Making and Breaking of Glycosidic bond: From Glycosylation to Glycan Editing

विद्या वाचस्पति की उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत शोध प्रबंध

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INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE

2024

Dedicated to ...

My Parents

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CERTIFICATE

Certified that the work incorporated in the thesis entitled "***Making and breaking of glycoside bond: From glycosylation to Glycan editing***" submitted by **Mr. Sumit Sen** 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.

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Date: 29-07-2024

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- ^{1}H NMR spectra were recorded on AV 200, AV 400, DRX-500 MHz, JEOL ECX 400 or Bruker Avance 500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in ppm units downfield to TMS.
- ^{13}C NMR spectra were recorded on AV 50, AV 100, DRX-125 MHz, JEOL ECX 100 or Bruker Avance 125 MHz spectrometer.
- High resolution mass spectroscopy (HRMS) was performed on Waters Synapt G2 and Maldi-TOF.
- IR spectra were recorded on Perkin-Elmer 1310 and Perkin-Elmer 1600 FT-IR spectrometers with sodium chloride optics and are measured in cm^{-1}.
- Specific rotations were measured on a JASCO P-1020 or Rudolph polarimeter and measured in degree.
- All reactions were monitored by Thin-Layer Chromatography carried out on precoated Merck silica plates (F254, 0.25 mm thickness); compounds were visualized by UV light or by staining with anisaldehyde spray.
- All reactions were carried out under nitrogen or argon atmosphere with dry freshly prepared solvents under anhydrous conditions and yields refer to chromatographically homogenous materials unless otherwise stated.
- All evaporators were carried out under reduced pressure on Büchi and Heildoph rotary evaporator below 45 °C unless otherwise specified.
- Silica gel (100-200) and (230-400) mesh were used for column chromatography.
- All gold and transition metal salts were purchased from multinational commercial vendors.
- Materials were obtained from commercial suppliers and were used without further purification.
- High resolution mass spectroscopy (HRMS) was performed using an ESI-TOF mass analyzer or MALDI-TOF mass analyzer.
- Normal Phase HPLC purifications were performed using Gilson's PLC 2050 series.
- Scheme, Figure and Compound numbers in abstract and individual chapters are different.

Abbreviations

Å – Angstrom
Ac – Acetate
AcBr – Acetyl bromide
AcCl – Acetyl chloride
AcOH – Acetic acid
Ac_2O – Acetic anhydride
AG – Arabinogalactan
Ara*f* – arabinofuranosyl/arabinofuranoside
Bn – Benzyl
BnBr – Benzyl bromide
Boc – *t*-butylcarbonyl
BSA – Bis(trimethylsilyl)acetamide
Bu_2SnO – Dibutyltin oxide
Bz – Benzoyl
BzCl – Benzoyl chloride
Calcd – Calculated
Cbz – Carbobenzoxy
cat. – catalytic
$CDCl_3$ – Chloroform-D
$CHCl_3$ – Chloroform
d – days
DBU – 1,8-Diazabicycloundec-7-ene
DDQ – 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DCM – Dichloromethane
DCE – Dichloroethane
DEPT – Distortion less Enhancement by Polarization Transfer
DIPEA – *N,N′*-Diisopropylethylamine
DIC – *N,N′*-Diisopropylcarbodiimide
DMAP – *N,N′*-Dimethylaminopyridine
DMF – *N,N′*-Dimethyl formamide
DMSO-d_6 – Dimethyl sulfoxide (deuterated)
D_2O – Deuterium oxide
δ – delta (in ppm)
eq. – equivalents
Et_3N – Triethyl amine
Et_2O – Diethylether
EDC– 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc – Ethyl acetate
Et_3SiH – Triethylsilane
g – gram
Gal*f* – galactofuranosyl / galactofuranoside
h – hour
HOAt – 1-Hydroxy-7-azabenzotriazole
HRMS – High-Resolution Mass Spectrometry
Hz – Hertz
Im. – Imidazole
IR – Infrared

J – coupling constant
Kg – Kilogram
LAM – Lipoarabinomannan.
Lev – Levulinoate
M – Molar
Man*p* – mannopyranosyl / mannopyranoside
MeI – Methyl Iodide
MeOD – Methanol-D4
mg – milligram
min. – minutes
MHz – Megahertz
mL – millilitre
mmol – millimolar
MS – Molecular sieves
Mtb – Mycobacterium tuberculosis
NaH – Sodium hydride
NAP – N-bromomethyl naphthalene
NGP – Neighbouring group participation
NIS – N-Iodosuccinimide
NMR – Nuclear Magnetic Resonance
NNGP – Non-neighbouring group participation
NPG – n-Pentenyl glycoside
PMB – p-Methoxy benzyl
PMBCl – p-Methoxy benzyl chloride
Py – Pyridine
PTSA, TsOH – *p*-Toluene sulfonic acid
ppm – parts per million
Rib*f* – ribofuranoside / ribofuranosyl
rt – room temperature
sat. – saturated
Tb – tuberculosis
TBAI – tetra-n-Butyl ammonium iodide
TBDMS – *t*-Butyldimethylsilyl
TBDMSCl – *t*-Butyldimethylchloride
TBDPS – *t*-Butyldiphenylsilyl
TBDPSCl – *t*-Butyldiphenylsilylchloride
TCA – Trichloroacetimidate
TfOH – trifluoromethanesulfonic acid
TFA – Triflouroacetic acid
THF – Tetrahydrofuran
TLC – Thin Layer Chromatography
TMSOTf – Trimethylsilyl trifluoromethanesulfonate
µg – microgram
µmol – micromolar
µL – microliter

Synopsis

The thesis entitled "***Making and Breaking of Glycoside Bonds: From Glycosylation to Glycan Editing"*** is divided into three chapters. Chapter one outlines the significance of glycoconjugates and glycosylation, delineates different types of glycosylations, examines the factors influencing the stereochemical outcome of glycosylation, and discusses various types of glycosyl donors and their respective glycosylation methods. Additionally, this chapter emphasizes the importance of gold-catalysed glycosylation, which has emerged as a particularly intriguing and promising method for constructing complex carbohydrate structures. Chapter two illustrates the effectiveness of the stable alkynyl glycosyl carbonate donor for synthesizing complex glycan motifs found on the cell wall of *Mycobacterium tuberculosis*, the pathogen causing tuberculosis (Tb). The synthesis of an arabinan motif with 31 interglycosidic linkages was successfully achieved using an alkynyl carbonate glycosyl donor strategy with [Au]/[Ag]-catalysis. In chapter three, continuing our exploration of oligosaccharide syntheses, we shifted our focus to carbohydrate editing. In this section, we successfully developed a novel molecular surgical editing approach called CIStER, designed for precise glycan editing. This method expedites the creation of diverse oligosaccharides, glycohybrids and glycan probes. Furthermore, this glycan editing concept was utilized to develop a transglycosylation strategy, enabling the formation of a diverse class of oligosaccharides starting from an easily accessible glycan unit.

Chapter 1: Chemical Glycosylation and synthesis of oligosaccharides

Glycoconjugates are crucial for all living organisms, found abundantly on cell surfaces and in secreted fluids as polysaccharides, glycoproteins, proteoglycans, and glycolipids. They play a vital role in regulating various biological processes, including viral and bacterial infections, tumour metastasis, angiogenesis, inflammation, cell-cell communication, hormone balance, and metabolism. The medicinal applications of glycoconjugates are well established, with numerous carbohydrate-based drugs and vaccines currently undergoing clinical trials. However, despite their biological and medical significance, the field of glycobiology faces challenges due to the scarcity of pure, structurally well-defined glycoconjugates and oligosaccharides. Natural sources yield only micro-quantities in heterogeneous forms, making purification and characterization difficult. In this context, the chemical synthesis of complex glycoconjugates with higher glycosylation yields and excellent stereoselectivity is crucial. Consequently, numerous glycosyl donors and glycosylation protocols have been developed over the past few decades. These range from highly acidic conditions (such as BF_3•Et_2O,

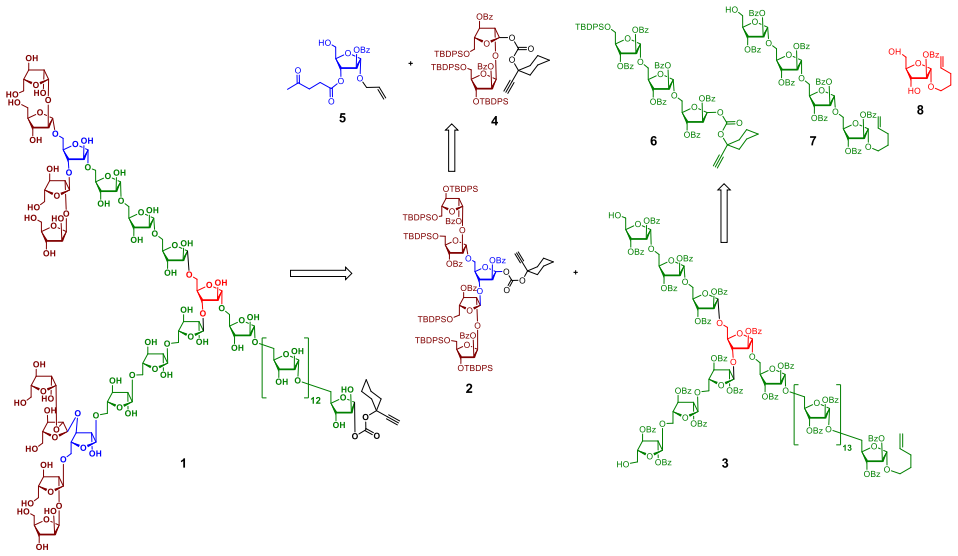
TMSOTf, and TfOH) to very mild conditions (including Au, Ag, Pd, Cu, and Zn catalysis). A wide variety of activators or promoters have been created to facilitate these processes. Gold catalysis has been instrumental in advancing oligosaccharide synthesis strategies. Over the past decade, the unique benefits of gold-catalyzed donor chemistry have enabled the efficient synthesis of numerous disaccharides, trisaccharides, and oligosaccharides. Moreover, the development of new reaction technologies for synthesizing oligosaccharides and glycoconjugates is crucial for progress across various scientific and industrial fields. These advancements facilitate more detailed biological studies, the creation of new therapeutics, efficient and scalable production processes, and the development of innovative materials and analytical techniques.

Chapter 2: Chemical Synthesis of 31-mer Arabinan of *Mycobacterium Tuberculosis* Cell wall

Arabinogalactan (AG), a major component of the *Mycobacterium tuberculosis* (Mtb) cell wall, offers potential for developing new therapeutic agents and a single-dose treatment for tuberculosis. Increasing number of TB infections due to multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR- TB) stains continue as a major public health crisis and urged immediate action on finding out new drug or vaccine candidates where the use of synthetic AG fragments is important. Despite significant efforts by several groups, the successful synthesis of different AG and LAM motifs has been limited by the use of less efficient glycosyl donors or glycosylation methods with poor overall yields. Various glycosyl donors, including the recently employed propargyl 1,2-orthoester donor, have been used for these oligosaccharide syntheses. In this premise, the recently discovred glycosyl alkynyl carbonate donor chemistry is quite promising for the synthesis of large oligosaccharides as the glycosylation is fast, high yielding, catalytic and the donor is quite easy to prepare. Drawing upon previous strands of research on the synthesis of mycobacterial arabinogalactan, this study was therefore conducted to synthesize the full length arabinan portion containing 31-Ara*f* residues using the versatility of the developed alkynyl carbonate donor.

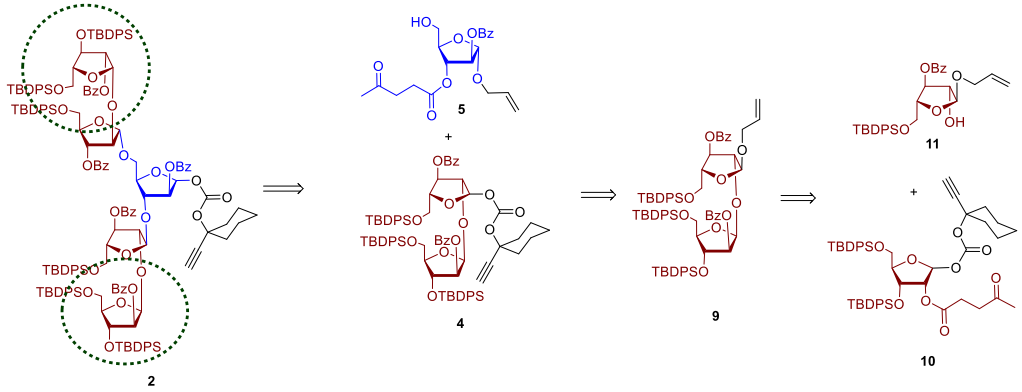
From the perspective of the types of linkages and branching in the Araf_{31} unit showcases enough complexity and challenges for its synthesis. The target polysaccharide arabinan **1** was disconnected into two sizeable fragments, that is, the terminal pentasaccharide unit **2** and the branched 21mer arabinan unit **3**. It was convinced that the desired 31mer arabinan motif can

be comprehended by a stereoselective glycosidation between a heneicosasaccharide **3** and two molar equivalents of a pentasaccharide-carbonate (**2**) in a [2x5+21] fashion. The larger component **3** was envisioned from the trisaccharide **6**, tetrasaccharide **7**, the monosaccharide **8**.



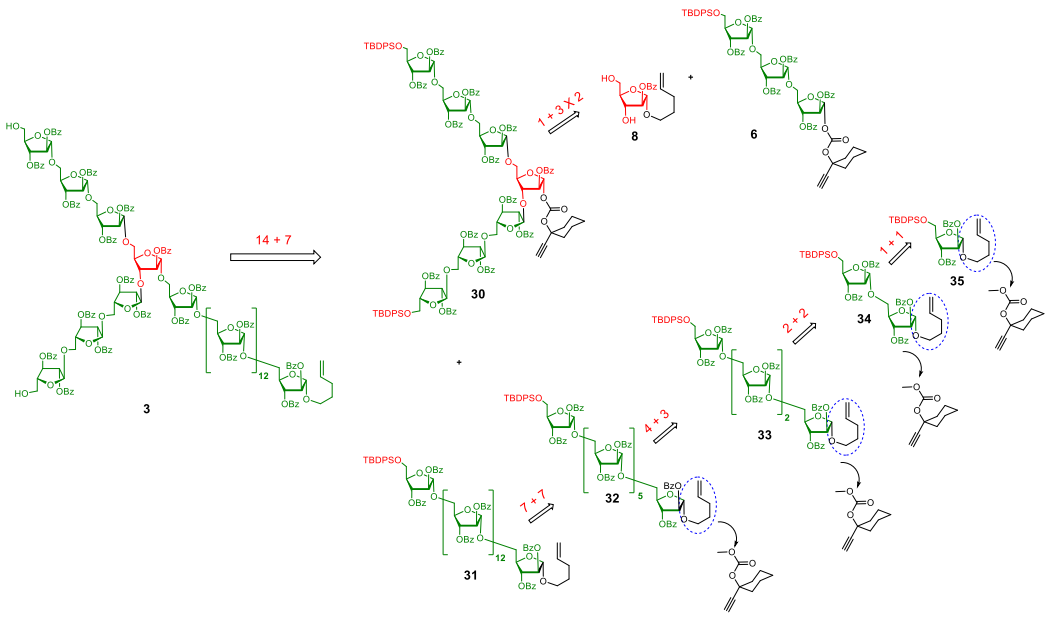
Scheme 1. Retrosynthetic analysis of the Ara*f*$_{31}$

Our synthetic journey started with the synthesis of most challenging terminal pentasaccharide unit which required installation of two *β*-Ara*f*-(1→2)-Ara*f* linkages that are notorious for their difficulty as well as three α-Ara*f*-(1→2)-Ara*f* linkages. For the 1,2-*trans* glycosylation between the two arabinose moieties, we intended to use the [Au/Ag]-catalysed activation of glycosyl carbonate donor chemistry through the neighbouring group participation of C2-ester group and for the 1,2-*cis* glycosylation we used our lab protocol of initiating the neighbouring group assisted 1,2-*trans* linkage followed by saponification and oxidation-reduction steps. We choose a retrosynthetic plan for this motif, a [(2+1)+2] glycosylation between the disaccharide donor **4** and the acceptor **5**, was thought to be the most convenient way to produce the desired pentasaccharide carbonate donor **2**.



Scheme 2. Retrosynthesis of the terminal pentasaccharide of MTb

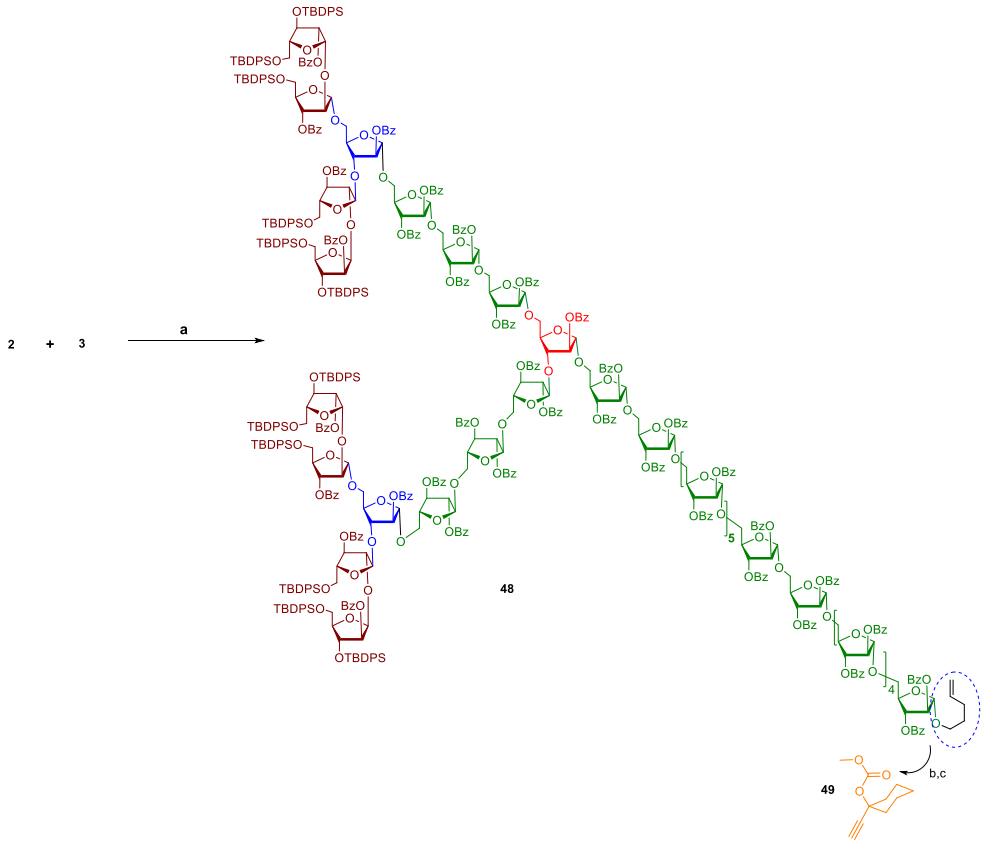
We envisioned the branched 21mer arabinan unit **3** by the [Au/Ag] catalysed coupling of linear 14mer arabinofuranosyl acceptor **31** and the branched heptasaccharide arabinofuranosyl donor **30**. The 14mer glycosyl acceptor can further be obtained by the coupling of the linear heptasaccharide donor and acceptor unit. Similarly, the branched heptasaccharide can be generated via the glycosylation of 2molar equivalents of the trisaccharide donor **6** and the monosaccharide arabinofuranosyl acceptor unit **8.**



Scheme 3. Retrosynthesis plan for the synthesis of Ara*f*$_{21}$ of MTb

Accomplishing the synthesis in sufficient quantities of all of these major structural motifs, pentasaccharide **2** and the branched heneicosasaccharide **3** were called upon to complete the assembly of the 31mer arabinan motif. The glycosidation was carried out by following the

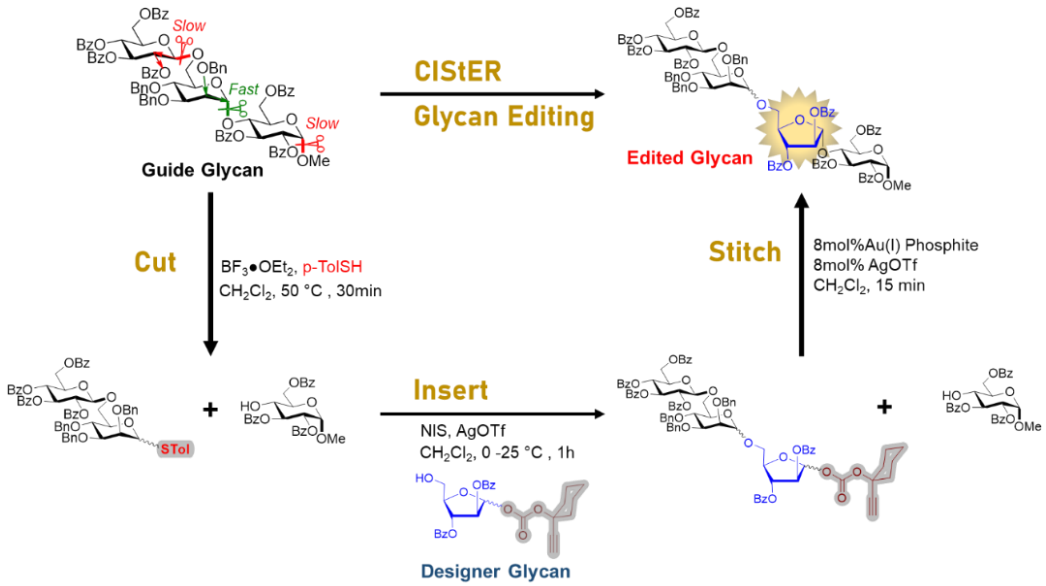
catalytic protocol containing 8 mol% each of Au-phosphite and AgOTf for 30 min. This route enabled us to synthesize 500mg in 80 steps with an overall yield of 0.012%. The presence of *n*-pentenyl moiety can be exploited for conjugating immunogenic proteins, biomarkers, and chemical probes for further biological explorations. Additionally, the anomeric pentenyl group was converted into the alkynyl carbonate donor in two steps, allowing for the installation of the linear galactan unit into the arabinan motif to synthesize the full-length 131-mer arabinogalactan of the *Mycobacterium tuberculosis* cell wall. Progress on coupling the arabinan unit with the linear galactan chain is ongoing in our lab.



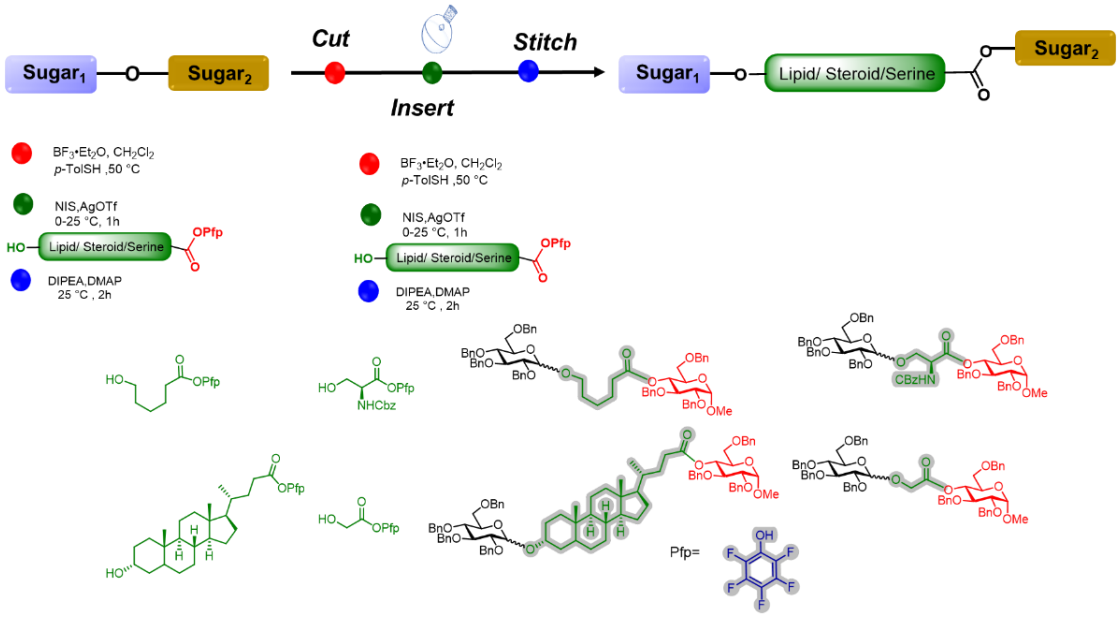
Chapter 3: Cut-Insert-Stitch Editing Reaction (CIStER) Sequence for Surgical Glycan Editing

Challenges encountered during the synthesis of Araf_{31} led us to understand that glycosidic bonds can be hydrolyzed in a site-specific manner by leveraging stereoelectronic factors. Thus, the focus shifted to the bond breaking aspect that can be utilized for *editing of carbohydrates*. 'Molecular' Editing is still in its infancy with a handful papers only though (i) atomic level editing is popular among organic chemists and (ii) genomic editing popular among bioorganic

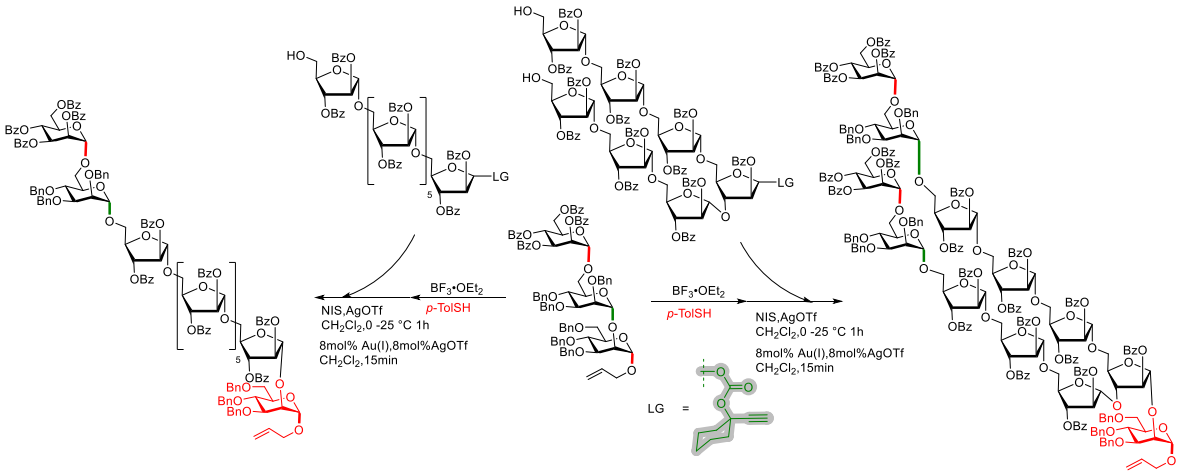
chemists**.** Tools to engineer the glycome at different hierarchal complexity levels, with thorough, up-to-date information, are offering a new trail to decode the fundamental biological activities that are imprinted in glycans, and bring new insights on glycotherapeutics, even though they have not been fully established. The process of metabolic oligosaccharide engineering (MOE), enzymatic addition of glycans (EAG) are rapidly growing for cell surface glycan engineering. However, there is no editing technique enables the insertion of a foreign glycan, lipid or protein into the already synthesized glycan via chemical or enzymatic routes. In this scenario, we hypothesized a three-step chemical glycan editing process that allow us to insert glycans, proteins, lipid to a pre-designed glycan unit (guide glycan) to generate various glycoconjugates. In this endeavour, a three-step chemical approach to enable the editing of the pre-designed glycan to generate various glycoconjugates was invented comprising three steps *viz.* (i) '**c**ut' step which cleaves the glycosidic bond selectively based on their reactivity and producing an intermediate that could be intercepted with 4-mercaptotoluene, (ii) '**i**nsertion' of the foreign glycan unit involving the orthogonal activation strategy of the glycosyl donor, and (iii) '**st**itching' by activating the alkynyl carbonate donor. All three steps were performed in a single pot using the **C**ut-**I**nsert **St**itch-**E**diting **R**eaction sequence (**CIStER**). The versatility of the CIStER technique was demonstrated by synthesis of oligosaccharides through the insertion of various pyranosides and furanosides, originating from the same designer trisaccharide.



Further, the CIStER strategy was successfully applied to insert various activated hydroxyl acids (serinyl, steroidal, lipids) into the naturally occurring disaccharides to generate various glycohydrids.



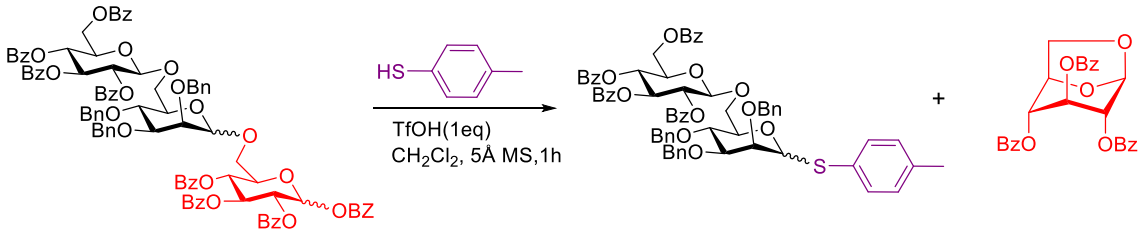
Along with this, we have demonstrated the potential of the developed *cut-insert-stitch editing reaction sequence* in the synthesis of different linear and branched lipoarabinomanan (LAM) analogues present in the cell wall of the *Mycobacterium tuberculosis*. Efforts to extend the CIStER methodology to small molecules is presently in progress.



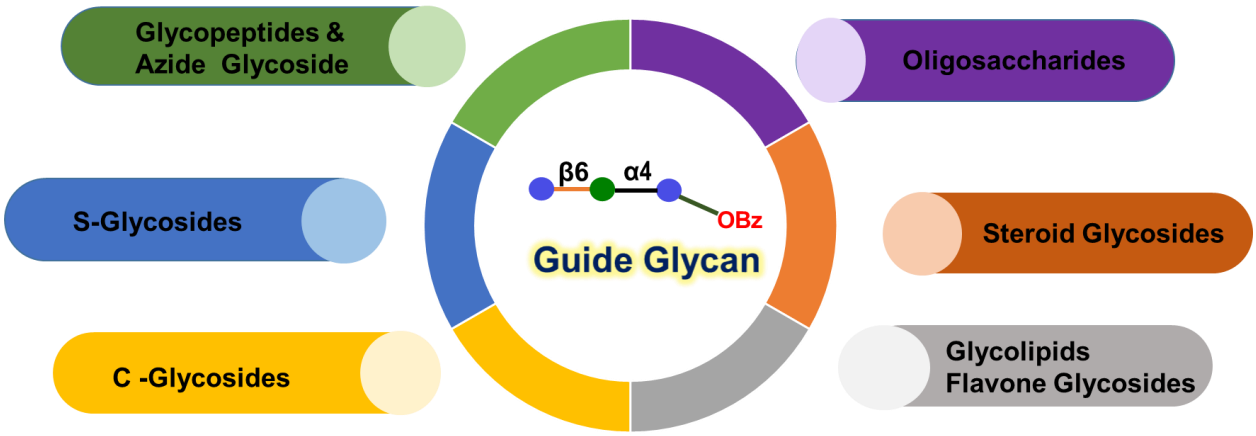
Chapter 4: Chemical Transglycosylation: Engineered by Glycan Swapping

Further, CIStER strategy has been expanded to the develop a chemical transglycosylation strategy with special focus on the generation of various oligosaccharides and glycoconjugates which regulate many biological processes such as viral and bacterial infection, tumor metastasis, angiogenesis, inflammation, cell-cell communication, hormone balance and metabolism. The transglycosylation is a versatile and essential biochemical reaction that involves transferring glycosyl residues from a donor to an acceptor molecule, forming new glycosidic bonds.

In this current work, we have successfully developed a method for chemical transglycosylation which involves the exchange of glycan units under acidic conditions. The simple ester functional group at the reducing end of the oligosaccharide facilitates the departure of the terminal residue of the glycan upon cleavage of the interglycosidic bond. The electrophilic intermediate formed during the cleave of the interglycosidic bond is attacked by an external nucleophile producing the desired glycosides and non-reactive 1,6-anhydro sugar as a byproduct.



This transglycosylation technique allows for the facile generation of a variety of glycoconjugates. The synthesis of different glycoconjugates using this strategy is noteworthy because it emphasizes the simple derivatization of glycan units and the use of milder acidic conditions for glycan activation. The production of different glycoconjugates using the current method is particularly noteworthy because enzymatic synthesis of these glycosides typically requires extensive molecular engineering of existing glycosidases and glycosynthases to enhance their specificity, making the process less cost-effective for large-scale applications. This method also prioritizes the simple derivatization of glycan units and the use of milder conditions for glycan activation. Efforts to extend the transglycosylation methodology to higher oligosaccharides is presently in progress.


Glycopeptides &
Azide Glycoside
Oligosaccharides
S-Glycosides
β6
α4
OBz
Guide Glycan
Steroid Glycosides
C -Glycosides
Glycolipids
Flavone Glycosides

Chapter 1

Chemical Glycosylation and synthesis of oligosaccharides

1.1 Carbohydrates: Simple to find, difficult to prepare:

Four major biomacromolecules play pivotal role in all lifeforms from the tiniest bacterium to the giant sperm whale viz. nucleic acids, proteins, carbohydrates, and lipids (or fats). Carbohydrates are classified as compounds constituted of carbon, oxygen and hydrogen. The term "carbohydrate" was coined by combining the words "carbon" and "hydrated," defining the atomic composition of these molecules. Carbohydrates were fundamental in establishing a direct connection between solar energy and chemical energy through photosynthesis when life arose on the Earth during the primordial era. As a source of chemical energy, carbohydrates are conserved by plants and many non-photosynthesizing organisms resort to this chemical energy (glycolysis) for survival and evolution through respiration. Carbohydrates are also widely utilized as molecular building blocks in plants (cellulose), arthropods (chitins), and the cell walls of a broad variety of organisms, ranging from simple single cells to enormously complex multicellular ones.[1] Carbohydrates have a significantly complicated structures than proteins and DNA, which can form linear chains only.[2] They range within degree of structural complexity from a monomer unit composed of a single monosaccharide such as glucose to hundreds of glucose monomers joined together generate cellulose, which is responsible for the fundamental structure of the plant cell wall.[3-4] Chitin, which results by further polymerization of N-acetyl glucosamine units, is another instance of this polysaccharide and provides the hard outer coat of insects and crabs in the animal kingdom.[5]

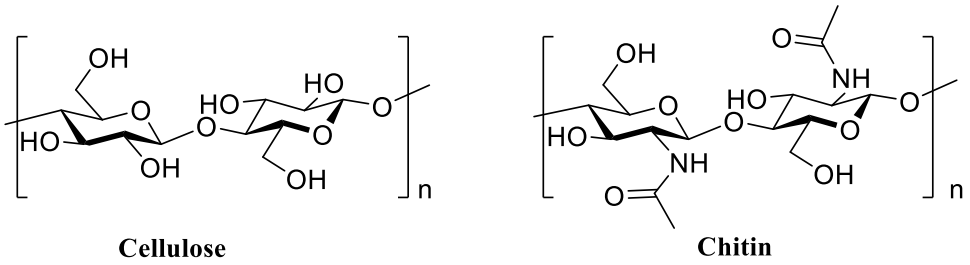


Fig 1.1.: Illustrations include common polysaccharides of glucose (cellulose) and N-acetyl glucosamine (Chitin)

The enormous structural diversity observed in carbohydrates was set up by the numerous reasons:

- Ring size variations, such as furanosyl and pyranosyl [6]
- Difference in the arrangement of monosaccharides at various chiral centers (e.g. gluco, manno, galacto, allo etc.)[7]

- Difference in anomeric configuration viz. α- and β- glycosides [8]
- The number of locations where a glycosidic connection can occur, resulting leading to various non-linear polysaccharide and oligosaccharide structures [9]
- Alternation of carbohydrates (e.g. sulfation, acetylation, phosphorylation)[9]

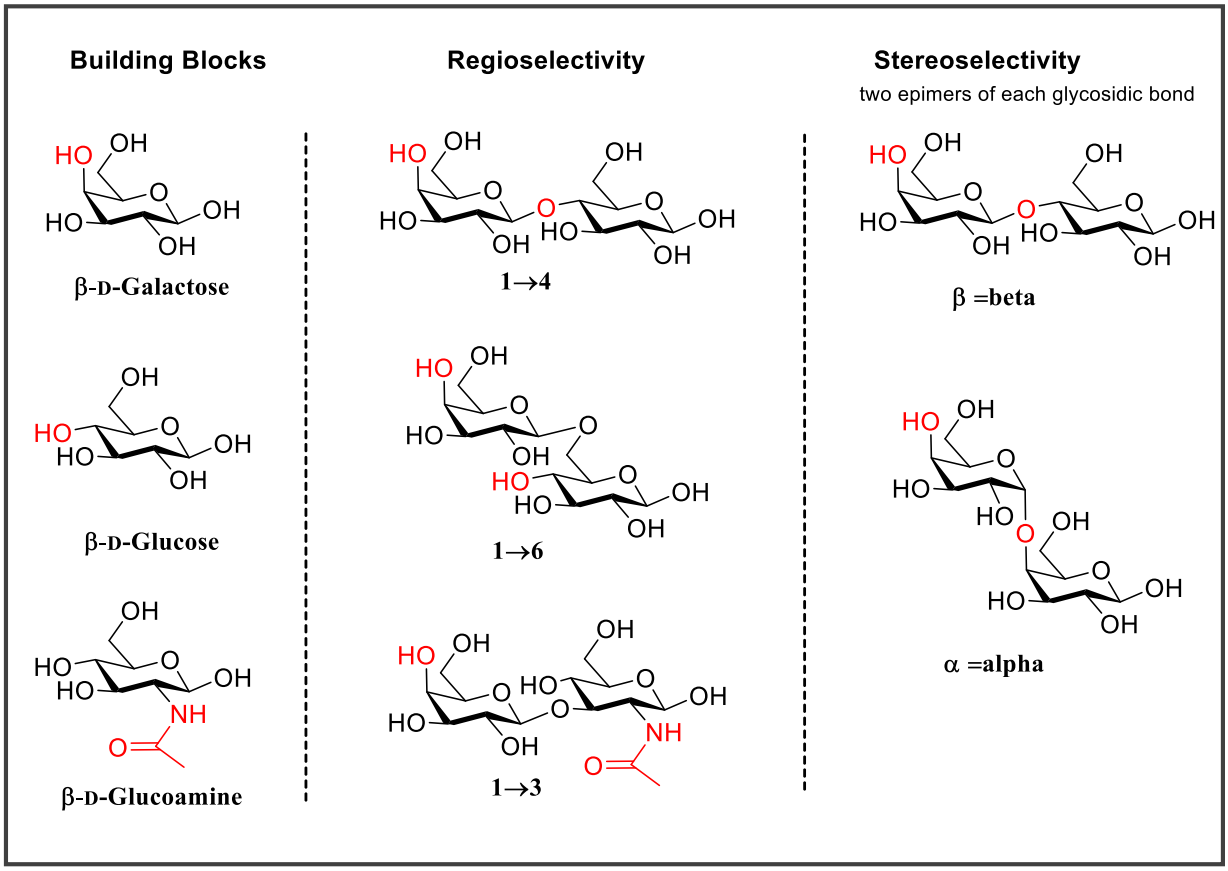


Figure 1.2. Types of structural parameters resulting in inherent complexity of carbohydrates

Because of this enormous structural diversity, carbohydrates serve show a wide range of roles in Nature and are indispensable for multiple biological activities.[10] Carbohydrates are primarily found in cells as oligosaccharides linked to protein and lipid molecules (termed to as glycoproteins and glycolipids, respectively), which are called as glycoconjugates. Glycoproteins are particularly interesting because half of all human proteins are *O*-, *N*-, or *C*-glycosylated (by serine /threonine, aspergine amino acid units, or the carbon atom of the lateral chain of tryptophan residues). The glycan moieties present in these glycoconjugates play a significant role for multiple biological processes, including cell proliferation, cell-cell adhesion and communication, immunological defense, fertilization, inflammation and brain development. Additionally, glycosylation of

proteins, either as co- or post- translational modification, influences protein folding.[11] Protein glycosylation also impacts the stability and solubility of glycoproteins or can affect susceptibility to protease and circularity lifespan.

The role of glycans in a variety of biological events persistently fascinated researchers in the domain of Glycobiology. However, the dearth of pure and structurally precise carbohydrates and glycoconjugates, on the other hand, appears to be a substantial impediment to further progress in this domain.[12] Glycoproteins, for example, are frequently discovered as combinations in which the same basic protein structure is connected to different distinct oligosaccharides, known as glycoforms. Although the biological actions of these materials can vary greatly, the similar structures and physical properties of these materials make it nearly hard to isolate specific protein glycoforms.[13] For this reason, the complete production of oligosaccharides by employing enzymatic, chemical, or chemoenzymatic methods is incredibly important.

1.2 Enzymatic Synthesis of Oligosaccharides:

In living organisms, glycosylation is regulated by various enzymes that carry out this complex reaction with precise stereo- and regioselectivity. Glycosylation occurs primarily at the ER and Golgi via numerous enzymes such as glycosidases, glycosynthases, and glycosyl transferases. In 1957, Leloir discovered glycosyl transferases, enzymes that produce oligosaccharides using sugar nucleotides as glycosyl donors. This category of glycosyl transferases are known as 'Leloir Enzymes'. Another type of glycosyl transferase termed as a 'non-Leloir' enzyme operates on phosphate containing sugar substrates. On the contrary, glycosidases and glycosynthases incorporate nitrophenyl glycosides and glycosyl halides as glycosyl donors, respectively.[14] During the last several decades, enzymatic methods had been used to perform the synthesis of different oligosaccharides and glycoconjugates.[15] These methods tend to be intriguing due to their key characteristics, which include (i) single step conversions (ii) high amount of regio- and stereo-selectivity resulting in almost no by-products. (iii) very mild and reactions in aqueous media. In 2008, Xia group reported facile enzymatic synthetic procedure of a vital tumor-assisted carbohydrate antigen Globo-H-hexasaccharide.[16] With the use of three glycosyltransferases (four enzymatic glycosylation stages), Globo-H hexasaccharide was synthesized enzymatically with a 57% yield.

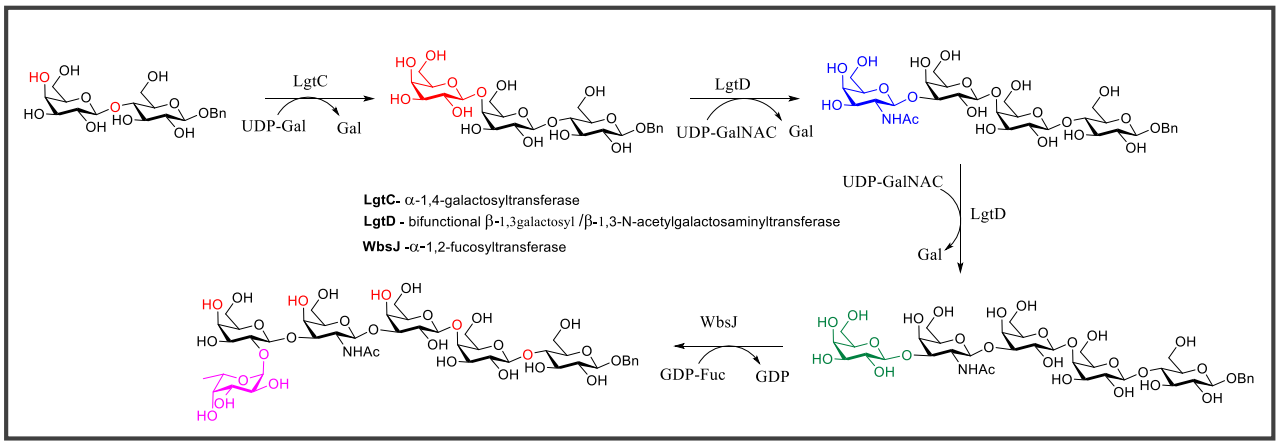


Fig.1.3. Enzymatic production of globo H hexasaccharide.

The intrinsic product hydrolysis in glycosidase-catalyzed transglycosylation is a significant problem. When employing glycosidases for synthetic purposes, excess amounts of glycoside is usually used as the donor substrate to drive the reaction and to accelerate the product formation because the transfer product can also serve as a substrate and be re-hydrolyzed from the glycosidase..[17] To solve the problem, a significant achievement in recent years has been the discovery of glycosynthases, a class of novel glycosidase mutants that do not possess product hydrolysis function but can stimulate glycosidic bond formation by activating a suitable glycosyl donor. To date, numerous glycosynthases have been successfully engineered in order to obtain beneficial oligosaccharides and glycoconjugates.[18] Numerous glycan-modifying enzymes, including acetyltransferases, oxidases, sulphotransferases, and epimerases, have recently been engineered for the post-glycosylation modification of glycoconjugates and oligosaccharides..[19-20] In 2001, Wong and co-workers made significant strides in this sector by developing the automated enzymatic procedures.[21] Over the past few years, this technique has been used widely to assemble various oligosaccharides and glycoconjugates. In 2015, Seeberger group demonstrated a method for producing sialylated oligosaccharides that involved automated glycan assembly and enzymatic sialylation. With only a few building blcoks, solid-phase chemistry is employed to quickly and efficiently produce the glycan backbones, whereas sialyltransferase is used to quickly and efficiently sialylate a wide range of substrates.[22]

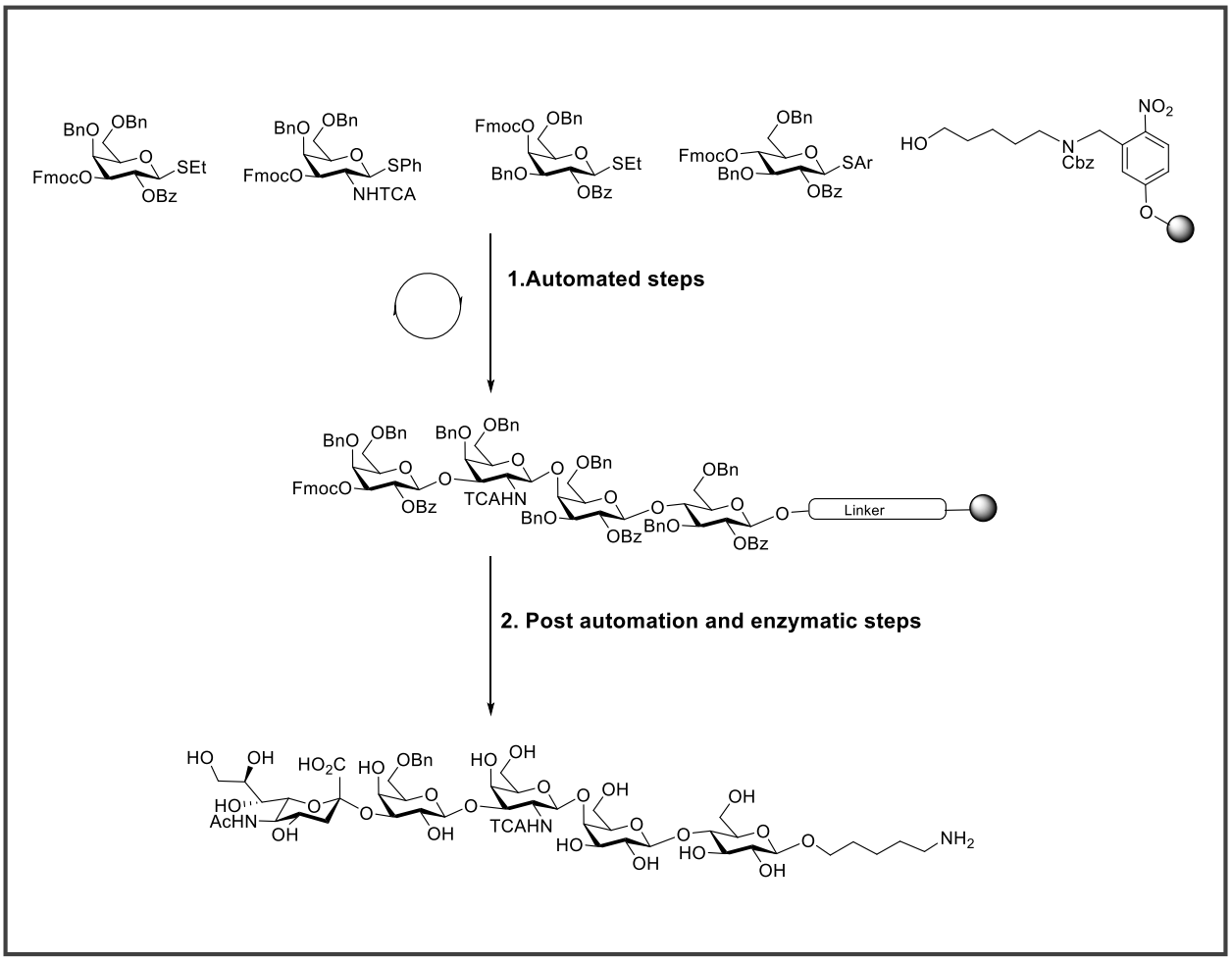


Fig 1.4: Synthesis of sialic acid containing glycans by automated glycan assembly strategy

Automated steps: a) glycosylation (NIS, TfOH, DCM,Dioxane), b)Fmoc deprotectuin (TEA,DMF), c)acid wash(TMSOTf,DCM) Post automation steps: a) photo-cleavage(hv,DCM) b) methanolysis(NaOMe,MeOH, DCM), c) hydrogenolysis(Pd/C,H2,MeOH,H2O,AcOH), d) sialylation(a(2,3)-sialyltransferase,CMP-Neu5Ac,alkalinephosphatase,Tris-HClbuffer)

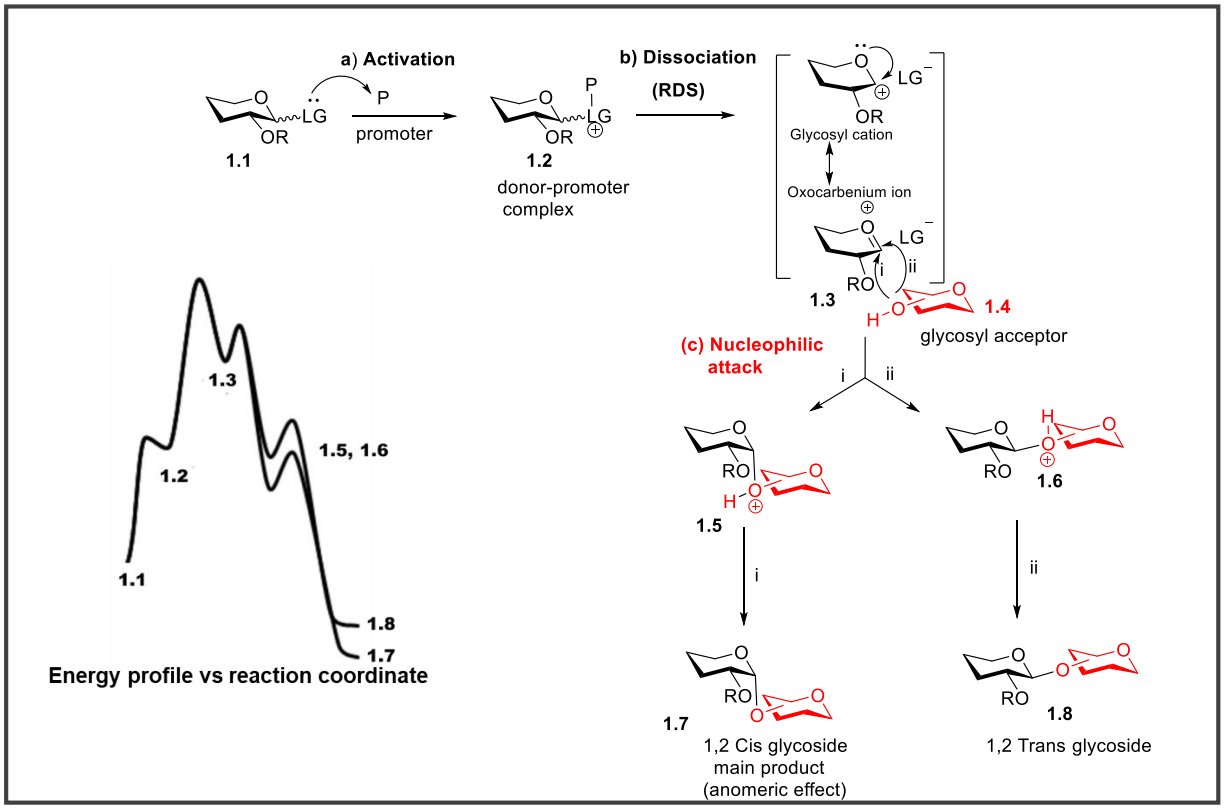
However the widespread use of enzymes in synthetic glycochemistry is still significantly hampered by certain factors (i) as specific enzymes are very selective for specific acceptors and with high amount of variability of the glycosidic linkages, needs a vast collection of varied expensive and unusual enzymes, (ii) enzyme tolerance with saccharides with atypical or unconventional sugar units and (iii) minor alterations to the catalytic region of enzymes produced by mutation can slow down the reaction pace.[23-24] Consequently, the primary power source

synthetic approach that the scientific community still has access to is the chemical synthesis of glycoconjugates and oligosaccharides

1.3 Chemical Synthesis of Oligosaccharides:[25]

A glycosidic bond is formed when a sugar alcohol or another nucleophile, with the appropriate promoters, nucleophilically displaces a leaving group at the anomeric carbon of a sugar unit. Glycosidic bond formation considered to involve a four separate phases:[26]

i. Generation of the donor-promoter complex **1.2**, which, depending on the system, may be irreversible or reversible
ii. The slowest stage (RDS) of the reaction is the ionization of the glycosyl donor, which results in the production of an oxocarbenium ion intermediate **1.3**;
iii. Glycosyl acceptor attacks the oxocarbenium ion intermediate to produce protonated glycoside **1.5**,**1.6**
iv. Transfer of proton delivering the glycoside **1.7** and**1.8**



Scheme1.1: General Mechanism of Glycosylation

Over the past decades, the majority of research directed at enhancing the efficiency and stereoselectivity of oligosaccharide syntheses concentrated on the identification of new activators, leaving groups,), and on the modification of condition of the reactions.[27] However, because even minor advancements have yet to fully regulate the end product of all glycosylation reactions, efforts have also been made to better understand the mechanisms and energies that drive this critical reaction. Nature precisely and consistently achieves the glycosylation process to build complex oligosaccharides. However, chemical construction of the glycosidic bond remains a difficulty. Numerous variables contribute to the difficulty of oligosaccharide synthesis.

1.3.1 Types of glycosidic linkages:[28-29]

There are numerous ways for an aglycon to attach itself to the reducing end of a sugar molecule, resulting in a variety of oligosaccharide and glycoconjugate structures. Based on the spatial relationship of two vicinal functional groups connected to the C1 and C2locations, glycosidic connections are classified into two forms: 1,2-cis and 1,2 -trans linkage. The linkage is known as a 1,2 -trans linkage if the two groups are not coplanar, and a 1,2-cis linkage if they are. When a saccharide unit (2-deoxy sugars) has no functional group at the C2 position, the linkage assignment is determined only by the orientation of the functional group at the reducing end.

The existence of a certain glycosidic linkages can have a significant impact on its biophysical characteristics or functions in a biological system. For example, starch and cellulose are both glucose polymers with α-(1→4) and β-(1→4) glycosidic connections, but only starch can be hydrolyzed to monomer glucose by enzymes and used as a source of energy in humans. The deoxy sugars and their glycosidic connections are also very essential because they are found in many biologically important natural scaffolds. As a result, oligosaccharide or glycoconjugate synthesis with specified regio- and stereo-specific connections are of tremendous interest in carbohydrates.

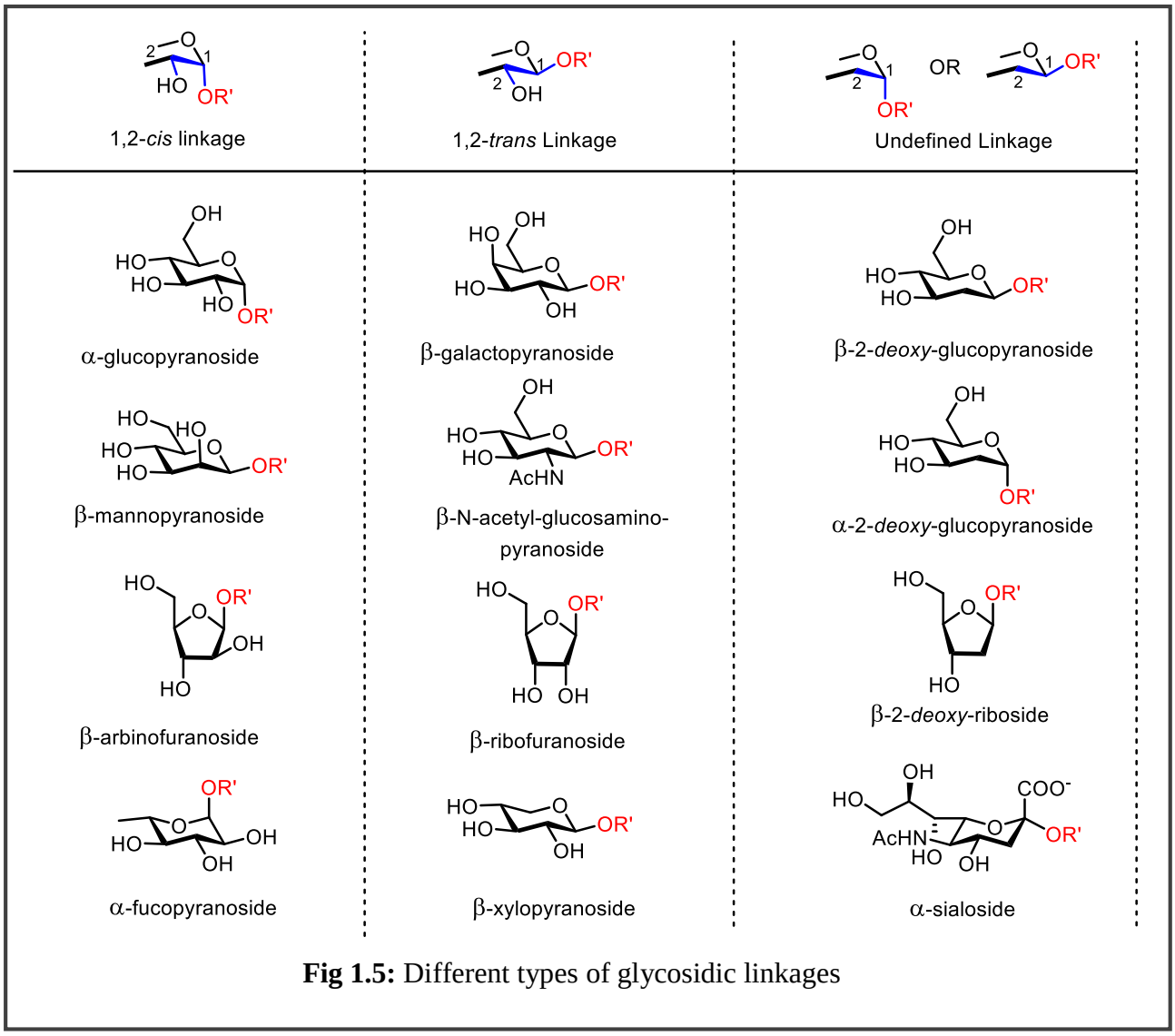


Fig 1.5: Different types of glycosidic linkages

Fig 1.5: Different types of glycosidic linkages

1.3.2 Factors in the stereoselectivity and regioselectivity in glycosylation reaction:

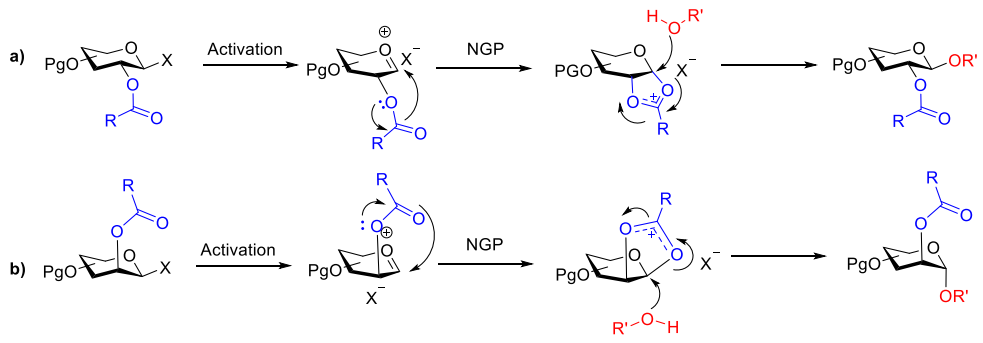
The development of a diverse range of orthogonal protective groups gives exposure to building units containing a single exposed hydroxyl group and as a result, regioselectivity concerns in glycosylation reactions are readily averted. The glycosylation reaction mechanism is complicated in many ways, and it is frequently difficult to distinguish between S_N1 and S_N2 nucleophilic substitution reactions. Nonetheless, it is usually assumed that the reaction conditions favour a

unimolecular S_N1 mechanism, therefore regulating the stereochemistry of the new anomeric bond is important.[30] The chemical synthesis of complex oligosaccharides are often a time-consuming, multi-step process that results in extremely low yields. Since each target oligosaccharide's monosaccharide component needs its own set of protective groups, every synthetic method uses a different strategy in order to ensure that the hydroxyl groups in each sugar unit are orthogonal to one another. Efforts have been undertaken to increase the speed and the efficiency of oligosaccharide synthesis by adopting one-pot multistep glycosylation procedures, polymer supported syntheses, and programmable one-pot methodologies.[31,32] These methods are frequently hampered by the same problem that bothers any glycosylation reaction: the resultant glycoside is typically produced as a mixture of both anomers. In particular, the stereocontrolled synthesis of 1,2-cis glycosyl linkages (e.g., α-glucosides, β–mannosides, β-arabinofuranosides) demonstrates the harder challenges. Numerous infections and cancerous cells have oligosaccharides on their surface, and these oligosaccharides containing 1,2-cis linked glycosides as important structural components. Numerous studies into the building of these links have been conducted in response to the potential use of these structures in carbohydrate-based vaccine candidates, but no generally accepted technique for their synthesis has yet been produced.

1.3.2.1 Stereoselective Glycosylation Strategies:

1, 2-trans glycosides:[33]

Unlike the difficulty in the synthesis of 1,2-cis glycosides, the synthesis of 1,2 -trans glycosides (e.g., - glucosides, - mannosides) can typically be accomplished with high levels of stereocontrol by utilizing classical neighbouring group participation (NGP) of 2-*O*-acyl protected glycosyl donors. Below is a schematic representation of the synthesis of a 1,2 -trans glucoside and a 1,2 -trans mannoside.



Scheme 1.2: Generation of 1,2 -trans -glycoside by classical NGP

When the glycosyl donor is activated, an oxocarbenium ion is formed. Subsequent acyl group participation at the 2-position results in the more stable dioxolenium ion. Since an S_N2-like attack by the alcohol of a glycosyl acceptor can now only occur on one face, the resulting glycosidic bond must be trans to the bond at the 2-position producing the β-glycoside in the case of glucosides. Same type of reaction pathway follows for the Manno-glycosyl donor, however, this time the β-face of dioxolenium ion is protected from attack, and so the α -glycoside is produced. By using an amide protecting group at C2 position, it is also feasible to generate 1,2 -trans glycosides from glucosamine donor. However, because the acetamide can react both with the glycosyl donor and the activator, N-acetylglucosamine derivatives frequently have low glycosylation efficiency. As a result, N-phthalimido group is frequently selected as the protective group for glucosamine donor. Because carbamate protecting groups can participate in nearby groups, N-troc protected glucosamine donors also supply 1,2 -trans -glycoside.

Apart from the usage of ester type protecting groupS in C-2 position for installation of 1, 2 trans selectivity, there has other reports like introducing 2-*O*-nitrobenzyl, 2-*O* -picolylether, 2-*O*-alkoxymethyl, 2-*O* -cyanomethyl ether groups.[34-37]

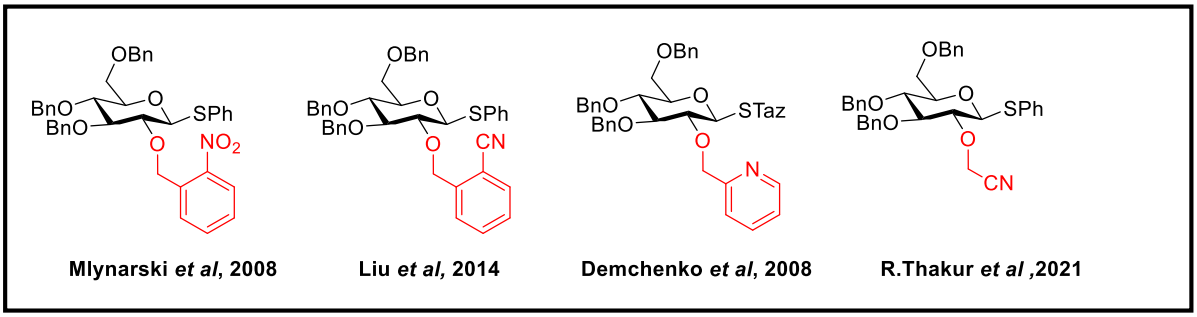


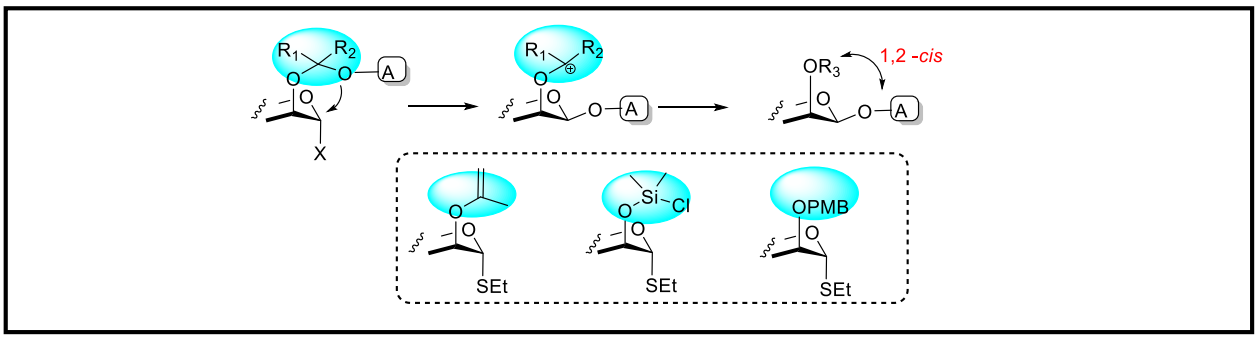
Fig 1.6: Ether type C-2 particiapting groups for 1,2 -trans -glycosides

This novel ether-based armed protective group has the potential to greatly expand the concept of using participating groups in glycosylation reactions. The ease with which these ether groups may be protected and deprotected further demonstrated their efficiency in oligosaccharide synthesis.

1, 2- cis glycoside:[38]

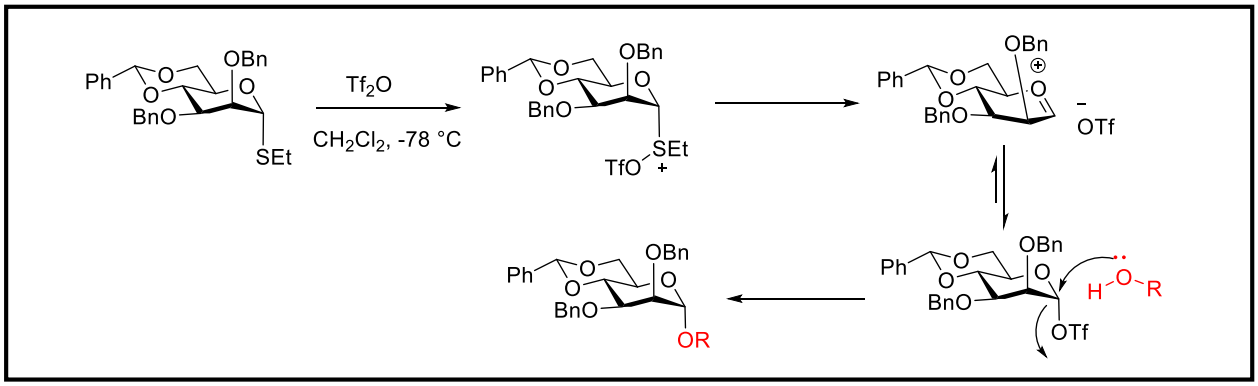
Construction of 1,2-cis glycosidic connections is more difficult than that of 1,2 -trans linkages. The existence of a non-participating group enhances the formation of 1,2 -cis glycosides, and the level of 1,2 -cis selectivity is determined by the reaction conditions and the reactivity of the glycosyl donor and the acceptor. Over the past several decades, a number of strategies for stereoselective 1,2 -cis glycoside synthesis have been reported. Two major approach for the synthesis of 1,2 -cis linkages are – (i) Intermolecular approach, (ii) Intramolecular approach.

In intramolecular techniques, the acceptor molecules are linked to the glycosyl donors via a linker or a spacer. The activation of glycosyl donor produces an oxocarbenium ion, which is trapped by the acceptor intramolecularly to produce 1, 2-cis glycosides. Hindsgaul *et al.* pioneered this effort by creating an isopropylidene linker to tether the acceptor molecules.[39] Hindsgaul, Stork, and Ogawa groups then reported separately chlorosilyl and PMB linkers for 1,2 -cis glycosylation.[40-41]



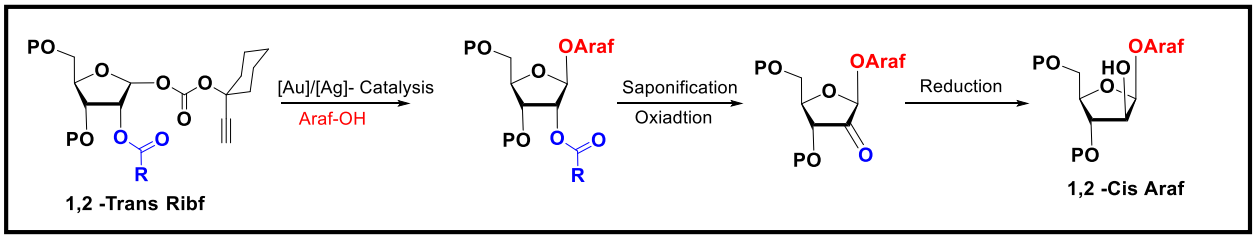
Scheme 1.3: Intramolecular aglycon delivery for synthesis of 1,2 -cis glycosides

In the example of intermolecular approach, Crich *et al.* used 4,6-benzylidene protecting group for the 1,2 -cis mannosylation.[42] The 4,6-benzylidene deactivated the sulfoxide glycosyl donor with the help of changes in electronics and torsional angles, while Tf_2O treatment stabilized the -OTf intermediate at lower temperatures. In the presence of acceptor, the intermediate α-triflate undergoes S_N2 displacement to generate 1, 2-cis mannopyranoside.



Scheme1.4: The synthesis of 1, 2-cis mannoside

Along with the the cyclic 4,6-benzylidene protecting group, many other cyclic protecting groups for 1, 2-cis glycosylation have been developed in recent years;[43] examples include 2,3- or 3,4-acetal, 3,5- or 4,6-di-^{t}butylsilylene, and 3,5- or 4,6-*O*- (1,1,3,3-tetra-isopropyl-1,3-disiloxanylidene). In this regard, Boons *et al*. carried out another significant research by incorporating chiral auxiliary as a protective group in the donor motif itself.[44] When the chiral auxiliary is in the *S*-configuration, a trans-Decalin intermediate is suggested. It was proposed that trans-decalin formed to prevent steric repulsion in the intermediate cis-Decalin. Acceptor molecules can only access the axial-face of the trans-decaline intermediate to produce 1,2 -cis glycosides. In case of furanosides, the epimerization of the C2-Hydroxyl group through stereoselective reduction after oxidation was recognized as a ground-breaking effort by the Hotha group.[45]



Scheme1.5: The synthesis of 1, 2 -*cis*- Arafuranoside

1.3.3 Importance of glycosyl donors in chemical synthesis of oligosaccharide:

As previously mentioned, one of the hardest challenges in building glycoconjugates and oligosaccharides is the creation of glycosidic linkage. Despite numerous glycosyl donors being developed in recent decades, a universal glycosylation method for oligosaccharide synthesis has not yet been established. Predicting the appropriate glycosylation technique for creating a specific glycosidic linkage remains challenging. As a result, each oligosaccharide synthesis becomes a

separate research endeavor that can take months or years to complete effectively. The narrative of glycosyl donors begin with Fisher glycosylation, in which unprotected cyclic hemiacetal and a huge excess of alcohol are refluxed under acidic conditions. Fisher glycosylation is not optimal for oligosaccharide assembly since use of excess alcohol might be expensive and hard to remove during the post-glycosylation stage. To broaden the scope of the glycosidation reaction, several glycosyl donors are being created for glycoconjugate and oligosaccharide synthesis.

Glycosyl Halide:[46]

Koenigs and Knorr created the first practical glycosyl donor in 1901, using glycosyl halides (bromides and chlorides). Different kinds of heavy silver salts are used as promoters in this glycosylation, including AgOTf, Ag_2O, Ag_2CO_3, $AgClO_4$ and $AgNO_3$. Later, Hilfriech et al. improved the glycosylation reaction's effectiveness by adding metal salts that are soluble in organic solvents, such as $HgBr_2$, $Hg(CN)_2$, HgO, $HgCl_2$, and HgI_2. Although less stable than glycosyl chlorides and bromides, glycosyl iodides are superior due to their effectiveness, speed of reaction, and stereoselectivity. Glycosyl iodides can afford β-glycosides in basic reaction media, whilst in-situ anomerization can produce α-glycosides. In 1981, Mukaiyama et al. reported the most stable glycosyl fluoride. While $SnCl_2$ and $AgClO_4$ were virtually the only activators utilized by the Mukaiyama group, Nicolaou and Noyori fine-tuned the reaction conditions by adding TMSOTf to activate the glycosyl fluoride donor. Despite the huge benefits of using glycosyl halides as donors, it is not generally employed to construct large-scale glycoside synthesis due to the utilization of exceedingly toxic promoter systems.

Glycosyl esters and orthoesters: [47]

Hilfriech and Kochetkov independently reported glycosyl esters and orthoesters as glycosyl donor. To activate glycosyl esters, a range of Lewis and Brønsted acids, including $ZnCl_2$, $SnCl_4$, $FeCl_3$, BF_3•OEt_2, TfOH, and TMSOTf were used. Although glycosyl esters have very good stability, their applications have been restricted by their poor reactivity and the requirement of a stoichiometric amount of activators, which can be very harsh for oligosaccharide synthesis. When glycosyl esters are treated with acid halide, they produce glycosyl halide, which can be converted into glycosyl 1, 2-orthoester in the presence of base. Among the numerous 1,2- orthoesters, Kochetkov initially created ^{t}-butyl orthoester and activated it with 2,6-dimethylpyridinium perchlorate to generate the

necessary glycoconjugates. However, 1,2-orthoesters can be activated by a catalytic amount of actiavtors, a direct glycoside side product frequently provides a constraint.

Thioglycosides:[48]

Thioglycosides are the most common and stable glycosyl donors in carbohydrate synthesis. In 1973, R. J. Ferrier reported phenyl-1-thioglycosides as glycosyl donors, using $HgSO_4$ or $Hg(OAc)_2$ for activation and demonstrating glycoside synthesis. Following this, numerous metal-based Lewis acids and electrophilic reagents were reported to successfully activate thioglycosides. The most practical and efficient approach, however, was devised in 1990 by Van Boom (NIS/TfOH) and Fraser-Reid (NIS/AgOTf), who demonstrated the applicability of thioglycoside for oligosaccharide synthesis with enhanced yield. Many stable thioglycoside donors, including -STol, SEt, SMe, SOPh, and SBox, as well as over 50 distinct activating reagents, have been identified so far. These thioglycoside donors are elegant because of (i) The donor can be produced simply by treating hemiacetals with dithiols in the presence of nBu$_3$P or PPh_3 and per-*O*-acetylated sugar derivatives and a mercaptan under Lewis acids, such as BF_3•OEt_2, TMSOTf, and $SnCl_4$ (ii) The donor can also be utilized as a temporary anomeric protective group because it is stable and immune to the many functional group transformations used in carbohydrates synthesis (iii) can be activated in extremely mild temperatures between 50 and -90 ^oC

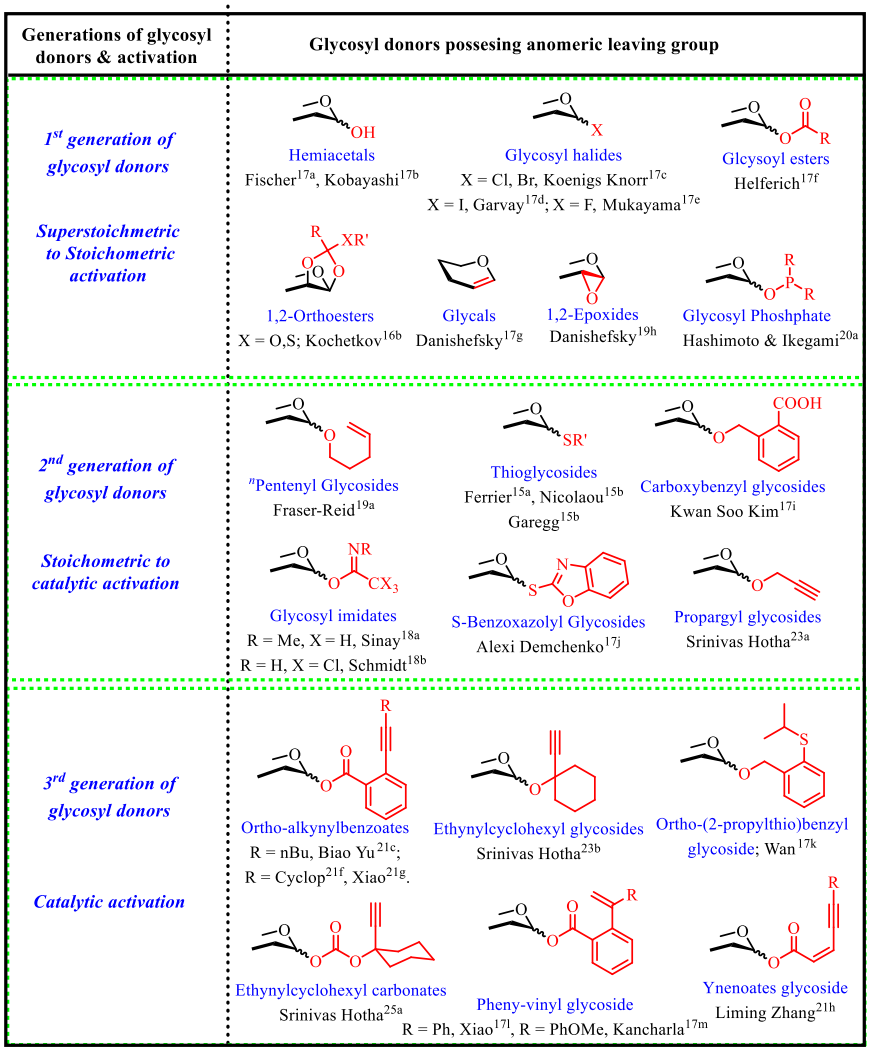


Fig. 1.7 List of glycosyl donors developed in carbohydrates syntheses

Glycosyl Imitates:[49]

Glycosyl imidates are among the most versatile and commonly utilized donors in carbohydrate synthesis. Sinäy invented the donor in 1976, and Schmidt and colleagues expanded on it (glycosyl trichloroacetamidates (TCA) and trifluoroacetaimidates (TFA). TCA donors that are thermally and chemically stable can be generated by reacting glycosyl hemiacetal with trichloroacetonitrile with addition of NaH, K_2CO_3, Cs_2CO_3, or DBU. The glycosylations can be performed effectively in presence of catalytic amounts of BF_3•OEt_2, TMSOTf, TfOH or PPTS. In addition to the promoters already described, Kunz and Schmidt have also reported the use of Au-catalysts to initiate TCA. The limited stability of glycosyl TCA and TFA, on contrary, laid the groundwork

for the invention of a more stable (*N*-phenyl)-trifluoroacetimidate glycosyl donor which can be kept for months and is catalytically activated by Lewis acids.

Alkenyl glycosides:[50]

In 1988, Bert Fraser-Reid group developed this *n*-pentenyl glycoside as a stable and potent glycosyl donor, which has been used extensively in the synthesis of glycoconjugates and oligosaccharides. Typically, *n*-pentenyl glycosyl (NPG) donors are synthesised by reacting glycosyl hemiacetal and *n*-pentenyl alcohol with acid at higher temperatures, or by treating glycosyl acetate with 4-pentene-1-ol in the presence of appropriate Lewis acids. During the activation of n-pentenyl glycosides with NIS-TfOH, the distant olefin moiety is iodinated, followed by cyclization to produce tetrahydrofurfuryl iodide and oxocarbenium ion intermediates, which are subsequently attacked by acceptor molecules to produce the appropriate glycoside. Both orthogonal activation glycosylations and several latent-active glycosylation techniques using this donor are developed.

Glycosyl phosphates:[51]

In 1980, glycosyl phosphates and phosphites were studied as glycosyl donor. Hashimoto and Ikegami created diphenyl phosphates and diphenyl phosphine imidates, respectively, to serve as glycosyl donor. Phosphate donors are easily generated from glycosyl trichloroacetimidates by treating with phosphorous acid. Seeberger et al. used a number of Lewis acids, such as TMSOTf, TBSOTf, and BF_3•OEt_2, to activate glycosyl phosphates, and discovered that TMSOTf was more efficient. Glycosyl phosphates are also used in solid-phase oligosaccharide syntheses and have shown to be promising building blocks because to their thermal stability and good leaving group ability when exposed to promoters. In 2019, Jacobsen found that chiral bis-thiourea catalyst can be used to activate glycosyl diphenylphosphates for stereospecific nucleophilic substitution processes producing *O*- and *S*-glycosides,

Glycals:[52]

Glycals have emerged as very important and adaptable glycosyl intermediates in the formation of a certain family of glycosides, namely 2-deoxy or 2,6 -dideoxy glycosides. Commercially available glycals include 3,4,6-tri-*O*-acetyl-D-glucal and 3,4,6-tri-*O*-acetyl-D-galactal, which can be used as is or modified with necessary protecting groups. Furthermore, zinc-assisted elimination of glycosyl

halides under acidic conditions or treatment with zinc dust and imidazole in ethyl acetate can yield glycals. Furthermore, Lemieux et al. used glycals to synthesize sucrose glycosides from 1,2-anhydro sugars. Danishefsky and colleagues made a breakthrough in glycal donor chemistry by oxidizing glycals with dimethyldioxirane (DMDO) to produce 1,2-anhydro sugars, which can react with $ZnCl_2$ to produce desirable glycoconjugates and oligosaccharides.The aforementioned donors have several limits in terms of stability, reaction output, and yields, allowing for the construction of a "universal glycosyl donor."

1.3.4 The gold catalysis era in glycosylation:[53-55]

In the last decade, the glycosylation process, involving cationic Au(I) and Au(III) complexes as catalysts has underwent ground-breaking advancements. As a result, several new varieties of glycosyl donors have been developed which can be activated by certain alkyne-containing gold species and have varying aglycons as anomeric leaving groups. In 2006, Hotha et al. reported the first gold-catalyzed glycosylation process involving the Ferrier-type glycosylation of 3-*O*-propargyl glycals in the presence of catalytic amount of $AuCl_3$. This novel glycosylation approach spurred other carbohydrate chemists to investigate and expand the application of gold catalysis in glycosylation processes. Significant developments in the field include reports of the Zhu group's $AuCl_3$-mediated activation of alkyne functionality comprising 2,2-dimethylbut-3-ynyl thioglycosides and the Hotha group's, glycosyl propargyl 1,2-orthoester donor for 1,2-trans glycosylation. The mild reaction conditions and 1,2-trans glycosylation method permitted this approach to be used to prepare furanosides, pyrimidine nucleosides, thioglycosides, glycosyl carbamates, aminooxy glycosides, and glycosyl acrylate/acrylamides. Later, Hotha and Kayastha discovered that propargyl glycoside donors are significantly less reactive than 1-ethynylcyclohexanyl glycoside donors since the former may be activated at room temperature using co-catalysts $AuCl_3$ and AgOTf. The use of these glycosylation methods to prepare 1,6-anhydro saccharides, thioglycosides, furanosides was reported.

In 2008, Yu group showed Au (I)-catalyzed glycosylation employing easily accessible and shelf-stable glycosyl *o*-hexynylbenzoates as potent glycosyl donor and $Ph_3PAuOTf$ or $Ph_3PAuNTf_2$ as catalyst. This glycosylation reaction can take place under mild conditions and can tolerate a very broad range of substrates. Mishra, Neralkar and Hotha discovered another very mild Au(I)/Ag(I) co-catalyzed glycosylation technique employing 1-ethynylcyclohexyl glycosyl carbonate donors.

The Au(I) catalyzed glycosylation is milder than all existing glycosylation procedures that frequently use powerful Lewis acid or Bronsted acid activators. As a result, these relatively novel approaches are gaining traction, as many normally complex glycoside syntheses could be easily accomplished by utilizing key properties of gold catalysis.

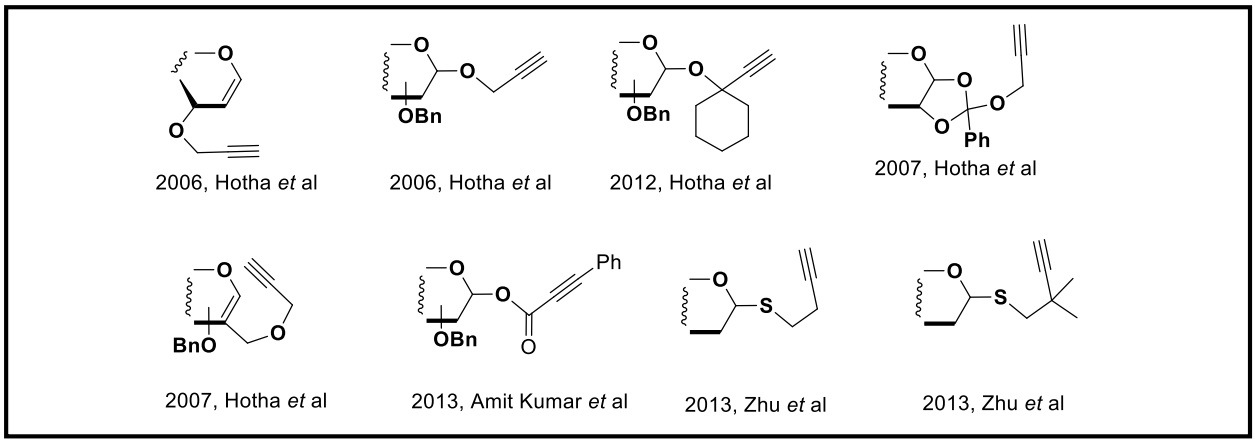


Fig. 1.7 Representative glycosyl donors that can be activated by a Au(III) catalyst.

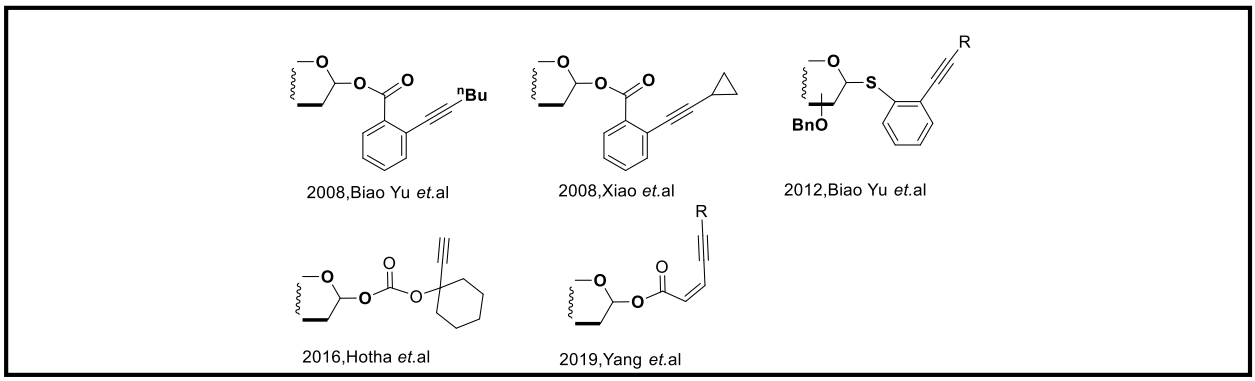


Fig. 1.8 Representative glycosyl donors that can be activated by Au (I) catalyst

1.3.5 Glycosyl ethynylcyclohexyl carbonate – A potential Glycosyl Donor for oligosaccharide synthesis:[55-61]

After the first report of gold catalyzed glycosylation from Hotha group, Mishra et al. developed a mild, easily scalable, catalytic, and fast glycosylation method using shelf-stable glycosyl 1-ethynylcyclohexyl carbonate donor and Au(I) phosphite and AgOTf as co-catalysts. This novel donor has demonstrated promise in the synthesis of different kinds of glycoconjugates and oligosaccharides. With this newly created approach, the *O*-glycosylation reaction takes 15 minutes to complete at room temperature. The toolbox of synthetic carbohydrate chemists today includes

a number of glycosyl donors that can be utilized to generate simple glycosides and disaccharides. Only a few glycosyl donors, however, have been used in the synthesis of higher oligosaccharides and complex glycosides thus far. The first oligosaccharide synthesis reported with this method was a tridecasaccharide resembling lipoarabinomannan from the cell wall of *Mycobacterium tuberculosis*. Finally, 21-mer arabinomannan and arabinogalactan motifs observed in the cell wall of M. tuberculosis were successfully synthesized. Hotha group have effectively synthesized more complex branched glycan fragment seen in the chlorovirus ATCV-1. Another advantage of adopting this alkynyl carbonate donor chemistry is that it produces high glycosylation yields even for larger oligosaccharide couplings. The development of difficult 1, 2- cis connections of glucose and galactose units employing this novel glycosyl donor has been thoroughly researched. This donor was used in the successful development of the shortest synthetic pathway for the core pentasaccharide of the life-saving medication Fondaparinux. This donor was employed in developing a unique solid-phase synthesis process for producing oligosaccharides in significantly less time. This donor has already gained importance due to promising oligosaccharide synthesis findings in a gentler manner than normal glycosyl donors. However, further refinement of this methodology is still needed to synthesize glycosides and glycoconjugates, which would otherwise be challenging.

1.4 Goal of the thesis:

Synthetic oligosaccharides and glycoconjugates are utilized as probes in biological research and as lead compounds in medicinal chemistry and vaccine development. However, the absence of universal procedures makes it difficult to prepare these necessary compounds on a regular basis. Recent advancements are beginning to address these challenges, including one-pot multistep protecting-group manipulations, the use of unified monosaccharide building units, the introduction of stereoselective glycosylation strategies, and convergent protocols for oligosaccharide assembly. Controlling and altering carbohydrates' stereochemistry is very difficult due to a variety of dependent as well as independent environmental variables, intrinsic and poorly understood molecular preferences, high sensitivity, and multiple competing side reactions. In recent years, various methods for catalytic glycosylation have shown great potential and made significant contributions to the realm of oligosaccharide synthesis.[62] Many of these techniques provide excellent yields and selectivities, but only for a limited set of substrates.

CHAPTER 1

Gold-catalysis has played a significant role in advancing oligosaccharide synthesis strategies. Its success is largely due to the mild, catalytic, and highly reactive nature of the donor chemistry, along with the noncompetitive leaving group. Over the past decade, many disaccharides, trisaccharides, and oligosaccharides have been efficiently generated using the distinctive advantages of gold-catalyzed donor chemistry. Additionally, the development of new reaction technologies for synthesizing oligosaccharides and glycoconjugates is essential for progress in various scientific and industrial fields. These advancements enable more detailed biological studies, the creation of new therapeutics, efficient and scalable production processes, and the development of novel materials and analytical techniques. In this context, the major goals of this thesis are as follows:

1) Synthesize the Arabinan 31-mer fragment, reminiscent of the *Mycobacterium tuberculosis* cell wall, using [Au]/[Ag]-catalysis
2) Apply 1-ethynylcyclohexyl carbonate donor for orthogonal and one-pot glycosylations
3) Develop new reaction strategies to address current challenges in the synthesis of oligosaccharides and glycoconjugates.
4) Utilize these new reaction strategies to create various glycan probes for the discovery of new biological pathways, medicines, and materials science.

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Chapter: 2

Chemical Synthesis of 31-mer Arabinan of *Mycobacterium Tuberculosis* Cell wall

2.1- *Tuberculosis*-Introduction:

Tuberculosis (TB) stands as one of humanity's oldest and most devastating adversaries, persistently afflicting millions across the globe, particularly in developing regions. Approximately 1.7 billion individuals, constituting about 23% of the global populace, carry latent TB infections, putting them at significant risk of developing active TB at any point in their lives. According to the 2017 World Health Organization (WHO) report, TB affects roughly 10 million people worldwide annually, resulting in 1.3 million deaths among HIV-negative individuals and an additional 300,000 deaths among those living with HIV.[1] The situation is compounded by the challenge of drug resistance, with multi drug-resistant (MDR) and extremely drug-resistant (XDR) strains posing serious public health threat. In 2017 alone, nearly 5,58,000 people developed rifampicin-resistant tuberculosis (RR-TB), with 82% progressing to MDR-TB and 8.5% to XDR-TB. To stem the tide of TB-related mortality and transmission, swift and accurate diagnosis is imperative, particularly for HIV-associated and drug-resistant strains.[2] The Xpert® MTB/RIF assay is currently endorsed by the WHO as the sole rapid diagnostic tool, furnishing results within two hours. Despite decades of devastation, effective treatments emerged only in the 1940s. Presently, the WHO advocates a six-month regimen comprising four first-line drugs — isoniazid, rifampicin, ethambutol, and pyrazinamide—for newly diagnosed pulmonary TB patients. Moreover, ongoing clinical trials are exploring an array of novel TB drugs and combination therapies. Following the adage "prevention is better than cure," the Bacilli Calmette-Guérin (BCG) vaccine was developed nearly a century ago, named after its creators Albert Calmette and Camille Guerin.[3] This vaccine has exhibited significant efficacy in safeguarding against severe forms of childhood TB and remains widely administered today.[4]

2.2 -*Mycobacterium tuberculosis* (Mtb)

On March 24, 1882, Dr. Robert Koch made a ground breaking announcement: he had identified the bacillus *Mycobacterium tuberculosis* (Mtb), the culprit behind tuberculosis (TB).[5] This pivotal moment is now commemorated annually as the World TB Day. M. tuberculosis belongs to the mycobacteriaceae family and primarily targets the mammalian respiratory system, particularly the lungs, although it can also affect other areas such as the central nervous system, lymph nodes, bones, and skin.[6] Within the *MTb* complex, comprising at least nine sub-species, M. tuberculosis, M. africanum, and M. bovis stand out as the primary agents responsible for human TB infections.[7] M. tuberculosis, characterized by its aerobic nature and

rod-like shape measuring 0.5 μm in width and 3 μm in length, is identified as acid-fast bacilli.[8] The bacterium's flexible and resilient metabolic features empower it to thrive in various surroundings and persist within human hosts for extended periods, potentially reactivating to induce post-primary tuberculosis.

2.3- Structure of Mtb Cell wall:

Every year, the number of multi drug-resistant (MDR) and extremely drug-resistant (XDR) tuberculosis cases are raising, and the compositional and architectural complexity of the Mtb cell wall is thought to have a significant impact. The Mtb cell wall differs significantly from both Gram-negative and Gram-positive bacteria. The high lipid content (40% of the dry mass of the cell) of the Mtb cell wall makes it abnormally less permeable to external elements, resulting in significant resistance to standard antibiotics and chemotherapeutic drugs. Given these facts, extensive research has been conducted to determine the chemical structure of the Mtb cell wall and its biosynthesis pathways in order to generate new therapeutic agents.

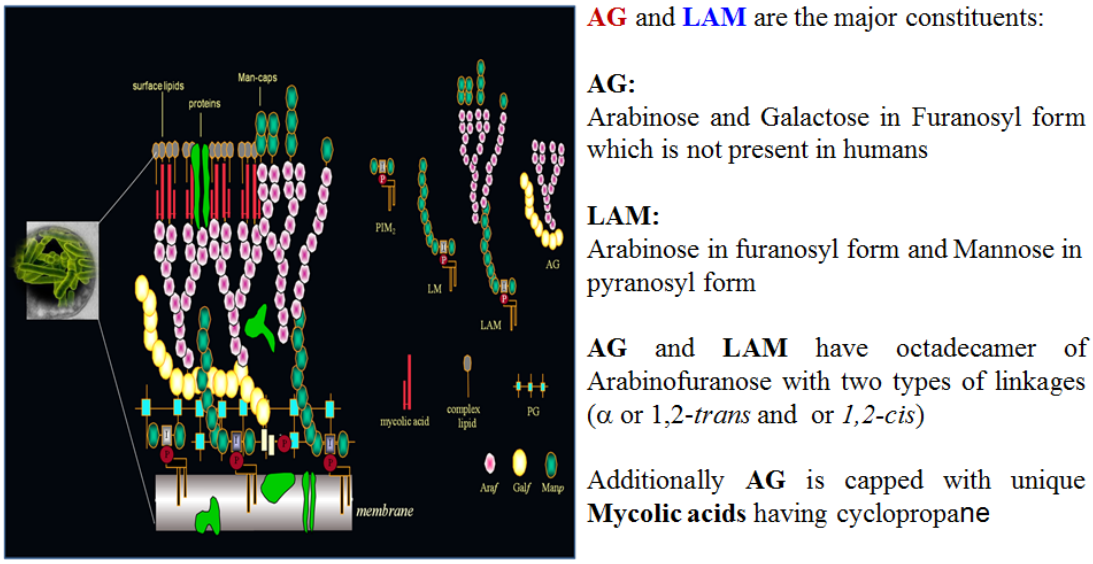


Figure 2.1: Cell wall envelope of *Mycobacterium Tuberculosis*

After extensive investigation and detailed analysis using mass spectroscopic studies, Brenan et al. proposed a tentative structure of the Mtb cell wall in 1980.[9] The cellular envelope consists of three main parts: the inner membrane (or plasma membrane), the cell wall core, and the outer membrane. The inner membrane is a phospholipid bilayer containing glycolipids that reach into the periplasmic space. The cell wall core comprises two major components: (i) the mycolyl-arabinogalactan-peptidoglycan complex, where the heteropolysaccharide arabinogalactan (AG) is linked to the peptidoglycan (PG) and to long-chain mycolic acid (MA),

and (ii) lipoglycans, which include lipomanan (LM) and lipoarabinomannan (LAM). The outer membrane of the *Mtb* cellular envelope is composed of various lipids, glycolipids, and proteins that are not covalently attached.[10]

2.4- Arabinogalactan (AG):

Mycolyl–arabinogalactan–peptidoglycan (mAGP) complex is a major constituent (~35%) of the Mtb cell wall and arabinogalactan (AG) is the key structural component of it.[11] One of the unique features about AG is that none of the sugar residues are in pyranose form and unlike other bacterial polysaccharides, it lacks simple monosaccharide repeating units.[11] Detailed analysis of the methylated oligomers, generated through partial depolymerization of per-*O*-alkylated AG residues using advanced spectroscopic techniques such as GC-MS, NMR and FABMS deciphered the complete structure of AG. With these techniques, the following generalization can be envisioned

1. Around 100 sugar residues make up the AG polymer, out of which 69 are arabinose and 31 are galactose.
2. All galactose and arabinose residues in AG are in the furanose form.[10a]
3. The non-reducing termini of arabinan have a branched pentaarabinofuranosyl structure [β-D-Ara*f*-(1→-α-D-Ara*f*]$_2$-3,-5-α-D-Ara*f*(1→5)-…
4. There are three types of connections according to the position of hydroxyl groups: i.e. major 1→5, four 1→2, and two 1→3 linkages are pressent
5. The arabinan chains are connected to the alternating 5-β-D-Gal*f* moieties at 8, 10 and 12 positions.[10c]
6. The galactan portion of AG is linked to the C-6 of muramyl residues of peptidoglycan *via* the glycophosphoryl bridge L-Rha*p*-(1→3)-D-GlcNAc-(1→PO_3)
7. Mycolic acids encapsulate the primary hydroxyl groups in the terminal non-reducing pentaarabinofuranoside.

2.5 - Drug development against TB and importance of AG AND LAM:

A few essential causes for targeting arabinogalactan (AG): i) **Vital for Survival:** AG is essential for the viability of MTb. ii) **Unique Sugars:** AG contains three sugars — D-arabinofuranose (D-Ara*f*), D-galactofuranose (D-Gal*f*), and L-rhamnose (L-Rha*p*) — which remain absent in humans. Prospective targets for drug discovery are the enzymes involved in the production of these sugars and their polymerization. It is essential to prioritize the isolation

and expression of these enzymes' genes top priority. By using high-throughput screening and detailed enzyme characterization, inhibitors can be discovered and rationally designed. The non-reducing ends of both AG and lipoarabinomannan (LAM) are capped with branched arabinofuranosides known as motif A, linked α-1→5 to the rest of the polymer. In LAM, these ends are further substituted with mannopyranosyl residues, which bind to mannose-binding proteins during early infection stages. In AG, the hydroxyl groups are changed with mycolic acid. The primary degradation products of AG, including motifs A–E, are important for the advancement of drug development. These are key to AG's antigenicity, with serological activity primarily found in motif A, which contains 2-linked arabinofuranosyl units. The primary humoral immunological epitope of AG is motif A. Monoclonal antibodies produced against LAM interact with the purified cell wall, demonstrating a common arabinose-containing epitope between LAM and AG.[12] Motif A in both LAM and AG is required for infection and survival in humans. Some current TB treatments, such as ethambutol, rifampicin, isoniazid, and streptomycin, have recently been identified as inhibitors of arabinosyltransferase.[13-14]

The key objective of this work was to synthesize the large, naturally occurring oligosaccharide motifs of AG to use as probes for investigating mycobacterial cell wall biosynthesis and characterizing enzymes involved in this process, which are promising targets for the discovery of new antitubercular drugs.

2.6 -Present work:

Arabinogalactan (AG), an integral part of the *Mycobacterium tuberculosis* (Mtb) cell wall, offers potential for developing new therapeutic agents and a single-dose treatment for tuberculosis. The structural complexity of the Mtb cell surface, formed by glycosyl tranferases with mycolic acids, arabinose, galactose, and mannose, complicates the understanding of Mtb cell biology. Consequently, the synthesis of various AG and LAM motifs has been a key focus in efforts to develop new therapeutics. Despite significant efforts by several groups,[15-19] the successful synthesis of different AG and LAM motifs has been limited by the use of less efficient glycosyl donors or glycosylation methods with poor overall yields. Various glycosyl donors, including the recently employed propargyl 1,2-orthoester donor, have been used for these oligosaccharide syntheses.[20] However, these methods have limitations such as longer reaction times, moderate yields, excessive activator use, and difficulty in donor preparation at the oligosaccharide level, restricting their effectiveness in synthesizing AG and LAM motifs.

In this premise, glycosyl alkynyl carbonate donor chemistry identified is quite promising for the synthesis of large oligosaccharides as the glycosylation is fast, high yielding, catalytic and the donor is quite easy to prepare.[21] The chemical synthesis of the full length arabinan motif from AG component was investigated using the versatility of the developed alkynyl carbonate donor chemistry.

2.6.1 Total synthesis of 31mer Arabinose motif from the AG part:

Drawing upon previous strands of research on the synthesis of mycobacterial arabinogalactan, this study was therefore conducted to synthesize the full length arabinan portion containing 31-Ara*f*- residues. A careful look at the pioneering work by the Brennan group identified five major structural fragments of AG complex. The arabinan motif consists of different kinds of glycosidic linkages between two arabinose moieties like α-Ara*f*-(1→5)-α-Ara*f*, α-Ara*f*-(1→3)-α-Ara*f* and β-Ara*f*-(1→2)-α-Ara*f*. Therefore, major challenges in the synthesis of hentriacontasa-saccharide are identified as: i) stereoselective installation of Ara*f*-(1→2)-Ara*f* linkage; ii) a robust furanosylation method that enables preparation of both 1,2-trans and 1,2-cis linkages; iii) minimum number of different reactions; and iv) final assembly of the 31 mer arabinan units from different building blocks.

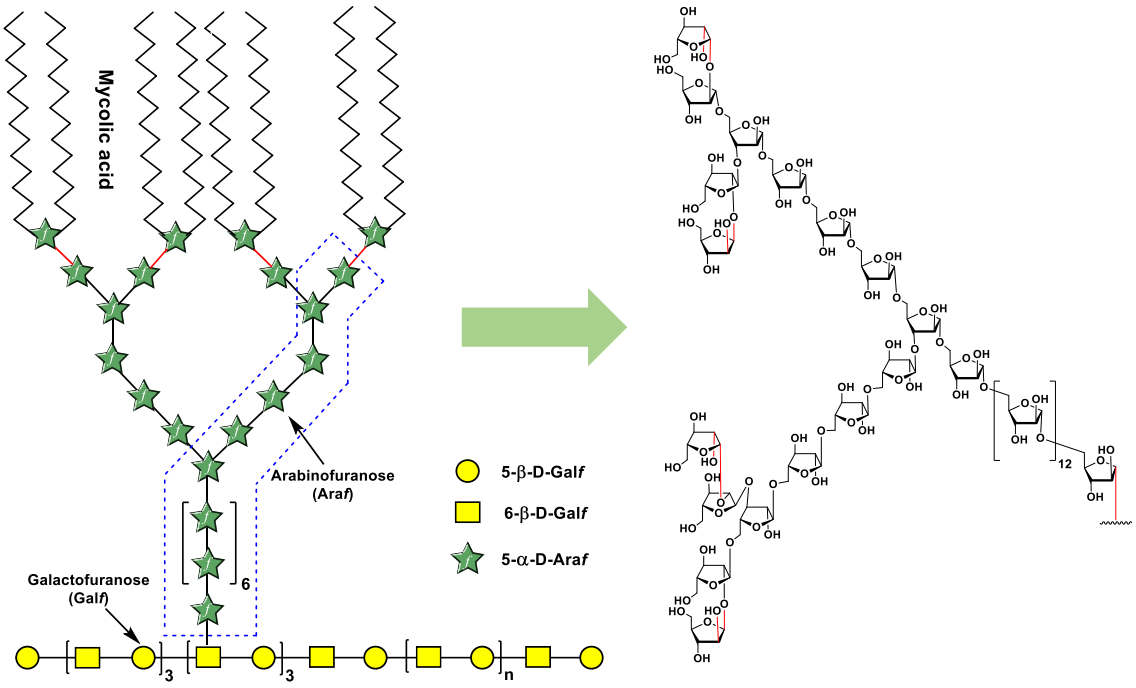
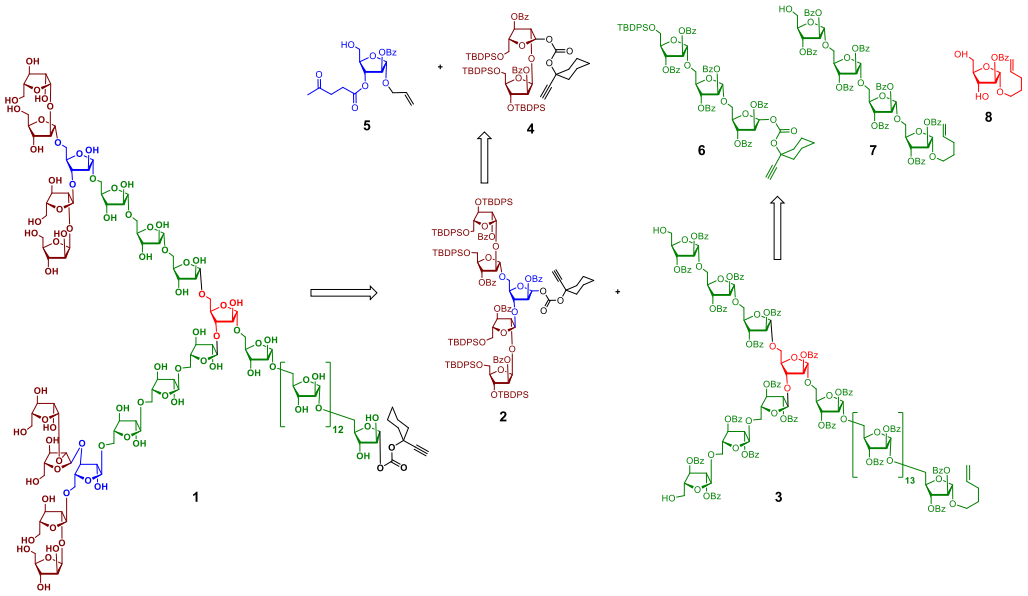


Figure 2.2: Cartoon representation of arabinogalactan (AG) from *M. tb* cell wall and the target 31 mer Arabinan motif

2.6.2 Retrosynthetic disconnection of targeted 31 mer Arabinan motif:

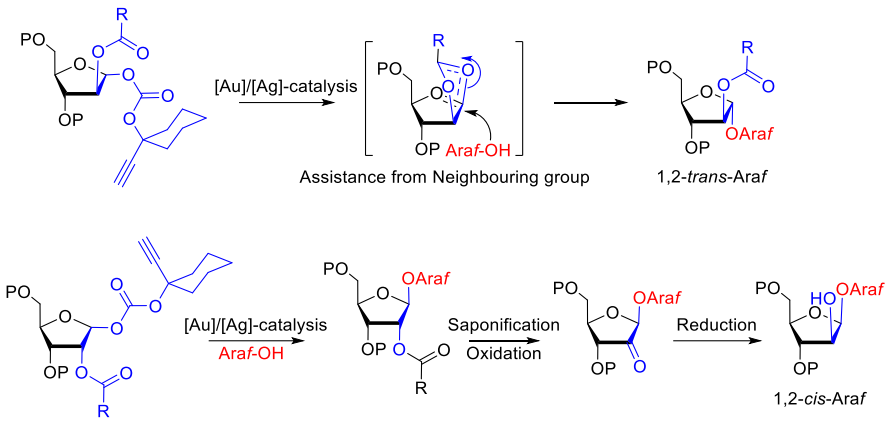
In terms of branching and linking types, the $Araf_{31}$ unit exhibits sufficient complexity and difficulties for its synthesis. The target polysaccharide arabinan **1** was disconnected into two sizeable fragments, that is, the terminal pentasaccharide unit **2** and the branched 21 mer arabinan unit **3**. It was conceived that the desired 31 mer arabinan motif can be constructed by a stereoselective glycosidation between a heneicosasaccharide **3** and two molar equivalents of a pentasaccharide-carbonate (**2**) in a {2 x 5 + 21} fashion. The larger component **3** was envisioned from the trisaccharide **6**, tetrasaccharide **7**, the monosaccharide **8** (Scheme 1). Overall, it was anticipated that the major challenge of our plan towards the total synthesis of arabinan **1** would rely on the efficiency a robust furanosylation strategy that allows preparation of both 1,2-trans and1,2-cis linakges in minimum number of complex reactions and protecting groups still enabling stereoselective assembly of the 31mer arabinan moiety from the motifs **2-8** (Scheme 1)



Scheme 1. Retrosynthetic analysis of the Araf$_{31}$ motif of the arabinogalactan of *Mycobacterium tuberculosis.*

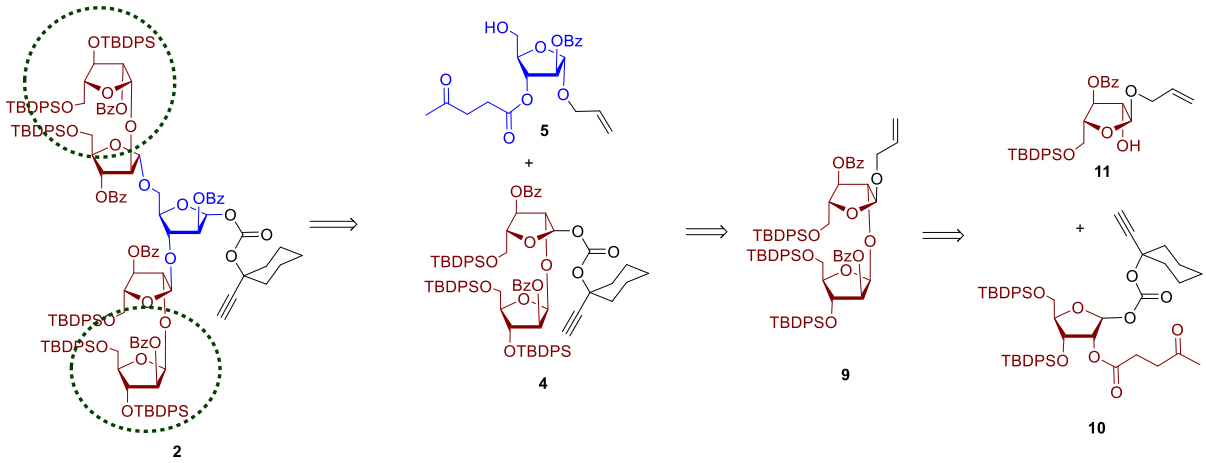
2.6.3 Synthesis of the terminal pentasaccharide motif 2:

One of the most complex portions of the Araf$_{31}$ motif is the terminal pentasaccharide, which requires installation of two β-Ara*f*-(1→2)-Ara*f* linkages that are notorious for their difficulty as well three α-Ara*f*-(1→2)-Ara*f* linkages. We started our synthetic effort by setting up the methods for the 1,2-cis and 1,2-trans glycosylation reactions. For 1,2-trans glycosylation between the two arabinose moieties, we intended to use the [Au/Ag]-catalyzed activation of glycosyl carbonate donor through neighbouring group participation of C2- ester group which was earlier reported by our group. We aimed to replicate the β-(1→2)-Ara*f* linkage formation process observed in mycobacteria.[22-23] Our protocol involves initiating the neighbouring group assisted 1,2-trans linkage on a Rib*f*- derivative. Through subsequent saponification and oxidation-reduction steps, we successfully attain the targeted 1,2-cis or β-(1→2)-Ara*f* linkage, as outlined in Scheme 1.[21,24]



Scheme 2. Synthesis protocol for installation of 1,2 cis- Araf and 1,2 –trans- Araf

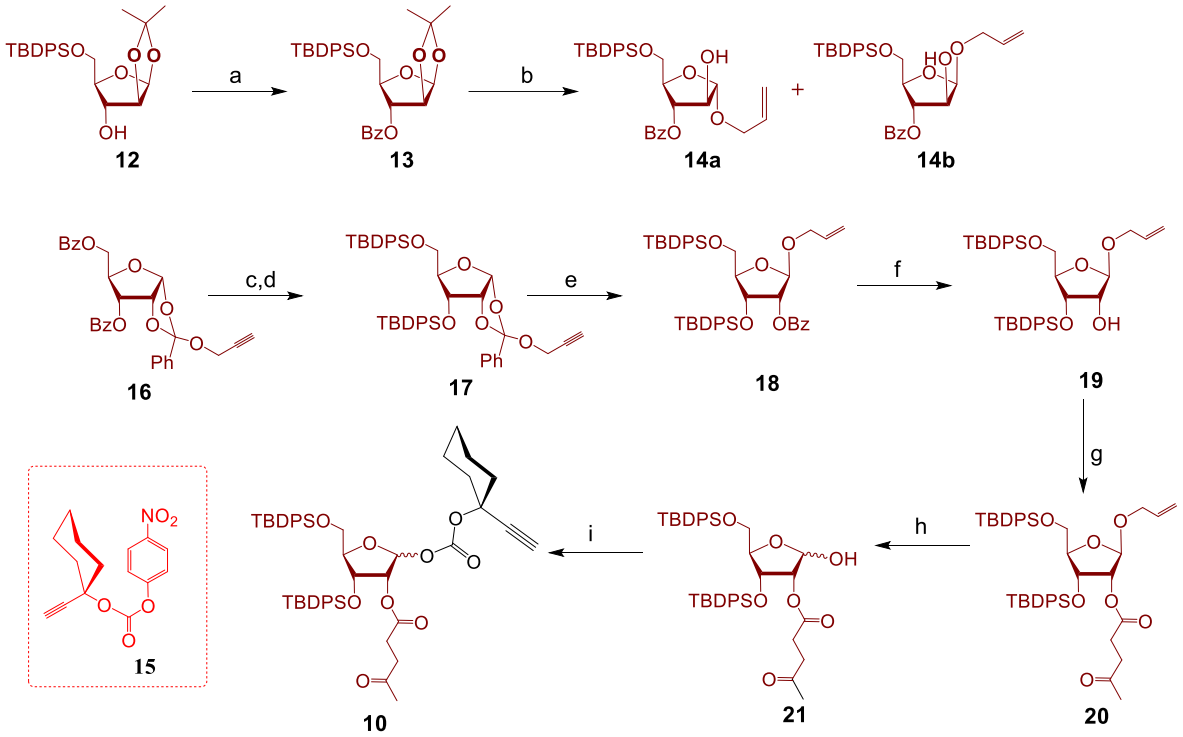
Having identified the methods for both 1,2-cis and 1,2-trans stereoselective Ara*f* glycosylations, we started to synthesize the desired pentasaccharide carbonate motif **2**. The carbonate donor **2** was envisioned from the disaccharide donor **4** and monosaccharide acceptor **5**. We choose a {(2+1) +2} glycosylation strategy between the disaccharide donor **4** and the acceptor **5** that was thought to be the most convenient way to produce the desired pentasaccharide carbonate donor **2**. The glycosyl donor can be easily synthesized from two starting substrates **10** and **11** (scheme 3).



Scheme 3: Retrosynthetic analysis of the Pentasaccharide carbonate donor 2.

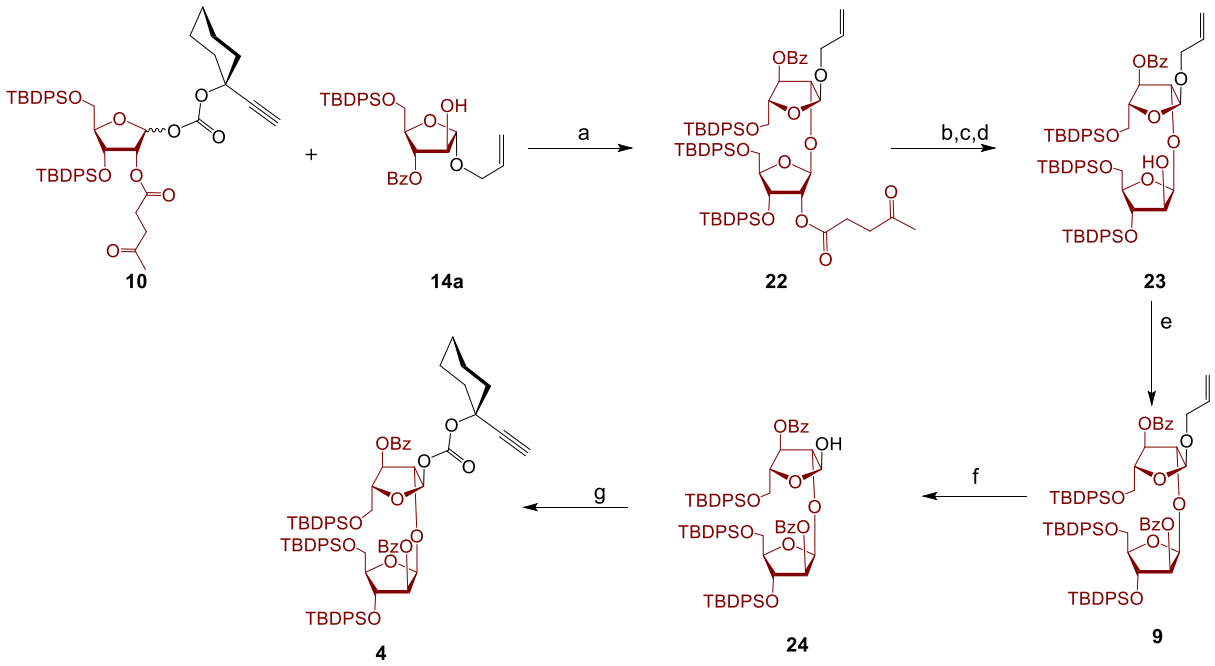
The synthetic effort started with a known arabinofuranoside **12**[25] whose isopropylidene group was opened with allyl alcohol under the acidic conditions to afford a separable mixture of allyl-arabinofuranoside **14a** and **14b** in 1:1 ratio, which are utilised as glycosyl acceptors later. 1,2-cis-Arabinofuranosylation was envisioned through oxidation-reduction strategy; hence, the 1, 2-trans-furanosyl donor of Rib*f*- was required. In addition, the protecting group at the C-2

position of the Rib*f*- donor needs to be orthogonal to the remaining appendages present in the molecule. Unmasking of acetates in the presence of benzoate esters was reported, and the installation of the acetate at the C-2 position is easier if we start with an orthoester **16**. Accordingly, propargyl-1,2-orthoacetate **16** was synthesised by invoking a protocol developed in our group.[25] After this, orthoacetate **16** was transformed into di-*O*-silyl ether **17**, which was treated with allyl alcohol and $AuBr_3$ to obtain allyl arabinofuranoside **18** with C2-benzoate. In continuation, benzoate was saponified easily under Zemplén conditions[26] and protected again with levulinic acid under DIC/DMAP conditions to obtain levulinoate **20**. Then the compound **20** was hydrolysed successfully to hemiacetal **21** by employing 1, 2-DME-CH_2Cl_2-MeOH (3:1:1)/ 25 °C in 83% yield.[27] Subsequently, hemiacetal **21** was conveniently converted into the desired carbonate donor **10** (Scheme 4).[28-30]



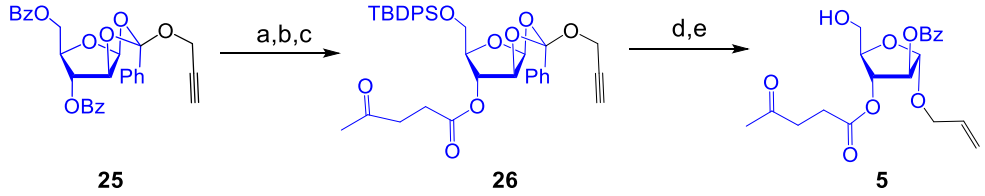
Scheme 4: Synthesis of building blocks for terminal pentasaccharide motif. Reagents a) Py, PhCOCl, CH_2Cl_2, 0–25 °C, 2 h, 94% b) PTSA (0.25 eq.), allylOH, CH_2Cl_2, 55 °C, 3 h, 44% for 14b and 46% for 14a c) NaOMe (0.3 eq.), CH_2Cl_2 -MeOH (1:1), 25 °C, 3 h; d) TBDPSCl, Im., DMF, 0–25 °C, 4 h, 68% over two steps; f) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 3h ,84% g) Levulinic acid, DIC, DMAP, CH_2Cl_2, 0–25 °C,3h, 86% h) $PdCl_2$ (0.2 eq.), 1,2-DME/CH_2Cl_2 /MeOH (3:1:1), 25 °C, 4 h, 83 %. i) **15**, DMAP, CH_2Cl_2, 4h, 88%.
PTSA: p-tolunesulphonic acid; DMAP: 4-dimethylaminopyridine; TBBPSCl: tert-butyldiphenylchlorosilane; DIC: N-N'-diisopropylcarbodiimide.

After synthesizing the desired building blocks, we proceeded with the preparation of the β-Ara*f*-(1→2)-Ara*f* disaccharide. Initially, donor **10** was treated with an excess of the 1,2-trans glycosyl acceptor **14a** under standard [Au]/[Ag]-catalyzed glycosidation conditions. This yielded disaccharide **22** in very high yield, confirmed to be structurally homogeneous through spectroscopic analysis. For example, the two anomeric protons of compound **22** were observed at δ 5.51 (d, *J* = 4.6 Hz, 1H) and 5.33 (s, 1H) ppm in the ^{1}H NMR spectrum, and at δ = 106.1 and 100.9 ppm in the ^{13}C NMR spectrum. Next, the levulinoate group was deprotected to produce the corresponding alcohol, which was then oxidized with PDC/Ac_2O to form the 2-ulose derivative which was smoothly reduced with $NaBH_4$ in EtOAc/EtOH to yield the desired β-Ara*f*-(1→2)-Ara*f* disaccharide moiety **23**. In the ^{1}H NMR spectrum of compound **23**, the two anomeric protons appeared as singlets at δ 5.22 and 5.33 ppm. Additionally, the ^{13}C NMR spectrum confirmed the presence of 1,2-cis and 1,2-trans linakges with resonances at δ 101.5 and 104.6 ppm, respectively. The successfully synthesized disaccharide **9** was then converted into carbonate donor **4** in three steps, as outlined in Scheme 5.



Scheme 5: Synthesis of key β-Araf-(1-2)-Araf disaccharide donor 4 Reagents: a) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, $CH2Cl_2$, 4 Å MS powder, 25 °C, 40 min, 81% for 28 b) 70% $N_2H_4 \cdot H_2O$ (4.5 eq), CH_2Cl_2 , AcOH (22 eq), Py (27 eq), 25 c, 2 h, 92% ; c) PDC (0.6 equiv), Ac_2O (3.3 eq), CH_2Cl_2 , 25 °C, 5 h; d) $NaBH_4$ (1.2 equiv), EtOH/EtOAc (2:1), 0 °C , 2 h, 78% for 23 over two steps; e) Py, PhCOCl, CH_2Cl_2 , 0–25 °C, 2 h, 93%; f) $PdCl_2$ (0.2 eq), CH_2Cl_2 -MeOH (1:4), 25 °C, 4 h; g) **15**, DMAP, CH_2Cl_2 , 25 °C, 5 h, 79% over two steps.

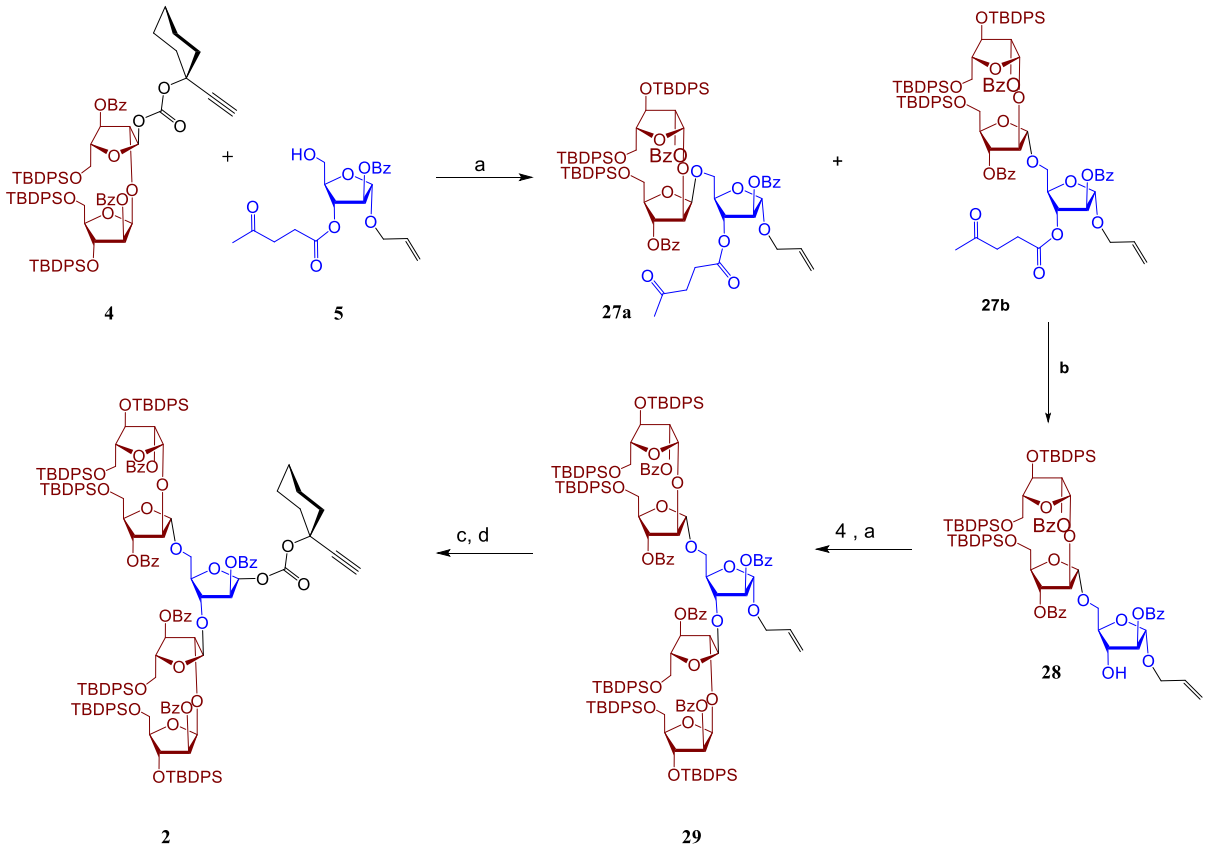
Having reached this key milestone, we advanced to the synthesis of the pentasaccharide donor **2**. The process began with converting the easily accessible propargyl orthoester **25**, which was saponified and then mono-silylated at the C5-OH position using 1.1 equivalents of TBDSCl at 0 °C. Next, a levulinate group was installed at the C3 position, yielding compound **26** with a 61% overall yield in three steps. The propargyl 1,2-orthoester was then reacted with allyl alcohol in the presence of catalytic amount of $AuBr_3$ to install an allyl moiety at the anomeric position. Finally, this compound was treated with HF, producing the desired monosaccharide acceptor unit **5** with an overall yield of 84%.



Scheme 6: Synthesis of the monosaccharide glycosyl acceptor unit 5. Reagents and conditions : a) NaOMe (0.3 eq), CH_2Cl_2-MeOH (1:1), 25 °C, 3h ,84% b) TBDPSCl (1eq.), Im., DMF, 0–25 °C, 4 h, c) Levulinic acid, DIC, DMAP, CH_2Cl_2, 0–25 °C,3h, overall 78% for compound 26 d) 10 mol% $AuBr_3$, allylOH, CH_2Cl_2, 4Å MS powder, 25 °C, 3 h, 86% 85% towards 26; e) Pyridine, HF·py, 0–25 °C, 5 h; overall 83% for 5

With the stage set for sequential gold/silver-catalyzed glycosidation in a {(2+1) +2} fashion, we first planned to install the disaccharide unit at the C5 position of the glycosyl acceptor, followed by the same disaccharide unit at the C3 position. To our satisfaction, the gold-phosphite and AgOTf catalysed glycosidation between donor **4** and acceptor **37** resulted in a 1:4 diastereomeric mixture of trisaccharides **27**. Furthermore, the two trisaccharides were easily separated by flash silica gel chromatography, allowing for thorough characterization. The three anomeric carbons of trisaccharide **27b** were observed at δ 105.5, 104.8, and 100.0 ppm, corresponding to two 1,2-trans linkages and one 1,2-cis linkage, respectively.

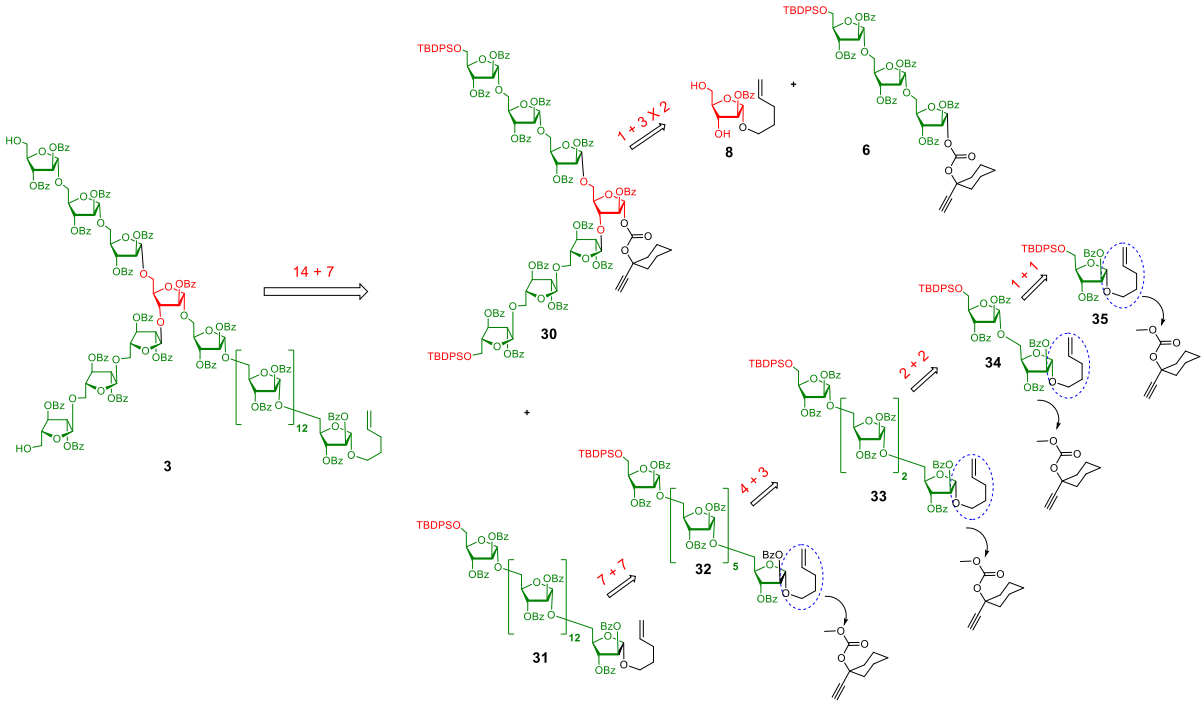
The major isomer of the trisaccharide **27b** was treated with hydrazine hydrate to afford alcohol **28** and further treated with glycosyl donor **4** under 8mol% each of Au-phosphite catalyst and AgOTf to deliver structurally homogenous pentasaccharide **29** in very high yield. In the ^{13}C NMR spectrum of pentasaccharide **40**, resonances of the five anomeric carbons were noted at δ 105.7, 105.1, 104.4, 99.6 (2C) ppm along with significant matching of the molecular ion in the mass spectrum (*m/z* calcd. for $C_{159}H_{174}NaO_{26}Si_6$: 2692.0874; found: 2692.0864). The successfully synthesised pentasaccharide **29** was converted easily into the corresponding carbonate donor **2** in two steps.



Scheme 7 : Synthesis of the pentaarabinofuarnosyl carbonate donor 2.Reagents and conditions : a) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, $CH2Cl_2$, 4 Å MS powder, 25 °C, 40 min, 19 % for 27a ,76% for 27b , 84 % for 29; b) 70% N_2H_4 ·H2O (4.5 equiv), CH_2Cl_2 , AcOH (22 equiv), Py (27 equiv), 25 °C, 2 h, 92% for 28; c) $PdCl_2$ (0.2 equiv), CH_2Cl_2 -MeOH (1:4), 25 °C, 4 h; d) 15, DMAP, CH_2Cl_2 , 25 °C, 5 h, 79% over two steps for compound 2

2.6.4- Synthesis of the branched heneicosasaccharide (21 mer) arabinan motif 3:

After the successful synthesis of the terminal pentaarabinofuranosyl donor **2**, we started to design the routes for generating the branched 21 mer arabinan unit **3**. The 21mer arabinan motif was thought to be generated from the [Au/Ag]-catalyzed coupling of a linear 14mer arabinofuranosyl acceptor **31** and the branched heptasaccharide arabinofuranosyl donor **30**. Now the 14mer glycosyl acceptor unit can further be obtained from the coupling of the linear heptasaccharide donor and acceptor unit. Similarly, the branched heptasaccharide unit can be generated via the glycosylation of 2 molar equivalents of the trisaccharide donor **6** and the monosaccharide arabinofuranosyl acceptor unit **8** as outlined in Scheme 8.

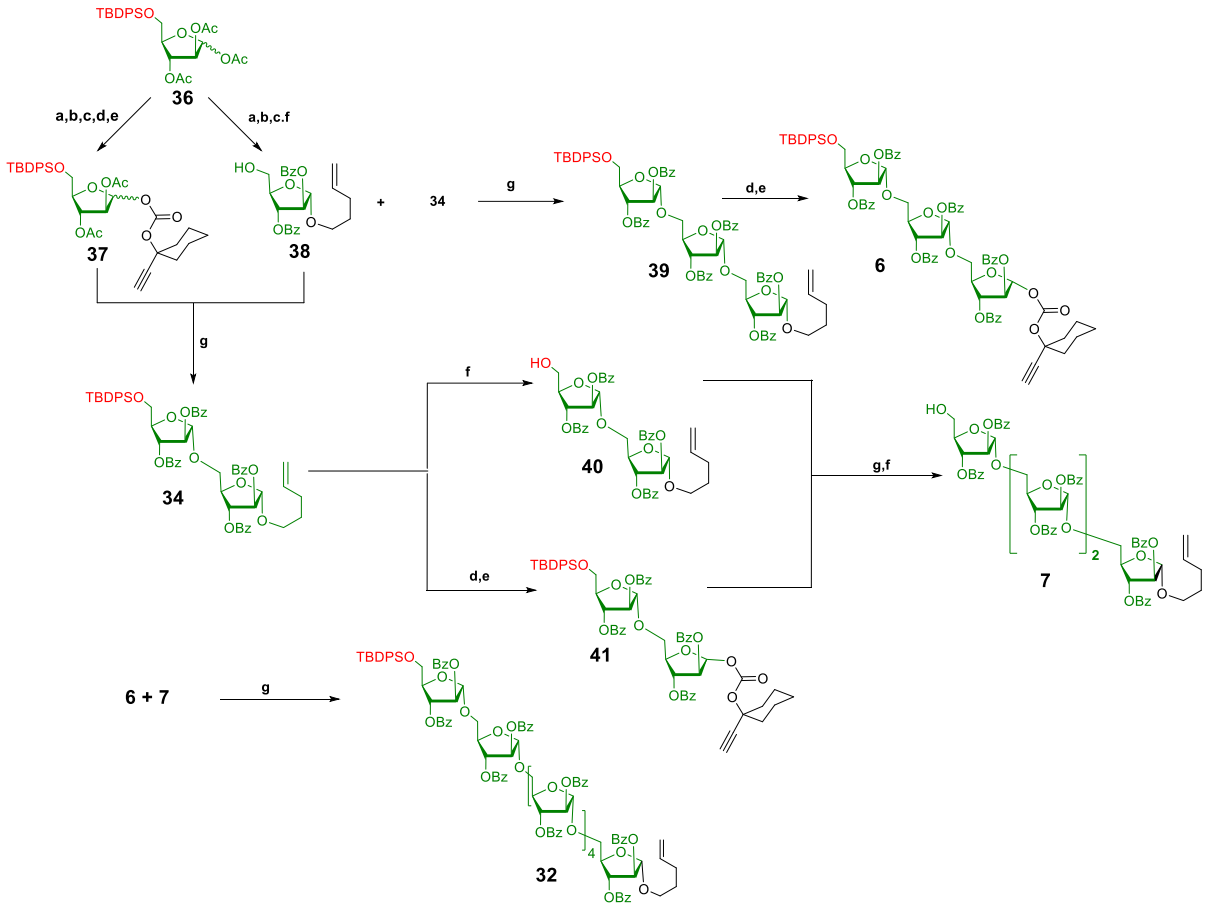


Scheme 8: Retrosynthesis of the branched 21 mer Arabinofuranosyl acceptor 3

2.6.4.1- Synthesis of the linear 14 mer acceptor unit 44:

At first, we started with the synthesis of the 14mer glycosyl acceptor unit necessary for assembling the desired 21mer arabinan unit. The above detailed Scheme 8 shows the strategy for generation of 14mer unit i.e. coupling of 7mer donor and acceptor unit. With these strategies we started to synthesize the linear heptasaccharide moiety. To start with, we generated two building blocks, the monosaccharide acceptor **38** and the donor **37** units from a known compound **36**. The first furanosylation between the donor **37** and acceptor **38** employing [Au]/[Ag]-catalytic conditions in CH_2Cl_2 smoothly resulted the disaccharide **34** in high yield. Consecutively, the TBDPS group was deprotected by HF•py to generate the disaccharide acceptor **40** and the same disaccharide was converted into glycosyl carbonate donor **41** in two steps, the deprotection of *n*-pentenyl group followed by its reaction with carbonate reagent afforded the desired donor **41**. Now the disaccharide donor and acceptor were further coupled with [Au]/[Ag]-combo catalytic system to generate the tetrasaccharide compound in excellent yield. This compound was further converted in to glycosyl acceptor **7** with the deprotection of the TBDPS group in presence of HF•py. Further, the disaccharide donor **34** was glycosylated with the furanosyl acceptor **38** to afford the trisaccharide **39** that was transformed into the carbonate donor **6** for further chain elongation.

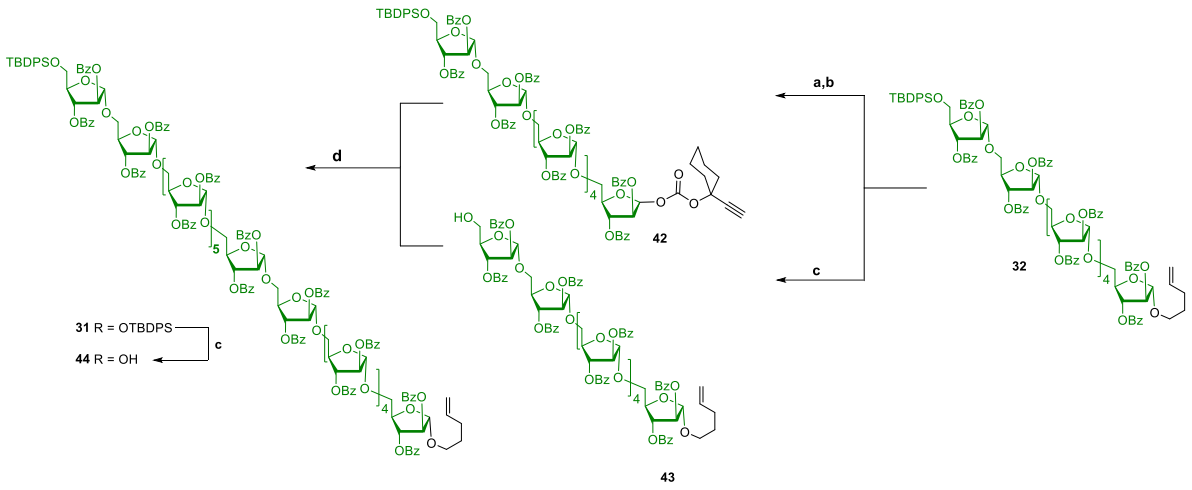
With the trisaccharide donor **6** and tetrasaccharide acceptor **7** in hand, we moved forward by glycosylating these two unit by using combination of [Au]/[Ag]-catalysts to afford the linear heptasaccharide **32** in excellent yield (Scheme 9).[31]



Scheme 9: Synthesis of the linear heptasaccharide motif 32 Reagents and conditions: a) 10 mol% $AuBr_3$, 4-pentene-1-ol, CH_2Cl_2, 4 Å MS powder, 25 °C, 3 h, b) NaOMe (0.3 equiv), CH_2Cl_2 -MeOH (1:1), 25 °C, 2 h c) Py, PhCOCl, CH_2Cl_2, 0–25 °C, 2 h, d) NIS (2.2 equiv), TfOH (0.2 equiv) CH_2Cl_2 /CH_3CN/H_2O (5:3:0.5), -10 °C to 0 °C, 1 h, e) 15, DMAP, CH_2Cl_2, 25 °C, 5 h, 76% over five steps for 37 ; f) Pyridine, HF:Py, 0–25 °C, 5 h; 87% over 4 steps for 38; g) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, CH_2Cl_2 , 4 Å MS powder, 25 °C, 40 min, 84 % for 34. NIS: N-iodosuccinamide TfOH: Triflic Acid

After this, the linear heptasaccharide moiety **32** was split into two portions, one portion was converted to the heptasaccharide acceptor **42** by the deprotection of the primary TBDPS group and second portion was converted to the linear heptasaccharide donor **43** in two aforementioned steps involving deprotection of *n*-pentenyl group followed by installation of the carbonate group at anomeric position. These two fragments **42** and **43** were coupled using the combination of [Au]/[Ag]-catalysts to generate the desired linear tetradecasaccharide **31**. Finally, the compound was treated with HF•py/pyridine to afford the linear 14mer acceptor

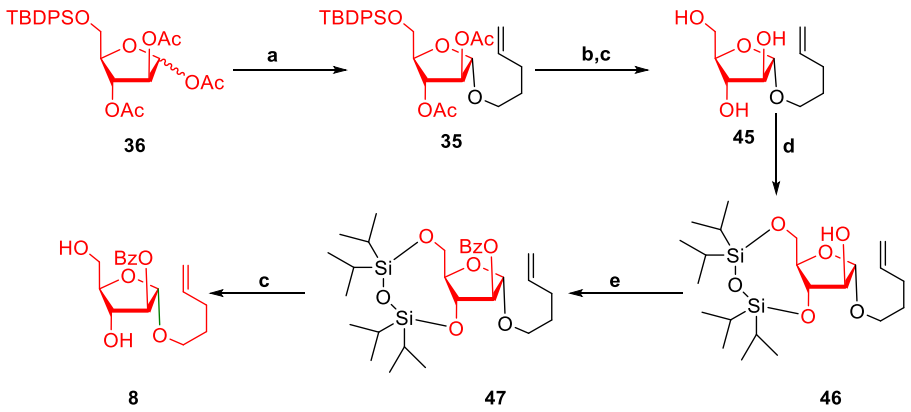
moiety **44** bearing a *n*-pentenyl unit at the anomeric position of the reducing end. Along with the ^{1}H and ^{13}C NMR spectroscopy, the efficient synthesis of this tetradecasaccharide compound **44** was checked by mass spectrometric analysis [calcd. for $C_{287}H_{252}O_{85}NaSi$, $[M+Na]^{+}$: 4876.4532, found 4876.4568.



Scheme 9: Synthesis of the linear tetradodecasaccharide motif 43 Reagents and conditions: a) NIS (2.2 equiv), TfOH (0.2 equiv) CH_2Cl_2 /CH_3CN/H_2O (5:3:0.5), -10 °C to 0 °C, 1h; b) **15**, DMAP, CH_2Cl_2, 25 °C, 5 h, 77% over two steps for **42**; c) Pyridine, HF: Py, 0–25 °C, 5 h; 96% for **43** and 79% for **44**; d) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, CH_2Cl_2, 4 Å MS powder, 25 °C, 40 min, 76 % for **31**

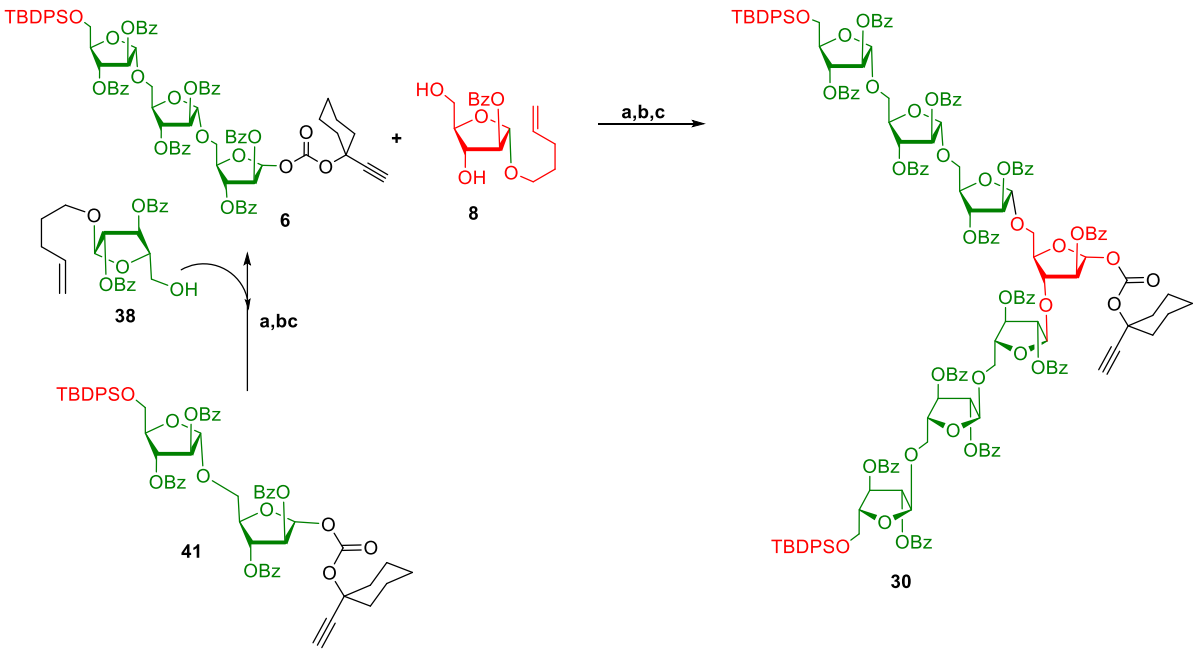
2.6.4.2- Synthesis of branched heptasaccharide Arabian Motif 30:

After successfully synthesizing the linear tetradecasaccharide motif **44**, we shifted our focus to generating the branched heptasaccharide donor motif **40**, following the retrosynthetic disconnection detailed in Scheme 10. We began with the previously synthesized compound **36**, treating it with BF_3•OEt_2 in the presence of 4-pentene-1-ol to produce the anomeric *n*-pentenyl arabinofuranoside **35**[32] that was treated with NaOMe in a binary solvent mixture of CH_2Cl_2 and MeOH (1:1) to deprotect the C-2 and C-3 hydroxyl groups, resulting in the triol **36**. Subsequently, compound **36** was reacted with 1,3-dichloro-tetraisopropyldisiloxane to install a rigid disiloxane moiety, yielding compound **46**. This compound was further protected with benzoyl chloride in pyridine, forming compound **47**. Finally, compound **47** underwent HF•pyridine mediated opening of the disiloxane group to afford the desired motif **8**, ready for further exploration.



Scheme 9: Synthesis of the monosaccharide diol acceptor motif 8; Reagents and conditions: a) $BF_3 \bullet OEt_2$, 4-pentene-1-ol, CH_2Cl_2, 4 Å MS powder, 25 °C, 4 h 83 % for 35; b) NaOMe (0.3 equiv), CH_2Cl_2 -MeOH (1:1), 25 °C, 2 h; c) Pyridine, HF: Py, 0–25 °C, 5 h; 88% over two steps for 45 and 82% for 8 d) 1, 3-Dichloro-tetraisopropyldisiloxane, Py, 0–25 °C, 6h; 82% for 46 e) Py, PhCOCl, CH_2Cl_2, 0–25 °C, 2 h

The synthesized diol **8** was then used as a glycosyl acceptor and coupled with 2 molar equivalents of the previously synthesized trisaccharide carbonate donor **6** to generate the heptasaccharide. The conversion of *n*-pentenyl glycoside into carbonate was achieved using the previously described two-step reaction strategy to afford compound **30**. The presence of the carbonate group in the branched heptasaccharide was confirmed by its transmittance at 1726 cm^{-1} in the IR spectra. Compound **30** was further characterized by ^{1}H and ^{13}C NMR spectroscopy. The structural authenticity of the heptasaccharide **30** was evident from the ^{1}H NMR spectrum where the presence of the seven anomeric protons were observed at δ=5.62, 5.50, 5.46, 5.40, 5.38, 5.26 ppm and the presence of two TBDPS moieties were confirmed by the characteristic peaks at δ=0.91 ppm. The ^{13}C NMR spectrum of the compound 30 confirmed the formation of the α linkages by displaying the resonances due the anomeric carbons at δ=106.1 (4C) and 105.8 (3C) ppm. Additionally, the presence of the carbonate moiety at the anomeric position showed the peak at δ=151.2 ppm which corresponds to the carbonyl carbon of the ester moiety. The compound 30 also showed significant matching of corresponding sodium adduct in the mass spectrum (*m/z* calcd. for $C_{167}H_{156}NaO_{44}Si_2$: 2944.9439; found: 2944.9578).

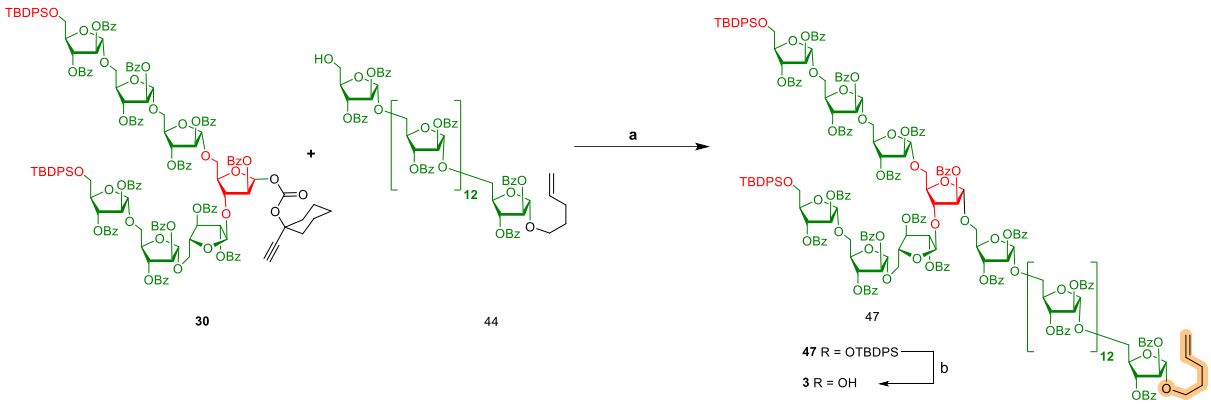


Scheme 10: Synthesis of the branched heptasaccharide donor motif 30 Reagents and conditions: a) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, CH_2Cl_2, 4 Å MS powder, 25 °C, 40 min; b) NIS (2.2 equiv), TfOH (0.2 equiv) CH_2Cl_2 /CH_3CN/H_2O (5:3:0.5), -10 °C to 0 °C, 1h; c) 15, DMAP, CH_2Cl_2, 25 °C, 5 h, 77% over three steps for 6; 84% three steps for 30

2.6.4.3-Synthesis of the branched heneicosasaccharide (21 mer) arabinan motif 3:

Accomplishing the synthesis of the two major structural motifs encouraged us to move forward towards the synthesis of the heneicosasaccharide **3**. In this direction, the glycosidation between the glycosyl donor **30** and the acceptor **44** was carried out by invoking the [Au]/[Ag]-catalyzed glycosylation procedure under argon atmosphere at 25 °C to afford the 21mer compound **47**. The two silyl ethers at the non-reducing end of the heneicosasaccharide **47** were unmasked by treatment with HF•py to afford the aglycon **3** in 79 % yield. (Scheme 11).

The ^{1}H NMR spectrum of the heneicosasaccharide **3** showed the presence of the *n*-pentenyl moiety by displaying characteristic resonances at δ 6.02 (m, 1H), 4.91 (m, 2H), 3.48 (m, 2H), 2.06 (m, 2H) and 1.59 (m, 2H). Many overlapping signals were noted in the anomeric and aromatic regions. Additionally, the ^{13}C NMR spectrum confirmed the presence of 21-saccharide residues by displaying 21 signals between 105.9-105.2 ppm. MALDI-TOF spectrum of the compound **3** revealed *m/z*= 7626.7539 for the corresponding di-sodium adduct (*m/z* calcd for $C_{429}H_{378}Na_2O_{126}Si_2$= 7628.2775).[25]

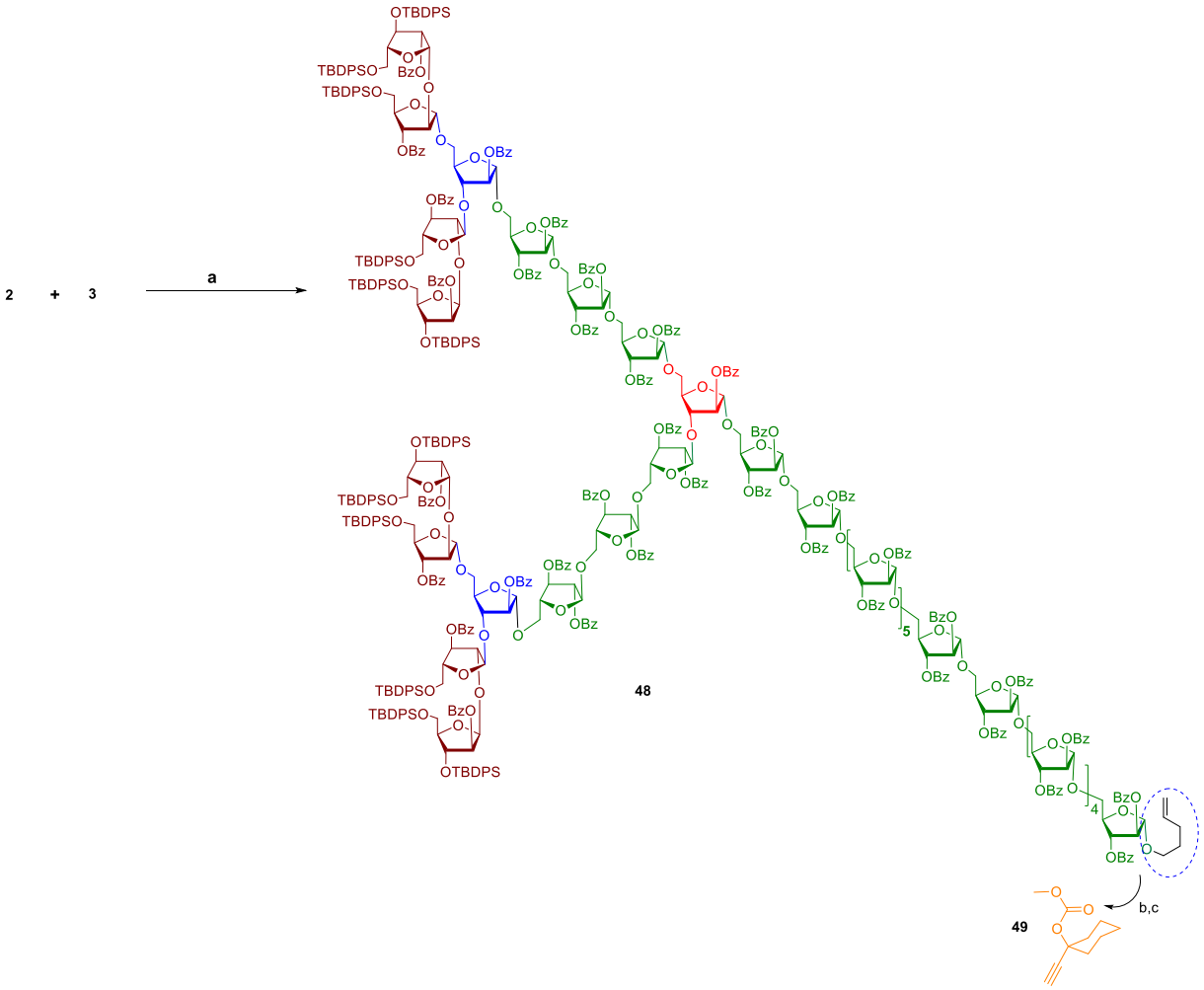


Scheme 11: Synthesis of the heneicosasaccharide motif 3 Reagents and conditions: a) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) and AgOTf, CH_2Cl_2, 4 Å MS powder, 25 °C, 40 min, 82% for compound 47; b) Pyridine, HF: Py, 0–25 °C, 5 h, 79% for compound 3

2.6.5- Synthesis of the 31 mer Arabinan motif 48:

The glycosylation in a {(2x5) +21} fashion was envisioned to generate the desired 31mer arabinan motif. Accordingly, pentasaccharide **2** and the branched heneicosasaccharide **3** were called upon to complete the assembly of the 31mer arabinan motif. The glycosidation was carried out by following the catalytic protocol containing 8mol% each of Au-phosphite and AgOTf for 35 min.

Further, 31mer arabinan **48** was carefully isolated by flash silica gel column chromatography. The presence of large numbers of benzoates and furanose ring protons obscured anomeric protons in the ^{1}H NMR spectrum; however, evidence of a *n*-pentenyl moiety at δ 6.02 (m,1H), 4.91 (m, 2H), 3.48 (m, 2H), 2.06 (m, 2H) and 1.59 (m, 2H) and ^{t}butyl of TBDPS-moieties at 0.99, 0.96,0.91, 0.88, 0.80 and 0.79 ppm as six singlets supported the successful formation of the 31mer arabinan **48**. Full confirmation and the structural integrity of the polysaccharide **48** were obtained from the high-field ^{13}C NMR spectrum (150.97 MHz). All the 31 anomeric carbons at the non-reducing end of the arabinan **48** were confirmed between 105.9-105.4 ppm. Furthermore, the compound showed satisfactory matching of the sodium adduct of the molecular ion. The 31mer arabinan **48** was synthesized as a viscous syrup (500 mg) in 80 steps with an overall yield 0.018%.



Scheme 12: Synthesis of the 31 mer arabinan motif 48 Reagents and conditions: a) 8 mol% each of chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) gold and AgOTf, CH_2Cl_2, 4 Å MS powder, 25 °C, 40 min, 80% for compound 48; b) NIS (2.2 equiv), TfOH (0.2 equiv) CH_2Cl_2 /CH_3CN/H_2O (5:3:0.5), -10 °C to 0 °C, 1h; c) **15**, DMAP, CH_2Cl_2, 25 °C, 5 h, 77% over two steps for 49

In summary, the synthesis of an arabinan motif with 31interglycosidic linkages was successfully achieved using the alkynyl carbonate glycosyl donor strategy under [Au]/[Ag] catalysis. Key features of this process include: (i) installation of all 1,2-trans linkages via neighbouring group participation from the C-2 acyl group, (ii) conversion of β- or 1,2-trans Rib*f* into β- or 1,2-cis Ara*f* through an oxidation-reduction strategy, (iii) a single glycosylation method for stapling glycans, (iv) effective use of silver-assisted gold-catalysed glycosidation, and (v) a highly modular and convergent assembly of the $Araf_{31}$ motif. The synthesis of the 31-mer arabinan motif was achieved with an overall yield of 0.0018%, producing 500 mg of the final protected 31-mer arabinan unit **48**. This unit is valuable for the synthesis of the full

length arabinogalactan and also will be useful for studying mycobacterial arabinogalactan biosynthesis. The *n*-pentenyl moiety present can be utilized for conjugating immunogenic proteins, biomarkers, and chemical probes for further biological studies. Additionally, the anomeric pentenyl group was converted into the alkynyl carbonate donor **49** in two steps, allowing for the installation of the linear galactan unit into the arabinan motif to synthesize the full-length 131-mer arabinogalactan of the *Mycobacterium tuberculosis* cell wall. Progress on coupling the arabinan unit with the linear galactan chain is ongoing in our lab.

2.7 Experimental section:

Synthesis of ethynylcyclohexylglycosyl carbonate donor (2,4,6,10,30,37,41,42,49):

To a rapidly stirring CH_2Cl_2 solution of arabinofuranosyl rhamnopyranosyl hemiacetal (1.0 mmol) and DMAP (1.5 mmol) at 25 ºC, ethynylcyclohexyl(4-nitrophenyl) carbonate (**15**) (1.2 mmol) was added and continued stirring at 25 ºC for 2 h. After complete consumption of the hemiacetal, the reaction mixture was concentrated in vacuo and purified by silica gel column chromatography (*n*-hexane/EtOAc) to afford ethynylcyclohexylglycosyl carbonate donors containing trace quantity of the 4-nitro phenol. Eluents containing the compound are concentrated and redissolved in CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ solution to get **2**,**4**,**6**,**10**,**30**,**37**,**41**,**42**,**49** in 85-90% yield.

Glycosylation using ethynylcyclohexyl glycosyl carbonate donors (**6, 7, 22, 27,29, 30,31,32, 34,39, 47,48**): To a solution of acceptor (1.0 mmol) and donor (1.0 mmol) in anhydrous CH_2Cl_2 (15 mL) was added freshly activated 4Å MS powder (0.4 g) at 25 °C. After stirring at 25 °C for 10 minutes chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold(I) (8mol%) and AgOTf (8mol%) were added to the reaction mixture and stirred for 15-25 minutes if the reaction was carried out at 25 °C and 5-8 h for reactions at -78 °C, the reaction was neutralized by adding Et_3N and filtered through a bed of Celite®. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using *n*-hexane/EtOAc as mobile phase to afford compounds **6**, **7**, **22**, **27**, **29**, **30**, **31**, **32**, **34**, **39**, **47**, **48** in 80-95% yield.

Protection of alcohol using TBDPS-Cl (**17**, **26**): A solution of the alcohol (1 mmol) and imidazole (1.3 mmol) in anhydrous DMF (5 mL) was cooled to 0 °C and *tert*butyldiphenyl-chlorosilane (1.2 mmol) was added dropwise under nitrogen atmosphere. The reaction mixture was gradually warmed up to 25 °C and stirred for 2 h. After completion, the reaction mixture was quenched by adding ice cold water. The whole mixture was transferred to a separatory funnel and extracted with ethyl acetate (3x25 mL). The combined organic layers were washed with cold water and brine solution. Organic layer was dried over anhydrous Na_2SO_4, the organic layer was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (ethyl acetate/hexanes) to obtain the desired product.

Deprotection of the TBDPS-ethers (**3**, **5**, **7**, **38**, **43**, **44**, **45**): HF•py (3 mmol) was added dropwise under inert atmosphere to a solution of silyl ether (1 mmol) in pyridine (3 times to the volume of HF•py) at 0 °C. The reaction mixture was stirred for 5 h at 25 °C, 6*N* HCl was

added to quench the reaction at 0 °C, diluted with EtOAc (25mL) and washed successively with ice cold water (2x10 mL), saturated solution of $NaHCO_3$(aq), and brine solution. Organic layer was dried anhydrous Na_2SO_4, concentrated *in vacuo* to obtain a residue that was purified by silica gel column chromatography (ethyl acetate/hexanes) to furnish the alcohol.

Synthesis of Allyl glycoside from 1, 2-orthoester (5, 18): Ally alcohol (4 mmol) followed by PTSA (0.2 mmol) were added to a vigorously stirred solution of the corresponding orthoester (1 mmol) in freshly distilled anhydrous CH_2Cl_2 (10 mL) at 25 °C. After completion of the reaction, Et_3N (2 mL) was added and the solvent was evaporated *in vacuo* and the crude residue was purified by conventional silica gel column chromatography (ethyl acetate/hexanes) to obtain the desired allyl glycosides.

Synthesis of 4-pentene-1-ol glycoside from 1, 2-*O*-orthoester (35): Pent-1-ene-ol (4 mmol) followed by PTSA (0.2 mmol) were added to a vigorously stirred solution of orthoester (1 mmol) in freshly distilled anhydrous CH_2Cl_2 (10 mL) at 25 °C. After completion of the reaction, Et_3N (2 mL) was added and the solvent was evaporated *in vacuo* and the crude residue was purified by conventional silica gel column chromatography (ethyl acetate/hexanes) to obtain the desired pentene-1-ol- glycoside.

Deprotection of allyl glycosides (2, 21, 24): To the solution of the allyl glycoside (1 mmol) in CH_2Cl_2 (10 mL), $PdCl_2$ (0.2mmol) in MeOH (40mL) was added in five portions at 25 °C and stirred for 4-5 h. After completion of the reaction, excess amount of Et_3N was added and the solid residue was filtered off through a pad of Celite®. The solvent was evaporated *in vacuo* and the resulting residue was purified by conventional silica gel column chromatography (ethyl acetate/hexanes) to obtain the desired hemiacetal.

Protection of levulinoate (20, 26): A solution of the alcohol (1 mmol), DMAP (0.5 mmol) and levulinic acid (1.3 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C and *N*, *N*'-diisopropylcarbodiimide(1.5 mmol) was added dropwise under nitrogen atmosphere. The reaction mixture was gradually warmed to 25 °C and stirred for 2 h. After completion of the reaction, CH_2Cl_2 was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexanes) to furnish the lev-ester.

Deprotection of levulinoate (23, 28): Hydrazine acetate (4 mmol) in THF (4 mL) was added to a solution of levulinoate (1 mmol) in MeOH (1 mL) at 25 °C and stirred for 45 minutes. The reaction was quenched by adding ice cold 1*N* HCl solution and washed successively with ethyl acetate (3x25 mL), water, saturated solution of $NaHCO_3$ (aq), and brine solution. After drying

over Na_2SO_4, the organic layer was concentrated under diminished pressure and the residue was purified by column chromatography (ethyl acetate/hexanes) to afford the alcohol.

Synthesis of benzoates (9, 47): A solution of the alcohol (1 mmol) in anhydrous pyridine (10 mL) and DMAP (0.1 mmol) was cooled to 0 °C under nitrogen atmosphere and benzoyl chloride (1.2 mmol per alcohol) was added dropwise and stirred for 5 h at 25 °C. The reaction mixture was poured into cold water with vigorous shaking, neutralized by the addition of 6*N* HCl solution, extracted with ethyl acetate (3x50 mL) and combined EtOAc layers were washed with cold water, sat. $NaHCO_3$, brine solution, and dried over Na_2SO_4. The EtOAc solution was decanted and evaporated *in vacuo* to obtain a reddish brown colored residue that was purified by column chromatography (ethyl acetate/hexanes) to obtain the required benzoate.

Saponification of esters (19, 26, 45): Freshly prepared NaOMe (0.1 mmol per benzoate) was added to a solution of the benzoate (1 mmol) in MeOH (10 mL) and stirred for 8 h at 25 °C. The reaction mixture was concentrated *in vacuo* to obtain a residue that was purified by column chromatography (ethyl acetate/hexanes) to obtain alcohol.

Synthesis of allyl arabinofuranoside from 1, 2-acetonide (14): Allyl alcohol (2.12 mL, 30.8 mmol) was added to a solution of acetonide **25** (8.0 g, 15.4 mmol) in CH_2Cl_2 (60 mL), stirred at 60 ^oC. After 5 min, PTSA (0.53 g, 3.1 mmol) was added and continued stirring for additional 1 h, the reaction mixture was neutralized by the addition of Et_3N (excess), diluted with water and extracted with CH_2Cl_2 (3x50 mL). Combined CH_2Cl_2 layers were washed with brine solution, dried over anhydrous sodium sulphate, decanted and concentrated *in vacuo* to obtain a residue that was partially purified by silica gel column chromatography (ethyl acetate/hexanes) to obtain α/β-allyl furanosides which were separated by flash chromatography by obtain diastereomerically pure **26** (3.6 g, 45%) as a pale yellow syrup.

Synthesis of propyn-3-yl 1, 2-Orthoesters: Per-*O*-acyl methyl arabino-, ribo- (1.0 mmol) were dissolved in anhydrous CH_2Cl_2 and cooled to 0 °C. Acetyl bromide (6.5 mmol) was added to the reaction mixture, followed by the dropwise addition of MeOH (4.5 mmol), and the mixture was stirred at 0 °C for 2 h. Once the starting material was completely converted, the reaction mixture was diluted with CH_2Cl_2, poured over an ice-water mixture, and extracted with CH_2Cl_2 (3x50 mL). The organic layers were washed with ice-cold saturated $NaHCO_3$ (25 mL) and brine solution (25 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford the corresponding furanosyl bromide as a white foam, which was immediately used without further purification.

To the solution of the crude furanosyl bromide in anhydrous CH_2Cl_2, propargyl alcohol (2.0 mmol), 2, 6-lutidine (2.2 mmol), and tetra-^{n}-butyl ammonium iodide (0.5 mmol) were added. The reaction mixture was stirred at 25 °C for 15 h. Afterwards, the reaction mixture was diluted with CH_2Cl_2 and water, extracted with saturated aqueous oxalic acid solution (3x25 mL), saturated $NaHCO_3$ (1x25 mL), and brine solution (1x25 mL), then dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using EtOAc and Hexane as the mobile phase to obtain the corresponding orthoester.

Allyl 3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranoside (14a):

$[\alpha]_D^{25}$ = +64.8 (*c* 1.0, $CHCl_3$); 1H NMR (399.78 MHz, $CDCl_3$): δ 8.06 (dt, *J* = 8.4, 1.6 Hz, 2H), 7.79 – 7.73 (m, 4H), 7.62 – 7.56 (m, 1H), 7.48 – 7.40 (m, 8H), 6.03 – 5.91 (m, 1H), 5.37 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.28 (dd, *J* = 3.6, 1.1 Hz, 1H), 5.25 (s, 1H), 5.22 (ddd, *J* = 10.5, 3.0, 1.4 Hz, 1H), 4.39 – 4.27 (m, 3H), 4.13 – 4.00 (m, 2H), 3.99 – 3.90 (m, 2H), 1.13 (s, 9H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 166.8, 135.8 (2C), 135.7 (2C), 134.3, 133.5, 132.5, 132.4, 130.1, 130.0, 129.9 (2C), 129.6, 128.5 (2C), 128.0 (2C), 127.9 (2C), 117.0, 107.7, 83.7, 80.7, 79.2, 68.0, 64.0, 26.9 (3C), 19.3; IR (cm^{-1} , $CHCl_3$): 3431.93, 3073.44, 2931.53, 2860.97, 1719.61, 1596, 1460, 1428, 1390, 1273, 1220, 1110, 1074, 993, 934, 874, 848, 820, 775, 742, 707; HRMS (ESI-MS) $[M+Na]^+$ *m/z* calcd. for $C_{31}H_{36}NaO_6Si$, 555.2179; found 555.2181.

3,5-Di-*O*-Benzoyl-α-D-ribofuranoside propyn-3-yl-1,2-orthoacetate (16):

$[\alpha]_D^{25}$ = +55.6(*c* 1.0, $CHCl_3$); ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 8.05 – 8.00 (m, 4H), 7.60 – 7.50 (m, 2H), 7.45 – 7.36 (m, 4H), 6.08 (d, *J* = 4.0 Hz, 1H), 5.17 – 5.14 (m, 1H), 5.05 (dd, *J* = 8.8, 5.3 Hz, 1H), 4.71 (dd, *J* = 11.7, 3.0 Hz, 1H), 4.58 – 4.45 (m, 2H), 4.12 (d, *J* = 2.5 Hz, 2H), 2.40 (t, *J* = 2.5 Hz, 1H), 1.76 (s, 3H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 166.2, 165.6, 133.7, 133.3, 129.9 (2C), 129.8 (2C), 129.5, 128.9, 128.6 (2C), 128.5 (2C), 124.5, 104.6, 79.5, 78.0, 76.4, 73.8, 73.1, 63.0, 50.7, 22.9; IR (cm^{-1} , $CHCl_3$): 3287, 2925, 2856, 1725, 1603, 1453, 1384, 1268, 1172, 1102, 1067, 1001, 902, 771, 711, 635; HRMS (ESI-MS) $[M+Na]^+$ *m/z* calcd. for $C_{24}H_{22}NaO_8$, 461.1212, found 461.1214.

3,5-Di-*O*-tert-butyldiphenylsilyl-α-D-ribofuranoside propyn-3-yl-1,2-orthoacetate (17):

$[\alpha]_D^{25}$ = +15.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 7.76 – 7.72 (m, 4H), 7.64 – 7.62 (m, 4H), 7.41 – 7.34 (m, 12H), 5.71 (d, *J* = 3.9 Hz, 1H), 4.33 (d, *J* = 2.3 Hz, 1H), 4.14 (dd, *J* = 8.4, 5.0 Hz, 1H), 4.03 – 3.97 (m, 2H), 3.95 (d, *J* = 2.4 Hz, 2H), 3.74 (dd, *J* = 11.8, 3.9 Hz, 1H), 2.36 (t, *J* = 2.4 Hz, 1H), 1.76 (s, 3H), 1.09 (s, 9H), 0.98 (s, 9H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 135.9 (2C), 135.8 (2C), 135.7 (4C), 133.7, 133.5, 133.4, 133.3, 130.1, 130.0,

130.0, 129.7, 127.9 (4C), 127.7 (4C), 124.2, 104.3, 82.0, 79.6, 73.5, 72.2, 62.2, 52.6, 50.3, 27.1 (3C), 26.9 (3C), 23.4, 19.5, 19.4; IR (cm^{-1}, $CHCl_3$): 2926, 2858, 1747, 1463, 1429, 1370, 1229, 1108, 1005, 939, 895, 851, 823, 771, 741, 701; HRMS (ESI-MS) $[M+H]^+$ *m/z* calcd. for $C_{42}H_{50}O_6Si_2$, 707.3146; found 707.3160.

Allyl 2-*O*-acetyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-ribofuranoside (18):

$[\alpha]_D^{25}$ = -10.3 (*c* 1.0, $CHCl_3$); ^1H NMR (399.78 MHz, $CDCl_3$): δ 7.75 – 7.73 (m, 2H), 7.64 – 7.59 (m, 6H), 7.42 – 7.32 (m, 12H), 5.82 – 5.70 (m, 1H), 5.15 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.10 (ddd, *J* = 10.5, 2.8, 1.3 Hz, 1H), 5.03 (d, *J* = 1.7 Hz, 1H), 4.80 (dd, *J* = 4.7, 1.7 Hz, 1H), 4.46 (dd, *J* = 6.0, 4.9 Hz, 1H), 4.23 (td, *J* = 6.0, 2.5 Hz, 1H), 4.08 (ddt, *J* = 13.1, 5.0, 1.5 Hz, 1H), 3.92 (ddt, *J* = 13.0, 6.1, 1.3 Hz, 1H), 3.59 (dd, *J* = 11.2, 2.6 Hz, 1H), 3.35 (dd, *J* = 11.2, 6.0 Hz, 1H), 2.07 (s, 3H), 1.06 (s, 9H), 1.02 (s, 9H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 170.3, 135.9 (2C), 135.8 (2C), 135.7 (4C), 134.0, 133.5, 133.4, 133.2, 132.9, 130.1 (2C) 129.8, 129.7, 127.9 (4C), 127.8 (4C), 117.2, 104.2, 84.8, 76.6, 71.8, 68.6, 65.1, 26.9 (6C), 21.1, 19.4, 19.3; IR (cm^{-1}, $CHCl_3$): 3071, 2931, 2858, 1747, 1467, 1428, 1368, 1227, 1110, 1007, 965, 931, 848, 823, 772, 743, 701; HRMS (ESI-MS) $[M+Na]^+$ *m/z* calcd. for $C_{42}H_{52}NaO_6Si_2$, 731.3200; found 731.3191.

Allyl- 2-*O*-(4-oxopentanoyl)-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-ribofuranoside (20):

$[\alpha]_D^{25}$ = -2.2 (*c* 1.0, $CHCl_3$); ^1H NMR (399.78 MHz, $CDCl_3$): δ 7.70 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.65 – 7.57 (m, 8H), 7.42 – 7.27 (m, 11H), 5.82 – 5.65 (m, 1H), 5.16 – 5.05 (m, 2H), 4.98 (d, *J* = 1.1 Hz, 1H), 4.76 (dd, *J* = 4.6, 1.2 Hz, 1H), 4.43 (dd, *J* = 6.5, 4.7 Hz, 1H), 4.22 (td, *J* = 6.3, 2.4 Hz, 1H), 4.05 (ddt, *J* = 12.9, 4.9, 1.4 Hz, 1H), 3.89 (ddt, *J* = 12.9, 6.0, 1.2 Hz, 1H), 3.59 (dd, *J* = 11.2, 2.4 Hz, 1H), 3.36 (dd, *J* = 11.2, 6.2 Hz, 1H), 2.76 – 2.53 (m, 4H), 2.20 (s, 3H), 1.05 (s, 9H), 1.01 (s, 9H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 206.3, 172.0, 135.9 (2C), 135.8 (2C), 135.7 (3C), 135.4, 134.0, 133.5 (2C), 133.1, 132.8, 130.2, 130.1 (2C), 129.7, 127.9 (4C), 127.7 (4C), 117.2, 104.1, 84.6, 76.7, 71.7, 68.5, 65.1, 38.0, 30.0, 28.0, 26.9 (6C), 19.4, 19.3; IR (cm^{-1} , $CHCl_3$): 3070, 2931, 2859, 1744, 1723, 1467, 1426, 1364, 1310, 1219, 1154, 1110, 994, 962, 848, 824, 771, 744, 702; HRMS (ESI-MS) $[M+Na]^+$ *m/z* calcd. for $C_{45}H_{56}NaO_7Si_2$, 787.3462; found 787.3470.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-*O*-(4-oxopentanoyl)-3,5-di-*O*-tertbutyldiphenylsi-lyl-β-D-ribofuranoside (10):

$[\alpha]_D^{25}$ = +9.0 (*c* 1.0, $CHCl_3$); ^1H NMR (400.31 MHz, $CDCl_3$): δ 7.69 – 7.54 (m, 8H), 7.47 – 7.23 (m, 12H), 6.05 (d, *J* = 1.6 Hz, 1H), 4.95 (dd, *J* = 4.7, 1.7 Hz, 1H), 4.75 – 4.66 (m, 1H),

4.23 – 4.18 (m, 1H), 3.66 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.43 (dd, *J* = 11.7, 4.0 Hz, 1H), 2.76 – 2.55 (m, 4H), 2.53 (s, 1H), 2.19 (s, 3H), 2.15 – 2.04 (m, 2H), 1.79 – 1.44 (m, 8H), 1.06 (s, 9H), 0.98 (s, 9H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 206.1, 171.7, 151.3, 135.8 (6C) 135.7 (2C), 133.4, 133.1, 133.0, 132.8, 130.2, 130.1, 129.7, 129.6, 127.9 (4C), 127.8 (2C), 127.7 (2C), 101.2, 85.9, 82.7, 78.1, 76.2, 75.3, 70.6, 63.0, 37.9, 36.9, 36.6, 30.0, 27.9, 26.9 (6C), 25.0, 22.7 (2C), 19.4, 19.2; IR (cm^{-1} , $CHCl_3$): 3048, 2934, 2860, 1753, 1721, 1466, 1426, 1363, 1272, 1228, 1154, 1111, 1073, 1009, 947, 912, 847, 828, 768, 703; HRMS (ESI-MS) $[M+Na]^+$ *m/z* calcd. for $C_{51}H_{62}NaO_9Si_2$, 897.3830; found 897.3828.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-*O*-(4-oxopentanoyl)-3,5-di-*O*-tertbutyldiphenylsi-lyl-β-D-ribofuranoside (10β):

$[\alpha]_D^{25}$ = +9.0 (c 1.0, CHCl3); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.69 – 7.54 (m, 8H), 7.47 – 7.23 (m, 12H), 6.05 (d, J = 1.6 Hz, 1H), 4.95 (dd, J = 4.7, 1.7 Hz, 1H), 4.75 – 4.66 (m, 1H), 4.23 – 4.18 (m, 1H), 3.66 (dd, J = 11.6, 2.4 Hz, 1H), 3.43 (dd, J = 11.7, 4.0 Hz, 1H), 2.76 – 2.55 (m, 4H), 2.53 (s, 1H), 2.19 (s, 3H), 2.15 – 2.04 (m, 2H), 1.79 – 1.44 (m, 8H), 1.06 (s, 9H), 0.98 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 206.1, 171.7, 151.3, 135.8 (6C) 135.7 (2C), 133.4, 133.1, 133.0, 132.8, 130.2, 130.1, 129.7, 129.6, 127.9 (4C), 127.8 (2C), 127.7 (2C), 101.2, 85.9, 82.7, 78.1, 76.2, 75.3, 70.6, 63.0, 37.9, 36.9, 36.6, 30.0, 27.9, 26.9 (6C), 25.0, 22.7 (2C), 19.4, 19.2; IR (cm^{-1} , $CHCl_3$): 3047 , 2934, 2859, 1752, 1721, 1465, 1426, 1362, 1272, 1227, 1154 , 1111 , 1072 , 1008 , 947 , 912 , 847 , 824 , 767, 703 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{51}H_{62}NaO_9Si_2$, 897.3830; found 897.3828.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-*O*-(4-oxopentanoyl)-3,5-di-*O*-tertbutyldiphenylsilyl-α-D-ribofuranoside (10α):

$[\alpha]_D^{25}$ = +34.9 (c 1.0, CHCl3); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.70 – 7.64 (m, 4H), 7.56 – 7.45 (m, 4H), 7.41 – 7.28 (m, 12H), 6.31 (d, *J* = 4.4 Hz, 1H), 5.15 (dd, *J* = 5.8, 4.4 Hz, 1H), 4.59 (dd, *J* = 5.8, 1.4 Hz, 1H), 4.22 (d, *J* = 1.4 Hz, 1H), 3.92 (s, 1H), 3.43 (dd, *J* = 11.5, 2.6 Hz, 1H), 2.99 (dd, *J* = 11.5, 2.5 Hz, 1H), 2.80 – 2.68 (m, 2H), 2.67 (s, 1H), 2.57 – 2.51 (m, 2H), 2.16 (s, 3H), 2.00 (t, J = 10.6 Hz, 2H), 1.79 – 1.41 (m, 7H), 1.10 (s, 9H), 0.94 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 206.4, 171.8, 152.0, 136.0 (4C), 135.7 (2C), 135.6 (2C), 133.6, 133.4, 133.0, 132.7, 130.0, 129.9, 129.8, 127.9 (2C), 127.8 (8C), 97.8, 88.7, 83.5, 74.8, 72.8, 70.6, 63.3, 38.1, 37.2, 36.7, 30.0, 27.9, 26.9 (3C), 26.8 (3C), 25.1, 22.4 (2C), 19.6, 19.2; IR (cm^{-1} , $CHCl_3$): 3284 , 3069 , 2934 , 2859 , 1751 , 1464 , 1426 , 1363 , 1274 , 1242 , 1158 ,

1110 , 1068 , 1009 , 946 , 906 , 848 , 822; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{51}H_{62}NaO_9Si_2$, 897.3830; found 897.3845.

Allyl 3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-2-*O*-(3,5-di-*O*-tert-butyldiphenylsilyl-β-D-ribofuranosyl)-α-D-arabinofuranoside (22):

$[\alpha]_D^{25}$ = -13.9 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.87 – 7.80 (m, 2H), 7.68 – 7.62 (m, 4H), 7.54 – 7.45 (m, 9H), 7.34 – 7.17 (m, 20H), 6.00 – 5.89 (m, 1H), 5.33 (dq, *J* = 17.1, 1.5 Hz, 1H), 5.22 – 5.15 (m, 3H), 5.12 (d, J = 2.2 Hz, 1H), 4.47 – 4.42 (m, 1H), 4.29 – 4.21 (m, 1H), 4.16 (dd, *J* = 10.3, 5.3 Hz, 2H), 4.07 – 3.99 (m, 2H), 3.90 – 3.77 (m, 3H), 3.34 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.16 (dd, *J* = 11.2, 6.2 Hz, 1H), 2.81 (d, *J* = 5.2 Hz, 1H), 1.04 (s, 9H), 0.97 (s, 9H), 0.85 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.5, 135.8 (8C), 135.7 (2C), 135.6 (2C), 134.4, 133.7, 133.6, 133.4 (2C), 133.0, 132.5, 132.3, 130.4, 130.3, 129.9 (2C), 129.7, 129.6 (2C), 128.3 (2C), 128.1 (2C), 128.0 (2C), 127.7 (10C), 117.1, 106.6, 104.5, 85.2, 84.5, 83.4, 78.6, 75.6, 73.6, 68.1, 65.1, 64.5, 27.1 (3C), 26.8 (6C), 19.3 (2C), 19.2; IR (cm^{-1} , $CHCl_3$): 3071 , 2932 , 2859 , 1726 , 1465 , 1427 , 1387 , 1362 , 1270 , 1219 , 1111, 1064 , 1023 , 964 , 849 , 823 , 771 , 744 , 704 ; HRMS (ESI-MS) [M+Na] + m/z calcd for $C_{68}H_{80}NaO_{10}Si_3$, 1163.4957; found 1163.4963.

Allyl 3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-2-*O*-(3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-α-D-arabinofuranoside (23):

$[\alpha]_D^{25}$ = -10.5 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.93 – 7.90 (m, 2H), 7.79 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.72 (dd, *J* = 7.7, 1.5 Hz, 4H), 7.67 – 7.64 (m, 2H), 7.61 – 7.55 (m, 6H), 7.44 – 7.30 (m, 20H), 6.00 (ddd, *J* = 16.7, 10.7, 5.5 Hz, 1H), 5.39 (ddd, *J* = 17.2, 3.1, 1.5 Hz, 1H), 5.35 – 5.30 (m, 2H), 5.26 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.22 (s, 1H), 4.55 (dd, *J* = 2.5, 1.1 Hz, 1H), 4.36 – 4.21 (m, 2H), 4.16 – 4.03 (m, 4H), 3.94 (ddd, *J* = 17.1, 11.2, 4.9 Hz, 2H), 3.48 (dd, *J* = 11.0, 3.7 Hz, 1H), 3.33 (dd, *J* = 11.0, 6.0 Hz, 1H), 2.51 (d, *J* = 8.5 Hz, 1H), 1.10 (s, 9H), 1.07 (s, 9H), 0.95 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.5, 136.0 (2C), 135.9 (2C), 135.8 (4C), 135.7 (2C), 135.6 (2C), 134.3, 133.6, 133.5 (2C), 133.4 (2C), 133.2, 133.1, 130.0, 129.9 (2C), 129.8, 129.7, 129.6 (2C), 128.3 (2C), 127.9 S27 (2C), 127.8 (6C), 127.7 (6C), 117.3, 104.6, 101.5, 85.7, 85.3, 82.7, 78.4, 78.2, 77.8, 68.2, 65.2, 64.4, 27.0 (3C), 26.9 (3C), 26.8 (3C), 19.4, 19.3, 19.2; IR (cm^{-1} , $CHCl_3$): 3426 , 3068 , 2930 , 2858 , 1728 , 1592 , 1464 , 1427 , 1365 , 1269 , 1219 , 1109 , 1045 , 1002 , 852 , 822 , 771 , 703 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{68}H_{80}NaO_{10}Si_3$, 1163.4957; found 1163.4941

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-2-*O*-(2-*O*-benzoyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-α-D-arabinofuranoside (4):

$[\alpha]_D^{25}$ = -49.6 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.93 – 7.88 (m, 2H), 7.59 – 7.52 (m, 12H), 7.49 – 7.45 (m, 4H), 7.39 – 7.33 (m, 3H), 7.27 – 7.19 (m, 15H), 7.13 – 7.09 (m, 4H), 5.91 (s, 1H), 5.60 (d, *J* = 4.7 Hz, 1H), 5.17 (d, *J* = 3.0 Hz, 1H), 5.09 (dd, *J* = 6.0, 4.7 Hz, 1H), 4.51 (t, J = 5.8 Hz, 1H), 4.45 (d, *J* = 1.2 Hz, 1H), 4.23 – 4.14 (m, 2H), 3.64 – 3.52 (m, 2H), 3.36 (qd, *J* = 10.7, 6.4 Hz, 2H), 2.59 (s, 1H), 2.17 – 2.04 (m, 2H), 1.95 – 1.82 (m, 2H), 1.61 – 1.45 (m, 6H), 0.99 (s, 9H), 0.92 (s, 9H), 0.87 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.8, 164.7, 151.1, 135.8 (4C), 135.7 (2C), 135.6 (6C), 133.6, 133.5 133.4 (2C), 133.1 (2C), 133.0, 132.9, 130.0 (2C), 129.9, 129.8 (2C), 129.6 (3C), 128.3 (2C), 128.1 (2C), 127.9 (2C), 127.8 (2C), 127.7 (12C), 102.1, 99.5, 85.8, 84.4, 83.3, 82.9, 80.0, 77.8, 77.6, 75.7, 75.2, 65.6, 63.6, 36.9 (2C), 26.9 (6C), 26.8 (3C), 25.1, 22.6 (2C), 19.3, 19.2 (2C); IR (cm^{-1} , $CHCl_3$): 3300 , 3068 , 2934 , 2859 , 1727 , 1594 , 1459 , 1427 , 1361 , 1273 , 1109 , 1067 , 1028 , 942 , 906 , 853, 823 , 769 , 703 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{81}H_{90}NaO_{13}Si_3$, 1377.5587; found 1377.5685.

Allyl -2-*O*-benzoyl-3-*O*-(4-oxopentanoyl)-α-D-arabinofuranoside (5):

$[\alpha]_D^{25}$ = +17.9 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.06 – 7.95 (m, 2H), 7.62 – 7.51 (m, 1H), 7.48 – 7.37 (m, 2H), 5.98 – 5.87 (m, 1H), 5.39 – 5.30 (m, 2H), 5.22 (ddd, *J* = 6.1, 3.7, 1.8 Hz, 3H), 4.29 – 4.17 (m, 2H), 4.08 (ddt, *J* = 13.1, 6.1, 1.3 Hz, 1H), 3.90 (ddd, *J* = 29.8, 12.1, 3.7 Hz, 2H), 2.86 – 2.68 (m, 2H), 2.68 – 2.55 (m, 2H), 2.18 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 206.4, 172.6, 165.4, 133.8, 133.7, 129.9 (2C), 129.2, 128.6 (2C), 117.7, 104.7, 83.4, 82.1, 77.6, 68.1, 62.2, 38.0, 29.9, 28.0; IR (cm^{-1} , $CHCl_3$): 3498 , 2926 , 1721 , 1452 , 1412 , 1363 , 1263 , 1218 , 1158 , 1107 , 1066 , 991 , 771 , 715 , 683 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{20}H_{24}NaO_8$, 415.1369; found 415.1372

Allyl-2-*O*-benzoyl-3-*O*-(4-oxopentanoyl)-5-*O*-(2-*O*-(2-*O*-benzoyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (27b):

This compound is prepared from 32 and 37 using the above delineated procedure I; Yield: 76%; $[\alpha]_D^{25}$ = -15.3 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.92 – 7.86 (m, 2H), 7.78 – 7.71 (m, 2H), 7.62 – 7.47 (m, 13H), 7.45 – 7.35 (m, 3H), 7.34 – 7.08 (m, 25H), 5.97 – 5.84 (m, 1H), 5.50 (d, *J* = 4.8 Hz, 1H), 5.35 – 5.28 (m, 2H), 5.26 (dd, *J* = 5.5, 1.1 Hz, 1H), 5.21 – 5.16 (m,

1H), 5.15 (s, 1H), 5.10 (dd, *J* = 4.8, 1.8 Hz, 1H), 5.03 (dd, *J* = 5.5, 5.0 Hz, 1H), 4.87 (s, 1H), 4.53 (t, J = 5.5 Hz, 1H), 4.31 (d, *J* = 1.7 Hz, 1H), 4.27 – 4.09 (m, 4H), 4.04 (ddt, *J* = 13.2, 6.0, 1.3 Hz, 1H), 3.86 (dd, *J* = 11.2, 4.9 Hz, 1H), 3.68 – 3.56 (m, 3H), 3.47 (d, *J* = 5.8 Hz, 2H), 2.69 (dd, J = 9.9, 4.1 Hz, 2H), 2.63 – 2.50 (m, 2H), 2.09 (s, 3H), 0.97 (s, 9H), 0.92 (s, 9H), 0.85 (s, 9H); **^{13}C NMR** (101 MHz, CDCl3): δ 206.3, 171.9, 165.8, 165.5, 165.1, 135.8 (4C), 135.7 (8C), 133.9, 133.7, 133.6, 133.5 (2C), 133.4, 133.2, 133.0, 132.9 (2C), 129.9 (5C), 129.8 (2C), 129.7, 129.6 (3C), 129.4, 129.2, 128.5 (2C), 128.2 (4C), 127.9 (2C), 127.7 (8C), 127.6 (4C), 117.6, 105.5, 104.8, 100.0, 85.0, 84.3, 83.0, 82.2, 81.4, 80.0, 78.4, 77.3, 77.2, 67.9, 66.1, 65.7, 64.2, 38.0, 29.9, 28.0, 26.9 (6C), 26.8 (3C), 19.3 (2C), 19.2; IR (cm^{-1} , $CHCl_3$): 3068 , 2928 , 2858 , 1724 , 1459 , 1426 , 1361 , 1269 , 1219 , 1107 , 1060 , 1028 , 854 , 822 , 770 , 704 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{92}H_{102}NaO_{18}Si_3$, 1601.6272; found 1601.6308.

Allyl-2-*O*-benzoyl-5-*O*-(2-*O*-(2-*O*-benzoyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (28):

$[\alpha]_D^{25}$ = -11.9 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, CDCl3): δ 8.04 – 7.97 (m, 2H), 7.78 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.65 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.62 – 7.50 (m, 14H), 7.48 – 7.39 (m, 4H), 7.37 – 7.19 (m, 19H), 7.14 (t, *J* = 7.3 Hz, 2H), 6.03 – 5.88 (m, 1H), 5.53 (d, *J* = 4.7 Hz, 1H), 5.35 (ddd, *J* = 17.2, 3.2, 1.6 Hz, 1H), 5.30 (s, 1H), 5.24 (ddd, *J* = 10.4, 2.7, 1.2 Hz, 1H), 5.18 – 5.04 (m, 3H), 4.92 (s, 1H), 4.52 (t, *J* = 5.8 Hz, 1H), 4.36 (dd, *J* = 2.3, 0.9 Hz, 1H), 4.30 (ddt, *J* = 13.0, 5.1, 1.5 Hz, 1H), 4.24 (dd, *J* = 10.7, 4.7 Hz, 1H), 4.16 (dt, *J* = 10.8, 5.3 Hz, 2H), 4.12 – 4.04 (m, 2H), 3.84 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.65 – 3.56 (m, 3H), 3.53 (d, *J* = 5.7 Hz, 2H), 3.28 (s, 1H), 1.01 (s, 9H), 0.94 (s, 9H), 0.89 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.8, 166.0, 165.1, 135.8 (4C), 135.7 (4C), 135.6 (4C), 133.9 (2C), 133.6, 133.5 (3C), 133.1, 133.0 (2C), 132.9, 130.0 (2C), 129.9 (2C), 129.8 (2C), 129.7, 129.6 (6C), 129.3, 129.2, 128.6 (2C), 128.2 (2C), 127.9 (2C), 127.7 (8C), 127.6 (4C), 117.8, 105.5, 104.5, 99.6, 86.1, 84.6, 84.1, 82.9, 82.6, 80.0, 78.2, 77.0, 75.8, 68.4, 66.4, 65.6, 64.3, 26.9 (6C), 26.8 (3C), 19.2 (3C); IR (cm^{-1} , $CHCl_3$): 3069 , 2930 , 2859 , 1724 , 1460 , 1427 , 1362 , 1270 , 1220 , 1108 , 1063 , 1031 , 855 , 822 , 771 , 705 ; HRMS (ESI-MS) [M+Na]+ m/z calcd for $C_{87}H_{96}NaO_{16}Si_3$, 1503.5904; found 1503.5914

Allyl-2-*O*-benzoyl-3,5-di-*O*-(2-*O*-(2-*O*-benzoyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (29):

$[\alpha]_D^{25}$ = -18.4 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.98 (d, *J* = 7.4 Hz, 2H), 7.83 (dd, *J* = 17.2, 7.5 Hz, 4H), 7.63 – 7.48 (m, 30H), 7.35 – 7.11 (m, 49H), 5.91 (ddd, *J* = 22.4, 10.7, 5.4 Hz, 1H), 5.62 (d, *J* = 4.6 Hz, 1H), 5.48 (d, *J* = 4.7 Hz, 1H), 5.36 – 5.28 (m, 1H), 5.23 (d, *J* = 13.7 Hz, 2H), 5.19 – (m, 5H), 5.06 – 4.97 (m, 1H), 4.91 (s, 1H), 4.54 (dt, *J* = 17.4, 5.5 Hz, 2H), 4.38 (d, *J* = 23.7 Hz, 2H), 4.27 – 4.11 (m, 6H), 4.05 (dd, *J* = 13.1, 6.0 Hz, 1H), 3.96 (dd, *J* = 10.4, 5.6 Hz, 1H), 3.79 (dd, *J* = 11.6, 5.2 Hz, 1H), 3.71 – 3.58 (m, 5H), 3.55 – 3.39 (m, 4H), 1.02 (s, 18H), 0.96 (d, *J* = 3.2 Hz, 18H), 0.86 (s, 18H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.7 (2C), 165.5, 165.0 (2C), 135.8 (8C), 135.7 (8C), 135.6 (8C), 134.2, 133.7 (2C), 133.5 (5C), 133.4, 133.2 (3C), 133.1 (3C), 133.0, 132.9, 132.8 (2C), 129.9 (9C), 129.8 (5C), 129.6 (3C), 129.5 (3C), 129.4, 128.4 (2C), 128.3 (2C), 128.2 (2C), 128.1 (4C), 127.8 (4C), 127.7 - 127.6 (26C), 117.3, 105.7, 105.1, 104.4, 99.6 (2C), 84.7, 84.3, 84.2 (2C), 83.6, 83.3, 83.0, 81.8, 80.7, 79.9 (2C), 78.4, 77.9, 76.3, 75.9, 67.7, 66.3, 65.7 (2C), 64.2, 64.0, 27.0 (3C), 26.9 (9C), 26.8 (6C), 19.3 (4C), 19.1 (2C); IR (cm^{-1} , CHCl3): 3069 , 2930 , 2859 , 1725 , 1460 , 1427 , 1362 , 1269 , 1219 , 1107 , 1061, 1028 , 855 , 822 , 769 , 702 ; HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{159}H_{174}NaO_{26}Si_6$, 2692.0874; found 2692.0864.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-*O*-benzoyl-3,5-di-*O*-(2-*O*-(2-*O*-benzoyl-3,5-di-*O*-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (2):

$[\alpha]_D^{25}$ = -14.2 (c 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.94 – 7.88 (m, 2H), 7.84 – 7.79 (m, 2H), 7.78 – 7.73 (m, 2H), 7.59 – 7.52 (m, 20H), 7.48 – 7.43 (m, 10H), 7.31 – 7.19 (m, 35H), 7.12 (ddd, *J* = 7.3, 5.0, 1.9 Hz, 14H), 6.34 (s, 1H), 5.62 (d, *J* = 4.7 Hz, 1H), 5.43 (d, *J* = 4.8 Hz, 1H), 5.32 (d, *J* = 1.3 Hz, 1H), 5.19 – 5.12 (m, 4H), 4.99 – 4.93 (m, 1H), 4.80 (s, 1H), 4.49 (dt, *J* = 8.5, 5.7 Hz, 2H), 4.39 (d, *J* = 1.8 Hz, 1H), 4.33 – 4.27 (m, 1H), 4.24 (d, *J* = 1.4 Hz, 1H), 4.22 – 4.12 (m, 4H), 4.03 (dd, *J* = 10.4, 5.7 Hz, 1H), 3.91 (dd, *J* = 10.4, 5.6 Hz, 1H), 3.70 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.62 – 3.56 (m, 4H), 3.46 – 3.36 (m, 4H), 2.64 (s, 1H), 2.23 – 2.12 (m, 2H), 1.84 – 1.60 (m, 8H), 0.99 (s, 18H), 0.92 (s, 18H), 0.81 (d, *J* = 2.0 Hz, 18H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.7 (2C), 165.1, 165.0, 164.9, 151.3, 135.8 (8C), 135.7 (4C), 135.6 (12C), 133.7, 133.4 (4C), 133.3 (2C), 133.0 (6C), 132.8 (4C), 130.0 - 129.9 (7C), 129.8 (6C), 129.7, 129.6 (7C), 129.5 (3C), 129.3, 129.2, 129.1, 128.5 (2C), 128.4 (2C), 128.3 (2C), 128.1 (2C), 127.9 (4C), 127.7 - 127.6 (22C), 105.6, 104.4, 102.9, 99.7, 99.6, 96.2, 84.7, 84.2 (2C), 84.1, 83.4, 83.1, 82.9, 82.4, 80.0 (4C), 78.2, 78.0, 77.7, 76.1, 75.8, 75.4, 65.9, 65.8, 65.7, 64.0, 63.8, 36.9, 36.8, 26.9 (12C), 26.7 (6C), 25.1, 22.7 (2C), 19.2 (4C), 19.1, 19.0; IR (cm^{-1} , CHCl3): 3068 , 2931 , 2859 , 1726 , 1459 , 1428 , 1364 , 1271 , 1221 , 1109 , 1063 , 944, 911

, 853 , 823 , 771 , 705 ; HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{165}H1_{80}NaO_{28}Si_6$, 2801.1208; found 2801.1285.

1-*O*-(((1-ethynylcyclohexyl)oxy) carbonyl) 2,3 di-*O*-benzoyl 5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl)-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofurano side **(6**):

$[\alpha]_D^{25}$ = -2º (*c* 0.16 , $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11 – 7.92 (m, 12H), 7.75 – 7.66 (m, 4H), 7.61 – 7.30 (m, 24H), 6.35 (s, 1H), 5.73 (dt, *J* = 5.1, 0.9 Hz, 2H), 5.68 – 5.62 (m, 2H), 5.61 (t, *J* = 1.0 Hz, 1H), 5.58 (d, *J* = 1.3 Hz, 1H), 5.41 – 5.37 (m, 2H), 4.68 – 4.60 (m, 2H), 4.51 (q, *J* = 4.6 Hz, 1H), 4.26 – 4.15 (m, 2H), 4.02 – 3.90 (m, 4H), 2.67 – 2.64 (m, 1H), 2.30 – 2.09 (m, 2H), 2.06 – 1.53 (m, 8H), 1.02 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.7, 165.79(2C), 165.4, 165.3, 165.2, 151.1, 135.8(2C), 133.8, 133.7, 133.5, 133.4, 133.3, 133.3, 133.2, 130.1, 130.1, 130.0, 129.9, 129.9, 129.8, 129.3(2C), 129.2(2C), 129.1(2C), 128.7, 128.6, 128.5, 128.3, 127.8, 126.2, 115.7, 106.1, 106.0, 102.4, 84.3, 83.3, 82.6, 82.3, 82.2, 81.6, 81.0, 78.5, 75.5, 65.9, 65.8, 63.5, 36.9, 36.8, 26.9(3C), 25.0, 22.8, 22.7, 19.4 ; **IR** (cm^{-1}, $CHCl_3$): 3297, 2864, 2939, 1727, 1599, 1453, 1363, 1261, 1107, 1020, 966, 852, 756, 708; **HRMS** (ESI-MS): $[M+Na]^+$ calculated for $C_{82}H_{78}O_{21}SiNa$: 1449.4703 found 1449.4725

Pent-4-enyl-2,3 di-*O*-benzoyl 5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl)- 5-*O*-(2,3-di-*O*-benzoyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside **(7**):

$[\alpha]_D^{25}$ = -21º (*c* 0.16 , $CHCl_3$)**; ^{1}H** NMR (400 MHz, $CDCl_3$) δ 8.09 – 7.84 (m, 16H), 7.63 – 7.16 (m, 24H), 5.87 – 5.76 (m, 1H), 5.69 – 5.61 (m, 6H), 5.52 (d, *J* = 1.3 Hz, 1H), 5.43 (s, 1H), 5.42 (s, 1H), 5.41 (s, 2H), 5.22 (s, 1H), 5.05 – 4.93 (m, 2H), 4.62 (dt, *J* = 4.5, 2.2 Hz, 2H), 4.50 – 4.41 (m, 2H), 4.24 – 4.14 (m, 3H), 3.95 (ddt, *J* = 13.7, 11.2, 2.7 Hz, 5H), 3.82 – 3.72 (m, 1H), 3.57 – 3.47 (m, 1H), 2.43 (dd, *J* = 8.0, 4.8 Hz, 1H), 2.24 – 2.13 (m, 2H), 1.73 (ddd, *J* = 8.6, 6.4, 4.4 Hz, 2H); **^{13}C** NMR (101 MHz, $CDCl_3$) δ 166.1, 165.7, 165.5, 165.2, 165.2, 138.1, 133.6, 133.5, 133.3, 133.3, 133.2, 129.9, 129.9, 129.8, 129.3, 129.2, 129.1, 129.0, 129.0, 128.6, 128.5, 128.4, 128.3, 115.0, 105.9, 105.9, 105.7, 105.7, 83.7, 82.1, 82.0, 81.9, 81.8, 81.7, 81.6, 81.6, 77.7, 77.3, 76.8, 66.7, 66.1, 66.0, 65.9, 62.3, 30.3, 28.8 ; **IR** (cm^{-1}, $CHCl_3$): 3297, 2864, 2939, 1727, 1599, 1453, 1363, 1261, 1107, 1020, 966, 852, 756, 708; **HRMS** (ESI-MS): $[M+Na]^+$ calculated for $C_{81}H_{74}O_{25}SiNa$: 1470.4450 found 1470.4448

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-α-D-(2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**32**):

$[\alpha]_D^{25}$ = -31º (*c* 0.16, $CHCl_3$)**;** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 7.87 (m, 28H), 7.74 (ddt, *J* = 7.6, 3.6, 1.7 Hz, 4H), 7.63 – 7.22 (m, 48H), 5.86 (ddt, *J* = 16.9, 10.3, 6.6 Hz, 1H), 5.69 (td, *J* = 5.5, 2.2 Hz, 12H), 5.61 (d, *J* = 1.8 Hz, 1H), 5.56 (d, *J* = 1.9 Hz, 1H), 5.44 (t, *J* = 5.8 Hz, 6H), 5.26 (d, *J* = 1.7 Hz, 1H), 5.11 – 4.95 (m, 2H), 4.72 – 4.44 (m, 7H), 4.27 – 4.18 (m, 6H), 4.07 – 3.91 (m, 8H), 3.82 (dt, *J* = 9.9, 6.7 Hz, 1H), 3.56 (dt, *J* = 9.9, 6.2 Hz, 1H), 2.21 (dt, *J* = 11.4, 5.7 Hz, 2H), 1.81 – 1.72 (m, 2H), 1.05 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.6(4C), 165.6, 165.5, 165.2(2C), 165.1(4C), 165.1(2C), 138.2, 135.7(2C), 135.7(2C), 133.7-133.1(18C), 130.0-129.7(24C), 129.3(2C), 129.3(4C), 129.1(4C), 129.1(4C), 128.7(2C), 128.6-128.2(24C), 127.7(12C), 115.0, 106.0 (4C), 105.9, 105.7 (2C), 83.3(2C), 82.6, 82.2(2C), 82.1(2C), 82.1(2C), 82.1(2C), 81.5(4C), 81.0, 78.4, 77.3(2C), 77.3(2C), 77.2, 75.4, 66.8, 66.1, 65.8(2C), 63.5, 30.4, 28.8, 26.8, 19.4 (3C) ;); **IR** (cm^{-1}, $CHCl_3$): 3016, 2941, 1718, 1453, 1264, 1109, 1071, 1027, 747, 707, 667; **HRMS** (ESI-MS) $[M+Na]^+$ calculated for $C_{158}H_{142}O_{45}SiNa$: 2729.8502 found 2729.8500.

1-*O*-(((1-ethynylcyclohexyl) oxy) carbonyl) 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-α-D-(2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**42**):

$[\alpha]_D^{25}$ = -10º (*c* 0.16 $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09 – 7.85 (m, 28H), 7.74 – 7.24 (m, 52H), 6.33 (d, *J* = 4.1 Hz, 1H), 5.72 (d, *J* = 1.7 Hz, 1H), 5.65 (d, *J* = 3.9 Hz, 3H), 5.63 (s, 3H), 5.62 (s, 3H), 5.61 (d, *J* = 3.7 Hz, 1H), 5.59 (d, *J* = 1.3 Hz, 1H), 5.55 (d, *J* = 1.4 Hz, 1H), 5.37 (d, *J* = 6.2 Hz, 6H), 4.66 – 4.47 (m, 7H), 4.25 – 4.10 (m, 7H), 4.00 – 3.86 (m, 8H), 2.64 (s, 1H), 2.29 – 1.99 (m, 2H), 1.95 – 1.63 (m, 8H), 1.00 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.6(4C), 165.6, 165.5, 165.2(2C), 165.1(4C), 165.1(2C), 150.9, 135.7(2C), 135.7(2C), 133.7-133.1(16C), 130.0-129.7(24C), 129.3(2C), 129.3(4C), 129.1(4C), 129.1(4C), 128.7(2C), 128.6-128.2(24C), 127.7(12C), 106.0(3C), 105.9(3C), 102.3, 84.3, 83.2(2C), 82.6, 82.2(2C), 82.1(2C), 82.1(2C), 82.1(2C), 81.5(4C), 81.0, 78.4, 77.3(2C), 77.3(2C), 77.2, 75.4, 65.8(6C), 63.4, 36.8, 36.7, 26.8(3C), 25.0, 22.7, 22.6, 19.3; **IR** (cm^{-1}, $CHCl_3$): 3016, 2941,

1718, 1453, 1264, 1109, 1071, 1027, 747, 707, 667; **HRMS** (ESI-MS) $[M+Na]^+$ calculated for $C_{158}H_{142}O_{45}SiNa$: 2810.8524 found 2810.8521.

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-α-D-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**43**):

$[\alpha]_D^{25}$ = -8º (*c* 0.16 $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.11 – 7.84 (m, 28H), 7.62 – 7.36 (m, 30H), 7.30 – 7.22 (m, 12H), 5.92 – 5.79 (m, 1H), 5.69 – 5.62 (m, 11H), 5.53 (d, *J* = 1.4 Hz, 1H), 5.42 (td, *J* = 6.0, 5.6, 2.1 Hz, 7H), 5.23 (s, 1H), 5.07 – 4.96 (m, 2H), 4.67 – 4.56 (m, 5H), 4.51 – 4.44 (m, 2H), 4.25 – 4.14 (m, 6H), 4.00 – 3.89 (m, 7H), 3.80 (dt, *J* = 9.6, 6.6 Hz, 1H), 3.53 (dt, *J* = 9.5, 6.2 Hz, 1H), 2.38 – 2.29 (m, 1H), 2.25 – 2.14 (m, 2H), 1.76 (m, 4H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.1, 165.7, 165.6(4C), 165.2(4C), 165.2(4C), 138.2, 133.7-133.2(10C), 130.1-129.9 (30C),129.3 (2C), 129.2 (2C), 129.1(4C), 129.1(4C), 129.0(2C), 128.6 -128.3(30C), 115.0, 106.0 (5C), 105.9, 105.7, 83.3(2C), 82.6, 82.2(2C), 82.1(2C), 82.1(2C), 82.1(2C), 81.5(4C), 81.0, 78.4, 77.3(2C), 77.3(2C), 77.2, 75.4, 66.8, 66.1, 65.8(2C), , 62.4, 30.4, 28.9; **IR** (cm^{-1}, $CHCl_3$): 3016, 2941, 1718, 1453, 1264, 1109, 1071, 1027, 747, 707, 667; HRMS (MALDI-TOF) $[M+Na]^+$ calculated for $C_{138}H_{122}O_{43}SiNa$: 2491.7325 found 2491.7225.

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl -α-D-arabinofuranoside (**31**):

$[\alpha]_D^{25}$ = -25º (*c* 0.16 $CHCl_3$); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.13 – 8.00 (m, 30H), 7.91 (dd, *J* = 26.1, 8.0 Hz, 26H), 7.73 (t, *J* = 6.5 Hz, 6H), 7.58 – 7.39 (m, 56H), 7.31 – 7.20 (m, 32H), 5.68 (d, *J* = 6.6 Hz, 27H), 5.57 (dd, *J* = 28.7, 9.0 Hz, 4H), 5.43 (d, *J* = 14.3 Hz, 15H), 5.02 (dd, *J* = 34.4, 13.7 Hz, 2H), 4.70 – 4.56 (m, 12H), 4.24 – 4.16 (m, 13H), 4.02 – 3.89 (m, 16H), 2.25 – 2.16 (m, 2H), 1.82 – 1.74 (m, 2H), 1.05 (s, 9H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 165.7, 165.7, 165.6, 165.5, 165.3, 165.2, 165.2, 138.2, 135.8, 135.7, 133.5, 133.4, 133.4, 133.4, 133.4, 133.3, 133.2, 133.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.7, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 127.8, 115.0, 106.1, 106.0, 105.9, 105.7, 83.3, 82.2, 82.2, 82.0, 81.9, 81.6, 66.8, 66.1, 65.9, 63.5, 30.4, 28.8, 26.9, 19.4; **IR** (cm^-

[1], $CHCl_3$): 3016, 2941, 1718, 1453, 1264, 1109, 1071, 1027, 747, 707, 667; HRMS (MALDI-TOF) $[M+Na]^+$ calculated for $C_{287}H_{252}O_{85}SiNa$: 5110.5164 found 5110.5061.

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl - α-D-arabinofuranoside (**44**):

$[\alpha]_D^{25}$ = -33º (*c* 0.16 $CHCl_3$);**^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.14 – 7.87 (m, 56H), 7.63 – 7.40 (m, 58H), 7.33 – 7.23 (m, 26H), 5.91 – 5.82 (m, 1H), 5.77 – 5.64 (m, 26H), 5.60 – 5.55 (m, 1H), 5.49 – 5.41 (m, 13H), 5.27 (d, *J* = 12.6 Hz, 1H), 5.11 – 4.94 (m, 2H), 4.71 – 4.60 (m, 12H), 4.54 – 4.48 (m, 2H), 4.30 – 4.18 (m, 13H), 4.07 – 3.92 (m, 15H), 3.87 – 3.79 (m, 1H), 3.61 – 3.54 (m, 1H), 2.52 – 2.38 (m, 1H), 2.32 – 2.16 (m, 2H), 2.00 (s, 1H), 1.84 – 1.73 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1-165.2 (12C), 165.2-165.2 (16C), 138.2, 133.6-133.2 (20C), 133.2-129.4 (40C), 129.2 -128.3 (24C), 115.0, 115.0, 106.0 -105.9 (12C), 105.7 (2C), 82.2- 81.6 (50C), 66.8, 66.2, 66.1, 65.9, 65.9, 62.4, 30.4, 30.4, 28.8. HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{274}H_{231}O_{85}Na$, : 4876.453; found 4876.368.

Pent-4-enyl- 2, 3 di-*O*-benzoyl- α-D-arabinofuranoside (**8**):

$[\alpha]_D^{25}$ = -45.1 (c 0.7, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11 – 8.01 (m, 4H), 7.58 (td, *J* = 7.3, 5.3 Hz, 2H), 7.49 – 7.40 (m, 4H), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.53 (d, *J* = 1.3 Hz, 1H), 5.44 (dd, *J* = 4.7, 0.8 Hz, 1H), 5.23 (s, 1H), 5.00 (ddd, *J* = 17.1, 3.5, 1.6 Hz, 1H), 4.95 (ddt, *J* = 10.2, 2.1, 1.2 Hz, 1H), 4.32 (dd, *J* = 8.4, 4.1 Hz, 1H), 4.06 – 3.93 (m, 2H), 3.78 (dt, *J* = 9.5, 6.6 Hz, 1H), 3.53 (dt, *J* = 9.5, 6.2 Hz, 1H), 2.52 (s, 1H), 2.23 – 2.11 (m, 2H), 1.80 – 1.68 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.2, 165.4, 138.1, 133.6 (2C), 130.0 (2C), 129.9 (2C), 129.3, 129.2, 128.6 (4C), 115.0, 105.6, 83.8, 81.9, 77.9, 66.8, 62.5, 30.3, 28.8; IR (cm^{-1}, $CHCl_3$): 3498 , 2932 , 1720 , 1599 , 1450 , 1358 , 1260 , 1175 , 1107 , 1062 , 969 , 913 , 865 , 711 ; **HRMS** (ESI-MS) [M+Na]+ m/z calcd for $C_{24}H_{26}NaO_7$, 449.1576; found 449.1582.

1-O-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-O-benzoyl-3,5-di-O-(2,3-di-O-benzoyl-5-O-(2,3-di-O-benzoyl-5-O-(2,3-di-O-benzoyl-5-O-tert-butyldiphenylsilyl-α-Darabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**30)**:

$[\alpha]_D^{25}$ = +12.7 (c 0.7, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.99 – 7.92 (m, 4H), 7.89 – 7.82 (m, 28H), 7.80 – 7.71 (m, 18H), 7.62 – 7.56 (m, 16H), 7.44 – 7.40 (m, 4H), 7.39 – 7.36 (m, 4H), 7.35 – 7.10 (m, 96H), 6.36 (d, *J* = 4.4 Hz, 1H), 6.24 (s, 1H), 5.66 – 5.61 (m, 2H), 5.59 – 5.51 (m, 14H), 5.50 (d, *J* = 7.5 Hz, 4H), 5.47 (d, *J* = 1.0 Hz, 2H), 5.46 – 5.44 (m, 2H), 5.44 – 5.42 (m, 2H), 5.33 – 5.18 (m, 11H), 4.53 – 4.24 (m, 16H), 4.14 – 4.03 (m, 8H), 4.00 – 3.83 (m, 11H), 3.82 – 3.66 (m, 10H), 2.54 (s, 1H), 2.26 (s, 1H), 2.16 – 1.86 (m, 4H), 1.80 – 1.71 (m, 4H), 1.64 – 1.36 (m, 10H), 1.34 – 1.24 (m, 2H), 0.91 (s, 36H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.8, 165.6 (7C), 165.5 (4C), 165.3 (2C), 165.2 (8C), 165.1 (4C), 151.2, 151.0, 135.7 (16C), 133.1 - 133.6 (34C), 130.1 - 129.6 (60C), 129.4 - 128.7 (26C), 128.6 - 128.2 (52C), 127.7 (16C), 106.2, 106.1, 106.0 (4C), 105.9 (3C), 105.8, 105.5, 105.3, 102.8, 96.5, 83.8, 83.2 (4C), 82.9, 82.7, 82.6, 82.5, 82.2 (9C), 81.9, 81.7, 81.6, 81.5 (4C), 80.3, 80.0, 78.5, 78.1, 77.7, 77.6, 77.4 (5C), 77.2 (5C), 77.0 (2C), 76.8 (2C), 76.6, 75.5, 75.1, 67.0, 65.8 (3C), 65.7 (3C), 65.6 (2C), 65.4, 63.4 (4C), 36.9, 36.7, 36.6, 36.5, 26.8 (12C), 25.0, 24.8, 22.7 (3C), 22.5, 19.4 (4C); IR (cm^{-1} , $CHCl_3$): 2940 , 1725 , 1599 , 1452 , 1363 , 1262 , 1175 , 1106 , 966 , 910 , 859 , 754 , 707 ; HRMS (MALDITOF) [M+Na]+ m/z calcd for $C_{167}H_{156}NaO_{44}Si_2$ 2944.9439; found: 2944.9578.

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2-*O*-benzoyl-3,5-di-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl -α-D-arabinofuranoside (**47**)

$[\alpha]_D^{25}$ = -19.2(c 0.5, $CHCl_3$); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.14 – 7.84 (m, 84H), 7.77 – 7.68 (m, 9H), 7.57 – 7.22 (m, 138H), 5.92 – 5.81 (m, 1H), 5.73 – 5.65 (m, 31H), 5.62 – 5.58 (m, 4H), 5.57 (p, *J* = 1.7 Hz, 4H), 5.44 (d, *J* = 5.2 Hz, 14H), 5.39 (d, *J* = 3.3 Hz, 2H), 5.36 (s, 1H), 5.28 (d, *J* = 8.8 Hz, 2H), 5.09 – 4.97 (m, 2H), 4.63 (p, *J* = 3.6 Hz, 15H), 4.56 – 4.42 (m, 7H),

4.29 – 4.15 (m, 18H), 4.04 – 3.88 (m, 21H), 3.86 – 3.78 (m, 2H), 3.56 (dt, *J* = 9.6, 6.2 Hz, 1H), 2.31 – 2.16 (m, 2H), 1.86 – 1.71 (m, 2H), 1.05 (s, 18H) ;**^{13}C NMR** (151 MHz, $CDCl_3$) δ 165.7, 165.7, 165.6 (5C), 165.5 (5C), 165.4, 165.3- 165.2 (28C) 164.9, 138.2, 135.7- 133.5 (80C), 133.5-133.3 (60C), 133.3-133.2 (136C), 115.0, 106.0-105.7 (16C), 105.9-105.3 (5C), 83.2 (4C), 82.7(5C), 82.2- 81.6 (47C), 66.7- 65.9 (19C), 63.4 (2C), 30.4, 28.8, 26.8 (2C), 19.3 (6C) ; IR (cm^{-1} , $CHCl_3$): 2923 , 2855 , 1723 , 1598 , 1453 , 1360 , 1262 , 1175 , 1107 , 966 , 864 , 771 , 709 , 556 ; HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{429}H_{378}NaO_{126}$ 7628.2885; found 7626.4557

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2-*O*-benzoyl-3,5-di-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl -α-D-arabinofuranoside (**3**)

$[\alpha]_D^{25}$ = +15.2(c 0.5, $CHCl_3$); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.07 – 7.77 (m, 84H), 7.53 – 7.16 (m, 126H), 5.88 – 5.75 (m, 1H), 5.67 – 5.55 (m, 34H), 5.52 – 5.45 (m, 5H), 5.42 – 5.34 (m, 19H), 5.30 (s, 1H), 5.25 (s, 1H), 5.21 (s, 1H), 5.05 – 4.92 (m, 2H), 4.65 – 4.53 (m, 13H), 4.50 – 4.33 (m, 8H), 4.21 – 4.07 (m, 17H), 3.96 – 3.82 (m, 21H), 3.79 – 3.71 (m, 2H), 3.50 (dq, *J* = 9.4, 6.4 Hz, 1H), 2.50 (s, 2H), 2.23 – 2.11 (m, 2H), 1.77 – 1.65 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.7, 165.7, 165.6 (5C), 165.5 (5C), 165.4, 165.3- 165.2 (28C) 164.9, 138.1, 135.7-133.5 (80C), 133.5-133.3 (60C), 133.3-133.2 (112C), 114.9, 106.0-105.7 (16C), 105.9-105.3 (5C), 83.2 (6C), 82.7(5C), 82.2- 81.6 (47C), 66.7- 65.9 (19C), 62.3 (2C), 30.3, 28.8; IR (cm^{-1} , $CHCl_3$): 2923 , 2855 , 1723 , 1598 , 1453 , 1360 , 1262 , 1175 , 1107 , 966 , 864 , 771 , 709 , 556 ; HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{397}H_{342}NaO_{126}$ 7150.0325; found 7150.2544.

Pent-4-enyl- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-

di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2-*O*-benzoyl-3,5-di-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*- (2- O-benzoyl-3-O-(2-O-(2-O-benzoyl-3,5-di-O-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)- 3-O-benzoyl-5-O-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-5-O-(2-O-(2-O-benzoyl-3,5-di-O-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-O-benzoyl-5-O-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranoside (**48**)

$[\alpha]_D^{25}$ = +3.8(c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 – 7.80 (m, 104H), 7.77 – 7.70 (m, 9H), 7.60 – 7.38 (m, 140H), 7.28 – 7.14 (m, 128H), 5.69 – 5.62 (m, 36H), 5.56 – 5.49 (m, 10H), 5.43 – 5.36 (m, 21H), 5.33 – 5.28 (m, 4H), 5.23 (s, 1H), 5.17 – 5.08 (m, 8H), 5.05 – 5.02 (m, 1H), 5.01 – 4.93 (m, 3H), 4.85 (d, *J* = 2.9 Hz, 2H), 4.62 – 4.55 (m, 17H), 4.50 (dt, *J* = 10.6, 5.8 Hz, 6H), 4.31 – 4.26 (m, 4H), 4.17 (q, *J* = 13.7, 10.0 Hz, 28H), 4.09 – 4.01 (m, 5H), 3.94 – 3.87 (m, 21H), 3.77 – 3.70 (m, 2H), 3.61 (dd, *J* = 22.1, 6.3 Hz, 9H), 3.42 (t, *J* = 4.8 Hz, 6H), 2.26 – 2.12 (m, 2H), 1.80 – 1.74 (m, 2H), 1.00 (s, 18H), 0.98 (s, 18H), 0.92 (s, 19H), 0.89 (s, 18H), 0.82 (s, 18H), 0.80 (s, 18H); **^{13}C NMR** (151 MHz, $CDCl_3$) 165.7, 165.7, 165.6 (5C), 165.5 (5C), 165.4, 165.3- 165.2 (38C) 164.9, 138.1, 135.7-133.6 (120C), 133.4- 132.9 (120C), 132.8-114.9 (140C), 106.1- 105.6 (23C), 105.2 (2C), 104.9 (2C), 99.8 (2C), 99.7 (2C), 84.8-83.4 (24C), 82.8- 81.8 (26C), 81.5- 78.1(40C), 77.9, 76.2, 66.7- 65.5 (27C), 63.9 (2C), 63.7 (2C), 30.3, 28.8, 26.8 (12C), 19.1(36C); IR (cm^{-1} , $CHCl_3$): 2928 , 2859 , 1723 , 1598 , 1453, 1427 , 1361 , 1265 , 1221 , 1174 , 1107 , 1066 , 1028.94, 967.32, 858.52, 824.61, 770 , 707; HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{709}H_{678}NaO_{176}Si_{12}$ 12346.1423; found 12346.14645.

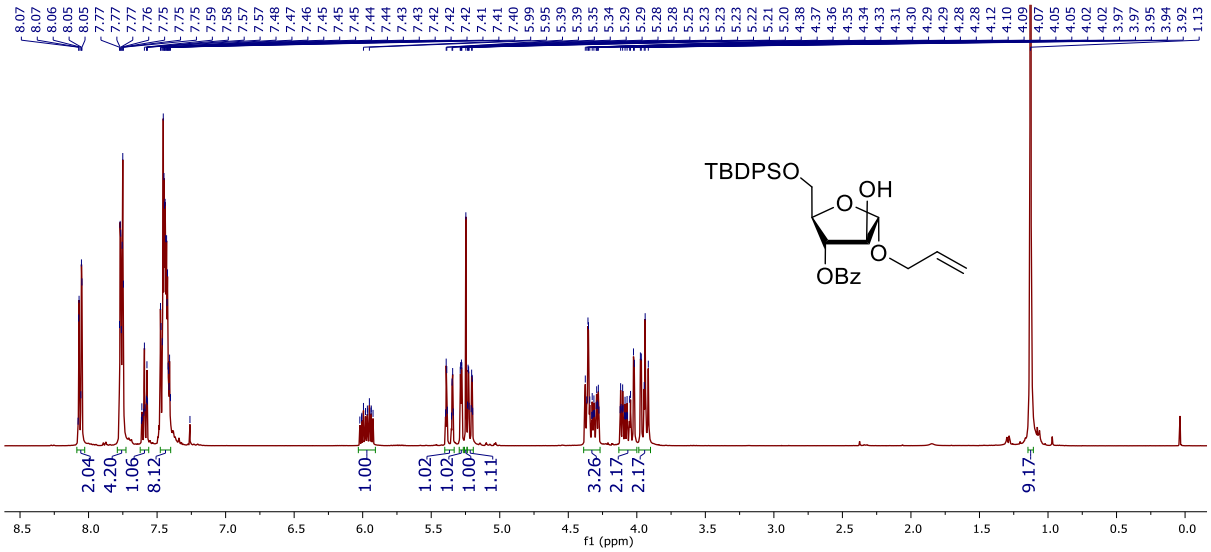
1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)- 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2-*O*-benzoyl-3,5-di-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*- (2- O-benzoyl-3-O-(2-O-(2-O-benzoyl-3,5-di-O-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)- 3-

O-benzoyl-5-O-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-5-O-(2-O-(2-O-benzoyl-3,5-di-O-tert-butyldiphenylsilyl-β-D-arabinofuranosyl)-3-O-benzoyl-5-O-tert-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl) α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranoside (**49**)

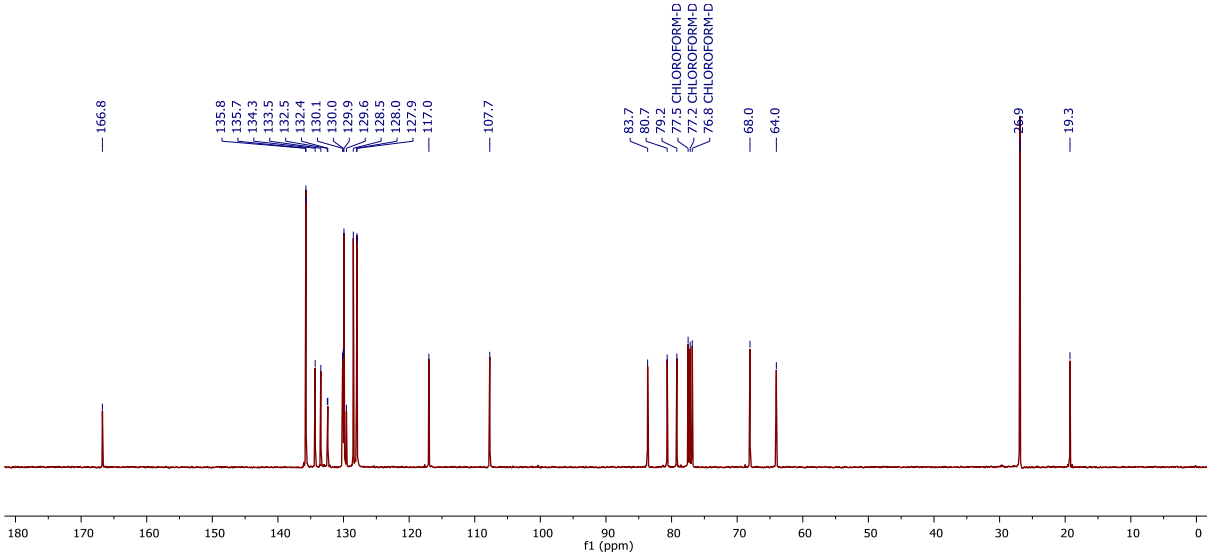
$[\alpha]_D^{25}$ = +23.5(c 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.11 – 7.77 (m, 104H), 7.76 – 7.69 (m, 9H), 7.60 – 7.36 (m, 144H), 7.25 – 7.01 (m, 124H), 5.68 – 5.59 (m, 35H), 5.57 – 5.51 (m, 9H), 5.43 – 5.37 (m, 19H), 5.34 – 5.30 (m, 11H), 5.16 – 5.05 (m, 7H), 5.00 – 4.94 (m, 2H), 4.84 (d, *J* = 2.9 Hz, 1H), 4.63 – 4.53 (m, 18H), 4.52 – 4.47 (m, 6H), 4.42 (d, *J* = 21.1 Hz, 3H), 4.28 (d, *J* = 13.8 Hz, 4H), 4.17 (dq, *J* = 12.0, 7.7, 7.0 Hz, 29H), 4.09 – 3.99 (m, 4H), 3.89 (d, *J* = 10.4 Hz, 20H), 3.72 (d, *J* = 11.6 Hz, 3H), 3.61 (dd, *J* = 22.2, 6.2 Hz, 8H), 3.45 – 3.40 (m, 5H), 3.35 (dd, *J* = 10.9, 5.9 Hz, 2H), 2.66 (s, 1H), 2.19 (d, *J* = 12.2 Hz, 8H), 0.98 (s, 36H), 0.91 (s, 36H), 0.80 (s, 36H); **^{13}C NMR** (151 MHz, $CDCl_3$) 165.7, 165.7, 165.6 (5C), 165.5 (5C), 165.4, 165.3- 165.2 (38C) 164.9, 150.9, 138.1, 135.7-133.6 (120C), 133.4- 132.9 (120C), 132.8-114.9 (140C), 106.1- 105.6 (23C), 105.2 (2C), 104.9 (2C), 99.8 (2C), 99.7 (2C), 84.8-83.4 (24C), 82.8- 81.8 (28C), 81.5- 78.1(40C), 77.9, 76.2, 66.7- 65.5 (27C), 63.9 (2C), 63.7 (2C), 36.8, 36.7 ,25.0, 24.9, 26.8 (12C), 22.1, 19.1(36C); IR (cm^{-1} , $CHCl_3$): 2929 , 2859 , 1723, 1598 , 1453 , 1424 , 1361 , 1265 , 1221 , 1174 , 1107 , 1066 , 1028 , 967 , 858 , 824 , 770, 707 ;HRMS (MALDI-TOF) [M+Na]+ m/z calcd for $C_{713}H_{680}NaO_{178}Si_{12}$ 12428.1245; found 12428.3245.

2.8 Spectral Charts of Representative Compounds

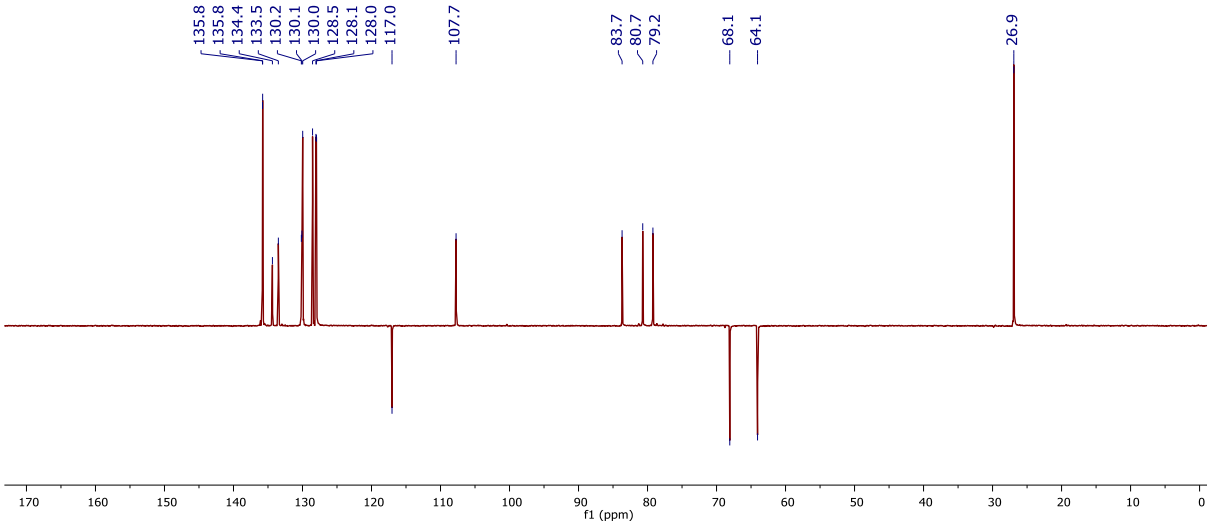
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of Compound **14a**



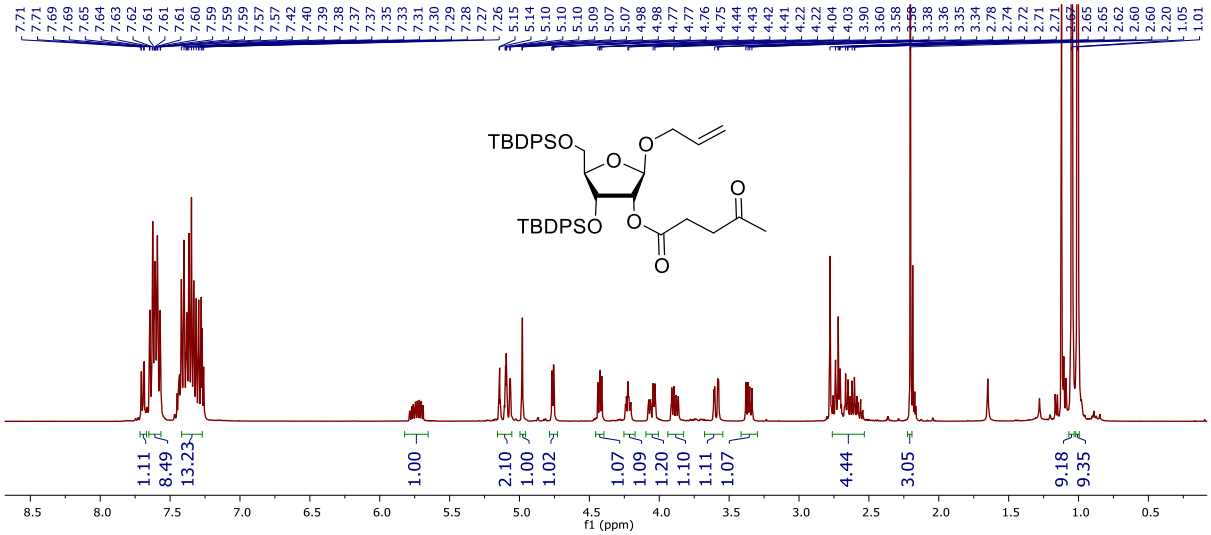
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of Compound **14a**



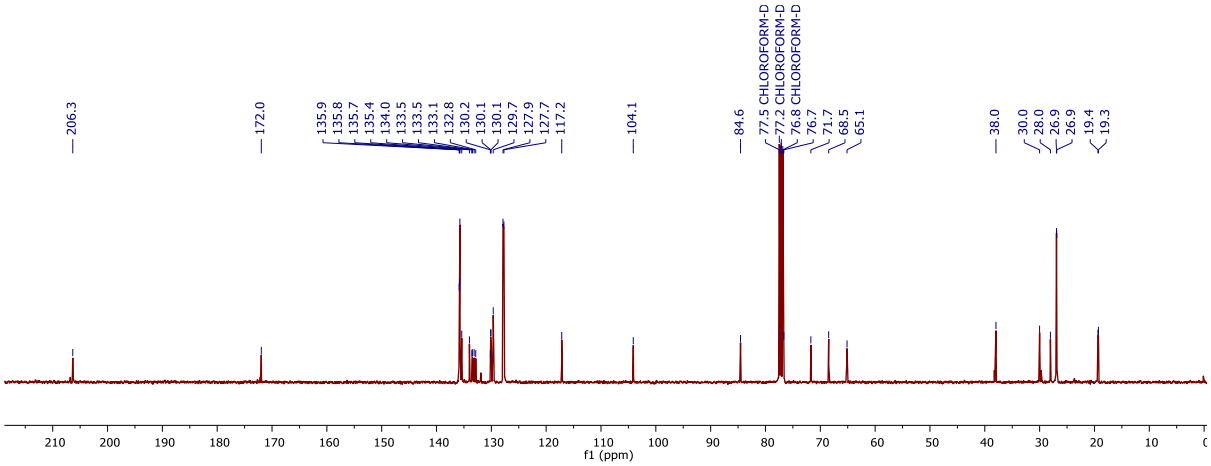
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of Compound **14a**



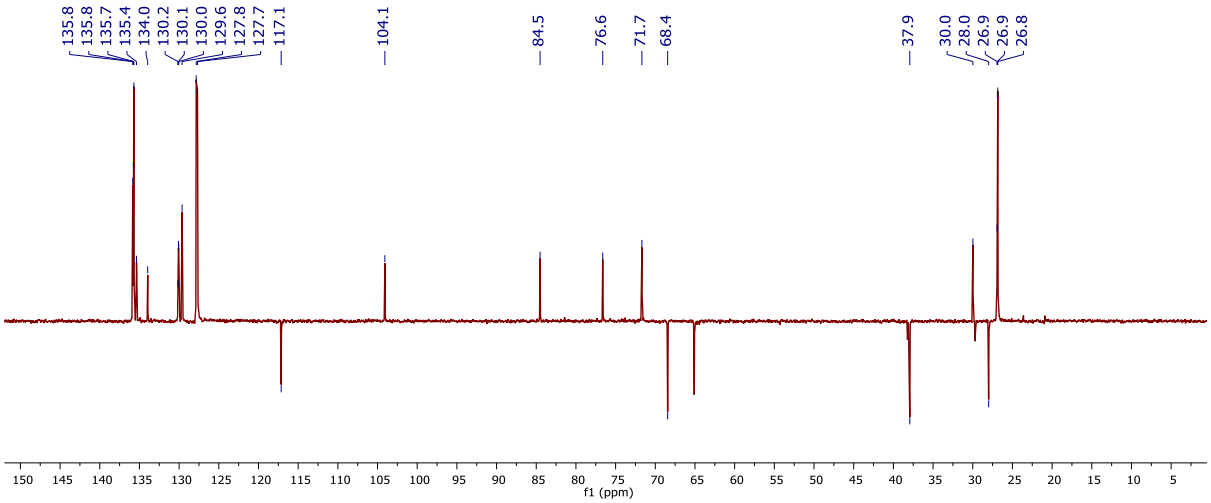
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of Compound **20**



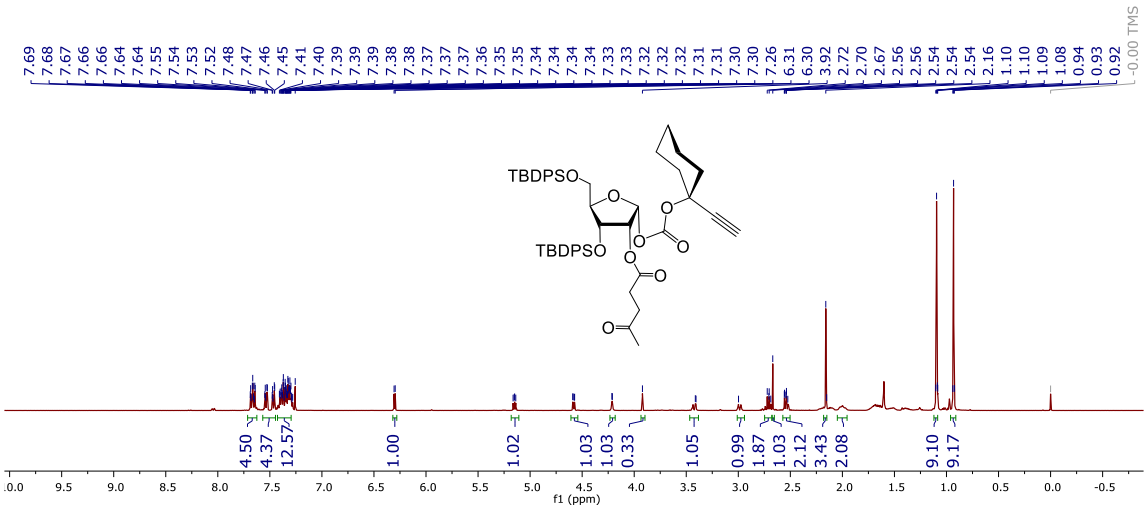
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of Compound **20**



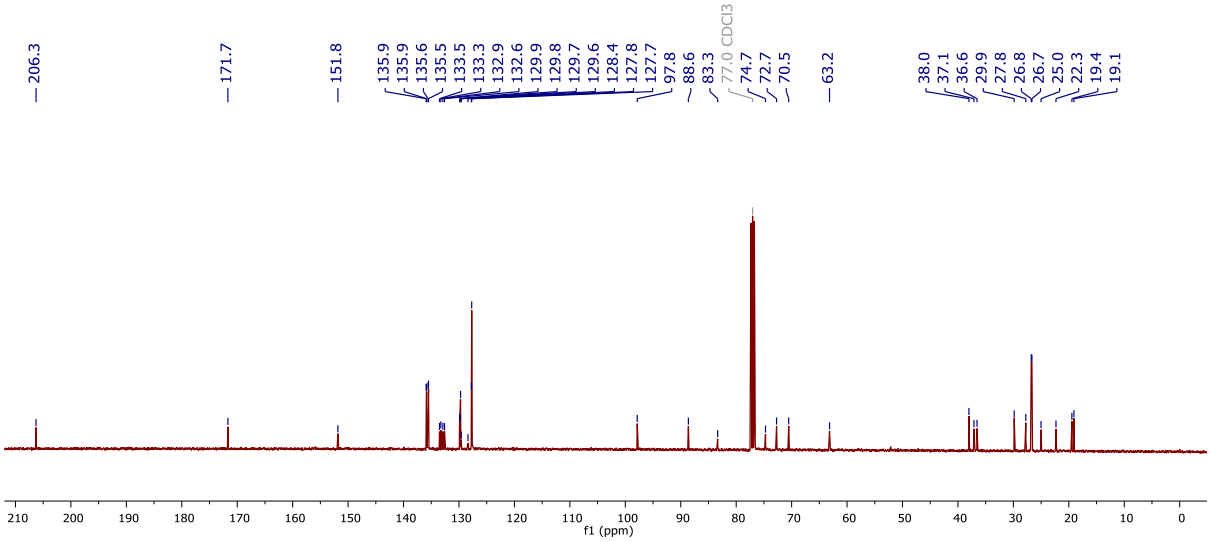
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of Compound **20**



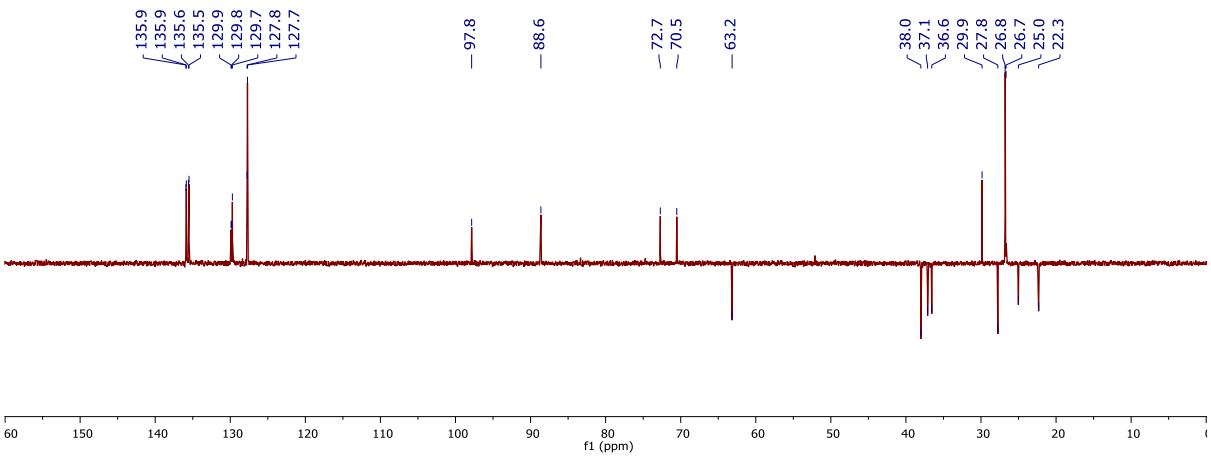
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of Compound **10α**



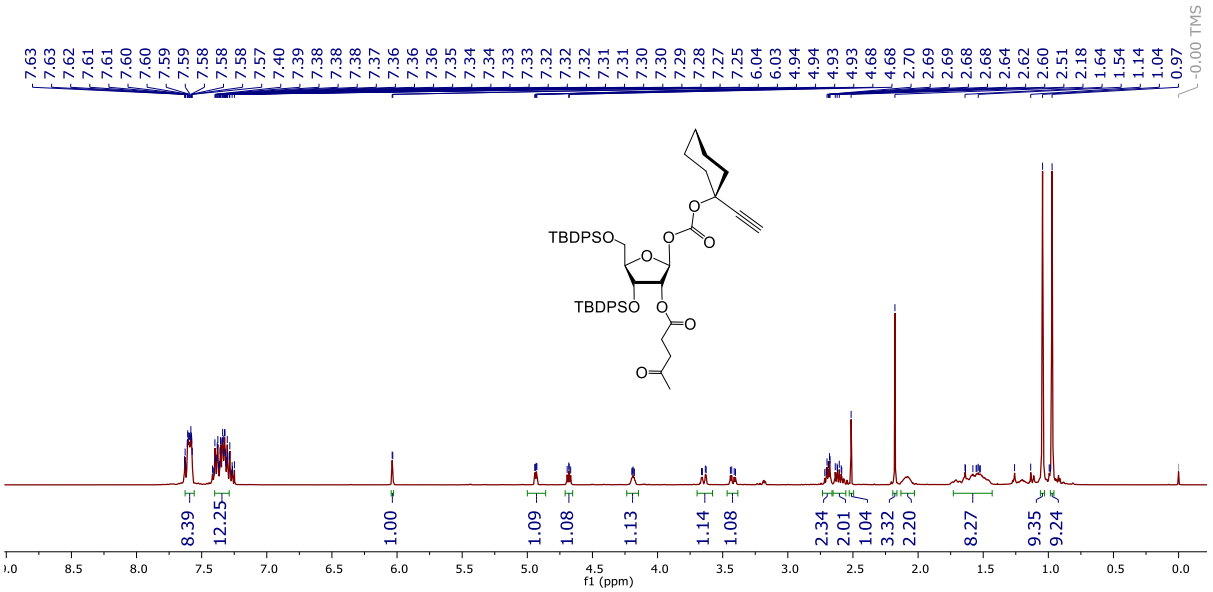
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of Compound **10α**



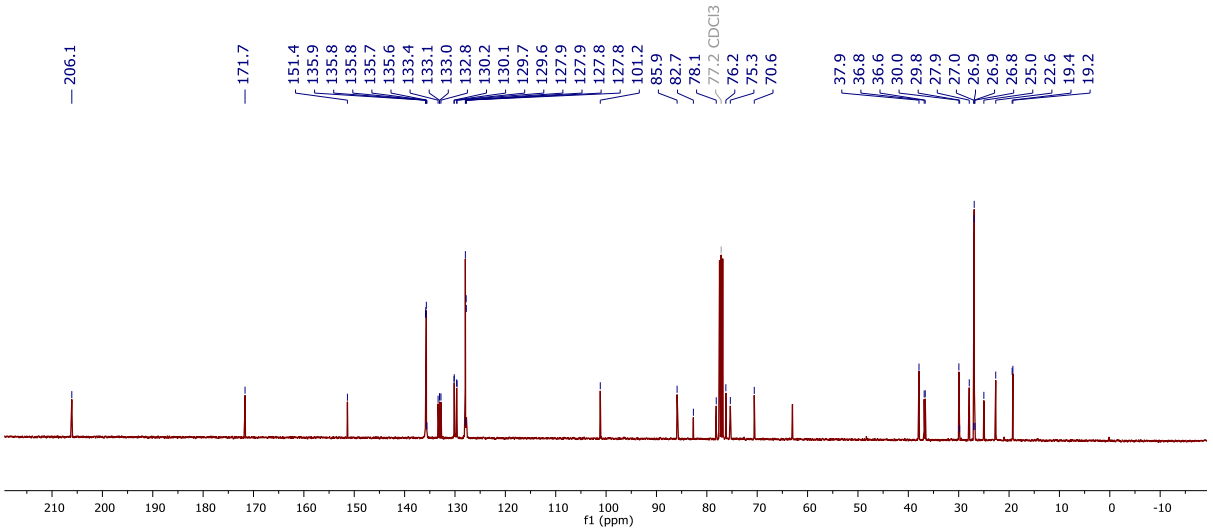
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of Compound **10α**



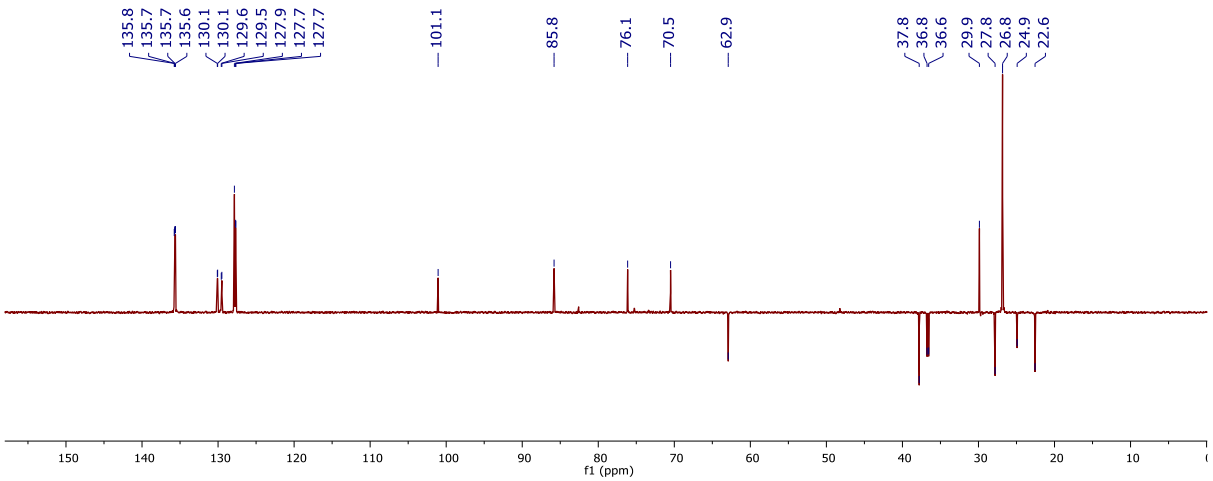
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of Compound **10β**



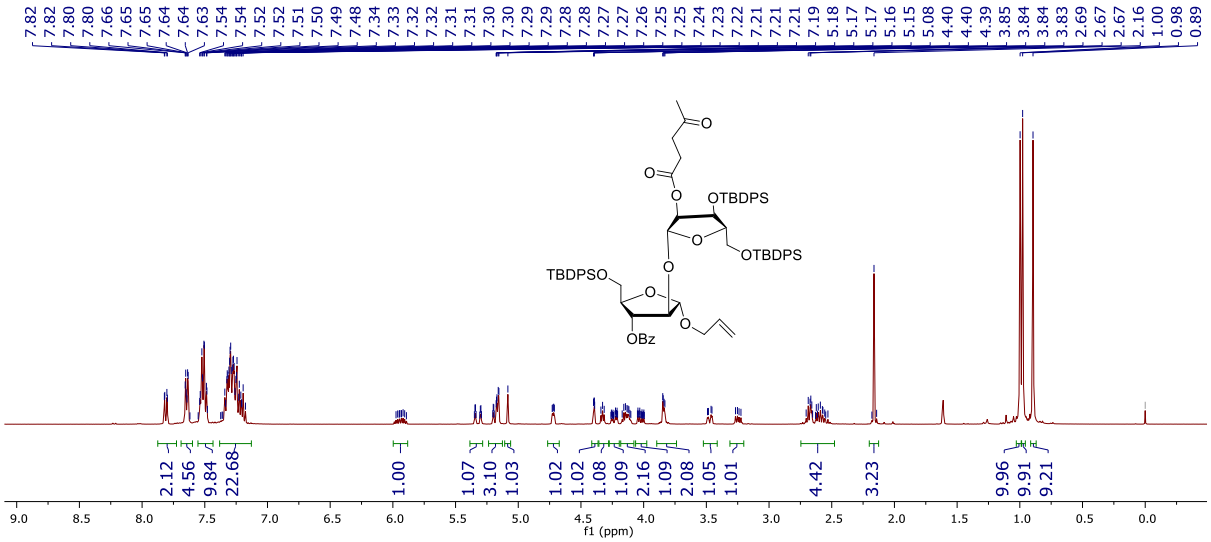
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of Compound **10β**



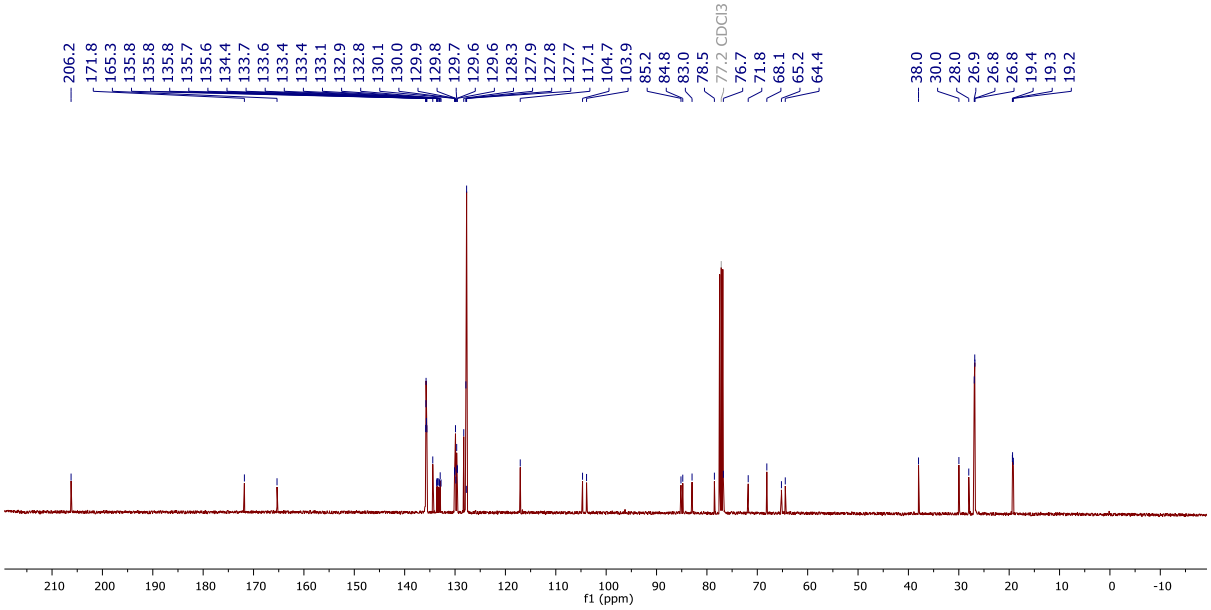
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of Compound **10β**



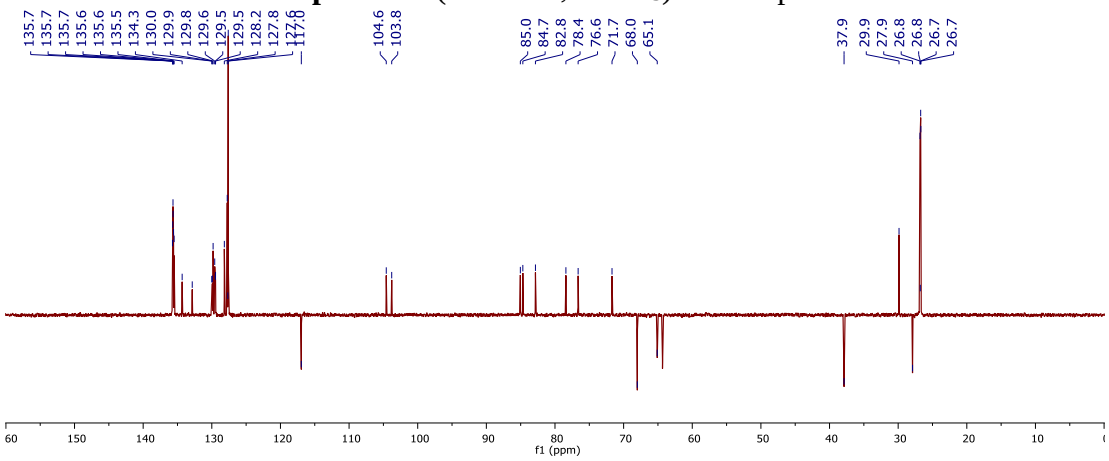
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of Compound **22**



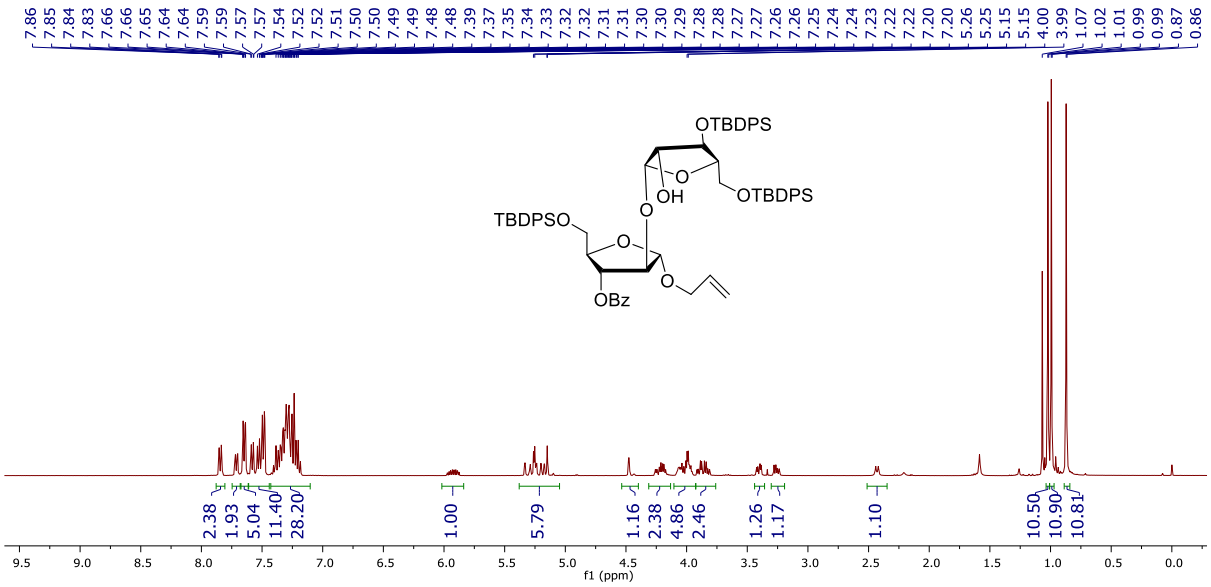
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of Compound **22**



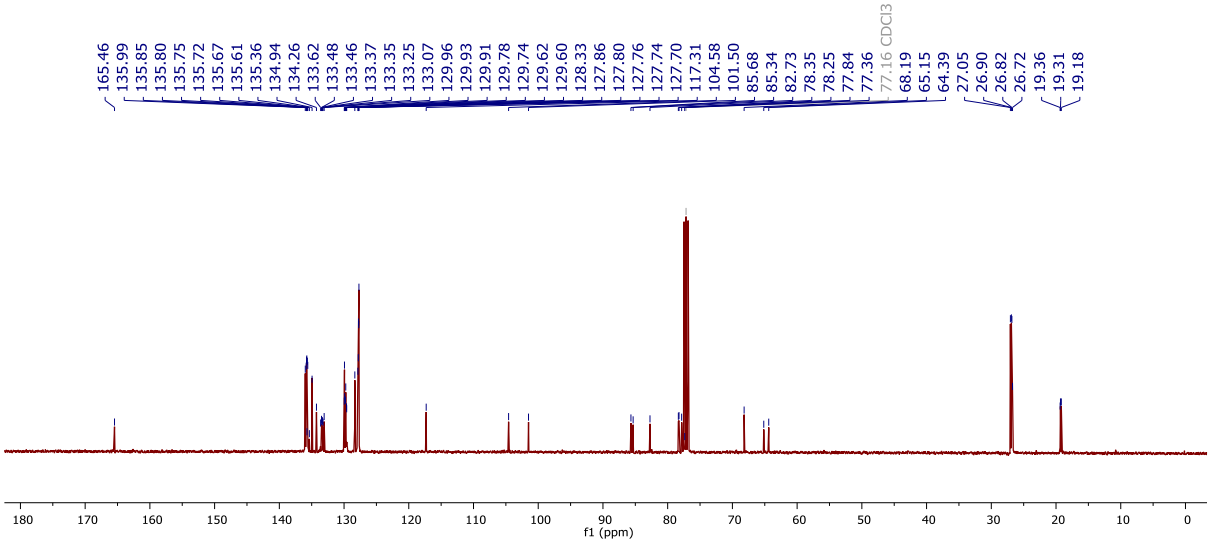
DEPT NMR Spectrum (101MHz, $CDCl_3$) of Compound **22**



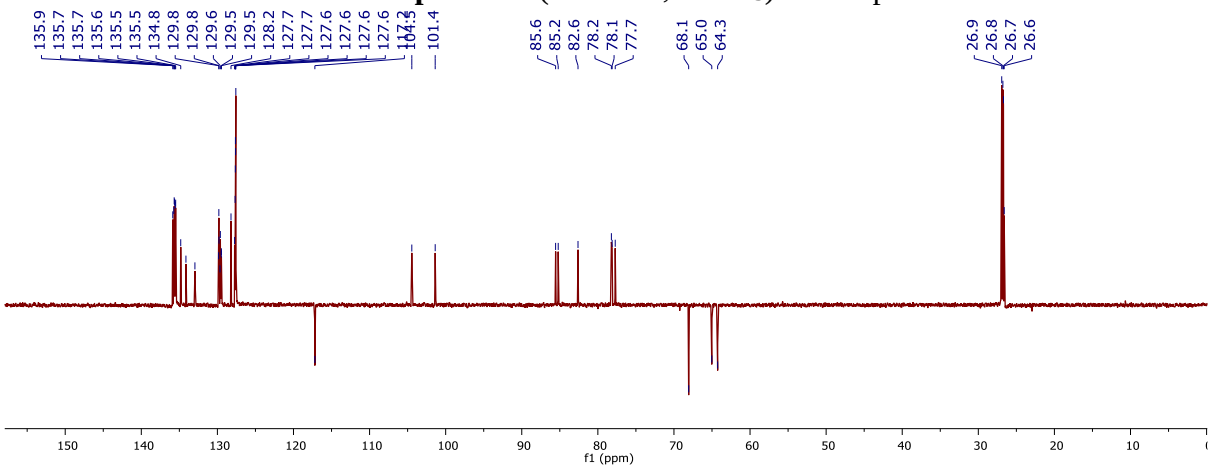
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **23**



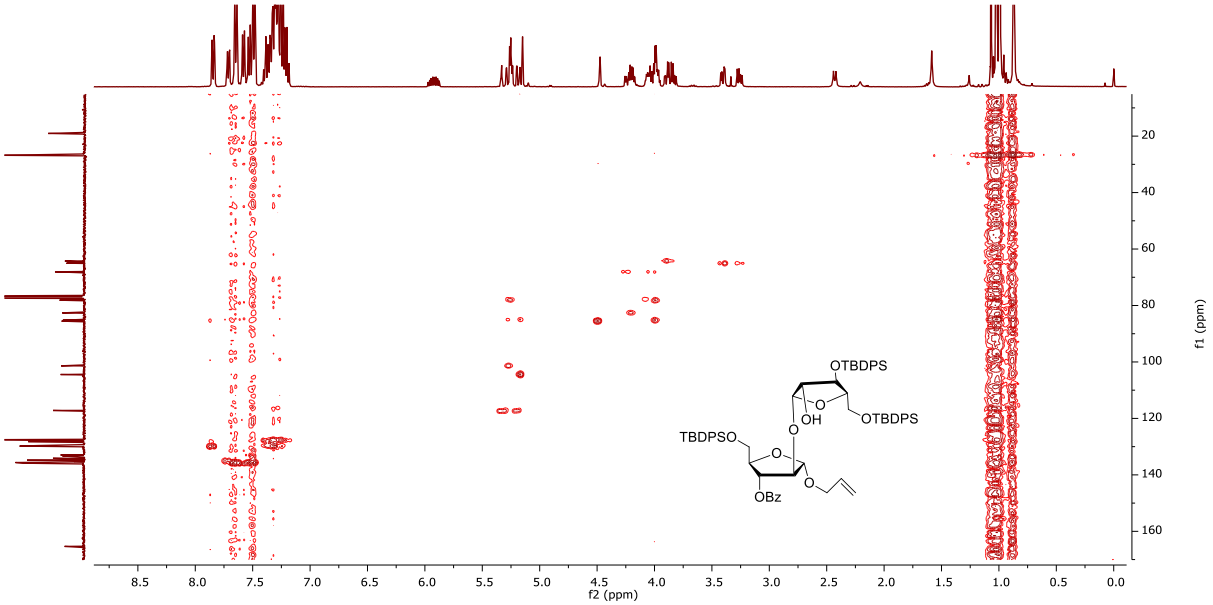
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **23**



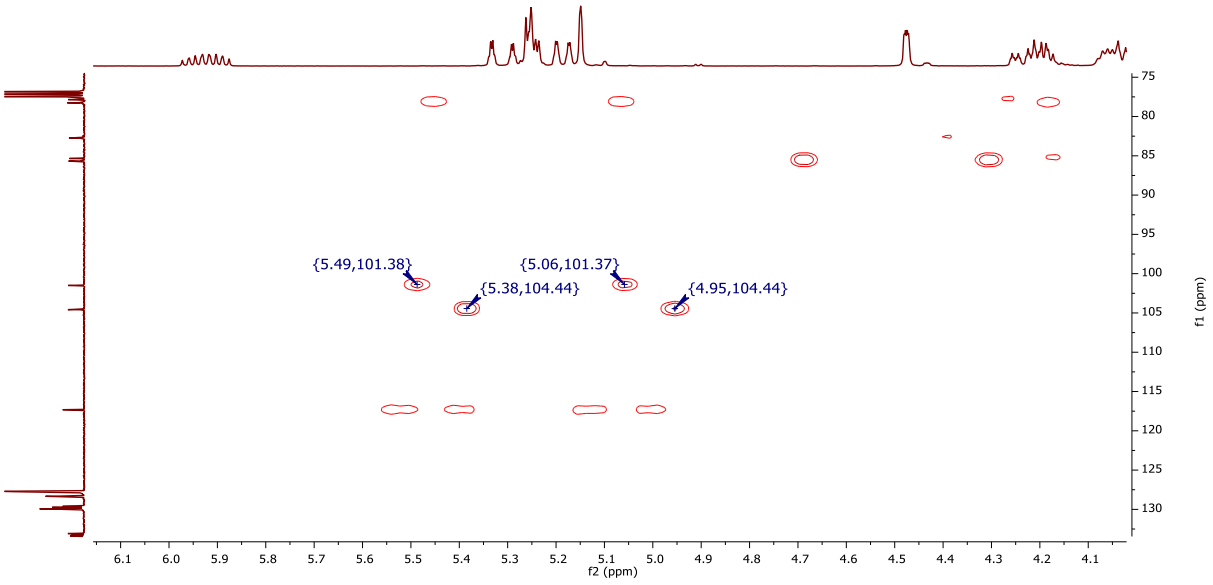
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **23**



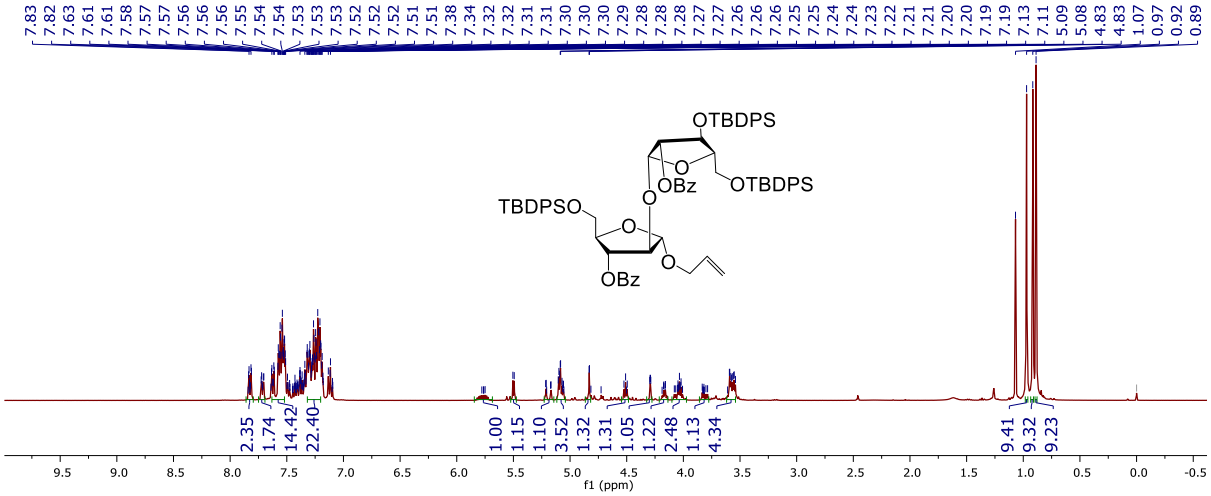
HSQC Spectrum (400/101 MHz, $CDCl_3$) of Compound **23**



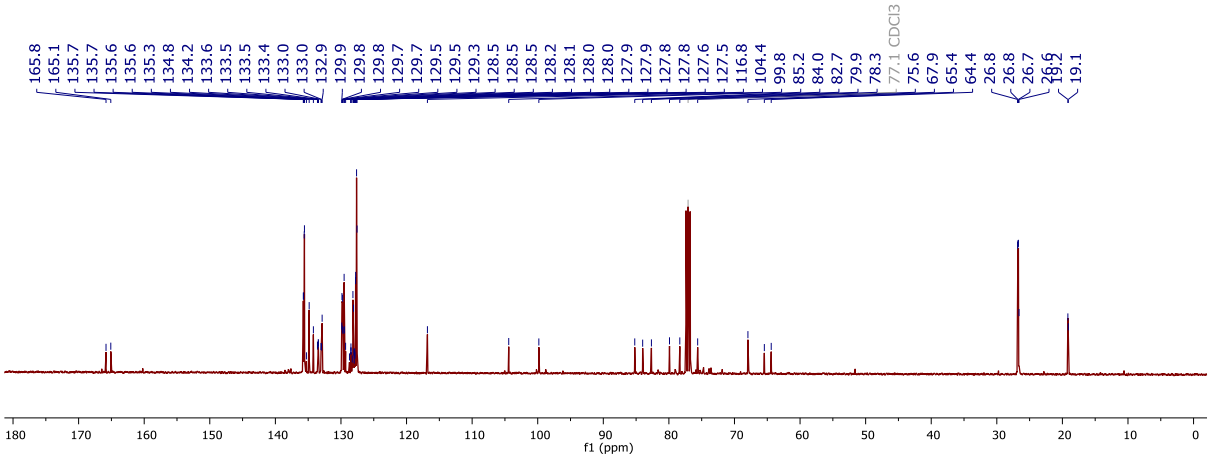
gHSQC Spectrum (400/100 MHz, $CDCl_3$) of Compound **23**



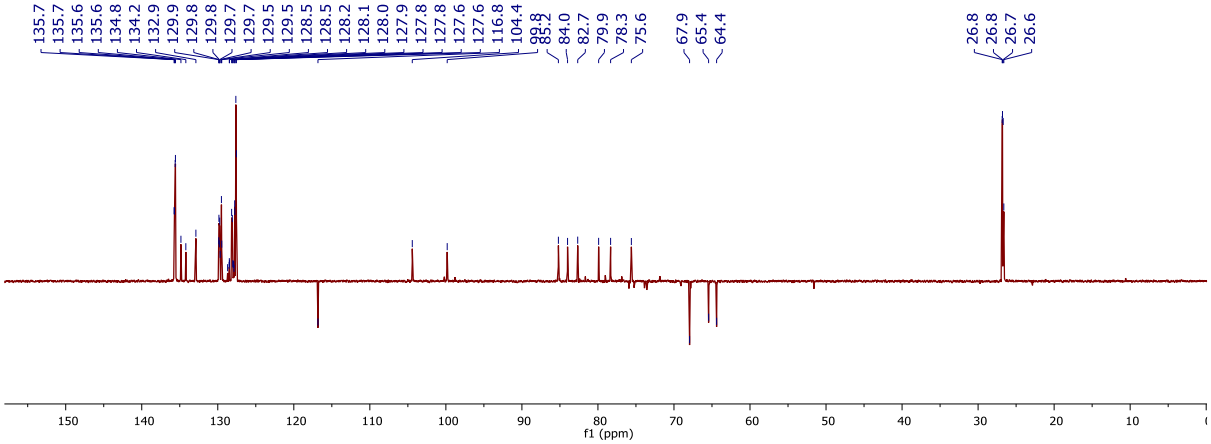
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **9**



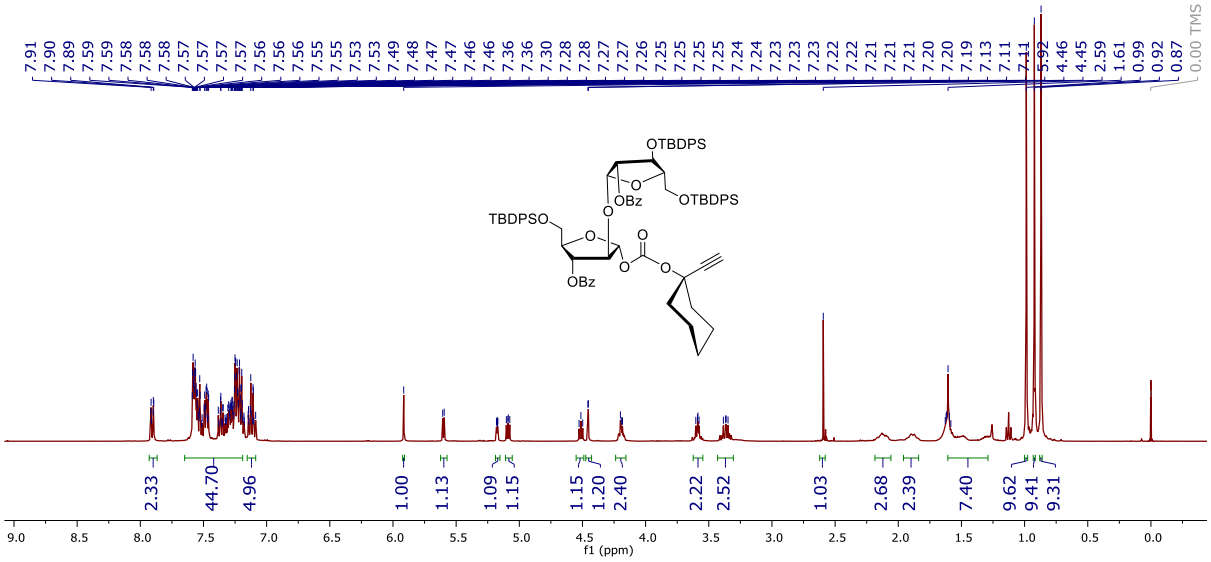
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **31**



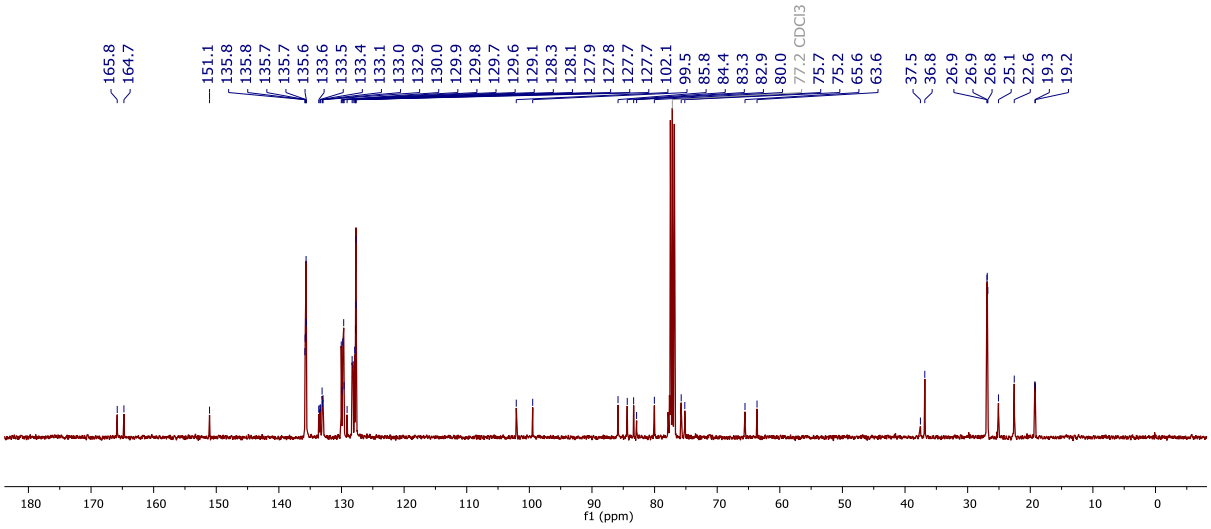
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **3**



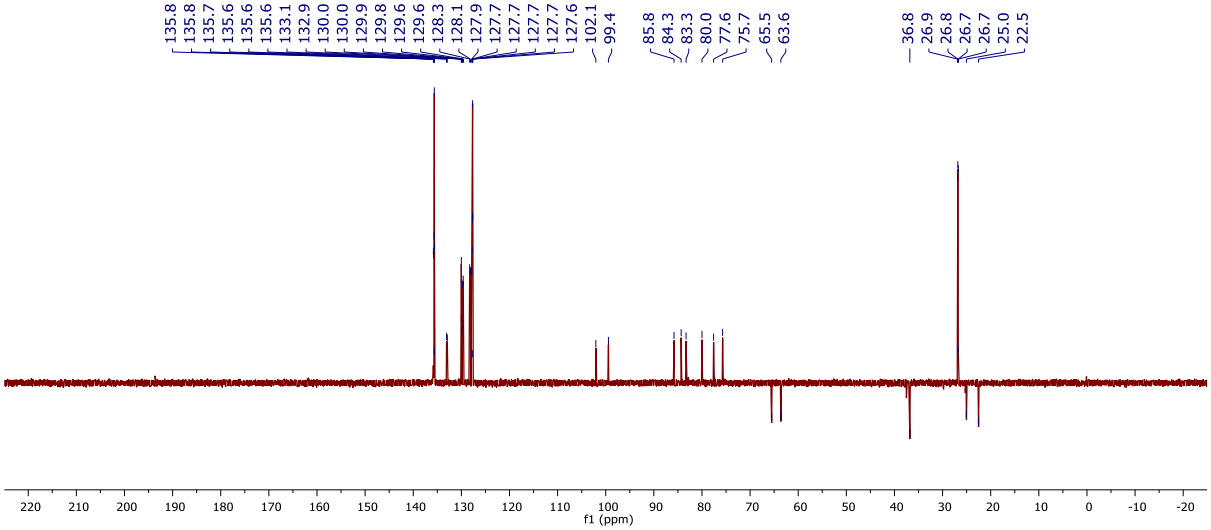
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **4**



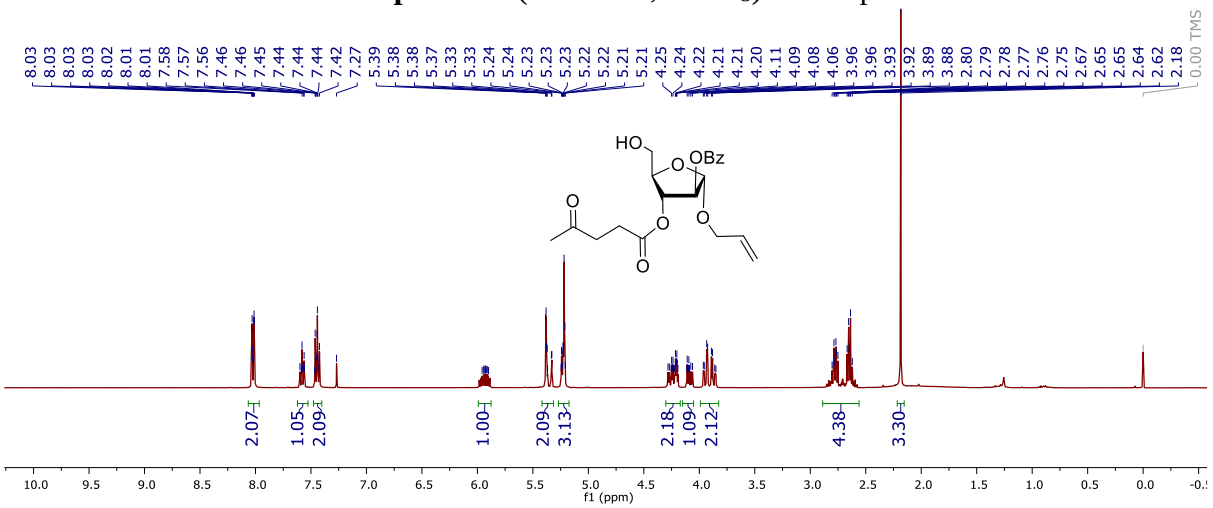
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **4**



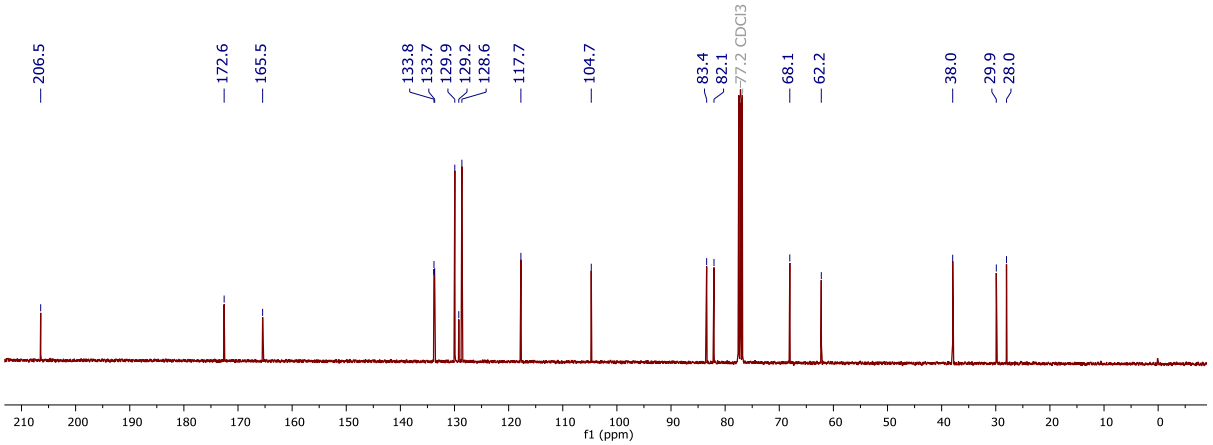
DEPT Spectrum (101 MHz, $CDCl_3$) of compound **4**



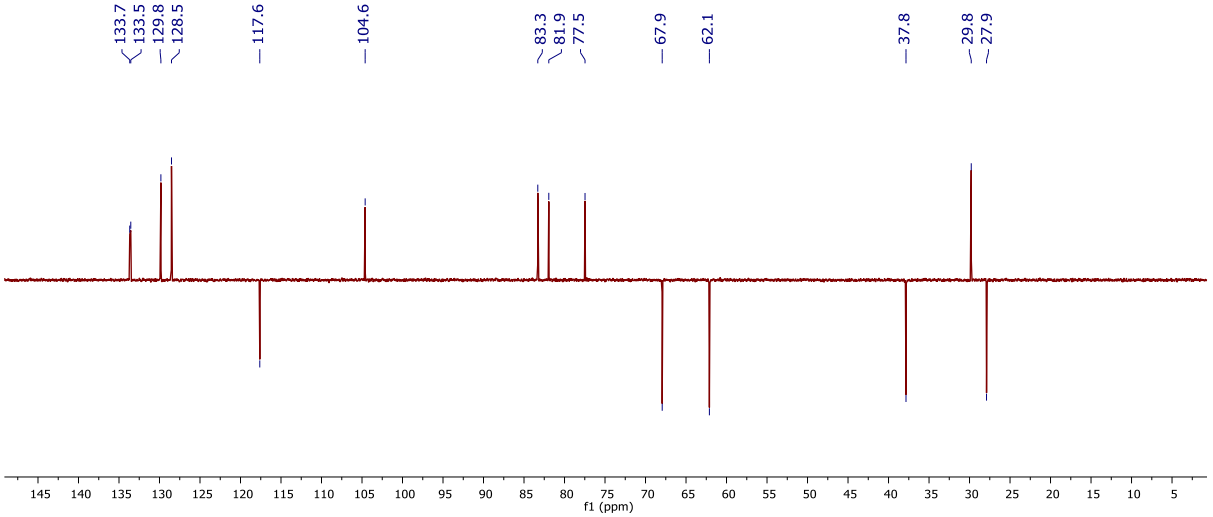
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **5**



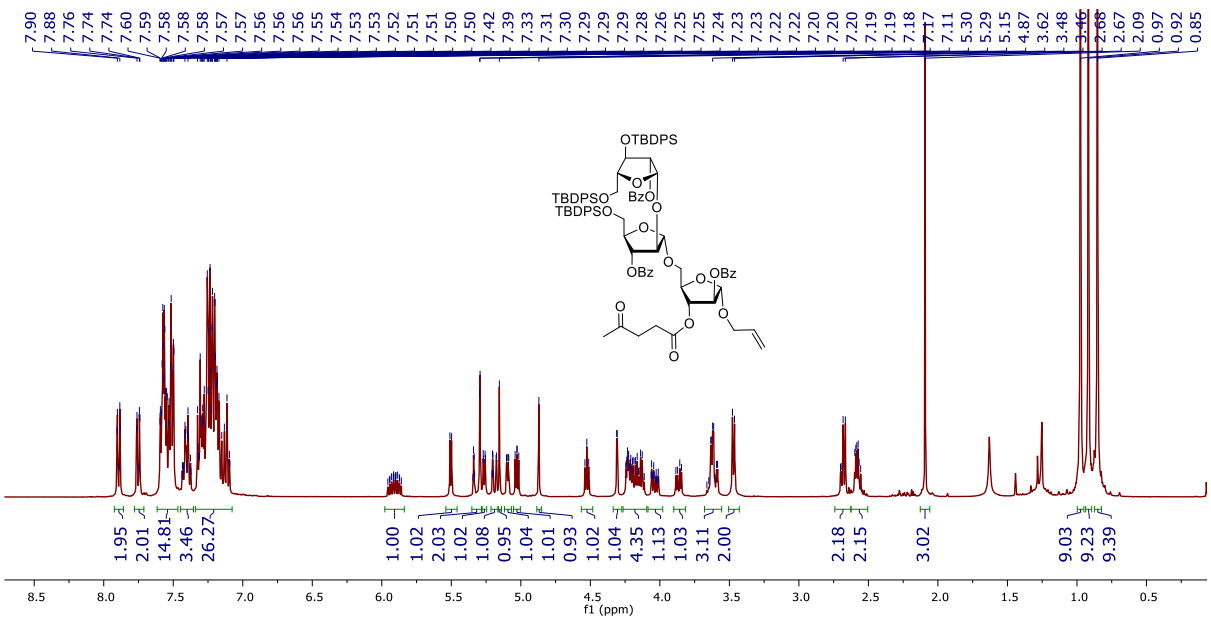
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **5**



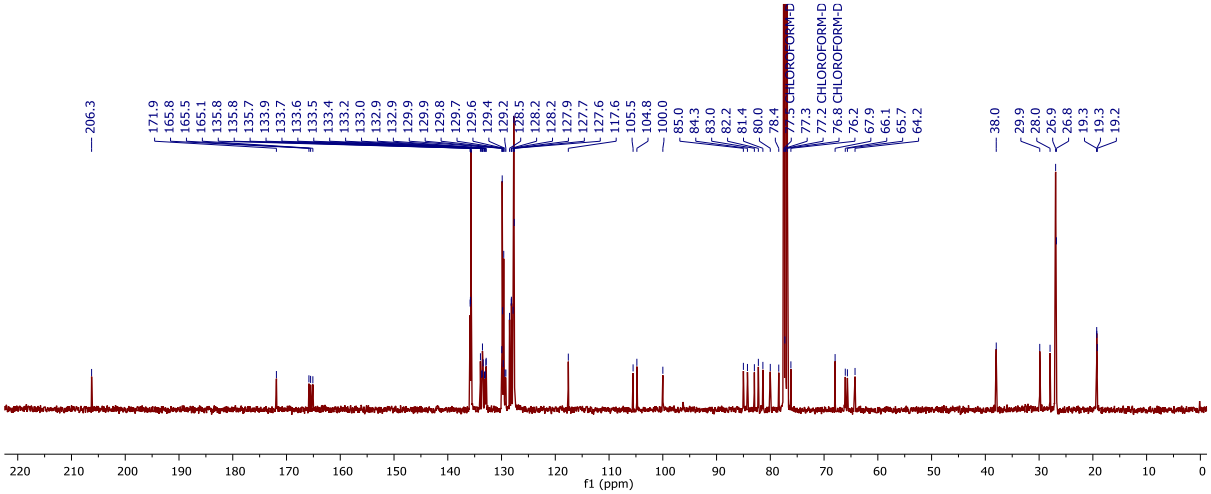
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **5**



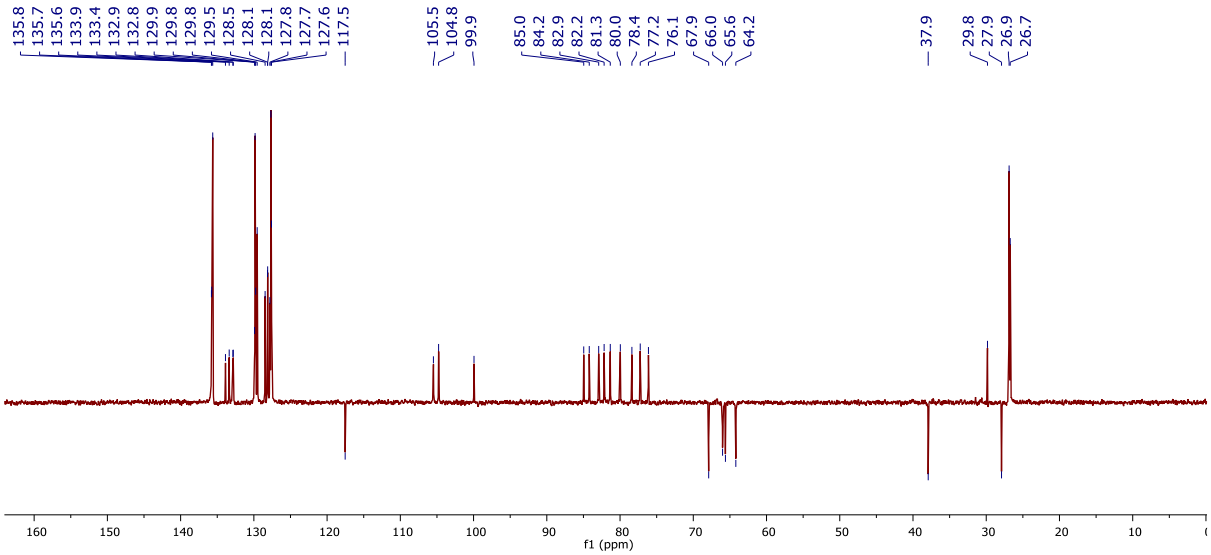
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **27b**



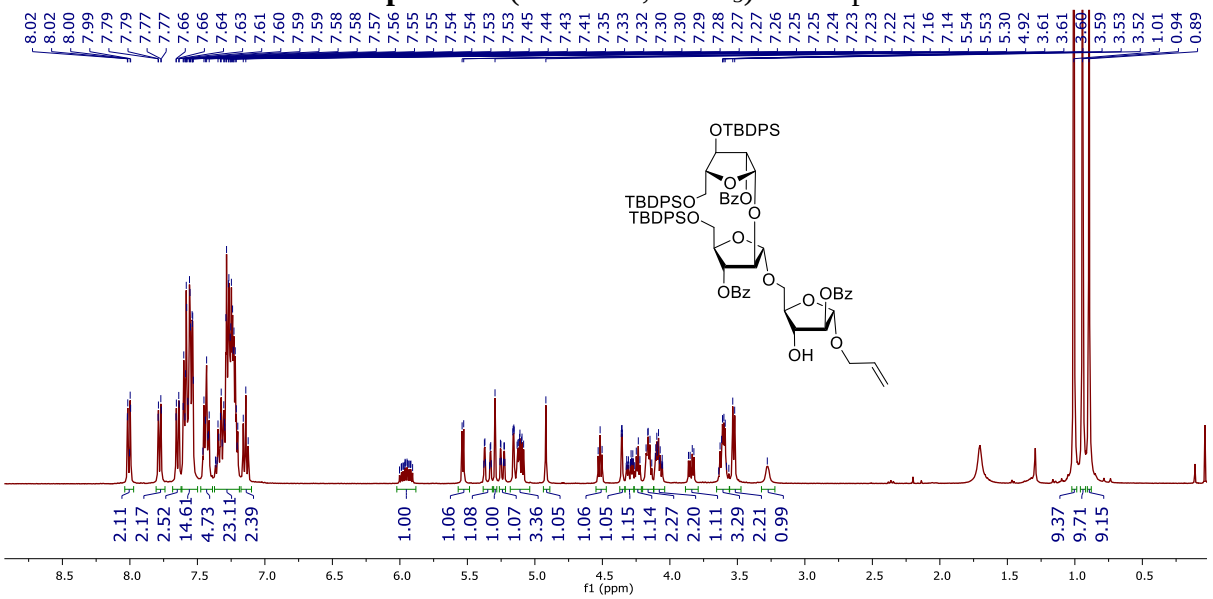
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **27b**



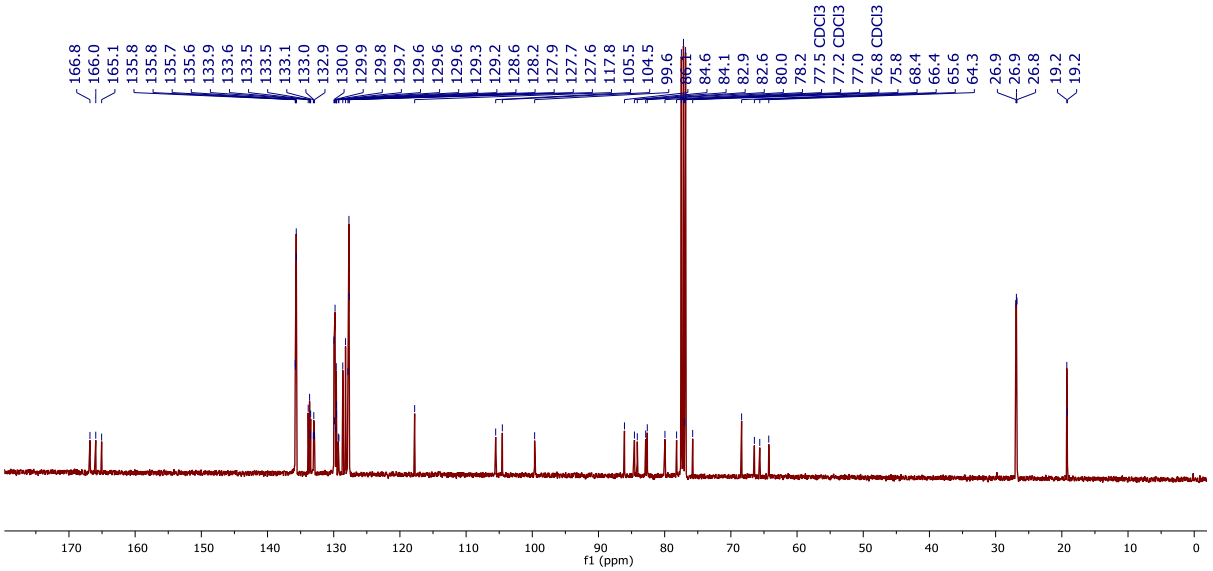
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **27b**



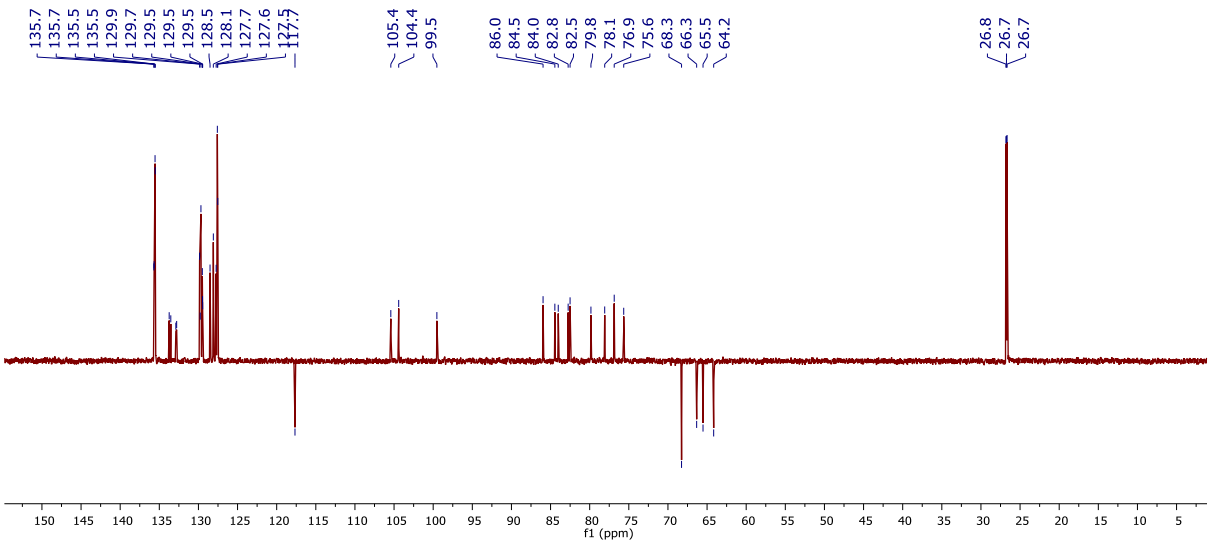
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **28**



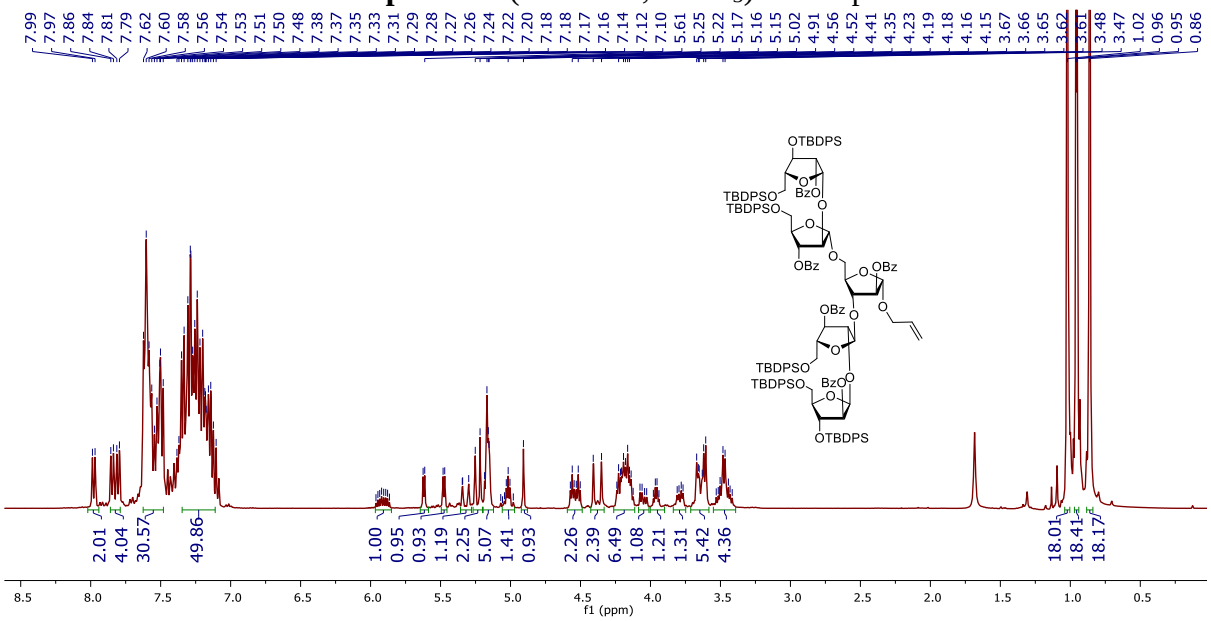
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **28**



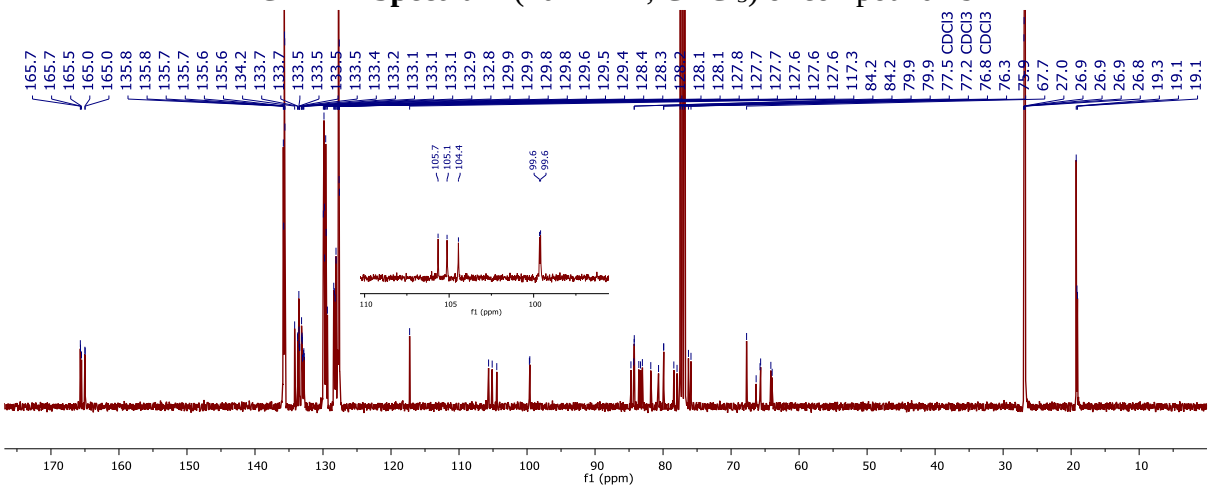
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **28**



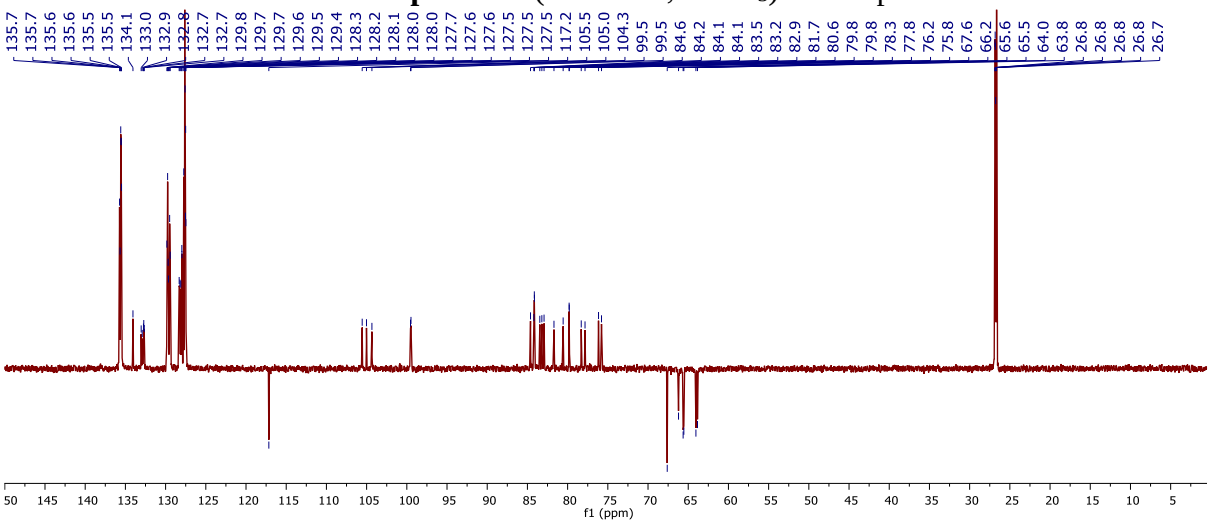
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **29**



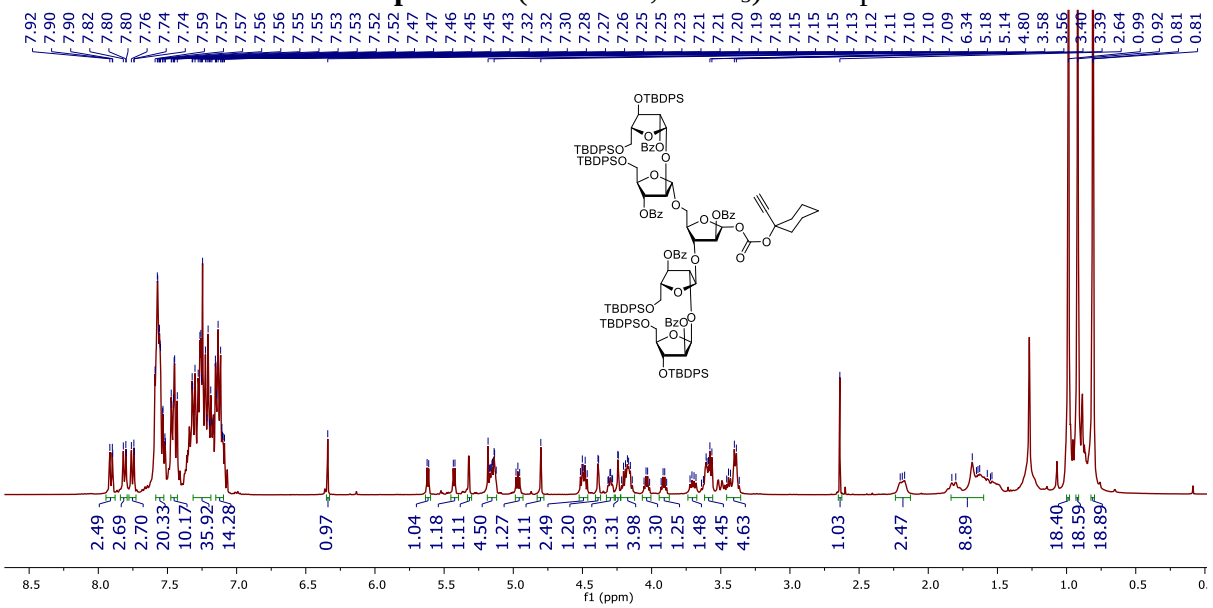
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **29**



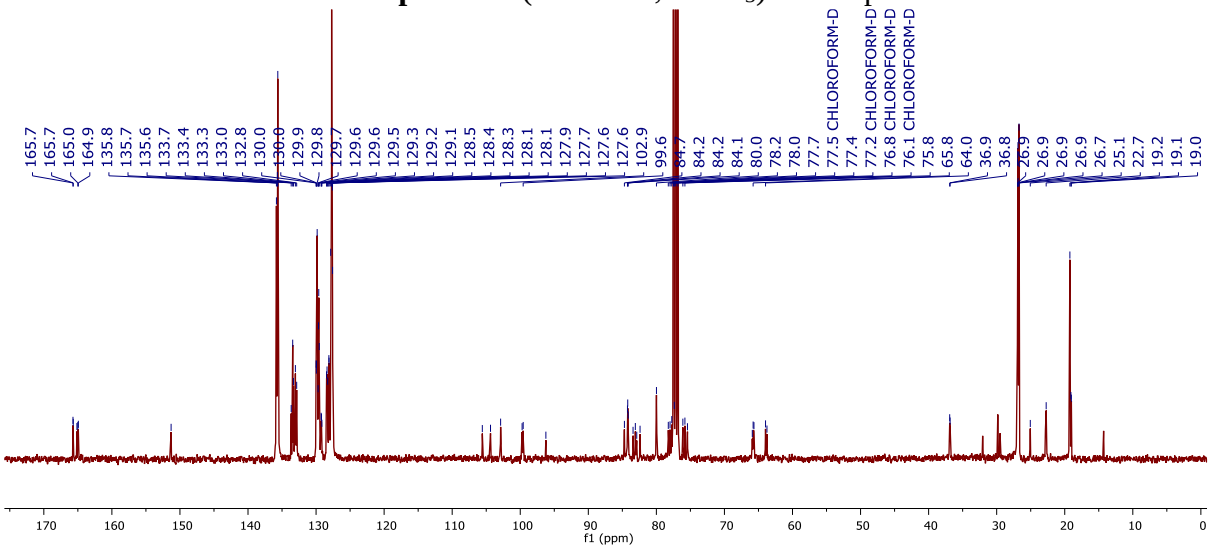
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **29**



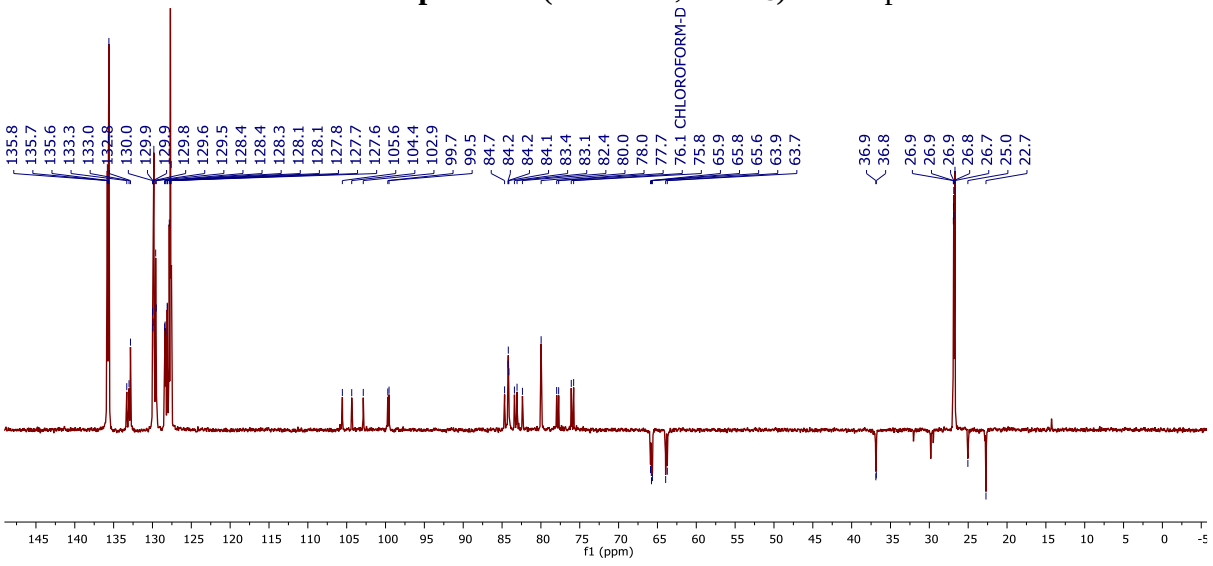
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **2**



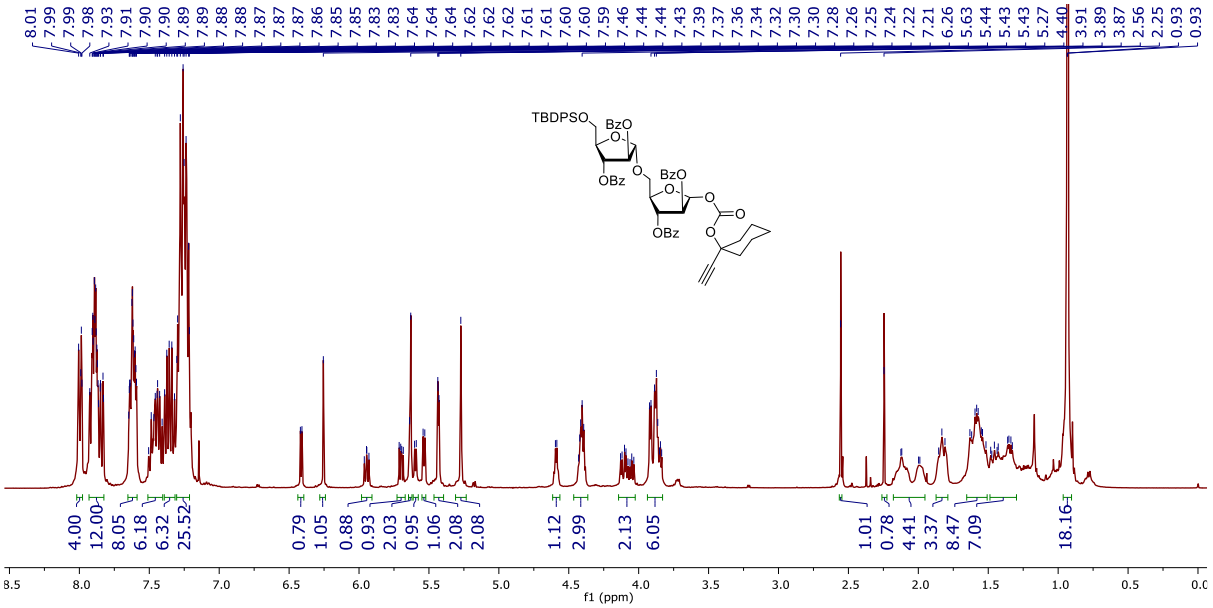
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **2**



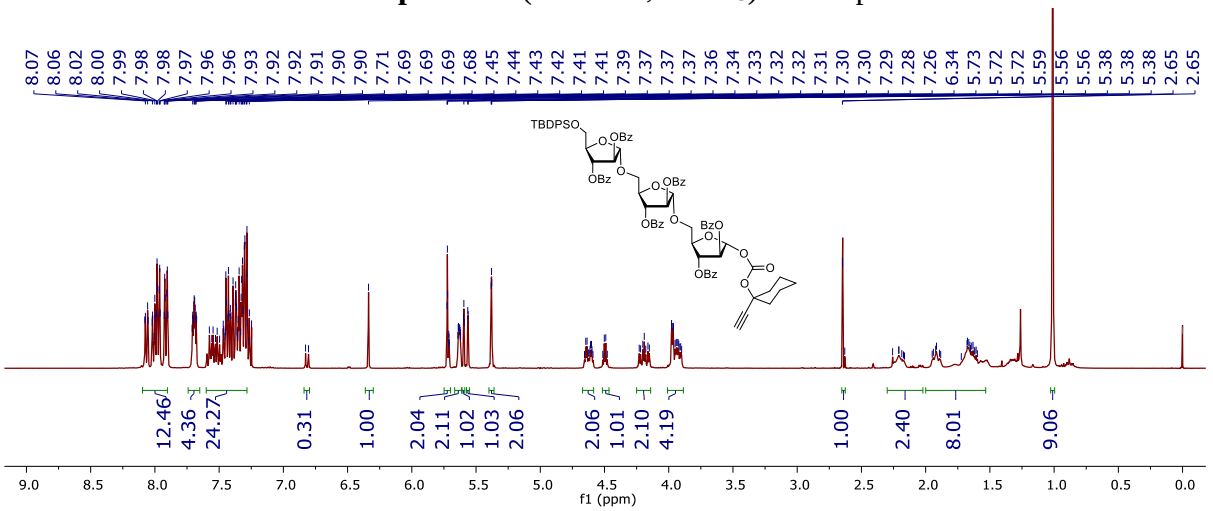
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **2**



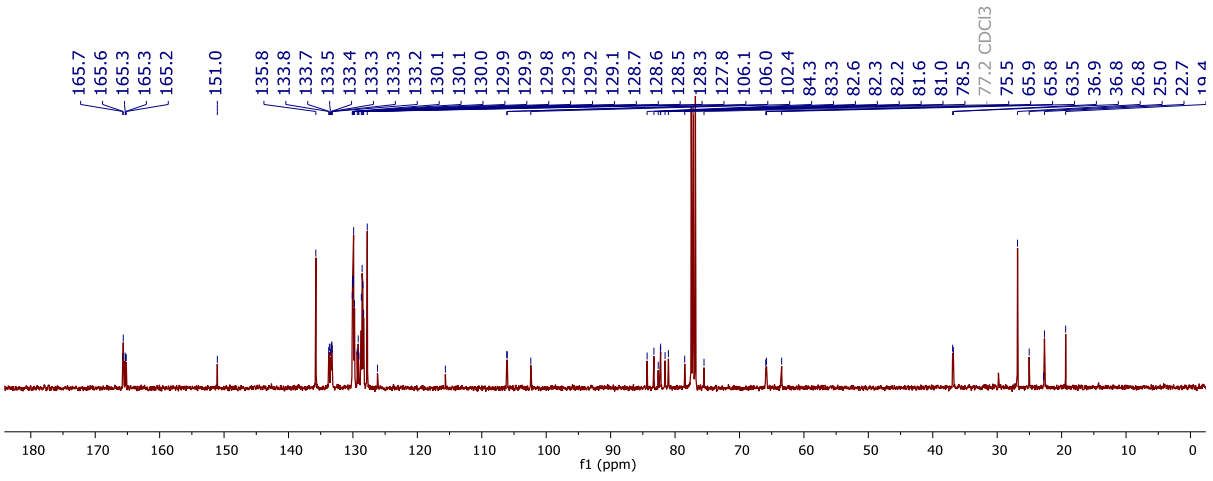
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **41**



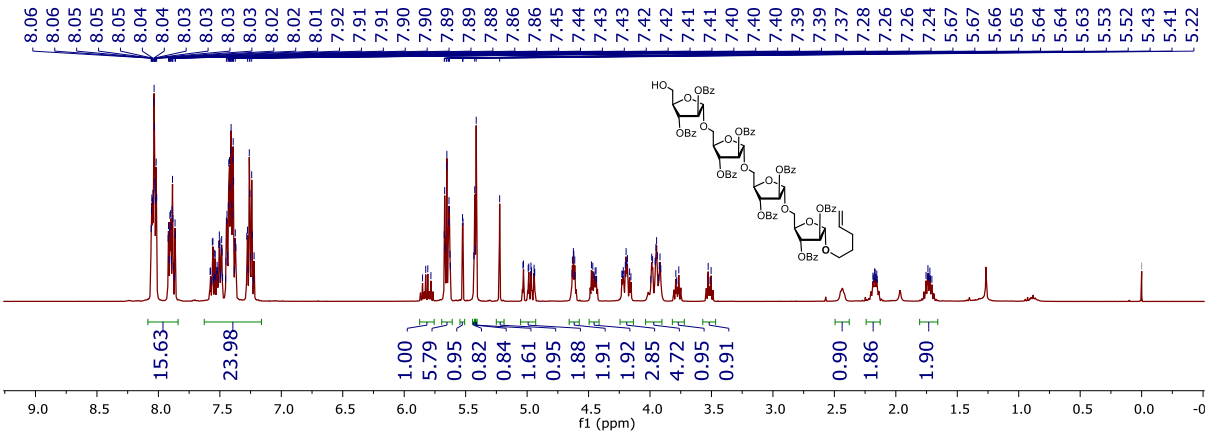
^{1}H NMR Spectrum (101MHz, $CDCl_3$) of compound **6**



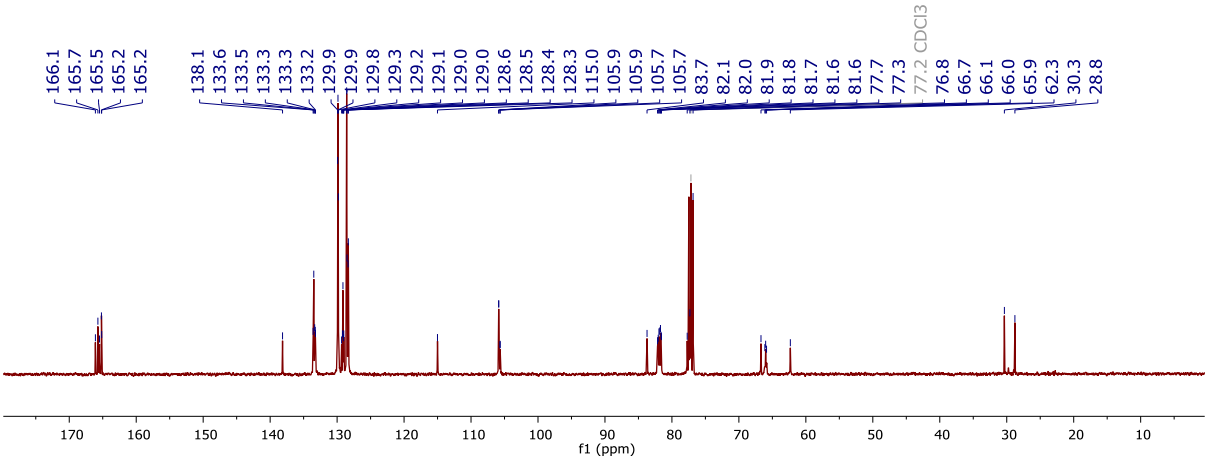
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **6**



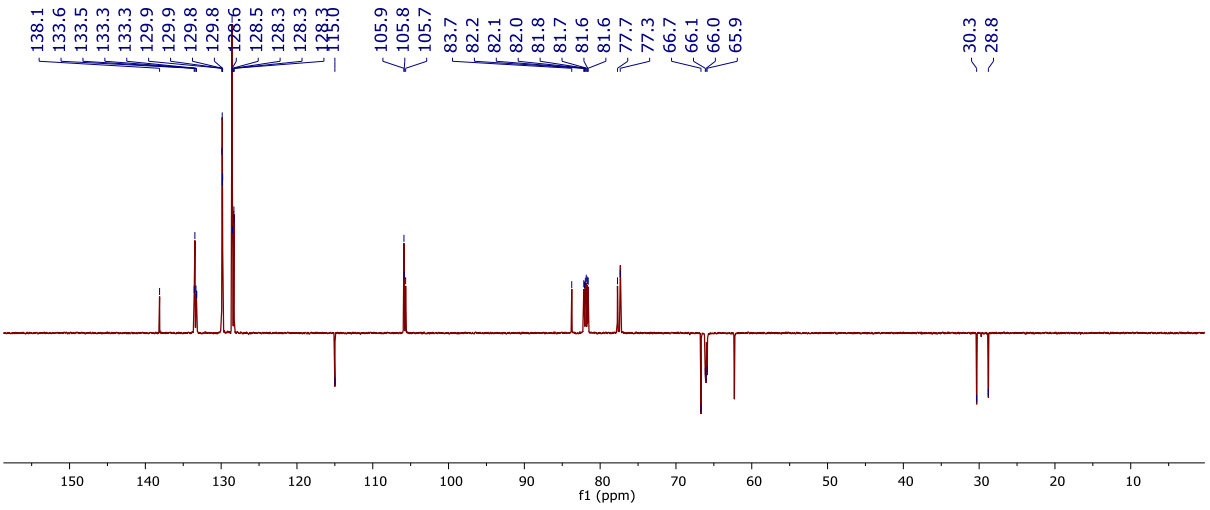
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **7**



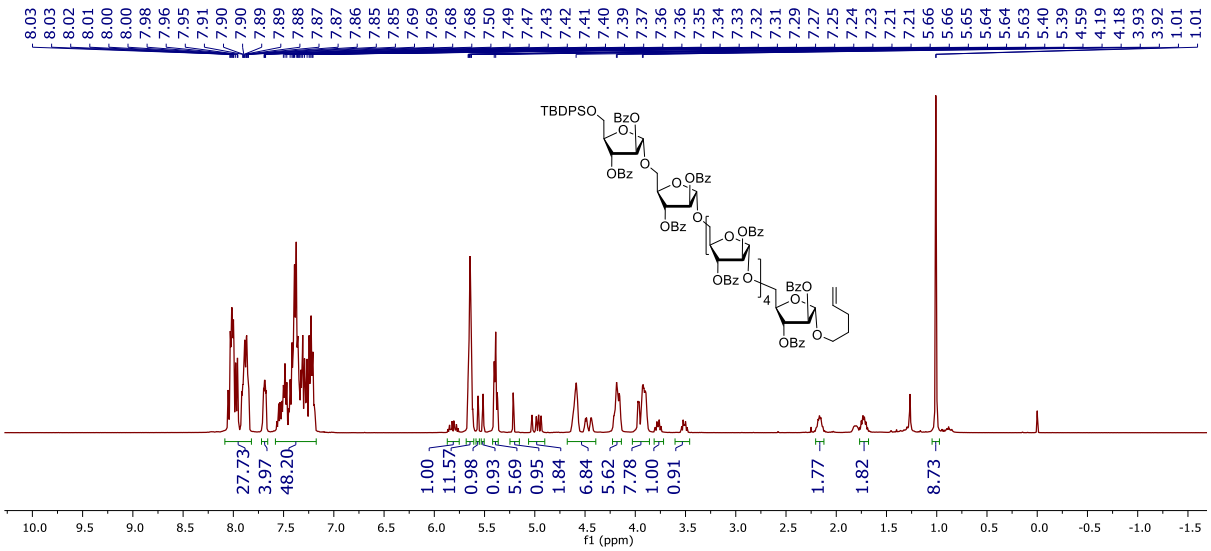
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **7**



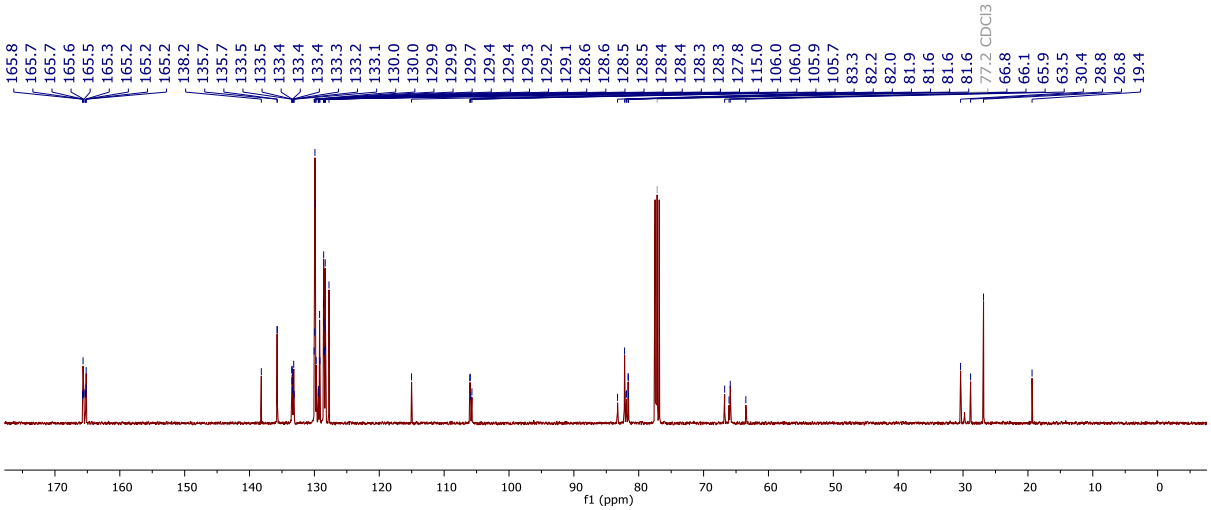
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **7**



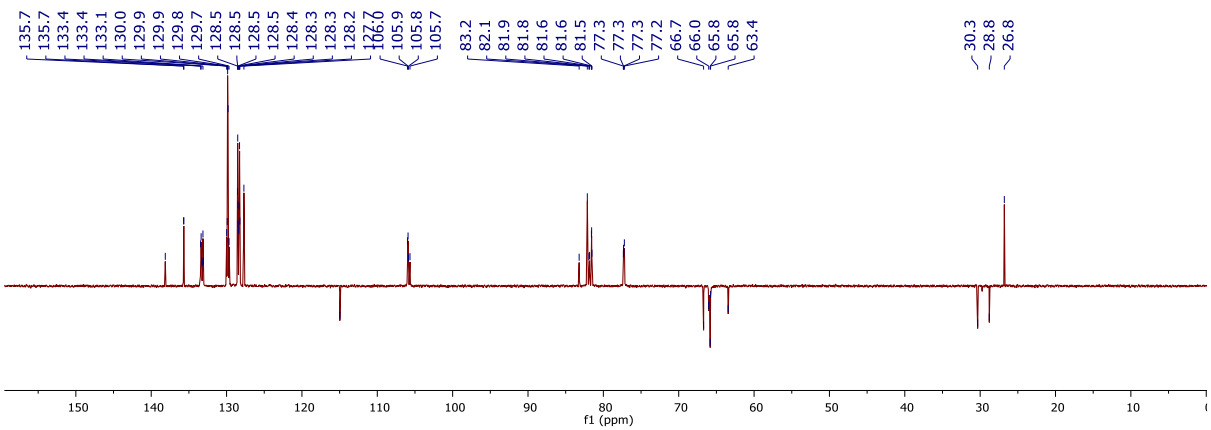
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **32**



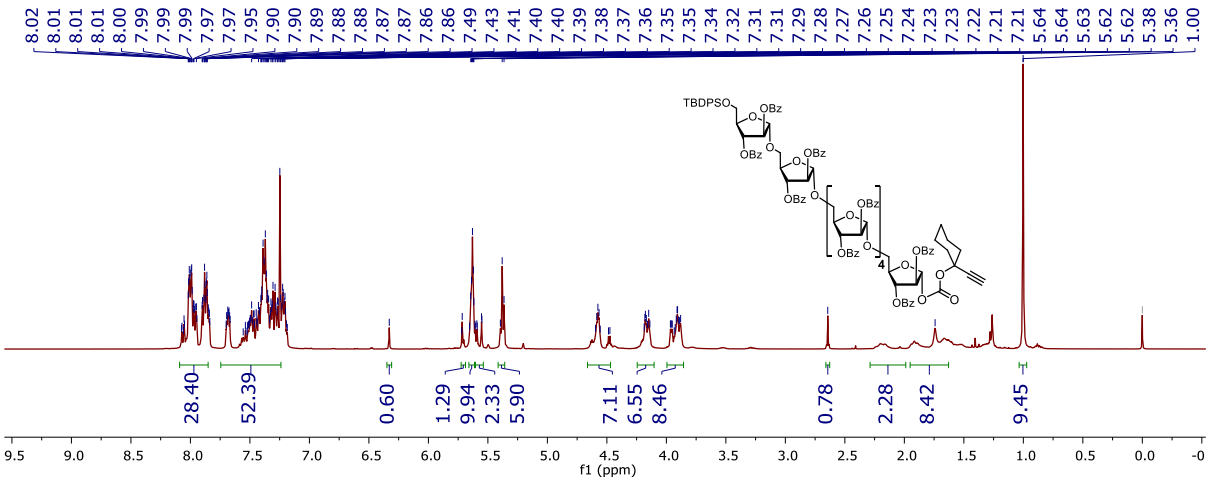
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **32**



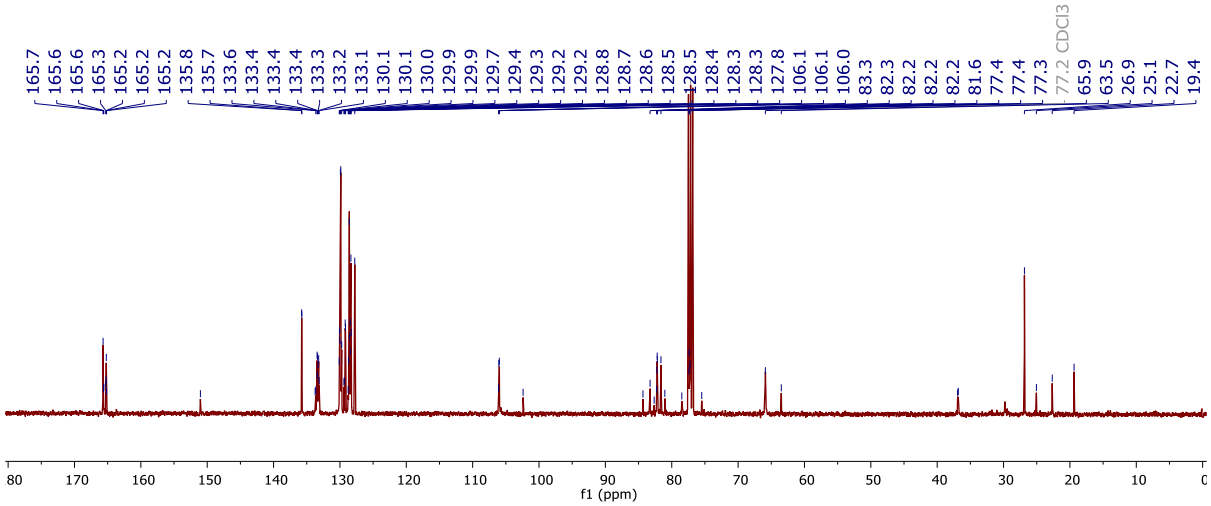
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **32**



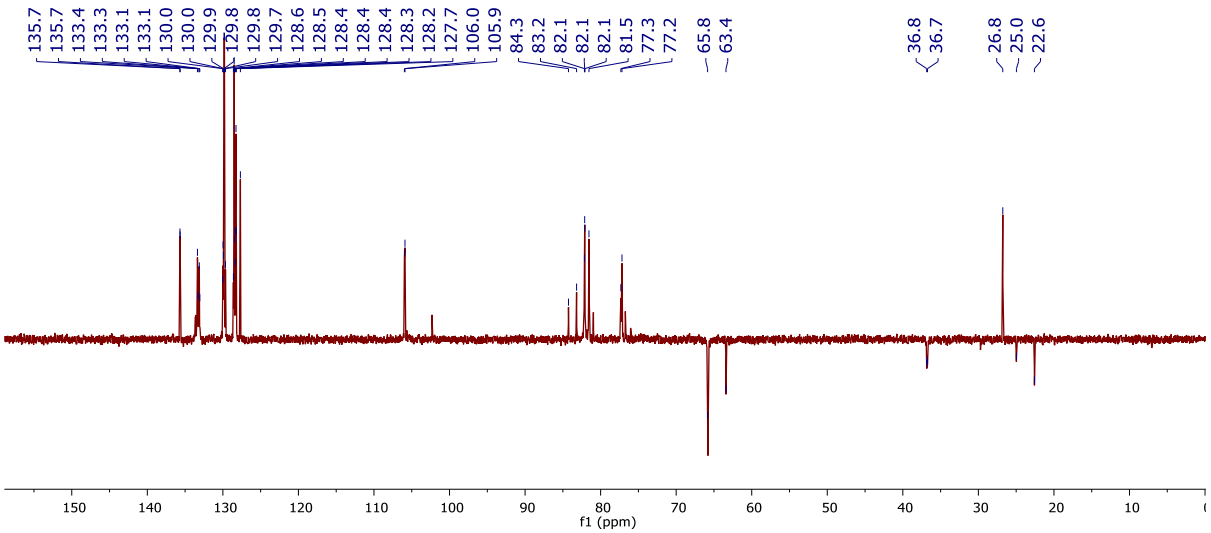
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **42**



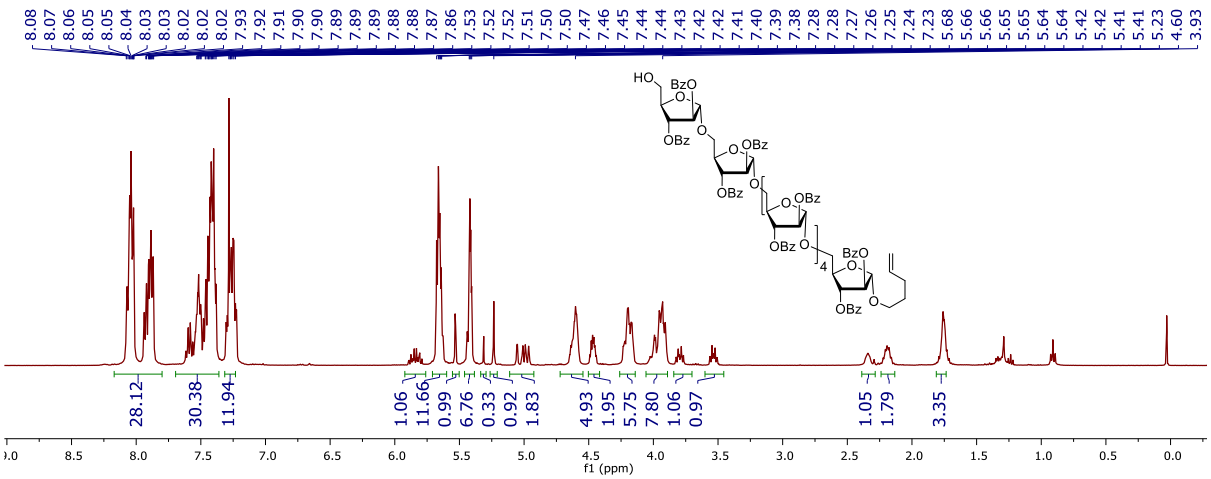
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **42**



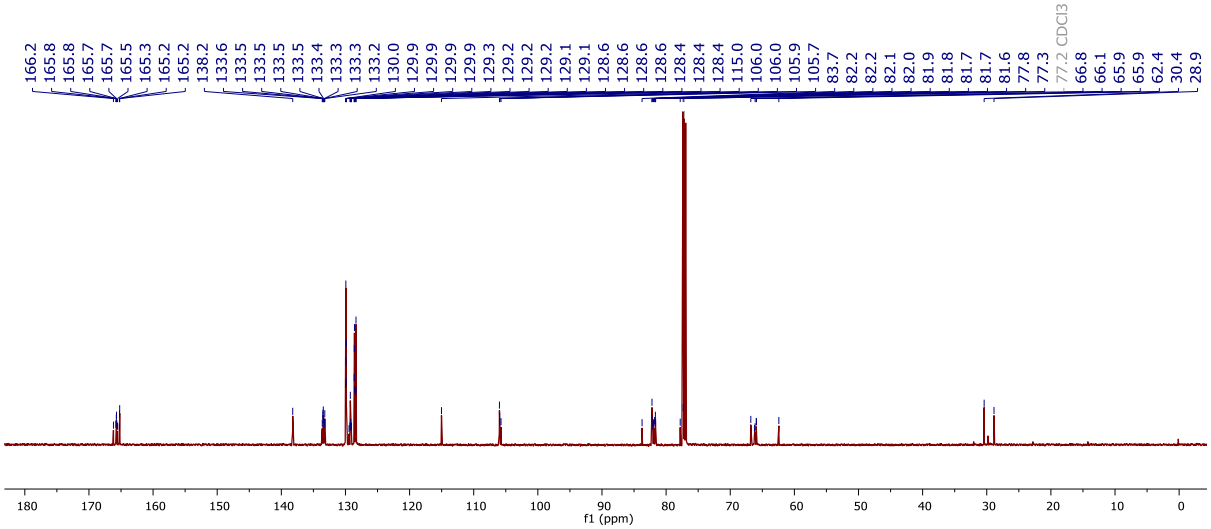
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **42**



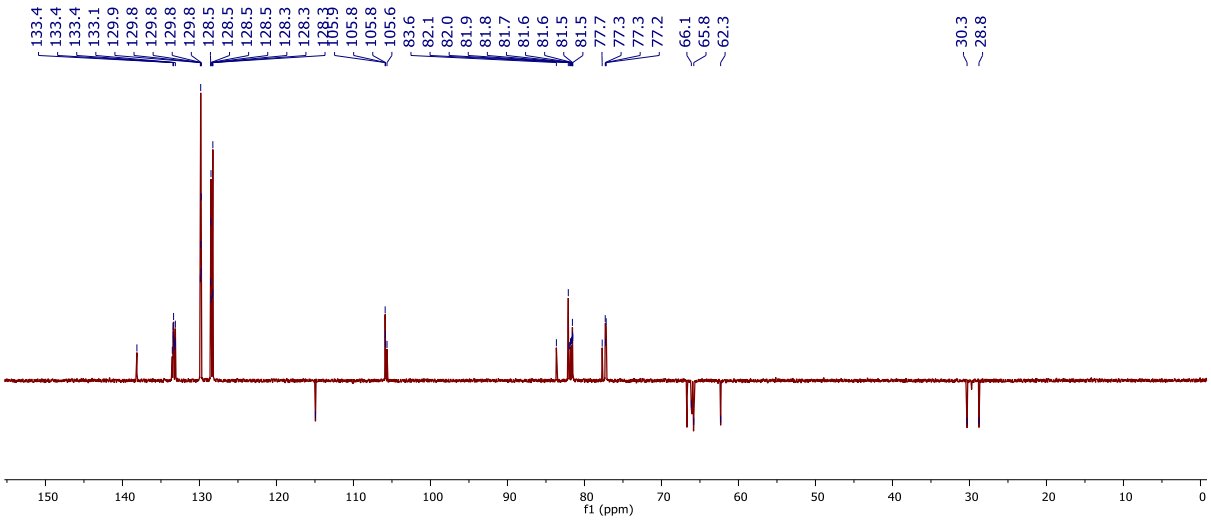
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **43**



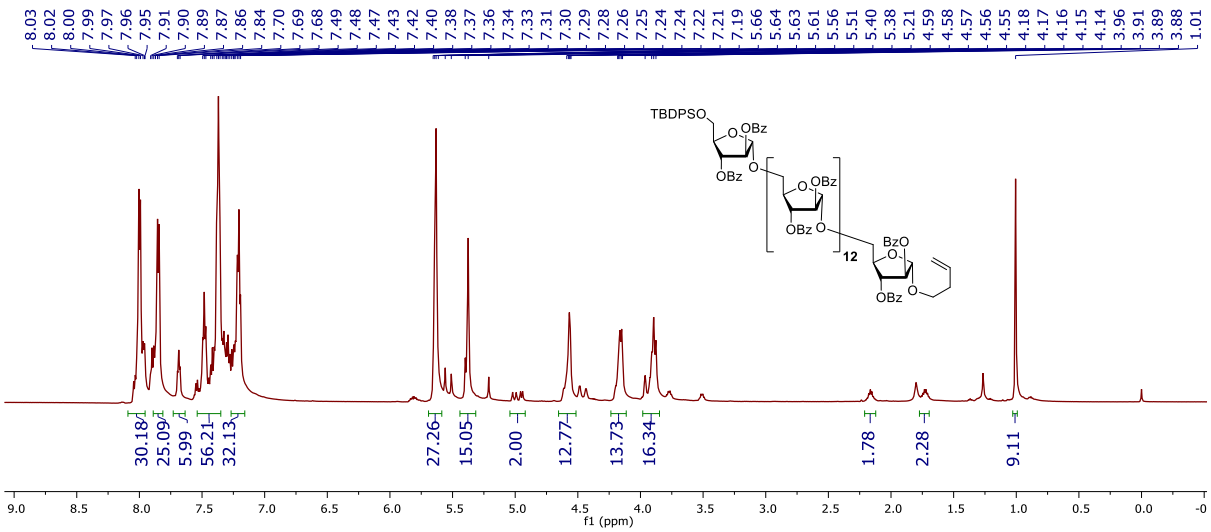
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **43**



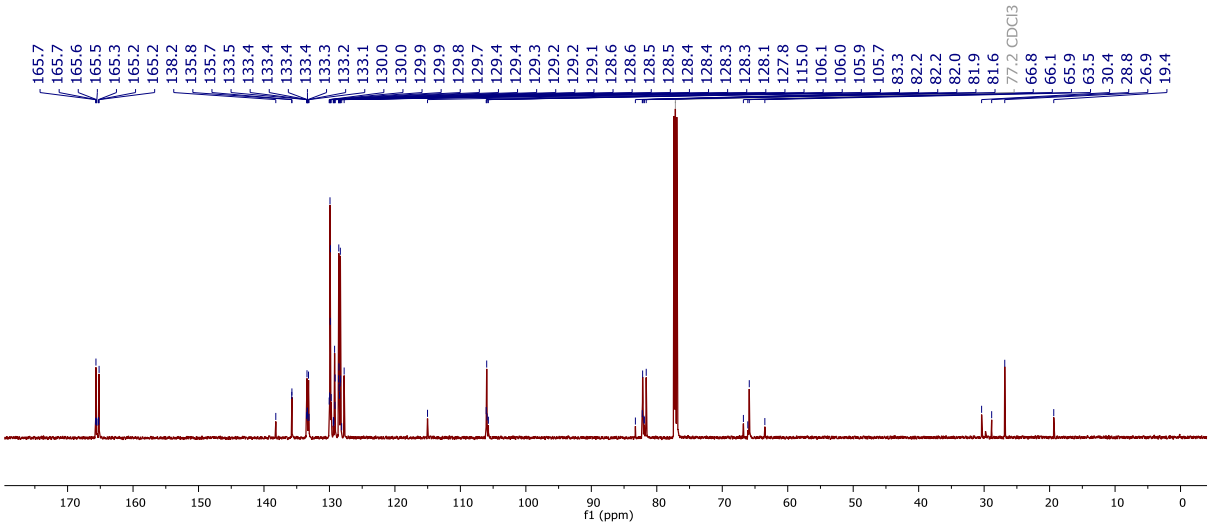
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **43**



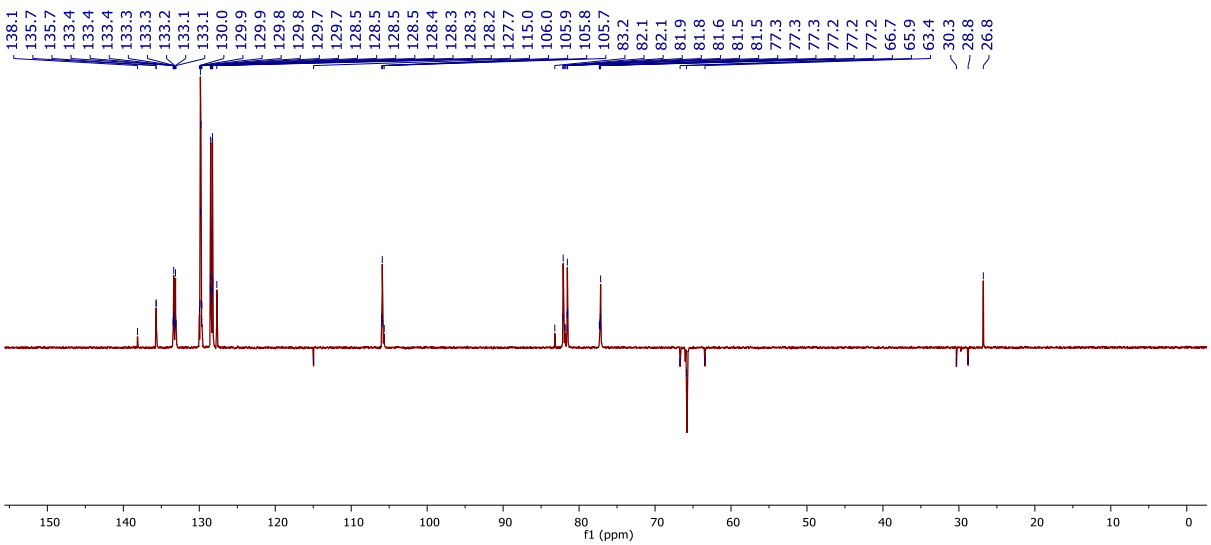
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **31**



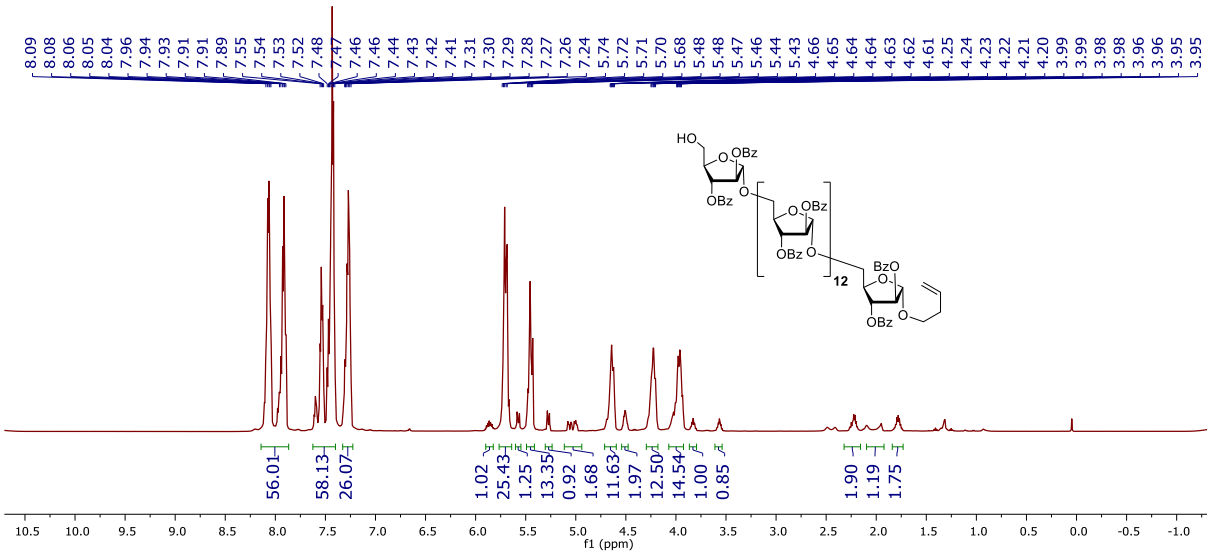
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **31**



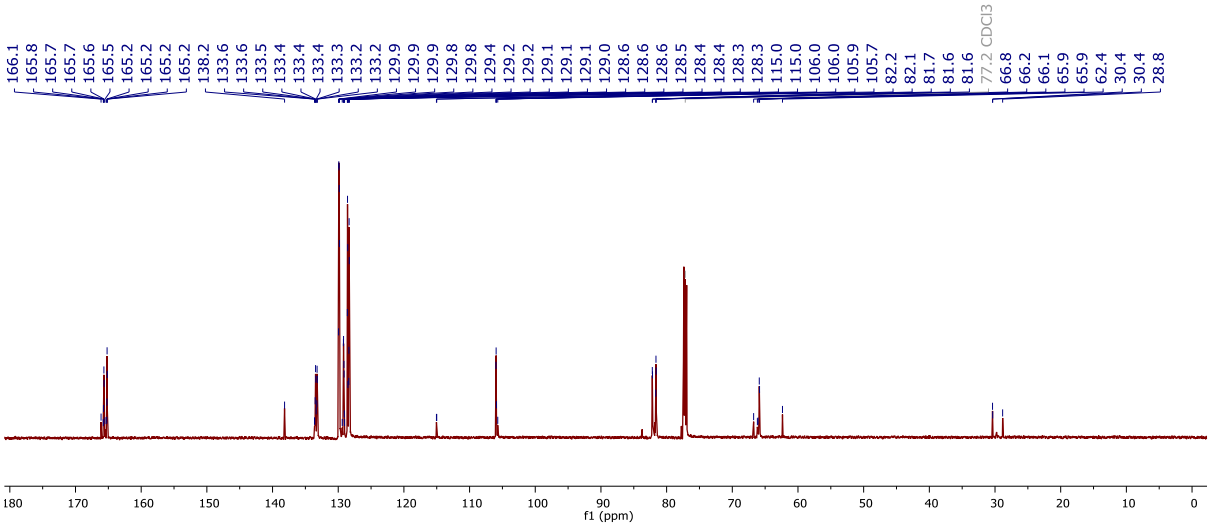
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **31**



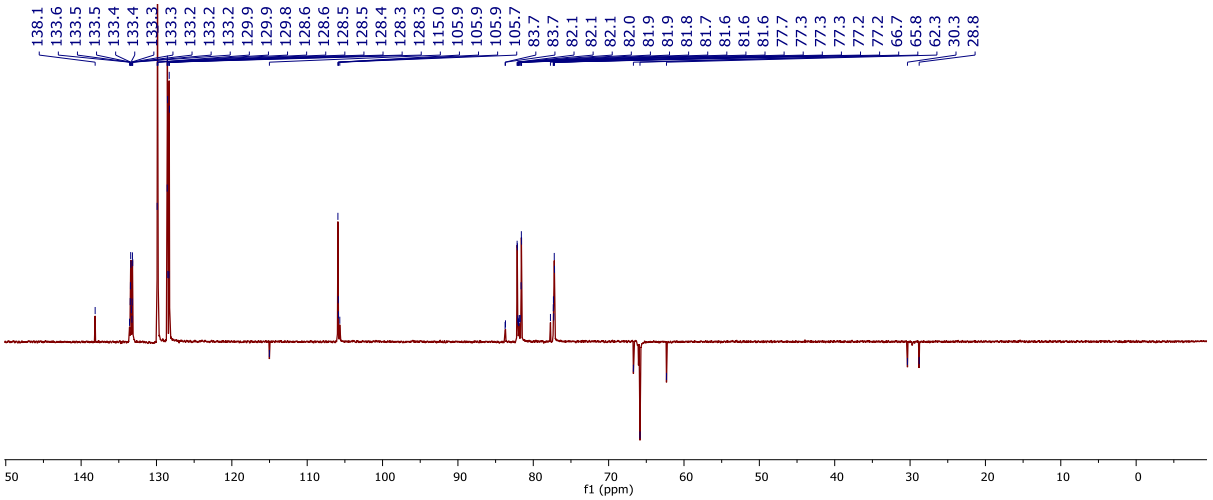
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **44**



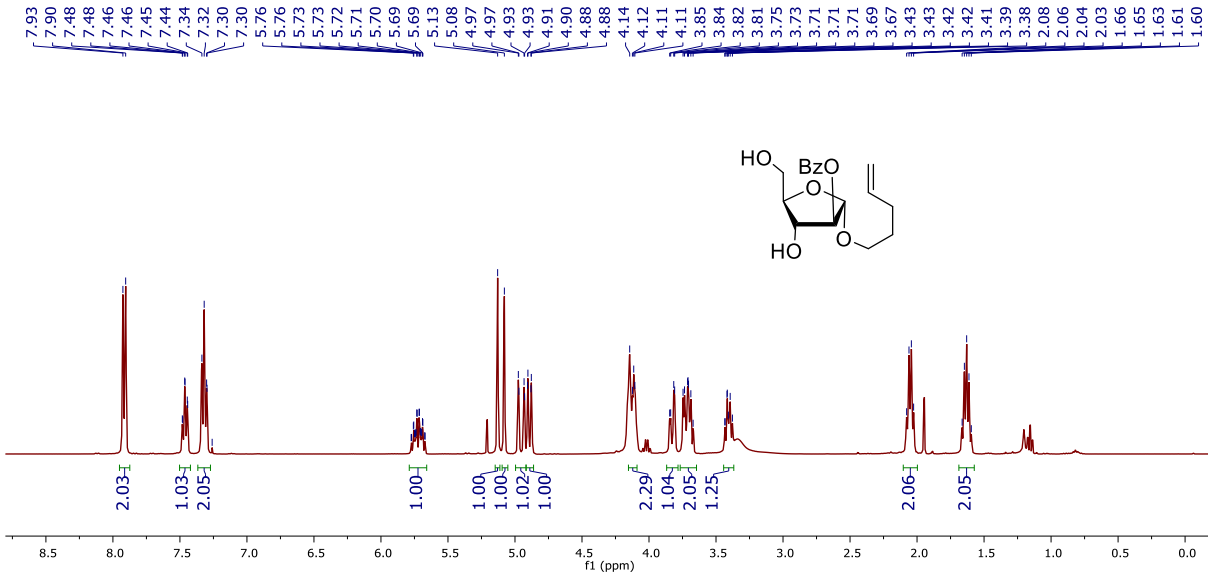
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **44**



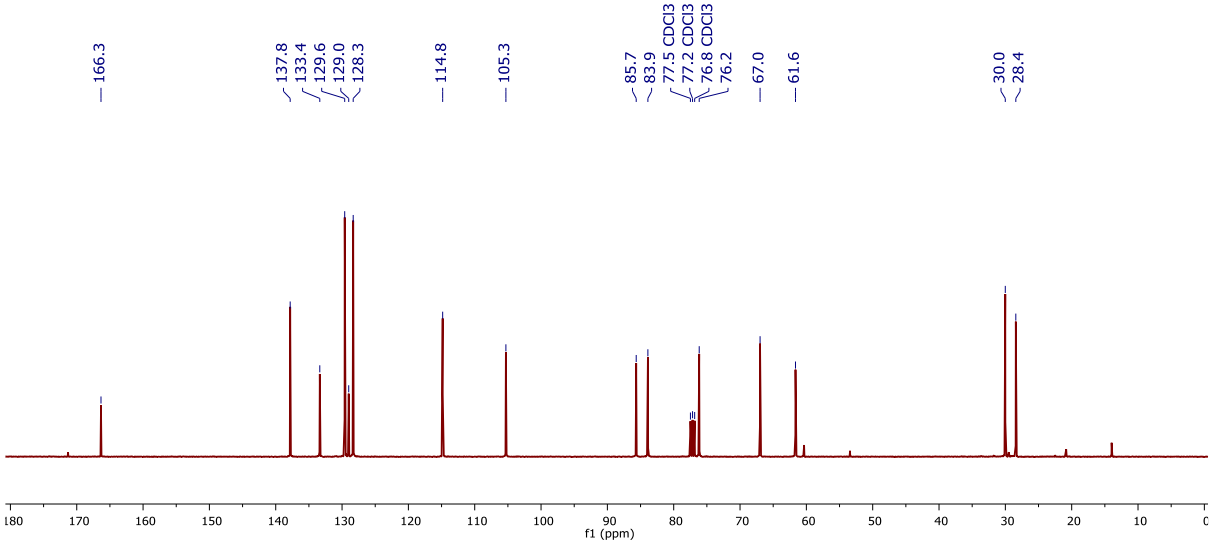
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **44**



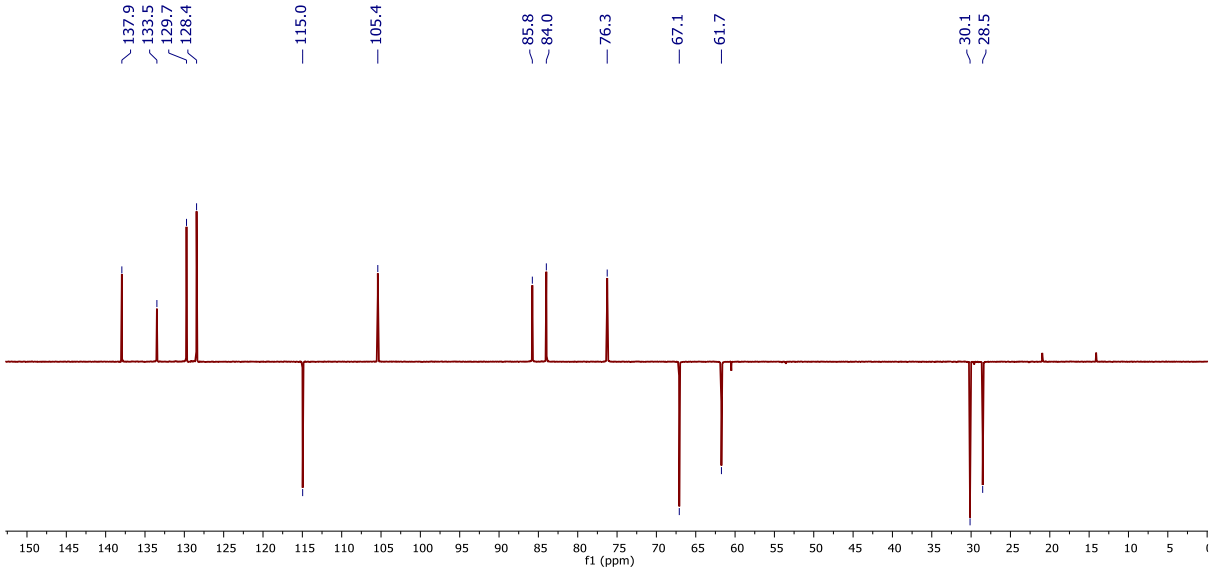
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8**



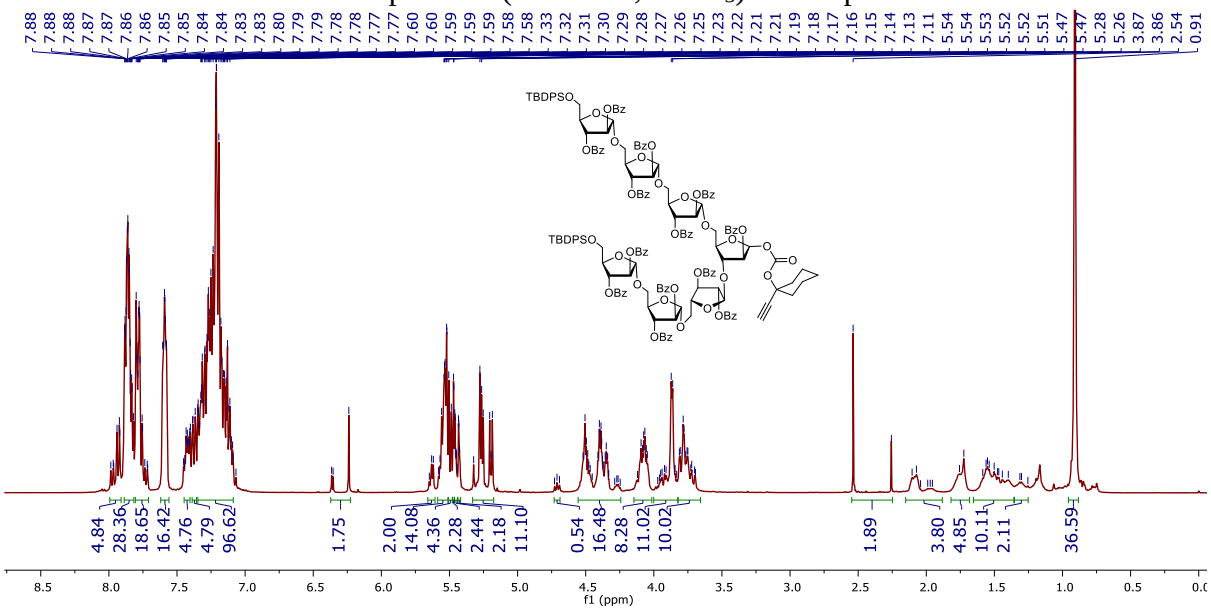
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8**



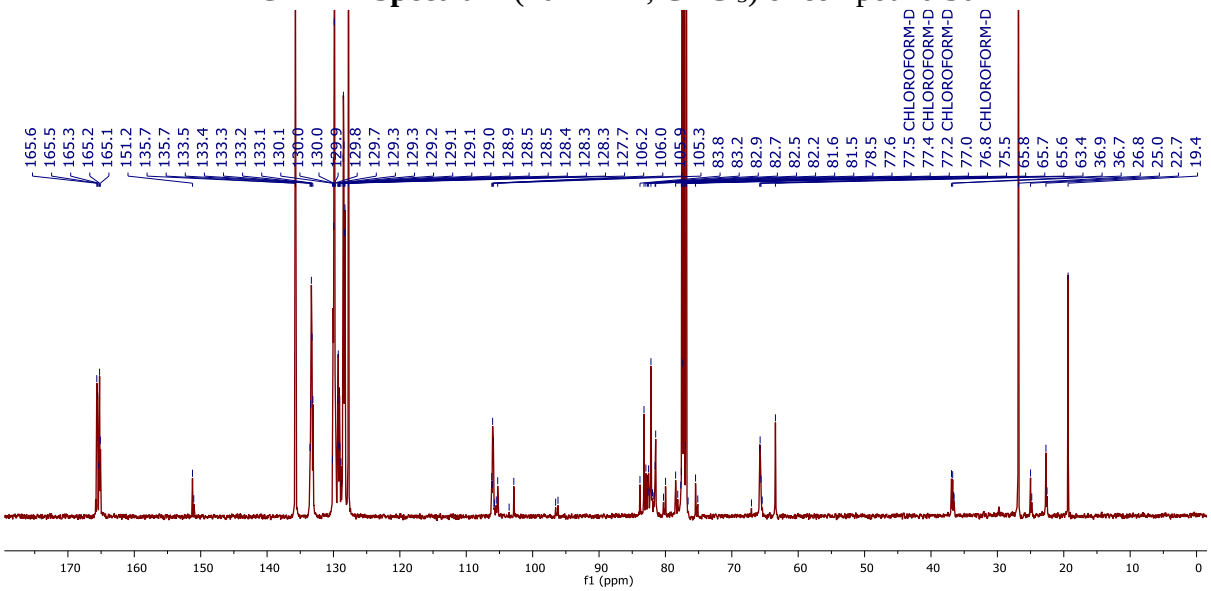
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8**



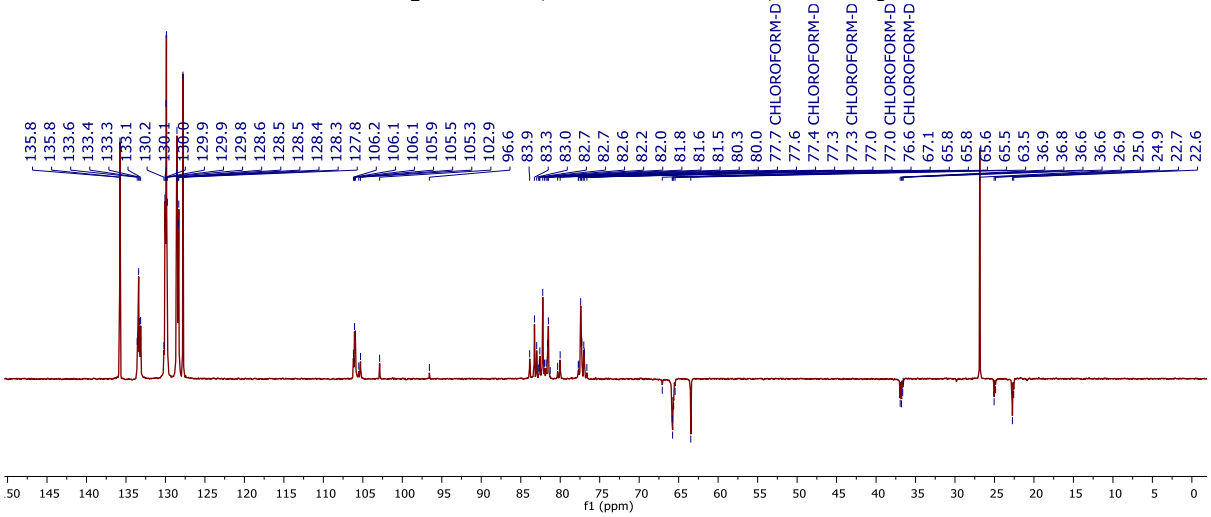
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **30**



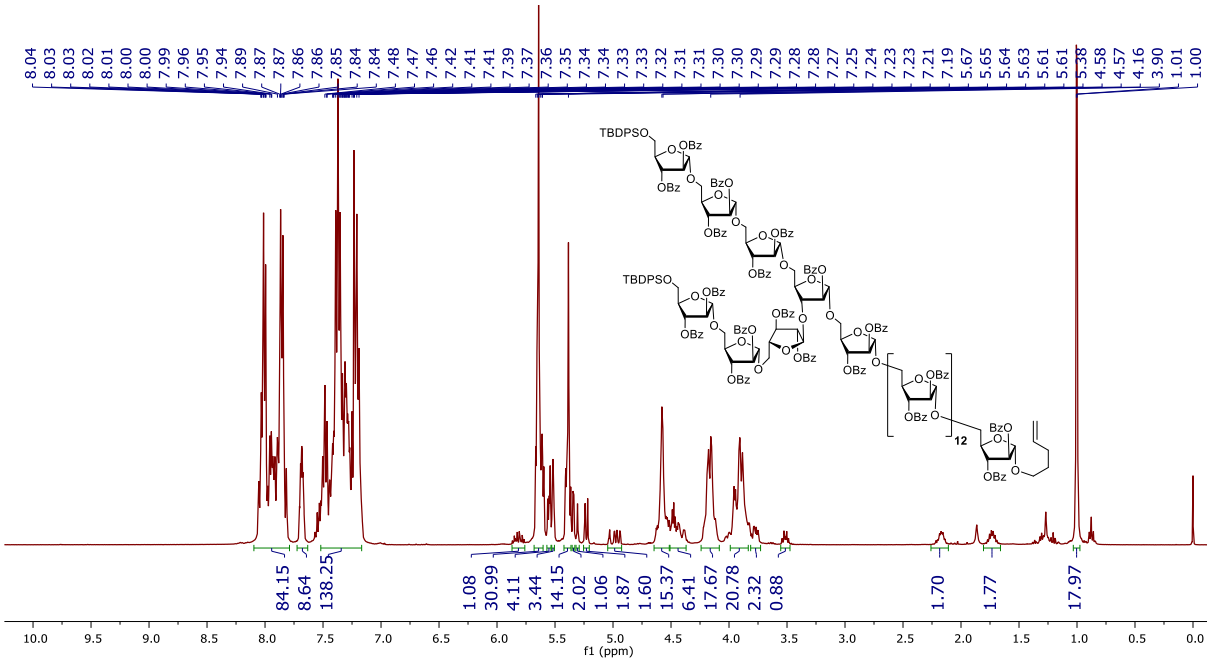
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **30**



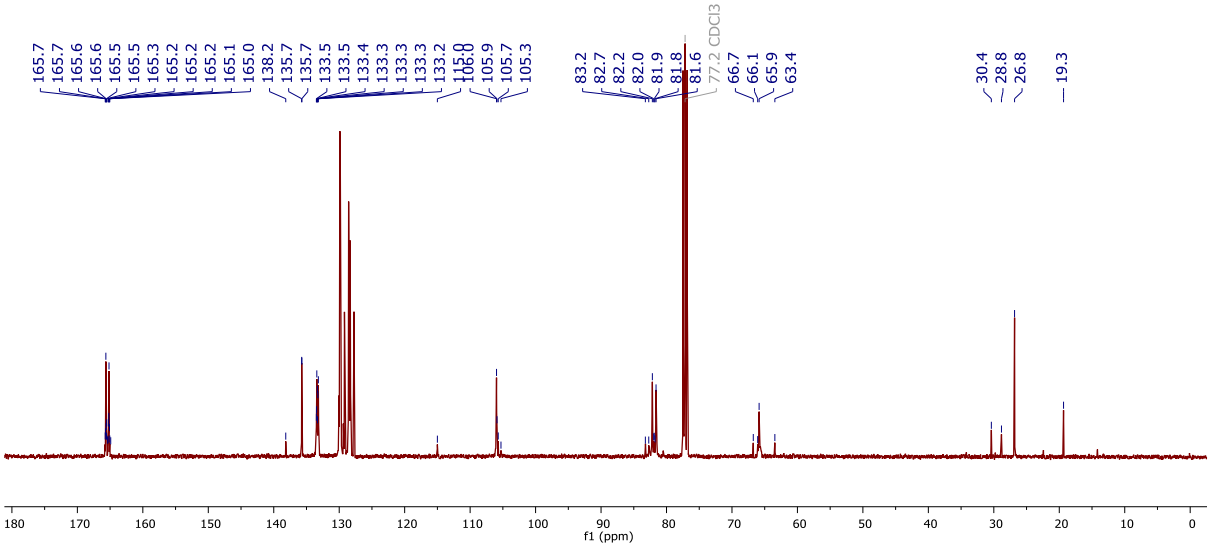
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **30**



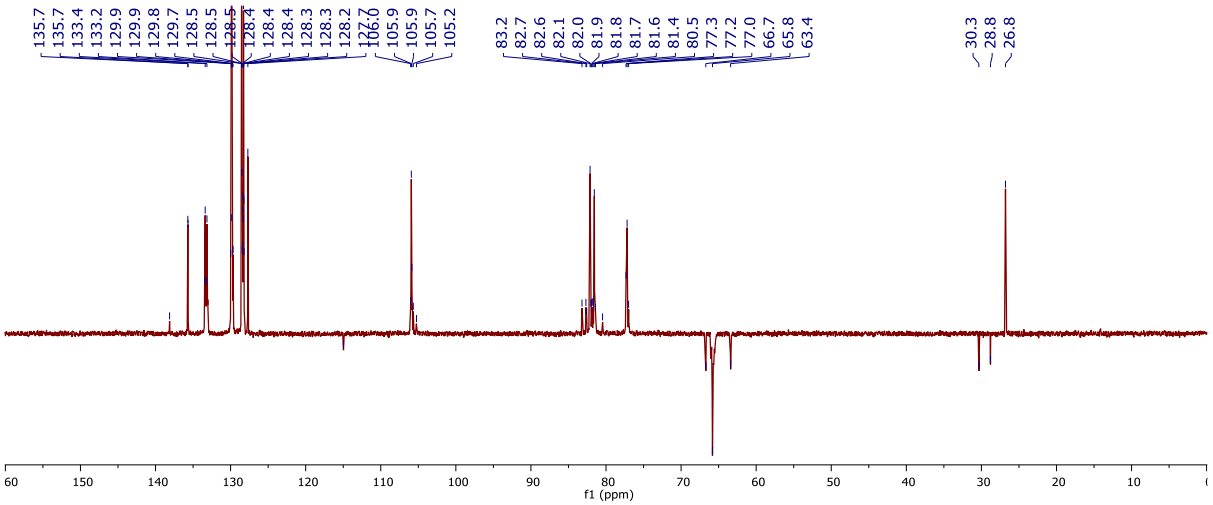
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **47**



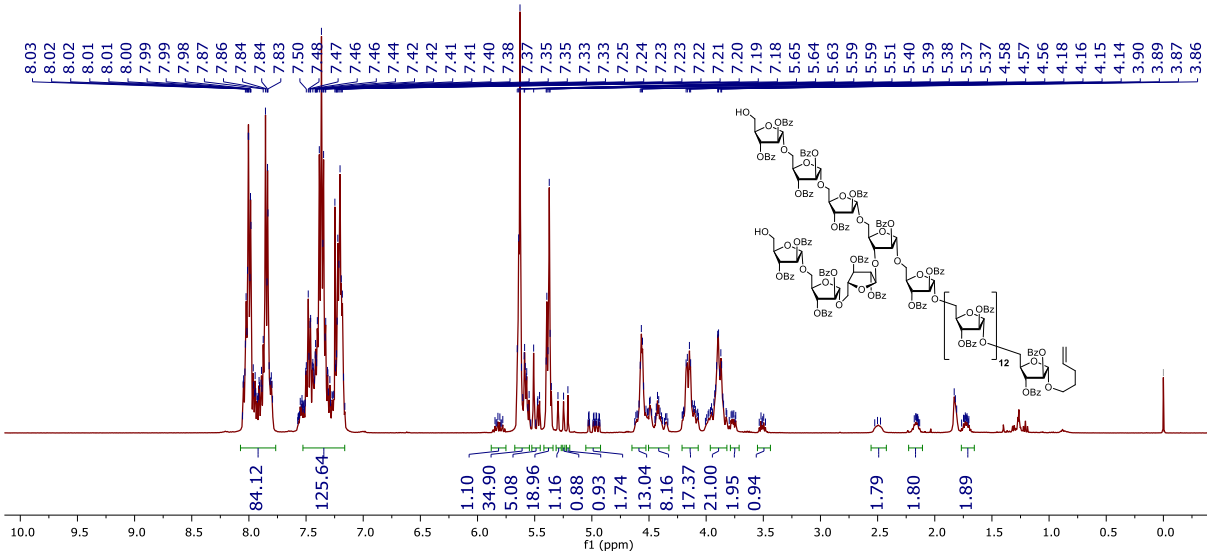
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **47**



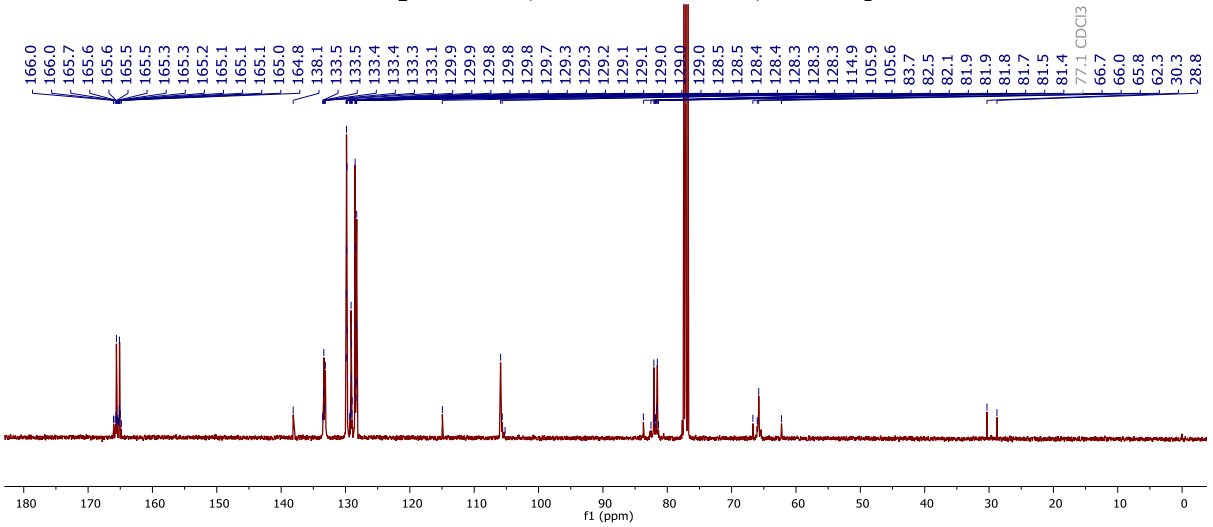
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **47**



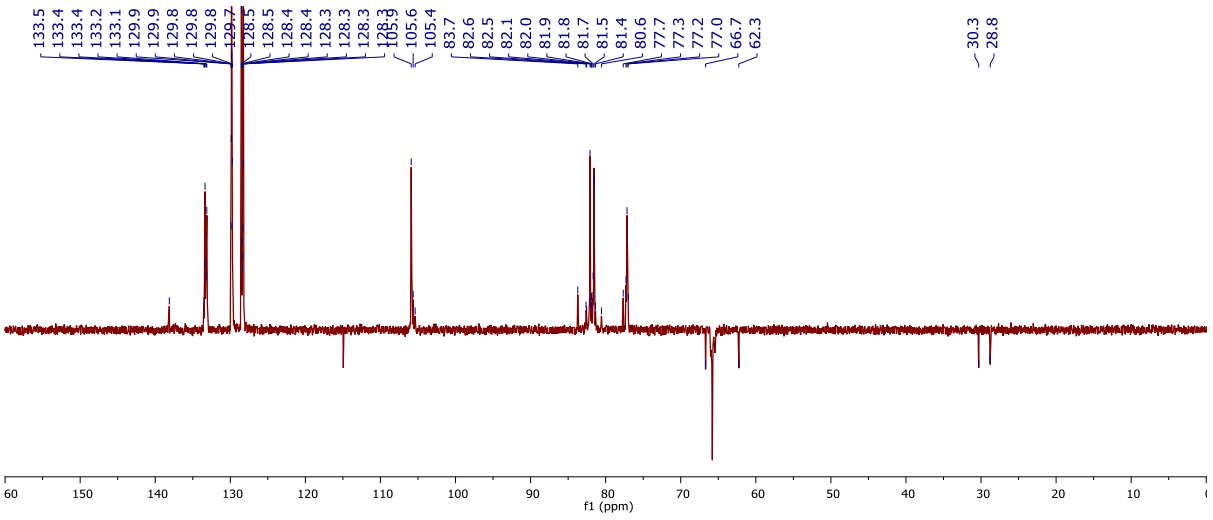
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **3**

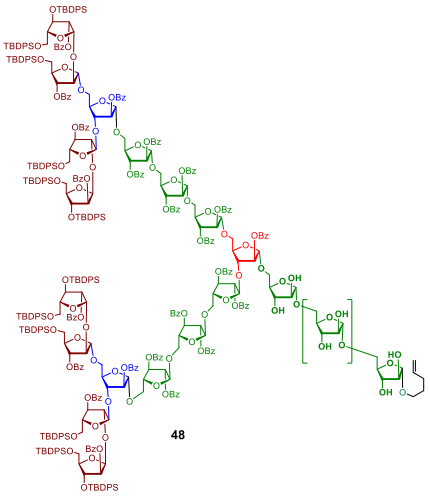


^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **3**

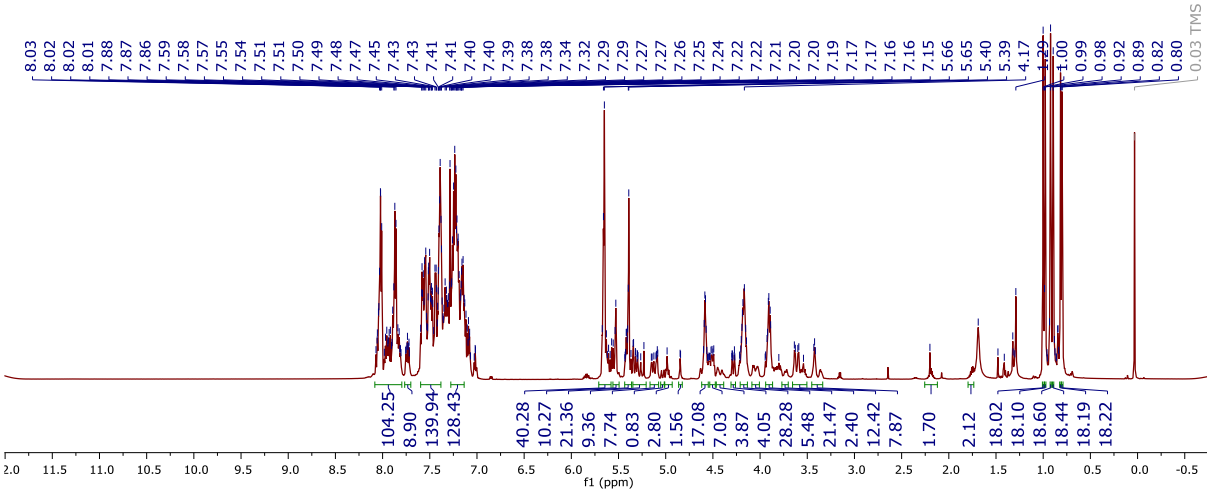


DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **3**

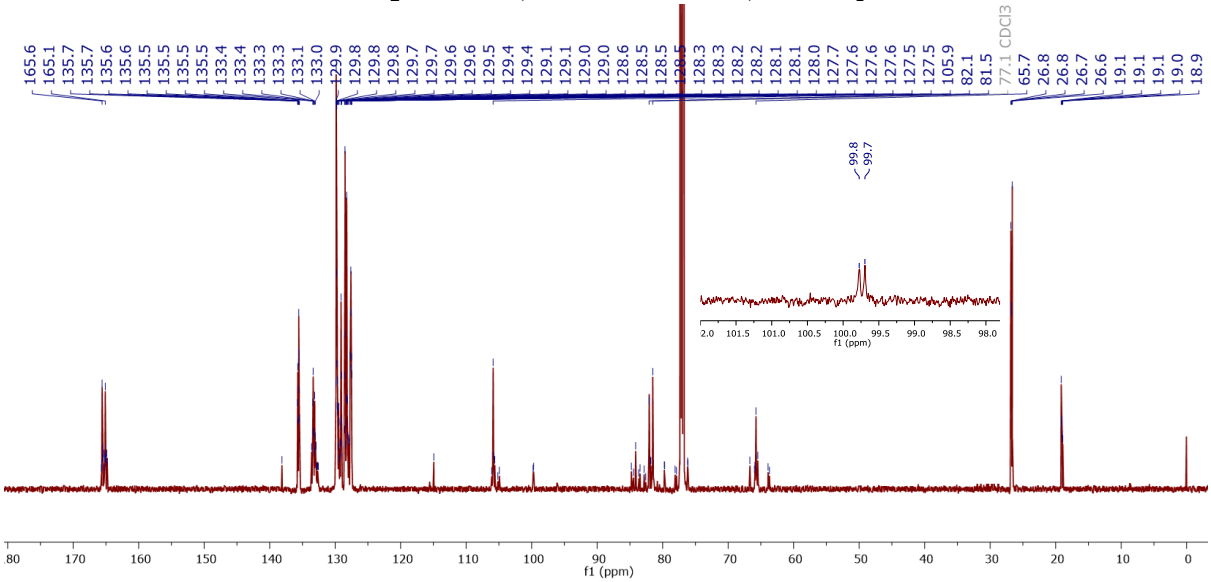




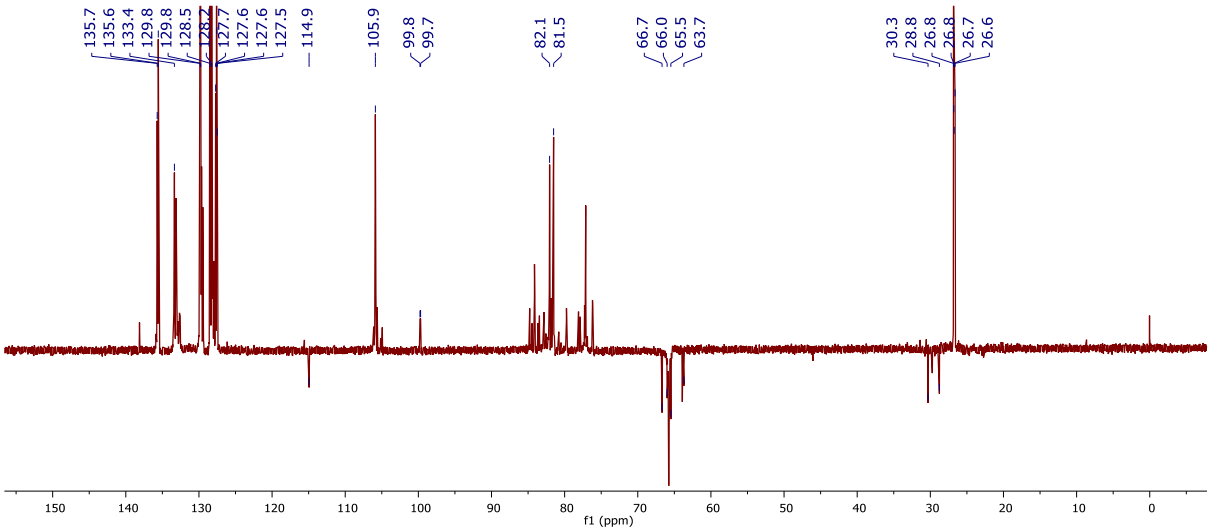
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **48**

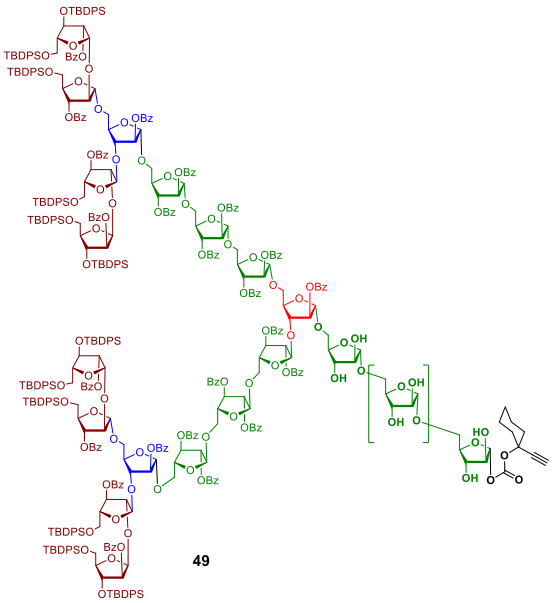


^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **48**



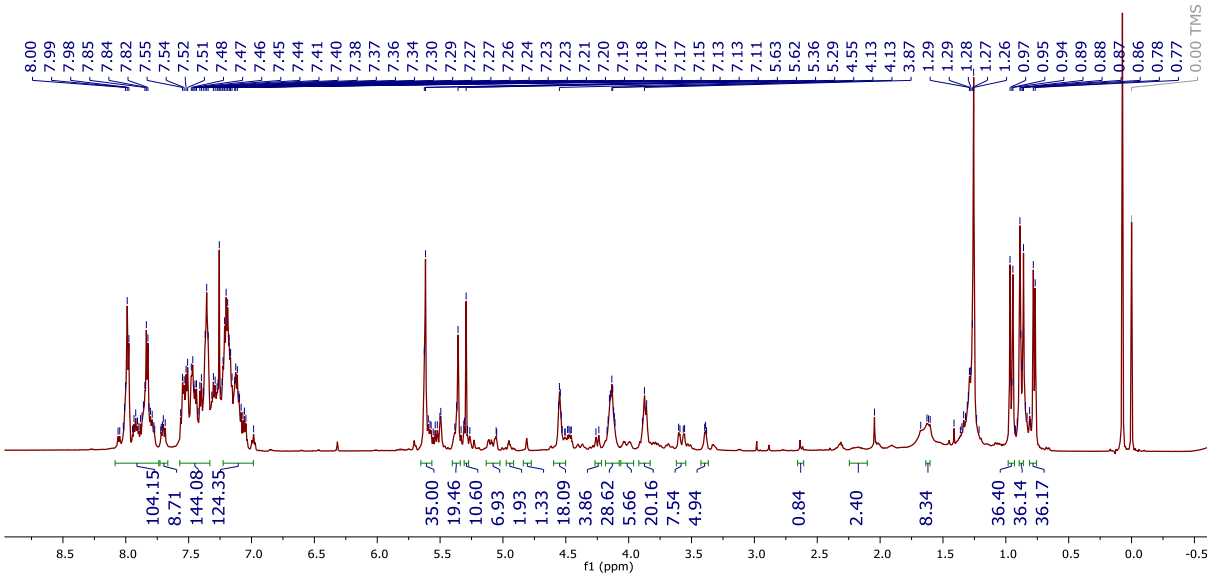
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **48**



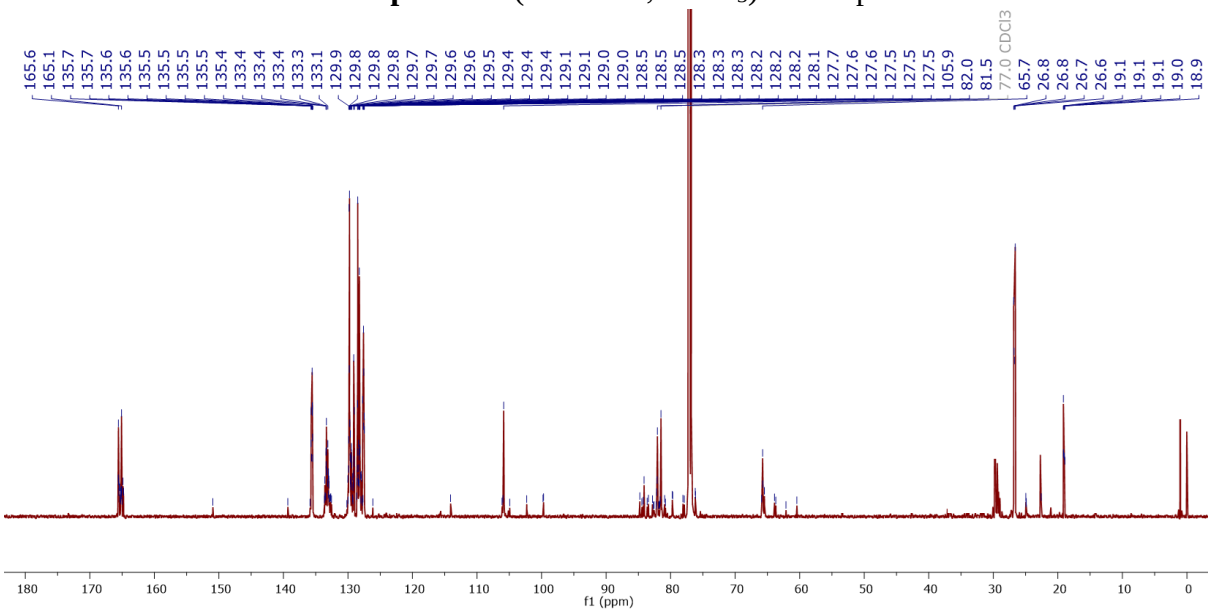


49

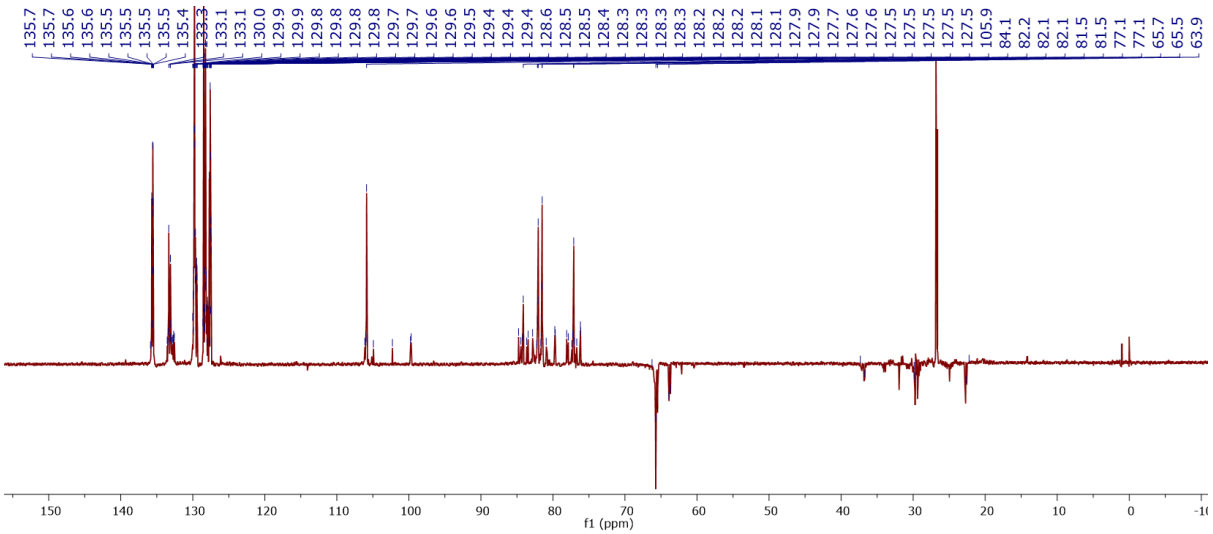
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **49**



^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **49**



DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **49**

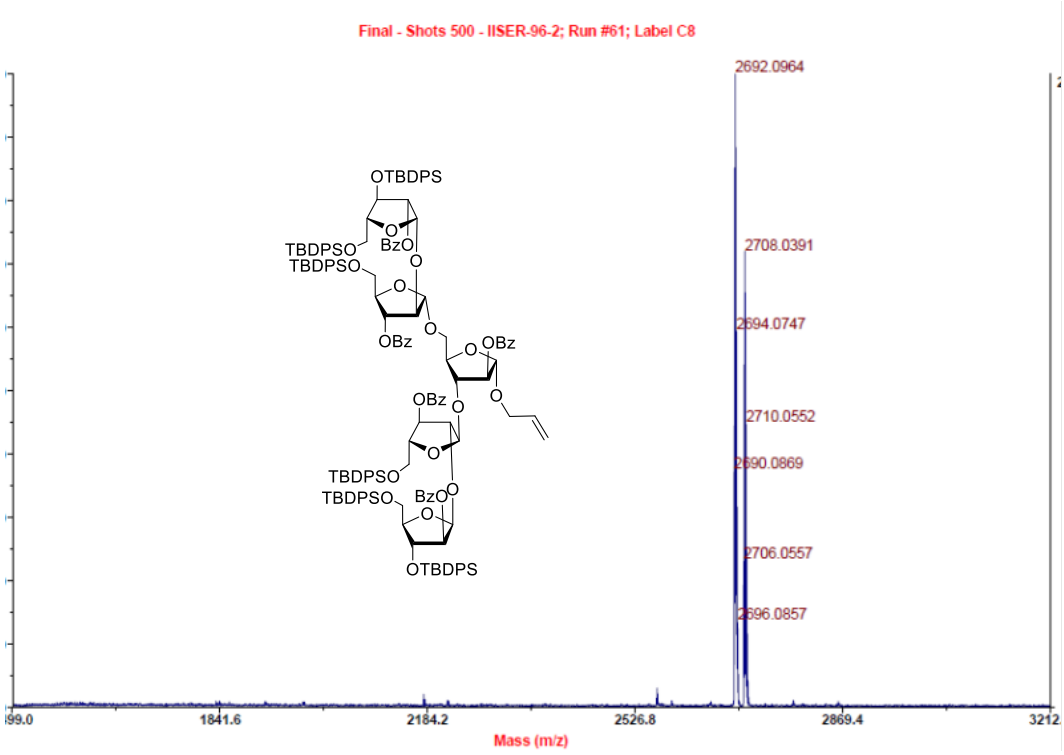


2.9 MALDI-ToF Spectra of Representative Compounds

Compound- **29**

Calculated mass: 2692.0874 ($C_{159}H_{174}NaO_{26}Si_6$)

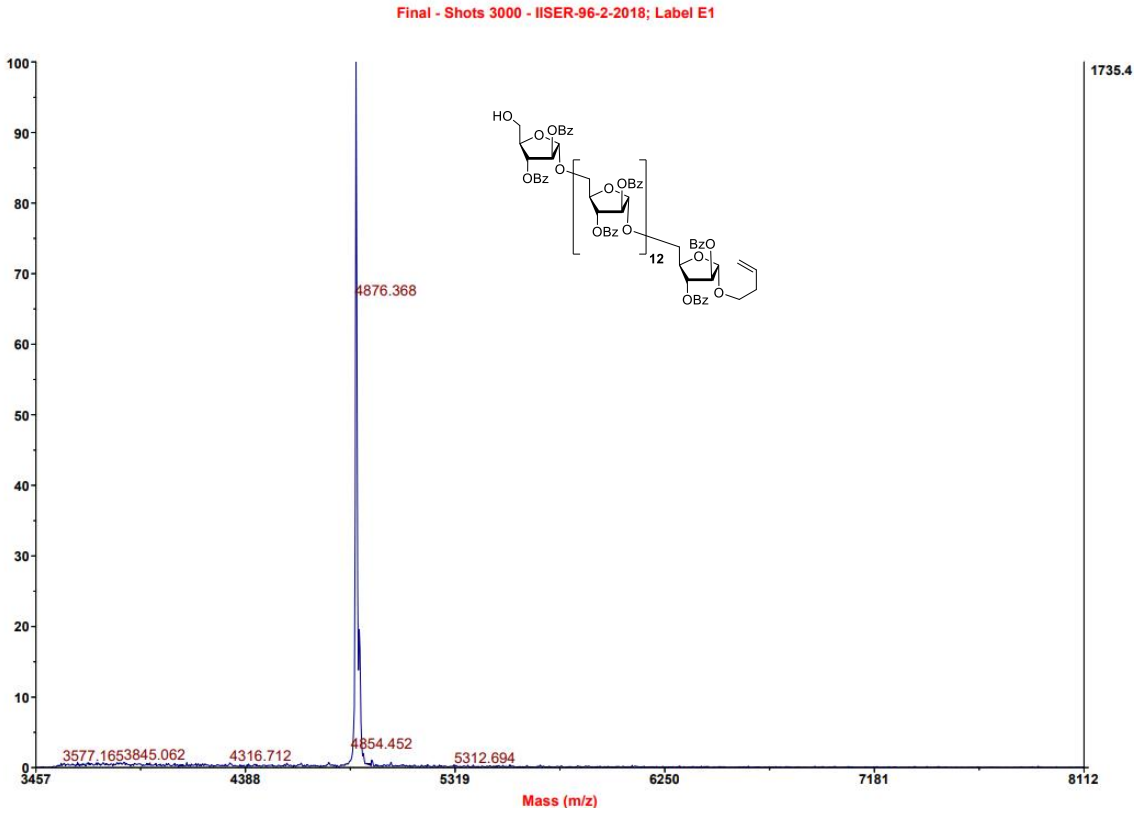
Observed mass: 2692.0964



Compound- **44**

Calculated mass: 4876.453 ($C_{274}H_{231}O_{85}Na$)

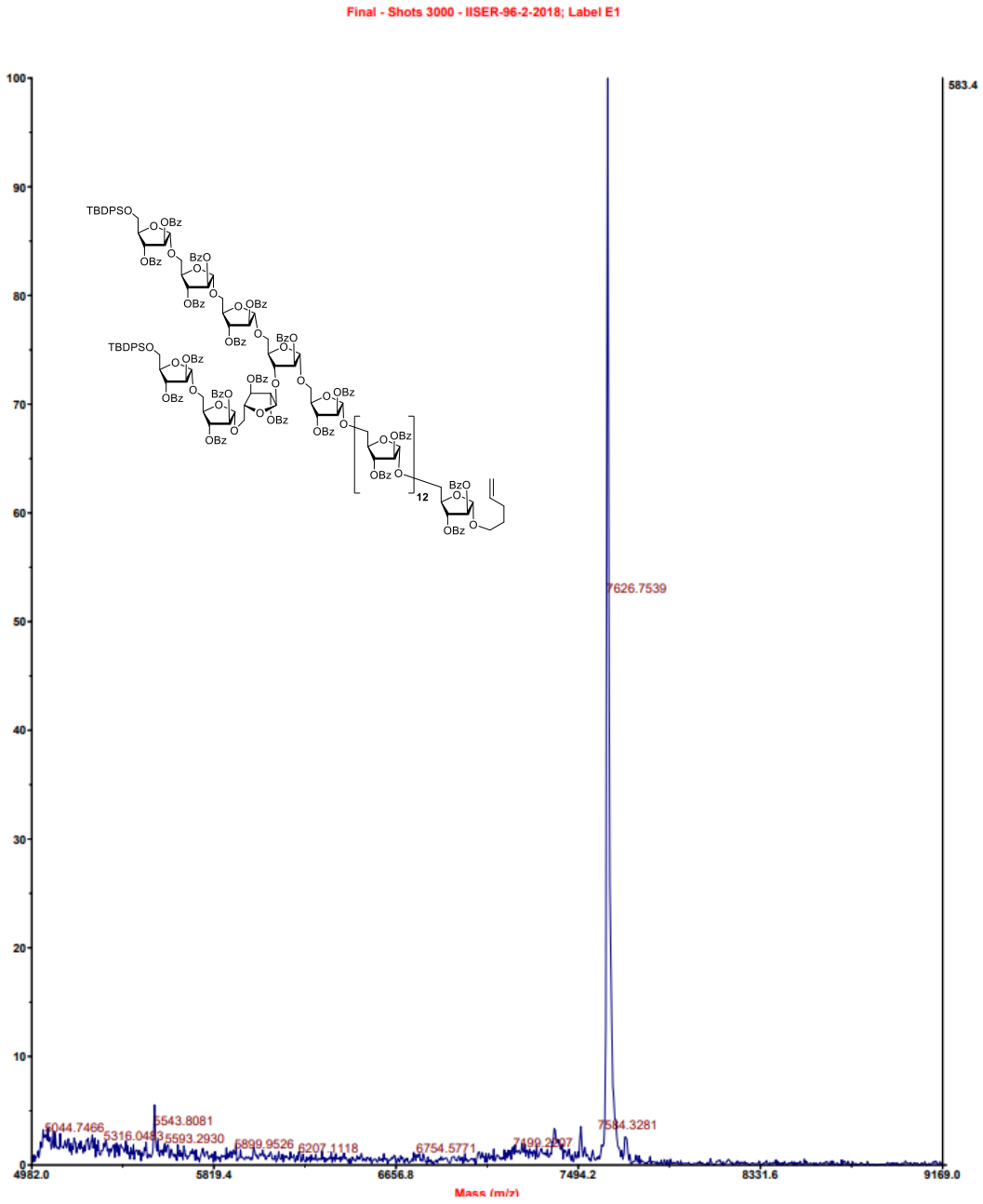
Observed mass: 4876.368



Compound- **47**

Calculated mass: 7626.8974 ($C_{429}H_{378}O_{126}Si_2Na$)

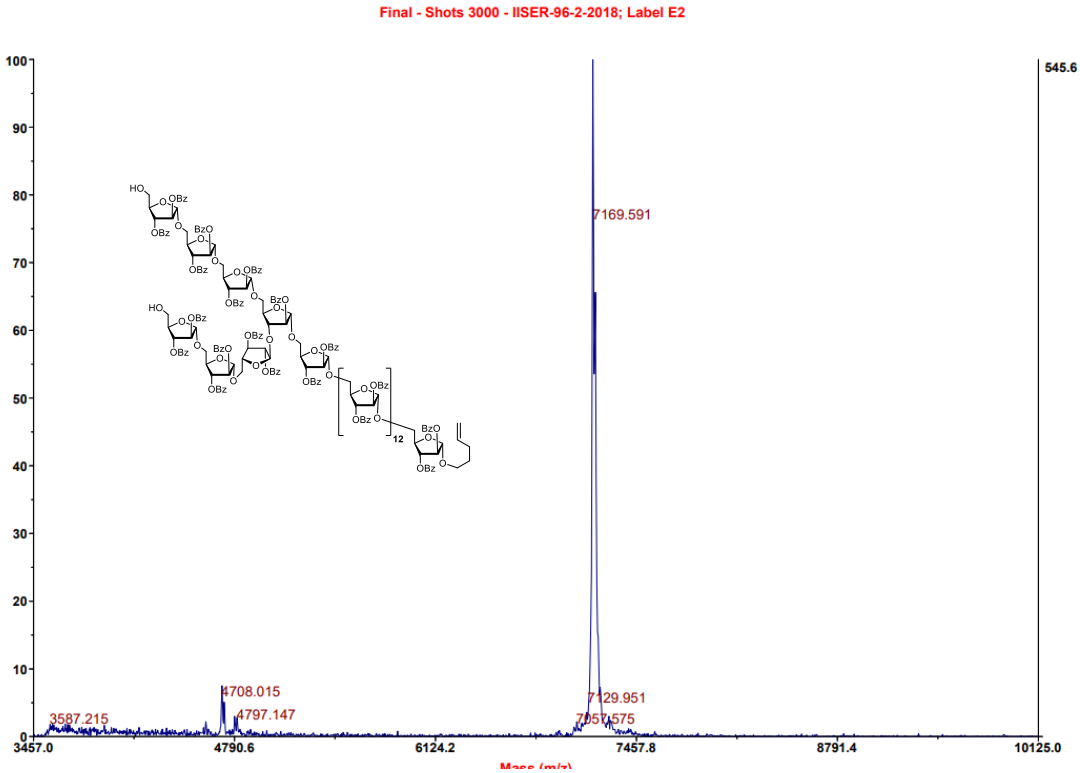
Observed mass: 7626.7539



Compound- **3**

Calculated mass:7169.458 ($C_{397}H_{342}O_{126}K$)

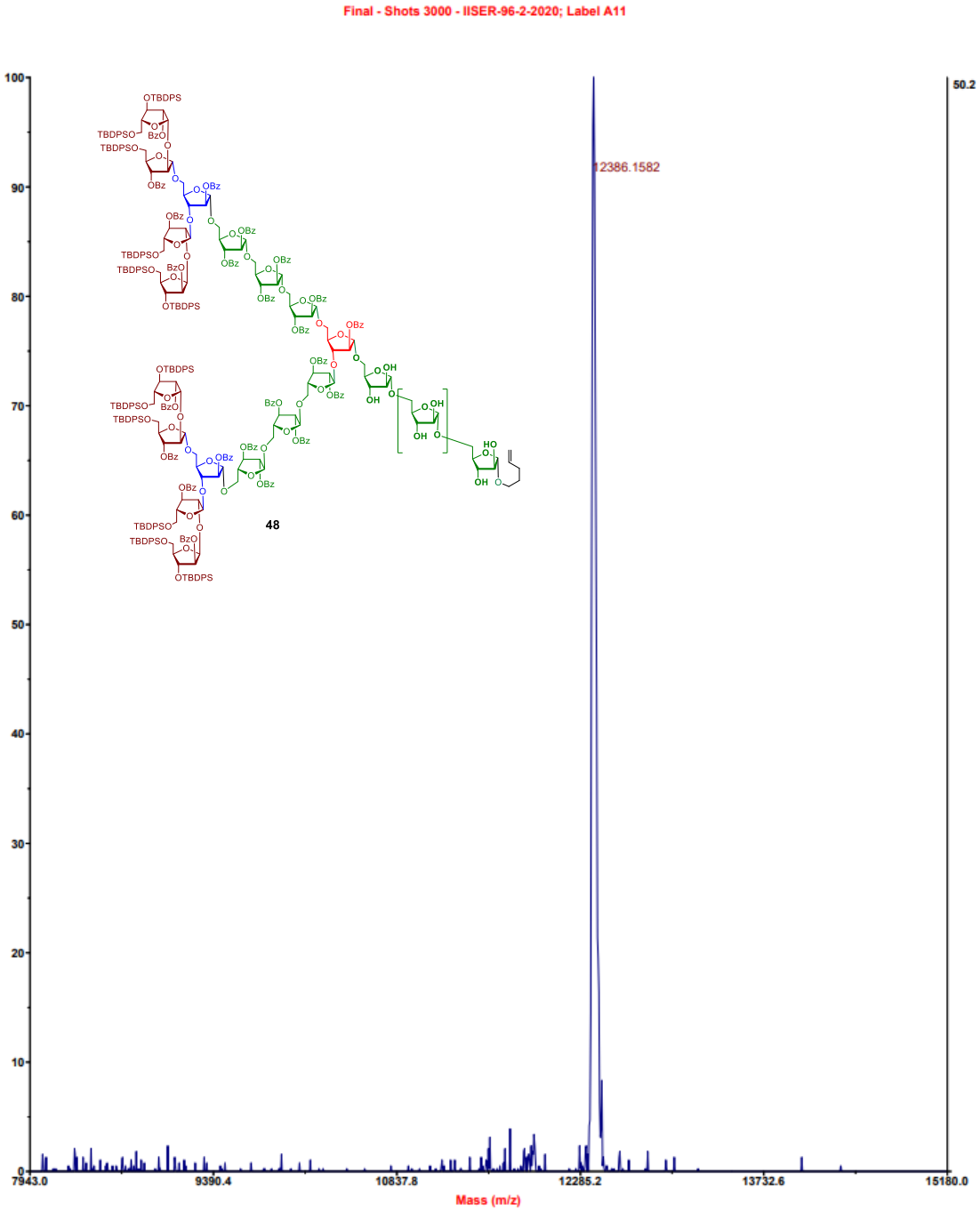
Observed mass: 7169.591



Compound- **48**

Calculated mass:12386.1139 ($C_{709}H_{678}O_{176}Si_{12}$ Na)

Observed mass: 12386.1582



2.10. References:

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Chapter 3

Cut-Insert-Stitch Editing Reaction (CIStER) Sequence for Surgical Glycan Editing

3.1 Introduction:

In the ever-evolving landscape of pharmaceutical research, synthetic Organic Chemistry stands as the artistic cornerstone of drug development.[1] Chemists use their understanding of disease mechanisms and biological targets to design molecules that can interact with and modulate these targets. Once a potential drug candidate is designed, chemists embark on the synthesis journey. They assemble atoms in a strategic manner to create complex molecular structures. This often involves multi-step processes, with each step carefully optimized to ensure high yields and purity. Over the past decades, several ground-breaking methodologies like photoredox Chemistry[2], new catalyst development for cross coupling reactions [3], direct C-H bond activation [4], and late stage C-H bond functionalization[5] have sparked the pharmaceutical industries to make and explore various life changing medicines.[6] Although these breakthroughs continue to have an impact in the field of drug discovery, organic synthesis remains a rate limiting factor from the standpoint of pharmaceutical industries.[7] Significant synthetic challenges arise from some of the key unsolved problems with potential implications in drug discovery includes selective saturation and functionalization of heteroaromatics;[8] concise synthesis of highly functionalized, constrained bicyclic amines [9]; and C-H functionalization for the synthesis of α,α,α-trisubstituted amines. Other topics, such as site-selective biomolecule modification[10] and the synthesis of noncanonical nucleosides,[11] are emerging as high-impact opportunities. To deal with these challenges, synthetic chemists need some of the new reactions that enable non-traditional disconnection, more C-H bond activation, stereoselective synthesis of substituted heterocyclic rings, C-X, C-C bonds. In the last few years, the field of organic chemistry has been captivated by intriguing idea of 'Molecular Editing'.[12] Molecular editing encompassing insertion, deletion, or exchange of atoms of highly functionalized compounds has emerged as a hotspot in new method development and has been applied in complex natural product synthesis.[13] The core of organic synthesis is the retrosynthetic analysis. The evaluation of newly discovered chemical transformations through retrosynthetic disconnections and the identification of underutilized synthetic changes are vital for advancing both the chemical sciences and related fields. Assessing progress in synthesis involves analysing available disconnections and strategically implementing innovative transformations for the development of purpose-built molecules.[14] Molecular editing and late stage functionalization have become attractive approaches for discovering these elusive reactions. These methods embody the

principles of contemporary synthesis, which involve the capacity to construct and accommodate complexity. More importantly the insertion, deletion or exchange of atoms in editing strategy enables the modification of molecular skeletons in single steps.[15]

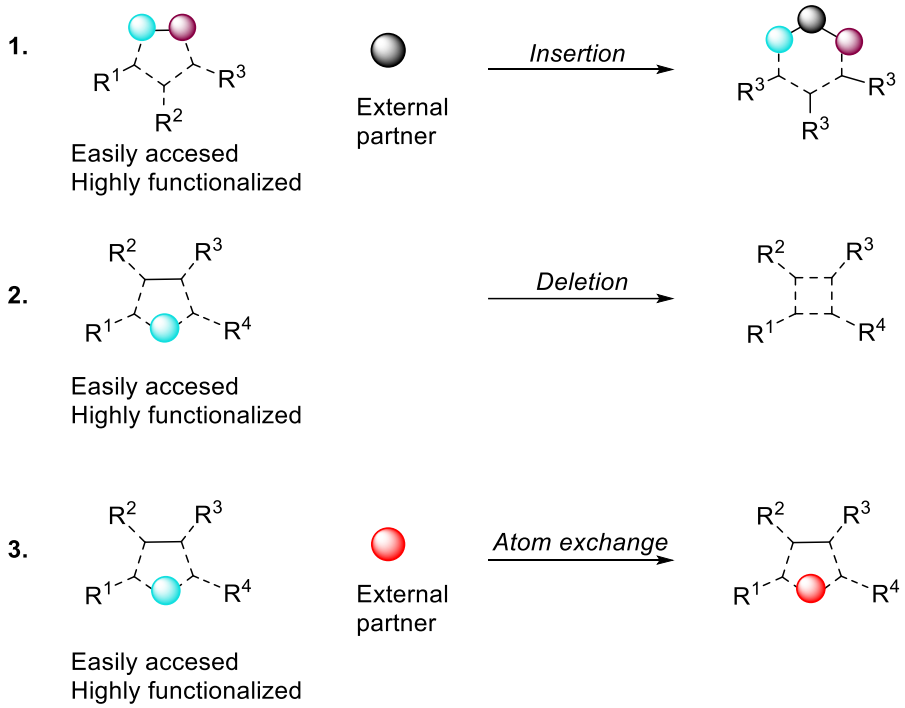


Fig 1: Schematic description of the concept of molecular editing

For instance, Levin and co-workers developed a method to insert a carbon atom that transforms pyrrole to pyridine.[16] Similarly, deletion of a heteroatom introduces a new strategy to produce shorter chains or ring-contracted products. In 2021, Levin group reported a method that snips nitrogen atom from a secondary amine, liberating N_2 and forming a C-C bond between the remaining molecular fragments.[17] Another class of skeletal editing is the swap of one particular atom for another in a single step. For some researchers, most interesting skeletal changes would swap one atom for another in a single step. In 2023, Levin and colleagues have come up with a method for site-selective carbon-to-nitrogen replacement in aromatic compounds.[18] Simply replacing one atom in an organic substance with another can be beneficial, for example, in medicinal chemistry. For example, making a pyridine derivative by replacing an aromatic carbon atom with a nitrogen atom can lead to therapeutic possibilities.

On the other hand, the editing in biomolecules is at the forefront of scientific and technological advancement, reshaping our approach to genetic modification and molecular manipulation. The editing mechanism of biomolecules focuses on the DNA, RNA, protein editing. The understanding of these biomolecule editing processes has led to development of various

molecular biology tools such as recombinant DNA technology, polymerase chain reaction, DNA amplification, CRISPR-Cas9 editing, and prime editing.[19–22] These tools have profound implications for biology, medicine, and biotechnology. The field of genetic engineering has been dramatically transformed by gene editing technologies, which empower precise and regulated alterations of organisms, cells, and their genetic material. These advancements find practical use in agriculture, medicine, and biotechnology.[23] The understanding and manipulation of editing mechanisms are central to biology, pharmaceutical development, and biotechnology, with the potential to improve human health, address genetic disorders, and advance our understanding of the fundamental processes of life.[24]

On the contrary, the state of editing within the realm of glycotechnology is still in its nascent stages. Glycotechnology, the study of carbohydrate-related molecules and their manipulation, is a rapidly evolving field with numerous implications in various areas of science, medicine, and industry.[25] The ability to edit and modify glycans, which are complex carbohydrates often attached to proteins and lipids, is essential for understanding and manipulating their functions. The intricacy of glycan structures and functions pose formidable obstacles in this field, necessitating sophisticated techniques and tools for examination and modification. One of the most powerful tools for editing of carbohydrates is the chemical transformation, in which chemist can design and synthesize carbohydrates with specific structures, enabling the creation of custom-designed carbohydrates for various applications. Chemical approach for saccharides editing includes skeletal editing via the acid-catalyzed transformation of furanosides to pyranosides[26], Ferrier rearrangement for converting carbohydrates to cyclitols[27], conversion of biomass carbohydrates into the rare sugar isomer through site selective epimerization reaction[28], transformation of the biomass-derived carbohydrates in the production of chiral synthetic building blocks through selective excission of C-O bonds.[29]

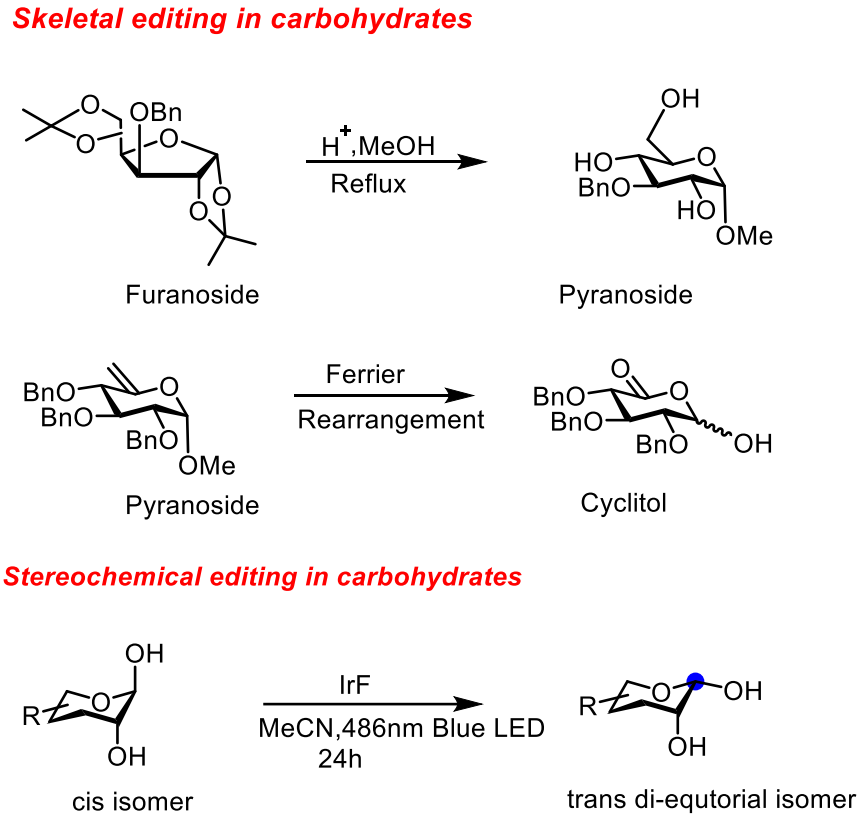


Fig 2: Chemical approaches towards editing of saccharides

Another approach for editing of carbohydrate is the enzymatic approach that involves the controlled manipulation of complex carbohydrate structures using specific enzymes. This process allows researchers to modify, create, or remove specific sugar residues in glycans, offering a range of applications across various scientific and medical fields. There are two key techniques in this category: metabolic oligosaccharide engineering (MOE)[30] and enzymatic addition of glycans (EAG).[31] Metabolic oligosaccharide engineering allows for precise tweaks to cellular sugars. In MOE, cells are cultured with a monosaccharide containing a reporter moiety (e.g., azides, alkynes, terminal or strained alkenes, diazo groups or nitrones), hypothesizing that the conjugate of the reporter moiety and monosaccharide are incorporated into the cellular processes, the incorporated moiety can guide the investigation of biological pathways including cell imaging using bioorthogonal chemistry. Metabolic oligosaccharide engineering has now been applied to myriad problems in glycobiology like disrupting glycan structures on cells, delivering bioorthogonal chemistry for cell surface modification, probing metabolic flux inside cells, and for proteomics analysis of glycosylation. Another approach for cell surface glycan engineering is chemo-enzymatic glycan editing which exploits exogenous glycosyltransferases to covalently transfer an artificial monosaccharide (typically containing a bioorthogonal tag for visualization) onto the glycan structure of interest. The signal outcomes

for the glycan of interest are provided via a complementary bioorthogonal process.

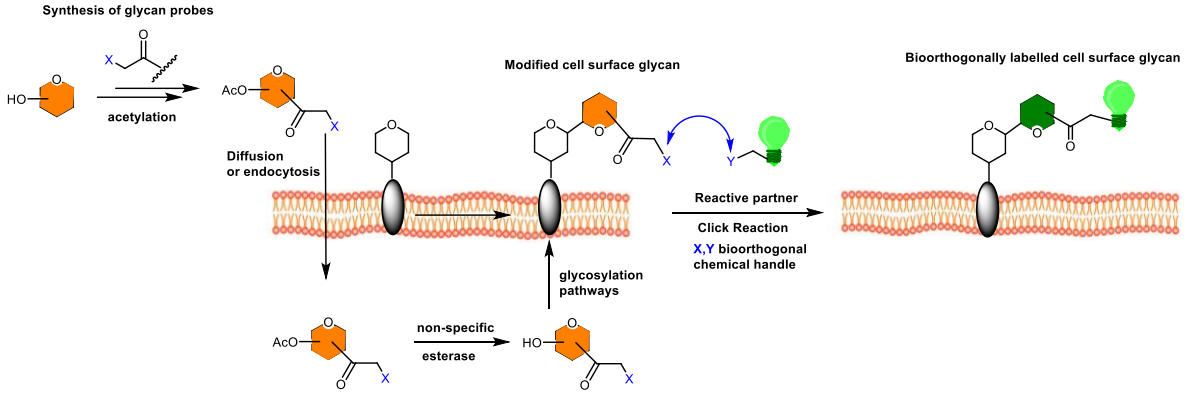
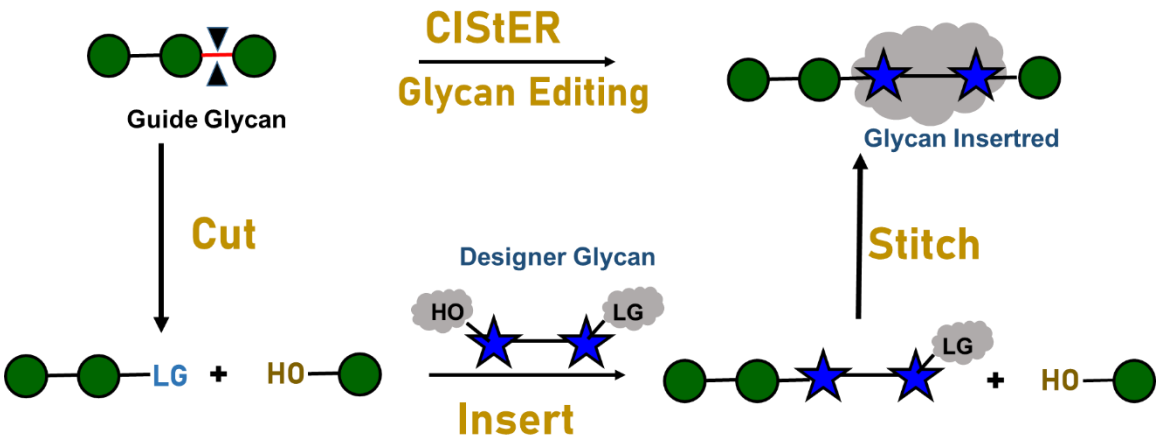


Fig 3: Chemo-enzymatic Glycan editing using metabolic oligosaccharide engineering

3.2 Current Work:

3.2.1 The major goal of the current work:

Tools to engineer the glycome at different hierarchical complexity levels, with thorough, up-to-date information, are offering a new trail to decode the fundamental biological activities that are imprinted in glycans, and bring new insights on glycotherapeutics, even though they have not been fully established. The process of MOE and EAG introduces modified sugar analogs or enzymes that can selectively manipulate glycan structures which helps for investigation of various cellular processes such as signalling, adhesion, or immunity. Although these editing techniques for cell surface glycan engineering is rapidly growing, there is no editing technique that enables the insertion of a foreign glycan, lipid or protein into the already synthesized glycan via chemical or enzymatic routes. In this scenario, we hypothesized a three-step chemical glycan editing process that allows insertion of glycans, proteins, or lipids to a pre-designed glycan unit (guide glycan) to generate various glycoconjugates. A significant feature of this editing process is that all three steps can be seamlessly executed in a single reaction vessel through the utilization of the cut-insert stitch-editing reaction (CIStER) sequence. Moreover, the CIStER strategy stands as a potent tool for the synthesis of diverse hybrid glycans and unnatural glycoconjugates, presenting a compelling alternative to numerous established methods for glycan assembly, including latent, iterative, catalytic, or orthogonal activation protocols.

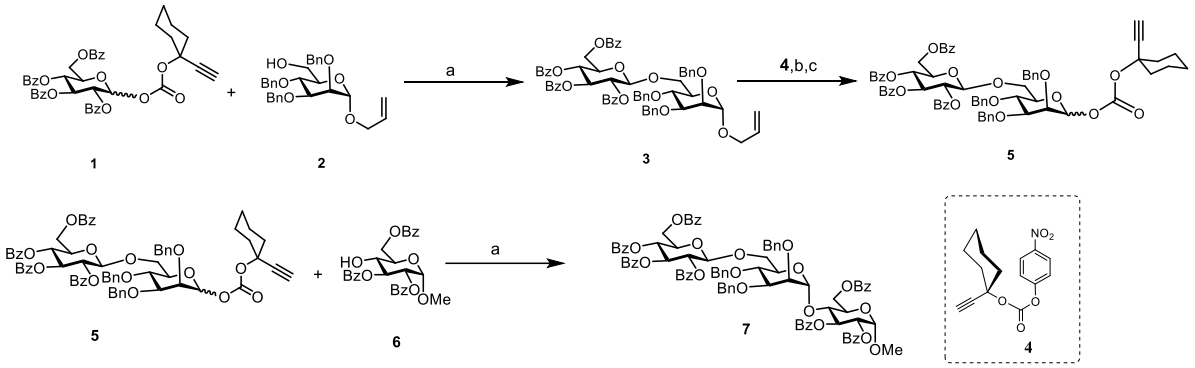


Scheme 1: Strategy for the editing of glycans by CIStER

3.2.2 Strategy for development of CIStER technique:

The first step for the CIStER requires the regiospecific cleavage of the glycosidic bond. To ensure the effective execution of site-specific cleavage, a notable disparity in reactivity within the glycosidic bond is imperative. In a glycosylation reaction, the reactivity of the glycosidic bond can be fine-tuned by the nature of protecting group present at the C2 position. The electron withdrawing groups imparts *–I* effect and thereby slowing down the rate of the leaving group extrusion from the anomeric position, whereas the electron donating moieties shows *+I* effect that favors the displacement of the leaving group at anomeric position, making the intermediate oxocarbenium ion more stable. The pioneering work by Prof. Bert Fraser-Reid showed that the armed and disarmed effect showed by the C2-protecting group can be utilized to activate selectively *n*-pentenyl orthoesters (NPOEs) over other *n*-pentenyl donors, and that this chemoselective process enables regioselective glycosylation.[32] This ground-breaking idea is widely used in modern oligosaccharide synthesis, and it is at the cornerstone of one-pot oligosaccharide protocols, which are an alternative to the conventional practice of extensive protection and deprotection techniques for oligosaccharide synthesis. We started the designing of the guide glycan for the CIStER method based on this pioneering “armed-disarmed” effect. As a result, we chose to synthesize the trisaccharide **7** as our guide glycan, which comprises of three sugar units, glucose, mannose, and glucose, and possesses three glycosidic bonds, of which one which is more reactive due to the presence of an electron donating benzyl ether group at the C2-position compared to the other two, which have an electron withdrawing

benzoyl ester group at the C2-position.With this concept, we started to synthesize the trisaccharide from the three easily accessible building blocks.

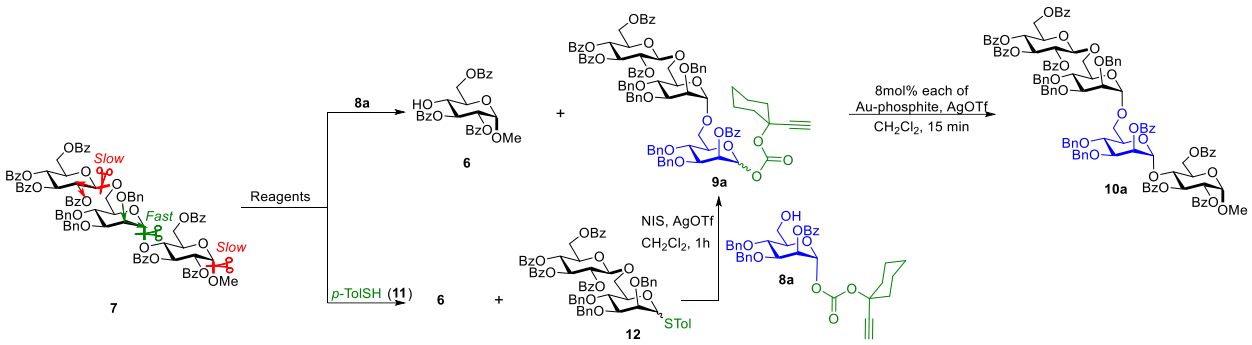


Scheme 2: Synthesis of linear trisaccharide 7; Reagents and conditions**:** a) 8 mol% each of chloro[tris(2,4-di-tert-butylphenyl) phosphite] gold and AgOTf, $CH2Cl_2$, 4 Å MS powder, 25 °C, 40 min, 92% for **3** and 90% for **8**; b) $PdCl_2$ (0.2 equiv), CH_2Cl_2 -MeOH (1:4), 25 °C, 4 h; c) 4, DMAP, CH_2Cl_2, 25 °C, 5 h, 85% over two steps for compound **5**

The glycosyl carbonate **1**[33] was synthesized from D-glucose by three steps, per-*O*-benzoylation of the D-Glucose, selective deprotection of anomeric benzoyl group using hydrazine acetate in DMF and finally converting the hemiacetal into glycosyl carbonate donor by the use of carbonate reagent (**4**) and DMAP. The mannose building block was synthesized from D-mannose following 4 steps: anomeric locking by heating D-mannose with allyl alcohol in DMF, following the selective protection of C6-OH as C6-OTBDPS, the benzyl ether protection at 2,3,4 positions and concluding with the deprotection of C6-TBDPS to generate the aglycon **2**. The other glucose building block was made from D-glucose involving four transformations: benzylidene protection commercially available of methyl α-D-glucopyranoside, installation of benzoyl ester group at C2, C3 by using benzoyl chloride and DMAP, followed by opening of benzylidene group by PTSA in CH_2Cl_2:MeOH followed by selective C6-OH benzoyl protection using the benzoic anhydride in triethyl amine and CH_2Cl_2 to generate the C4-OH glucose acceptor **6**. After this the compounds **1** and **2** were subjected to the glycosylation using Au/Ag catalysts to generate the disaccharide **3**, which was converted into glycosyl donor in two steps: first, deprotection of allyl group and converting the resulting hemiacetal into carbonate donor **5**. Finally, the donor **5** and acceptor **6** were coupled to give our desired guide glycan trisaccharide **7**. The compound was characterized by ^{1}H, ^{13}C NMR spectral analysis. Also the linkage in the trisaccharide was confirmed by coupled HSQC spectra.

3.2.3 Execution of molecular surgery on the guide glycan:

After synthesizing our guide glycan, we moved forward towards the execution of molecular surgery on it, keeping our target constant to make diverse class of edited glycoconjugates from the same guide glycan in a single reaction vessel. The first step of our CIStER strategy is the cleavage of the glycosidic bond. Initially we hypothesized that during the cut stage, the intermediate oxocarbenium ion can be trapped by the external glycan unit **8a** to give the trisaccharide component **9a** and the acceptor **6**. Accordingly, we screened various Lewis acids. At first we used 5mol% of $AuCl_3$ for this cleavage, but unfortunately the starting material remained unreacted as the external glycan unit decomposed to produce 1,6-anhydro sugar which is similar to the result reported by Kayastha and Hotha in 2013.[34] When the reagent was switched from $AuCl_3$ to TMSOTf, an improvement was observed, and 10% of product **9a** and monosaccharide **6** were formed. On the other hand, trisaccharide **9a** is not produced when TfOH is applied (Scheme 3). When BF_3•Et_2O is used as the Lewis acid, the yield raised to 30%; three Lewis acid equivalents only slightly improved the yield of trisaccharide **9a** to 35% at 25 °C; nevertheless, breakdown happened at 50 °C. Synthesis of trisaccharide **9a** from trisaccharide **7** is notable since the former could produce tetrasaccharide **10a** when 8 mol% of AgOTf and Au-phosphite were present. We thought that a two-step procedure would increase this process' efficiency; specifically, it was suggested that a more nucleophilic species could trap the oxocarbenium ion intermediate from the cleavage of trisaccharide **7**, and then activate the resulting glycoside to produce trisaccharide **9a**. Thus, trisaccharide **7** and pTolSH (**11**) are treated with 1 equivalent of BF_3•Et_2O, as indicated in Scheme 3, to produce thiotolyl gentiobiose **12** in a 35% yield at 25 °C. After treating trisaccharide **7** and *p*TolSH with three equivalents of BF_3•Et_2O, yield got improved to 60%; after 30 minutes at 50 °C, 85% yield is observed. After performing the cut step, we move forward for the next step of the surgery i.e insertion step where we planned to install an external glycan unit **8a** having the carbonate moiety in the anomeric position. The CIStER technique necessitates the development of two orthogonally activable stable glycosyl donors capable of producing the necessary glycosidic linkages in a chemo-specific and quicker kinetic manner. Next, we have successfully investigated the orthogonality relationship between thioglycosyl and ethynylcyclohexyl glycosyl carbonate donors.

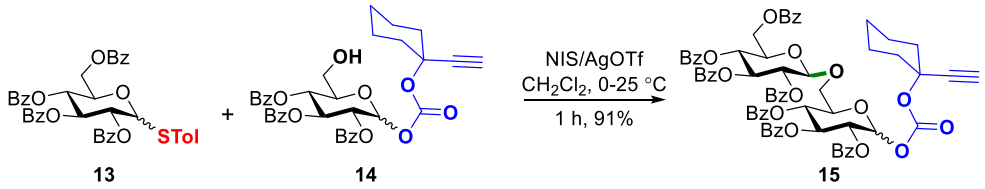


NuH		$AuCl_3$	CuOTf	TMSOTf	TfOH	BF_3•OEt_2	BF_3•OEt_2	
CH_2Cl_2	Product	1 eq.	1 eq.	1 eq.	1 eq.	1 eq.	3 eq.	
6 h		25 °C	25 °C	25 °C	25 °C	25 °C	25 °C	50 °C
8a	**9a**	NR*	ND	10%*	NR	30%	35%*	NR*
11	**10a**	NR	NR	30%	25%	35%	60%	85%**

* **8a** *reacted to produce 1,6-anhydrosugar or decomposed;* ***30 min*

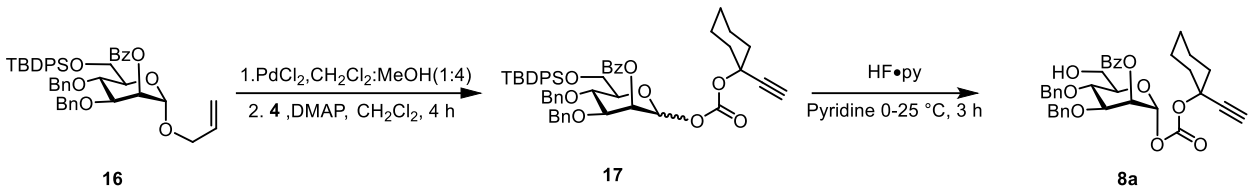
Scheme 3: Optimization of reaction condition for the '*Cut*' step of CIStER reaction sequence

In the presence of *N*-iodosuccinimide/silver triflate (NIS/AgOTf), the thiotolyl per-*O*-benzoyl-D-glucopyranoside **13**,[35] and aglycon **14** were reacted to generate gentiobioside **15** in a 91% yield, indicating that the alkynyl glycosyl carbonate moiety remained stable during the activation of the thiotolyl group.[36]



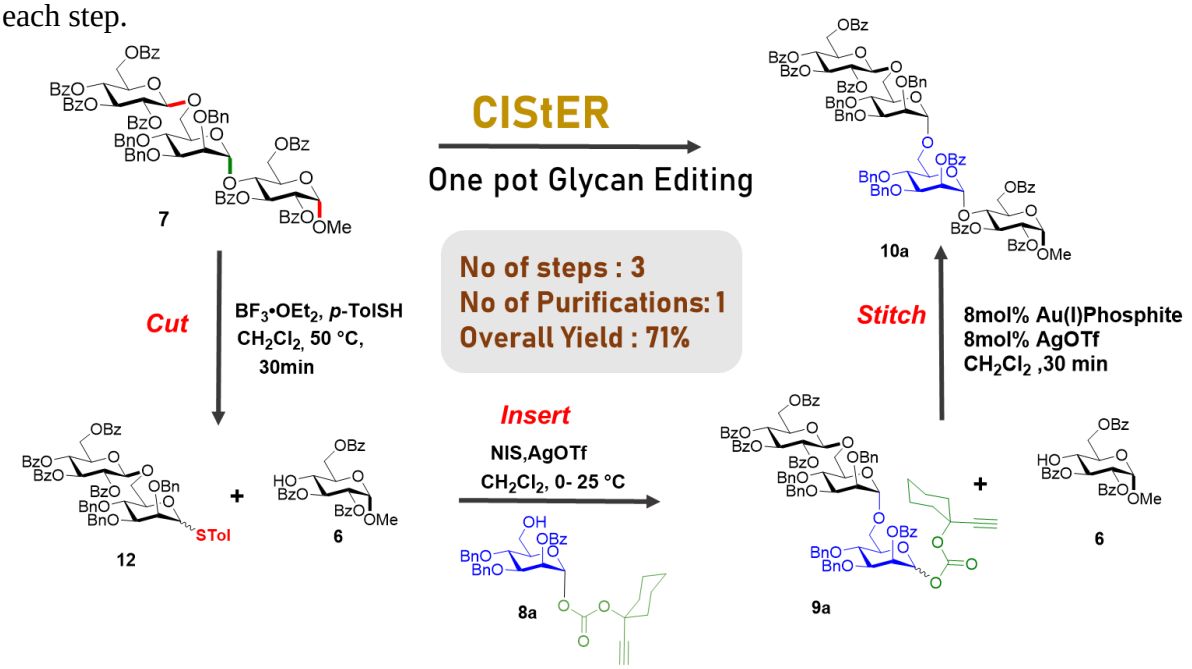
Scheme 4: Activation of STol donor in presence of ethynylcyclohexyl carbonate donor

These findings led us to place the carbonate moiety at the reducing end. Accordingly, we started the synthesis of the aglycon **8a** starting from the previously reported building block **16** in following 3 steps: i) deprotection of allyl group into hemiacetal ii) followed by conversion of the hemiacetal into carbonate donor using carbonate reagent **4** and DMAP iii) finally, deprotection of the C6-TBDPS group by HF•py in Pyridine to generate the C6-OH mannose acceptor **8a** in 88% yield. The compound was characterized by analysing the ^{1}H, ^{13}C NMR spectra.



Scheme 5: Synthesis of C6-OH Mannose acceptor 8a

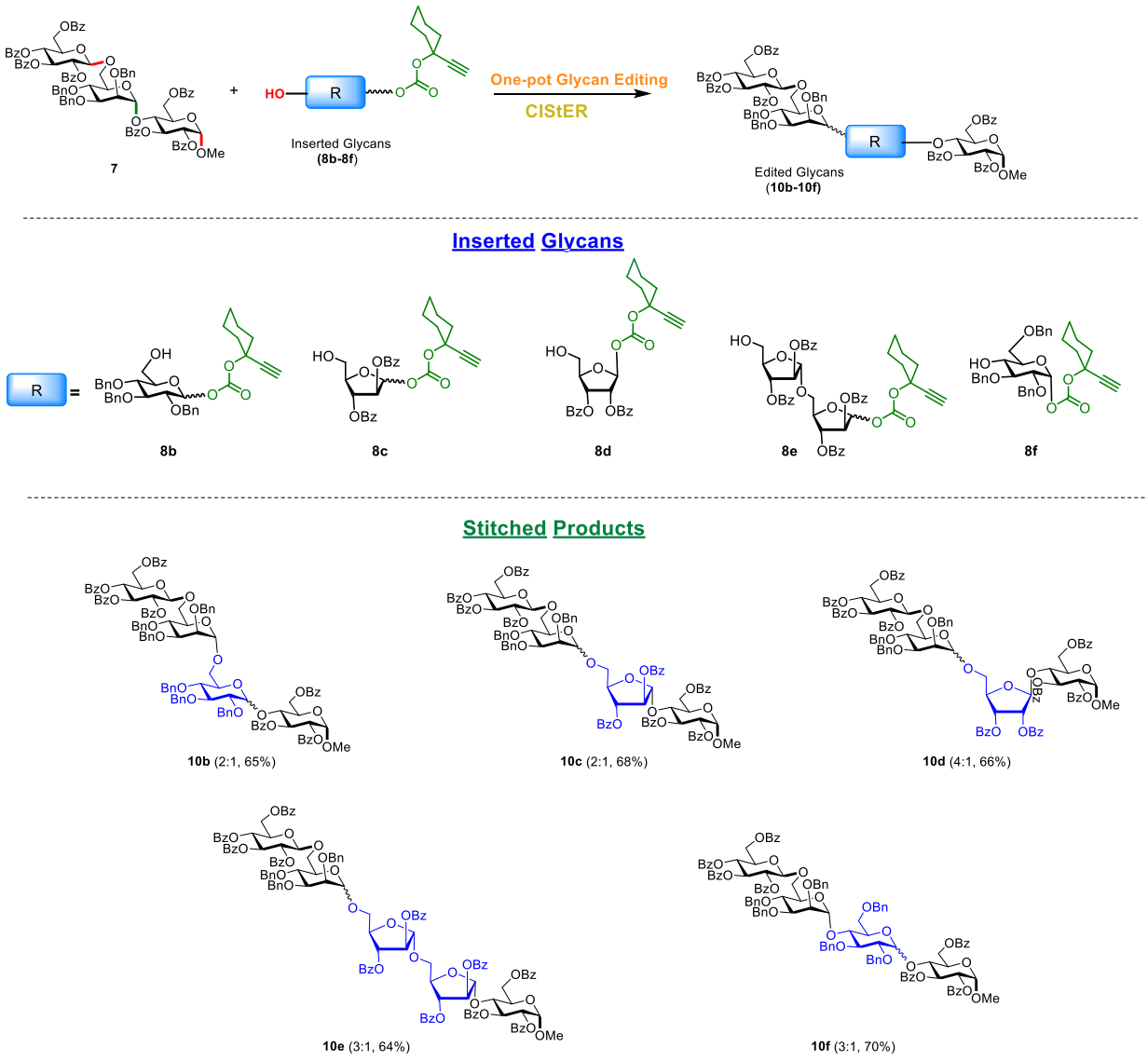
Next Gentiobiose **12** is converted to trisaccharide **9a** within 1h in a facile manner in the presence of *N*-iodosuccinimide/silver triflate (NIS/AgOTf). The reactivity difference between the external glycan unit **8a** (1°-acceptor) and the initially cleaved alcohol (2° acceptor) **6** was utilized strategically to perform the insert step in a specific manner. Finally, the trisaccharide donor **9a** was stitched with the acceptor **6** invoking the Au/Ag-catalyzed glycosylation conditions to produce the edited tetrasaccharide **10a**. This editing reaction, in which the interglycosidic bond of trisaccharide **7** was cut to insert a monosaccharide **8a** and stitched to produce tetrasaccharide **10a**, amply illustrated the potential of CIStER. The molecular surgery (Cut-Insert-Stitch) were performed in a single pot to avoid chromatographic purification after each step.



Scheme 6: Editing of linear trisaccharide 7 by using CIStER reaction sequence

These initial results led us to install various external glycan units into the trisaccharide **7** to obtain diverse class of glycans. The reactivity differential between the in-pot cleaved acceptor moiety and the external glycan unit is crucial for the effective insertion of the external glycans. This thought has prompted us to employ the reactive external glycan units (**8b-8f**). Consequently, the glycans were synthesized (scheme **16-20** detailed in the experimental section) and their structures were determined by using ^{1}H and ^{13}C NMR spectral analysis. After

this, the intermediate disaccharide thiotolyl compound **12** was reacted with the glucosyl acceptor **8b** to produce the edited tetrasaccharide **10b** in 70% yield. The CIStER reaction is also suitable for the insertion of reactive secondary acceptor **8f** to generate the edited tetrasachahride **10f** in 70% yield. In a similar way, furanosyl substrates **8c**, **8d**, **8e** were also inserted to the linear trisaccharide to afford the tetrasaccharides **10c, 10d** and the pentasaccharide **10f** in 68, 66, and 64% yield respectively.



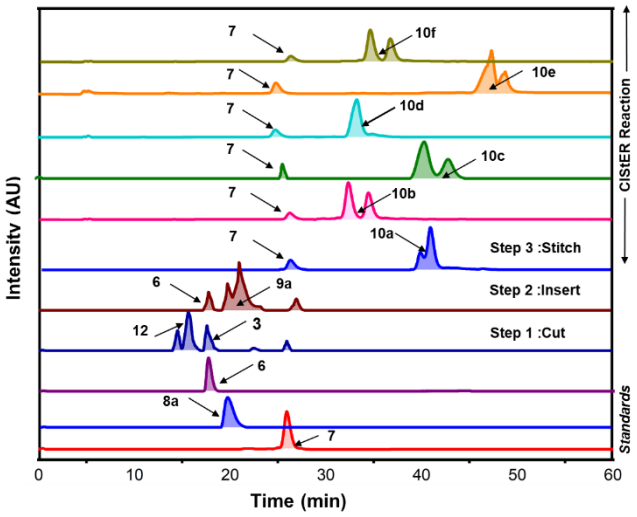
Scheme 7: Insertion of different glycans in to the guide glycan 7 by CIStER reaction sequence

Progress of the one-pot CIStERs was monitored by using semi-preparative HPLC system equipped with a normal phase silica gel diol column. Initially, mobile phase conditions were optimized by injecting the three standards samples **7**, **6**, and **8a** so that the peaks are well resolved for identification purpose. Subsequently, aliquots of CIStER reaction were injected

under above optimized mobile phase conditions. Products **10a**-**10f** and intermediates during the CIStER were collected and characterized.

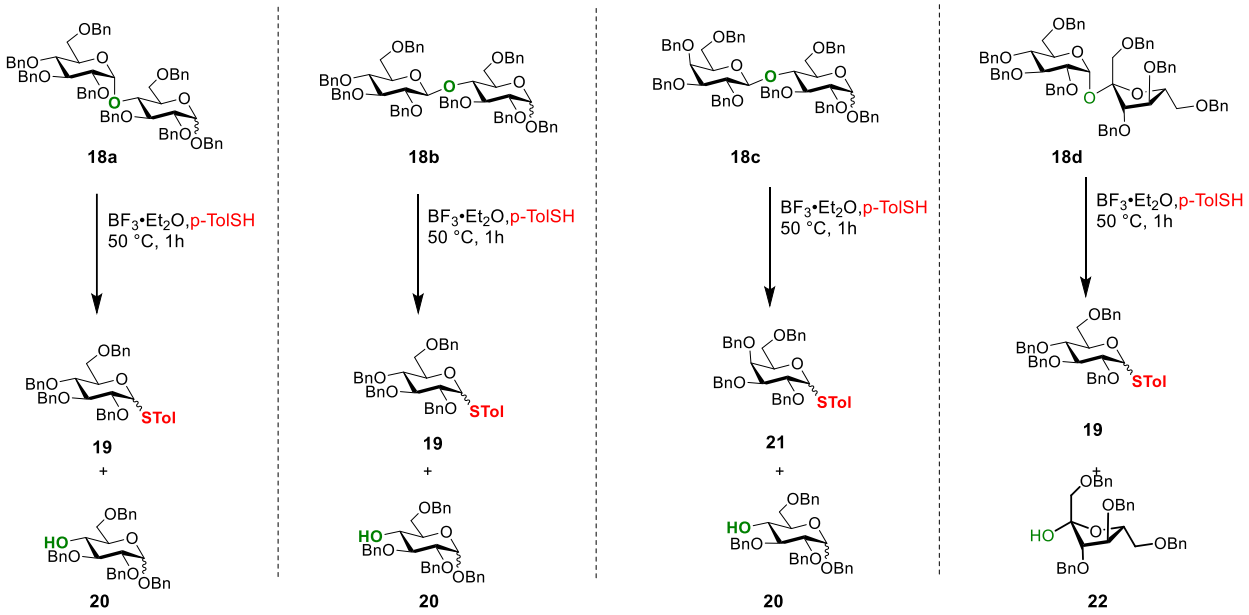
Figure 4: LC Profile of CIStER:

Hplc system: Gilson's PLC 2050 series
Column: Chromasol ONYX DIOL column (5µ, 4.6 mm ×250 mm)
Mobile Phase A: Ethyl Acetate
Mobile Phase B: n-hexane
Column temp: 25 °C
Sample temp: 25 °C
Flow Rate: 1ml/min
Run time: 60 min
Uv-detection: 270nm
Mobile Phase: 20% ethyl acetae/ n-hexane (Isocratic 20% 60 min)



3.2.4 Application of CIStER strategy in naturally occurring Disaccharides:

The effective implementation of the CIStER strategy necessitates careful consideration of the selection of the guide glycan. In our pursuit of enhancing the versatility of CIStER reactions, we have devised a strategy to incorporate naturally occurring disaccharides such as maltose, cellobiose, lactose, and sucrose as guide glycans. The utilization of these disaccharides as guide glycans promises not only a reduction in the number of synthetic steps involved in glycan manipulation but also the generation of a diversified repertoire of glycoconjugates. This diversification is poised to pave the way for novel developments in the field of glycan editing. Our initial approach involved using four per-*O*-benzyl disaccharides as the starting materials. These benzylated derivatives (**18a**-**18d**) were synthesized in a single step from D-maltose, D-cellobiose, D-lactose, and D-sucrose. D-Maltose derivative **18a** underwent the cleavage of the interglycosidic bond to afford the glycosyl donor **19** and the C4-OH glucoside **20** in 90% overall yield. In a similar vein, per-*O*-benzyl cellobiose (**18b**) led to the formation of donor compound **19** and alcohol **20**. Furthermore, we delved into the lactose derivative **18c** by artfully cleaving its interglycosidic bond with the assistance of BF_3•Et_2O at 50 °C resulting in the formation of thiotolyl galactopyranoside **21**, as well as alcohol **20**. Concurrently, the non-reducing sugar disaccharide **18d** was also subjected to same cleavage conditions yielding thiotolyl glucoside **19** and the fructose derivative **22** containing a tertiary hydroxyl group.

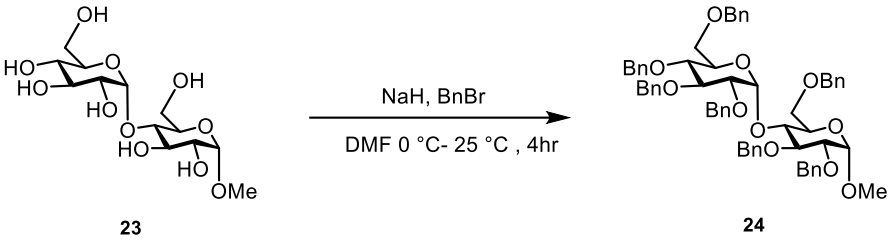


Scheme 8: Cutting of interglycosidic bond of naturally occurring disaccharides

Progress of the cleaving of the per-*O*-benzylated disaccharides was monitored by using semi-preparative HPLC system equipped with a normal phase silica gel diol column. Initially, mobile phase conditions were optimized by injecting the four standards samples **18a-18d** so that the peaks are well resolved for identification purpose. Subsequently, aliquots of cleaving reactions were injected under above optimized mobile phase conditions. Products **19,20,21,22** were collected and characterized. The spectra of the compounds were matched with the previous reports. The effective cleavage of the interglycosidic bond, resulting in the formation of thiotolyl glucoside and galactoside, constitutes a pivotal advancement in the CIStER reactions. This achievement will open avenues for the synthesis of a diversified array of glycoconjugates, as the generated thioglycosides can be readily activated with a wide range of aglycon units which will be discussed in the next section.

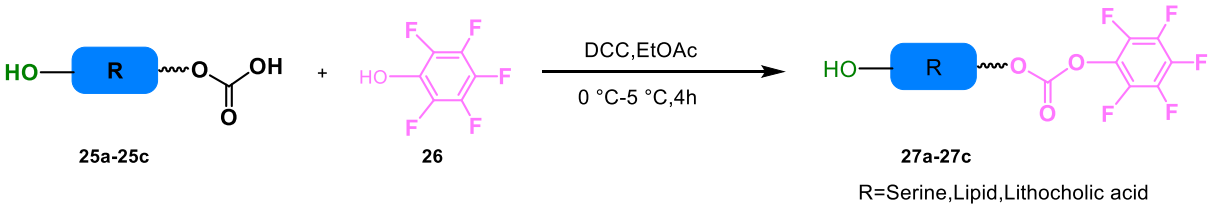
3.2.5 Synthesis of unnatural glycoconjugates by CIStER strategy:

The insights gained from prior studies directed our choice of naturally occurring disaccharides as the guide glycans for the CIStER strategy. To showcase the adaptability of CIStER reactions, we aimed to incorporate a range of pre-designed aglycons such as lipids, amino acids, and steroids. This approach promises to yield a diverse array of glycohybrids, introducing an innovative dimension to the realm of glycan editing. With this idea, we thought to take per-*O*-benzylated maltopyranoside **24** as our guide glycan. This was synthesized in a single step by treating commercially available α-D-maltopyranoside with benzyl bromide and NaH.



Scheme 9: Synthesis of per-*O*-benzylated Maltopyranoside 24

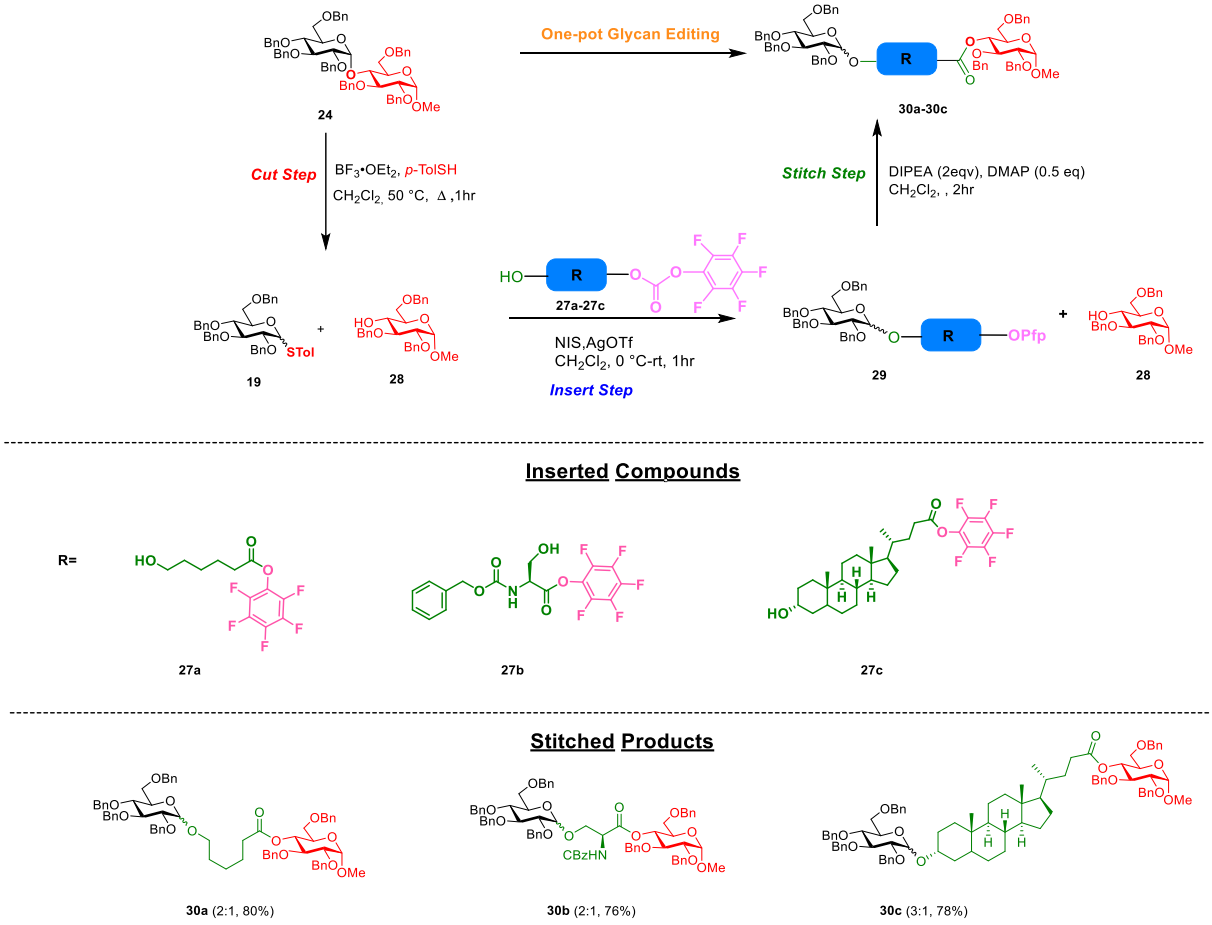
After this we started to synthesize the aglycon motifs which we planned to use as inserts. Here, we thought to make the bifunctional acceptor moiety containing the activatable pentafluorophenol ester moieties. We synthesized the desired pentafluorophenol ester compounds of 6-hydroxycaproic acid **27a**, *N*-Cbz L-serine **27b**, lithocholic acid **27c** by treating the compounds (**25a-25c**) with pentaflurophenol **26** in presence of DCC in ethyl acetate. The desired pentaflourophenol esters (**27a-27c**) were obtained in 85-90% yield and were characterized by ^{1}H, ^{13}C, ^{19}F NMR spectra. The presence of 3 peaks at -152.9, -158.4, -162.5 ppm in the ^{19}F spectra of all the three compounds (**27a-27c**) corresponding to the 5 'F'atoms of the pentafluorophenol ester moiety.



Scheme 10: General Procedure for synthesis of OPfp ester of compounds 25a-25c

After synthesizing the aglycon motifs, we next started to investigate the molecular surgery of our guide glycan i.e the per-*O*-benzylated maltopyranoside. A three-step molecular surgery was performed on methyl per-*O*-benzyl maltopyranoside **24** in a single pot using inserts **27a-27c** to afford CIStER products **30a-30c**. The interglycosidic bond of the maltose derivative **24** was cut in presence of BF_3•Et_2O at 50°C resulting the formation of thiotolyl glucopyranoside **19** and the C4-acceptor **28**. After this, the aglycon motifs **27a-27c** were reacted with the compound **19** in presence of NIS and AgOTf to afford the desired glycosides. Finally, the stitching reaction was performed by subjecting the glycosylated pentafluroester compounds to the esterification under DMAP/DIPEA at 25 °C to afford the glycoconjugates **30a-30c** in 76-80% yields. The stitched compounds **30a-30c** were characterized by ^{1}H, ^{13}C, DEPT NMR spectra. This current approach highlights the adaptability of the CIStER technique in utilizing a range of naturally sourced disaccharides as templates for generating a diverse library of novel

glycohybrids. These glycohybrids serve as potent tools for exploring undiscovered biological pathways and further enriching our comprehension of them.



Scheme 11: Synthesis of glycohybrids by CIStER strategy

3.2.6 Application of CIStER strategy in gm scale synthesis of Human Milk Oligosaccharide:

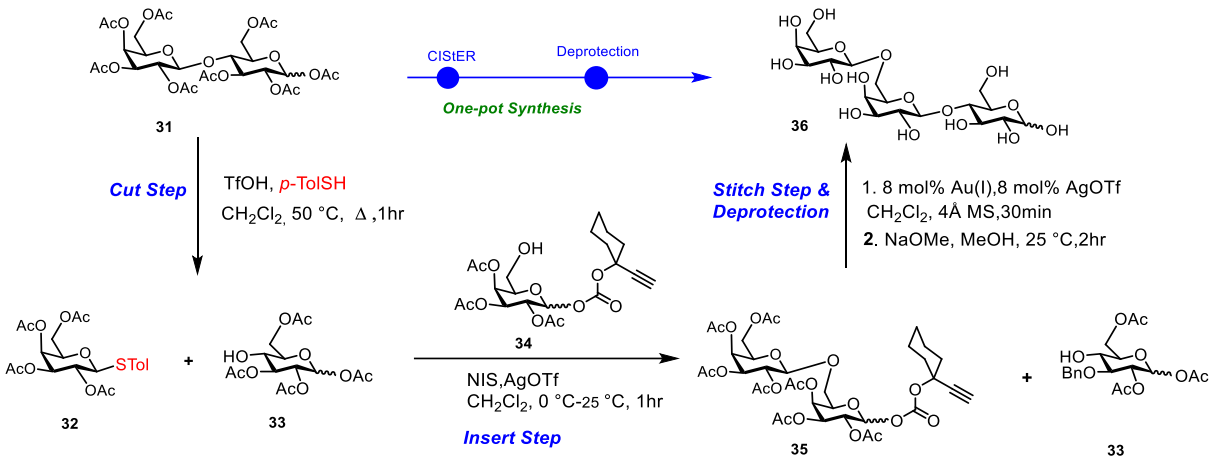
To further demonstrate the versatility of the CIStER technique, we initiated the synthesis of human milk oligosaccharides through the implementation of this editing strategy. Human breast milk has adapted over time to fulfil all the essential requirements of a newborn's body. Recognizing its efficacy, the World Health Organization (WHO) advocates for exclusive breastfeeding for the initial six months of life. Human milk called as "living tissue" because it provides infants with the ideal blend of nutrients and a wealth of bioactive elements, including immunoglobulins, hormones, oligosaccharides, and various other components.[37] Human Milk Oligosaccharides (HMO), multifunctional glycans, are the third largest component of breast milk after lactose (55−70 g/L) and fat (15−40 g/L). They are particularly interesting because of their quantity and structural diversity. HMOs are made of five basic monosaccharides: glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc) and sialic acid

(SA). Almost all HMOs contain lactose (Gal-B1, 4-Glc) at the reducing end, which can be extended with lacto-*N*-biose I (Gal-B1, 3GlcNAc) or lactosamine (Gal-B1, 4-GlcNAc). Branching can be linear or branched through bonds β-1→3 or β 1→6. The sequence can be further modified by the addition of Fuc and/or SA monosaccharides through α- 1→2, 3, 4 and alpha 2→3,6 bonds due to the action of fucosyltransferases and sialyltransferases.[38] Traditionally, research on HMOs has been limited due to insufficient amounts, which is caused by challenges in isolating native HMOs from human milk and unscalable syntheses. It is thus up to the chemical community to provide a feasible programme for accessing a varied array of HMOs and new probes at glycobiology-enabling sizes.[39]

All HMOs are based on a lactose molecule (a disaccharide composed of a galactose molecule connected by a β-1→4-glycosidic bond to a glucose molecule). Previously described studies of our developed CIStER strategy shows that the molecular surgery can be performed on the naturally occurring disaccharides to generate various glycoconjugates. Here in this case, we thought to implement the CIStER strategy to the synthesis of HMO. The main focus for implementing the cut-insert-stitch editing reaction sequence was to simplify the synthesis of some of those HMO and producing the oligosaccharides in gram scale quantity.

We began our investigation by taking the commercially available lactose octaacetate (**31**) as our starting material. The previous understanding of the CIStER strategy gave us the insight that the electron donating protecting group is very much essential for the successful cleavage of the glycosidic bond. However, the presence of the acetate group at C2- position of per-*O*-acetyl lactose shows the disarming effect i.e disfavors the formation of the oxocarbenium ion making the glycosidic bond strong. For this reason, we started to investigate the bond cleaving condition in presence of various acidic promoters. Interestingly, we discovered that to successfully cleave the glycosidic bond bearing the electron-withdrawing C2-acetyl group, more acidic conditions are necessary, such as those induced by TfOH-mediated cleavage. Accordingly, the glycosidic bond of the lactose octacetate **31** was treated with TfOH and *p*-TolSH in CH_2Cl_2 at 50 °C for 1 h, generating thiotolyl galactosyl derivative **32** and the C4-OH glucose acceptor **33** in the reaction mixture. After making our desired compound **34** (synthesis is discussed in the experimental section), it was subjected to the glycosylation with the thiogalactoside **32** in the presence of NIS and AgOTf to obtain a disaccharide moiety with carbonate moiety at the reducing end. Finally, the unreacted acceptor **33** generated by the cleaving of the guide glycan **31** was stitched with the disaccharide carbonate donor **35** using 8 mol% of [Au]/[Ag]-catalysts to obtain the trisaccharide compound. After this the trisaccharide

compound was subsequently deprotected under Zemplén conditions to afford our desired compound 6′-galactosyllactose **36** in 71% overall yield.



Scheme 12: Application of CIStER technique in the synthesis of Human milk oligosaccharide

The compound **36** was thoroughly characterized by the ^{1}H, ^{13}C, HSQC NMR spectra. These experiments clearly demonstrate the power of the CIStER strategy for the gram scale synthesis of important HMOs. Furthermore, the present editing strategy enables the potential generation of structurally well-defined HMOs in substantial quantities. This, in turn, accelerates ongoing studies on the structure-activity relationship of these HMOs and enhances the value of target-directed carbohydrate synthesis in the pursuit of novel molecules with clinical/nutritional significance.

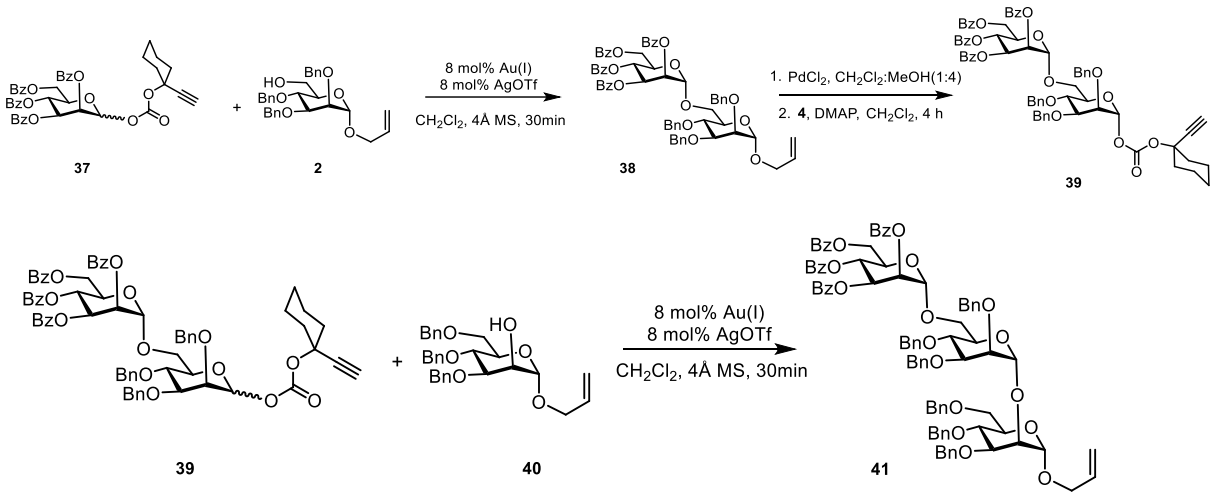
3.2.7 Application of CIStER strategy in synthesis of linear and branched oligosaccharides reminiscent of MTb. cell wall:

After the successful execution of the CIStER strategy in generating diverse class of glycohybrids, we next started to use this powerful and innovative strategy in the realm of higher oligosaccharide synthesis. Oligosaccharides are highly intricate structures and, as biopolymers found throughout life, are thought to play a significant part in a variety of biological systems and signal recognition processes. Because of their crucial roles, oligosaccharides have received a lot of interest in the biological sciences.[41] However, it is difficult to obtain homogeneous oligosaccharide samples during the glycosylation processes. Historically, chemical synthesis has been used as an exploratory research strategy to achieve homogeneous samples and prepare homogenous oligosaccharide.[42] Over the past few decades there has been an increasing number of studies on the synthesis of oligosaccharides by chemical methods.[43] These include versatile techniques such as use of proximal protecting groups,[44] distal auxiliary groups for

stereoselective glycosylation,[45] metal-catalysed glycosylation reaction,[46] non-metal catalyzed glycosylation reaction,[47] one pot glycosylation reaction[48] and solid phase glycosylation reactions.[49] In this context, we were interested to check the potential of the developed *cut-insert-stitch editing reaction sequence* to generate different oligosaccharides. The major focus of using the current CIStER sequence in the development of oligosaccharide synthesis was to streamline a one-pot chemical transformations to rapidly assemble stereoselective glycosylation. Along with this, CIStER strategy judiciously uses the guide glycan as the starting point from which different glycan units can be surgically inserted to generate longer oligosaccharides.

We were very much interested to check the application of this current surgical chemical glycan editing technique in the synthesis of different linear and branched lipoarabinomannan (LAM) analogues present in the cell wall of the *Mycobacterium tuberculosis*. LAM stands out as one of the most antigenic epitopes found in the cell wall of Mtb. Presently, it is under thorough investigation as a prime target for the advancement of vaccines and diagnostic tools. LAM is structurally more diverse where a mannose backbone having a α-1,2 and α-1,6 linked mannopyranoside residues are attached to arabinofuranosides.[50] Based on our previous knowledge of importance of arming and disarming effect of protecting group for successful execution of CIStER, we envisioned that the trimannosaccharide **41** could be our starting point for assembling the higher oligosaccharide sequence. We began our studies by synthesizing the guide glycan **41** from the three easily accessible building blocks.

3.2.7.1: Synthesis of Mannotrisaccharide 41:



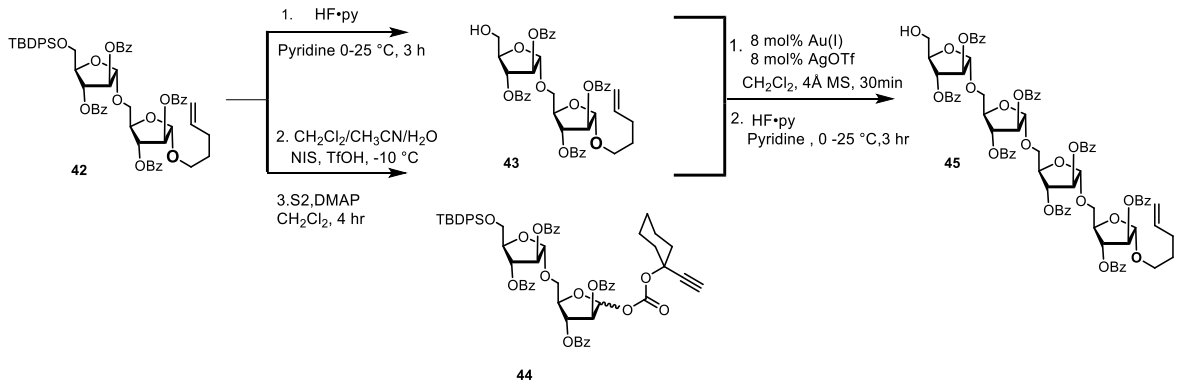
37 2 38 39 39 40 41

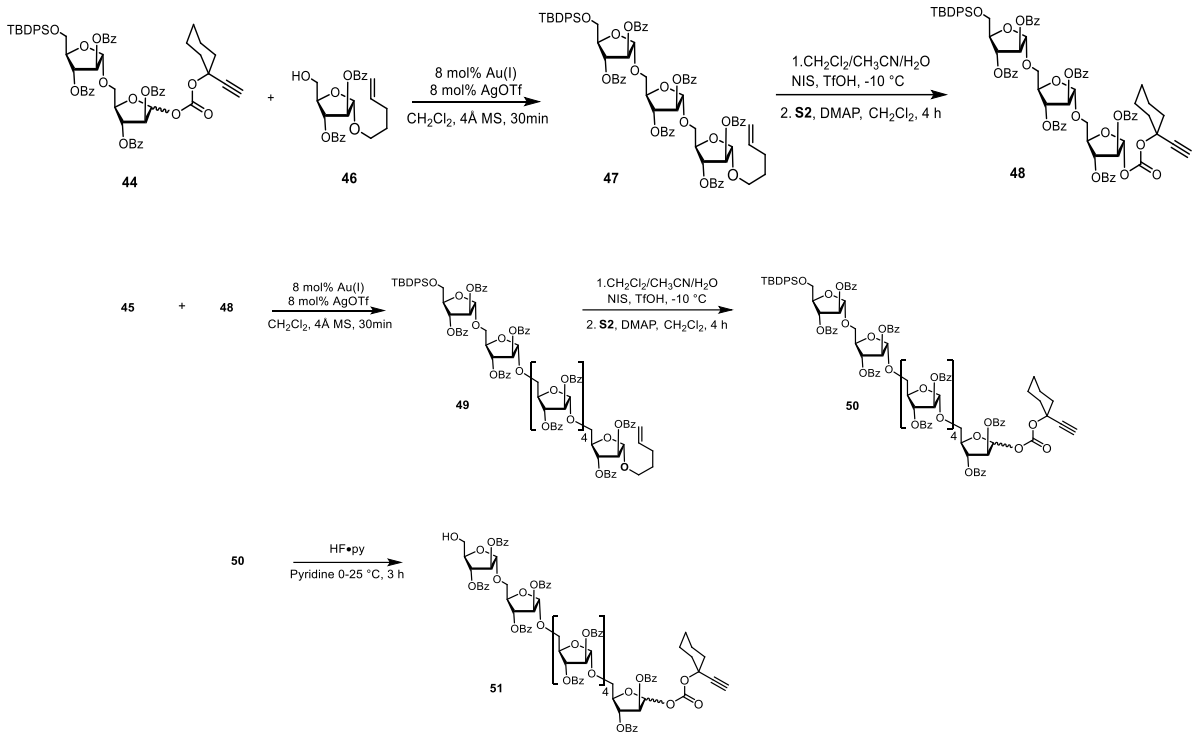
Scheme 13: Synthesis of mannotrisaccharide 41

The per-*O*-benzoylated mannosyl donor **37** was coupled with the previously synthesized C6-OH mannose acceptor **2** using Au/Ag catalysts to generate the disaccharide **38**, which was converted into glycosyl donor by two steps: allyl group deprotection and converting into carbonate donor **39**. Further the glycosyl donor **39** and the acceptor **40** were glycosylated to give our desired guide glycan **41**. The compound **41** was characterized by ^{1}H, ^{13}C NMR spectra. Also the linkage in the trisaccharide was confirmed by coupled HSQC spectra. Next for successful synthesis of LAM component using the CIStER strategy, we started to synthesize the desired glycan motifs *i.e* the linear and branched heptasaccharide units.

3.2.7.2: Synthesis of linear heptasaccharide motif:

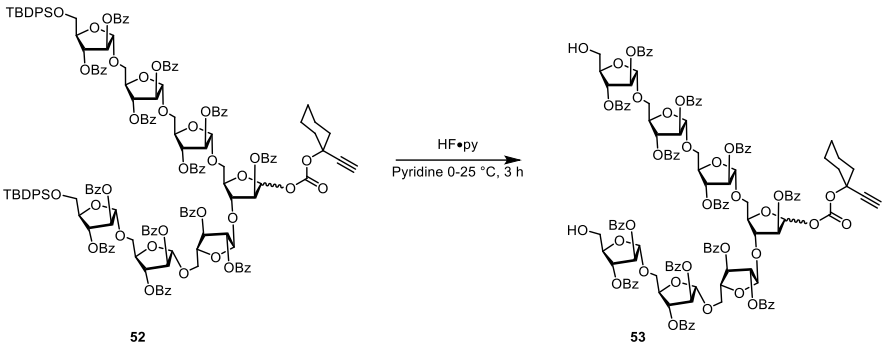
The synthesis of the linear heptasaccharide (**51**) was initiated from the generation of the disaccharide donor **44** and acceptor **43** from a common disaccharide **42**. After this, donor **44** and acceptor **43** were glycosylated to generate the tetrasaccharide unit which was deprotected further using HF•py to make the tetrasaccharide acceptor **45**. The trisaccharide glycosyl donor **48** was generated from two building blocks **44** and **46** in 3 steps viz. i) glycosylation of donor **44** and acceptor **46** to generate the disaccharide **47**, ii) deprotection of the pentenyl moiety, and iii) conversion of hemiacetal into the carbonate donor. After that, the trisaccahride donor **48** and the tetrasacccharide acceptor **45** were glycosylated under Au/Ag catalysis to generate the heptasaccharide unit **49** in 80% overall yield. In the ^{13}C NMR spectrum of compound **49**, the presence of the seven anomeric carbons between δ 106.1- 102.4 ppm and further distinctive carbonyl carbon of carbonate moiety at δ 151 ppm with the five methylene carbons of the cyclohexyl ring at δ 36.9, 36.8, 25.0, 22.7, and 22.7 ppm confirmed the successful synthesis of the linear heptasaccharide **49**. Finally, the TBDPS group of the compound **50** was subjected to HF•py deprotection to provide the desired glycan **51** in 78% yield.





Scheme 14: Synthesis of linear heptasaccharide aglycon motif 51

3.2.7.3: Synthesis of branched heptasaccharide motif 53:

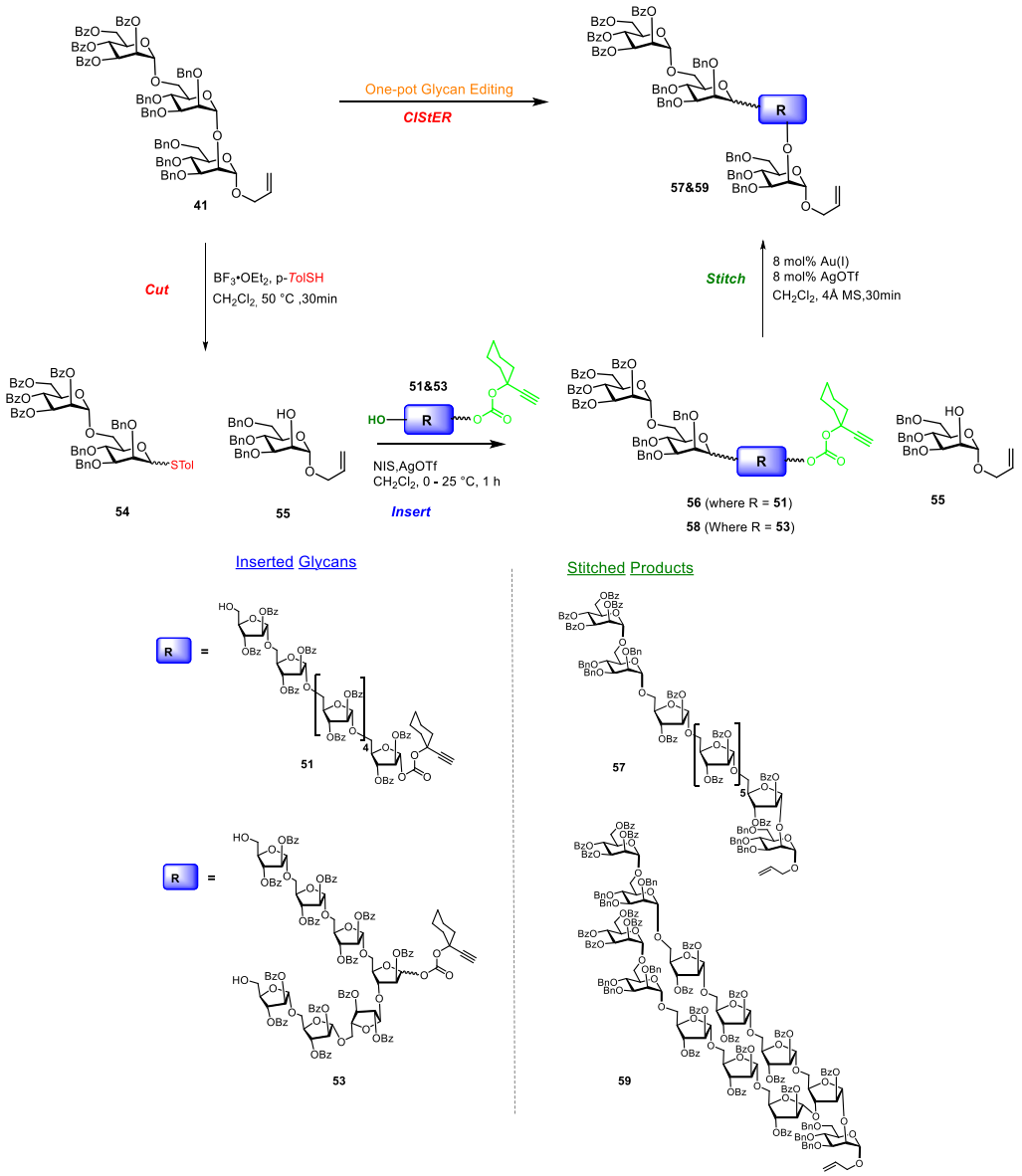


The synthesis of the branched heptasaccharide motif was started from the previously synthesized heptasaccharide compound **52** (The procedure was detailed in Chapter 2). The two TBDPS groups were deprotected by using the HF•py to provide the desired glycan **53** in 81% yield. The compound was thoroughly characterized by ^{1}H and ^{13}C NMR spectra analysis.

3.2.7.4: One pot Editing of Mannose trisaccharide by CIStER:

After the successful synthesis of our desired guide glycan **41** and the glycan inserts **51** and **53**, we moved forward to the execution of molecular surgery by CIStER strategy to generate the desired LAM components. At first, we performed the cleavage reaction on the trisaccharide **41** by treating it with 3 eq. of BF_3•Et_2O in presence of *p*-TolSH. In this case, the most acid

labile glycosidic bond of the trisaccharide was selectively cleaved and trapped to generate the disaccharide **54** with an anomeric sulphide and the monosaccharide acceptor **55** with a free secondary axial alcohol at the C2- position. At this stage, the insert linear heptasaccharide unit **51** was reacted with the disaccharide thiotolyl donor **54** in presence of NIS and AgOTf to produce the compound **56** having the carbonate moiety at reducing end. Notably, in this step the initially cleaved secondary acceptor remain unreacted in the reaction mixture. Finally, the stitching step was performed using the Au/Ag-catalyzed activation of ethynylcyclohexyl carbonate moiety. Here the unreacted acceptor unit **55** was coupled with the compound **56** to produce the decasaccharide **57** in just 15 min in an overall of 51% yield. Similarly, the two molar equivalent of branched heptasaccharide moiety **53** was also reacted with the common disaccharide thiotolyl donor to generate the compound **58** in the reaction mixture. Finally, this compound was similarly stitched with the monosaccharide acceptor unit by invoking the Au/Ag catalysis to generate the dodecasaccharide **59** in overall 45% yield.



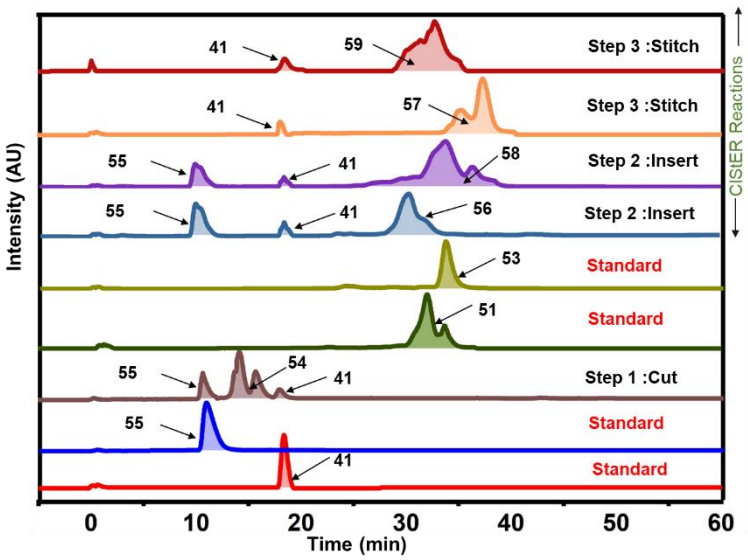
Scheme 15: Synthesis of linear and branched oligosaccharides using CIStER

The cut-insert-stitch reaction sequence was monitored by using semi-preparative HPLC system equipped with a normal phase silica gel diol column. Initially, mobile phase conditions were optimized by injecting the four standards samples **41**, **51**, **53**, and **55** so that the peaks are well resolved for identification purpose. Subsequently, aliquots of CIStER reaction were injected under above optimized mobile phase conditions. Products and intermediates during the CIStER were collected and characterized. The produced higher oligosaccharide compounds were thoroughly characterized by ^{1}H, ^{13}C NMR spectra. In addition, MALDI (TOF) spectra clearly showed the formation of both the decasaccharide **57** and the dodecasaccharide 59.

Figure 5: LC Profile of CIStER:

Gilson's PLC 2050 series
Column: Chromasol ONYX DIOL column (5µ, 4.6 mm ×250 mm)
Mobile Phase A: Ethyl Acetaate
Mobile Phase B: n-hexane
Column temp: 25 °C
Sample temp: 25 °C
Flow Rate: 1ml/min
Run time: 60 min
Uv-detection: 270nm
Mobile Phase:

Time (min)	%A	%B
0	20	80
15	20	80
20	30	70
30	30	70
40	40	60
50	100	00
60	100	00



The above described results clearly illustrate the potential of the CIStER strategy in generating longer oligosaccharides in one-pot manner. The judicial selection of the guide glycan and the protecting groups are very much essential for the successful execution the CIStER technique.

In summary, these findings effectively showcase the surgical editing of glycans using CIStER. Such CIStER procedures demand meticulous planning of the target glycan and subtle stereoelectronic considerations, leading to modifications in their interglycosidic bonds susceptible to hydrolysis. CIStER operates by activating glycosyl donors through trapping the oxocarbenium ion intermediate with a nucleophile (*p*TolSH), which can be selectively activated alongside an aglycon furnished with another leaving group (ethynylcyclohexyl carbonate) to yield a novel glycoside. Subsequently, ethynylcyclohexyl carbonate is activated to link the glycan and generate a collection of higher oligosaccharides in a precise manner. Through the CIStER approach, a repertoire of novel glycohybrids can be generated, serving as potent tools for elucidating new biological pathways and enhancing our current comprehension thereof. Efforts to extend the CIStER methodology to small molecules are presently in progress.

3.3 Experimental Section:

Procedure A: Deprotection of Allyl Glycosides: To a solution of the allyl glycoside (1.0 mmol) in CH_2Cl_2/MeOH (1:4 in 10 mL), a solution of $PdCl_2$ (0.3 mmol) in MeOH (6 mL) was added and the reaction mixture was stirred at 25 °C for 5 h. After complete conversion, the reaction mixture was neutralized by the addition of excess Et_3N (~2 mL) and the solid residue was filtered off through a pad of Celite®. The volatile organics were evaporated *in vacuo* and the crude residue was purified by silica gel column chromatography to obtain desired hemiacetals.

Procedure B: Deprotection of Pent-4-ene-1-ol Glycosides: The pent-4-enyl glycoside (1.0 mmol) was dissolved in a mixture of solvents $CH_2Cl_2/CH_3CN/H_2O$ (5.0:3.0:0.5) and the reaction mixture was cooled to -10 °C. After 15 min, NIS (2.2 mmol) and TfOH (0.2 mmol) were added to the reaction mixture simultaneously and the reaction was stirred for 1 h at 0 ° C. After completion, the reaction mixture was quenched with $NaHCO_3$ solution and diluted with CH_2Cl_2. The organic layer was successively washed with water (2x25 mL), brine solution (1x25 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated in *vacuo* to obtain a crude residue that was purified by silica gel column chromatography using ethyl acetate and hexane to furnish corresponding hemiacetals.

Procedure C: Synthesis of Ethynyl Cyclohexyl Glycosyl Carbonate Donors: To a solution of hemiacetal (1.0 mmol) in anhydrous CH_2Cl_2 (5 mL), ethynyl cyclohexyl (4-nitrophenyl) carbonate **4** (1.2 mmol) and DMAP (1.1 mmol) were added and the reaction mixture was stirred at 25 °C for 5 h. the reaction mixture was concentrated and purified by silica gel column chromatography (EtOAc/Hexane as mobile phase) to obtain ethynylcyclohexyl glycosyl carbonate donors.

Procedure D: Deprotection of the TBDPS-ethers: To a solution of the silylated ether (1.0 mmol) in anhydrous pyridine and THF (4 mL, 3:1), HF•Py (3 mmol per TBDPS) was added at 0 °C and the reaction mixture was stirred at 25 °C for 3 h. After completion, the reaction mixture was quenched by the drop-wise addition of 2*N* HCl at 0 °C and diluted with 25 mL of ethyl acetate. The organic layer was successively washed with 1*N* HCl (25 mL), saturated aqueous solution of $NaHCO_3$ (25 mL) and brine solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using ethyl acetate and hexane to furnish corresponding alcohol.

Procedure E: Glycosidation using Ethynyl Cyclohexyl Glycosyl Carbonate Donors:

To a solution of acceptor (1.0 mmol) and donor (1.1 mmol) in anhydrous CH_2Cl_2 (5 mL) was added freshly activated 4Å MS powder (0.200 g) at 25 °C under argon atmosphere. After 15 min of vigorous stirring at 25 °C, chloro[tris(2,4-di-*tert*butylphenyl)phosphite]gold(I) (8 mol%) and AgOTf (8 mol%) were added simultaneously to the reaction mixture and stirred for 30 min to 1 h. After consumption of the glycosyl acceptor, the reaction mixture was filtered through a bed of Celite® and the filtrate was concentrated *in vacuo*, the residue was purified by silica gel column chromatography using EtOAc and Hexane as mobile phase.

General reaction procedure for one-pot Glycan Editing methodology: To a solution of trisaccharide **7** (1eq., 300 mg) in 3 mL of anhydrous CH_2Cl_2, BF_3•OEt_2 (3 eq, 74 µL) was added at 0 °C. After 10 min, reaction mixture was refluxed at 50 °C for 30 min. The reaction mixture was brought to 25 °C, 1 equivalent of glycosyl acceptor **8a-8f** [**8a** (121mg), **8b** (118 mg), **8c** (100 mg), **8d** (100 mg,), **8e** (167 mg),8f (175mg)] were added to the reaction mixture and cooled to 0 °C. Freshly activated 4Å MS powder (80mg) was added at 0 °C under nitrogen atmosphere and kept for vigorous stirring for another 15 min. Then NIS (1.5 eq, 66 mg), AgOTf (1.5 eq, 10 mg) were added simultaneously. The reaction mixture was stirred for 15 min at 0 °C and gradually warmed to 25 °C and stirred for 45 min. After this, chloro[tris(2,4-di^{t}butylphenyl)phosphite] gold(I) (0.08 eq, 13 mg), AgOTf (0.1 eq, 5 mg) were added simultaneously to the reaction mixture and stirred for another 30 min. Et_3N was added to quench the reaction mixture and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography (30% - 40% ethyl acetate/hexane) to afford the compounds **10a-10f**.

One-pot Glycan Editing methodology for the synthesis of glycohybrids:

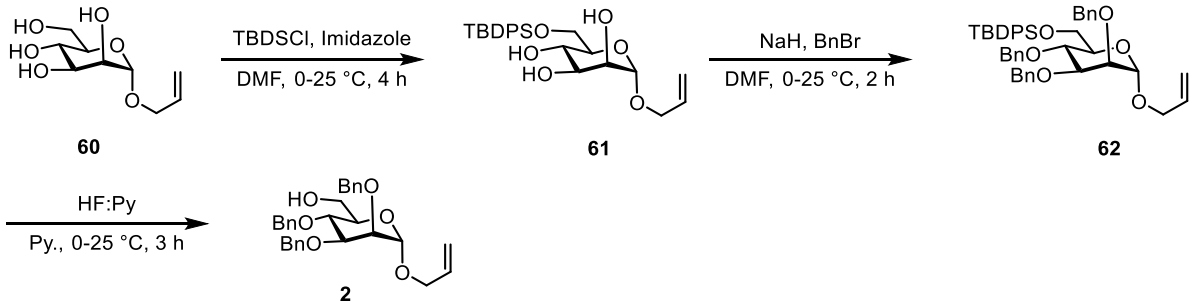
To a solution of disaccharide **24** (1eq, 500 mg) in 4 mL of anhydrous CH_2Cl_2, BF_3•OEt_2 (3 eq, 188 µL) was added at 0 °C. After 10 min, reaction mixture was refluxed at 50 °C for 1hr. The reaction mixture was brought to 25 °C, 1 equivalent of compounds **27a-27c** [**27a** (170mgmg), **27b** (310mg), **27c** (232 mg)] were added to the reaction mixture and cooled to 0 °C. Freshly activated 4Å MS powder (80mg) was added at 0 °C under nitrogen atmosphere and kept for vigorous stirring for another 15 min. Then NIS (1.5 eq, 194 mg), AgOTf (0.25 eq, 36 mg) were added simultaneously. The reaction mixture was stirred for 15 min at 0 °C and gradually warmed to 25 °C and stirred for 45 min. After this, DIPEA (2eq, 164 µL), DMAP (0.5 eq, 29 mg) were added simultaneously to the reaction mixture and stirred for another 2 hr. After completion, the reaction mixture was concentrated under reduced pressure. The crude residue

was purified by silica gel column chromatography (15% - 20% ethyl acetate/hexane) to afford the compounds **30a-30c**

Procedure for one-pot Glycan Editing methodology of trimannosaccharide:

The trisaccharide **41** (300 mg, 0.266 mmol) was dissolved in 3mL of anhydrous CH_2Cl_2. The reaction vessel was cooled to 0 °C and BF_3•OEt_2 (98.7 µL, 0.799 mmol) was added. The reaction mixture was refluxed at 50 °C for 30 min, brought to 25 °C and external glycan acceptor (1 eq. for **51** 0.5 eq. for **53**) [**51** (674 mg,) or **53** (323 mg)] were added to the reaction mixture and the reaction vessel was stirred at 0 °C. To this reaction mixture, freshly activated 4Å MS powder (80 mg) was added at 0 °C under nitrogen atmosphere and kept for vigorous stirring for another 15 min. Then NIS (1.5 eq., 89 mg), AgOTf (0.2 eq., 13 mg) were added simultaneously and stirred for 15 min at 0 °C and kept at 25 °C for another 45 min. After this to this reaction mixture, chloro[tris(2,4 di^{t}butylphenyl)phosphite] gold(I) (0.08 eq., 14 mg), AgOTf (0.1 eq., 5 mg) were added simultaneously to the reaction mixture and stirred for another 30 min. Et_3N was added to arrest the reaction and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography (30% - 40% ethyl acetate/hexane) to afford the oligosaccharides **57** and **59**.

Allyl 2,3,4 tri-*O*-benzyl α-D-mannopyranoside (2):



Scheme 16: Synthesis of C6-OH Mannose acceptor 2

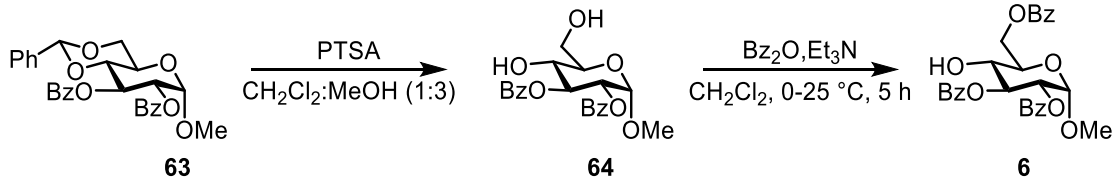
To a solution of DMF (25 mL) of the compound **60**[51] (5.00 g, 22.91 mmol) at 0 °C imidazole (3.82 g, 45.82 mmol), TBDPSCl (7mL, 24.97 mmol) were added and resulting solution was kept 15 min at 0 °C and then heated to 25 °C. After 3 h, the reaction was arrested by the addition of ice-cold water, extracted with ethyl acetate (3x400 mL). Combined organic layers were washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, the crude was the purified by silica gel column chromatography (40% ethyl acetate in hexane) to provide compound **61** as a thick syrup (92%). Further, compound **61** (9.82 g, 21.08 mmol) in 150 mL of DMF was cooled to 0 °C, NaH (3.03 g, 75.84 mmol) was added portion wise over a period

of 10 min, and stirred. After 15 min, benzyl bromide (11.26 mL, 94.20 mmol) was slowly added at 0 °C and stirred at 25 °C. After 2 h, the reaction mixture was poured into ice cold water, extracted with ethyl acetate (2x50 mL), the organic layer was washed with brine solution, combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Resulting crude residue was purified by silica gel column chromatography (15% ethyl acetate/hexane) to give the compound **62** as a yellow syrup (88%).

In continuation, the compound **62** (5.0 g, 6.86 mmol) was dissolved in anhydrous pyridine (30 mL), HF•py (1.84 mL, 20.58 mmol) was added at 0 °C and the reaction mixture was stirred at 25 °C for another 3 h. Upon completion of the reaction, the reaction mixture was quenched by the addition of 1*N* HCl at 0 °C and diluted with ethyl acetate. The organic layer was successively washed with saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified with silica gel column chromatography (25% ethyl acetate/hexane) to furnish the compound **2** as a white solid (85%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 – 7.27 (m, 15H), 5.88 – 5.77 (m, 1H), 5.24 – 5.13 (m, 2H), 4.94 (dd, *J* = 10.9, 1.7 Hz, 1H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.82 – 4.59 (m, 6H), 4.17 – 4.07 (m, 1H), 4.01 – 3.94 (m, 2H), 3.94 – 3.87 (m, 1H), 3.84 – 3.76 (m, 3H), 3.66 (td, *J* = 7.0, 3.8 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 138.6, 138.5, 138.3, 133.7, 128.6(2C), 128.5(4C), 128.2(2C), 128.0(2C), 127.9, 127.9, 127.7, 127.7(2C), 117.5, 97.5, 80.3, 75.4, 75.0, 74.9, 73.1, 72.4, 72.3, 68.0, 62.5; **mp:** 129 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = +62° (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3445, 2919, 1454, 1027, 747, 696, 665; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{30}H_{34}NaO_6$: 513.2253; found 513.2250

Methyl 2,3,6 tri-*O*-benzoyl α-D-glucopyranoside (**6**):



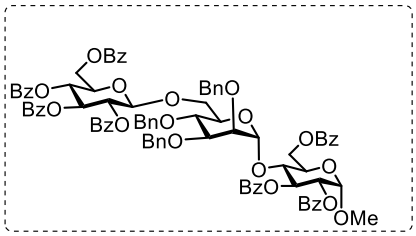
63 **64** **6**

Scheme 17: Synthesis of C6-OH Glucose acceptor 6

To a solution of compound **63**[52] (5.0 g, 10.19 mmol) in 80 mL of CH_2Cl_2:MeOH (1:3), *p*-toluenesulphonic acid (PTSA) (877 mg, 5.10 mmol) was added and stirred at 25 °C. After 4 h, the reaction mixture was neutralized by addition of Et_3N, extracted with CH_2Cl_2 (2x25 mL), the organic layer was washed with sodium bicarbonate and brine solution. Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, the resulting crude

residue was purified by silica gel column chromatography (45% ethyl acetate/hexane) to obtain the compound **64** as a thick syrup (85%). Further, compound **64** (3.49 g, 8.66 mmol) was taken in 40 mL of anhydrous CH_2Cl_2 and Et_3N (6.93 mL, 49.70 mmol) and benzoic anhydride (Bz_2O) (2.70 gm, 11.93 mmol) were added simultaneously at 0 °C. The reaction mixture was brought to 25 °C and stirred for 2 h, extracted with CH_2Cl_2, the organic layer was washed with sodium bicarbonate and brine solution. Collected organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated to obtain a crude residue that was purified by silica gel column chromatography (20% ethyl acetate/hexane) to afford the compound **6**as a white solid (76%) ;**^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.13 – 8.07 (m, 2H), 8.06 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.98 (dd, *J* = 8.4, 2.5, 2H), 7.60 – 7.33 (m, 9H), 5.83 (dd, *J* = 10.1, 9.2 Hz, 1H), 5.30 – 5.25 (m, 1H), 5.16 (d, *J* = 3.7 Hz, 1H), 4.81 – 4.75 (m, 1H), 4.66 (dd, *J* = 12.1, 2.3 Hz, 1H), 4.13 (s, 1H), 3.92 (d, *J* = 4.6 Hz, 1H), 3.59 (d, *J* = 4.9 Hz, 1H), 3.45 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 167.3, 167.0, 166.1, 133.5, 133.4, 133.4, 130.0(2C), 129.9(2C), 129.9(2C), 129.8, 129.3, 129.2, 128.6(2C), 128.5(2C), 128.5(2C), 97.2, 73.9, 71.6, 70.1, 69.7, 63.6, 55.5; **mp**: 74 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = +149° (*c* 0.18 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3455, 3024, 1719, 1452, 1270, 1094, 1068, 1026, 748, 707, 666; **HRMS** (ESI-MS): $[M+H]^+$ m/z calculated for $C_{28}H_{27}O_9$: 507.1655 found 507.1658

Methyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α-D-mannopyranosyl) α-D-glucopyranoside (**7**):



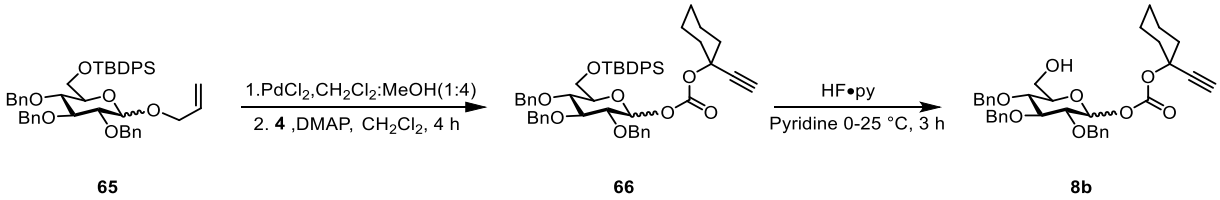
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, *J* = 7.8 Hz, 2H), 8.01 – 7.71 (m, 12H) ,7.52 – 7.14 (m, 36H), 5.99 (t, *J* = 9.5 Hz, 1H), 5.88 (t, *J* = 9.6 Hz, 1H), 5.63 (q, *J* = 9.8 Hz, 2H), 5.12 (d, *J* = 3.4 Hz, 1H), 5.09 (d, *J* = 3.2 Hz, 1H), 5.07 (d, *J* = 9.6 Hz, 1H), 4.80 (d, *J* = 7.7 Hz, 1H), 4.70 (d, *J* = 11.1 Hz, 1H), 4.59 – 4.34 (m, 6H), 4.23 (dd, *J* = 16.3, 10.8 Hz, 2H), 4.12 – 3.97 (m, 3H), 3.97 – 3.85 (m, 3H), 3.75 (q, *J* = 10.0, 9.5 Hz, 3H), 3.56 (s, 1H), 3.40 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3(2C), 166.0(2C), 165.6, 165.3, 138.7, 138.5, 138.3, 133.7(2C), 133.4(4C), 133.3(2C), 133.2(2C), 133.1, 133.1, 130.0, 130.0, 129.9, 129.9, 129.9, 129.7, 129.6, 129.3, 129.2, 129.0, 128.8(3C), 128.5, 128.4,(4C) 128.4(6C), 128.3(4C), 128.1(4C), 127.8(4C), 127.6, 127.4(4C), 127.2(4C), 101.8, 100.1, 96.7, 79.6, 76.1, 75.9, 74.7, 74.1, 73.2, 73.1, 72.6, 72.4, 72.2, 72.1, 71.9, 71.7, 70.0, 69.3, 68.5, 63.9, 63.5, 55.5; **mp**: 104 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = +45° (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (ESI-MS): $[M+H]^+$ m/z calculated for $C_{89}H_{81}O_{23}$: 1517.5169 found 1517.5168

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl) 2-*O*-benzoyl, 3,4-di-*O*-benzyl α-D-mannopyranoside (**8a**):

Compound **16**[53] was converted to carbonate donor **17** according to general experimental procedure A, C. Next, the compound **17** was subjected to the experimental procedure D to obtain the compound **8a** (yield 88% ,white solid).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 – 8.04 (m, 2H), 7.64 – 7.45 (m, 3H), 7.36 – 7.24 (m, 10H), 6.08 (d, *J* = 2.1 Hz, 1H), 5.70 (dd, *J* = 3.2, 2.1 Hz, 1H), 4.95 – 4.57 (m, 4H), 4.20 – 3.76 (m, 6H), 2.65 (s, 1H), 2.18 (d, *J* = 8.1 Hz, 2H), 2.07 – 1.58 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.5, 150.5, 138.1, 137.8, 133.6, 130.1(2C), 129.6, 128.7(2C), 128.5(2C), 128.5(2C), 128.2(2C), 128.1(2C), 128.0, 127.8, 94.3, 82.5, 78.8, 77.9, 75.5, 75.5, 74.2, 73.4, 71.9, 67.9, 61.8, 37.0, 36.7, 25.0, 22.8, 22.6; **mp**: 60 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 3° (*c* 0.16, $CHCl_3$); **IR** (cm^{-1},$CHCl_3$): 3440, 2941, 1760, 1724, 1453, 1264, 1239, 1095, 1011, 902, 745, 709, 666; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{38}NaO_9$: 637.2414 found 637.2413.

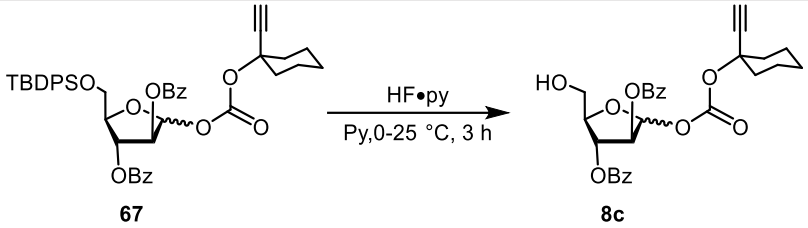
1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranoside (**8b**):



Scheme 18: Synthesis of C6-OH glucose acceptor 8b

Compound **65**[54] was converted to carbonate donor **66**according to general experimental procedure A,C. Next, the compound **66** was subjected to the experimental procedure D to obtain the compound **8b** (yield 92%, thick syrup)(α:β=2.5:1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.37 – 7.29 (m, 15H), 6.14 (d, *J* = 3.5 Hz, 1H), 4.99 – 4.68 (m, 6H), 4.02 (t, *J* = 9.3 Hz, 1H), 3.90 – 3.84 (m, 1H), 3.84 – 3.62 (m, 4H), 3.59 – 3.45 (m, 1H), 2.62 (d, *J* = 9.1 Hz, 1H), 2.23 – 2.09 (m, 2H), 1.95 – 1.58 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 151.5, 151.2, 138.6, 138.3, 138.0, 137.9, 137.9, 137.7, 128.6, 128.5, 128.5(4C), 128.4(4C), 128.1(4C), 128.1, 128.1(4C), 128.0(4C), 127.9(4C), 127.9, 127.8, 127.7, 97.4, 93.4, 84.4, 82.8, 82.5, 81.4, 81.0, 79.0, 78.6, 78.1, 76.9, 76.6, 76.1, 76.1, 75.8, 75.8, 75.4, 75.2, 75.2, 75.1, 73.5, 73.3, 61.5, 61.4, 36.8, 36.8, 36.8, 36.7, 25.0, 24.9, 22.6, 22.6, 22.5, 22.4; **Optical Rotation**: $[\alpha]_D^{25}$ = + 44° (*c* 0.23, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3447, 2938, 1755, 1454, 1268, 1239, 1070, 1006, 895, 848, 749, 695; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{40}NaO_8$: 600.2621 found: 600.2610.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl) 2,3-di-*O*-benzoyl α/β-D-arabinofuranoside (**8c**):

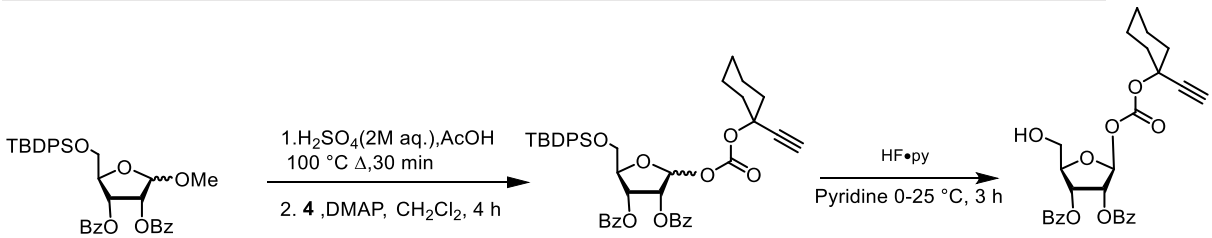


67 8c

Scheme 19: Synthesis of C5-OH arabinose acceptor 8c

Compound **67**[55] was converted to compound **8c** according to the general experimental procedure D (Yield 92%, white solid) (α:β =3.7:1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.12 – 8.01 (m, 4H), 7.63 – 7.41 (m, 6H), 6.33 (s, 1H), 5.89 – 5.77 (m, 1H), 5.72 (d, *J* = 1.6 Hz, 1H), 5.54 (dd, *J* = 4.6, 1.6 Hz, 1H), 4.52 (q, *J* = 4.1 Hz, 1H), 4.06 – 3.95 (m, 2H), 2.69 (s, 1H), 2.20 (dq, *J* = 13.1, 6.6, 5.5 Hz, 2H), 1.93 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3, 165.9, 165.4, 165.1, 151.0, 150.8, 133.8(2C), 133.7(2C), 133.7(2C), 130.0(2C), 130.0(2C), 129.9(2C), 129.0, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5(4C), 128.4(2C), 102.1, 96.7, 85.9, 83.3, 82.5, 81.0, 78.5, 78.4, 77.4, 77.0, 76.2, 75.5, 75.2, 75.1, 63.6, 61.9, 36.8, 36.8, 36.7, 36.5, 24.9, 24.8, 22.7, 22.5, 22.4, 22.3; **mp**: 59 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = -41 (*c* 0.16, $CHCl_3$); **IR**(cm^{-1}, $CHCl_3$): 3440, 2939, 1725, 1240, 1109, 1026, 902, 746, 708, 666; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{28}H_{28}NaO_9$: 531.1631 found 531.1637.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2,3-di-*O*-benzoyl β-D-ribofuranoside (**8d**):



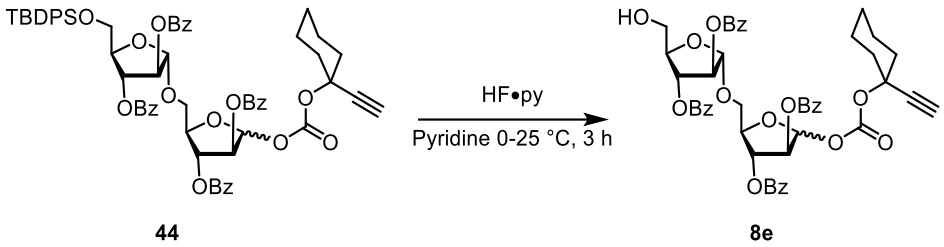
68 69 8d

Scheme 20: Synthesis of C5-OH ribose acceptor 8d

To a solution of compound **68** (4gm, 6.55 mmol) in glacial acetic acid (80 mL) and aqueous H_2SO_4 (2M, 40 mL) was heated to 100 °C for 30 min. After completion of starting material the reaction mixture was diluted with cold water (70 ml) and ethyl acetate (70ml). The organic layer was washed several times with cold water to remove AcOH and then with ice cold saturated $NaHCO_3$. The combined organic layer was concentrated under vacuum to get a syrup. This syrup was purified using silica gel column chromatography (20% ethyl acetate/ hexane)

to obtain the hemiacetal., which was transformed to the glycosyl donor **69** according to general experimental C. (82% yield over two steps, thick syrup). Finally, the TBDPS group was deprotected using general experimental procedure D to obtain compound **8d** (78%, viscous syrup); **^{1}H NMR** (400 MHz, $CDCl_3$) :δ 8.06 – 7.99 (m, 2H), 7.91 – 7.83 (m, 2H), 7.61 – 7.28 (m, 6H), 6.31 (d, *J* = 8.4 Hz, 1H), 5.81 – 5.76 (m, 2H), 4.54 (q, *J* = 5.8, 4.7, 3.7 Hz, 1H), 4.02 – 3.80 (m, 2H), 2.68 (s, 1H), 2.36 (s, 1H), 2.27 – 2.10 (m, 2H), 1.97 – 1.53 (m, 8H).**^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.6, 165.0, 150.8, 133.7, 133.5, 129.9, 129.8, 128.9(2C), 128.8(2C), 128.6(2C), 128.4(2C), 101.3, 83.7, 82.4, 78.8, 75.5, 75.3, 71.0, 62.6, 36.8, 36.7, 24.9,22.7, 22.6. **Optical Rotation**: $[\alpha]_D^{25}$ = -11º (*c* 0.16, $CHCl_3$) **IR** (cm^{-1}, $CHCl_3$): 3410.29, 2940.26, 2860.45, 1729.43, 1602.06, 1451.80, 1264.54, 1238 , 1178, 1107, 1010 , 902, 748, 706, 660; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{28}H_{28}NaO_9$: 531.1631, found 531.1636.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2,3 di -*O*-benzoyl -5- *O*-(2,3,-di- *O*-benzoyl – α-D-arabinofuranosyl)- α/β-D-arabinofuranoside (**8e**) :

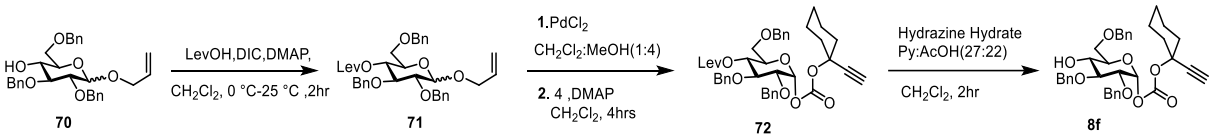


Scheme 21: Synthesis of C5-OH disaccharide acceptor 8e

The compound **44**[55] was converted to compound **8e** according to general reaction procedure D. (Yield 88 %, white foam). (α:β=4.5:1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.13 – 7.89 (m, 8H), 7.61 – 7.28 (m, 12H), 6.36 (d, *J* = 4.5 Hz, 1H), 5.74 (d, *J* = 1.4 Hz, 1H), 5.72 (d, *J* = 4.6 Hz, 1H), 5.59 (d, *J* = 4.9 Hz, 1H), 5.48 – 5.43 (m, 1H), 5.40 (s, 1H), 4.66 (d, *J* = 4.0 Hz, 1H), 4.46 (m, 1H), 4.21 (dd, *J* = 11.3, 4.3 Hz, 1H), 3.98 (m, 3H), 2.68 (s, 1H), 2.22 (s, 1H), 2.19 – 1.92 (m, 2H), 1.91 – 1.22 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.2, 166.0, 165.7, 165.6, 165.5, 165.2, 165.2, 165.1, 151.1, 151.0, 133.7(2C), 133.7(2C), 133.6(2C), 133.4(2C), 130.1, 130.1, 130.0, 129.9(4C), 129.9(4C), 129.8, 129.8(4C), 129.2, 129.1, 129.1, 129.1, 129.0, 128.9, 128.8, 128.8, 128.7(4C), 128.6(4C), 128.5(4C), 128.4(4C), 106.0, 105.6, 102.3, 96.6, 84.3, 84.0, 83.9, 82.6, 82.0, 81.6, 81.0, 80.7, 78.4, 78.4, 77.7, 77.4, 77.6, 76.8, 76.1, 75.5, 75.2, 74.5, 67.3, 65.9, 62.3, 62.2, 36.8, 36.7, 36.6, 36.6, 24.9, 24.8, 22.7, 22.6, 22.4, 22.4; **Optical Rotation**: $[\alpha]_D^{25}$ = -15º (*c* 0.16 $CHCl_3$); **IR (**cm^{-1}, $CHCl_3$): 3316, 2934, 1722 ,

1452 , 1264 , 1109, 1070 , 1026 , 709; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{47}H_{44}O_{11}Na$ 872.2611found 872.2618.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)- 2,3,6 -tri-*O*-benzyl-α-D-glucopyranoside (**8f)**

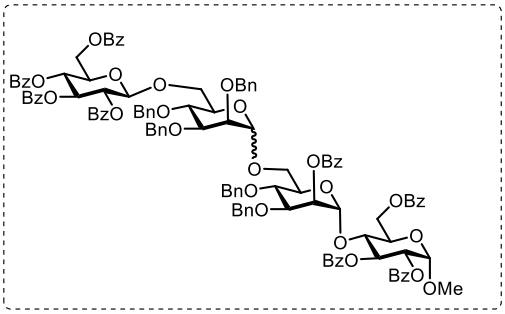


Scheme 22: Synthesis of C4-OH glucose acceptor 8f

To a solution of compound 70[56] (**1**mmol, 1gm) in anhydrous CH_2Cl_2 (10ml), DMAP (1mmol, 250mg), Levulinic acid (1.5 mmol, 356mg), N, N'-Diisopropylcarbodiimide (1.5 mmol, 474µL) were added at 0 °C under nitrogen atmosphere and the reaction was allowed to stir for another 2 hr. After complete conversion of starting alcohol, the reaction mixture was concentrated in *vaccu* and the crude reaction mixture was purified by silica gel column chromatography with ethyl acetate and hexane as eluent (25-30 % e.a/ hexane) to afford the compound 71. After this the compound was converted to the compound 72 according the general reaction procedure A, C. Finally, the compound 72 (1mmol, 1.5 gm) was dissolved in 10 ml of anhydrous CH_2Cl_2. To this solution a buffer solution of py/AcOH (27:22 mol) and 80% hydrazine hydrate (6mmol, 651 µL) were added and the reaction mixture was stirred for 2hr.After completion, the reaction mixture was quenched by addition of acetone (2ml) and the reaction mixture was extracted with ethyl acetate. The organic layer was further washed by 1(N) HCl, $NaHCO_3$ (2X 50 ml), brine (3x25 ml), dried over Na_2SO_4 and concentrated in *vaccu*. The crude reaction mixture was purified in silica gel column chromatography (15-20 e.a/hexane) to afford the compound the 8f. (88 % yield, thick syrup); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.27 (m, 15H), 5.52 (d, *J* = 7.8 Hz, 1H), 4.93 – 4.68 (m, 4H), 4.62 – 4.43 (m, 2H), 3.78 – 3.66 (m, 3H), 3.59 – 3.49 (m, 3H), 2.76 (s, 1H), 2.60 (s, 1H), 2.24 – 2.09 (m, 2H), 1.98 – 1.56 (m, 8H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.3, 138.5, 138.0, 137.8, 128.5-127.8 (15C), 97.5, 83.9, 82.6, 80.5, 78.4, 75.4, 75.3, 75.0, 75.0, 73.6, 71.2, 69.5, 36.8, 36.7, 24.9, 24.7, 22.5. **Optical Rotation** $[\alpha]_D^{25}$ = + 70º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{40}NaO_8$: 623.2621 found 623.2610

Methyl-2, 3, 6-tri-*O*-benzoyl-4-*O*-(2-*O*-benzoyl 3,4-di-*O*-benzyl 6-*O*-(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D- mannopyranosyl)-α-D-mannopyranosyl)-α-D-glucopyranoside **(10a)** :

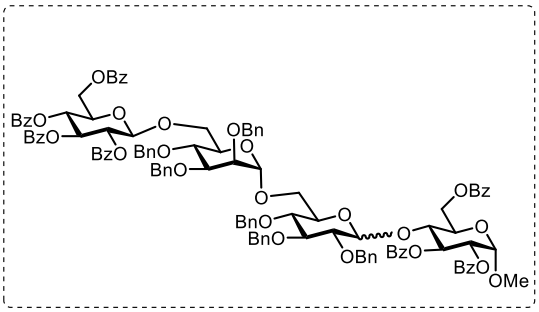
This compound was synthesized according the one pot glycan editing procedure mentioned above (61% overall yield, white solid) (α :β = 2:1).



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 – 7.69 (m, 16H), 7.50 – 7.13 (m, 49H), 6.13 (d, *J* = 9.9 Hz, 1H), 5.92 (t, *J* = 9.7 Hz, 1H), 5.67 (t, *J* = 9.7 Hz, 1H), 5.62 – 5.52 (m, 1H), 5.31 (s, 1H), 5.20 (s, 1H), 5.16 (s, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 4.92 (d, *J* = 13.0 Hz, 1H), 4.84 – 4.76 (m, 2H), 4.73 – 4.06 (m, 18H), 3.98 (d, *J* = 6.6 Hz, 2H), 3.90 – 3.74 (m, 4H), 3.68 (d, *J* = 8.3 Hz, 2H), 3.59 (t, *J* = 11.2 Hz, 1H), 3.37 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3, 166.3, 166.3, 166.2, 166.1, 166.0, 166.0, 165.9, 165.9, 165.8, 165.7, 165.7, 165.3, 165.2, 165.1, 164.9, 138.8, 138.7, 138.6, 138.5, 138.5, 138.4, 138.3, 138.3, 138.0, 137.9, 133.6 -133.0 (18C), 130.1- 129.7 (40C) , 129.5 (2C), 129.2 (2C), 129.1 (2C), 129.0 (2C), 129.0 (2C), 128.7-128.1 (40C), 128.0 (2C), 127.9 (2C), 127.8-127.4 (30C), 102.0, 101.5, 101.4, 100.5, 100.5, 98.1, 97.2, 96.9, 80.0 (2C), 78.0 (2C), 77.7 (2C) , 77.4 (2C), 75.5 (2C), 75.2 (2C), 74.9 (2C), 74.6 (2C), 73.9 (2C), 73.6 (2C), 73.2 (2C), 72.7 (2C), 72.3 (2C), 72.2 (2C), 72.1 (2C), 72.1 (2C), 71.6 (2C), 71.5 (2C), 71.4 (2C), 71.2 (2C), 70.5 (2C), 69.9 (2C), 69.1 (2C), 68.8 (2C), 65.6 (2C), 63.7 (2C), 63.4(2C), 55.9, 55.6; **mp**: 99 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = 43º (*c* 0.20, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2921, 1722, 1452, 1263, 1092, 1067, 1026, 750, 707; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{116}H_{106}O_{29}Na$ 1986.675 found 1986.635.

Methyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-mannopyranosyl)-α/β-D-glucopyranosyl)-α-D-glucopyranoside **(10b)**:

This compound was synthesized according the one pot glycan editing procedure mentioned above (65% overall yield, thick syrup) (α :β = 2:1).

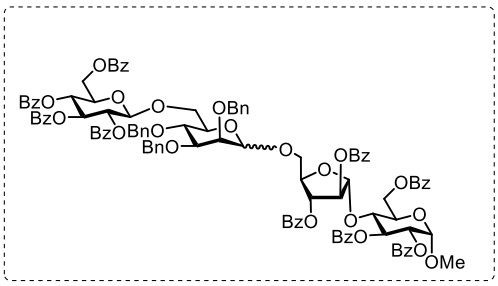


^{1}H NMR (600 MHz, $CDCl_3$): δ 8.18 – 7.86 (m, 24H), 7.56 – 7.08 (m, 106H), 6.28 (t, *J* = 10.2 Hz, 1H), 6.00 (t, *J* = 9.6 Hz, 1H), 5.74 (t, *J* = 9.8 Hz, 1H), 5.61 (t, *J* = 8.9 Hz, 1H), 5.37 (dd, *J* = 10.3, 3.7 Hz, 1H), 5.22 (d, *J* = 3.7 Hz, 1H), 5.14 (dd, *J* = 11.9, 4.0 Hz, 1H), 5.08 (d, *J* = 8.2 Hz, 1H), 4.97 (d, *J* = 11.3 Hz, 1H), 4.92 (d, *J* = 4.0 Hz, 2H), 4.89 (d, *J* = 6.3 Hz, 1H), 4.86 (d, *J* = 10.4 Hz, 2H), 4.80 (d, *J* = 8.7 Hz, 5H), 4.76 – 4.39

(m, 24H), 4.39 – 4.23 (m, 7H), 4.23 – 4.17 (m, 2H), 4.14 (t, *J* = 11.0 Hz, 2H), 4.10 (d, *J* = 11.4 Hz, 2H), 3.96 (dd, *J* = 11.1, 7.8 Hz, 4H), 3.82 – 3.65 (m, 11H), 3.63 – 3.56 (m, 3H), 3.54 – 3.47 (m, 3H), 3.45 (s, 1H), 3.44 (s, 3H), 3.32 (t, *J* = 9.5 Hz, 2H), 3.24 – 3.14 (m, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$): δ 166.3, 166.2, 166.1, 166.1, 166.1, 166.0, 166.0, 165.9, 165.9, 165.7, 165.3, 165.2, 165.2, 165.1, 139.2, 138.8, 138.6, 138.6, 138.5, 138.5, 138.4, 138.4, 138.3, 138.2, 138.2, 137.9, 133.4 - 133.0 (20C), 130.2-129.6 (30C), 129.2 (2C), 129.1 (2C), 129.1 (2C), 129.0 (2C), 128.9 (2C), 128.9 (2C), 128.7 (2C), 128.6, 128.5 - 128.1 (50C), 127.8 - 127.4 (30C), 101.5, 101.3, 101.0, 100.9, 99.2, 97.9, 96.9, 96.8, 82.7, 81.5, 81.4, 79.6, 79.4, 79.2, 79.1, 76.4, 75.6, 75.3, 75.2, 74.8, 74.7, 74.6, 74.2, 74.1, 73.5, 73.1, 73.0, 73.0, 72.7, 72.7, 72.5, 72.3, 72.3, 72.1 , 72.1, 72.1, 72.0, 71.9, 71.8, 71.7, 71.7, 71.6, 71.6, 71.5, 71.2, 69.9, 69.8, 69.3, 69.0, 68.8, 68.6, 68.4, 68.1, 65.6, 65.1, 63.8, 63.6, 63.4, 63.3, 55.4, 55.3; **Optical Rotation**: $[\alpha]_D^{25}$ = + 47º (*c* 0.16 $CHCl_3$); **IR (**cm^{-1}, $CHCl_3$): 2922, 1723, 1452, 1263, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{116}H_{108}O_{28}Na$ 1972.696 found 1972.028.

Methyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,-di-*O*-benzoyl-5-*O*-(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α/β-D-mannopyranosyl)-α-D-arabinofuranosyl)-α-D-glucopyranoside (**10c**):

This compound was synthesized according the one pot glycan editing procedure mentioned above. (68% overall yield, white solid). (α :β = 2:1).

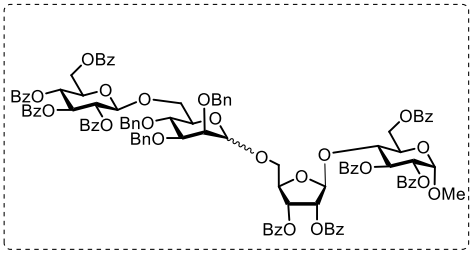


^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 – 7.74 (m, 18H), 7.58 – 7.15 (m, 42H), 6.27 – 6.06 (m, 1H), 5.90 (t, *J* = 9.7 Hz, 1H), 5.76 – 5.59 (m, 2H), 5.54 (d, *J* = 7.5 Hz, 1H), 5.49 (d, *J* = 9.5 Hz, 1H), 5.40 (d, *J* = 8.2 Hz, 2H), 5.21 (d, *J* = 5.2 Hz, 1H), 5.17 (s, 1H), 4.88 (d, *J* = 7.6 Hz, 1H), 4.86 – 4.73 (m, 1H), 4.71 – 4.43 (m, 8H), 4.39 (d, *J* = 4.9 Hz, 1H), 4.33 (d, *J* = 11.6 Hz, 1H), 4.28 (d, *J* = 11.2 Hz, 1H), 4.25 – 4.19 (m, 3H), 4.15 (d, *J* = 13.9 Hz, 1H), 3.90 (s, 1H), 3.74 (dd, *J* = 19.0, 9.8 Hz, 4H), 3.61 (t, *J* = 9.7 Hz, 1H), 3.44 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3, 166.2, 166.1, 166.1, 166.1, 165.9, 165.7, 165.4, 165.4, 165.3, 165.3, 165.2, 165.2, 165.1, 164.9, 164.8, 164.7, 164.7, 138.7, 138.6, 138.6, 138.5, 138.1, 138.0, 133.5-132.8(20C), 130.1- 129.5(20C), 129.4(2C), 129.3(2C), 129.2(2C), 129.1(2C), 128.9 (2C), 128.9(2C), 128.8(2C), 128.8(2C), 128.7(2C), 128.6-128.1(60C), 127.9-127.3(20C), 107.8, 107.4, 101.7(2C), 101.3, 98.2, 97.0(2C), 83.3, 82.2(2C), 82.1, 82.0, 80.2, 77.7, 77.3, 75.2, 74.8, 74.7, 74.5, 74.5, 74.2, 74.0, 73.0, 72.8, 72.4(4C), 72.4, 72.3, 72.1, 71.9, 71.9, 71.6, 71.2, 71.1, 69.9(2C), 69.6, 69.3, 68.9, 68.8, 68.8,

68.6, 66.1, 63.4 (2C), 63.3 (2C), 55.5, 55.5; **mp**: 111 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 34º (*c* 0.23 $CHCl_3$); **IR (**cm^{-1}, $CHCl_3$): 3010 ,1727, 1268, 1215, 1095, 745, 708, 667; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{108}H_{96}O_{29}Na$ 1880.596 found 1882.640.

Methyl-2, 3, 6-tri-*O*-benzoyl-4-*O*-(2, 3-di-*O*-benzoyl 5-*O*-(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D- mannopyranosyl)-β-D-ribofuranosyl)-α-D-glucopyranoside (**10d)**:

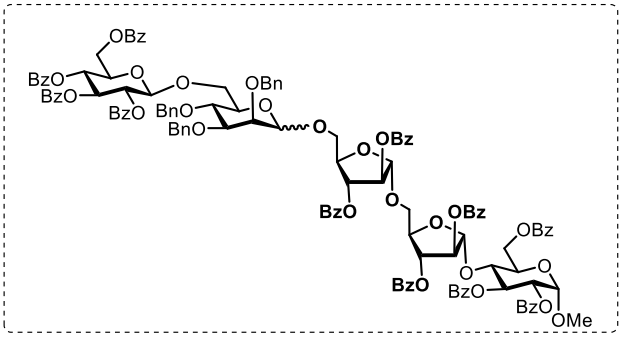
This compound was synthesized according the one pot glycan editing procedure mentioned above. (66% overall yield, white solid). (α :β = 4:1)



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 – 7.79 (m, 18H), 7.49 – 7.16 (m, 42H), 6.09 (t, *J* = 9.8 Hz, 1H), 5.91 (t, *J* = 9.7, 1H), 5.68 (dd, *J* = 11.7, 9.4 Hz, 1H), 5.61 – 5.52 (m, 2H), 5.45 (d, *J* = 4.9 Hz, 1H), 5.39 (d, *J* = 3.6 Hz, 1H), 5.20 (d, *J* = 3.6 Hz, 1H), 5.17 (d, *J* = 2.0 Hz, 1H), 4.95 (d, *J* = 4.4 Hz, 1H), 4.91 (d, *J* = 9.0 Hz, 1H), 4.86 (d, *J* = 6.3 Hz, 1H), 4.79 (d, *J* = 6.9 Hz, 1H), 4.75 (d, *J* = 3.4 Hz, 1H), 4.71 (s, 1H), 4.69 – 4.44 (m, 7H), 4.37 (dd, *J* = 11.6, 6.3, 1H), 4.27 – 4.08 (m, 5H), 4.05 – 3.98 (m, 1H), 3.94 – 3.84 (m, 1H), 3.82 – 3.69 (m, 2H), 3.68 – 3.60 (m, 1H), 3.36 (s, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$): δ :166.4, 166.3, 166.3, 166.2, 166.1, 166.0, 166.0, 165.7, 165.5, 165.5, 165.4, 165.4, 165.3, 165.2, 165.1, 165.1, 165.1, 165.1, 139.3, 139.3, 139.1, 138.9, 138.5, 138.4, 133.6 - 133.1 (20C), 130.1 -129.9 (40C) , 129.4 (2C), 129.3 (2C), 129.2 (2C), 129.2 (2C), 129.1 (2C), 129.0 (2C), 129.0 (2C), 128.7 - 128.4 (40C), 128.2 (2C), 128.1 (2C), 127.9 - 127.4 (20C), 107.7, 106.7, 101.9, 101.7, 101.6, 98.6, 97.2 (2C), 82.3, 81.9, 81.1, 80.4, 79.4, 78.6, 76.4, 76.0, 75.2, 75.2, 74.9, 74.9, 74.6, 74.6, 74.5, 73.3, 73.2, 73.2, 73.0, 72.5, 72.4, 72.3, 72.1, 72.0, 71.9, 71.8, 71.8, 71.5, 71.2, 70.3, 70.1, 70.0, 69.9, 69.3, 69.1, 68.7, 68.4, 66.7, 63.4, 63.4 , 63.0 (2C), 55.7 (2C); **mp**: 104 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 49º (*c* 0.17, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2942, 1726, 1452, 1264, 1093, 1068, 1026, 747, 705, 666; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{108}H_{96}O_{29}Na$ 1880.596 found 1879.900.

Methyl-2, 3, 6-tri-*O*-benzoyl-4-*O*-(2, 3-di-*O*-benzoyl 5-*O*- (2, 3-di-*O*-benzoyl 5-*O* -(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α/β-D-mannopyranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-glucopyranoside (**10e)**:

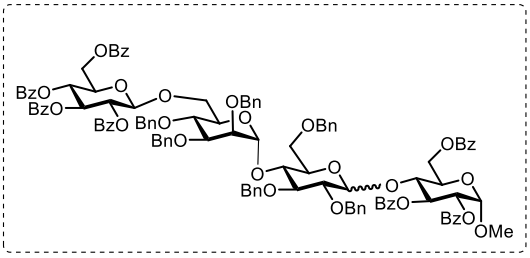
This compound was synthesized according the one pot glycan editing procedure mentioned above. (64% overall yield, white solid). (α :β = 3:1)



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 – 7.74 (m, 22H), 7.50 – 7.15 (m, 48H), 6.17 (t, *J* = 9.5 Hz, 1H), 5.88 (t, *J* = 9.7 Hz, 1H), 5.67 – 5.58 (m, 3H), 5.50 (s, 1H), 5.48 (s, 1H), 5.41 (s, 1H), 5.36 (d, *J* = 4.9 Hz, 1H), 5.19 (s, 2H), 5.08 (s, 1H), 4.88 (d, *J* = 8.1 Hz, 1H), 4.78 (d, *J* = 9.4 Hz, 1H), 4.74 – 4.44 (m, 9H), 4.35 (d, *J* = 11.4 Hz, 1H), 4.31 – 4.18 (m, 6H), 4.13 – 4.00 (m, 2H), 3.90 (s, 1H), 3.84 – 3.69 (m, 5H), 3.64 (d, *J* = 8.8 Hz, 1H), 3.45 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.4, 166.3, 166.3, 166.2, 166.1, 165.9, 165.8, 165.7, 165.5, 165.5, 165.5, 165.4, 165.4, 165.3, 165.2, 165.2, 165.2, 165.1, 165.1, 164.9, 164.6, 138.5(2C), 138.5(2C), 138.4, 137.9, 133.5 - 132.7(20C), 130.0 - 129.6(30C), 129.5(2C), 129.5(2C), 129.4(2C), 129.4(2C), 129.4(2C), 129.2(2C), 129.2(2C), 129.1(2C), 128.9(2C), 128.9(2C), 128.8(2C), 128.6 -128.1(50C), 127.8 - 127.3(45C), 107.5, 107.4, 105.9, 105.9, 101.8(2C), 101.2, 98.2, 97.1(2C), 83.2, 83.2, 83.1, 82.0, 81.9, 81.8, 81.8, 81.7, 81.6, 80.2, 77.6, 76.9, 75.2, 74.7, 74.7, 74.4, 74.4, 74.2, 73.6, 73.5, 73.0, 72.7, 72.5, 72.4(4C), 72.3(4C), 72.1, 71.9, 71.9, 71.7, 71.2, 71.2, 69.9, 69.4, 69.4, 68.6, 68.5, 68.5, 66.2, 65.6, 65.6, 63.3, 63.0, 55.5, 55.5; **mp**: 107 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 33° (*c* 0.18 ,$CHCl_3$); **IR (**cm^{-1}, $CHCl_3$): 2922, 1724, 1452, 1268, 1096, 1027, 707; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{127}H_{112}O_{35}Na$ 22219.688 found 2219.599.

Methyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-mannopyranosyl)-α/β-D-glucopyranosyl)-α-D-glucopyranoside **(10f)**:

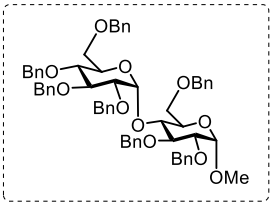
This compound was synthesized according the one pot glycan editing procedure mentioned above (70% overall yield, thick syrup) (α :β = 3:1).



^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 – 7.78 (m, 14H), 7.42 – 7.18 (m, 51H), 5.98 – 5.77 (m, 1H), 5.74 – 5.65 (m, 1H), 5.61 – 5.49 (m, 1H), 5.33 – 5.27 (m, 1H), 5.17 (dd, *J* = 4.8, 3.0 Hz, 2H), 4.87 (dd, *J* = 11.6, 2.7 Hz, 2H), 4.84 – 4.81 (m, 2H), 4.60 – 4.36 (m, 13H), 4.26 – 4.11 (m, 6H), 4.09 – 4.01 (m, 2H), 3.83 – 3.71 (m, 6H), 3.66 – 3.58 (m, 3H), 3.45 (s, 3H).**^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.3, 166.2, 166.2, 166.2, 166.1, 166.0, 165.9, 165.8, 165.3, 165.3, 165.1, 165.0, 165.0, 165.0, 138.9, 138.8, 138.8, 138.7, 138.7, 138.6, 138.5, 138.4, 138.3, 138.0, 138.0,

137.8, 133.4 - 133.0 (20C), 130.2-129.6 (30C), 129.2 (2C), 129.1 (2C), 129.1 (2C), 129.0 (2C), 128.9 (2C), 128.9 (2C), 128.7 (2C), 128.6, 128.5 - 128.1 (50C), 127.8 - 127.4 (30C) 101.8, 101.7, 100.6, 99.7, 99.7, 98.6, 96.9, 96.9, 82.7, 81.5, 81.4, 79.6, 79.4, 79.2, 79.1, 76.4, 75.6, 75.3, 75.2, 74.8, 74.7, 74.6, 74.2, 74.1, 73.5, 73.1, 73.0, 73.0, 72.7, 72.7, 72.5, 72.3, 72.3, 72.1 , 72.1, 72.1, 72.0, 71.9, 71.8, 71.7, 71.7, 71.6, 71.6, 71.5, 71.2, 69.9, 69.8, 69.3, 69.0, 68.8, 68.6, 68.4, 68.1, 65.6, 65.1, 63.8, 63.6, 63.4, 63.3, 55.4, 55.3 **Optical Rotation** $[\alpha]_D^{25}$ = + 46º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS (MALDI-TOF)** [M+Na]+ m/z calculated for $C_{116}H_{108}O_{28}Na$ 1973.101 found 1973.321

Methyl – α- D –Maltopyranoside (**24**):



To a solution of the compound 23 (1gm, 2.81 mmol) was dissolved in 10ml of DMF and cooled to 0 °C. To this, NaH (566 mg, 23.57 mmol) was added portion wise over a period of 5 min. The resulting solution was allowed to stir for another 15 min. After that benzyl bromide (2.4 ml, 19.65mmol) was slowly added. The reaction mixture was kept at 0 °C for 30min and after that it was shifted to 25 °C. After completion, the reaction mixture was poured into ice cold water. Then it was extracted with ethyl acetate. The organic layer was further washed with brine solution. Next the organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The crude product was purified by silica gel column chromatography (10% ethyl acetate / hexane) to give the compound **24**. (96% yield, syrup).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.14 (m, 35H), 5.03 – 4.65 (m, 9H), 4.63 (d, *J* = 3.6 Hz, 2H), 4.61 – 4.43 (m, 6H), 3.99 (t, *J* = 9.3 Hz, 2H), 3.78 – 3.53 (m, 9H), 3.36 (s, 3H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.8 (2C), 138.3, 138.2 (2C), 137.9 (2C), 128.4-127.6 (42C), 98.2 (2C), 82.1 (2C), 79.9 (2C), 77.7, 75.7 (2C), 75.0 (2C), 73.5 (2C), 73.4 (2C), 70.1 (2C), 68.5 (2C), 55.2. (2C). **Optical Rotation** $[\alpha]_D^{25}$ = + 40 º (c 0.25, $CHCl_3$) IR **(**cm^{-1} $CHCl_3$): 3446, 2937, 1453, 1268, 1239, 1167, 1005, 801, 847, 749, **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{62}H_{66}O_{11}Na$: 1010.4536 found 1010.4533

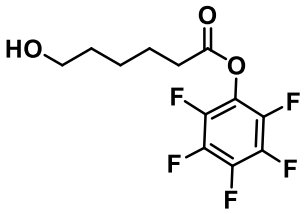
General Procedure for synthesis of OPfp ester:

To a solution of compounds 6-hydroxycaproic acid, Lithocholic acid, N-CBz L-Serine (1mmol) in anhydrous EtOAc (10ml) DCC was added (1.1 mmol) added at 0 °C under nitrogen atmosphere. After stirring for 10 min, pentaflurophenol (1mmol) was added and the reaction was allowed to stir for 4 hr at 25 °C. After completion of reaction, the reaction mixture was filtered filtered off through a pad of Celite®. The filtrate part containing the product was evaporated *in vacuo* and the crude residue was purified by silica gel column chromatography

to obtain desired OPfp ester compounds 27a-27c. (Purified in 10-20e.a/hexane). (85-90% yield)

Perfluorophenyl 6-hydroxyhexanoate :(**27a**)

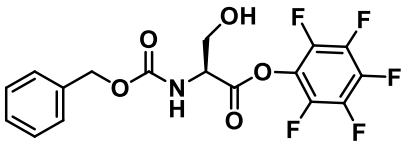
This compound was synthesized according the general procedure for OPfp ester synyhesis mentioned above. (88% yield, syrup)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 – 3.64 (m, 2H), 2.69 (t, *J* = 7.3 Hz, 2H), 1.98 (s, 1H), 1.86 – 1.78 (m, 2H), 1.68 – 1.60 (m, 2H), 1.56 – 1.48 (m, 2H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.5, 142.3, 140.7, 139.8, 139.1, 138.2, 136.7, 62.4, 33.2, 32.1, 25.1, 24.5. **Optical Rotation** $[\alpha]_D^{25}$ = + 45 º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 969, 1023, 895, 847, 749, **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{12}H_{11}F_5O_3Na$: 321.0526 found 321.0524

Perfluorophenyl N-(benzyloxy)carbonyl)-L-serinate :(**27b**)

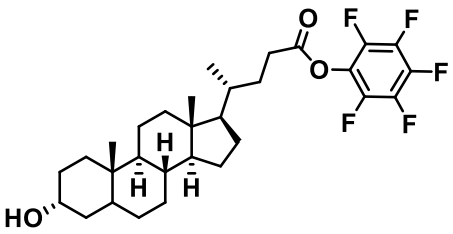
This compound was synthesized according the general procedure for OPfp ester synyhesis mentioned above. (88% yield, white powder)



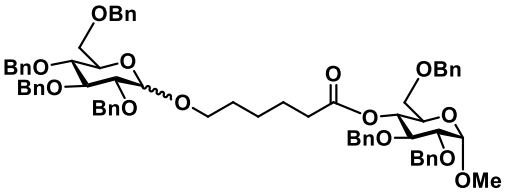
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 5H), 5.94 (d, *J* = 8.3 Hz, 1H), 5.15 (s, 2H), 4.82 (dd, *J* = 8.0, 3.9 Hz, 1H), 4.22 (dd, *J* = 11.4, 3.5 Hz, 1H), 4.04 (dd, *J* = 11.3, 3.4 Hz, 1H), 2.68 (s, 1H).**MP**: 63 °C **Optical Rotation** $[\alpha]_D^{25}$ = +23 º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{17}H_{12}F_5NO_3Na$: 428.0533 found 428.0530

Perfluorophenyl (4R)-4-((3R,8R,9S,10S,13R,14S,17R)-3-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl) pentanoate :(**27c**)

This compound was synthesized according the general procedure for OPfp ester synyhesis mentioned above. (90% yield, White powder)**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (tt, *J* = 11.0, 4.6 Hz, 1H), 2.75 – 2.54 (m, 2H), 2.03 – 1.09 (m, 27H), 0.98 (d, *J* = 6.2 Hz, 3H), 0.93 (s, 3H), 0.67 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 142.3, 140.6, 139.8, 139.1, 138.1, 136.5, 71.8, 56.5, 55.9, 42.8, 42.1, 40.4, 40.2, 36.4, 35.9, 35.4, 35.3, 34.6, 30.9, 30.5, 30.4, 28.2, 27.2, 26.4, 24.2, 23.4, 20.8, 18.1, 12.0. **MP**: 78 ºC **Optical Rotation** $[\alpha]_D^{25}$ = + 8 º (c 0.25, $CHCl_3$) IR **(**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{30}H_{39}F_5O_3Na$: 565.2713 found 565.2710



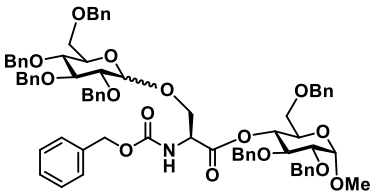
Methyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl glucopyranosyloxyhexanoyl)-α-D-glucopyranoside (**30a**):This compound was synthesized according the one pot glycan editing procedure mentioned above (80% overall yield, thick syrup) (α: β = 2:1)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.13 (m, 35H), 5.03 – 4.74 (m, 9H), 4.70 – 4.61 (m, 2H), 4.58 (d, *J* = 3.3 Hz, 2H), 4.57 – 4.44 (m, 3H), 4.36 (d, *J* = 7.8 Hz, 1H), 4.26 (d, *J* = 3.3 Hz, 2H), 4.05 – 3.90 (m, 2H), 3.81 (d, *J* = 9.9 Hz, 1H), 3.73 – 3.42 (m, 8H), 3.36 (s, 3H), 2.26 (td, *J* = 7.8, 3.0 Hz, 2H), 1.68 – 1.57 (m, 4H), 1.46 – 1.33 (m, 2H).**^{13}C NMR** (101 MHz , $CDCl_3$) δ 173.3, 173.3, 138.9, 138.7, 138.6, 138.5, 138.4, 138.4, 138.3, 138.3, 138.2, 138.2, 138.1, 138.0, 137.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 103.7, 98.0 (2C), 97.0, 84.8, 84.7, 82.3, 82.1, 82.1, 82.0, 80.1, 80.0, 80.0, 77.9, 77.8, 77.6, 77.6, 75.9, 75.8, 75.7, 75.2, 75.1, 75.0, 75.0, 74.9, 74.8, 73.6, 73.5, 73.4, 73.4, 73.2, 70.2, 69.8, 69.8, 69.0, 68.7, 68.7, 68.6, 67.9, 63.0, 62.9, 55.2, 55.2, 34.0, 29.5, 25.8, 24.7. **Optical Rotation** $[\alpha]_D^{25}$ = +51 º(c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (Maldi-TOF) $[M+Na]^+$ m/z calculated for $C_{68}H_{76}O_{13}Na$: 1123.518 found 1123.824

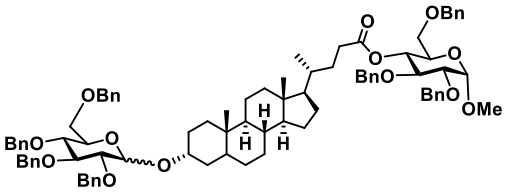
Methyl-2,3,6-tri-O-benzyl-4-O-(2S-((benzyloxy)carbonyl)amino-(3-(2,3,4,6-tetra-O-benzyl glucopyranosyloxy))propanoyl) α-D-glucopyranoside (**30b**):

This compound was synthesized according the one pot glycan editing procedure mentioned above (76% overall yield, thick syrup) (α: β = 2:1)



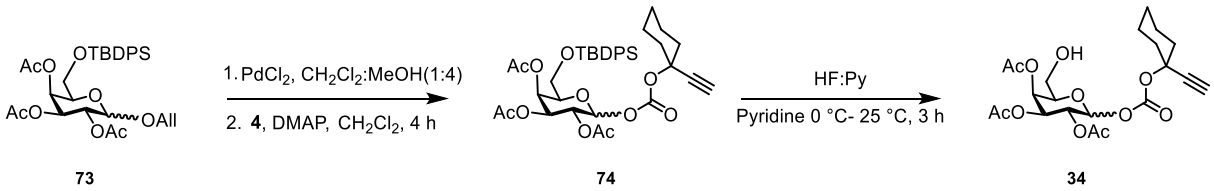
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.18 (m, 40H), 6.09 (dd, *J* = 28.6, 8.7 Hz, 1H), 5.12 – 4.64 (m, 11H), 4.59 – 4.50 (m, 5H), 4.46 – 4.28 (m, 5H), 4.17 – 4.08 (m, 1H), 3.97 (td, *J* = 9.2, 2.7 Hz, 1H), 3.87 – 3.78 (m, 2H), 3.71 – 3.44 (m, 6H), 3.37 (d, *J* = 9.3 Hz, 1H), 3.30 (s, 3H), 3.27 – 3.20 (m, 1H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.0, 169.9, 156.3, 155.8, 139.0, 139.0, 138.8, 138.7, 138.6, 138.4, 138.4, 138.3, 138.3, 138.2, 138.1, 137.9, 137.9, 137.8, 136.3, 136.2-127.7 (96C), 104.1, 99.0, 98.1, 97.9, 84.5, 82.5, 82.2, 82.0, 81.8, 81.6, 80.3, 80.1, 79.8, 78.2, 78.0, 77.9, 76.0, 75.7, 75.5, 75.4, 75.1, 74.7, 73.8, 73.6, 73.4, 73.3, 72.9, 71.0, 70.5, 70.5, 70.3, 69.1, 68.9, 68.7, 68.3, 68.3, 67.3, 67.2, 64.5, 64.4., 55.3,55.4. **Optical Rotation** $[\alpha]_D^{25}$ = +15 º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1680, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (Maldi-TOF) $[M+Na]^+$ m/z calculated for $C_{73}H_{77}O_{15}NNa$: 1230.519 found 1230.910

Methyl 2,3,6-tri-O-benzyl-4-O-((4R)-4-((3R,8R,9S,10S,13R,14S,17R)-3-(2,3,4,6-tetra-O-benzyl glucopyranosyloxy)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoyl) α-D-glucopyranoside (**30c**):



This compound was synthesized according the one pot glycan editing procedure mentioned above (78% overall yield, thick syrup) (α: β = 3:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 35H), 5.01 (d, *J* = 2.6 Hz, 1H), 4.99 – 4.62 (m, 11H), 4.60 (d, *J* = 1.2 Hz, 3H), 4.57 – 4.54 (m, 2H), 4.50 – 4.43 (m, 2H), 4.28 – 4.25 (m, 2H), 4.00 (td, *J* = 9.2, 3.5 Hz, 2H), 3.91 – 3.71 (m, 3H), 3.71 – 3.43 (m, 8H), 3.37 (s, 3H), 2.41 – 1.29 (m, 29H), 0.90 (s, 6H), 0.61 (d, *J* = 2.8 Hz, 3H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.0, 174.0, 139.0, 138.7, 138.7, 138.6, 138.6, 138.3, 138.3, 138.3, 138.2, 138.1, 138.1, 138.0, 138.0, 137.9, 128.5- 127.6 (84C), 102.4, 98.1, 94.7, 85.0, 84.9, 82.5, 82.3, 82.2, 82.1, 80.3, 80.2, 80.0, 79.9, 78.1, 78.0, 77.6, 76.5, 76.0, 75.9, 75.9, 75.7, 75.1, 75.0, 75.0, 74.9, 73.5, 73.5, 73.4, 70.1, 70.0, 69.3, 69.2, 68.7, 68.5, 68.4, 62.9, 62.8, 56.5, 56.4, 56.0, 55.3, 55.2, 42.9, 42.8, 42.2, 42.1, 40.2, 40.0, 35.9, 35.9, 35.6, 35.3, 34.8, 34.7, 32.3, 32.0, 31.1, 30.9, 29.7, 29.7, 28.2, 27.4, 27.4, 27.2, 26.4 , 24.2, 23.5, 23.3, 20.8, 20.8, 18.3, 18.3, 12.1, 12.1. **Optical Rotation** $[\alpha]_D^{25}$ = +39 ° (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (Maldi-TOF) $[M+Na]^+$ m/z calculated for $C_{86}H_{104}O_{13}Na$: 1368.740 found 1368.028

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)- 2,3,4 -tri-*O*-acetyl-α/β-D-glucopyranoside **(34):**

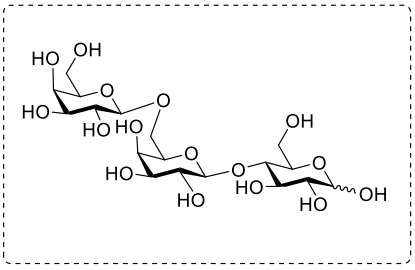


73 74 34

Scheme 23: Synthesis of C6-OH glucose acceptor 34

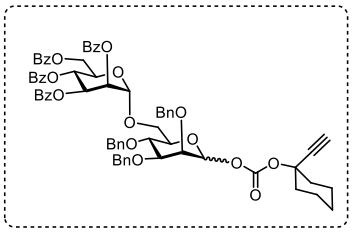
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 (d, *J* = 8.2 Hz, 1H), 5.12 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.42 – 4.16 (m, 1H), 3.94 – 3.89 (m, 1H), 3.75 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.55 (dd, *J* = 11.7, 6.4 Hz, 1H), 2.67 (s, 1H), 2.17 (s, 4H), 2.14 – 2.10 (m, 2H), 2.07 (s, 4H), 2.01 (s, 3H), 1.92 – 1.56 (m, 8H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.0, 170.9, 170.0, 170.0, 169.5, 169.4, 151.0, 150.9, 95.3, 92.9, 82.3, 78.9, 78.7, 75.4, 75.3, 74.5, 73.1, 71.8, 70.9, 70.4, 68.1, 67.5, 66.7, 66.4, 60.7, 60.4, 36.8, 36.7, 36.6, 36.6, 24.9, 24.9, 24.8, 24.7, 22.5, 22.4, 20.8, 20.8, 20.7, 20.6, 20.6, 20.6. **Optical Rotation** $[\alpha]_D^{25}$ = + 69º (c 0.25, $CHCl_3$) **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1755, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, 695 **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{21}H_{28}O_{11}Na$: 479.1529 found 475.1524

β- D -galactopyranosyl-(1 → 6)-β-D -galactopyranosyl-(1 → 4)- α /β D –glucopyranose **(36):**



^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, *J* = 3.7 Hz, 1H),4.59 (d, *J* = 7.9 Hz, 1H), 4.44 – 4.34 (m, 4H), 4.19 – 4.03 (m, 2H), 4.01 – 3.29 (m, 37H), 3.23 – 3.12 (m, 1H).**^{13}C NMR** (151 MHz, D_2O) δ 103.3 (2C), 103.3, 103.2, 96.0, 92.1, 81.0, 75.6, 75.1, 74.9, 74.0, 73.9, 73.9, 72.7, 72.7, 72.5, 72.5, 71.4, 70.8, 70.7, 70.7, 70.4, 69.5, 69.5, 68.9, 68.9, 68.9, 68.7, 68.6, 67.3, 61.0. **MP:** 185 °C **Optical Rotation** $[\alpha]_D^{25}$ = + 16 º (c 0.23, H_20 **IR (**cm^{-1} $CHCl_3$): 3446, 2937, 1453, 1268, 1239, 1069, 1005, 895, 847, 749, **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{18}H_{32}NaO_{16}$: 527.1588 found 527.1583

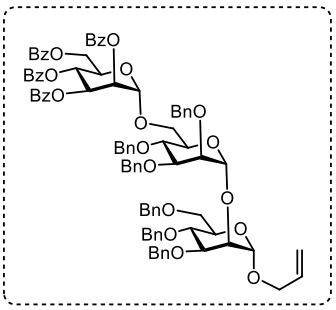
1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6- tetra-*O*-benzoyl α -D-mannopyranosyl) – α- D-mannopyranoside (**39**) :



^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.15 – 8.09 (m, 2H), 8.06 – 7.99 (m, 4H), 7.86 – 7.78 (m, 2H), 7.51 – 7.24 (m, 27H), 6.14 (t, *J* = 10.0 Hz, 1H), 6.07 (d, *J* = 2.0 Hz, 1H), 5.88 – 5.75 (m, 2H), 5.14 (d, *J* = 1.3 Hz, 1H), 5.04 (d, *J* = 11.2 Hz, 1H), 4.85 – 4.52 (m, 7H), 4.50 – 4.42 (m, 1H), 4.06 – 3.89 (m, 6H), 2.70 (s, 1H), 2.21 (m, 2H), 1.94 – 1.49 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.6, 165.2, 165.2, 150.8, 138.2, 138.2, 137.8, 133.3, 133.3, 133.1, 132.9, 130.1, 130.0(2C), 129.9(2C), 129.9(2C), 129.8(2C), 129.7, 129.5, 129.2, 129.2, 128.6(2C), 128.5(2C), 128.5-128.3(12C), 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 97.9, 94.4, 82.7, 79.5, 78.4, 75.4, 75.2, 74.1, 73.6, 73.2, 72.6, 72.2, 70.4, 70.3, 68.6, 67.3, 66.7, 62.7, 37.2, 36.3, 24.9, 22.7, 22.6; **Optical Rotation**: $[\alpha]_D^{25}$ = + 4º (*c* 0.26, $CHCl_3$); **IR (**cm^{-1}, $CHCl_3$): 2990, 1728, 1265, 1216, 1109, 1027, 903, 743, 709, 667; **HRMS** (ESI-MS) $[M]^+$ m/z calculated for $C_{70}H_{67}O_{17:}$ 1179.4378 found 1179.4367

Allyl-3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl) α-D-mannopyranosyl) α-D-mannopyranoside (41):

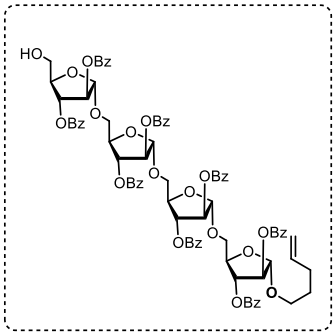
The glycosyl donor **39** and the acceptor **40**[30] was coupled according to general experimental procedure E to give compound **41** (Yield 88%, yellow syrup).



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 – 8.00 (m, 4H), 7.95 – 7.77 (m, 4H), 7.61 – 7.16 (m, 42H), 6.10 (t, *J* = 10.0 Hz, 1H), 5.94 (d, *J* = 7.0 Hz, 1H), 5.88 – 5.76 (m, 2H), 5.27 (d, *J* = 3.2 Hz, 1H), 5.24 (d, *J* = 3.2 Hz, 1H), 5.21 – 5.15 (m, 1H), 5.08 – 5.00 (m, 2H), 4.95

(d, *J* = 3.2 Hz, 1H), 4.86 – 4.37 (m, 14H), 4.17 (d, *J* = 9.0 Hz, 2H),), 4.02 -3.97 (m, , 6H), 3.92 – 3.85 (m, 1H), 3.83 – 3.76 (m, 3H), 3.76 – 3.65 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3, 165.6, 165.3, 165.2, 138.7, 138.6, 138.6, 138.6, 138.4, 138.3, 134.0, 133.5, 133.1, 130.1, 130.0(3C), 129.9(3C), 129.9(3C), 129.9(3C), 129.6, 129.4, 129.2, 128.7-127.5(36C), 117.3, 99.0, 98.4, 98.0, 80.4, 79.9, 75.3, 75.2, 74.9, 74.7, 74.5, 73.8, 73.5, 72.6, 72.1, 72.1, 72.0, 72.0, 70.5, 70.2, 69.3, 68.9, 68.2, 67.4, 67.1, 62.8; **Optical Rotation**: $[\alpha]_D^{25}$ = +12º (*c* 0.20 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3005, 1728, 1453, 1264, 1093, 1027, 745, 709, 665; **HRMS** (ESI-MS): $[M+H]^+$ m/z calculated for $C_{91}H_{89}O_{20}$: 1501.5947 found 1501.594

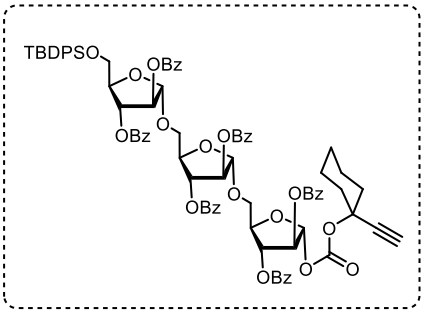
Pent-4-enyl-2,3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D- arabinofuranosyl)- α-D-arabinofuranoside (**45**):



Then the donor **44**[29] and the acceptor **43**[29] were coupled according to the general glycosylation method E to obtain compound the tetrasaccharide. After that primary TBDPS group was deprotected following the general experimental method D to obtain compound **45** (84 % overall yield, thick syrup).

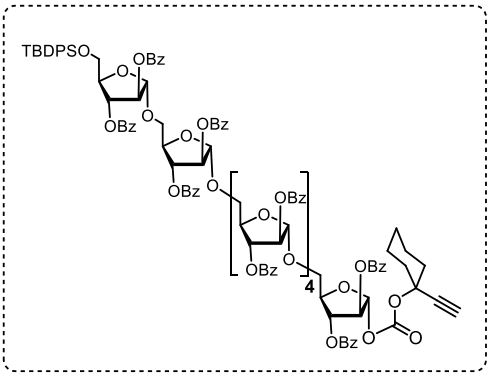
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 – 7.84 (m, 16H), 7.63 – 7.16 (m, 24H), 5.87 – 5.76 (m, 1H), 5.69 – 5.61 (m, 6H), 5.52 (d, *J* = 1.3 Hz, 1H), 5.43 (s, 1H), 5.42 (s, 1H), 5.41 (s, 2H), 5.22 (s, 1H), 5.05 – 4.93 (m, 2H), 4.62 (dt, *J* = 4.5, 2.2 Hz, 2H), 4.50 – 4.41 (m, 2H), 4.24 – 4.14 (m, 3H), 3.95 – 3.85 (m, 5H), 3.82 – 3.72 (m, 1H), 3.57 – 3.47 (m, 1H), 2.43 (s, 1H), 2.24 – 2.13 (m, 2H), 1.81 – 1.66 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.2, 165.8 (2C), 165.5, 165.3(2C), 165.2(2C), 138.2, 133.6, 133.5(4C), 133.4, 133.3, 133.3, 130.0(4C), 129.9(8C), 129.9(4C), 129.4, 129.3, 129.29(4C), 129.1, 129.1 128.6(8C), 128.6(2C), 128.4(2C), 128.4(4C), 115.1, 105.9, 105.9, 105.7, 105.7, 83.8, 82.2, 82.1, 82.0, 81.9, 81.8, 81.7, 81.6, 77.8, 77.4, 76.9, 66.8, 66.2, 66.1, 65.9, 62.4, 30.4, 28.8; **Optical Rotation**: $[\alpha]_D^{25}$ = - 9º (*c* 0.23, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3453, 3022, 1720, 1264, 1216, 1109, 1027, 745, 702, 667; **HRMS** (ESI-MS) $[M+Na]^+$ calculated for $C_{81}H_{74}O_{25}$: 1469.4417 found 1469.4412

1-*O*-(((1-ethynylcyclohexyl)oxy) carbonyl) 2,3 di-*O*-benzoyl 5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl)-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofurano side **(48)**:



The glycosyl donor **44** and acceptor **46**[29] was coupled according to general glycosylation method E to obtain compound **47**. The obtained compound **47** was converted to compound **48**according to general experimental procedure A, C (88% overall yield, viscous syrup); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11 – 7.92 (m, 12H), 7.75 – 7.66 (m, 4H), 7.61 – 7.30 (m, 24H), 6.35 (s, 1H), 5.73 (dt, *J* = 5.1, 0.9 Hz, 2H), 5.68 – 5.62 (m, 2H), 5.61 (t, *J* = 1.0 Hz, 1H), 5.58 (d, *J* = 1.3 Hz, 1H), 5.41 – 5.37 (m, 2H), 4.68 – 4.60 (m, 2H), 4.51 (q, *J* = 4.6 Hz, 1H), 4.26 – 4.15 (m, 2H), 4.02 – 3.90 (m, 4H), 2.67 – 2.64 (m, 1H), 2.30 – 2.09 (m, 2H), 2.06 – 1.53 (m, 8H), 1.02 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.7, 165.79(2C), 165.4, 165.3, 165.2, 151.1, 135.8(2C), 133.8, 133.7, 133.5, 133.4, 133.3, 133.3, 133.2, 130.1, 130.1, 130.0, 129.9, 129.9, 129.8, 129.3(2C), 129.2(2C), 129.1(2C), 128.7, 128.6, 128.5, 128.3, 127.8, 126.2, 115.7, 106.1, 106.0, 102.4, 84.3, 83.3, 82.6, 82.3, 82.2, 81.6, 81.0, 78.5, 75.5, 65.9, 65.8, 63.5, 36.9, 36.8, 26.9(3C), 25.0, 22.8, 22.7, 19.4; **Optical Rotation**: $[\alpha]_D^{25}$ = -2º (*c* 0.16 , $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3297, 2864, 2939, 1727, 1599, 1453, 1363, 1261, 1107, 1020, 966, 852, 756, 708; **HRMS** (ESI-MS): $[M+Na]^+$ calculated for $C_{82}H_{78}O_{21}SiNa$: 1449.4703 found 1449.4725.

1-*O*-(((1-ethynylcyclohexyl) oxy) carbonyl) 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-α-D-(2,3-di-*O*-benzoyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**50**):

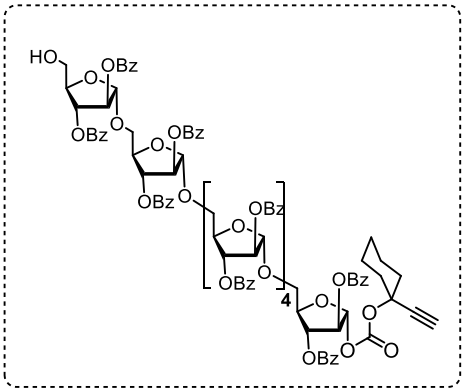


The donor **48** and acceptor **45**were coupled according to general glycosylation procedure E to obtain the product **49**, which was converted to carbonate donor **50** according to general experimental procedure A, C (80 % overall yield, viscous syrup).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 – 7.85 (m, 28H), 7.74 – 7.24 (m, 52H), 6.33 (d, *J* = 4.1 Hz, 1H), 5.72 (d, *J* = 1.7 Hz, 1H), 5.65 (d, *J* = 3.9 Hz, 3H), 5.63 (s, 3H), 5.62 (s, 3H), 5.61 (d, *J* = 3.7 Hz, 1H), 5.59 (d, *J* = 1.3 Hz, 1H), 5.55 (d, *J* = 1.4 Hz, 1H), 5.37 (d, *J* = 6.2 Hz, 6H), 4.66 – 4.47 (m, 7H), 4.25 – 4.10 (m, 7H), 4.00 – 3.86 (m, 8H), 2.64 (s, 1H), 2.29 – 1.99 (m, 2H), 1.95 – 1.63 (m, 8H), 1.00 (s, 9H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.6(4C), 165.6, 165.5, 165.2(2C), 165.1(4C), 165.1(2C), 150.9, 135.7(2C), 135.7(2C),

133.7-133.1(16C), 130.0-129.7(24C), 129.3(2C), 129.3(4C), 129.1(4C), 129.1(4C), 128.7(2C), 128.6-128.2(24C), 127.7(12C), 106.0(3C), 105.9(3C), 102.3, 84.3, 83.2(2C), 82.6, 82.2(2C), 82.1(2C), 82.1(2C), 82.1(2C), 81.5(4C), 81.0, 78.4, 77.3(2C), 77.3(2C), 77.2, 75.4, 65.8(6C), 63.4, 36.8, 36.7, 26.8(3C), 25.0, 22.7, 22.6, 19.3; **Optical Rotation**: $[\alpha]_D^{25}$ = -10º (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3016, 2941, 1718, 1453, 1264, 1109, 1071, 1027, 747, 707, 667; **HRMS** (ESI-MS) $[M+Na]^+$ calculated for $C_{158}H_{142}O_{45}SiNa$: 2810.8524 found 2810.8521.

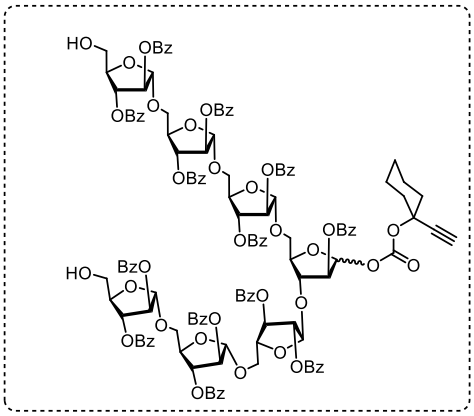
1-*O*-(((1-ethynylcyclohexyl) oxy) carbonyl) 2, 3 di-*O*-benzoyl 5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (**51**):



The compound **51** was synthesized from compound **50** according to general experimental method D (78% yield, white solid).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 – 7.84 (m, 28H), 7.60 – 7.24 (m, 42H), 6.33 (s, 1H), 5.76 – 5.57 (m, 13H), 5.43 – 5.35 (m, 6H), 4.67 – 4.41 (m, 8H), 4.25 – 4.10 (m, 6H), 4.02 – 3.85 (m, 8H), 2.65 (s, 1H), 2.34 (s, 1H), 2.26 – 2.11 (m, 2H), 2.07 – 1.63 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.1, 165.7, 165.6(4C), 165.2(4C), 165.2(4C), 151.0, 133.7-133.2(10C), 130.1-129.9 (30C),129.3 (2C), 129.2 (2C), 129.1(4C), 129.1(4C), 129.0(2C), 128.6 - 128.3(30C), 106.1, 106.0(5C), 102.4, 84.3, 83.7(2C), 82.7, 82.3, 82.2(4C), 82.1, 81.8, 81.7, 81.6(4C), 81.0, 78.5, 77.8, 77.4(2C), 77.3, 75.5, 66.2, 65.9(5C), 62.4, 36.9, 36.8, 25.0, 22.7, 22.7; **mp**: 96.5 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 5° (*c* 0.17, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3016, 2940, 1720, 1452, 1264, 1105, 1070, 1026, 747, 707, 666; **HRMS** (ESI-MS) $[M+Na]^+$ calculated for $C_{142}H_{124}O_{45}SiNa$ 2572.7346 found 2572.7385.

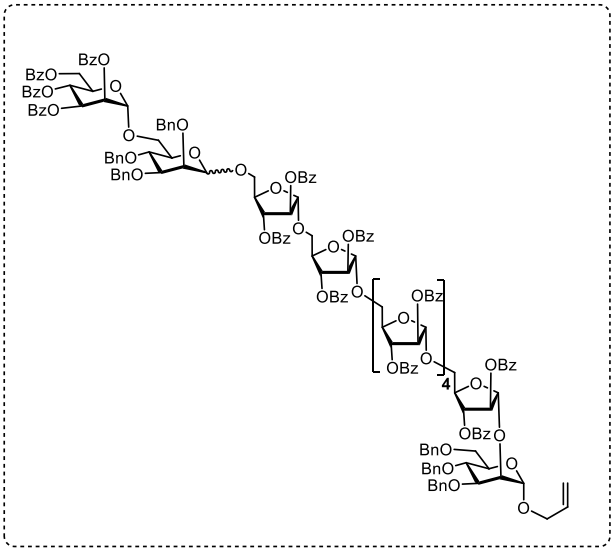
1-*O*-(((1-ethynylcyclohexyl) oxy) carbonyl) 2-*O*-benzoyl-3,5-di-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α/ β -D-arabinofuranoside (**53)**:



The compound **52**[55] was subjected to di-TBDPS deprotection according to general reaction procedure D to afford the compound **53**. (Yield 81%, white solid) (α :β = 2:1).

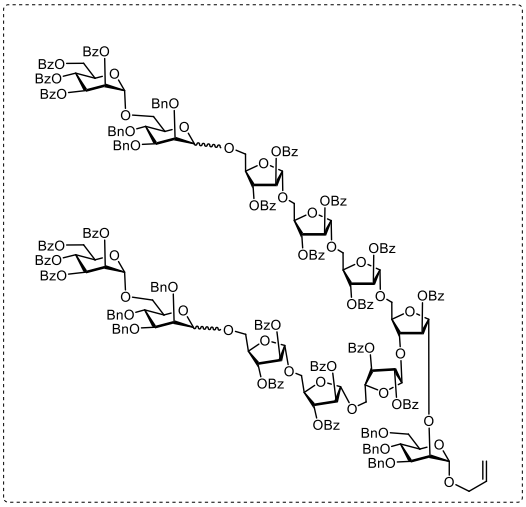
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 – 7.78 (m, 26H), 7.58 – 7.22 (m, 39H), 6.31 (s, 1H), 5.68 (d, *J* = 4.8 Hz, 1H), 5.59 (t, *J* = 4.9 Hz, 7H), 5.55 (d, *J* = 10.6 Hz, 1H), 5.52 (d, *J* = 2.8 Hz, 2H), 5.45 – 5.31 (m, 6H), 5.28 (s, 2H), 4.57 (d, *J* = 15.2 Hz, 4H), 4.46 – 4.34 (m, 4H), 4.22 – 4.06 (m, 4H), 4.03 – 3.94 (m, 3H), 3.94 – 3.76 (m, 7H), 2.63 (s, 1H), 2.53 – 2.31 (m, 2H), 2.25 – 1.99 (m, 2H), 1.95 – 1.50 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.0 (2C), 166.0 (2C), 165.6 (4C), 165.6 (4C), 165.4, 165.3 (2C), 165.2 (5C), 165.2, 165.1 (5C), 151.2, 151.0, 133.5-133.2 (30C), 130.1 (2C), 130.0-129.7 (50C), 129.2 (4C), 129.2 (4C), 129.1 (4C), 129.0 (4C), 129.0 (6C), 128.8 (2C), 128.5-128.3 (50C), 106.0 (2C), 105.9 (4C), 105.8 (4C), 105.3 (2C), 102.8, 102.7, 83.7 (8C), 82.9 (2C), 82.6 (2C), 82.5 (2C), 82.4, 82.3, 82.1 (2C), 81.9, 81.7 (8C), 81.6 (2C), 81.4, 81.4 (2C), 80.0 (2C), 78.5 (2C), 77.7 (4C), 77.3 (6C), 77.0 (2C), 75.4 (2C), 66.0 (2C), 65.6 (2C), 65.6 (2C), 65.5 (2C), 65.3 (2C), 62.3 (4C), 36.9, 36.7, 36.6, 36.5, 25.0, 24.8, 22.7, 22.6, 22.6, 22.5; **mp**: 106 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = -5º (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3390, 2935, 1719, 1602, 1452, 1249, 1178, 1107, 1069, 1027, 754, 708; **HRMS** (ESI-MS): $[M+Na]^+$ calculated for $C_{135}H_{120}O_{44}$ 2468.7084 found 2468.7088

Allyl-3,4,6-tri-*O*-benzyl-2-*O*-(2, 3 di-O-benzoyl -5-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α/β-D-mannopyranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D- Mannopyranoside (**57**)):



This compound was synthesized according to above mentioned one pot glycan editing procedure (51% overall yield, white solid). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.15 – 7.75 (m, 36H), 7.57 – 7.14 (m, 84H), 6.11 (t, *J* = 10.1, 3.5 Hz, 1H), 5.97 (t, *J* = 10.2, 6.8, 3.3 Hz, 1H), 5.81 – 5.72 (m, 2H), 5.69 – 5.62 (m, 12H), 5.62 – 5.53 (m, 3H), 5.46 – 5.36 (m, 7H), 5.30 – 5.14 (m, 2H), 5.02 (t, *J* = 5.9 Hz, 2H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.80 – 4.73 (m, 1H), 4.70 – 4.49 (m, 14H), 4.42 – 4.34 (m, 1H), 4.30 (d, *J* = 11.1 Hz, 2H), 4.25 – 4.13 (m, 10H), 4.12 – 4.02 (m, 2H), 4.02 – 3.81 (m, 16H), 3.75 – 3.64 (m, 2H), 3.63 – 3.55 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 166.3-165.1(36C), 138.7, 138.7, 138.7, 138.5, 138.5, 138.5, 138.5, 138.5, 138.4, 138.4, 138.2, 138.1, 133.9(2C), 133.6 -133.0(40C), 130.1 - 129.8(80C), 129.7-129.2(36C), 128.6 - 128.1(80C), 128.0 - 127.5(40C), 117.3(2C), 108.5, 106.8, 106.2, 106.0-105.9(12C), 101.9, 98.5, 98.5, 98.0, 97.8, 82.7- 81.6(42C), 80.6, 80.1, 79.8, 78.0, 77.8, 75.3, 75.2(2C), 75.1, 75.0, 74.8(2C), 74.7(2C), 74.5, 74.2(2C), 73.9(2C), 73.6, 73.3, 72.7(2C), 72.2, 71.9, 71.9(2C), 71.7, 71.1, 70.6, 70.5, 70.2, 70.1, 69.6(2C), 68.9, 68.9, 68.0(2C), 67.0(2C), 66.9, 66.0(14C), 62.8(2C); **mp**: 97 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = + 3° (*c* 0.15, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2918, 1722, 1452, 1264, 1109, 1026, 708 ; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{224}H_{200}O_{62}Na$ 3905.243 found 3905.878

Allyl-3,4,6-tri-*O*-benzyl-2-*O*- (3, 5-di-*O*-(2, 3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3-di-*O*-benzoyl-5-*O*-(2,3,4,-tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α/β-D-mannopyranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-Mannopyranoside (**59**):



This compound was synthesized according to above mentioned one pot glycan editing procedure. (45% overall yield, white solid).

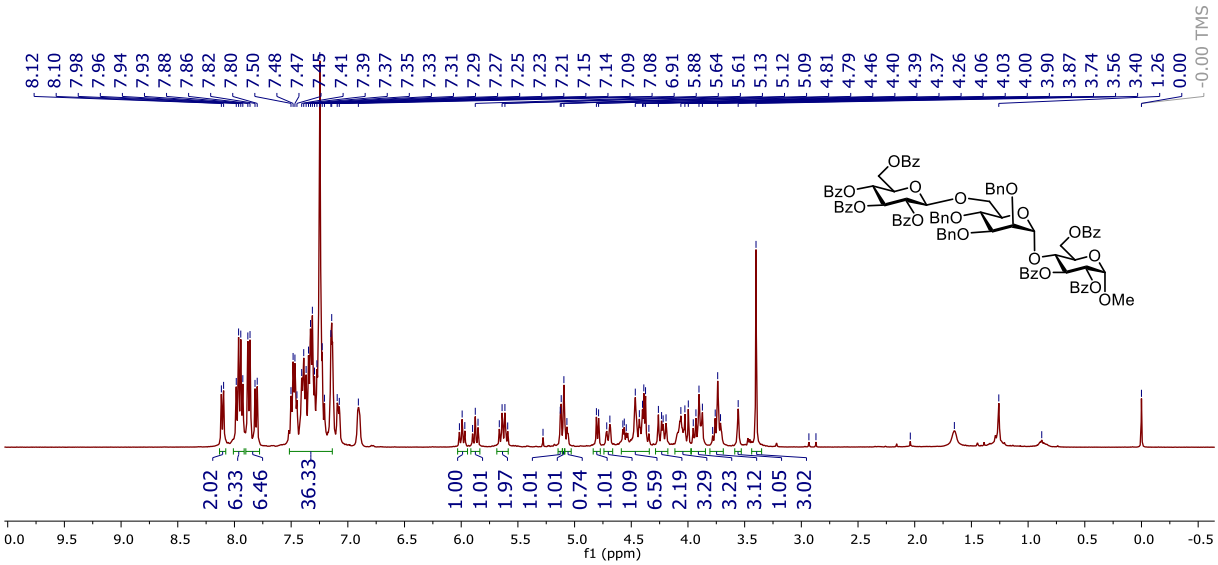
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14 – 7.80 (m, 44H), 7.61 – 7.11 (m, 111H), 6.10 (t, *J* = 10.2 Hz, 2H), 5.97 (d, *J* = 9.4 Hz, 2H), 5.77 (d, *J* = 18.9 Hz, 2H), 5.71 (s, 1H), 5.69 – 5.62 (m, 6H), 5.61 – 5.53 (m, 5H), 5.49 (dd, *J* = 15.0, 6.1 Hz, 2H), 5.40 (q, *J* = 9.4, 8.6 Hz, 3H), 5.30 (d, *J* = 8.9 Hz, 1H), 5.27 (d, *J* = 10.5 Hz, 1H), 5.21 (d, *J* = 5.2 Hz, 1H), 5.14 (d, *J* = 9.3 Hz, 1H), 5.04 – 5.00 (m, 3H), 4.97 – 4.91 (m, 1H), 4.82 (d, *J* = 10.7 Hz, 1H), 4.74 (dd, *J* = 23.4, 10.9 Hz, 4H), 4.68 – 4.40 (m, 20H), 4.36 (dd, *J* = 11.8, 7.8 Hz, 3H), 4.31 – 4.13 (m, 11H), 4.11 – 4.02 (m, 5H), 3.98 (m, 5H), 3.93 – 3.82 (m, 13H), 3.76 (d, *J* = 4.0 Hz, 1H), 3.63 – 3.55 (m, 1H); **^{13}C NMR** (151 MHz, $CDCl_3$): δ 166.3 (8C), 165.7 (8C), 165.6 (8C), 165.5,165.4, 165.3 (8C), 165.1 (8C), 138.7, 138.7, 138.6, 138.5, 138.5 (4C), 138.5 (4C), 138.4, 138.3, 138.1 (4C), 134.1, 134.0, 133.5, 133.5-133.0 (20C), 130.0-129.8 (120C), 129.7- 129.1 (44C), 128.6 -128.3 (120C), 128.0 -127.4 (50C), 117.3, 117.2, 106.2-106.0 (10C), 105.3, 105.1, 101.9 (2C), 98.8, 98.6, 98.5, 98.0, 97.8, 97.8, 82.7-81.4 (24C), 80.6-80.1 (12C), 78.1, 78.1, 78.0, 77.8, 75.3-75.0 (14C), 74.7-74.5 (16C), 74.2 (2C), 73.9, 73.5, 73.3 (4C), 73.3, 72.7 (4C), 72.3 - 71.7 (8C), 71.1, 70.6 ,70.5 , 70.0, 69.7 (2C), 69.3 (2C), 68.9, 68.9, 67.9 (4C), 67.1, 67.0, 66.9, 66.8, 66.7 (4C), 65.9-65.5 (12C), 62.8, 62.8; **mp**: 100 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = -5° (*c* 0.11, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2925, 1722, 1452, 1263, 1107, 1027, 709. **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{278}H_{250}O_{75}Na$ 4813.247 found 4813.147

Note: Characterization data and full spectral charts for all compounds can also be found in the Supporting information of the following publication. Sen, S.; Kundu, S.; Pasari, S.; Hotha, S. *Commun. Chem.* **2024**, *7* (1), 1–6. It can be downloaded free of charge from the following link: Supplementary Data 1: communication chemistry

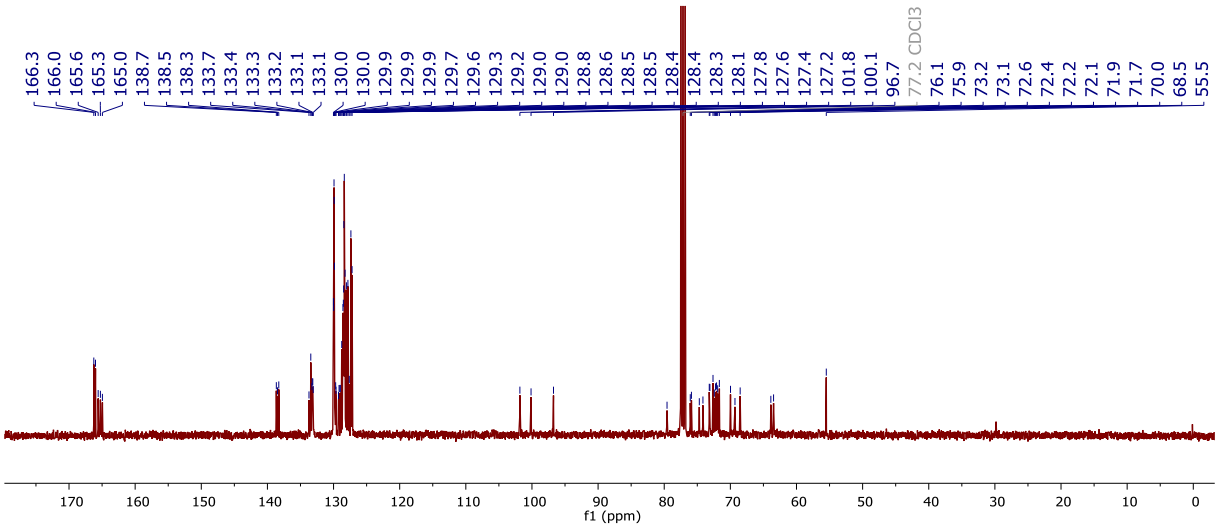
Spectral charts of some of the important compounds are provided here below.

3.4- Spectral Charts of Representative Compounds

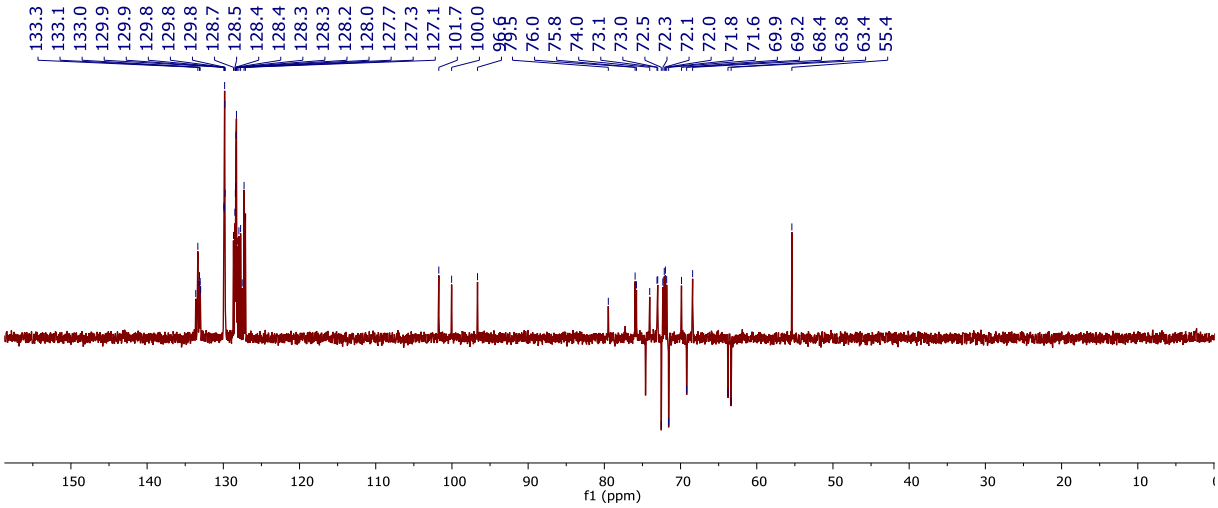
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **7**



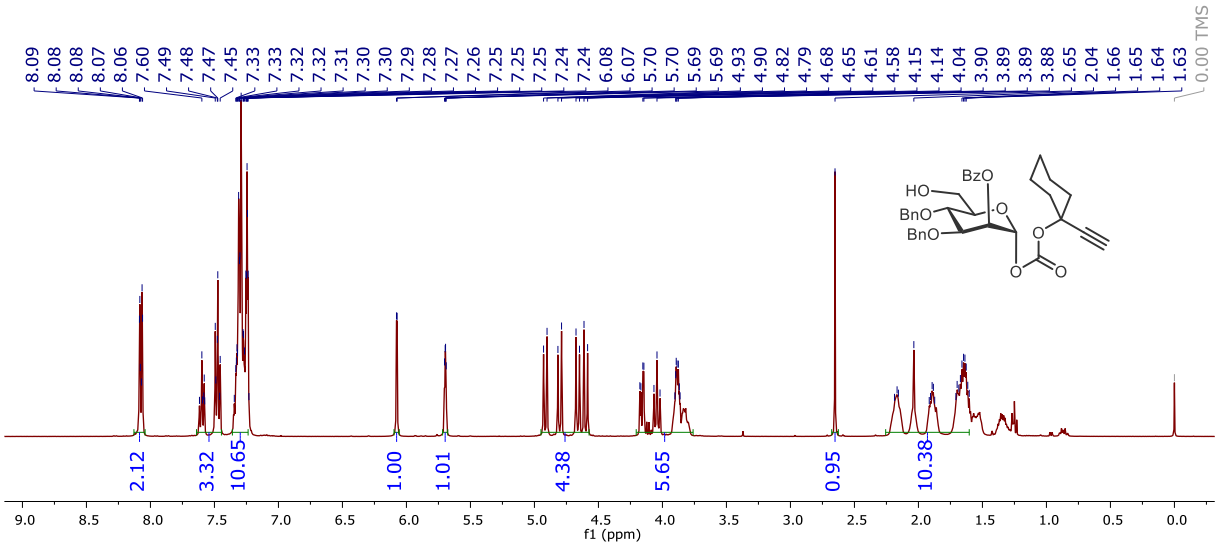
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **7**



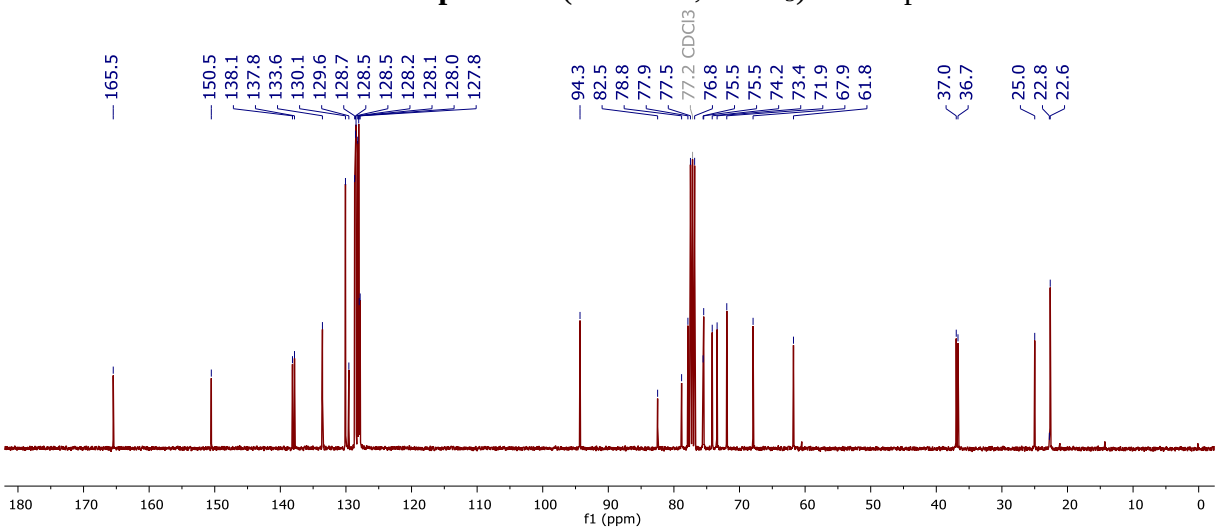
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **7**



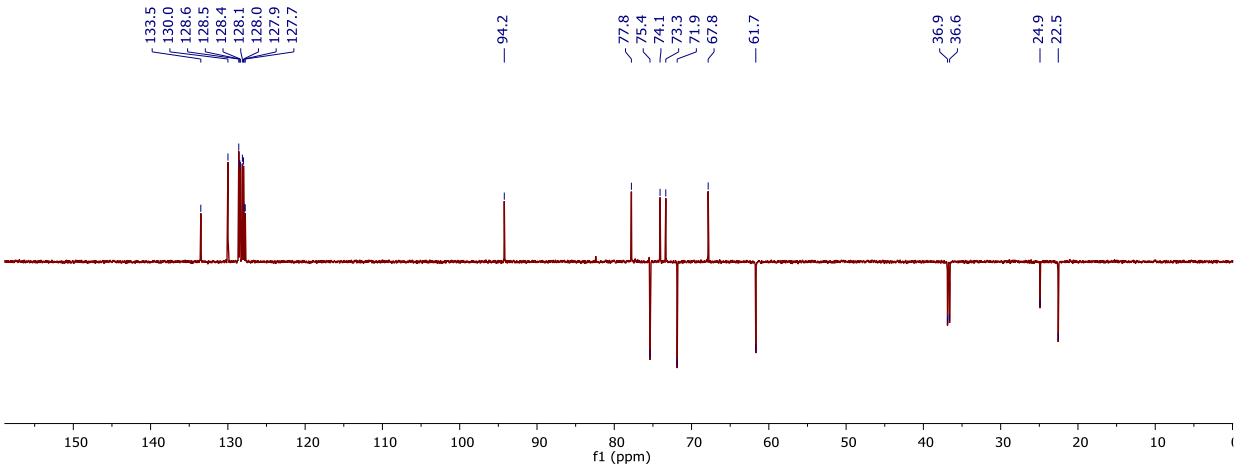
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8a**



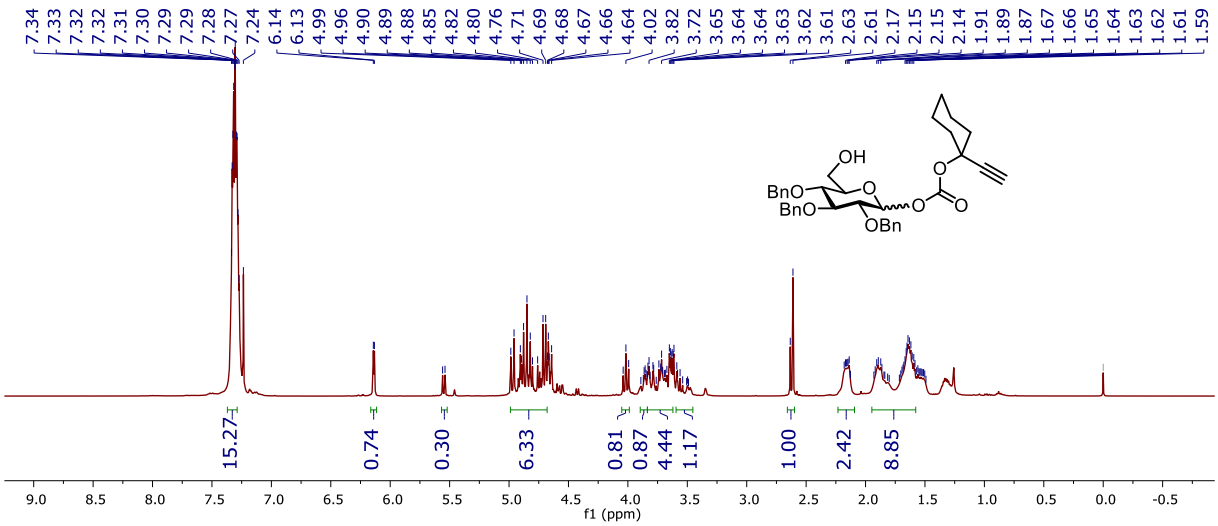
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8a**



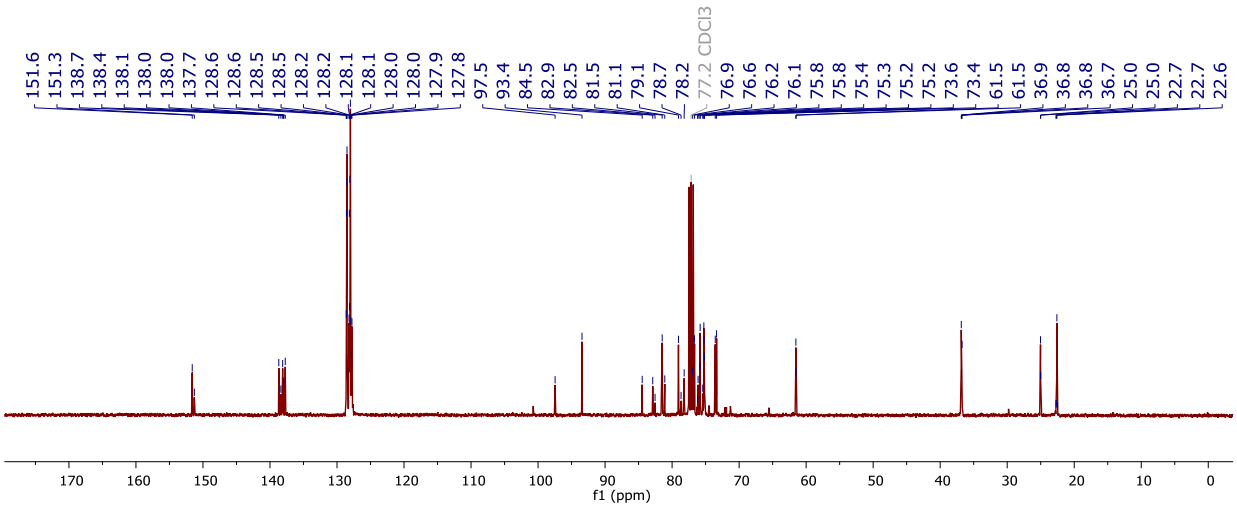
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8a**



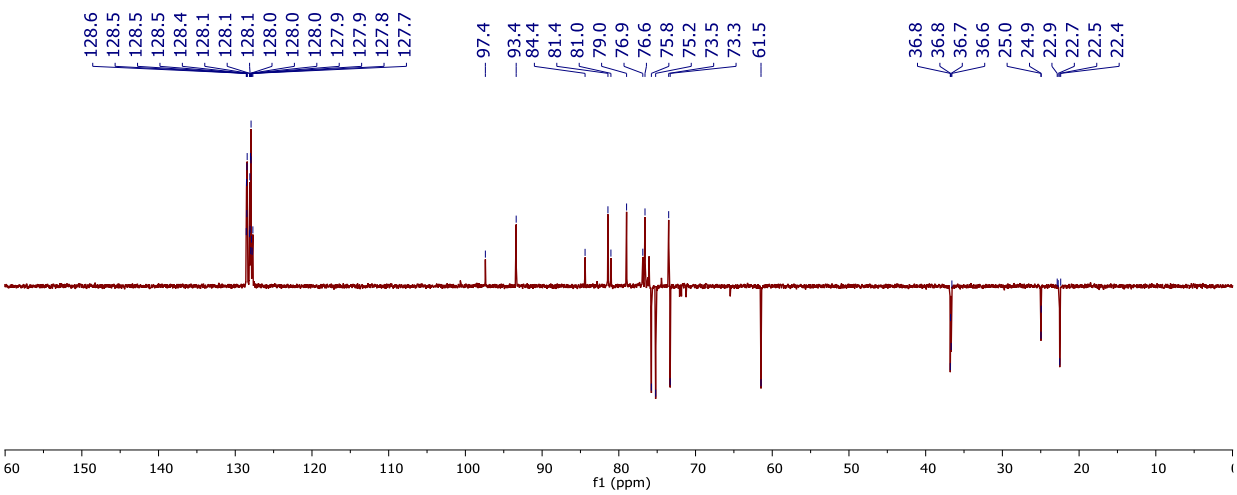
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8b**



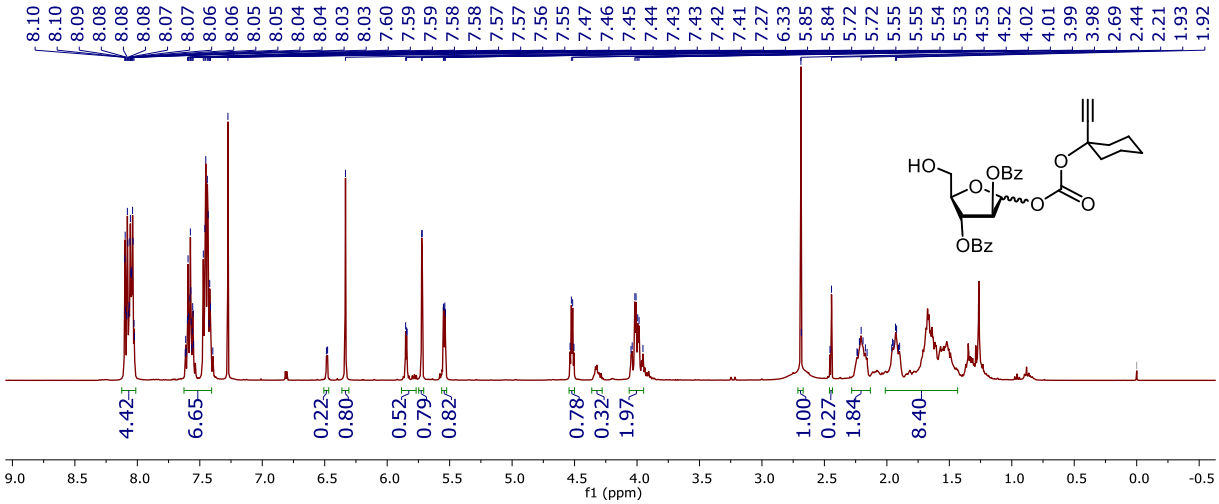
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8b**



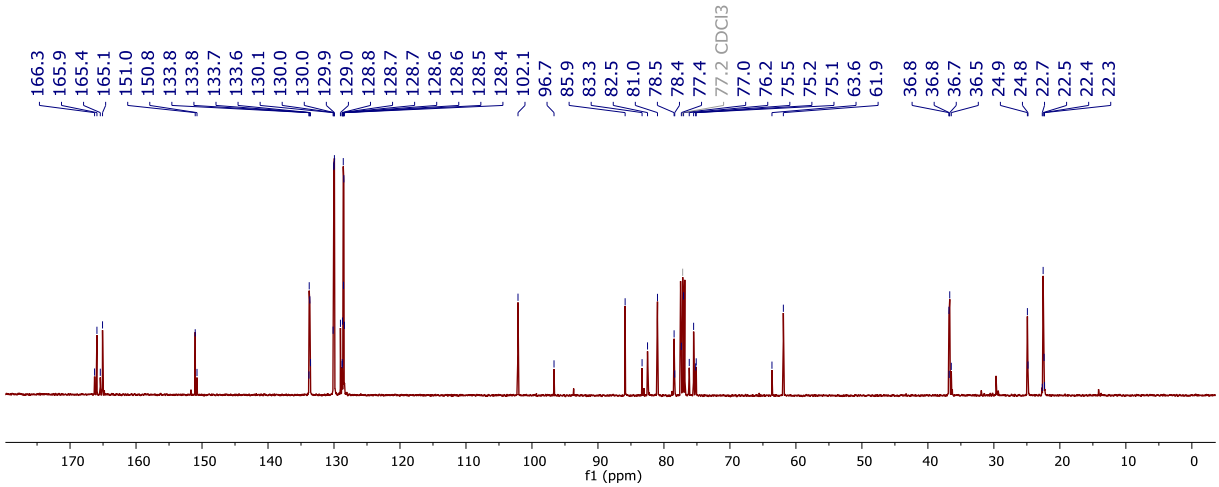
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8b**



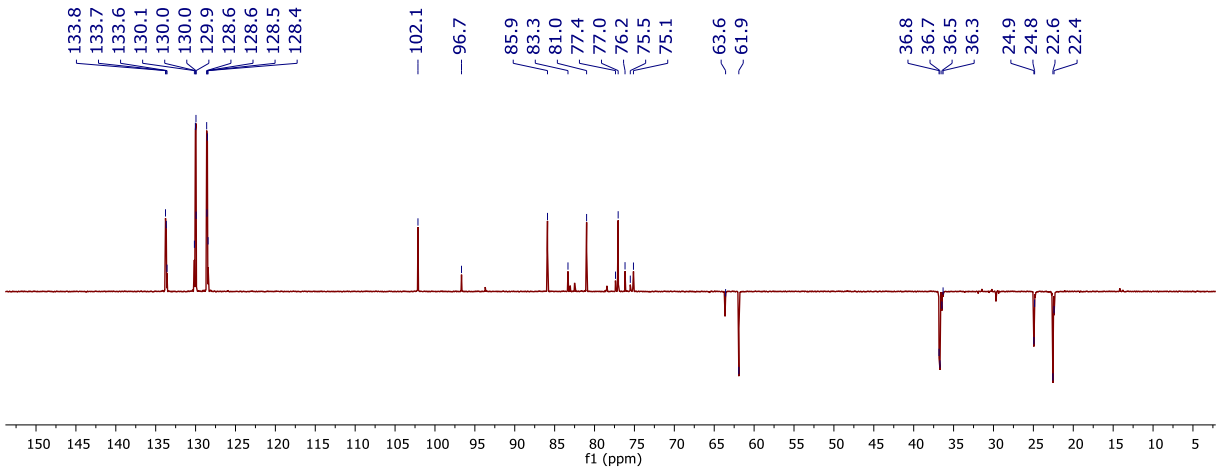
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8c**



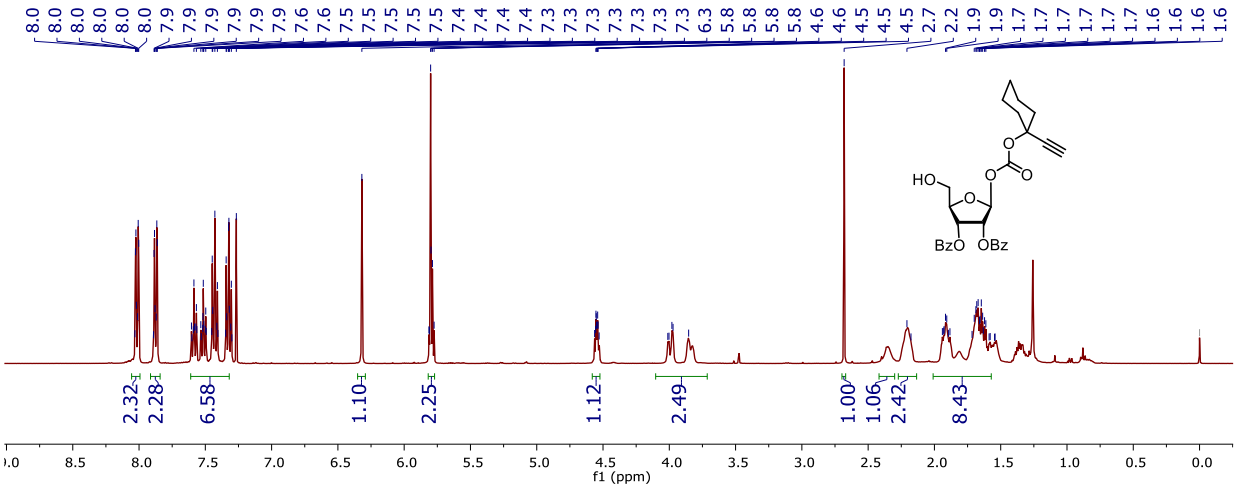
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8C**



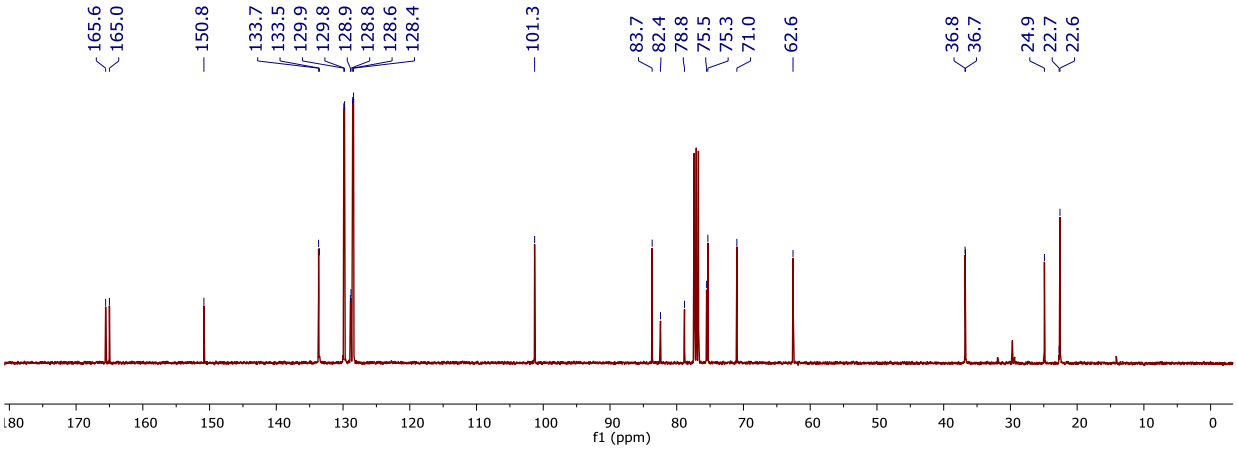
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8c**



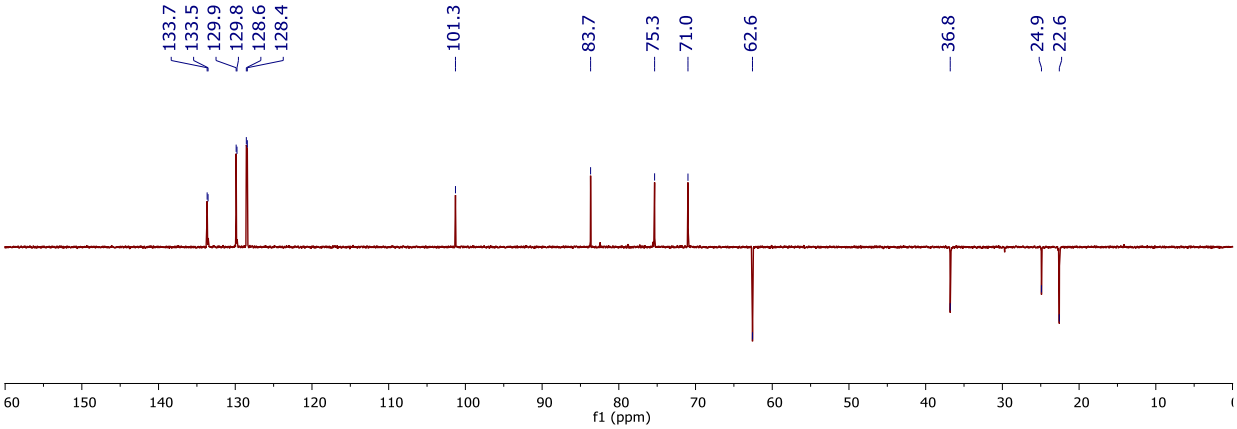
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8d**



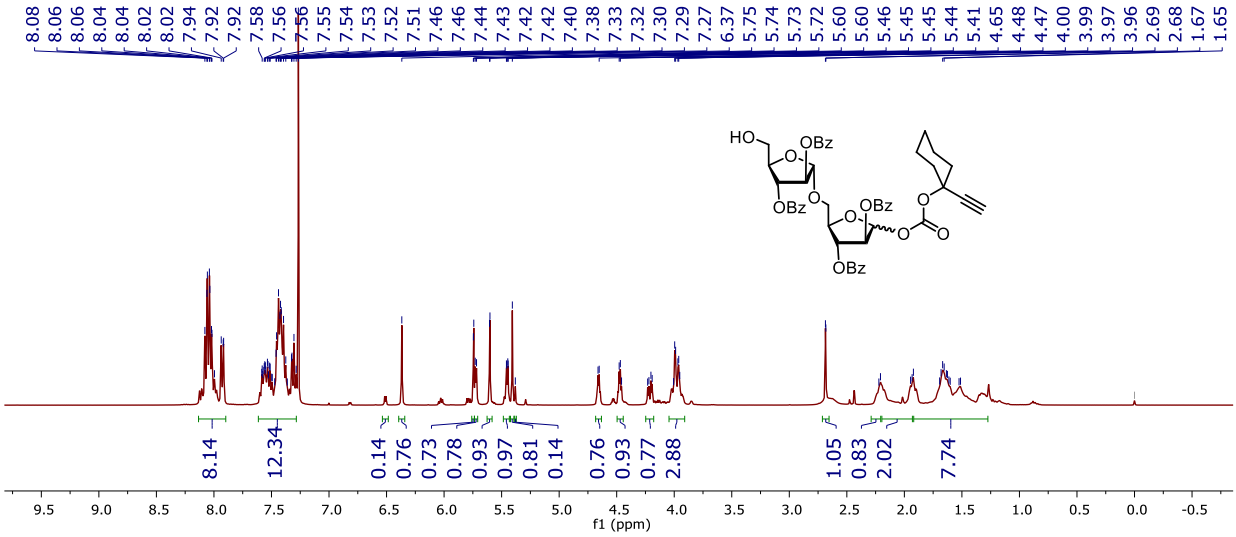
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8d**



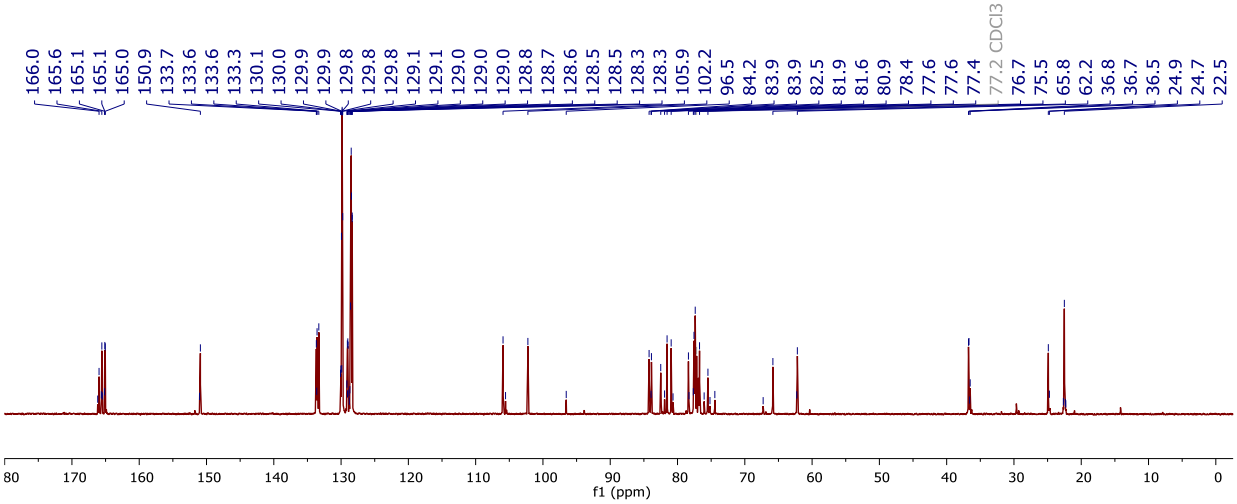
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8d**



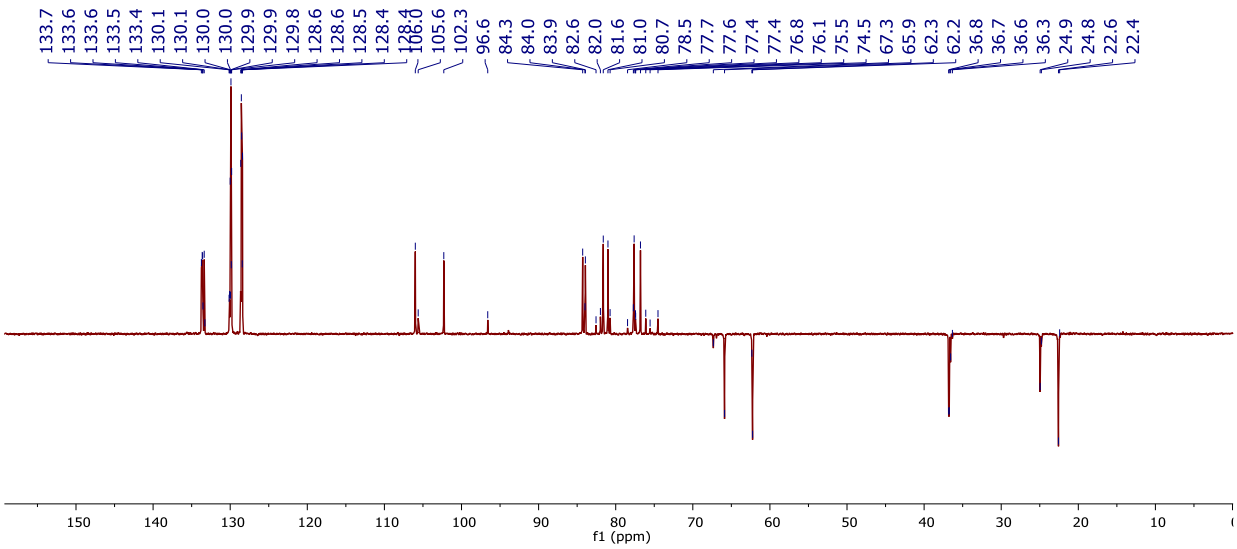
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8e**



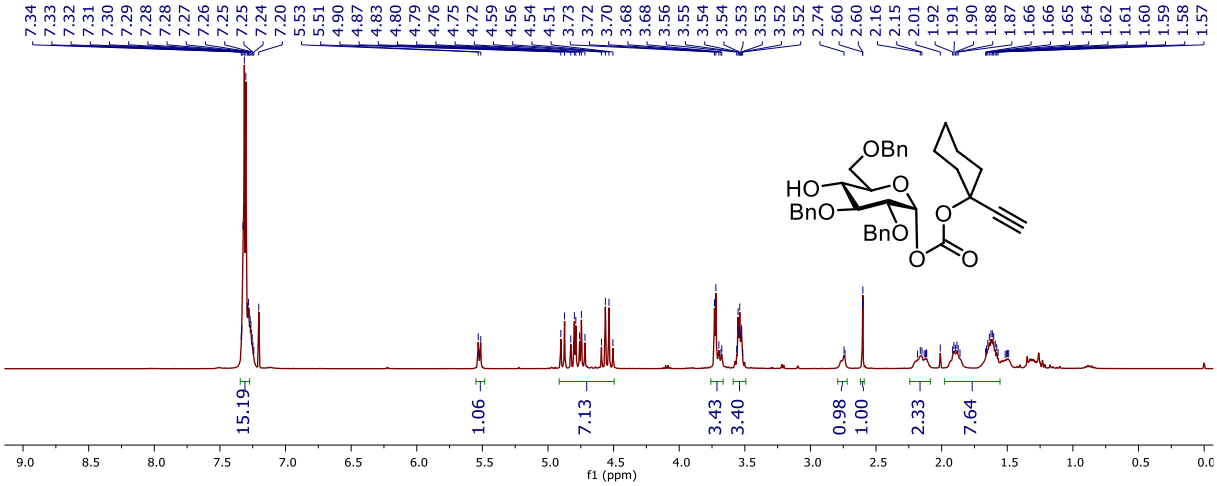
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8e**



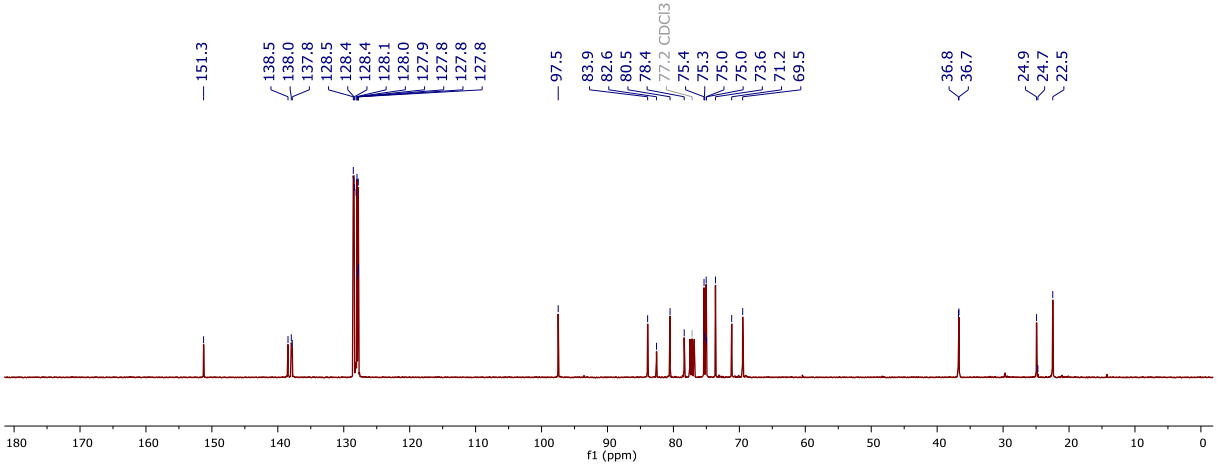
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8e**



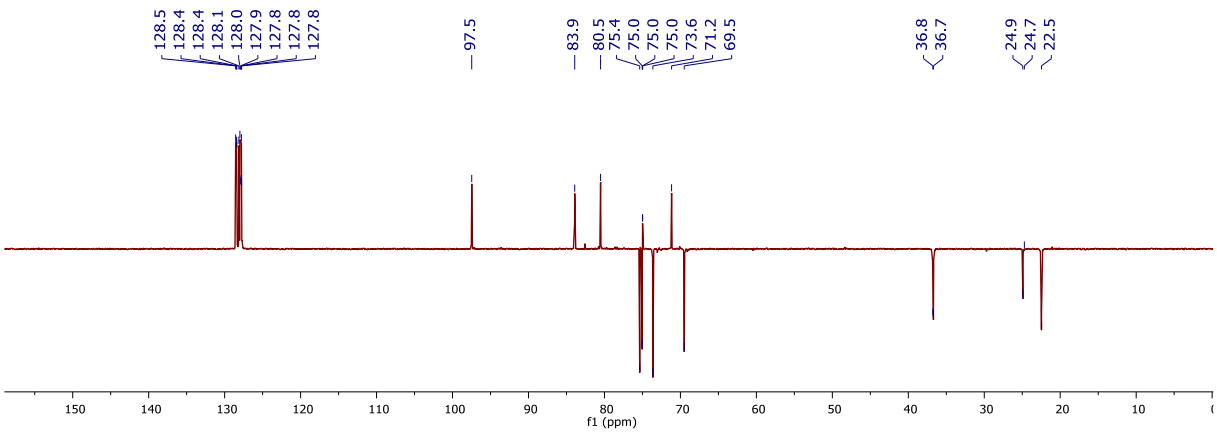
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8f**



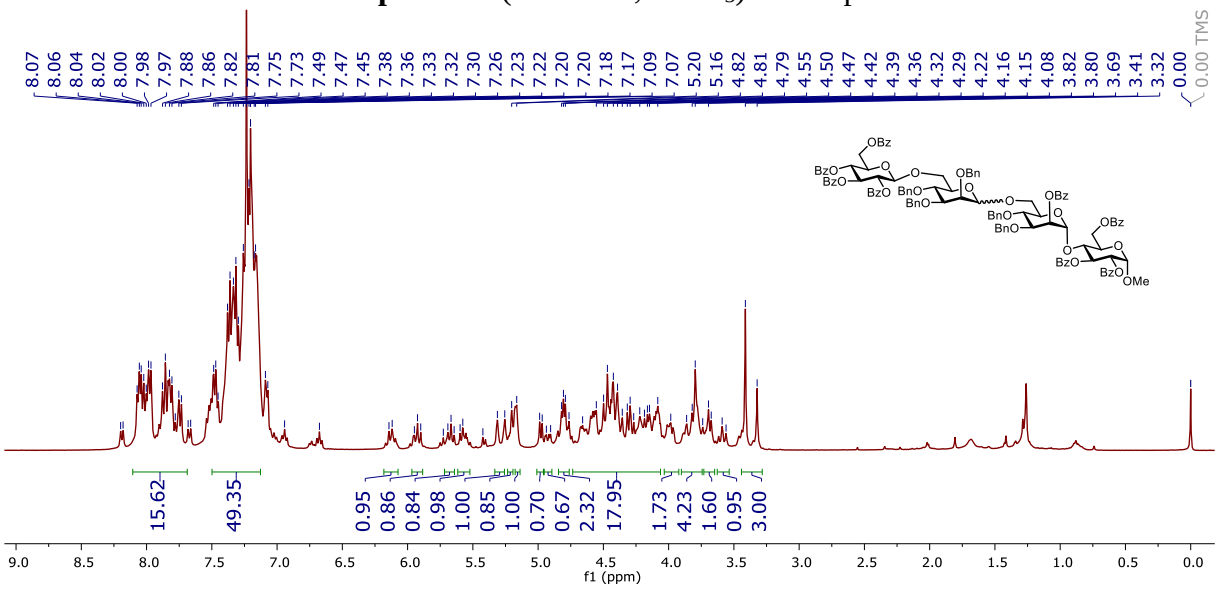
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8f**



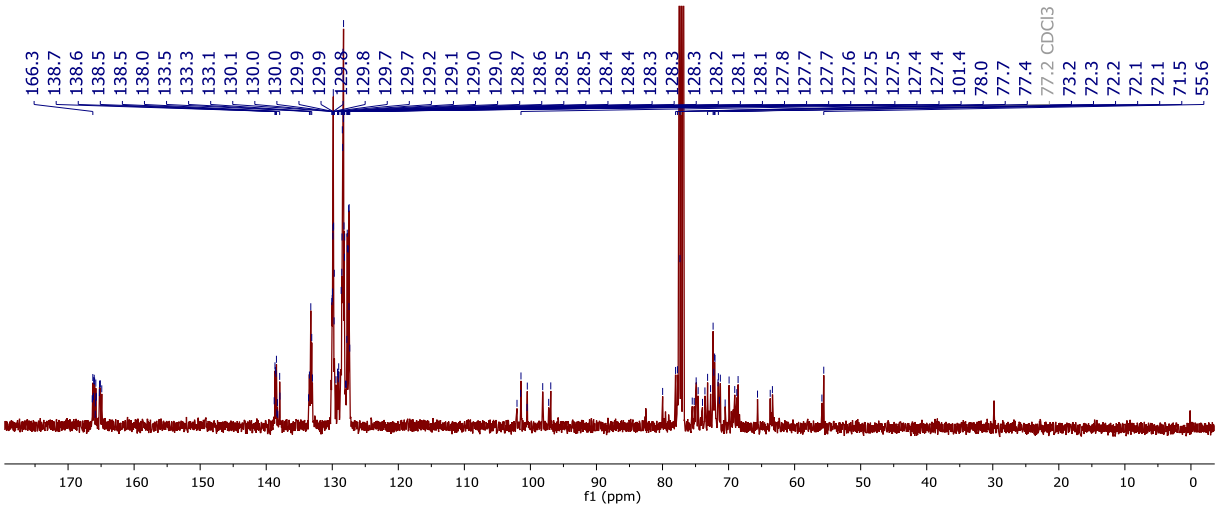
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **8f**



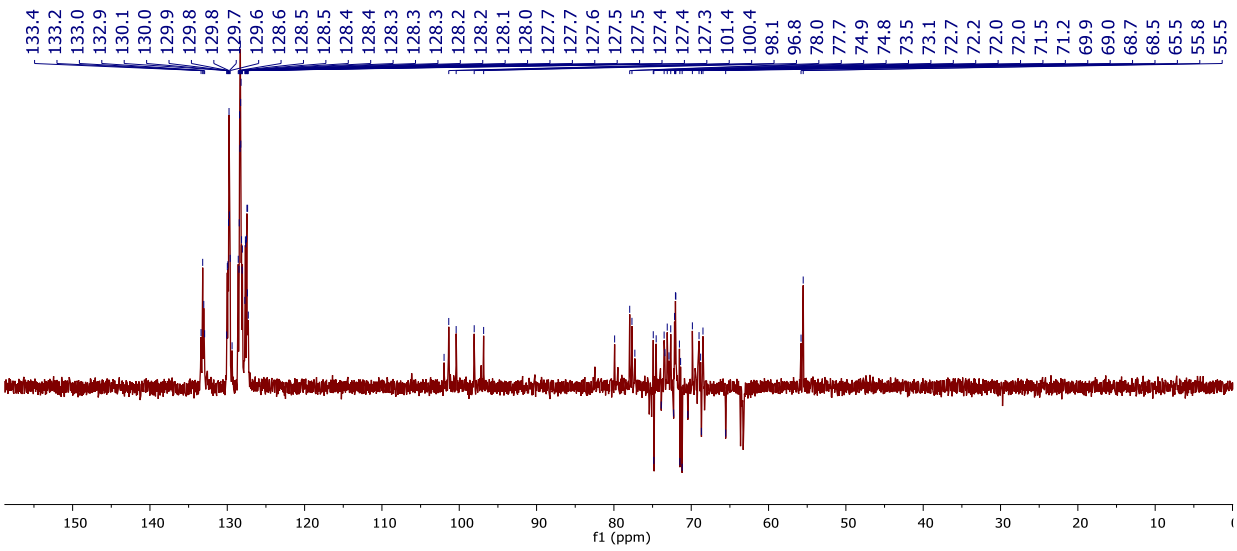
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **10a**



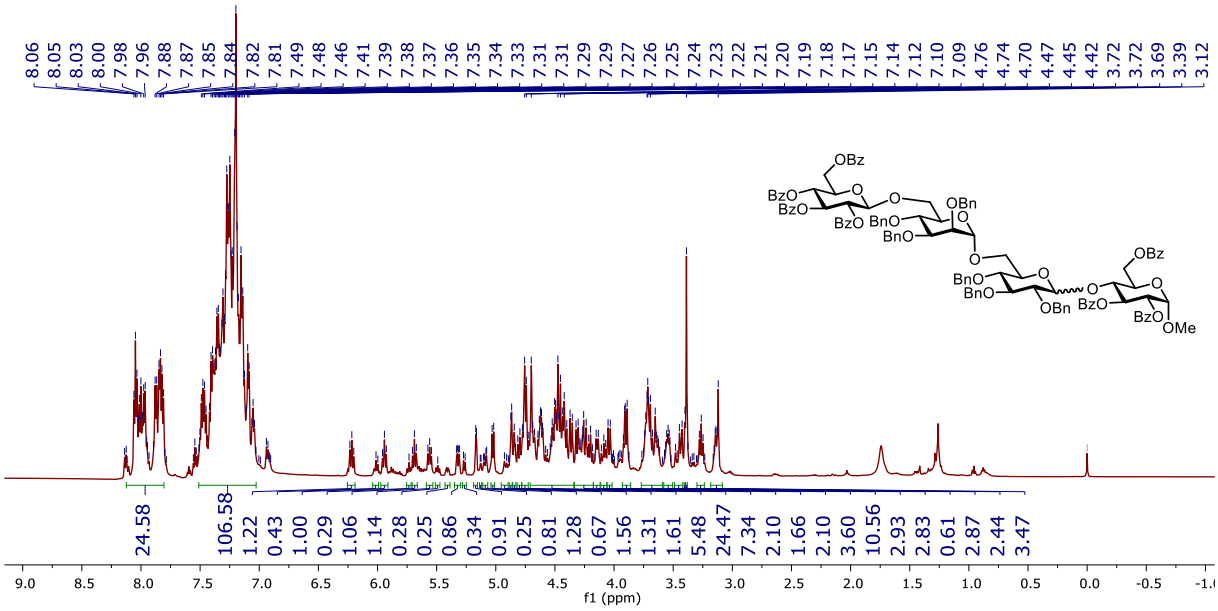
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **10a**



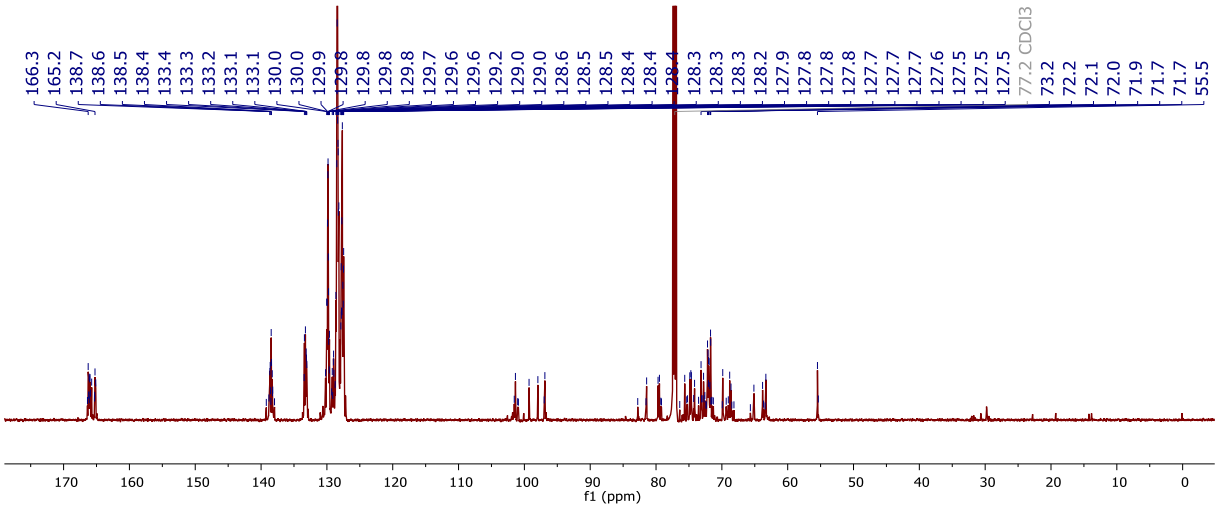
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **10a**



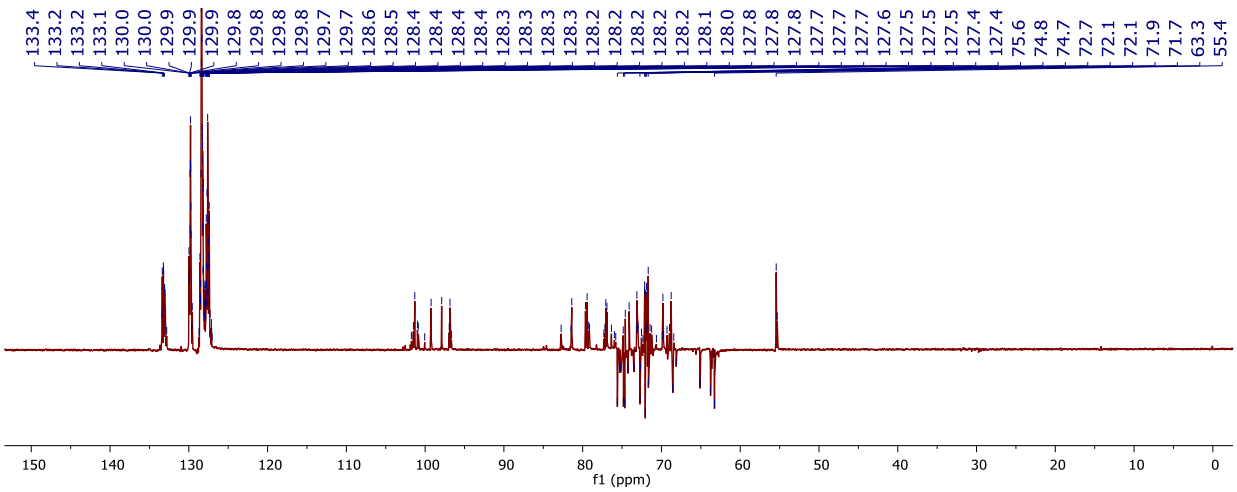
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **10b**



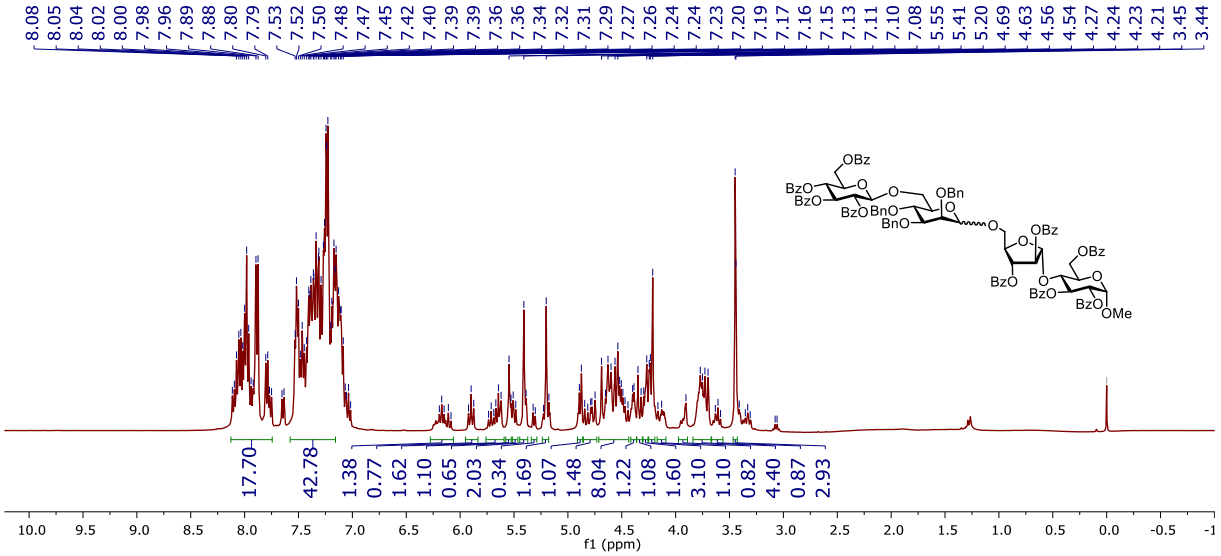
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **10b**



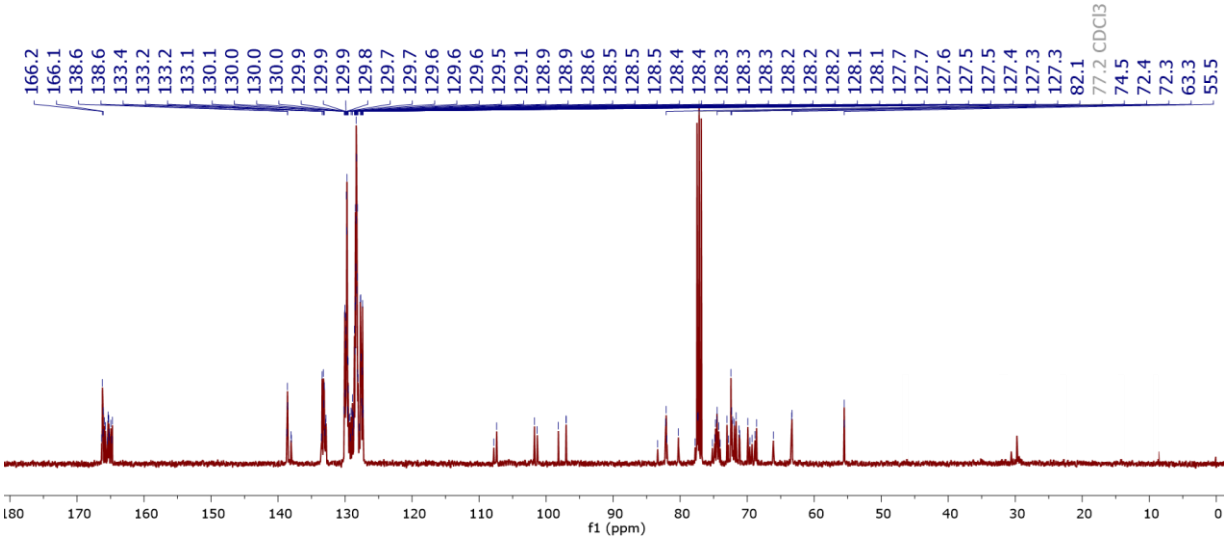
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **10b**



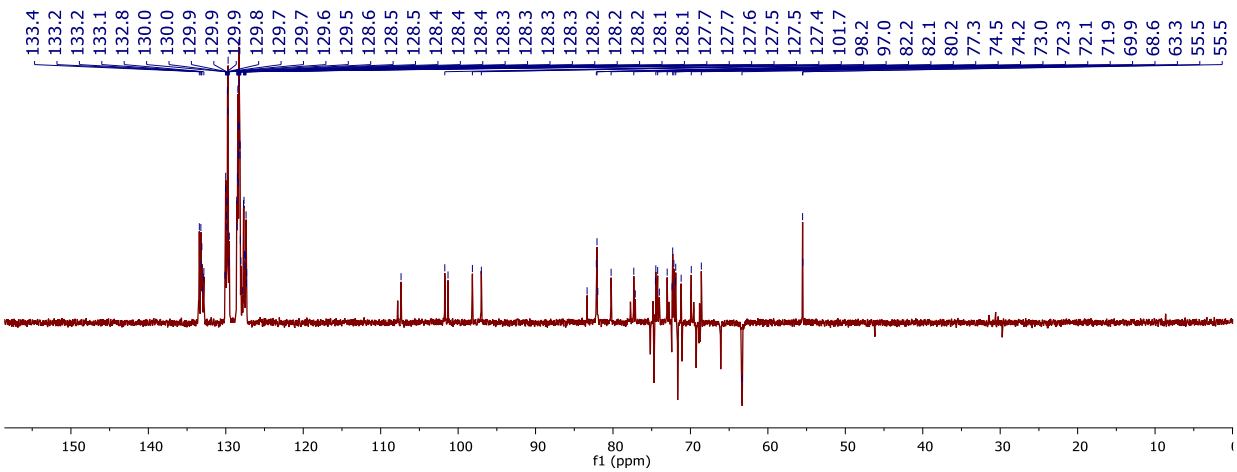
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **10c**



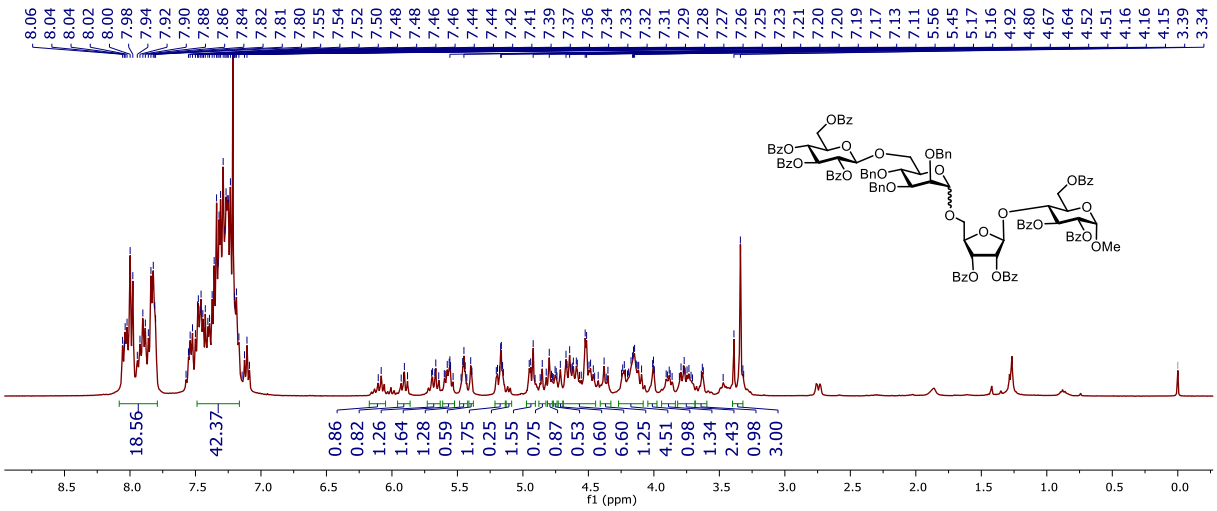
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **10c**



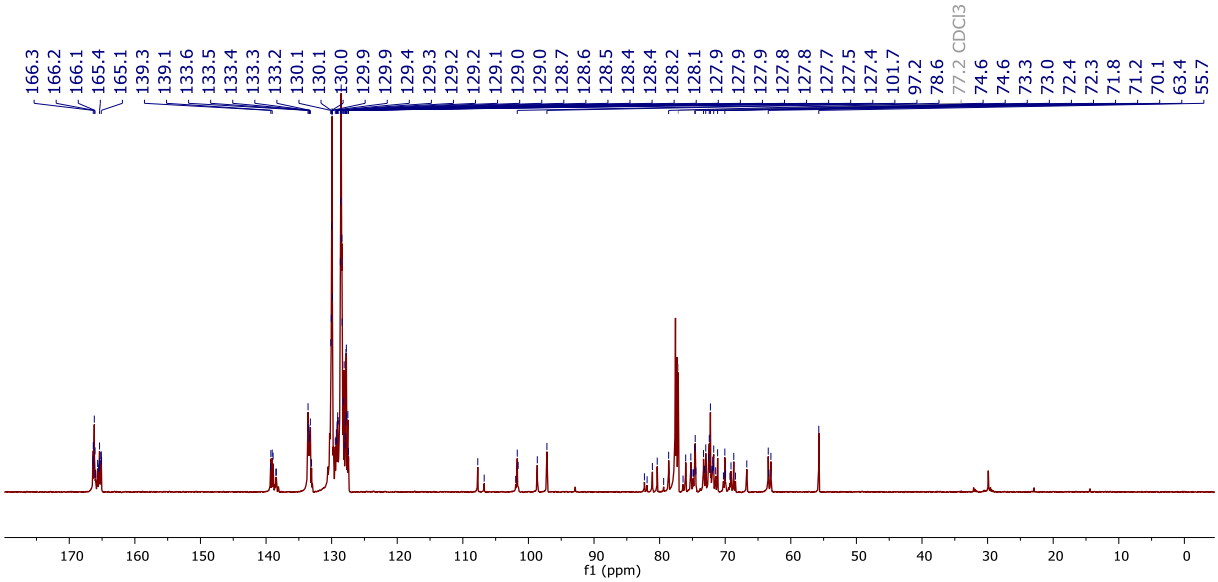
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **10c**



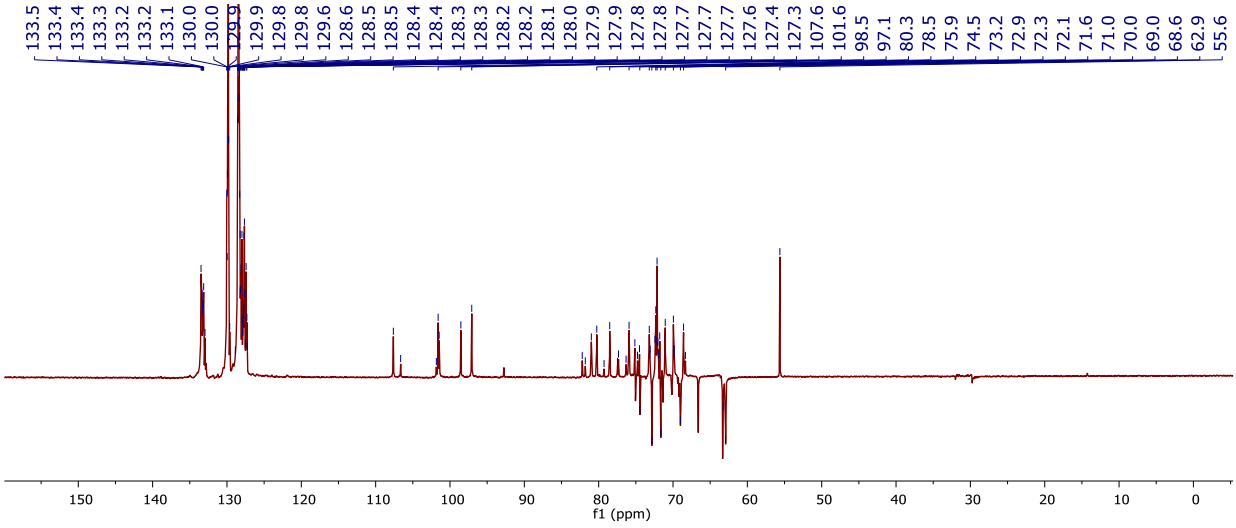
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **10d**



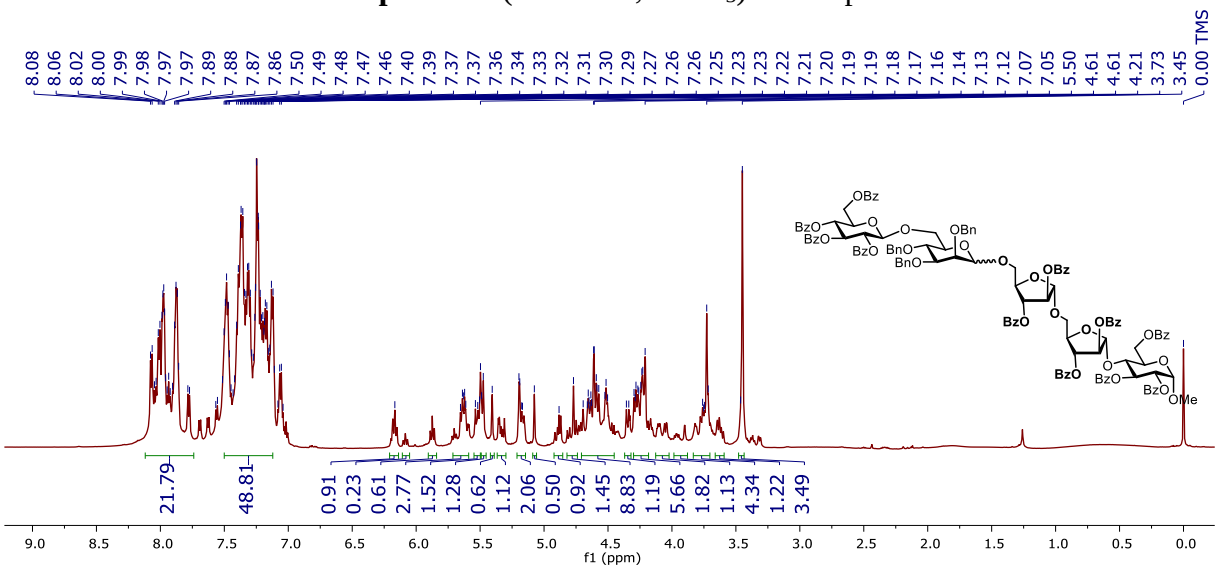
^{13}C NMR Spectrum (151MHz, $CDCl_3$) of compound **10d**



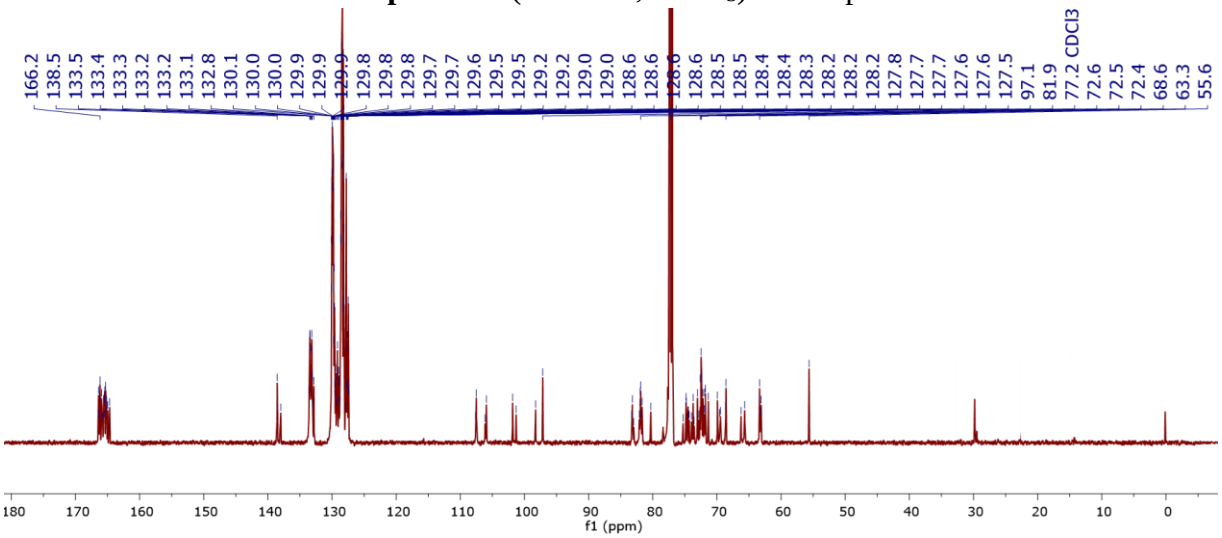
DEPTNMR Spectrum (151MHz, $CDCl_3$) of compound **10d**



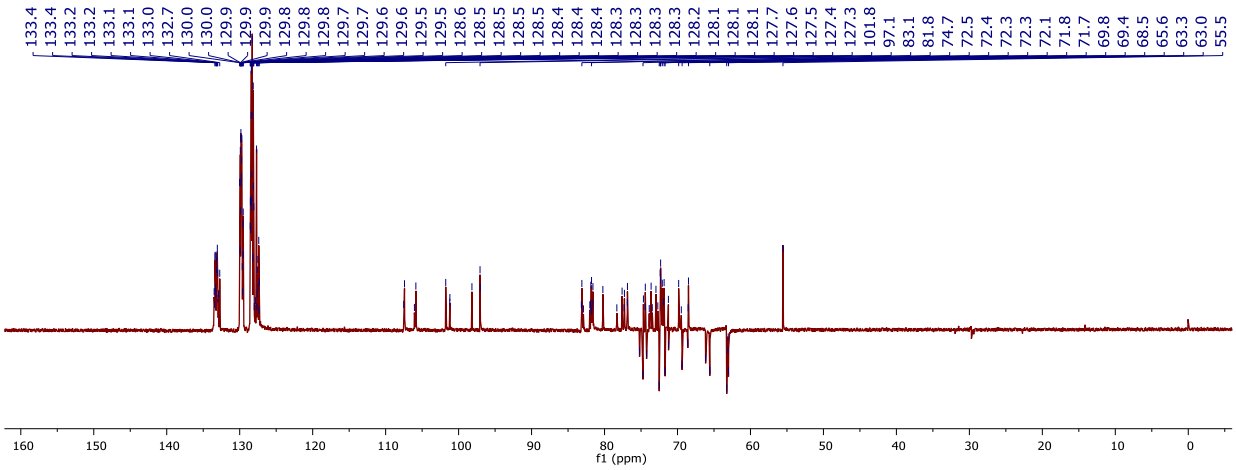
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **10e**



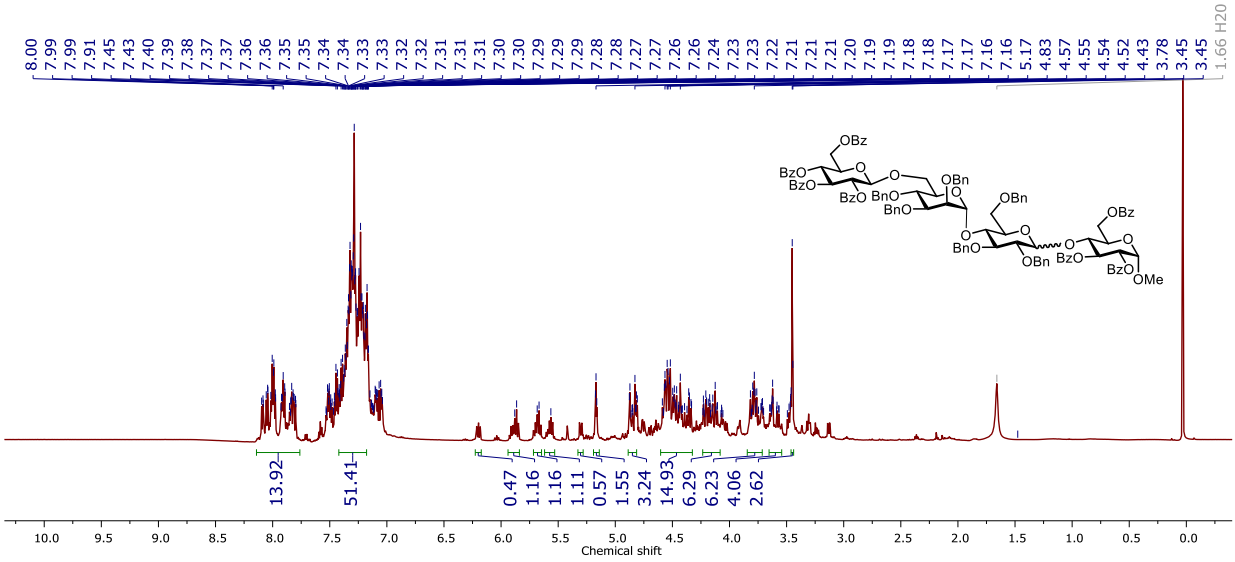
^{13}C NMR Spectrum (151MHz, $CDCl_3$) of compound **10e**



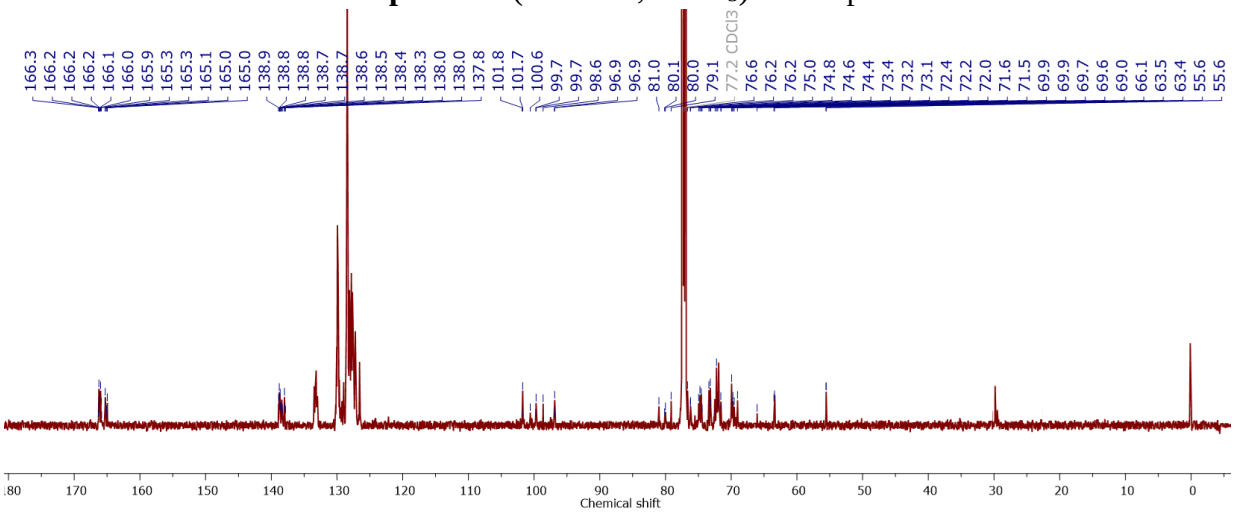
DEPT NMR Spectrum (151MHz, $CDCl_3$) of compound **10e**



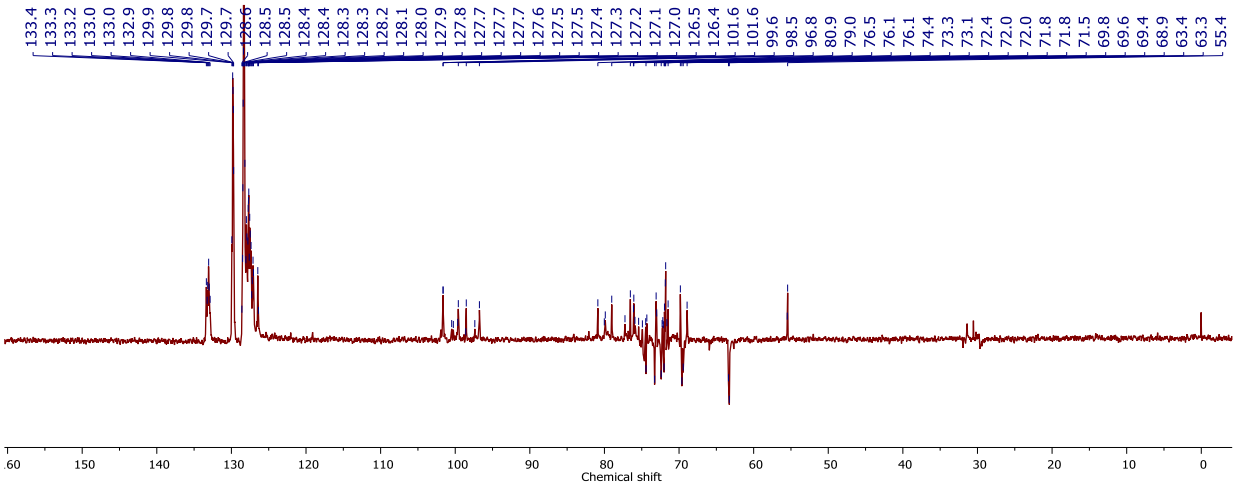
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **10f**



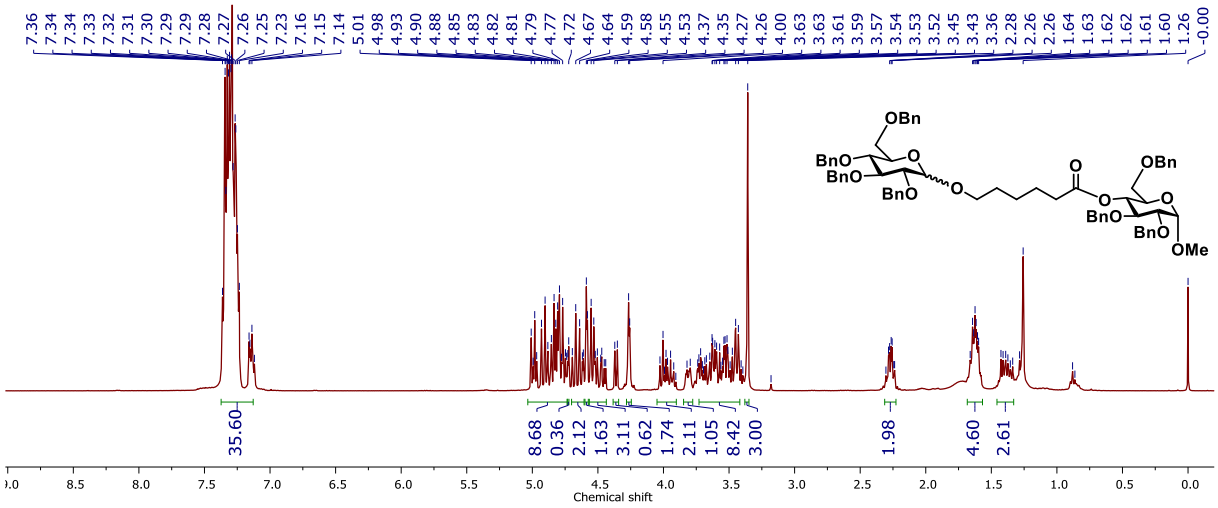
^{13}C NMR Spectrum (151MHz, $CDCl_3$) of compound **10f**



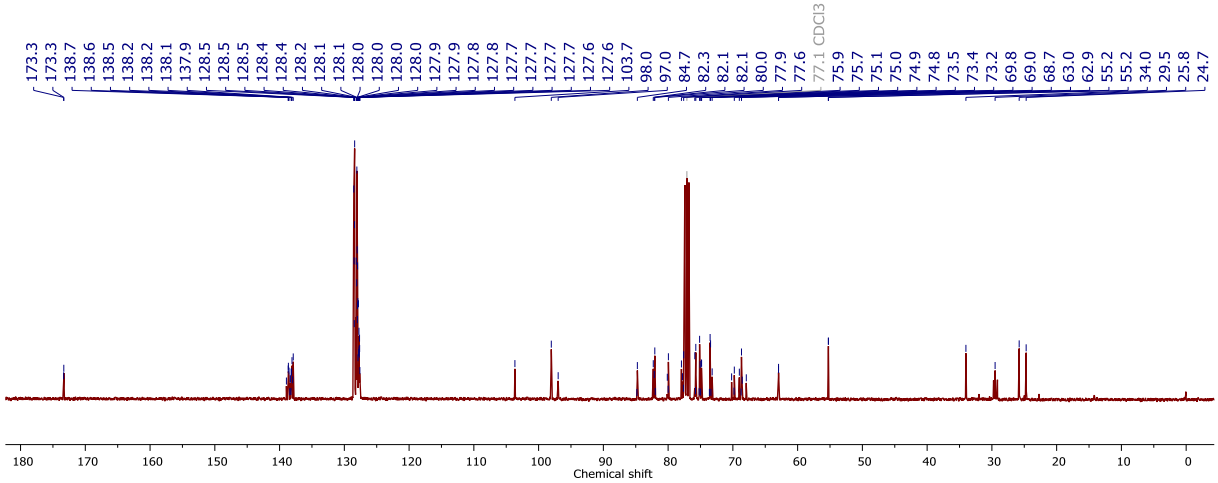
DEPT NMR Spectrum (151MHz, $CDCl_3$) of compound **10f**



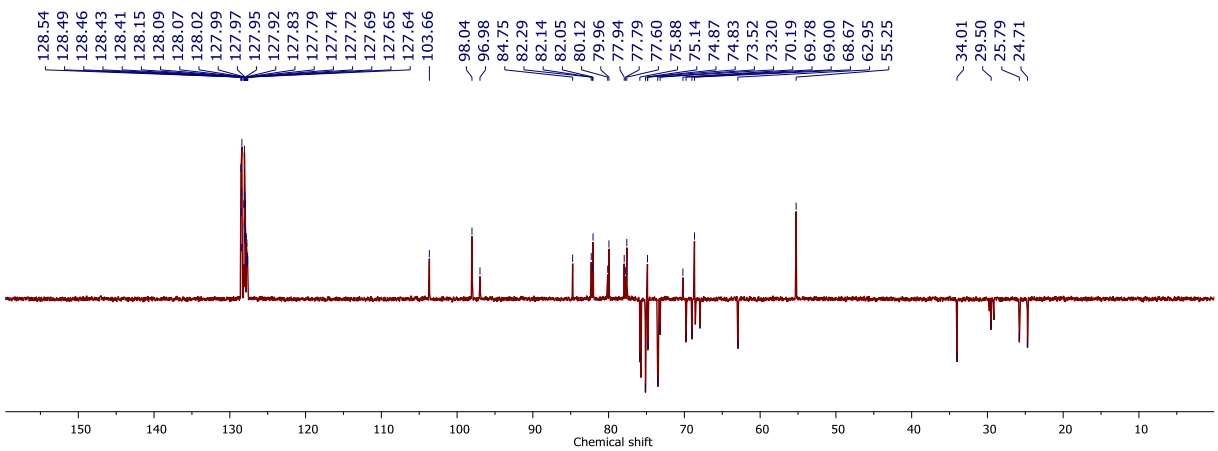
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **30a**



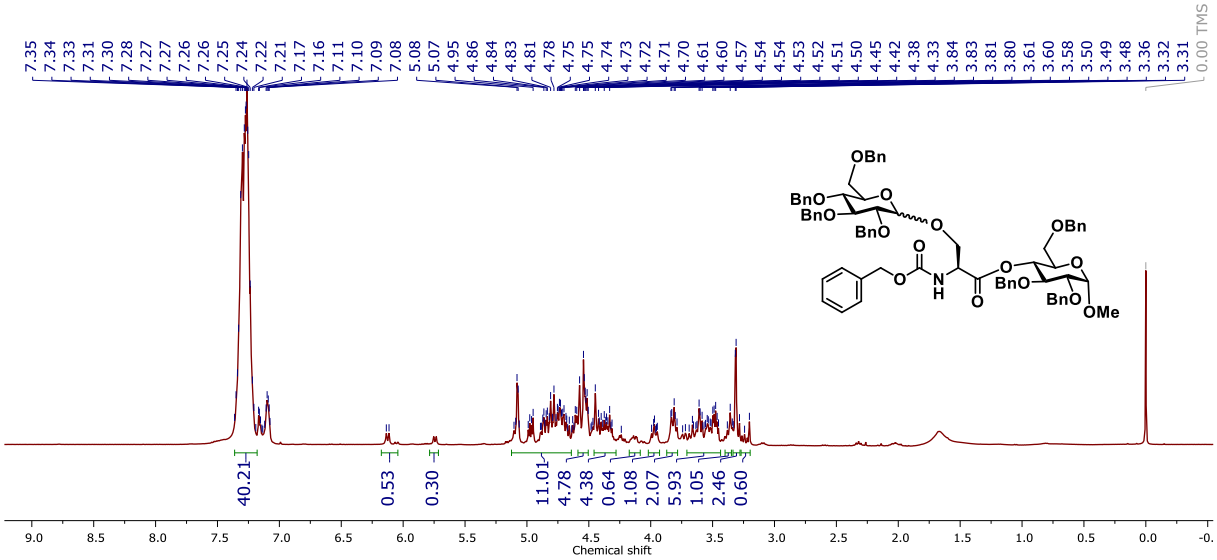
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of compound **30a**



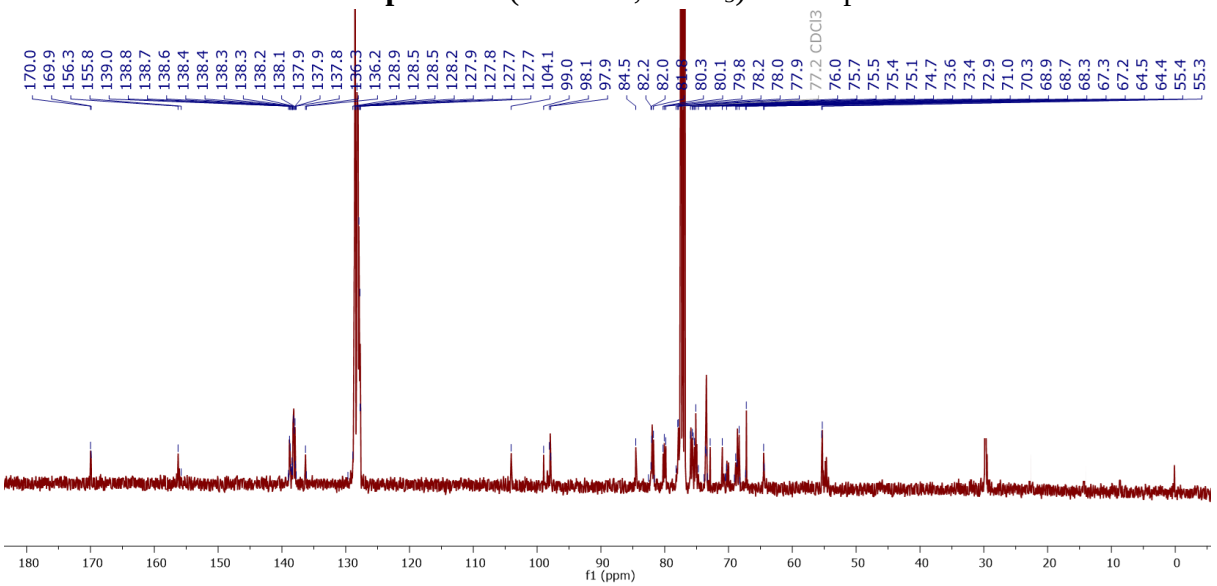
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **30a**



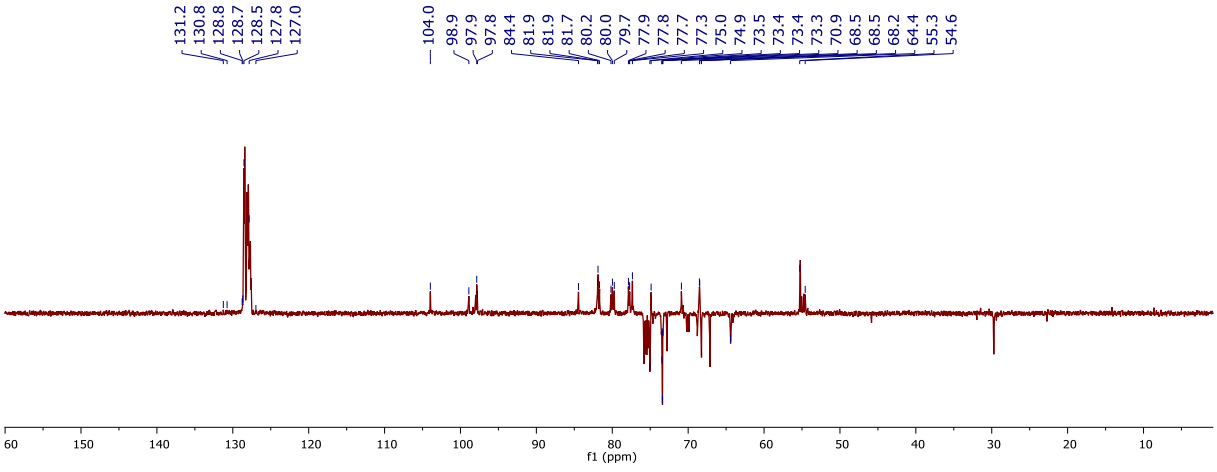
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **30b**



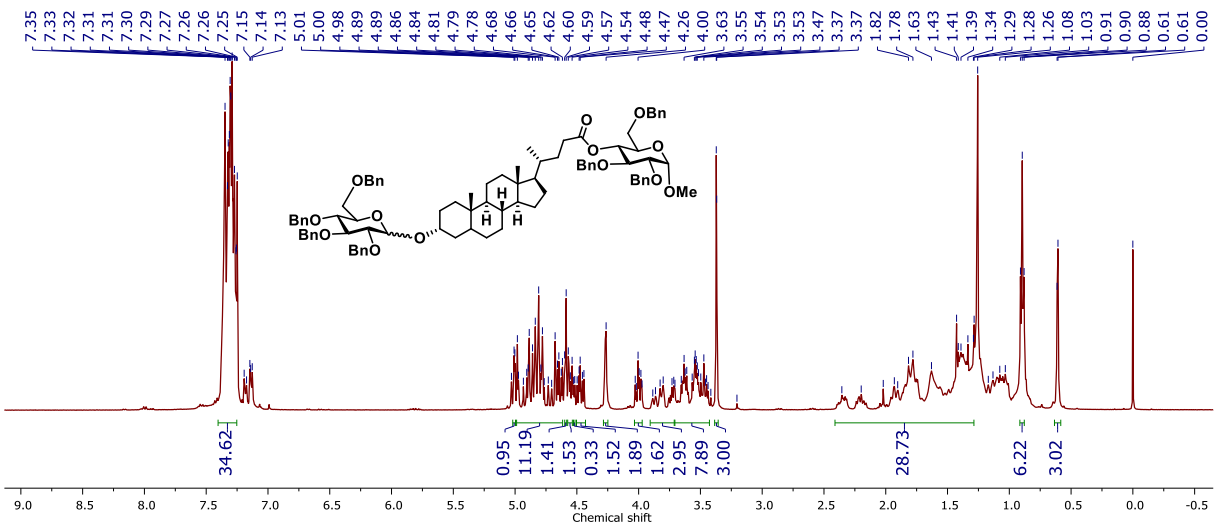
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of compound **30b**



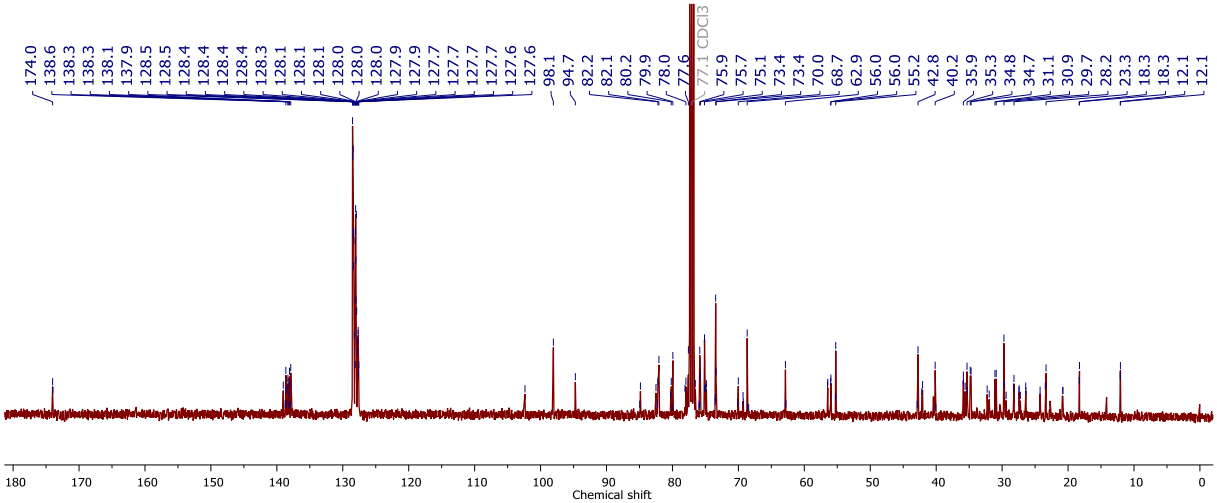
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **30b**



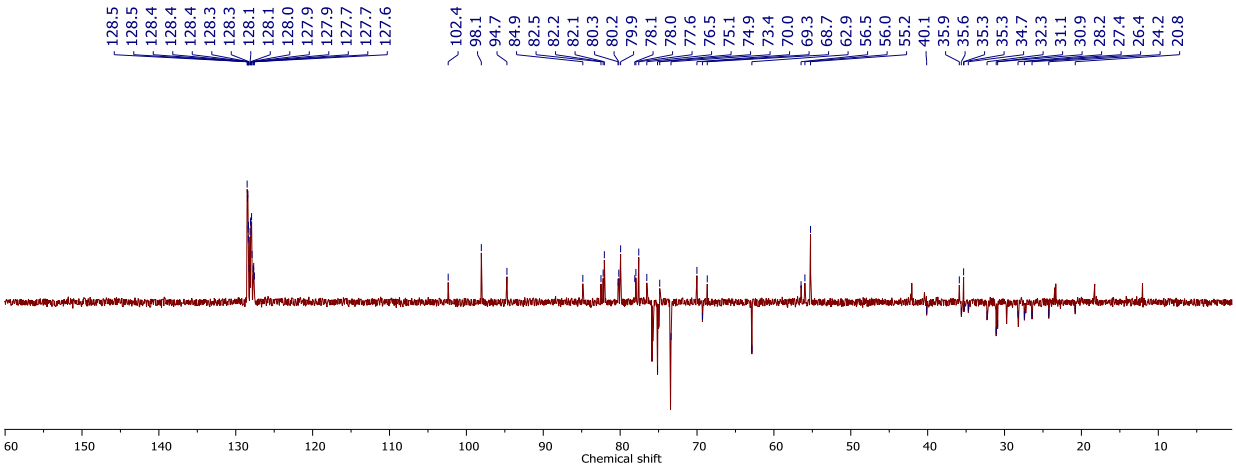
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **30c**



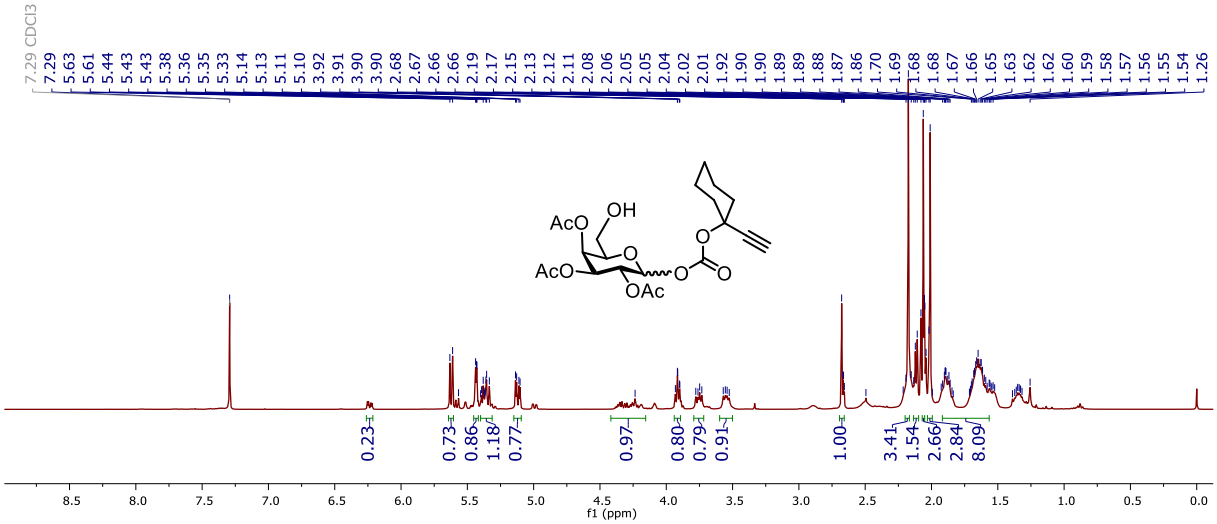
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of compound **30c**



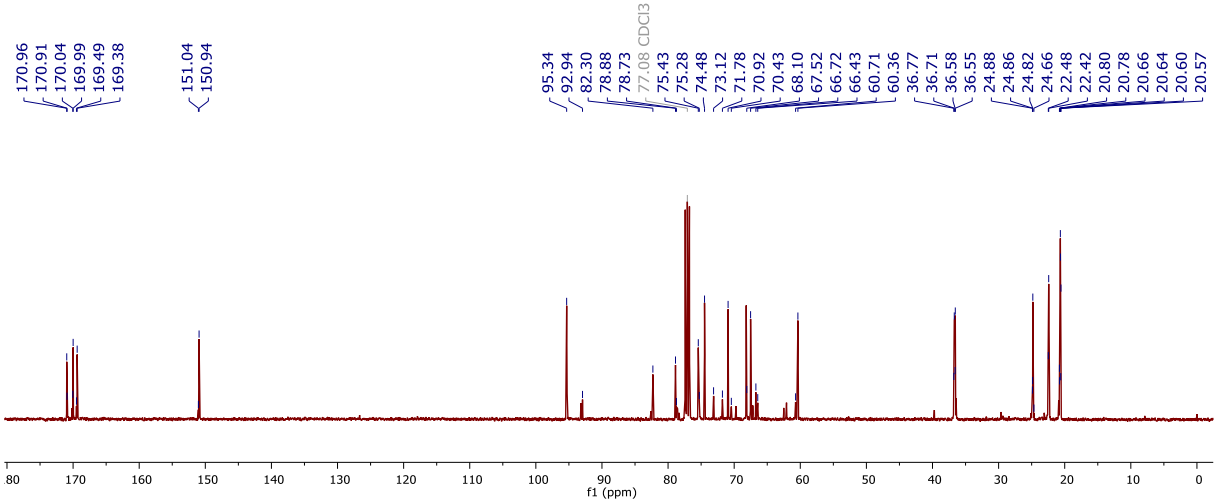
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **30c**



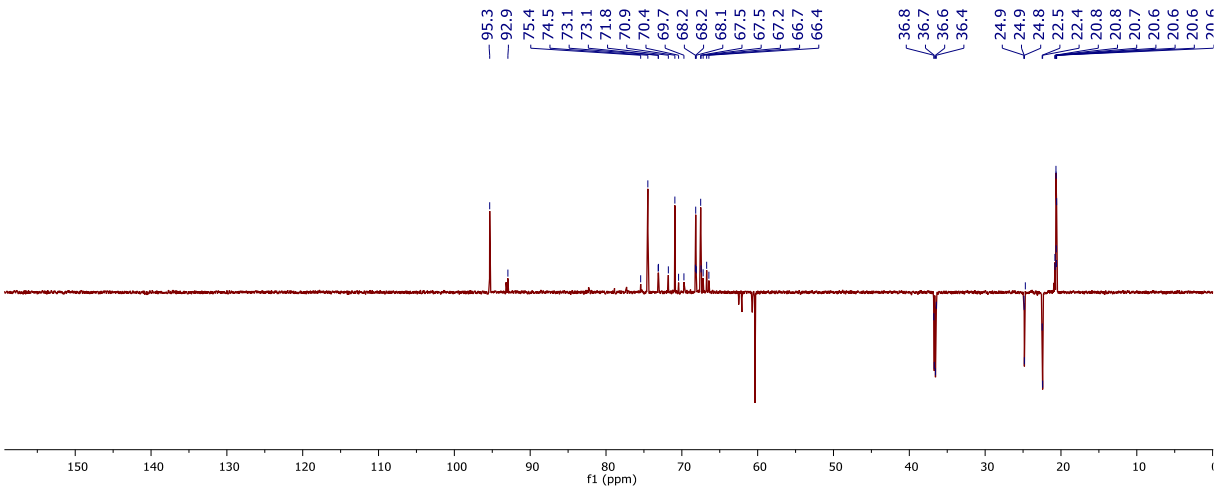
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **34**



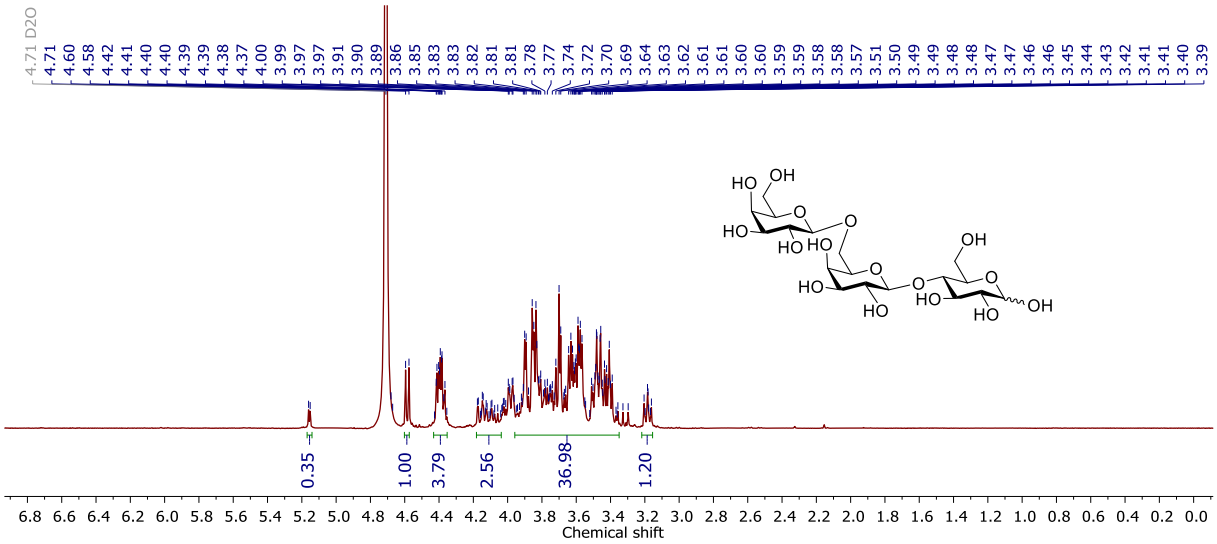
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of compound **34**



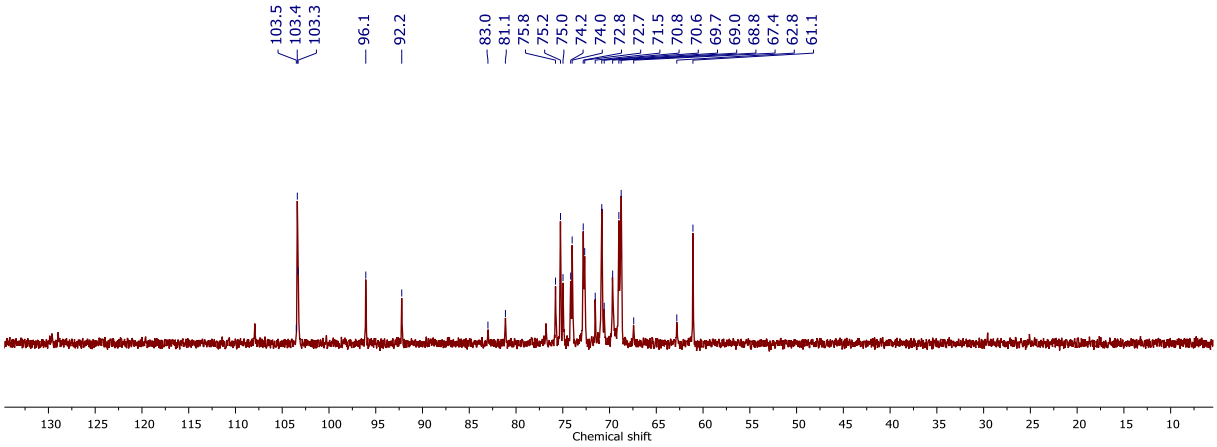
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **34**



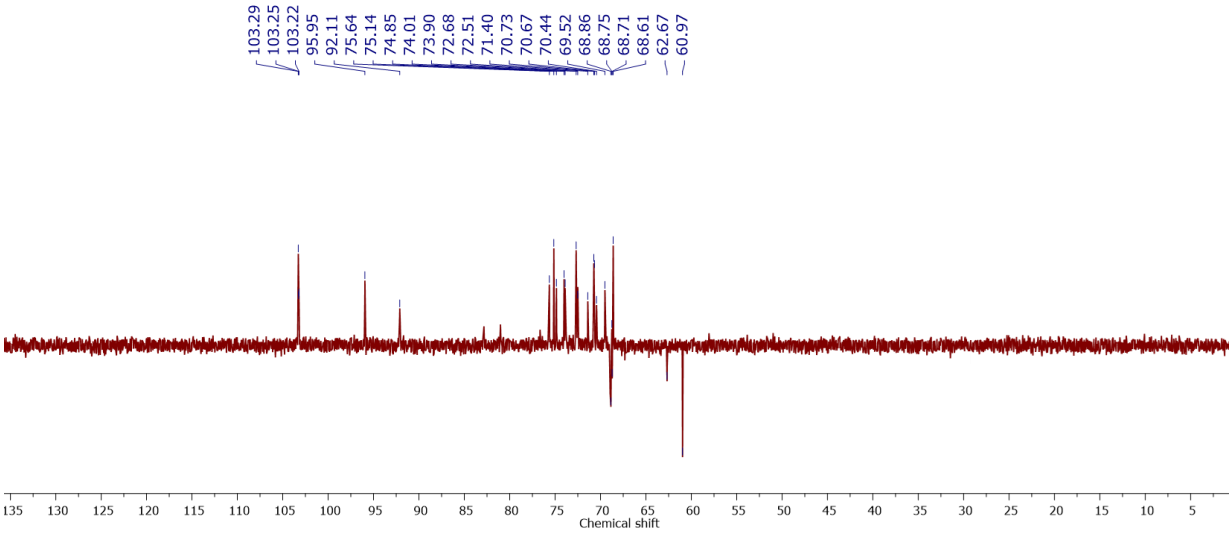
^{1}H NMR Spectrum (600MHz, D_2O) of compound 36



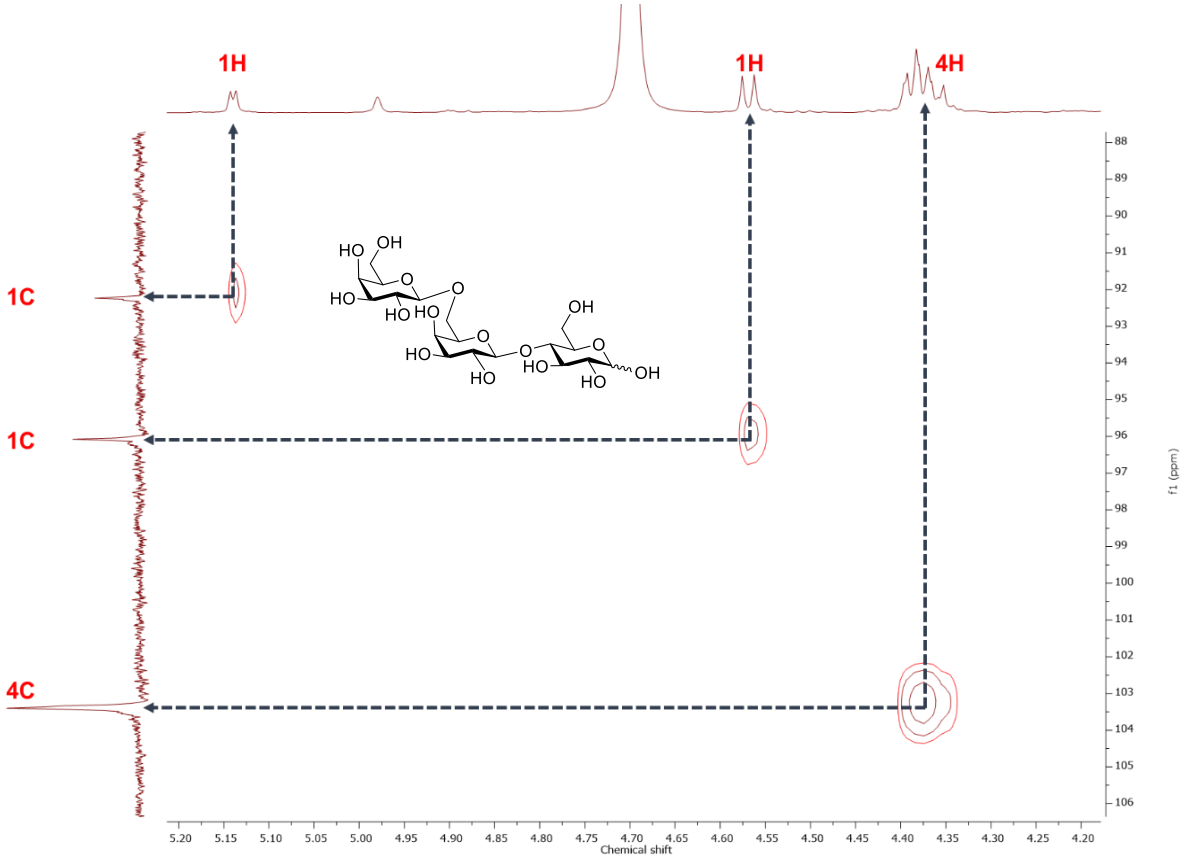
^{13}C NMR Spectrum (151MHz, D_2O) of compound 36



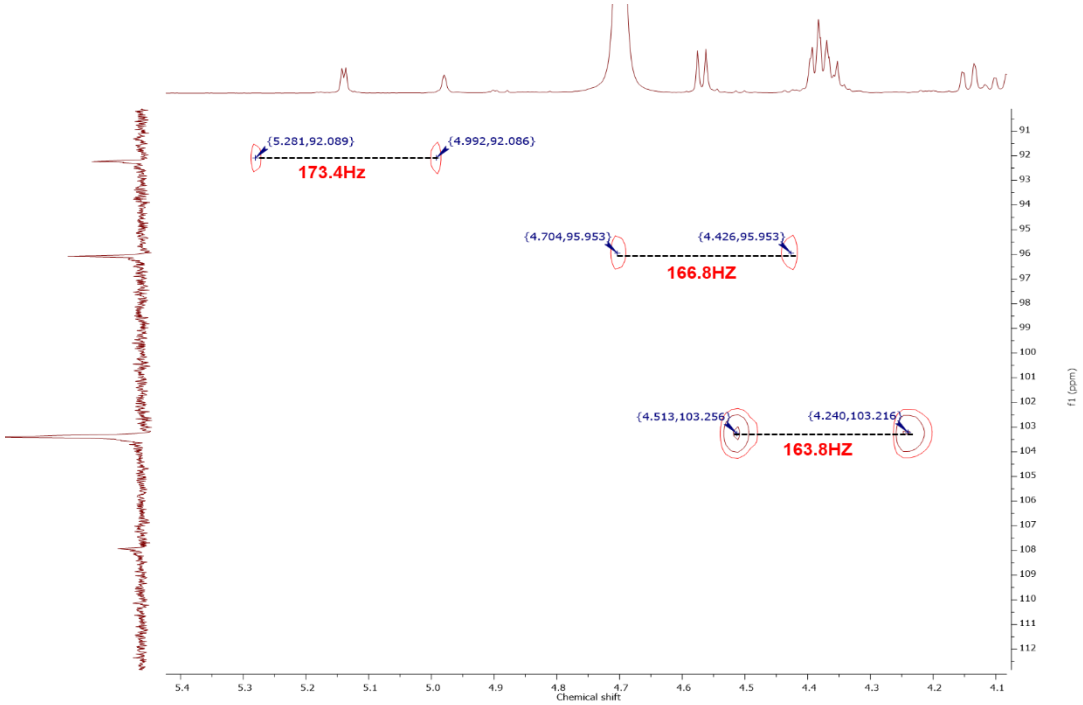
DEPT NMR Spectrum (151MHz, D_2O) of compound 36



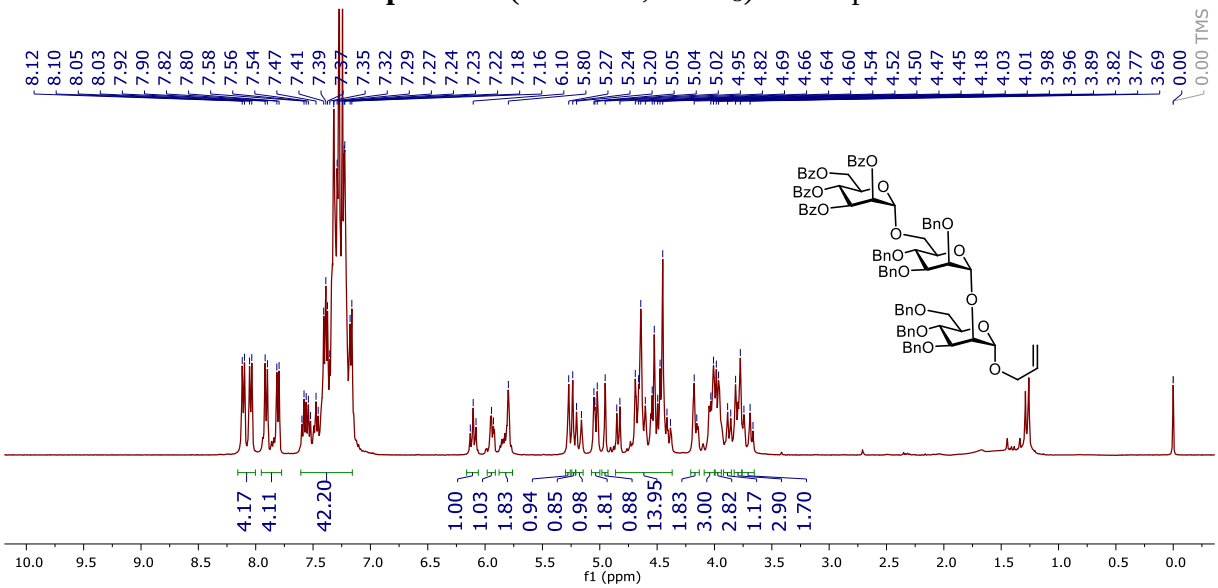
^{1}H-^{13}C HSQC NMR Spectrum (600 MHz, 151 MHz D_2O) of Compound 36 (Anomeric Expansion)



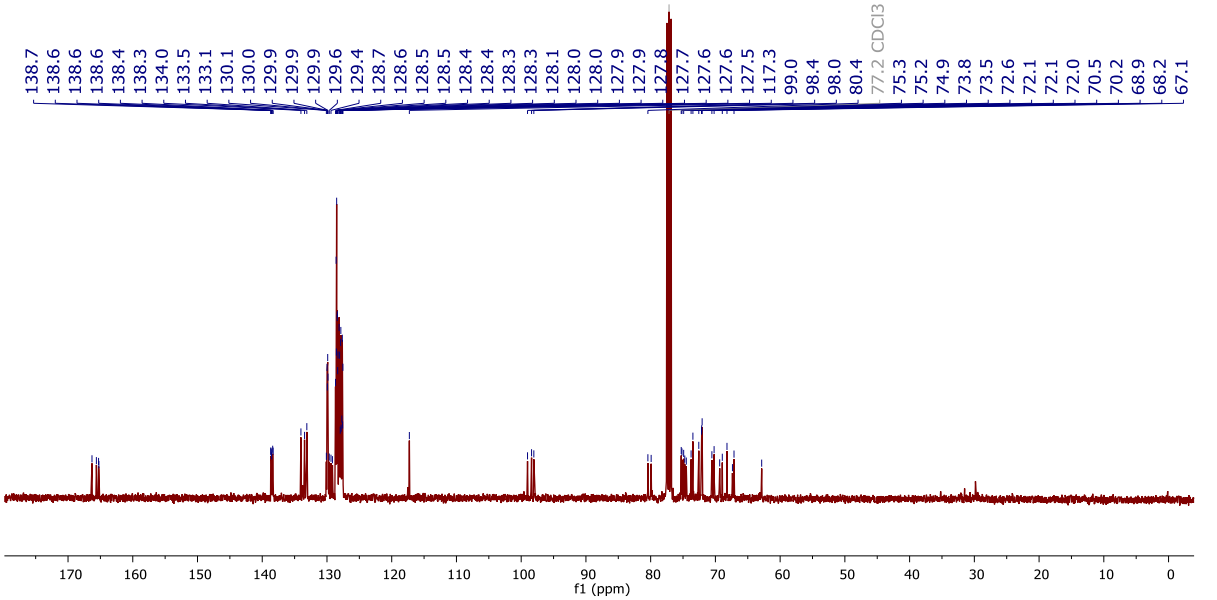
^{1}H-^{13}C HSQC-Coupled NMR Spectrum (600 MHz, D_2O) of Compound 36 (Anomeric expansion)



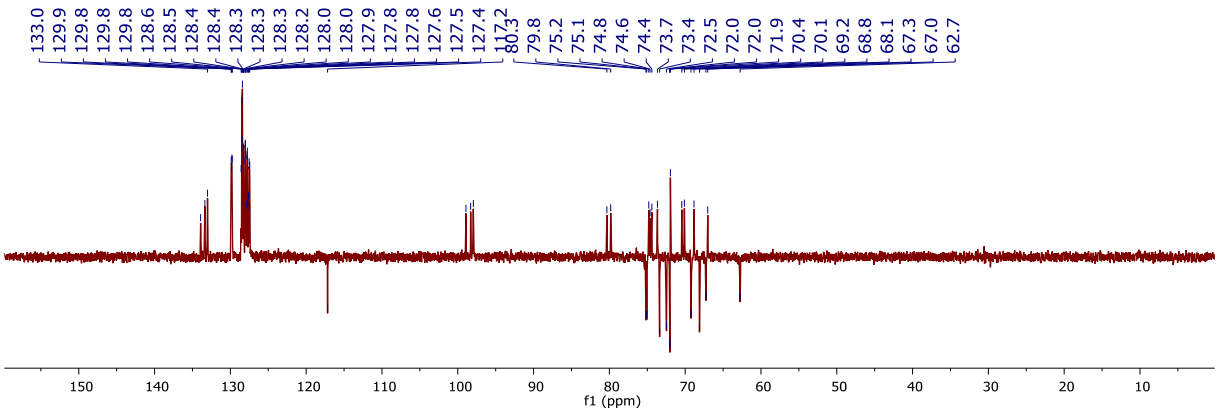
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **41**



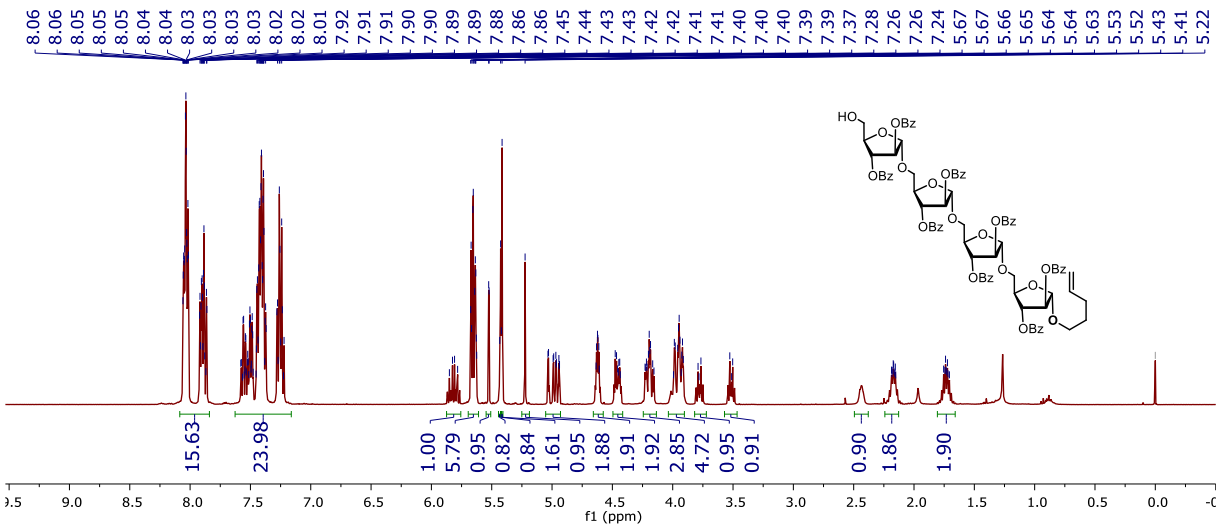
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **41**



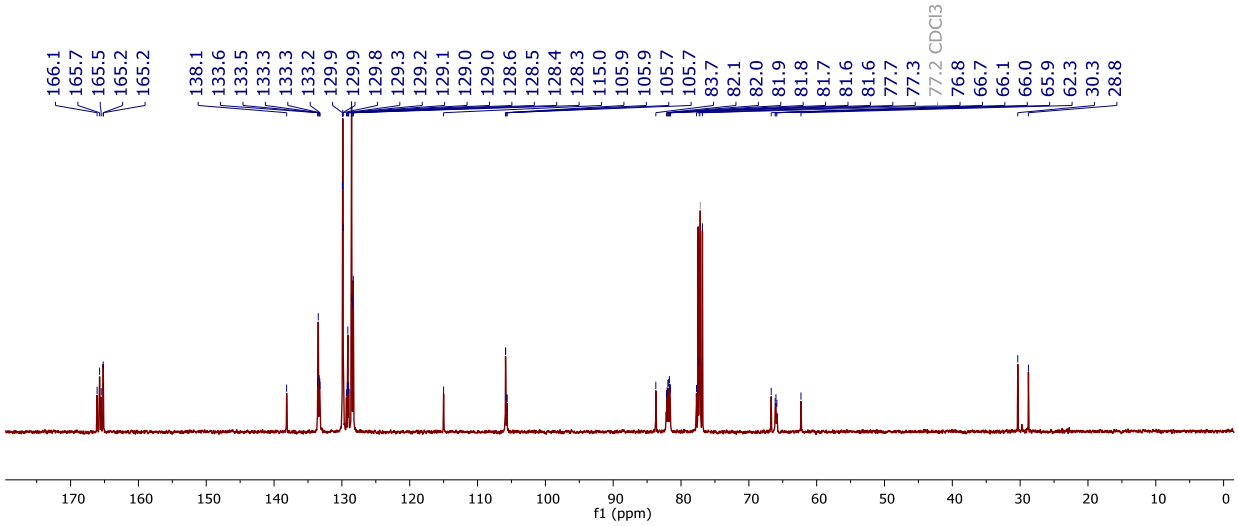
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **41**



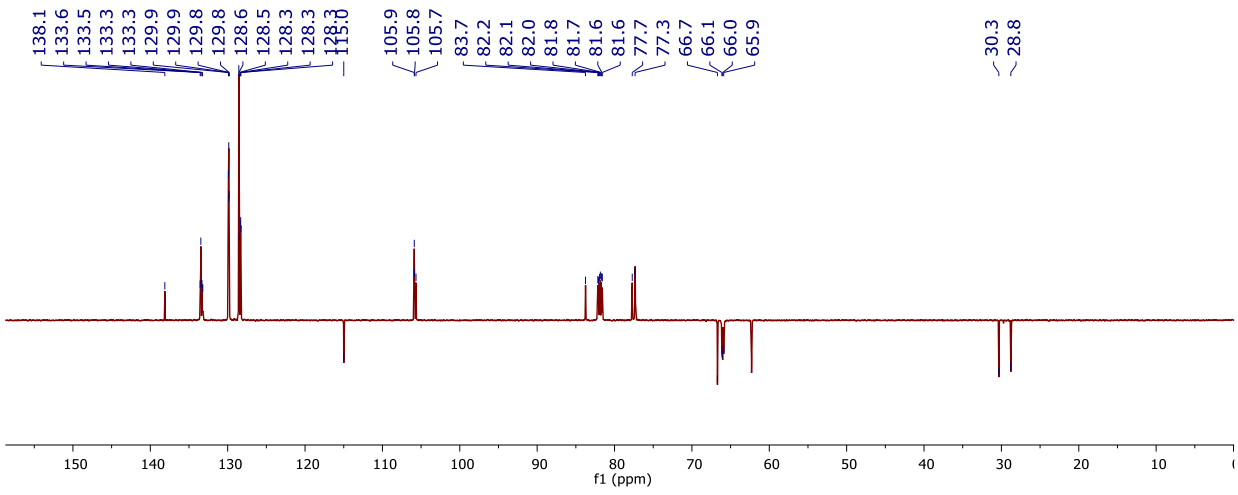
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **45**



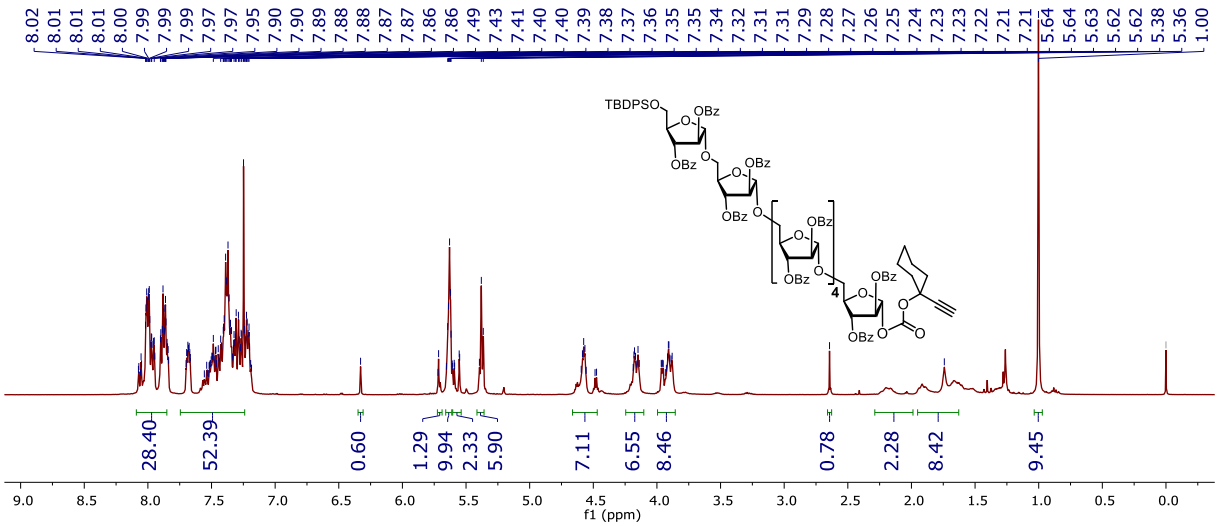
^{13}C NMR Spectrum (101MHz, $CDCl_3$) of compound **45**



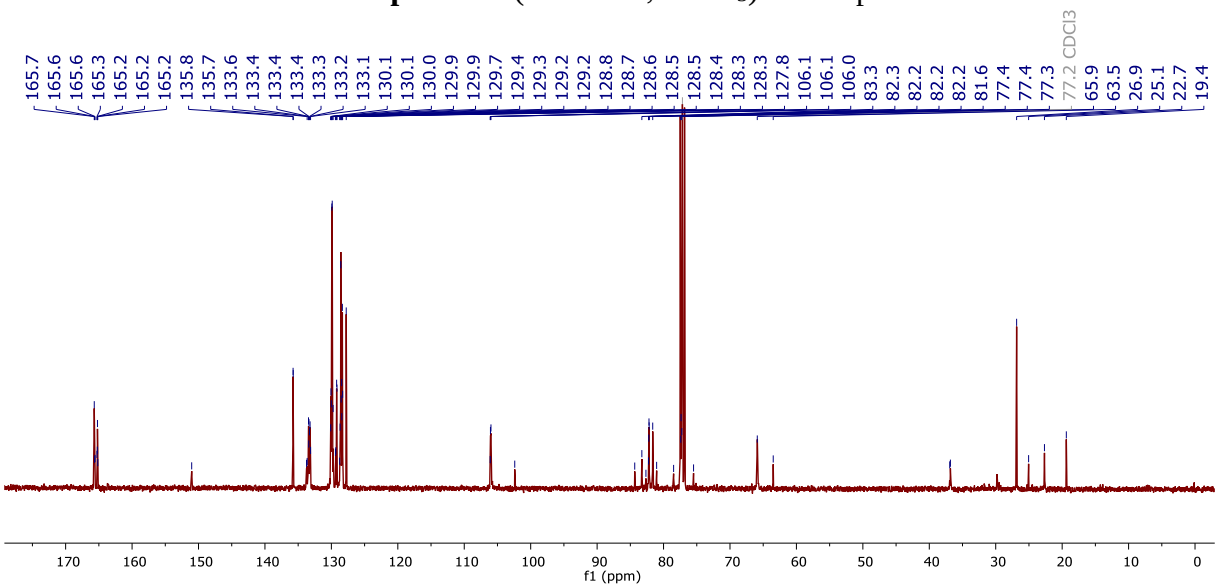
DEPT NMR Spectrum (101MHz, $CDCl_3$) of compound **45**



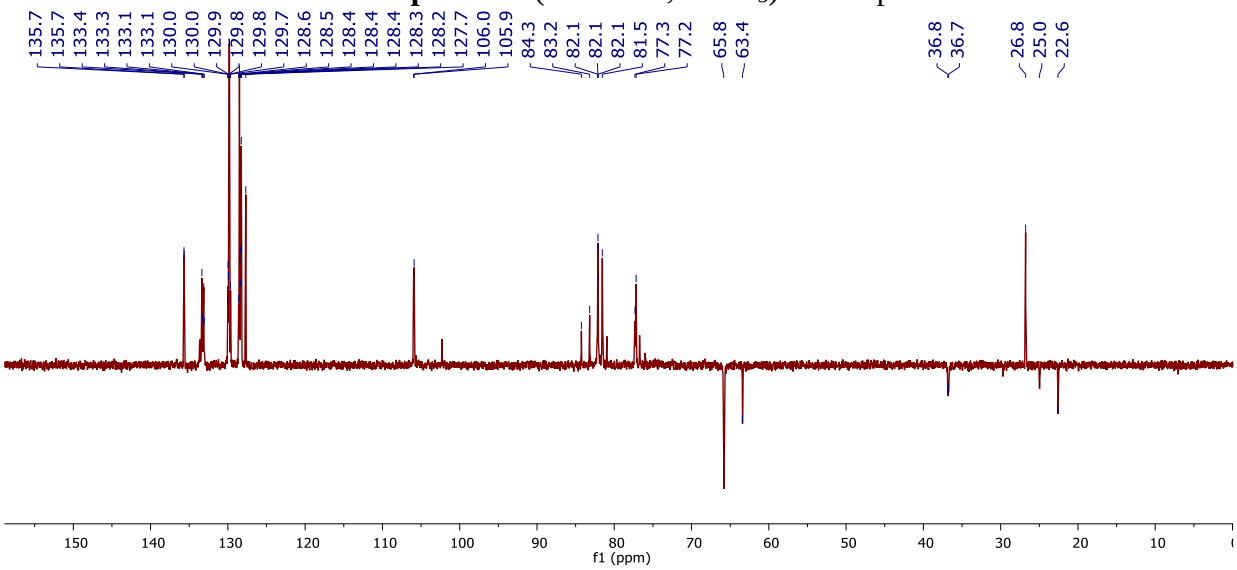
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **50**



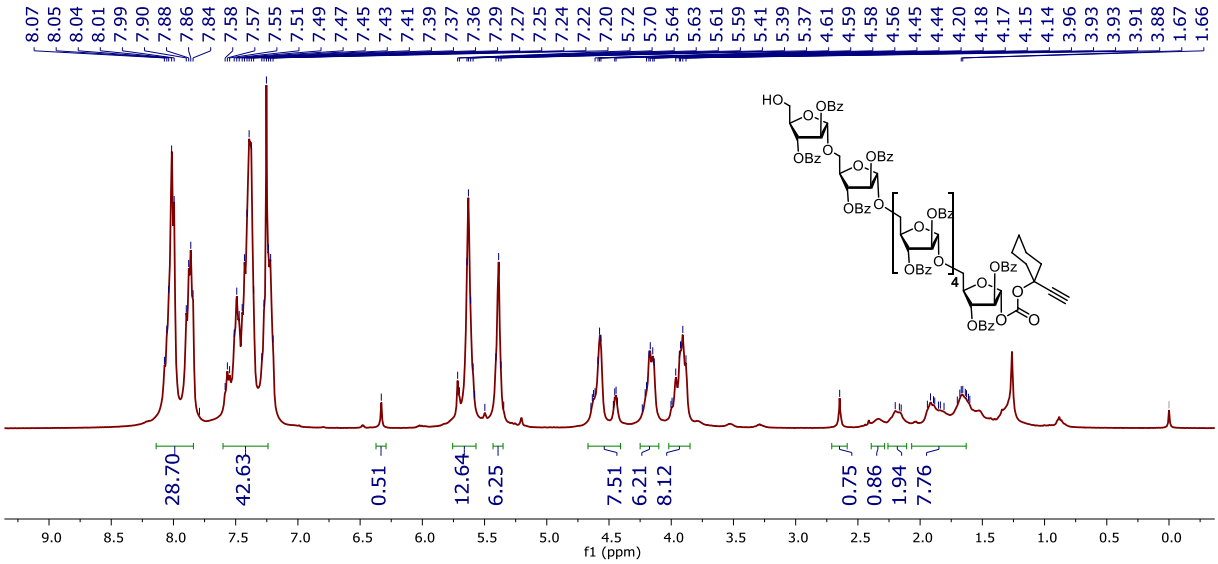
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **50**



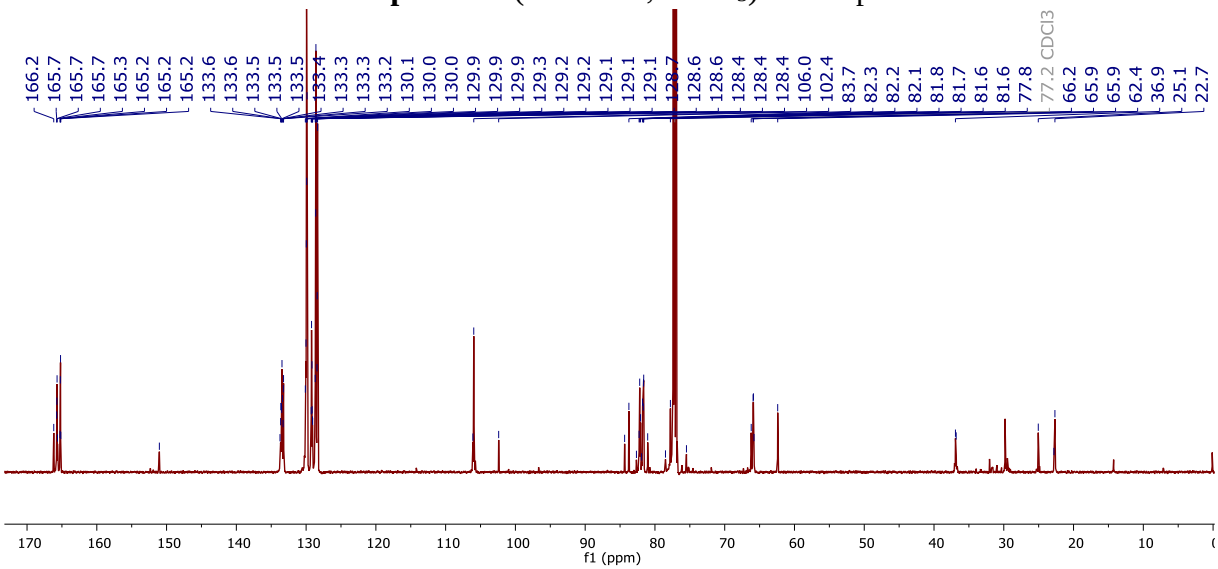
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **50**



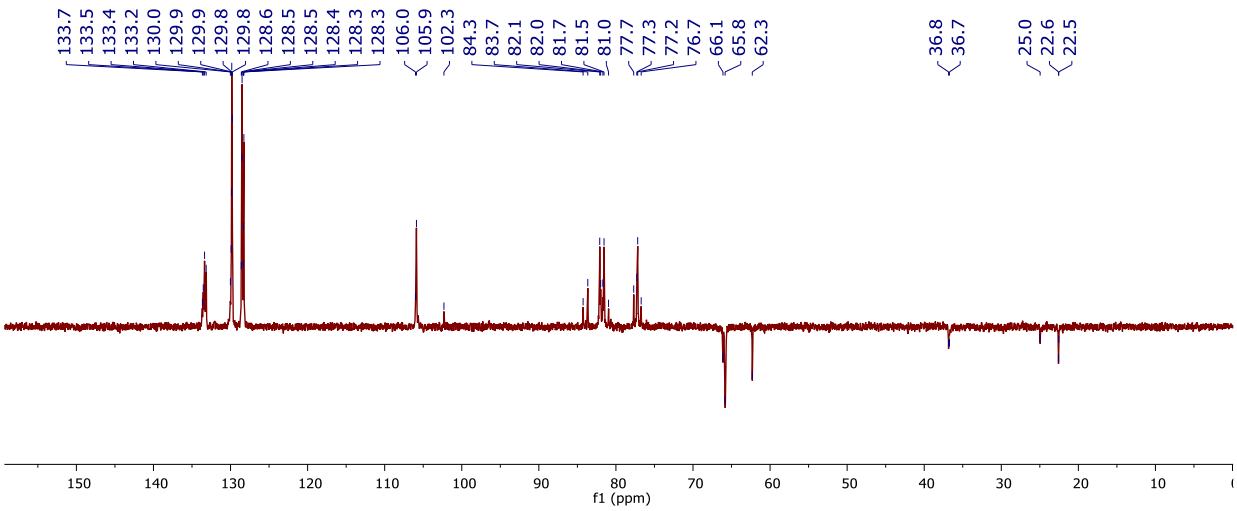
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **51**



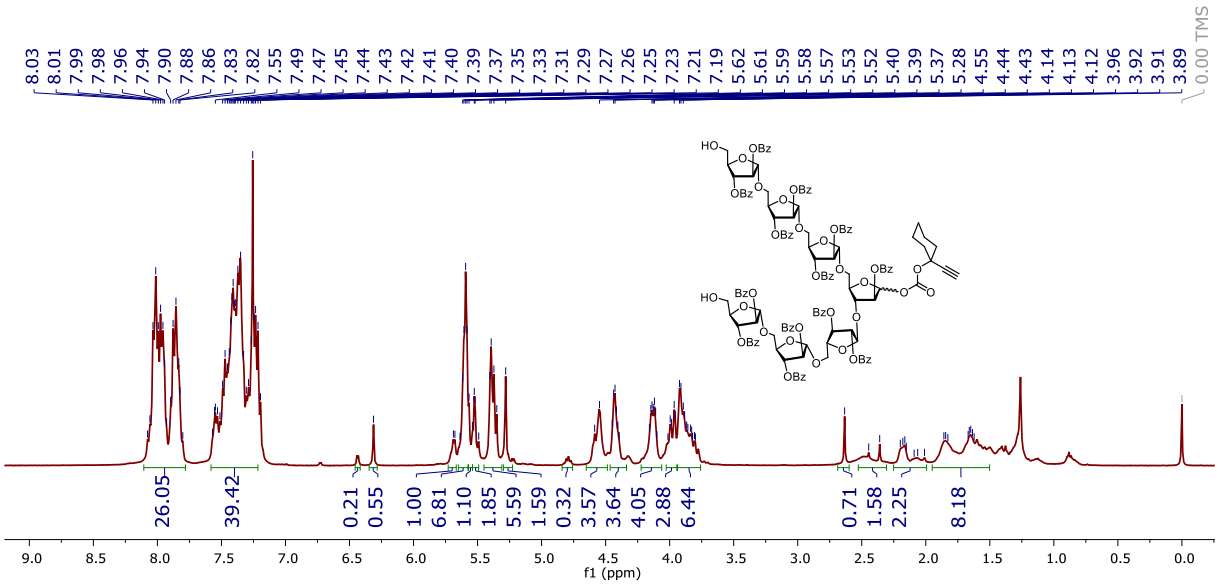
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **51**



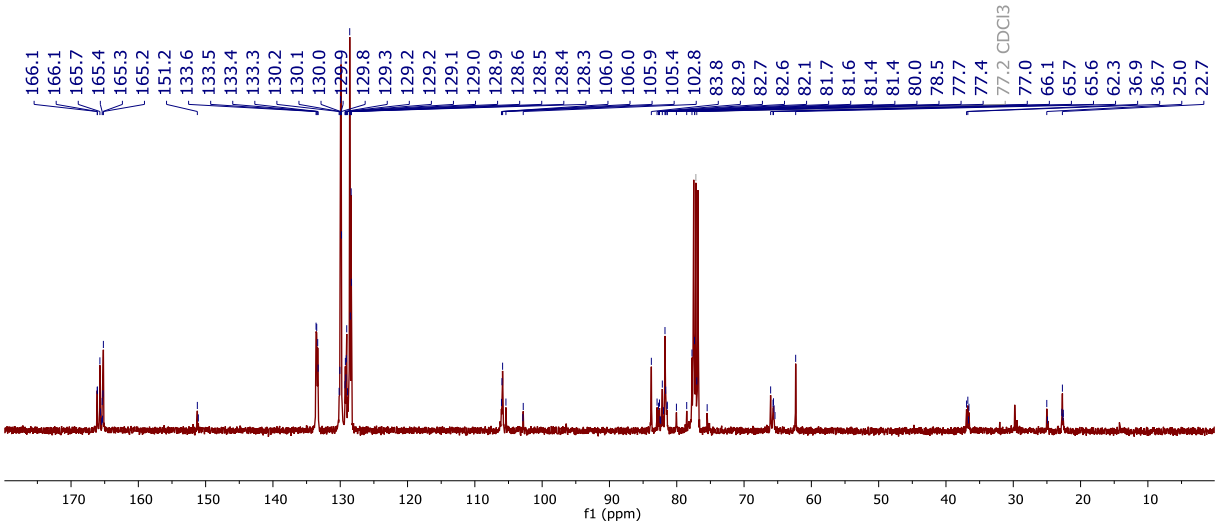
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **51**



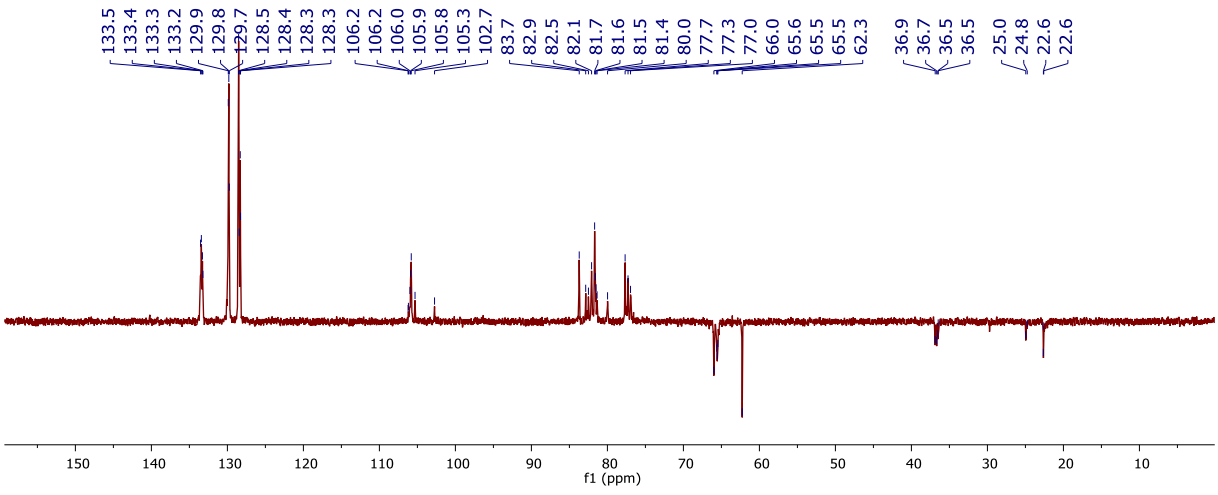
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **53**



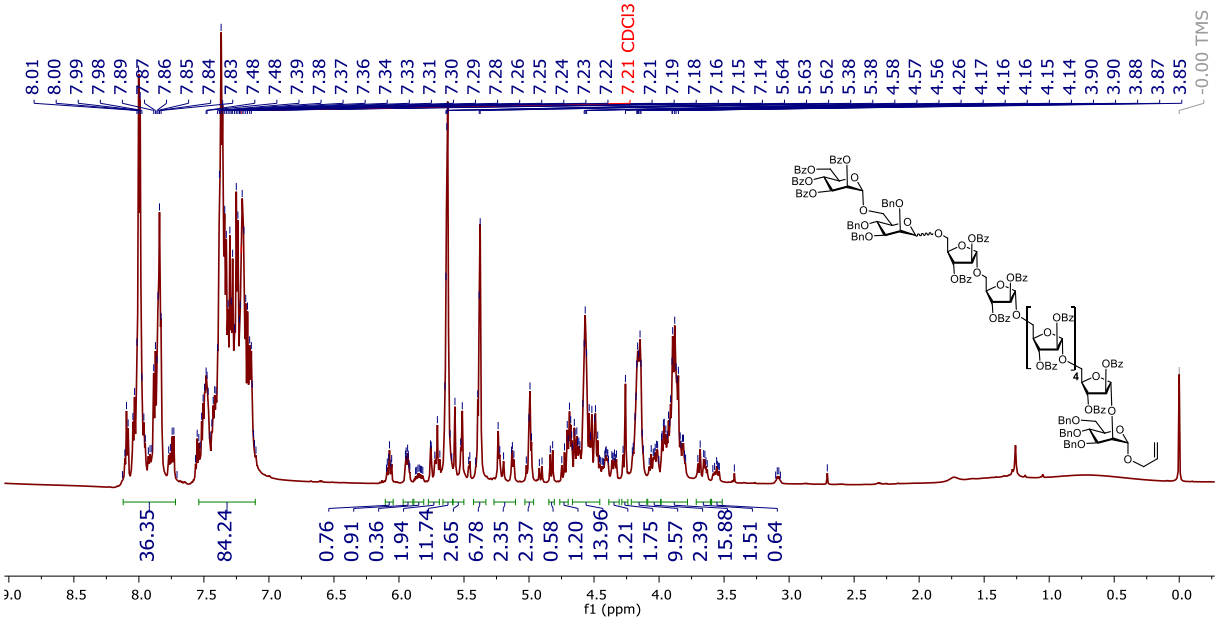
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **53**



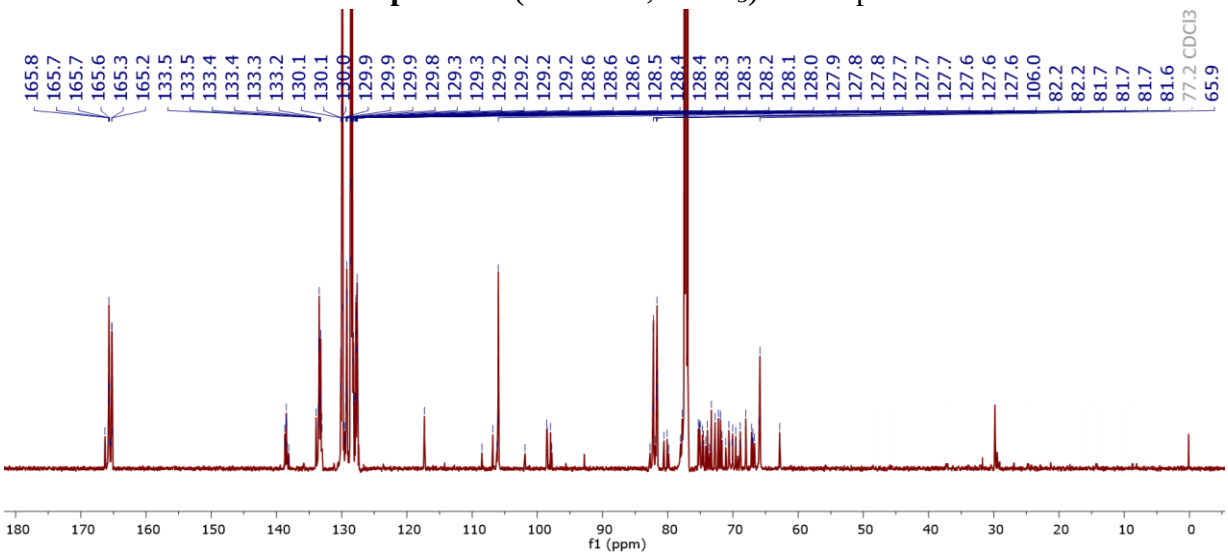
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **53**



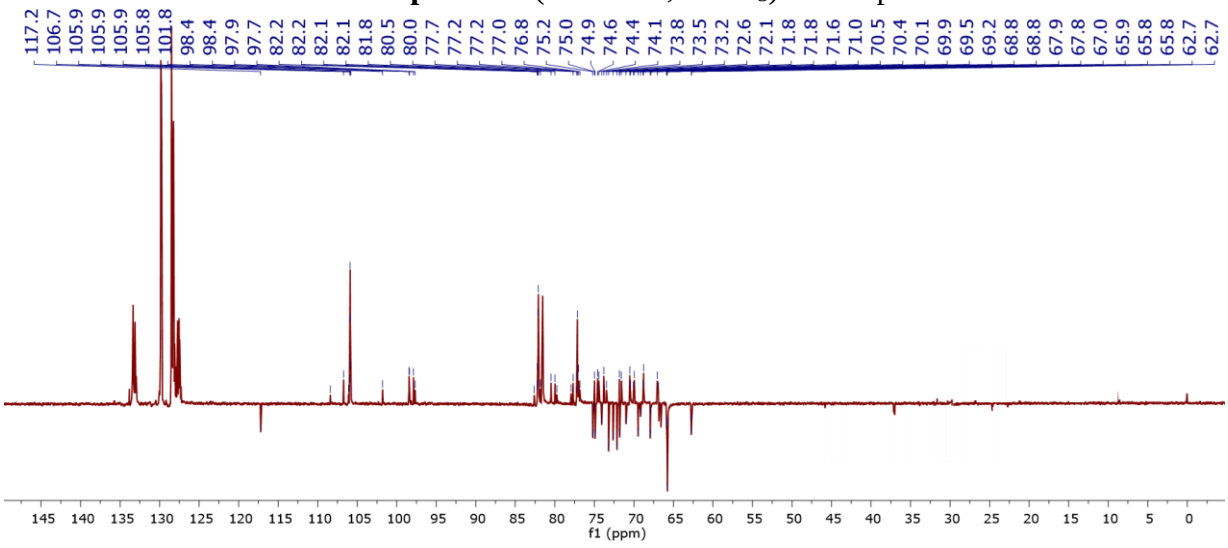
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **57**



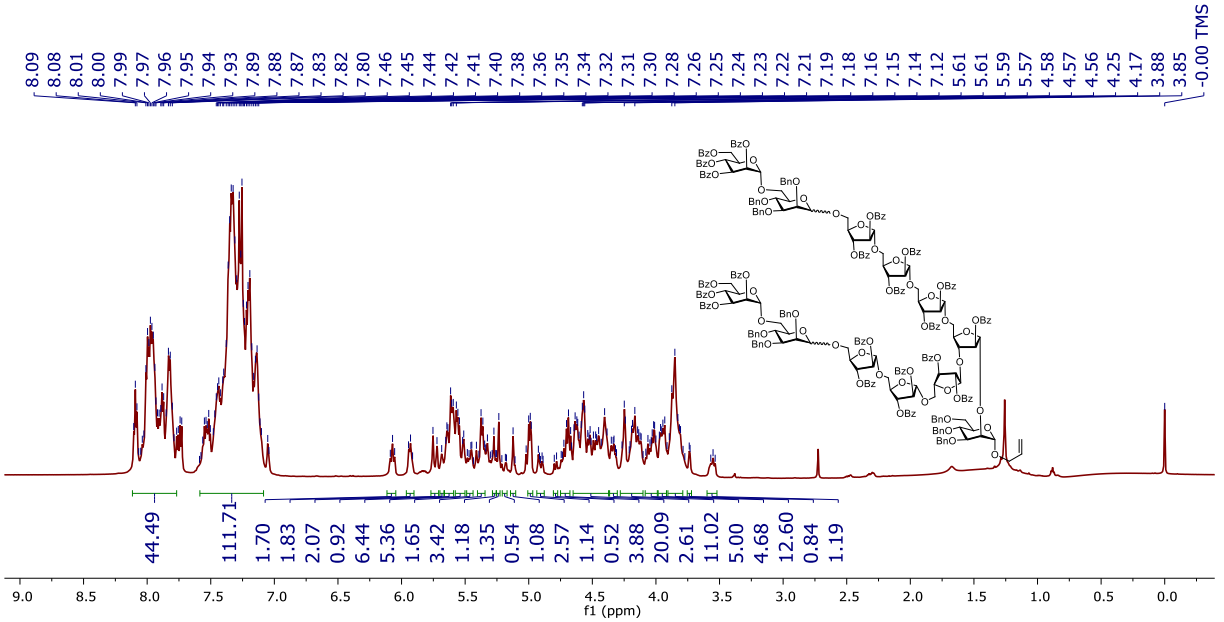
^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **57**



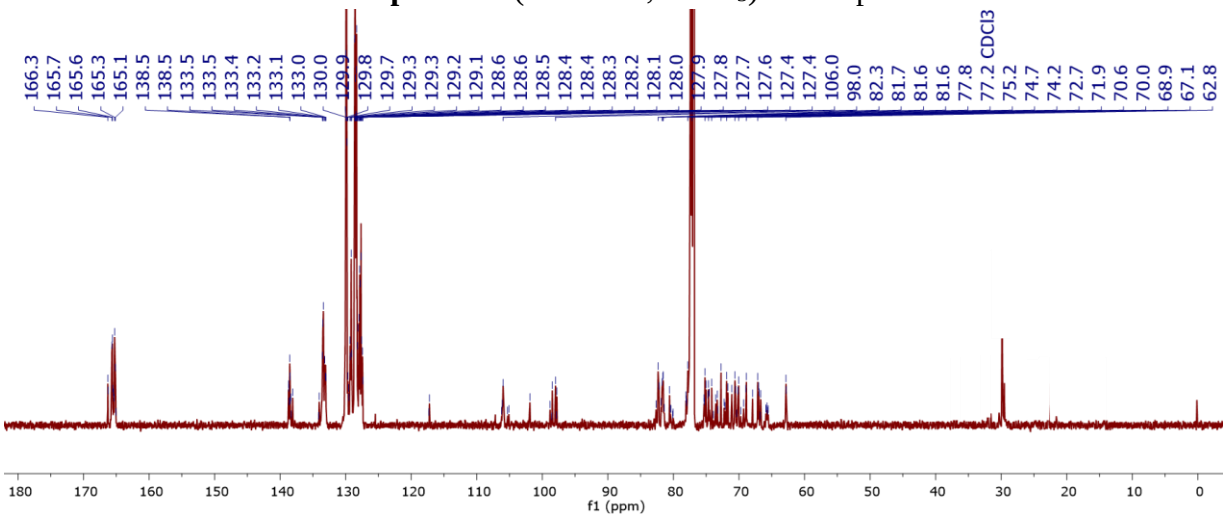
DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **57**



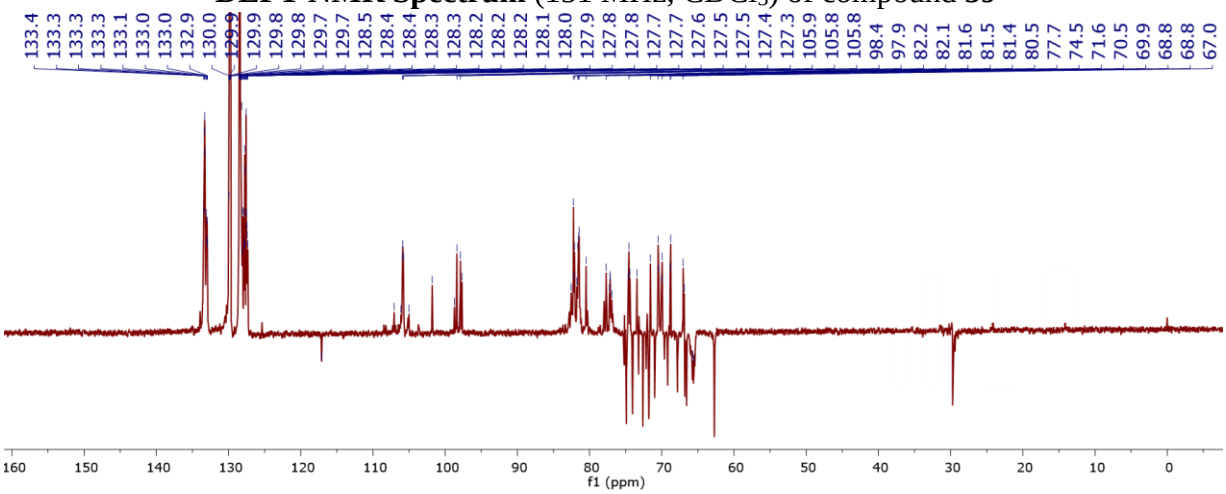
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **59**



^{13}C NMR Spectrum (151 MHz, $CDCl_3$) of compound **59**



DEPT NMR Spectrum (151 MHz, $CDCl_3$) of compound **59**

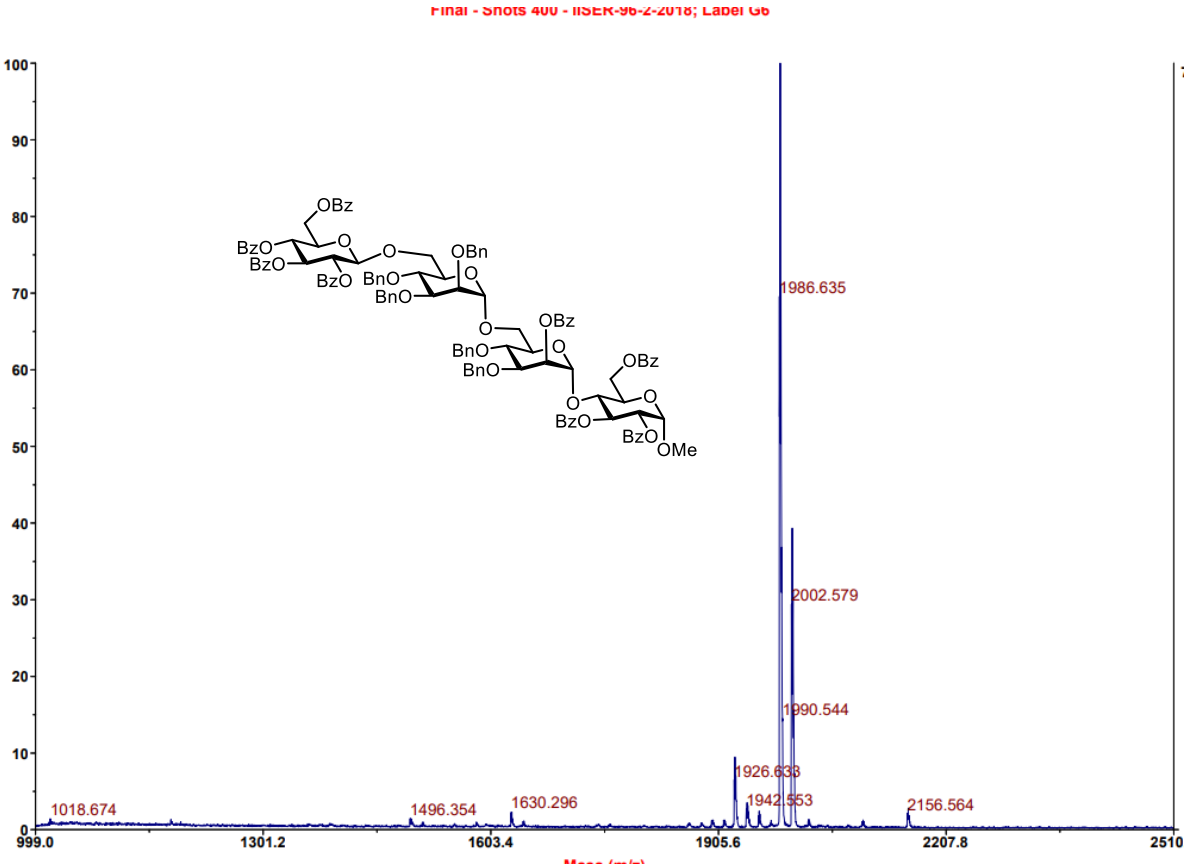


3.5 MALDI-ToF Spectra of Representative Compounds

Compound- **10a**

Calculated mass: 1986.675 ($C_{116}H_{106}O_{29}Na$)

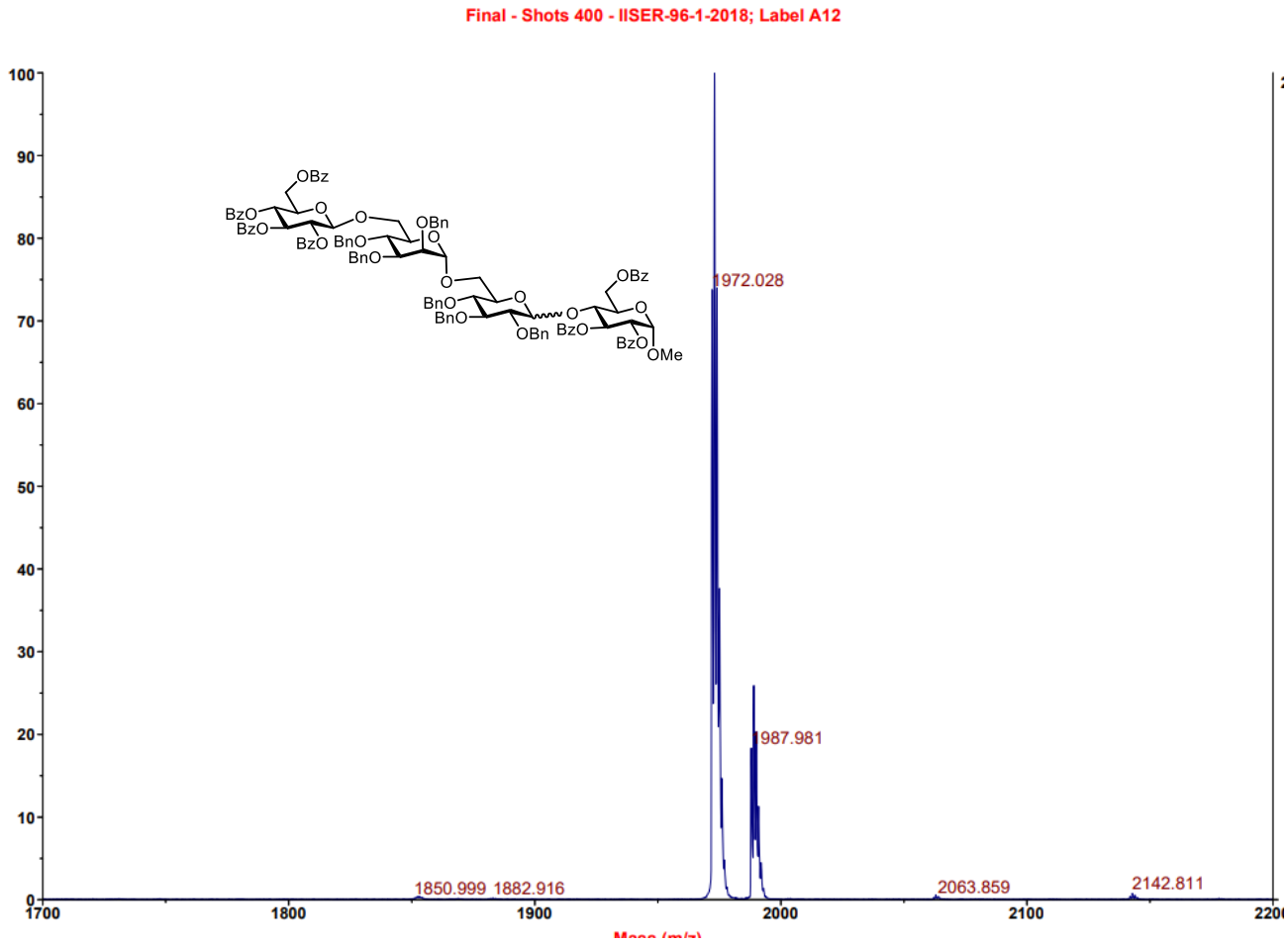
Observed mass: 1986.635



Compound- **10b**

Calculated mass : 1972.696 ($C_{116}H_{108}O_{28}Na$)

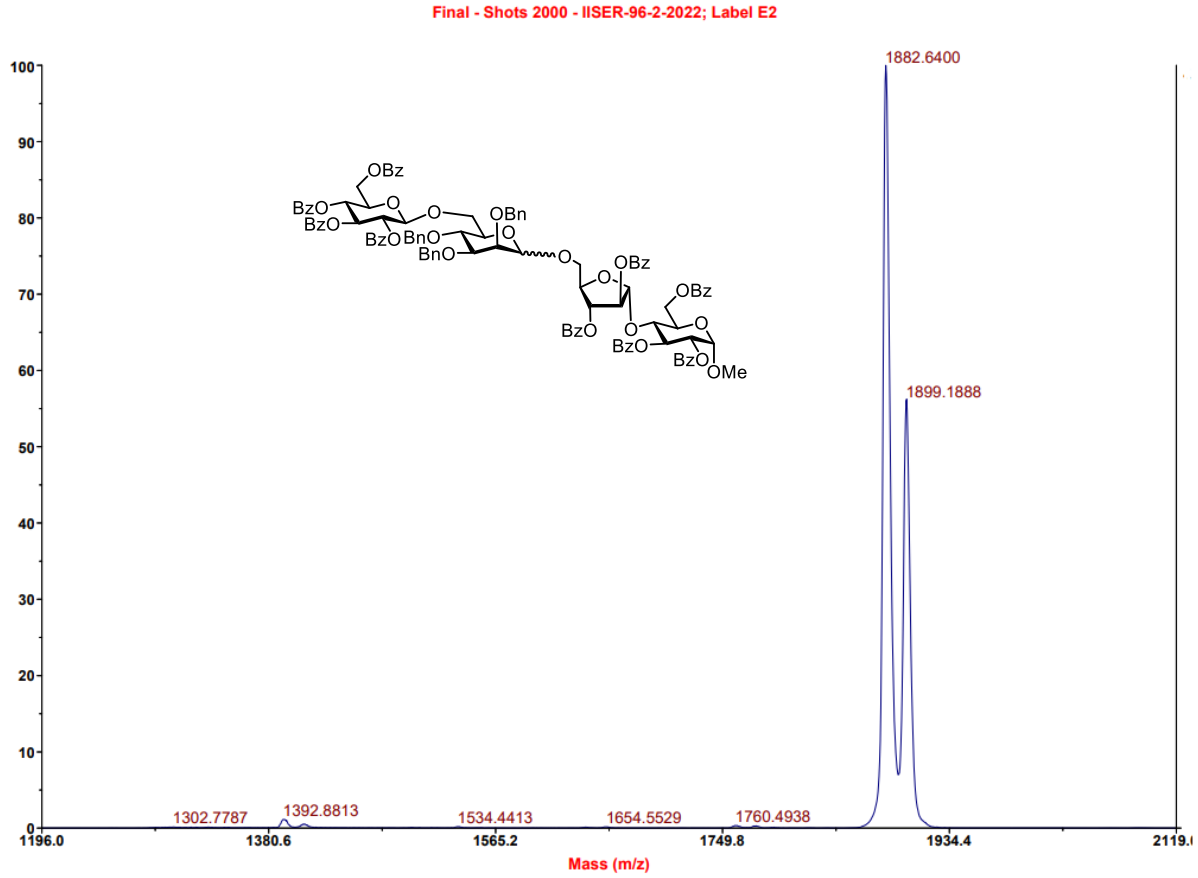
Observed mass : 1972.028



Compound- **10c**

Calculated mass: 1880.5969 ($C_{108}H_{96}O_{29}Na$)

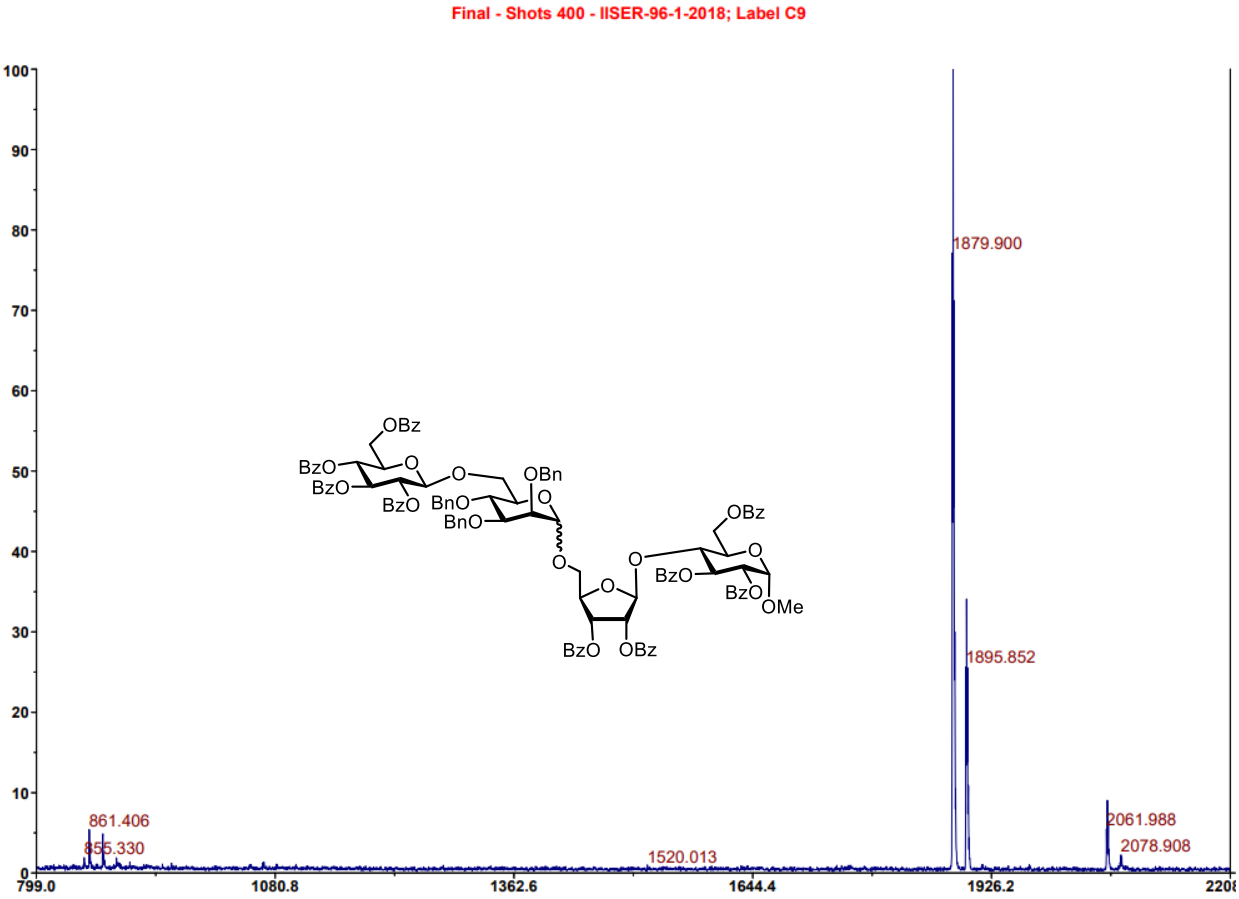
Observed mass:1882.6400



Compound- **10d**

Calculated mass: 1880.5969 ($C_{108}H_{96}O_{29}Na$)

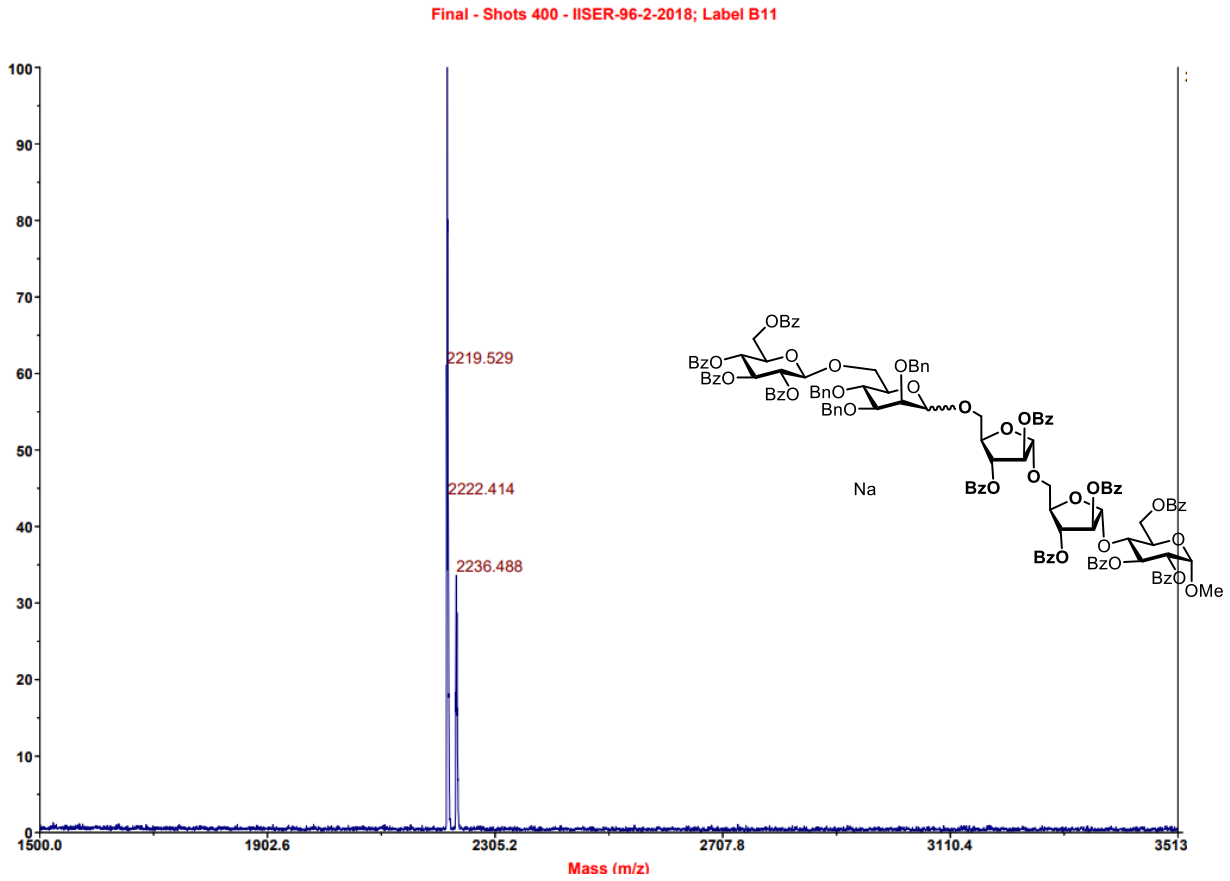
Observed mass: 1879.900



Compound- **10e**

Calculated mass: 2219.688 ($C_{127}H_{112}O_{35}Na$)

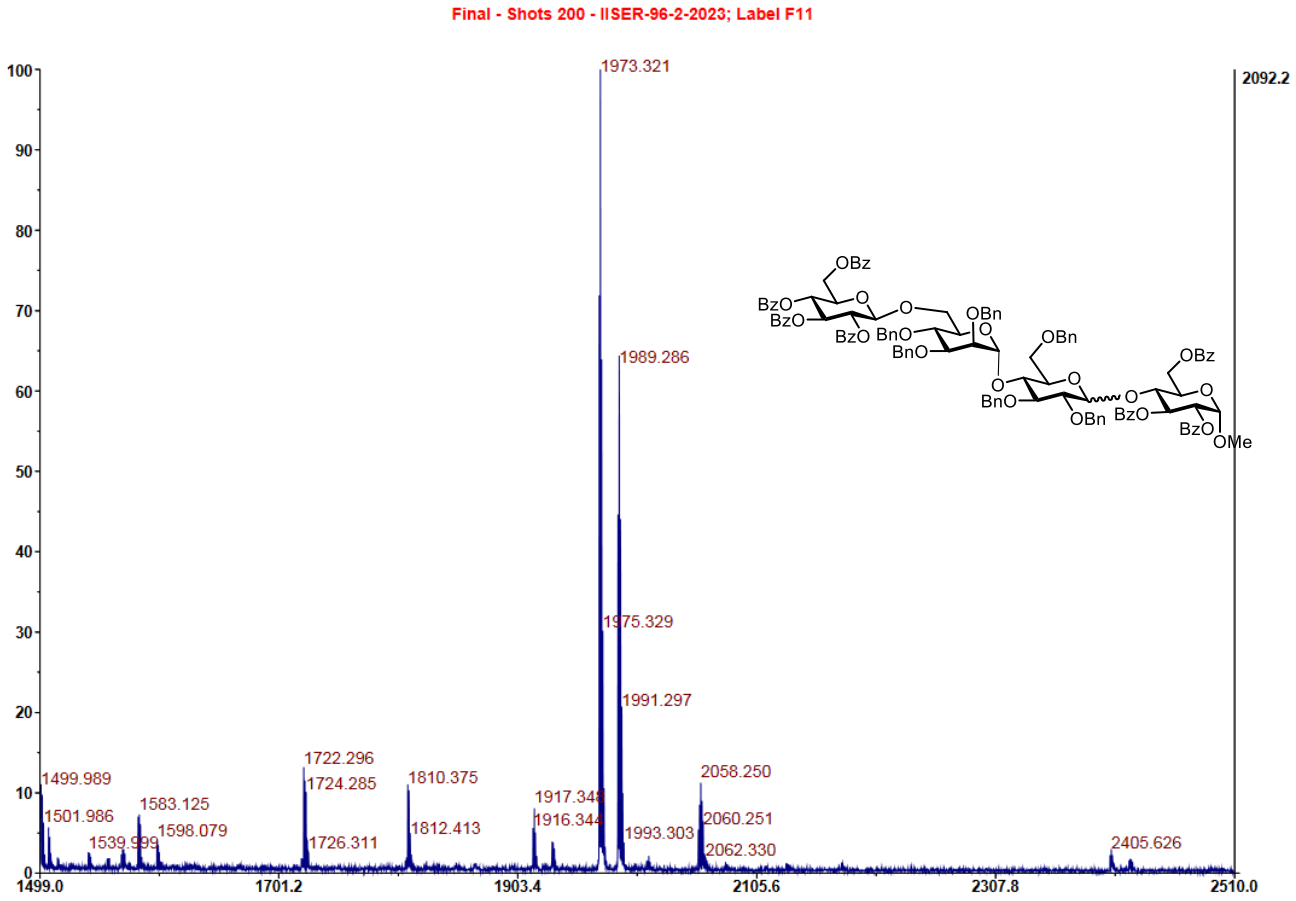
Observed mass: 2219.529



Compound- **10f**

Calculated mass: 1973.101 ($C_{116}H_{108}O_{28}Na$)

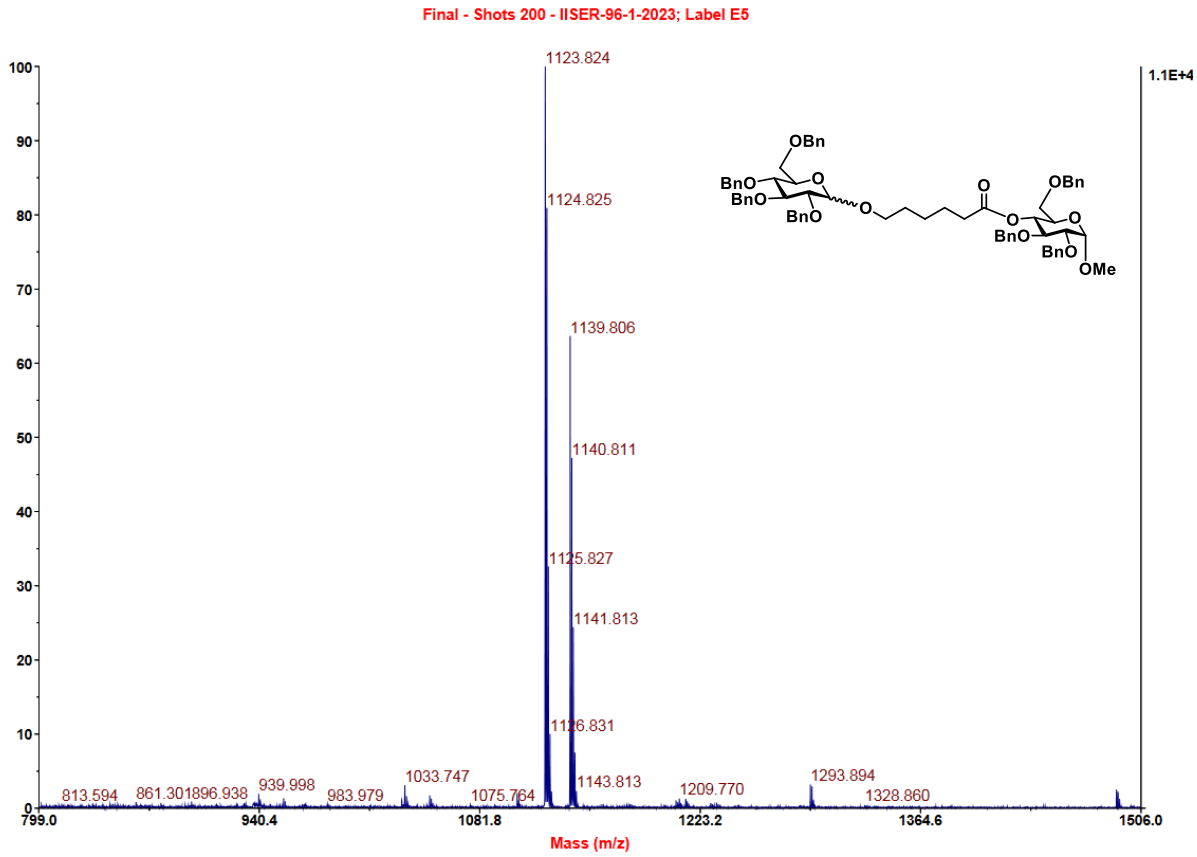
Observed mass: 1973.321



Compound- 30a

Calculated mass: 1123.518 ($C_{68}H_{76}O1_3Na$)

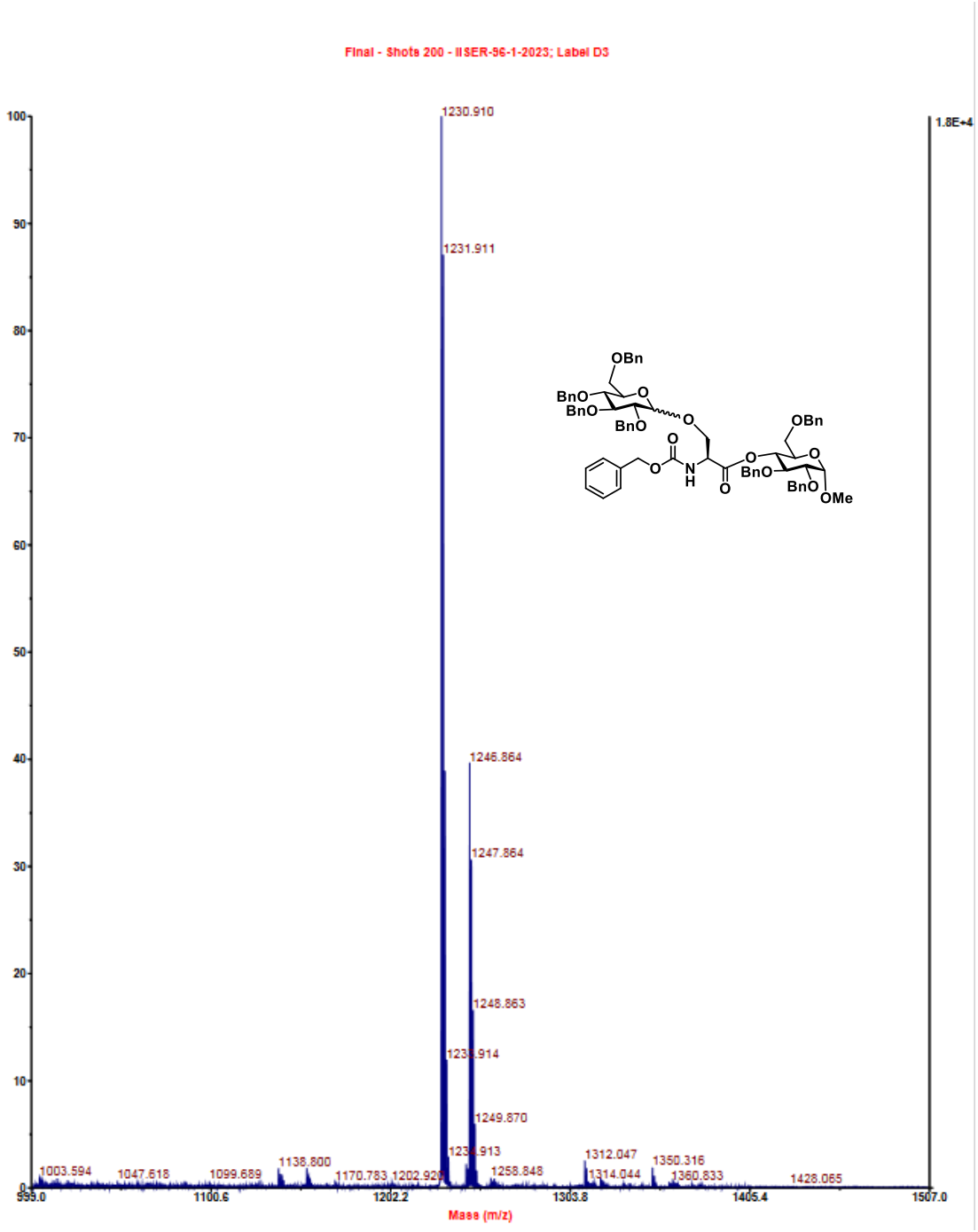
Observed mass: 1123.824



Compound- 30b

Calculated mass: 1230.519 ($C_{73}H_{77}O_{15}NNa$)

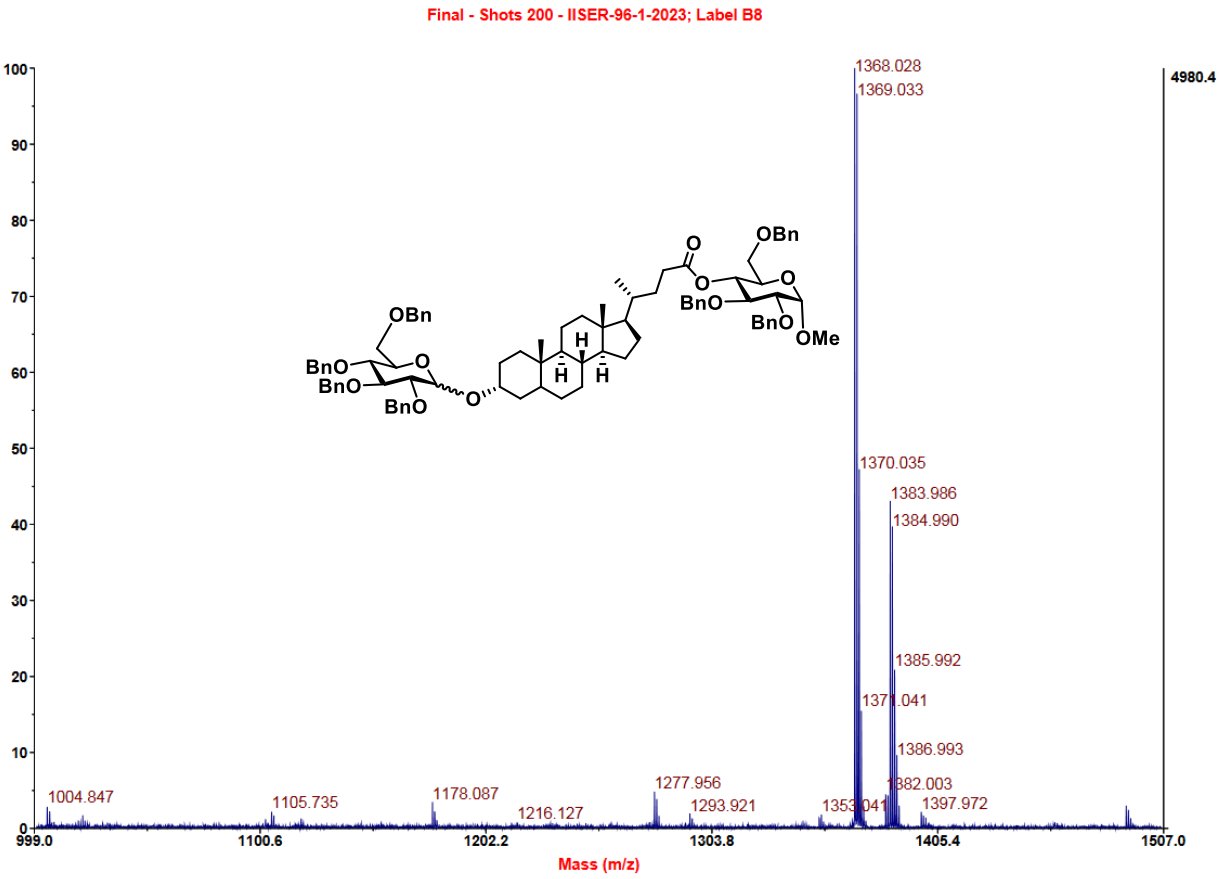
Observed mass: 1230.910



Compound- 30c

Calculated mass: 1368.740 ($C_{86}H_{104}O_{13}Na$)

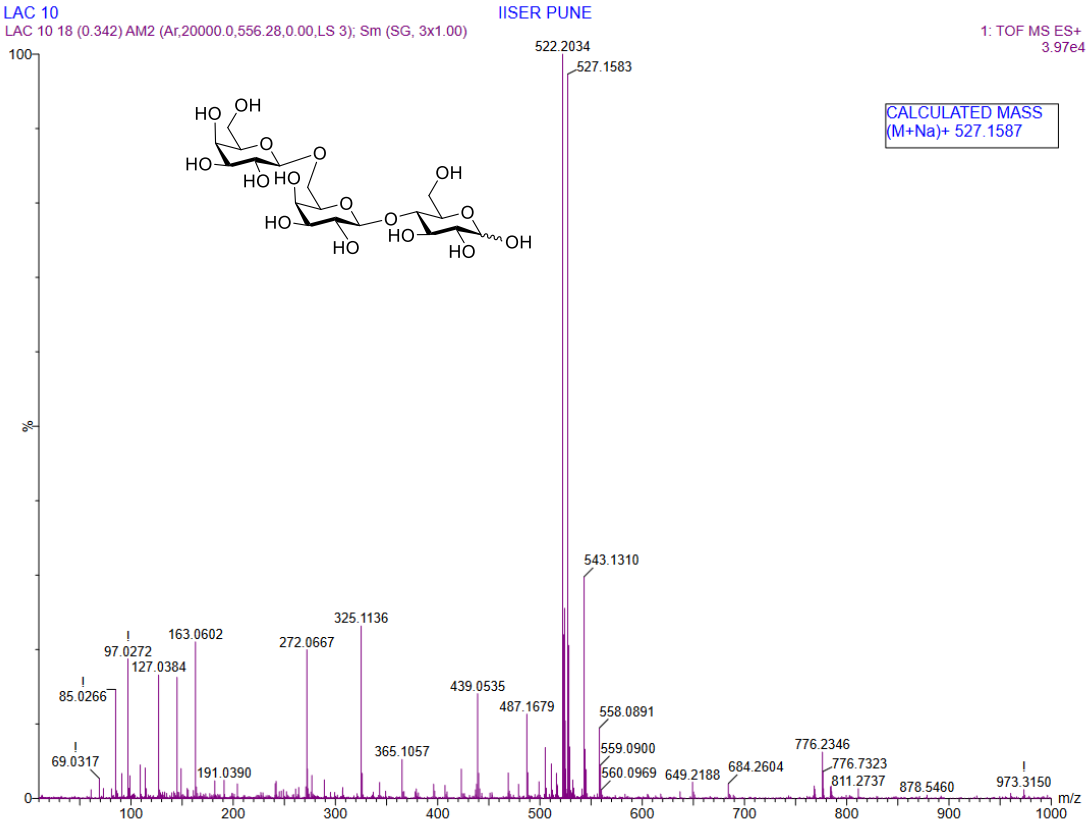
Observed mass: 1368.028



Compound- 36

Calculated mass: 527.1587 ($C_{73}H_{77}O_{15}NNa$)

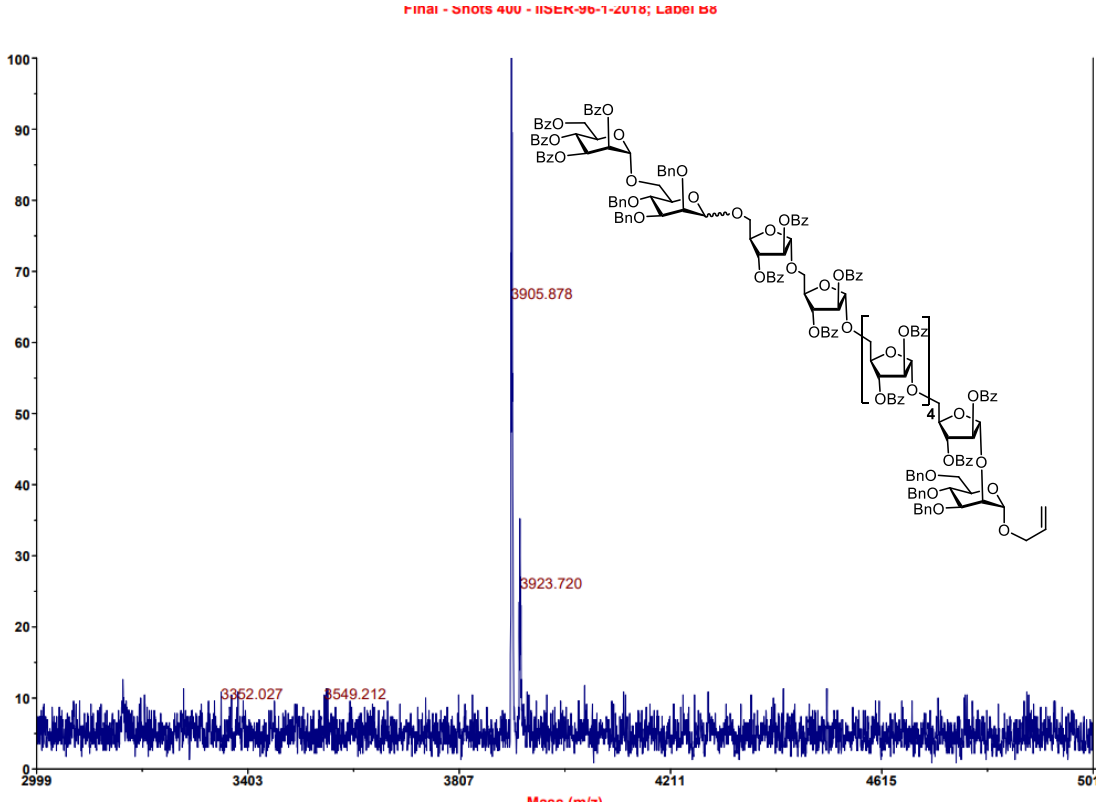
Observed mass: 527.1583



Compound- **57**

Calculated mass**:** 3905.243 **(**$C_{224}H_{200}O_{62}Na$**)**

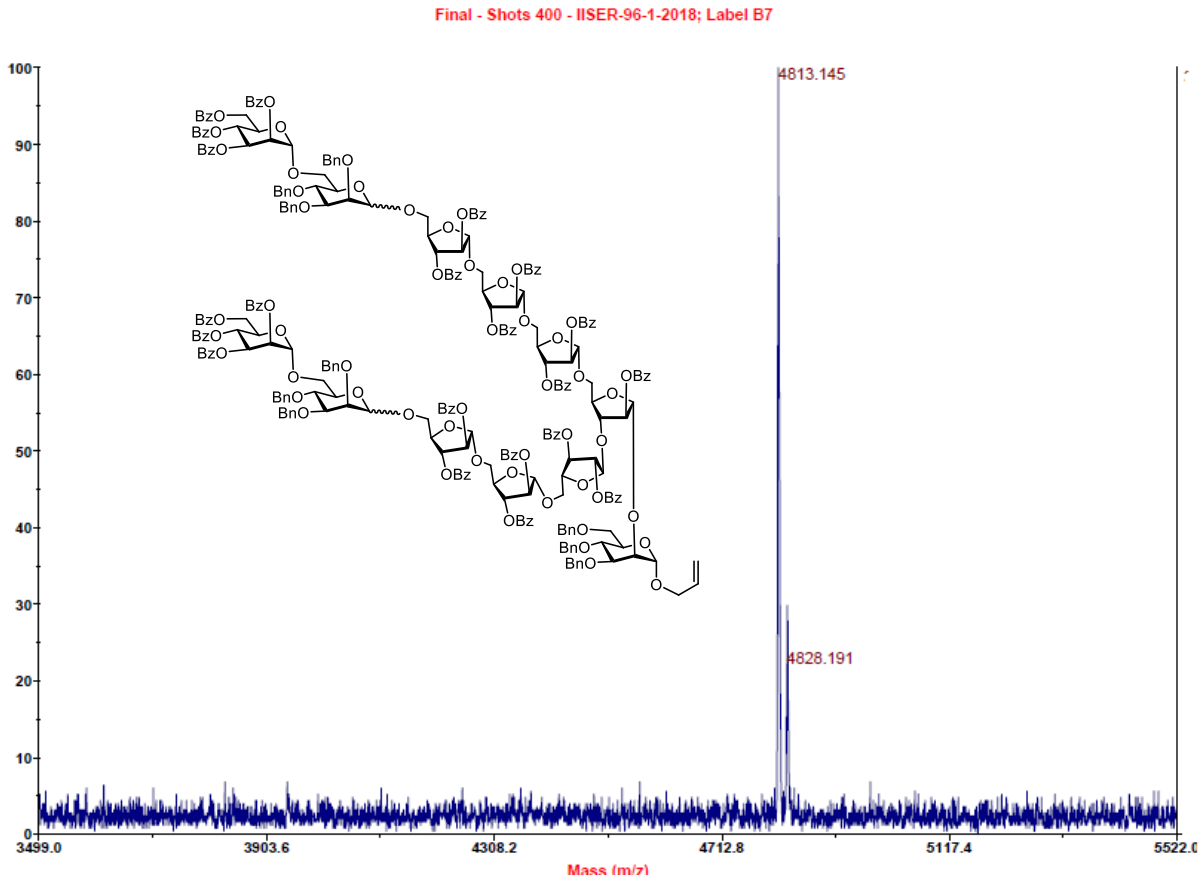
Observed mass: 3905.878



Compound- **59**

Calculated mass : 4813.241($C_{278}H_{250}O_{75}Na$)

Observed mass: 4813.145



3.6- References:

(1) Campos, K. R.; Coleman, P. J.; Alvarez, J. C.; Dreher, S. D.; Garbaccio, R. M.; Terrett, N. K.; Tillyer, R. D.; Truppo, M. D.; Parmee, E. R. *Science* **2019**, *363* (6424)

2. Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C. *Chem. Rev.* **2013**, *113* (7), 5322–5363

3.Yamamoto, K.; Li, J.; Garber, J. A. O.; Rolfes, J. D.; Boursalian, G. B.; Borghs, J. C.; Genicot, C.; Jacq, J.; van Gastel, M.; Neese, F.; Ritter, T. *Nature* **2018**, *554* (7693), 511–514.

4. Ruiz-Castillo, P.; Buchwald, S. L. *Chem. Rev.* **2016**, *116* (19), 12564–12649.

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Chapter: 4

Chemical Glycan Swapping

4.1 Introduction:

Glycosylation is a post-translational modification employed by Nature to enhance the biological functionality of biomolecules, adding a new layer of structural diversity. Glycans within glycoconjugates modulate the physicochemical properties of these molecules and directly engage in crucial biological processes, including cell adhesion, cancer progression, host-pathogen interactions, and immune responses.[1-4] Understanding the roles of glycans within glycoconjugates is therefore essential. However, the study of glycomic functions is often impeded by the difficulty in obtaining homogeneous glycoconjugates in pure forms from natural sources. To address the demand for structurally defined oligosaccharides and glycoconjugates, a range of sophisticated chemical and enzymatic synthesis methods have been developed.[5-8] Conventional chemical synthesis, which requires intricate protection/deprotection steps to achieve regioselectivity and stereoselectivity, contrasts with enzymatic glycosylation, which offers precise control over anomeric configuration and high regioselectivity without the need for protecting groups. Enzymatic glycosylation is particularly advantageous due to its precise control of anomeric configuration and regiochemistry. Both glycosyltransferases and glycosidases have been extensively studied and utilized for synthetic purposes.[9-10] Glycosyltransferases, the natural enzymes for constructing glycosidic bonds, are challenging to apply broadly for large-scale synthesis due to their membrane-associated nature, instability, difficulty in expression, strict substrate specificity, and the need for expensive donor substrates like sugar nucleotides (Leloir pathway) or sugar phosphates (non-Leloir pathway).[9]

Transglycosylation, however, is a versatile and essential biochemical reaction that involves transferring glycosyl residues from a donor to an acceptor molecule, forming new glycosidic bonds. This reaction is catalyzed by enzymes such as glycosyltransferases and glycosidases and is fundamental to the biosynthesis and remodeling of glycoconjugates, including polysaccharides, glycoproteins, and glycolipids. These processes play critical roles in various biological functions, underlining the importance of transglycosylation in both natural and synthetic contexts.[10]

4.1.1Enzymatic transglycosylation for glycosidic bond formation:[11]

For a typical retaining β-glycosidase, catalysis typically follows a two-step, a double displacement mechanism involving two key carboxylate residues that are spatially close (usually within ~ 6.0Å). In this process, the nucleophilic residue attacks the anomeric center to form an enzyme-substrate

complex, while the general acid/base residue protonates the glycosidic oxygen and subsequently activates water (in hydrolysis) or a sugar acceptor (in transglycosylation). For reactions catalyzed by family GH18 chitinases, GH20 hexosaminidases, or GH85 endo-β-*N*-acetylglucosaminidases (ENGases), a substrate-assisted catalysis mechanism has been proposed. Here, the 2-acetamido group in the substrate acts as the nucleophile, promoting the breakdown of the glycosidic bond and forming an oxazolinium ion intermediate, which then reacts with water or a sugar acceptor. Transglycosylation efficiency hinges on the enzyme's ability to favorably accommodate a sugar acceptor to compete with water. Additionally, product hydrolysis presents a significant challenge when using glycosidases for synthesis. Early strategies to address these issues included incorporating organic solvents into the reaction media to reduce water activity and using activated species (e.g., glycosyl fluoride and synthetic sugar oxazoline) as donors to achieve better kinetic control. For instance, a variety of oligosaccharides and artificial polysaccharides have been synthesized through the transglycosylation of disaccharide glycosyl fluoride catalyzed by endoglycosidases (cellulase, α-amylase, 1,3 or 1,4-β-glucanase, and xylanase). Artificial chitin and other glycosaminoglycan derivatives have been created by polymerizing modified disaccharide oxazolines using family GH18 chitinase and GH56 hyaluronidase as catalysts. Moreover, employing synthetic sugar-oxazoline in ENGase-catalyzed transglycosylation has significantly expanded the synthetic capabilities for constructing homogeneous N-glycopeptides and N-glycoproteins.

In 1998, Withers and colleagues reported the first glycosynthase (Abg E358A) by engineering the β-glycosidase from Agrobacterium sp., achieved by mutating the nucleophile residue E358. Additionally, Moracci and co-workers described another innovative glycosynthase approach, utilizing an activated glycosyl species as the substrate while restoring the transglycosylation activity of the non-hydrolyzing glycosidase mutant with exogenous sodium formate or azide as an external nucleophile. Glycosynthases derived from inverting glycosidases were also reported, similarly requiring an activated sugar donor with an opposite anomeric configuration to facilitate transglycosylation.

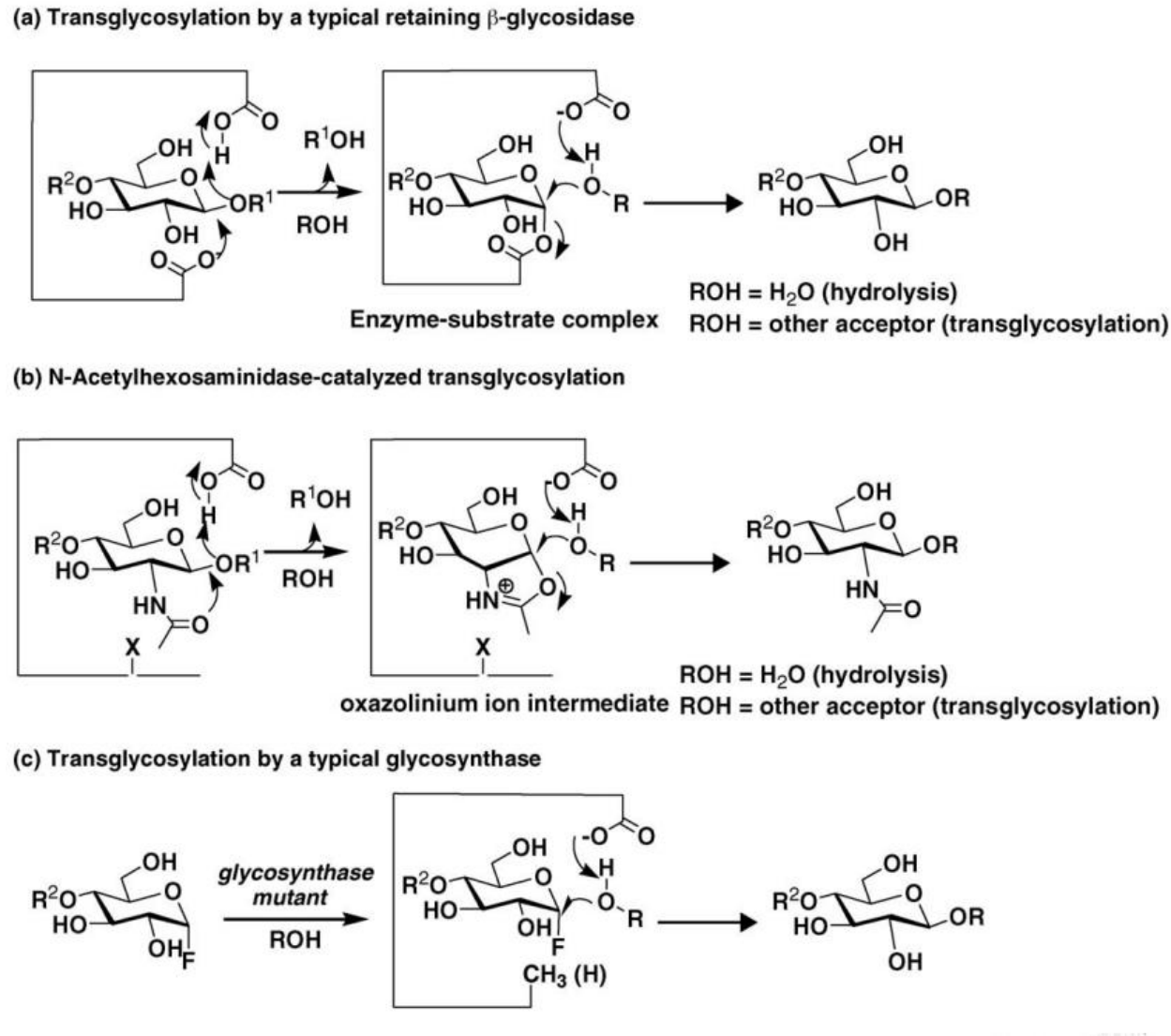


Fig 1: Mechanism for glycosidase catalyzed transglycosylation (Adapted from reference 11)

4.1.2 Synthesis of glycoconjugates via enzymatic transglycosylation:

In recent years, chemoenzymatic synthesis involving enzymatic transglycosylation has become a powerful method for producing complex oligosaccharides and glycoconjugates, which are highly valuable for functional studies and biomedical applications.[12] The advent of glycosynthases, which address the limitations of traditional glycosidase-catalyzed transglycosylation, marks a significant advancement in this field.[13-15] Synthesizing homogeneous glycoproteins with well-defined oligosaccharides remains a significant challenge for synthetic chemists.[16] Wang and colleagues explored synthetic sugar oxazolines as donor substrates for ENGase-catalyzed N-glycopeptide synthesis.[17-19] They found that the oligosaccharide oxazoline corresponding to the N-glycan core

$Man_3GlcNAc$, along with its truncated and selectively modified forms, could serve as donors for transglycosylation, yielding the corresponding N-glycopeptides in good to excellent yields. Notably, the resulting glycopeptides were resistant to hydrolysis due to structural modifications on the N-glycans. This chemoenzymatic approach was successfully extended to the glycosylation remodeling of glycoproteins. An efficient chemoenzymatic synthesis of various homogeneous glycoforms of ribonuclease B, incorporating azido tags and oligosaccharide ligands, was reported, highlighting the versatility and potential of this method in glycoprotein synthesis.[20-21]

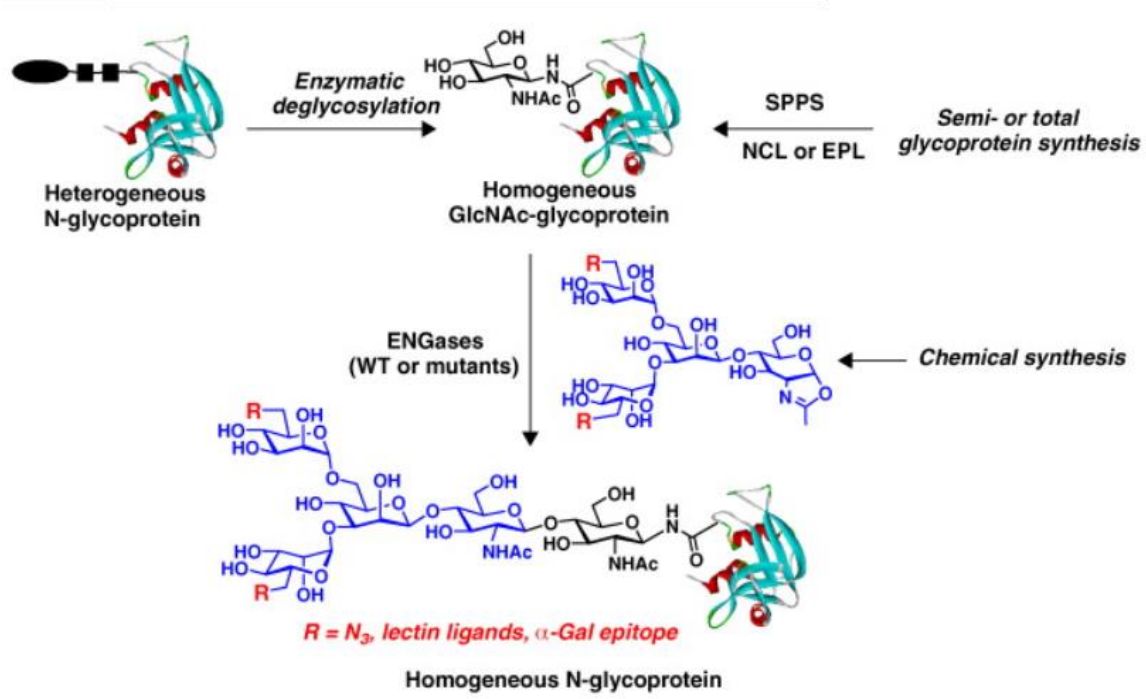


Fig 2: Synthesis of glycoprotein by ENGase-catalyzed transglycosylation (Fig Adapted from reference 11)

Withers and colleagues created the first glycosynthase for glycosphingolipid synthesis by engineering the endoglycoceramidase II (EGC II) from Rhodococcus sp., an enzyme that hydrolyzes glycosphingolipids. They discovered that mutating the putative nucleophile E351 eliminated the hydrolysis activity of EGC II, but the mutants could utilize synthetic 3'-*O*-sialyl-α-D-lactosyl fluoride as a donor to glycosylate D-*erythro*-sphingosine.[22] Among the mutants, the E351S variant was identified as the most efficient glycosynthase, capable of promoting the formation of the corresponding lyso-GM3 ganglioside product with an over 90% yield.

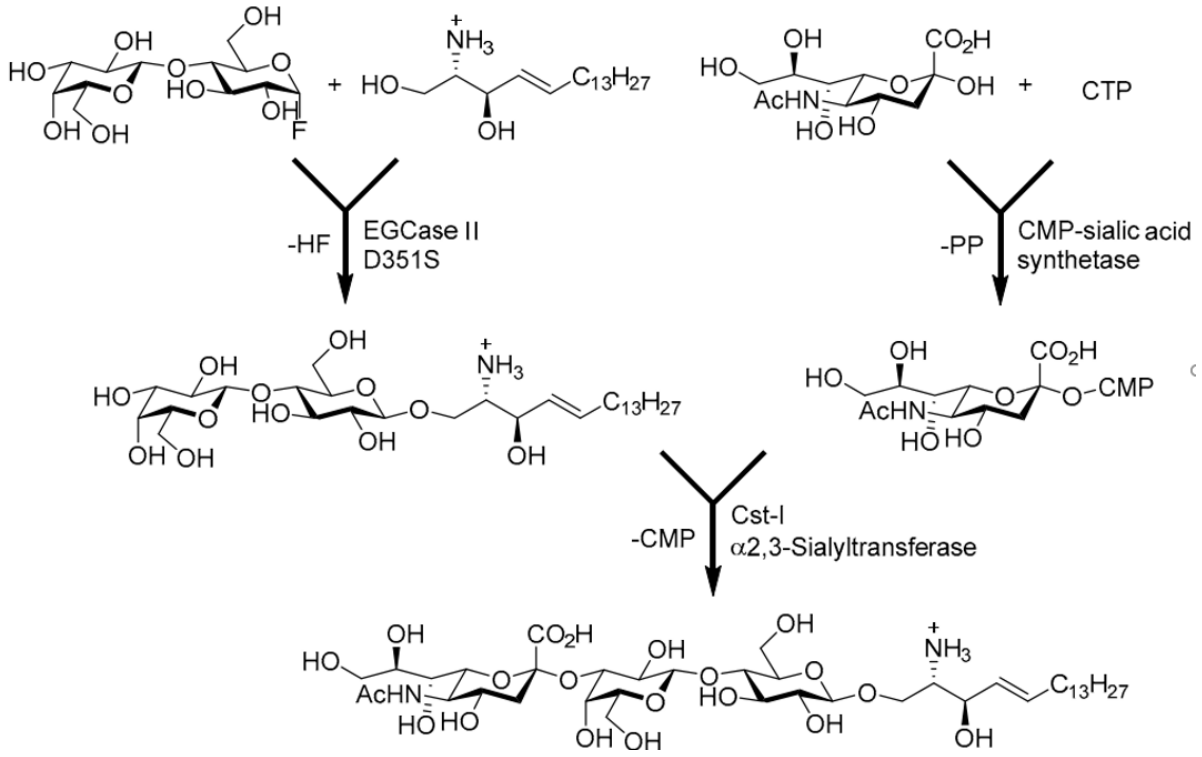


Fig 3: Synthesis of glycosphingolipid by combining GTs and EGC II (Fig Adapted from reference 22a)

4.1.3 Synthesis of nucleosides via enzymatic transglycosylation:

Enzymatic transglycosylation has become a crucial tool for synthesizing complex nucleosides, bolstered by advancements in enzyme engineering technologies such as directed evolution, semi-rational design, artificial synthesis, computer-assisted design, and artificial intelligence alongside traditional chemical synthesis.[23] Historically, transglycosylations have been catalyzed by two types of enzymes: nucleoside 2′-deoxyribosyltransferases (NDTs) and nucleoside phosphorylases (NPs). NDTs catalyze the one-step exchange of 2′-deoxyribose moieties between purine and/or pyrimidine nucleosides, using one nucleoside as the donor and another nucleobase as the acceptor.[24-25] NPs, a family of transferases (EC 2.4 and EC 2.7.7), catalyze the reversible cleavage of the N-glycosidic bond of β-nucleosides in the presence of inorganic orthophosphate as a co-substrate. This reaction produces a free nucleobase and α-pentofuranose-1-phosphate. The NP-catalyzed transfer of α-pentofuranose-1-phosphate to a second nucleobase results in the formation of a new β-nucleoside with near-perfect regioselectivity and stereoselectivity.[26]

Recently, Merck introduced a novel biocatalytic pathway termed path IV (lipase/kinase/NP) to directly synthesize molnupiravir, as depicted in Figure 4.[27] The process began with the selective esterification of ribose using isobutyric anhydride, catalyzed by Novozyme 435, a commercial lipase. This cycle relied on five enzymes: 5-*S*-methylthioribose (MTR) kinase, uridine phosphorylase (UP), pyruvate oxidase (PO), acetate kinase (AcK), and catalase. MTR kinase from *Klebsiella* spp. was identified as suitable for the 1-phosphorylation of ribose-hemiacetal, despite its natural absence in nucleoside biosynthesis. UP sourced from E. coli (URP-003 from Codexis) was chosen for the transglycosylation . Through multiple rounds of evolution, optimized mutants of MTR kinase and UP were developed to enhance enzyme activities. Additionally, PO and AcK facilitated ATP regeneration, while catalase managed hydrogen peroxide decomposition, crucial for constructing the fully enzymatic cascade. Finally, treatment with hydroxylamine reagent enabled the synthesis of molnupiravir in three steps, achieving a 69% yield. Furthermore, enzymatic transglycosylation could be used for the synthesis of 2′-*deoxy* ribonucleosides and is ready for scale up with high efficiency. Comparing the three strategies, it can be seen that all these methods could achieve good α/β selectivities in most cases for the five natural nucleobases, while enzymatic transglycosylation is the most suitable method for 2′-*deoxy* nucleoside synthesis. These methods are also amenable for building the base moiety early in the synthesis of modified nucleosides, and there are few reports for building the nucleobase late in the β-nucleoside synthesis, which is frequently used in C-nucleoside synthesis.[28]

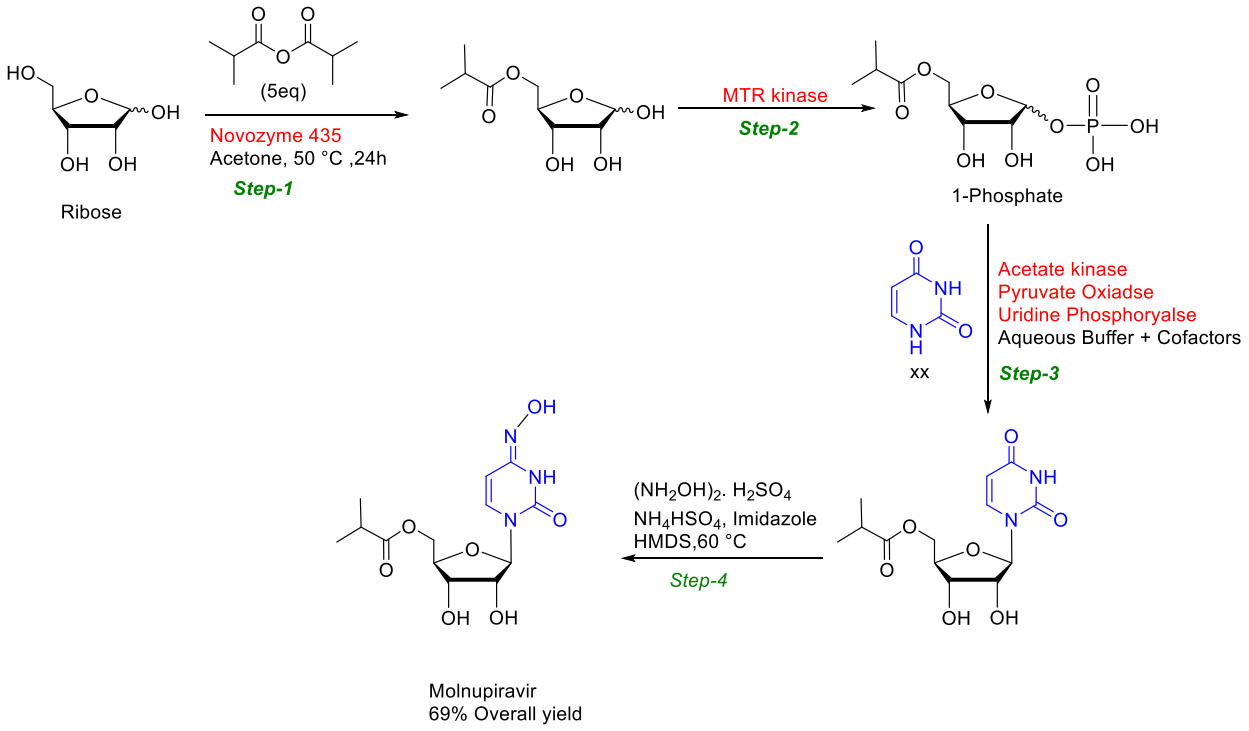


Fig 4: Chemoenzymatic synthesis of molnupiravir

4.1.4 Challenges in enzymatic transglycosylation for the synthesis of glycoconjugates:

Enzymatic transglycosylation is a valuable technique in modern biotechnology, with extensive applications in the synthesis of complex carbohydrates, glycoproteins, and glycolipids, all of which play crucial roles in the pharmaceutical, food, and cosmetic industries. This process utilizes the inherent specificity and efficiency of enzymes to form glycosidic bonds, providing an alternative to traditional chemical synthesis methods that often require harsh conditions and multiple steps. Despite its potential, enzymatic transglycosylation faces several challenges that need to be addressed for its effective and widespread use in the synthesis of oligosaccharides and glycoconjugates. These key challenges are outlined below.

- **Improving the efficiency of transglycosylation**:

In glycosidase-catalyzed synthesis, the product often serves as a substrate for hydrolysis, typically resulting in moderate yields. A significant breakthrough to address this challenge involved developing nucleophile mutants, known as glycosynthases. These mutants lose hydrolytic activity while retaining transglycosylation activity. The first glycosynthase was derived from the GH1-glycosidase (Abg) of Agrobacterium sp., which exhibits both β-glucosidase and β-galactosidase activities. By replacing the catalytic nucleophile glutamate at position 358 with an alanine residue, the enzyme mechanism was completely altered.[29] Additionally, site-directed mutagenesis outside the catalytic residue can enhance transgalactosylation properties, although some hydrolytic activity may remain. Another method to reduce hydrolytic activity is semi-rational site-saturation mutagenesis, which can modify enzyme properties unfavorable for synthesis. For instance, truncated mutation has been shown to transform the hydrolytic β-galactosidase of B. bifidum DSM20215 into an efficient transgalactosylase. Deleting the C-terminal 580 amino acid residues involved in the galactose-binding motif resulted in the truncated enzyme using approximately 90% of reacted lactose for GOS synthesis.[30]

- **Glycosyl donor specificity of enzymes:**

Enzymes often have high specificity for certain glycosyl donors, which limits the variety of possible transglycosylation reactions. For example, β-galactosidases generally exhibit strict specificity for the galactose part of the glycosyl donor, except for those in the GH1 family. Similarly, glycosyl transferases (GTs) use nucleotide-activated donors like nucleoside diphosphate (e.g., UDP), nucleoside monophosphate, or dolichol phosphate. While these transferases are efficient, their industrial use is hindered by the high cost of their nucleotide-activated donors. However, enzyme engineering can alter this specificity. Through this process, β-galactosidases can be converted into other glycosidases, such as β-fucosidase and β-glucuronidase, which act on substrates containing fucose and glucuronic acid, respectively. For instance, in 2012, Shim et al. showed that molecular evolution of β-galactosidases enables the enzyme to effectively process engineered donor substrates like *p*-nitrophenyl 3-*O*-methyl-β-D-galactopyranoside (3-MeOGalpNP), which is modified by methylation at the C-3 position of the galactose ring. Structural analysis of Abg from Agrobacterium sp. identified residues Gln24, His125, Trp126, Trp404, Glu411, and Trp412 as constraining binding around the 3-OH group.[31] Site-directed saturation mutagenesis of these residues revealed that two mutants (Q24S-W404L and Q24N-

W404N) exhibited enhanced hydrolytic activity toward 3-MeOGalpNP. Integrating Q24S and W404L into the engineered glycosynthase 2F6 significantly increased the synthesis rate of 3-*O*-methylated trisaccharide by 40 times when using 3-*O*-methyl glucopyranosyl fluoride as a glycosyl donor and *p*NP-cellobioside as an acceptor. This engineered enzyme is valuable for producing naturally methylated glycoconjugates or artificial analogs.

- **Specificity of enzymes towards glycosyl acceptors**:

Despite their flexibility towards acceptor structures, enzymes exhibit different preferences for various acceptors. For instance, β-galactosidases catalyse the formation of O-glycosidic bonds between galactose and the alcoholic hydroxyl groups of acceptors. Enzymatic glycosylation of phenolic hydroxyl groups is more challenging than that of alcoholic hydroxyl groups due to the lower nucleophilicity of phenolic hydroxyls.[32] Natural glycosidases and glycosynthases typically glycosylate acceptors at hydroxyl groups to produce O-glycosidic linkages. However, modifying thiosugars is difficult despite the importance of *S*-glycosides.[33] This difficulty may be attributed to electrostatic repulsion between acid/base residues and thiolate or steric hindrance caused by larger sulfur atoms. In recent years, numerous pioneering efforts have been reported to address the specificity problem of glycosyl acceptors, employing various rational and non-rational engineering strategies such as site-directed mutagenesis, truncation, site-saturation mutagenesis, random mutagenesis, DNA shuffling, and monobody modifications of enzyme proteins.[34] A notable advancement is the rational design of L. bulgaricus L3 β-galactosidase, which has improved its preference for phenolic hydroxyl groups. This breakthrough facilitates enzymatic galactosylation of challenging phenols. Many valuable phenolic compounds possess attractive bioactivities but are limited by poor water solubility and instability under photochemical irradiation. Interestingly, site-directed mutagenesis of catalytic acid/base residues has led to the creation of a new class of catalysts, known as thioglycoligases, which can synthesize *S*-glycosidic linkages.

In addition to the aforementioned challenges, several factors impact enzymatic transglycosylation strategies. These include the thermal stability of the enzymes, reaction conditions, the cost of enzymes for scalability, and the ability to achieve specific glycosidic linkages (e.g., α or β) with high regioselectivity and stereoselectivity. Overcoming these challenges requires ongoing research

and development in enzyme engineering, process optimization, and the development of cost-effective production methods.[35]

4.1.5 Chemical transglycosylation for the synthesis of nucleosides and nucleosides analogues:

Despite the dominance of enzymatic transglycosylation strategies for synthesizing oligosaccharides and glycoconjugates, several reports highlight the use of chemical transglycosylation methods for generating nucleoside and modified nucleoside analogues. Chemical transglycosylation between purines and pyrimidines has been achieved using the Vorbrüggen glycosylation method.[36-38] This method couples silylated nucleobases with glycosyl donors in the presence of Lewis acids ($HgBr_2$, $SnCl_4$, $TMSClO_4$, and TMSOTf), resulting in a mixture of protected purine nucleosides with yields ranging from very low to moderate. The most commonly used glycosyl donors in these strategies are ribose acetates, although other activated ribose derivatives have also been reported. However, nucleosides that feature complex ribose components pose greater challenges for the conventional glycosylation approach using ribose acetates as glycosyl donors. Addressing these challenges typically demands substantial investment in synthesizing unnatural ribose acetates or modifying natural/simple nucleosides.

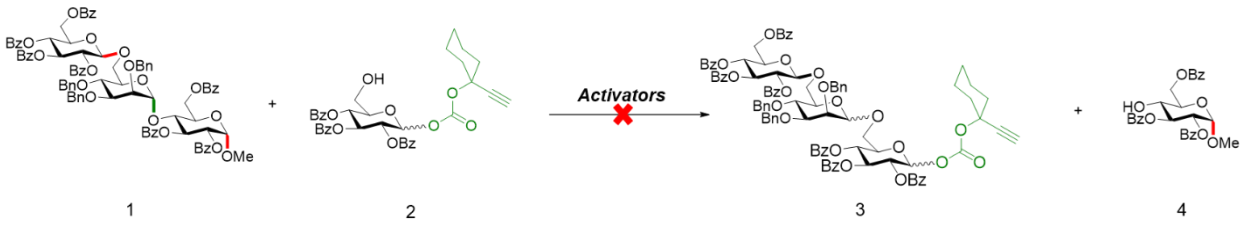
In 2020, Gong et al. developed a chemical method for transglycosylation. They discovered that under optimized Vorbrüggen conditions (BSA/TMSOTf/MeCN, with microwave irradiation), 5-nitrouridine could efficiently facilitate reversible nucleobase exchange with various 5-substituted uracil, yielding moderate to high yields. The strong electron-withdrawing ability of key nitro-activating groups enhances the leaving ability of the silyloxy-nitropyrimidine group. The resulting cationic intermediate, formed from 5-nitrouridine, is readily accessible due to the equilibrium constant K_{nitro} being greater than K_H. Any newly introduced silylated nucleobase can undergo reversible reaction to produce a new nucleoside. For instance, when treated with BSA/TMSOTf/MeCN under microwave irradiation, the desired nucleoside was synthesized (yielding 76%), which could alternatively be obtained via oil bath heating (yielding 83%).[39]

However, much work remains to be done in this field. For instance, the generation of various oligosaccharides and glycoconjugates using chemical glycosylation strategies remains largely unexplored. Methods for synthesizing oligosaccharides should prioritize the simple derivatization

of glycan units and the use of milder conditions for glycan activation. With these considerations in mind, we embarked on developing a chemical transglycosylation strategy based on exchanging glycan units between donor and acceptor units.

4.2 Present Work:

We began our investigation based on a result observed in our previous work with the CIStER strategy for surgical chemical glycan editing (Chapter 3). During our initial optimization study of generating the edited glycan from the protected guide glycan, we noticed that when the external glycosyl acceptor unit containing the anomeric ethylcyclohexyl carbonate moiety was used to react with the guide glycan **1** in the presence of various Lewis acids, the reaction failed in all cases.[40] This prompted us to investigate the fate of the external acceptor unit under the Lewis acidic conditions used. To begin these experiments, we took one of the previously synthesized glycan units, specifically the glucose moiety **2** with a free OH at the C-6 position and an anomeric carbonate appendage. This compound was then subjected to various acidic conditions, including BF_3•Et_2O, TMSOTf, $AuCl_3$, and TfOH. In the presence of all these activators, we observed the disappearance of the starting glycan unit **2** in the TLC, resulting in the formation of either 1,6-anhydro compound **5** or the hemiacetal **6** along with the formation of cyclohexyl propargyl alcohol **7**. The identity of compound **7** was confirmed through HRMS spectra. The reaction mixtures were purified, and both compounds (**5**, **6**) were isolated. The structure of compound **5** was further verified through ^{1}H and ^{13}C NMR spectra, matching with that of previously reported data.



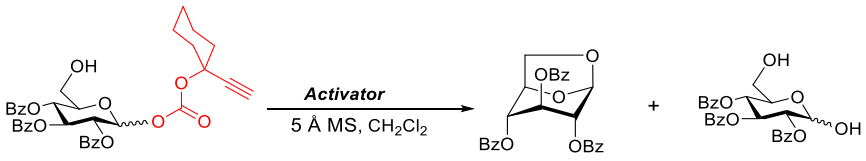
Scheme1: Insertion of external glycan acceptor **2** into the trisaccharide **1**

NuH		$SnCl_4$	CuOTf	TMSOTf	TfOH	BF_3•OEt_2	BF_3•OEt_2	
CH_2Cl_2	Product	1 eq.	1 eq.	1 eq.	1 eq.	1 eq.	3 eq.	
6 h		25 °C	25 °C	25 °C	25 °C	25 °C	25 °C	50 °C
2	**3**	NR*	NR*	10%*	NR*	30%	35%*	NR*

NR** 2 reacted to produce 1,6-anhydrosugar or hemiacetal; **30 min

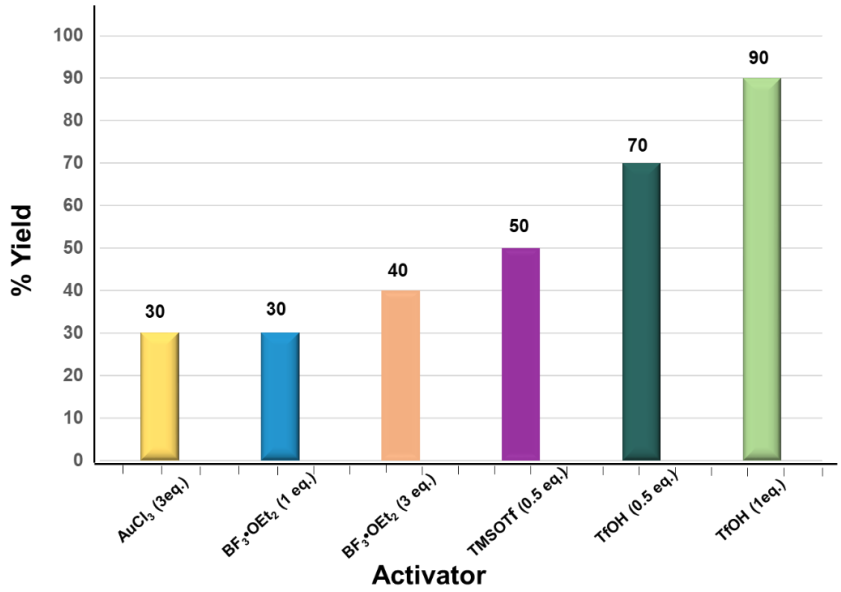
Table 1: Optimal reaction conditions for inserting acceptor 2 into the guide glycan 1.

4.2.1 Optimization study for the formation of 1, 6-anhydro compound in presence of acidic activators:



Scheme 2: Synthesis of 1, 6-anhydroglucopyranose 5 under various acidic activators

Following this, we investigated the reactivity of the glucose acceptor unit in the presence of various acidic activators. We reacted the glucose compound **2** with different acidic activators in anhydrous CH_2Cl_2 (4 mL) containing 5Å MS powder. Using 3 eq. of $AuCl_3$ as an activator, the reaction produced 30% of the 1, 6-anhydro compound **5** in 5 hours at 25 °C. Changing to 1 equivalent of BF_3•Et_2O did not improve the yield of compound **5**, but using 3 equivalents of this Lewis acid slightly increased the yield to 40%. Employing more acidic conditions with TMSOTf and TfOH further improved the yield. Ultimately, using 1 equivalent of TfOH resulted in the formation of 90% of the 1, 6-anhydro compound **5** within 1 hour.



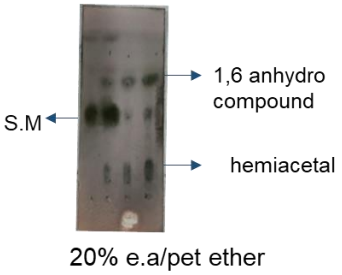
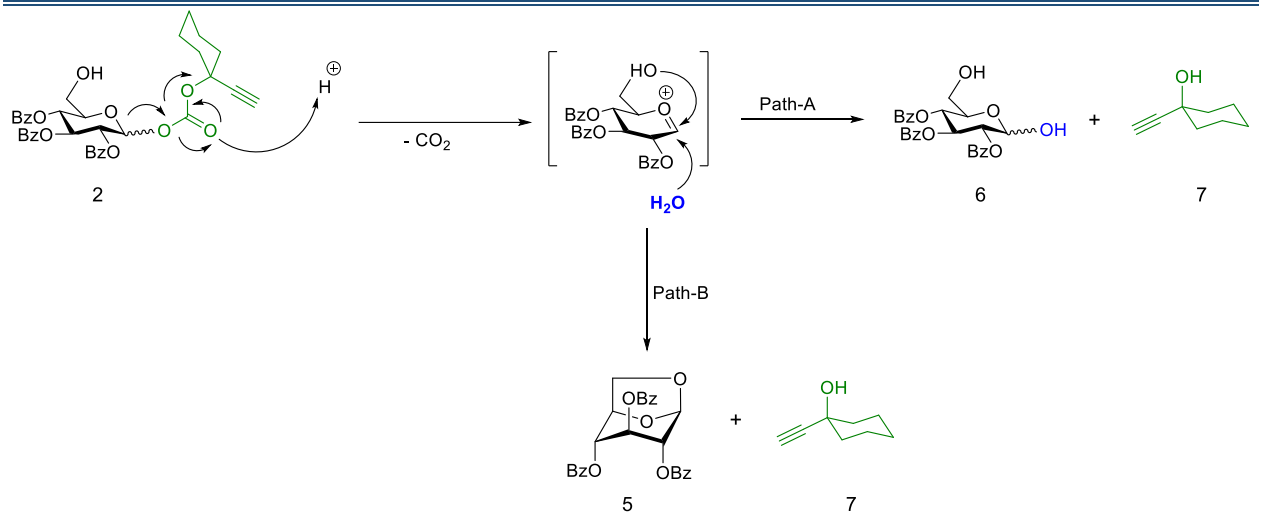


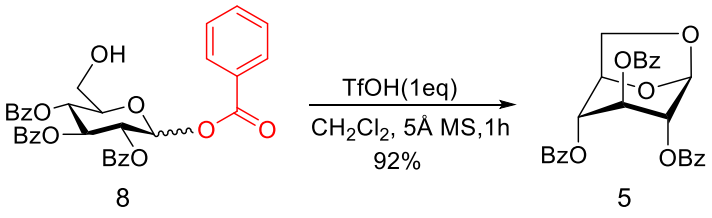
Fig 5: Evolution of conditions for the synthesis of compound **5**; Yields were determined by ^1H NMR spectroscopic analysis($CDCl_3$) of unpurified reaction mixtures using a quantitative internal standard; **Fig 6**: TLC of the reaction mixture depicted in scheme 2

The generation of the compound **5** and **6** along with byproduct **7** in the presence of different acidic activators in the reaction mixture led us to following probable pathway for generating either the 1,6-anhydro compound or the per-*O*-benzoylated glucose hemiacetal



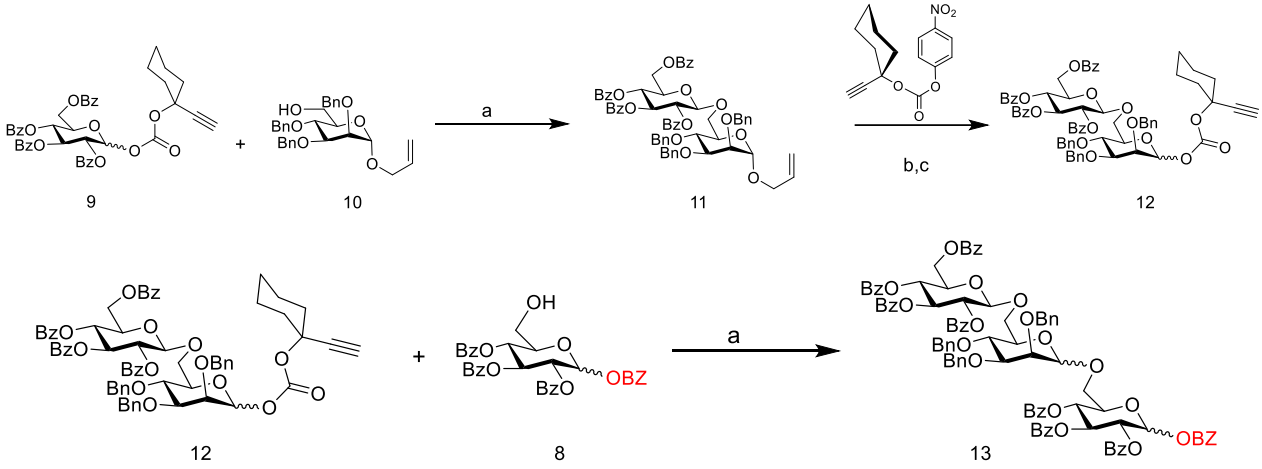
Scheme 3: Probable pathway of formation of the 1,6-ahydro glucopyranose **5**

The reaction conditions and probable pathway for the degradation of the external glycan provided insights into the formation of 1,6-anhydro sugar. This led us to investigate the potential of other C-1 leaving groups to form the same 1,6-anhydro sugar using acidic activation conditions. We decided to install a simple ester functionality at the anomeric position by introducing a benzoate group at the C-1 position. Installing the benzoate group was significantly easier than the ethynylcyclohexyl carbonate moiety, which requires the synthesis of the 1-ethynylcyclohexyl *p*-nitrophenyl carbonate reagent (see Chapter 2 for the reagent synthesis). To test this idea, we began by synthesizing the glucose unit **8**. This compound was prepared from the previously known compound **48** in two steps (see experimental section). Subsequently, compound **8** was treated with 1 eq. of TfOH in anhydrous CH_2Cl_2 containing 5Å MS powder. The TfOH-mediated activation of the glucose compound **8** resulted in the formation of per-*O*-benzoylated 1,6-anhydro sugar **5** in 92% yield, as previously observed in Scheme **3**. The structure of compound **5** was confirmed by ^{1}H and ^{13}C NMR spectra.



Scheme 4: Synthesis of the compound 5 from the compound **8**

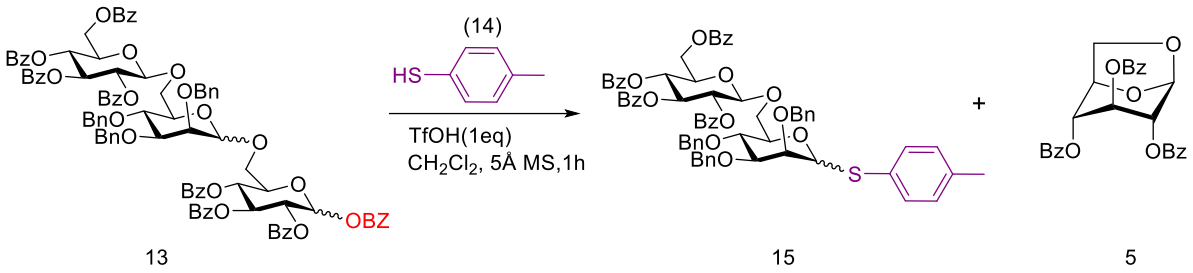
Next, we aimed to introduce the anomeric benzoate-containing glucose unit at the reducing end of a linear trisaccharide compound. To achieve this, we synthesized the desired trisaccharide **13** through the following steps. Initially, we glycosylated the previously known carbonate donor **9** with the acceptor unit **10** using Au/Ag as a catalyst to produce compound **11**. This intermediate was then converted into a glycosyl donor via two steps: deprotection of the allyl group and conversion into carbonate donor **12**. Finally, we coupled donor **12** with acceptor **8** to obtain the desired guide glycan trisaccharide **13**. The compound was characterized by ^{1}H and ^{13}C NMR spectra, and the linkage in the trisaccharide was confirmed by coupled HSQC spectra.



Scheme 5: Synthesis the starting trisaccharide **13** Reagents and conditions: a) 8 mol% each of chloro[tris(2,4-di-tert-butylphenyl) phosphite]gold and AgOTf, $CH2Cl_2$, 4 Å MS powder, 25 °C, 15min, 94% for 11 and 92% for 13 b) $PdCl_2$ (0.2 eq), CH_2Cl_2 -MeOH (1:4), 25 °C, 4 h; c) 15, DMAP, CH_2Cl_2 , 25 °C, 5 h, 84% over two steps for 12

4.2.2 Synthesis of *S*-glycosides by transglycosylation strategy:

Next, we evaluated the reactivity of the synthesized trisaccharide **13** in the presence of acidic activators. Building on our previous CIStER technique study, we planned to introduce a more nucleophilic species **14** to trap the oxocarbenium ion intermediate resulting from the cleavage of trisaccharide **13**. Thus, the trisaccharide **13** was reacted with 1 equivalent of *p*-TolSH (**14**) in the presence of 1 equivalent of TfOH in anhydrous CH_2Cl_2 containing 5Å MS powder. This reaction smoothly produced the disaccharide thiotolyl compound **15** and the 1,6-anhydro compound **5** within 1h. The progress of the reaction was checked by TLC analysis. After 1 hour, the starting trisaccharide was completely consumed, yielding the two non-polar compounds **15** and **5**.



Scheme 6: Synthesis of *S*-glycoside **15** by transglycosylation method

The crude reaction mixture was the purified and the both the compounds were isolated and characterized by the NMR spectra as well as MALDI-TOF spectra. Further, the structure of the compound **15** was examined by the ^{1}H, ^{13}C NMR spectra. In the ^{13}C NMR spectra of the compound **15** two peaks at δ 101.1 ppm corresponding to the *O*-glycosidic linkage and two peaks at δ 87.8 and 87.4 ppm belongs to the *S*-glycosidic linkage of the compound **15**. In addition, MALDI (TOF) spectra clearly showed the formation of the compound **15** as the observed mass matched with the calculated mass of the compound.

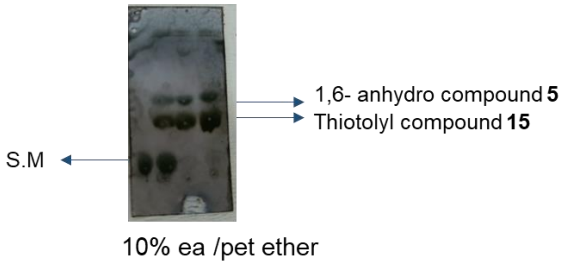
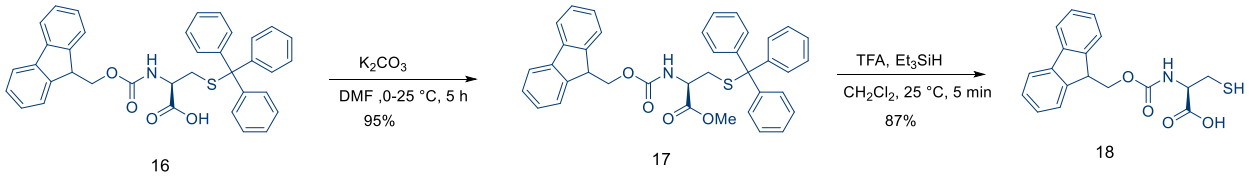


Fig 7: TLC of the reaction mixture depicted in scheme 6

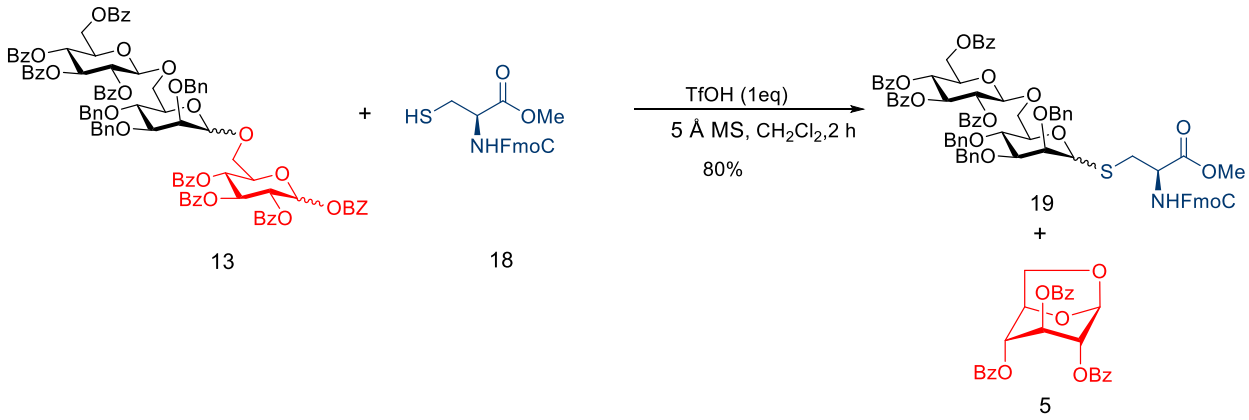
The successful synthesis of *S*-glycosidic linkage by the transglycosylation strategy prompted us to generation of *S*-glycosylated peptides. *S*-glycosylated peptides have garnered significant interest in drug synthesis and delivery due to their compatibility with biological systems, enhanced chemical stability, and resistance to proteolytic enzymes.[41]Various approaches have been developed for the chemical synthesis of *S*-linked glycopeptides, often leveraging the exceptional nucleophilicity of the thiol group.[42-45] Many of these methods involve site-directed glycoconjugation on peptide substrates with chemical functionalities that readily react with appropriate nucleophilic thiol-sugar derivatives. To check the potentiality of the transglycosylation strategy for generating *S*-linked glycopeptides, we started by generating the peptide moiety first.

The synthesis of the compound **18** was started from the previously synthesized compound **16**.[46] The Fmoc- Cys(trt)-OH was then reacted with K_2CO_3 in presence of DMF to convert it into Fmoc-Cys(trt)-OMe **17**. Finally, the trityl group in the compound **17** was deprotected with the trifluoroacetic acid and triethylsilane to generate the desired compound **18**. The compound **18** was characterized by the analysis of ^{1}H, ^{13}C NMR Spectra and it matched with that of previous reports. In addition, MALDI (TOF) spectra clearly showed the formation of the compound **18** as the observed mass matches with the calculated mass of the compound.



Scheme 7: Synthesis of Fmoc-Cys-OMe **18**

After that the compound **18** was reacted with the trisaccharide compound **13** in presence of the TfOH. The reaction proceeded smoothly within 1 h generating the desired *S*-glycoside compound **19** in 80% yield. The synthesized compound was thoroughly characterized by the ^{1}H,^{13}C, DEPT NMR spectra analysis. In the ^{13}C NMR spectrum, the anomeric *O*-glycosidic linkage shows two peaks at δ 101.5 and 101.2 ppm whereas the *S*-glycosidic linkage at δ 84.7 and 84.6 ppm.



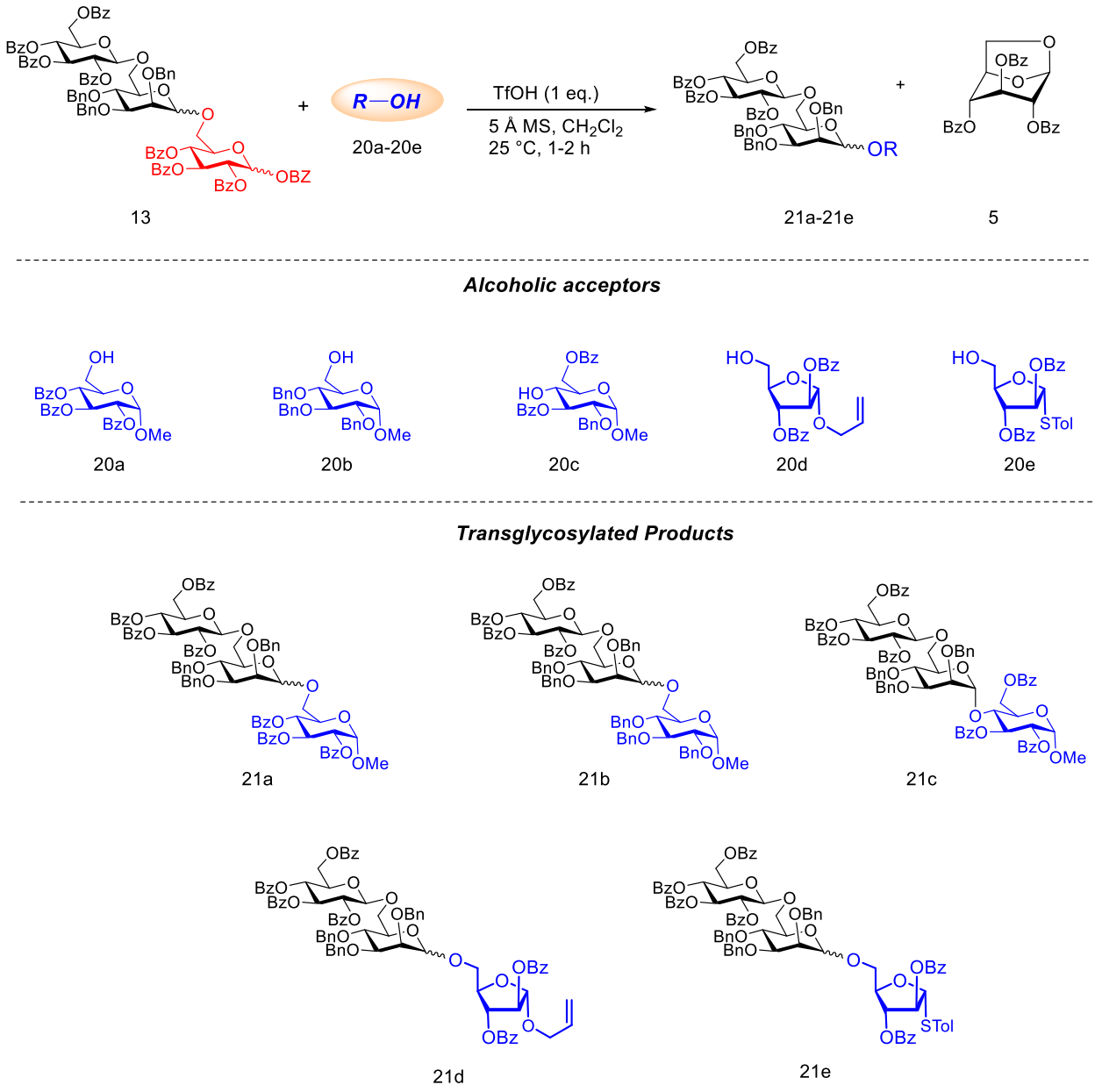
Scheme 8: Synthesis of *S*-glycopeptide **19** by transglycosylation method

These results emphasize the critical role of having the benzoate moiety at the reducing end of the trisaccharide **13**. The benzoate group reacts with TfOH, resulting in the breaking of the interglycosidic bond and the production of an oxocarbenium ion. This oxocarbenium ion

intermediate was then trapped by the nucleophile **14, 18** producing the compound **15**, **19**. Additionally, the terminal glucose unit of the trisaccharide compound forms 1,6-anhydro sugar **5**.This study clearly demonstrates that the trisaccharide compound **13** can serve as a glycosyl donor for chemical transglycosylation, facilitated by the presence of the ester group at the reducing end of the trisaccharide.

4.2.2 Synthesis of *O*-glycosides by transglycosylation strategy:

Based on the results described above, we planned to use this transglycosylation strategy to synthesize *O*-glycosidic bonds. We used various glycosyl acceptors to react with the trisaccharide compound **13** in the presence of TfOH. Interestingly, in all the cases depicted below, we obtained the transglycosylated product in good to excellent yields. The compound **13** reacted with the primary glucose acceptor **20a** in the presence of 1 equivalent of TfOH, generating the trisaccharide compound **21a** within 1 hour in 86% yield. During the reaction, the reducing end glucose moiety produced the by-product 1, 6-anhydro sugar **5**. Similarly, the per-*O*-benzylated glucose acceptor **20b** reacted under similar conditions, generating the trisaccharide compound **21b** in 82% yield. Additionally, the C4-glucose acceptor **20c** was used to generate the trisaccharide **21c** within 2 hours at an 80% yield using this strategy. The primary arabinose acceptor **20d** was also used to synthesize the trisaccharide **21d** with an 88% yield. Additionally, we employed a primary arabinose acceptor unit **20e** with a thiotolyl moiety at the anomeric end. This compound reacted smoothly in the presence of TfOH, generating the trisaccharide compound **21e** with an 85% yield. Interestingly, the thiotolyl protecting group was well tolerated under the reaction conditions. It is worth noting the anomeric groups like thiotolyl present in the acceptor unit are well tolerated in this protocol which expands the utilty of this strategy for orthogonal and one-pot synthesis of oligosaccharides. The generated trisaccharide compounds were throughly characterizzed by ^{1}H,^{13}C, HSQC NMR spectra.The generated compounds were also characterized by the MALDI-TOF spectra. For example ^{13}CNMR spectra of compound **21e** shows two peaks at δ 101.4 ppm which corresponds the glucose glycosidic linkage and the peaks at δ 91.7 , 91.4 ppm belong to the *S*-glycosidic linkages. The other two peaks at δ 97.2 and 103.5 ppm corresponding to the mannose *O*-glycosidic linkage. The successful synthesis of this trisaccharide compound **21e** was confirmed by the mass spectrometric analysis [calculated for $C_{87}H_{78}O_{20}SNa$, $[M+Na]^+$: 1497.4705, found 1497.4785.



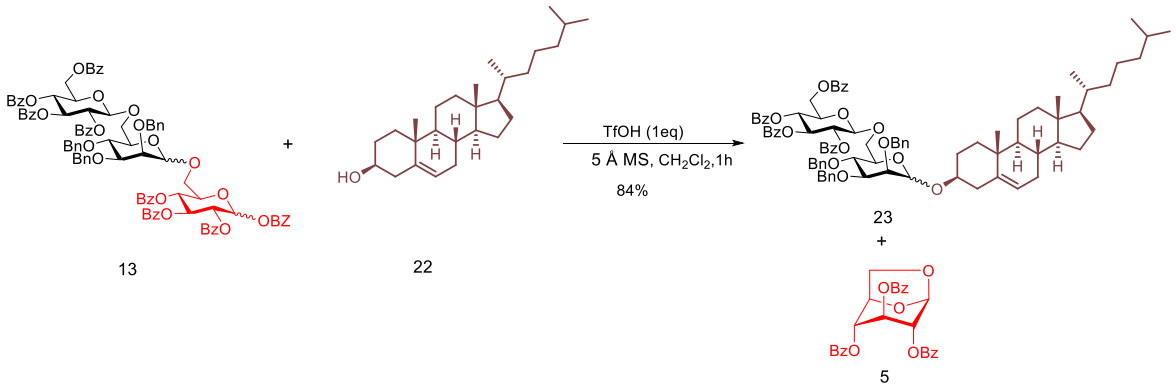
Scheme 8: Synthesis of *O*-glycosides by transglycosylation method

4.2.3 Synthesis of *Steroid & Flavonoid-glycosides* by transglycosylation strategy:

Steroids are a group of triterpenic, tetracyclic compounds known for their particularly bitter and toxic nature. They are typically oxygenated at various sites on the molecule's nucleus. Steroidal glycosides play a crucial role in medicine, agriculture, biotechnology, and the economy due to

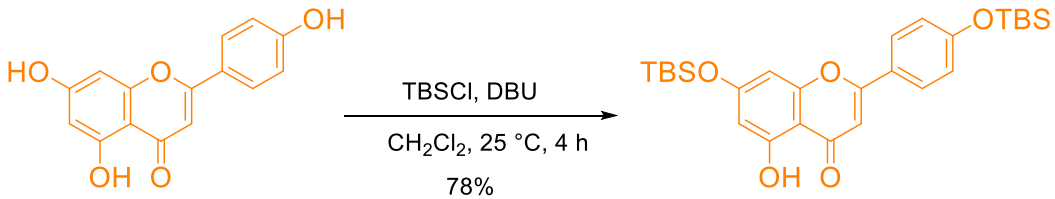
their diverse and potent biological activities. Their therapeutic potential, combined with their natural occurrence, makes them invaluable resources across various scientific and industrial fields.[47-48] over the past few years, considerable chemical efforts have been devoted toward the synthesis of cardiac oligosaccharides with various positions of steroids. However, the chemical glycosylation of cardiac glycosides remains restricted by several disadvantages, e. g., poor regio- and stereo-selectivity and tedious protection and deprotection of the functional groups. Enzymatic glycosylation of by specific glycosyltransferases (GTs) could alleviate these disadvantages.[49] In the past few years, cardiac oligosaccharides significant progress has been achieved in the discovery of GTs from microbes for CTS glycosylation, such as OleD and YjiC1.[50] Previously, reported the glycosylation of CTS using a plant GT, UGT74AN1, which exhibited robust capabilities for the regiospecific C-3 glycosylation of CTS.[51]

The previous results of our transglycosylation strategy for generation of *O*-glycosides motivated us to generate the steroid glycoside using this protocol. Accordingly, we used the Cholesterol **22** to react with the trisaccharide unit **13**. The reaction proceeds smoothly in presence of TfOH to generate the steroid glycoside **23** in excellent yield. The generation of the 1,6-anhydro sugar **5** in the reaction mixture supported the proposed reaction pathway i.e. cleavage of the Interglycosidic bond of the trisaccharide **13** to generate the compound **23**. The generated compound **23** was thoroughly characterized by ^{1}H,^{13}C NMR Spectra. In the ^{13}C NMR spectrum of the compound **23**, the anomeric region showed 4 peaks - δ 101.6, 101 ppm corresponding to the glucose glycosidic linkage whereas the peaks at δ 100.2 and 95.4 ppm belonging to the mannose glycosidic linkage. The molecule demonstrated a strong matching of the molecular ion in the mass spectrum. (m/z calcd for $C_{88}H_{100}NaO_{15}$: 1420.2443; found: 1420.2444).



Scheme 9: Synthesis of Steroid glycoside **23** by transglycosylation method

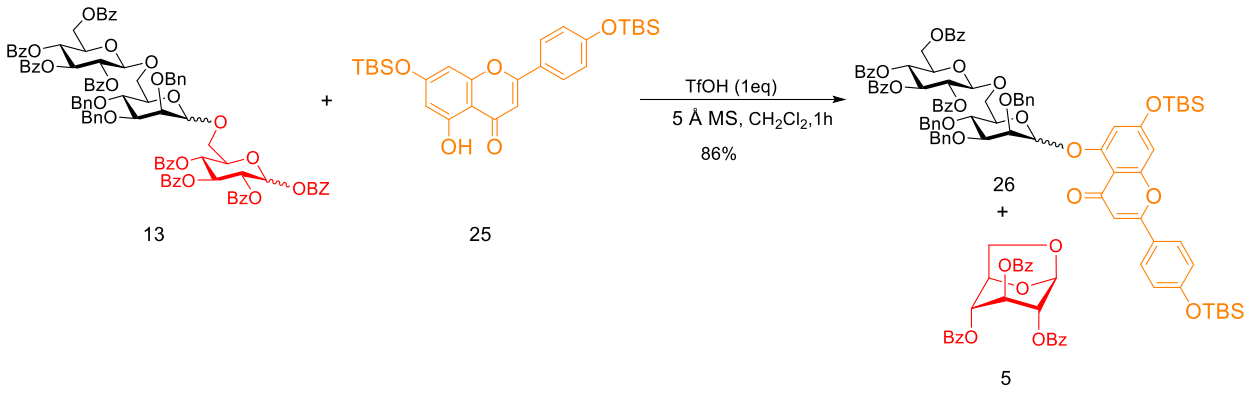
Then we move forward to the synthesis of another important class of molecule-flavonoid glycosides. Flavonoid *O*-glycosides are widely found in the plant kingdom,[52] and they play avariety of important roles in the growth and development of plants, e.g. as interspecies signal transferring molecules. In particular, they have also been demonstrated to possess a wide spectrum of activities beneficial to humans, such as antimicrobial, anticancer, and radical-scavenging activities.[53] Structurally, flavonol 5-*O*-glycosides are distinguished from their cousins by the existence of flavonol 5-*O*-glycosidic linkages, which pose a considerable challenge for synthetic chemists equipped with modern synthetic arsenals. The difficulty associated with the construction of 5-OH glycosidic linkages resides in the low reactivity of these phenolic OHs since they are involved in the formation of intramolecular H-bonds with the C-4 carbonyl group. This kind of intramolecular H-bond is so strong that it makes simple protecting group manipulations very difficult, let alone sophisticated glycosylation reactions. Of course, the electron-withdrawing properties of 4-carbonyl groups can further diminish the nucleophilicity of 5-OHs.[54] Previous studies employed glycosyl trichloroacetimidate donor with activation by TMSOTf,[55] glycosyl bromide as donors demonstrated very less to moderate yield of these 5-*O*-flavonoid glycosides. [56-59] However, a highly efficient approach to forge flavonol-5-*O*-glycosidic linkages is always in high demand. Here in this case we thought to incorporate the developed transglycosylation strategy to generate the flavonol-5-*O*-glycosidic linkages. At first we started to synthesize our aglycone compound **25** from the Apigenin **24**. At first, **24** treated with TBSCl and DBU was thoroughly silylated to produce the compound **25** in 89% yield.[60]



24 **25**

Scheme 10: Synthesis of Flavone acceptor **25**

After this, the compound **25** was reacted with the trisaccharide **13** in presence of 1 eq. of the TfOH to generate the desired flavonol-5-*O*-glycoside **26** in 86 % yield along with the formation of the 1,6-anydro compound **5**. The compound **26** was characterized by ^{1}H, ^{13}C, HSQC NMR spectra.

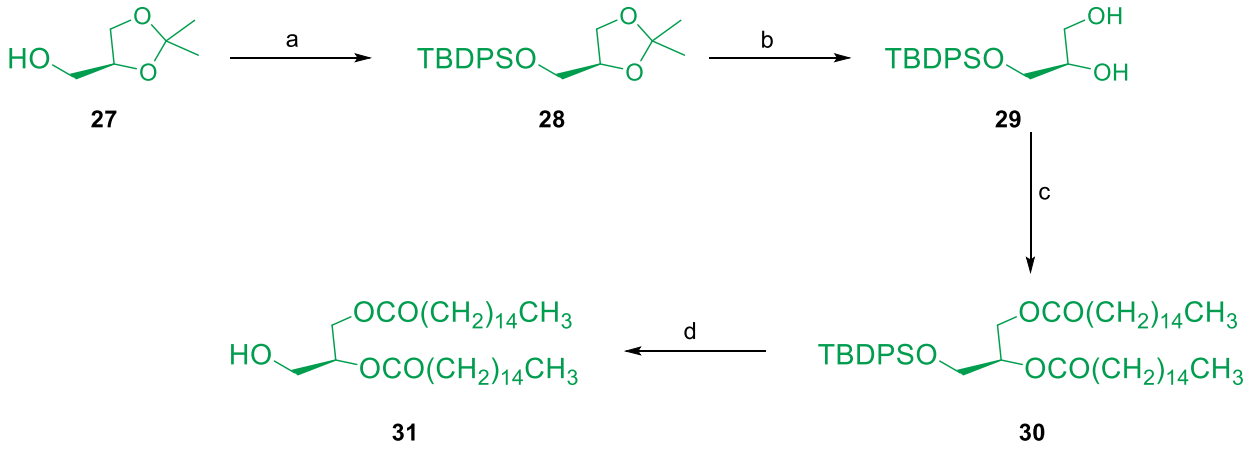


Scheme 10: Synthesis of flavonoid glycoside **26** by transglycosylation strategy

4.2.4 Synthesis of *Glycolipids& Glycopeptides* by transglycosylation strategy:

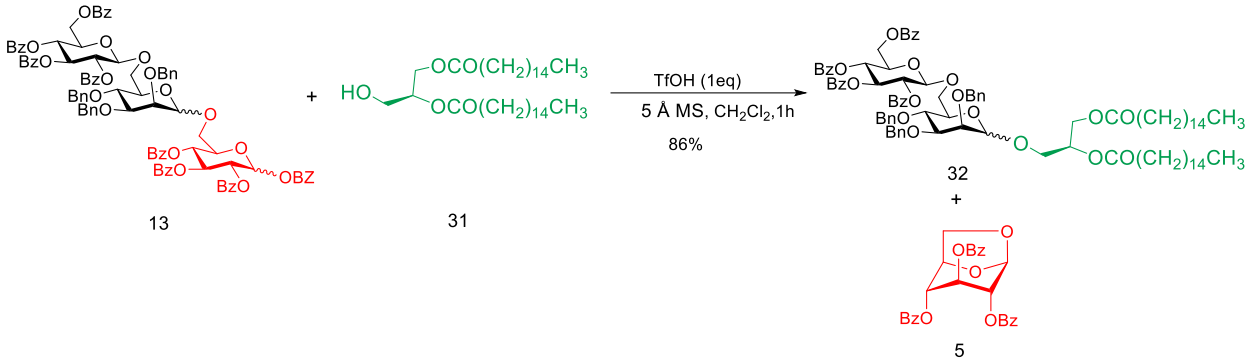
Next, we proceeded to synthesize another significant class of glycoconjugates: glycolipids. These molecules are essential components of cellular membranes, consisting of a hydrophobic lipid tail and one or more hydrophilic sugar groups connected by a glycosidic bond. Glycolipids are widely distributed across all cells and are primarily, though not exclusively, localized in the plasma membrane. The basic structure of a glycolipid includes a mono- or oligosaccharide group linked to either a sphingolipid or a glycerol group (which can be acetylated or alkylated) with one or two fatty acids. These form the classes of glycosphingolipids and glycoglycerolipids, respectively. Glycoglycerolipids have demonstrated a range of bioactivities, including anti-tumor, antiviral, and anti-inflammatory properties.[61-65] However, obtaining pure glycoglycerolipids from natural sources is challenging due to their limited availability. Additionally, the similar structures of glycoglycerolipids, with identical polarity and size, complicate their separation into individual compounds. Therefore, synthetic glycoglycerolipids are essential for biological evaluation. The primary challenge in synthesizing glycoconjugates lies in controlling the stereoselectivity of the glycosylation reaction. When synthesizing glycoglycerolipids, it is crucial to consider both the anomeric configuration of the glycosidic linkage and the chirality of the glycerol moiety. Over the past decade, various total synthetic strategies for producing glycoglycerolipids have been reported. These syntheses employ various glycosyl donors, such as glycosyl halides, trichloroacetimidates, thioglycosides, and orthoalkylbenzoate donors, to react with suitable glycerol derivatives. These approaches generated diverse classes of glycoglycerolipids, including mono- and diacylglyceroglycosides, sulfoquinovosylacylglyceroglycosides, and aminoglycerolipids.[66-70]

To apply the transglycosylation strategy for the synthesis of the glycoglycerolipids, we began by generating the desired aglycone motif **31**. At first (*S*)-isopropylidene glycerol **27** reacted with the chloro^{t}butyldiphenyl silane and imidazole to make 3-O-^{t}butyldiphenylsilylglycerol **28** that was treated with PTSA to generate the diol compound **29**. Introduction of two palmitoyl groups at C1 and C2 position by condensation of **29** with palmitic acid in presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 4-*N*,*N*'-dimethylaminopyridine (DMAP) gave the compound **30** in 87 % yield. Finally, the primary TBDPS group was removed by HF•py to generate the desired aglycon motif **31**.[71]



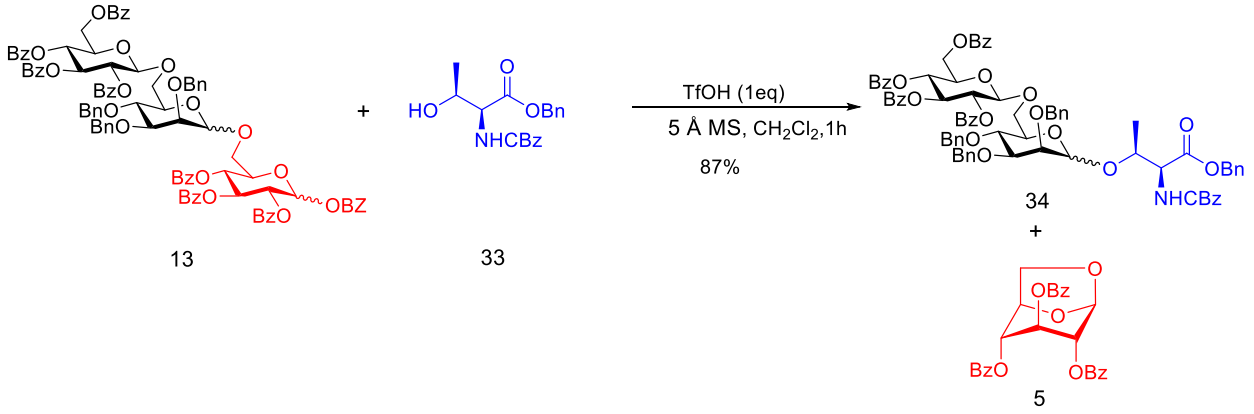
Scheme 11: Synthesis of aglycon motif **31 Reagents and conditions a)** TBDPSCl (1eq.), Im., DMF, 0–25 °C, 4 h, 88% for **28**; b) PTSA (0.5 eq), THF: H_2O (2:1), 5h, 83 % for **29**; c) Palmatic acid (2eq.), EDCI (2.5 eq.), DMAP, CH_2Cl_2, 25 °C, 3 h, 86% for **30**; d) Pyridine, HF·py, 0–25 °C, 5 h; 85% for **31**

Then this compound **31** was allowed to react with the previously synthesized trisaccharide compound **13** under the activation of 1 eq. of TfOH. The reaction proceeded smoothly to generate the glycoglycerolipid **32** within 1h in 86% yield. The compound **32** was characterized by ^{1}H, ^{13}C, HSQC NMR spectra.



Scheme 12: Synthesis of the glycoglycerolipid **32** by transglycosyaltion strategy

Next, we aimed to apply transglycosylation strategy for the generation of glycopeptides. Native glycosylation of peptides and proteins typically occurs in two forms: *O*-glycosylation, where the reducing end of an oligosaccharide is attached to the hydroxyl group of a serine, threonine, or occasionally tyrosine residue, and *N*-glycosylation, wherein the oligosaccharide is attached to the carboxamide group of an asparagine residue. The nature of the glycosidic bond to the peptide differs inherently between these two types of glycosylation. Additionally, the general structures of *N*-linked and *O*-linked glycans are fundamentally distinct, each presenting unique synthetic and biological challenges.[72]



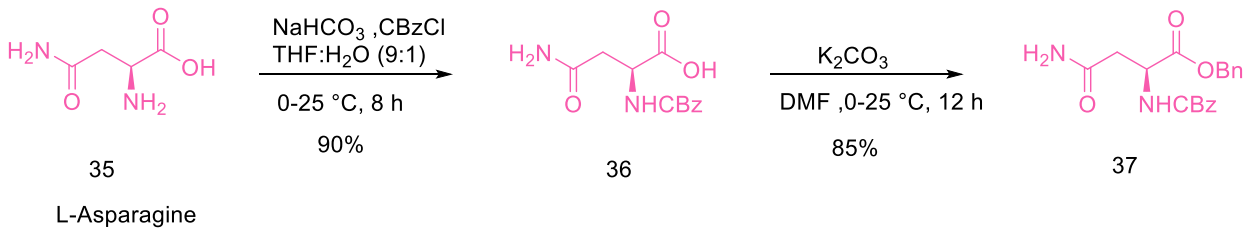
Scheme 13: Synthesis of the *O*-glycopeptide **34** by transglycosyaltion strategy

So keeping in mind these challenges, we thought of implementing the transglycosylation concept for successful synthesis of the *O*-linked glycopeptides. The synthesis started by generating a threonine acceptor moiety utilizing a previously described method.[73]After this the compound **33**

was used to transglycosylated our designed trisaccharide compound **13** in presence of TfOH. The reaction proceeded smoothly producing the desired *O*-linked threonine glycoconjugate. The compound **34** was analyzed by ^{1}H, ^{13}C, NMR spectra. The presence of two peaks at δ 156.2 ppm corresponding to the carbonyl carbon of the amide bond present in the acceptor unit and peaks at δ 18.6 ppm belonging to the -CH_3 group present on the aglycon unit confirmed the structure. The formation of the compound was also confirmed by MALDI-TOF spectrum analysis which showed a good matching of the molecular ion to the mass spectrum. (m/z calcd for $C_{80}H_{73}NaKO_{18}$: 1376.4498; found: 1376.4484).

4.2.5 Synthesis of *N*- glycosides by transglycosylation strategy:

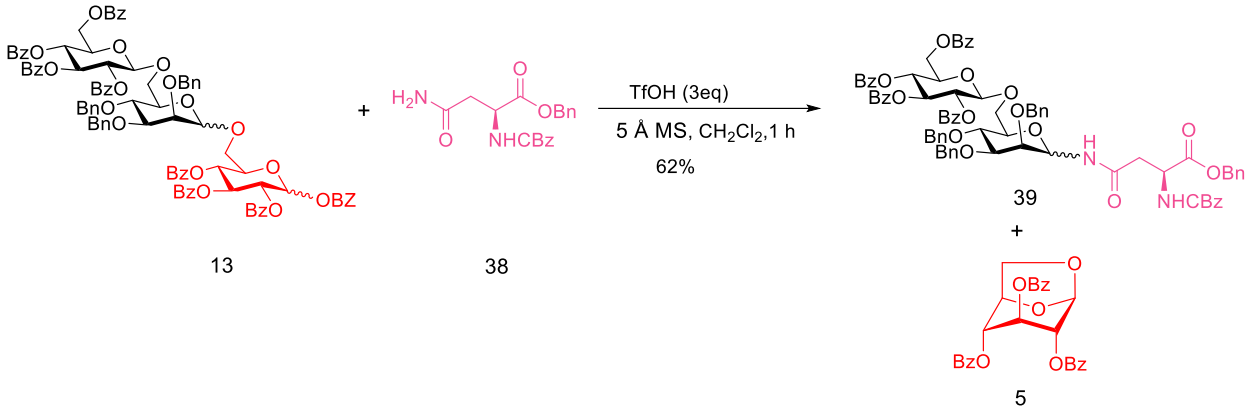
Moving next, we focused on the *N*-linked glycopeptides which is generally more challenging than the synthesis of *O*-glycosylated peptides, due to the high complexity of even "simple" *N*-glycans. In contrast to the synthesis of *O*-glycosylated peptides, where the glycosidic linkage between amino acid and oligosaccharide is often introduced in an early stage, most syntheses of *N*-glycosylated amino-acids or peptides produce the crucial glycoside-asparagine linkages at a late stage in the synthesis. Over the last few decades, convergent chemical synthesis,[74] chemoenzymatic synthesis[75] and semi-synthesis strategies[76] have attracted extensive attentions towards the preparation of structurally defined glycopeptides and glycoproteins. Other activation methods like direct *N*-glycosylation of amides have also been reported utilizing the mild activation of glycosyl trichloroacetamidate donor,[77] silver assisted gold catalyzed activation of glycosyl carbonate donor.[78] In this case, we thought to use this transglycosylation strategy for the generation of some of the important N-linked glycosides. To facilitate the amide glycosylation, we at first started to synthesize the acceptor moiety. The compound **37** was synthesized from L-asparagine in two previously reported steps.[78]



Scheme 14: Synthesis of the Cbz-Asn-OBn **37**

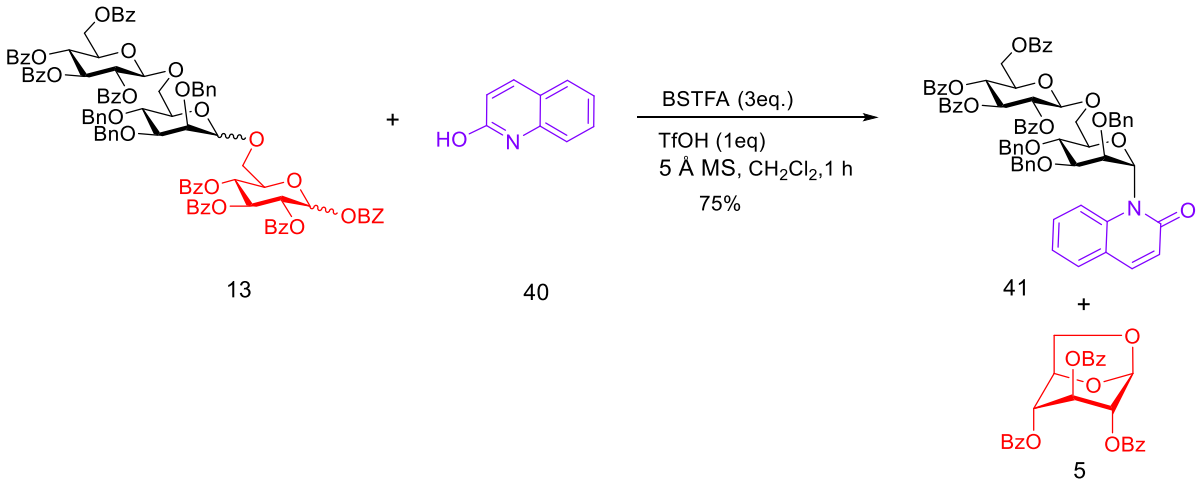
The protected asparagine Cbz-Asn-OBn was allowed to react with the trisaccharide compound **13** in presence of 3 eq. of TfOH to generate the *N*-linked asparagine glycoside in 62% yield. The compound **39** was identified by ^{1}H, ^{13}C, NMR spectra. The presence of two peaks at δ 156.2 ppm corresponding to the carbonyl carbon of the amide bond present in the acceptor unit, the resonances at 38.2 ppm also belongs to the -CH_2 group present on the aglycone unit. The formation of the

compound was also confirmed by MALDI-TOF spectrum analysis as it shows a good match of the molecular ion in the mass spectrum. (m/z calcd for $C_{80}H_{73}NaKO_{18}$: 1391.1142; found: 1391.0625).



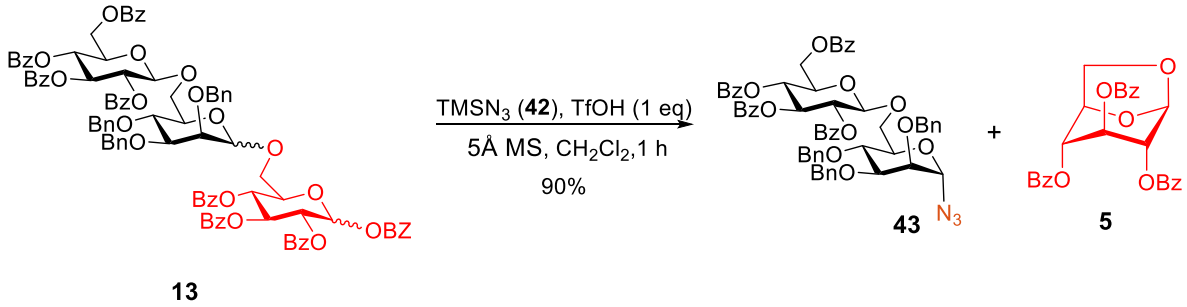
Scheme 15: Synthesis of the amide glycoside **39** by transglycosyaltion strategy

Similarly, silylation followed by TfOH mediated chemical transglycosylation of the commercially available 2-hydroxyquinoline **40** furnished in 1 h the medicinally important quinolin-2-one derivative **41** that was otherwise synthesized in a multistep endeavor involving intramolecular *N*-arylation under palladium catalysis.



Scheme 16: Synthesis of the quinolone glycoside **41** by transglycosyaltion strategy

Furthermore, the transglycosylation strategy was also proven to be useful to generate the azido glycoside in presence of azidotrimethyl silane (**42**). The compound **43** was formed smoothly within 1h from the trisaccharide compound **13** under the activation of TfOH. The structure synthesized compound **43** was confirmed through ^{1}H, ^{13}C, NMR spectra. Additionally, the presence of the azide group in the branched heptasaccharide unit was confirmed by its transmittance at 2107 cm^{-1} in the IR spectrum.



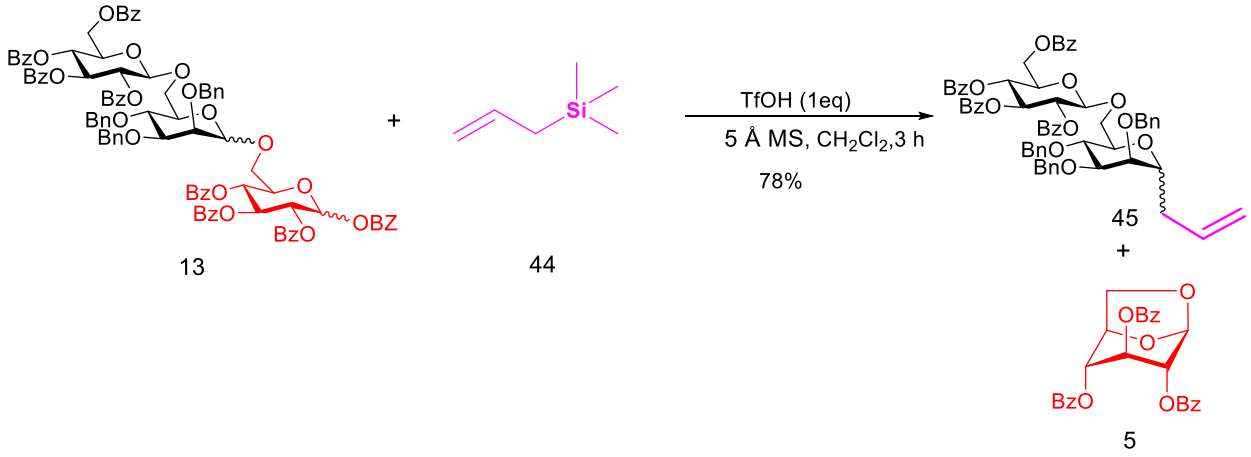
Scheme 17: Synthesis of the azido glycoside **43** by transglycosylation strategy

4.2.5 Synthesis of *C –glycosides* by transglycosylation strategy:

Moving forward, we got interested in synthesizing another important class of glycosides: *C*-glycosides. *C*-Glycosides and their derivatives constitute a unique category of carbohydrate motifs found abundantly in various natural products, potential bioactive drugs, and fine chemicals.[79-82] Their notable biological activities and resistance to metabolic degradation make them highly desirable compounds.[83-86] However, constructing *C*-glycosides is more challenging compared to

O- and *N*-glycosides. It requires substantial effort to achieve high regio- and stereoselectivity in the synthesis of complex and biologically important compounds. Literature indicates that multiple approaches have been developed to form C-C glycosidic bonds through various cross-coupling reactions, including Heck, Suzuki, Stille, and Negishi-type reactions.[87-91] Furthermore, a variety of *C*-glycoside-based compounds have been synthesized using different glycosyl halides,[92] glycosyl esters,[93] alkynyl glycosides,[94] alkenyl glycosides, and other precursors. These compounds have been synthesized through various catalytic pathways, involving glycosyl cationic and anionic intermediates, as well as radical species, regardless of the use of transition metal catalysis. Enzymatic C-glycosylation, mediated by specific *C*-glycosyltransferases (CGTs), is regarded as a promising approach for synthesizing C-glycosides.[95] Consequently, we were keen to explore the potential of the designed transglycosylation strategy for the synthesis of C-glycosides. Accordingly, allyltrimethylsilane **44** was reacted with the trisaccharide **13** in the presence of 1 equivalent of TfOH under reflux conditions. Within 4 h, the reaction was complete, yielding the desired *C*-glycoside **45** in 73% along with the formation of 1,6-anhydro sugar. The compound **45** was characterized by ^{1}H, ^{13}C, and DEPT NMR spectral analysis. The formation of the compound

was also confirmed by MALDI-TOF spectrum analysis, which showed a good match of the molecular ion in the mass spectrum (m/z calcd for $C_{64}H_{60}KO_{14}$: 1091.7834; found: 1091.7724).



Scheme 18: Synthesis of the C- glycoside **45** by transglycosylation strategy

In summary, the current transglycosylation strategy effectively demonstrates the synthesis of various classes of glycosides, including *O*-, *S*-, *N*-, and *C*-glycosides. This method involves the exchange of glycan units under acidic conditions. The simple ester functional group at the reducing end of the oligosaccharide initiates the cleavage of the interglycosidic bond, generating an oxocarbenium ion. This ion is then trapped by an external nucleophile, producing the desired glycosides and 1,6-anhydro sugar as a byproduct. This transglycosylation technique allows for the facile generation of a variety of glycoconjugates. The synthesis of different glycoconjugates using this strategy is noteworthy because it emphasizes the simple derivatization of glycan units and the use of milder acidic conditions for glycan activation. The production of *N*-, *S*-, and-glycosides using the current method is particularly noteworthy because enzymatic synthesis of these glycosides typically requires extensive molecular engineering of existing glycosidases and glycosynthases to enhance their specificity, making the process less cost-effective for large-scale applications. Efforts to extend the transglycosylation methodology to higher oligosaccharides are presently in progress.

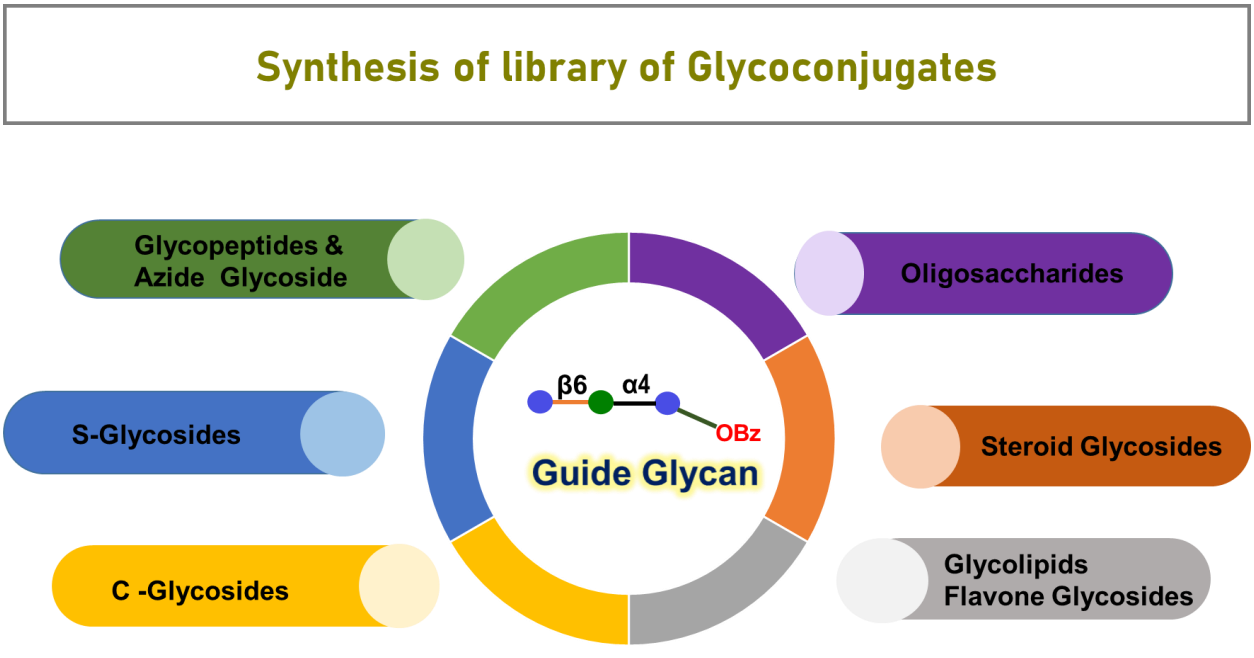


Fig 8: Synthesis of diverse class of glycoconjugates utilizing the transglycosylation strategy

4.3 Experimental Section:

Procedure A: Deprotection of Allyl Glycosides: A solution of allyl glycoside (1.0 mmol) in CH_2Cl_2/MeOH (1:4 in 10 mL) was mixed with a solution of $PdCl_2$ (0.3 mmol) in MeOH (6 mL), and the reaction was agitated at 25 °C for 5 hours. After conversion, extra Et_3N (~2 mL) was added to neutralize the reaction mixture, and the solid residue was filtered out using a Celite® pad. The volatile organics were removed in *vacuo*, and the crude residue was purified using silica gel column chromatography to get the required hemiacetals.

Procedure B: Synthesis of Ethynyl Cyclohexyl Glycosyl Carbonate Donors: A solution of hemiacetal (1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was mixed with ethynyl cyclohexyl (4-nitrophenyl) carbonate **4** (1.2 mmol) and DMAP (1.1 mmol), and the reaction underwent stirring for 5 hours at 25 °C. The reaction mixture was concentrated and purified using silica gel column chromatography (EtOAc/Hexane as the mobile phase) to produce ethynylcyclohexyl glycosyl carbonate donors.

Procedure C: Synthesis of Glycosyl BenzoateDonors: To a solution of hemiacetal (1.0 mmol) in anhydrous CH_2Cl_2 (5 mL), benzoyl chloride (1.1 mmol) and DMAP (1.1 mmol) were added and the reaction mixture was stirred at 25 °C for 5 h. the reaction mixture was concentrated and purified by silica gel column chromatography (EtOAc/Hexane as mobile phase) to obtain ethynylcyclohexyl glycosyl carbonate donors.

Procedure D: Deprotection of the TBDPS-ethers: To a solution of the silylated ether (1.0 mmol) in anhydrous pyridine and THF (4 mL, 3:1), HF•Py (3 mmol per TBDPS) was added at 0 °C and the reaction mixture was stirred at 25 °C for 3 h. After completion, the reaction mixture was quenched by the drop-wise addition of 2*N* HCl at 0 °C and diluted with 25 mL of ethyl acetate. The organic layer was successively washed with 1*N* HCl (25 mL), saturated aqueous solution of $NaHCO_3$ (25 mL) and brine solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using ethyl acetate and hexane to furnish corresponding alcohol.

Procedure E: Glycosidation using Ethynyl Cyclohexyl Glycosyl Carbonate Donors:

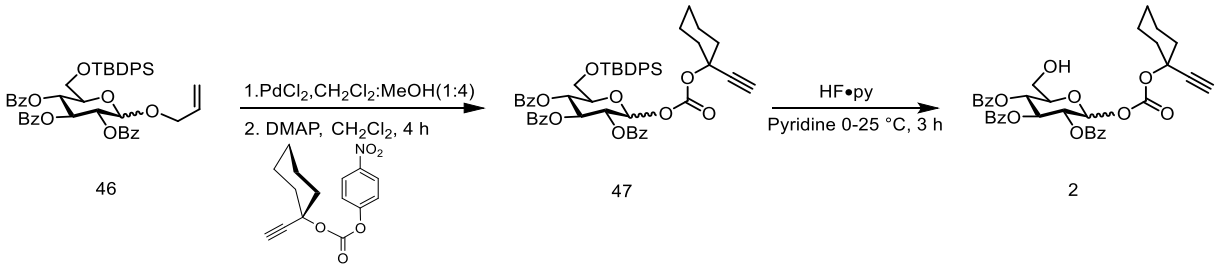
To a solution of acceptor (1.0 mmol) and donor (1.1 mmol) in anhydrous CH_2Cl_2 (5 mL) was added freshly activated 4Å MS powder (0.200 g) at 25 °C under argon atmosphere. After 15 min of vigorous stirring at 25 °C, chloro[tris(2,4-di-*tert*butylphenyl)phosphite]gold(I) (8 mol%) and

AgOTf (8 mol%) were added simultaneously to the reaction mixture and stirred for 30 min to 1 h. After consumption of the glycosyl acceptor, the reaction mixture was filtered through a bed of Celite® and the filtrate was concentrated *in vacuo*, the residue was purified by silica gel column chromatography using EtOAc and Hexane as mobile phase.

General reaction procedure for synthesis of *O*-glycosides by transglycosylation strategy:

The trisaccharide compound (1eq.) was dissolved in 3 ml of anhydrous CH_2Cl_2. To this solution external glycosyl acceptor **20a-20e** (1eq.) were added. Following this, TfOH (1eq.) was added in to the reaction mixture and kept at stirring for 1 hour. The progress of the reactions were monitored by the TLC analysis. After the starting material was consumed, the reaction mixtures were quenched by the addition of Et_3N and solvent were removed by under reduced pressure. The crude residue was purified by silica gel column chromatography (20% - 30% ethyl acetate/hexane) to afford the compounds **21a-21e**

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2,3,4-tri-*O*-benzoyl-α/β-D-glucopyranoside (**2a**):

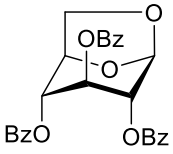


The compound **46** was converted to the compound **47** according to general experimental procedure A, B mentioned above. After that compound **47** was subjected to primary TBDPS deprotection according to general experimental procedure D to obtain the compound **2** (Yield 87 %, white solid) (α :β = 1:1.3).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 – 7.77 (m, 6H), 7.65 – 7.09 (m, 9H), 6.55 (d, *J* = 3.7 Hz, 1H), 6.28 (t, *J* = 10.0 Hz, 1H), 6.09 – 5.90 (m, 1H), 5.71 – 5.47 (m, 2H), 4.03 –3.69 (m, 2H), 2.81 (s, 1H), 2.49 (s, 1H), 2.27 – 1.94 (m, 2H), 1.93 – 1.18 (m, 8H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.1, 165.9, 165.8, 165.7, 165.3, 164.9, 151.0, 150.7, 133.8, 133.7, 133.4, 133.3, 130.0 (2C), 129.9 (2C), 129.9 (2C), 129.8 (2C), 129.7 (2C), 128.9 (4C), 128.7 (4C), 128.5 (2C), 128.4 (4C), 128.3 (4C), 128.3 (4C), 95.2, 92.7, 82.3, 82.2, 78.9, 78.8, 75.6, 75.4, 75.3, 72.8, 72.7, 70.9, 70.4, 70.0, 68.9, 68.7, 60.9, 60.7, 36.8, 36.7, 36.5, 36.4, 24.9, 24.7, 22.7, 22.5, 22.3, 22.3; **mp:** 101 °C; **Optical**

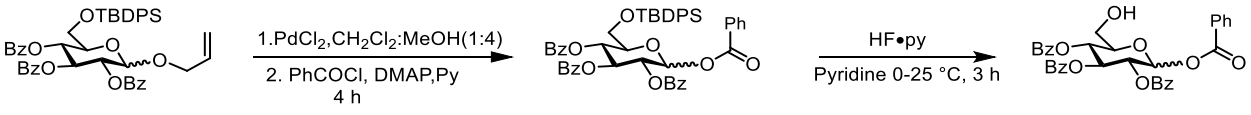
Rotation: $[\alpha]_D^{25}$ = +42.048 (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3444, 2919, 1453, 1027, 747, 696, 664; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{34}NaO_{11}$: 665.1999; found 665.1997.

1-6 anhydro 2,3,4,5-tetra-O-benzoyl-glucopyranoside: (**5**):



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.03 (m, 6H), 7.65 – 7.57 (m, 3H), 7.54 – 7.38 (m, 6H), 5.75 (d, *J* = 1.6 Hz, 1H), 5.45 (t, *J* = 1.7 Hz, 1H), 5.09 (dq, *J* = 9.7, 1.5 Hz, 2H), 4.90 (d, *J* = 5.6 Hz, 1H), 4.39 (dd, *J* = 7.8, 1.0 Hz, 1H), 3.98 (dd, *J* = 7.7, 5.7 Hz, 1H); **Optical Rotation**: $[\alpha]_D^{25}$ = + 21° (*c* 0.23, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3447, 2938, 1755, 1454, 1268, 1239, 1070, 1006, 895, 848, 749, 695; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{40}NaO_8$: 497.4548 found: 497.4547.

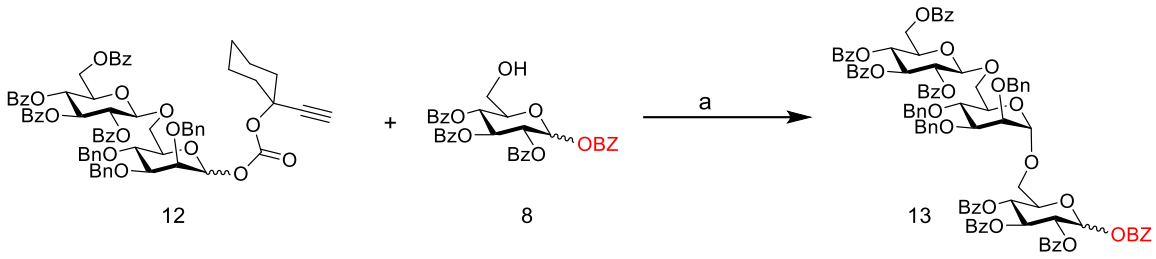
1-*O*-benzoyl-2,3,4-tri-*O*-benzoyl-α/β-D-glucopyranoside (**8**):



48 49 8

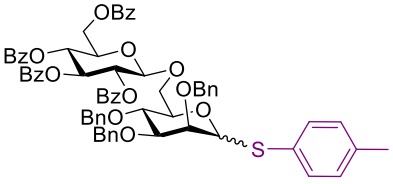
Compound **48**[54] was converted to benzoate **49** according to general experimental procedure A, C. Next, the compound **49** was subjected to the experimental procedure D to obtain the compound **8** (yield 92%, thick syrup) (α:β=6:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.14 (m, 2H), 8.03 – 7.99 (m, 2H), 7.93 – 7.87 (m, 4H), 7.58 (dt, *J* = 45.5, 7.5 Hz, 4H), 7.46 – 7.28 (m, 8H), 6.88 (d, *J* = 3.7 Hz, 1H), 6.38 (t, *J* = 10.0 Hz, 1H), 5.76 – 5.70 (m, 1H), 5.70 – 5.65 (m, 1H), 4.34 – 4.26 (m, 1H), 3.94 – 3.71 (m, 2H), 2.82 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 166.0, 165.8, 165.4, 165.4, 165.2, 164.8, 164.6, 134.0, 133.8, 133.5, 133.4, 130.1, 130.0, 129.8, 129.8, 128.8, 128.6, 128.6, 128.5, 128.4, 92.8, 90.2, 75.7, 72.9, 70.9, 70.5, 70.3, 69.2, 68.9, 61.0, 60.9 ; **Optical Rotation**: $[\alpha]_D^{25}$ = + 48° (*c* 0.23, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3447, 2938, 1755, 1454, 1268, 1239, 1070, 1006, 895, 848, 749, 695; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{36}H_{40}NaO_8$: 619.5578 found: 619.5574.

Benzoyl-2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α-D-mannopyranosyl) α/ β -D-glucopyranoside **(13)**:



The glycosyl donor **12** and acceptor **8** was coupled according to the general glycosylation procedure E mentioned above to generate the trisaccharide **13** (Yield 92 %, viscous syrup) (α:β=1.5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 – 7.73 (m, 16H), 7.55 – 7.06 (m, 39H), 6.33 – 6.13 (m, 1H), 5.88 – 5.75 (m, 2H), 5.70 – 5.54 (m, 2H), 5.46 (t, *J* = 8.7 Hz, 1H), 4.85 (dd, *J* = 15.0, 10.6 Hz, 1H), 4.75 – 4.42 (m, 8H), 4.36 – 4.13 (m, 2H), 4.07 – 3.49 (m, 6H), 3.45 – 3.24 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2 (2C), 166.0 (2C), 165.9 (2C), 165.3 (2C), 165.2 (2C), 165.0 (2C), 164.9 (2C), 164.5 (2C), 139.1, 139.1, 138.7, 138.6, 138.4, 138.3, 133.9, 133.4, 133.3, 133.2, 133.1, 130.2, 130.1, 130.1, 130.0, 129.9, 129.8, 129.8, 129.6, 129.2, 129.1, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.5, 127.4, 127.4, 101.5, 101.2, 97.2, 97.0, 92.8, 90.0, 81.6, 81.5, 80.0, 79.9, 75.5, 74.8, 74.5, 74.3, 73.3, 73.3, 73.2, 73.2, 73.0, 72.9, 72.9, 72.1, 72.0, 71.8, 71.8, 71.4, 71.0, 71.0, 70.9, 70.7, 69.8, 69.5, 69.5, 68.5, 68.2, 66.1, 66.1, 63.3, 63.2; **Optical Rotation**: $[\alpha]_D^{25}$ = + 12° (*c* 0.23, $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 3447, 2938, 1755, 1454, 1268, 1239, 1070, 1006, 895, 848, 749, 695; **HRMS** (ESI-MS) $[M+Na]^+$ m/z calculated for $C_{95}H_{82}NaO_{24}$: 1630.5127 found: 1630.5144.

p-thiotolyl-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α/β-D-mannopyranoside **(15)**:

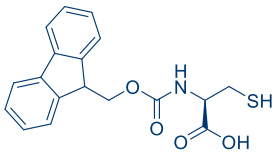


The trisaccharide compound **13** (1eq, 100mg) was dissolved in 3 ml of anhydrous CH_2Cl_2. To this solution external acceptor **14** (1eq., 8mg) was added. To this reaction mixture, freshly activated 5Å MS powder (40 mg) was added under nitrogen atmosphere

and kept for vigorous stirring for another 15 min. Following this, TfOH (1eq., 6µL) was added in to the reaction mixture and kept at stirring for 1 hour. The progress of the reactions was monitored by the TLC analysis. After the starting material was consumed, the reaction mixtures were quenched by the addition of Et_3N and solvent were removed by under reduced pressure. The crude residue was purified by silica gel column chromatography (10% - 20% ethyl acetate/hexane) to afford the compounds **15.** (Yield 89%, yellow syrup) (α:β=2.8:1); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.02 – 7.81 (m, 8H), 7.53 – 7.21 (m, 29H), 7.07 (d, *J* = 8.5 Hz, 2H), 5.85 (t, *J* = 9.6, 2.7 Hz, 1H), 5.64 (t, *J* = 11.2, 8.5 Hz, 1H), 5.58 (d, *J* = 4.1 Hz, 1H), 4.91 (dd, *J* = 11.8, 3.1 Hz, 1H), 4.86 – 4.73 (m, 1H), 4.78 – 4.28 (m, 9H), 4.15 – 4.02 (m, 2H), 3.89 (dd, *J* = 10.9, 3.0 Hz, 1H), 3.76 (dt, *J* = 5.9, 3.0 Hz, 2H), 3.50 (d, *J* = 8.9 Hz, 1H), 2.31 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3,

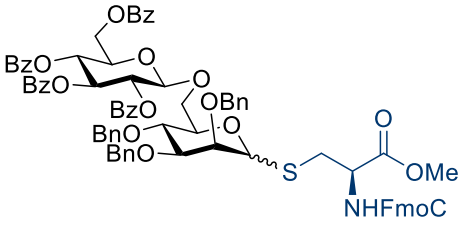
166.2, 165.9, 165.9, 165.3, 165.3, 165.1, 165.1, 138.9, 138.8, 138.4, 138.2, 138.0, 137.8, 137.6, 137.2, 133.5, 133.5, 133.2, 133.1, 132.4(2C), 130.5, 130.5, 130.2-129.9(32C), 129.7, 129.6, 129.4, 129.4, 129.2, 129.2, 128.7 -127.8(40C), 101.1, 101.1, 87.8, 87.4, 82.4, 80.7, 79.8, 77.4, 75.7, 75.5 (2C), 75.0, 74.8(2C), 73.2(2C), 73.0(2C), 72.6(2C), 72.3(2C), 72.0, 71.9(2C), 70.7(2C), 69.9, 68.1, 68.0, 63.4, 63.2, 21.3, 21.2 ; **Optical Rotation**: $[\alpha]_D^{25}$ = +8º (*c* 0.26, $CHCl_3$) ; **IR** (cm^{-1}, $CHCl_3$): 2990, 1728, 1265, 1215, 1108, 1027, 903, 742, 708, 666; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{68}H_{62}O_{14}S$ 1158.3791 found 1158.3785.

methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-cysteinate (**18**):



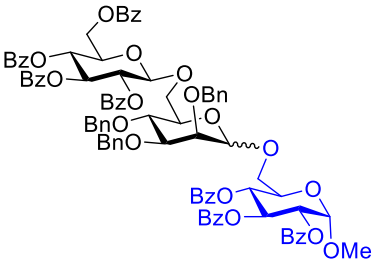
The compound 18 was synthesized from the compound 16 according to a previously reported procedure. (Yield 93%, white foam); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 2H), 5.73 (d, *J* = 7.8 Hz, 1H), 4.66 (dt, *J* = 8.3, 4.2 Hz, 1H), 4.43 (dq, *J* = 7.7, 3.8 Hz, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 3.78 (s, 3H), 3.05 – 2.93 (m, 2H), 1.37 (t, *J* = 8.9 Hz, 1H) **Optical Rotation**: $[\alpha]_D^{25}$ = +12º (*c* 0.26, $CHCl_3$) ; **IR** (cm^{-1}, $CHCl_3$): 2902, 1728, 1265, 1215, 1108, 1027, 903, 742, 708, 666; **HRMS** $[M+Na]^+$ m/z calculated for $C_{18}H_{17}NO_4SNa$ 366.6488 found 366.6247

methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-cysteinate-2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-1-thio-α/β-D-mannopyranoside (**19**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **18** according the previous transglycosylation strategy mentioned above. (Yield 80%, syrup) (α:β=1.8:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.04 – 7.85 (m, 8H), 7.83 – 7.73 (m, 4H), 7.61 (dd, *J* = 19.7, 7.4 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.34 – 7.18 (m, 27H), 6.00 – 5.81 (m, 2H), 5.59 (dt, *J* = 17.9, 9.2 Hz, 2H), 5.25 (d, *J* = 3.7 Hz, 1H), 4.86 – 4.60 (m, 6H), 4.51 – 4.27 (m, 6H), 4.25 – 3.99 (m, 5H), 3.82 (s, 1H), 3.70 (s, 3H), 3.54 – 3.33 (m, 2H), 3.12 – 2.99 (m, 1H), 2.88 (dd, *J* = 14.4, 4.6 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.1(2C), 166.1 (2C), 165.9(2C), 165.2(2C), 165.0(2C), 155.8 (2C), 144.0(2C), 143.8(2C), 141.3(2C), 138.7(2C), 138.4, 138.2, 137.8, 137.7, 133.5-133.1 (20C), 129.8-127.5(60C), 127.2-125.2 (16C), 120.0(2C), 101.5, 101.2, 84.7, 84.6, 82.1(2C), 82.0(2C), 79.4 (2C), 75.6, 75.6, 74.9, 74.8(2C), 72.9(2C), 72.0, 70.5(2C), 69.7(2C), 68.7, 68.2, 67.3(2C), 67.2(2C), 63.3(2C), 54.4, 54.1, 52.8, 52.7, 47.2, 47.1, 32.8(2C) ; **Optical Rotation**: $[\alpha]_D^{25}$ = +8º (*c* 0.26, $CHCl_3$) ; **IR** (cm^{-1}, $CHCl_3$): 2990, 1728, 1265, 1215, 1108, 1027, 903, 742, 708, 666; **HRMS** (MALDI-TOF) $[M+Na]^+$ m/z calculated for $C_{80}H_{73}NO_{18}SNa$ 1390.8446 found 1390.9060.

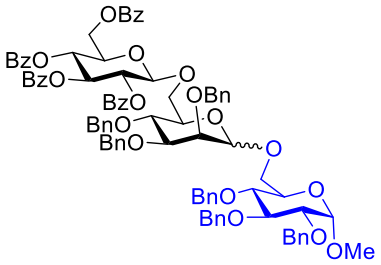
Methyl-2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α/ β -D-mannopyranosyl) α-D-glucopyranoside (**21a**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor 20a according the general transglycosylation strategy mentioned above. (Yield 88%, syrup) (α:β=1.8:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 – 7.82 (m, 14H), 7.50 – 7.18 (m, 36H), 6.04 (t, *J* = 9.8 Hz, 1H), 5.84 (t, *J* = 9.6 Hz, 1H), 5.74 – 5.46 (m, 3H), 5.41 – 5.28 (m, 1H), 5.22 – 5.17 (m, 1H), 5.13 (d, *J* = 4.2 Hz, 1H), 5.01 – 4.86 (m, 1H), 4.78 (d, *J* = 4.6 Hz, 1H), 4.70 – 4.41 (m, 7H), 4.35 (t, *J* = 11.3 Hz, 1H), 4.14 (d, *J* = 10.7 Hz, 1H), 4.02 – 3.97 (m, 2H), 3.94 – 3.78 (m, 2H), 3.68 – 3.58 (m, 3H), 3.45 (d, *J* = 42.8 Hz, 3H), 3.17 (dd, *J* = 11.5, 3.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 166.1, 166.1, 166.1, 165.9, 165.8, 165.7, 165.4, 165.2, 165.2, 165.1, 165.1, 164.9, 164.9, 138.7, 138.6, 138.5, 138.1, 138.0, 138.0,

133.5-130.0 (20C), 129.9- 129.2 (40C), 129.1- 128.3 (40C), 128.2- 127.5 (14C), 102.1, 101.4, 101.4, 98.1, 97.0, 97.0, 82.3, 79.9, 75.1, 74.8, 74.7, 74.7, 74.6, 74.2, 73.4, 73.0, 72.9, 72.6, 72.3, 72.3, 72.0, 72.0, 72.0, 71.4, 71.3, 71.3, 71.2, 70.7, 70.6, 70.5, 69.9, 69.6, 69.6, 69.5, 69.2, 69.0, 68.8, 68.1, 68.0, 65.4, 63.3, 63.3, 55.7, 55.7; **mp**: 104 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = +45° (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF): $[M+Na]^+$ m/z calculated for $C_{89}H_{80}O_{23}Na$: 1539.5838 found 1517.5837

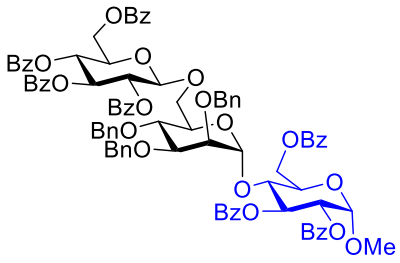
Methyl-2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α/ β -D-mannopyranosyl) α-D-glucopyranoside (**21b**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **20b** according the general transglycosylation strategy mentioned above. (Yield 84%, syrup) (α:β=1.8:1)**; ^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.01 – 7.79 (m, 8H), 7.46 – 7.21 (m, 42H), 5.91 – 5.78 (m, 1H), 5.71 – 5.62 (m, 1H), 5.60 – 5.46 (m, 1H), 5.09 – 5.02 (m, 1H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.88 – 4.42 (m, 17H), 4.34 (d, *J* = 11.2 Hz, 1H), 4.18 – 3.89 (m, 4H), 3.76 – 3.62 (m, 4H), 3.52 – 3.41 (m, 2H), 3.31 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 166.2, 165.9, 165.8, 165.2, 165.2, 165.1, 164.9, 139.0, 138.8, 138.6, 138.5, 138.5, 138.5, 138.4, 138.3, 138.3, 138.2, 138.1, 138.0, 133.5-130.0 (10C), 129.9- 129.2 (40C), 129.1- 128.3 (40C), 128.2- 127.5 (18C) , 101.6, 101.5, 100.9, 98.2, 97.9, 97.9, 82.4, 82.3, 82.2, 80.0, 79.7, 79.5, 76.3(2C), 75.8, 75.6, 75.1, 74.8(2C), 74.8, 74.5, 74.4, 73.6, 73.3, 73.3, 73.2, 73.0 (2C), 72.9 (2C), 72.4(2C), 72.1, 72.1, 72.0, 71.8, 71.7, 71.3, 69.9, 69.8, 69.6, 69.5, 69.2, 69.0, 67.4, 65.6, 63.2, 63.2, 55.4, 55.2; **Optical Rotation**: $[\alpha]_D^{25}$ = +15° (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF): $[M+Na]^+$ m/z calculated for $C_{89}H_{86}O_{20}Na$: 1497.6368 found 1497.6360

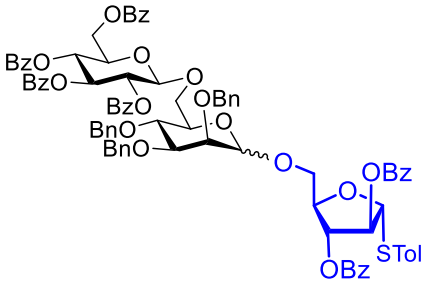
Methyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α-D-mannopyranosyl) α-D-glucopyranoside **(21c)**

This compound was synthesized from the trisaccharide 13 and glycosyl acceptor 20c according the general transglycosylation strategy mentioned above. (Yield 86%, white solid)



^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, *J* = 7.8 Hz, 2H), 8.01 – 7.71 (m, 12H) ,7.52 – 7.14 (m, 36H), 5.99 (t, *J* = 9.5 Hz, 1H), 5.88 (t, *J* = 9.6 Hz, 1H), 5.63 (q, *J* = 9.8 Hz, 2H), 5.12 (d, *J* = 3.4 Hz, 1H), 5.09 (d, *J* = 3.2 Hz, 1H), 5.07 (d, *J* = 9.6 Hz, 1H), 4.80 (d, *J* = 7.7 Hz, 1H), 4.70 (d, *J* = 11.1 Hz, 1H), 4.59 – 4.34 (m, 6H), 4.23 (dd, *J* = 16.3, 10.8 Hz, 2H), 4.12 – 3.97 (m, 3H), 3.97 – 3.85 (m, 3H), 3.75 (q, *J* = 10.0, 9.5 Hz, 3H), 3.56 (s, 1H), 3.40 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 166.3(2C), 166.0(2C), 165.6, 165.3, 138.7, 138.5, 138.3, 133.7(2C), 133.4(4C), 133.3(2C), 133.2(2C), 133.1, 133.1, 130.0, 130.0, 129.9, 129.9, 129.9, 129.7, 129.6, 129.3, 129.2, 129.0, 128.8(3C), 128.5, 128.4,(4C) 128.4(6C), 128.3(4C), 128.1(4C), 127.8(4C), 127.6, 127.4(4C), 127.2(4C), 101.8, 100.1, 96.7, 79.6, 76.1, 75.9, 74.7, 74.1, 73.2, 73.1, 72.6, 72.4, 72.2, 72.1, 71.9, 71.7, 70.0, 69.3, 68.5, 63.9, 63.5, 55.5; **mp**: 104 °C; **Optical Rotation**: $[\alpha]_D^{25}$ = +45° (*c* 0.16 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (ESI-MS): $[M+H]^+$ m/z calculated for $C_{89}H_{81}O_{23}$: 1517.5169 found 1517.5168

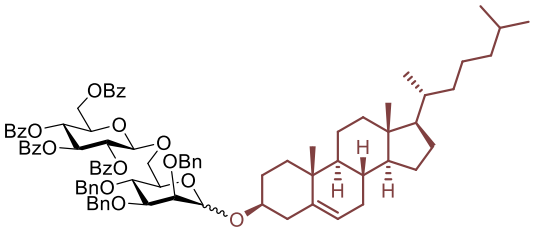
p-tolyl-2,3-di-*O*-benzoyl-5-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) α/ β -D-mannopyranosyl) 1-thio-α-D-arabinofuranoside**(21e)**



This compound was synthesized from the trisaccharide 13 and glycosyl acceptor 20e according the general transglycosylation strategy mentioned above. (Yield 90%, syrup) (α:β=1.5:1)**; ^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.15 – 7.79 (m, 12H), 7.50 – 7.16 (m, 37H), 5.88 (t, *J* = 9.6 Hz, 1H), 5.76 (d, *J* = 1.9 Hz, 1H), 5.68 (dt, *J* = 9.0, 2.6 Hz, 3H), 5.61 – 5.55 (m, 1H), 5.50 (d, *J* = 5.0 Hz, 1H), 4.91 – 4.81 (m, 1H), 4.79 – 4.39 (m, 10H), 4.26 (d, *J* = 11.4 Hz, 1H), 4.16 (dd, *J* = 11.2, 1.9 Hz, 1H), 4.10 – 4.02 (m, 1H), 3.96 (dd, *J* = 11.0, 6.1 Hz, 1H), 3.88 (t, *J* = 9.2 Hz, 1H), 3.74 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.46 – 3.38 (m, 2H), 2.20 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 166.2, 166.0, 165.9, 165.9, 165.6, 165.5, 165.5, 165.3, 165.3, 165.2, 165.0, 139.0, 138.6, 138.5, 138.4, 138.3, 138.1, 138.0, 137.8, 133.7, 133.5-130.0 (20C), 129.9- 129.2 (40C), 129.1-

128.3 (40C), 128.2- 127.5 (12C) 103.5, 101.4 (2C), 97.2, 91.7, 91.4, 84.4, 82.5, 82.3, 82.2, 82.0, 81.8, 81.3, 79.9, 78.6, 78.1, 77.7, 77.4, 75.8, 75.6, 75.4, 75.1, 74.8, 74.7, 73.0, 72.9, 72.8, 72.3, 72.2, 71.9, 69.9, 69.7, 69.7, 69.4, 68.4, 68.3, 67.5, 67.5, 63.4, 63.3, 21.2, 21.1 ; **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF):$[M+Na]^+$ m/z calculated for $C_{87}H_{78}O_{20}SNa$: 1497.4705 found 1497.4470

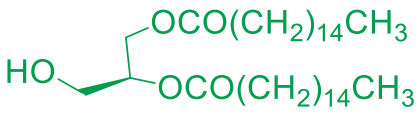
Cholesteryl-2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-1-thio-α/β-D-mannopyranoside (**23**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **22** according the general transglycosylation strategy mentioned above. (Yield 84%, syrup) (α:β=2.2:1)**; ^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.14 – 7.79 (m, 8H), 7.56 – 7.17 (m, 27H), 5.93 – 5.78 (m, 1H), 5.71 – 5.58 (m, 2H), 5.33 (dd, *J* = 24.9, 5.2 Hz, 1H), 4.98 – 4.77 (m, 2H), 4.69 – 4.33 (m, 7H), 4.28 – 4.04 (m, 2H), 3.94 – 3.78 (m, 3H), 3.70 – 3.61 (m, 1H), 3.52 – 3.27 (m, 2H), 2.34 – 2.14 (m, 2H), 2.08 – 1.94 (m, 3H), 1.87 – 1.39 (m, 14H), 1.17 – 0.99 (m, 10H), 0.95 – 0.84 (m, 12H), 0.68 (d, *J* = 10.9 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.9, 165.6, 165.2, 165.1, 164.9, 140.7, 140.4, 138.7, 138.7, 138.6,

138.6, 138.1, 138.1, 122.0, 121.8, 101.6, 101.0, 100.2, 95.5, 82.4, 80.3, 79.7, 76.3, 75.1, 74.9, 74.9, 74.7, 74.1, 73.9, 73.2, 73.1, 72.4, 72.3, 72.2, 72.0, 71.9, 71.4, 71.0, 69.9, 69.8, 69.2, 68.7, 63.3, 63.2, , 56.9, 56.9, 56.3, 50.3, 50.2, 42.5, 40.0, 39.9, 39.6, 39.2, 37.4, 37.2, 36.9, 36.9, 36.3, 35.9, 32.1, 32.1, 32.0, 30.1, 28.4, 28.1, 27.6, 24.4, 23.9, 23.0, 22.7, 21.2, 19.5, 19.5, 18.9, 12.0; $[\alpha]^{25}_D$ ($CHCl_3$, *c* 1.0): +25.8º; IR (cm^{-1}, $CHCl_3$): 2938, 2860, 1456, 1376, 1074, 737; **HRMS** (MALDI-TOF) : m/z calcd for $C_{88}H_{100}O_{15}Na$ $[M+Na]^+$: 1420.6993, Found: 1420.6925.

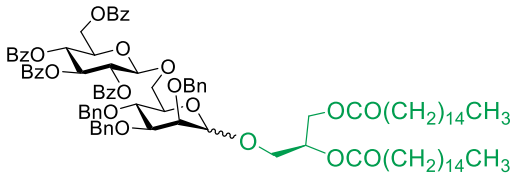
1,2-Di-hexadecanoyl-(S)-glycerol: (**31**):



The compound 31 was generated from the (S)-isopropyleneglycerol according to a reported procedure. (Yield 85%, white solid)**; ^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.12 – 5.05 (m, 1H), 4.33 (dd, *J* = 11.9, 4.4 Hz, 1H), 4.22 (dd, *J* = 11.9, 5.8 Hz, 1H), 3.76 – 3.69 (m,

2H), 2.41 – 2.28 (m, 5H), 1.67 – 1.57 (m, 4H), 1.32 – 1.24 (m, 48H), 0.88 (t, *J* = 6.7 Hz, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 14.35, 23.01 , 25.27 , 29.44 , 29.52 , 29.69 , 29.75 , 32.13 , 34.33 , 34.52 , 65.26 , 67.87 , 72.42 , 174.16 , 174.21 ; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{35}H_{68}O_5Na$: 591.4964 found 591.4967

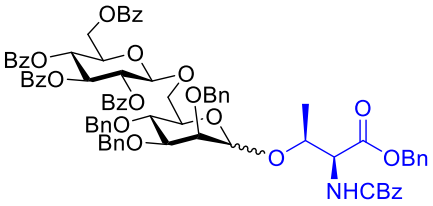
(S)-3-hydroxypropane-1,2-diyl dipalmitate-2,3,4-tri-*O*-benzyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-α/β-D-mannopyranoside (**32**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **31** according the general transglycosylation strategy mentioned above. (Yield 86%, syrup) (α:β=1.2:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 – 7.78 (m, 8H), 7.52 – 7.17 (m, 27H), 5.89 (dd, *J* = 11.4, 6.8 Hz, 1H), 5.73 – 5.53 (m, 2H), 4.92 – 4.81 (m, 1H), 4.77 (d, *J* = 7.7 Hz, 1H), 4.73 – 4.49 (m, 6H), 4.46 – 4.23 (m, 3H), 4.20 – 4.03 (m, 3H), 3.85 (t, *J* = 9.2 Hz, 1H), 3.70 (dddd, *J* = 32.0, 13.0, 7.2, 4.1 Hz, 2H), 3.56 – 3.28 (m, 3H), 2.35 – 2.23 (m, 4H), 1.60 (dq, *J* = 12.3, 5.9 Hz, 6H), 1.26 (d, *J* = 2.9 Hz, 48H), 0.89 (d, *J* = 6.2 Hz, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 173.4, 173.3, 173.0, 172.9, 166.1, 165.9, 165.8, 165.2, 165.0, 164.9, 164.9 (2C), 138.9, 138.5, 138.4, 138.3, 138.3, 138.3, 138.0, 133.4, 133.3, 133.1, 130.1, 129.9, 129.8, 129.8, 129.8, 129.6, 129.2, 128.8, 128.8, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 101.3, 101.3, 97.7, 97.1, 84.4, 81.8, 81.6, 80.0, 79.9, 75.4, 74.7, 73.1, 73.0, 72.9, 72.3, 71.9, 69.8, 69.7, 68.1, 66.4, 65.8, 63.3, 62.5, 62.5, 62.1, 34.3, 34.2, 34.1, 34.1, 32.0, 31.5, 31.4, 30.6, 29.8, 29.7, 29.7,

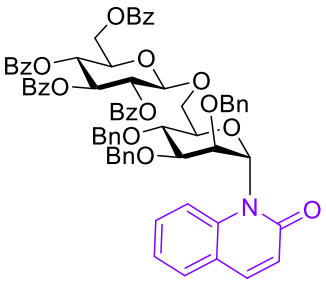
29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 29.2, 29.2, 27.3, 25.0, 24.9, 24.7, 22.7, 14.2; **Optical Rotation**: $[\alpha]_D^{25}$ = +20° (*c* 0.1 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF): $[M+Na]^+$ m/z calculated for $C_{96}H_{122}O_{19}Na$: 1601.8478 found 1601.8422

Benzyl N-((Benzyloxy)carbonyl)- L-allothreoninate-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl- β -D-glucopyranosyl)-α/β-D-mannopyranoside **(34):**



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **33** according the general transglycosylation strategy mentioned above. (Yield 87%, syrup) (α:β=3.3:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 – 7.81 (m, 8H), 7.51 – 7.22 (m, 37H), 5.91 (t, *J* = 17.1, 8.7 Hz, 1H), 5.78 – 5.55 (m, 2H), 5.29 – 5.17 (m, 1H), 5.13 (d, *J* = 12.7 Hz, 2H), 5.02 (dd, *J* = 12.3, 3.8 Hz, 1H), 4.91 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.69 – 4.41 (m, 8H), 4.28 – 4.10 (m, 3H), 3.97 – 3.85 (m, 1H), 3.82 – 3.59 (m, 4H), 3.50 – 3.37 (m, 1H), 1.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.5, 166.2, 165.9, 165.9, 165.2, 165.0, 156.8, 156.6, 138.6, 138.3, 138.2, 138.1, 138.0, 136.1, 135.1, 133.5, 133.4, 133.3, 133.2, 129.9, 129.8, 129.8, 129.6, 128.9, 128.9, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 101.6, 101.1, 99.4, 96.9, 79.4, 76.2, 76.1, 75.1, 75.0, 74.9, 74.8, 74.4, 73.1, 72.4, 72.2, 71.9, 71.7, 71.6, 71.6, 69.9, 69.6, 67.4, 67.2, 63.3, 58.7 , 18.7, 18.6 ; **Optical Rotation**: $[\alpha]_D^{25}$ = +54° (*c* 0.1 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (MALDI-TOF): $[M+Na]^+$ m/z calculated for $C_{80}H_{73}O_{18}K$: 1376.7534 found 1376.7531

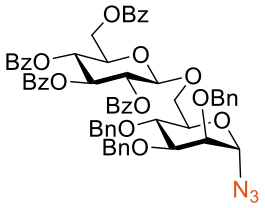
1-(2-*O*-2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-mannopyranosyl-) quinolin-2-one (**41**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **40** according the general transglycosylation strategy mentioned above. (Yield 75%, syrup); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.3 Hz, 3H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.83 – 7.72 (m, 6H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.48 (dd, *J* = 9.3, 6.1 Hz, 4H), 7.34 – 7.08 (m, 25H), 6.75 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 2.0 Hz, 1H), 5.75 – 5.45 (m, 3H), 4.93 (d, *J* = 7.7 Hz, 1H), 4.87 – 4.59 (m, 4H), 4.54 (dd, *J* = 8.2, 3.3 Hz, 1H), 4.49 – 4.47 (m, 1H), 4.46 – 4.40 (m, 2H), 4.18 (d, *J* = 10.8 Hz, 1H), 4.09 – 4.00 (m, 2H), 3.97 – 3.85 (m, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2, 165.8, 165.2, 165.0, 159.7, 146.2, 139.5, 138.4, 138.3, 138.3, 133.4, 133.1, 133.0, 132.8, 129.9, 129.8, 129.8, 129.8, 129.7, 129.6, 129.4, 128.9, 128.6, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 125.5, 124.6, 112.6, 101.2, 93.4, 79.6, 74.9, 74.5, 73.7, 73.4, 73.1, 73.0,

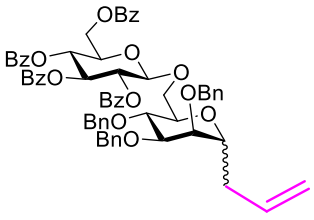
72.4, 72.1, 71.9, 69.8, 68.8, 63.3 ; **Optical Rotation**: $[\alpha]_D^{25}$ = +11° (*c* 0.1 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2946, 1722, 1452, 1264, 1092, 1068, 1026, 749, 707; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{70}H_{61}NO_{15}Na$: 1178.7865 found 1178.7856

1-azido-2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α/β-D-mannopyranoside (**43**):



This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **42** according the general transglycosylation strategy mentioned above.**^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.06 – 7.80 (m, 8H), 7.50 – 7.03 (m, 27H), 5.90 (t, *J* = 9.6, 2.4 Hz, 1H), 5.68 (t, *J* = 8.4 Hz, 1H), 5.65 – 5.56 (m, 1H), 5.17 (d, *J* = 3.4 Hz, 1H), 4.84 (d, *J* = 10.6 Hz, 1H), 4.80 (d, *J* = 1.9 Hz, 1H), 4.76 – 4.40 (m, 6H), 4.26 (dd, *J* = 11.2, 2.3 Hz, 1H), 4.22 – 4.06 (m, 2H), 3.90 – 3.73 (m, 3H), 3.58 – 3.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 165.9, 165.2, 165.0, 138.6, 138.1, 137.7, 133.5, 133.3, 133.3, 133.3, 133.2, 129.9, 129.8, 129.8, 129.8, 129.6, 129.1, 128.8, 128.8, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.2, 128.2, 128.2, 127.9, 127.8, 127.7, 127.7, 127.6, 127.4, 101.2, 88.0, 81.6, 79.5, 76.6, 75.6, 74.8, 73.8, 72.9, 72.4, 71.9, 71.8, 69.8, 67.8, 63.3 ; **Optical Rotation**: $[\alpha]_D^{25}$ = +79° (*c* 0.1 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2916, 2881, 2114, 1460, 1385, 1085, 734; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{61}H_{55}N_3O_{14}Na$: 1076.3582 found 1076.3544

3-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α/β-D-mannopyranosyl)-1-propene (**45**):

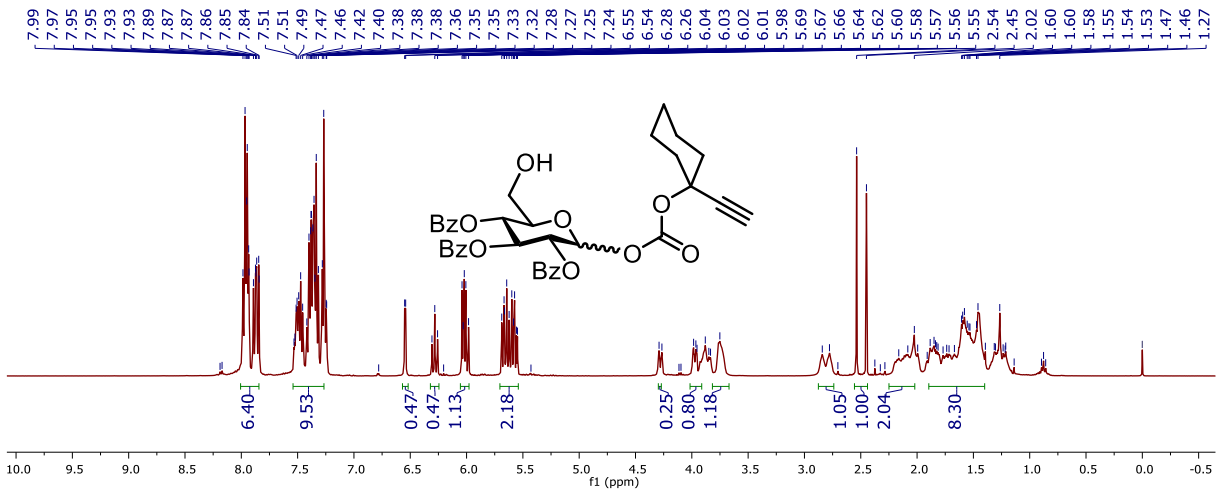


This compound was synthesized from the trisaccharide **13** and glycosyl acceptor **42** according the general transglycosylation strategy mentioned above.**^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.04 – 7.79 (m, 8H), 7.51 – 7.10 (m, 27H), 5.94 – 5.85 (m, 1H), 5.74 – 5.65 (m, 1H), 5.59 (ddd, *J* = 15.1, 9.8, 7.8 Hz, 1H), 5.09 – 4.95 (m, 1H), 4.88 – 4.81 (m, 2H), 4.70 – 4.44 (m, 6H), 4.30 (d, *J* = 11.0 Hz, 1H), 4.17 – 4.01 (m, 4H), 3.93 (dd, *J* = 10.9, 6.2 Hz, 1H), 3.80 – 3.55 (m, 3H), 3.41 (t, *J* = 9.2 Hz, 1H), 2.41 (dd, *J* = 10.7, 6.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 166.2, 165.9, 165.9, 165.2, 165.2, 165.1, 165.0, 138.8, 138.7, 138.3, 138.3, 138.2, 137.8, 137.2, 134.7, 133.4, 133.3, 133.2, 133.1, 129.9, 129.9, 129.8, 129.8, 129.7, 129.6,

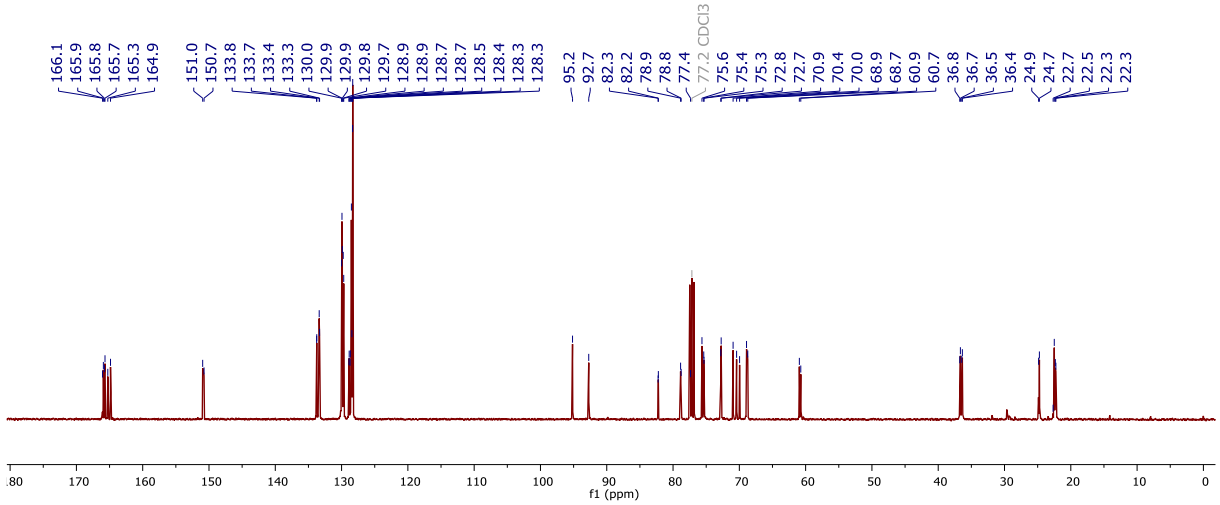
129.4, 129.3, 128.9, 128.8, 128.4, 128.4, 128.4, 128.3, 128.3, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.5, 127.3, 116.8, 101.8, 101.2, 82.1, 79.9, 77.7, 75.7, 75.2, 74.9, 74.8, 73.7, 73.6, 73.1, 73.0, 72.9, 72.0, 71.9, 70.9, 70.8, 68.6, 68.2, 63.4, 63.2, 29.8, 29.7; **Optical Rotation**: $[\alpha]_D^{25} = +5°$ (*c* 0.1 $CHCl_3$); **IR** (cm^{-1}, $CHCl_3$): 2916, 2881, 2114, 1460, 1385, 1085, 734; **HRMS** (ESI-MS): $[M+Na]^+$ m/z calculated for $C_{64}H_{60}O_{14}K$: 1091.7630 found 1091.7734

4.4- Spectral Charts of Representative Compounds

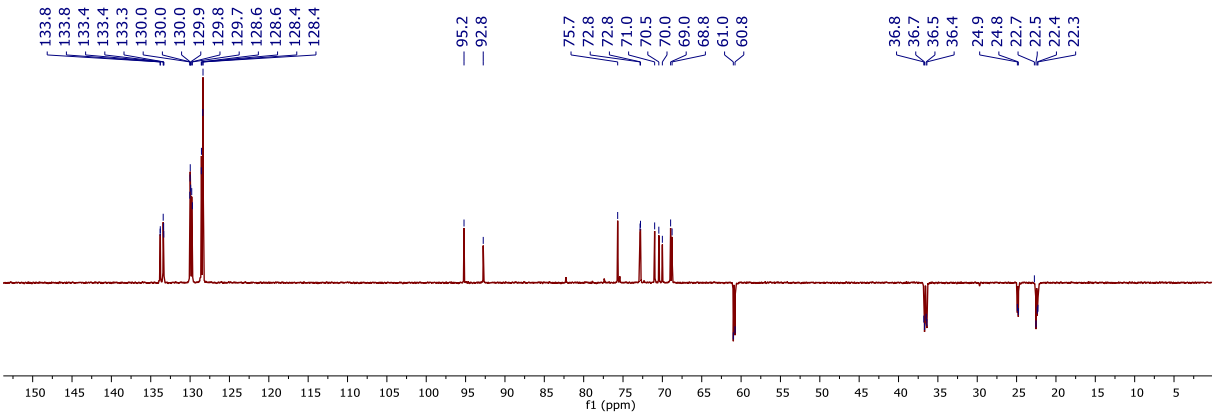
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **2**



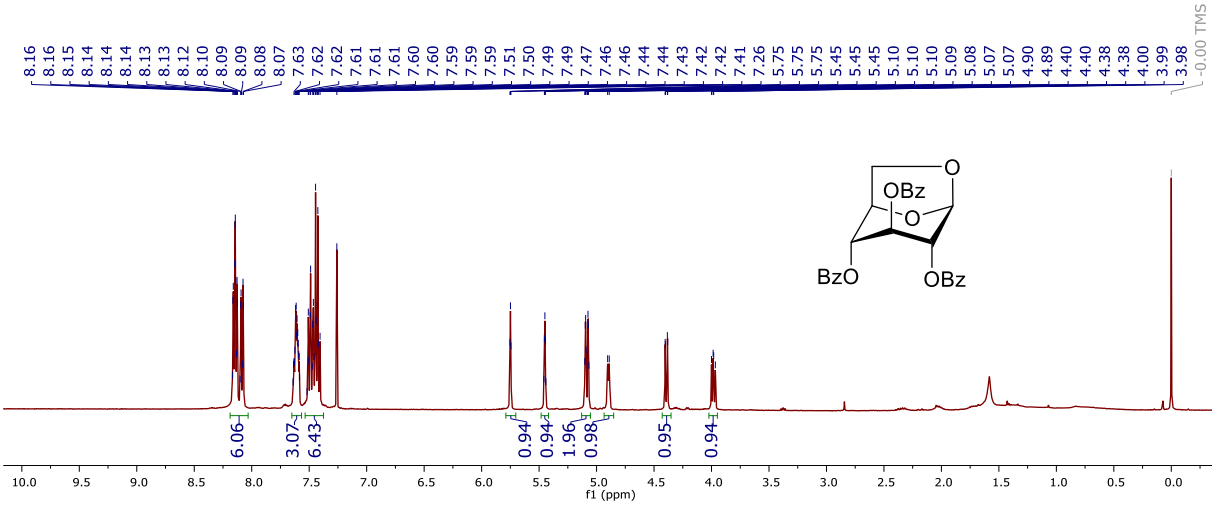
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **2**



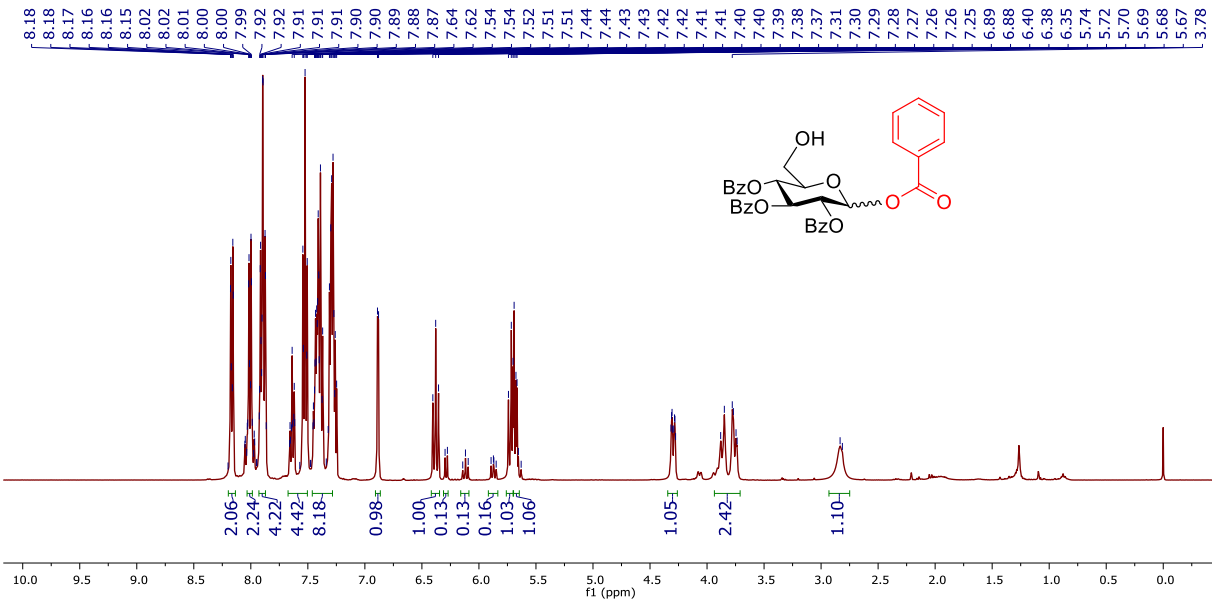
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **2**



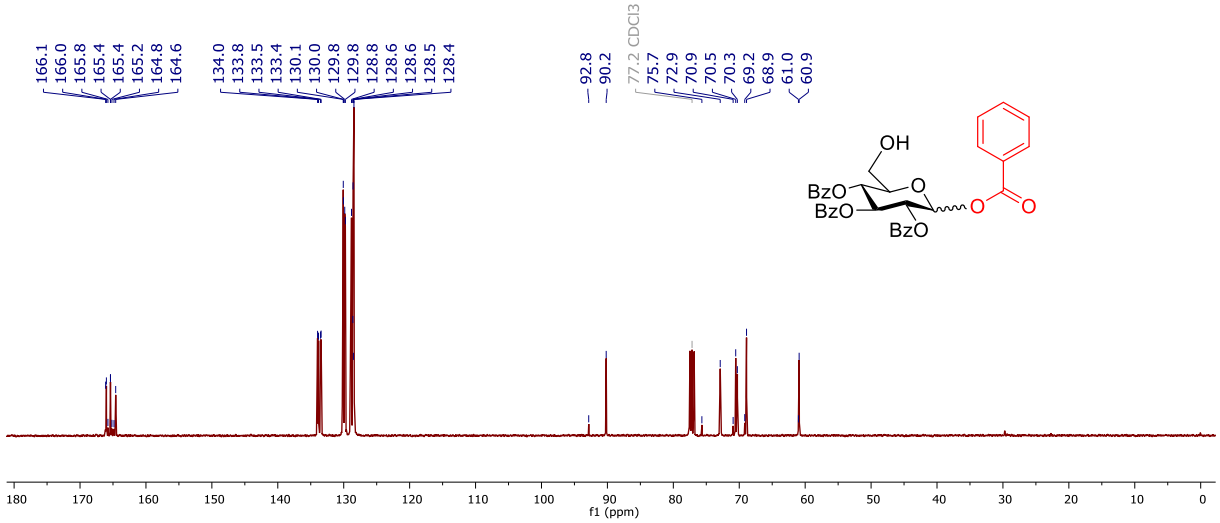
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **5**



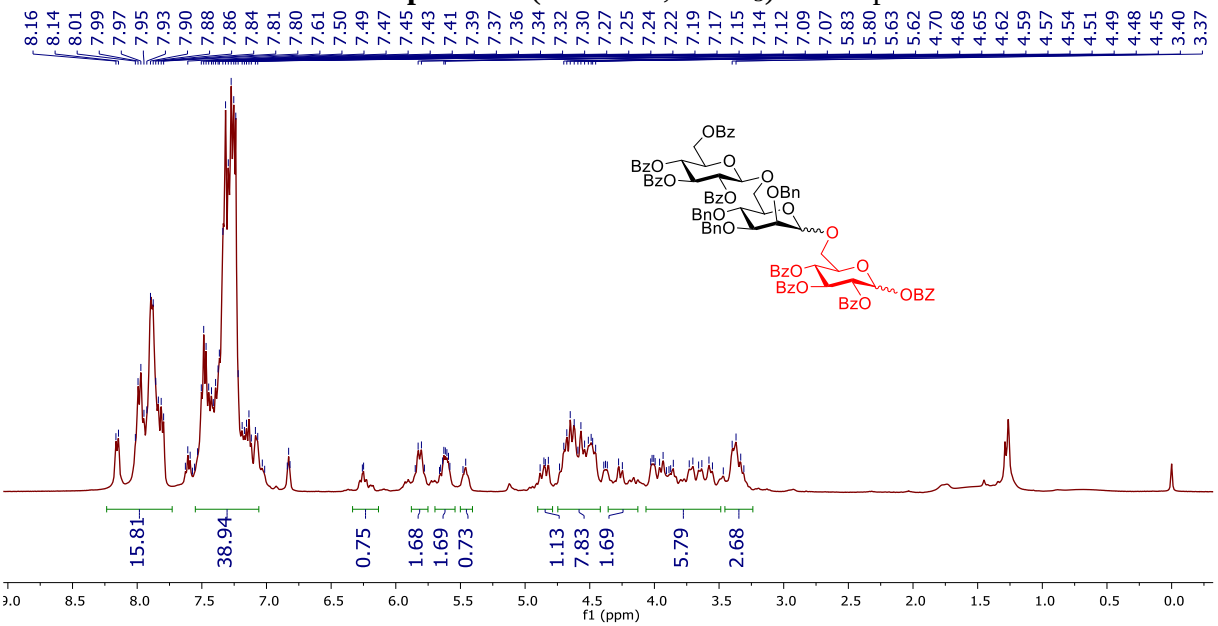
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **8**



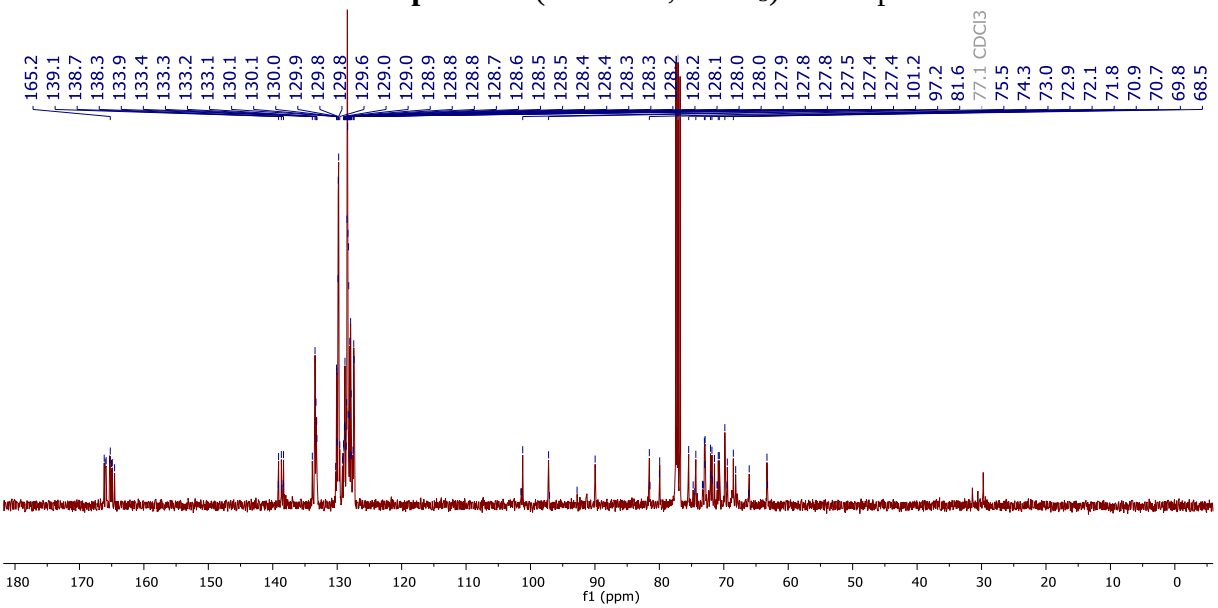
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **8**



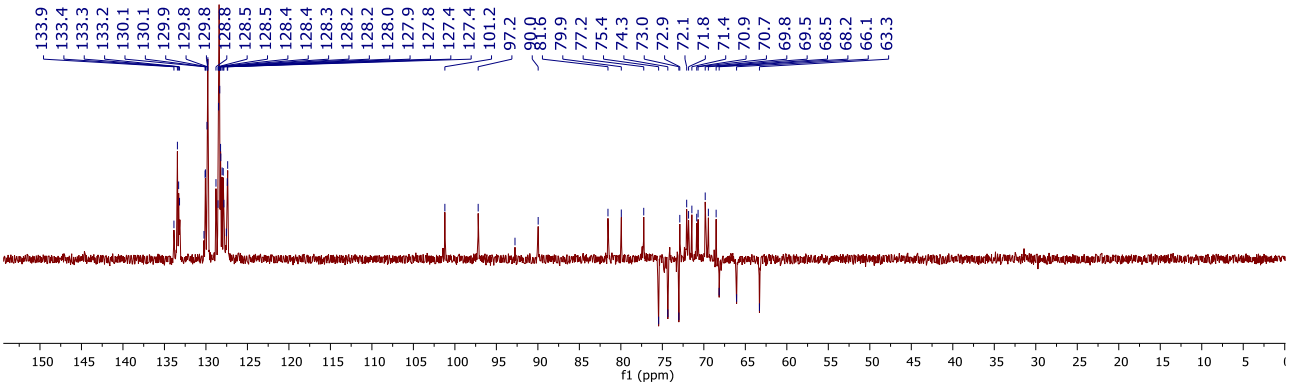
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **13**



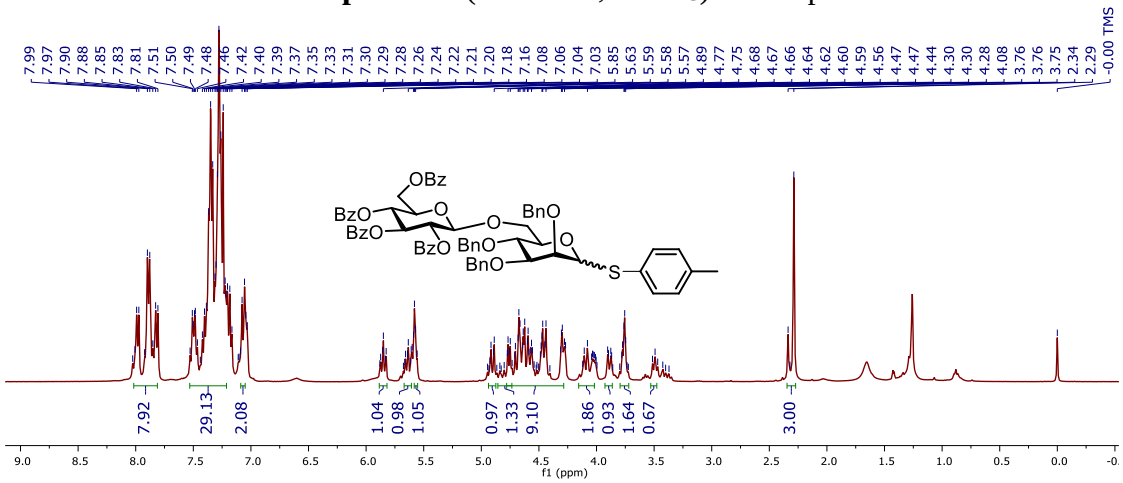
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **13**



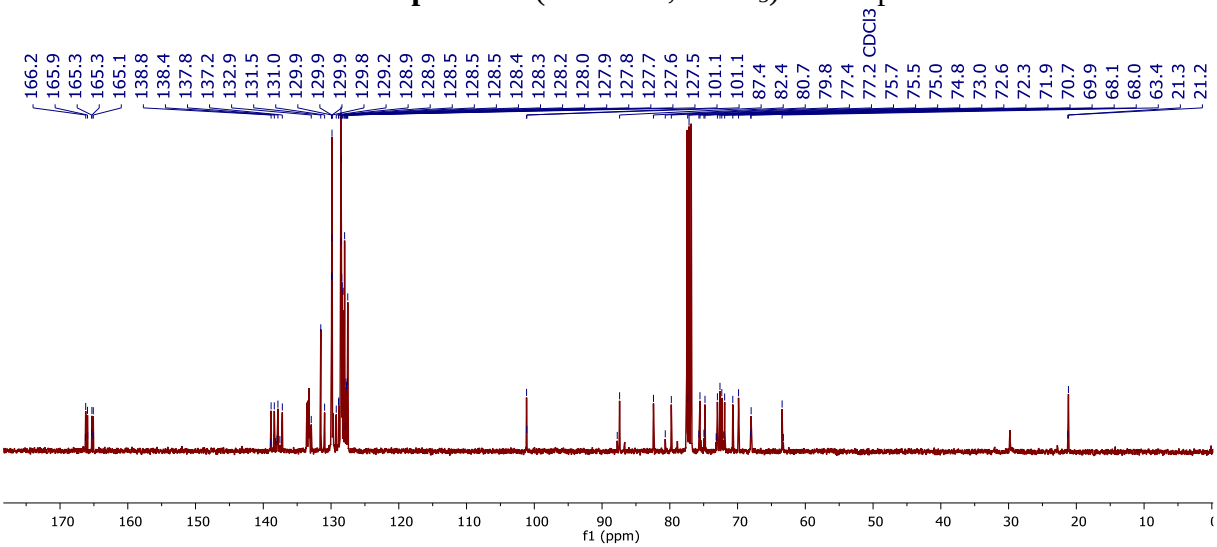
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **13**



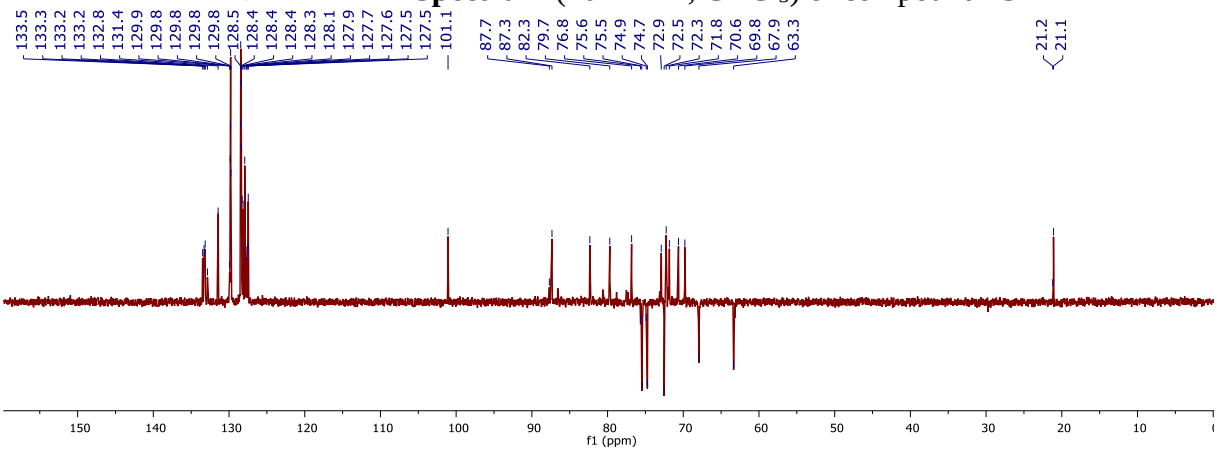
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **15**



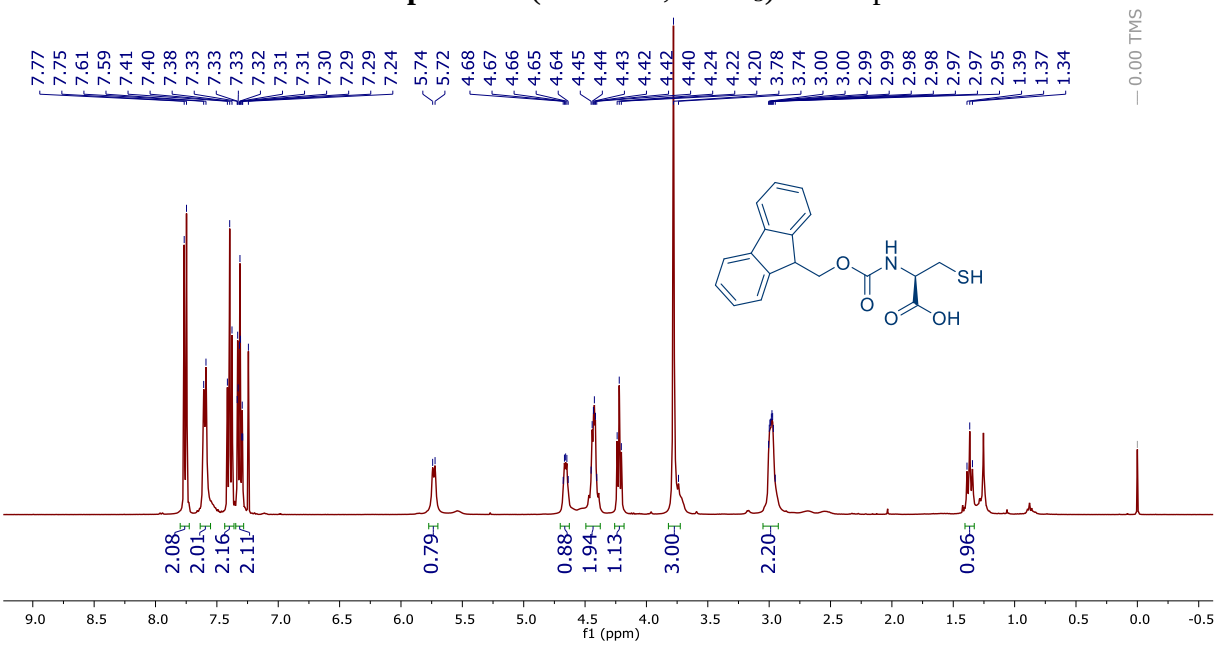
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **15**



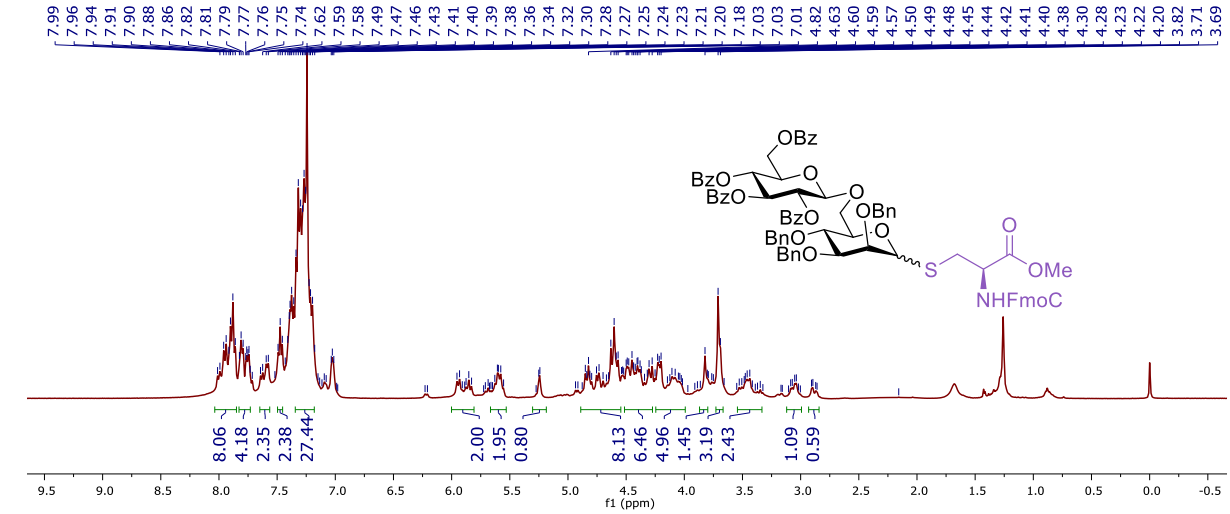
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **15**



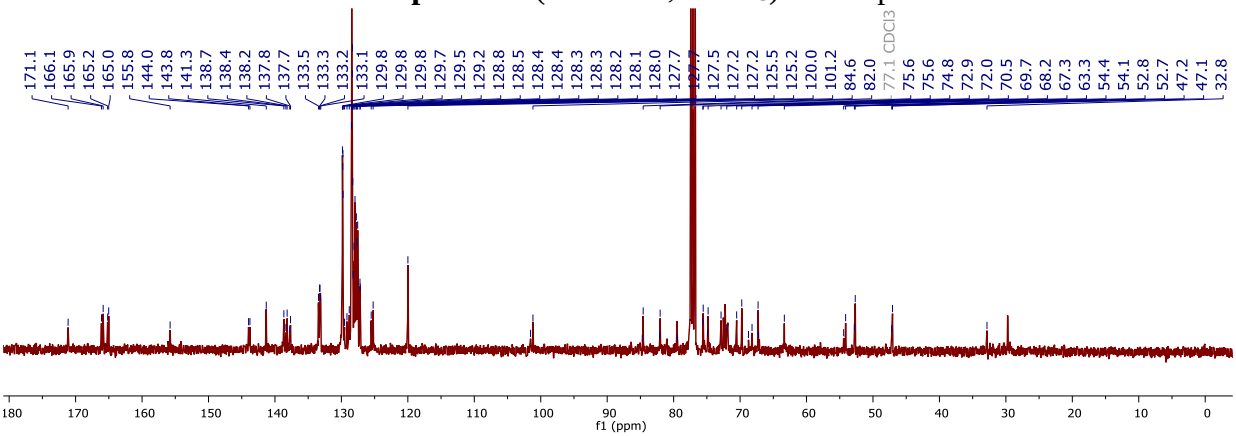
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **18**



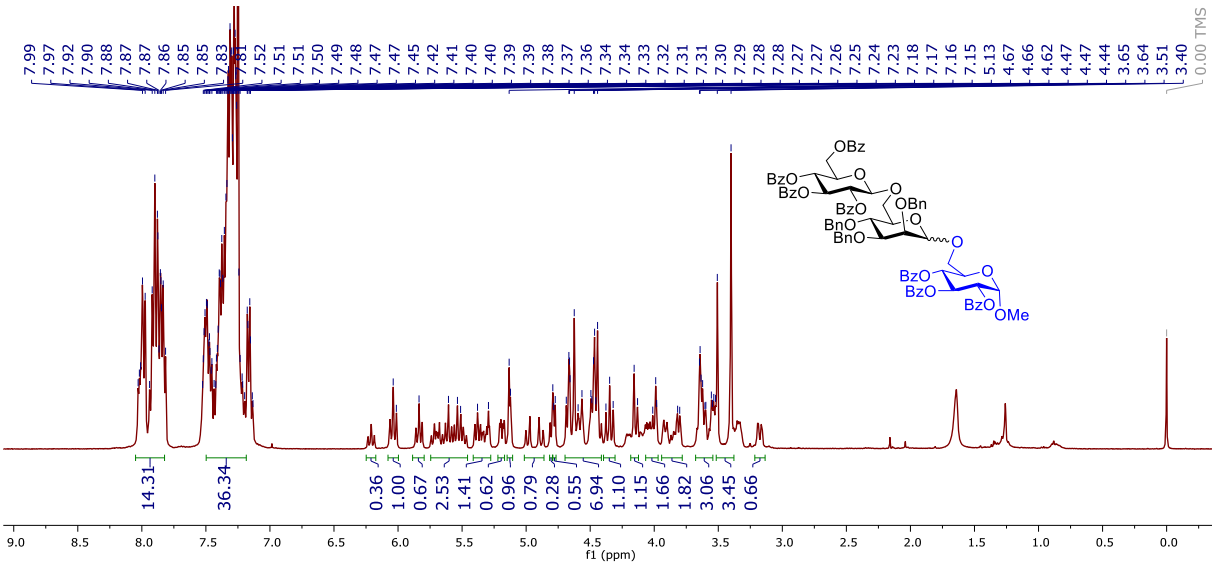
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **19**



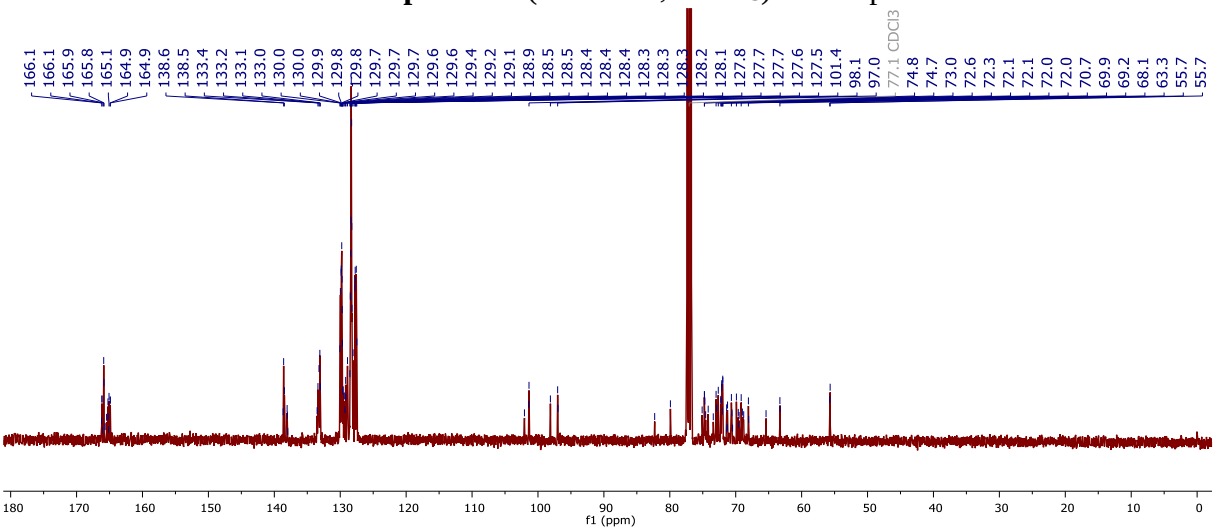
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **19**



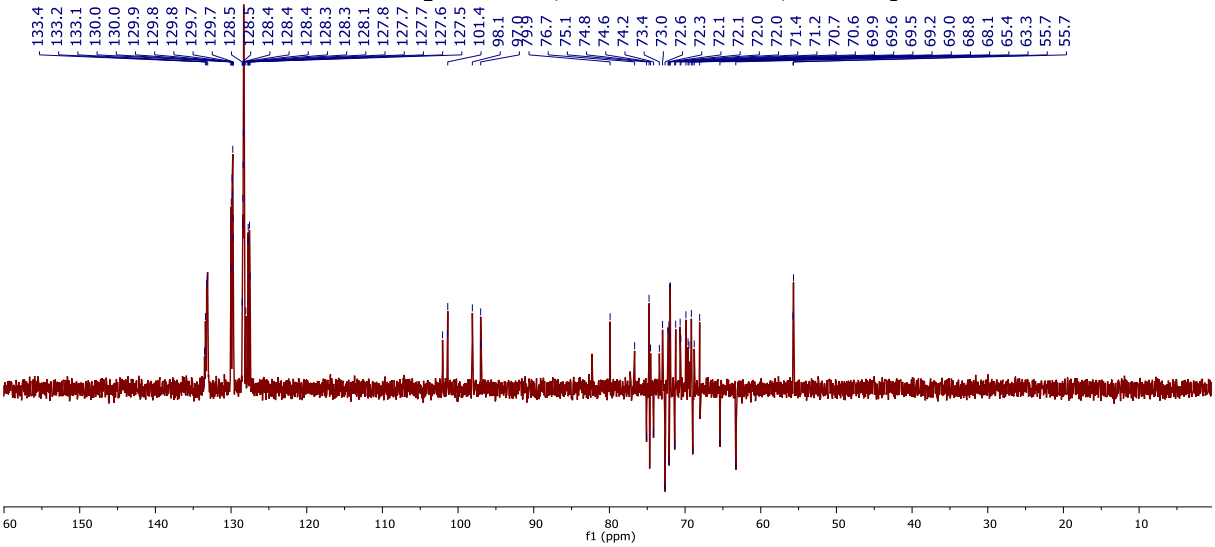
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **21a**



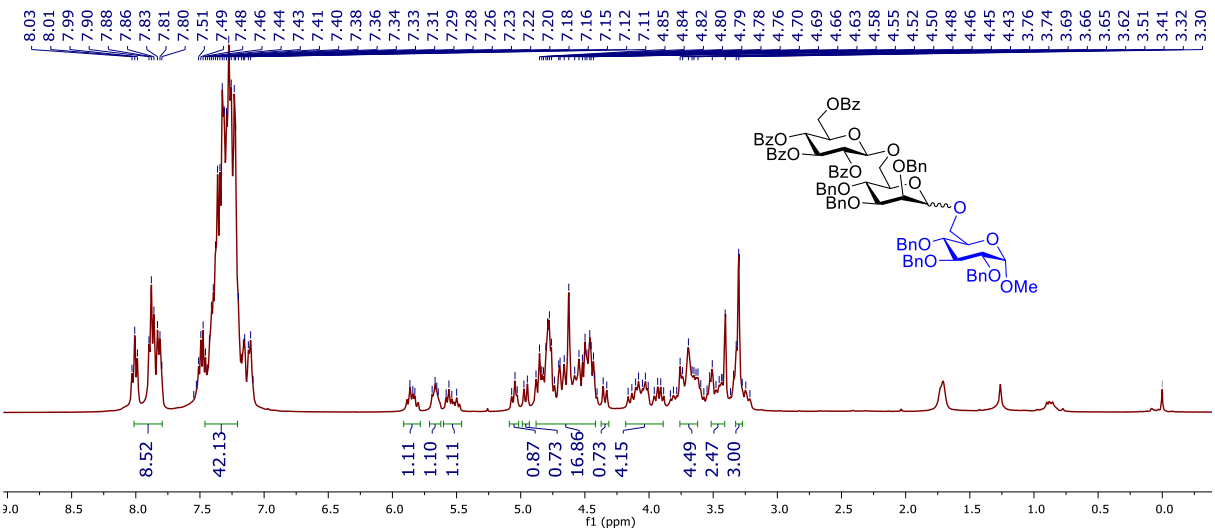
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **21a**



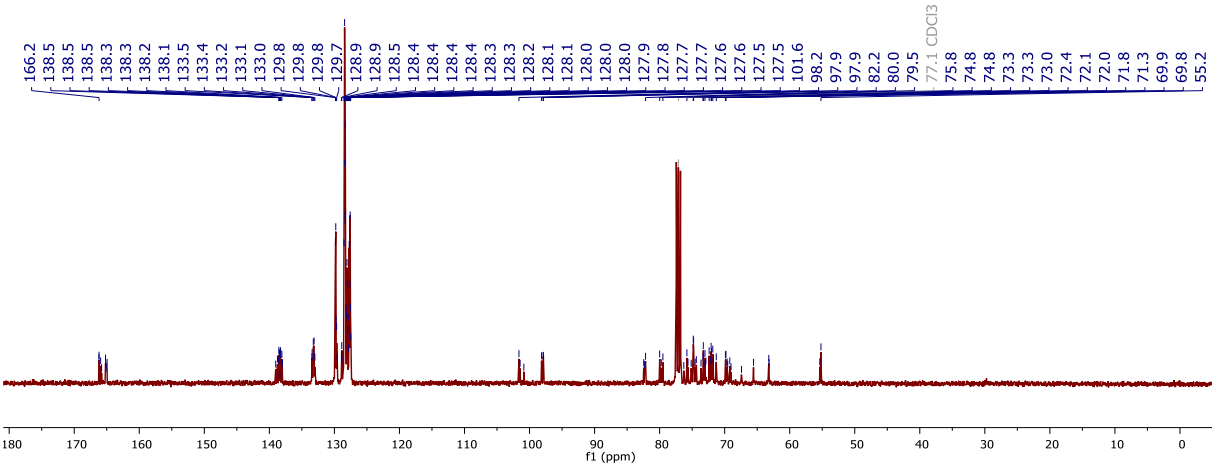
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **21a**



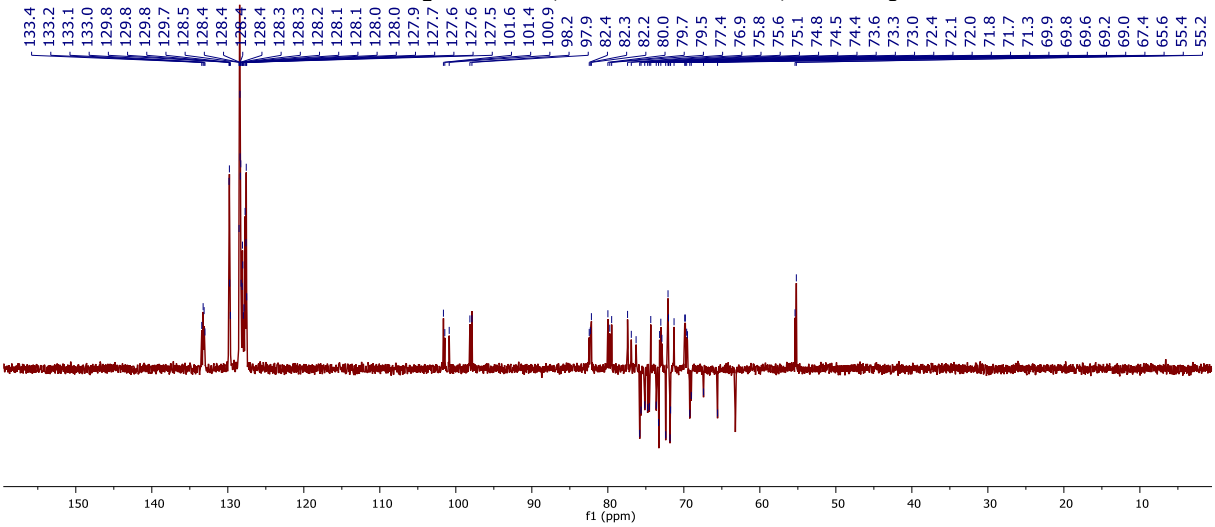
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **21b**



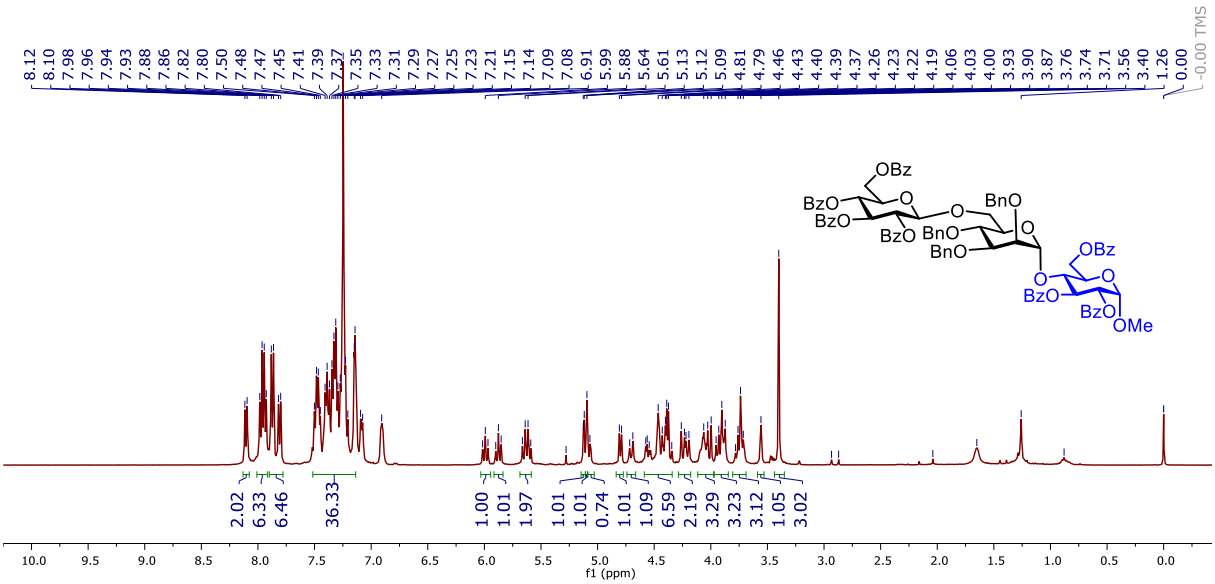
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **21b**



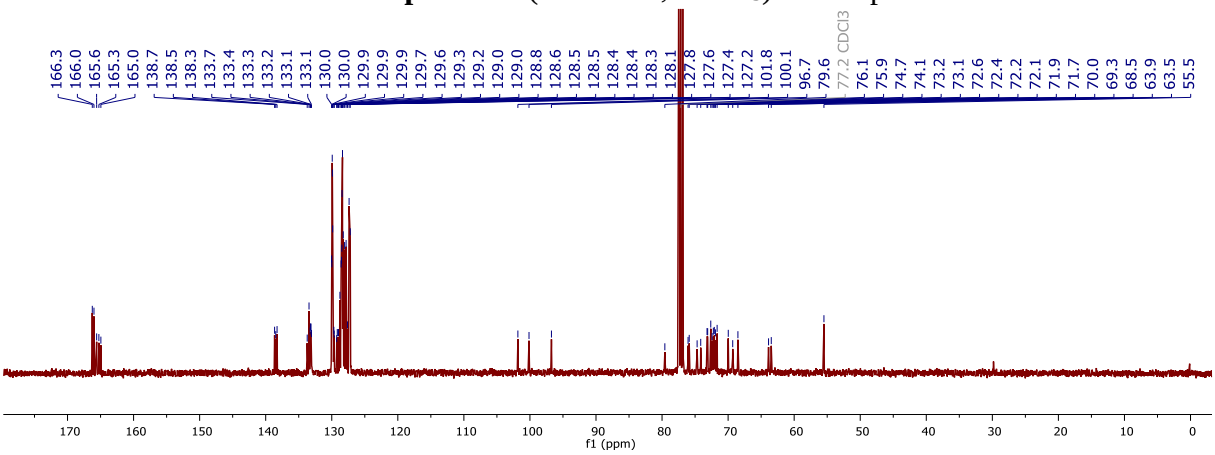
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **21b**



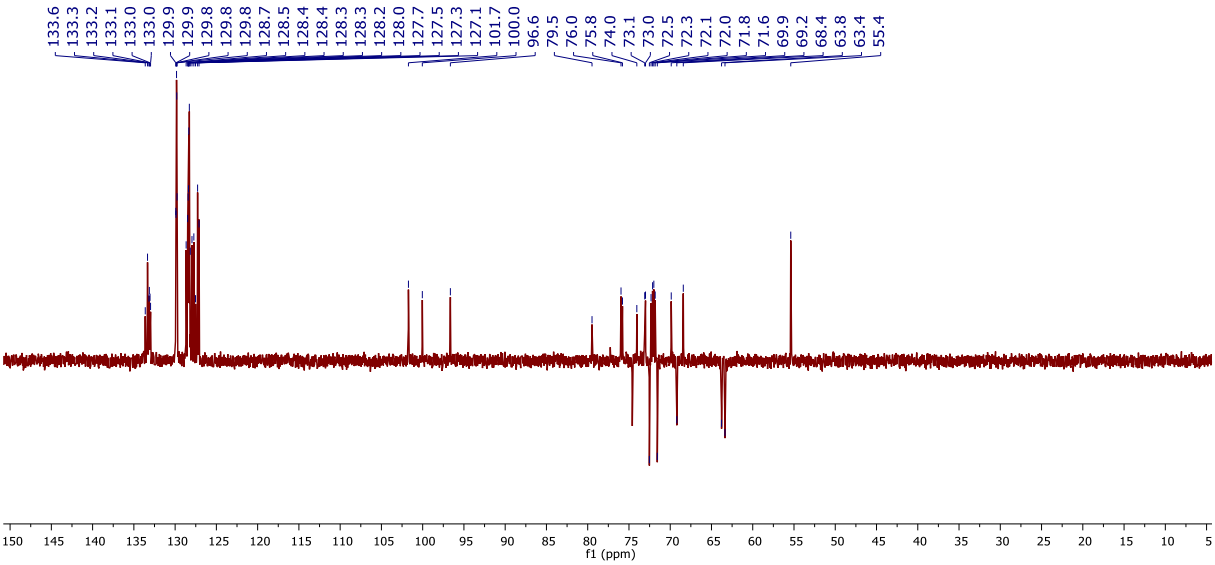
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **21c**



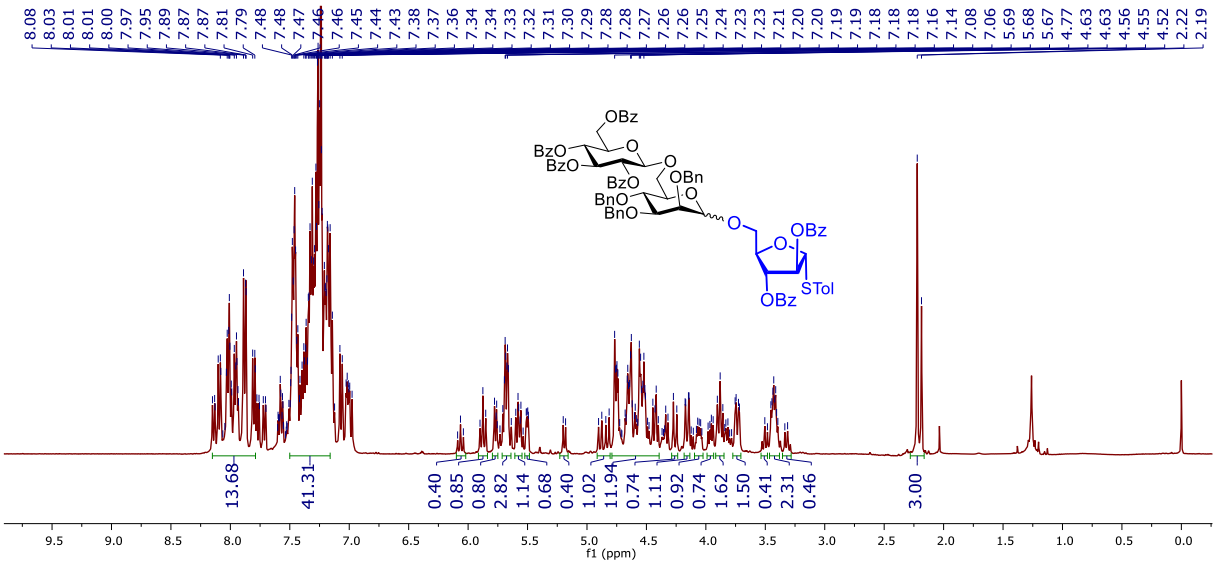
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **21c**



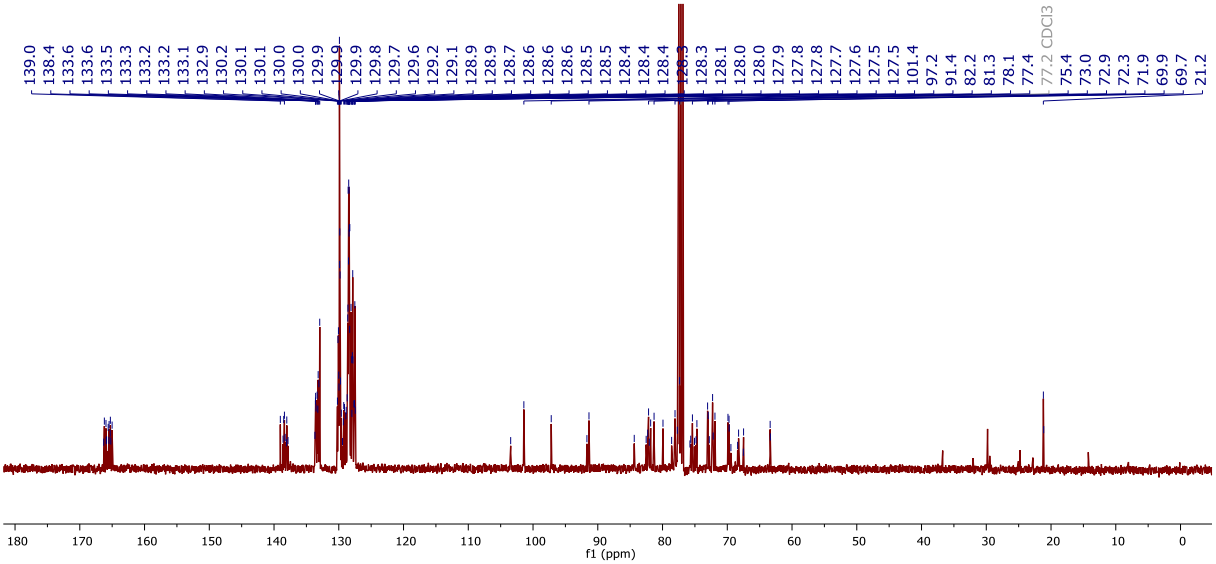
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **21c**



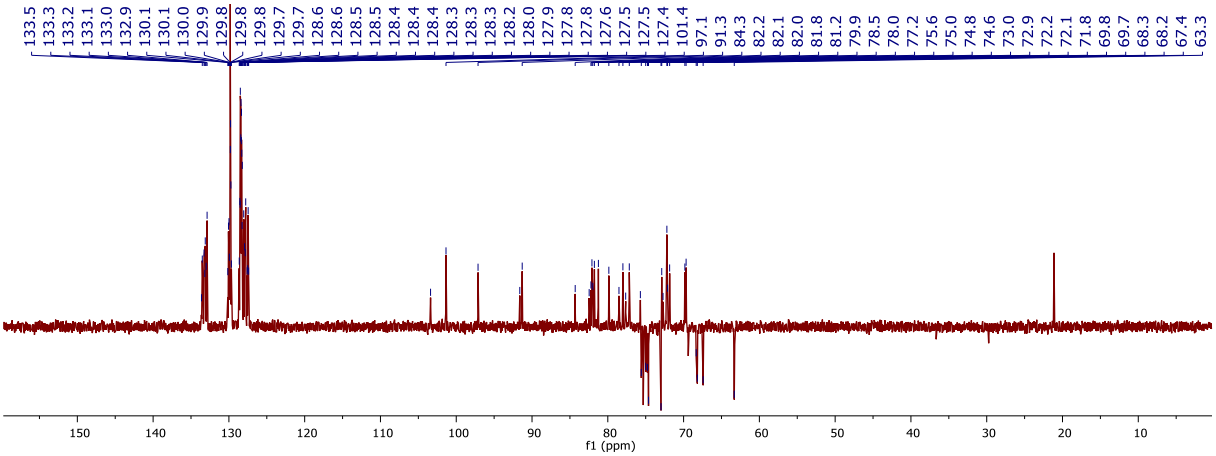
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **21e**



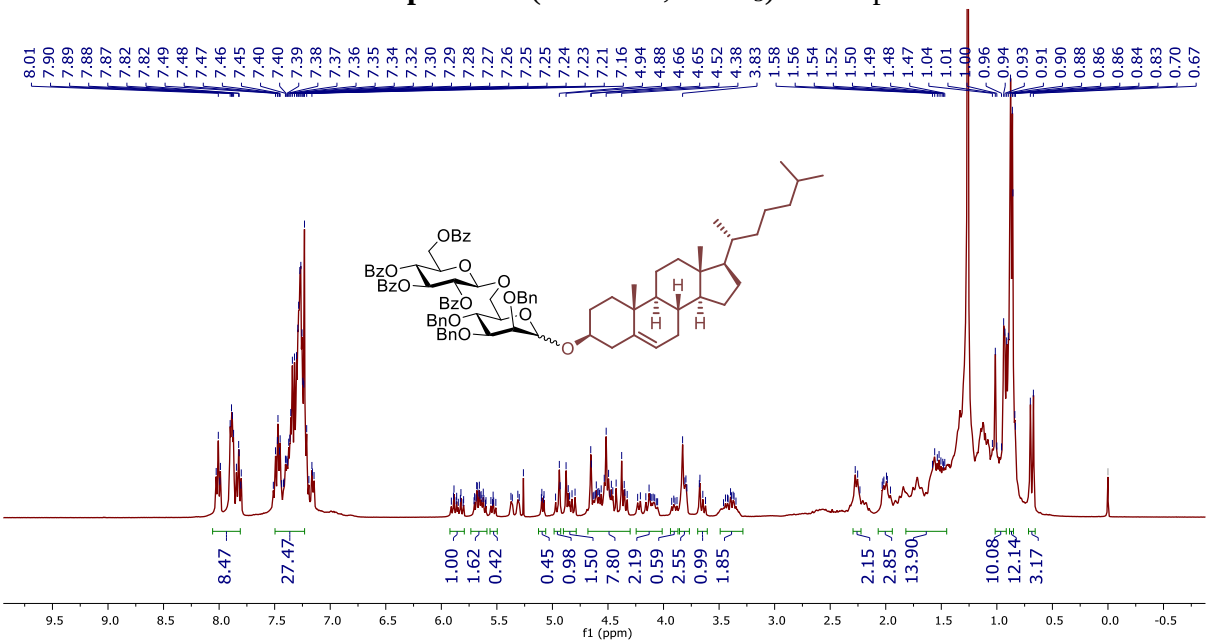
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **21e**



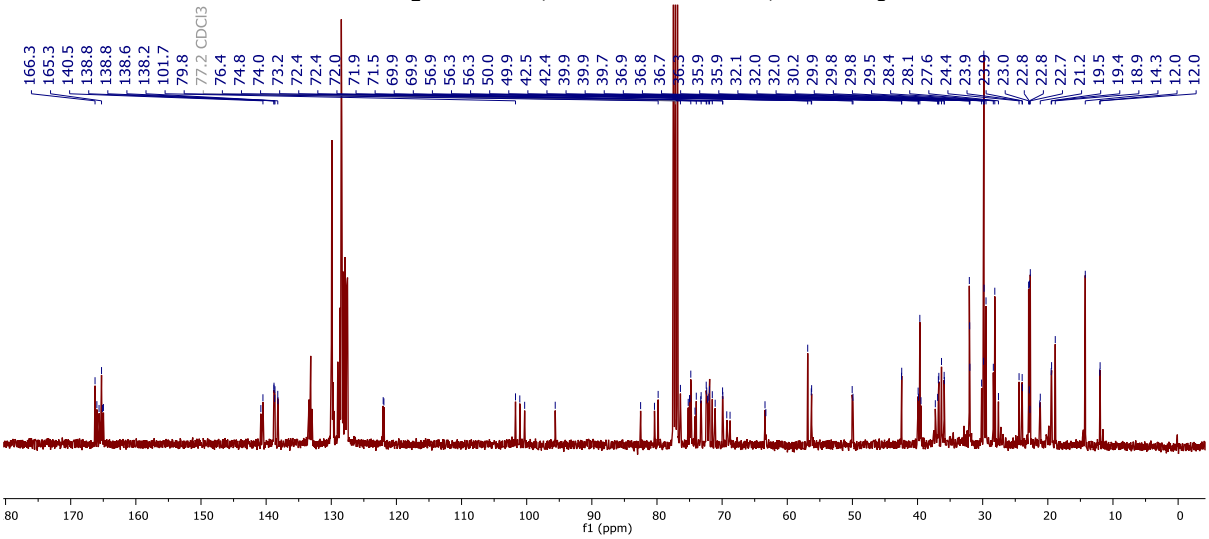
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **21e**



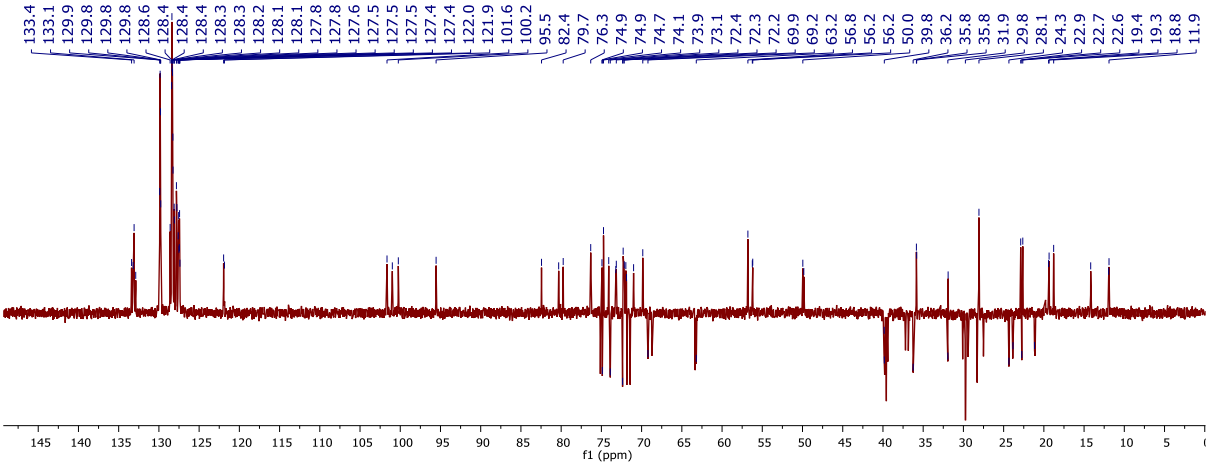
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **23**



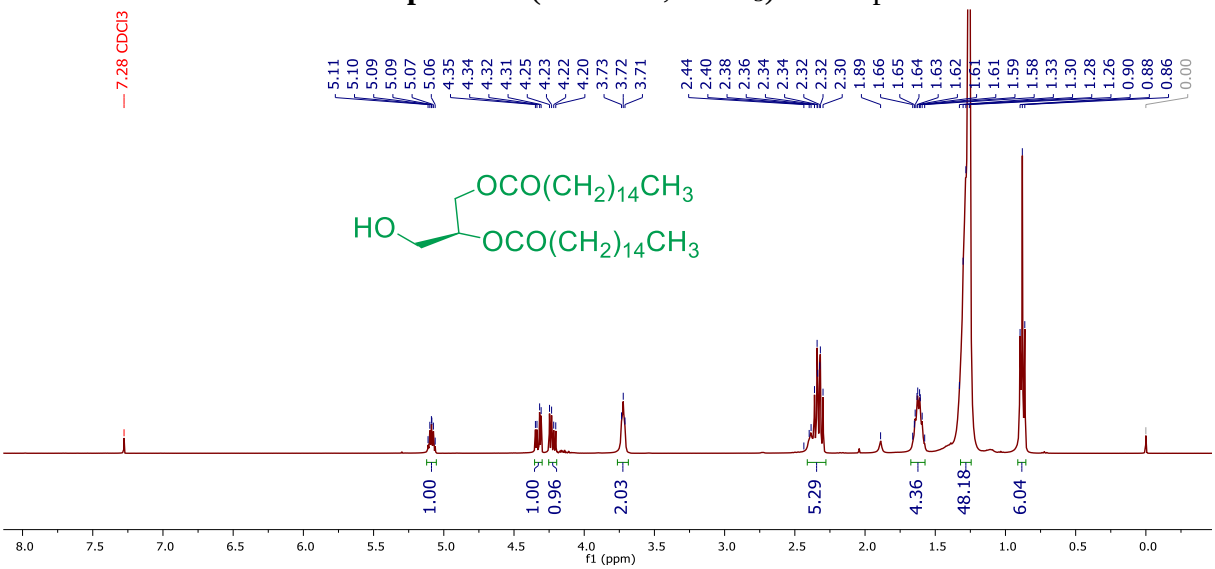
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **23**



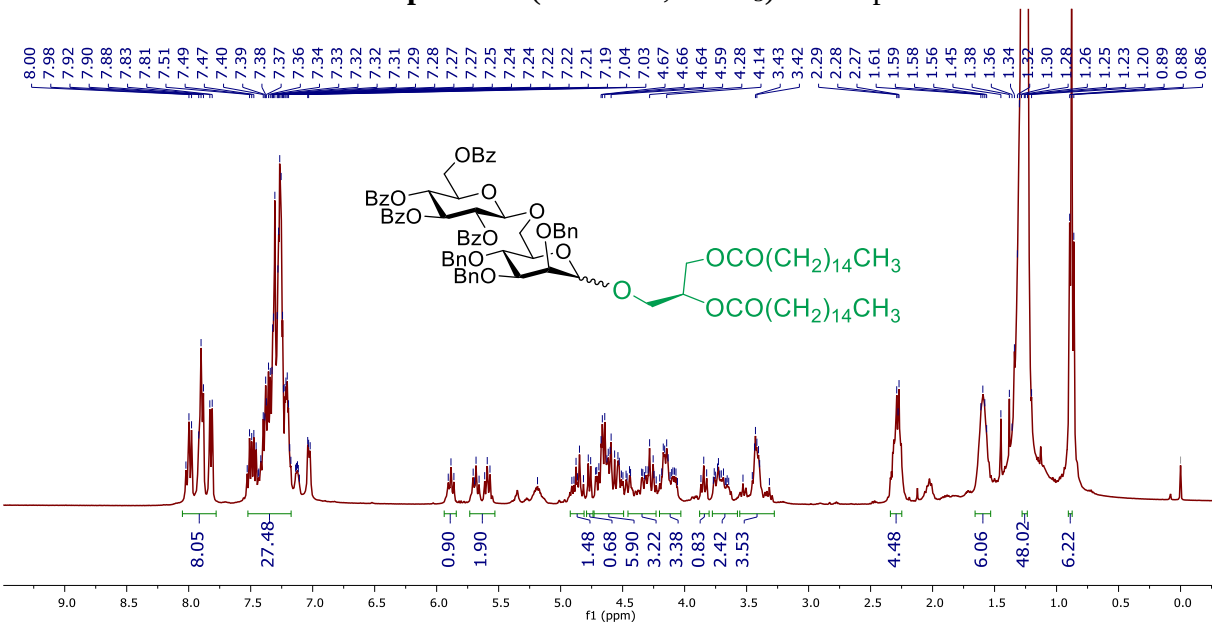
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **23**



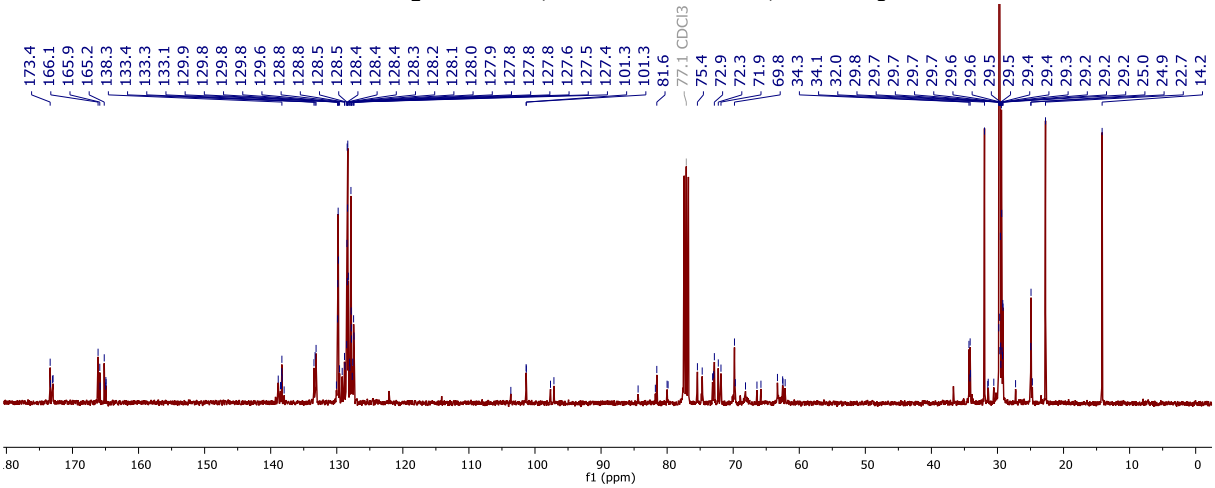
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **31**



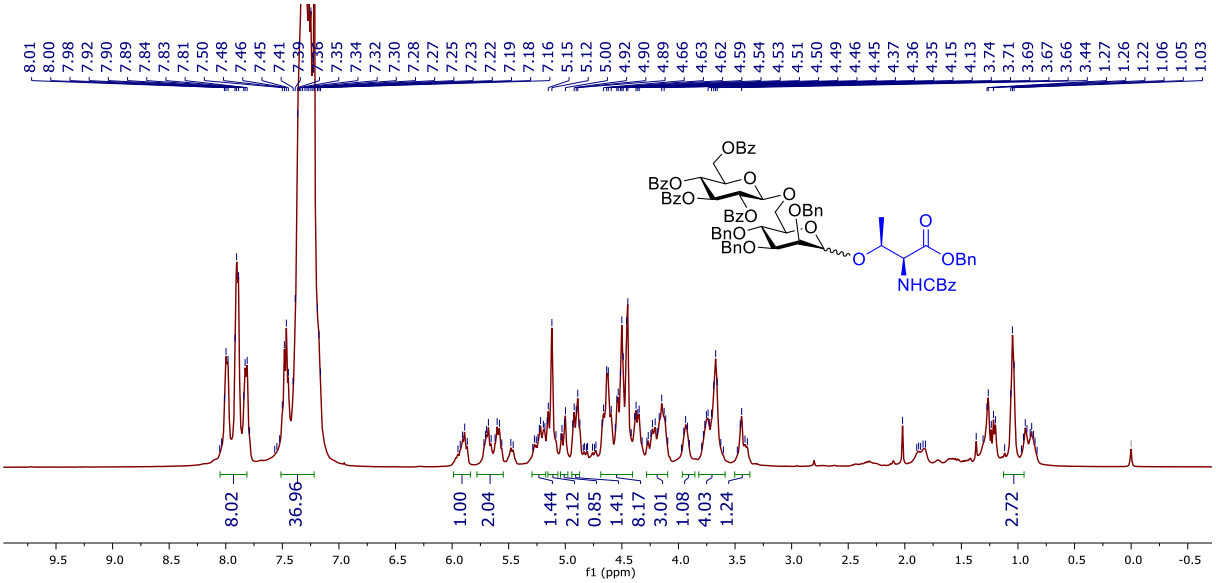
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **32**



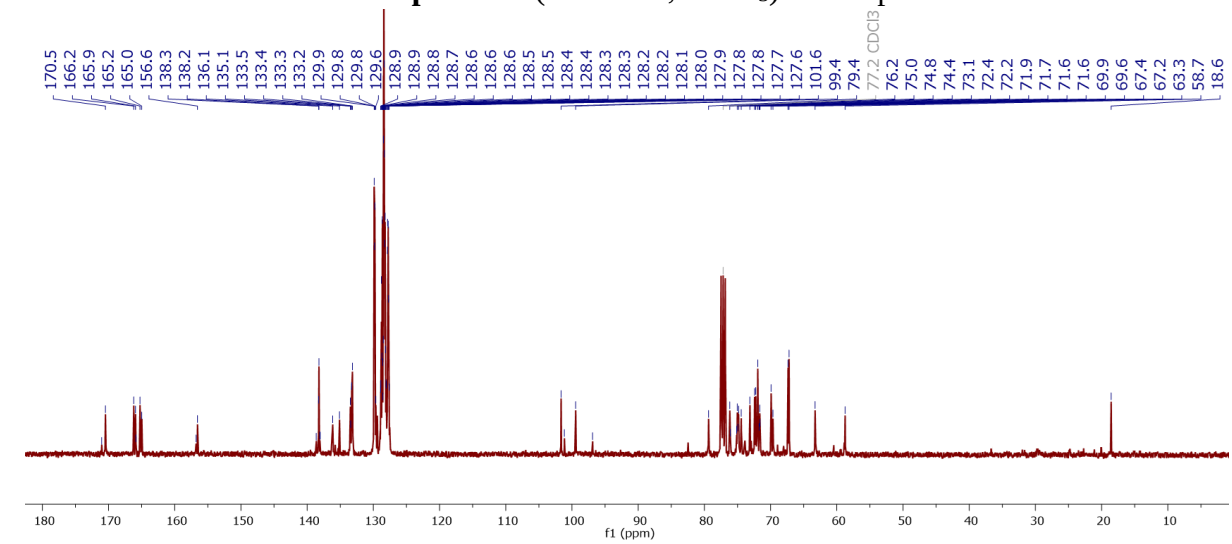
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **32**



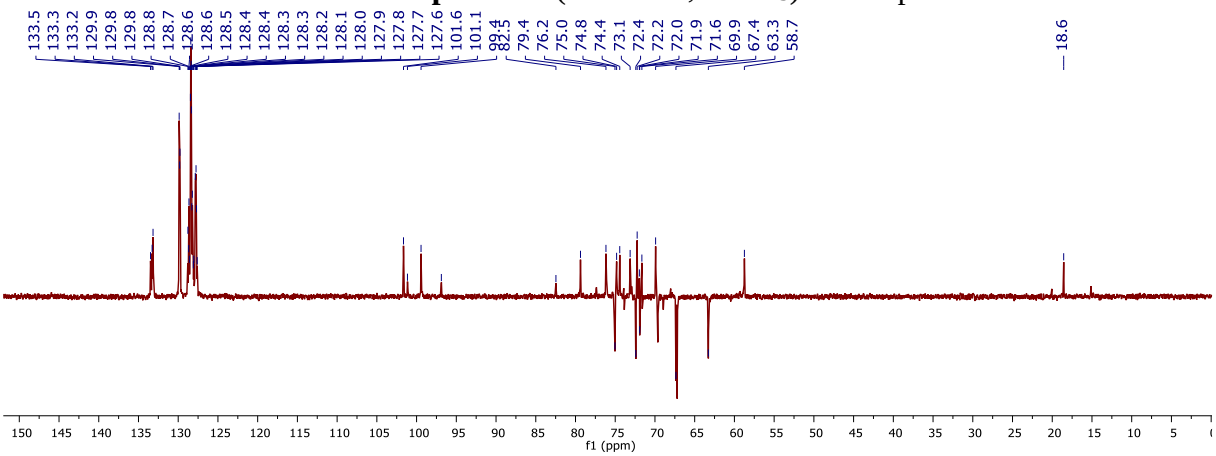
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **34**



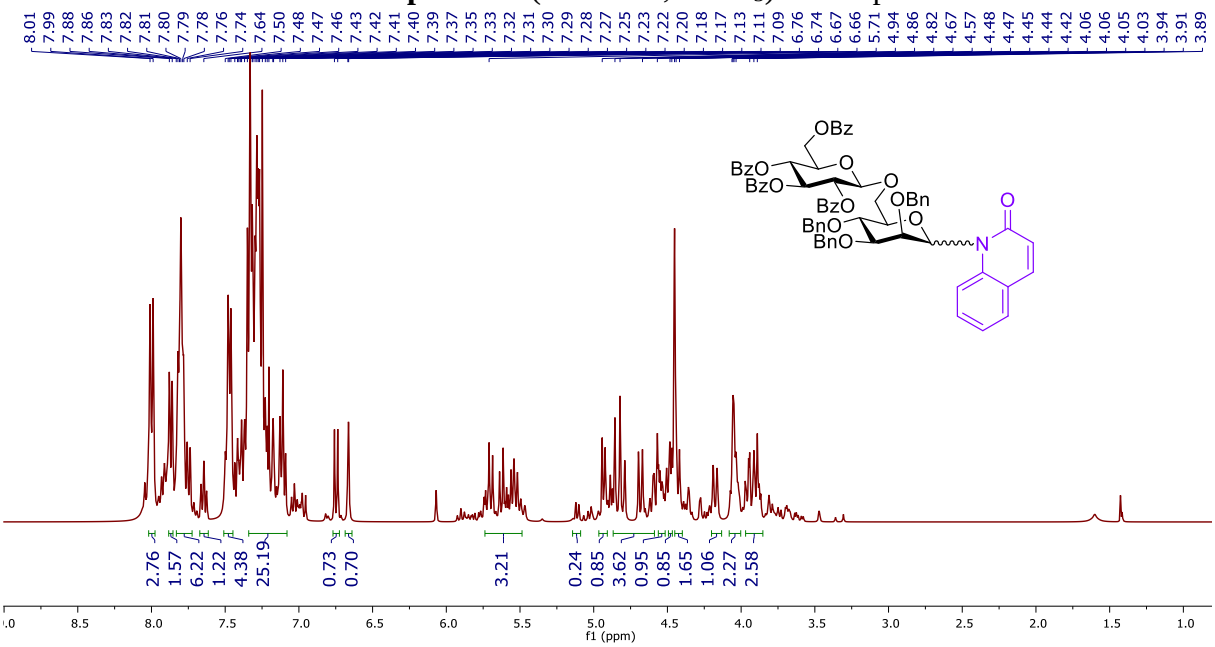
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **34**



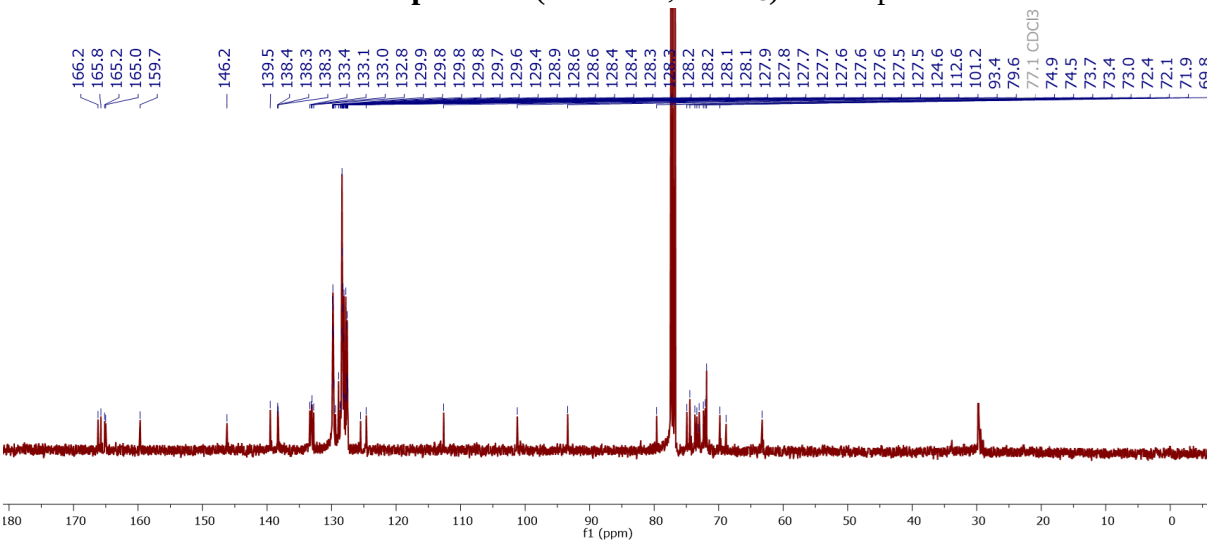
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **34**



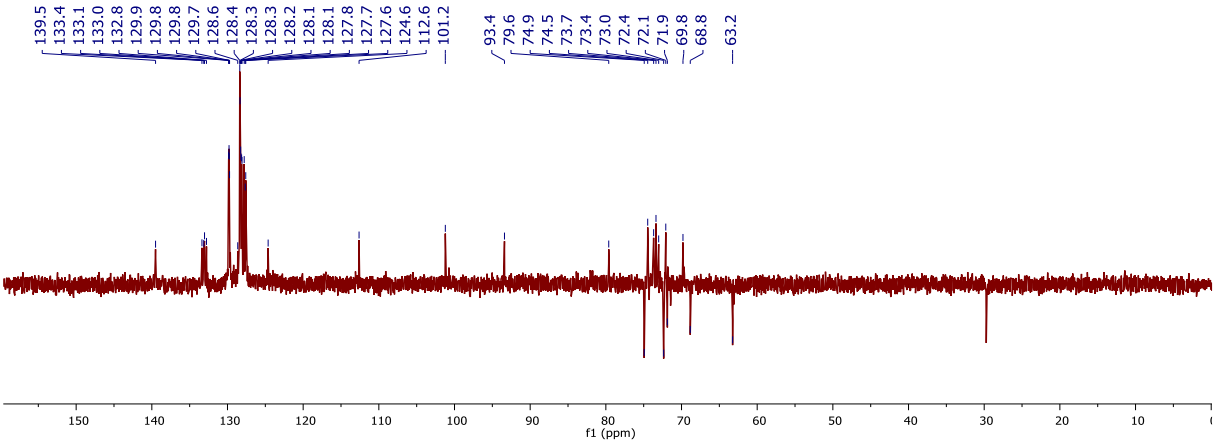
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **41**



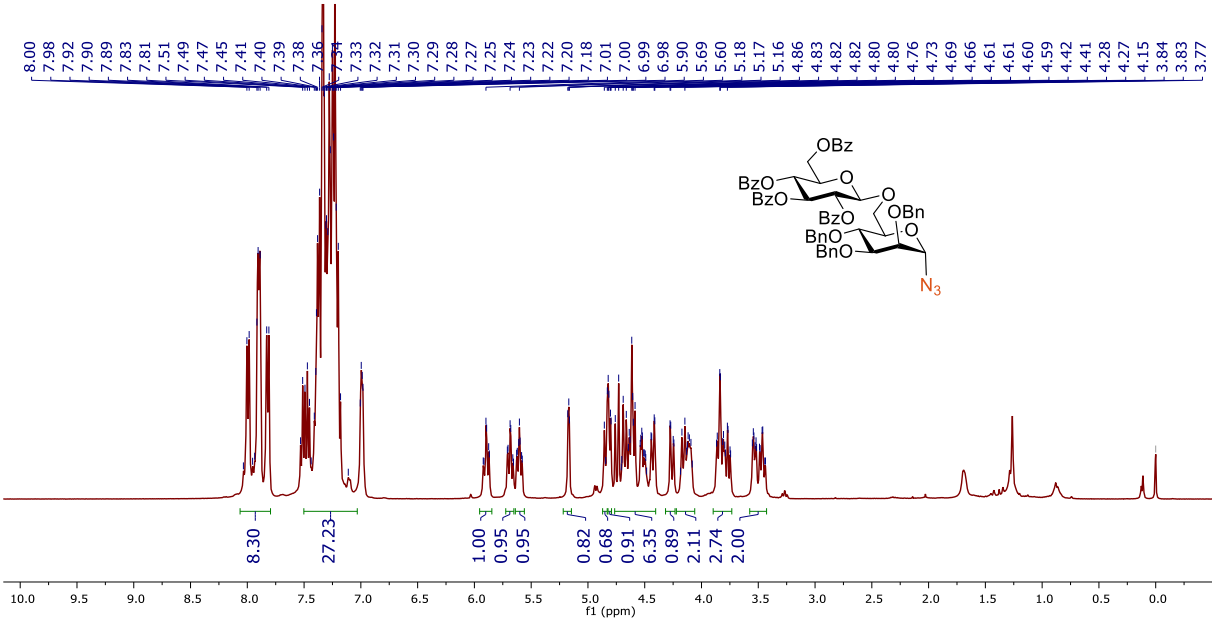
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **41**



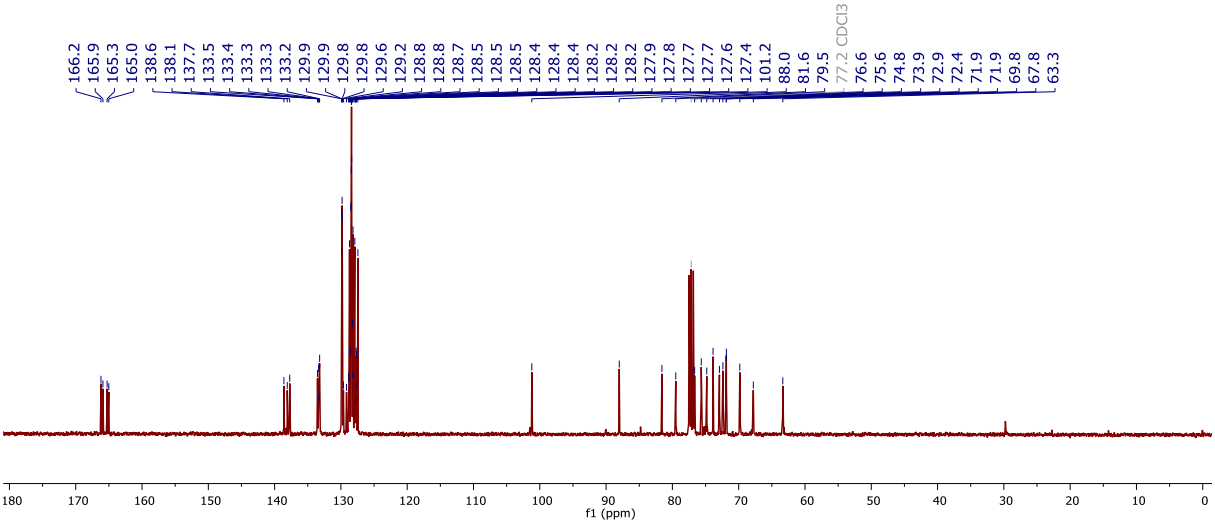
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **41**



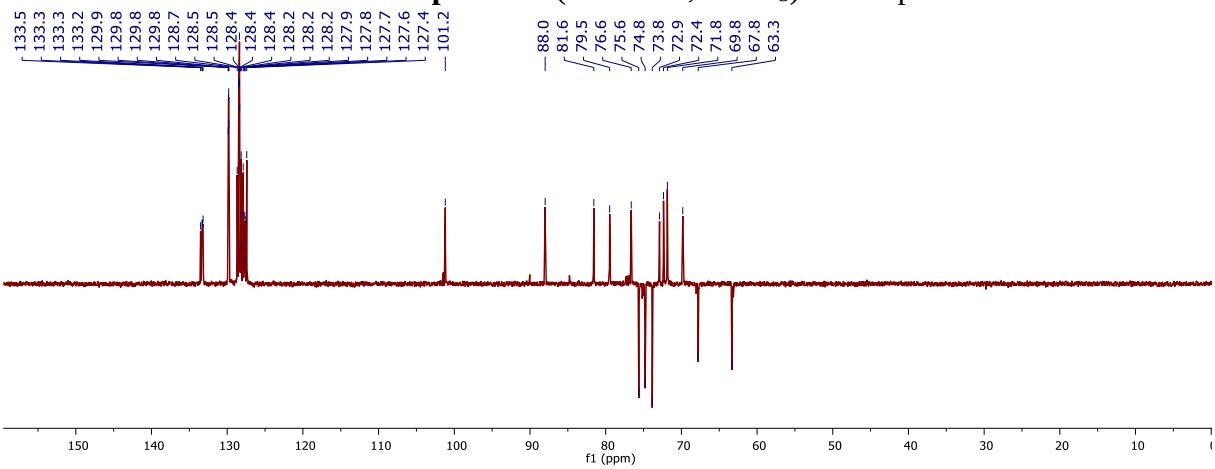
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **43**



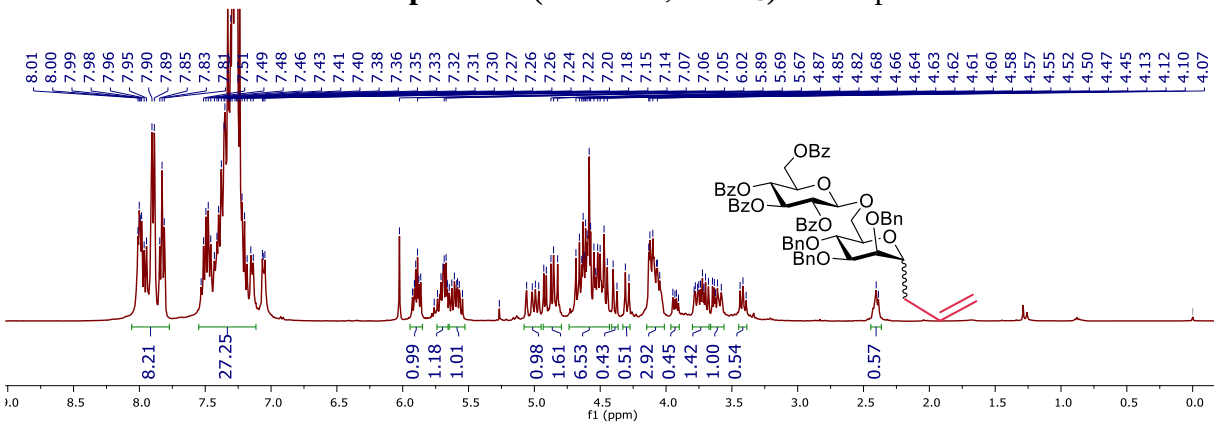
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **43**



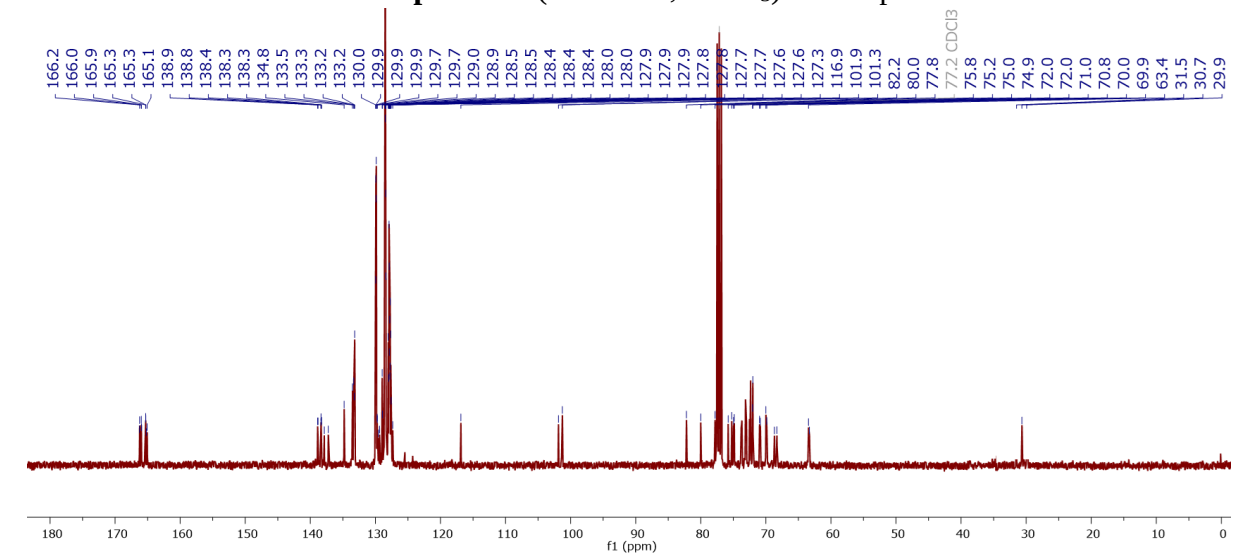
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **43**



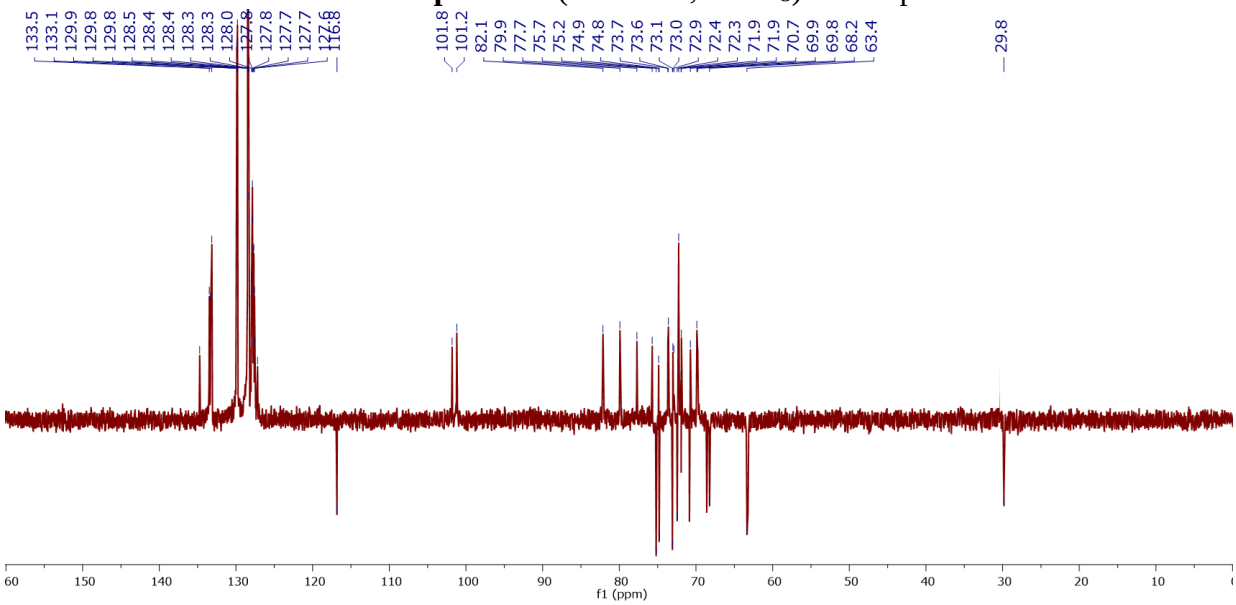
^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of compound **45**



^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **45**



DEPT NMR Spectrum (101 MHz, $CDCl_3$) of compound **45**

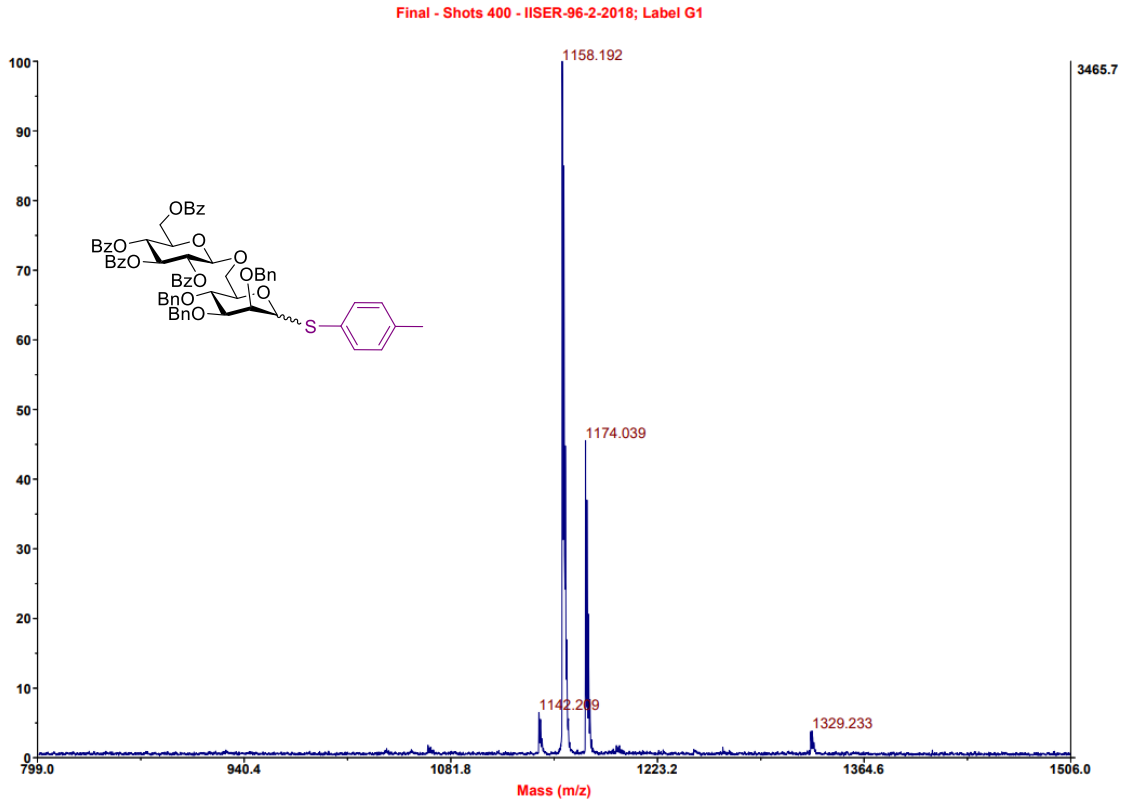


4.5 MALDI-ToF Spectra of Representative Compounds

Compound- 15

Calculated mass: 1158.195 ($C_{68}H_{62}O_{14}S$)

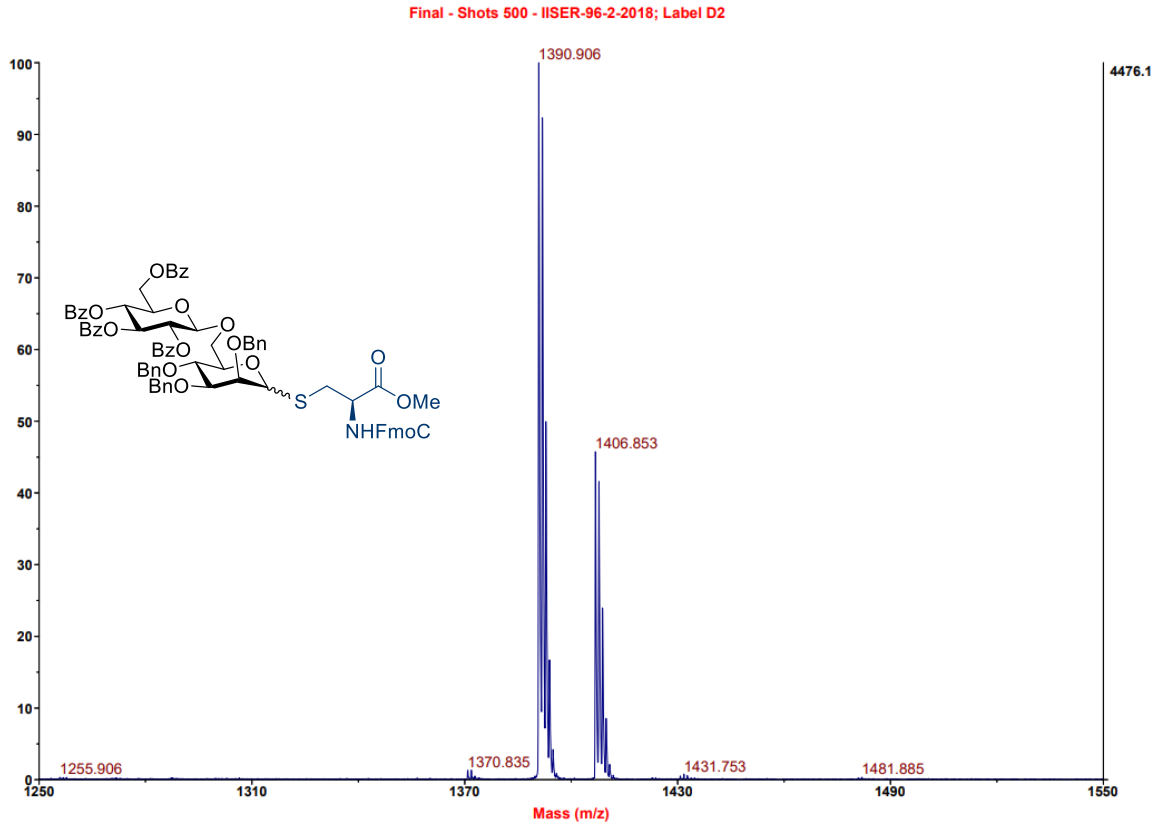
Observed mass: 1158.192



Compound- **19**

Calculated mass: 1390.916 ($C_{80}H_{73}NO_{18}SNa$)

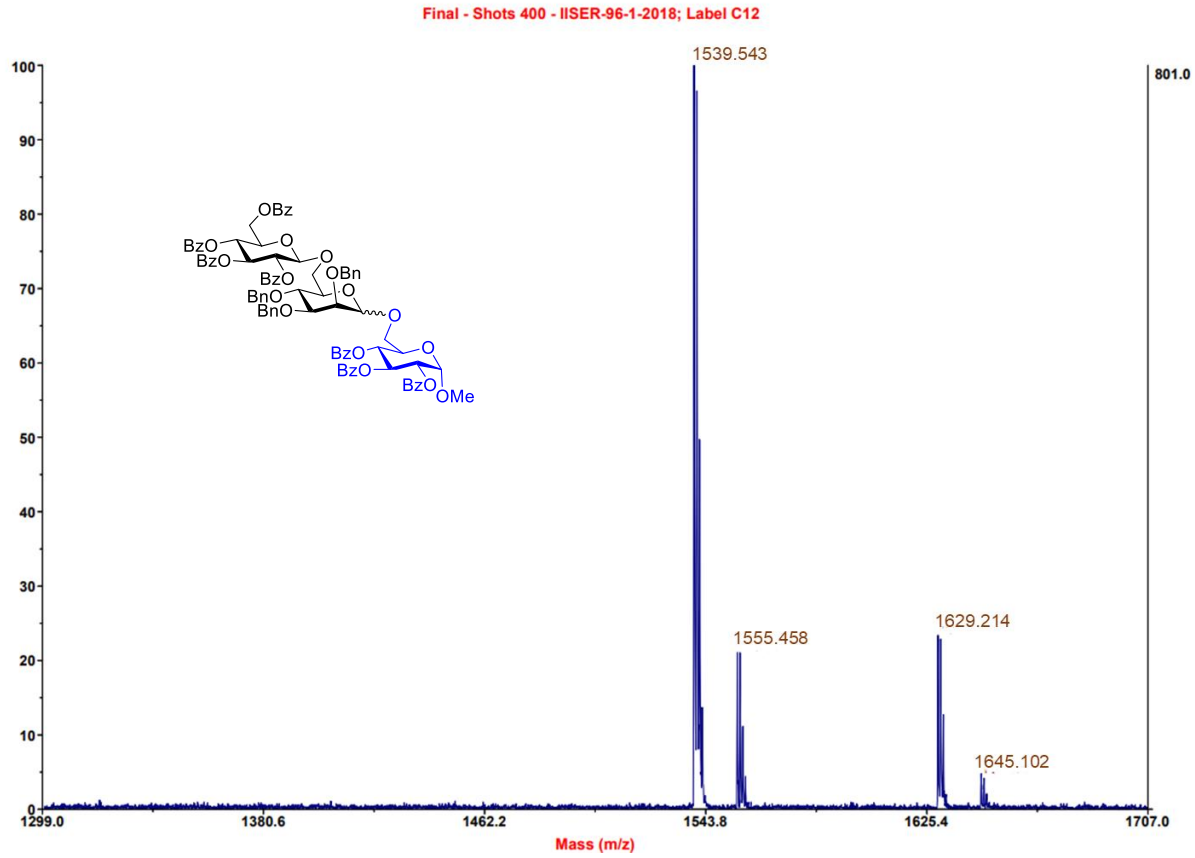
Observed mass: 1390.906



Compound- **21a**

Calculated mass: 1539.5838 ($C_{89}H_{80}O_{23}Na$)

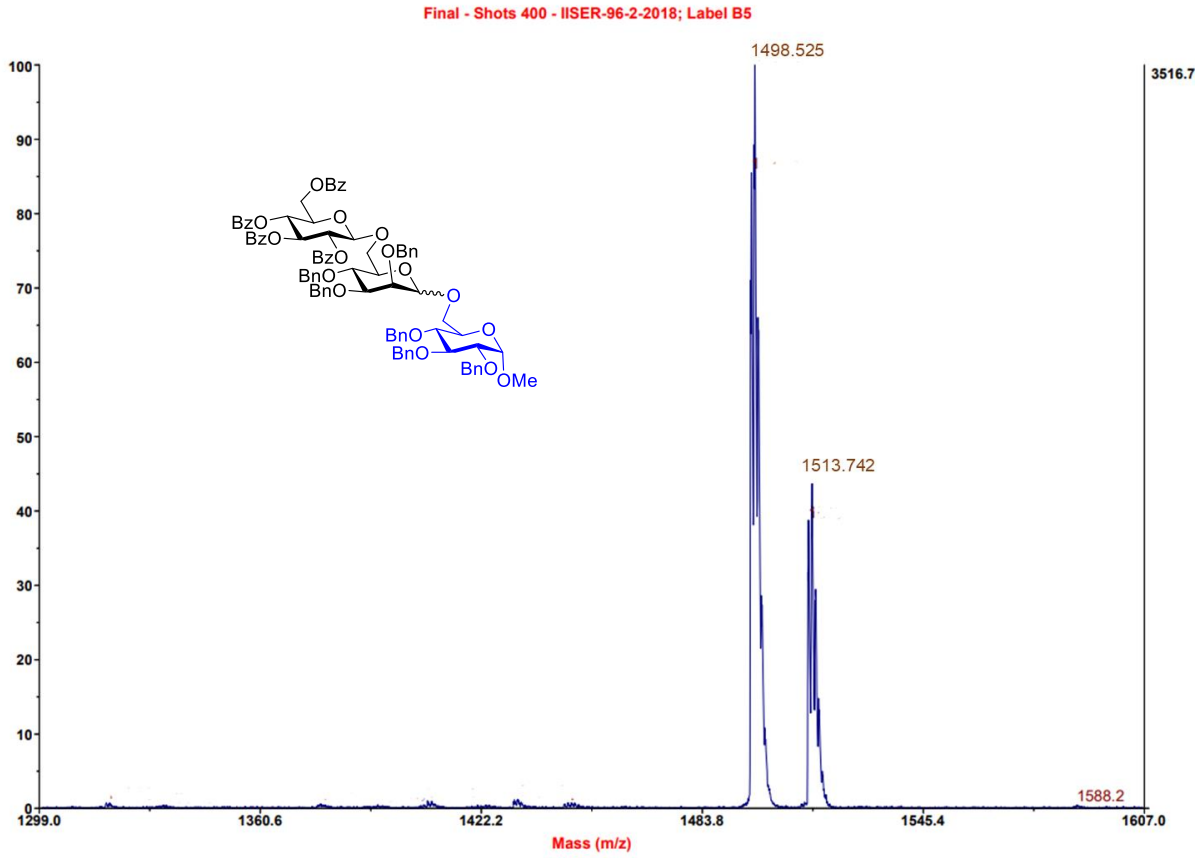
Observed mass: 1539.5837



Compound- **21b**

Calculated mass: 1498.539 ($C_{89}H_{86}O_{20}Na$)

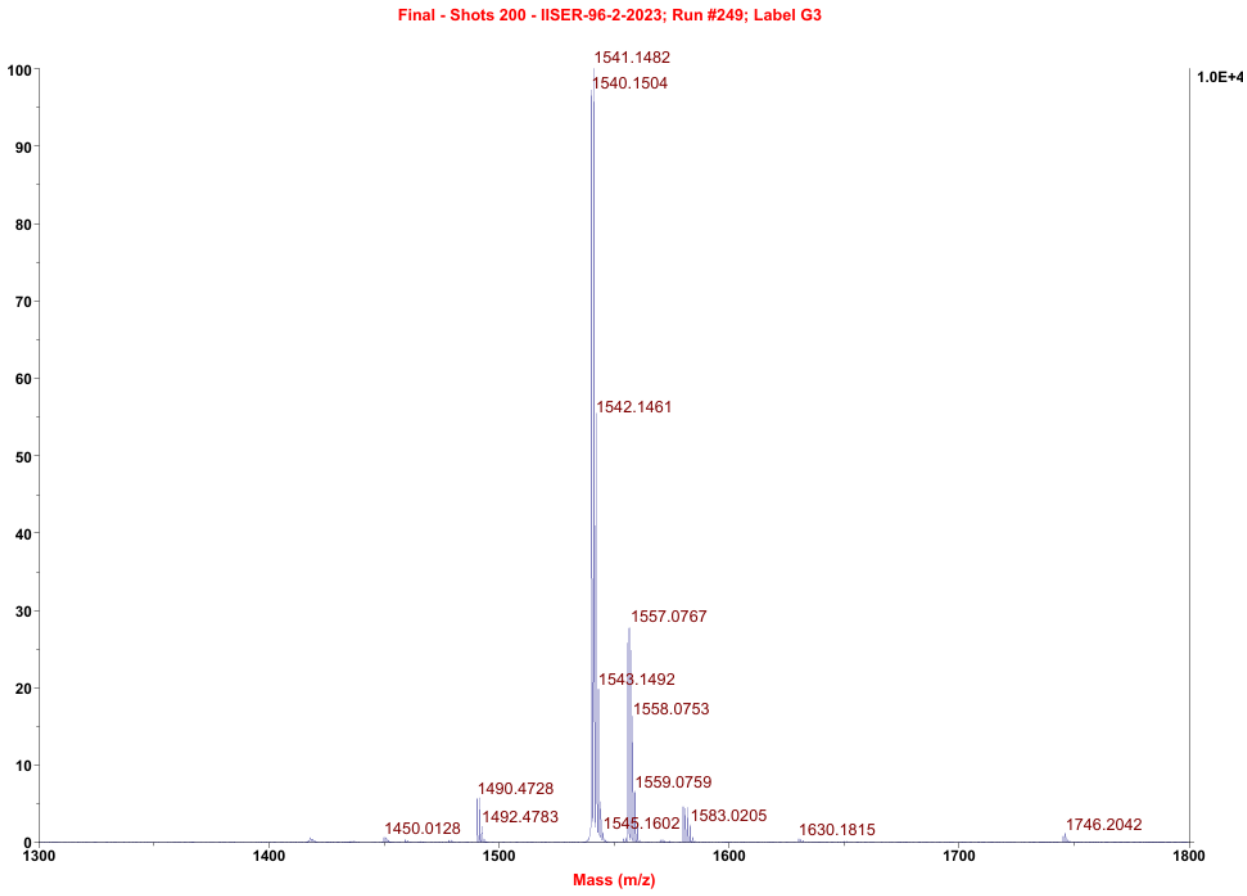
Observed mass: 1498.525



Compound- **21c**

Calculated mass: 1541.1495 ($C_{89}H_{86}O_{20}Na$)

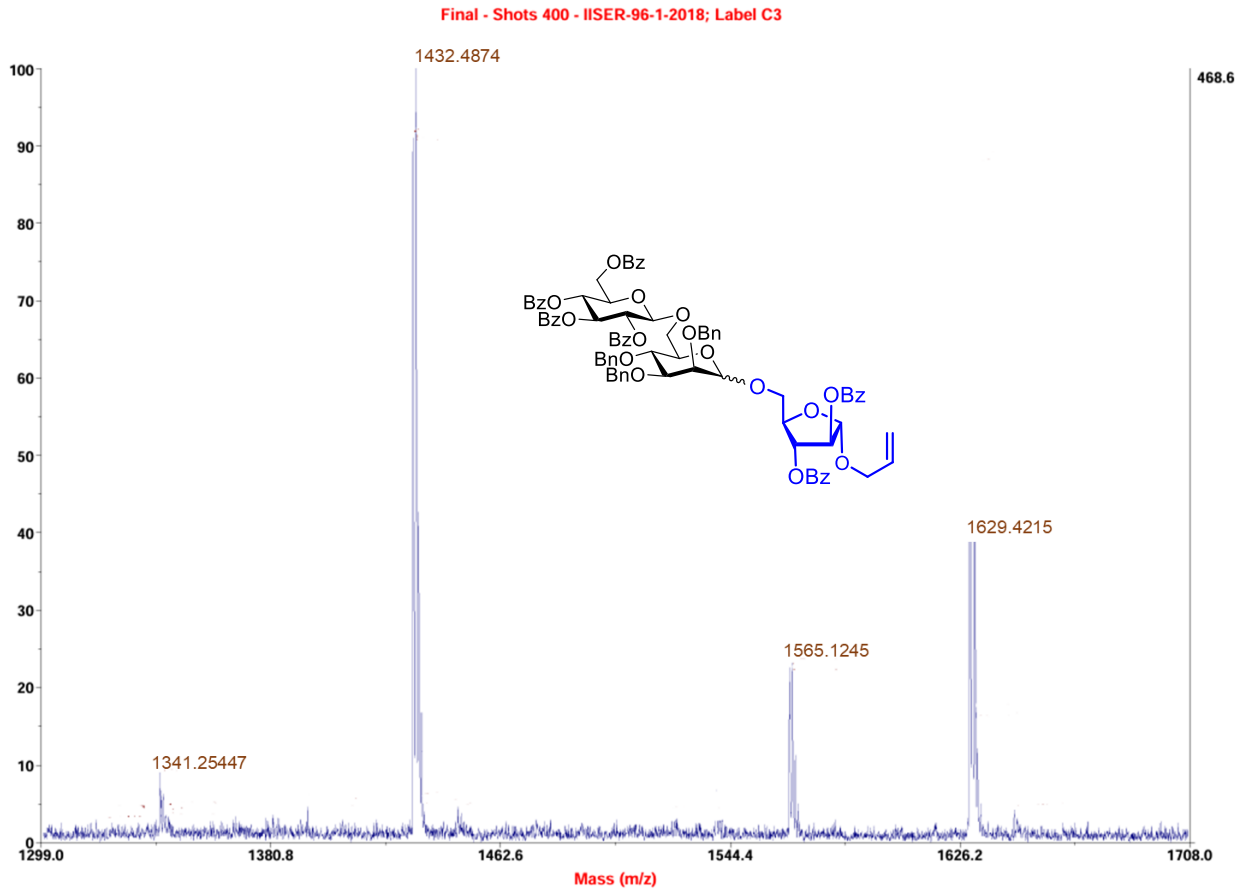
Observed mass: 1541.1482



Compound- **21d**

Calculated mass: 1432.4898 ($C_{83}H_{76}O_{21}Na$)

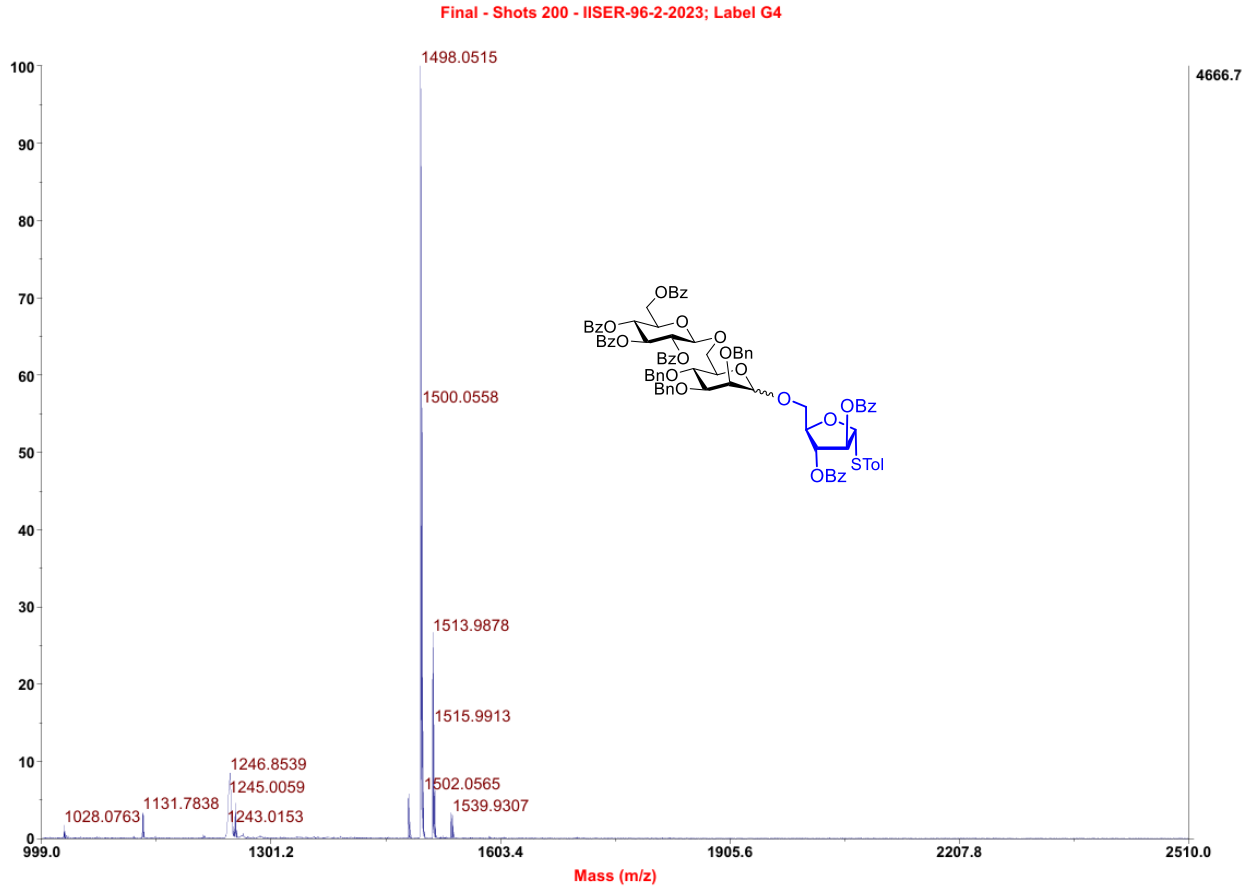
Observed mass: 1432.4874



Compound- **21e**

Calculated mass: 1498.0524 ($C_{87}H_{78}O_{20}SNa$)

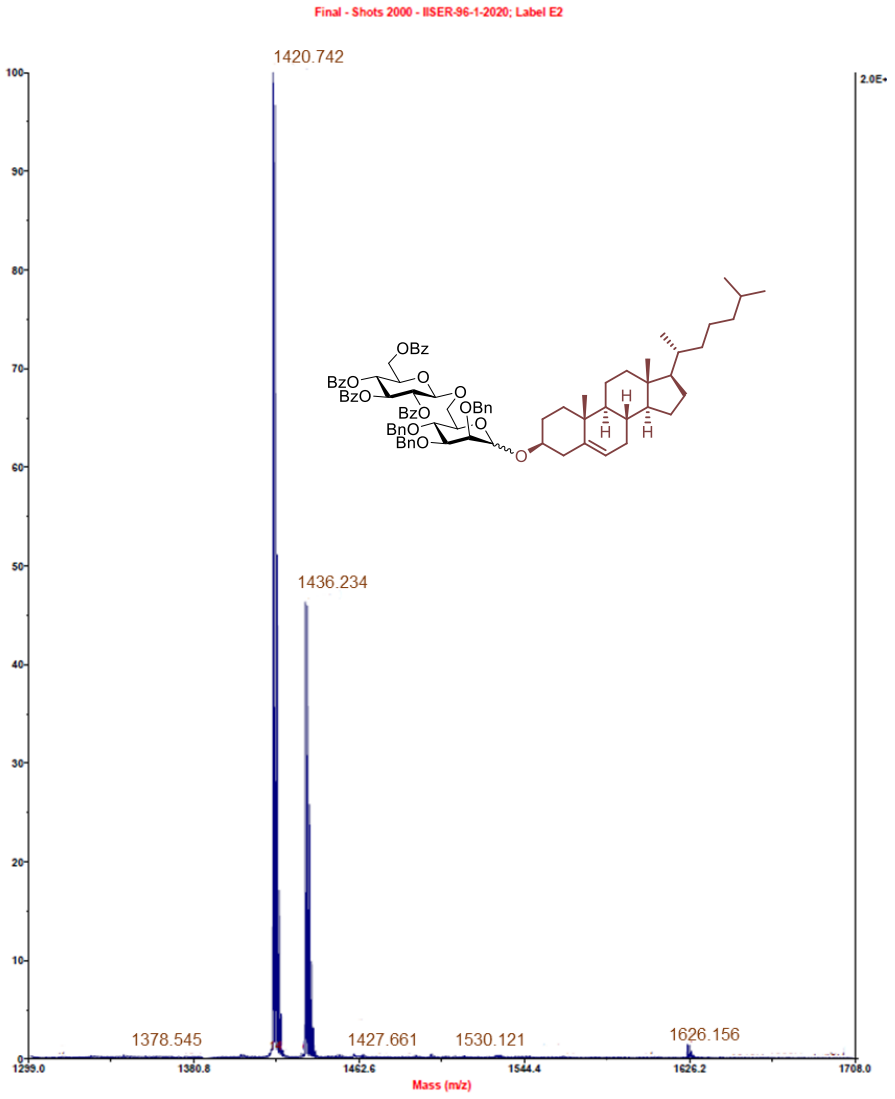
Observed mass: 1498.0515



Compound- **23**

Calculated mass: 1420.752 ($C_{88}H_{100}O_{15}Na$)

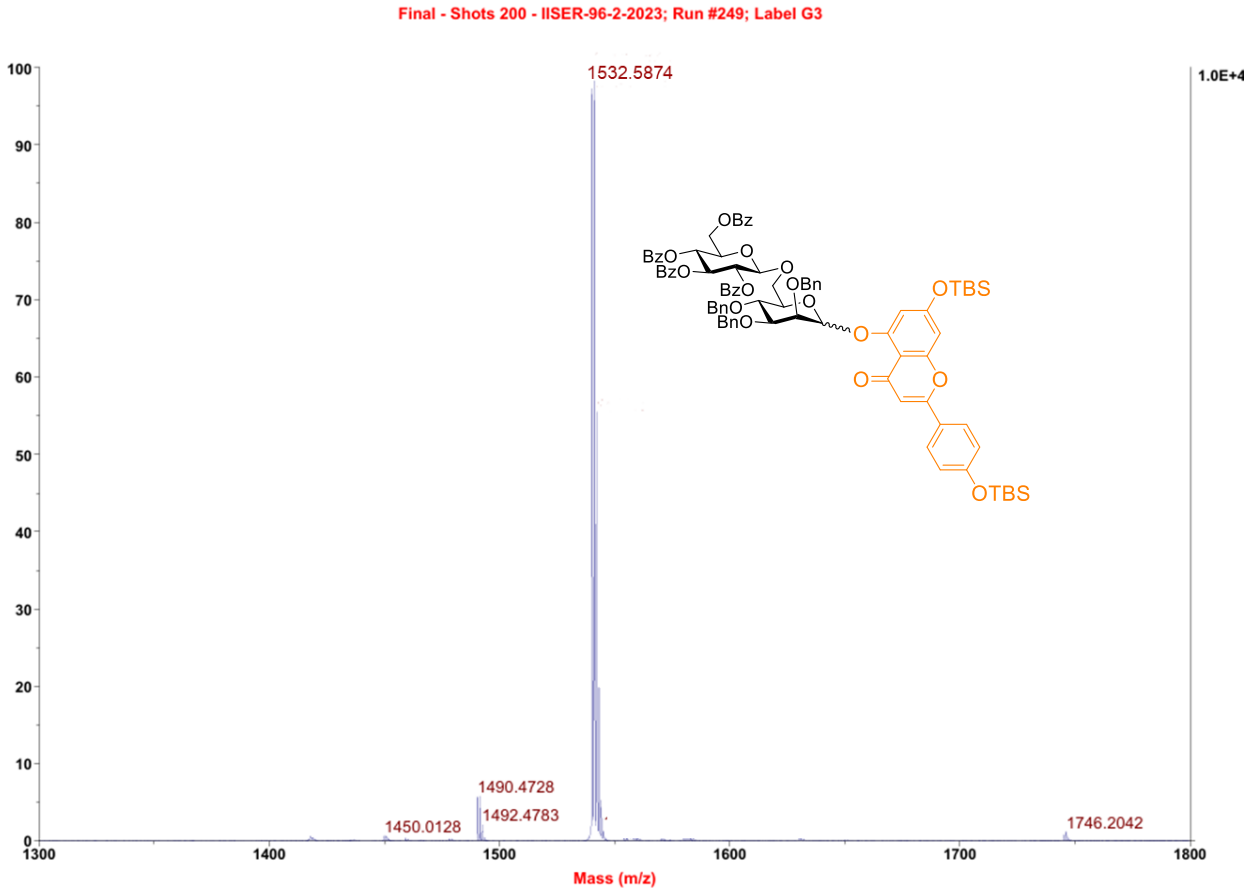
Observed mass: 1420.742



Compound- **25**

Calculated mass: 1532.5892 ($C_{88}H_{92}O_{19}Na$)

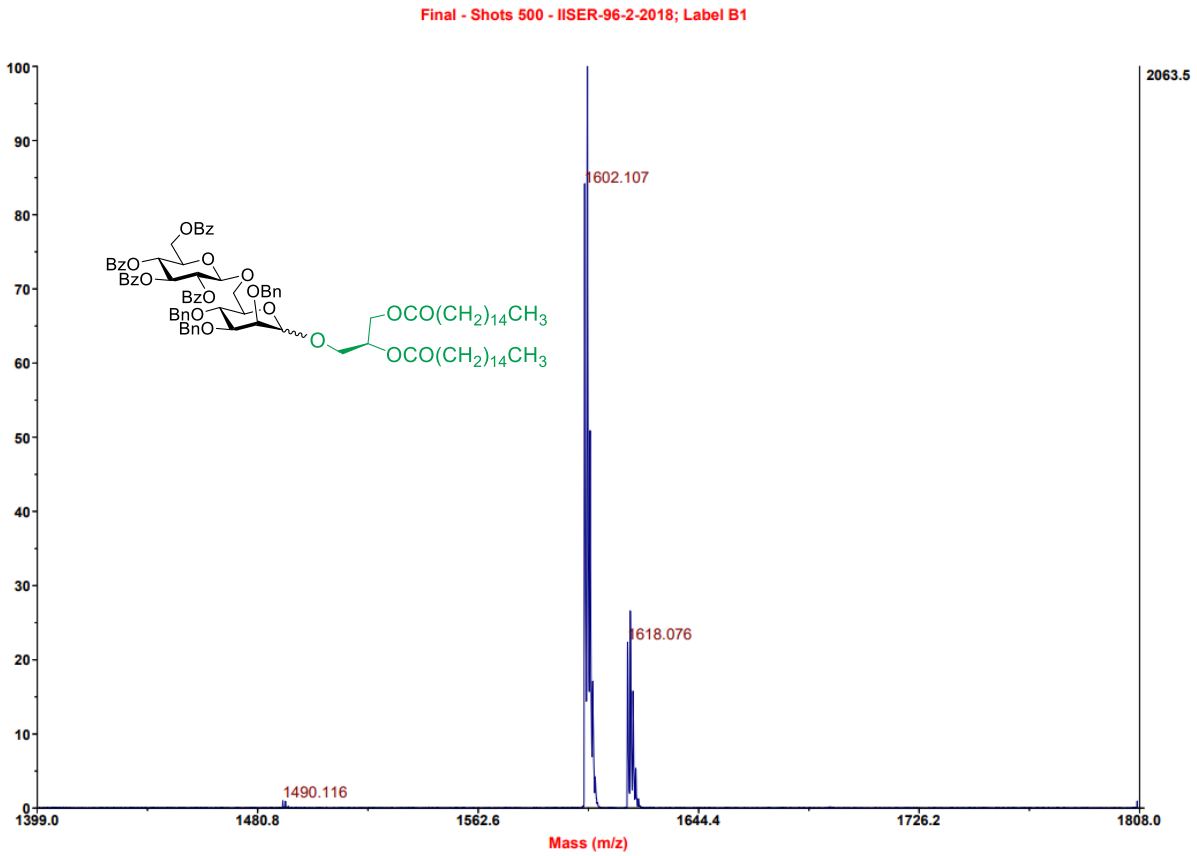
Observed mass: 1532.5874



Compound- **32**

Calculated mass: 1602.118 ($C_{96}H_{122}O_{19}Na$)

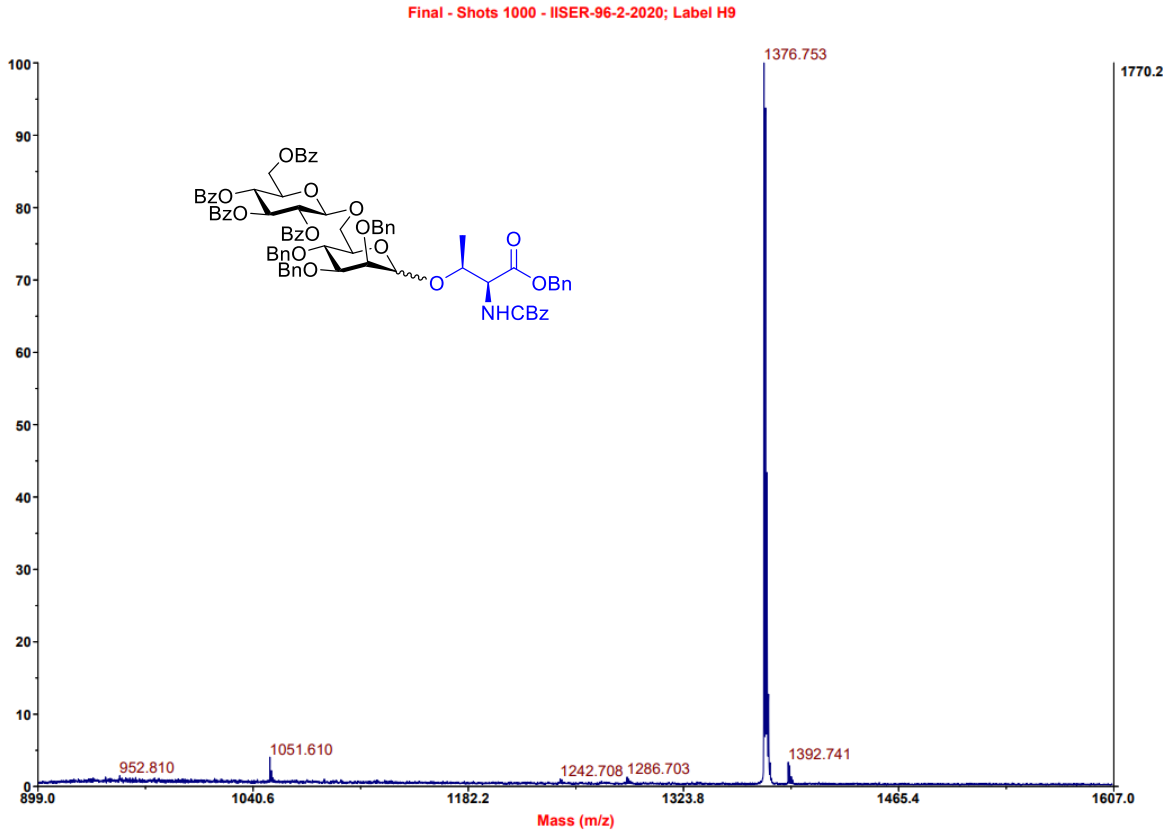
Observed mass: 1602.107



Compound- **34**

Calculated mass: 1376.762($C_{80}H_{73}O_{18}K$)

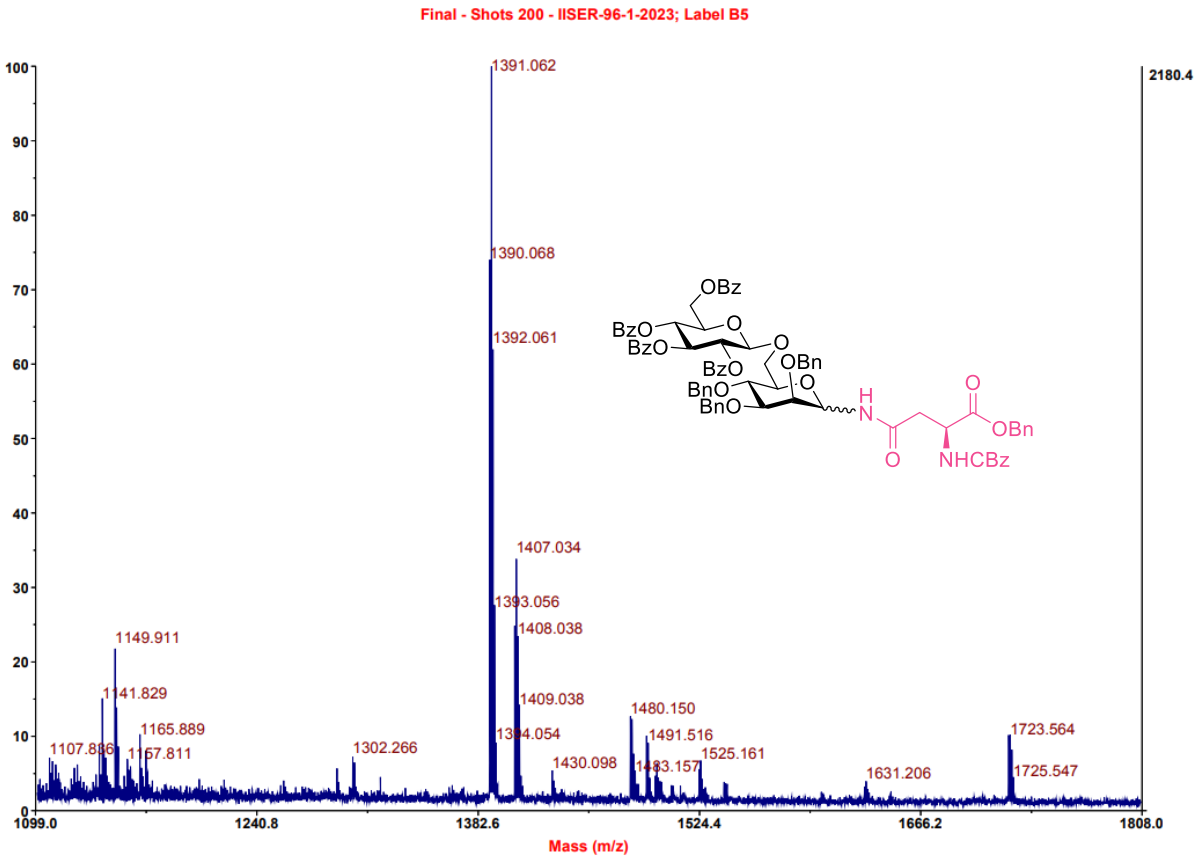
Observed mass: 1376.753



Compound- **39**

Calculated mass: 1391.114($C_{70}H_{61}NO_{15}Na$)

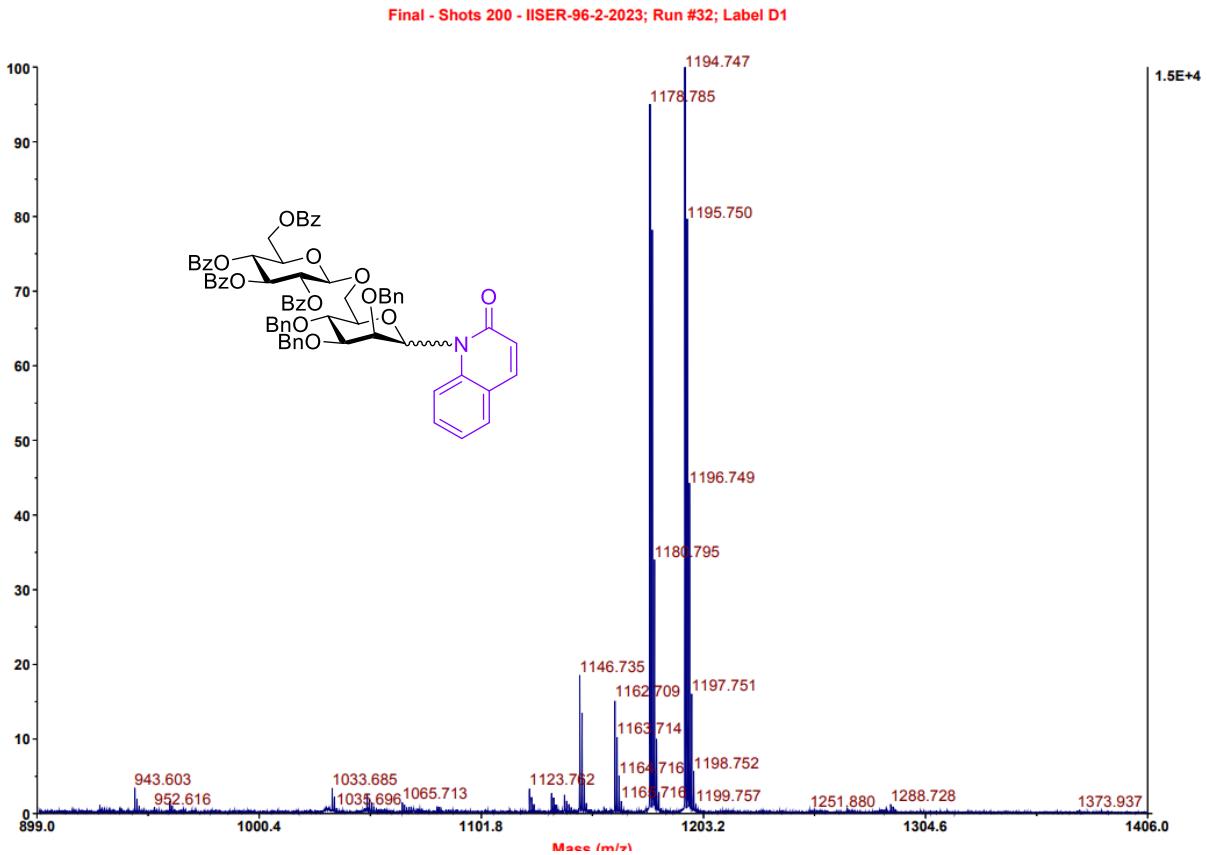
Observed mass: 1391.062



Compound- **41**

Calculated mass: 1178.786($C_{70}H_{61}NO_{15}Na$)

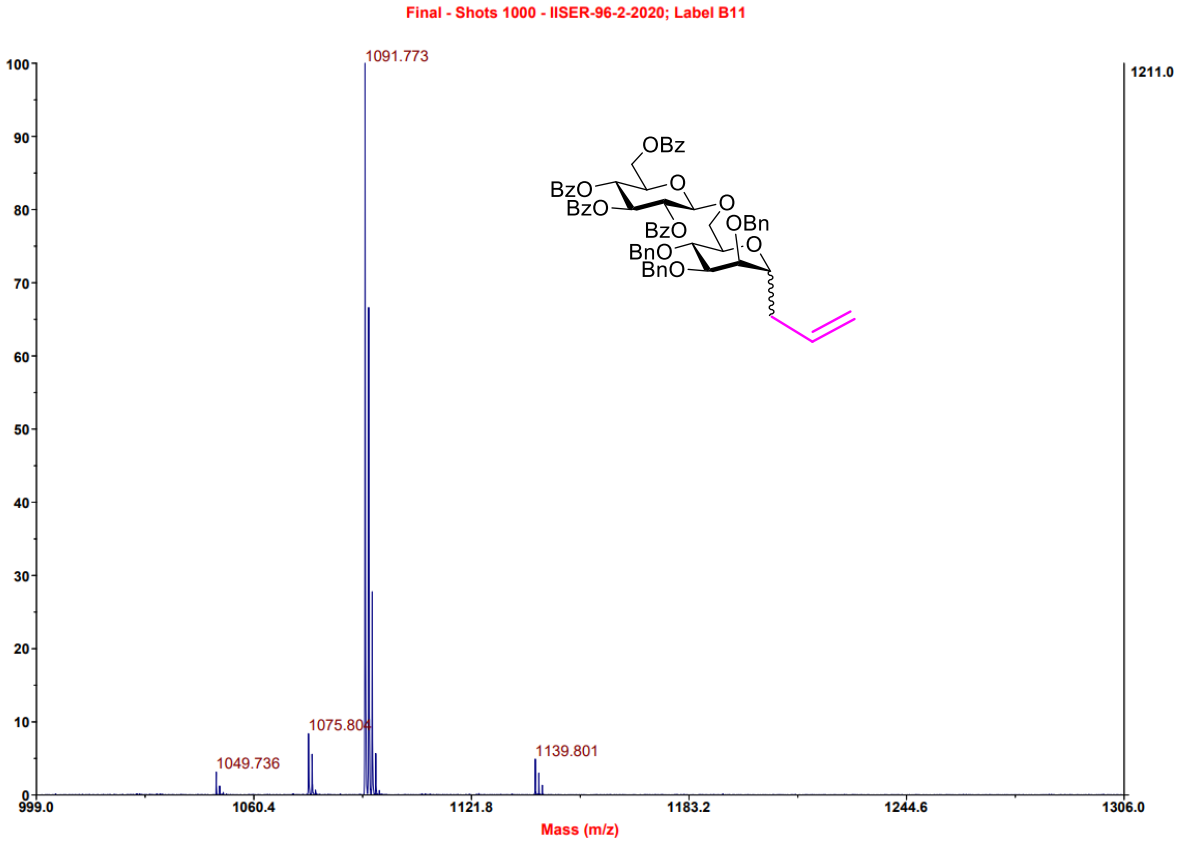
Observed mass: 1178.785



Compound- **45**

Calculated mass: 1091.783 ($C_{64}H_{60}O_{14}K$)

Observed mass: 1091.773



4.6: References:

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List of Publications

1. Cut-insert-stitch editing reaction (CIStER) sequence for surgical chemical glycan editing Sumit Sen, Suman kundu, Sandip Pasari and Srinivas Hotha* In *Commun Chem* 7, 73 (2024). DOI: https://doi.org/10.1038/s42004-024-01152-z
2. Molecular Surgery: Cut-Insert-Stitch Editing Reaction (CIStER) Technique: Srinivas Hotha, Sumit Sen, Suman Kundu, Sandip Pasari - A Patent has been filed: IN 2024 21006807 dt. 01.Feb.2024. Assignee: Indian Institute of Science Education and Research
3. Synthesis of Arabinogalactan Portion of *Mycobacterium tuberculosis* Cell wall Sumit Sen, Sandip Pasari, and Srinivas Hotha* (Manuscript under preparation)
4. Chemical synthesis of glycoconjugates by transglycosylation strategy Sumit Sen, Suman Kundu, Pooja Joshi and Srinivas Hotha*(Manuscript under preparation)
5. Nucleobase Editing of Nucleosides Purines→Pyrimidines/Purines/O-/S-/N- Glycosides Sumit Sen, Suman Kundu, and Srinivas Hotha* (Manuscript under preparation), Patent filing is in progress
6. Othogonal one-pot synthesis of Oligosaccharides Based on Glycosyl ethylcyclohexyl carbonate donor Sumit Sen, Suman Kundu, and Srinivas Hotha*(Manuscript under preparation)
7. Chemical Synthesis of Calix[4]arene *O*-Glycosides by silver assisted gold catalyzed glycosylation strategy Sumit Sen, Pooja Joshi, Gede Kamada and Srinivas Hotha*(Manuscript under preparation)
8. Editing of Trehalose derivatives and synthesis of Methyl Protodioscin Sumit Sen, Suman Kundu, and Srinivas Hotha* (Work in under progress)

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Article

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Cut-insert-stitch editing reaction (CIStER) sequence for surgical chemical glycan editing

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Sumit Sen, Suman Kundu, Sandip Pasari & Srinivas Hotha

Post-synthetic surgical editing enables synthesizing diverse molecules from a common scaffold. Editing carbohydrates by inserting a foreign glycan is still a far-reaching goal for synthetic chemists. In this study, a one-pot-three-step chemical approach was employed to edit glycoconjugates. It is comprised of three steps: the first is a 'cut' step, cleaving one of the interglycosidic bonds and producing an intermediate that could be intercepted with 4-mercaptotoluene; second step activates the thiotolyl glycoside in the presence of an aglycon containing an orthogonally activatable ethynylcycloxyl carbonate moiety; and the third step involves 'stitching' by activating the carbonate donor. The cut-insert stitch-editing reaction (CIStER) is demonstrated by inserting branched and linear arabinans reminiscent of M. tuberculosis cell wall from the same designer trimannoside. Glycosylating an activated hydroxyacid (serinyl, steroidal, and lipid) after cutting the interglycosidic bond and stitching in the presence of base extendes the CIStER approach to the synthesis of glycohybrids.

Nucleic acids, proteins, carbohydrates, and lipids are among the numerous biomolecules produced by living organisms that occur in myriad sizes and structures[1]. These biomolecules perform several biological functions and have been extensively investigated[2]. Additionally, nucleic acid chemistry has advanced owing to the development of excellent molecular biology tools, such as recombinant DNA technology, polymerase chain reaction, DNA amplification, CRISPR-editing, and prime editing[3–7]. These tools have had an oblique impact on the synthesis of proteins as a direct consequence of the central dogma[8], which states that a detailed residue-by-residue transfer of sequential information is possible in a unidirectional way from DNA to RNA, to Protein. Genomic editing techniques play a pivotal role in many fields such as agriculture, the discovery of pharmaceuticals and diagnostic tools, the production of biofuels, and to development of a better understanding of disease biology[9]. In this study, we report a surgical editing strategy (CIStER) for glycans that involves cutting an interglycosidic bond in a regiospecific manner, inserting a foreign glycan, and stitching the glycosidic bond to obtain a hybrid glycan by fine-tuning the subtle reactivity differences and inherent susceptibility to hydrolysis.

Carbohydrates are ubiquitous molecules that perform several biological functions, including signal transduction, cell-cell communication, and cellular development[10]. Carbohydrates exist as conjugates of glycans and aglycons, wherein the aglycon can be a protein, as in glycoproteins; a lipid, as in glycolipids; or a steroid, as in saponins[11]. The isolation of carbohydrates from natural systems is complicated because natural systems are innately microheterogeneous, and post-synthetic correction, amplification, and modification of synthetic or natural glycans are still in their nascent stages.

Some of the most powerful approaches for saccharide editing in glycotechnology include skeletal editing *via* the acid-catalyzed transformation of furanosides to pyranosides[12], Ferrier rearrangement for converting carbohydrates to cyclitols[13], sugar-to-sugar transformations via oxidation followed by reduction[14], uncontrolled editing of cell surface glycans by metabolic oligosaccharide engineering (MOE)[15], and enzymatic addition of glycans (EAG)[16]. In MOE, cells are cultured with a monosaccharide containing a reporter moiety (e.g., alkyne/azide); hypothesizing that the conjugate of the reporter moiety and monosaccharide are incorporated into the cellular processes, the incorporated moiety can guide the investigation of biological pathways including cell imaging using bioorthogonal chemistry (Fig. 1A)[15]. This technique has been utilized for cell imaging and probing biosynthetic machinery. In contrast, EAG exploits the site-specific cleavage of glycosidic bonds using designer glycosidases, and the resulting hydrolyzed glycans are subjected to post-synthetic modifications using glycosyl transferases to attach new glycans/probes (Fig. 1B)[16]. To the best of our knowledge, no editing technique enables the insertion of a foreign glycan into the already synthesized glycans via chemical or enzymatic routes (Fig. 1C).

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