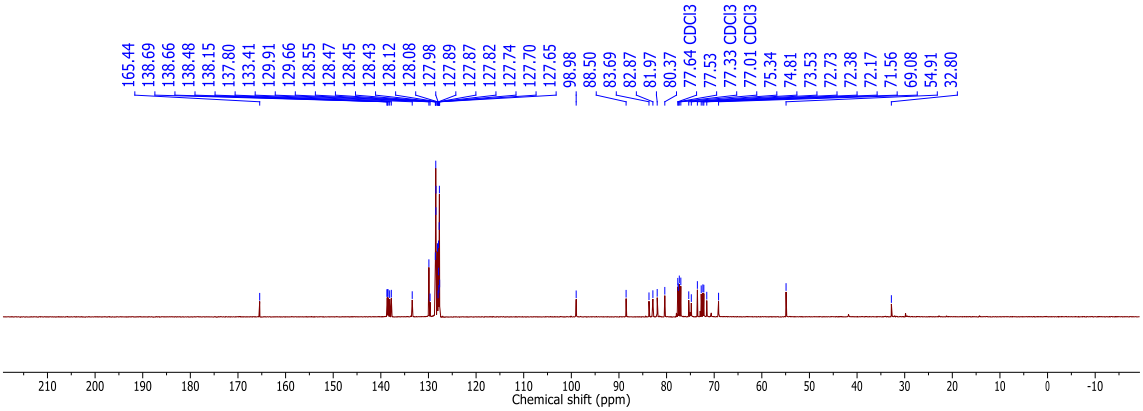
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(59)**



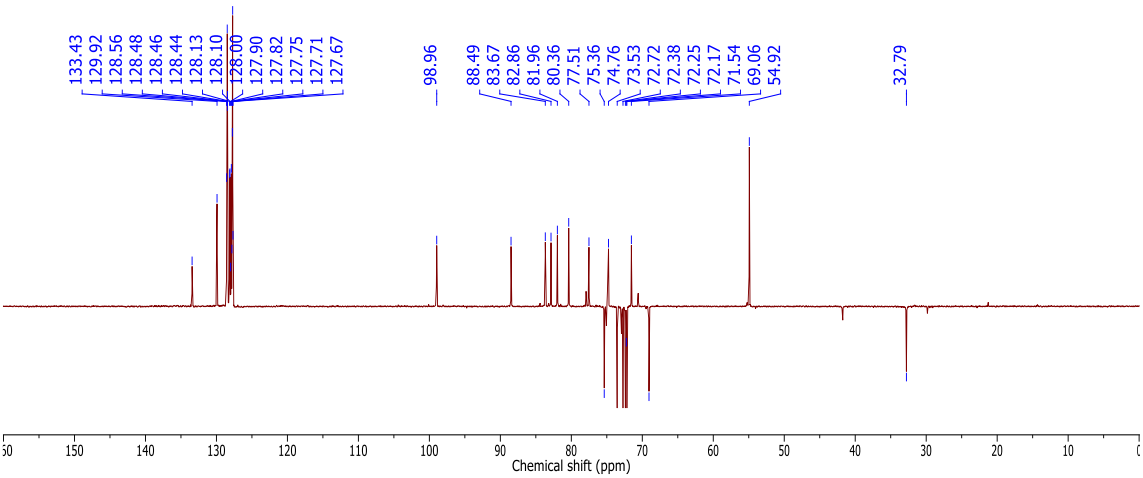
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(60)**



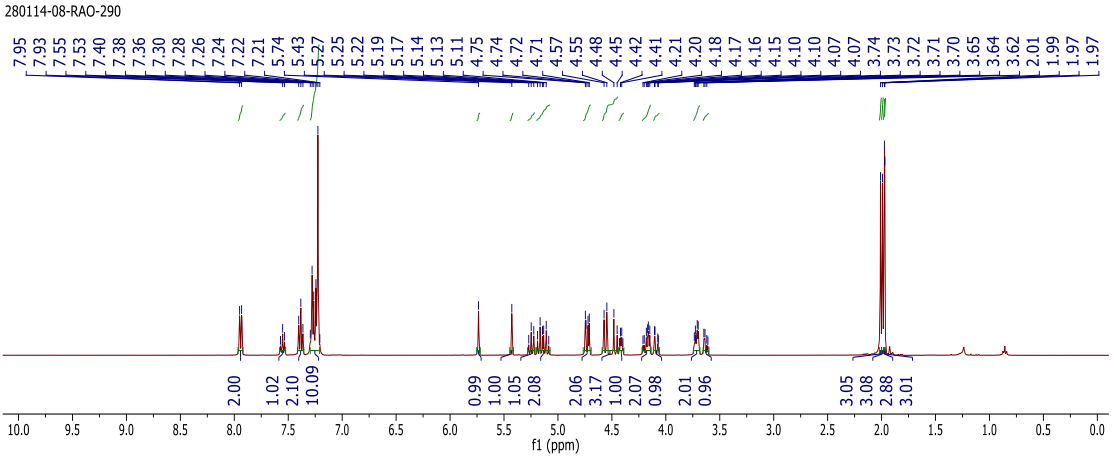
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(60)**



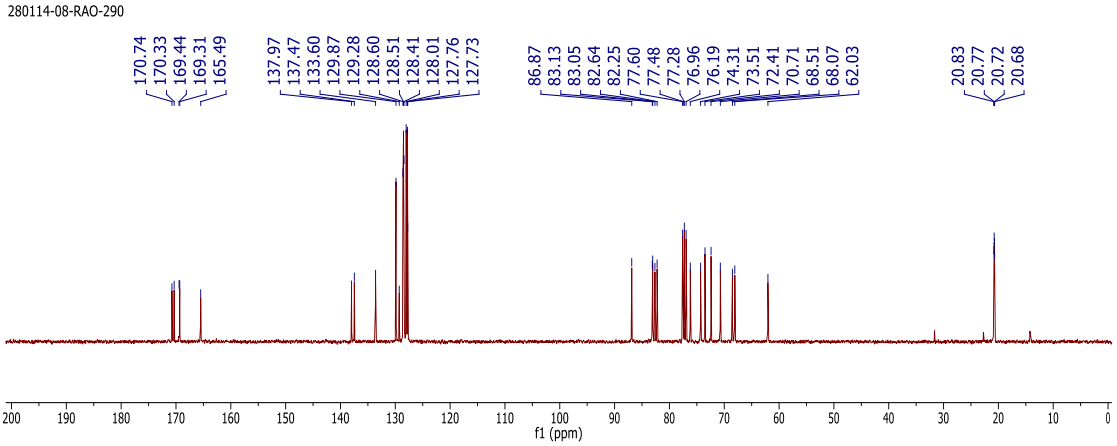
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(60)**



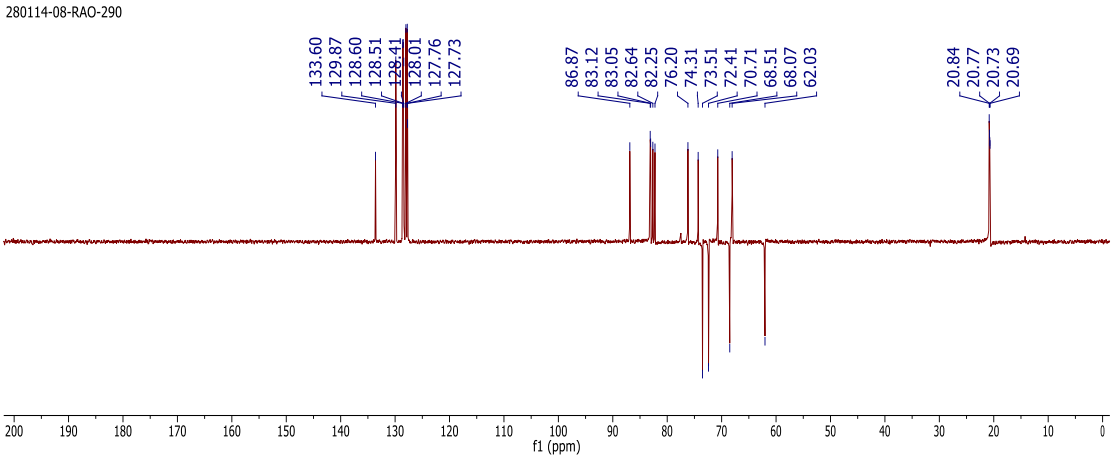
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(61)**



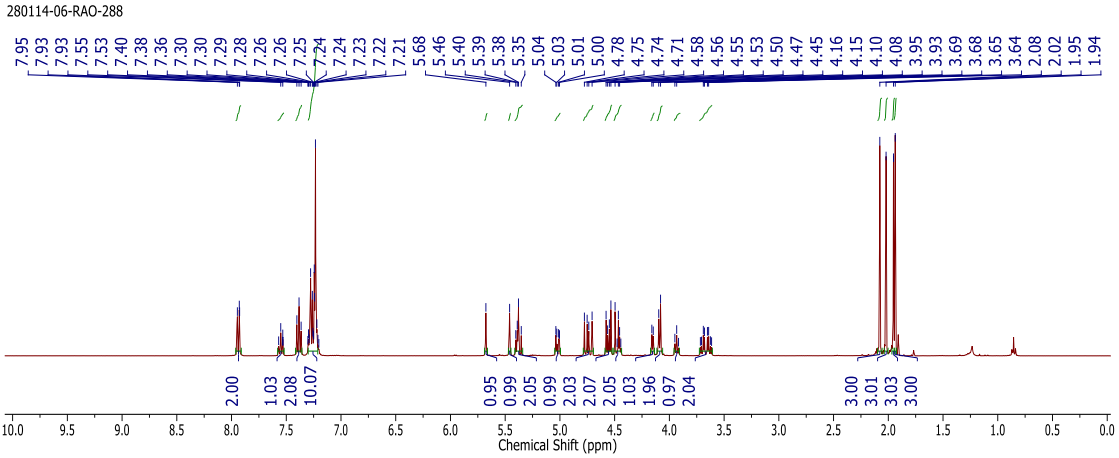
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(61)**



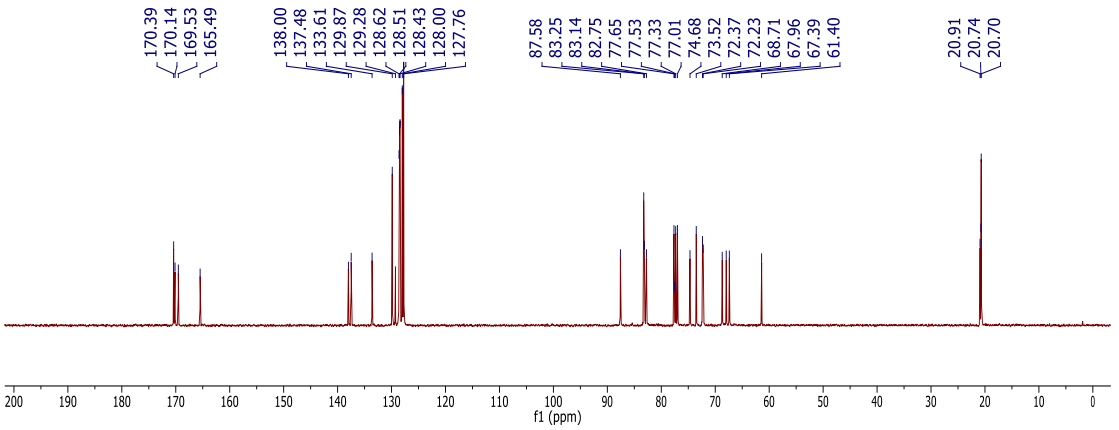
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(61)**



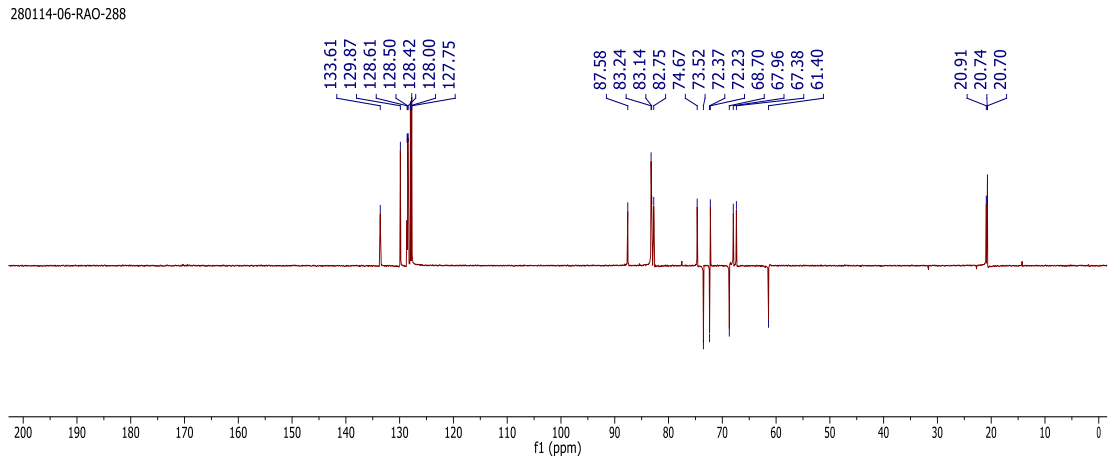
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(62)**



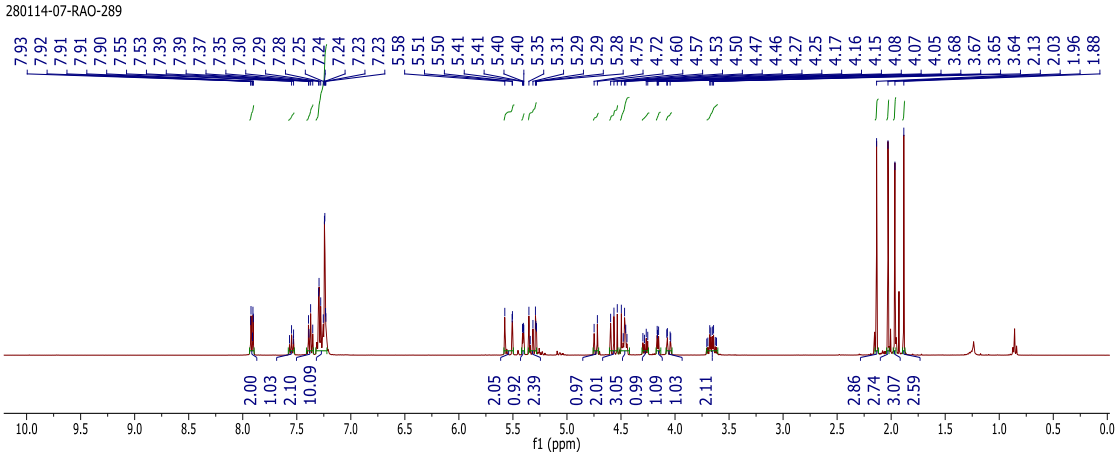
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(62)**



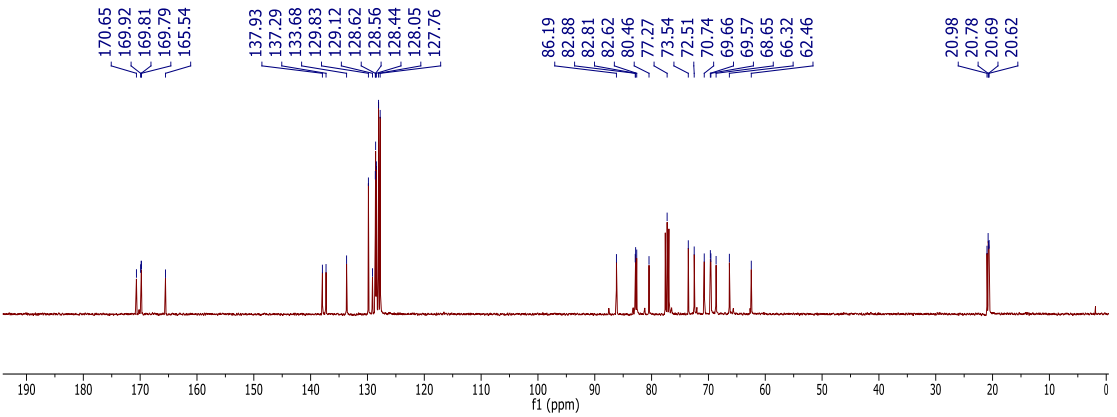
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(62)**



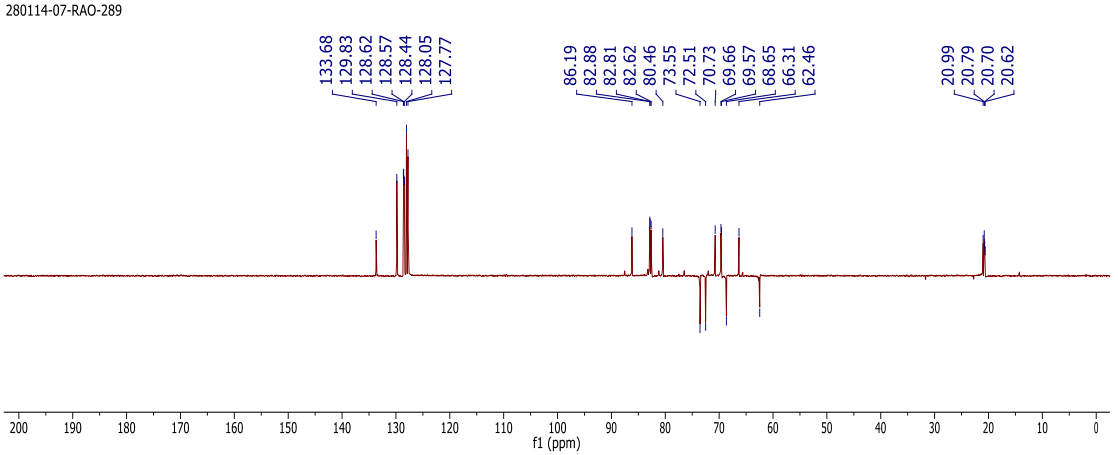
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(63)**



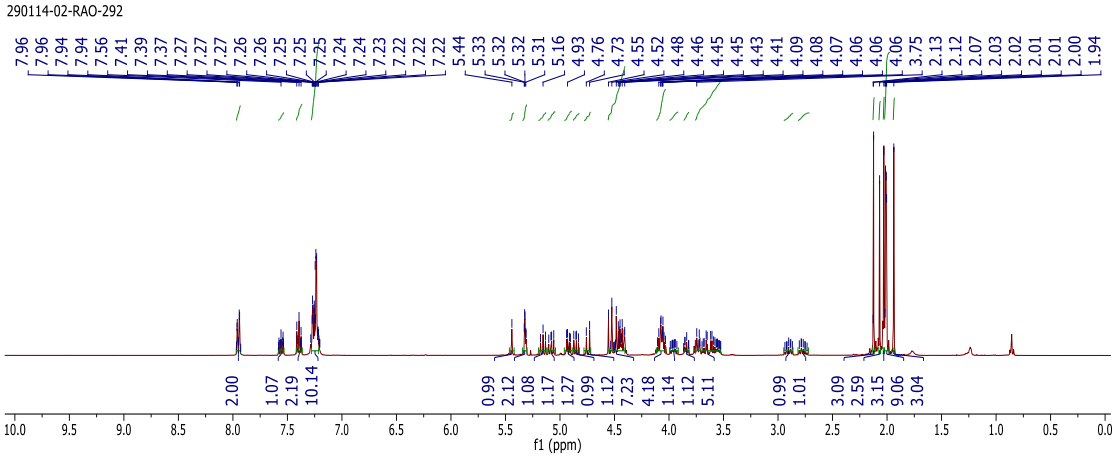
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(63)**



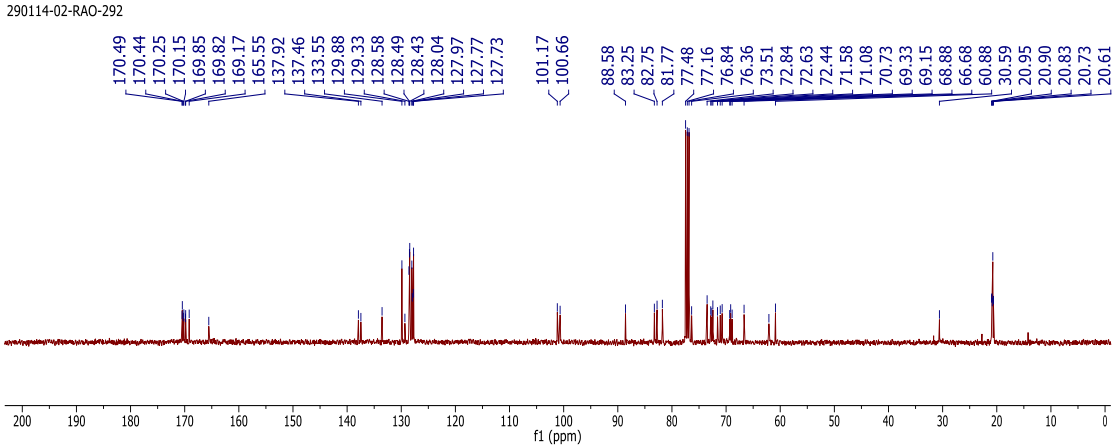
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(63)**



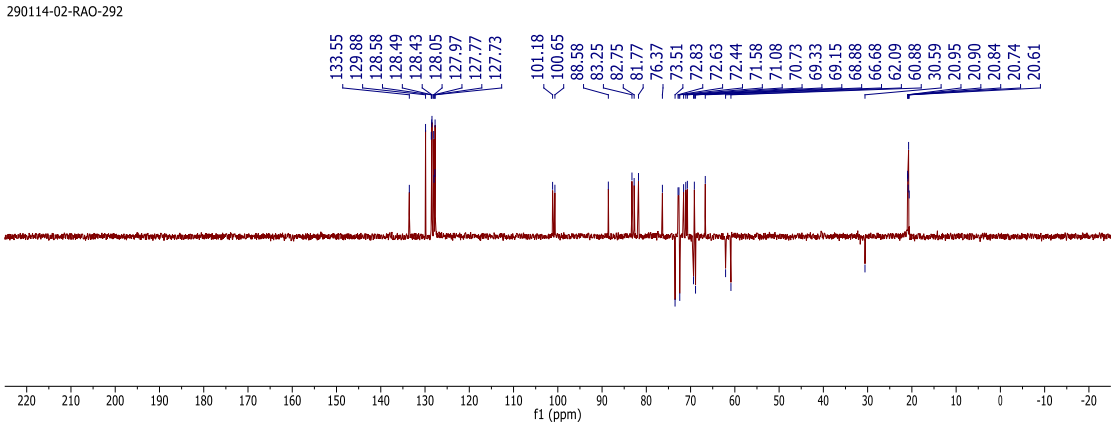
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(64)**



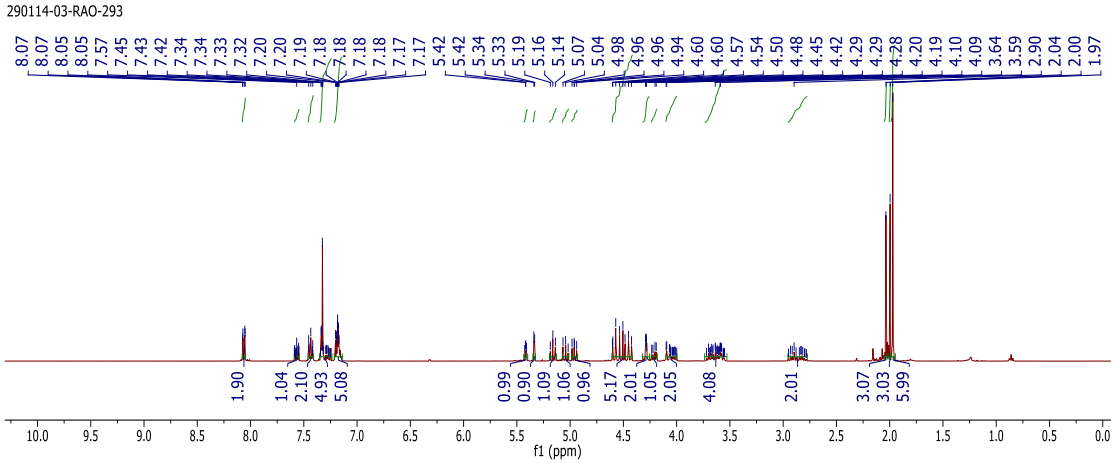
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(64)**



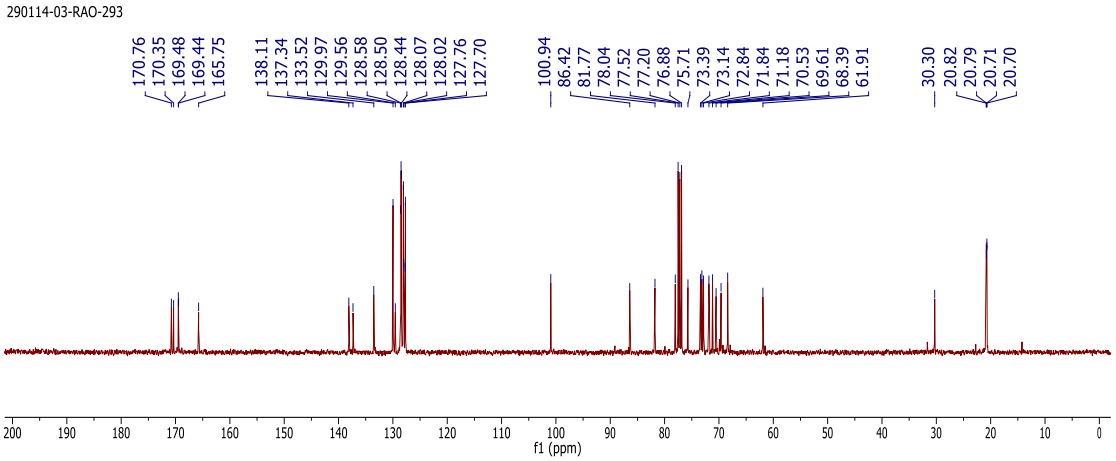
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(64)**



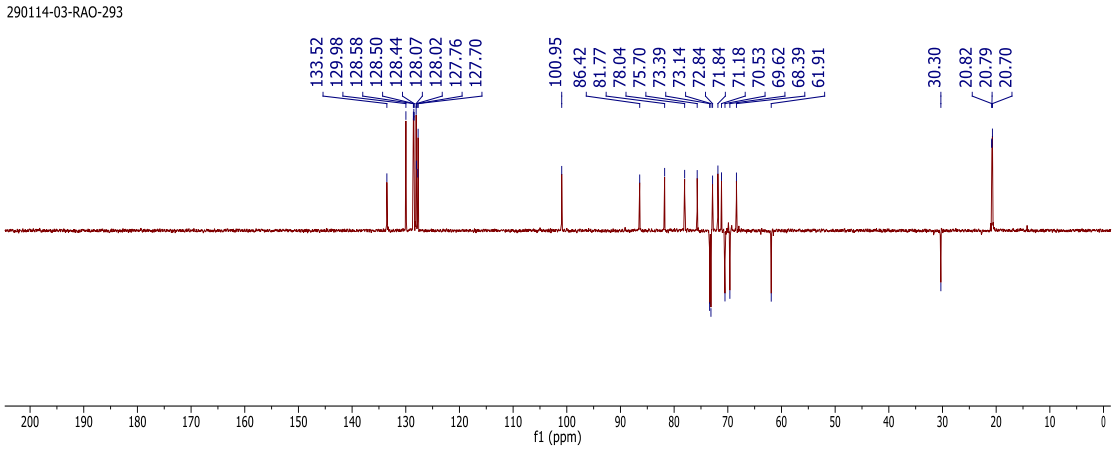
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(65)**



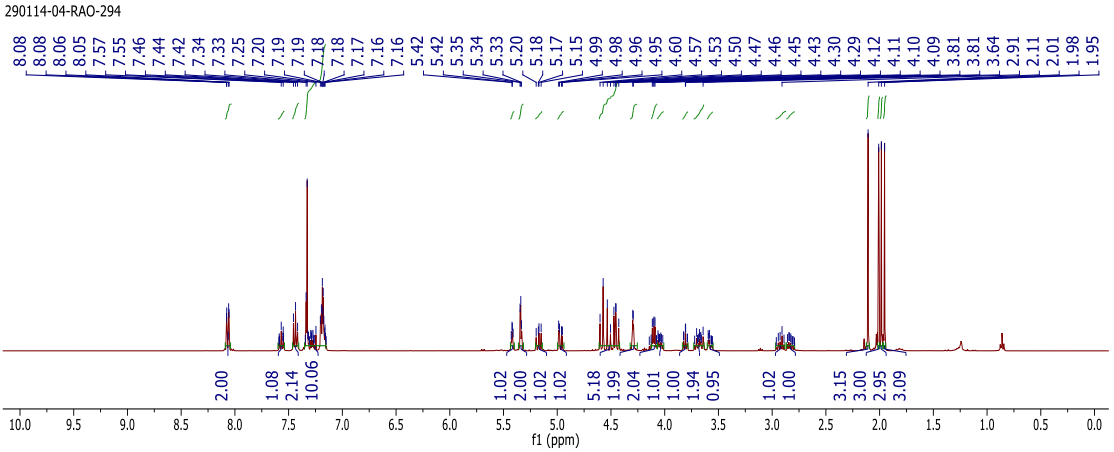
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(65)**



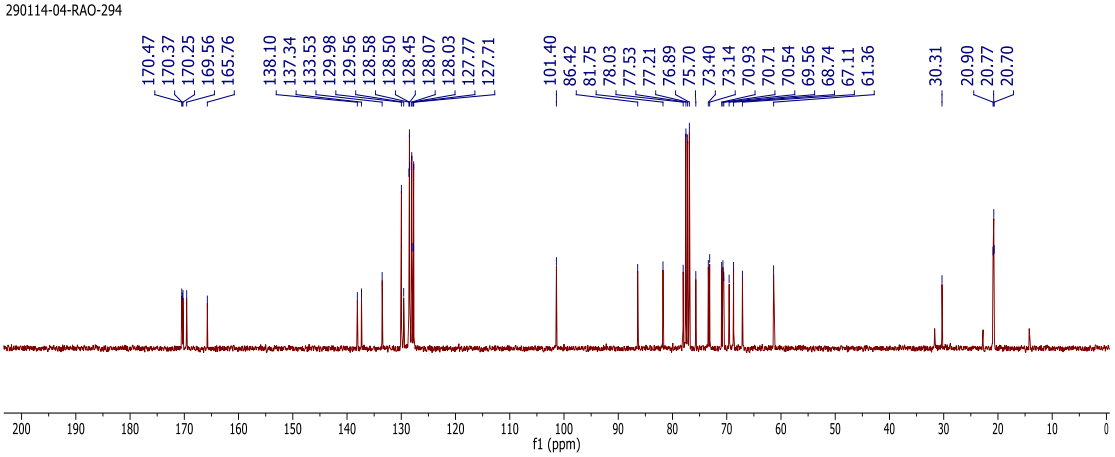
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(65)**



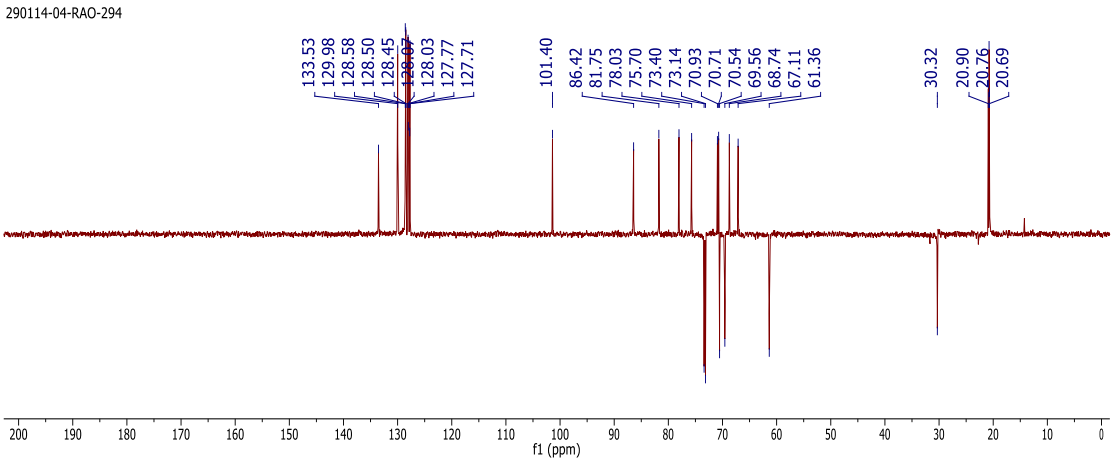
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **(66)**



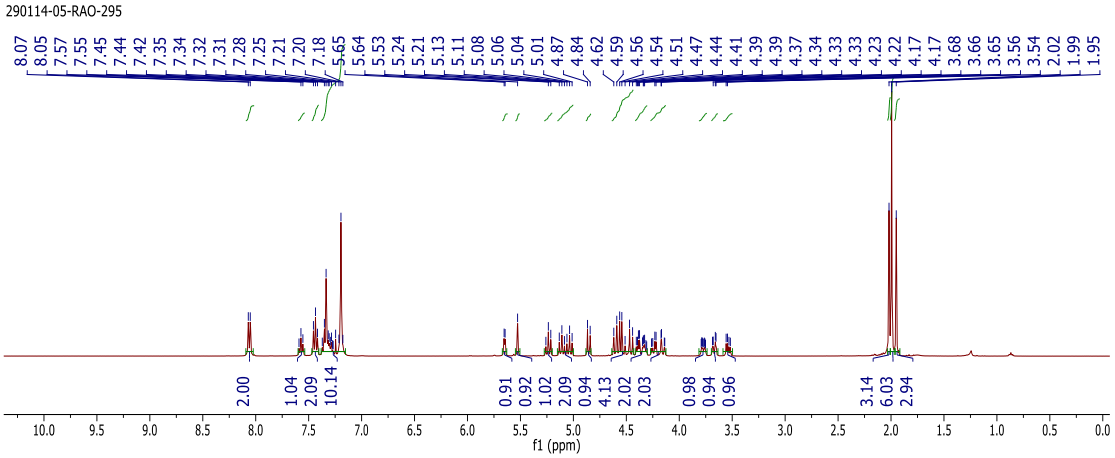
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(66)**



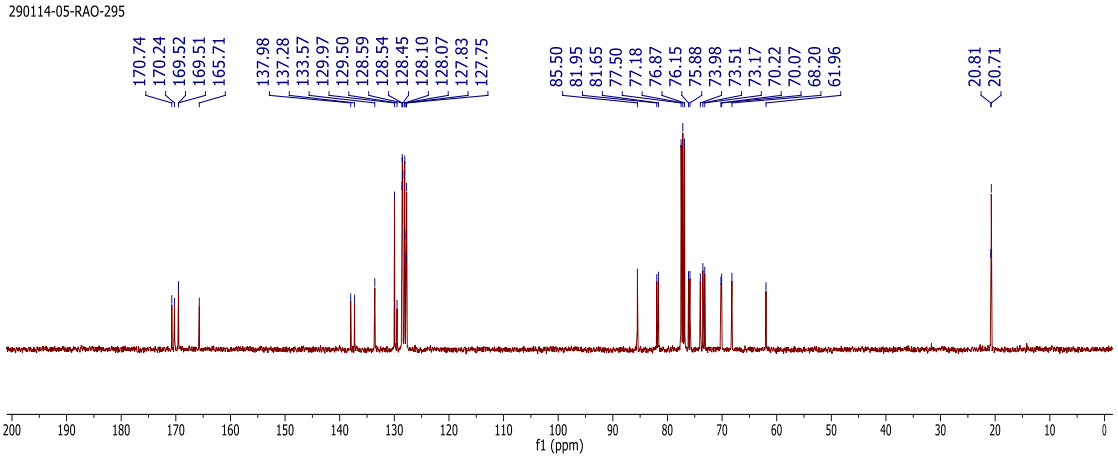
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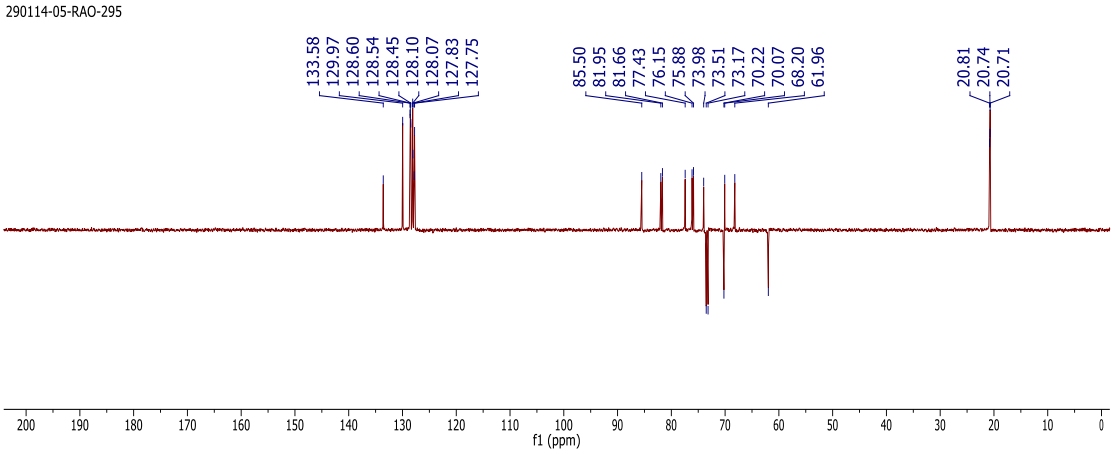
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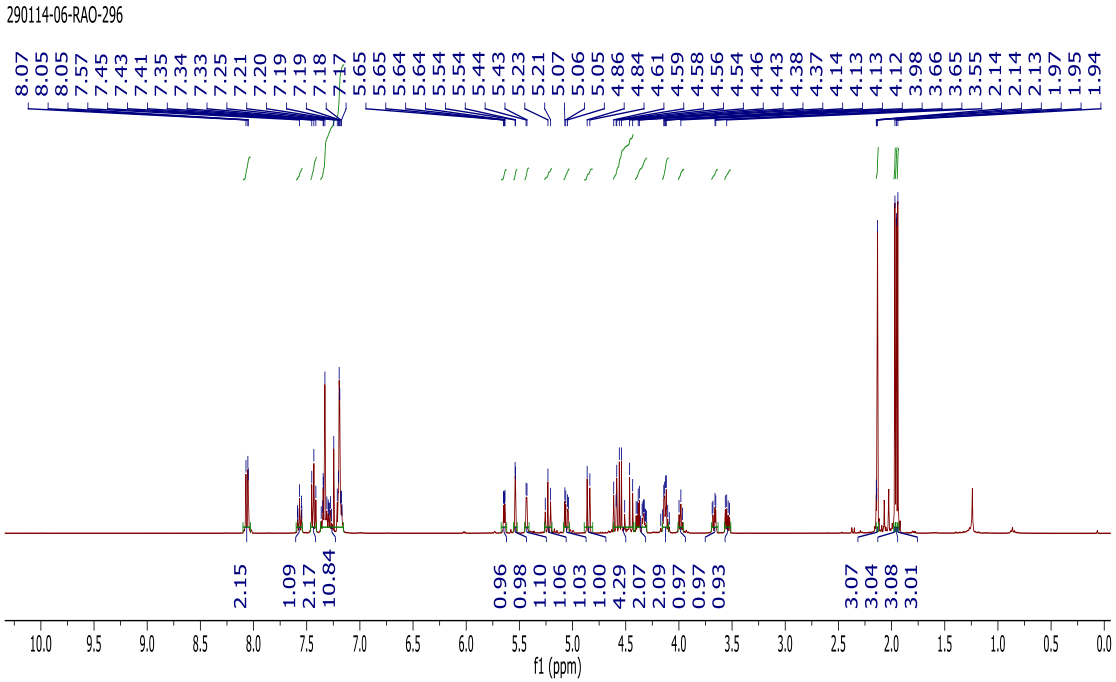
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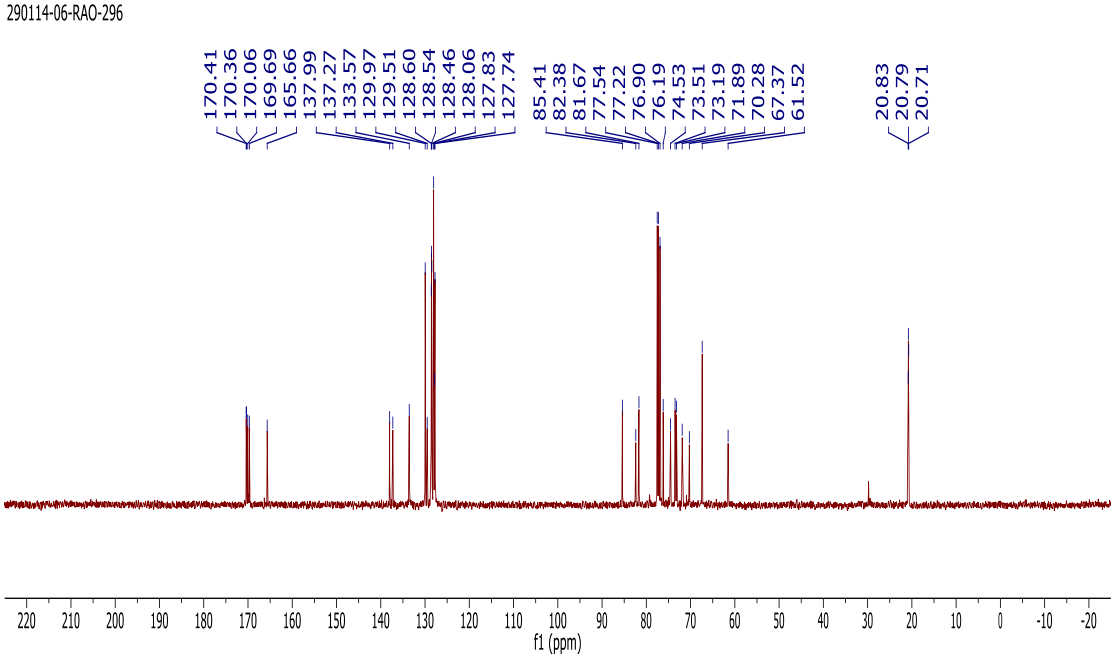
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(67)**



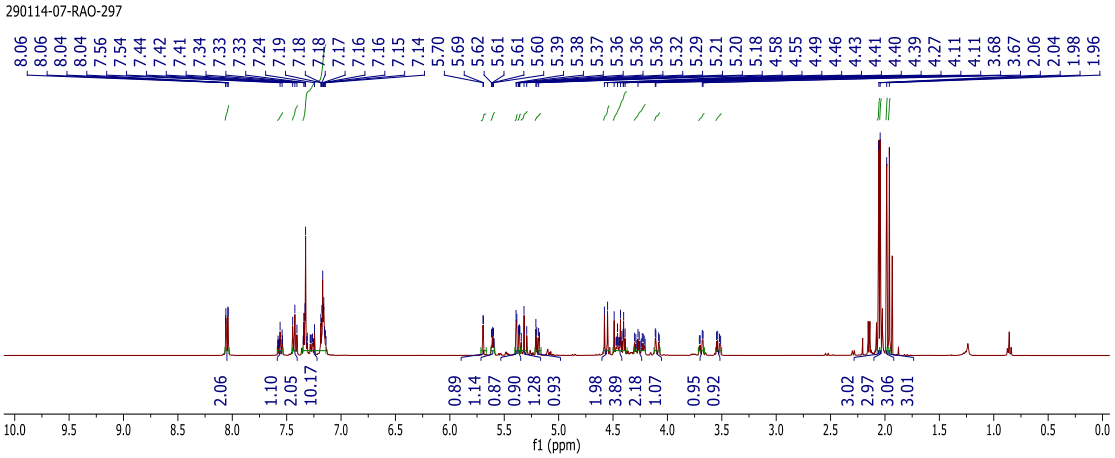
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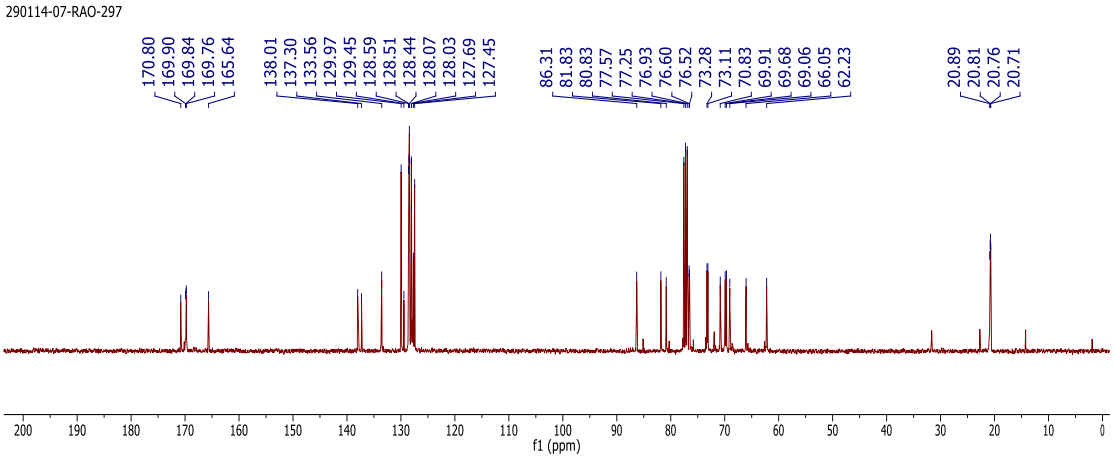
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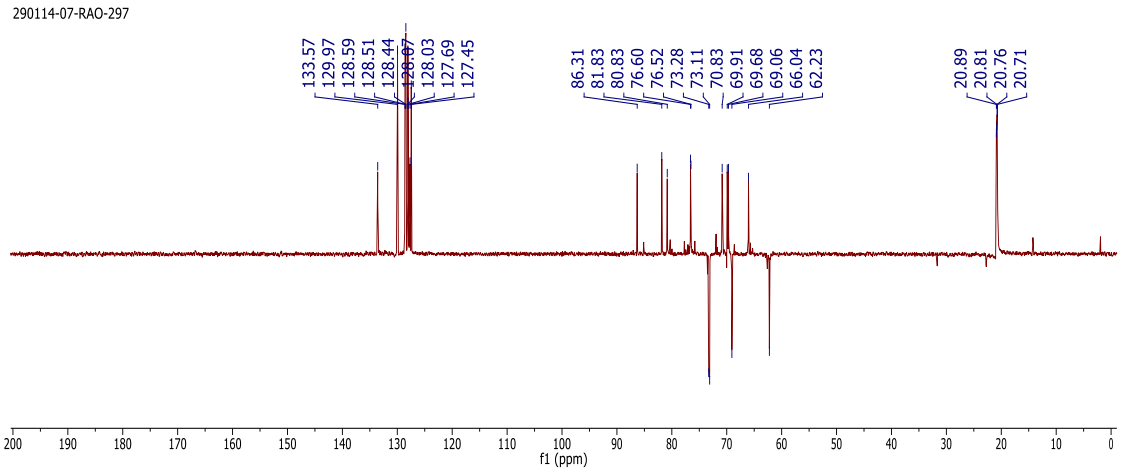
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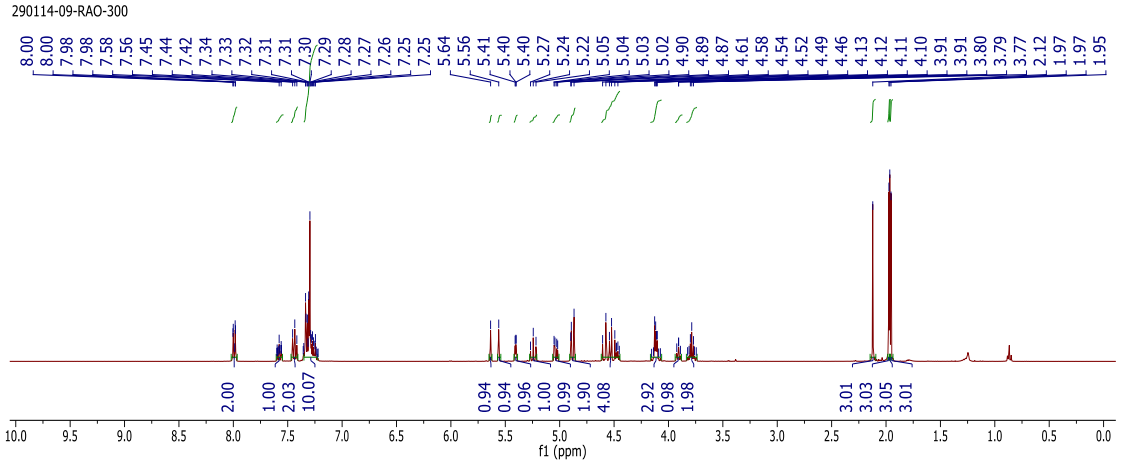
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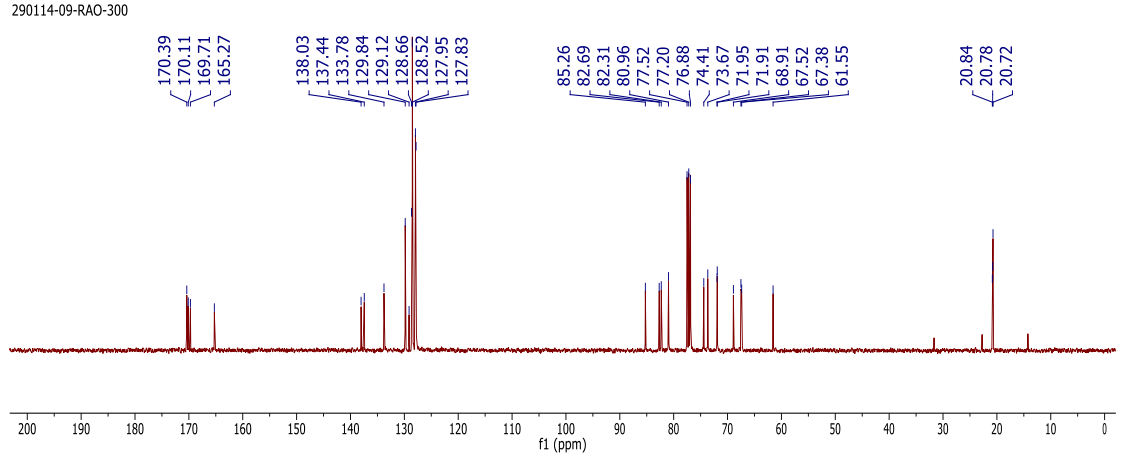
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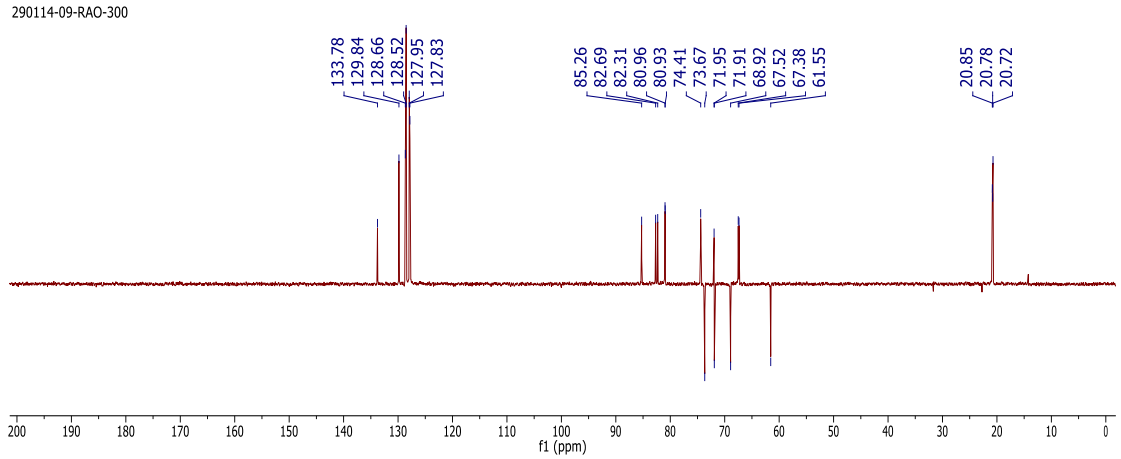
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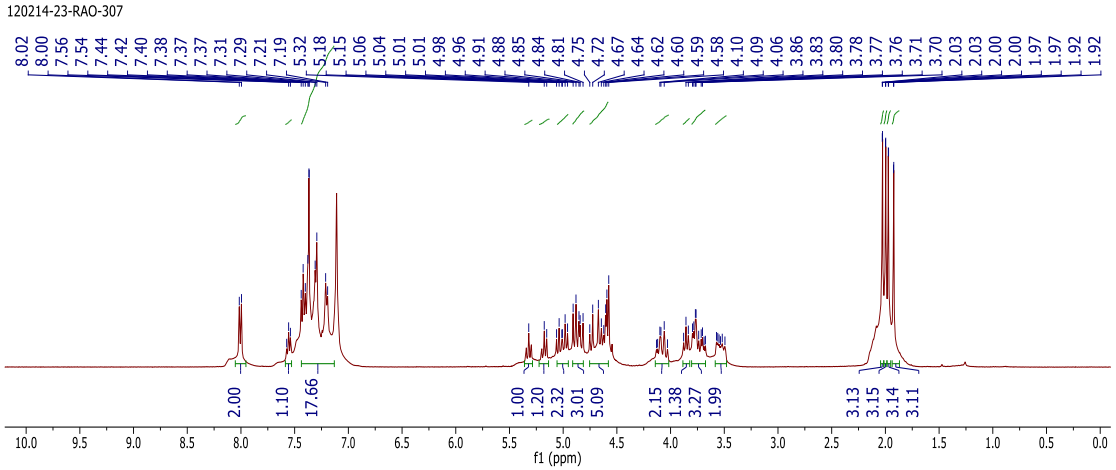
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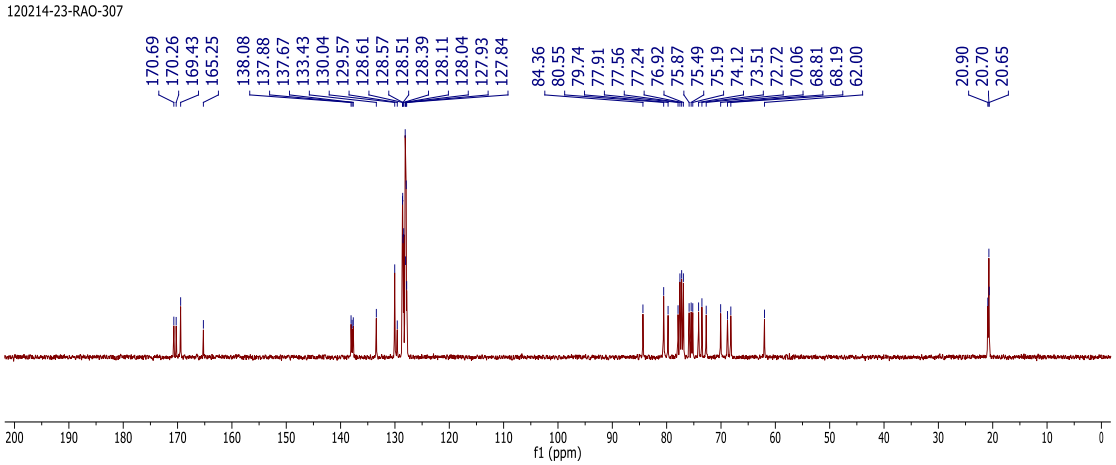
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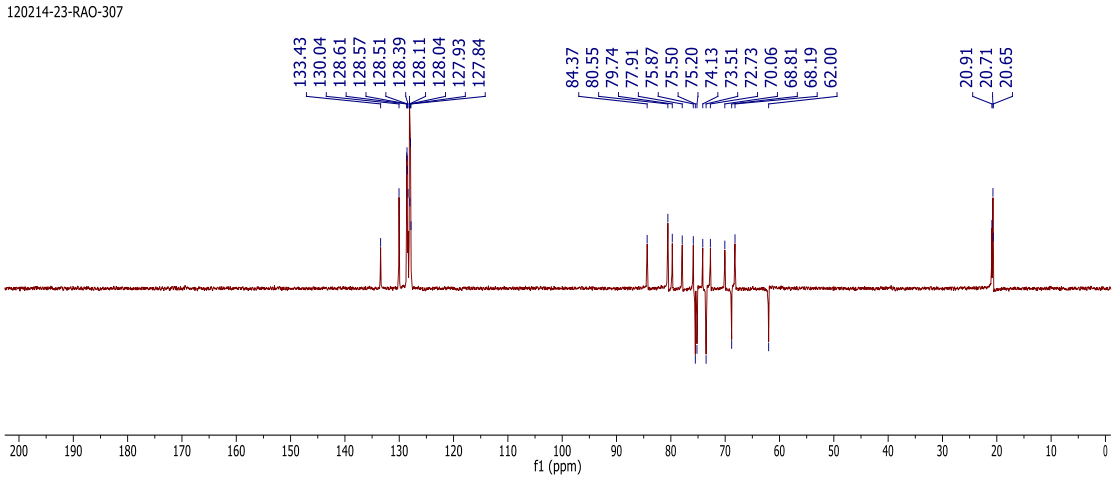
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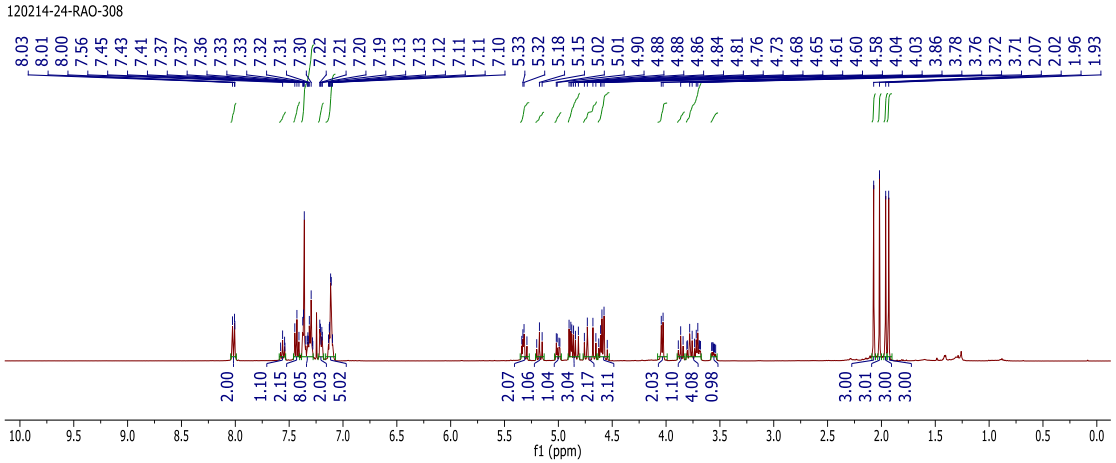
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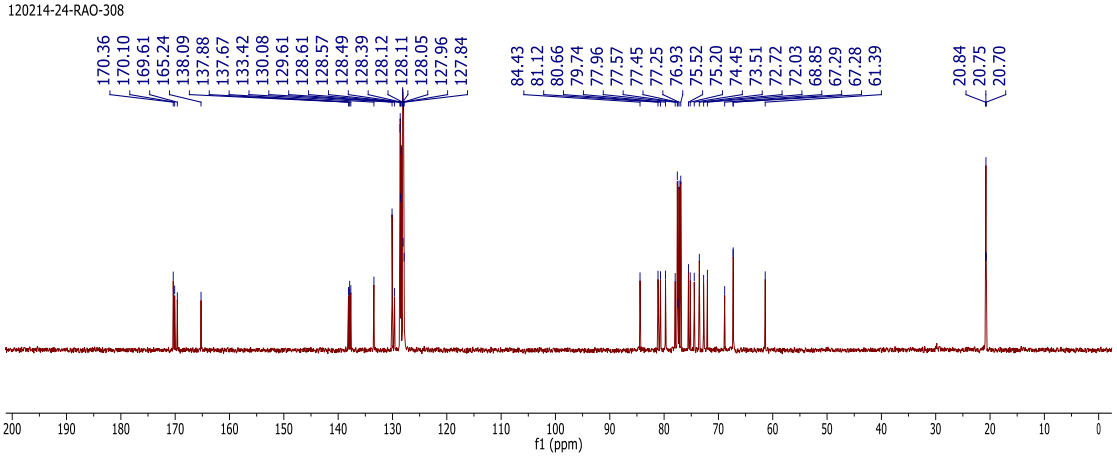
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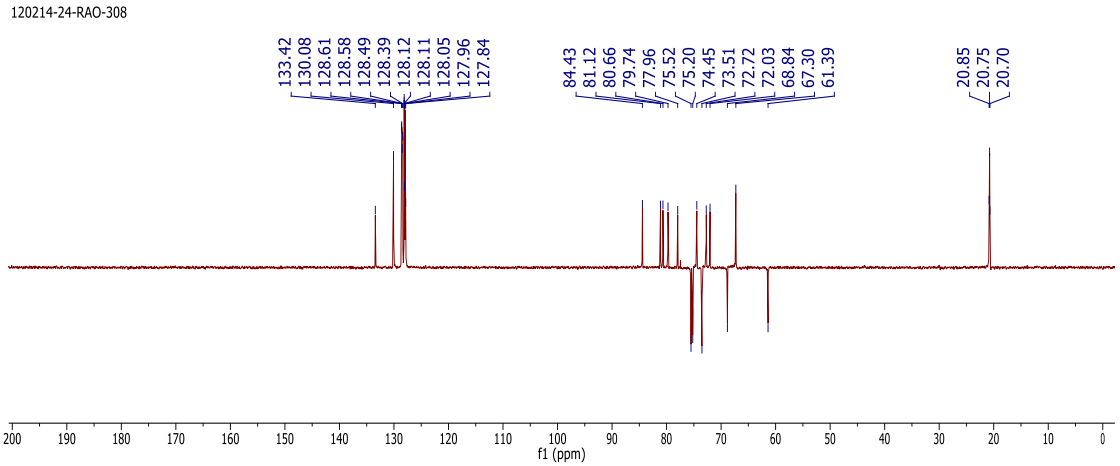
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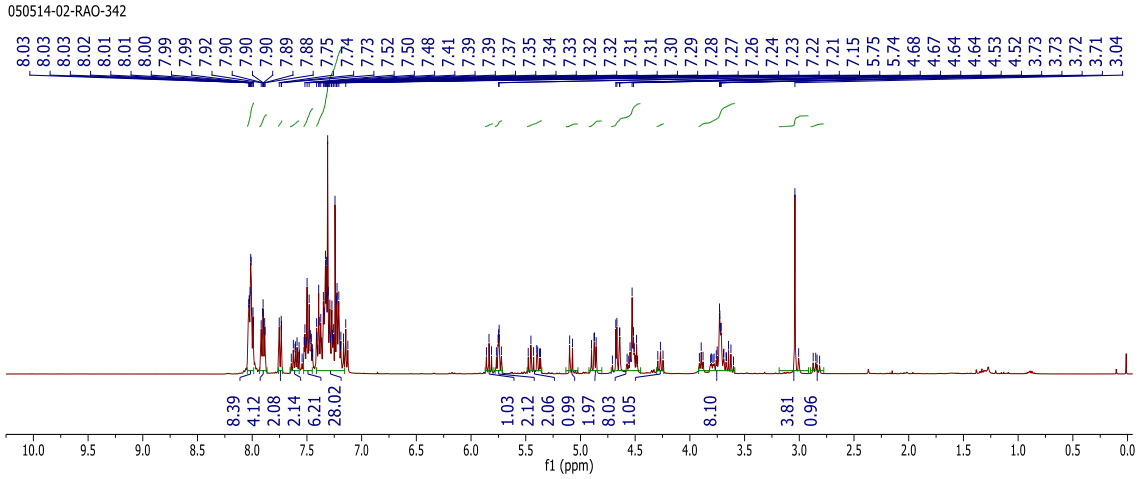
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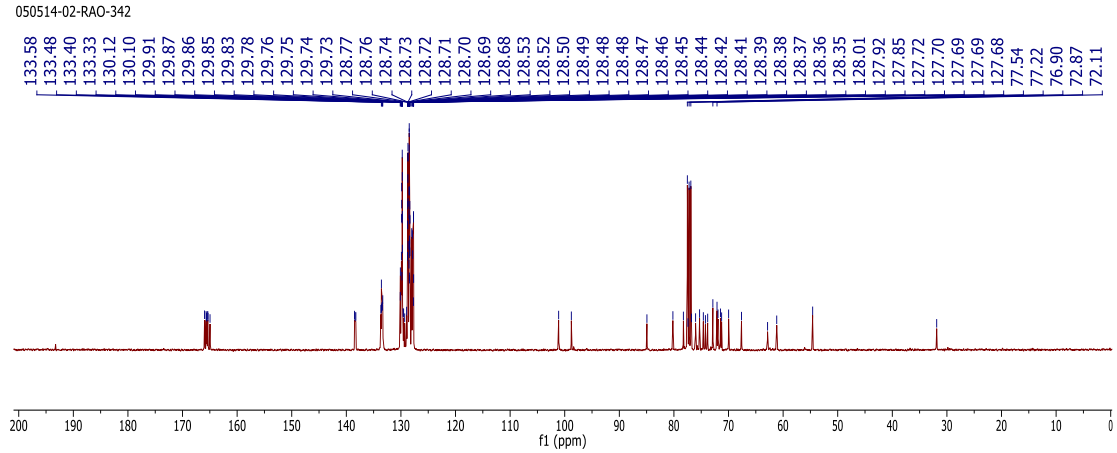
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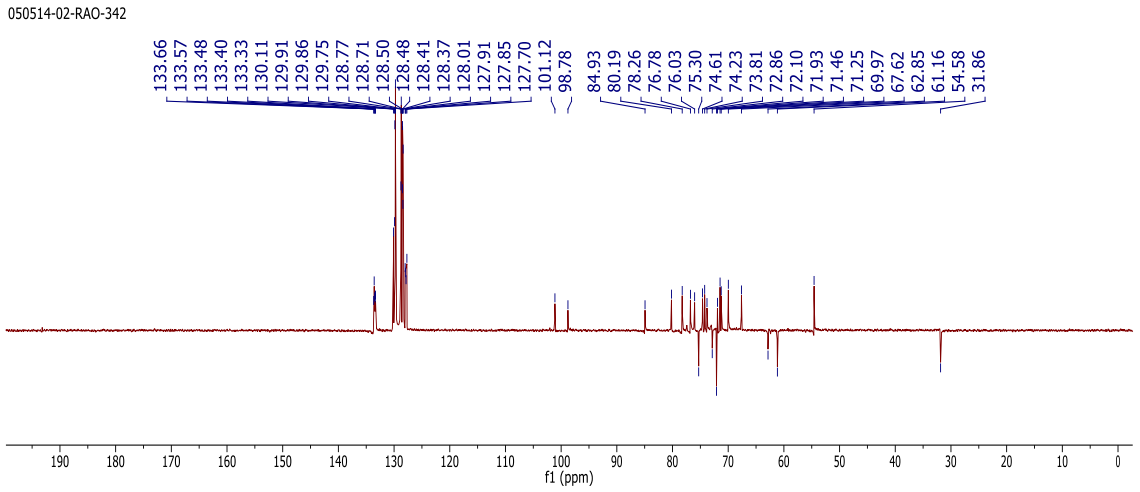
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^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(80)**



DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **(80)**



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CHAPTER 3

A Tetrazine Templated Method for the Synthesis of Ternary Conjugates

3.1 Introduction

Conjugation is one of the most fundamental techniques involving attachment of two or more biomolecules together by covalent bond.[1] Conjugations have facilitated the discovery of new biomolecules which are otherwise difficult to achieve by other tools and sometimes they are the key components in the preparation of medical diagnostics and materials.[2] Generally conjugation reactions are used to modify biomolecules with chemical probes such as fluorescent tags or nanoparticles.[1,3] Subsequently these conjugates are utilized for various purposes such as tracking of cellular events, revealing the function of an enzyme, determining protein bio-distribution, imaging of specific cellular parts, delivering drugs to targeted cells etc.[2-4]

Decades of research led to the discovery of excellent tools for achieving binary conjugates and poly-valent display of a single molecules (e.g. dendrimers,[5] nanoparticles,[6] calixarenes[7]). However, biological processes are very complex and often times require deployment of more than two molecules (glyco-, protein, nucleic acids, etc...) in a single process. A ternary or quaternary conjugate will be desirable if one wishes to probe interactions of more than two different molecules. Methods to get conjugates of more than two are rare in the literature; cyanuric chloride has been reported for the synthesis of ternary conjugates[8] but conditions employed to achieve ternary conjugate are not suitable really for making glycoconjugates.[8]

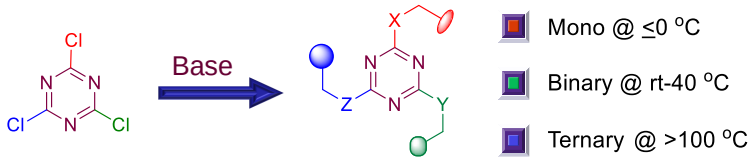


Figure 3.1 Ternary conjugation with cyanuric chloride

The three chlorine atoms on cyanuric chloride can undergo S_NAr reactions with various nucleophiles such as thiol, amino and hydroxyls to form stable linkages.[9] The primary conjugation with these nucleophiles undergoes at ≤0 °C depending on the nucleophile.[10] The second chlorine atom can be substituted with another nucleophile to get binary conjugate at 25–40 °C in alkaline pH.[10] But the third chlorine atom demands stronger nucleophile such as thiolate or amine at high temperature and pH (Figure 3.1).[9-10] In spite of these limitations, cyanuric chloride has been affectively used in the preparation of functional materials.[10-11] The requirement of high temperature for ternary conjugation reaction and instability of cyanuric chloride in aqueous media limited its use in chemical biology.

3.2 Tetrazine Chemistry

3.2.1 Tetrazine Chemistry for ternary Conjugation

Stepwise conjugation of molecules to get ternary complexes is still a formidable challenge under mild conditions though many applications for such ternary conjugates can be envisioned (figure 3.2). Any new method for the ternary conjugation should happen in a modular fashion so that a suite of molecules can react.

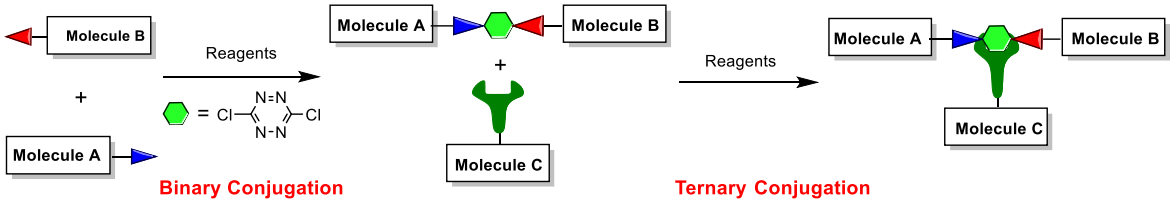
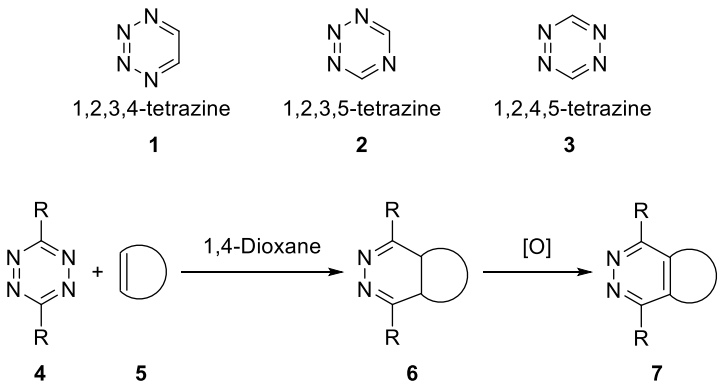


Figure 3.2 Cartoon representation of the ternary conjugation

Synthesis of ternary bioconjugates using dichlorotetrazine as a templating scaffold has been investigated. We hypothesized that chlorines on the dichlorotetrazine moiety can undergo S_NAr reaction with nucleophiles to result in a binary conjugate; further the tetrazine nucleus can participate in the iEDDA reaction to afford a ternary conjugate.

3.2.2 Introduction to Tetrazines

Tetrazine is a six membered cyclic molecule, wherein "tetraza" refers to four nitrogen atoms and "ine" represents a six membered ring. Tetrazine can exist in three possible isomers of which 1,2,3,4-terazines (**1**) are more useful in the synthesis of biologically active heterocyclic compounds[12] whereas 1,2,3,5-tetrazines (**2**) are lesser known isomers.[13] The third category of isomers are *s*-tetrazines or 1,2,4,5-tetrazines (**3**) which undergo 4+2 inverse-Electron-Demand-Diels–Alder (iEDDA) cycloaddition reaction, known as Carboni–Lindsey reaction.[14] iEDDA reaction was first applied to biomolecules concurrently by Fox[15] and Hilderbrand[16] (Scheme 3.3).

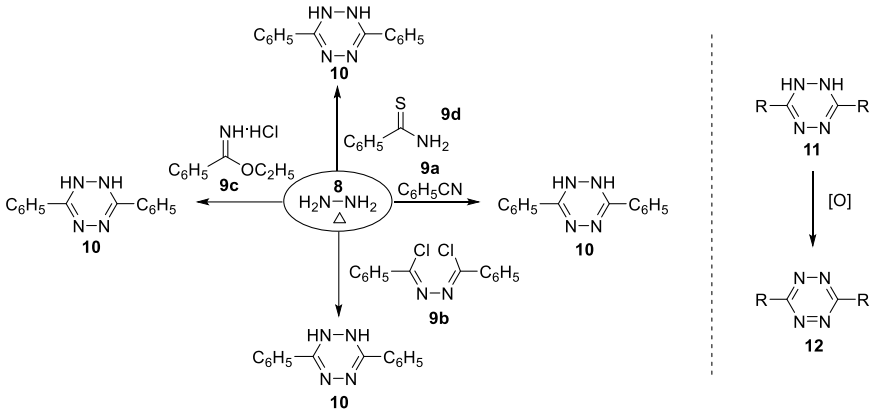


Scheme 3.3 Tetrazine isomers

s-Tetrazines gained significant interest in various fields such as chemical biology,[17] material science,[18] coordination chemistry,[19] natural product chemistry,[12] photochemistry[13] and explosives[20] due to their unique physical, chemical properties and chemical reactivity as well as their stability. The recent discovery of ultrafast biorthogonal reaction of *s*-tetrazine with *trans*-cyclooctene resulted in the increased use of *s*-tertazine for biorthogonal live-cell imaging.[21]

3.2.3 Synthesis of *s*-tetrazines

Direct synthesis of s-tetrazine is not yet known and are prepared by the oxidation of dihydrotetrazine precursors using mild oxidative reagents such as nitrites, bromine, air, ferric chloride, hydrogen peroxide or chromic oxide.[22] Pinner reported the synthesis of *s*-tetrazine from the reaction of aromatic nitriles with hydrazine in an aqueous solution of ammonium hydroxide to result dihydrotetrazine which was further oxidized to *s*-tetrazine.[23] In the literature few other methods also reported to synthesize dihydrotetrazines (Scheme 3.4).[24]



Scheme 3.4 General methods for the synthesis of *s*-tetrazines

Pinner's synthetic route is helpful in the synthesis of symmetric-aromatic *s*-tetrazines but has moderate yields. Hence it has been modified in several ways to improve yields and the time required of the reaction.

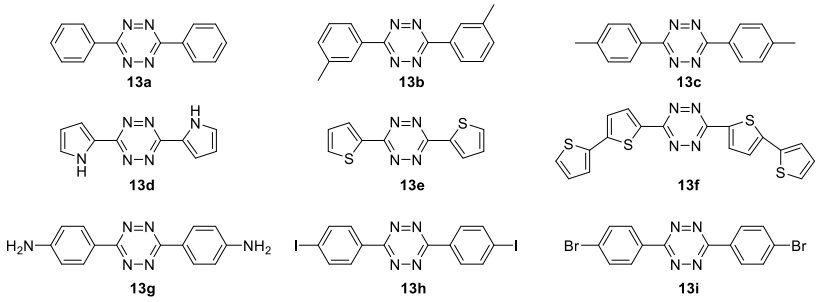
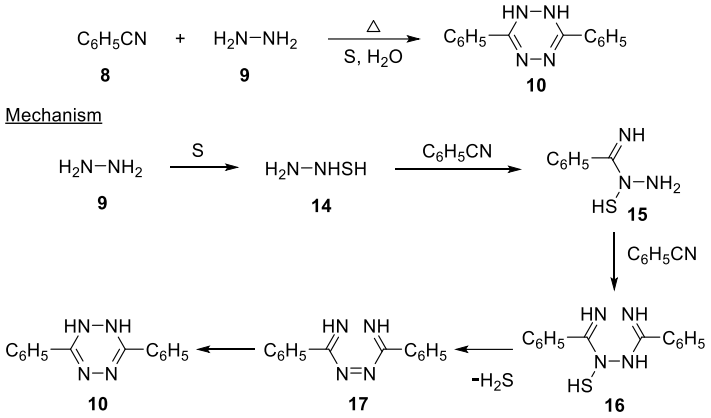


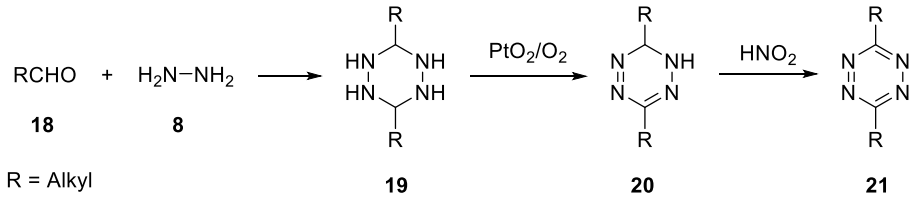
Figure 3.5 Various aryl *s*-tetrazines

Wiley *et al.*[12] reported improved yields by carrying out the reaction using triethylamine in methanol which was also used by Soloducho *et al.* to synthesize a series of linear s-tetrazines (Figure 3.5).[25] Improvement to the Pinner's route was reported by Audebert *et al.*[13] by the addition of sulfur which increased yields of the reaction and proposed the possible mechanism on the basis of evolution of H_2S gas during the course of the reaction (Scheme 3.6).[13]



Scheme 3.6 Sulfur promoted Pinner synthesis

The Pinner synthetic route is inefficient to synthesize aliphatic *s*-tetrazines as aliphatic nitriles are less reactive than aromatic nitriles.[23] In such cases, the aliphatic nitrile can be replaced with an aldehyde to get hexahydro-*s*-tetrazine, which can be oxidized to *s*-tetrazine subsequently in two steps albeit in poor yields (Scheme 3.7).[26] However, aliphatic *s*-tetrazines are noticed to be volatile making their isolation difficult.[26]



Scheme 3.7 Synthesis of alkyl *s*-tetrazines

3.2.4 Metal-catalyzed synthesis of *s*-tetrazines

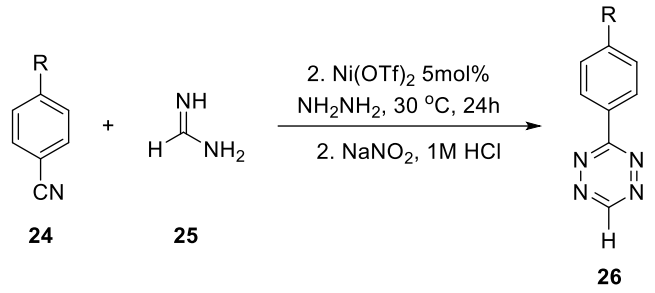
In the synthesis of *s*-tetrazines, the nucleophilic attack of hydrazine on the nitrile initiates the reaction to form an amidrazone, which then reacts with another nitrile to form dihydroterazine. This nucleophilic attack can be promoted by the addition of Lewis acid catalyst. Recently, Neal's group reported the metal-catalyzed one-pot synthesis of *s*-tetrazines directly from nitriles and hydrazines.[22] The reaction of benzyl cyanide with neat hydrazine was studied by adding various Lewis acid catalysts and found that the

addition of 5 mol% of nickel triflate ($Ni(OTf)_2$) leads to the near quantitative yield of *s*-tetrazines (Scheme 3.8).[22]



Scheme 3.8 Metal-catalyzed synthesis of the *s*-tetrazines

However, the synthesis of unsymmetrical *s*-tetrazines are difficult than symmetrical tetrazines. Neal's group also explored the utility of $Ni(OTf)_2$ to the synthesis of *s*-tetrazines to prepare unsymmetrical *s*-tetrazines with aromatic nitriles and formamidine (Scheme 3.9).[22]



Scheme 3.9 Synthesis of unsymmetrical *s*-tetrazines

The reaction yields were reported between 60%-74% with various nitriles.[22] This metal-catalyzed one-pot synthesis of *s*-tetrazines has broadened the range of accessible unsymmetrically substituted *s*-tetrazines.

3.2.5 Applications of *s*-tetrazines in co-ordination chemistry

As the four nitrogen atoms of the tetrazine ring serve as ancillary ligands,[27] tetrazine is substituted with pyridyl, dipyridyl and carboxylate functional groups for the coordination with metals (Figure 3.10).[28]

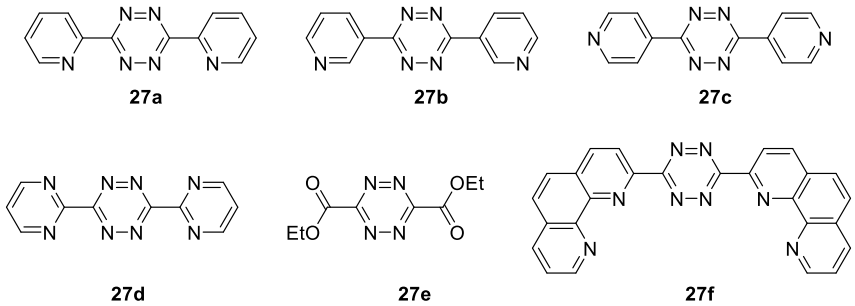


Figure 3.10 Tetrazine derivatives for co-ordination chemistry

Coordination polymer, based on ruthenium and iron using tetrazine to produce 1-D conducting polymer was reported by Hanack *et al.*.[29] The conductance was achieved due to the excellent electron affinity of the tetrazine and allowed charge transfer.[29] The

tetrazine ring provides the electronic connection between the metal centre and conjugation systems.[29] The conductivities of these polymer materials were reported up to ca. 0.1 Scm^{-1} which fit quite well in the range to that of standard poly-pyrrole materials (Figure 3.11).[29]

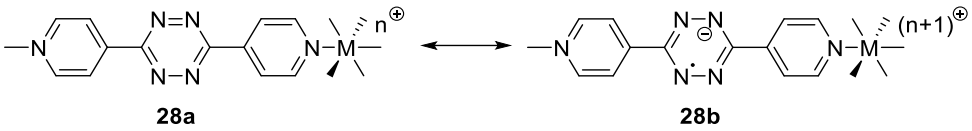
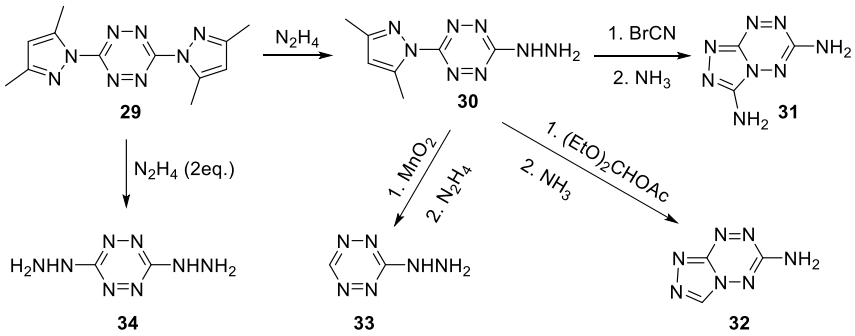


Figure 3.11 1-D conducting polymer of tetrazine

3.2.6 Applications of *s*-tetrazines in explosives

Tetrazine derivatives have been investigated as prominent energetic materials due to their high nitrogen content, large positive heats of formation, high thermal stability and low mechanical sensitivity.[30] In addition tetrazines are rich in spectroscopic properties, such as fluorescence, strong absorption of visible light, and reversible electrochemistry.[13] Chavez and Hiskey prepared many high nitrogen containing tetrazine derivatives starting from readily available tetrazine compound **29** (Scheme 3.12 and Figure 3.13).[31]



Scheme 3.12 Tetrazine containing traditional explosives

The thermal decomposition of nitrogen-rich tetrazole and tetrazine compounds were investigated by Löbbecke and coworkers. The thermal decomposition behaviour of these samples was probed by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and evolved gas analysis (EGA).[32]

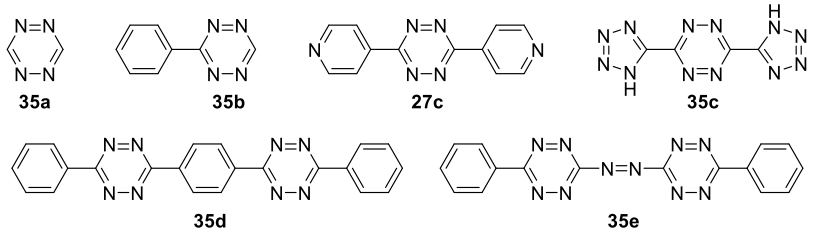
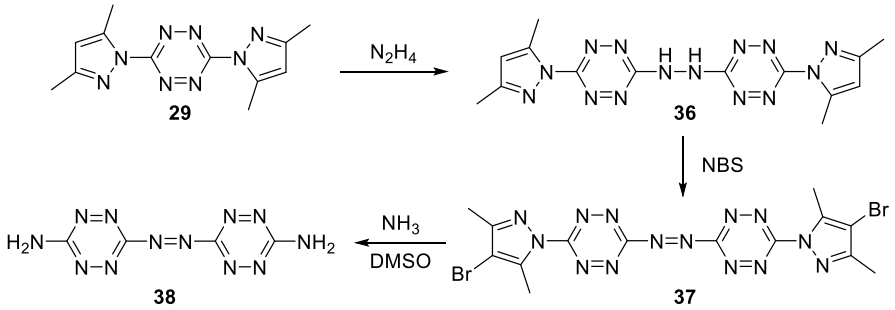


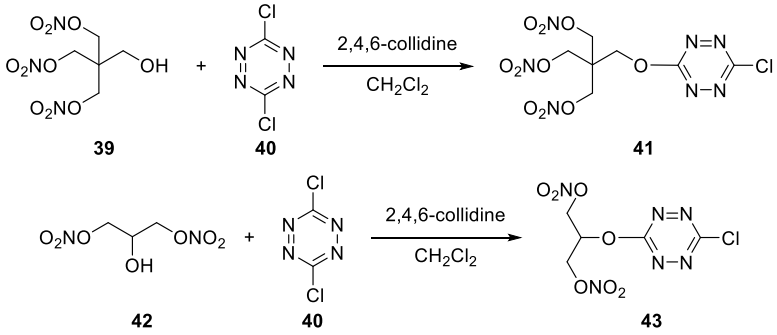
Figure 3.13 Tetrazine containing traditional explosives

The thermal decomposition of the tetrazine derivatives resulted in the opening of the heterocyclic ring to give nitriles, N_2, and carbon soot.[32] The molar decomposition enthalpy measured for **35c** is remarkably high and resembles that of a high explosive.[32] Hiskey and co-workers reported the synthesis a novel high nitrogen containing energetic compound **38** from **29** in three steps (Scheme 3.14).[33] The hydrazo compound **36** was obtained by the treatment of **29** with 0.5 equivalents of hydrazine. The oxidation of compound **36** to **37** was achieved by NBS and the resulting bromide **37** was treaed with ammonia in DMSO affording the high nitrogen containing compound **38**, which was found to be thermally stable up to 252 ºC.[33]



Scheme 3.14 Tetrazine containing explosives

Tetrazines are also derivatized with a variety of other high nitrogen containing compounds. Traditional explosive compounds, such as 3,3′- dinitroazetidine (DNAZ) and trinitropentaerythritol (PETrN) were coupled with tetrazine leading to the formation of electroactive explosive chromophores (Scheme 3.15).[20a,34]



Scheme 3.15 Tetrazine containing traditional explosives

3.2.7 Applications of *s*-tetrazines in chemical biology

Tetrazines react with electron rich alkene/alkyne in an inverse-electron demand [4 + 2] Diels−Alder cycloaddition. In the first step, alkene-tetrazine reaction yields a cycloadduct which undergoes a *retro* Diels−Alder reaction by losing N_2 to produce a 4,5-dihydropyridizine which then isomerises to 1,4-diydropyridazine in protic solvents.[12] In 1990, Sauer reported the kinetics of electron-deficient tetrazines with various dienophiles

in organic solvents and found that the reaction with *trans*-cyclooctene was very fast.[35] In 2008, the scope of this reaction was investigated by two groups in aqueous media for labelling of biomolecules.[36] For this purpose, a water soluble 3,6-diaryl-*s*-tetrazine was utilized, the iEDDA reaction was performed with *trans*-cyclooctene (**44**) to notice extraordinarily fast kinetics with a rate constant of 2000 $M^{-1}S^{-1}$ Fox *et al.* and successfully applied it in the modification of proteins (thioredoxin) *in vitro* and *in vivo* under a range of aqueous buffer conditions (Figure 3.16).[36-37]

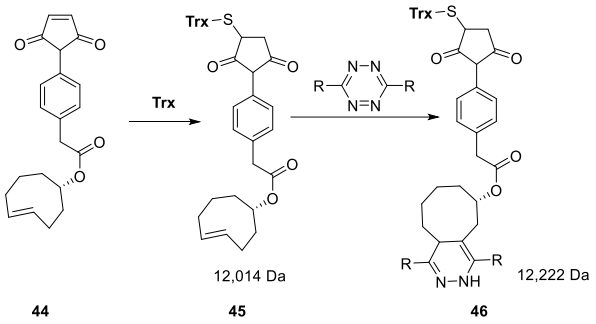


Figure3.16 Functionalization of thioredoxin with cyclooctyne further orthogonal reaction with tetrazine[36]

The iEDDA reaction of norbornene with tetrazine to yield the dihydropyridazine ligated product after losing dinitrogen was reported at the same time by Hilderbrand *et al.*[38] The rate of thes reaction ($k_2 \approx 2\ M^{-1}S^{-1}$) was lower than the ligation reaction between *trans*-cyclooctene and tetrazine, but still considerably faster than strain-promoted click reactions (10^{-3} to $10^{-1}\ M^{-1}S^{-1}$).[38] The orthogonality of this reaction was demonstrated in live cells by conjugating a red fluorophore (rhodamine) to the norbornene and to a monoclonal antibody.[38] Subsequently, this antibody was exposed to cells expressing the corresponding antigen. Tetrazine-near-infrared (NIR) dye conjugate was synthesised which was then incubated with cells to obtain a dye conjugate and a robust colocalization between rhodamine and the NIR dye VT680 (Figure 3.17).[38]

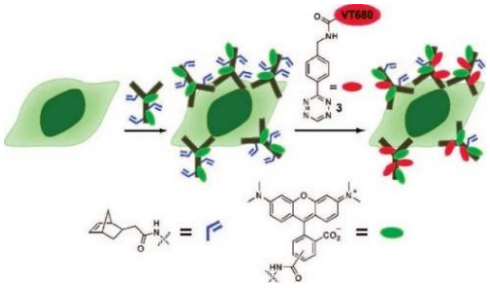


Figure 3.17 A)SKBR3 cells with norbornene and tetramethylrhodamine co-labeled trastuzumab, B) tagging live cells with tetrazine-VT680[38]

Based on these initial reports on tetrazine-based iEDDA cycloadditions for the orthogonal bioconjugation, many developments and new applications of the reaction especially for live cell labelling were reported.[21] Tetrazine based ligations were used to modify and image proteins *in vivo* both in bacteria and in mammalian cells,[39] to image chemotherapeutics inside living cells,[40] to label biomarkers on cells with magneto-fluorescent nanoparticles,[18] and to conjugate quantum dots.[41]

Potential biomedical applications include tetrazine based PET imaging and ^{18}F scintillographic imaging.[42] The main advantage of this reaction is that it can be made fluorogenic by conjugating tetrazine to a red fluorophore, where the fluorescence is quenched by the tetrazine but activated upon conjugation with alkyne/alkene (Figure 3.18).[42]

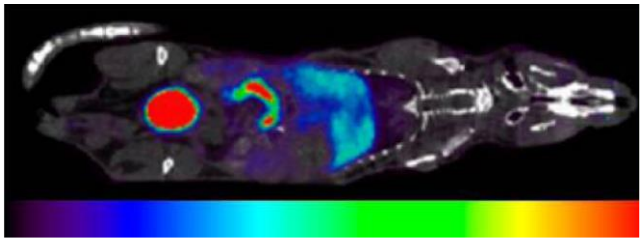


Figure 3.18 PET-CT image showing the biodistribution of radioactivity in mouse model using ^{18}F-labelled tetrazine derivative.[42]

Recently stable methylcyclopropene derivatives are also explored as reactive dienophiles towards tetrazine bioorthogonal reaction.[43] Phospholipids were modified with methylcyclopropene and are then labelled with fluorogenic tetrazine-BODIPY probe in live human cancer cells.[43] Unnatural amino acids, bearing various strained alkene and alkyne functionalities such as *trans*-cyclooctenes, norbornenes, bicyclononynes, and cyclopropenes, have been synthesized which can be incorporated site specifically into proteins *in vivo* by expanding the genetic code (Figure 3.19).[43]

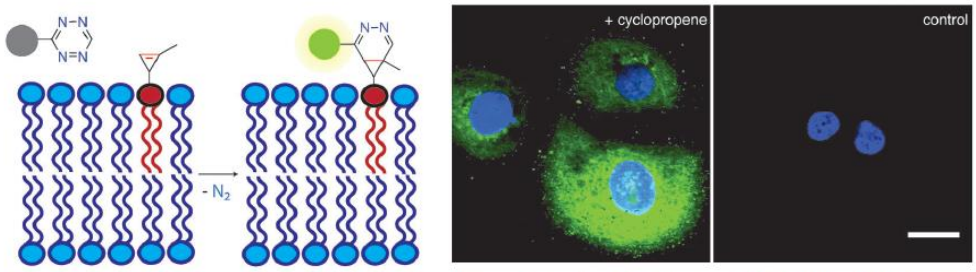


Figure 3.19 Live-Cells Imaging with the fluorogenic tetrazine-BODIPY probe[43]

These reactions are very rapid and specific. Primarily most of the tetrazine fluorophores are weakly fluorescent, but show strong fluorescence once attached to the protein of

interest through bioorthogonal reaction and improve the signal to noise ratio in the overall ligation process.[44] Using expanded genetic code, cyclooctyne and *trans*-cyclooctene modified unnatural amino acids (**47, 49**) were introduced in *E. coli* at a specific site in the protein and were derivatized using a tetrazine-diacetylfluorescein (Figure 3.20).[44]

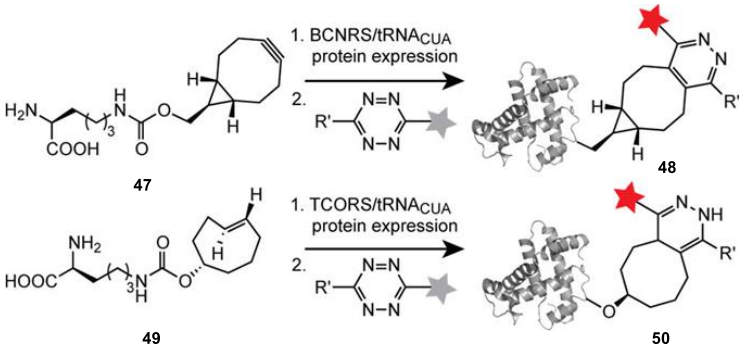


Figure 3.20 Genetic Encoding and Fluorogenic Labeling of Unnatural Amino Acids[44]

Very recently Fox group converted dihydrotetrazine (**51**), photocatalytically and enzymatically, to tetrazine (**52**) under physiological conditions. This report increased the scope of tetrazine ligation to a range of applications in cell imaging, prodrug activation, and gene activation (figure 3.21).[45]

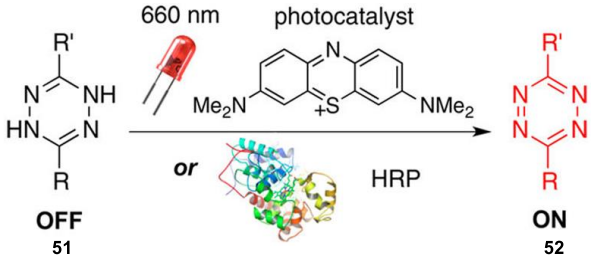
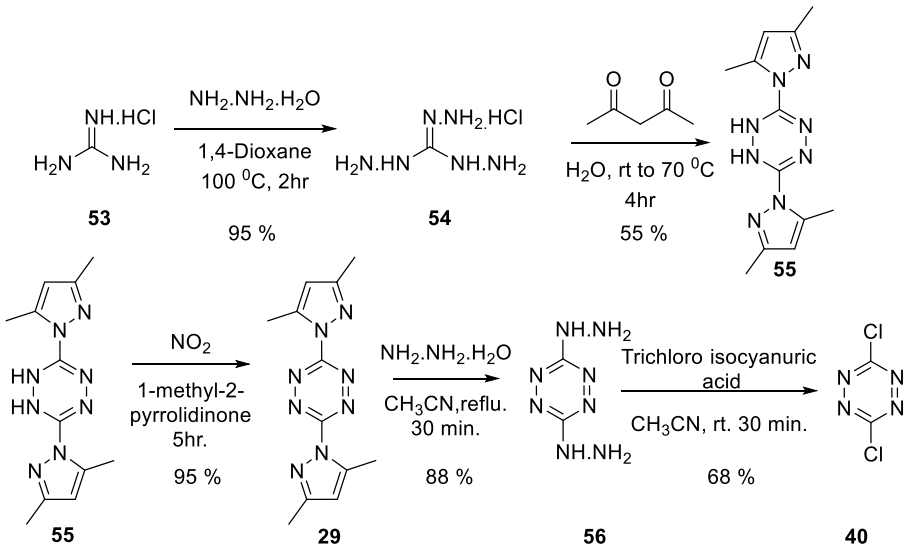


Figure 3.21 Turn-on through enzyme or light[45]

3.3 Present Work

To initiate our research work, an easy access to dichlorotetrazine was necessary as it is not available from commercial sources. The dichlorotetrazine was synthesized starting from the guanidine hydrochloride in five steps. The guanidine hydrochloride **53** was treated with hydrazine hydrate in 1,4-dioxane under reflux condition to obtain triaminoguanidine hydrochloride **54** in good yield. The condensation reaction of triaminoguanidine hydrochloride **54** with acetyl acetone at 70 ºC in water resulted in substituted dihydrotetrazine **55** in moderate yield. The purity of substituted dihydrotetrazine **55** is crucial for the next reaction; however, the high nitrogen content of **55** made its purification difficult by conventional column chromatography. Hence we attempted recrystallization from hot ethanol, which resulted in the formation of yellow needle like crystals of **55** with high purity (Scheme 3.22).



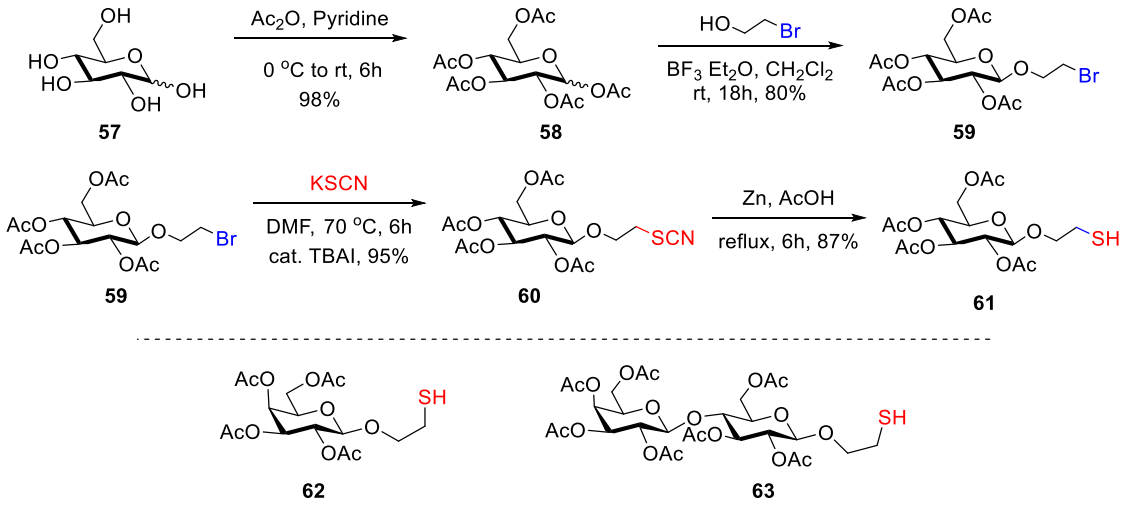
Scheme 3.22 Synthesis of *s*-dichlorotetrazine

Among the various available oxidizing agents we have chosen NO_2 for the oxidation of **55** since it produces less side products. The NO_2 gas was generated by the dropwise addition of conc.HNO_3 to copper powder in a two-neck round bottom flask. The generated NO_2 gas was then carefully transferred to the solution of dihydrotetrazine **55** in 1-methyl-2-pyrrilodinone until complete conversion of dihydrotetrazine to the diazole tetrazine **29** as adjudged by TLC analysis and change in the colour of the reaction mixture. The purity of this compound **29** was improved by repeated washing with ice cold water. The compound **29** was then reacted with hydrazine hydrate in acetonitrile to obtain hydrazine substituted tetrazine **56** as a brown colour solid in good yield. The purification of the compound **56** was difficult by conventional column chromatography due to the presence

of hydrazine moieties. Nevertheless, it has been observed that compound **56** was sparingly soluble in methanol; hence, cold methanol was used to wash out impurities from the crude compound **56** resulting in pure di-hydrazine substituted tetrazine **56** which can be converted to required dichlorotetrazine by treating it with Cl_2 gas or by the reaction with trichloroisocyanuric acid. Between these two reagents, trichloroisocyanuric acid was preferred to avoid the toxicity of Cl_2 gas. Accordingly, compound **56** was reacted with trichloroisocyanuric acid in acetonitrile to obtain dichlorotetrazine **40** in 68% yield (Scheme 3.22). Finally, dichlorotetrazine was purified by sublimation. Dichlorotetrazine sublimated at 55 ºC under vacuum and the sublimated compound was collected at the cold finger kept at -20 ºC. In the ^{13}C spectrum of dichlorotetrazine, only one resonance at δ 168.22 ppm confirmed the formation of dichlorotetrazine.

Most of the biomolecules such as proteins contain cysteines; the side chain thiol functionality of cysteine is generally utilized for the labelling of various tags to proteins. Similarly the hydroxyl group of serine and threonine are used to functionalize proteins. To test our hypothesis, we chose carbohydrate molecules as biomolecules and introduced thiol and hydroxyl functional groups to carbohydrate derivatives which serve as model substrates to demonstrate our hypothesis. Accordingly, we have synthesized thiol and hydroxyl containing carbohydrate derivatives.

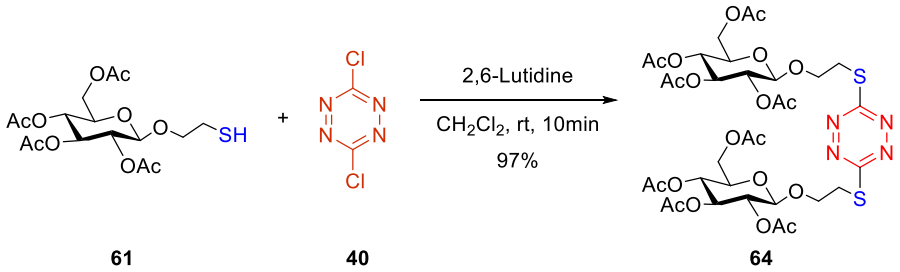
For this purpose, α-D-glucose **57** was converted to per-*O*-acetyl glucopyranose **58** by treating with acetic anhydride and pyridine. Compound **58** was then reacted with 2-bromoethanol in presence of $BF_3{\cdot}Et_2O$ in dichloromethane to form 2-bromoethyl glucopyranoside **59** in good yield.



Scheme 3.23 Synthesis of thioethyl glycosides

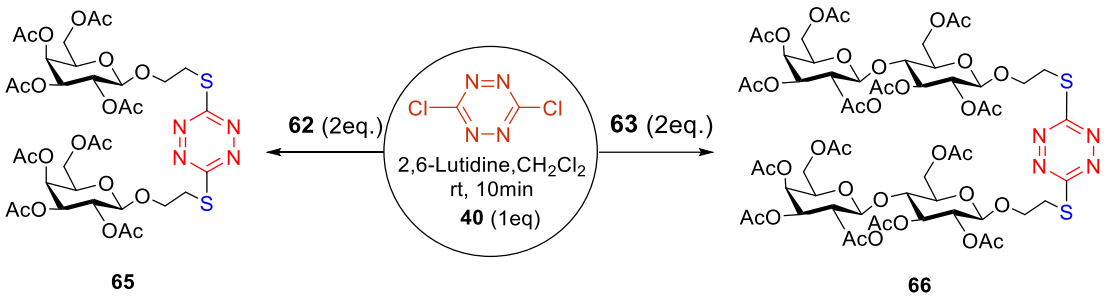
Further compound **59** was converted to thioisocyanate **60** using KSCN in DMF at 70 ºC. Thiocyanate **60** was reduced with Zn/AcOH at 70 ^{0}C to afford 2-thioethyl glucopyranoside **61** in 87% yield (Scheme 3.23). The same procedure was employed for the synthesis of 2- thioethyl galactoside **62** and 2-thioethyl lactoside **63** from α-D-galactose and α-D-lactose respectively.

To begin our investigation, 2 equivalents of 2-thioethyl glucopyranoside **61** was reacted with one equivalent of dichlorotetrazine **40** in the presence of hindered organic base such as 2,6-lutidine using dichloromethane as a solvent. The progress of the reaction was monitored by conventional TLC and the reaction was noticed to complete in 10min producing the binary conjugate **64** in excellent yield (Scheme 3.24).



Scheme 3.24 Synthesis of binaryconjugate

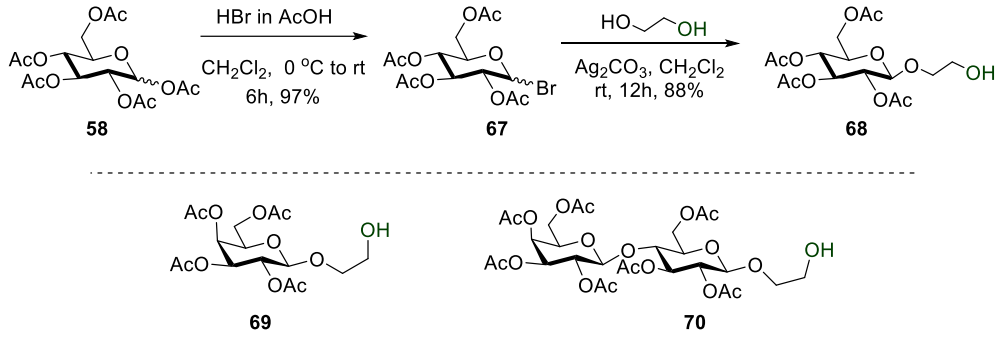
Next, we have examined homodimeric conjugation reaction of 2-thioethyl galactoside **62** and 2-thioethyl lactoside **63**. Glycosides **62** and **63** were treated with dichlorotetrazine **40** under aforementioned conditions to afford *pseudo*- di- (**65**) and tetra- (**66**) saccharide binary conjugates in 98% and 95% yields respectively (Scheme 3.25).



Scheme 3.25 Synthesis of binary conjugates

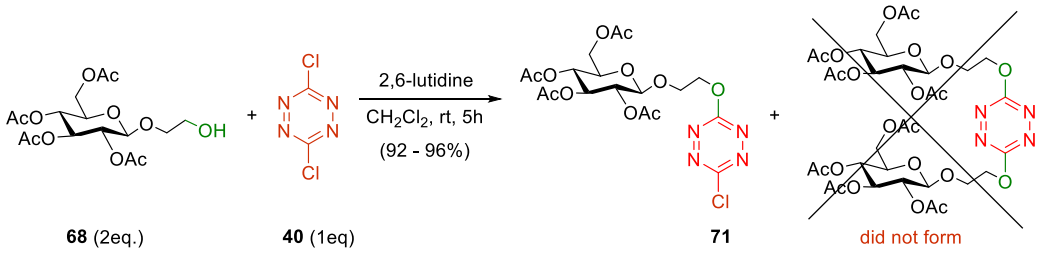
Now, we focussed our attention on the synthesis of hydroxyl functional carbohydrate derivatives. The per-*O*-acetyl glucopyranoside **58** was treated with HBr in acetic acid to afford glucosyl bromide **67**. The resulting bromide **67** was treated with ethylene glycol and silver carbonate in dichloromethane to form 2-hydroxyethyl glucoside **68** in good yield. Similar protocol was enabled the synthesis of 2-hydroxyethyl galactoside **69** and 2-hydroxy ethyl lactoside **70** (Scheme 3.26).

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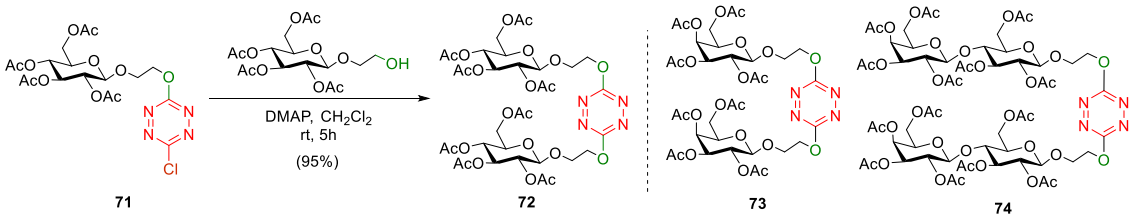
Scheme 3.26 Synthesis of hydroxyethyl glycosides

Next, the binary conjugation strategy was examined on hydroxyl containing sugar derivatives. Compound **68** (2 eq.) was treated with dichlorotetrazine (1eq.) in presence of 2,6-lutidine in dichloromethane. Even after several hours, the reaction did not proceed to form the binary conjugate, but the conjugation stopped at the mono conjugation level (Scheme 3.27).



Scheme 3.27 Synthesis of tetrazine mono-conjugate

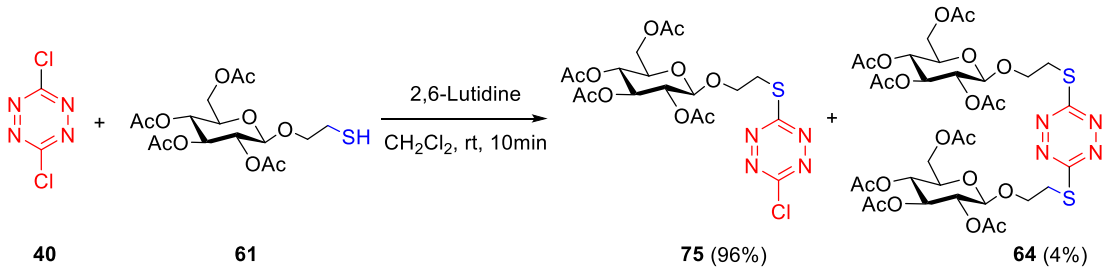
Reaction mixture was heated to reflux in order to drive the reaction; but, without any success. However, addition of one molar equivalent of nucleophilic DMAP led to the formation of binary conjugate **72** in near quantitative yield. Similar protocol was followed for the synthesis of binary conjugates **73** and **74** in 92 and 90% yields from 2-hydroxyethyl galactoside **69** and 2-hydroxyethyl lactoside **70** respectively (Scheme 3.28).



Scheme 3.28 Synthesis of binaryconjugates

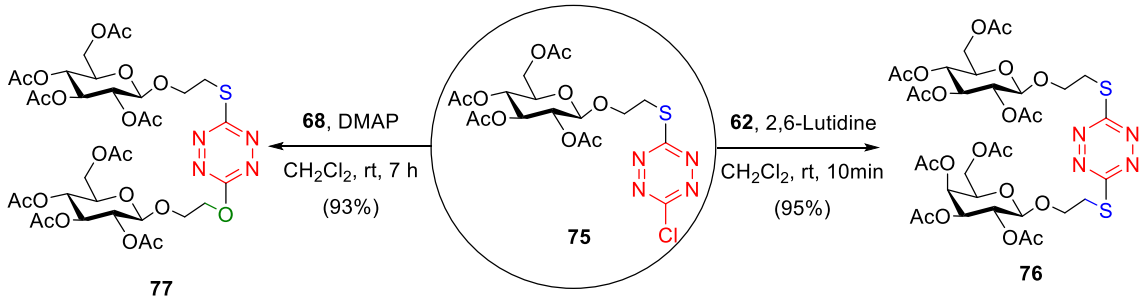
Successful identification of conditions for homo binary conjugation encouraged us to investigate the *s*-tetrazine chemistry for the synthesis of hetero-binary conjugates. There

can be two types of binary conjugates *viz.* homo- and hetero- linked conjugates. Heteroconjugates can differ by the point of attachment or the type of molecule that is participating in the conjugation step. The conjugation can be arrested at the mono conjugation level and thus formed mono conjugate can be subjected to the binary conjugation with different sugar derivatives to result in heterodimeric conjugates. Accordingly, 2-thioethyl glucoside **61** (1eq.) was treated with dichlorotetrazine (1eq.) in dichloromethane by the slow addition of 2,6-lutidine to form the mono-conjugated product **75** in high yield (96%) along with a minor formation of binary conjugate **64** (Scheme 3.29). Compounds **75** and **64** could be easily separated by simple silica gel column chromatography.



Scheme 3.29 Synthesis of mono-conjugate

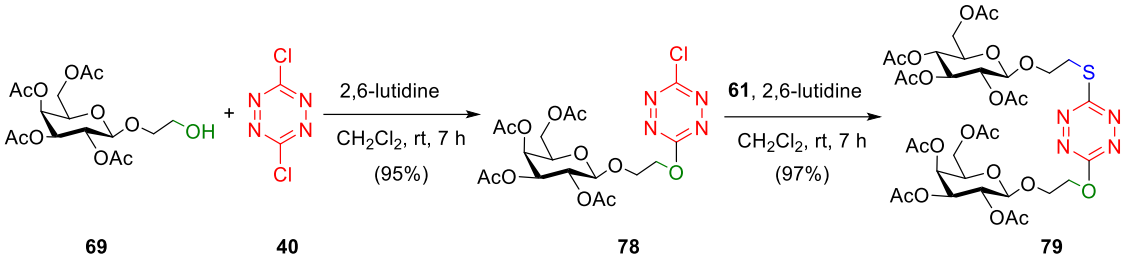
The mono-conjugate **75** was again subjected to further conjugation reaction with 2-thioethyl galactoside **62** under 2,6-lutidine condition to form tetrazine-linked gluco-galacto hybrid binary conjugate **76** in 95% yield; and was also reacted with 2-hydroxyethyl glucoside **68** under DMAP condition to afford tetrazine-linked gluco-gluco hybrid binary conjugate **77** in 93% yield (Scheme 3.30).



Scheme 3.30 Synthesis of heterodimeric glycoconjugates

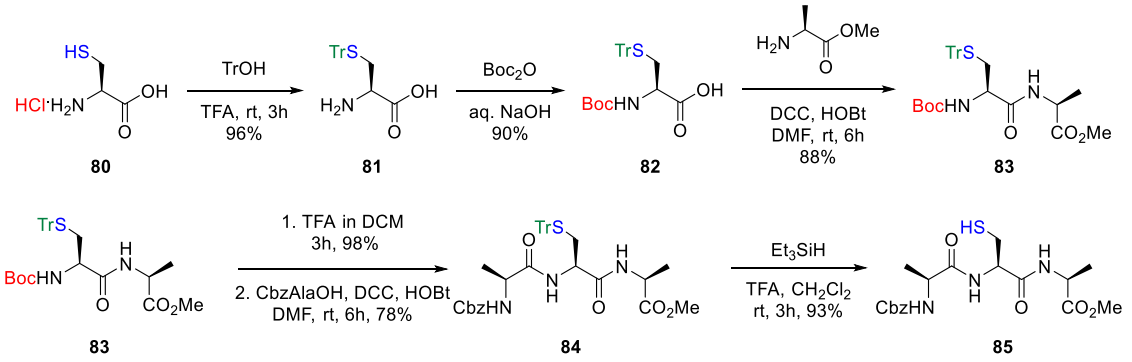
Next, we had tuned the heterodimerization protocol by using only mild 2,6-lutidine. The mono-conjugate of 2-hydroxyethyl galactoside **78** was prepared using 2,6-lutine which was then treated with 2-thioethyl glucoside **62** to form gluco-galacto hybrid conjugate **79** in 97% yield. It is interesting to note that heterodimers **76** and **79** differ by the point of

attachment, dimers **77** and **79** differ by the type of sugar and the dimers **76** and **77** have the difference of both type of sugars and the point of attachment as well (Scheme 3.31).



Scheme 3.31 Synthesis of heterodimeric glycoconjugates

Successful synthesis of homo and heterodimeric tetrazine conjugates of sugar derivatives encouraged us to utilize this protocol to synthesize peptide containing conjugates. A model tripeptide (Cbz.Ala.Cys.Ala.OMe), containing cysteine along with alanine on both sides, was chosen. Accordingly, cysteine hydrochloride **80** was reacted trityl alcohol in trifloroacetic acid to obtain trityl protected cysteine **81**. The primary amine group on **81** was protected as NH-Boc using Boc_2O in aqueous NaOH to obtain trityl and Boc protected cysteine **82**. The protected compound **82** was coupled with Ala-OMe using DCC and HOBt conditions in DMF to afford dipeptide **83** in 88% yield. The dipeptide **83** was treated with TFA in dichloromethane to get free amine dipeptide in excellent yield.

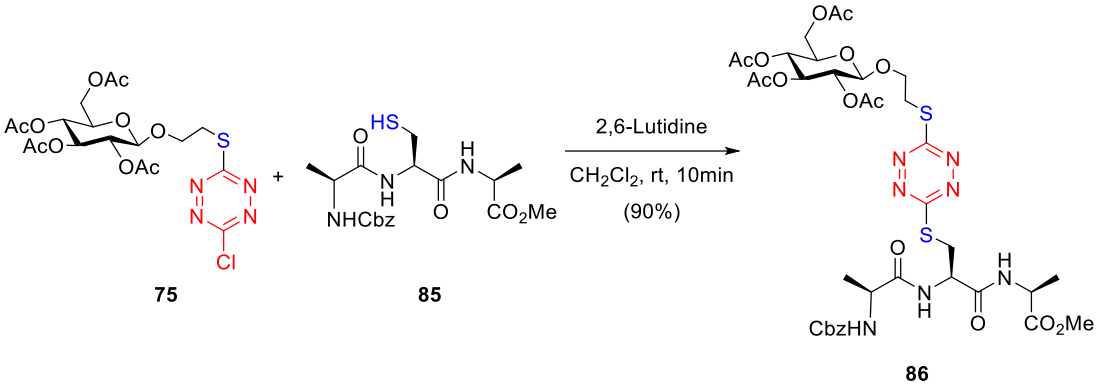


Scheme 3.32 Synthesis of a model tripeptide

The free amine dipeptide **83** was coupled with Cbz-Ala-OH using DCC and HOBt conditions in DMF to obtain a model S-protected tripeptide **84** in 78% yield. The trityl protecting group of the compound **84** was selectively deprotected using TFA and Et_3SiH condition in dichloromethane to obtain tripeptide **85** in 93% yield. The addition of Et_3SiH is necessary to deprotect the trityl group (Scheme 3.32).

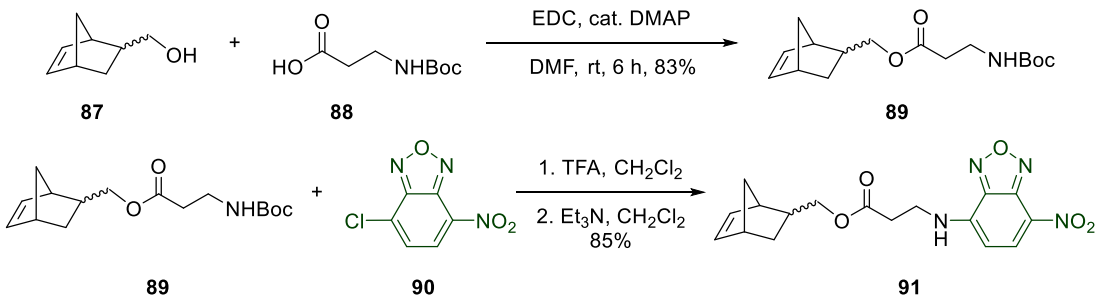
The successful synthesis of Cbz.Ala.Cys.Ala.OMe tripeptide **85** allowed us to examine the binary conjugation reaction of it with mono-conjugated glucosyl derivative **75** in

dichloromethane in the presence of 2,6-lutidine at 25 °C for 10 minutes resulting a sugar-peptide tetrazine binary conjugate **86** (SPC) in 90% yield (Scheme 3.33).



Scheme 3.33 Synthesis of sugar-peptide binary conjugates (SPC)

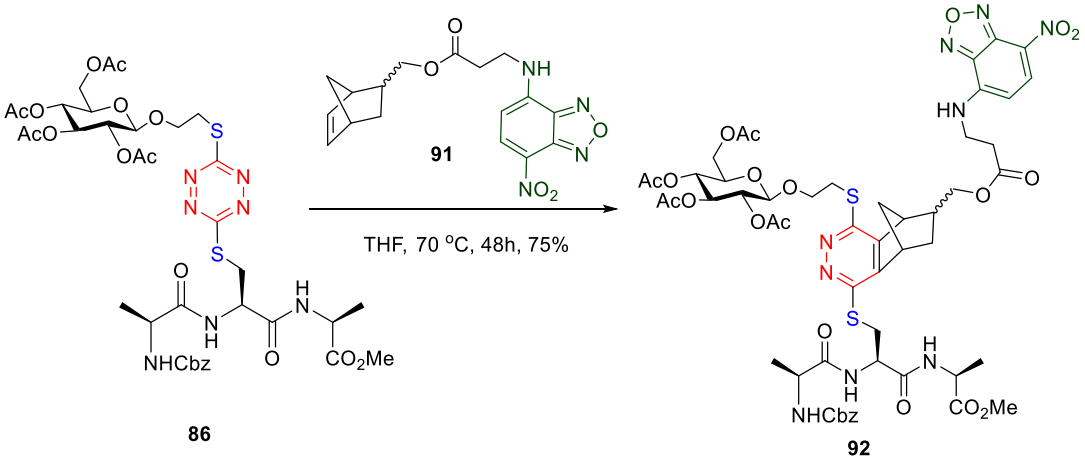
SPC serves as a model substrate for the iEDDA reaction to perform ternary conjugation. The first reports, where norbornene and *trans*-cyclooctyne biorthogonal reactions with tetrazine *via* iEDDA manner were reported by Fox and Hilderbrand respectively at the same time. Between these two molecules, commercially available endo, exo mixture of norbornene alcohol **87** was selected as a third reacting partner. The ternary conjugation reaction can be monitored easily by placing a fluoro tag to the third reacting partner. Among the available fluorescent tags, NBD-Chloride was chosen. Accordingly, norbornene alcohol **87** was coupled to Boc protected β-alanine **88** using EDCI, cat. DMAP condition in DMF to obtain the Boc-protected compound **89**. tert-Butoxycarbomate was deprotected using TFA in dichloromethane to obtain free amine of the norbornene compound which was immediately coupled to the NBD-Cl to afford fluorescently labelled strained alkene **91** in good yield (Scheme 3.34).



Scheme 3.34 Synthesis of fluorescent tag containing norbornene

Ternary conjugation reaction was performed by taking binary SPC conjugate **86** (2eq.) and norbornene **91** (1eq.) in THF at room temperature. The bulky nature of the binary conjugate made the reaction to occur very slow and thus heated the reaction mixture to

70 ºC for 48h to obtain the ternary conjugate as a mixture of endo, exo-isomers (Scheme 3.35).



Scheme 3.35 iEDDA reaction for the synthesis of ternary conjugates

Formation of the exo and endo products can be explained since the Diels-Alder reaction between **86** and **91** can occur in an *exo*- or *endo*- manner; subsequently, undergoes retro-Diels Alder reaction to release nitrogen and further gets oxidized to give an aromatic nucleus. The crude reaction mixture was partially purified to separate starting materials and other minor impurities from the mixture of ternary conjugates and then the mixture was then purified by UPLC coupled MS using C_{18} column. The mixture was supposed to have four compounds as reported by Hilderbrand. But the mixture gave only three fractions (1:2:1) containing ternary conjugates and further attempts to resolve into four fractions failed (Figure 3.36).

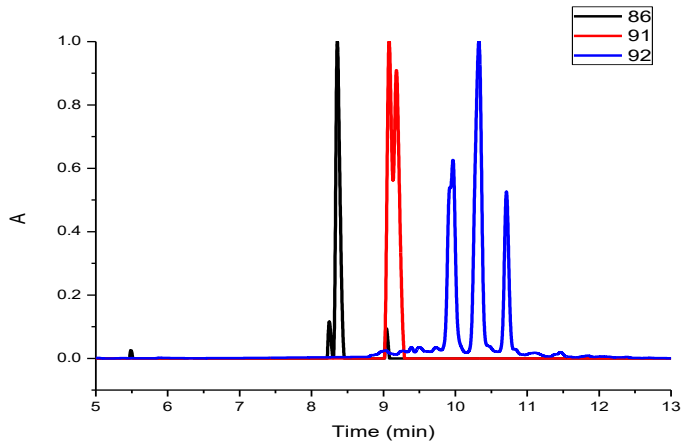
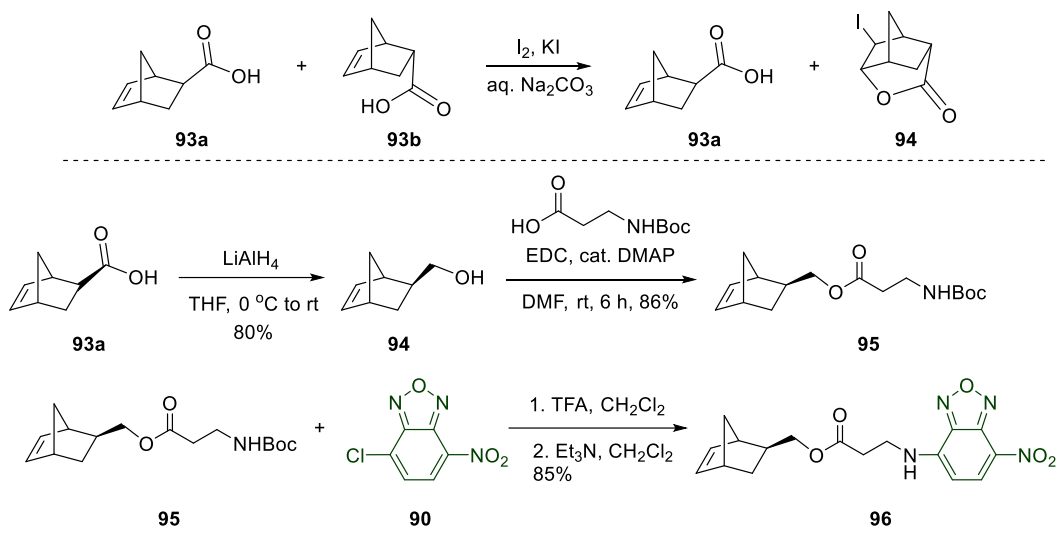


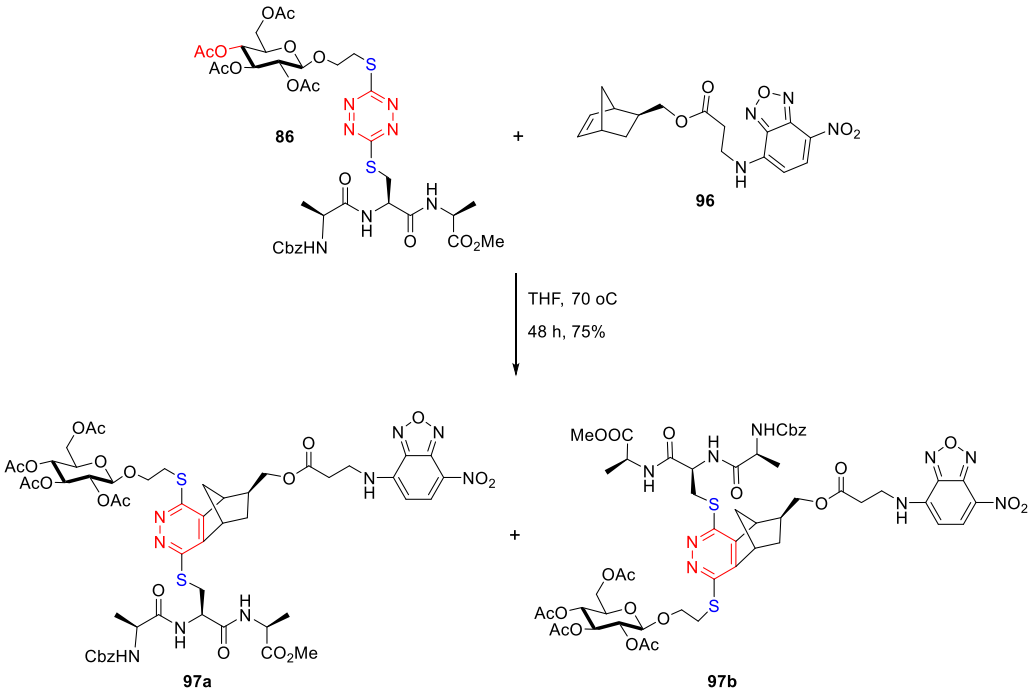
Figure 3.36 Normalized overlay LC traces spectra of **86**, **91** and **92**

In order to simplify this reaction we have separated *endo* and *exo* norbornene. For this purpose, commercially available endo/exo mixture of norbornene carboxylic acids (**93a** and **93b**) was separated by an iodolactonization reaction. The acid mixture was dissolved in a solution of sodium bicarbonate, and a solution of iodine and potassium iodide was added dropwise until a dark brown colour persisted which indicates complete conversion of endo-norbornene carboxylic acid to its idolactone **94**. The resulting mixture was extracted with diethyl ether and purified on column chromatography to afford exo-norbornene acid as a pale yellow crystalline solid. In continuation, the exo-norbornene carboxylic acid **93a** was converted to exo-norbornene alcohol **94** and tagged with NBD to afford fluorescenty labelled chirally homogenous NBD compound **96** in 85% yield (Scheme 3.37).



Scheme 3.37 Synthesis of fluorescent labeled exo-narbornene alkene

Now, the binary SPC conjugate **86** (2eq.) and pure exo-norbornene **96** (1eq.) in refluxing THF underwent iEDDA reaction in 48h giving 75% yield of the ternary conjugate (Scheme 3.38).



Scheme 3.38 Ternary conjugation reaction

Two fractions were obtained in the analytical UPLC-MS analysis as anticipated. Further purification of the reaction mixture on preparative HPLC using C_{18} column gave two fractions containing ternary conjugates (Figure 3.39).

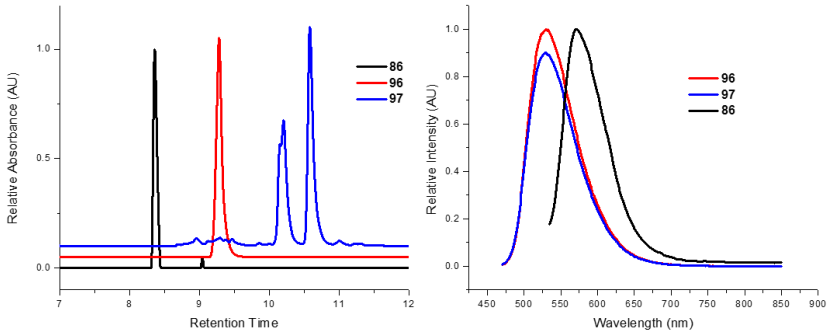
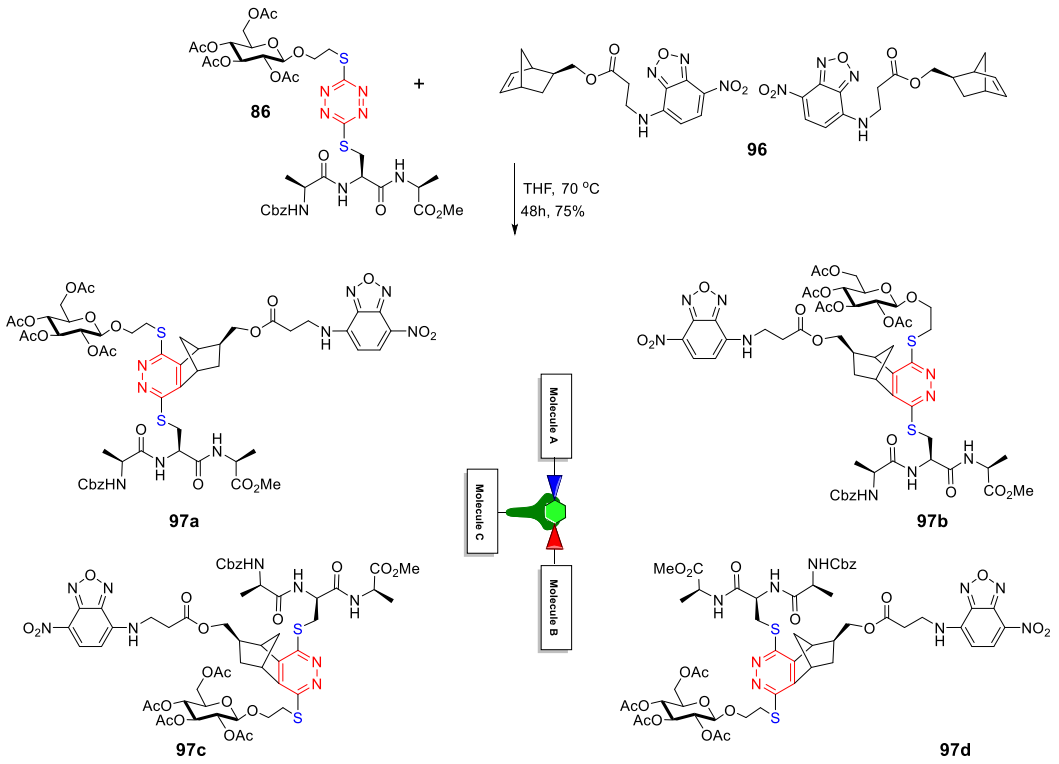


Figure 3.39 Normalized overlay LC traces and fluorescence emission spectra of 86 (λ_{exc} = 412 nm), 96 (λ_{exc} = 460 nm) and **97** (λ_{exc} = 460 nm).

Separated HPLC fractions were subjected to exhaustive NMR analysis, in which we have observed that each fraction contains two compounds. Then we found that we have used enantiomeric mixture of **96** that made the binary conjugation to happen in a non regiospecific manner resulting in ternary conjugates as a mixture of four compounds (Scheme 3.40).

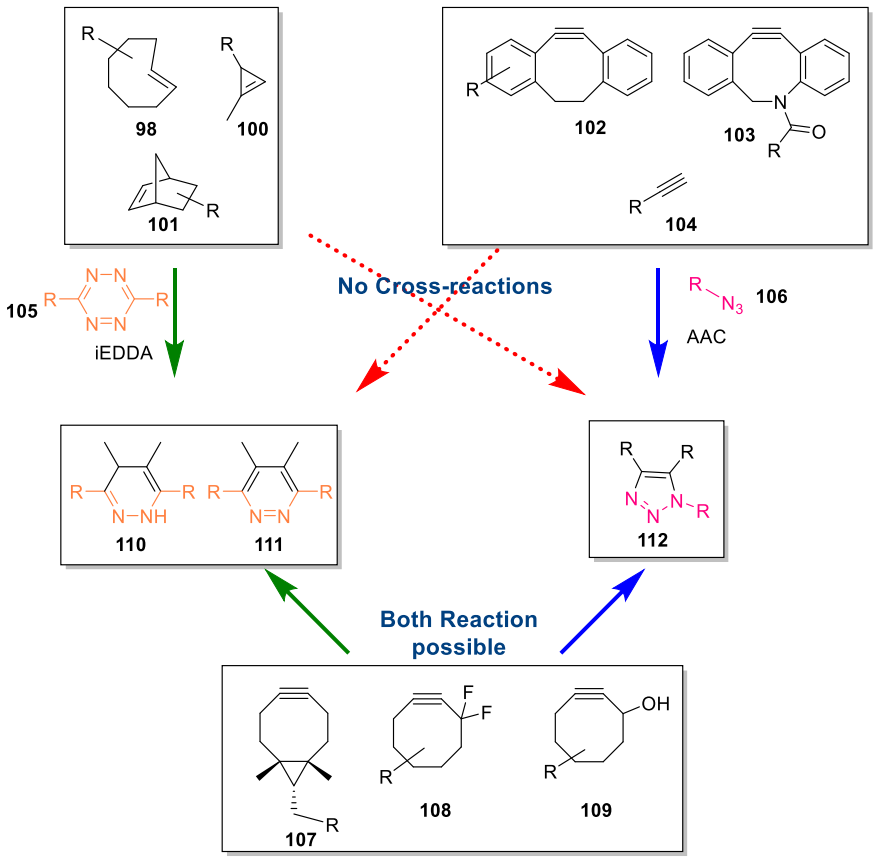


Scheme 3.40 Ternary conjugation with exo norbornene

The outcome of the ternary conjugation reaction is confirmed by HRMS and NMR analysis. In the NMR spectra, resonances corresponding to all the three conjugating partners were observed and the molecular weights of those fractions were found to be identical [calcd m/z $C_{53}H_{63}N_9O_{21}S_2$: 1225.3580 and found: 1226.3663 (M^++H)]. Furthermore, ternary conjugates **97** showed fluorescence emission λ_{max} at 528nm matching with that of the NBD-ester **96**. It is appealing to observe that the fluorescence emission λ_{max} 572nm corresponding to the tetrazine moiety of binary conjugate **86** disappeared and thereby confirmed the formation of ternary conjugate **97** (Scheme 3.39).

The choice of Alkyne\Alkene for ternary conjugation

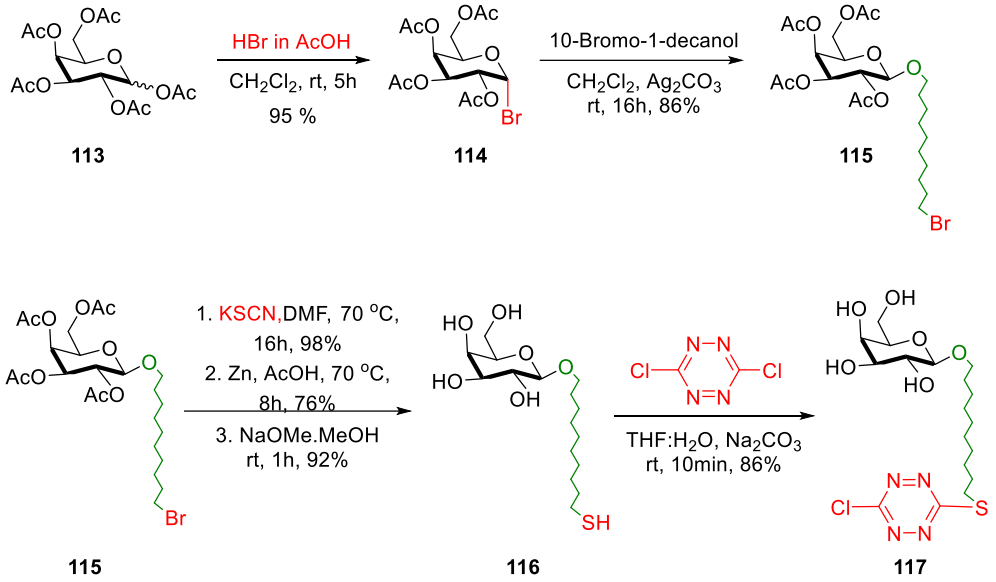
The click reaction with *trans*-cyclooctyne and norbornene usually results in a mixture of diastereo and regioisomers that are difficult to separate by normal chromatographic techniques. A recent review[46] by Knall reported the relative reactivity of selected alkynes and alkenes with azides and tetrazines (Scheme 3.42) which encouraged us to choose symmetrical alkyne **107,** which should react with symmetrical tetrazine giving only a single product.



Scheme 3.42 Suitable pairs for mutually orthogonal azide–alkyne/iEDDA click chemistry[46]

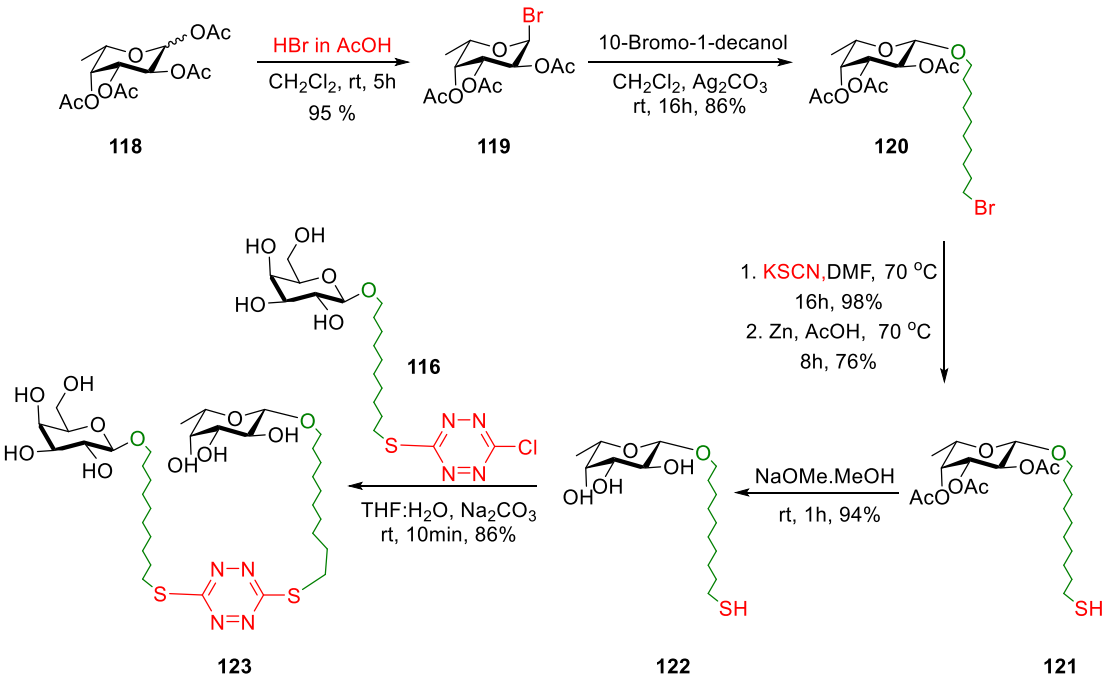
Tetrazine Chemistry for the synthesis of Ternary Glycoconjugates:

The per-*O*-acetyl galactopyranoside **113** was treated with HBr in acetic acid to obtain galactosyl bromide **114** which was then reacted with 10-bromo-decanol using silver carbonate as a glycosyl promoter in dichloromethane to form bromide **115** in 86% yield**.** Bromide **115** was converted to thiocyanate using KSCN in DMF at 70 ºC; further, thiocyanate galactoside was reduced to mercaptan by Zn in acetic acid at 70 ºC which upon Zemplèn deacetylation using NaOMe/MeOH afforded thiol containing galactosyl derivative **116**. Thiols react faster with dichlorotetrazine compare to hydroxyl groups. Mono conjugated tetrazine compounds are stable to mild bases and protic solvents. Taking advantage of the faster reactivity of thiol, thiol containing galactosyl derivative **116** was coupled with dichlorotetrazine **40** in THF/water mixture using Na_2CO_3 as base to form mono galactosyl conjugated product **117** in 86% yield (Scheme 3.43).



Scheme 3.43 Synthesis of tetrazine galactose mono-conjugate

After successful synthesis of monoconjugate **117**, the synthesis of fucosyl thiol derivative was considered. Commercially available L-fucose was converted to per-*O*-acetyl fucose by treating with Ac_2O in pyridine. Per-*O*-acetyl fucose **118** was then treated with HBr in AcOH to form bromide **119** that was treated with 10-bromodecanol in the presence of silver carbonate to obtain bromide **120** in 86% yield (Scheme 3.44).

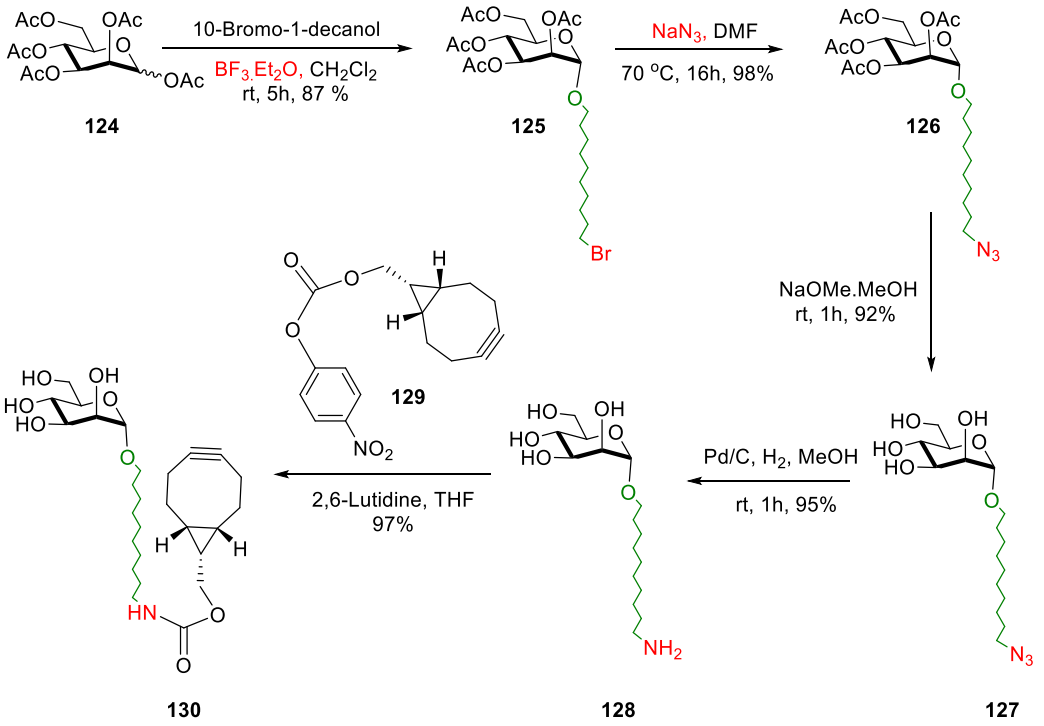


Scheme 3.44 Synthesis of Fuc*p*-Gal*p* binary conjugate

In continuation, bromide **120** was converted to thiocyanate compound using KSCN in DMF at 70 ºC. The resulted thiocyanate compound was converted to thiol linked fucosyl

derivative **121** by employing Zn in acetic acid conditions at 70 ºC. Finally, the deprotection of acetyl groups of compound **121** under Zemplèn conditions afforded fucosyl mercaptan **122** in 94% yield. The thiol containing fucosyl derivative **122** was selectively coupled with galactosyl tetrazine mono conjugate **117** in THF/water mixture in the presence of Na_2CO_3 to obtain Fuc*p*-Gal*p* binary conjugate **123** in 86% yield (Scheme 3.44).

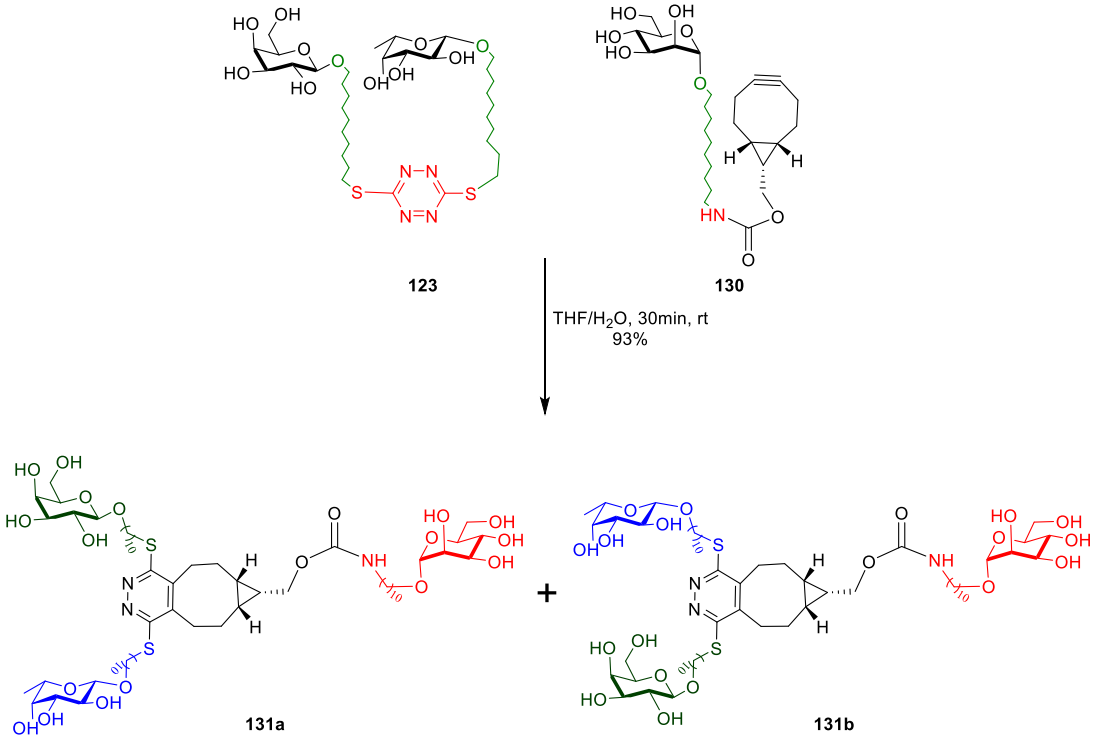
The successful synthesis of binary conjugate of **122** encouraged us to attached the third sugar partner that is mannosyl derivative containing reactive cyclooctyne. The per-*O*-acetyl mannose **124** was reacted with 10-bromodecanol in the presence of $BF_3{\cdot}Et_2O$ in dichloromethane to obtain mannose derivative **125** in 87% yield. Subsequently, compound **125** was converted to azide **126** by treating with NaN_3 in DMF at 90 ºC. The acetyl protecting groups of compound **126** were deprotected using NaOMe/MeOH to afford 10-azido decane linked mannosyl derivative **127** in 92% yield.



Scheme 3.45 Synthesis of octyne-mannose conjugate

The azide functionality was reduced to amine by hydrogen in the presence of cat. Pd/C in methanol to obtain free amine **128** in 95% yield. The free anime **128** was coupled to cyclooctyne **129** using 2,6-lutidine as a hindered base in THF at room temperature to get the desired mannosyl conjugated partner **130** for the ternary conjugation reaction (Scheme 3.45).

Finally, the Fuc*p*-Gal*p* binary conjugate **123** was subjected to the ternary conjugation with cyclooctyne containing mannosyl compound **130** in THF/H_2O at room temperature to afford the ternary conjugate in 93% yield (Scheme 3.46).



Scheme 3.46 Synthesis of ternary glycoconjugates

The resulting ternary conjugate was confirmed to be a single diastereomer by the HPLC and NMR analysis. In the ^{13}C spectrum of ternary conjugate **131**, resonances at δ 104.89, 104.72 and 101.46 ppm confirmed the presence of the three Gal*p*-, Fuc*p*- and Man*p*-sugars and resonance at δ 139.98 ppm confirmed presence formation of diazine nucleus.

In Conclusion, salient features of tetrazine nucleus were explored for the synthesis of ternary conjugates. Several binary homo- and hetero- dimeric glycoconjugates are prepared by the reactions of mercaptoethyl or hydroxyethyl saccharides. The ternary conjugation was performed under inverse electron demand Diels-Alder reaction on the tetrazine nucleus. Mild and aqueous mediated ternary conjugation protocol was identified. Selectively thiol functionality over hydroxyl was utilized to prepare mono and binary conjugates in the aqueous medium. The model ternary conjugation reaction was performed in the THF/H_2O system. And the lectin binding ability of galactosyl-fucosyl-

mannosyl ternary conjugate is currently under investigation. The method is modular and thus a suite of molecules can participate in ternary conjugation.

3.4 Experimental Section

Triaminoguanidine monohydrochloride[47]**(54)**: To a suspension of guanidine monohydrochloride (50 g, 523.39 mmol) in 1,4-dioxane (300 mL) was added 104 mL of 98% hydrazine monohydrate solution (2.09 mol). The reaction mixture was heated to reflux for 2 h during which a white precipitate formed. After cooling the reaction mixture to ambient temperature, the solid was collected by suction filtration, washed with 1,4-dioxane and dried in high vacuum overnight to afford 12.1 g of the title compound ii as a white solid (95%, m.p. 232-234 ^{o}C).

1,4-Dihydro-3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine[47] **(55):** To a slurry of **54** (70 g, 498 mmol) in 400 mL distilled water, was added dropwise acetylacetone (624.44 mmol) while stirring at room temperature over 30 min. The solution turned pale yellow and then a clear orange solution was observed, followed by precipitate formation. The reaction mixture was then heated to 70 ^{o}C for 4h and the resulting solid was filtered, washed with water and dried *in vacuo*, and recrystallised from ethanol to compound **55** (70%). ^{1}H NMR (399.78 MHz, CDCl3): δ 8.07 (s, 2H), 5.96 (s, 2H), 2.48 (s, 6H), 2.22 (s, 6H); ^{13}C NMR (100.53 MHz, CDCl3): δ 13.6, 13.6, 13.9, 13,9, 110.0, 110.0, 142.4, 142.4, 145.9, 145.9, 150.1, 150.1; MALDI (TOF): calcd. for $C_{12}H_{17}N_8^+$, [M+H$^+$]: 273.16; found: 273.30; m.p.148-150 ^{o}C.

3,6-Bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine[47] **(29)**: To a solution of compound **55** (45 g, 165.25 mmol) in 150 mL 1-methy-2-pyrrolidinone was stirred for 15 min. NO_2 gas was generated by dissolving 30 g of copper powder in 30 mL of conc.HNO_3. The gas was bubbled for 30 min. Ice cold water was added to the solution which was filtered and washed with cold water, dried under vacuum to obtain compound **29** as an orange-red solid (98%, m.p. 222 ^{o}C, MALDI (TOF): calcd. for $C_{12}H_{15}N_8^+$, [M+H$^+$]: 271.14; found: 271.56).

3,6-Dihydrazinyl-1,2,4,5-tetrazine[31] **(56):** To a slurry of compound iv (45 g, 166.5 mole) in CH_3CN (300 mL), was added hydrazine monohydrate (18.7 mL, 366.3 mole) drop wise at ambient temperature. After the addition was complete, the reaction mixture was refluxed for 20 min and then cooled to room temperature, filtered, and washed with CH_3CN to give compound **56** as a brown solid (85%, m.p. 158-160 ^{o}C). ^{1}H NMR (399.78 MHz, CDCl3): δ 8.37 (s, 2H), 4.24 (s, 4H); ^{13}C NMR (100.53 MHz, CDCl3): δ 163.4; MALDI (TOF): calcd. for $C_2H_7N_8^+$, [M+H$^+$]: 143.13; found: 143.26.

3,6-Dichloro-1,2,4,5-tetrazine[48] **(40).** A 300 mL CH_3CN solution of trichloro isocyanuric acid (65.4 g, 281.5 mmol) was added drop wise over 30 min to a slurry of compound **56** (20 g, 140.7 mmol) in CH_3CN (500 mL) at 0 ^oC. After the addition was completed, the reaction vessel was allowed to warm upto room temperature and stirred for additional 20 min. The resulting white precipitate was removed by filtration and the organic volatiles were removed *in vacuo* to afford crude 3,6-dichloro-*s*-tetrazine which was purified by sublimation at 55 ^oC under nitrogen atmosphere and orange crystals of 3,6-dichloro tetrazine **40** was collected from cold finger (68%). ^{13}C NMR (100.53 MHz, CDCl3): δ 168.2.

General Procedure for the synthesis of homodimers with sulphur linkage (64-66): To a solution of compound 2a (200 mg, 0.489 mmol) in CH_2Cl_2 (5 mL), 3,6-dichloro-1,2,4,5-tetrazine 1 (73 mg, 0.489 mmol) was added, stirred for one minute and then 2,6-lutidine (1 mL) was added drop wise under nitrogen atmosphere. Stirred at rt for 10 min and then solvent was evaporated, crude residue was purified by column chromatography to yield homodimer 4a. Same procedure was adopted for compounds 4b and 4c. All homodimers are found to be red fluorescent solids.

General Procedure for the synthesis of homodimers with oxygen linkage (72-74): To a solution of compound 5a (200 mg, 0.509 mmol) in CH_2Cl_2 (5 mL), 3,6-dichloro-1,2,4,5-tetrazine(77 mg, 0.510 mmol) was added. Subsequently, 2,6-lutidine (1 mL) was added, stirred for 30 min at room temperature and after complete consumption of 3,6-dichloro-1,2,4,5-tetrazine, DMAP (55 mg, 0.513 mmol) was added and stirred for 8h; volatiles were evaporated, crude was purified by silica gel column chromatography to yield homodimer 7a. Same procedure was followed for 7b and 7c and all are found to be pink coloured fluorescent solids (83-86%).

Synthesis of heterodimers (76, 77 &79): Binary conjugation for homodimers are arrested at the mono-conjugation level (e.g. **75**) and can be subjected to the heterodimerization. Accordingly, after the completion of conjugation reaction between **40** and 1 eq. of **61**, the chlorotetrazine linked glucoside 3 was isolated and subjected to the conjugation reaction with (i) another galactose-derived mercaptan **62** to form tetrazine-linked gluco-galacto hybrid molecule **76** (95%); (ii) compound **68** to give tetrazine-linked gluco-gluco hybrid molecule **79** (93%); (iii) galactose derivative **69** to result in gluco-galacto hybrid molecule **79** (90%).

Synthesis of Tripeptide 85:

***S*-Trityl-L-Cysteine[49] (81)**: Triphenylmethanol (7.4 g, 28.5 mmol) was added to a solution of cysteine chloro hydrate (5.0 g, 28.5 mmol) in TFA (50 mL) and the mixture was stirred for 3 h. After cooling to 0 ^{0}C, 4 N NaOH and diethyl ether (40 mL) were added until pH is adjusted to 4-5 and then 10% aqueous sodium acetate solution was added until pH 5-6. Resulting precipitate was filtered, washed with fresh Et_2O and dried to obtain the desired product (10.0 g, 96 % yield).

***N*-Boc-*S*-trityl-cysteine[49] (82):** To a solution of *S*-trityl-L-cysteine(8 g, 22.0 mmol) in 2 N NaOH (130 mL) was added Boc_2O (7.2 g, 33.0 mmol) and the reaction mixture was stirred at room temperature for 24 h. The aqueous solution was then acidified with conc. HCl until pH 2 and extracted with CH_2Cl_2, washed with brine, dried and concentrated under reduce pressure to give *N*-Boc-*S*-trityl-cysteine (9.5 g, 90 %).

***N*-Boc-*S*-trityl-cys-L-ala-OMe (83) [50]:** L-alanine methyl ester (2.3 g, 22.5 mmol) and *N*-Boc-*S*-trityl-cysteine (9.0 g, 18.8 mmol) were taken in 10 mL of DMF and stirred for 5 minutes at rt. Subsequently, HOBt (2.5 g, 18.77 mmol) and DCC (3.9 g, 18.77 mmol) were added and the reaction mixture was stirred for 6 h, extracted with ethyl acetate and washed with dilute HCl solution. Washed with saturated aq. Na_2CO_3 solution, brine and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography to give *N*-Boc-*S*-trityl-cys-L-ala-OMe (8.9 g, 88 %) as white solid.

***N*-Cbz-ala-*S*-trityl-cys-L-ala-OMe (84) [50]:** *N*-Boc-*S*-trityl-cys-L-ala-OMe (8.0 g, 14.6 mmol) was treated with 60 mL of 1:1 DCM:TFA solution and stirred for 3 h. The reaction mixture was concentrated, diluted with 200 mL of ethyl acetate and washed with saturated aq. Na_2CO_3 solution and brine, dried over anhydrous Na_2SO_4 and organic layers were concentrated *in vacuo*. The crude residue was directly dissolved in DMF and treated with a solution of *N*-Cbz-alanine (3.3 g, 14.6 mmol). HOBt (3.0 g, 14.6 mmol) and DCC (2.0 g, 14.6 mmol) were added, stirred for 6 h and extracted with ethyl acetate. Organic layers were washed with aq. HCl solution and then aq. Na_2CO_3 solution. Washed with brine solution and dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by silica gel column chromatography to give *N*-Cbz-ala-*S*-trityl-cys-L-ala-OMe (9.1 g, 78 %) as a white solid.

***N*-Cbz-ala-SH-cys-L-ala-OMe (85)[50]:** Triethylsilane (0.6 mL, 3.7 mmol) was added to a solution of *N*-Cbz-ala-*S*-trityl-cys-L-ala-OMe (2.0 g, 3.1 mmol) in CH_2Cl_2 (10 mL). Subsequently, TFA (3 mL) was added drop wise under nitrogen atmosphere and stirred

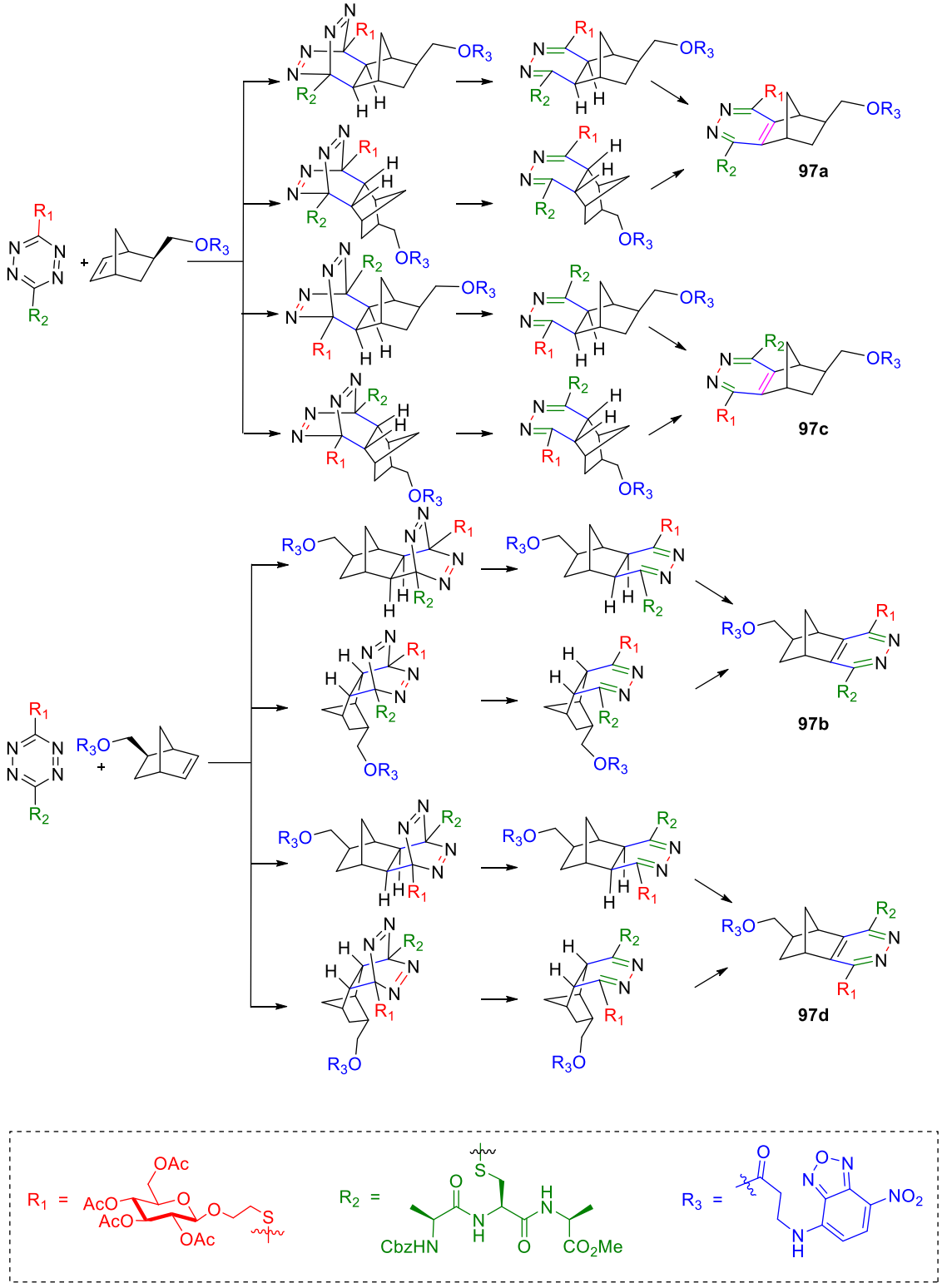
for for 30 min at room temperature. The reaction mixture was concentrated and the crude residue was purified by flash silica gel column chromatography to afford *N*-Cbz-ala-SH -cys-L-ala-OMe (1.2 g, 93 % yield) as a white solid.

Sugar-Peptide-Tetrazine-hybrid (86): 2, 6-lutidine (1 mL) was added to a mixture of compound **85** (0.5 g, 1.2 mmol) and compound **75** (0.6 g, 1.22 mmol) in CH_2Cl_2 (10 mL). After stirring for 10 min at room temperature, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography to afford compound **86** (1.0 g, 89 %) as a fluorescent red solid. $[\alpha]_D^{25}$ = -24.7 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3313, 2948, 2360, 2255, 1746, 1657, 1613, 1452,1371, 1221, 1167, 1041, 907, 724; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.23 – 1.30 (6 H, m), 1.90 (3 H, s), 1.93 (3 H, s), 1.95 (3 H, s), 1.98 (3 H, s), 3.35 – 3.45 (2 H, m), 3.60 (3 H, s), 3.61 – 3.77 (4 H, m), 4.00 – 4.20 (4 H, m), 4.21 – 4.32 (1 H, m), 4.41 (2 H, p, *J* 6.6), 4.50 (1 H, d, *J* 7.9), 4.88 – 5.03 (5 H, m), 5.13 (1 H, t, *J* 9.5), 6.06 (1 H, d, *J* 7.0), 7.22 (5 H, s), 7.53 (1 H, d, *J* 6.2), 7.72 (1 H, d, *J* 6.9); ^{13}C NMR (100.53 MHz, CDCl3): δ 17.6, 18.6, 20.4 (2C), 20.6, 20.6, 29.8, 48.1, 52.3, 61.7, 66.7, 67.5, 68.2, 70.8, 71.6, 72.4, 77.4, 100.6, 127.8, 127.9, 128.3 (2C), 136.1, 155.9, 169.0, 169.3, 169.4, 170.0, 170.6, 172.0, 172.2, 172.6, 173.1; HRMS (TOF): calcd. for $C_{36}H_{47}N_7NaO_{16}S_2^+$, [M+$Na^+$]: 920.2413; found: 920.2457. m. p. 88-89 ºC.

Synthesis of NBD ester of alkene (96): *exo*-5-Norbornen-2-ol[51] (1.3 g, 10.6 mmol) in DMF (3 mL), cat. DMAP and DCC (2.2 g, 10.6 mmol) were to a mixture of *N*-Boc-β-alanine (2.0 g, 10.6 mmol) and stirred for 6 h. The reaction mixture was diluted with water and extracted with ethyl acetate, washed with aq. Na_2CO_3 solution, brine and dried over anhydrous Na_2SO_4. Combined organic layers were concentrated *in vacuo*, purified by silica gel column chromatography to give exo-5-norbornen-2-ol ester of *N*-Boc-β-alanine as a colourless liquid. Boc deprotection was carried out using TFA in CH_2Cl_2 using above delineated procedure and the resulting residue was redissolved in CH_2Cl_2. NBD-Chloride (2.0 g, 10.0 mmol) and triethyamine (3 mL) were added to the above solution and stirred at room temperature. At the end of the reaction (~10 h), the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography to afford dark yellow solid compound **96** as a enantiomeric mixture (2.8 g, 76 %). ^{1}H NMR (399.78 MHz, CDCl3): δ 1.16 (1 H, dt, J 11.8, 3.9), 1.24 – 1.31 (2 H, m), 1.33 – 1.39 (1 H, m), 1.73 (1 H, tt, J 9.2, 5.3), 2.67 (1 H, s), 2.81 – 2.86 (3 H, m), 3.84 (2 H, q, J 6.0), 4.05 (1 H, dd, J 10.7, 9.3), 4.23 (1 H, dd, J 10.9, 6.5), 6.08 (2 H, p, J 5.6), 6.23 (1 H, d, J 8.6), 6.73 (1 H, s), 8.49 (1 H, d, J 8.6); ^{13}C NMR (100.53 MHz, CDCl3): δ 29.6, 33.0,

37.9, 39.4, 41.6, 43.7, 45.0, 69.6, 98.8, 124.2, 136.1, 136.6, 137.2, 143.7, 143.9, 144.4, 171.5. [HRMS (TOF): calcd. for $C_{17}H_{18}N_4NaO_5^+$, [M+Na^+]: 381.1169; found: 381.1194. **Click Reaction (97):** A mixture of sugar-peptide-tetrazine hybrid **86** (100 mg, 0.11 mmol) and compound **96** (20 mg, 0.06 mmol) was refluxed for 48 h in THF and then cooled to room temperature. THF was removed *in vacuo*, the crude residue was partially purified by silica gel column chromatography to obtain a mixture of compounds 14a-d (46 mg, 75%). Further purification of diastereomeric mixture was carried out by preparative HPLC using YMC-ODS-A-30x500mm C18 column.

Plausible mechanistic details for the formation of 97a-97d



2-mercaptoethyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside) **(61):** $[\alpha]_D^{25}$ = -8.2 ($CHCl_3$, c 1.00); IR(cm^{-1}): 2947, 2362, 1743, 1211, 1032, 753; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.47 (dd, J = 8.9, 7.9 Hz, 1H), 1.92 (3 H, s), 1.94 (3 H, s), 1.97 (3 H, s), 2.00 (3 H, s), 2.51 – 2.70 (2 H, m), 3.54 (1 H, ddd, *J* 10.1, 7.5, 6.2), 3.64 (1 H, ddd, *J* 10.0, 4.7, 2.4), 3.88 – 3.96 (1 H, m), 4.05 (1 H, dd, *J* 12.3, 2.3), 4.18 (1 H, dd, *J* 12.3, 4.7), 4.48 (1 H, d, *J* 8.0), 4.91 (1 H, dd, *J* 9.6, 8.0), 4.99 (1 H, t, *J* 9.7), 5.13 (1 H, t, *J* 9.5); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.5 (2C), 20.7 (2c), 24.2, 61.8, 68.2, 71.1, 71.6, 71.7, 72.6, 100.8, 169.3, 169.3, 170.2, 170.6; HRMS (TOF): calcd. for $C_{16}H_{24}NaO_{10}S^+$, [M+Na^+]: 431.0987; found: 431.0985. m.p. 85-86 ºC.

2-mercaptoethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside) **(62):** $[\alpha]_D^{25}$ = -8.2 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3023, 2974, 1744, 1215, 1045, 748; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.35 – 1.49 (m, 1H)1.85 (3 H, s), 1.92 (3 H, s), 1.94 (3 H, s), 2.02 (3 H, s), 2.47 – 2.67 (2 H, m), 3.50 (1 H, ddd, *J* 10.1, 7.5, 6.4), 3.83 (1 H, t, *J* 6.7), 3.86 – 3.93 (1 H, m), 3.96 – 4.06 (2 H, m), 4.41 (1 H, d, *J* 7.8), 4.90 (1 H, dd, *J* 10.5, 3.7), 5.06 (1 H, dd, *J* 10.4, 7.9), 5.25 (1 H, d, *J* 3.3); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.4, 20.5 (2C), 20.6, 24.1, 61.1, 66.8, 68.5, 70.5, 70.6, 71.4, 101.1, 169.2, 169.8, 170.0, 170.1; HRMS (TOF): calcd. for $C_{16}H_{24}NaO_{10}S^+$, [M+Na^+]: 431.0987; found: 431.0996. m.p. 82-83 ºC.

2-mercaptoethyl [2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside] **(63):** $[\alpha]_D^{25}$ = +12.6 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3419, 2984, 1645, 1217, 1046, 907, 646; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.47 – 1.54 (m, 1H), 1.94 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 2.57 – 2.76 (m, 2H), 2.95 – 3.10 (m, 2H), 3.59 (dt, J = 10.0, 6.8 Hz, 2H), 3.78 (t, J = 9.3 Hz, 1H), 3.85 (t, J = 6.7 Hz, 1H), 3.94 (dt, J = 11.4, 5.8 Hz, 1H), 4.09 (ddt, J = 12.1, 7.3, 4.8 Hz, 4H), 4.48 (dd, J = 9.7, 8.0 Hz, 3H), 4.84 – 4.98 (m, 2H), 5.05 – 5.12 (m, 1H), 5.18 (t, J = 9.3 Hz, 1H), 5.32 (d, J = 3.4 Hz, 1H); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6, 20.7 (3C), 20.8, 20.9, 20.9, 24.3, 42.3, 60.9, 62.0, 66.7, 69.2, 70.7, 71.0, 71.6, 71.7, 72.7, 76.3, 100.7, 101.1, 169.2, 169.8, 169.8, 170.1, 170.2, 170.4 (2C); HRMS (TOF): calcd. for $C_{28}H_{40}NaO_{18}S$+, [M+Na^+]: 719.1832; found: 719.1838. m. p. 62-63 ºC.

3,6-di((2,3,4,6-tetra-*O*-acetyl-β-D-glucoopyranosyl)oxyethyloxy)-1,2,4,5-tetrazine (**64**): $[\alpha]_D^{25}$ = +6.0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3022, 2884, 1746, 1370, 746; δ H (400 MHz, $CDCl_3$) 1.89 (6 H, s), 1.91 (6 H, s), 1.94 (6 H, s), 1.97 (6 H, s), 3.37 – 3.46 (4 H, m), 3.63 (2 H, ddd, J 10.0, 4.6, 2.4), 3.74 (2 H, dt, J 10.5, 6.7), 4.03 (2 H, dd, J 12.3, 2.3), 4.13 (4 H, ddd, J 17.9, 11.6, 5.0), 4.49 (2 H, d, J 8.0), 4.87 (2 H, dd, J 9.6, 8.1), 4.96 (2 H, t, J

9.7), 5.09 (2 H, t, J 9.5). δ C (101 MHz, $CDCl_3$) 20.4 (4C), 20.6 (2C), 20.6 (2C), 29.8 (2C), 61.6 (2C), 67.6 (2C), 68.1 (2C), 70.8 (2C), 71.7 (2C), 72.4 (2C), 100.7 (2C), 169.2 (2C), 169.2 (2C), 170.0 (2C), 170.4 (2C), 172.2 (2C). HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{20}S_2^+$, [M+$Na^+$]: 917.2044; found: 917.2050. m. p. 66-67 ºC.

3,6-di((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxyethyloxy)-1,2,4,5-tetrazine **(65):** $[\alpha]_D^{25}$ = +10.6 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3021, 1745, 1217, 1047, 744; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.96 (6 H, s), 2.02 (6 H, s), 2.05 (6 H, s), 2.13 (6 H, s), 3.45 – 3.58 (4 H, m), 3.81 (2 H, ddd, *J* 10.4, 7.7, 5.8), 3.90 (2 H, t, *J* 6.7), 4.12 (4 H, qd, *J* 11.3, 6.7), 4.22 (2 H, dt, *J* 10.6, 5.4), 4.51 (2 H, d, *J* 7.9), 4.99 (2 H, dd, *J* 10.5, 3.4), 5.19 (2 H, dd, *J* 10.5, 8.0), 5.37 (2 H, d, *J* 3.3).); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (2C), 20.7 (4C), 20.9 (2C), 30.1 (2C), 61.3 (2C), 67.0 (2C), 67.7 (2C), 68.5 (2C), 70.8 (2C), 70.9 (2C), 101.5 (2C), 169.6 (2C), 170.2 (2C), 170.3 (2C), 170.4 (2C), 172.4 (2C); HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{20}S_2^+$, [M+$Na^+$]: 917.2044; found: 917.2050. m. p. 67-68 ºC.

3,6-di((2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl)oxyethylmercapto)-1,2,4,5-tetrazine **(66):** $[\alpha]_D^{25}$ = +9.2 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3023, 2360, 1744, 1216, 1043, 745; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.99 (12 H, s), 2.00 (6 H, s), 2.01 (12 H, s), 2.06 (6 H, s, *J* 4.2), 2.10 (6 H, s, *J* 3.8), 3.43 – 3.48 (2 H, m), 3.57 (2 H, ddd, *J* 9.9, 4.8, 2.0), 3.80 (6 H, dt, *J* 18.8, 8.2), 3.99 – 4.15 (10 H, m), 4.47 (6 H, dd, *J* 15.4, 8.0), 4.88 (4 H, ddd, *J* 17.6, 10.0, 5.8), 5.05 (2 H, dd, *J* 10.4, 7.9), 5.14 (2 H, t, *J* 9.3), 5.29 (2 H, d, *J* 3.1); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.5 (2C), 20.7 (6C), 20.8 (4C), 20.9 (2C), 30.1 (2C), 60.8 (2C), 61.8 (2C), 66.6 (2C), 67.7 (2C), 69.1 (2C), 70.7 (2C), 71.0 (2C), 71.3 (2C), 72.6 (2C), 72.7 (2C), 76.1 (2C), 100.7 (2C), 101.1 (2C), 169.1 (2C), 169.7 (2C), 169.8 (2C), 170.1 (2C), 170.2 (2C), 170.4 (4C), 172.4 (2C); HRMS (TOF): calcd. for $C_{58}H_{78}N_4NaO_{36}S_2^+$, [M+$Na^+$]: 1493.3734; found: 1493.3719. m. p. 75-78 ºC.

2-hydroxyethyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside) **(68):** $[\alpha]_D^{25}$ = -11.60 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3557, 3022, 2950, 1746, 1370, 1214, 1032, 749; ^{1}H NMR (399.78 MHz, CDCl3): δ 2.00 (3 H, s), 2.02 (3 H, s), 2.05 (3 H, s), 2.08 (3 H, s), 2.51 (1 H, s), 3.63 – 3.79 (5 H, m), 3.83 (2 H, t, *J* 4.3), 4.18 (2 H, d, *J* 4.0), 4.54 (1 H, d, *J* 7.9), 4.97 – 5.08 (2 H, m), 5.21 (1 H, t, *J* 9.5); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.5 (2C), 20.6 (2C), 61.7, 61.9, 68.3, 71.2, 71.7, 72.6, 72.7, 101.2, 169.4, 169.5, 170.1, 170.6;

HRMS (TOF): calcd. for $C_{16}H_{24}NaO_{11}^+$, [M+Na^+]: 415.1215; found: 415.1211. m. p. 106-107 ºC.

2-hydroxyethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside) **(69):** $[\alpha]_D^{25}$ = -1.4 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 2944, 2256, 1744, 1217, 1047, 908, 724; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.89 (3 H, s), 1.96 (3 H, s), 1.98 (3 H, s), 2.06 (3 H, s), 2.78 (1 H, s), 3.62 (2 H, s), 3.68 – 3.82 (2 H, m), 3.89 (1 H, t, *J* 6.6), 4.06 (2 H, d, *J* 6.6), 4.44 (1 H, d, *J* 7.9), 4.94 (1 H, dd, *J* 10.5, 3.4), 5.11 (1 H, dd, *J* 10.4, 7.9), 5.30 (1 H, d, *J* 3.3); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.5, 20.5 (2C), 20.7, 61.5, 61.7, 67.0, 68.8, 70.7, 70.7, 72.8, 101.7, 169.7, 170.0, 170.2, 170.4; HRMS (TOF): calcd. for $C_{16}H_{24}NaO_{11}^+$, [M+Na^+]: 415.1215; found: 415.1211. m. p. 84-86 ºC.

2-hydroxyethyl (2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside) **(70):** $[\alpha]_D^{25}$ = -8.0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3022, 2972, 1745, 1216, 1045, 146; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.95 (3 H, s), 2.02 – 2.04 (9 H, m), 2.04 (3 H, s), 2.11 (3 H, s), 2.13 (3 H, s), 2.61 (1 H, s), 3.66 (3 H, dq, *J* 7.9, 5.2, 4.0), 3.72 – 3.80 (2 H, m), 3.81 – 3.89 (2 H, m), 4.01 – 4.15 (3 H, m), 4.46 – 4.55 (3 H, m), 4.92 (2 H, ddd, *J* 17.5, 9.9, 5.7), 5.09 (1 H, dd, *J* 10.3, 7.9), 5.19 (1 H, t, *J* 9.4), 5.33 (1 H, d, *J* 3.3); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6, 20.8 (3C), 20.9 (3C), 60.9, 62.1, 66.7, 69.1, 70.8, 71.0, 71.7, 72.7, 72.8, 73.6, 76.5, 101.2, 101.4, 169.2, 169.8, 169.9, 170.2, 170.3, 170.5, 170.5; HRMS (TOF): calcd. for $C_{28}H_{40}NaO_{19}^+$, [M+Na^+]: 703.2060; found: 703.2076. m. p. 65- 66 ºC.

(3-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethyloxy)-6-chloro)-1,2,4,5-tetrazine **(71):** $[\alpha]_D^{25}$ = -4.8 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 2948, 2361, 2256, 1747, 1217, 1033, 907, 724; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.97 (3 H, s), 1.99 (3 H, s), 2.00 (3 H, s), 2.06 (3 H, s), 3.70 (1 H, ddd, *J* 10.0, 4.5, 2.3), 4.01 (1 H, ddd, *J* 11.0, 7.7, 2.8), 4.11 (1 H, dd, *J* 12.3, 2.2), 4.18 – 4.29 (2 H, m), 4.59 (1 H, d, *J* 7.9), 4.75 (2 H, ddd, *J* 11.9, 4.6, 2.9), 4.87 – 4.97 (2 H, m), 5.04 (1 H, t, *J* 9.7), 5.18 (1 H, t, *J* 9.5); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.7 (3C), 20.8, 61.8, 67.0, 68.3, 68.9, 70.9, 72.1, 72.6, 100.9, 164.6, 166.7, 169.5, 169.5, 170.3, 170.7; HRMS (TOF): calcd. for $C_{18}H_{23}ClN_4NaO_{11}^+$, [M+$Na^+$]: 529.0948; found: 529.0952.

3,6-di ((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethyloxy)-1,2,4,5-tetrazine **(72):** $[\alpha]_D^{25}$ = -12.2 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3022, 1746, 1512, 1216, 1040, 745; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.97 (6 H, s), 1.99 (12 H, s), 2.06 (6 H, s), 3.70 (2 H, ddd, *J* 9.9, 4.5, 2.4), 3.99 (2 H, ddd, *J* 11.2, 7.8, 3.1), 4.10 (2 H, dd, *J* 12.3, 2.3), 4.16 – 4.28 (4

H, m), 4.61 (2 H, d, *J* 7.9), 4.63 – 4.71 (2 H, m), 4.77 (2 H, ddd, *J* 11.0, 7.7, 3.0), 4.96 (2 H, dd, *J* 9.6, 7.9), 5.05 (2 H, t, *J* 9.7), 5.18 (2 H, t, *J* 9.4); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (4C), 20.7 (2C), 20.8 (2C), 61.8 (2C), 67.4 (2C), 68.2 (2C), 68.3 (2C), 71.0 (2C), 72.0 (2C), 72.6 (2C), 101.0 (2C), 166.1 (2C), 169.5 (2C), 169.5 (2C), 170.3 (2C), 170.7 (2C); HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{22}^+$, [M+Na$^+$]: 885.2527; found: 885.2503.

3,6-di ((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxyethyloxy)-1,2,4,5-tetrazine **(73):** $[\alpha]_D^{25}$ = -7.8 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3021, 1747, 1218, 1042, 744; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.95 (6 H, s), 2.01 (6 H, s), 2.03 (6 H, s), 2.13 (6 H, s), 3.91 (2 H, t, *J* 6.7), 4.00 (2 H, ddd, *J* 11.1, 7.9, 3.0), 4.07 – 4.17 (4 H, m), 4.25 (2 H, dt, *J* 11.4, 3.7), 4.57 (2 H, d, *J* 8.0), 4.66 (2 H, dt, *J* 11.8, 3.6), 4.76 – 4.82 (2 H, m), 5.00 (2 H, dd, *J* 10.4, 3.4), 5.18 (2 H, dd, *J* 10.4, 7.9), 5.37 (2 H, d, *J* 3.4); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (2C), 20.7 (4C), 20.8 (2C), 53.5 (2C), 61.3 (2C), 67.0 (2C), 67.4 (2C), 68.2 (2C), 68.5 (2C), 70.8 (2C), 70.9 (2C), 101.5 (2C), 166.1 (2C), 169.7 (2C), 170.2 (2C), 170.3 (2C), 170.5 (2C); HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{22}^+$, [M+Na$^+$]: 885.2527; found: 885.2527. m. p. 60- 61 ºC.

3,6-di ((2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl)oxyethyloxy)-1,2,4,5-tetrazine **(74):** $[\alpha]_D^{25}$ = -11.8 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3619, 2360, 2257, 1748, 1170, 1050, 904, 721; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.93 (6 H, s), 1.98 (6 H, s), 2.00 (6 H, s), 2.01 (6 H, s), 2.02 (6 H, s), 2.08 (6 H, s), 2.11 (6 H, s), 3.60 (2 H, ddd, *J* 9.8, 4.8, 2.0), 3.77 (2 H, t, *J* 9.5), 3.84 (2 H, t, *J* 7.1), 3.92 – 3.99 (2 H, m), 4.01 – 4.12 (6 H, m), 4.19 (2 H, ddd, *J* 11.4, 4.7, 3.2), 4.42 – 4.49 (4 H, m), 4.56 (2 H, d, *J* 7.9), 4.63 (2 H, ddd, *J* 11.6, 4.6, 3.2), 4.74 (2 H, ddd, *J* 10.5, 7.4, 3.0), 4.85 (2 H, dd, *J* 9.5, 7.9), 4.92 (2 H, dd, *J* 10.4, 3.4), 5.06 (2 H, dd, *J* 10.4, 7.9), 5.16 (2 H, t, *J* 9.3), 5.31 (2 H, d, *J* 2.6); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (2C), 20.7 (8C), 20.8 (2C), 20.9 (2C), 60.8 (2C), 61.8 (2C), 66.6 (2C), 67.3 (2C), 68.2 (2C), 69.1 (2C), 70.7 (2C), 71.0 (2C), 71.4 (2C), 72.6 (2C), 72.8 (2C), 76.1 (2C), 100.7 (2C), 101.1 (2C), 166.1 (2C), 169.1 (2C), 169.8 (4C), 170.1 (2C), 170.2 (2C), 170.4 (4C); HRMS (TOF): calcd. for $C_{58}H_{78}N_4NaO_{38}^+$, [M+Na$^+$]: 1461.4191; found: 1461.4177. m. p. 105-107 ºC.

(3-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethylmercapto)-6-chloro)-1,2,4,5-tetrazine **(75):** $[\alpha]_D^{25}$ = +6.4 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3023, 1747, 1370, 1213, 1036, 746; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.95 (3 H, d, *J* 1.2), 1.97 (3 H, d, *J* 1.1), 1.99 (3 H, d, *J* 1.2), 2.03 (3 H, d, *J* 1.1), 3.47 – 3.61 (2 H, m), 3.67 (1 H, ddd, *J* 9.9, 4.4, 2.3), 3.79

(1 H, ddd, *J* 10.3, 7.6, 5.5), 4.09 (1 H, dd, *J* 12.3, 2.2), 4.15 – 4.23 (2 H, m), 4.51 (1 H, d, *J* 8.1), 4.91 (1 H, ddd, *J* 9.5, 8.2, 1.3), 4.98 – 5.05 (1 H, m), 5.14 (1 H, td, *J* 9.6, 1.2); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (2C), 20.7, 20.8, 30.4, 61.7, 67.2, 68.2, 70.8, 72.0, 72.5, 100.8, 165.9, 169.4 (2C), 170.2, 170.7, 175.4; HRMS (TOF): calcd. for $C_{18}H_{23}ClN_4NaO_{10}S^+$, [M+Na$^+$]: 545.0720; found: 545.0726. m. p. 155- 166 ºC.

(3-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethylmercapto)-6-((2,3,4,6-tetra-*O*-acetyl-β-D-glalactopyranosyl)oxyethylmercapto))-1,2,4,5-tetrazine **(76):** $[\alpha]_D^{25}$ = +7.2 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3744, 3022, 1746, 1512, 1216, 1040, 745; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.94 (3 H, s), 1.96 (3 H, s), 1.98 (3 H, s), 2.00 (3 H, s), 2.02 (3 H, s), 2.04 (3 H, s), 2.04 (3 H, s), 2.12 (3 H, s), 3.42 – 3.56 (4 H, m), 3.67 (1 H, ddd, *J* 10.0, 4.6, 2.4), 3.79 (2 H, ddd, *J* 9.1, 7.7, 5.5), 3.89 (1 H, td, *J* 6.6, 0.6), 4.05 – 4.25 (6 H, m), 4.52 (2 H, dd, *J* 14.0, 8.0), 4.93 – 5.07 (3 H, m), 5.13 – 5.19 (2 H, m), 5.35 (1 H, d, *J* 2.8); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.6 (4C), 20.7, 20.8, 20.8, 20.9, 30.0, 30.1, 61.3, 61.8, 67.0, 67.7 (2C), 68.3, 68.5, 70.8, 70.8, 71.0, 72.0, 72.6, 100.9, 101.5, 169.4 (2C), 169.6, 170.2, 170.3 (2C), 170.4, 170.7, 172.4, 172.4; HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{20}S_2^+$, [M+Na$^+$]: 917.2044; found: 917.2048. m. p. 57-58 ºC.

(3-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethylmercapto)-6-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethyloxy))-1,2,4,5-tetrazine **(77):** $[\alpha]_D^{25}$ = -7.9 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3022, 2966, 1746, 1371, 1214, 1032, 748; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.98 (6 H, s), 2.00 (3 H, s), 2.01 (6 H, s), 2.04 (3 H, s), 2.07 (3 H, s), 2.07 (3 H, s), 3.50 (2 H, dd, *J* 9.2, 3.7), 3.70 (2 H, tdd, *J* 10.7, 4.5, 2.3), 3.77 – 3.84 (1 H, m), 4.01 (1 H, ddd, *J* 11.1, 7.7, 2.9), 4.09 – 4.16 (2 H, m), 4.16 – 4.29 (4 H, m), 4.53 – 4.57 (1 H, m), 4.62 (1 H, d, *J* 8.0), 4.68 (1 H, ddd, *J* 11.8, 4.2, 3.1), 4.81 (1 H, ddd, *J* 10.8, 7.8, 2.8), 4.94 – 5.01 (2 H, m), 5.07 (2 H, td, *J* 9.7, 2.6), 5.19 (2 H, q, *J* 9.4); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.7 (6C), 20.8 (2C), 30.4, 61.8 (2C), 67.4, 67.9, 68.0, 68.3 (2C), 71.0 (2C), 71.9, 72.0, 72.6 (2C), 101.0 (2C), 166.1, 169.5 (4C), 169.6, 170.3 (2C), 170.8 (2C); HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{21}S$, [M+Na$^+$]: 901.2267; found: 901.2276.

(3-((2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyethylmercapto)-6-((2,3,4,6-tetra-*O*-acetyl-β-D-glalactopyranosyl)oxyethyloxy))-1,2,4,5-tetrazine **(79):** ^{1}H NMR (399.78 MHz, CDCl3): δ 1.95 (3 H, s), 1.97 (3 H, s), 1.99 (3 H, s), 2.01 (3 H, s), 2.02 (6 H, s), 2.05 (3 H, s), 2.12 (3 H, s), 3.49 (2 H, t, *J* 6.1), 3.67 (1 H, ddd, *J* 10.0, 4.5, 2.4), 3.80 (1 H, dt, *J* 10.4, 6.7), 3.88 – 3.94 (1 H, m), 3.99 (1 H, ddd, *J* 11.3, 7.9, 3.0), 4.05 – 4.29 (6 H, m), 4.55 (2 H, dd, *J* 11.5, 7.9), 4.67 (1 H, dt, *J* 11.8, 3.2), 4.80 (1 H, ddd, *J* 11.3, 7.9,

2.9), 4.92 – 5.08 (3 H, m), 5.12 – 5.21 (2 H, m), 5.37 (1 H, d, *J* 3.3); ^{13}C NMR (100.53 MHz, CDCl3): δ 20.7 (3C), 20.8 (3C), 20.9 (2C), 30.5, 61.3, 61.9, 67.1, 67.4, 67.9, 68.1, 68.4, 68.6, 70.8, 70.9, 71.0, 72.0, 72.7, 101.0, 101.6, 166.2, 169.5 (2C), 169.7, 170.2, 170.3 (2C), 170.5, 170.8, 171.3; HRMS (TOF): calcd. for $C_{34}H_{46}N_4NaO_{21}S^+$, [M+$Na^+$]: 901.2267; found: 901.2280

N-Cbz-ala-SH-cys-L-ala-OMe **(85):** ^{1}H NMR (399.78 MHz, CDCl3): δ 7.33 (5 H, s), 7.11 (1 H, d, *J* 30.6), 5.52 (1 H, d, *J* 6.7), 5.11 (2 H, s), 4.67 (1 H, s), 4.57 – 4.47 (1 H, m), 4.30 (1 H, s), 3.73 (3 H, s), 2.99 (1 H, d, *J* 38.1), 2.74 (1 H, s), 2.04 (1 H, s), 1.74 – 1.62 (1 H, m), 1.41 (3 H, s), 1.40 (3 H, s); ^{13}C NMR (100.53 MHz, CDCl3): δ 17.8, 18.3, 26.7, 48.4, 52.5 (2C), 54.1, 67.2, 76.7, 77.0, 77.0, 77.3, 128.0 (2C), 128.3, 128.6 (2C), 136.0, 169.1, 172.9 (2C); HRMS (TOF): calcd. for $C_{18}H_{25}N_3NaO_6S^+$, [M+Na^+]: 434.1356; found: 434.1361.

Peptide-Tetrazine-Sugar conjugate (PTS Conjugate) **(86):** $[\alpha]_D^{25}$ = -24.7 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3743, 3313, 2948, 2360, 2255, 1746, 1657, 1613, 1452,1371, 1221, 1167, 1041, 907, 724; ^{1}H NMR (399.78 MHz, CDCl3): δ 1.23 – 1.30 (6 H, m), 1.90 (3 H, s), 1.93 (3 H, s), 1.95 (3 H, s), 1.98 (3 H, s), 3.35 – 3.45 (2 H, m), 3.60 (3 H, s), 3.61 – 3.77 (4 H, m), 4.00 – 4.20 (4 H, m), 4.21 – 4.32 (1 H, m), 4.41 (2 H, p, *J* 6.6), 4.50 (1 H, d, *J* 7.9), 4.88 – 5.03 (5 H, m), 5.13 (1 H, t, *J* 9.5), 6.06 (1 H, d, *J* 7.0), 7.22 (5 H, s), 7.53 (1 H, d, *J* 6.2), 7.72 (1 H, d, *J* 6.9); ^{13}C NMR (100.53 MHz, CDCl3): δ 17.6, 18.6, 20.4 (2C), 20.6, 20.6, 29.8, 48.1, 52.3, 61.7, 66.7, 67.5, 68.2, 70.8, 71.6, 72.4, 77.4, 100.6, 127.8, 127.9, 128.3 (2C), 136.1, 155.9, 169.0, 169.3, 169.4, 170.0, 170.6, 172.0, 172.2, 172.6, 173.1; HRMS (TOF): calcd. for $C_{36}H_{47}N_7NaO_{16}S_2^+$, [M+$Na^+$]: 920.2413; found: 920.2457. m. p. 88-89 ºC.

((1R,2S,4R)-bicyclo[2.2.1]hept-5-en-2-yl)methyl 3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanoate **(96):** ^{1}H NMR (399.78 MHz, CDCl3): δ 1.16 (1 H, dt, J 11.8, 3.9), 1.24 – 1.31 (2 H, m), 1.33 – 1.39 (1 H, m), 1.73 (1 H, tt, J 9.2, 5.3), 2.67 (1 H, s), 2.81 – 2.86 (3 H, m), 3.84 (2 H, q, J 6.0), 4.05 (1 H, dd, J 10.7, 9.3), 4.23 (1 H, dd, J 10.9, 6.5), 6.08 (2 H, p, J 5.6), 6.23 (1 H, d, J 8.6), 6.73 (1 H, s), 8.49 (1 H, d, J 8.6); ^{13}C NMR (100.53 MHz, CDCl3): δ 29.6, 33.0, 37.9, 39.4, 41.6, 43.7, 45.0, 69.6, 98.8, 124.2, 136.1, 136.6, 137.2, 143.7, 143.9, 144.4, 171.5. [HRMS (TOF): calcd. for $C_{17}H_{18}N_4NaO_5^+$, [M+Na^+]: 381.1169; found: 381.1194.

10-bromodecyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside) **(115):** $[\alpha]_D^{25}$ = -11.0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3844, 3744, 3678, 3619, 2929, 2856, 1747, 1646, 1514, 1369,

1219, 1173, 1135, 955, 905, 139, 644; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.28 (s, 10H), 1.37 – 1.47 (m, 2H), 1.51 – 1.63 (m, 2H), 1.85 (p, J = 7.0 Hz, 2H), 1.99 (s, 3H), 2.06 (s, 6H), 2.15 (s, 3H), 3.41 (t, J = 6.8 Hz, 2H), 3.44 – 3.51 (m, 1H), 3.86 – 3.93 (m, 2H), 4.10 – 4.23 (m, 2H), 4.46 (d, J = 7.9 Hz, 1H), 5.02 (dd, J = 10.5, 3.3 Hz, 1H), 5.21 (dd, J = 10.3, 8.1 Hz, 1H), 5.39 (d, J = 3.1 Hz, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$) δ 20.7, 20.8, 20.8, 20.9, 25.9, 28.2, 28.8, 29.4, 29.5, 29.5, 29.5, 32.9, 34.1, 61.4, 67.2, 69.0, 70.4, 70.7, 71.1, 101.5, 169.5, 170.3, 170.4, 170.5; HRMS (TOF): calcd. for $C_{24}H_{39}NaO_{10}Br^{+}$, [M+$Na^{+}$]: 589.1619; found: 589.1629 .

10-mercaptodecyl (β-D-galactopyranoside) **(116):** $[\alpha]_D^{25}$ = -20.4 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3931, 3745, 3648, 3375, 2921, 2852, 2371, 2317, 1831, 1738, 1703, 1649, 1541, 1511, 1460, 1425, 1371, 1274, 1148, 1068, 901, 855, 760, 711, 665; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.26 (s, 12H), 1.33 (t, J = 7.7 Hz, 1H), 1.59 (p, J = 7.2 Hz, 4H), 2.50 (q, J = 7.4 Hz, 2H), 3.43 – 3.56 (m, 2H), 3.60 (s, 2H), 3.81 (dq, J = 21.3, 9.8, 8.7 Hz, 3H), 3.92 (s, 1H), 4.02 (s, 1H), 4.22 (d, J = 4.3 Hz, 1H), 4.44 (s, 1H), 4.62 (s, 1H), 4.98 (s, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$)δ 24.7, 26.0, 28.5, 29.2, 29.6, 29.7, 29.7, 29.7, 34.1, 61.0, 68.7, 70.6, 71.3, 73.7, 74.3, 103.4; HRMS (TOF): calcd. For$C_{16}H_{32}NaO_6S^{+}$, [M+Na^{+}]: 375.1812; found: 375.1819

(3-((β-D-glucopyranosyl)oxydecylmercapto)-6-chloro)-1,2,4,5-tetrazine **(117):** $[\alpha]_D^{25}$ = +54.9 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3898, 3853, 3676, 3646, 3616, 3563, 2923, 2854, 2730, 2572, 2498, 2143, 2091, 1919, 1868, 1739, 1700, 1649, 1516, 1461, 1421, 1372, 1241, 1060, 885, 758, 682, 649; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.25 (s, 12H), 1.39 – 1.50 (m, 2H), 1.53 – 1.66 (m, 2H), 1.77 (p, J = 7.5 Hz, 2H), 3.28 (t, J = 7.4 Hz, 2H), 3.49 (s, 2H), 3.60 (s, 2H), 3.67 – 3.87 (m, 3H), 4.03 (s, 1H), 4.22 (s, 1H), 4.58 (s, 1H), 4.88 (s, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$)δ 26.0, 28.5, 28.9, 29.2, 29.6, 29.6, 29.7, 29.7, 31.1, 61.0, 68.8, 70.6, 71.2, 73.6, 74.2, 103.4, 165.6, 176.3; HRMS (TOF): calcd. for $C_{18}H_{31}ClN_4NaO_6S^{+}$, [M+$Na^{+}$]: 489.1545; found: 489.1549.

10-bromodecyl (2,3,4-tri-*O*-acetyl-β-L-fucopyranoside) **(120):** $[\alpha]_D^{25}$ = +4.0 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3837, 3743, 3677, 3616, 2928, 2856, 2317, 1748, 1649, 1511, 1431, 1369, 1211, 1174, 1136, 1065, 975, 907, 726, 644; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.12 (d, J = 6.4 Hz, 3H), 1.18 (s, 10H), 1.28 – 1.37 (m, 2H), 1.39 – 1.53 (m, 2H), 1.71 – 1.80 (m, 2H), 1.88 (s, 3H), 1.95 (s, 3H), 2.07 (s, 3H), 3.31 (t, J = 6.8 Hz, 2H), 3.33 – 3.39 (m, 1H), 3.68 – 3.75 (m, 1H), 3.80 (dt, J = 9.6, 6.2 Hz, 1H), 4.34 (d, J = 7.9 Hz, 1H), 4.92 (dd, J = 10.5, 3.4 Hz, 1H), 5.07 (dd, J = 10.4, 7.9 Hz, 1H), 5.13 (d, J = 3.4 Hz, 1H); ^{13}C

NMR (100.53MHz, $CDCl_3$) δ 16.0, 20.5, 20.6, 20.7, 25.7, 28.0, 28.6, 29.1, 29.2, 29.3, 29.3, 32.7, 33.9, 68.9, 68.9, 69.9, 70.2, 71.2, 101.0, 169.3, 170.1, 170.6; HRMS (TOF): calcd. for $C_{22}H_{37}NaO_8Br^+$, [M+Na^+]: 531.1564; found: 531.1569.

10-mercaptodecyl (2,3,4-tri-*O*-acetyl-β-L-fucopyranoside) **(121):** $[\alpha]_D^{25}$ = +8.6 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3860, 3743, 3678, 3619, 2927, 2855, 2367, 1748, 1646, 1515, 1221, 1174, 1136, 1064, 976, 907, 726, 673; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.16 (d, J = 6.4 Hz, 3H), 1.21 (s, 12H), 1.28 (t, J = 7.7 Hz, 1H), 1.43 – 1.61 (m, 4H), 1.92 (s, 3H), 1.99 (s, 3H), 2.11 (s, 3H), 2.46 (q, 2H), 3.32 – 3.45 (m, 1H), 3.75 (q, J = 6.4 Hz, 1H), 3.84 (dt, J = 9.7, 6.2 Hz, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.88 – 5.00 (m, 1H), 5.06 – 5.17 (m, 1H), 5.13 – 5.20 (m, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$)δ 16.1, 20.7, 20.7, 20.8, 24.6, 25.8, 28.3, 29.0, 29.3, 29.4, 29.4, 29.5, 34.0, 69.0, 69.0, 70.0, 70.3, 71.4, 101.1, 169.4, 170.3, 170.7; HRMS (TOF): calcd. for $C_{22}H_{38}NaO_8S^+$, [M+Na^+]: 485.2180; found: 485.2184.

10-mercaptodecyl (β-L-fucopyranoside) **(122):** $[\alpha]_D^{25}$ = +11.82 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3834, 3730, 3396, 2924, 2856, 1726, 1456, 1375, 1299, 1171, 1071, 995, 902, 854, 758, 671; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.22 (s, 15H), 1.29 (t, J = 7.9 Hz, 1H), 1.51 – 1.60 (m, 4H), 2.47 (q, J = 7.4 Hz, 2H), 3.45 (q, J = 7.5 Hz, 1H), 3.49 – 3.56 (m, 2H), 3.60 (t, J = 8.6, 7.9 Hz, 1H), 3.66 (s, 1H), 3.79 (q, J = 7.4 Hz, 1H), 4.08 (s, 1H), 4.13 (d, J = 7.3 Hz, 1H), 4.51 (s, 1H), 4.96 (s, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$)δ 16.3, 24.6, 25.8, 28.3, 29.0, 29.4, 29.4, 29.5, 29.5, 34.0, 70.0, 70.5, 70.8, 71.6, 74.0, 103.0; HRMS (TOF): calcd. for $C_{16}H_{32}NaO_5S^+$, [M+Na^+]: 359.1863; found: 359.1870.

(3-((β-D-galactopyranosyl)oxydecylmercapto)-6-((β-L-fucopyranosyl)oxydecyl mercapto)) -1,2,4,5-tetrazine **(123) :** $[\alpha]_D^{25}$ = -1.72 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3733, 3396, 2924 , 2857, 2312, 1648, 1455, 1375, 1239, 1067; ^{1}H NMR (500.20MHz, MeOH-D4) δ 1.37 (d, J = 6.5 Hz, 3H), 1.42 (d, J = 24.3 Hz, 22H), 1.57 (p, J = 7.2 Hz, 4H), 1.71 (p, J = 6.6 Hz, 4H), 1.88 (p, J = 7.5 Hz, 4H), 3.37 (t, J = 7.3 Hz, 3H), 3.54 – 3.64 (m, 6H), 3.68 – 3.73 (m, 2H), 3.81 – 3.87 (m, 2H), 3.90 – 4.01 (m, 3H), 4.25 – 4.28 (m, 1H), 4.30 (d, J = 7.4 Hz, 1H); ^{13}C NMR (125.79MHz, MeOH-D4);δ 16.7, 26.7, 26.8, 29.6, 29.7, 29.9, 30.2, 30.2, 30.3, 30.5, 31.1, 31.2, 62.1, 69.8, 70.6, 70.6, 71.5, 72.0, 72.2, 72.6, 74.6, 74.8, 76.0, 78.5, 78.5, 78.7, 78.8, 79.0, 79.0, 79.0, 104.3, 104.5, 173.6, 173.6; HRMS (TOF): calcd. for $C_{16}H_{32}NaO_5S^+$, [M+Na^+]: 767.3935; found:767.3946.

10-bromodecyl (2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside) **(125):** $[\alpha]_D^{25}$ = +43.6 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3851, 3743, 3676, 3647, 3616, 2928, 2856, 2362, 1748, 1649, 1512, 1222, 1135, 977, 796, 641; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.23 (s, 10H), 1.31 –

1.40 (m, 2H), 1.46 – 1.57 (m, 2H), 1.73 – 1.83 (m, 2H), 1.92 (s, 3H), 1.97 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 3.28 – 3.42 (m, 3H), 3.55 – 3.65 (m, 1H), 3.86 – 3.95 (m, 1H), 3.98 – 4.06 (m, 1H), 4.21 (dd, J = 12.2, 5.2 Hz, 1H), 4.73 (s, 1H), 5.10 – 5.31 (m, 3H); ^{13}C NMR (100.53MHz, $CDCl_3$) δ 20.7, 20.7, 20.7, 20.9, 26.0, 28.1, 28.7, 29.2, 29.2, 29.3, 29.3, 32.8, 34.0, 62.5, 66.2, 68.3, 68.5, 69.1, 69.7, 97.5, 169.7, 169.8, 170.0, 170.6; HRMS (TOF): calcd. for $C_{24}H_{39}NaO_{10}Br^+$, [M+Na^+]:589.1619; found:589.1616 .

10-azidodecyl (2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside) **(126):** $[\alpha]_D^{25}$ = + ($CHCl_3$, c 1.00); IR(cm^{-1}): ; ^{1}H NMR (399.78MHz, $CDCl_3$)δ 1.28 (s, 12H), 1.58 (p, J = 6.9 Hz, 4H), 1.98 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.14 (s, 3H), 3.24 (t, J = 6.9 Hz, 2H), 3.43 (dt, J = 9.6, 6.6 Hz, 1H), 3.66 (dt, J = 9.5, 6.7 Hz, 1H), 3.96 (ddd, J = 9.7, 5.2, 2.4 Hz, 1H), 4.09 (dd, J = 12.2, 2.4 Hz, 1H), 4.27 (dd, J = 12.2, 5.3 Hz, 1H), 4.78 (d, J = 1.5 Hz, 1H), 5.21 (dd, J = 3.3, 1.8 Hz, 1H), 5.26 (t, J = 9.9 Hz, 1H), 5.33 (dd, J = 10.0, 3.4 Hz, 1H); ^{13}C NMR (100.53MHz, $CDCl_3$)δ 20.8, 20.8, 20.9, 21.0, 26.2, 26.8, 28.9, 29.2, 29.4, 29.4, 29.5, 29.5, 51.6, 62.6, 66.4, 68.5, 68.6, 69.2, 69.8, 97.7, 169.9, 170.0, 170.2, 170.8; HRMS (TOF): calcd. for $C_{24}H_{39}N_3NaO10^+$, [M+Na^+]: 552.2528; found: 552.2534.

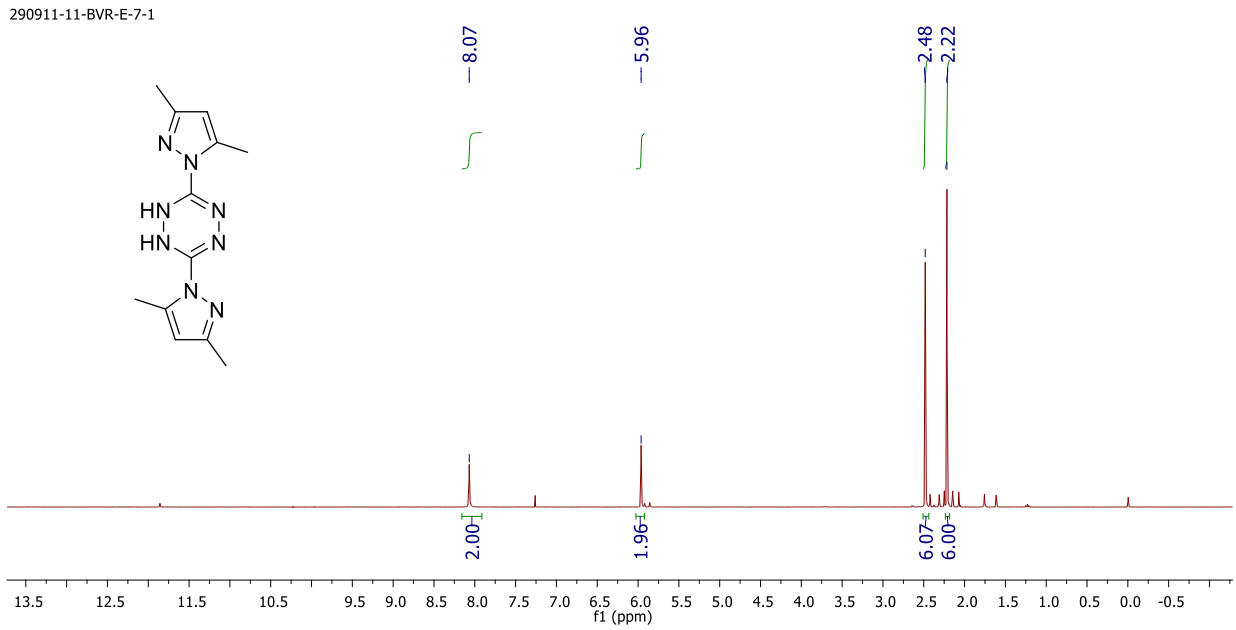
((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (4-nitrophenyl) carbonate **(129):** $[\alpha]_D^{25}$ = +33.8 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3736, 3389, 2923, 2856, 1698, 1529, 1454, 1369, 1251, 1130, 1061, 761, 678; ^{1}H NMR(400 MHz, $CDCl_3$) δ 1.02 – 1.14 (m, 2H), 1.52 (p, J = 8.6 Hz, 1H), 1.59 – 1.70 (m, 2H), 2.22 – 2.41 (m, 6H), 4.42 (d, J = 8.3 Hz, 2H), 7.37 – 7.44 (m, 2H), 8.27 – 8.33 (m, 2H); ^{13}C NMR (100.53MHz, $CDCl_3$); 17.2, 20.5, 20.5, 21.4, 21.4,29.0, 29.0, 68.0, 98.7, 98.7, 121.8, 121.8, 125.3, 125.3, 145.3, 152.6, 155.6; HRMS (TOF): calcd. for $C_{16}H_{32}NaO_5S^+$, [M+Na^+]: 359.1863; found: 359.1869.

10-(α-D-mannopyranosyl)oxydecyl (((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl)carbamate **(130):** $[\alpha]_D^{25}$ = +20.4 ($CHCl_3$, c 1.00); IR(cm^{-1}): 3382, 2924, 2857, 1699, 153 1, 1458, 1375, 1253, 1130, 1063, 679; ^{1}H NMR (500.20MHz, $CDCl_3$) δ 0.93 (t, J = 9.2 Hz, 2H), 1.26 (s, 12H), 1.41 – 1.68 (m, 7H), 2.15 – 2.37 (m, 6H), 3.09 – 3.19 (m, 2H), 3.37 (d, J = 6.1 Hz, 1H), 3.50 (d, J = 9.4 Hz, 1H), 3.61 (d, J = 7.3 Hz, 1H), 3.75 (d, J = 11.3 Hz, 1H), 3.82 (d, J = 8.0 Hz, 1H), 3.87 – 4.00 (m, 3H), 4.13 (d, J = 7.8 Hz, 2H), 4.46 (s, 1H), 4.78 (d, J = 15.3 Hz, 2H), 4.85 – 5.17 (m, 3H).^{13}C NMR (125.79MHz, $CDCl_3$); δ 17.9, 20.2, 21.4, 21.6, 26.2, 26.9, 29.1, 29.2, 29.2, 29.2, 29.3, 29.4, 29.4, 29.5, 29.6, 30.1, 41.2, 61.1, 62.7, 66.4, 68.0, 71.3, 71.8, 72.3, 99.0, 100.1, 156.9; HRMS (TOF): calcd. for $C_{16}H_{32}NaO_5S^+$, [M+Na^+]: 512.3223; found: 512.3229.

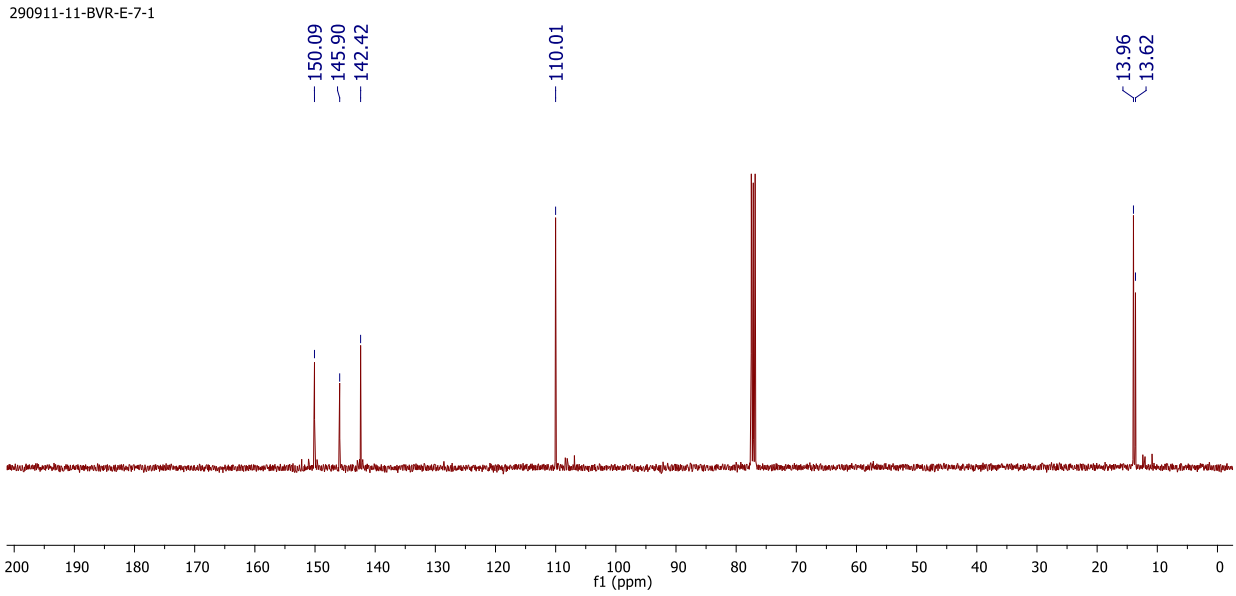
Galactosyl-Fucosyl-Mannosyl Ternary Conjugate **(131):** ^{1}H NMR (399.78MHz, MeOH-D4) δ 0.74 – 0.94 (m, 2H), 1.08 – 1.26 (m, 2H), 1.27 – 1.41 (m, 36H), 1.44 – 1.69 (m, 14H), 1.75 (p, J = 7.4 Hz, 4H), 2.27 – 2.44 (m, 2H), 2.86 – 3.02 (m, 3H), 3.10 (t, J = 6.9 Hz, 2H), 3.19 – 3.30 (m, 5H), 3.32 – 3.35 (m, 1H), 3.41 – 3.59 (m, 9H), 3.63 – 3.93 (m, 12H), 4.15 (d, J = 7.7 Hz, 1H), 4.18 – 4.23 (m, 1H), 4.24 (d, J = 7.2 Hz, 1H), 4.59 (s, 1H); ^{13}C NMR (100.53MHz, MeOH-D_4); 13C NMR (101 MHz, METHANOL-D3) δ 9.2, 16.7, 16.8, 27.0, 27.1, 27.1, 27.3, 27.3, 27.8, 27.8, 27.9, 28.0, 30.0, 30.0, 30.1, 30.1, 30.3, 30.3, 30.3, 30.4, 30.5, 30.6, 30.6, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 30.9, 31.5, 31.5, 47.8, 49.8, 49.8, 62.4, 62.7, 68.5, 68.6, 70.2, 70.8, 70.8, 71.8, 72.2, 72.2, 72.5, 72.6, 73.0, 74.4, 74.9, 75.1, 76.4, 101.5, 104.7, 104.9, 140.0, 140.0, 160.1, 160.1; HRMS (TOF): calcd. for $C_{61}H_{107}N_3NaO_{19}S_2^+$, [M+$Na^+$]: 1250.7018; found: 1250.7025.

3.5 Spectral Charts

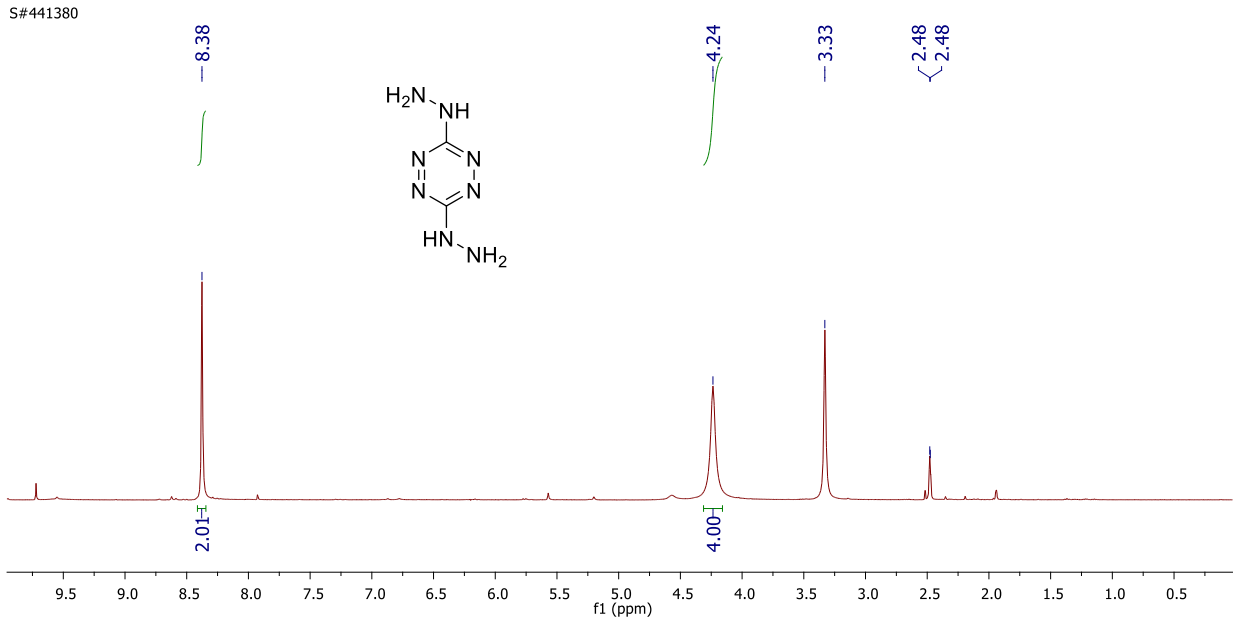
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **55**



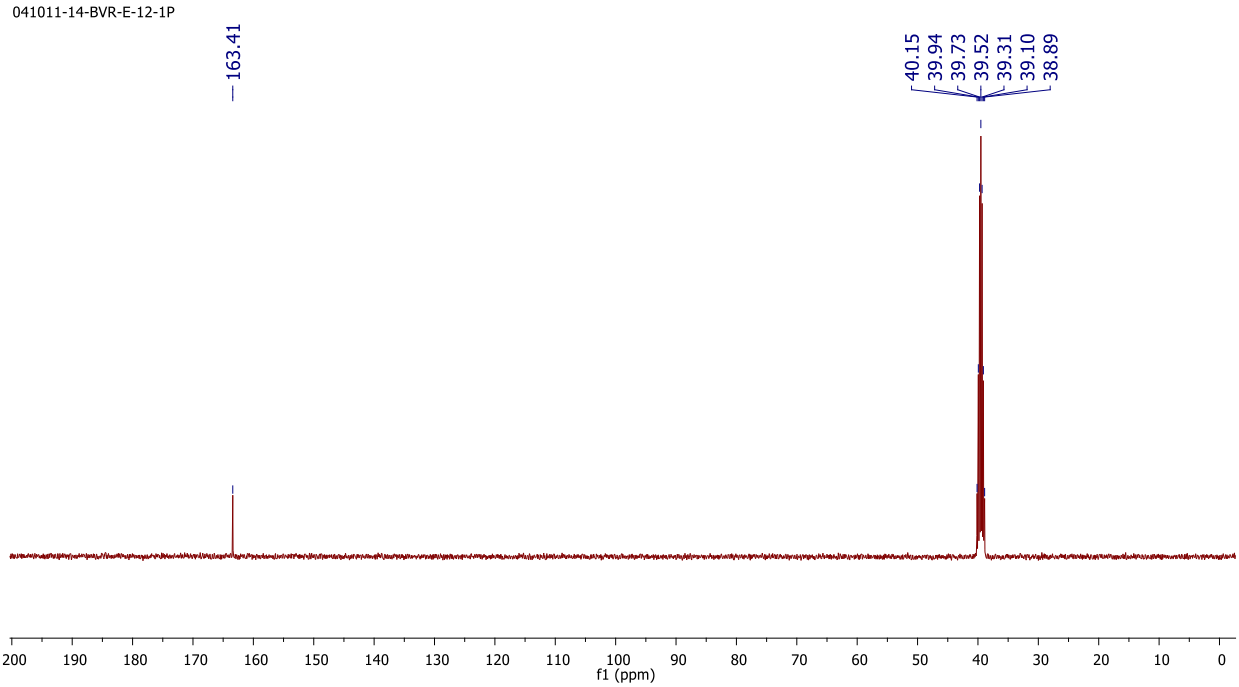
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **55**



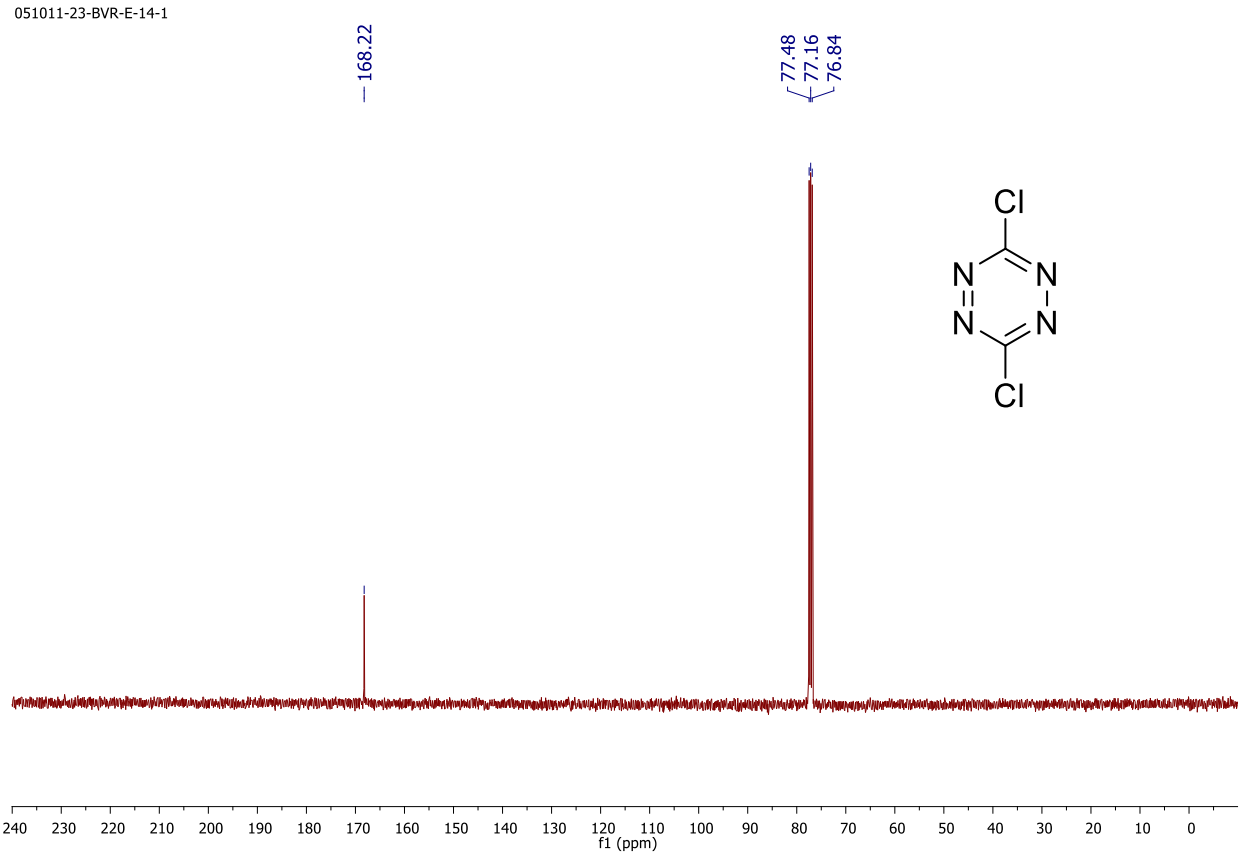
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **56**



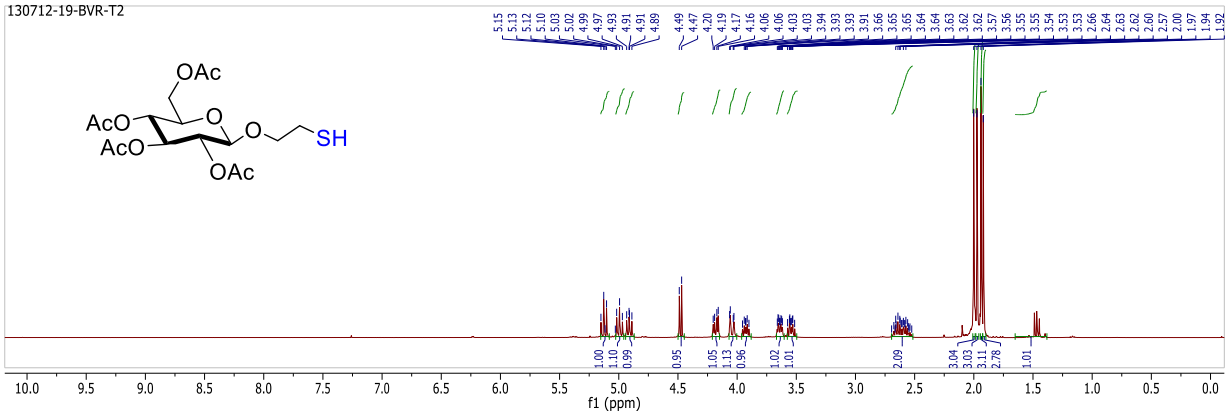
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **56**



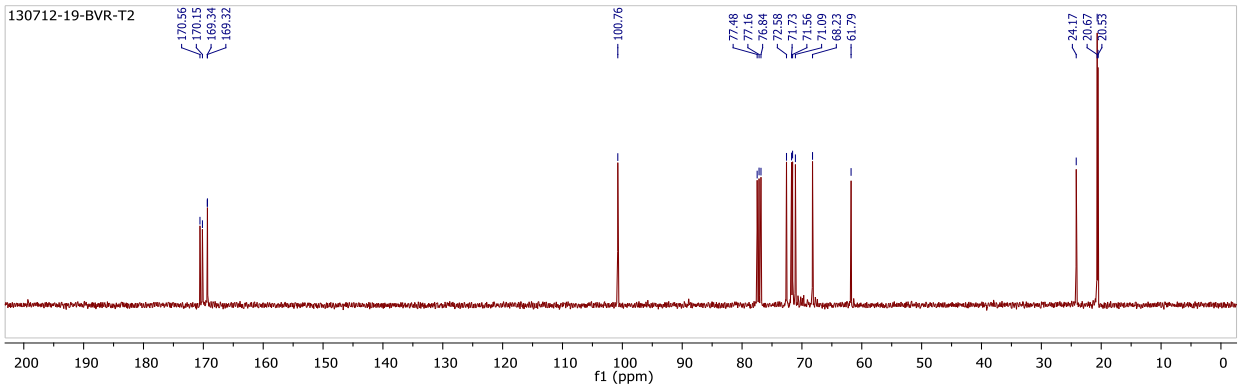
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **40**



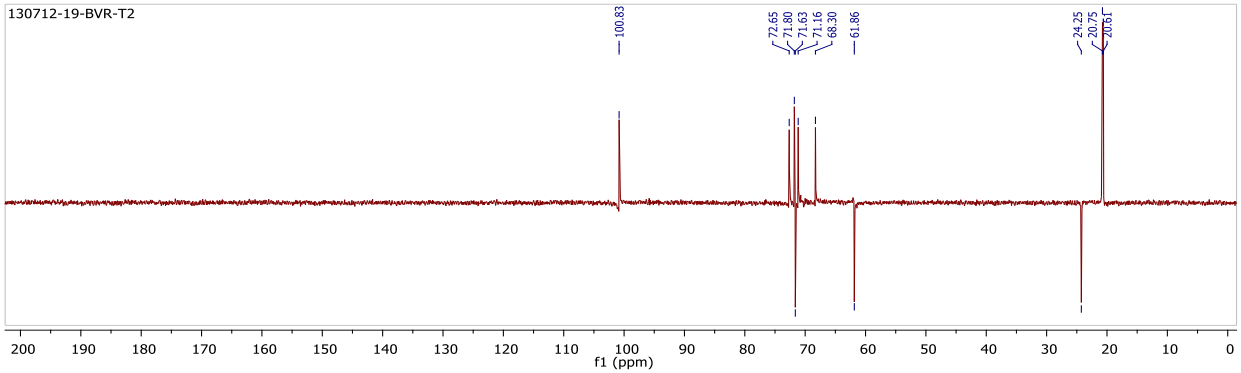
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **61**



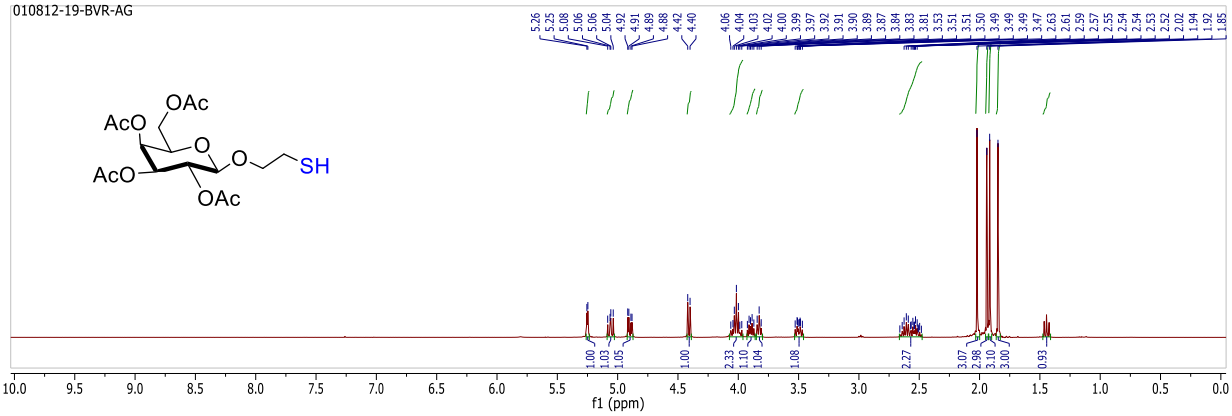
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **61**



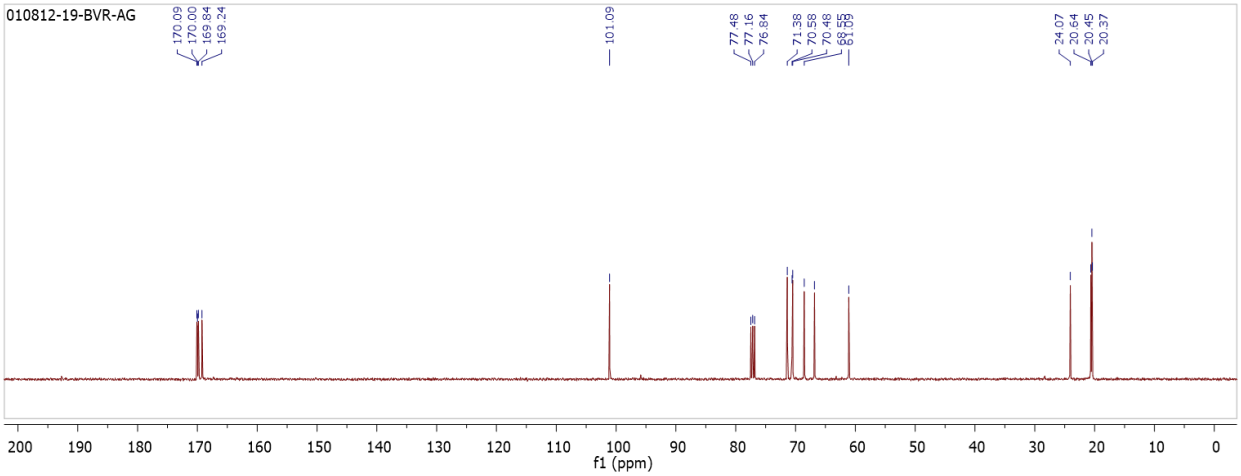
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **61**



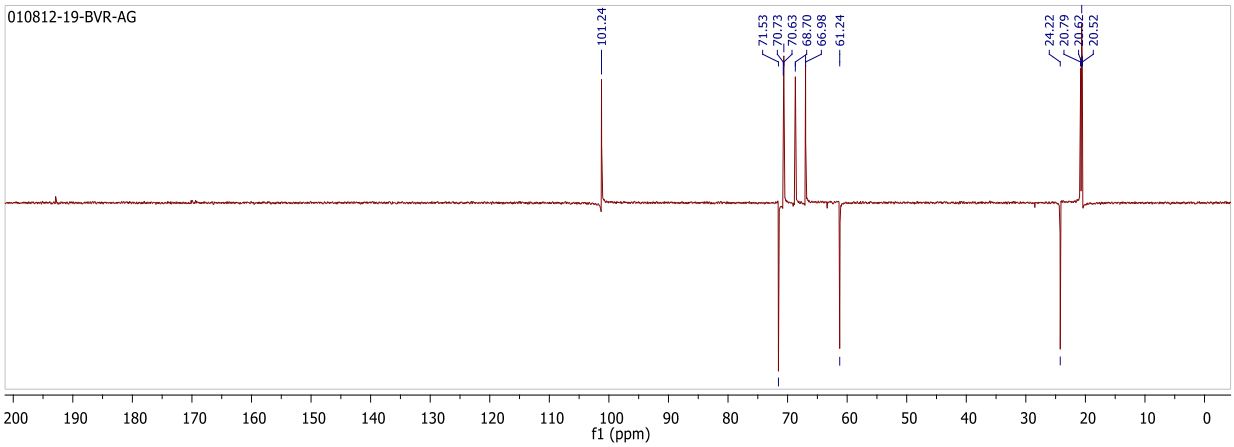
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **62**



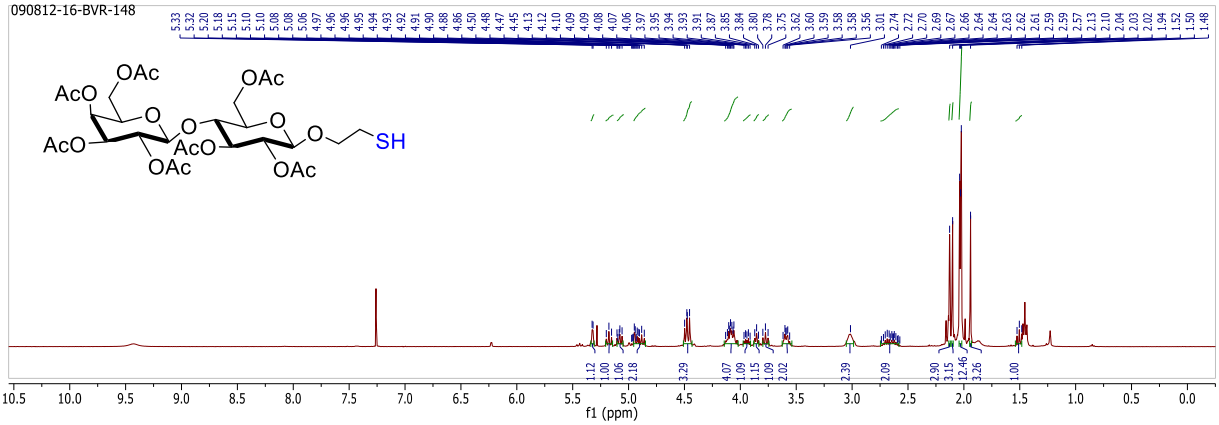
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **62**



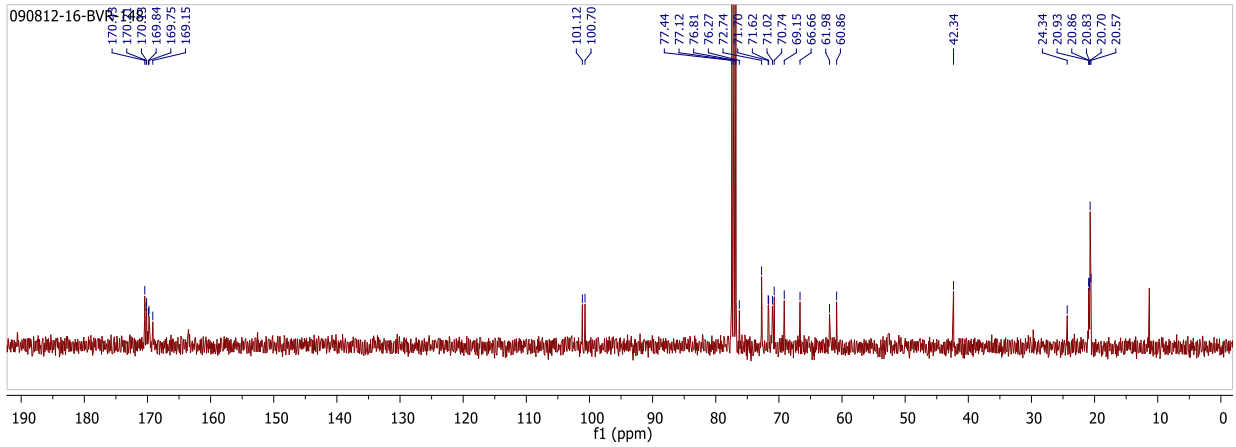
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **62**



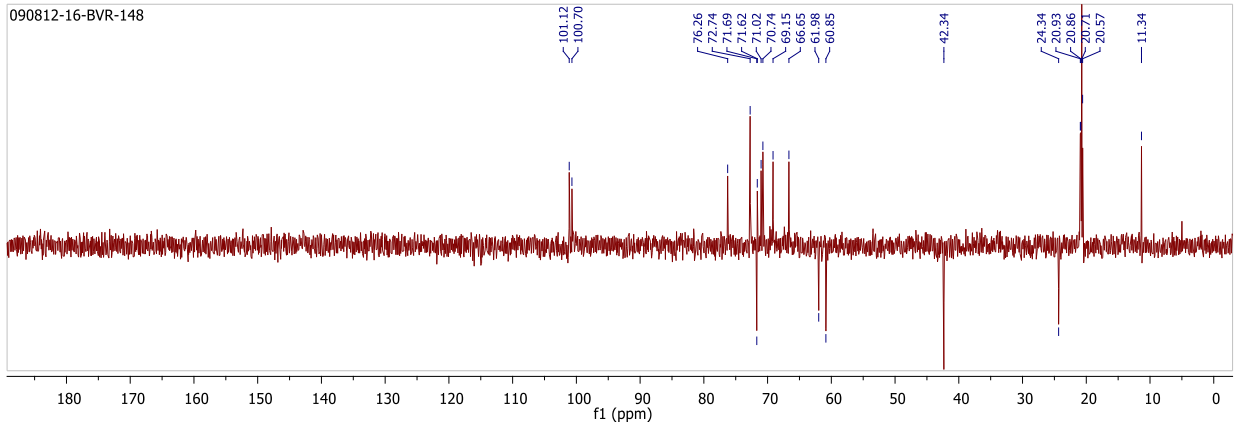
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **63**



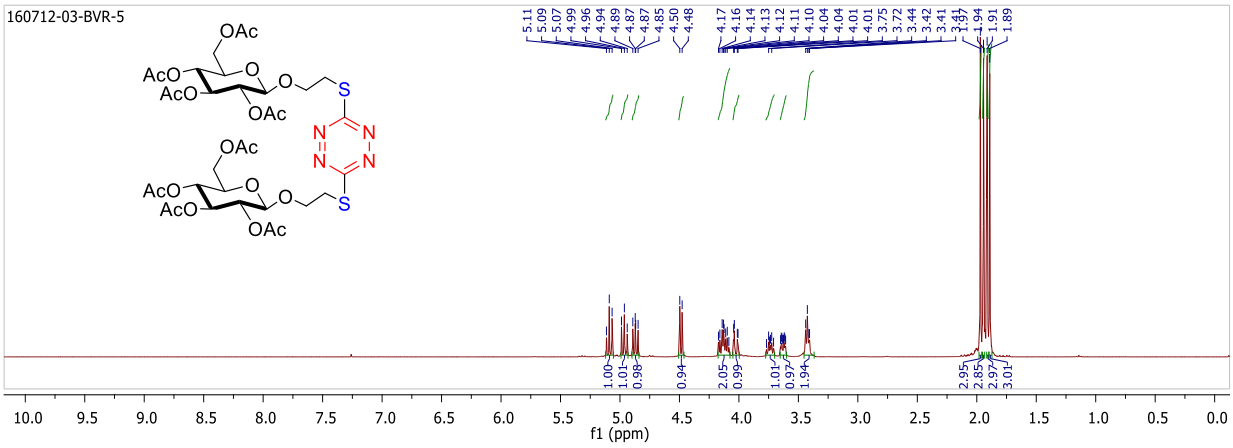
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **63**



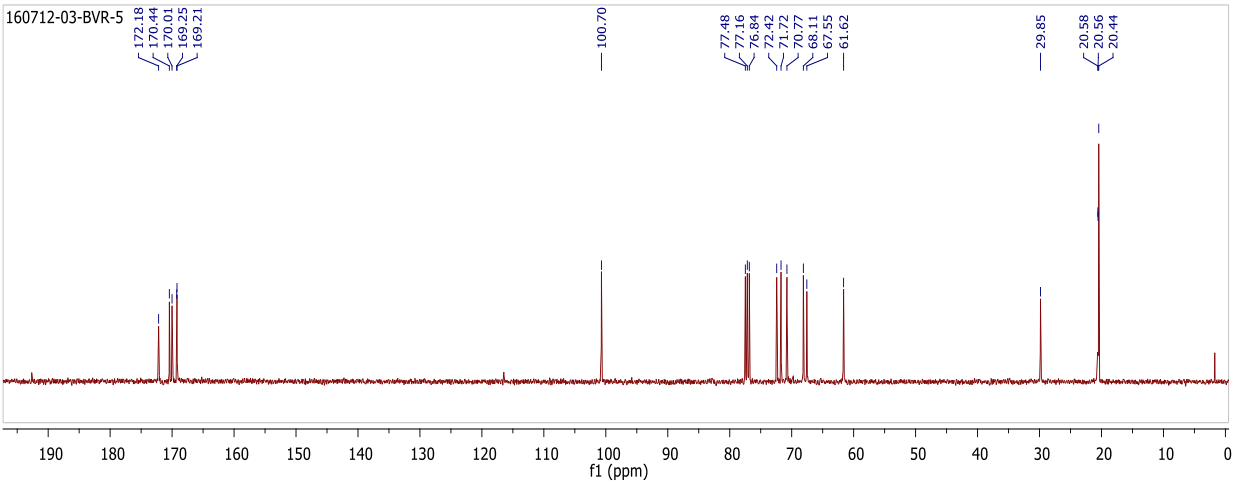
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **63**



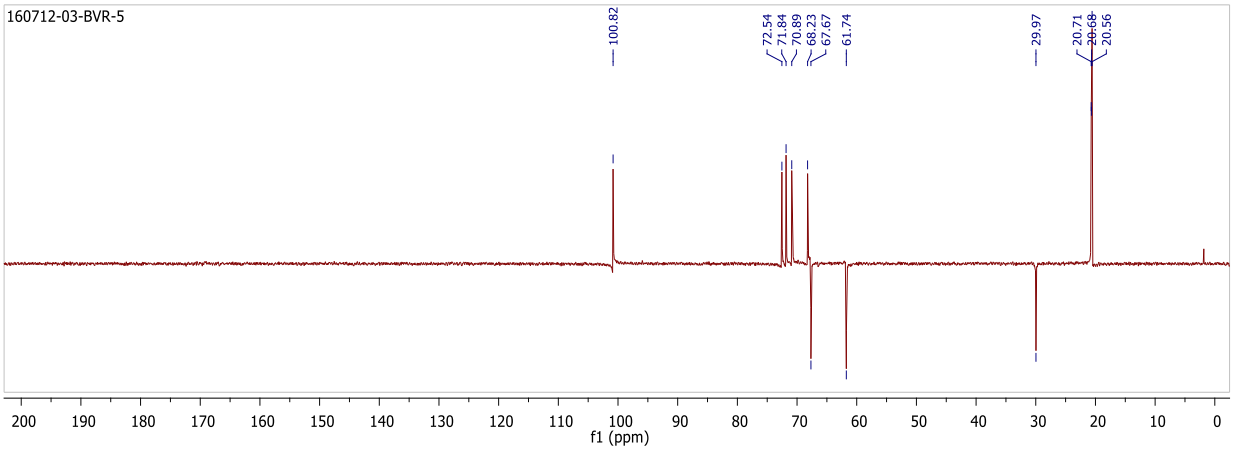
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **64**



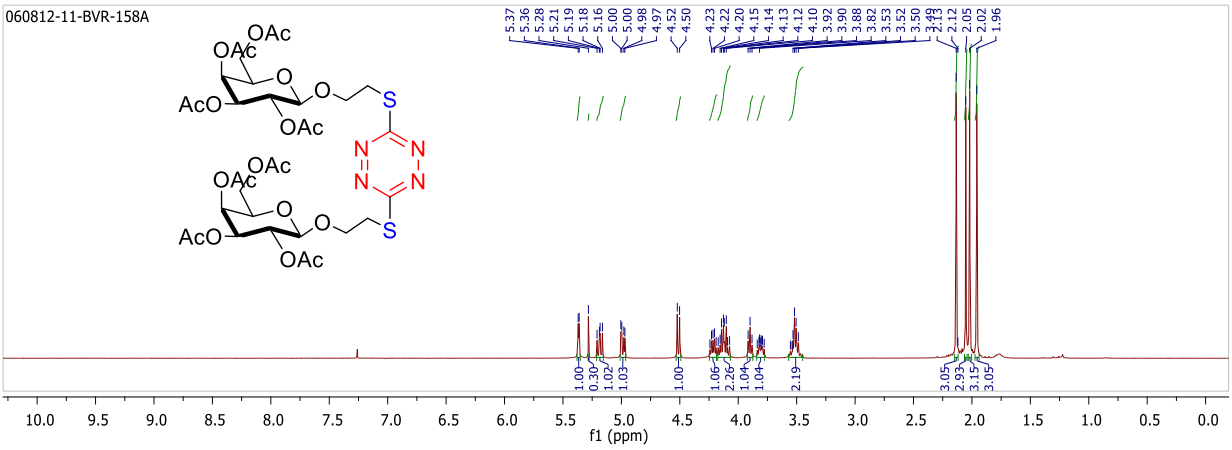
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **64**



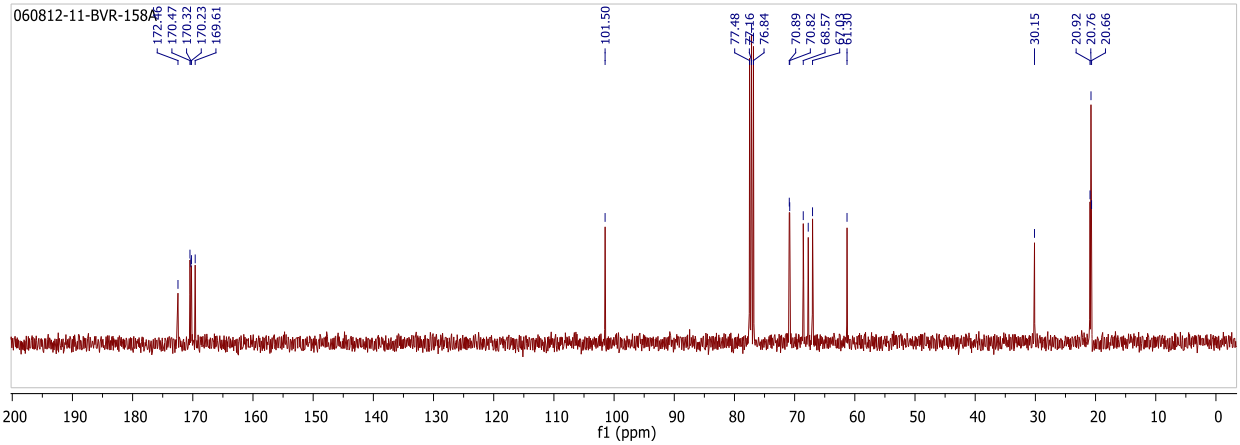
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **64**



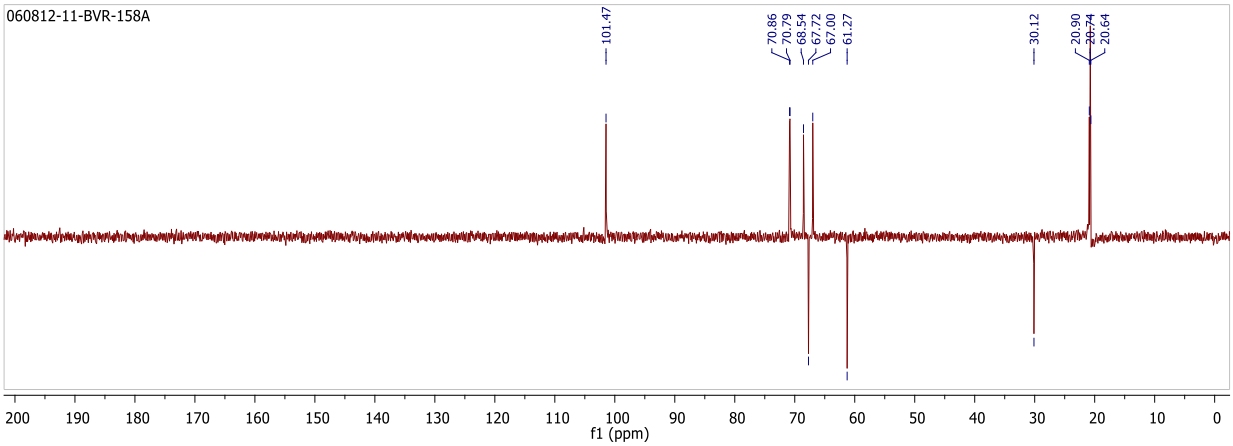
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **65**



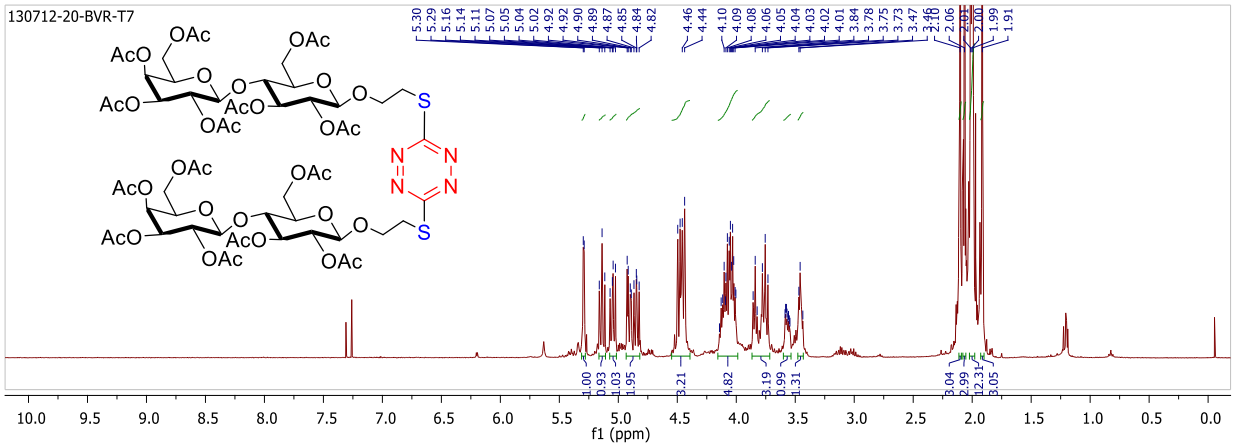
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **65**



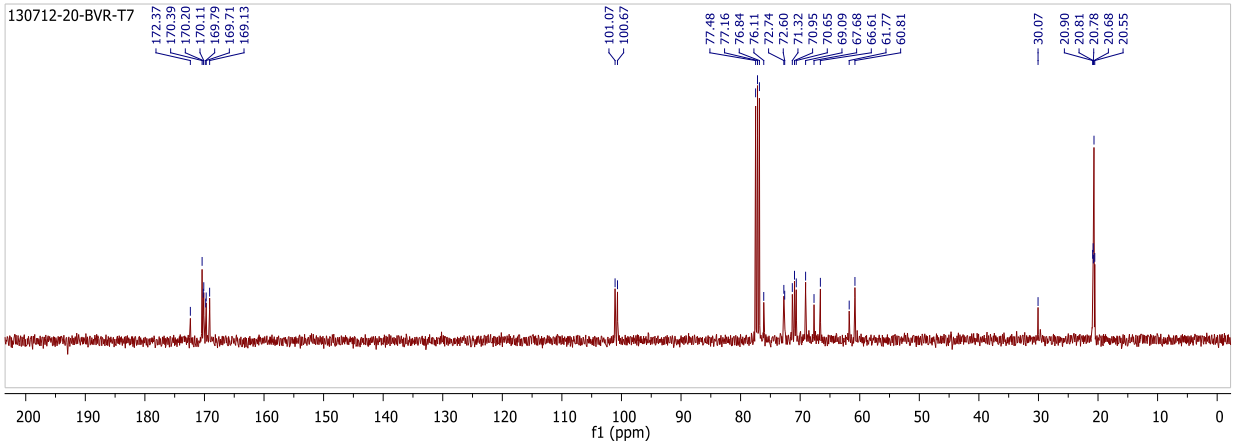
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **65**



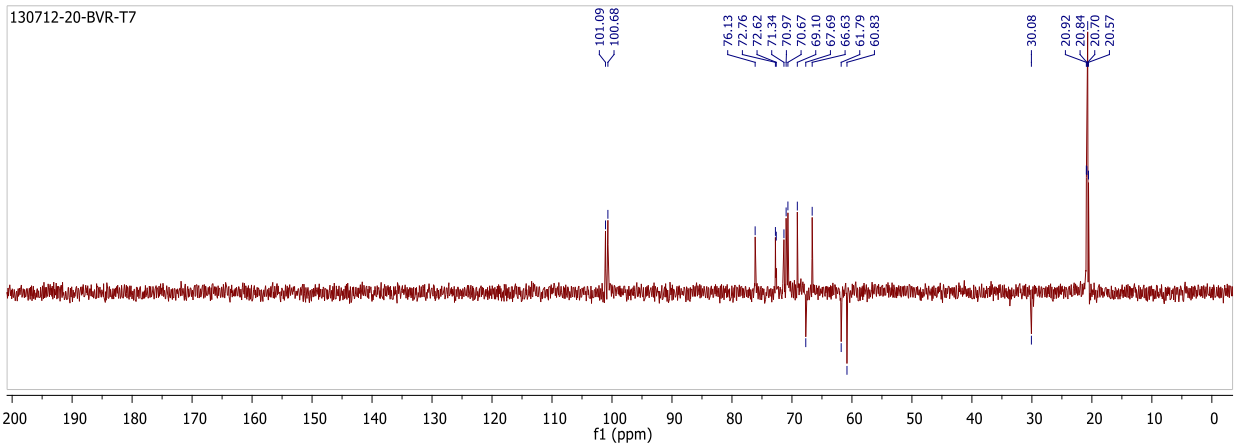
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **66**



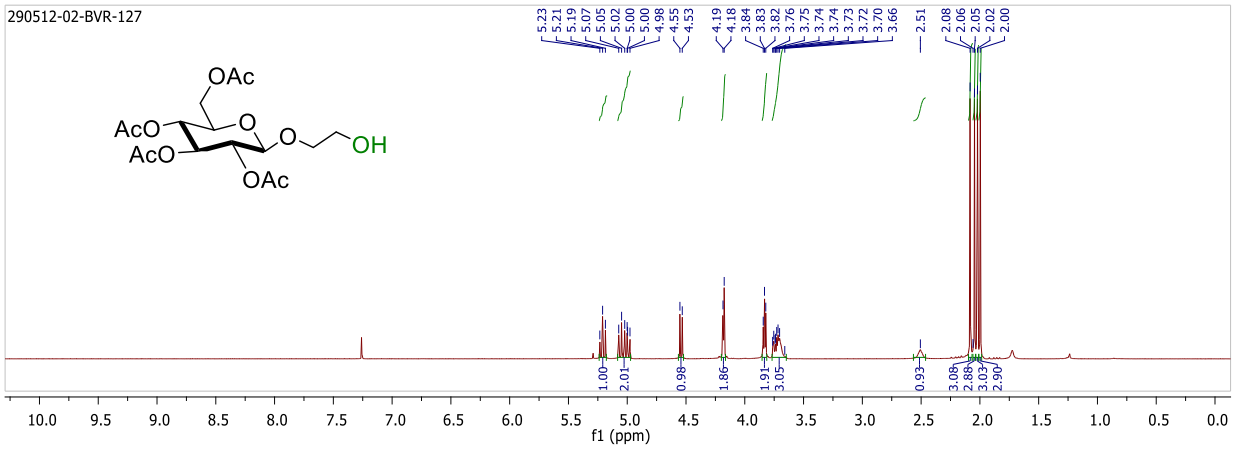
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **66**



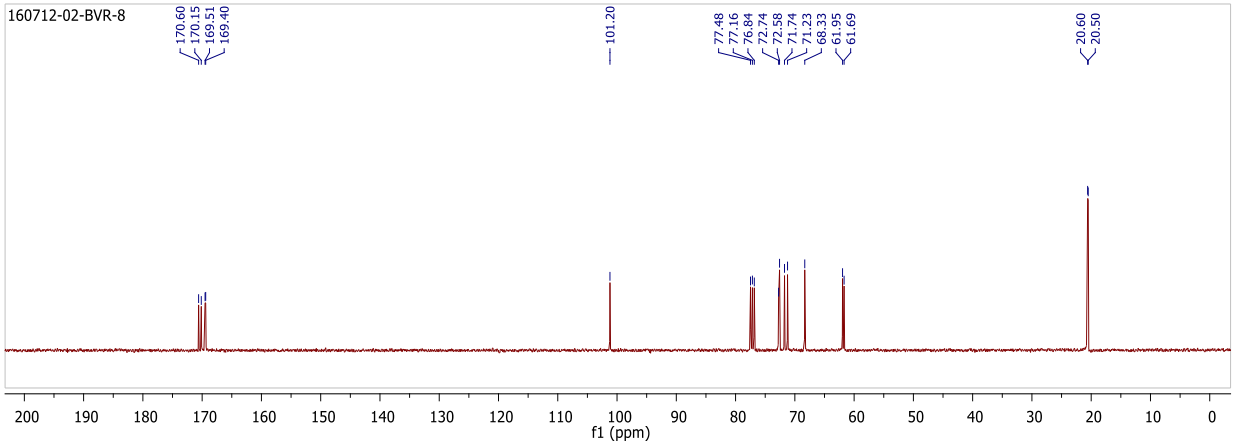
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **66**



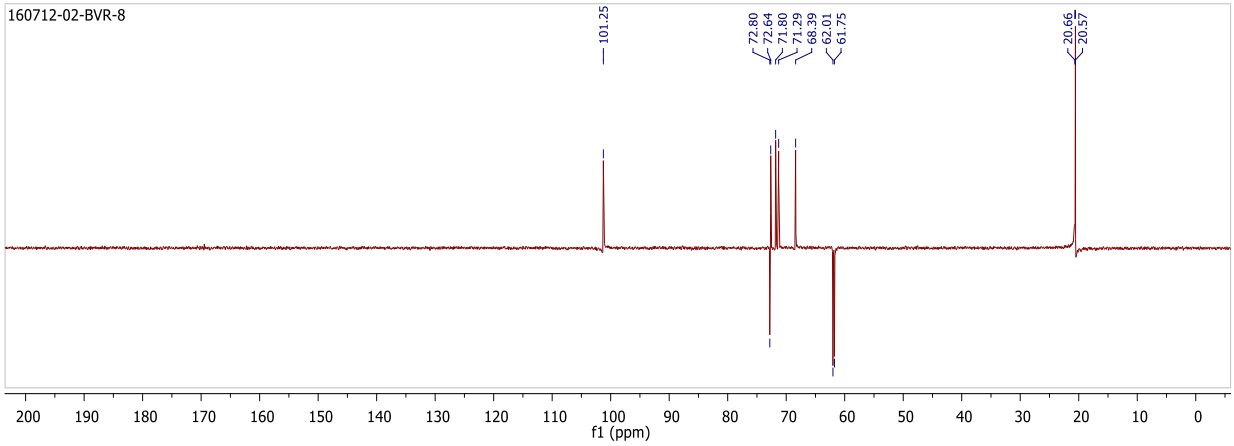
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **68**



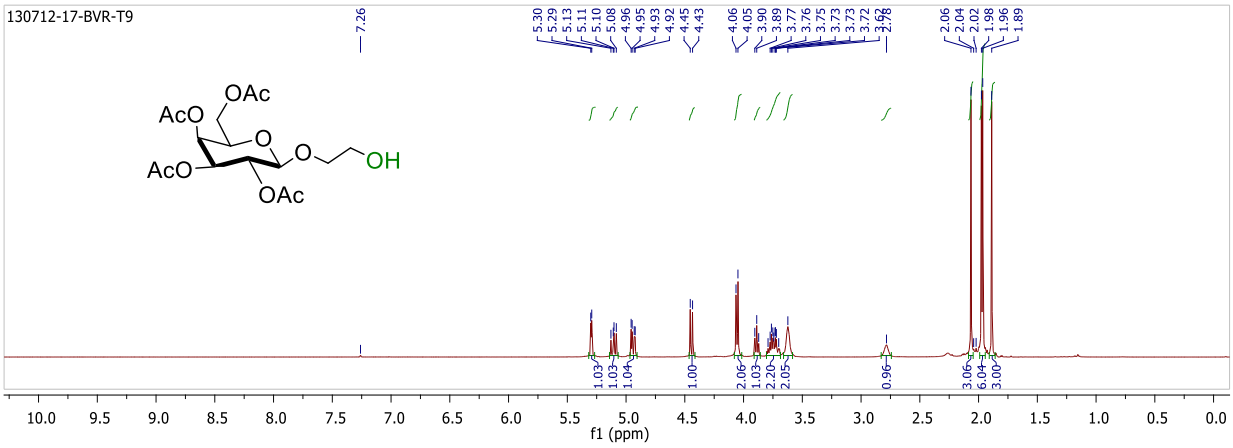
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **68**



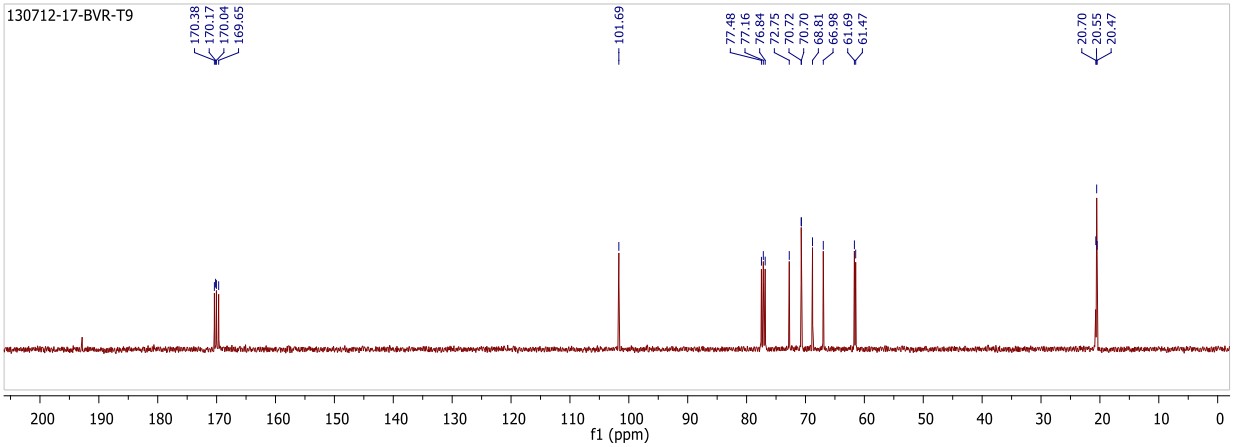
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **68**



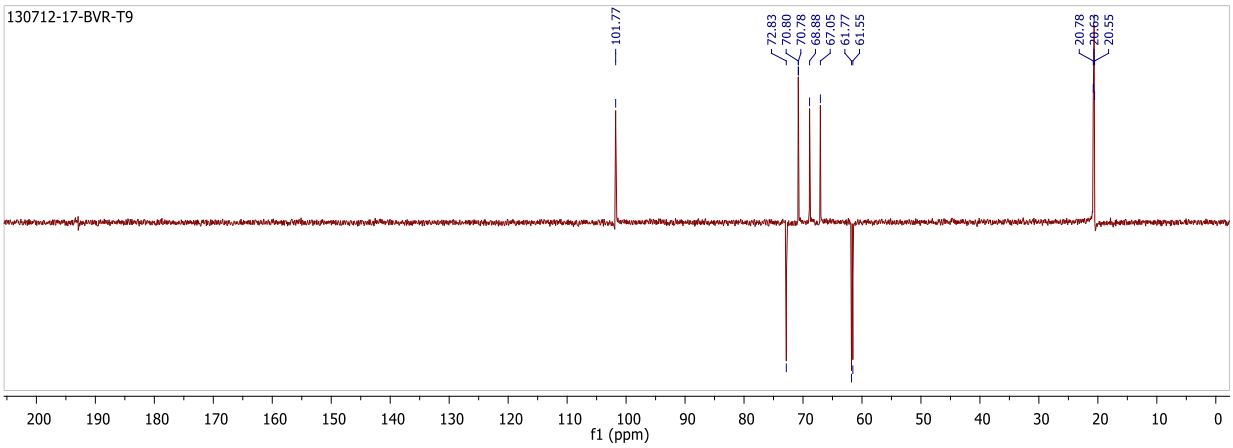
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **69**



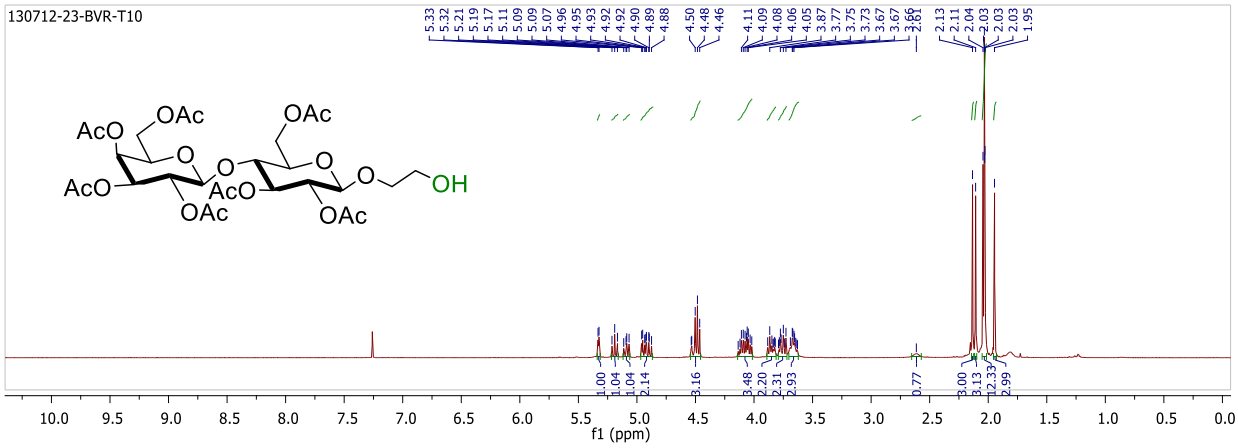
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **69**



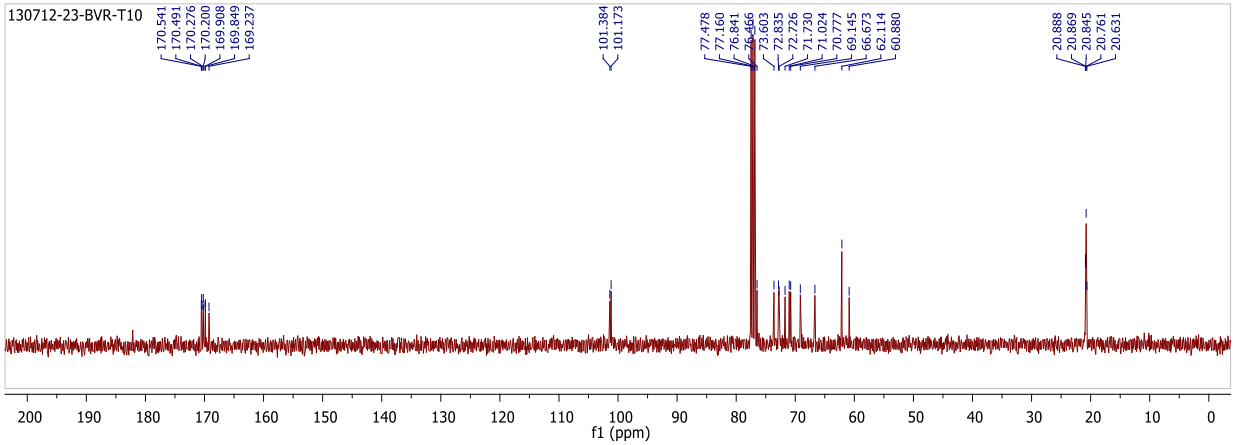
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **69**



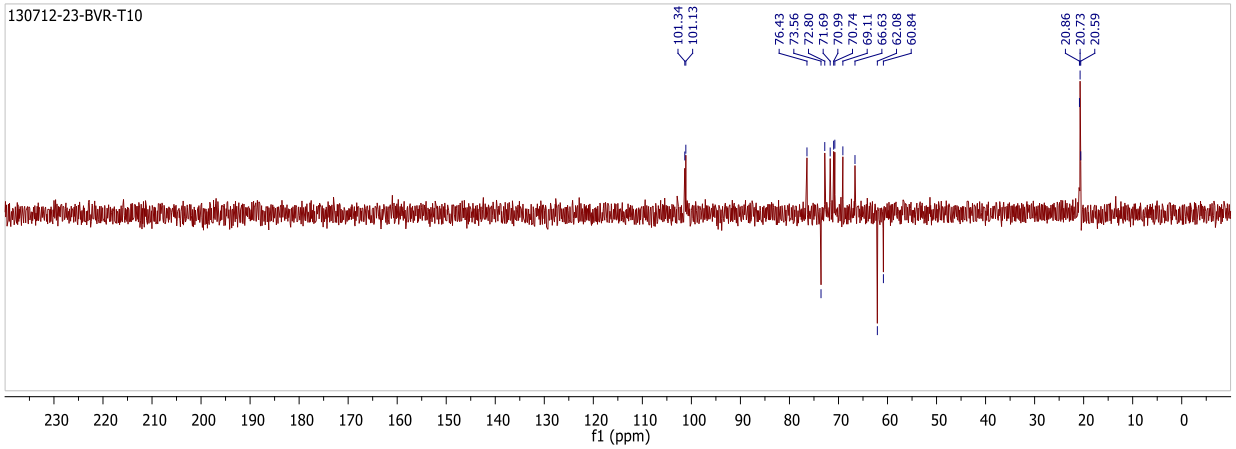
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **70**



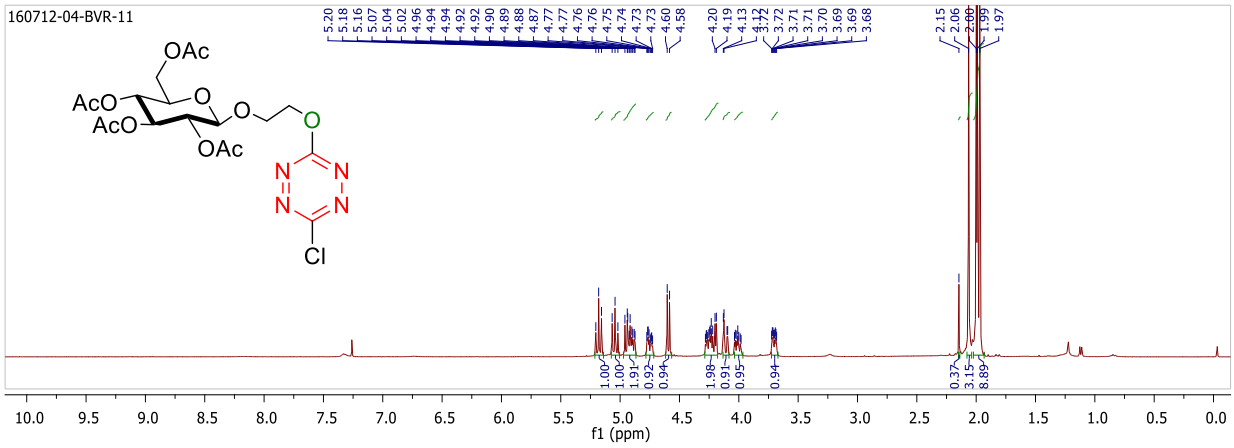
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **70**



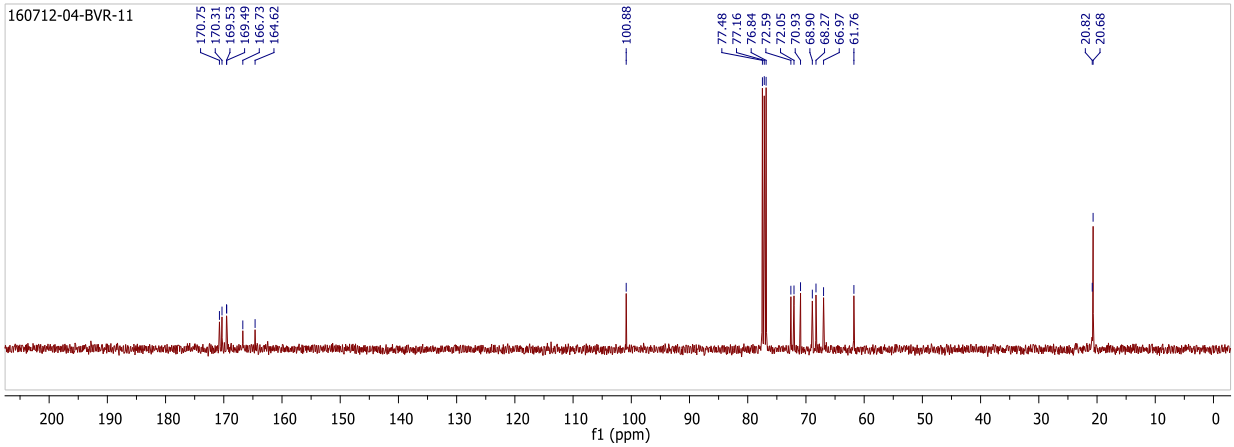
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **70**



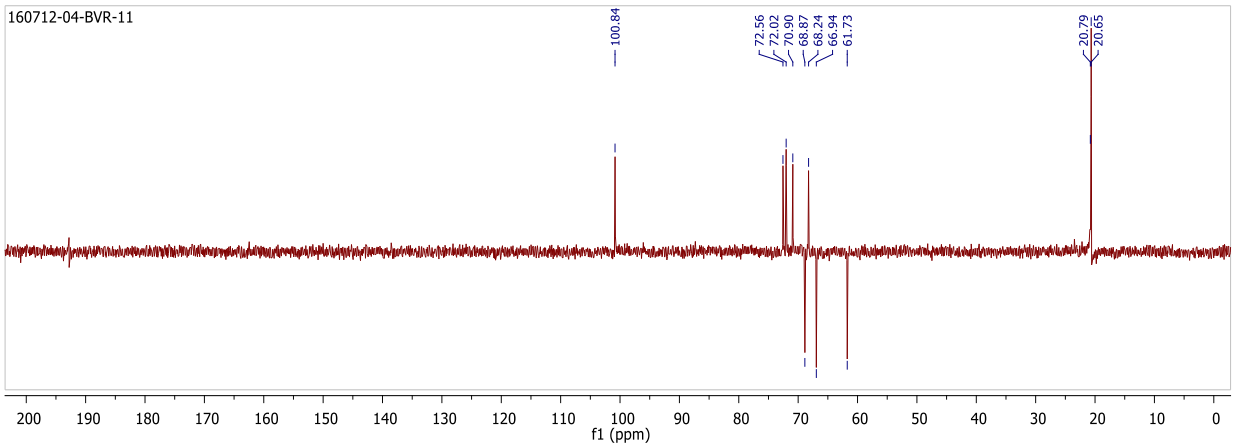
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **71**



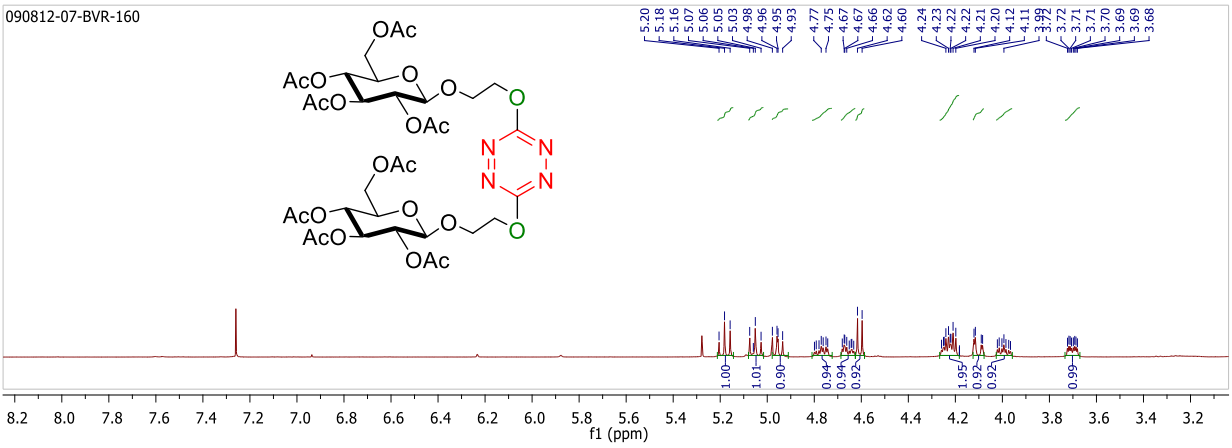
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **71**



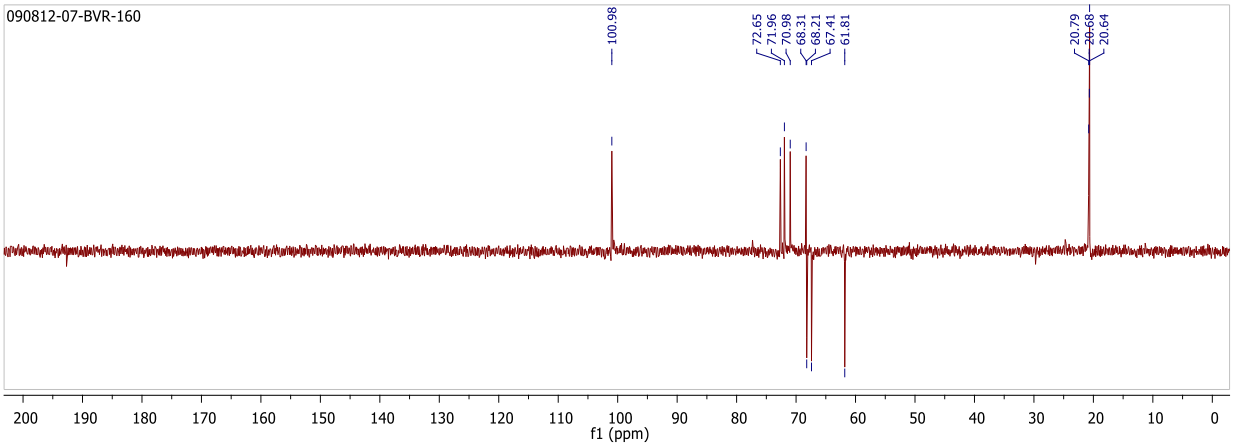
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **71**



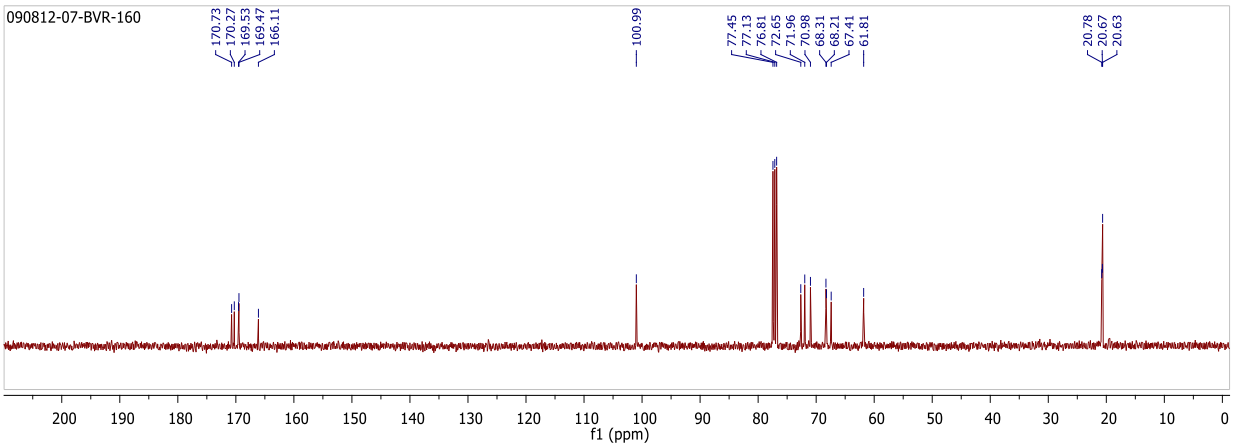
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **72**



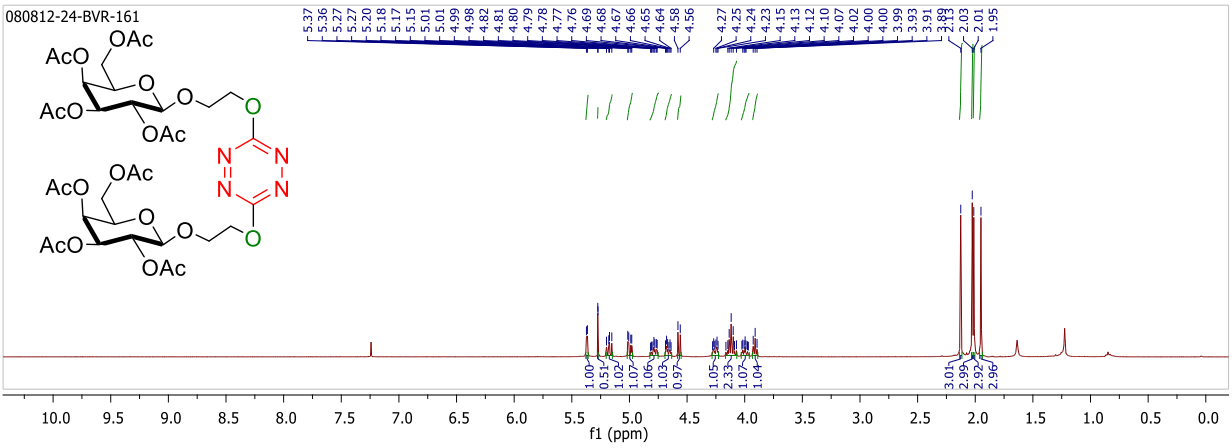
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **72**



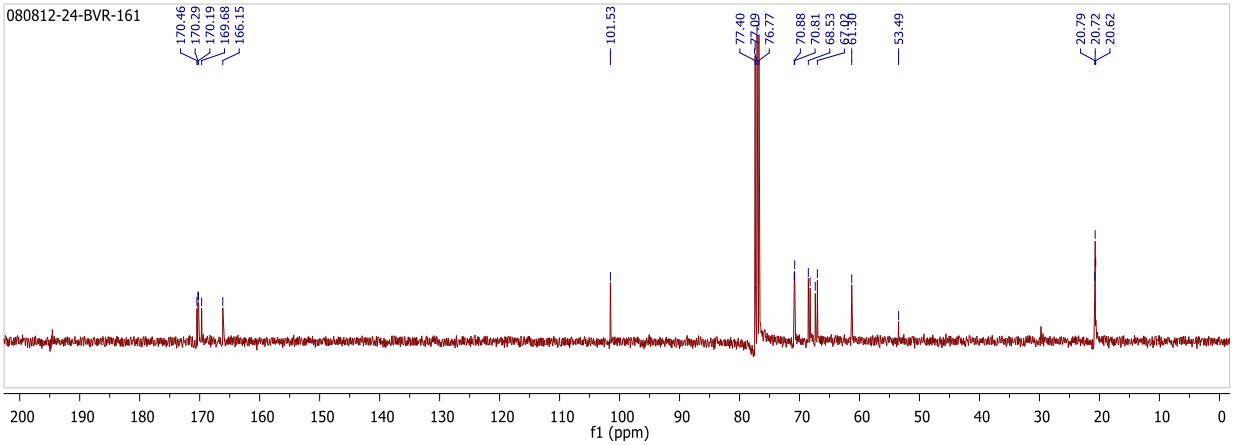
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **72**



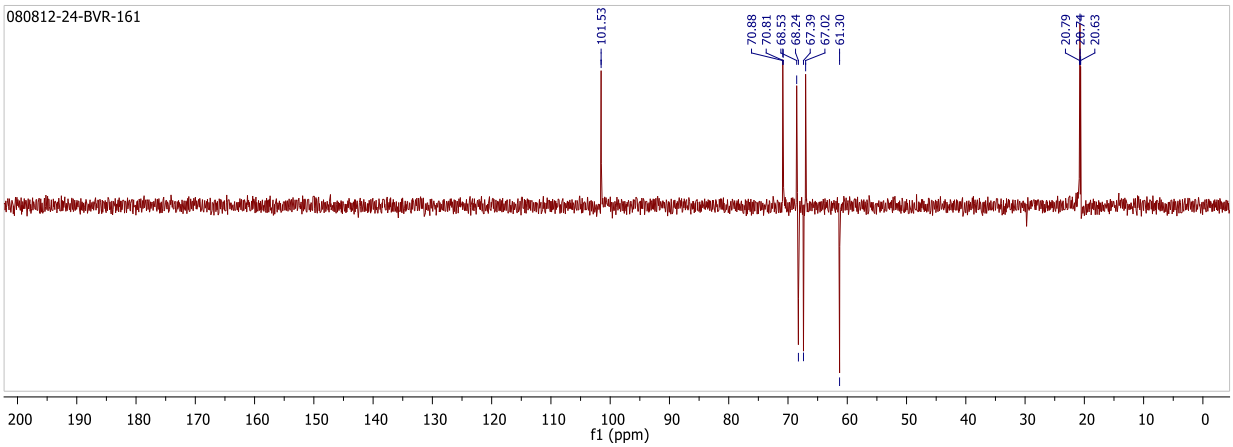
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **73**



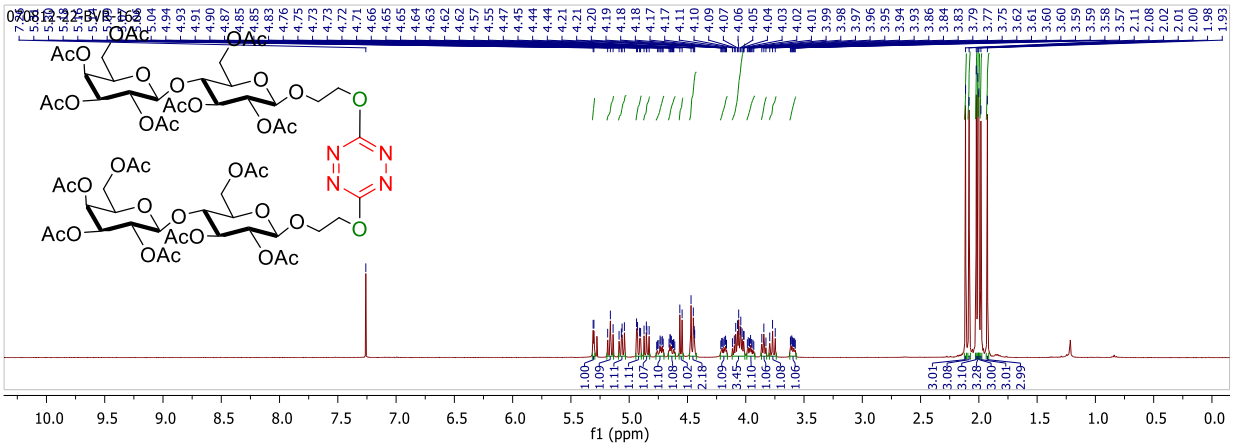
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **73**



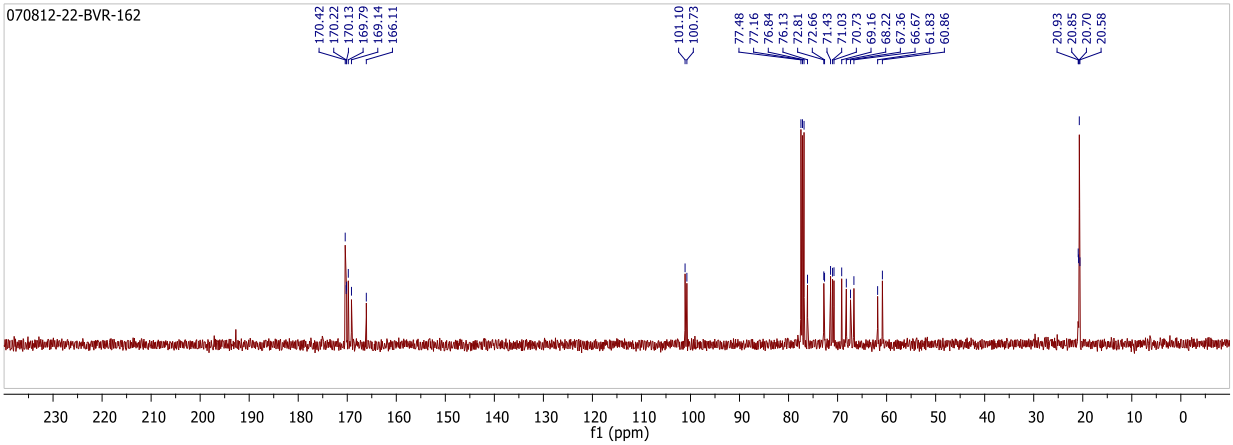
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **73**



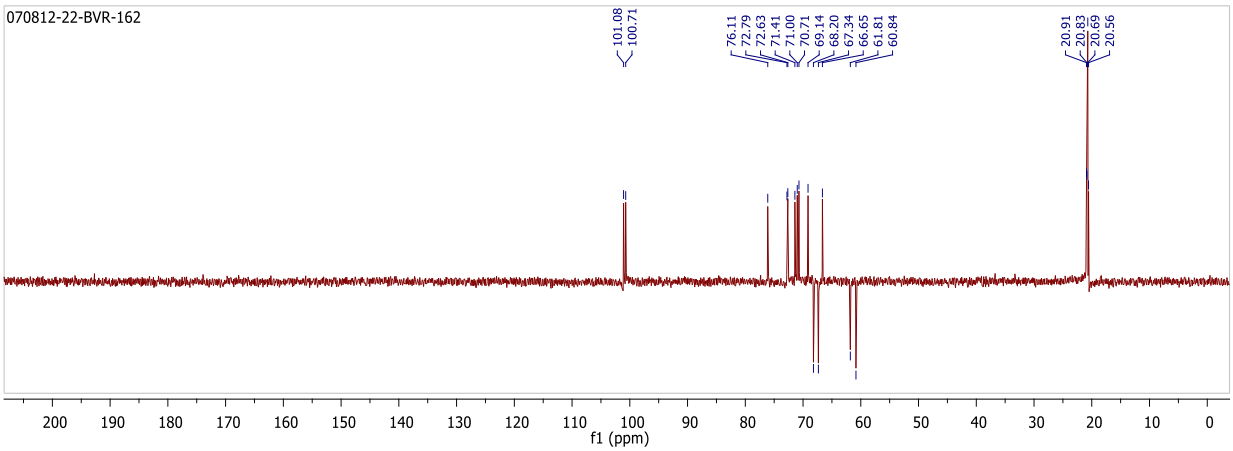
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **74**



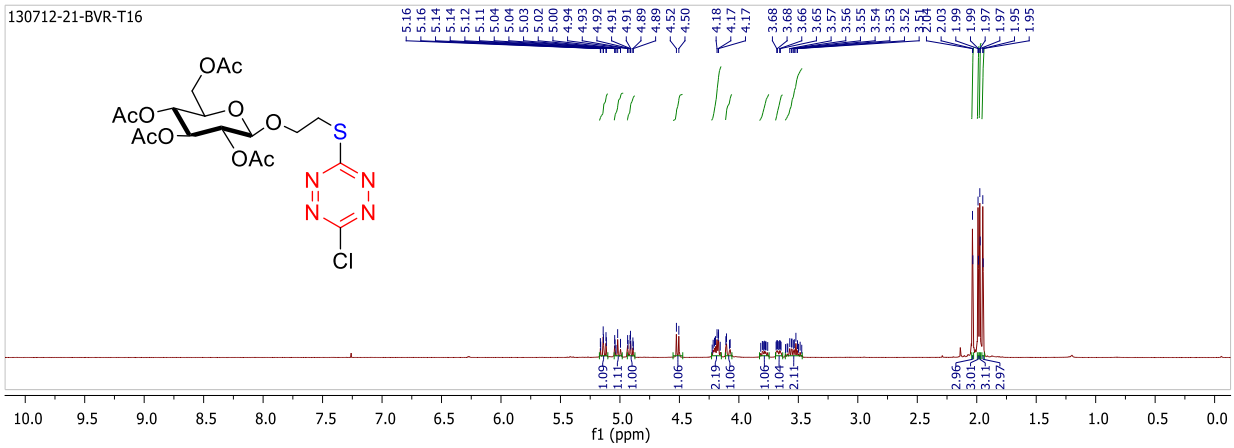
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **74**



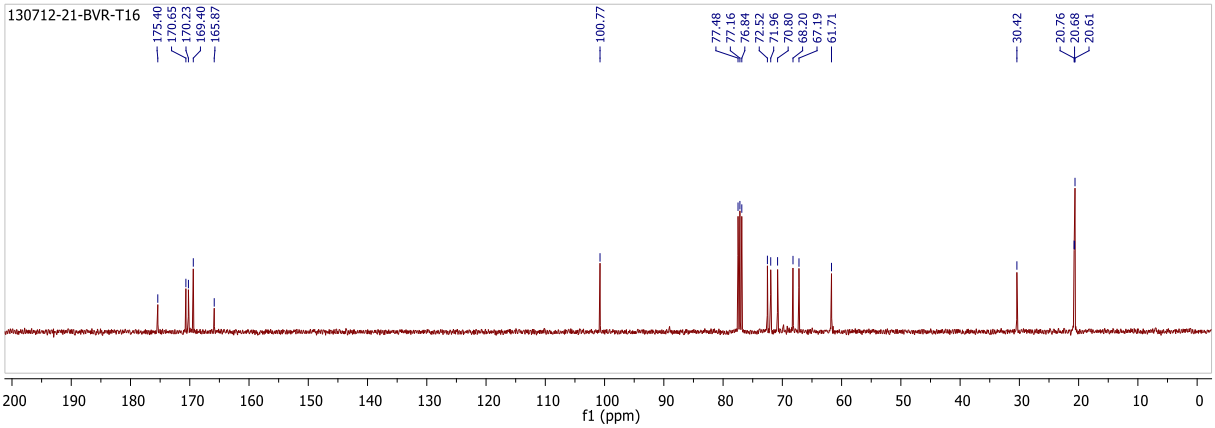
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **74**



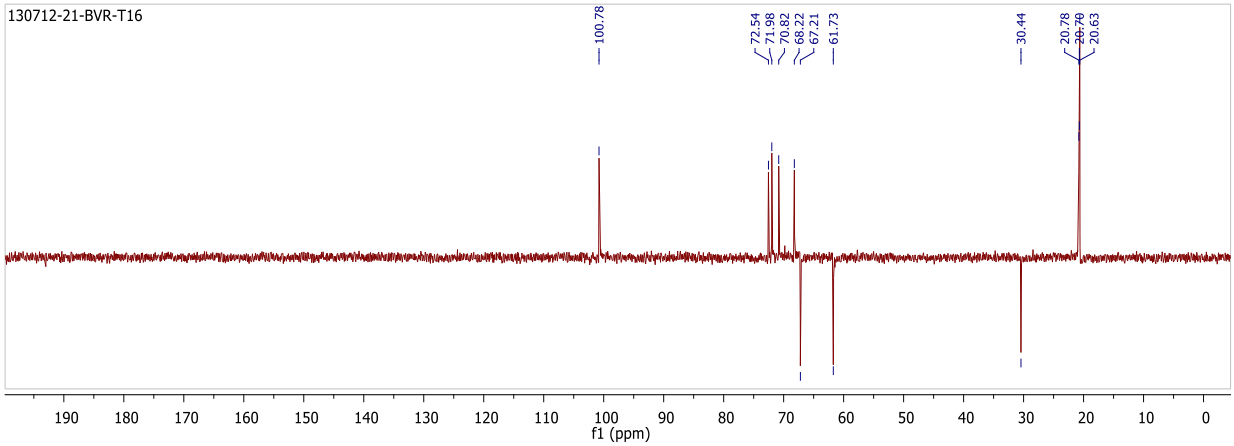
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **75**



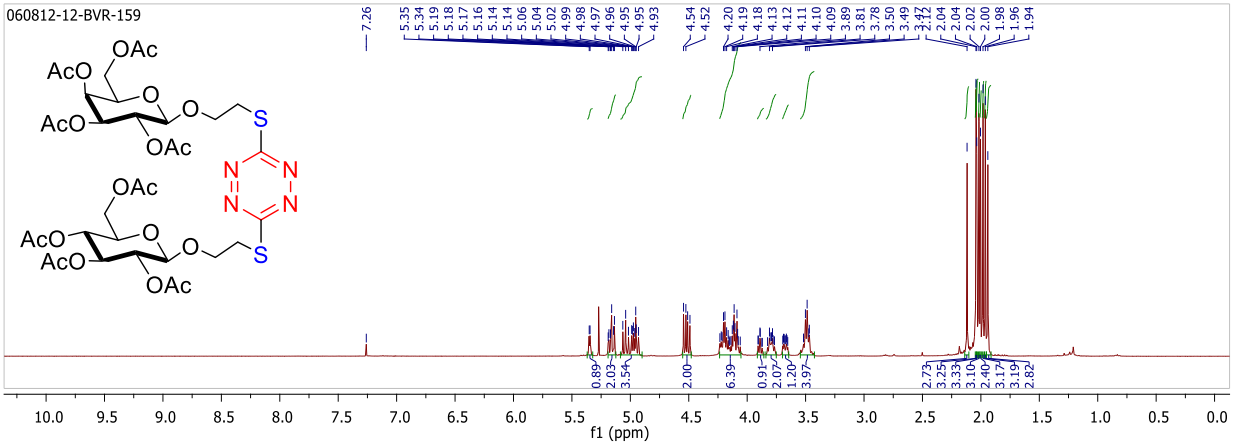
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **75**



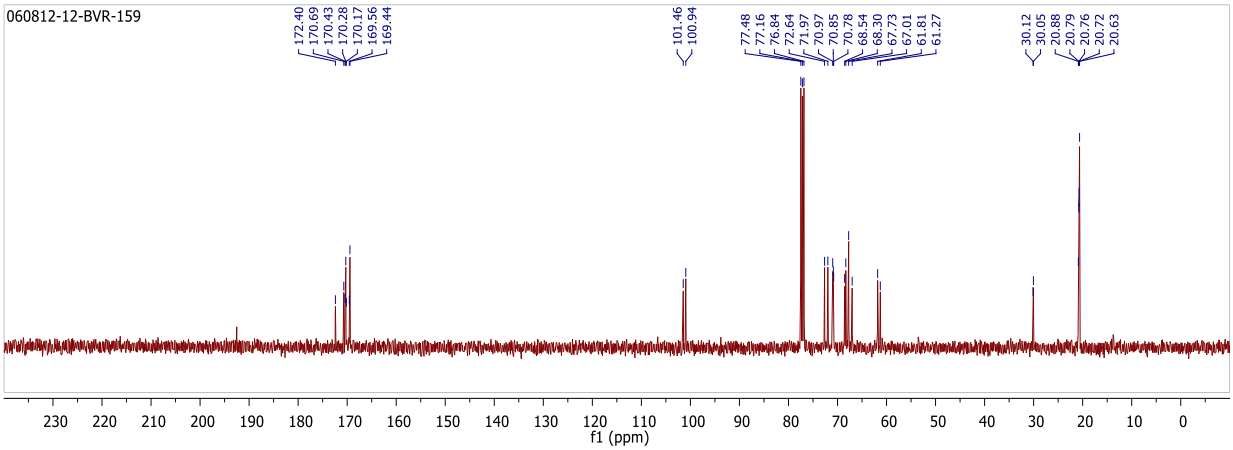
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **75**



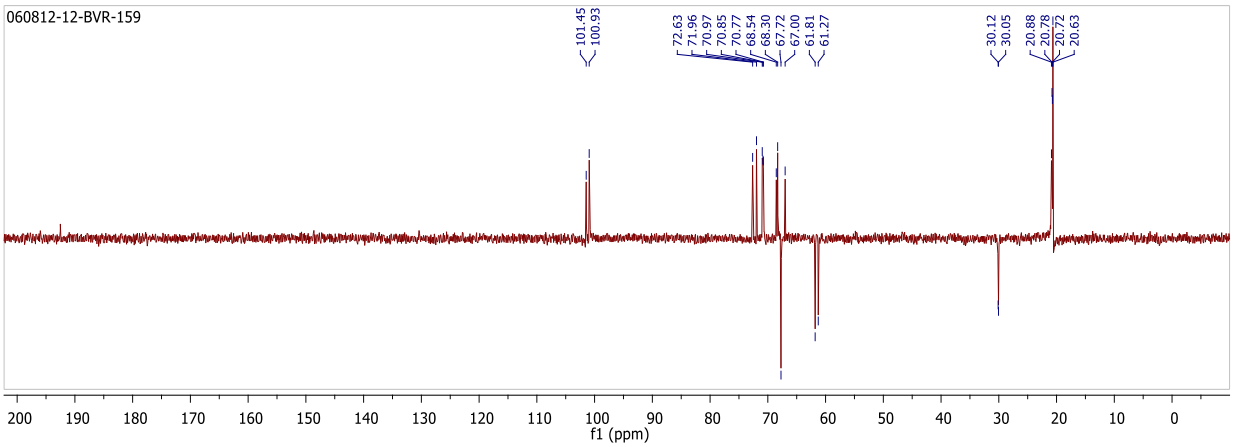
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **76**



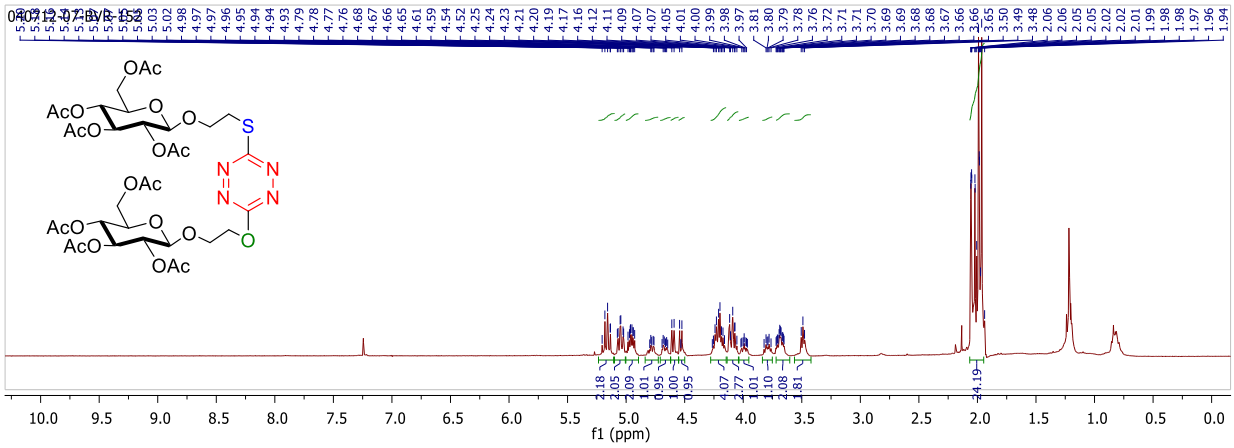
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **76**



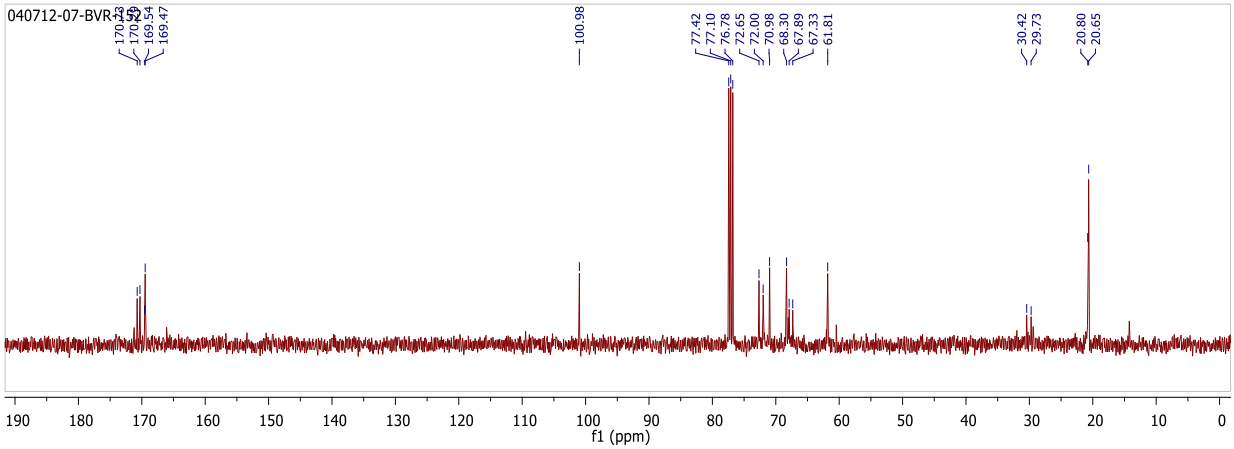
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **76**



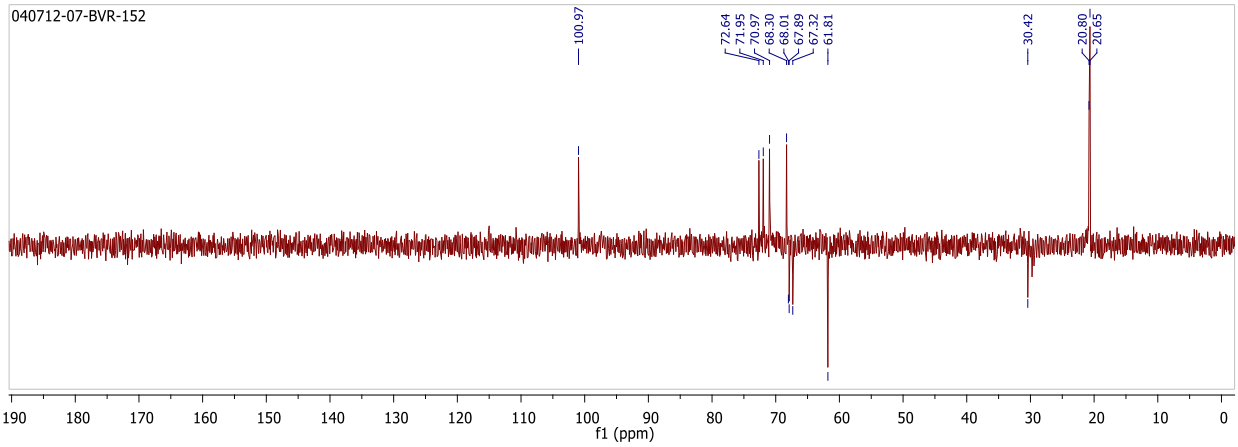
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **77**



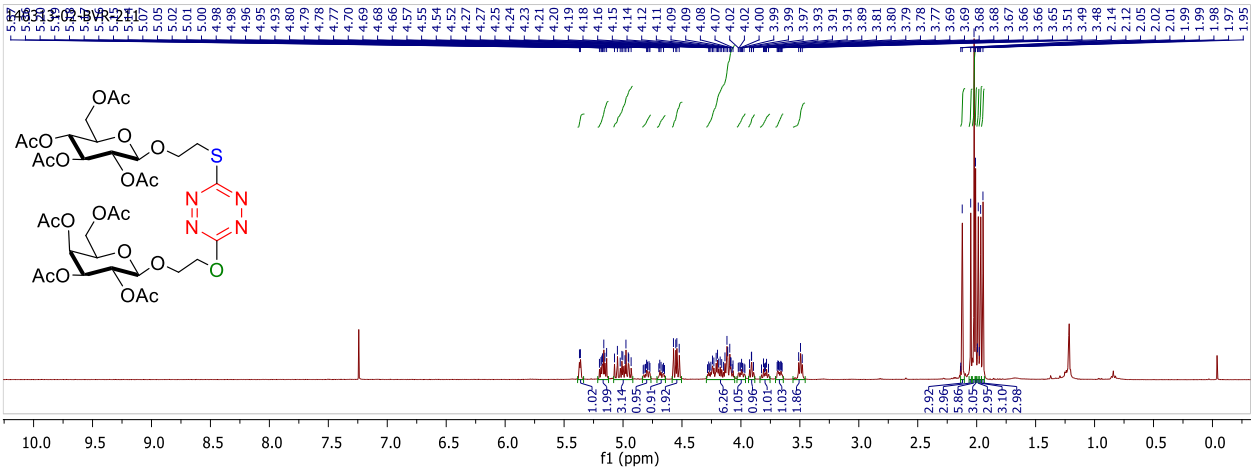
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **77**



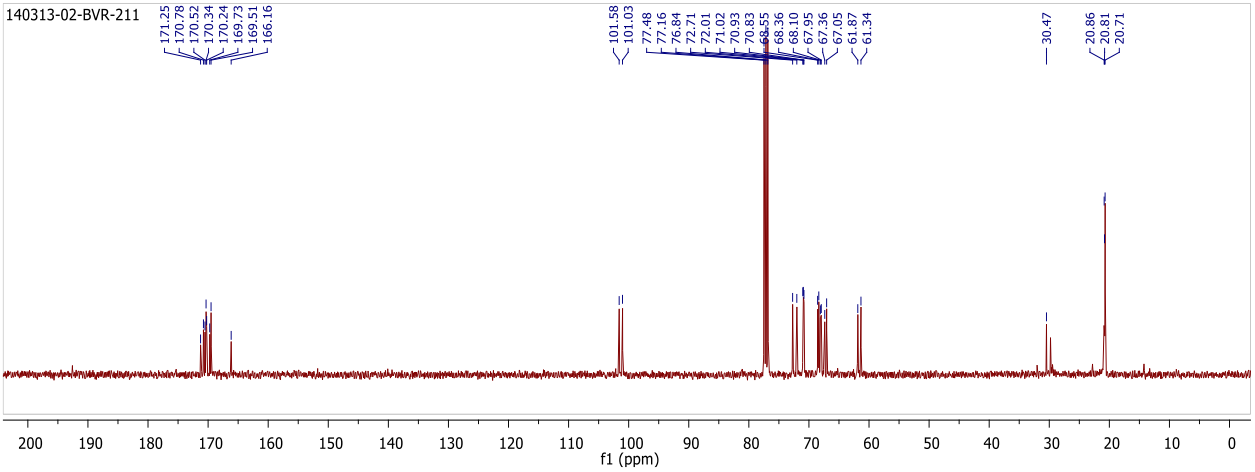
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **77**



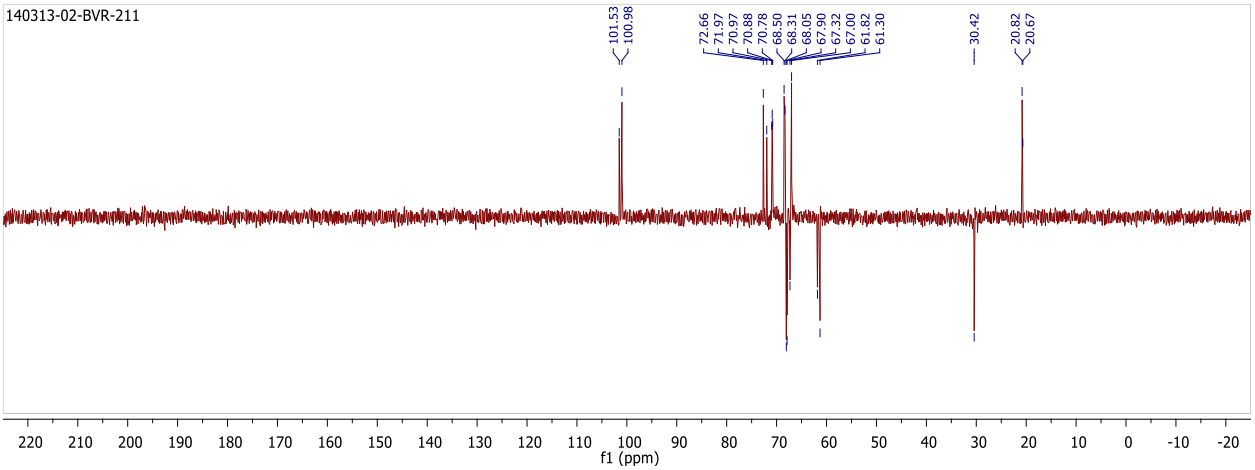
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **79**



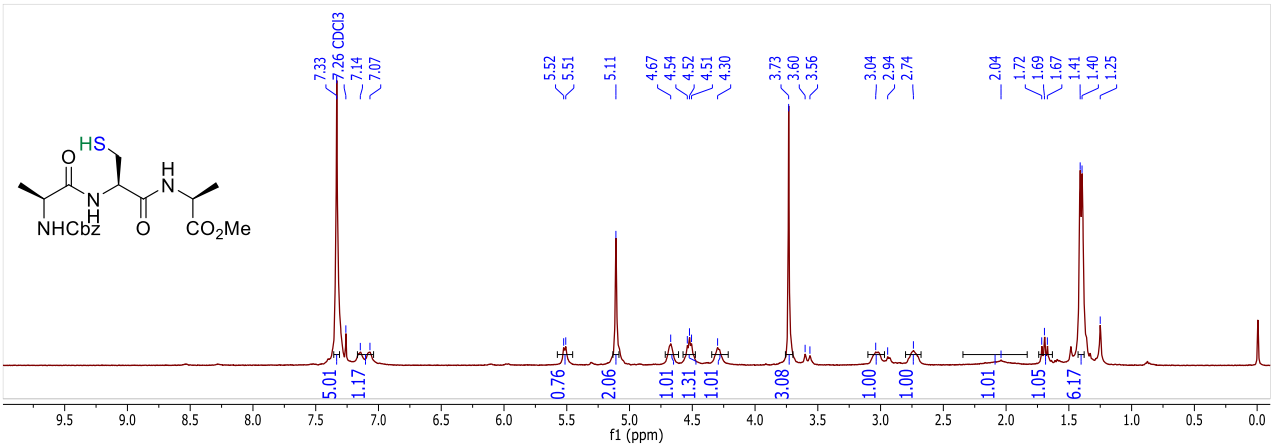
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **79**



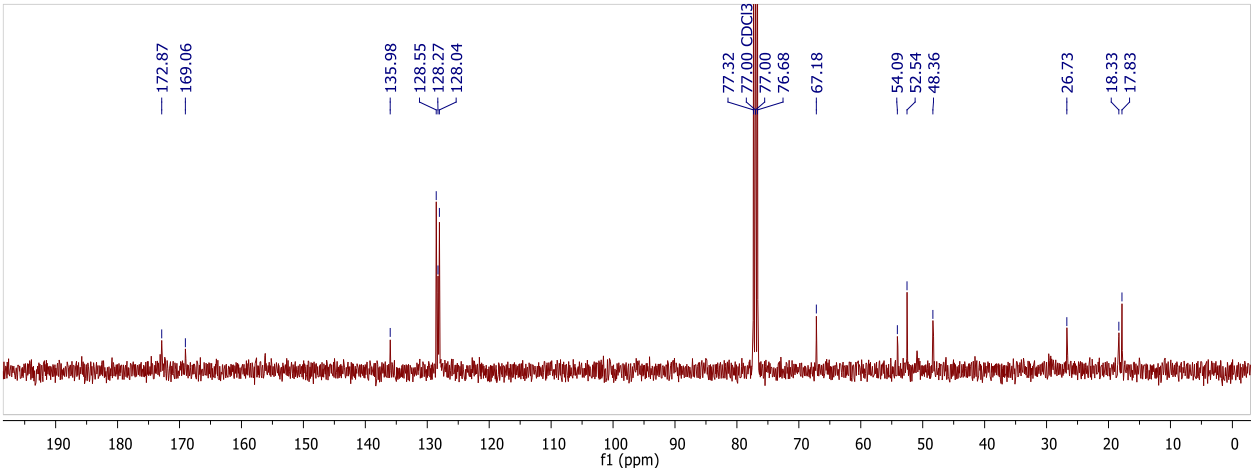
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **79**



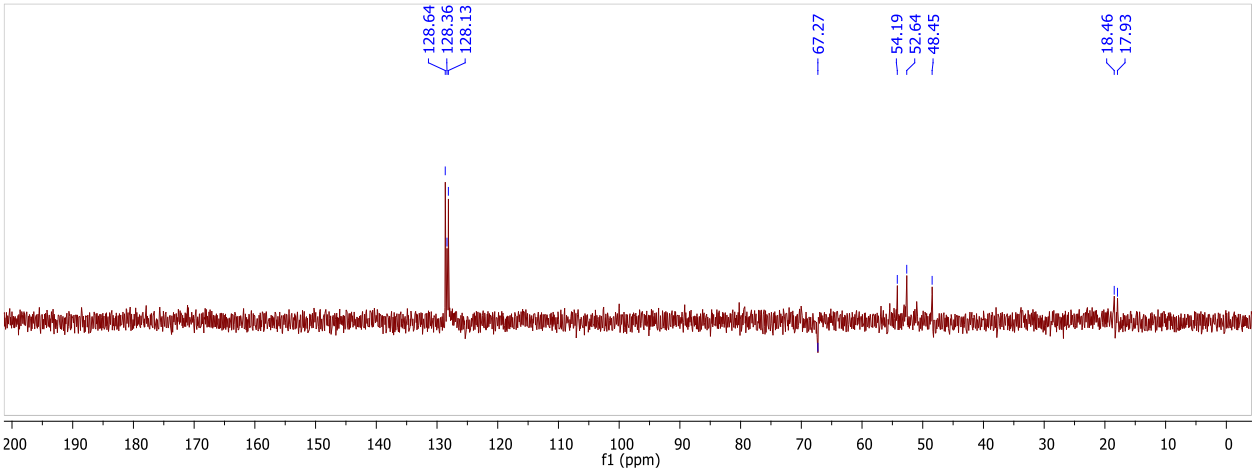
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **85**



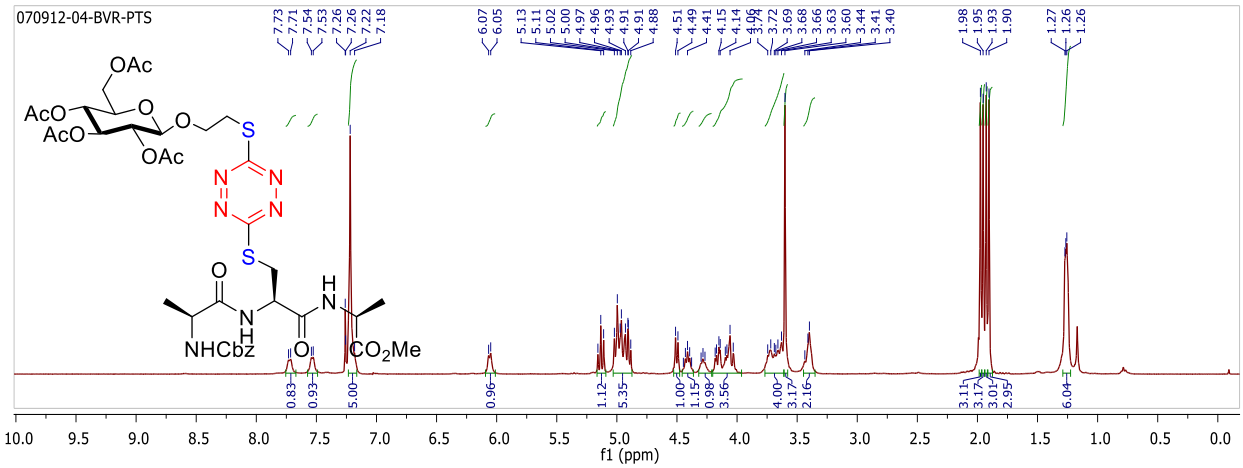
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **85**



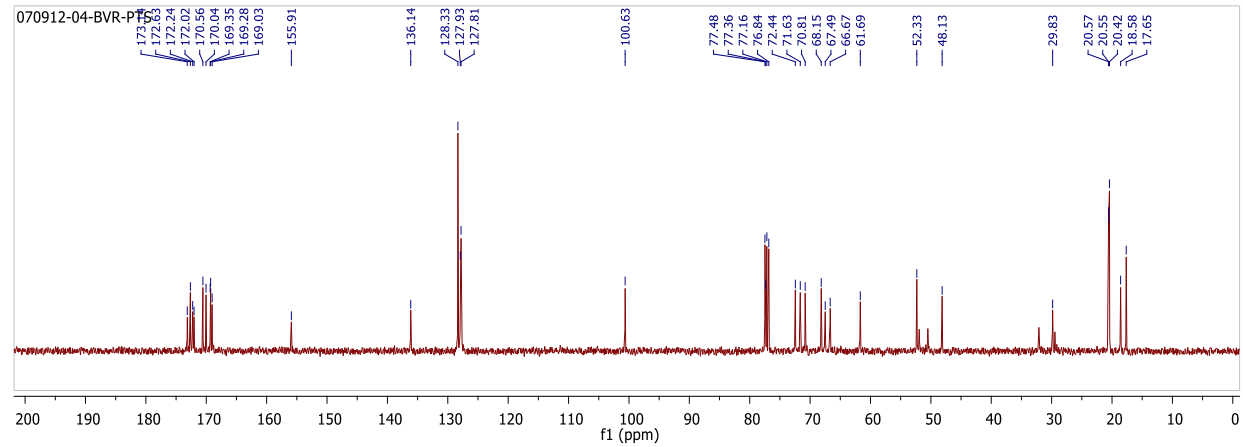
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **85**



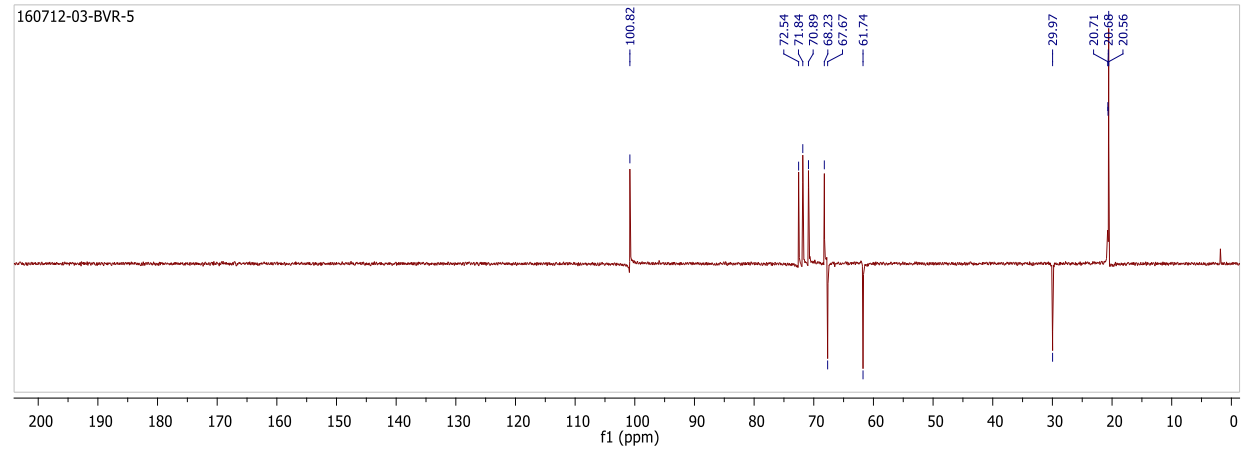
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **86**



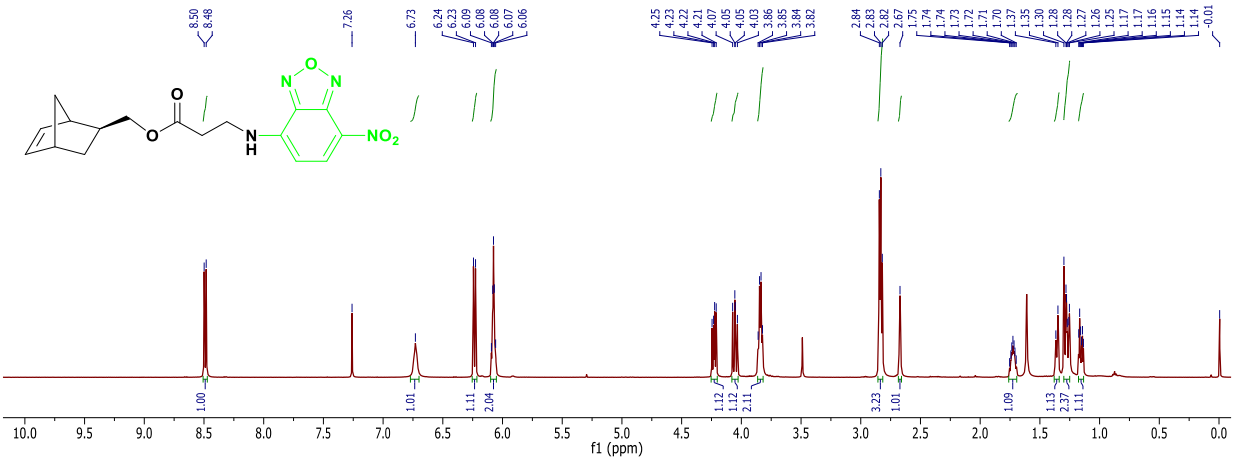
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **86**



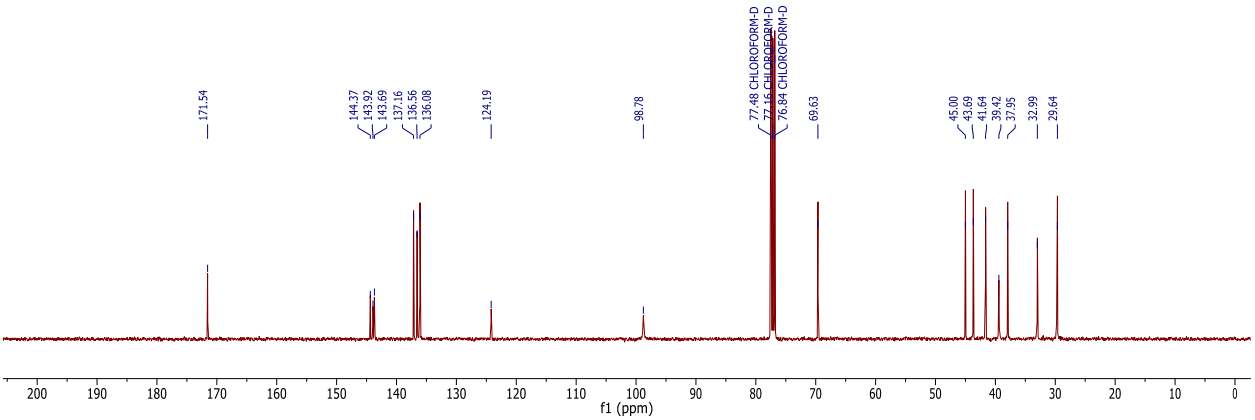
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **86**



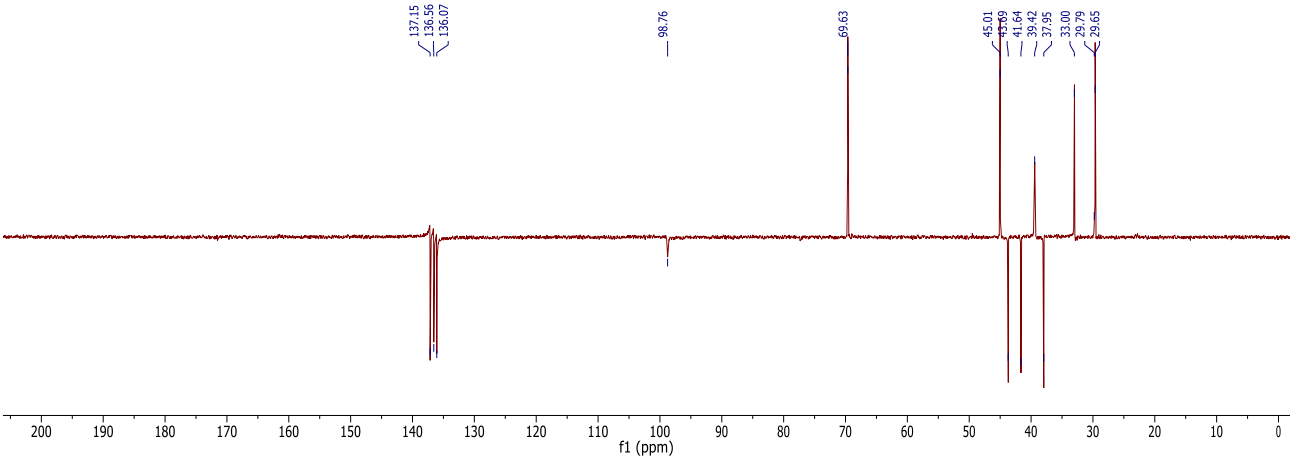
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **96**



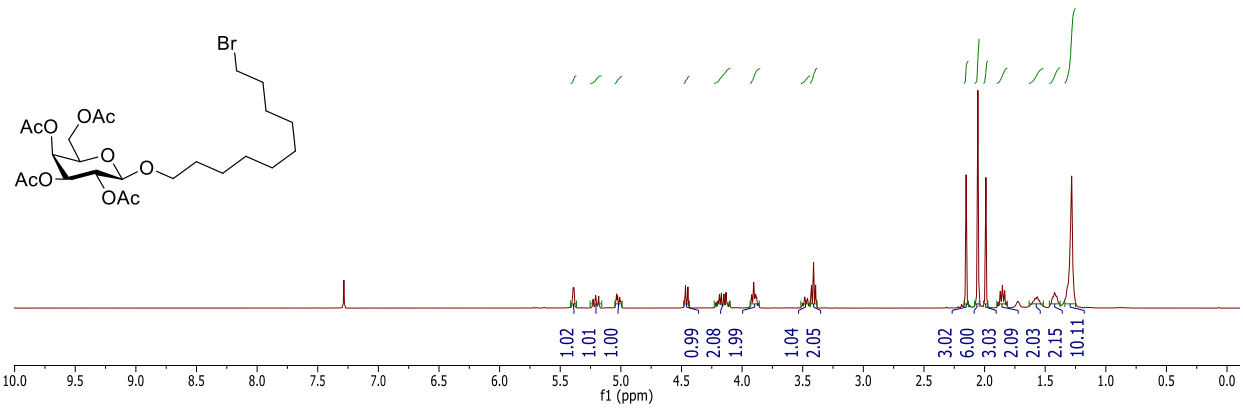
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **96**



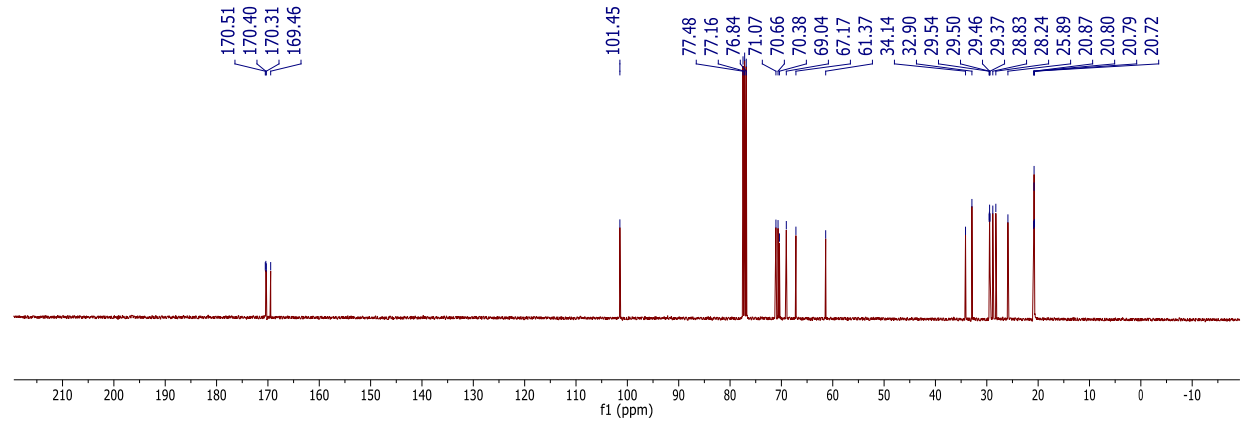
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **96**



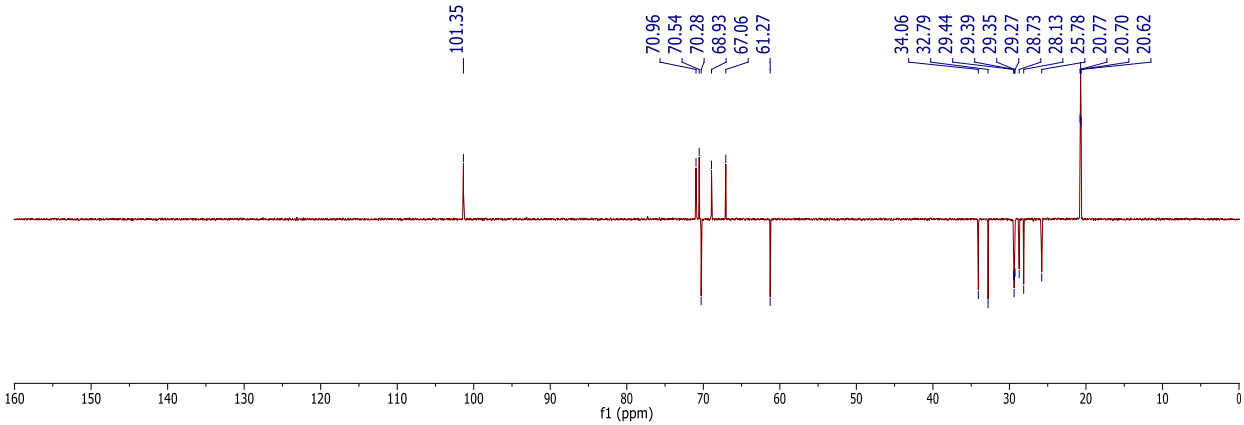
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **115**



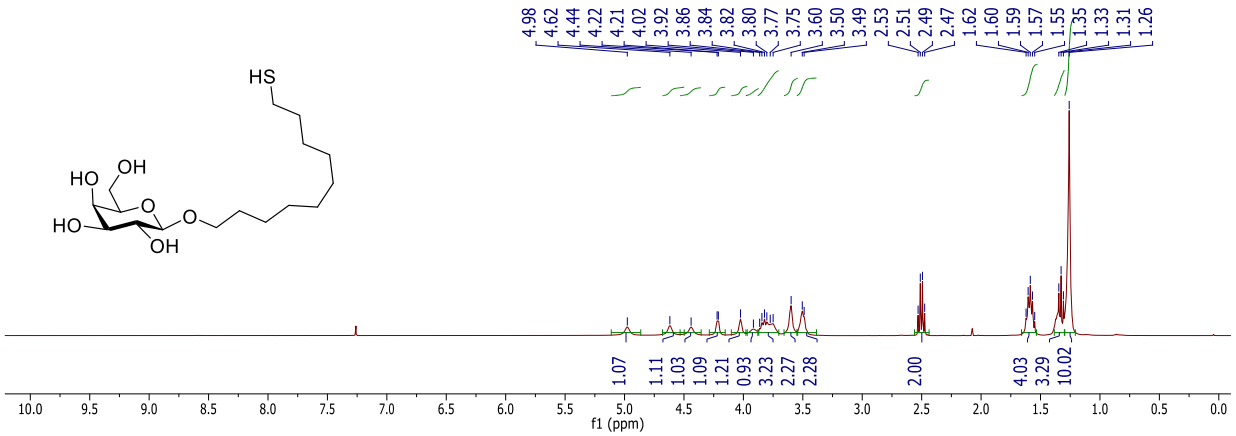
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **115**



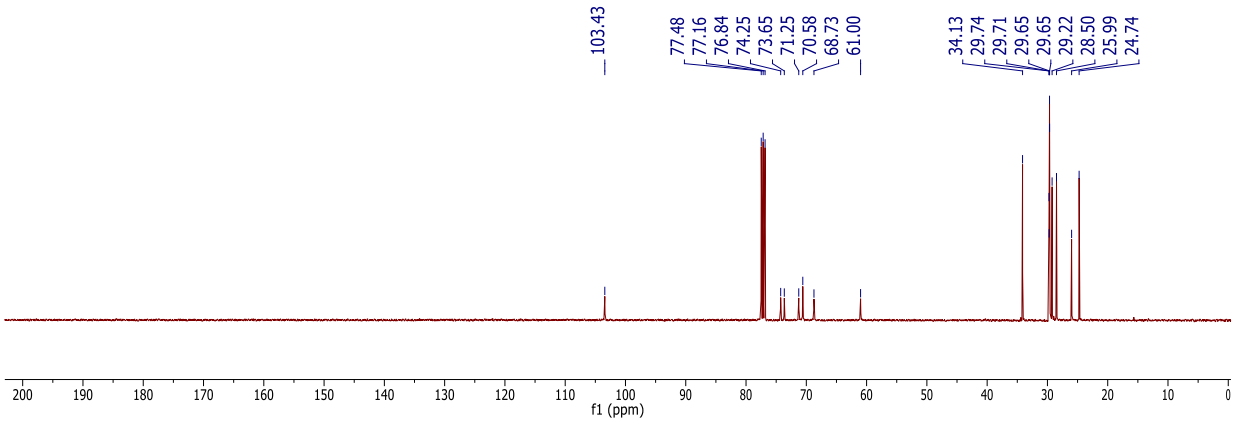
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **115**



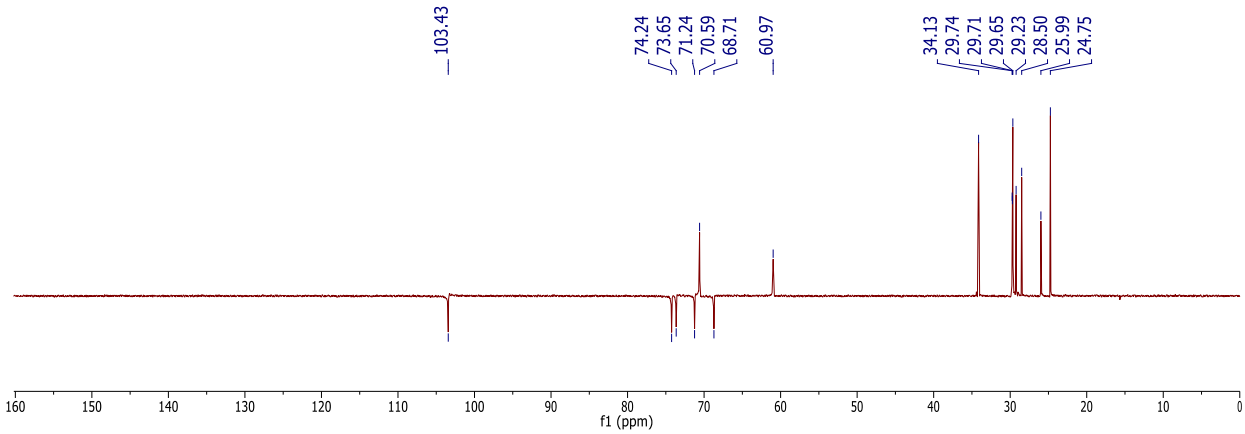
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **116**



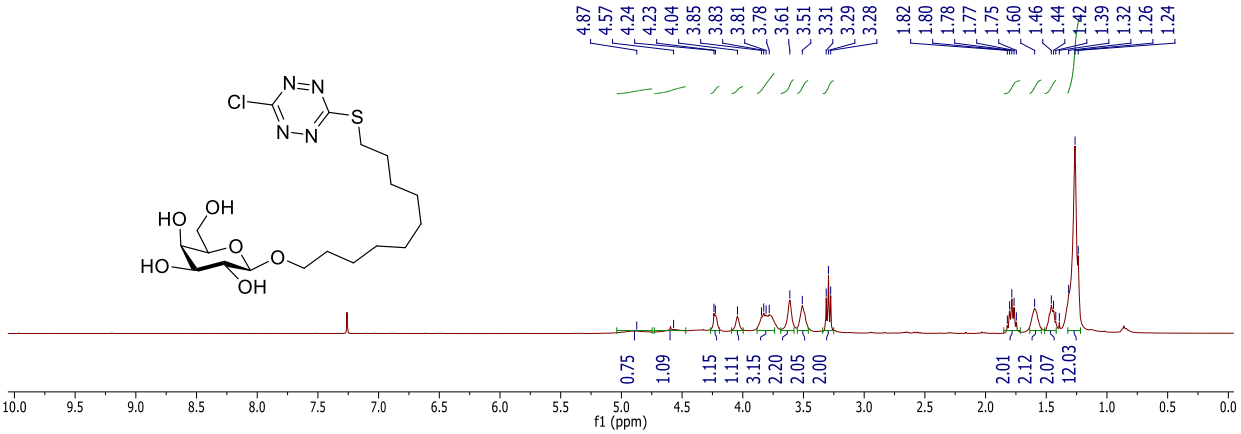
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **116**



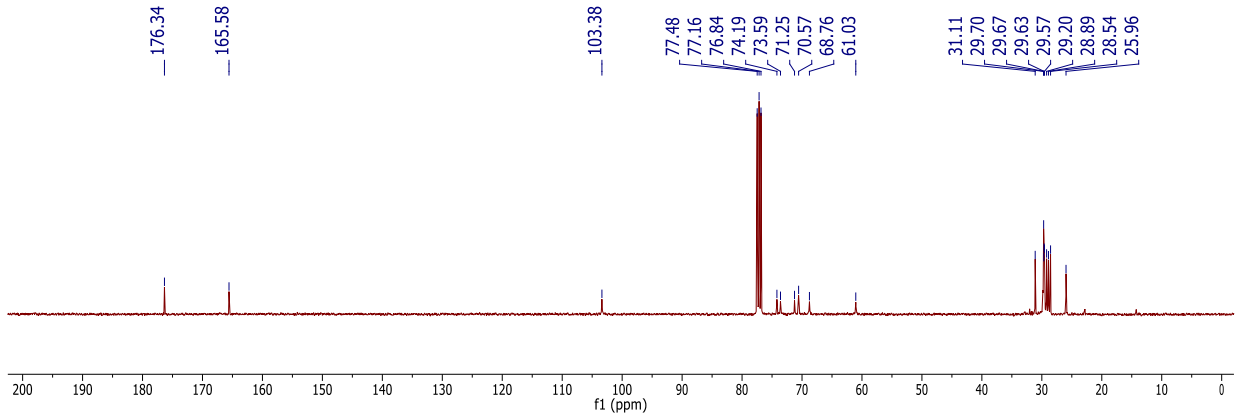
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **116**



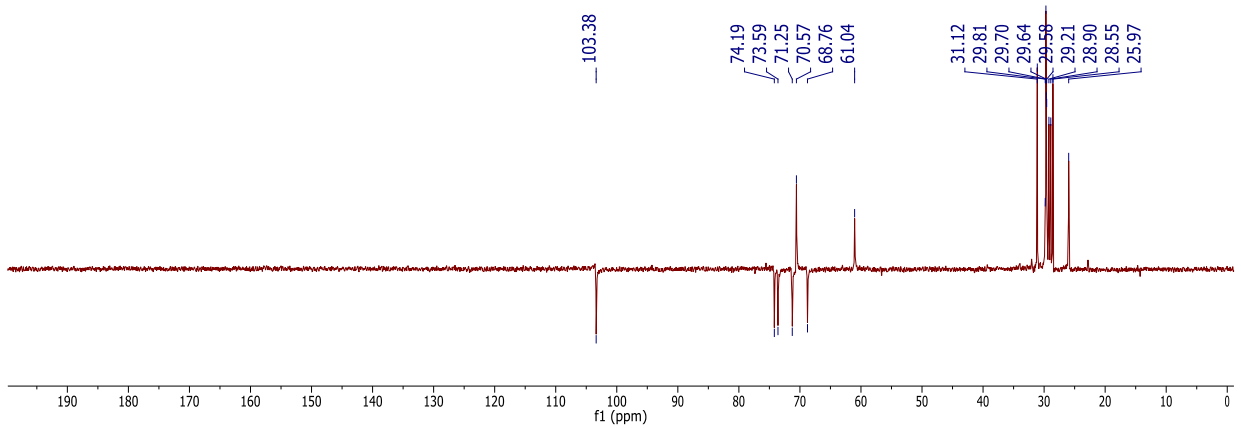
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **117**



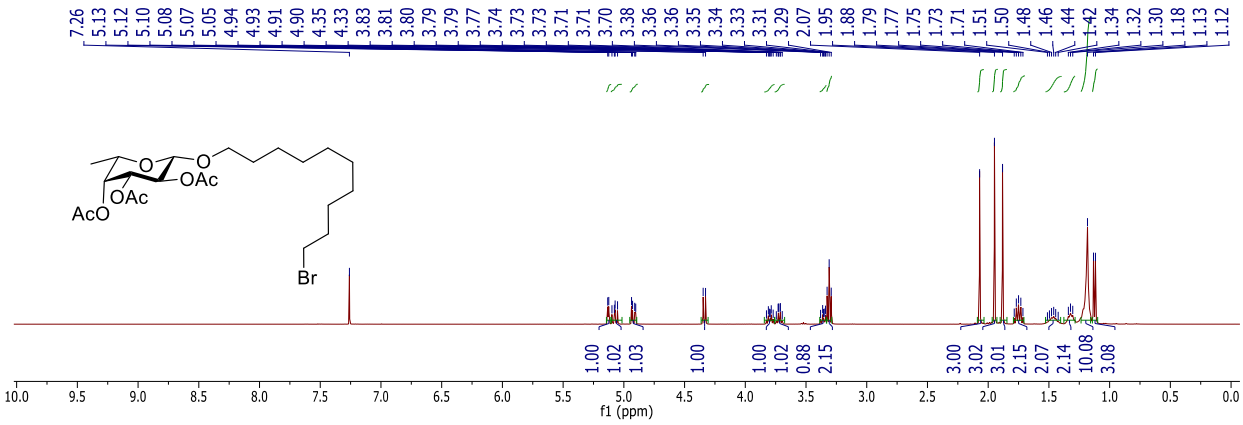
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **117**



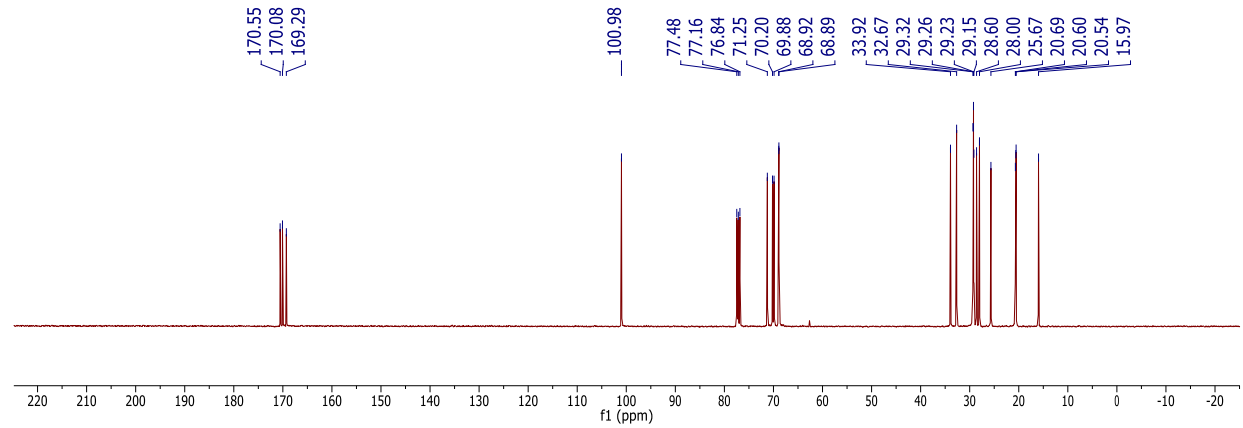
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **117**



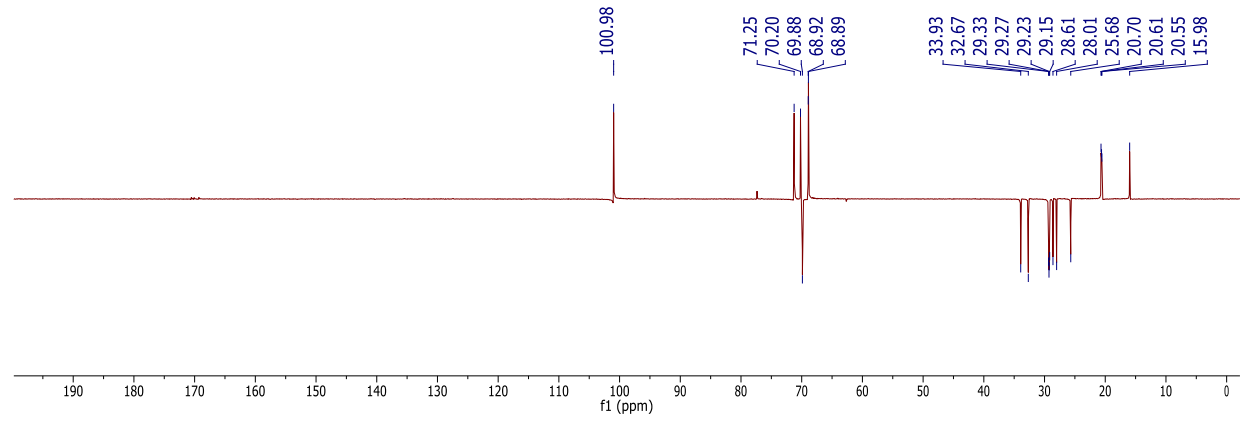
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **120**



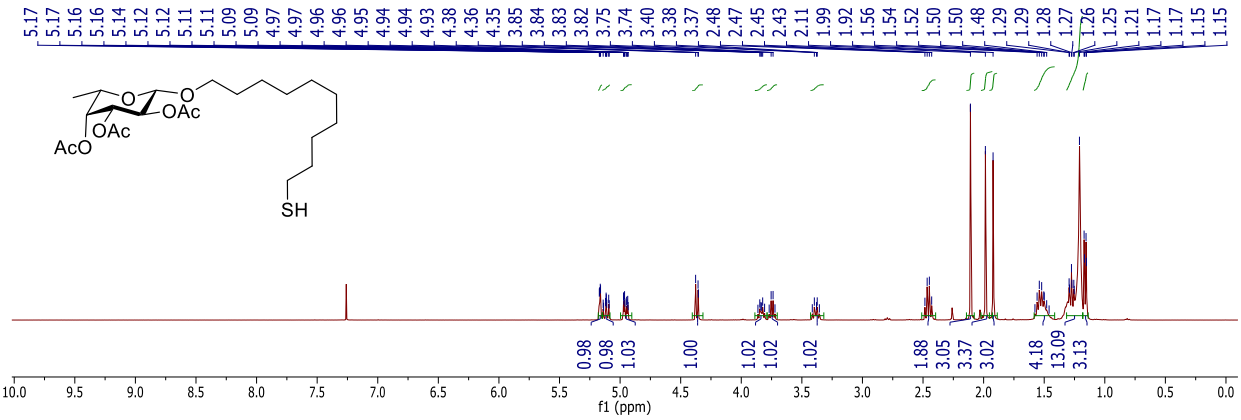
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **120**



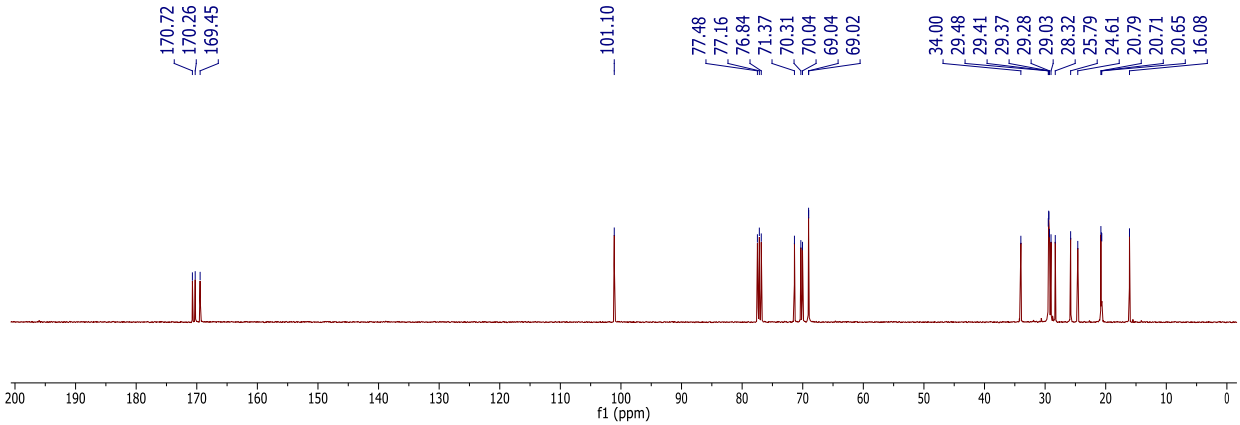
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **120**



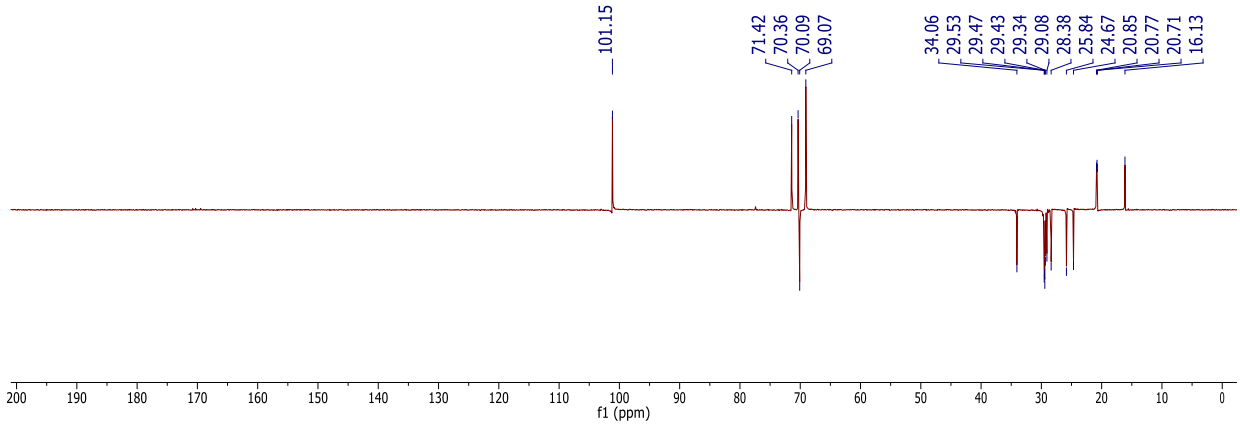
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **121**



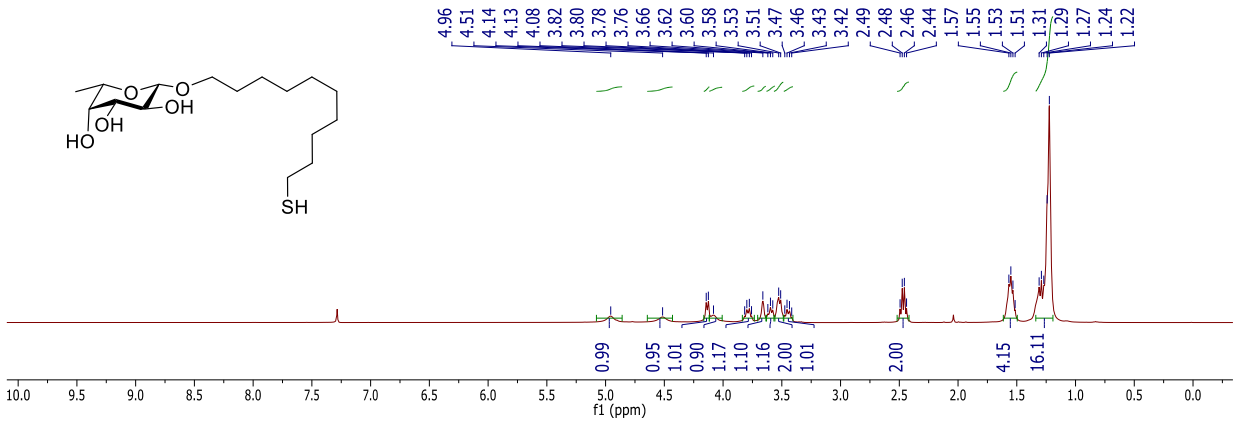
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **121**



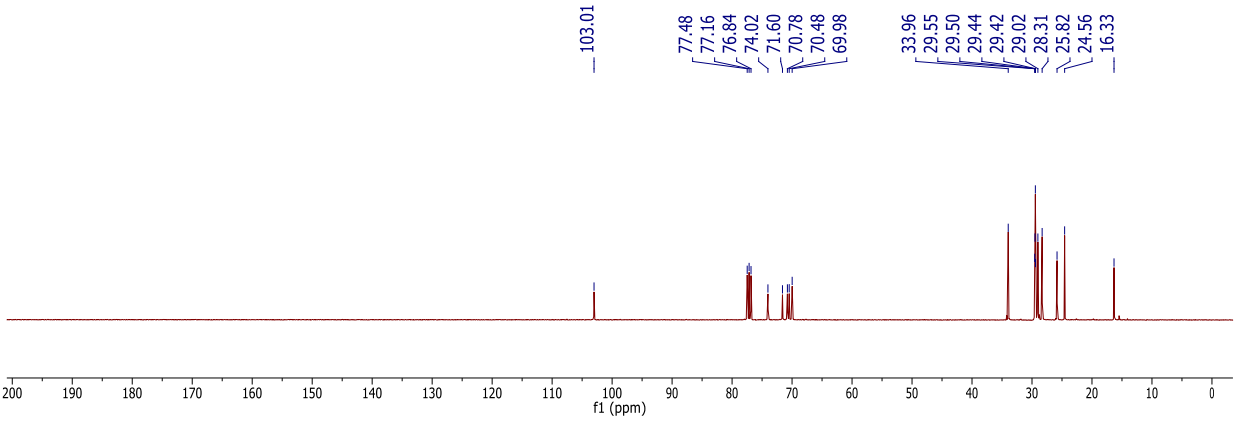
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **121**



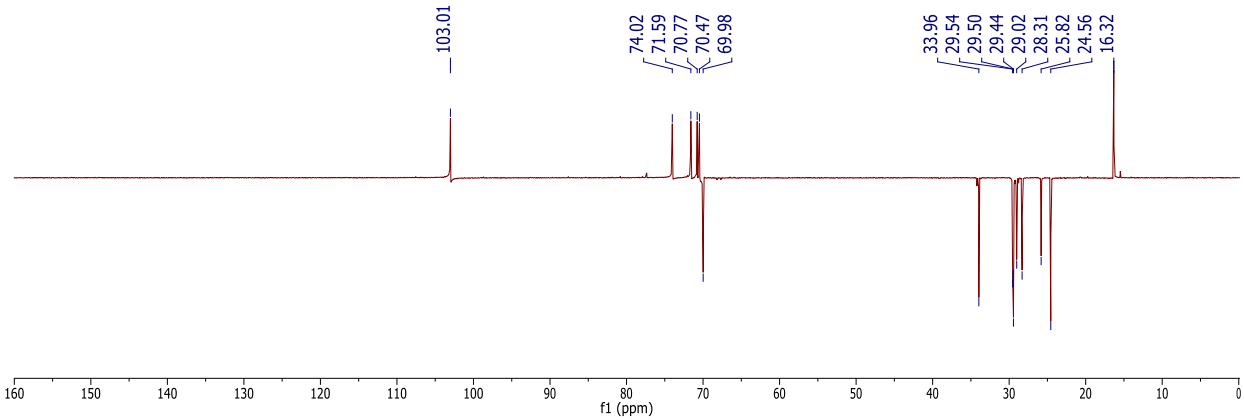
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **122**



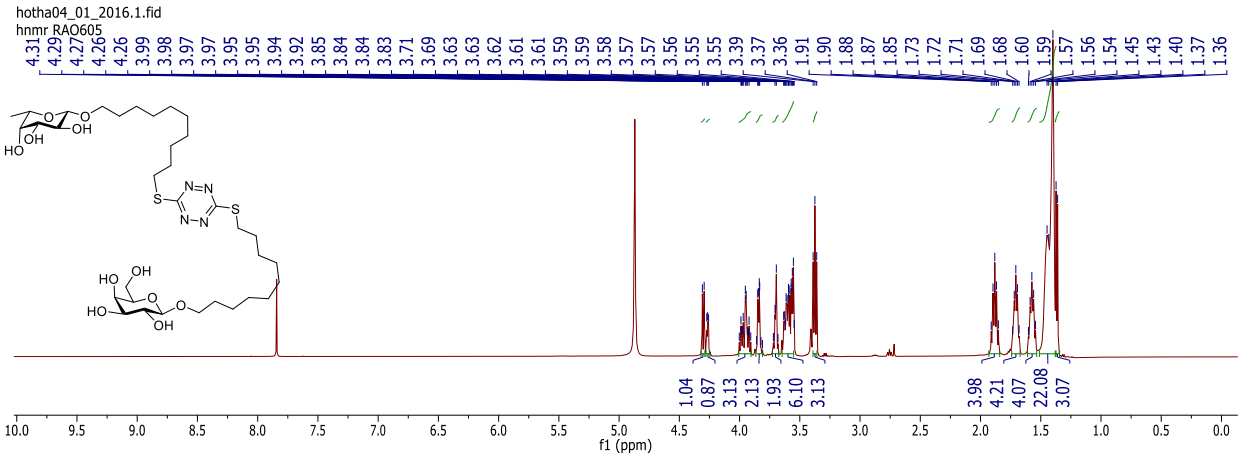
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **122**



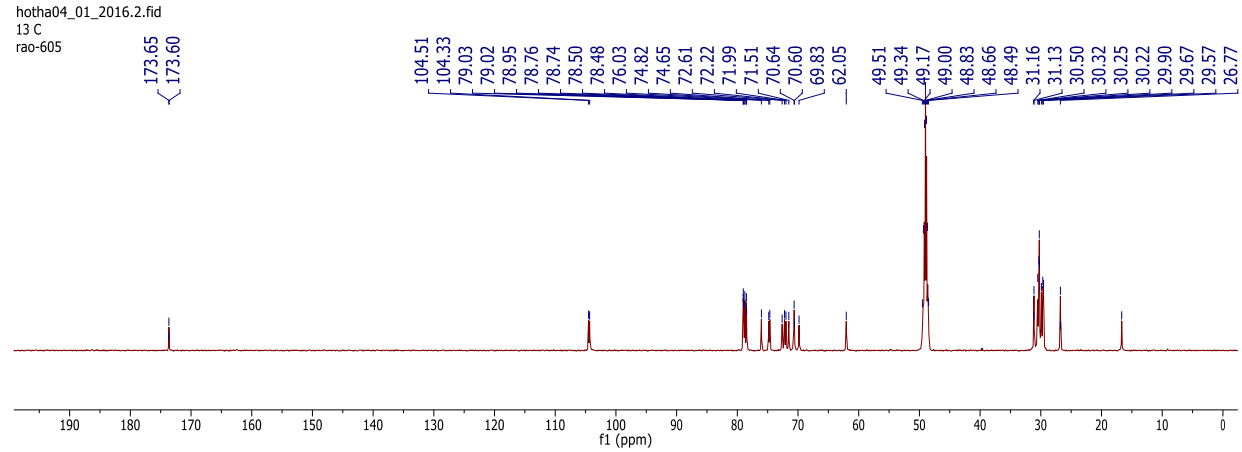
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **122**



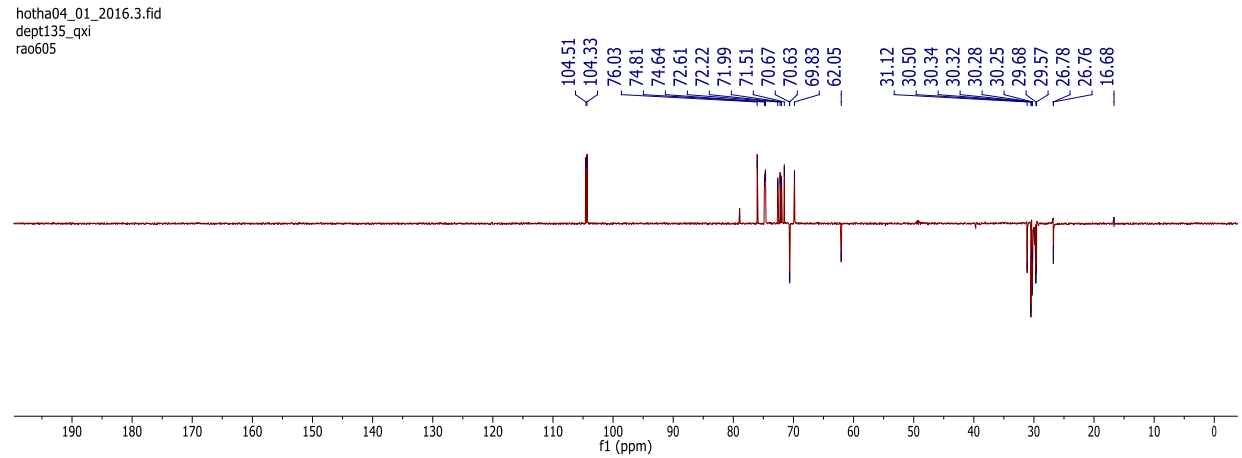
^{1}H NMR Spectrum (500.20MHz, $CDCl_3$) of Compound **123**



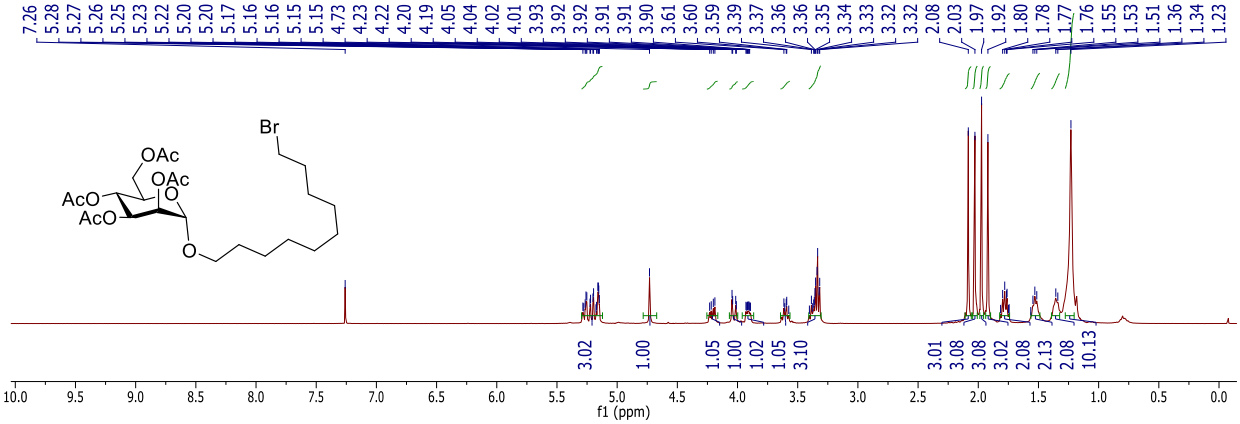
^{13}C NMR Spectrum (125.79MHz, $CDCl_3$) of Compound **123**



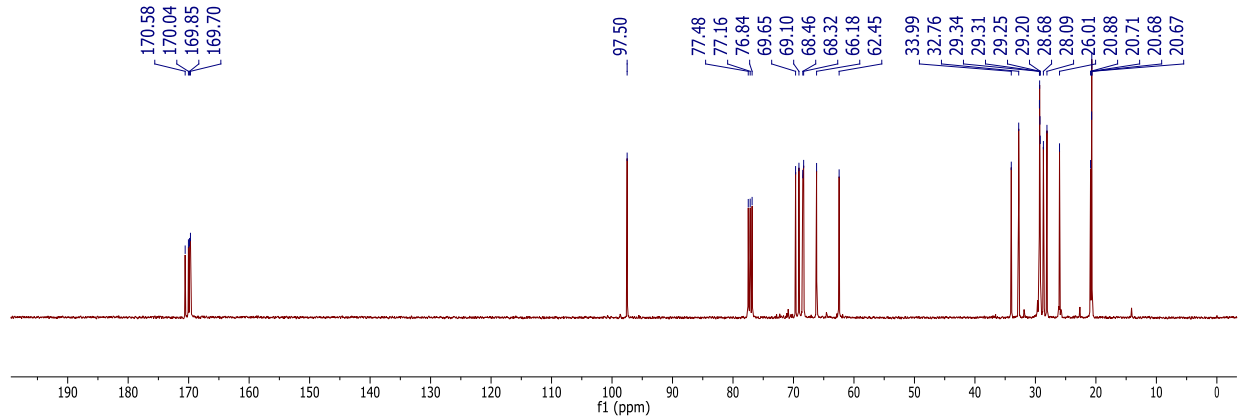
DEPT NMR Spectrum (125.79MHz, $CDCl_3$) of Compound **123**



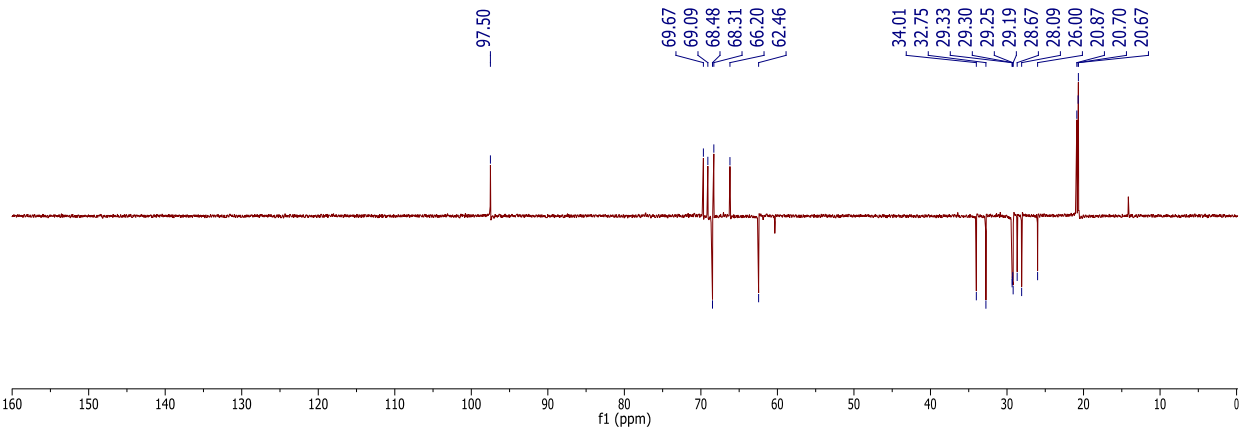
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **125**



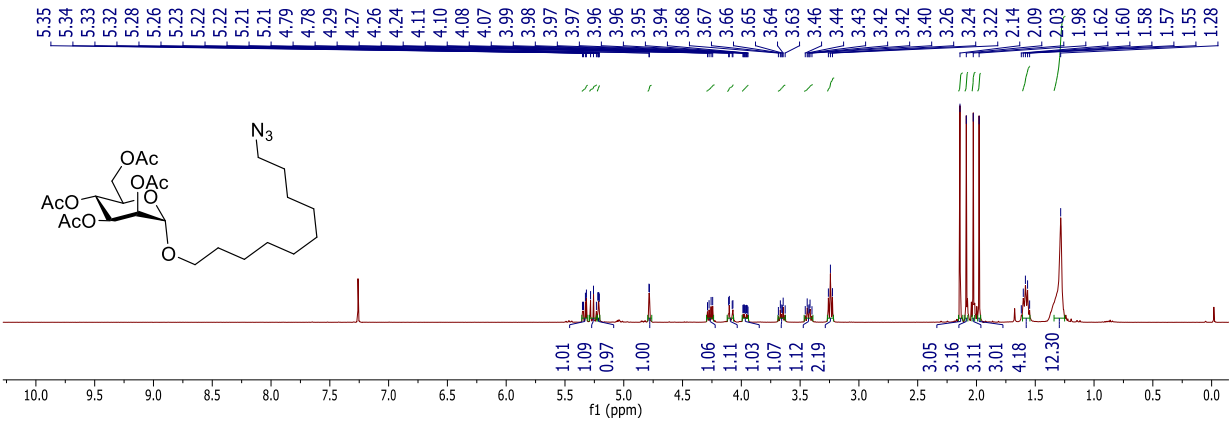
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **125**



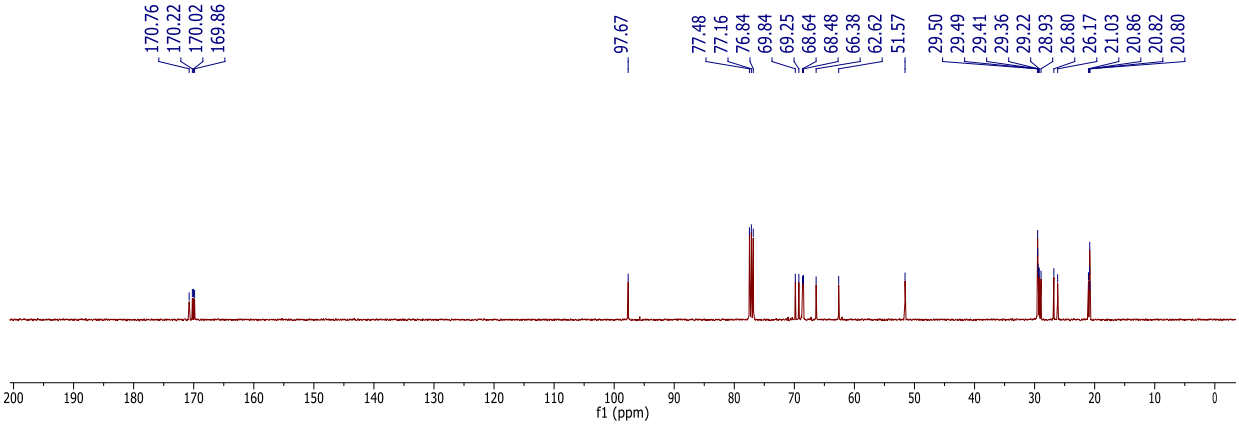
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **125**



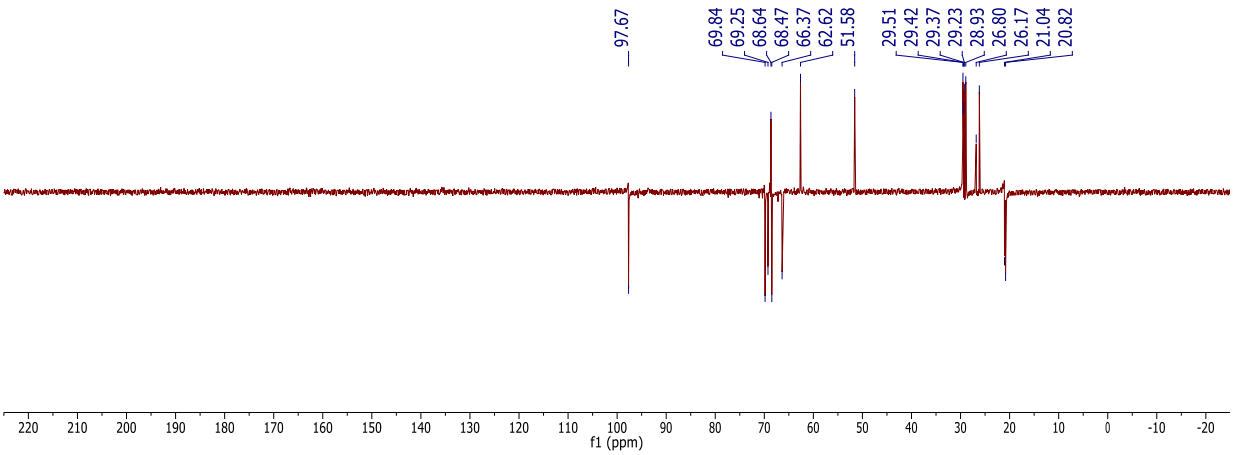
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **126**



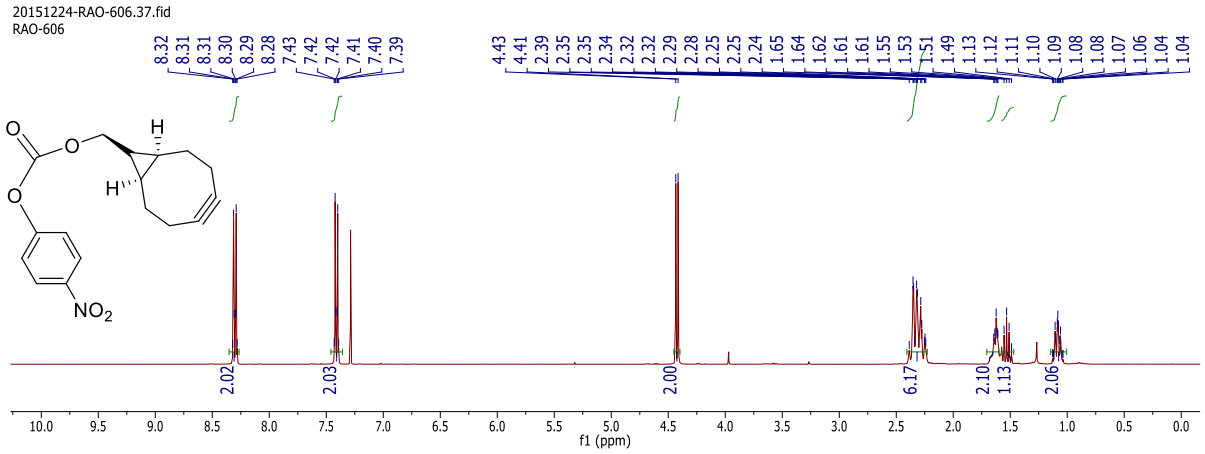
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **126**



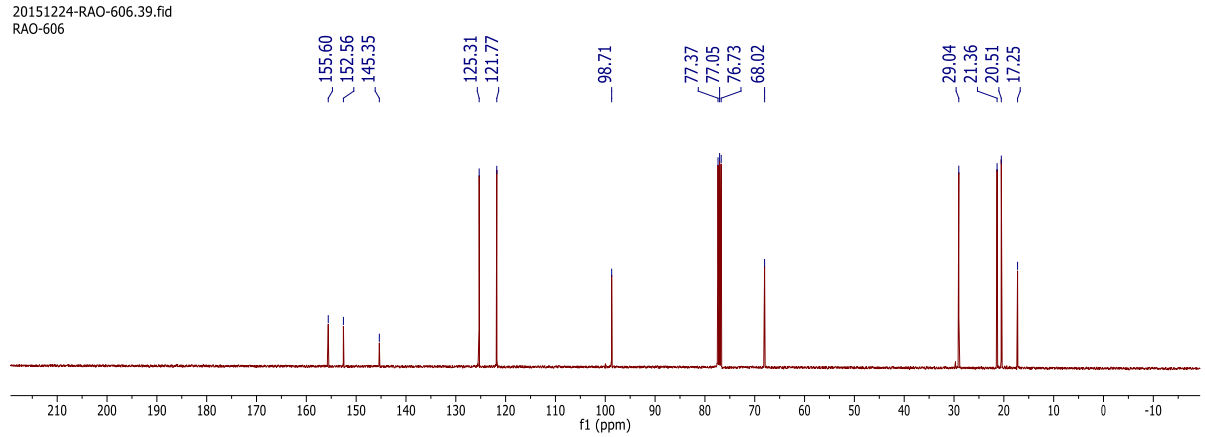
DEPTNMR Spectrum (100.53MHz, $CDCl_3$) of Compound **126**



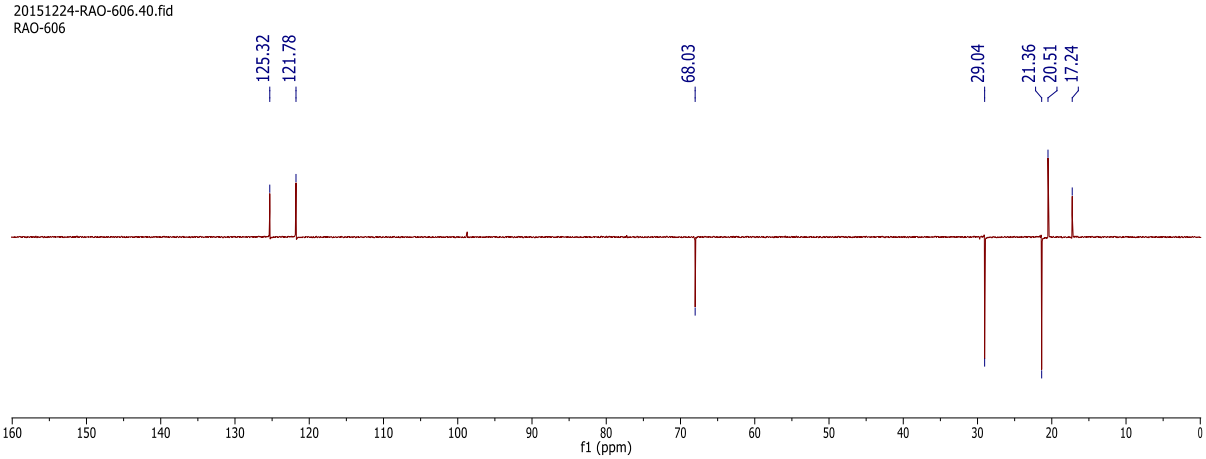
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **129**



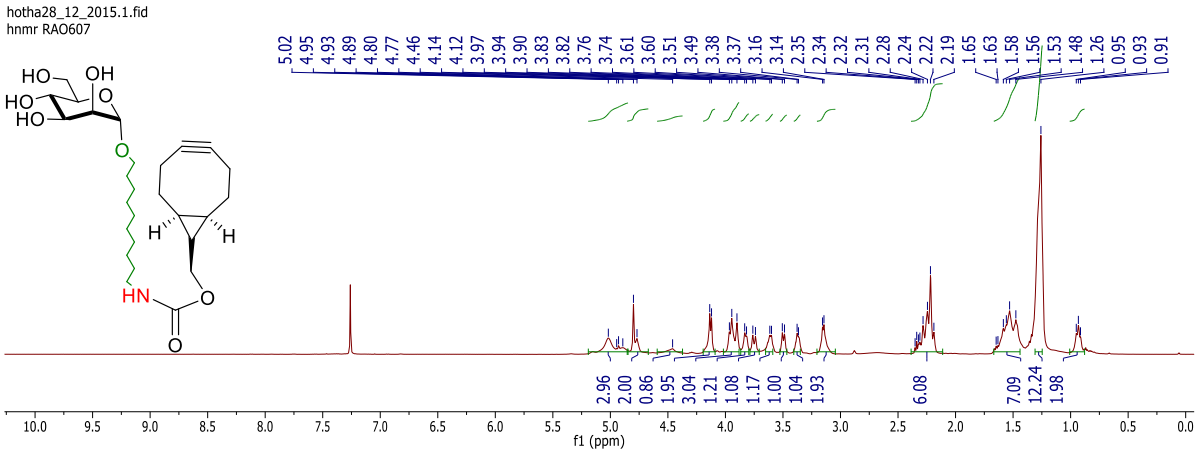
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **129**



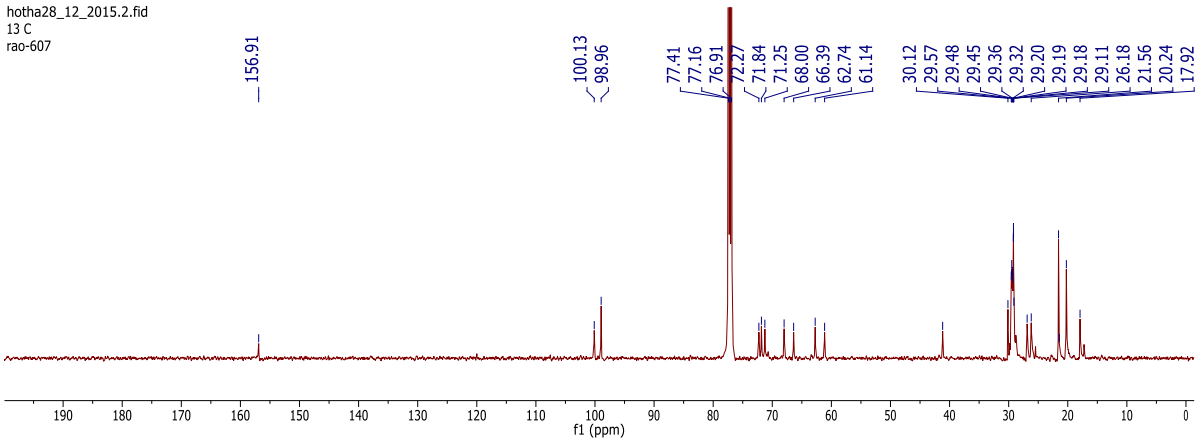
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **129**



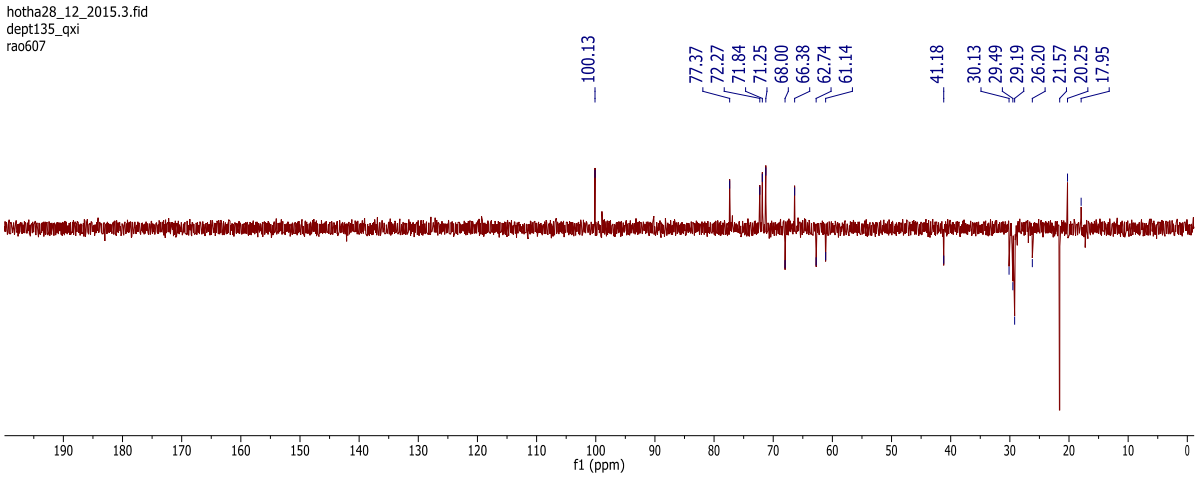
^{1}H NMR Spectrum (500.20MHz, $CDCl_3$) of Compound **130**



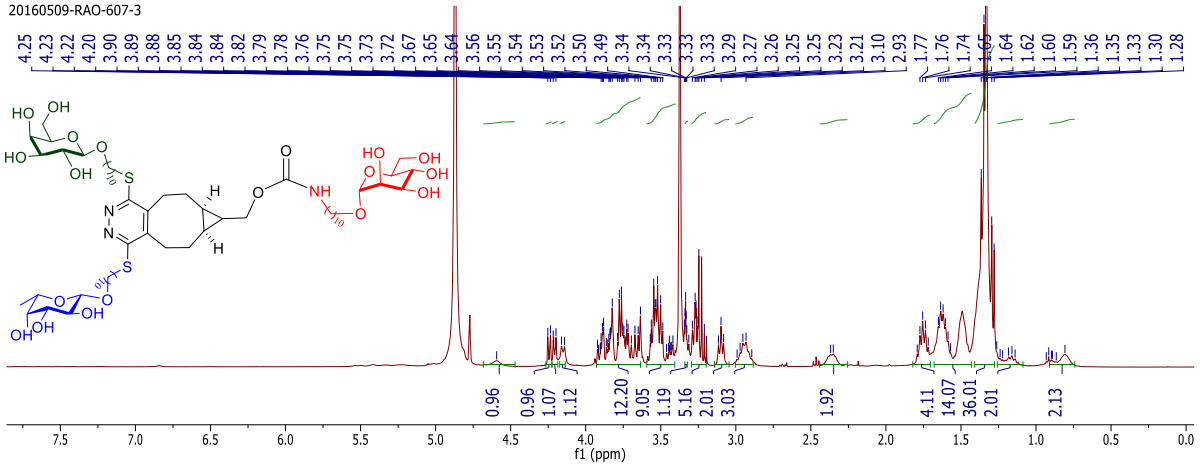
^{13}C NMR Spectrum (125.79MHz, $CDCl_3$) of Compound **130**



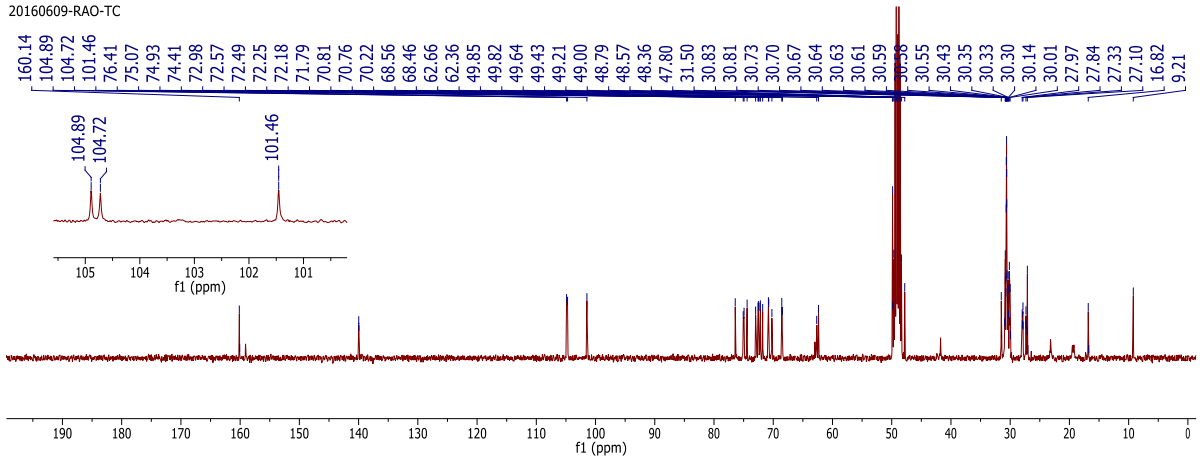
DEPT NMR Spectrum (125.79MHz, $CDCl_3$) of Compound **130**



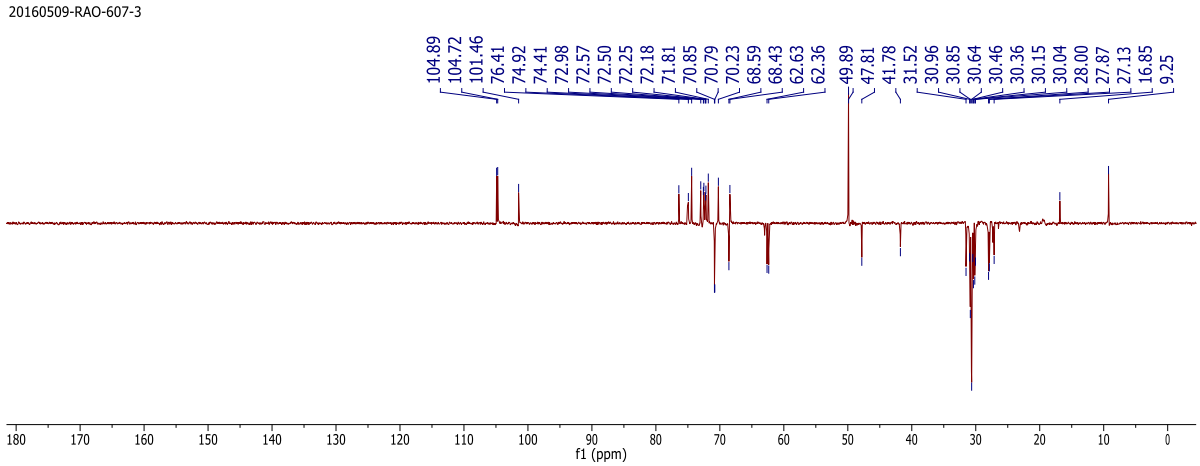
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **131**



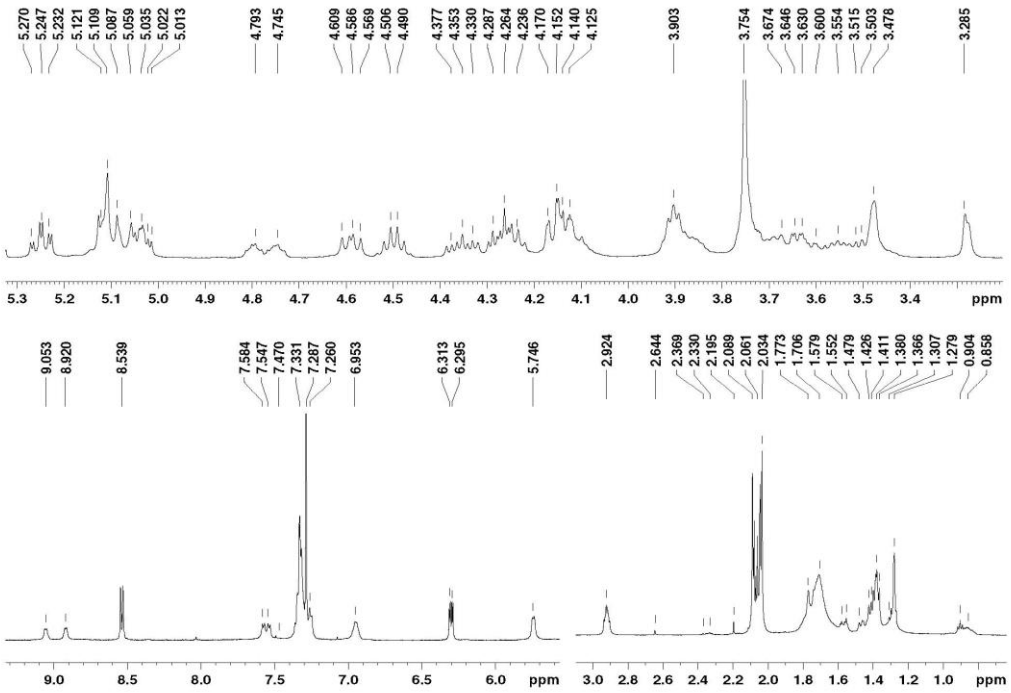
^{13}C NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **131**



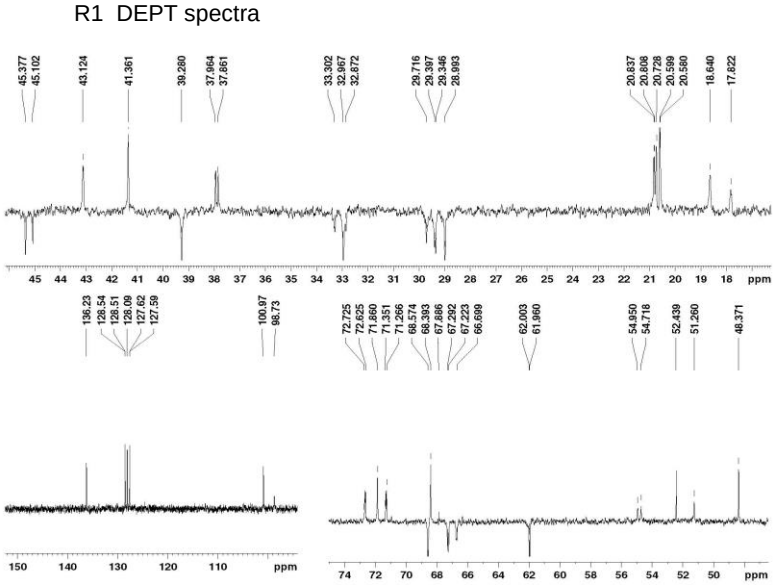
DEPT NMR Spectrum (100.53MHz, $CDCl_3$) of Compound **131**



^{1}H NMR Spectrum (500.13 MHz, $CDCl_3$) of Compound **97** (Fraction 1)



DEPT NMR Spectrum (125.76 MHz, $CDCl_3$) of Compound **97** (Fraction 1)



^{13}C NMR Spectrum (125.76MHz, $CDCl_3$) of Compound **97** (Fraction 1)

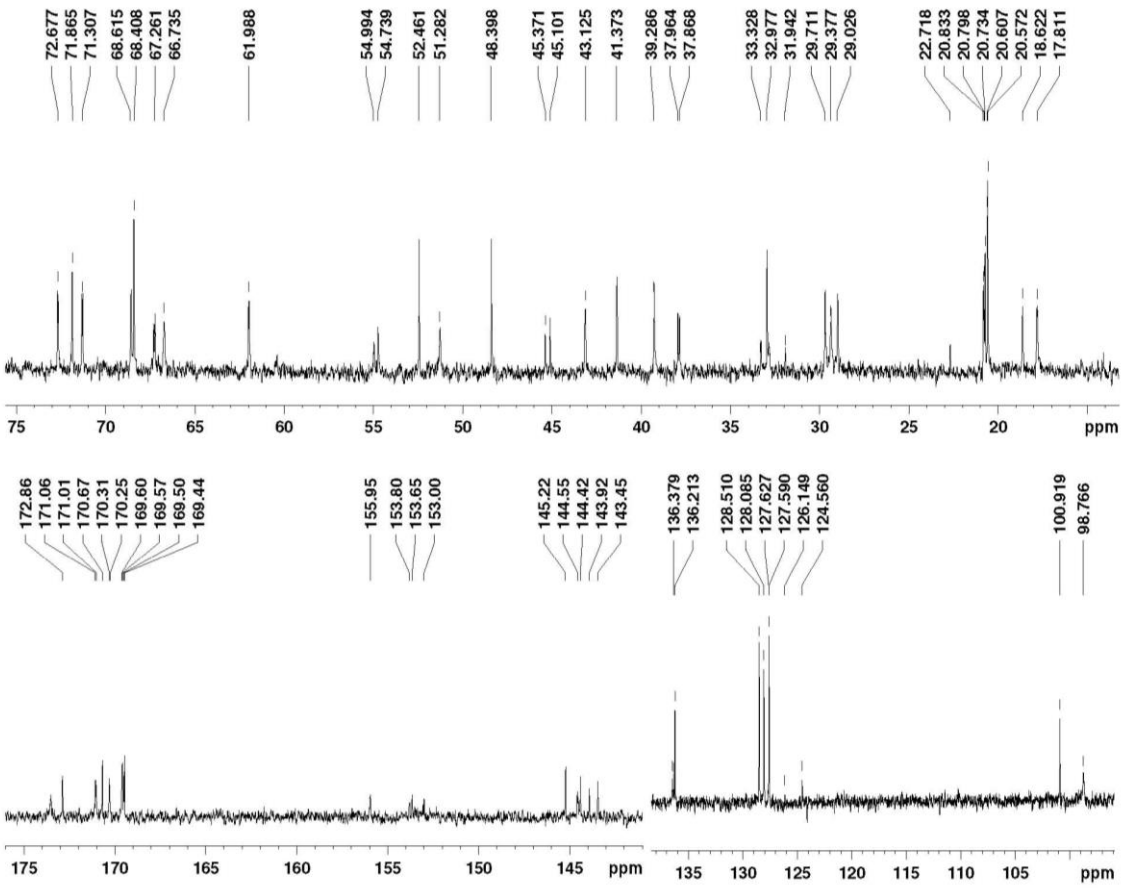


Figure S. Partial COSY spectra of Fraction 1 (500.13 MHz, CDCl3)

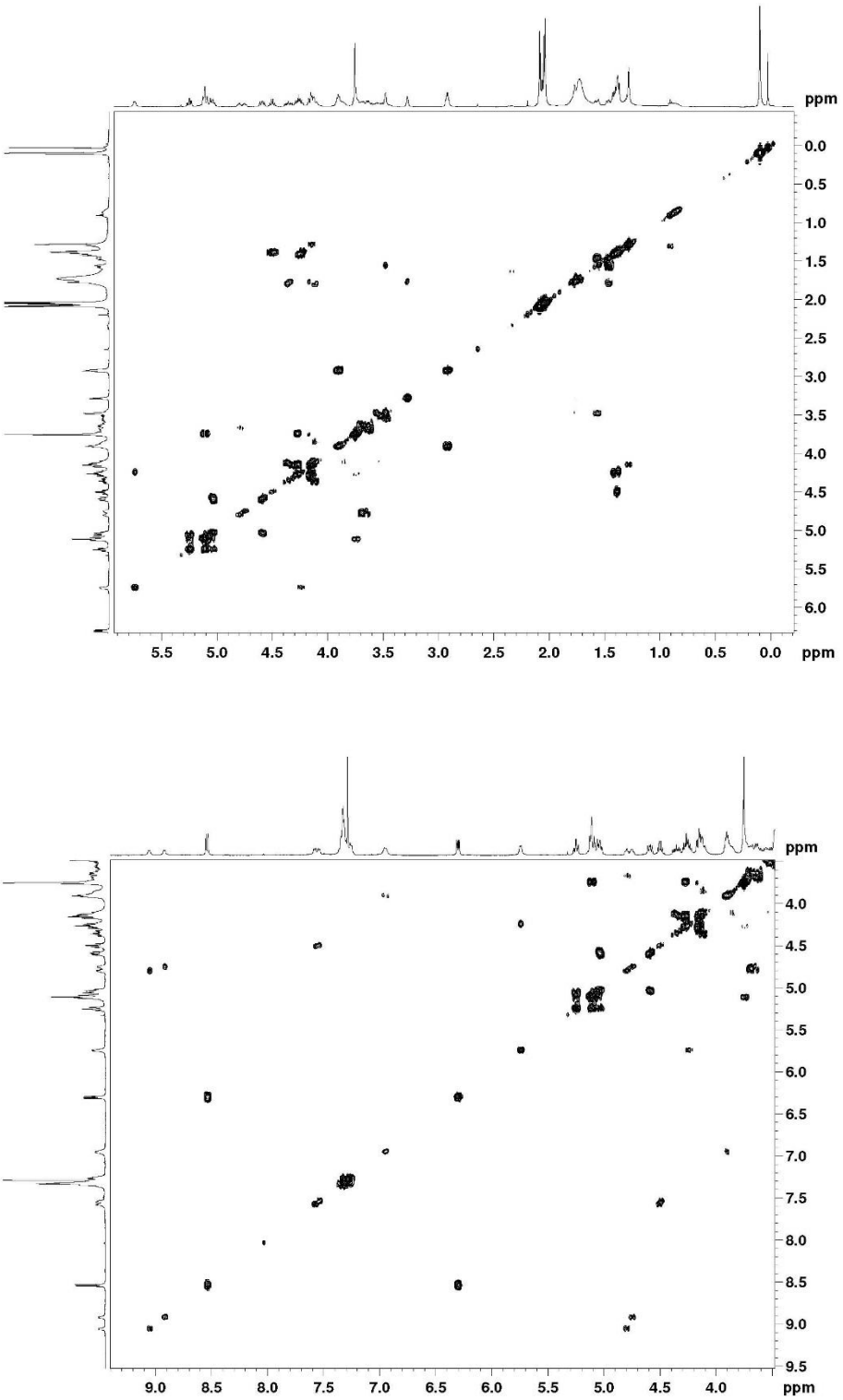


Figure S. Partial TOCSY spectra of Fraction 1 (500.13 MHz, CDCl3)

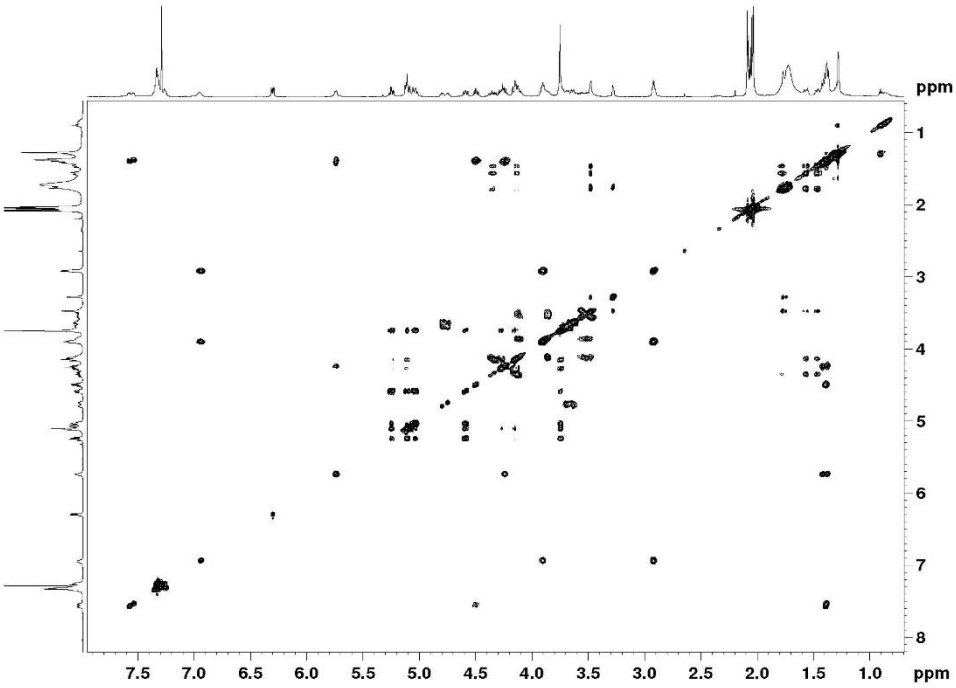


Figure S. Partial NOESY spectra of Fraction1 (500.13 MHz, CDCl3)

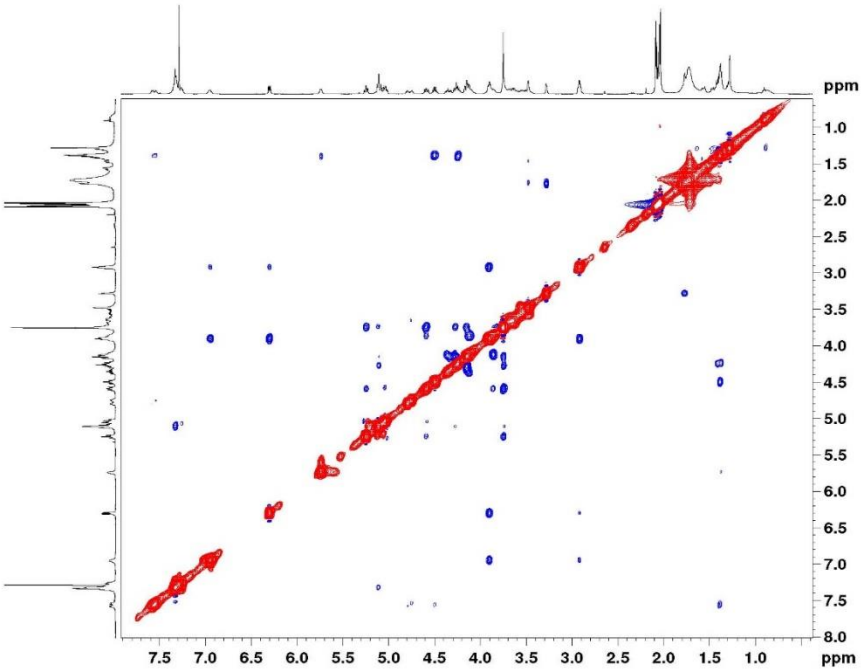


Figure S. Partial HSQC spectra of Fraction1 (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, CDCl3)

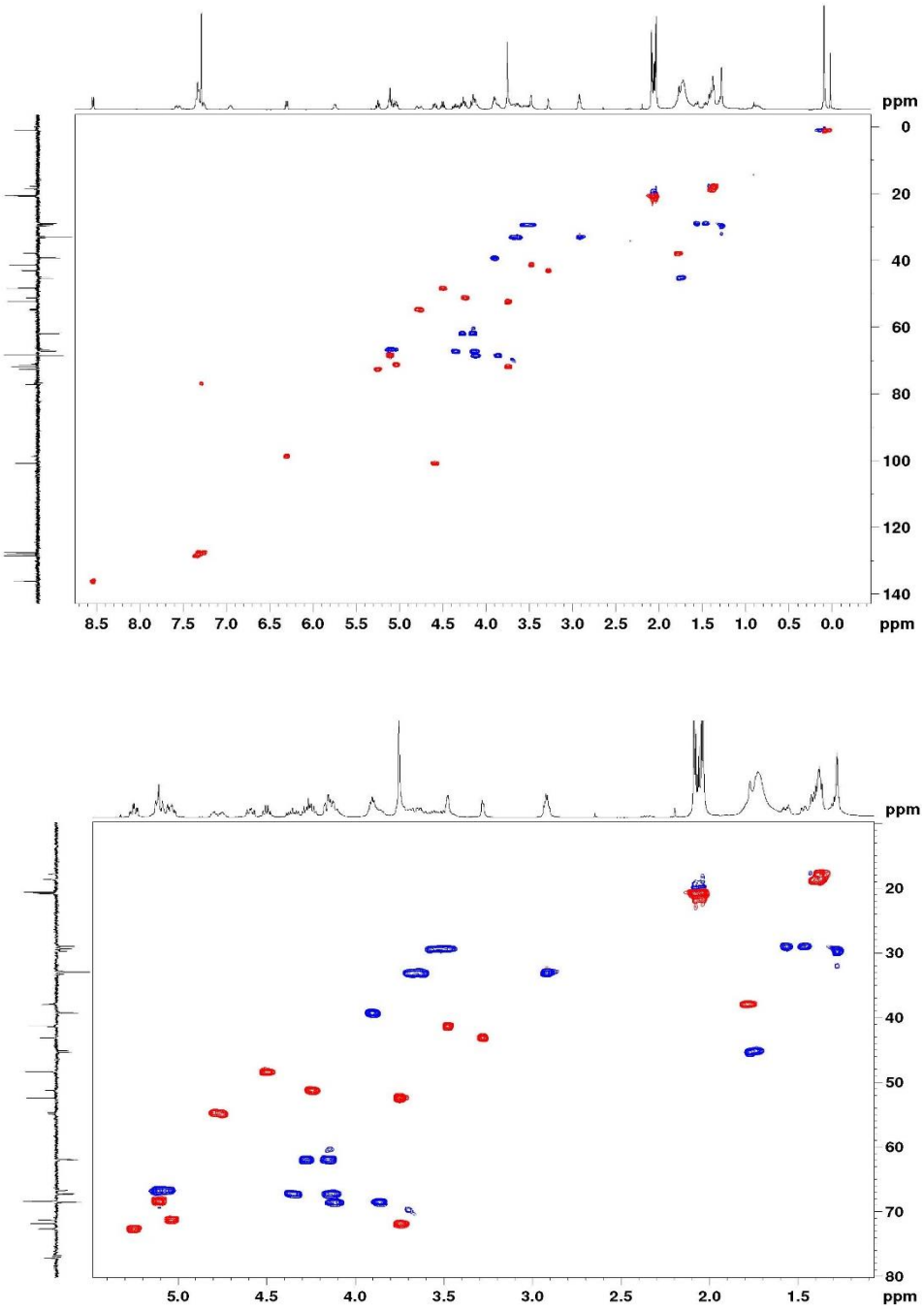
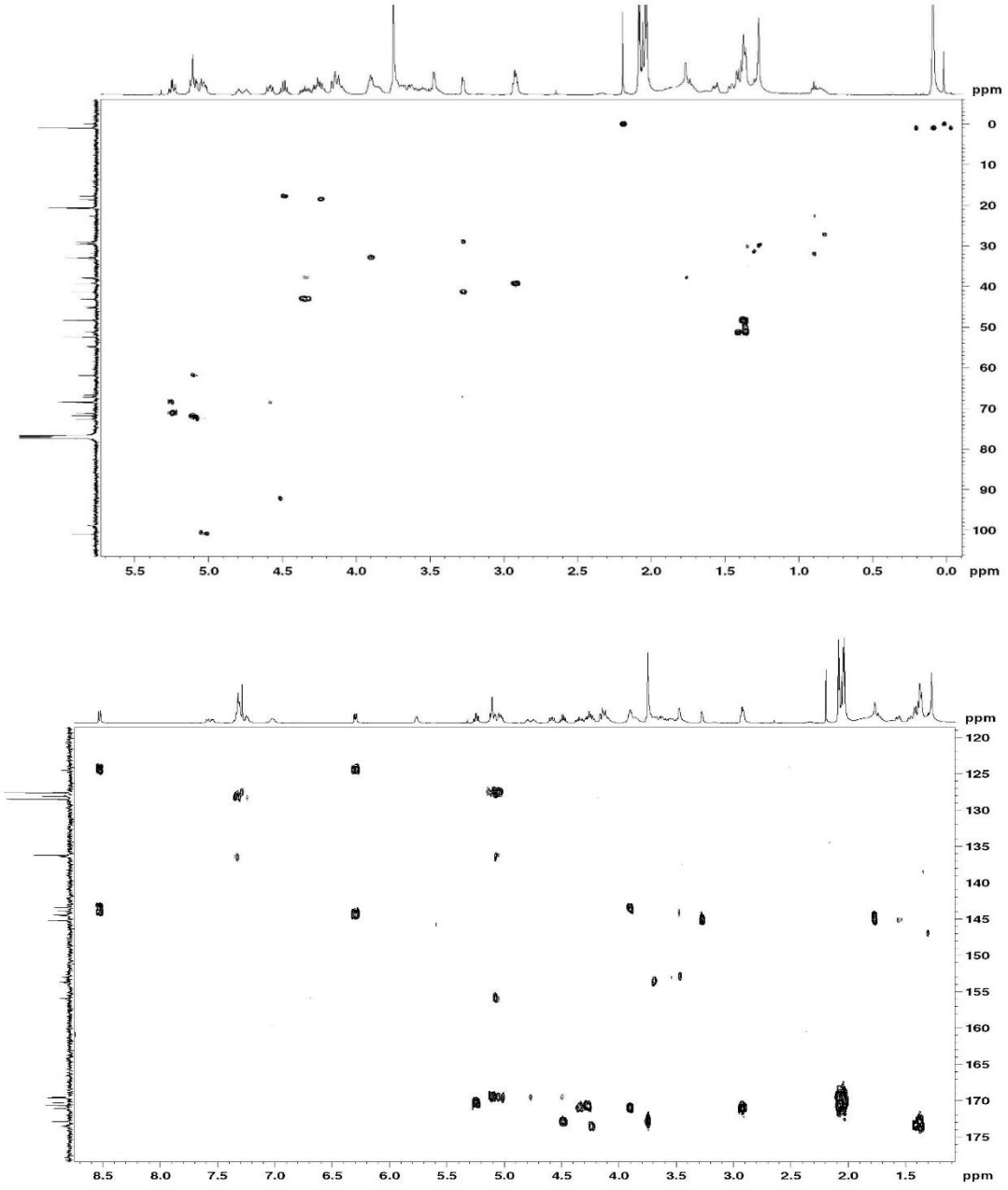
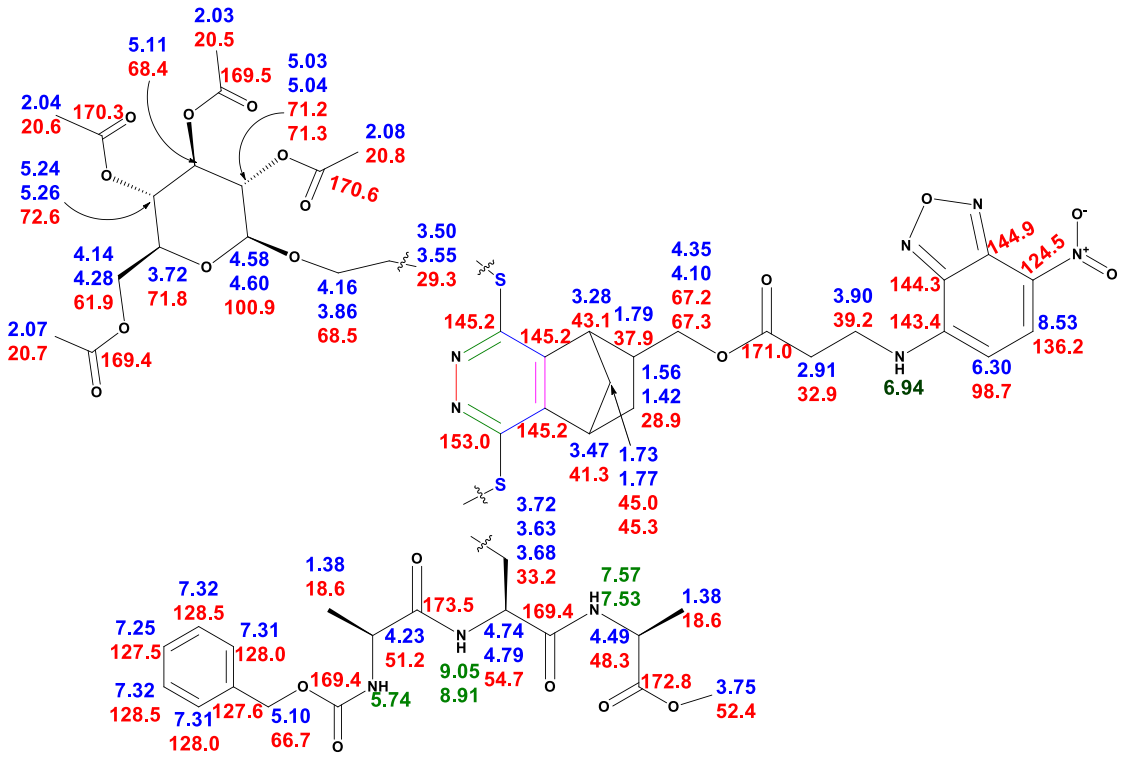


Figure S. Partial HMBC spectra of Fraction1 (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, CDCl3)



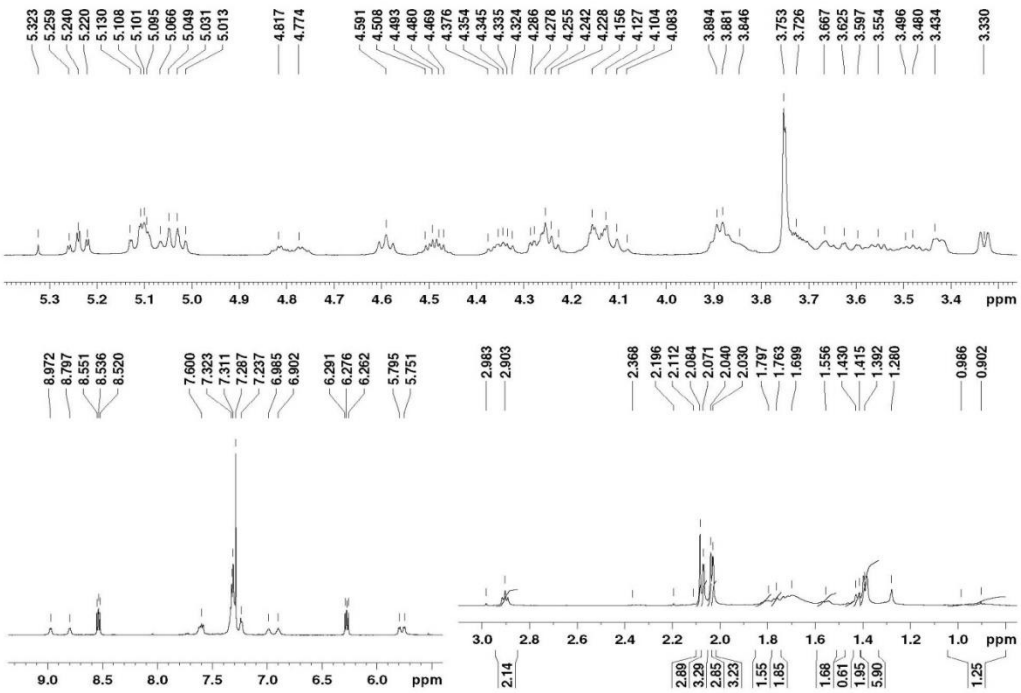
Based on the above Spectral data, the following assignments were obtained

Fraction 1

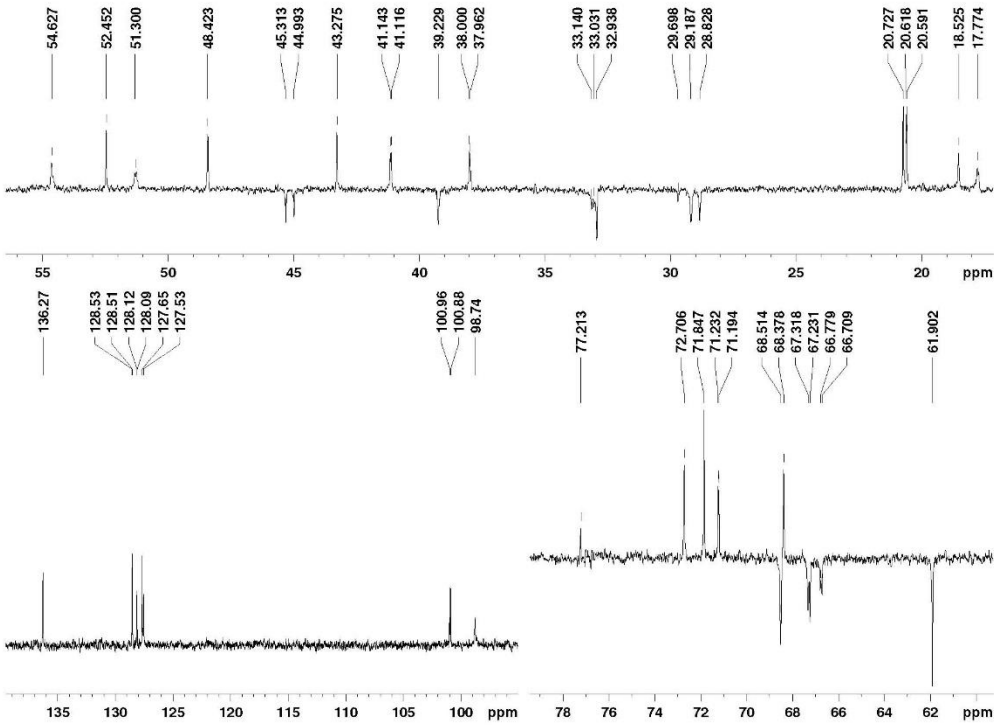


We could not conclusively assign further the position of sugar and peptide moiety relative to the fluorescent due to poor HMBC Correlations.

^{1}H NMR Spectrum (500.13 MHz, $CDCl_3$) of Compound **97** (Fraction 2)



DEPT NMR Spectrum (125.76 MHz, $CDCl_3$) of Compound **97** (Fraction 2)



^{13}C NMR Spectrum (125.76 MHz, $CDCl_3$) of Compound **97** (Fraction 2)

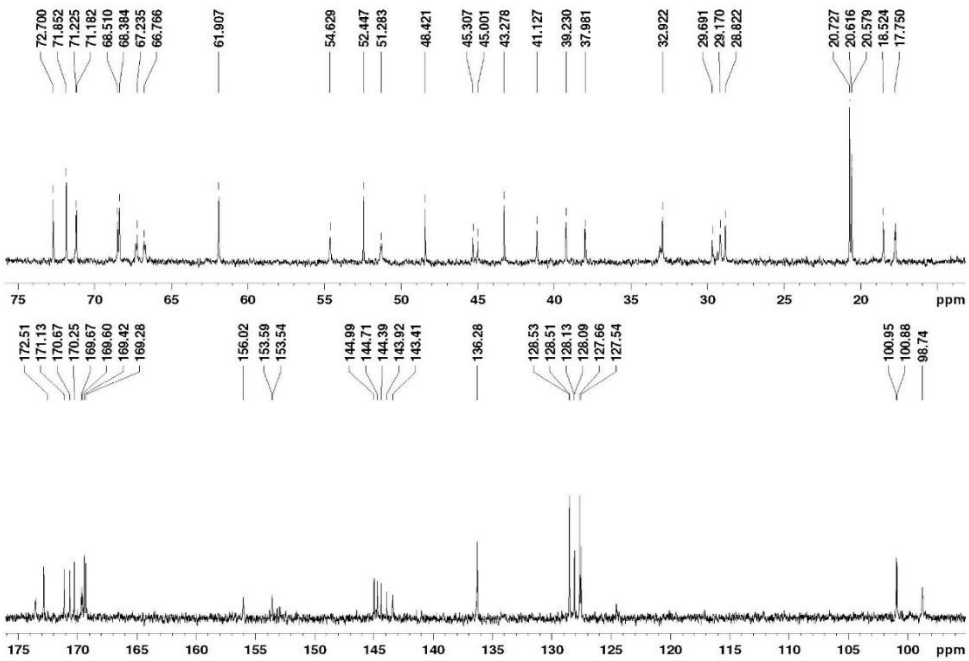


Figure S. Partial COSY spectra of Fraction 2 (500.13 MHz, CDCl3)

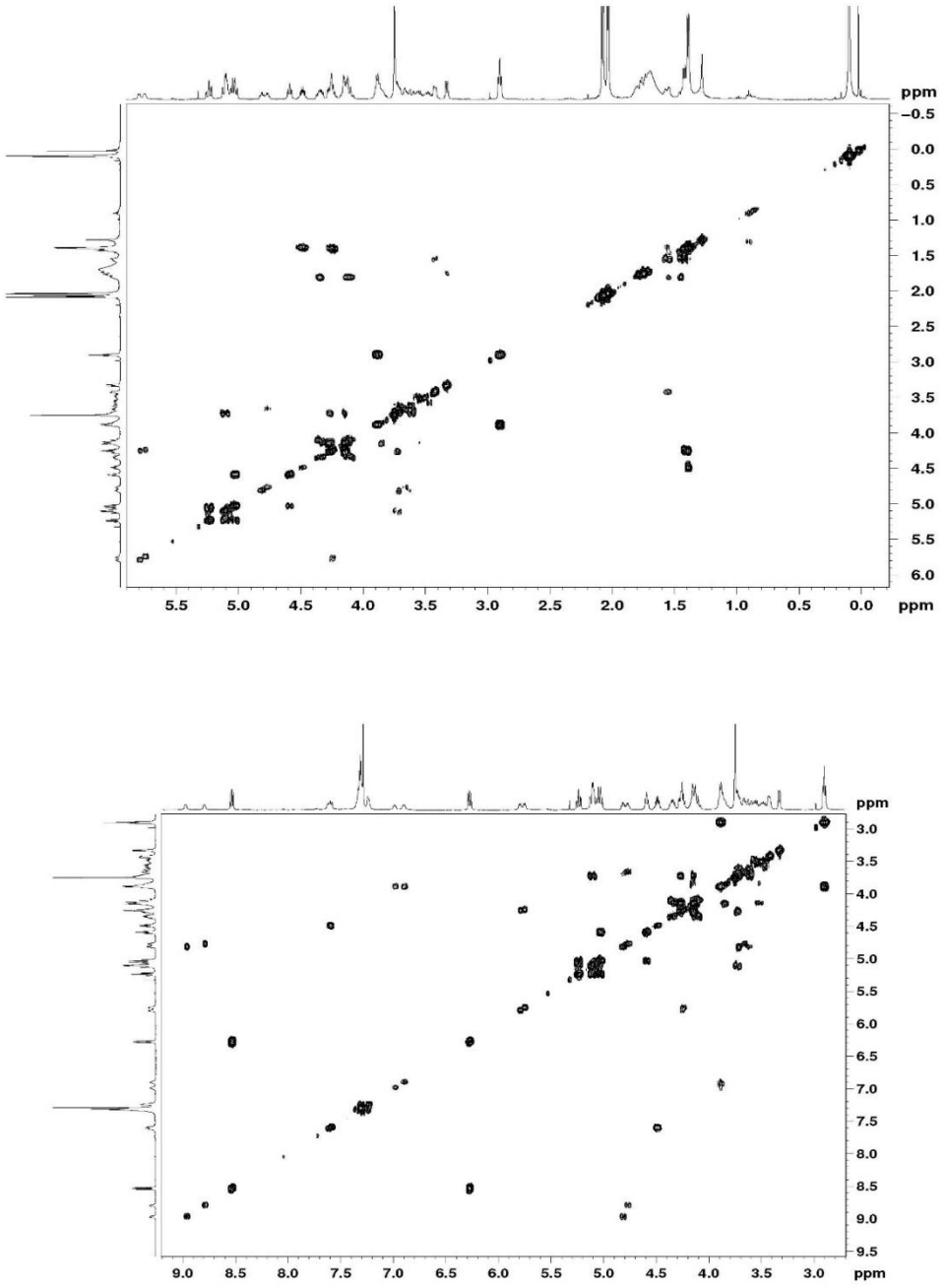


Figure S. Partial TOCSY spectra of Fraction 2 (500.13 MHz, CDCl3)

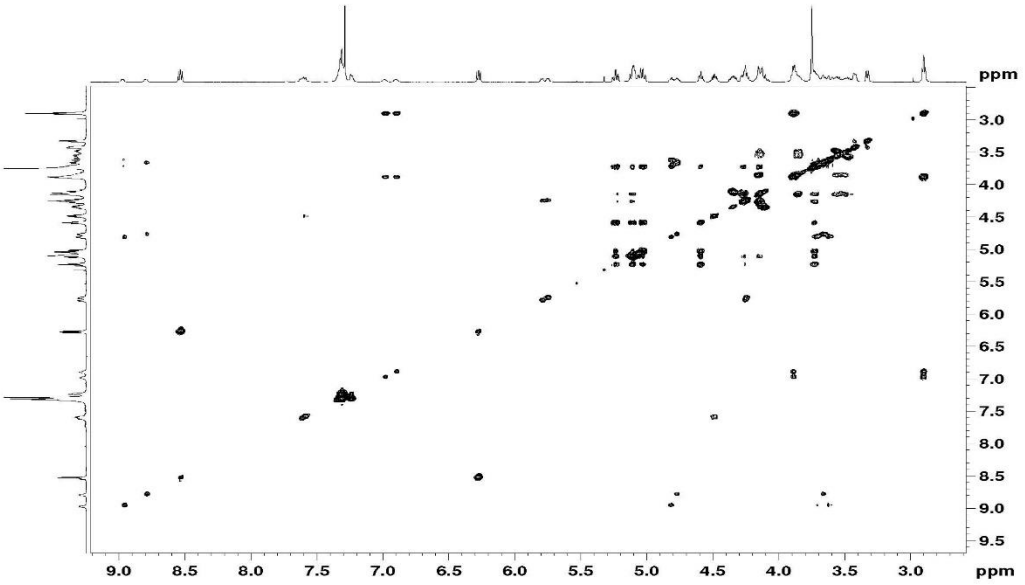


Figure S. Partial NOESY spectra of Fraction 2 (500.13 MHz, CDCl3)

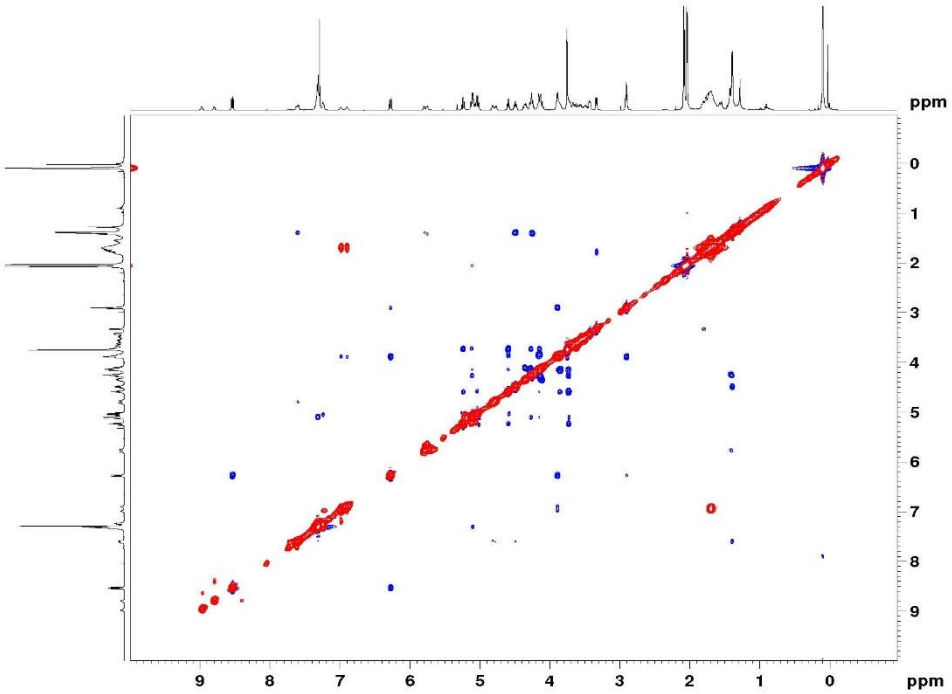
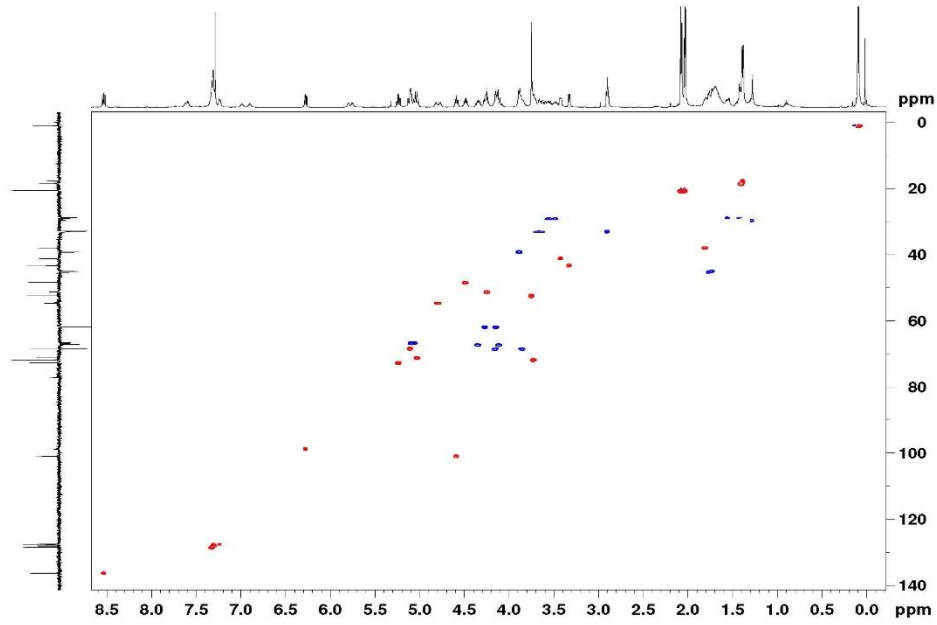


Figure S. Partial HSQC spectra of Fraction 2 (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, CDCl3)



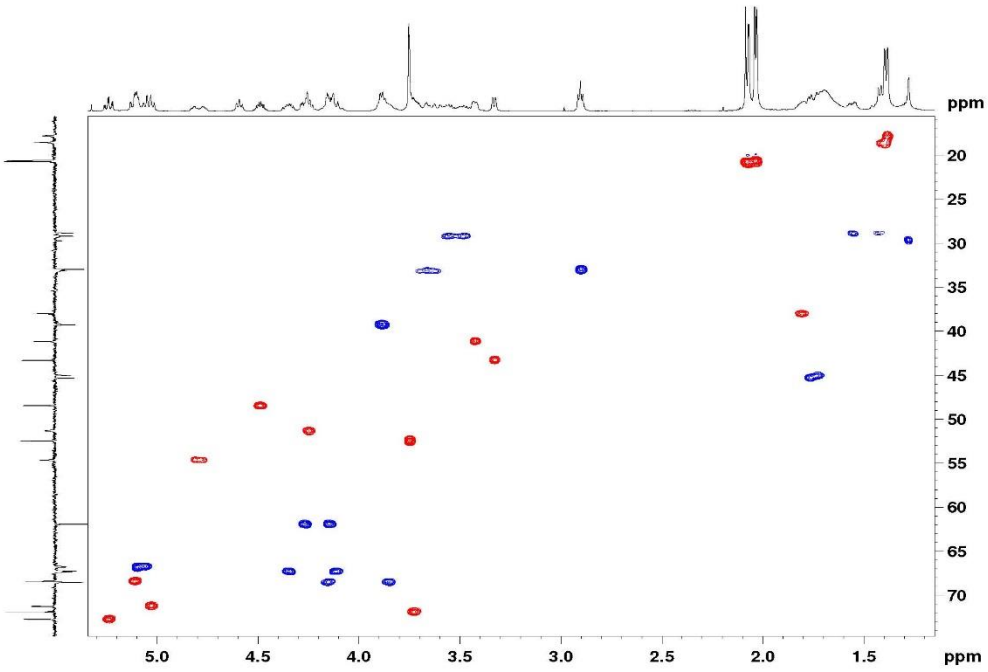
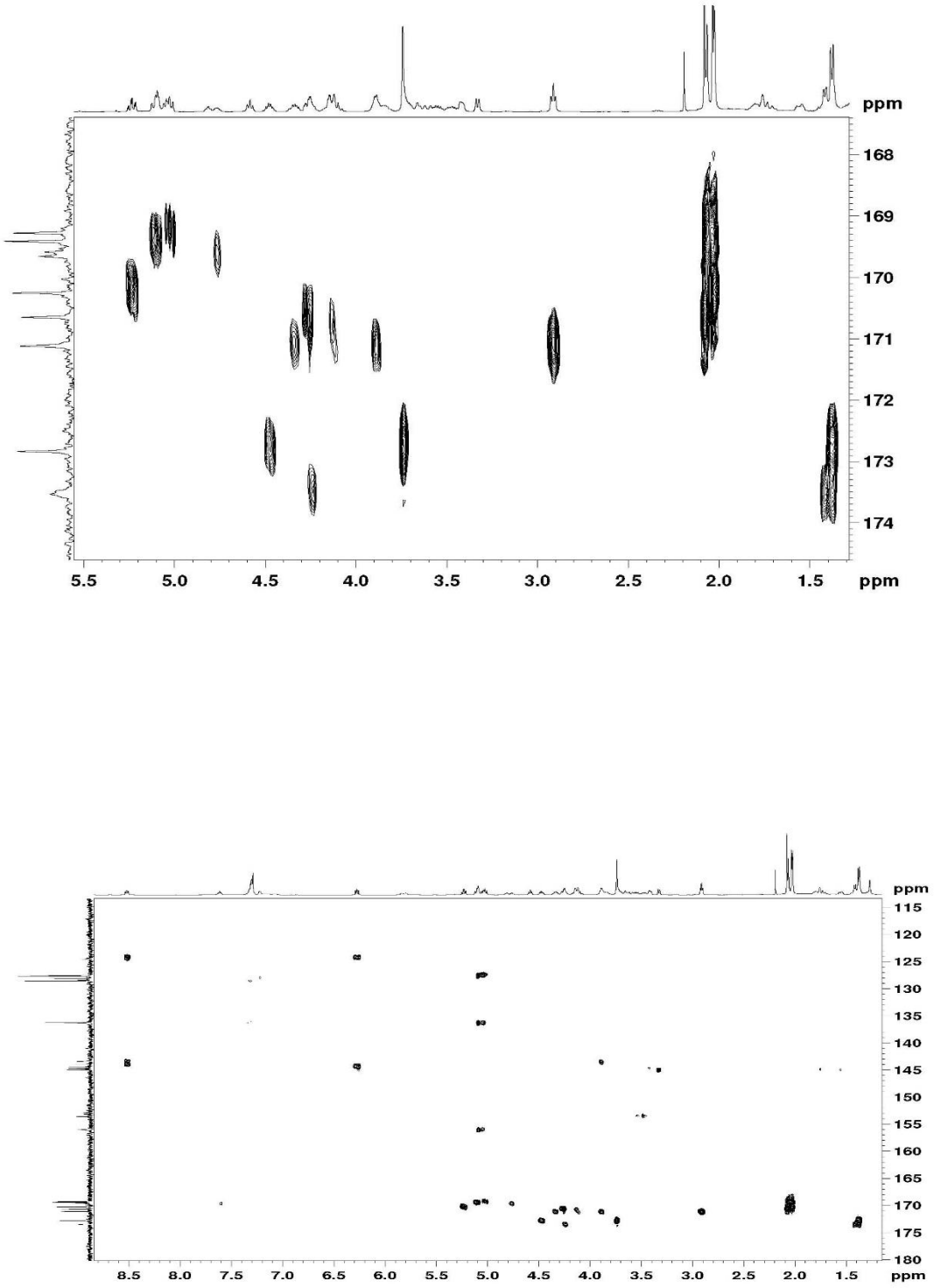
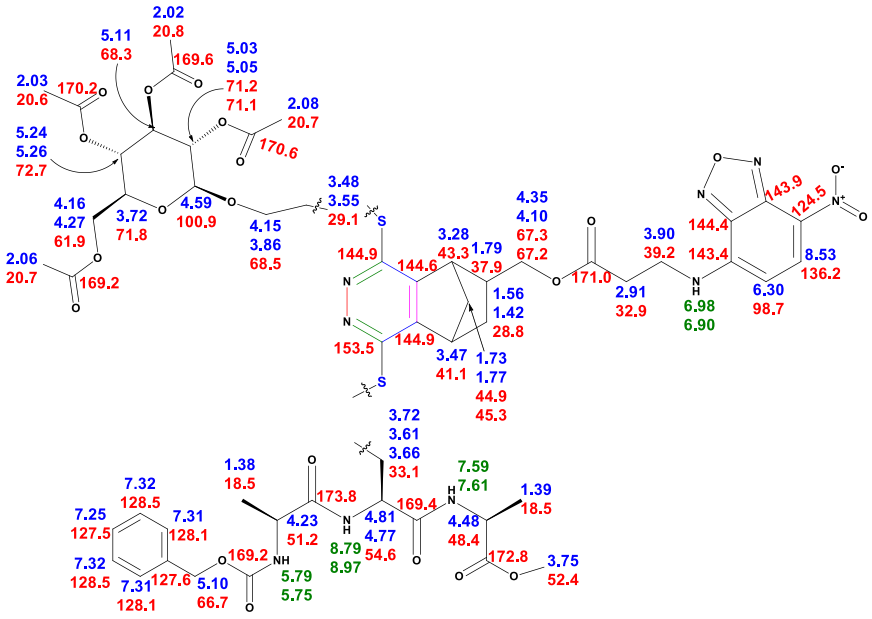


Figure S. Partial HMBC spectra of fraction 2 (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, CDCl3)



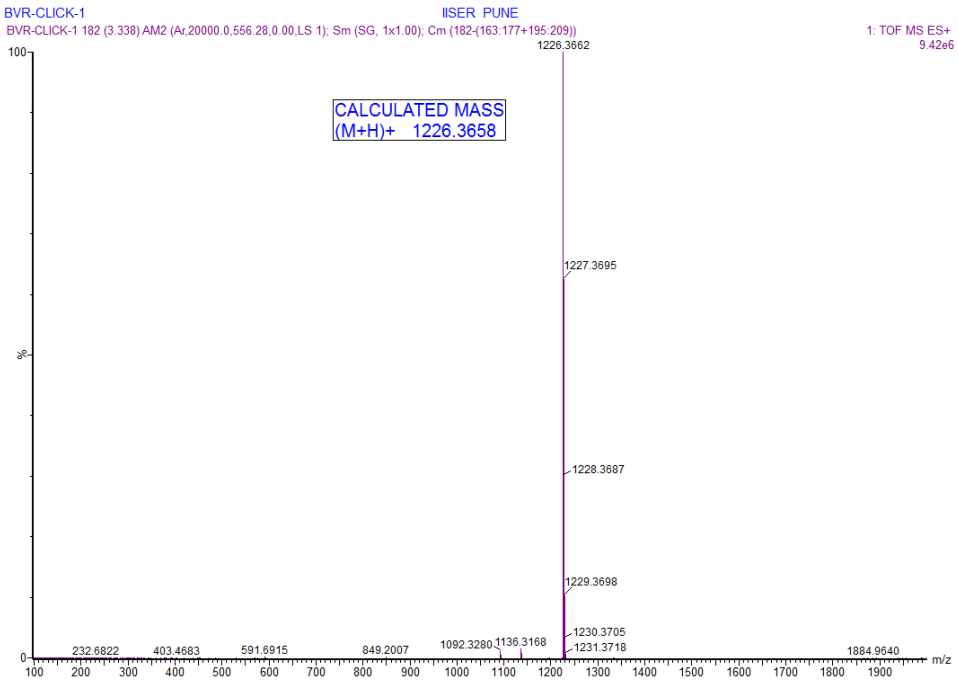
Based on the above Spectral data, the following assignments were obtained

Fraction 2

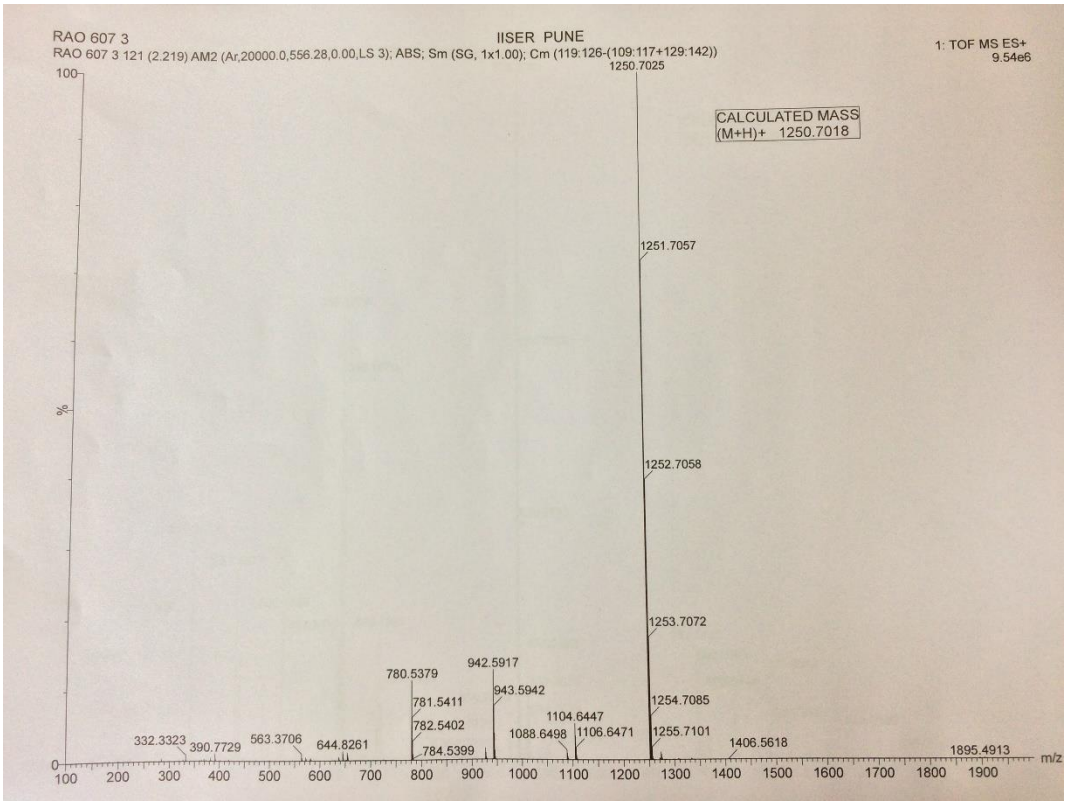


We could not conclusively assign further the position of sugar and peptide moiety relative to the fluorescent due to poor HMBC Correlations.

HRMS spectrum of Ternary Conjugate **97**



HRMS spectrum of Ternary Conjugate **131**



3.6 References

(1) (a) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435-446. (b) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 6974-6998.

(2) Oh, J. K.; Drumright, R.; Siegwart, D. J.; Matyjaszewski, K. *Prog. Polym. Sci.* **2008**, *33*, 448-477.

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List of Publications:

1. **Rao, B. V.**; Dhokale, S.; Rajamohanan, P. R.; Hotha, S., A tetrazine templated method for the synthesis of ternary conjugates. *Chem. Commun.* **2013,** *49*, 10808-10810. DOI: 10.1039/C3CC46634E

2. **Rao, B. V.**; Manmode, S.; Hotha, S., Propargyl 1,2-Orthoesters for a Catalytic and Stereoselective Synthesis of Pyrimidine Nucleosides. *J. Org. Chem.* **2015,** *80*, 1499-1505. DOI: 10.1021/jo502413z

3. **Rao, B. V.**; Manmode, S.; Hotha, S., Propargyl 1,2-orthoesters for stereoselective synthesis of thioglycosides and 1-thiotrehaloses. *Carbohydr. Res.* **2015,** *417*, 103-108. DOI:10.1016/j.carres.2015.09.009

4. Nikita, M. P.; Alexander, I. Z.; **Rao, B. V.**; Leonid, O. K., Improved Large-Scale Synthesis of β-d-Arabinofuranose 1,2,5-Orthobenzoate. *Proven Synthetic Methods,* **2015**, 3, 147-154. DOI: 10.1201/b18400-22

5. **Rao, B. V.**; Hotha, S., Smart Extraction Unit for Mass Spectrometry Named as **S**oftware **A**ssisted **D**irect **E**xtraction and **S**ampling **I**nterphase. **(SWADESI) Indian Patent Filed:** N-0000123839

6. Thadke, S. A.; Mishra, B.; **Rao, B. V.**; Islam, M.; Hotha, S., [Au]/[Ag]-Catalysed expedient synthesis of branched heneicosafuranosyl arabinogalactan motif of Mycobacterium tuberculosis cell wall *(Submitted).*

7. **Rao, B. V.**; Hotha, S., Photo-cleavable fluorescent glyco-calixarenes *(In preparation).*

8. **Rao, B. V.**; Hotha, S., Lectin Binding Studies on Hybrid Glycoconjugates *(In preparation).*