Designing of Linkers and Solid-phase Oligosaccharide Synthesis by Silver-assisted Gold-catalyzed Glycosidations

A thesis submitted in partial fulfillment

of the requirements of the degree of

Doctor of Philosophy

By

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2022

Dedicated to….

My friends and family….

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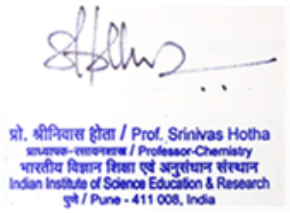
CERTIFICATE

Certified that, the work incorporated in the thesis entitled, "***Designing of Linkers and Solid-phase Oligosaccharide Synthesis by Silver-assisted Gold-catalyzed Glycosidations***" submitted by Yogesh Sutar was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institution.



Date: 19 Dec 2022
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Prof. Srinivas Hotha
(Research supervisor)

DECLARATION

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

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Yogesh Sutar

Contents

General remarks

- ^{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 or Applied Biosystems MALDI-ToF-ToF spectrometer.
- IR spectra were recorded on Perkin-Elmer 1310 or 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 and measured in degrees.
- All reactions were monitored by Thin-Layer Chromatography carried out on pre-coated Merck silica plates (F_{254}, 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 freshly prepared anhydrous solvents under anhydrous conditions, and yields refer to chromatographically homogenous materials unless otherwise stated.
- All evaporations were carried out under reduced pressure on Büchi and Heidolph rotary evaporators 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.
- Scheme, Figure, and Compound numbers in abstract and individual chapters are different.

Abbreviations

Å – Angstrom
Ac – Acetate
Ac_2O – Acetic anhydride
AcBr – Acetyl bromide
AcCl – Acetyl chloride
AcOH – Acetic acid
AG – Arabinogalactan
AGA- automated glycan assembly
AgOTf- silver trifluoromethane sulphonate
Ara*f* – arabinofuranosyl/arabinofuranoside
$AuBr_3$- gold tribromide
$AuCl_3$- gold trichloride
BB: Building Block
$BF_3.OEt_2$- Boron trifluoride diethyl etherate
Bu: Butyl
Bz – Benzoyl
BzCl – Benzoyl chloride
Calcd – Calculated
cat. – catalytic
Cbz – Carbobenzoxy
$CDCl_3$ – Chloroform-D
$CHCl_3$ – Chloroform
d – days
D_2O – Deuterium oxide
DBU – 1,8-Diazabicycloundec-7-ene
DCC- Dicyclohexylcarbodiimide
DEPT – Distortion less Enhancement by Polarization Transfer
DIC – *N,N′*-Diisopropylcarbodiimide
DIPEA – *N,N′*-Diisopropylethylamine
DMAP – *N,N′*-Dimethylaminopyridine
DMDO- Dimethyldioxirane
DMF – *N,N′*-Dimethyl formamide
DMSO-d_6 – Dimethyl sulfoxide (deuterated)
DMTST- Dimethyl(methylthio)-sulfonium triflate
DTBP- di-*tert*-butylpyridine
eq. – equivalents
Et_3N – Triethyl amine
Et_3SiH – Triethylsilane
Fmoc: Fluorenylmethyloxycarbonyl
g – gram
Gal*f* – galactofuranosyl / galactofuranoside
h – hour
HF- Hydrogen flouride
HPLC: High Performance Liquid Chromatography

Abbreviations

HRMS – High-Resolution Mass Spectrometry
HSQC: Heteronuclear single quantum coherence spectroscopy
Hz – Hertz
hν : photocleavage
IR – Infrared
J – coupling constant
LAM – Lipoarabinomannan.
LC: Liquid Chromatography
Lev – Levulinoate
LevOH: Levulinic acid
LG: Leaving Group
M – Molar
m/z: mass-to-charge ratio
MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization-Time of Flight
MeOD – Methanol-D4
MeOTf- Methyl trifluoromethane sulphonate
mg – milligram
MHz – Megahertz
min. – minutes
mL – milliliter
mmol – millimolar
MS – Molecular sieves
MS: Mass Spectrometry
Mtb – Mycobacterium tuberculosis
NaH – Sodium hydride
NaOMe- Sodium methoxide
NGP – Neighbouring group participation
NIS – N-Iodosuccinimide
NMR – Nuclear Magnetic Resonance
NNGP – Non-neighbouring group participation
NPG – n-Pentenyl glycoside
PDA- Photodiode Array
Ph: Phenyl
ppm – parts per million
Py – Pyridine
RP: Reversed Phase
rt – room temperature
sat. – saturated
SPOS- Solid-Phase Oligosaccharide Synthesis
Tb – Tuberculosis
TBAI – tetra-n-Butyl ammonium iodide
TBDPS – *t*-Butyldiphenylsilyl
TBDPSCl – *t*-Butyldiphenylsilylchloride
TCA – Trichloroacetimidate

Abbreviations

TESOTf- Triethylsilyl trifluoromethanesulfonate
TFA – Triflouroacetic acid
TfOH – trifluoromethanesulfonic acid
THF – Tetrahydrofuran
TIPDSCl– 1,3-dichlorotetraisopropyldisiloxane chloride
TLC – Thin Layer Chromatography
TMSOTf – Trimethylsilyl trifluoromethanesulfonate
UPLC: Ultra Performance Liquid Chromatography
UV: Ultraviolet
$ZnCl_2$- Zinc dichloride
δ – delta (in ppm)
μL – microliter

Synopsis

The thesis entitled **"*Designing of Linkers and Solid-phase Oligosaccharide Synthesis by Silver-assisted Gold-catalyzed Glycosidations*"** is divided into three chapters. The first chapter introduces a brief idea about the solid phase and other methods to synthesize the oligosaccharides. The second chapter demonstrates the synthesis and the utility of the olefin metathesis, photocleavable, and modified photocleavable linker for the glycosylation reaction on solid supports. In the third chapter, the development of a silver-assisted gold-catalysed solid phase synthesis method for synthesizing linear and branched oligosaccharides was delineated.

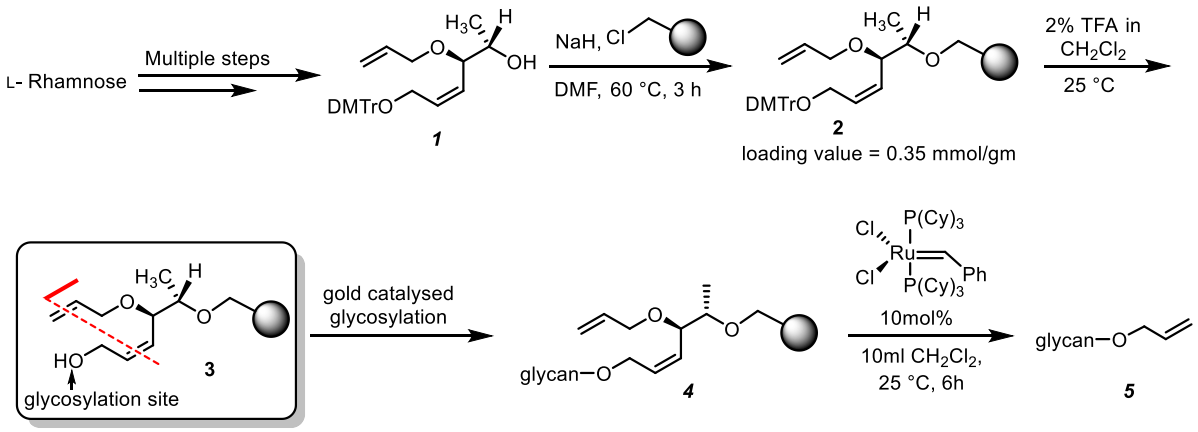
Chapter 1: Introduction to the Solid Phase Oligosaccharide Synthesis

The critical fact about carbohydrates is that they exist in biological systems as less explored biopolymers and are involved in many fundamental processes that occur routinely in all forms of life. Unlike other biopolymers, carbohydrate molecules are a major cause of many diseases and have played an integral role in biological processes. They exist as micro-heterogeneous forms, and their isolation from Nature is tough. Therefore, chemical synthesis is one of the best methods to obtain them in sufficient quantities. In that direction, various chemical and enzymatic methods have been introduced so far in the growing field of carbohydrates. Among all the reported methods, the solid phase method has shown potential advantages concerning simplicity in purification and overall synthetic strategy. Recently, the advancement in automation for glycan synthesis has reached certain heights. But the success of the solid phase technique depends on the choice of solid support, linker, and glycosylation method. The Merrifield resin has been used as the most reliable support for solid phase oligosaccharide synthesis. But, the selection of the linker and glycosylation method has maintained remarkable trouble for the glycochemists as both of the entities contain reactive functionalities. Further advancements in linker development has brought out the olefin metathesis, and the photocleavable linker, as the most frequently used and compatible linkers for the available glycosylation methods. Moreover, their utilization for the various glycosylation and deprotection reaction conditions have some drawbacks. Among the plethora of glycosylating agents, trichloroacetimidate donor, thioglycosyl donor, and phosphate donors have proven their strength for synthesizing the range of glycans using solid phase chemistry. But the reaction temperature, time, and number of donor equivalents are some of the obstacles.

Chapter 2: Synthesis of Linkers for the Solid Phase Oligosaccharide Synthesis

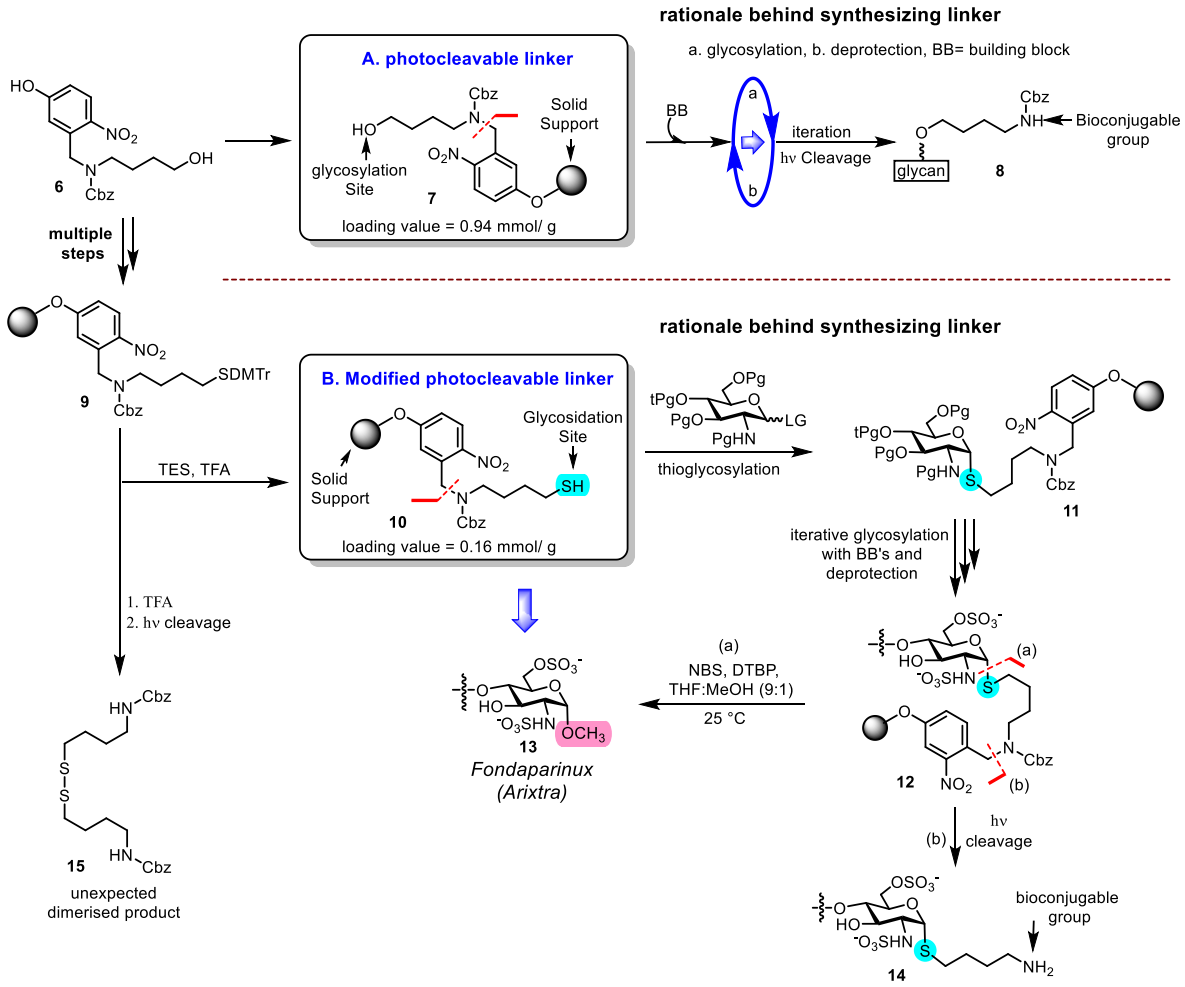
The choice of linker plays a major role for the successful implementation of the gold-catalyzed glycosidations to the solid phase chemistry. The solid phase oligosaccharide synthesis involves various building blocks, activators, and other reagents for the glycosylation and deprotection steps, under which the survival of the linker is essential. Additionally, its cleavage, whenever required, should offer a higher yield. Therefore, choosing such characteristic linkers for glycosylation chemistry over solid support has remained a prime obstacle.

Among the variable types of linkers, the olefin metathesis cleavable linkers have shown a significant impact in synthesizing a range of glycans over the solid support. Fréchet and Schuerch's allyl alcohol linker attached support, Schmidt's metathesis cleavable linker, and Seeberger's octane diol linkers are noteworthy. Out of these, The Schmidt linker was found to be advantageous as it involves Grubbs catalyst I alone for the cleavage. But the synthetic protocol for synthesizing the linker is not very user-friendly. We thought of developing a new simple, scalable route for the similar linker from L-rhamnose. After multiple steps, the sugar ring was opened to get the desired diene **1** and was attached to the Merrifield resin using sodium hydride in DMF (loading 0.35 mmol/g). Subsequently, the DMTr moiety was deprotected and the gold-catalyzed glycosylation was performed using Au-phosphite and AgOTf in CH_2Cl_2 at room temperature. The glycan was released from the solid support by employing the ring-closing metathesis (RCM) using Grubbs' metathesis reaction. Thus liberated glycan will have the olefin functionality at the reducing end, which was confirmed thoroughly by various spectroscopic techniques.



Attaching biomolecules to the olefin functionality at the reducing end is a cumbersome task. Hence, we sought for linkers that will enable the synthesis of glycans with amino functionality at the reducing end so that those can be directly used for the bioconjugation or for printing

them onto glass or microarrays. In this connection, yet another linker that can be cleaved off by using light is explored. The linker and the photocleavage conditions will be orthogonal to most of the glycosylation methods and deprotection cycles. Therefore, we envisioned the synthesis of a photocleavable linker as the most suitable for gold-catalyzed glycosylation. We synthesized the designer linker by adopting features of the known protocol reported by the Seeberger group. Thus, the synthesized linker was attached to the solid support by aforementioned conditions, and the loading value was calculated. The utility of this photocleavable solid support was utilized in Chapter 3, or the synthesis of arabinan.

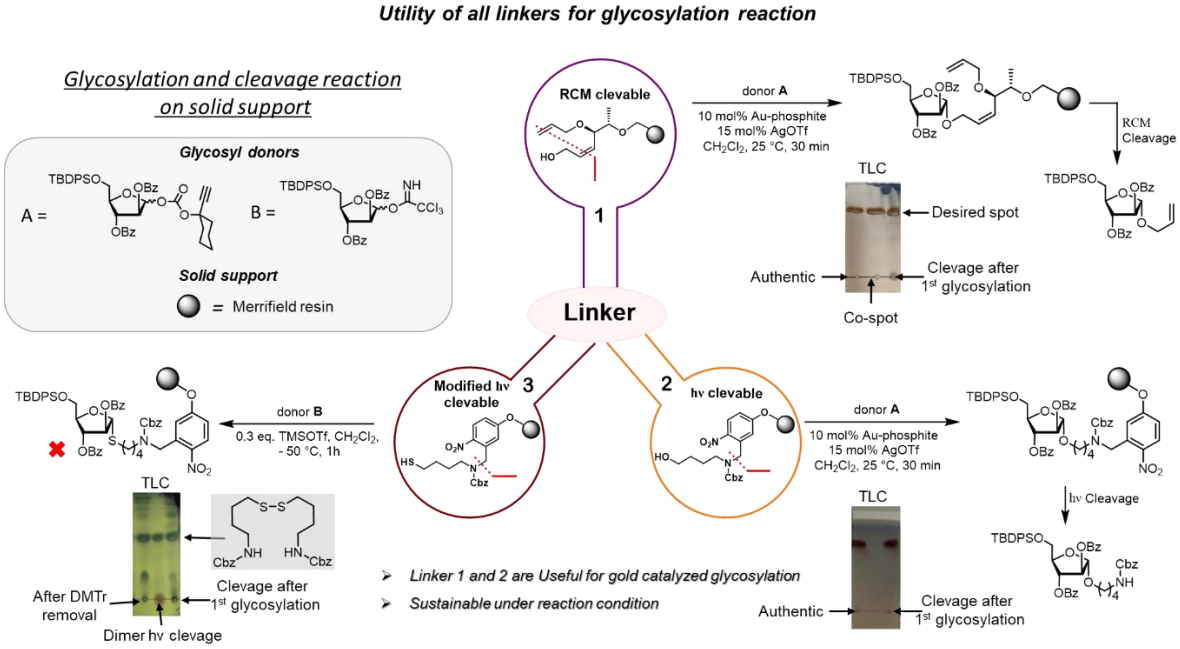


Furthermore, we envisioned that the thioglycosyl linkers would be beneficial for the installing group at the reducing end of the glycan. In this direction, the dual cleavage strategy would be highly useful for two reasons: (i) the routine monitoring of the reaction progress on the solid support can be performed under photocleavable conditions, (ii) the final release of the glycan from the solid support can be accomplished using thiophilic reagents in the presence of methanol. Also, the thio-glycoside can be explored to probe its medicinal value as it is isostere to that of the *O*-glycoside.

Accordingly, the photocleavable linker **6** was transformed to the corresponding thiol derivative under acidic conditions. The thiol linker could produce the α-selective *S*-glycosidic product that can be cleaved off using NBS/MeOH to obtain the α-OMe glycosidic product **13**. The functional group transformation from –OH to –SH under basic conditions afforded the dimerized product. To overcome this, triethylsilane and trifluoroacetic acid were added to suppress the formation of the dithio-compound.

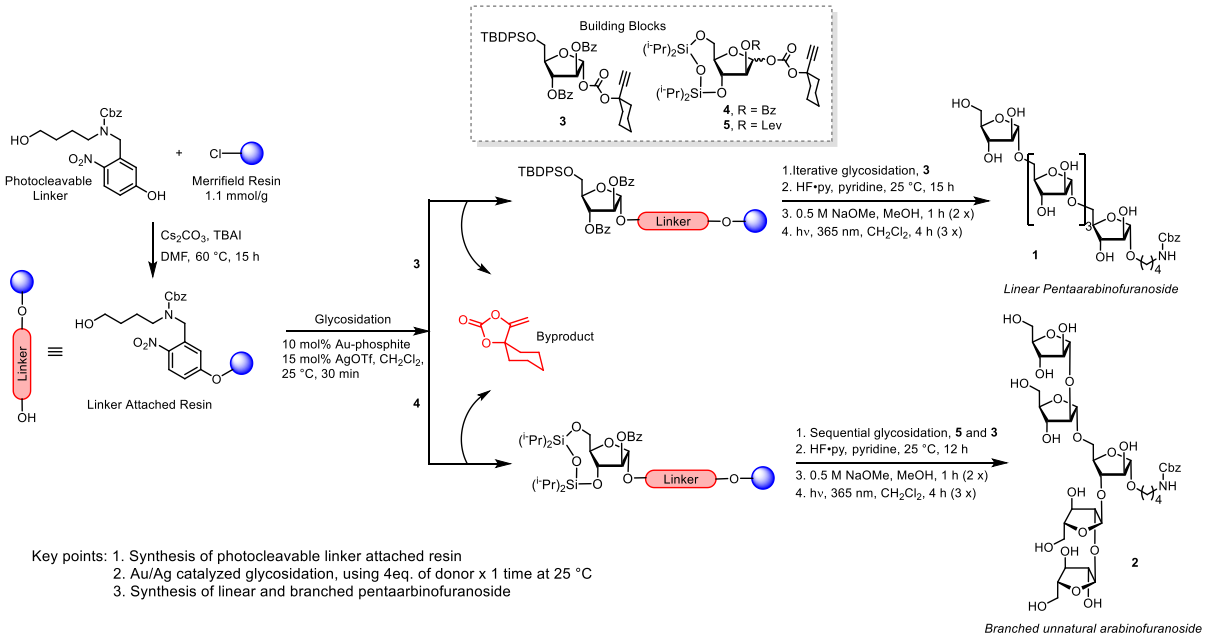
The linker was successfully attached to the chloromethyl Merrifield resin and calculated the loading value. However, to our dismay, the glycosylation failed on the solid support **10** at -50 °C using a trichloroacetimidate donor and the TMSOTf. Further experiments showed the lack of free thiols on the solid support, which was confirmed after releasing the glycan from the solid support. This was further confirmed by conducting the glycosylation with the O-linked solid support which cleanly afforded the desired product.

In addition, the photocleavage reaction after the DMTr removal using TFA alone as well as in the presence of TFA and TES afforded the dimerized linker. The formation of the dimerized product **15** was confirmed by various spectroscopic techniques. This experimental evidence corroborates that the glycosylation failure on the solid support is because of the non-availability of the free thiols. In conclusion, the synthesized support **10** can be used for the thio-glycosylation only when the free thiol is available.



Chapter 3: Silver-assisted Gold-catalysed Solid Phase Synthesis of Linear and Branched Arabinans

One of the major research themes in our group is the synthesis of large fragments of the mycobacterial cell wall. In this connection, we have successfully synthesized oligosaccharides having 21, 25, and 21-sugar residues which are part of the cell wall of mycobacteria. The *Mycobacterium tuberculosis* cell wall outer layer is composed of arabinogalactan and peptidoglycan. The AG and LAM are also major components, which are the long chain of the arabinofuranosides in a linear or branch fashion. As part of the major research program directed towards understanding the biophysical and biochemical significance of these AG and LAM, a library of glycolipids is required. In this direction, we thought of using solid phase chemistry to synthesize oligosaccharide fragments. Over the last two decades, various furanosides and pyranoside compounds have been well-tackled by solid-phase chemistry. Earlier efforts from our group showed that the gold-catalyzed solution phase glycosylation method is superior as it is faster, high yielding, and homogenous in conditions.



Linker attached Merrifield resin obtained in Chapter 2 was utilized to explore the silver-assisted gold-catalyzed glycosidation for the solid phase oligosaccharide chemistry. Initially, the number of equivalents of the donor and its concentration, catalytic loading of the activators, and total reaction time to find out the appropriate condition was investigated. Using 4 equivalents of alkynyl carbonate donor solution (300 mg in 2 mL) with the 10 mol% of the gold-phosphite and the 15 mol% of the silver triflate in dry dichloromethane at room temperature are optimized to furnish the desired glycosidic product over the solid support.

Moreover, we have also developed the protocol for the deprotection of the temporary protecting groups such as TIPS, TBDPS, Bz, and Levilonoyl moieties. Utilizing these developed protocols, the linear synthesis of pentaarabinofuranoside in a stepwise manner was accomplished using a single building block **3**. The progress of the reaction at each step was monitored using TLC and UPLC-MS analysis.

After the completion of linear pentasaccharide synthesis, the portion of the resin was cleaved using 360 nm wavelength light, and the remaining amount was treated with the HF•Py and the NaOMe reagent to carry out the global deprotection over the solid support. Then the cleavage was performed to get a fully deprotected glycan molecule bearing aliphatic linker equipped with the amino functionality.

The synthesis of branched pentasaccharide was also accomplished with a 1+2+2 strategy using the standardized gold catalysed condition. Here, in this case, the simultaneous glycosylation using 8 eq. of the donor helped in the completion of the reaction, indicating that the developed protocol is potent and reliable. The progress of the reaction was monitored by TLC and UPLC-MS analysis. Sufficient quantities of the glycans were obtained for recording the NMRs for the fully protected trisaccharide and the pentasaccharide fragments. After performing global deprotection over the resin, the cleavage reaction was performed to obtain the fully deprotected branched pentaarbinofuranoside. NMR and mass analysis identified the formation of all these products.

In conclusion, efficient solid phase syntheses of linear and branched pentaarabinofuranosides were accomplished using Merrifield resin, photo-cleavable linker, and orthogonally protected building blocks. On bead deprotection of silyl and benzoate protecting groups and the use of gold-catalyzed glycosidations are the key features of this endeavour. All the glycosidations were performed with four equivalents of the donor and noticed that one-time coupling was sufficient to afford excellent coupling efficiency. Thus synthesized glycans are equipped with protected amino functionality that can be exploited for ligating biomolecules such as proteins.

In summary, three new linkers were synthesized, immobilized onto solid supports, and explored for the silver-assisted gold-catalyzed glycosidation conditions. Further, the synthesis of linear and branch pentasaccharides on solid supports is accomplished. The linker chemistry enabled the release of the glycans from the solid supports either by using ring-closing metathesis or the light of 365 nm wavelength.

Chapter: 1

Introduction to the Solid Phase Oligosaccharide Synthesis

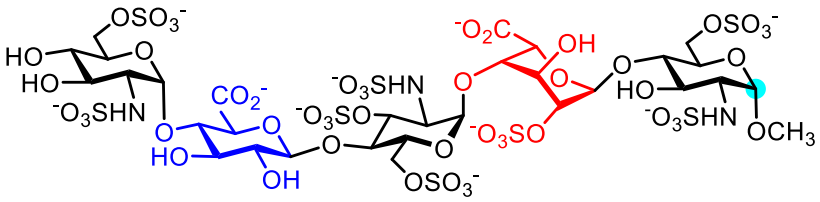
Chapter 1

Introduction to carbohydrates as a crucial cell wall component

The living system comprises three essential biopolymers: oligonucleotides, oligopeptides, and oligosaccharides.[1] Out of which oligonucleotides, oligopeptides biopolymers have reached a significant height in the development of science owing to the rapid and easy access. In the first instance, carbohydrates were identified as the energy source in living organisms. But then, these contingent carbohydrates are characterized as a long chain of monomeric units called "oligosaccharides." They are strange micro-heterogeneous, very complex in character, and along the same line, their isolation from nature is very effortful. These oligosaccharides have made their natural existence unique in structure, biological role, and composition. Recently, they have gained profound attention owing to the fundamental understanding that oligosaccharides play a crucial role in processes such as inflammation, immunological response, metastasis, and fertilization.[2] It has now been well established that carbohydrates on the cell surface are biological markers for different cancers and act as sites at which other substances, including pathogens, can attach.[3]

Biological Significance of Glycoconjugates

Glycoconjugates are oligosaccharides that occur in the living organism. They are either attached to macromolecules such as glycolipids and glycoproteins or long linear or branched oligomeric chains of the self-monomeric units. The glycoconjugates on the cell surface form a thick layer of various glycans. The cell walls of humans and pathogenic bacteria consist of a dense layer of complex glycocalyx, such as lipopolysaccharide and polysaccharide. These glycan surfaces are very characteristic and possess high diversity in glycans. Importantly, these complex glycans act as a protective antigen and are a significant virulence factor for the disease. During the pathogenesis, pathogens cause initial adhesion to host cells, and ultimately cause invasion of organism. According to biological investigation, this surface is responsible for recognizing, communicating, invading, and so forth; therefore, it could be used to diagnose and treat diseases.[4, 5] In recent years, several diseases have been successfully treated using naturally occurring carbohydrates and their derivatives for example, the carbohydrate-based drug heparin acts as an anticoagulant agent, thereby valuable for antithrombotic therapy.



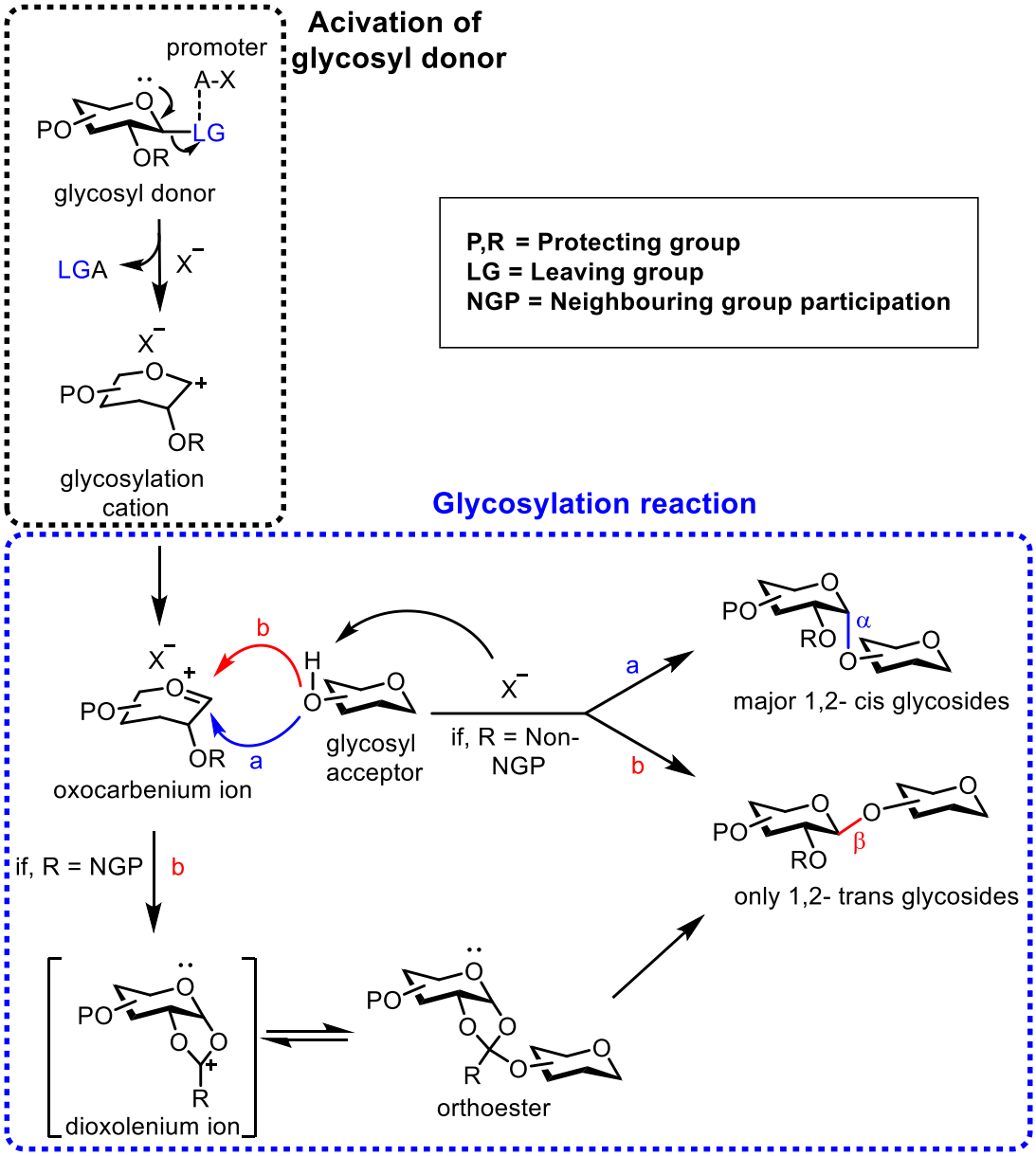
Fondaparinux (Arixtra)

Figure1.1: Chemically synthesized ultra-low molecular weight heparin

This molecule's structure was described as complex and heterogeneous in 1916 after first being isolated from dog liver. Heparin can bind to proteins, thereby showing its biological activity. The chemically synthesized third-generation heparin is ultra-low molecular weight, a negatively charged, and highly sulphated linear polysaccharide structure, in which D-glucosamine (GlcN) and D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) are held in place by 1,4-glycosidic bond. It is commonly known as fondaparinux (Arixtra®). In this fondaparinux sodium, the K_d value of 41–58 nmol/L and have a high binding affinity for AT–III. Upon binding, AT-III undergoes an irreversible conformational change, which inhibits factor Xa and induces thrombin and fibrin formation.

Chemical glycosylation

Chemical glycosylation is a reaction between the glycosyl donor and acceptor involving a promoter or an activator. The activator promotes the leaving ability of the anomeric group attached to C1 of the glycosyl donor and results in the formation of the glycosyl cation. This glycosyl cation gets stabilized by the endocyclic oxygen to form an oxacarbenium ion. Depending upon the presence or absence of participating (NGP group) at the C2 position, this glycosylation reaction produces either 1,2-*cis* or 1,2-*trans* products. If the C2 position has an ester, i.e., participating group, it stabilizes the oxacarbenium ion through the carbonyl group, thereby forming a trioxolenium ion. This intermediate, in turn, forms an orthoester and subsequently gives only *trans*-glycosidic product as the ring's lower face is blocked. On the other hand, if the non-participating group, such as –OBn, is present at C2, then the approaching glycosyl acceptor connects either from a top face or a bottom face and offers a mixture of cis/trans isomers. But in some instances, the 'anomeric effect' leads to a cis-selective product as a major product.



Scheme 1.1: Glycosylation reaction mechanism

So, the protecting group present on the reacting partner and the type of reaction mechanism involved in the reaction decide the glycosylation reaction's outcome. Glycoconjugates or oligosaccharides are nothing but the long chain of the monomeric glycan unit that happens through the glycosylation reaction. This reaction is concerned with different building blocks used, and many studies have been done on the leaving ability of the leaving group. Different approaches, like directing or neighbouring group participation at the C_2 position or remote participation, induced torsional effects, etc., have been introduced concerning the stereoselective glycosidic product. In addition to the reactivity of glycosyl acceptor, several other factors determine the outcome of the chemical glycosylation, such as temperature, solvent, amount, and type of promoter used.

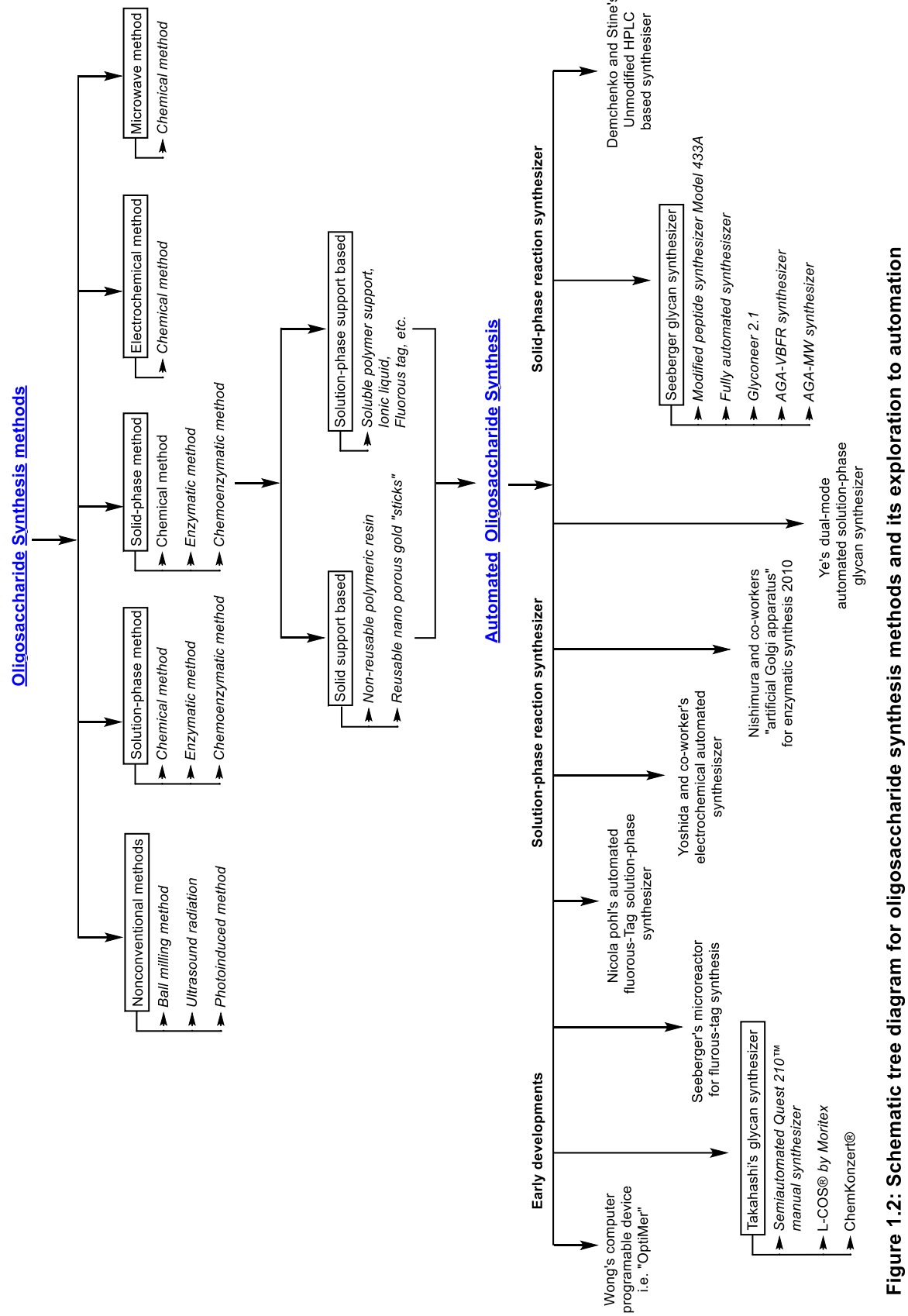


Figure 1.2: Schematic tree diagram for oligosaccharide synthesis methods and its exploration to automation

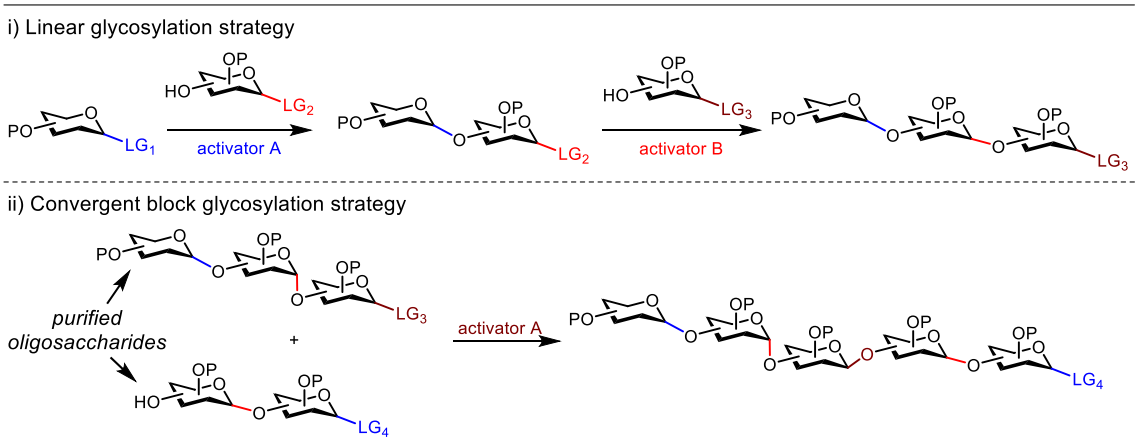
Methods for oligosaccharide synthesis:

1) Solution phase methods:

The synthesis of oligosaccharides in the solution phase can be done either in a chemical, enzymatic, or chemo-enzymatic way.

a) Chemical glycosylation method: The solution phase oligosaccharide synthesis occurs via interaction between the homogeneous mixture of glycosyl donor and the glycosyl acceptor with the Brønsted or Lewis acid as activators. The glycosyl donor usually contains anomeric leaving group, whereas the glycosyl acceptor possesses free hydroxyl, which acts as a nucleophile. The additional interfering reactive –OH, - NH_2 functionalities present over both reacting partners become non-interfering because of the protection strategy and remain intact throughout the reaction. For example, the Koenigs-Knorr reaction.[6]

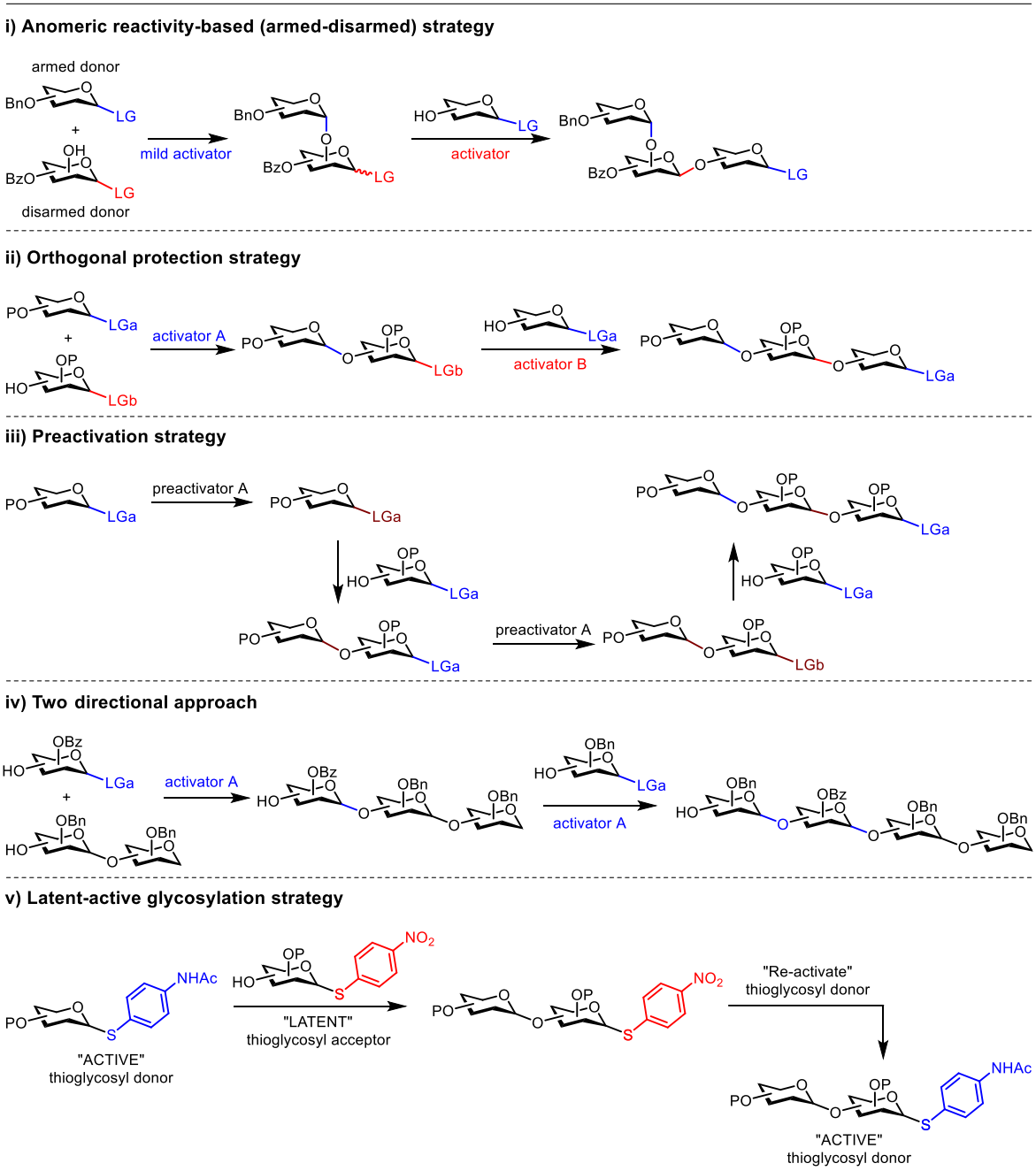
Linear and convergent block strategy: The oligosaccharide synthesis can be achieved either by a traditional way of connecting reacting partners in a stepwise linear fashion or a convergent manner. Stepwise synthesis involves linear connection of building blocks that, in turn, result in the drawback of purification at every stage (i.e., after each glycosylation and deprotection) during the process, leading to an effortful and time-intensive method. On the contrary, the convergent synthetic method relaxes these problems to some extent. It becomes a faster way of synthesizing the oligosaccharides, which involves a reaction between separately synthesized oligomer partners. This method also allows the synthesis of complex linkages by offering a higher yield. The blood-group determinant H-type II pentasaccharide[7] and the starfish ganglioside GP3,[8] synthesis have been successfully carried out by linear and convergent synthetic strategies, respectively.



Scheme 1.2: Traditional approaches for oligosaccharide synthesis

One-pot glycosylation method: One-pot glycosylation strategy is an expedited strategy consisting of sequential glycosylation reactions with a minimal number of deprotection steps.

In this method, the whole synthesis happens in one flask without the purification of intermediate products. The purification occurs only at the final stage of the synthesis.



Scheme 1.3: One pot glycosylation strategies and latent activation strategy

This one-pot glycosylation can be carried out in four different ways. i) Anomeric reactivity-based strategy, in which the electron donating and withdrawing groups present over the glycosyl donor differentiate its anomeric reactivity. The donor with an electron donating group (armed glycosyl donor) is more reactive than those with an electron withdrawing group (disarmed glycosyl donor). This is based on the Fraser-Reid[9] concept of armed-disarmed, which was further explored by Ley,[10] Wong,[11] Boons,[12] Bols,[13] and Demchenko[14-16] to predict

the reactivity of donors. ii) In the orthogonal protection strategy, selective activation of one glycosyl donor in the presence of another glycosyl donor without affecting the protecting groups helps to construct oligosaccharides. For example, Ogawa and co-workers have shown the activation of a thiophenyl glycosyl donor in the presence of a fluoride donor.[17] iii) In the preactivation strategy, the preactivated glycosyl donor reacts with the glycosyl acceptor, which has the same leaving group. The resultant glycosyl donor can be activated again similarly until the desired oligosaccharide is formed. This strategy offers high benefits because leaving the group does not require fine-tuning. Using this strategy, Ye and co-workers have recently synthesized the 92-mer mycobacterial arabinogalactan fragment.[18] iv) In the two directional approaches, the building blocks capable of acting as a glycosyl donor first act as an acceptor later in the synthesis. In this, the electron-withdrawing group at the surrounding position suppressed the reactivity of the glycosyl donor, thereby generating regioselectivity differently in the reacting partners. Zhu and Boons have carried out the pentasaccharide synthesis using this strategy.[19] v) The latent-active glycosylation is another helpful strategy for synthesizing oligosaccharides. In this, a stable anomeric functional group is converted into the good leaving group using interconversion reaction.[20, 21]

b) Enzymatic and chemo-enzymatic glycosylation method:

Although the chemical synthesis method is the most reliable method for synthesizing structurally well-defined oligosaccharides, in some instances, it creates complexity and becomes very challenging and less efficient. For example, connecting sialic acid residue by the chemical method is a daunting task, but that can be efficiently achieved using unprotected glycans in a very stereo and regioselective manner by the enzymatic method.[22] Therefore, the enzymatic method is another alternative to the chemical method. This reaction occurs without eliminating toxic side products and requires an aqueous solution with particular PH, a slight temperature, and, sometimes, the presence or absence of metal ions. The effectiveness of the enzymatic method can be well understood by Globo H hexasaccharide synthesis, whose chemical synthesis needed 11 steps with a 2.6% yield. [23] In contrast, enzymatic synthesis requires only four glycosylation steps, offering a 57% yield.[24] However, the major bottleneck is the poor availability of enzymes to tackle the diverse natural and unnatural glycan structures. Additionally, the enzymes work in a very specific way toward reacting partner. For example, E. coli O-antigen synthesis requires acceptors with a lipid tail.[25]

Dolichol phosphate sugar is used as a glycosyl donor in the N-glycan synthesis, which is insoluble in water and, therefore, difficult to prepare. To deal with this problem, the chemo-

enzymatic method has arisen, which combines chemical and enzymatic methods. Chemo-enzymatic synthesis can be done in three different ways: i) chemical synthesis of core oligosaccharides structure and then enzymatic extension - this is useful for oligomeric structures of very diverse and complex low molecular weight N-glycans.[26-29] ii) enzymatic synthesis followed by chemical synthesis – it is useful in ligating oligosaccharides to protein or printing them on microarray.[30] iii) modification of sugar residue with unnatural groups by enzymes – this is useful for producing oligosaccharide derivatives, which can be used as probes for studying protein-carbohydrate interactions and developing carbohydrate-based vaccines.[31]

2) Electrochemical glycosylation method:

Electrochemical glycosylation is the novel platform that Ryoji Noyori and Isao Kurimoto introduced in 1986 for oligosaccharide synthesis.[32] Later on, it was proved that chalcogenoglycosides could be easily activated selectively based on the reactivity control of glycosyl donor and acceptor by an electrochemical method.[33-38] A specially designed H-type divided cell with a carbon-felt anode and a platinum plate cathode facilitates the reaction. Customary activation of glycosyl donor happens at the anode; undergoes preactivation, which results in an unstable intermediate that forms a stable glycosidic bond when allowed to react with the glycosyl acceptor. This reaction occurs at a very low temperature (-80° C) and requires around 1.00 F/mol of electricity. In recent years, by using an automated electrochemical synthesizer Nokami and co-workers have synthesized linear hexamer using a thioglycoside donor in the solution phase.[39] Along with the thioglycoside donor, its glycosyl sulfonium ion has also been utilized as a valuable intermediate for this chemistry because it is highly stable and reactive at elevated temperatures.[40] Furthermore, they can be used as glycosyl donors in conjunction with thioglycosidic acceptors, thereby offering a platform for the elongation of the oligosaccharide chain in an orthogonal fashion.[41] Electrochemically induced glycosylation using glycopyranose orthobenzoates by Noshiyama and co-workers,[42] generation of "cation pool" from phenylthioethers by Suzuki et al.[43] and the electrochemical generation of $ArS(ArSSAr)^{+}B(C_6F_5)_4^{-}$ as an activator for thioglycosides by Yoshida and co-worker are noteworthy in this method.[44]

3) Microwave-assisted method:

This method generally uses microwave radiations of 300MHz to 300GHz for carbohydrate reactions. The microwave radiation generates heat during the reaction, accelerates the reaction rate, and completes the reaction with a high yield without producing much side products. The

initial efforts of Poulsen and co-workers[45] proved that alkyl and aryl glycoside derivatives D-glucose, D-galactose, and D-mannose, as well as *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine, could be synthesized within a short time without the use of solvents. Subsequently, Lin and co-workers[46] have also demonstrated microwave-assisted glycoside formation from *exo*-glycal as a donor without using a catalyst. In contrast, Yoshimura et al.[47] found that $Yb(OTf)_3$ could activate methyl glycosides. Nishimura et al.[48] have demonstrated the microwave radiation method by synthesizing Lewis X oligosaccharide at low temperatures without the formation of unwanted by-products, which was the only case for the reaction at low temperatures without the use of microwave irradiation.

4) Nonconventional methods:

A few nonconventional methods are also helpful for oligosaccharide synthesis. The mechanochemical ball milling method is another valuable tool for glycosylation chemistry. Metal carbonates mediated synthesis of O-glycosides by Kartha et al. and In(III) triflate mediated diastereoselective synthesis of thioglycosides are examples of this method. A few groups have synthesized glycosyl azides[49] and thioglycosides[50] from glycosyl chlorides/bromides without using solvent and activator.

Another way by which carbohydrate reactions have been performed is the use of ultrasound radiation. Chang group[51] demonstrated the excellency of this technique by performing functional group transformations using carbohydrates. Recently, a wide variety of reactions such as acetylation of free and hindered sugars, removal of protecting group, synthesis of thioglycosides from acetylated sugars using Lewis acids (TMSOTf, BF_3•OTf, TfOH, and AgOTf), construction of glycosyl azides from glycosyl bromides, etc. have been carried out successfully under ultrasonic light within a shorter time.[52] The use of solvent has shown a significant role in these reactions. Recently, the Galan group[53] has demonstrated the synthesis of an α-linked aminopropyl spacer attached to mucin-type O-glycan probes using the sonication method.

The use of light has also shown fewer results in carbohydrate reactions as it generates a diverse class of intermediates. The first report for O- glycoside synthesis was from Griffin et al.,[54] who introduced the generation of glycosyl cation from thioglycosides using 1,4-dicyanonapthalene (DCN). Later, Nakanishi et al.[55] reported UV radiation-mediated cleavage of unprotected thioglycosides in the presence of DDQ. The recent developments in this area include the activation of donors using photo light and the triflate salts that act as photo-

catalysts, activation using UV radiation and a specific activator, and activation via a visible light catalyst, which permits single electron transfer (SET) mechanism, and so on.

Solid phase method: This technique is now well-developed and popular for oligopeptides and oligonucleotide synthesis. However, oligosaccharide synthesis is still forthcoming and continuously evolving concerning automation and glycosylation methods used for the synthesis over the last 50 years. All other methods, including solution-phase, are associated with work-up followed by chromatographic separation to obtain the pure form of glycan. This purification process is the most time-consuming and laborious for all synthetic glycochemists. In the solid-phase method, the reaction happens over the insoluble support, and the washing step can easily remove the excess reagent and by-product formed in the reaction. This reduces overall reaction time in its synthetic strategy at each step. Consequently, that makes the solid-phase method superior to any other existing methods. The schematic representation of the whole process is shown below.

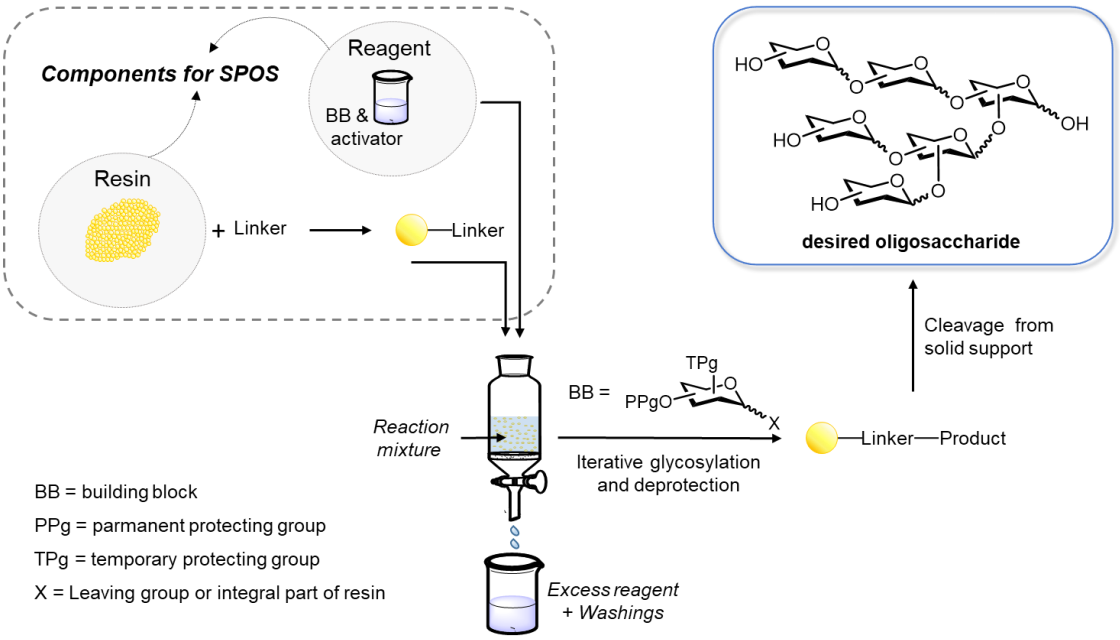


Figure 1.3: Schematic diagram for solid phase oligosaccharide synthesis

Solid-phase oligosaccharide synthesis:

❖ Advantages of Solid-Phase Synthesis:

The solid-phase method involves a correct combination of solid support, linker, and glycosylation method for synthesizing the desired glycan. After an appropriate choice of the linker and the solid support, the connection of the first sugar building block can be performed through a glycosylation reaction or organic reaction (for donor-bound strategy). Further, we can extend the chain by removing the temporary protecting group (deprotection) or leaving

group (glycosylation). So, sequential glycosylation and deprotection steps could help to achieve various glycans with stereospecificity. At last, the oligosaccharide can be detached from the solid support using the prerequisite cleavage condition and purified using HPLC. The purification at each step can be achieved by simple filtration since only the developing molecule remains bound to the support. That automatically eliminates the characterization step at each intermediate step involved in the synthesis. Additionally, the reaction yield can be improved with excess reagents. Furthermore, the process can be automated[56] due to its simplicity which helps non-specialists to synthesize oligosaccharides. One major advantage of solid-phase oligosaccharide synthesis is the ability to construct libraries of oligosaccharides.

❖ **Disadvantages or Problems in Solid-Phase Synthesis:**

Despite all the pros of solid-phase chemistry, this technique requires extensive effort to translate the solution-phase reaction to a solid-supported reaction. Also, before designing such reactions, choosing a suitable linker and reliable solid support is essential; sometimes, that brings limitations, and in most cases, it demands synthesis. In addition, this chemistry necessitates additional mandatory steps, i.e., linker attachment and cleavage of the final glycan from the solid support, and is generally suitable for preparing the final product in small quantities (~100mg).

❖ **Why solid-phase synthesis method is so important in oligosaccharide synthesis?**

The synthesis of oligosaccharides is quite challenging in comparison to oligopeptides and oligonucleotides. Both biomolecules exist as linear structures, whereas glycan majorly exists as a very complex branched structure. In addition, the monomeric unit of oligomer associated with -OH and $–NH_2$ functionality can lead to the unwanted glycosidic product while performing glycan synthesis. In that respect, the optimistic strategy of protection and deprotection always helps and favours desirable connections. However, achieving such reactions through traditional methods in the solution phase has remained daunting and time-consuming. As well as, the reaction response typically ends up with by-products, of which removal from the pure product is necessary at every intermediate stage to get its pure form. Moreover, the assortment in the number of steps involved in synthesis seeks a technique that can perform the same business effectively within a shorter time. The solid phase technique has attained this requirement exceptionally well and has proven its power in genomics and proteomics.

Factors affecting oligosaccharide synthesis

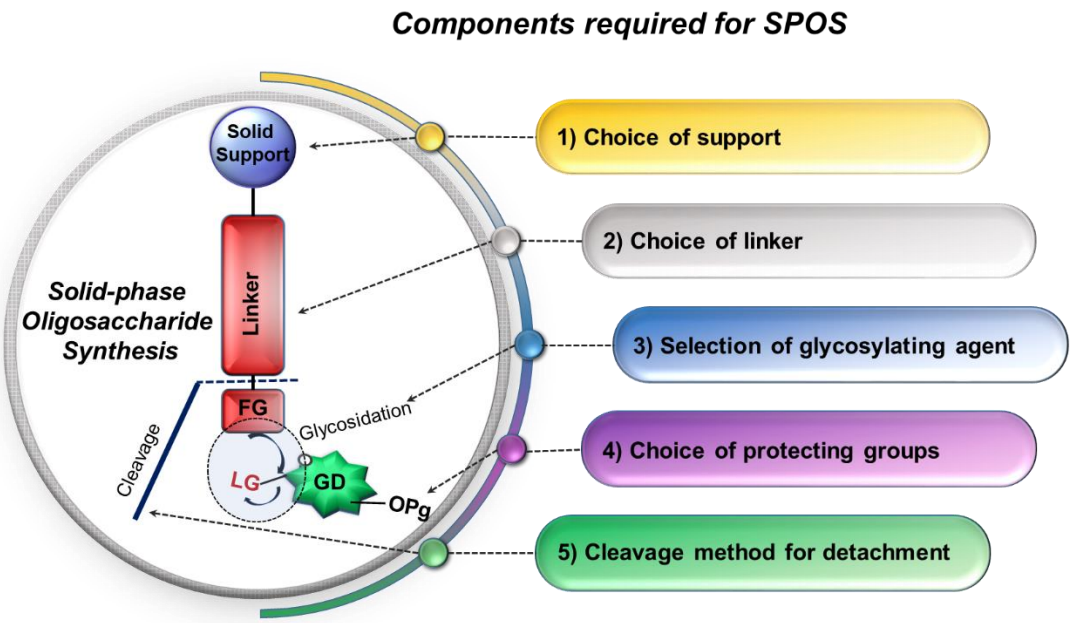


Figure 1.4: Components required to perform Solid-Phase Oligosaccharide Synthesis

1) **Choice of support:** In solid-phase chemistry, solid support acts as the support for the reagents and/or substrate used in the reaction. Two types of supports have been used in oligosaccharide synthesis, i.e., i) soluble support or ii) insoluble support.

Insoluble Supports: The functionalized insoluble polymer support used in the reaction must undergo appropriate swelling or solvation under the reaction condition. Additionally, it should offer adequate pore size, volume, and chemical and mechanical stability to the resin under the reaction conditions. Importantly, it should be stable under the cleavage condition employed for the cleavage of the desired substrate from the solid support. Nowadays, there are various types of polymeric solid supports available. There are two main types of solid supports: polystyrene (PS) and polyethylene glycol (PEG). Merrifield[57] resin [polystyrene (PS), cross-linked with 1% divinylbenzene] is most widely known in solid-phase oligosaccharide synthesis owing to its better swelling property, possessing high loading capacity, chemical inertness to most reaction conditions, and low price. This polystyrene resin swells only in non-polar solvents. The grafted versions of polystyrene resin have been introduced (TentaGel, ArgoGel, HMBA-AM, and Hypogel).[58, 59] They are more frequently utilized for oligosaccharide synthesis with non-polar and polar organic solvents like DMF. PEG has been used as good support for enzymatic reactions as they swell in water and is also helpful in studying enzymatic activity and inhibition,[60-65] protein ligation.[66] To facilitate synthesis and enzymatic reactions, radical

polymerized forms of long-chain PEG have been made, such as PEG-polyacrylamide (PEGA) copolymer[67, 68] or PEG-crosslinked oligostyrene (POEPS-3) resin.[69] Morten Meldal *et al.* introduced two novel PEG polymerized solid supports, POEPOP[70] and SPOCC[71], which possess high mechanical and chemical stability with optimum hydrophilic and hydrophobic balance. Very recently, Stine and Demchenko introduced STICS technology that uses nanoporous gold as a stable solid support.[72] Non-swelling controlled pore glass (CPG) supports have also been investigated for oligosaccharide synthesis with trichloroacetimidate donors.

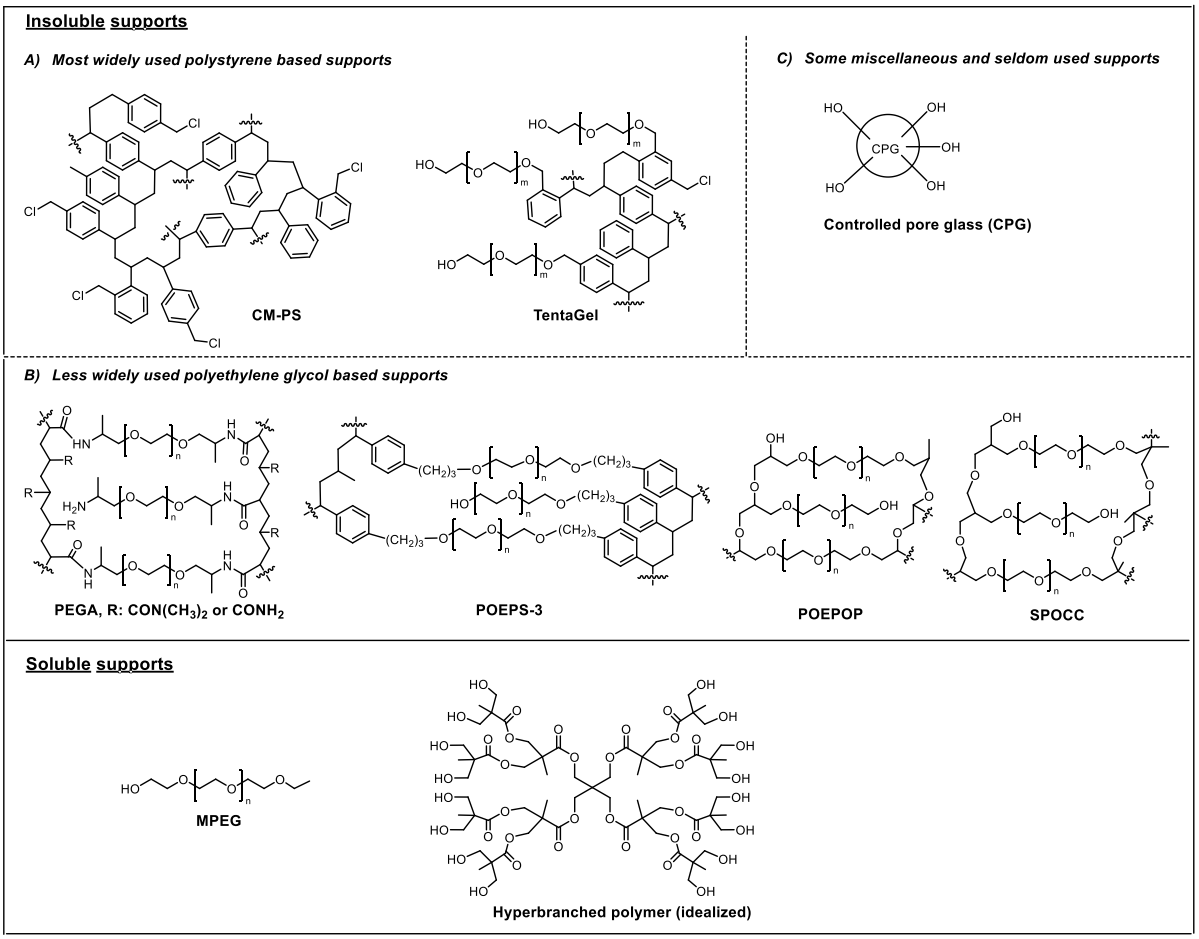
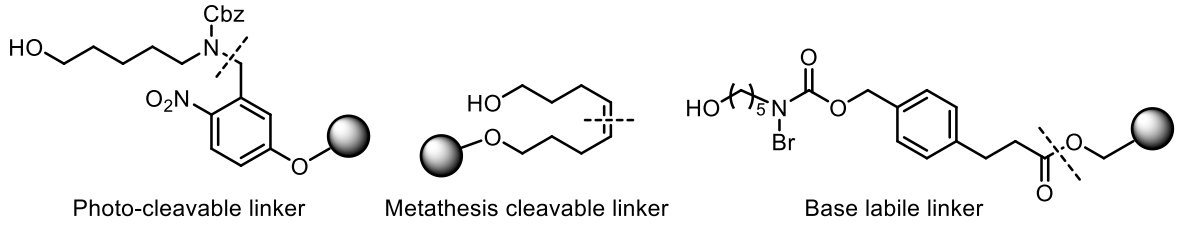


Figure 1.5: Chemical structure of insoluble and soluble solid supports.

Soluble Supports: Soluble supports offer a solution-phase regime and, therefore, render easiness in chemical transformations. Excess reagent removal with the aid of the precipitation method followed by filtration makes it advantageous over the insoluble solid supports. MPEG supports[73, 74] are commonly used, whereas a polydisperse soluble hyperbranched polyesters[75] are recently discovered as supports for oligosaccharide syntheses. However, these supports have the potential downside of precipitation as a purification method that lowers overall yield and restricts the temperature range under which to use of the soluble polymer can be efficiently

(above -45 °C). This implies that insoluble solid supports are more advantageous than soluble supports.

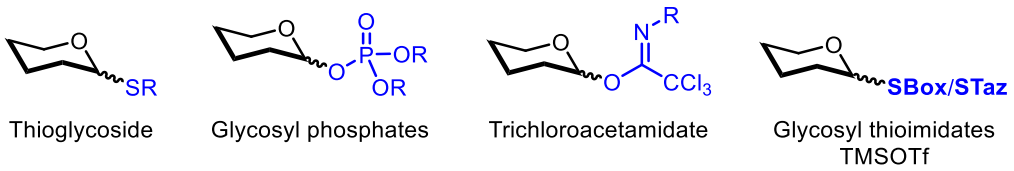
1) Choice of linker: The synthesis happens over the solid support through the handle called 'linker.' The linker plays a vital role in the solid phase synthesis because it has to remain stable during all the synthetic steps encountered in synthesizing the desired glycan. At the end of the synthesis, it should undergo easy cleavage in high yields to obtain the support-free targeted molecule. The linker choice is critical in formulating a synthetic strategy because the linker cleavage reaction should be orthogonal to the rest of all synthetic steps. The photo-cleavable linker, metathesis cleavable linker, and base cleavable linkers are three preferred classes of linkers in the carbohydrate field.



Scheme 1.4: Structures of most frequently used linker attached solid support

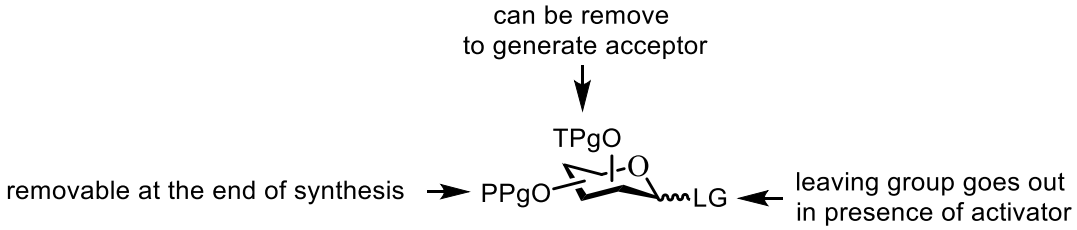
Various other linkers have emerged in the last few years, such as succinoyl, alkoxybenzyl, silyl-based linkers, and hydrogenation-removable linkers. But, the most recent developments include Reichardt's spacer/linker,[76] as well as Seeberger's Lenz linker, [77] safety catch linker,[78] and photocleavable linker.[79]

2) Selection of glycosylating agent: The oligosaccharide synthesis over solid support requires a sugar building block that bears a leaving group at the anomeric position, known as a glycosylating agent. This glycosylating agent forms the glycosidic bonds with the glycosyl acceptors attached to the solid support (acceptor-bound strategy) under the influence of activators. The crucial point is that under the conditions applied, the linker attached to the solid support and the corresponding reacting partner should not undergo miserable reactions. Among the plethora of glycosylating agents, the most frequently used for solid-phase chemistry are thioglycosides, glycosyl trichloroacetimidate donors, and glycosyl phosphate donors. Along the same line, a few other donors, such as glycosyl halides, glycals, glycosyl sulphoxides, and *n*-pentenyl glycosides, have been used for this chemistry. The recently introduced glycosyl thioimidates have shown their impact in developing glycosylation conditions for oligosaccharide synthesis.



Scheme 1.5: Popular donors used for solid phase chemistry

3) Choice of Protecting group: During the glycan synthesis, protecting groups on the sugar building blocks decide the glycosylation reaction's stereochemical outcome. For example, if the C2 benzoyl group (Bz) is present, then stereospecifically, it offers 1,2-*trans* glycosidic product. In carbohydrate chemistry, three types of protecting groups have been observed. The group present at the anomeric position (at the C1 position) is called the 'leaving group' that gives rise to a glycosylating agent or glycosyl donor. The second type is the 'temporary protecting group' (TPg), which needs to be removed during oligosaccharide synthesis to get free -OH/-NH_2 functionality that acts as an acceptor over the solid support. The third category is the 'permanent protecting group'(PPg). These protecting groups are generally very stable and rigid under the glycosylation reaction condition or other reagent conditions employed for synthesizing target glycan. Ppg will be removed after completing the synthesis of the targeted glycan.



Scheme 1.6: Building block used for solid phase chemistry

The most commonly used TPg is the 9-Fluorenylmethyleneoxycarbonyl (Fmoc) group, whereas the levulinoyl (Lev) group act as an orthogonal protecting group. Additionally, other groups like chloroacetyl (ClAc), 2-(azidomethyl)benzoyl (Azmb), 2-naphthylmethyl (Nap), 4-methoxybenzyl (PMB), and *tert*-butyldiphenylsilane (TBDPS) have been used as successful TPg for SPOS chemistry. Whereas, Benzyl (Bn) ether, benzoyl (Bz) esters, and cyanopivalolyl (PivCN) are used as PPg for the protection of the –OH group and the protecting group such as azides (N_3), *N*-trichloroacetyl (TCA) are generally well considered as PPg for amino functionality. Therefore, a proper retrosynthetic plan and appropriately protected building blocks are mandatory for successful solid support synthesis.

4) Cleavage method for detachment: This is the final step of the synthetic protocol during the SPOS. The desired synthetic targeted glycan can be released from the solid support using a cleavage reaction. Importantly, this cleavage reaction should be orthogonal to all other reactions employed during the synthesis to mandate the safety of the synthesized glycan. This cleavage always offers glycan with free hydroxyls (in the case of a traceless linker) or some other reactive functionality at the reducing end of the glycan that allows the installation of thiol or amino group as a terminal entity.

5) Purification/ isolation and quality control of the final product

The desired product isolation from other unwanted deletion sequences or impurities is also essential on completing the oligosaccharide synthesis.

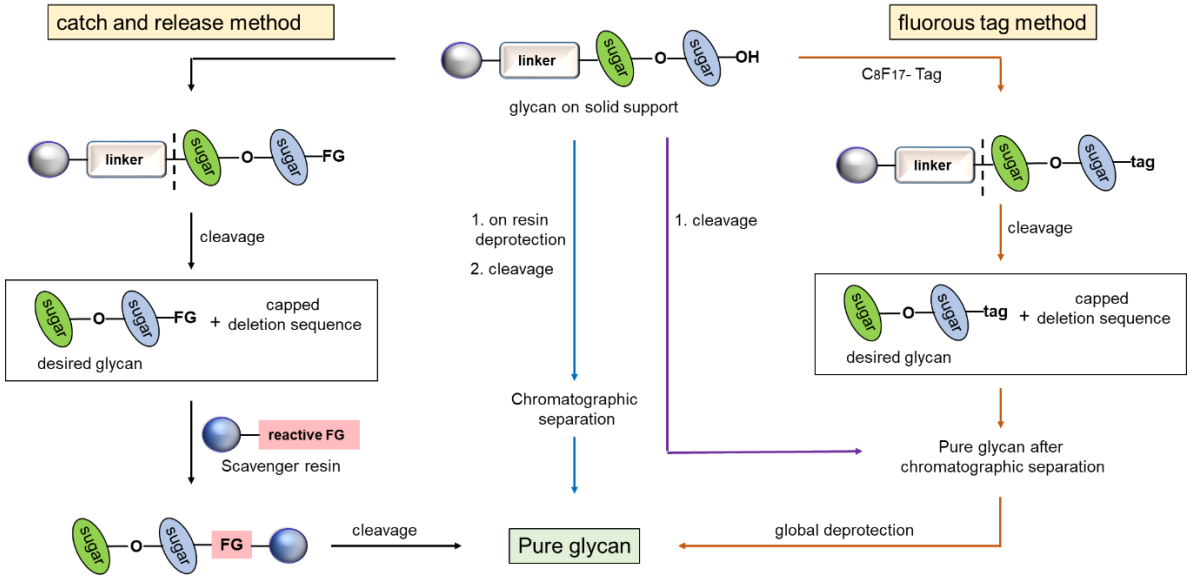


Figure 1.6: Isolation/ purification strategies after synthesis of desired glycan over solid support

There are four ways by which purification can be done. In this regard, two uncommon strategies have been created: ' fluorous tagging' and the "catch and release" protocol, viz. i) Capping the deletion sequence by acetyl group and installing a fluorous tag on the desired substrate prior its release from the solid support is called the fluorous tag method and found as a beneficial method for purification.[80] ii) Another way is the "catch and release" model that involves the installation of a chemical handle over the desired substrate prior to its cleavage from the support, which permits the temporary covalent immobilization to an appropriately functionalized scavenger resin. Finally, this loaded scavenger resin can release captured product on cleavage reaction.[81] This immobilization of oligosaccharide on scavenger resin has been done by reactions like amide formation, Staudinger ligation,[82, 83] oxime formation,[84] S_N2

displacement of α-haloesters.[85] iii) Cleavage of the glycan from the solid support, then the resultant fully protected glycan can be purified by HPLC purification technique to get highly pure glycan. Further, on treatment with various reagents, all remaining partially or permanent protecting groups can be removed to get fully deprotected glycan. iv) On resin, deprotection is another way that can facilitate high throughput synthesis. But, as in most cases, benzyl ethers are used as a permanent protection groups, of course removal of them from solid supports is not easy with commonly used resins. In this direction, Kanie and Wong have shown the usage of Pd-nanoparticles for Tenta Gel and PEGA solid support.[86] In contrast, Takahashi *et al.* have demonstrated the use of a birch reduction to remove the benzyl group with Argo Pore resin.[87] The purification of the fully deprotected glycan is complicated as it does not contain any UV active functional group that must pass through the size exclusion chromatography column and must be dialysed to remove the accumulated salts.

Solid-phase oligosaccharide synthesis strategies

The solid phase synthesis begins by coupling the first building block to the linker bearing solid support, then removing the temporary protecting group. This second step offers an accessible –OH group on the solid support to carry out the next glycosylation. Reagents and building blocks can be used excessively to prevent partial or incomplete reactions. After repeating the cycle of glycosylation and deprotection steps, the desired partially protected, or fully protected glycan can be made. Depending on the requirement, orthogonal temporary protecting groups can help to synthesize the branched oligosaccharides. This elongation of the oligosaccharide chain on the solid support can be done with different strategies. Basically, solid-phase oligosaccharide synthesis is divided into two approaches, which are distinguished by the activation state of the bound molecules. The first approach is the acceptor-bound strategy,[88] in which the reducing end of the glycosyl acceptor is bound to the solid support through the linker, which then offers reactive –OH functionality on deprotection reaction, and then on reacting with the glycosylating agent results in new glycosidic bond (Figure 5A). This strategy generally ends with the completion of the reaction with excess use of the glycosylating agent. Another strategy,[89-91] in which the non-reducing end of the glycosyl donor is bound to the solid support through the linker and keeps the anomeric group free to react is called as the donor-bound strategy (Figure 5B). As the donor is present in a limited amount that sometimes undergoes decomposition in the presence of the activator, that majorly leads to a decrease in the overall yield.

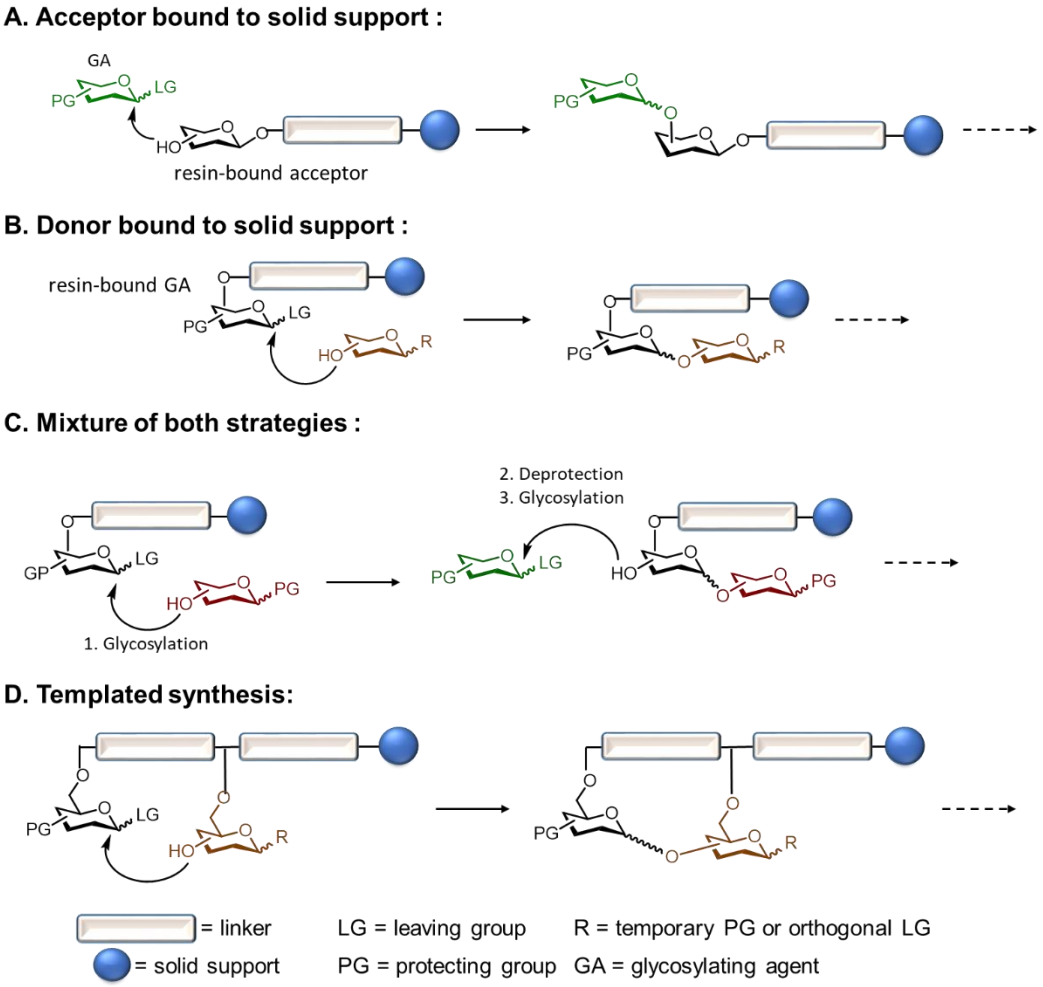


Figure 1.7: Strategies for solid-phase oligosaccharide synthesis

Therefore, the acceptor-bound strategy is found superior to the donor-bound strategy. The oligosaccharide synthesis can also be performed by combining both strategies (Figure 5C).[17, 92, 93] In support-bound reactions, the acceptor containing a probable leaving group is first allowed to react under certain reaction conditions with the donor. Then on performing selective removal of the temporary protecting group, the resultant acceptor can be made to react with another glycosylating agent. Also, the template approach has been investigated,[94, 95] wherein both components are joined to one polymer support, as outlined in (Figure 5 D).

Exploration of solid phase oligosaccharide chemistry

A) Solid support based synthesis

Chemical glycosylation: This solid support-based chemical glycosylation method was first pioneered by Fréchet and Schuerch in 1970 in the field of glycomics. They performed trisaccharide synthesis in comparable yield by reacting glycosyl halide with allyl alcohol containing polystyrene resin.[96] Further, this chemistry was explored by Danishefsky and

Bilodeau[89] and Guthrie *et al.* for donor-bound strategy.[90] The donor-bound approach has reflected fewer reports. Recently, Demchenko and co-workers have illustrated that glycosyl thioimidate donors, i.e., *S*-benzoxazolyl (-SBox) and *S*-thiazolinyl (-STaz), are useful for both glycosyl acceptor-bound and glycosyl donor-bound strategy.[97] They have shown essential characteristics for activating the thioimidoyl moiety in the presence of alkyl/aryl thioglycosides.

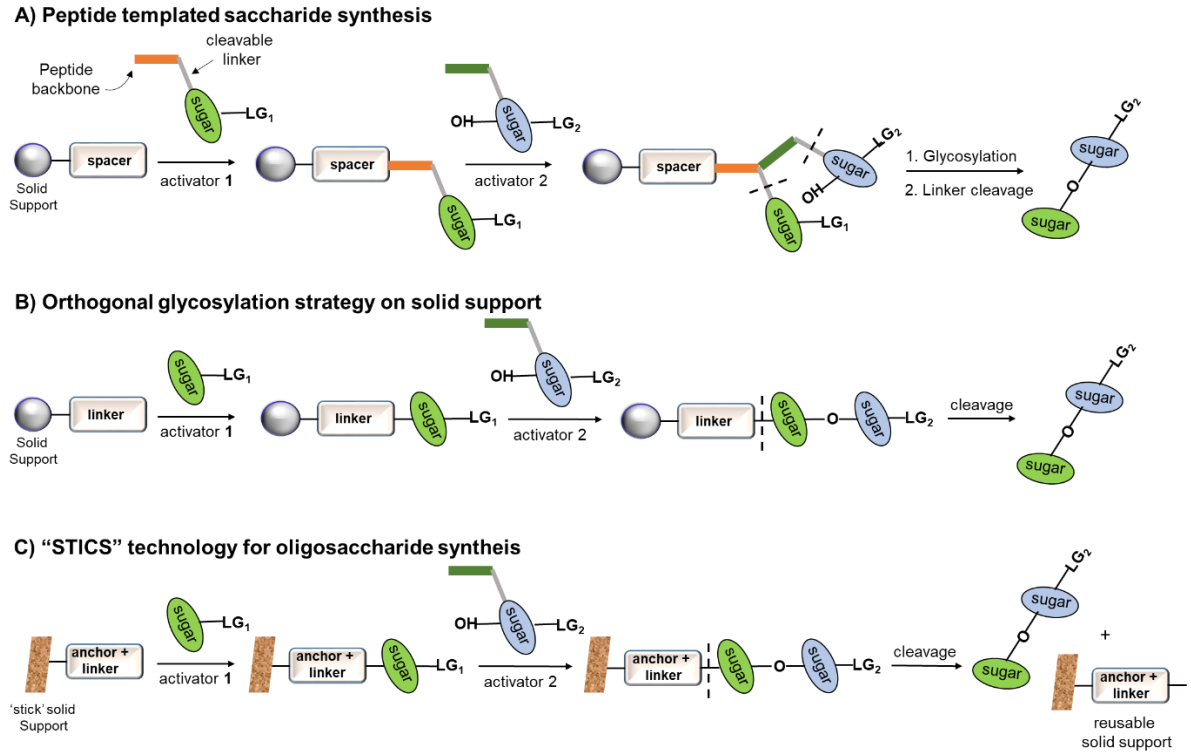


Figure 1.8: A) Strategy for the synthesis of stereoselective glycosidic bond over solid support, B) Orthogonal glycosylation strategy to tackle the problem of donor bound strategy C) Strategy for reusable solid support for oligosaccharide synthesis

The first linear heptamannoside was synthesized by Seeberger *et al.* using a novel metathesis cleavable linker and glycosyl phosphate donor in 9 % overall yield.[98] Later, Boons *et al.* reported the chiral auxiliary mediated fusion of a branched α-glucan that contains multiple 1,2-*cis* linkages.[99] Ogawa used the support-based orthogonal glycosylation strategy in 1996, utilizing the donor-bound approach.[100] Then, in 2006, Kanie *et al.* also successfully extended this orthogonal glycosylation strategy for the combinatorial oligosaccharide synthesis of trisaccharides.[101, 102] Initial peptide templated regio- and stereo-selective glycosylation reaction was introduced by Fairbanks and coworkers[103, 104] Further, they performed peptide templated glycosylation reaction over the solid support.[95] Around the same time, Warriner et al. also demonstrated disaccharide synthesis using a similar concept.[94] A new surface-tethered iterative

carbohydrate synthesis (STICS) technology was presented for the first time by Demcheko group in 2009.[72] This technology elaborates advantageous features of the traditional solid-phase synthesis by allowing the cost-efficient and straightforward synthesis of oligosaccharide chains using surface functionalized 'stick' that made up of chemically stable high surface area porous gold.

Enzymatic glycosylation: Like solution-phase chemistry, enzymatic glycosylation using solid support has many advantages over solid support-mediated chemical glycosylation. Two types of automated enzymatic glycosylations were proposed in 2001 by Wong and co-workers: immobilized enzymes and immobilized substrates.[105] The immobilized enzyme strategy is not ideal because the oligosaccharide product remains associated with regents used and by-products. There are two approaches for useful immobilized substrates strategy. In the first approach, an enzymatic reaction occurs on a solid-support bearing substrate. In the second, an enzymatic reaction occurs in the solution phase, followed by specialized technologies that captures and release the resulting product. Automation has also been achieved in enzymatic solid-phase glycosylation reactions. The conversion rate at each step and overall performance of the strategy depends on significant factors such as i) enzymes, ii) glycosyl donors, (iii) supports (resin/polymer-support/tag), and (iv) linkers and spacers.

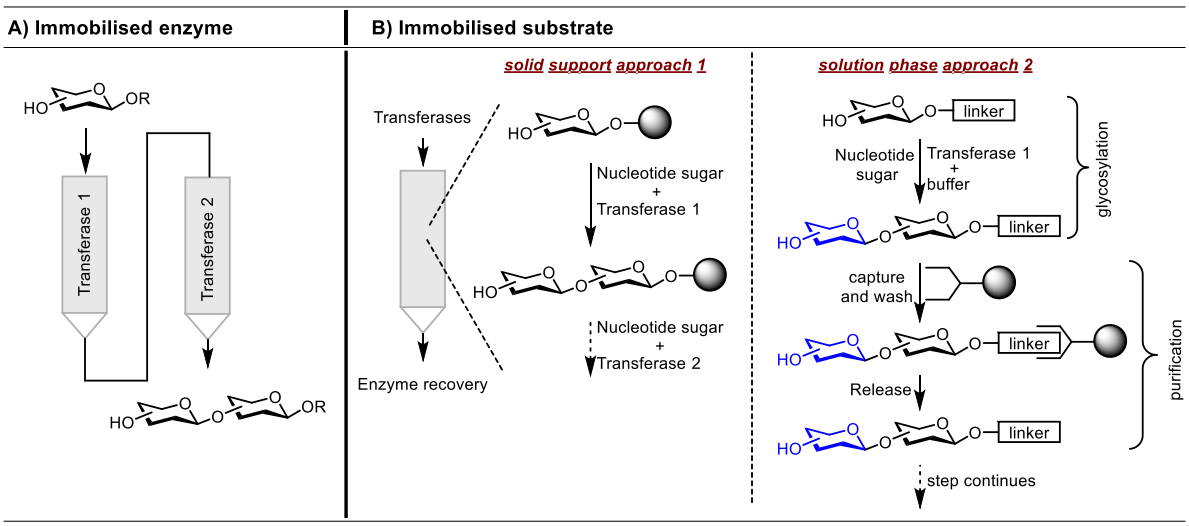


Figure 1.9: Enzymatic glycosylation Strategies

Important mammalian glycan sialosides are less yielding and low stereoselective in solution and solid phase by chemical methods. This is because of the unreactive nature of the anomeric carbon. Although satisfactory results were obtained for the installation of α-(2,6) sialosides using automated glycan assembly (AGA), the crucial structure of α-(2,6) sialosides with GlcNAc in the backbone and for α-(2,3) linkages remains less significant in yield. Such types

of targets can be accomplished using enzymes easily. Wong and co-workers reported the enzymatic elongation of the sugar chain over the amino silica-based solid support in 1994, wherein they demonstrated the chemo-enzymatic synthesis of a sialyl Lewis X glycopeptide.[106]

Chemo-enzymatic glycosylation: The problem associated with the chemical and enzymatic-based methods can be solved by combining both chemo-enzymatic approaches. In such approaches, the glycosidic bonds, which are difficult to tackle alone using a chemical way, can be made by enzymatic way[107] and vice versa. For example, Oliver Seitz and Chi-Huey Wong synthesized glycopeptides with an O-linked sialyl-Lewis-X (SLe^x) tetrasaccharide through the chemo-enzymatic solution and solid phase. In this, glycopeptide substrate synthesis was first carried out using the chemical method on the solid support. Then, after removing all acid-sensitive shielding groups of the peptide spine, the resultant deprotected glycopeptide was further extended using glycosyltransferase enzyme to obtain O-Sialyl-LacNAc Octapeptide.[108]

Solution-phase support based synthesis (Soluble Polymer support, Ionic, Fluorous tag):

A soluble polymer support method emerged to address the mismatch reaction phase associated with the solid-phase method. It offers advantages such as higher yield and easy purification recovery of materials. Nishimura and co-workers[109] demonstrated the use of water-soluble support polyacrylamide. In contrast, Boons,[110] has shown the utilization of water and organic soluble poly ethylene glycol support because they can be easily precipitated out simply by adding diethyl ether or other suitable solvents. In some instances, nanofiltration or a size-exclusion separation has been used to separate polymer-bound molecules from the reaction mixture.

The ionic liquid (IL) method is another promising method by which oligosaccharide synthesis can be expedited, as chromatographic purification of intermediates is not required. After finishing, the desired reaction over the ionic liquid support, simply by concentrating the reaction mixture and subsequent treating it with a non-polar solvent, the excess reagents and unreacted acceptor can be taken out easily from IL attached substrate. The inorganic reagents can be washed out simply by aqueous treatment. So, the desired glycan can be synthesized by performing phase separation clipping of IL. Chan and co-worker demonstrated the trisaccharide synthesis using an imidazolium salt ([mim][BF_4]) as an ionic tag.[111] In contrast, Gouthier and co-workers have reported a two-directional approach for selective α-(1→4)-glycosylation using 1-butyl-3-methylimidazolium hexafluorophosphate salt ([bmim]-[PF_6]).[112]

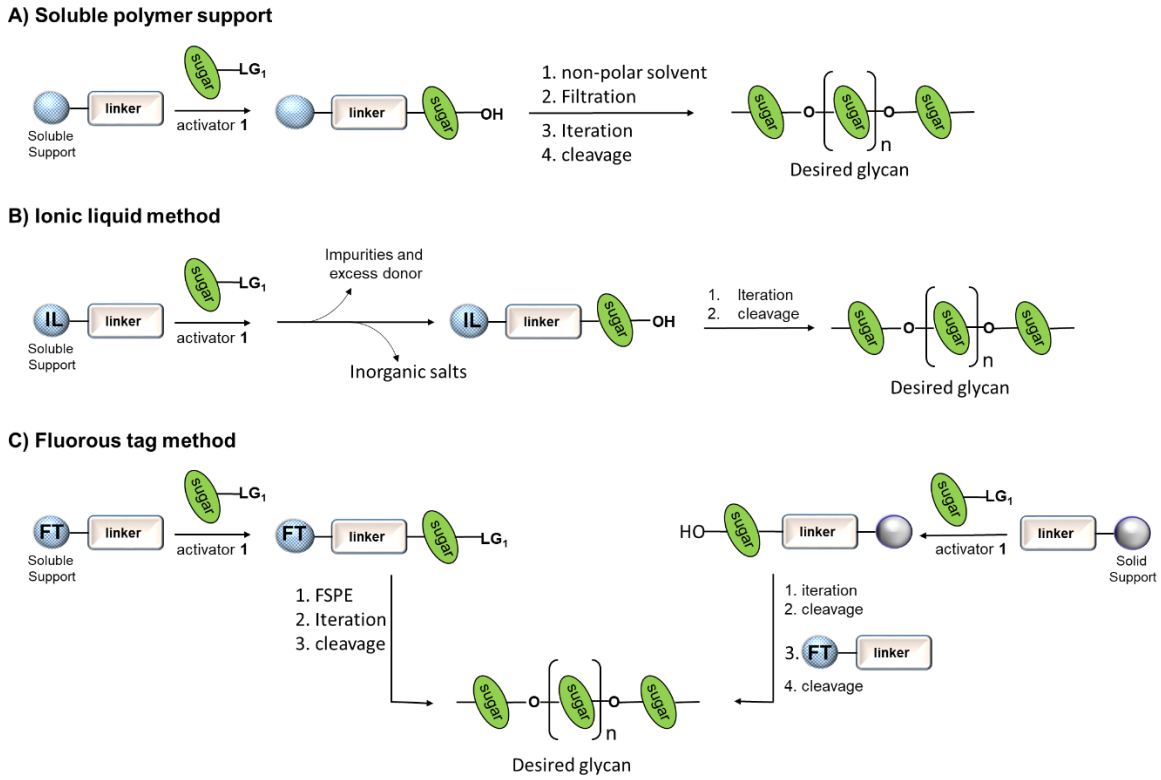


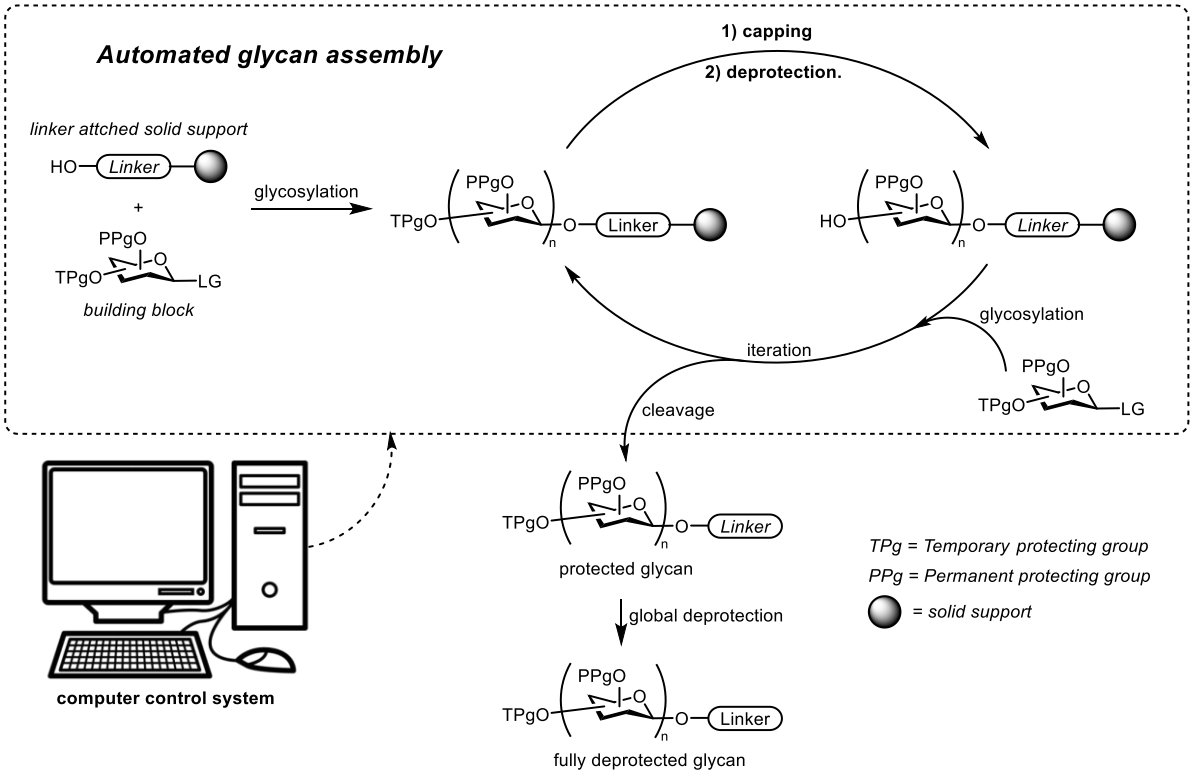
Figure 1.10: Oligosaccharide synthesis using soluble polymer supports

A fluorous tag method allows fluorine-containing components to be separated from the non-fluorinated ones through a different phase partitioning principle between per-fluoroalkenes and methanol. Sometimes these tags have solubility issues in reaction solvents, which can be solved either by increasing the solubility of the tag-associated linker or by introducing the soluble linker in the synthetic strategy, which would allow "catch and release" protocol. Jaipuri and Pohl investigated that linker designing is crucial for the automated fluorous-phase approach.[113] Huang and co-workers performed one-pot oligosaccharide synthesis, where they isolated the compound using a fluorous tag method involving "catch-release" protocol.[114]

Automation in solution and solid phase oligosaccharide synthesis

Although synthesizing oligosaccharides using the solid phase technique is less tedious owing to exclusions of column chromatography, shifting all the required synthetic manipulations to the machine-driven process has opened up a new field in carbohydrate chemistry called automated glycan assembly (AGA). In addition to the purification step, the remaining process of performing glycosylation and subsequent deprotection step necessitates experimental spatial arrangement that consumes ample time. Automated platforms often include computer interfaces and liquid handling equipment; thereby doing so, improvement in the reproducibility

of results and minimization of human error is possible. The principal motto of developing automation technology in carbohydrate chemistry was i) acquiring a rapid supply of all types of glycans in a short period, and ii) non-expert could also be able to synthesize the desired glycan.



Scheme 1.6: Representation of automated glycan assembly process

Solution phase synthesizers: To minimize the labor-intensive process of synthesizing complex oligosaccharides, Wong and Takahashi made the very first efforts to automate solution-phase chemistry, because of which they were able to introduce a computer program called "OptiMer." The program, fitted with the relative reactivity values (RRVs) for many thioglycoside donors and donor−acceptors, proposed the best building block combinations for synthesizing the desired glycan in a pot.[11] The example is the synthesis of Globo H hexasaccharide, whose synthetic route was designed using this computer program.[115] In 2000, Takahashi et al. developed Quest 210™ manual synthesizer,[116] and conducted a solution-phase one-pot synthesis of trisaccharides. Further, two more synthesizers such as L-COS®[117] and 'ChemKonzert,®[118] were utilized by Takahashi for controlling stirring, reaction temperature, and rate of reagent addition during the synthesis.

For accelerating fluorous-tag synthesis, Seeberger introduced a solution phase microreactor based on the combined effect of the fluorous-tag synthesis method and the benefits of a microreactor.[119] The Pohl group utilized the commercially available automated liquid handler with few modifications to accommodate cartridges for developing the FSPE. [113] Additionally, Pohl and co-workers have introduced the "recycling high-performance liquid chromatography" (R-HPLC) technique for the glycoconjugate isolation and purification eluted from an automated solution-phase synthesizer.[120] They used an alternate pump system for recycling the analyte through two identical columns using a 10-port switch valve[121] and further applied it to synthesize β-Mannuronan and β-Mannan.[122]

Yoshida and co-workers[39] introduced the automated platform for electrochemical glycosylation with an instrument equipped with easily accessible devices such as a syringe pump, a chiller, a power supplier for constant current electrolysis, and an electrochemical reaction system equipped with a divided electrolysis cell. Further, Nokami and co-workers proved its potential application by synthesizing TGM-chitotriomycin.[38, 123]

Nishimura and co-workers developed an HPLC-based "artificial Golgi apparatus" for glycan synthesis.[124, 125] This synthesizer employs an enzymatic approach and a dendrimer as ideal support. This computerized automated machine aids in mixing reagents by autosampler and ultrafiltration process using a hollow fiber module. Using this synthesizer, they successfully synthesized sialyl Lewis X tetrasaccharide derivatives from a simple N-acetyl D-glucosamine linked to an aminooxy-functionalized G7 dendrimer.

Solid-phase oligosaccharide synthesizers: Seeberger group introduced quite a sound synthesizer, i.e., AB 433, a modified form of peptide synthesizer, in 2001.[126] The instrument was able to deliver nine reagents and was equipped with an external chiller that could adjust the reaction temperature because which they were able to synthesize the linear heptasaccharide and Globo-H hexasaccharide.[127] In 2012, Seeberger and co-workers developed the second generation "first fully automated solid-phase oligosaccharide synthesizer".[128] This instrument was built with a syringe pump-driven part for reagent delivery and a solenoid valve-driven part for delivering solvents, mixing reaction solutions, and allowing rapid pressure-driven washing. Additionally, this machine's central creation is a double-jacketed reaction vessel with temperature control ranging from -50° C to +90° C using cryogenic fluid circulation and argon distribution manifold. Further, to assist the complexities of glycan assembly, Seeberger and co-workers incorporated flexible hardware and software into the second generation machine,

which includes computerized control of argon pressurized delivery of solvents and reagents, cytostatic temperature control of triple-jacketed reaction vessel, carousel feature with a two-way needle to deliver both solvents and reagents. Furthermore, they installed a provision for online monetaring of UV active compounds for coupling quantification and a fraction collector for separating specific reaction mixtures from the main waste stream. So, with this modification, they introduced the commercially available most complete and sophisticated glycan synthesizer called "Glyconeer 2.1".[129] By utilizing this synthesizer, Seeberger et al. successfully synthesized oligomannan containing 30 (triantamer)[130] and 50 (penindamer)[131] monosaccharide residues and complex glycan including sialic acids,[107] furanosides,[132] 1,2-*cis* glycosides,[133] glycopeptides,[134] and branched oligosaccharides.[135-138] In 2020, Seeberger and co-workers[139] developed automated glycan assembly by incorporating a variable-bed flow reactor (VBFR) system. This efficient platform allows real-time monitoring of each step by tracking the resin growth pattern, which depends on the solvation state of the monosaccharides during the synthesis. In 2021, Seeberger and co-workers designed a dual temperature regulation reaction (DTRR) system by combining a coolant circulating jacketed reaction vessel with the heating power of microwave radiation.[140] This microwave-assisted automated glycan assembly allows rapid temperature adaptation from −40 to +100 °C to control glycosylation at low temperatures and essential capping, deprotection, and glycan modification at high temperatures. This new instrument has drastically shortened the reaction time and expanded the orthogonal temporary protecting group portfolio, allowing the synthesis of highly branched glycan in less time.

Demchenko and co-workers developed another instrument for solid-phase oligosaccharide synthesis: Omnifit column fitted unmodified HPLC instrument.[141] They synthesized pentasaccharide by utilizing this machine in combination with acceptor-bound Tenta Gel-NH_2 polymeric resin and different glycosyl donor solutions. In 2016, they further implemented the autosampler to the same instrument for delivering reagents and solvents in an automated manner.[142] Furthermore, they introduced the surface-tethered iterative carbohydrate synthesis (STICS) technology to overcome the drawbacks of the solid phase synthesis,.[72] and then they applied this (STICS) technology to HPLC based machine and conducted pentasaccharide synthesis using an acceptor-bound longer spacer attached to nanoporous gold particles and different glycosyl donor solutions.[143] Following are some examples of representative glycans synthesized using AGA.

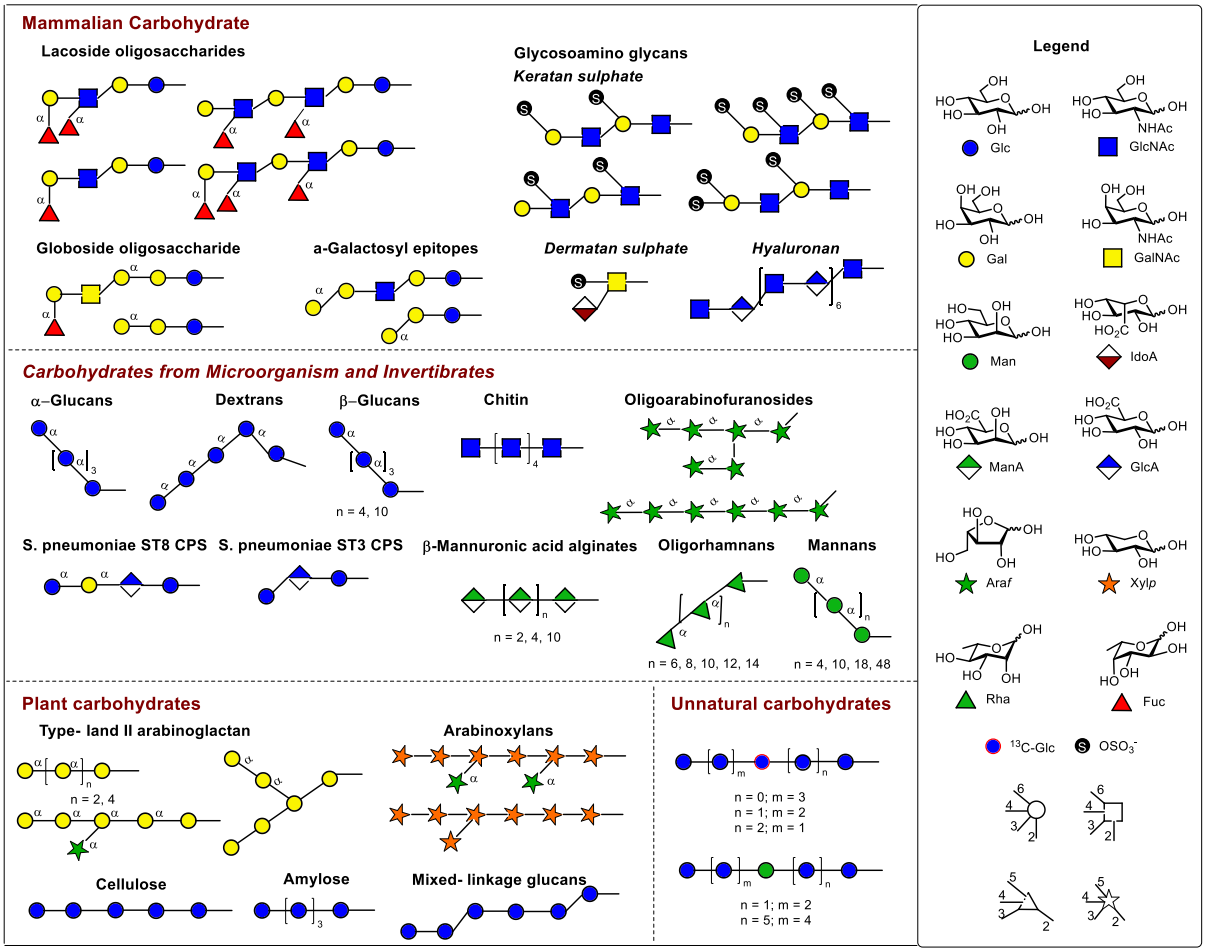


Figure 1.11: Representative oligosaccharides synthesized using AGA and their representation following SNFG nomenclature. The stereochemistry of the glycosidic linkage is *β* for pyranoses with *gluco* configuration and *α* for pyranoses with *manno* configuration at C2, unless indicated otherwise.

Why SPOS chemistry requires a new glycosylation method and linker synthesis?

In the case of the enzymatic solid phase method, the enzyme availability is still limited when we compare it with the diversity of glycan structure. However, enzymatic synthesis using automation is a promising avenue to date with few set examples; therefore, the scope for finding a new method or improvement remains open.

Many oligosaccharides have been synthesized in a sole chemical way using solid-phase techniques. But the finding of the general chemical glycosylation method for synthesizing regio- and the stereospecific product has remained challenging not only in the solution phase but also in the solid phase chemistry since the beginning of carbohydrate

chemistry. In particular, no universal glycosylation method to date can lead to all glycosidic bonds with high selectivity and yield over solid support. Therefore, the chemical glycosylation method still has scope for improvement concerning the time, temperature, and equivalence of donor use. The existing chemical glycosylation methods currently rely on the frequently used trichloacetimidate, phosphate, and thioglycoside donor. All these donors require a higher equivalent (2 or 3 times 5 eq.) of donor treatment to complete the reaction on the solid support. The thioglycosides and phosphate donors are sufficiently stable at ambient temperature, but their usage requires a stoichiometric amount of activator reagents and a lower temperature (-30° C) for a stereoselective product. The trichloacetimidate donor is not stable enough and also requires a low temperature for its usage. The remaining donors are not at all well recommended for solid support for the following reasons: i) the glycosylation method should be easily adaptable to solid phase chemistry, ii) it should not produce insoluble solid by-product/side product in the reaction, iii) its implementation on the automated machine should not create much complexity. This opens up the scope for finding a new glycosylation method that could make this process very smooth.

The success of solid-phase chemistry depends on the linker used in the process, which clarifies how the choice of a linker is essential. While synthesizing glycan over the support, the linker must stay stable and undergo effective cleavage. Although photo-cleavable and metathesis-cleavable linkers are well explored in this support-based chemistry, their finding as a manipulative linker has been felt now more than ever, and that will remain in the scope of discovery.

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Chapter: 2

Synthesis of Linkers for the Solid Phase Oligosaccharide Synthesis

Chapter 2

2.1 Introduction:

Synthetic carbohydrate chemists have been intrigued by synthesizing complex oligosaccharides for the last five decades owing to their involvement in various biological processes. Therefore, synthesizing such biomolecules is of primary importance, irrespective of their complexity. Importantly, their synthesis is necessary because the availability of such molecules from nature is significantly less, and their synthesis is also challenging. Synthesis of such complex molecules includes two major concerns; i) differentiation of similarly reactive functionalities and ii) generation of a new stereogenic center each time. Despite that, carbohydrate chemistry is very time-consuming and laborious in its synthesis. Various protecting groups and leaving groups chemistry (donor development chemistry) have also been developed to obtain a high-yielding, stereoselective, and reliable glycosylation method to smooth the synthesis. Nevertheless, carbohydrate chemistry poses many challenges in synthesizing large and branched oligosaccharides.

Many glycosylation techniques have been introduced to simplify the synthetic process, including electrochemical, ball milling, chemical, enzymatic, and solid phase methods. Bruce Merrifield invented the solid phase method for peptide chemistry, and later it was explored for oligonucleotide chemistry and benefited greatly. The solid phase method has a unique impression due to its synthetic steps, which are well discussed in the first chapter. In the case of oligosaccharide synthesis, the solid phase technique involves solid support, linker, and glycosylation method to accomplish the desired glycan synthesis. The synthetic protocol for synthesizing oligonucleotide and oligopeptides are well established concerning the reaction conditions helpful in stacking the molecule and the other components like linker and solid support. But in the case of oligosaccharides, the chemistry over the solid support is still challenging and under development.

What is a linker?

Figure 2.1: Components of solid phase chemistry

A linker is crucial because it is an intermediate between the solid support and the growing molecule. Therefore, the synthetic step is always associated with adding two additional steps; i) attachment of the linker to the support and ii) cleavage of the linker at the end of the synthesis

to get the support-free desired molecule. Becoming a useful linker, the linker should have some characteristics; i) the linker should orthogonal to protecting group used in building block synthesis and the glycosylation reaction condition, ii) it should remain intact during the synthetic cycles and undergo cleavage whenever required without affecting the synthesized glycan, iii) it should offer bioconjugatable group or the functional group which could be convertible to bioconjugatable group, iv) in the recent development of linker chemistry, it has shown that linker cleavage should offer hemiacetal form of glycan on cleavage reaction so that it can be further converted to the glycosylating agent and will automatically help in extending the oligosaccharide chain. In the last few decades, many research groups have introduced a variety of linkers that can be cleaved through chemical and enzymatic methods. to make solid-phase oligosaccharide chemistry successful.

2.2 Linkers used in oligosaccharide synthesis

The linker development has emerged various types of linkers in enzymatic-based and chemical-based SPOS chemistry.

2.2.1 Enzymatically cleavable linkers:

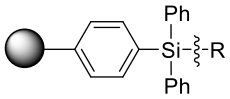
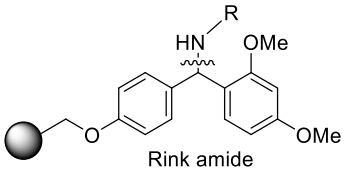
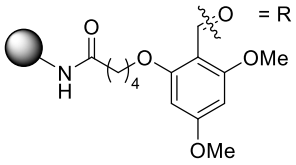
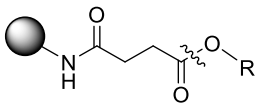
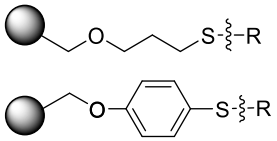
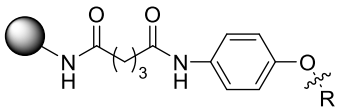
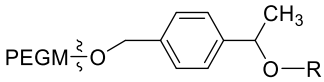
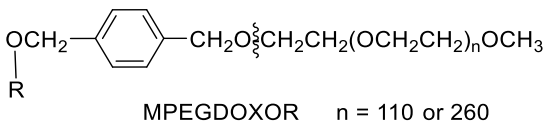
Although the availability of specific enzymes is the fundamental requirement for the development of enzymatic solid phase chemistry, the choice of the suitable linker and its utilization act as another obstacle to solid phase chemistry.[1] This chemistry sometimes requires a spacer along with the linker to increase substrate accessibility. The protease cleavable linkers,[2] photocleavable linkers,[3] disulfide linkers,[4] acid- or hydrazine-sensitive linkers,[5] and safety-catch linkers[6] are the linkers have been utilized to run this enzymatic solid-phase chemistry. Although these many linkers are available, the easy utilization under the enzymatic synthetic process and cleavage at the end of the synthesis is the major limitation in finding a linker.

2.2.2 Chemically cleavable linker

The chemical solid phase chemistry also has limitations concerning the linker availability and its choice. Even though performing chemical solid phase synthesis is preferable over enzymatic synthesis, the linker component required for connecting the substrate to the solid support must survive during the synthetic process and remain unaffected and stable under the reagents.

Silyl Ether Linkers: The initially used silyl linkers are of great importance because they are compatible with various glycosylating agents such as trichloroacetimidate, fluorides, glycosyl

sulfoxides, and thioglycosides. Moreover, that can be cleaved under orthogonal fluoridolysis condition,[7] but the difficulties come when hindered alcohol is used as an acceptor. Later, this problem was solved to some extent, but nowadays, they are less frequently preferred.

Sr. no	Linker	Cleavage condition
1	Silyl Ether Linkers 	TBAF, acetic acid
2	Acid labile linker  Rink amide 	20% TFA in CH_2Cl_2 TFA, H_2O
3	Base labile linker 	32% Aqueous NH_3
4	Thioglycoside linker 	DTBP, MeOH $Hg(OCOCF_3)_2$
5	Oxidative cleavable linker 	DDQ
6	Linkers Cleaved by Hydrogenation   MPEGDOXOR n = 110 or 260	$Sc(OTf)_3$, Ac_2O H_2, Pd black, EtOH
7	Metathesis cleavable linker	Grubbs catalyst, ethylene

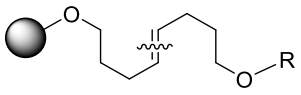
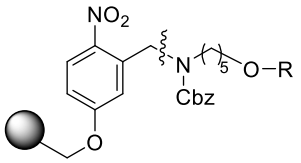
		
8	Photocleavable linker 	360nm wavelength

Figure 2.2: Likers used for solid phase glycosylation chemistry

Acid and base labile linkers: Similar to peptide chemistry, acid labile linkers are also used in solid-phase. The amino-functionalized rink linker[8] and benzylidene acetal-type linkage producing linker[9] were introduced by Silva et al. and Hanessian et al., respectively. They used TFA in CH_2Cl_2 for the linker cleavage. Very recently, TFA acid labile safety-catch linker called tris(alkoxy)-benzylamine (BAL) has been developed by Jakob F. Tolborg and Knud J. Jensen.[10]

Base labile linker also has been employed in oligosaccharide synthesis**.** The succinoyl linker[11] was utilized for disaccharide synthesis and cleaved using 32% aqueous ammonia at the end of the synthesis. In contrast, the peptide attached to hydroxymethyl benzoyl (HMBA) linker[12] was used by Meldal et al. for glycosylation reaction, which was further cleaved by sodium methoxide.

Thioglycoside linkers: Thioglycoside linkers are a very type of linkers as they can be activated in the presence of the thiophilic reagents only. Schmidt utilized 1-thiopropan-3-ol linker attached Merrifield resin for synthesizing the α-mannosidic oligomer, and further, they cleaved it using di-tert-butyl pyridine (DTBP) and methanol.[13] p-hydroxythiophenyl glycoside was used to make linker attached solid support, and then after synthesis, it was cleaved by mercuric trifluoroacetate.[14]

Oxidative cleavable linker: The p-methoxybenzyl group was introduced by Fukase as a useful linker for glycosylation reaction over support, and they cleaved it using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) reagent.[15] Importantly, the linker must be attached to the non-anomeric position of the first sugar unit to initiate the synthesis.

Linkers Cleaved by Hydrogenation: Linkers of these types are mostly used with soluble polymer. Krepinsky et al. introduced a MPEG-DOX support linker system for oligosaccharide synthesis, and they utilized hydrogenolysis conditions (H_2, Pd black, EtOH) for cleavage.[16]

Whereas, Whitfield et al. utilised $Sc(OTf)_3$, Ac_2O followed by H_2/Pd, AcOH for the cleavage of similar linker.[17] Further, Chan et al. used *p*-carboxamide benzyl containing MPEG-DOX related linker, and then they cleaved it under hydrogenolysis condition at the end of the synthesis.[18]

Most commonly used recent linkers: In chemical solid-phase oligosaccharide synthesis, metathesis cleavable linkers such as octenediol[19] and butenediol[20] functionalized linkers have been used successfully so far because they can be cleaved easily using metathesis catalyst and ethylene atmosphere. But the major drawback of these linkers is that they cannot be used under electrophilic reagents (especially in the case of thioglycoside donors). So, to overcome this difficulty, base-labile bifunctional linker[21] was designed by Seeberger et al. in 2012. This linker withstands mild basic conditions and can be cleaved using sodium methoxide followed by hydrogenolysis. Additionally, Seeberger et al. introduced one more class of linker, i.e., photocleavable linker,[22] in 2013 for oligosaccharide synthesis on the solid support. This was a very distinguishable linker as it offers orthogonal cleavage conditions for all of the protecting groups and the leaving group used in this glycosylation chemistry. Moreover, it provides the conjugation-ready linker at the reducing end of the synthesized glycan on cleavage. In addition to these linkers, Reichardt's linker, Seeberger's "Lenz linker," safety catch linker, and photocleavable linker are a few other linkers that also have been used successfully over the solid support for glycan synthesis.

2.3 Why linker development chemistry still needs to be improved?

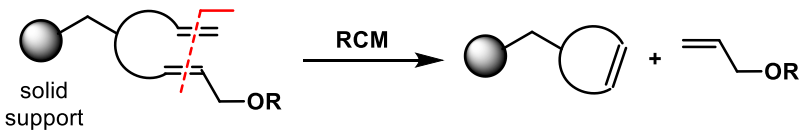
Various solid support-based linkers have been designed so far in the last two decades to heighten the solid phase chemistry. The choice of the linker mainly depends on compatibility with the reaction condition and the reagent used for removing the temporary protecting group in the glycosylation chemistry. Synthetic carbohydrate and organic chemists have put tremendous efforts into finding the suitable one, because of which the uneven classes of the linkers have emerged. The silyl ether linker has the limitation of using the hindered acceptor. In contrast, acid-base linker usage mandates the appropriate design of the building block and sometimes gives restrictions. In the case of the thioglycoside type of linkers, it limits the donor scope as the electrophilic thio-activating reagent does not support synthetic design. The acid and base cleavable linker may interfere with the designed route as it can affect the protecting group on the building block used in the stacking process. The recent developments in linker chemistry have proven their strength concerning variable reaction conditions and stability for

making longer oligosaccharides. However, the demand for a better structural version for efficient and simple cleavage still needs to be developed.

2A. Synthesis and Utility of Metathesis Cleavable Linker for Gold-catalysed Solid Phase Glycosylation Reaction

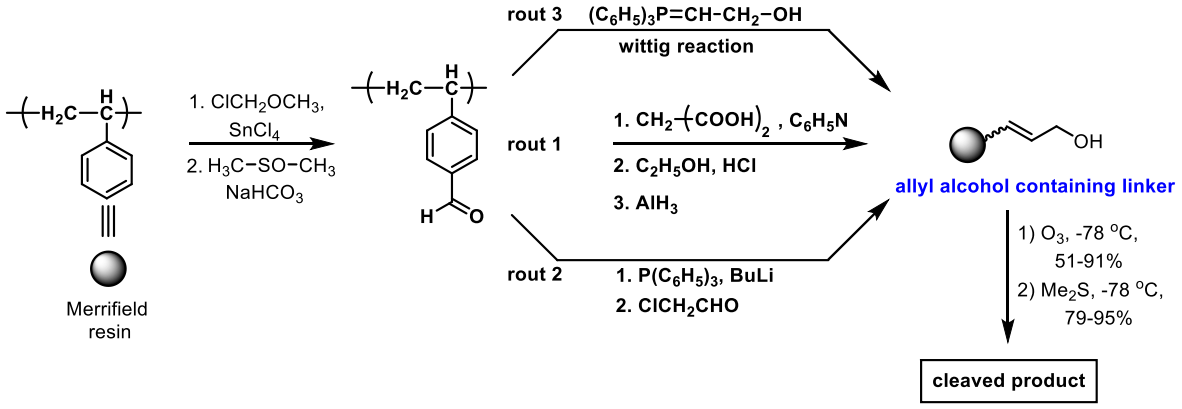
2A.1 Introduction:

The cleavage condition utilized in the synthetic strategy must be the most favorable and orthogonal to the glycosylation reaction. The variable-protecting groups used in protecting the relatively similar reactive hydroxyl groups, either permanently or temporarily, while synthesizing building blocks should not interfere with the reaction conditions applied for the cleavage and vice versa. Among all types of linkers, the metathesis cleavable linker is a unique linker concerning cleavage conditions. The reagent used for this cleavage reaction is very regioselective towards C=C and therefore does not hamper any other functionalities during the synthetic cycle. Generally, Grubbs catalysts are seen as more effective for this purpose, and the cleavage mechanism involved in it is either RCM (ring closing mechanism) or ROM (ring opening mechanism). Mechanistically this cleavage reaction demands two olefinic systems in the reaction partner.



Scheme 2A.1: Ring-closing cleavage reaction on a solid support

On resin linker synthesis: In 1971, Jean M. Fréchet and Conrad Schuerch introduced the synthetic strategy for synthesizing an allyl alcohol linker attached to the solid support. Then, they utilized it for synthesizing oligosaccharides.[23] To tackle the synthesis of this ready support, three different synthetic routes have been introduced, starting from the Merrifield resin (chloromethylated polystyrene-divinylbenzene).

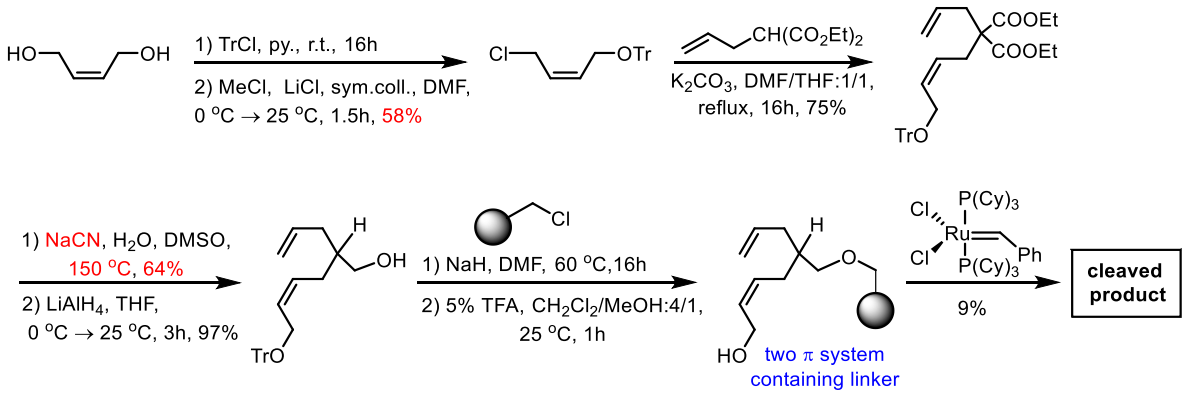


Scheme 2A.2: Synthesis of allyl alcohol containing linker attached solid support

As the support attached linker has only one double bond, the cleavage of the synthesized disaccharide from the support was performed using an ozonolysis reaction.

1) Two π systems containing linker attachment to solid support:

In 1999, Schmidt and co-workers introduced a metathesis cleavable linker with two double bonds in their composition.[24] In the strategic synthesis of linker attached solid support, they first synthesized the linker and then connected it to the Merrifield resin.

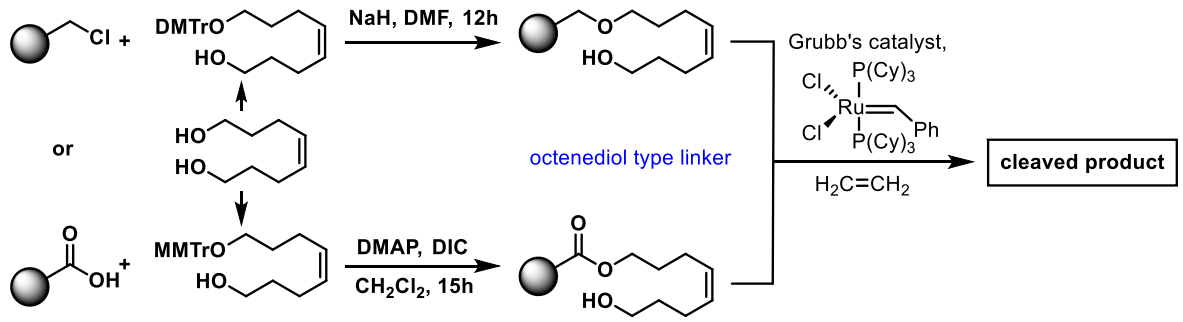


Scheme 2A.3: Synthesis of two double bond containing linker attached solid support

Using this ready support, they successfully synthesized tetrasaccharide and cleaved it from solid support at room temperature using 9 mol% of Grubbs catalyst I in 6 h (two times).

2) π system containing linker attachment to solid support:

Seeberger introduced the octane diol linker containing ether[25] and ester group[26] attached to resin in 2001. The advantages of these linkers are i) the cleavable double bond in the linker is inert under glycosylation conditions except thioglycosides, ii) the linker can be selectively cleaved under metathesis condition, iii) the resulting pentenyl group can be used for the glycosylation or hydrolysis or functionalization to get proper bioconjugable functionality.



Scheme 2A.4: Synthesis of one double bond containing linker attached solid support

The cleavage of the linker was performed using a Grubbs catalyst and gaseous ethylene. The synthesis of various oligosaccharides such as polymannan up to decasaccharide, phytoalexin elicitor hexasaccharide, Leishmania cap tetrasaccharide, and triglucosamine was carried out

using ether-linked support by the Seeberger group.[19] Additionally, they have synthesized N-Linked glycoprotein core pentasaccharide using ether-linked linker[27] whereas, the synthesis of Lewis^X pentasaccharide, Lewis^y hexasaccharide, and Le^y-Le^x nonasaccharide synthesis was carried out using an ester-linked support.[28]

2A.6 Rationale:

Schuerch's strategy of synthesizing the linker attached substrate includes the stepwise connections through functional group transformation on the support itself. The variable reagents that are generally used for functional group transformation involve the use of harsh reagents. Under such circumstances, constructing a linker over solid support may cause a core compositional change in resin or undergo unwanted damage or change in its physicochemical properties in some instances.

Although Schmidt's two π containing linker offers good yield on the cleavage at the end of the synthesis, the synthetic strategy they advanced for the linker was not so friendly, including multiple steps, harsh conditions, and the reagents for assembling the structure.

Seeberger's octanediol linker has been utilized for synthesizing a variety of linear and branch oligosaccharides with almost comparable yields. Still, the major disadvantage was the requirement of additional ethylene gas cylinder for the cleavage of the linker. Moreover, the cleavage requires 18 h treatment of 0.1 eq. of Grubbs catalyst two times and ethylene cylinder for completion. Therefore, the solid phase synthesis technique requires a suitable linker due to the use of various reagents during the glycosylation and deprotection steps in the synthetic cycle. On top of it, the designed strategy for the synthesis of linker attached solid support includes the multitude of reactions on the solid support before starting the actual synthesis is not a good practice which was seen in Schuerch's strategy. Seeberger's octanediol linker cleavage requires additional provision of an ethylene cylinder which mandates the particular arrangement of reaction setup requirements that may lead to a primary obstacle in developing a smooth protocol. So, both of these π bond-containing linkers attached supports are vulnerable in developing linker hooked solid support glycochemistry, which ultimately leads to lesser accessibility of glycans. On the other hand, Schmidt's two π containing linker can be readily cleaved using Grubbs catalyst alone. Therefore, in that perspective, we envisioned synthesizing a similar linker attached solid support could help in facile cleavage. Additionally, providing an easy synthetic route for synthesizing a similar linker from the commercially available simple

sugar unit will be more advantageous in reducing the number of steps is a good idea. With these perspectives, we designed a new linker attached to a solid support, as shown below.

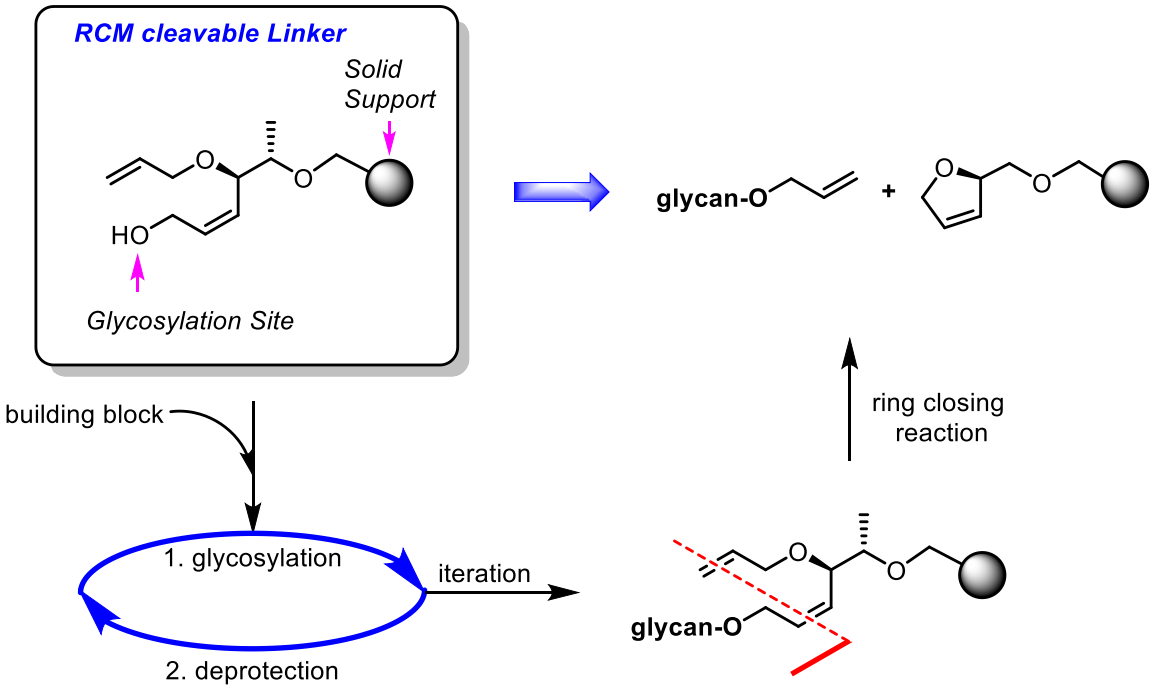


Figure 2A.3: Metathesis cleavable linker attached solid support

The valuable features of the designed linker are: i) it contains the reactive allylic hydroxyl group, which could act as an acceptor to bind the first sugar building block on the support, ii) it has two double bonds in their structural composition and, therefore can be cleaved easily using Grubbs catalyst I without the use of ethylene cylinder iii) the presence of ethereal linkage and the methyl substituent in the structure could help in offering excellent yield as per the previous literature reports.[29] Moreover, with the similar electronic environment in the linker structure has shown excellent yield for the cleavage reaction. iv) except for the electrophilic reagent environment, the linker can remain unaffected under all other conditions.

2A.7 Present work

After designing a metathesis cleavable linker, our synthetic endeavour started with synthesizing a metathesis cleavable linker. Although the designed linker molecule has fewer carbon atoms, the synthetic strategy typically includes multiple steps and unusual reagents. We envisioned that constructing a metathesis cleavable linker with two stereocenters, two hydroxyl functionality, and two double bonded system should be so easy that one can effortlessly build the molecule in a lesser number of steps, with commercially available reagents and minimal efforts.

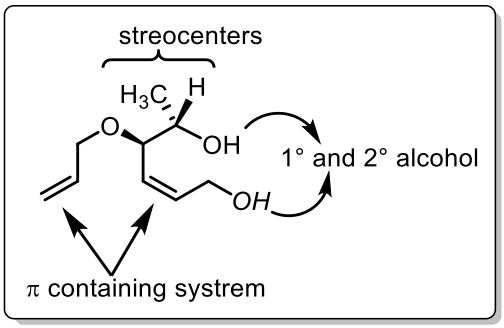
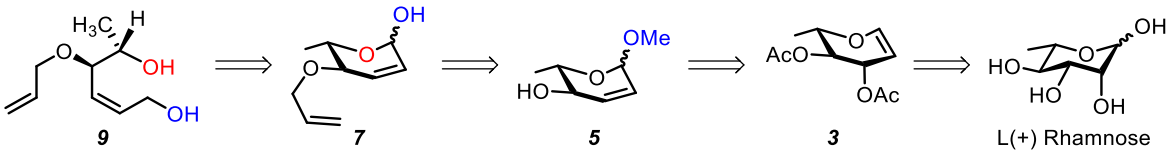


Figure 2A.4: Structure of the metathesis cleavable linker

2A.7.1 Retrosynthesis of metathesis cleavable linker

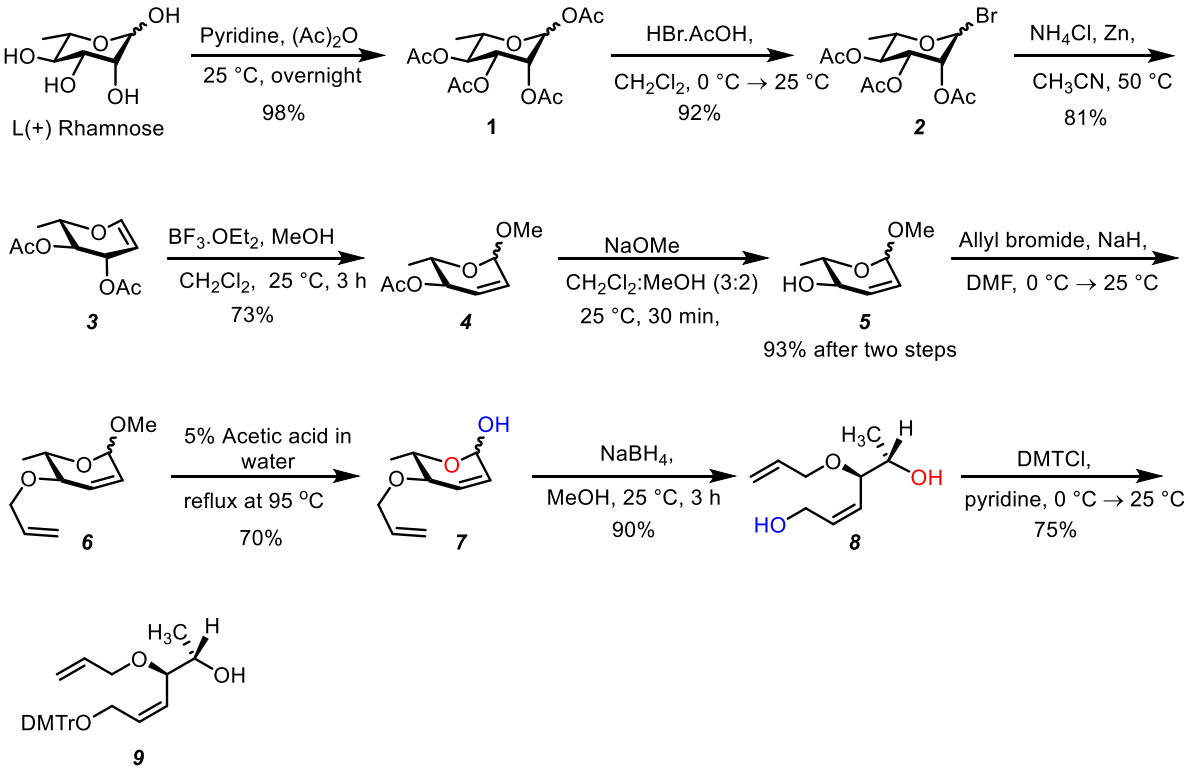
Before designing the best retrosynthetic approach, our first consideration was the functionalities present in the molecule, which can be built easily by connecting the small entities or choosing the precursor, which has useful inbuilt functionalities that could be moulded easily with simple reactions. As the molecule is rich in 'O' atoms with two stereogenic centers and two double bonds, it was apparent that we chose the sugar molecule as a starting precursor.



Scheme 2A.5: Retrosynthetic disconnection of the RCM cleavable linker

To synthesize the linker **9**, we thought of having the precursor intermediate **7**, which can be easily synthesized from the rhamnal **3** by carrying out the Ferrier reaction, followed by deacetylation and allylation. This intermediate **7** resembles the required carbon backbone with stereospecific carbon centers, and intermediate **7** can be smoothly converted to its open chain form by reduction reaction. The synthesis of rhamnal from L-rhamnose is the least challenging as the protocols are very well set. After carrying out the appropriate disconnection for the metathesis cleavable linker, our first efforts started with constructing the required linker for the solid phase oligosaccharide chemistry.

2A.7.2 Synthesis of the RCM cleavable linker



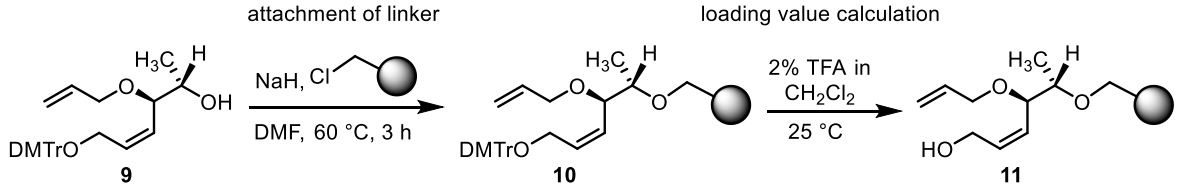
Scheme 2A.6: Synthesis of metathesis-cleavable linker

To begin the synthesis of the linker, we performed per-*O*-acetylation of L- rhamnose[30] using pyridine and acetic anhydride at room temperature and subsequently converted it to corresponding bromoside[31] using HBr in acetic acid. Further, without purification, the resultant bromoside was converted to rhamnal using ammonium chloride and activated zinc dust in acetonitrile at 50 °C to yield compound **3** in 81%.[32] The synthesized compound **3** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The resultant rhamnal **3** was treated with boron trifluoride etherate and methanol at room temperature to perform the Ferrier reaction to obtain α/β mixture of *O*-Methyl glycoside product **4** in 70% yield.[33] Then, deacetylation[33] was carried out using the known protocol of sodium methoxide to get the secondary hydroxyl free and subsequently converted to its allyl derivative using allyl bromide **6**. This ethereal bond formation reaction was performed using sodium hydride and the allyl bromide in DMF solvent to afford product **6** in quantitative yield.[34] At this stage, we thoroughly characterized this mixture of isomeric products **6** using spectroscopic techniques. In ^{1}H NMR spectrum, the signal at δ 5.96 – 5.82 (m, 2H), 5.27 (dq, *J* = 17.2, 1.6 Hz, 2H), and 5.17 (dq, *J* = 10.4, 1.4 Hz, 2H) ppm indicated the presence of the allylic substituent and the olefinic ring double bond protons were noticed at δ 5.96 – 5.82 (m, 2H) and 5.80 – 5.69 (m, 2H) ppm. The *O*-methyl protons at

the C-1 position and the ring C-5 methyl substituent appeared around δ 3.40 and 1.29 ppm. ^{13}C NMR spectrum showed similar functional groups, wherein the peaks at δ 134.9, 134.8, 131.1, 130.1, 128.9, 126.6, 117.5, 117.3 ppm corresponding to the olefinic group, and the anomeric methyl and methyl at the C-5 position were seen at δ 55.7, 55.1, 18.7, 18.3 ppm respectively.

The O-methyl glycosidic product **6** was further treated with 5% acetic acid in water at 95 °C to get hemiacetal[35] **7** in 70% yield. After chromatographic purification, we subjected compound **7** to the ring opening reaction using sodium borohydride to obtain the diol **8** in 90% yield.[36] Both the product **7** and **8** were characterized using ^{1}H, ^{13}C NMR and HRMS analysis (see the experimental section 2A). Further, the selective protection of allylic alcohol in the presence of secondary alcohol was performed using dimethoxy trityl chloride/pyridine to yield 75% of the desired product **9**. Compound **9** was confirmed using ^{1}H and ^{13}C NMR spectroscopy, the proton NMR signals at δ 7.48 – 7.44 (m, 2H), 7.38 – 7.33 (m, 4H), 7.32 – 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 6.87 – 6.81 (m, 4H) and 3.80 (s, 6H) ppm corresponding to the DMTr group and the δ values at 6.08 – 5.99 (m, 1H), 5.87 – 5.76 (m, 1H), 5.49 (ddt, *J* = 11.0, 9.3, 1.6 Hz, 1H), 5.19 – 5.11 (m, 1H), 5.11 – 5.06 (m, 1H) corresponding to five protons of two olefinic bonds confirmed the assigned structure. The signal for the terminal methyl substituent was also seen at the δ 1.06 (d, *J* = 6.2 Hz, 3H) ppm. Similar groups were identified by ^{13}C NMR spectroscopy as well. The peaks at δ 158.6(2C), 145.0, 136.2, 136.2, 134.8, 132.9, 130.1(2C), 130.1(2C), 128.5, 128.2(2C), 127.9(2C), 126.9, 117.0, 113.2(4C) and 55.3(2C) ppm confirms the presence of DMTr group and the two double bonds. The remaining two methylenes were found at δ 69.3, 60.1 ppm in the DEPT spectra, and the terminal methyl group at 55.3(2C) confirms the formation of compound **9**. This was further supported by HRMS analysis, ESI-MS: m/z calcd. for $[C_{30}H_{34}O_5Na]^+$: 497.2304; found: 497.2303.

Coupling of linker to the Merrifield resin and determination of loading value



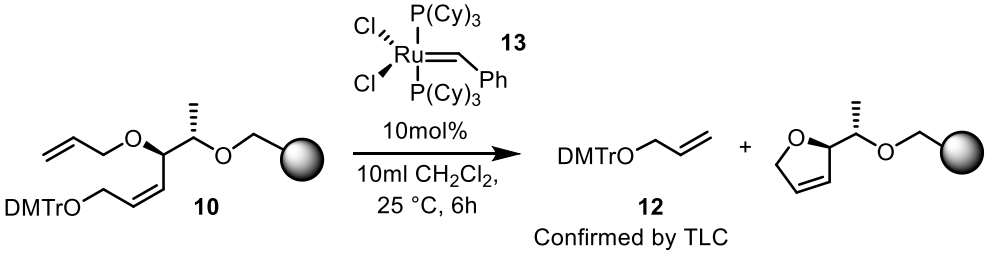
Scheme 2A.7: Attachment of linker to support and calculation of loading value

After standardizing all the reactions of the designed synthetic protocol for the linker **9,** we tested its viability by performing the large-scale synthesis. Further, to obtain the linker attached solid support **10**, we chose the Merrifield resin (loading value 1.1 mmol/g) as a suitable solid support as it has shown the applications in solid-phase oligosaccharide syntheses.

To synthesize the linker attached solid support **10**, we performed the reaction between the excess linker and the Merrifield resin in DMF solvent. The substitution reaction over the solid support was carried out at 0 ^{o}C in the presence of sodium hydride base and the catalytic amount of tetrabutylammonium iodide and then stirred overnight at room temperature to obtain the desired linker attached support **10**. After washing and drying, the resultant support was treated with 2% trifluoroacetic acid in a dichloromethane solution to remove the DMTr group. Using this standard reaction protocol, we made a free hydroxyl containing linker attached solid support **11**, which can act as an acceptor in the glycosylation reaction to connect the sugar unit over the solid support. Furthermore, we have also calculated the loading value determination experiment for the linker attached support **10**. On treating the resin **10** with 2% TFA solution in CH_2Cl_2, the resultant trityl ion concentration was measured at 495 nm wavelength using the spectrophotometry.[37] The loading value calculated for resin **10** was 0.395 mmol/g.

Conformation of compound 10 by cleavage reaction from the solid support:

After the attachment of the metathesis cleavable linker to the Merrifield resin, our first attempt was made to check whether the ring-closing metathesis reaction[24] for this π-containing linker is applicable or not. The resin was treated with the Grubbs catalyst I using the cleavage protocol III (see experimental section), and the compound was analyzed by TLC and LC-MS analysis, which confirmed the desired product **12**.



Scheme 2A.8: Metathesis cleavage reaction on solid support

TLC of the RCM cleavage reaction after linker attachment to support

LCMS run for the RCM cleaved product

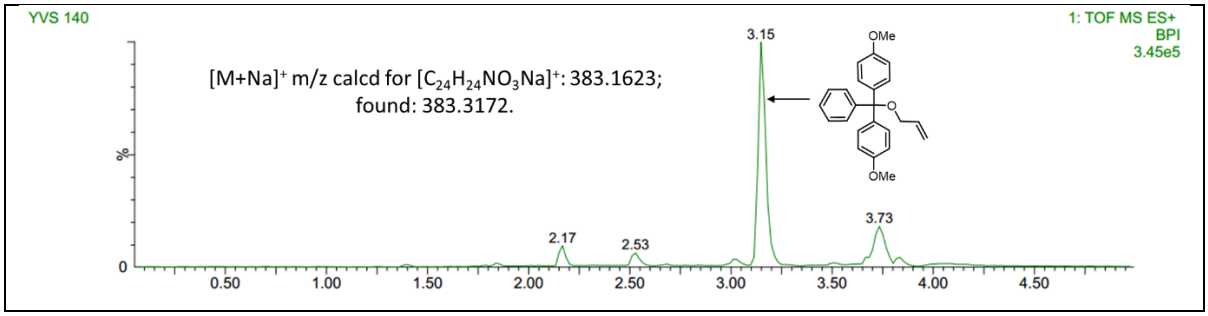
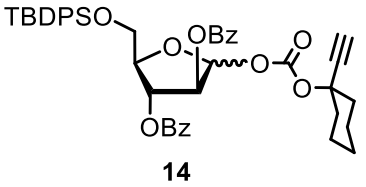


Figure 2A.5: Product analysis using TLC and LCMS

TLC analysis clearly showed the exact matching of the Rf value of the cleaved product with the authentic one. The LC-MS analysis also matched the *m*/*z* value with the dimethoxytrityl allyl ether. The HRMS analysis, $[M+Na]^+$ *m*/*z* calcd for $[C_{24}H_{24}NO_3Na]^+$: 383.1623; found: 383.3172. Therefore, this cleavage reaction and product analysis gave evidence for the presence of DMTr allyl ether and olefinic functionality over the solid support.

Synthesis of required donor: The synthesis of alkynyl glycosyl carbonate donor **14** was accomplished using a known protocol established by Maidul and co-authors in our group.[38]



14

Figure 2A.6: Structure of the alkynyl glycosyl carbonate donor

2A.7.3 Reagent preparation and general protocol for synthesis

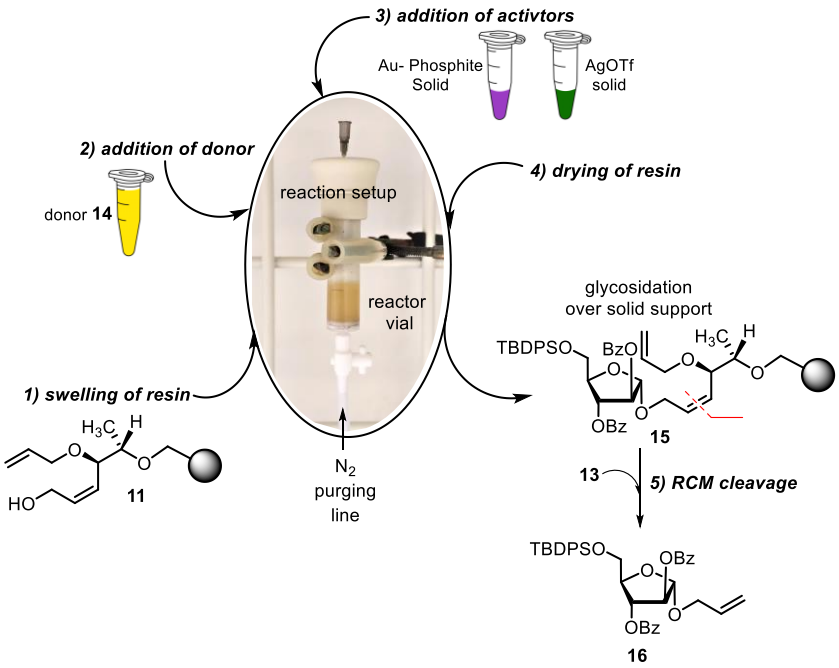
Reagent Preparation

After successfully synthesizing the linker attached solid support **11** and the required donor **14**, we focused on preparing all essential reagents necessary for solid phase glycosylation chemistry. For that, the building block and activator reagents were weighed accurately and enclosed into Eppendorf tubes just before their use. The donor solution was prepared by adding dry CH_2Cl_2 into an accurately weighed donor, and the activators, gold-phosphite and silver triflate, were used as such in their dry solid form. The Grubbs generation I catalyst was considered and used directly in its solid form to perform the metathesis reaction.

General Protocol for Synthesis

After loading the functionalized resin into the reaction vessel, dry CH_2Cl_2 was added, and the resin was kept for swelling with nitrogen bubbling for 5 min. Then after draining the solvent under nitrogen pressure, the solution of the building block (4 eq. in CH_2Cl_2) was added to the reaction vessel. Then the resulting biphasic mixture was again kept under bubbling with N_2 flow. After 5 min, both the activators, gold-phosphite (10 mol %); silver triflate (15 mol%), were added to the reaction vessel in their solid form under the inert atmosphere. The reaction mixture was bubbled with nitrogen flow for 30 min. After that, the excess reagents were drained out and washed the resin successively with washing solvents (CH_2Cl_2, DMF, and CH_2Cl_2) to get a reagent-free substrate attached to the solid support. Then, the resin was dried using high vacuum. Furthermore, the deprotection of the temporary protecting group, i.e., the TBDPS group, was performed easily by adding appropriate reagents to get free –OH over the solid support. The reaction progress at each step was checked by performing the RCM reaction using the Grubbs catalyst I under an argon atmosphere in a dry CH_2Cl_2 solvent. The resultant cleaved product was monitored by TLC analysis. The cleavage was performed two times to offer the cleaved anomeric allyl containing molecule, which was further isolated using silica gel column chromatography or HPLC technique. The isolated product was characterized completely using NMR and HRMS analysis.

2A.7.4 Gold-catalysed glycosylation reaction over support



Scheme 2A.9: Gold catalysed glycosylation reaction over the solid support 11

To show the utility of the metathesis cleavable linker, the first glycosylation was performed using gold-catalyzed chemistry (the glycosylation method is discussed in chapter 3). The reaction vessel (10 mL PTFE vial) was charged with the functionalized resin **11** (50 mg; loading 0.395 mmol/g; 0.055 mmol), and CH_2Cl_2 (2 mL) was added for swelling. To start the synthesis, the resin was washed with dry CH_2Cl_2 (2x), and coupling was performed, as depicted in Table 1.

Table 1: First glycosylation over solid support

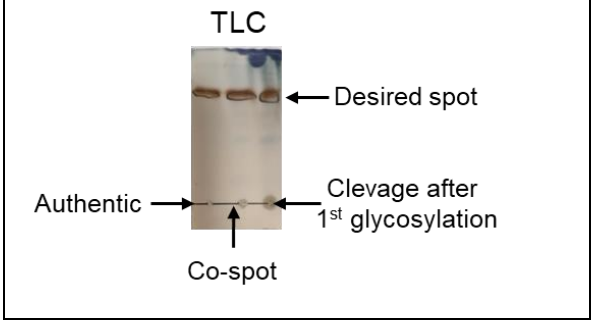
Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	2	4 eq. of donor **14** in 2 mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				

It is important to note that one time coupling of the donor was found sufficient to complete the reaction over the solid support, which contains allylic alcohol as an acceptor.

Cleavage, purification and analysis of product **16**:

After completing the first glycosylation reaction, the reaction conversion was checked by cleavage reaction. The cleavage reaction was performed using the cleavage protocol III, and the cleaved product was confirmed by TLC analysis.

TLC of the first glycosylation



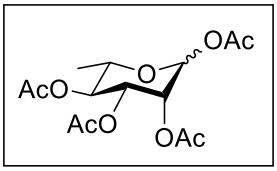
After confirmation of the product by TLC analysis, the whole 50 mg of resin was left using cleavage protocol III and purified using protocol IV. The cleavage reaction afforded (7 mg, 56% yield) desired product, which NMR and HRMS analysis confirmed.

2A.8 Conclusions: We successfully synthesized a metathesis cleavable linker starting from L-rhamnose sugar. The synthetic strategy is impressive concerning designing, reaction yields, number of steps and ease of synthesis. The synthesised linker was attached to the Merrifield resin and subsequently utilized for glycosylation reaction under gold-catalyzed conditions. Overall reaction yields were found effective concerning glycosylation and cleavage reaction, indicates that linker can be explored further for the synthesis of oligosaccharides and small organic molecules. The methyl substituent in the linker structure was not studied in detail and seems efficient in the overall cleavage process.

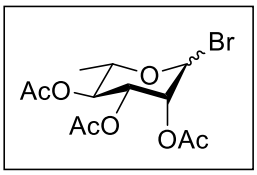
2A.9 Experimental section:

2A.9.1 Preparation of the linker and its attachment to the Merrifield resin

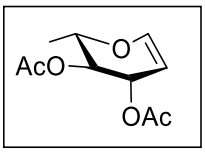
Synthesis of metathesis-cleavable linker



Synthesis of 1,2,3,4-Tetra-*O*-acetyl-L-rhamnose (1): L-Rhamnose monohydrate (20.0 g, 121.83 mmol) was dissolved in acetic anhydride (132 mL, 140 mol) and pyridine (132 mL, 164 mol) and then stirred for 12 h. After completion of the reaction, the reaction mixture was neutralized with diluted HCl and extracted in CH_2Cl_2, washed with $NaHCO_3$, and saturated NaCl solution. Finally, the solvent was evaporated on a rotary evaporator and then obtained crude residue was used for the next step without purification. The received product **1** was a colourless oil with 95 % yield (38.5 g). R_f = 0.6 (ethyl acetate:hexane 40:60).



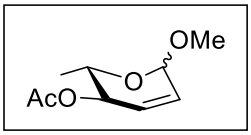
Synthesis of 1-Bromo-2,3,4-Tri-*O*-acetyl-L-rhamnopyranosyl bromide (2): Per-*O*-acetyl L-rhamnose **1** (19.50 g, 58.68 mmol) was dissolved in anhydrous CH_2Cl_2 (110 ml) and cooled to 0 °C. HBr (33% in AcOH, 29.76 ml) was added dropwise, and the reaction mixture was stirred for one hour and then shifted to room temperature for 2 h. The reaction was poured onto an ice-water mixture and stirred for 15 min. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with saturated aq. $NaHCO_3$, then brine, and finally dried over Na_2SO_4. After that, the extracted volume was filtered through cotton, and finally, the solvent was removed under reduced pressure to give a viscous oil as the desired compound **2** with 97% yield (20.15 g). R_f = 0.35 (ethyl acetate:hexane 20:80).



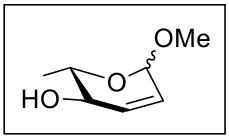
Synthesis of 3,4-Di-*O*-acetyl L-rhamnal (3): Under the N_2 gas atmosphere, the rhamnopyranosyl bromide **2** (20.15 g, 57.06 mmol) was dissolved in 200 mL of CH_3CN, and then zinc dust (27.98 g, 427.92 mmol) and ammonium

chloride (22.89 g, 427.92 mmol) were added at 50 °C under stirring. Upon completion of the reaction within 45 min (monitored by TLC), inorganic salts and excessive zinc dust were removed by filtration through Celite®. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel flash column chromatography to afford the corresponding rahmnal in pure form. The product **3** was isolated in 80% yield (20.15 g). R_f = 0.5 (ethyl acetate:hexane 20:80); $[\alpha]_D^{25}$ = 56.667 (*c* = 0.33, $CHCl_3$); IR ($CHCl_3$); 3624, 2950, 1742, 1649, 1452, 1375, 1251, 1171, 1043 cm^{-1}; ^1H NMR (399.78 MHz, $CDCl_3$) δ 6.43 (dd, *J* = 6.1, 1.5 Hz, 1H), 5.33 (m, 1H), 5.03 (dd, *J* = 8.2, 6.1 Hz, 1H), 4.77 (dd, *J* = 6.1, 3.0 Hz, 1H), 4.15 – 4.06 (m, 1H), 2.08 (d, 3H), 2.04 (d, 3H), 1.31 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100.53 MHz, $CDCl_3$) δ 170.8, 170.1, 146.1, 98.9, 72.6, 71.9, 68.4, 21.2, 21.1, 16.7. HRMS (ESI-MS): m/z calcd. for $[C_{10}H_{14}O_5Na]^+$: 237.0739; found: 237.0740.

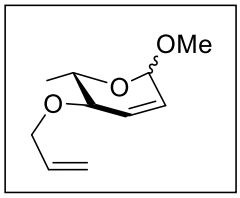
Synthesis of Methyl 4-*O*-acetyl-2,3,6-trideoxy-α/β-L-*erythro*-hex-2-enopyranoside [α:β(1:0.21)](4): To a solution of 3,4-di-*O*-acetyl-L-rhamnal **3** (16.30 g, 76.09 mmol) and methanol (46.18 mL, 1.14 mol) in CH_2Cl_2 (170 mL), 4Å molecular sieves were added under N_2 atmosphere. After 15 min, BF_3•OEt_2 (15.03 mL, 0.014 g, 121.75 mmol) was added dropwise at room temperature under stirring conditions. The reaction was stirred and monitored for 4 h, and then the reaction mixture was quenched with $NaHCO_3$ solution. The reaction mixture was subsequently extracted with CH_2Cl_2 and washed with saturated NaCl solution. The combined organic layers were dried over $NaSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (hexane:ethyl acetate) to yield desired compound **4** (10.39 g, 73%) as a colorless oil. R_f = 0.55 (ethyl acetate:hexane 10:90); IR ($CHCl_3$); 2924, 1741, 1453, 1376, 1241, 1179, 1045, 968 cm^{-1}; ^1H NMR (399.78MHz, $CDCl_3$) δ 5.97 – 5.87 (m, 1H), 5.89 (d, *J* = 1.1 Hz, 1H), 5.85 (dt, *J* = 10.4, 1.1 Hz, 1H), 5.84 – 5.74 (m, 1H), 5.09 – 5.01 (m, 1H), 5.03 (d, *J* = 1.6 Hz, 2H), 4.85 (t, *J* = 2.7 Hz, 1H), 3.93 (dq, *J* = 9.0, 6.1 Hz, 1H), 3.86 (p, *J* = 6.4 Hz, 1H), 3.46 (s, 3H), 3.43 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 1.31 (d, *J* = 6.5 Hz, 3H), 1.23 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (100.53 MHz, CDCl3) δ 170.7, 170.6, 130.3, 129.9, 128.0, 127.8, 96.9, 95.5, 71.4, 71.0, 69.6, 64.9, 55.9, 55.1, 21.2, 18.7, 18.1, 18.1. HRMS (ESI-MS): m/z calcd. for $[C_9H_{14}O_4Na]^+$: 209.0790; found: 209.0770.



Synthesis of Methyl 2,3,6-trideoxy-α/β-L-*erythro*-hex-2-enopyranoside (5): Under N_2 atmosphere, the methyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside **4** (8.82g, 47.38 mmol) was dissolved in CH_2Cl_2:MeOH (2:3). At room temperature, NaOMe (0.3 mmol) was added pinch

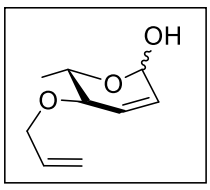


wise and then stirred for ½ an hour. After completion of the reaction, the solvent was evaporated on a rotary evaporator. The reaction mixture was then redissolved in CH_2Cl_2; washed with water and brine. The extracted organic solvent was then dried over Na_2SO_4 and evaporated using a rotary evaporator, and the crude compound was used as such for the next step. The crude compound **5** was obtained (6.26 g, 92%) as a colourless oil. R_f = 0.35 (ethyl acetate:hexane 30:70).



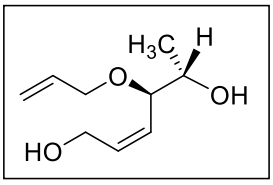
Synthesis of Methyl 4-*O*-allyl-2,3,6-trideoxy-α/β-L-*erythro*-hex-2-enopyranoside [α:β(1:0.07)](6): To a solution of Methyl 2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside **5** (6.26 g, 43.40 mmol) in dry DMF (50 mL), NaH (4.34 g, 108.50 mmol) and benzyl bromide (5.63 mL, 65.10 mmol) and a catalytic amount of tetrabutylammonium iodide (small pinch) were added at 0 °C. The mixture was vigorously stirred for an initial 0.5 h at 0 °C and then gradually shifted to room temperature. The reaction progress was monitored using TLC. After the consumption of starting material, the reaction mixture was diluted with chilled water (50 mL). The reaction mass was then extracted in CH_2Cl_2. The combined organic phases were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give a crude residue, which was subjected to silica gel column chromatography to yield compound **6** (7.45 g, 93.0%) as a colorless liquid. R_f = 0.7 (ethyl acetate:hexane 20:80); IR ($CHCl_3$); 2911, 1653, 1455, 1395, 1305, 1254, 1066, 966 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 6.05 – 5.97 (m, 2H), 5.96 – 5.82 (m, 2H), 5.80 – 5.69 (m, 2H), 5.27 (dq, *J* = 17.2, 1.6 Hz, 2H), 5.17 (dq, *J* = 10.4, 1.4 Hz, 2H), 5.02 (q, *J* = 1.7 Hz, 1H), 4.80 (dt, *J* = 2.8, 1.1 Hz, 1H), 4.15 – 4.08 (m, 2H), 4.06 – 3.96 (m, 2H), 3.82 (dq, *J* = 9.0, 6.2 Hz, 1H), 3.74 – 3.64 (m, 2H), 3.62 (dq, *J* = 9.0, 1.6 Hz, 1H), 3.45 (s, 3H), 3.40 (s, 3H), 1.34 (d, *J* = 6.1 Hz, 3H), 1.29 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100.67 MHz, CDCl3) δ 134.9, 134.8, 131.1, 130.1, 128.9, 126.6, 117.5, 117.3, 97.7, 95.6, 76.3, 75.4, 72.4, 70.5, 69.9, 65.8, 55.7, 55.1, 18.7, 18.3 HRMS (ESI-MS): m/z calcd. for $[C_{10}H_{16}O_3Na]^+$: 207.0997; found: 207.0987.

Synthesis of 4-*O*-allyl-2,3,6-trideoxy-α/β-L-*erythro*-hex-2-enopyranoside [α:β(1:0.45)](7): Compound **6** (7.34 g, 39.85 mmol) was dissolved in 5% aqueous acetic acid (350 mL) and refluxed under stirring at 95 °C for half an hour. After cooling the reaction mixture, sodium bicarbonate solution was added to quench the acidic reaction mass and extracted in CH_2Cl_2. Then all the combined organic volumes were washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was evaporated on a rotary evaporator, and then desired compound was isolated by silica gel

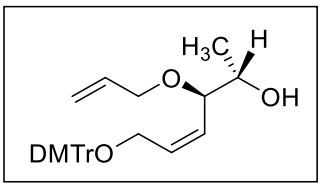


column chromatography using (hexane:ethyl acetate) system to afford the title compound **7** (5.03 g, 74%, α/β:1:0.45) as a colourless liquid. R_f = 0.47 (ethyl acetate:hexane 30:70). IR ($CHCl_3$); 3417, 2916, 1731, 1388, 1298, 1079, 978 cm^{-1}; ^1H NMR (399.78 MHz, $CHCl_3$) δ 6.07 – 6.00 (m, 2H), 5.97 – 5.85 (m, 2H), 5.85 – 5.78 (m, 2H), 5.39 – 5.32 (m, 2H), 5.32 – 5.26 (m, 2H), 5.22 – 5.17 (m, 2H), 4.18 – 4.10 (m, 2H), 4.06 – 3.99 (m, 2H), 3.92 (dq, *J* = 8.9, 6.2 Hz, 1H), 3.72 – 3.64 (m, 2H), 3.61 (dq, *J* = 8.8, 1.6 Hz, 1H), 2.99 (d, *J* = 8.5 Hz, 1H), 2.91 (d, *J* = 5.9 Hz, 1H), 1.36 (d, 3H), 1.31 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100.53 MHz, $CHCl_3$) δ 134.6, 134.6, 131.1, 130.4, 129.8, 127.4, 117.7, 117.6, 91.9, 89.0, 76.0, 75.6, 72.9, 70.6, 70.2, 66.0, 18.7, 18.4. HRMS (ESI-MS): m/z calcd. for $[C_9H_{14}O_3Na]^+$: 193.0841; found: 193.0831.

Synthesis of (4*R*,5*S*,*Z*)-4-(allyloxy)hex-2-ene-1,5-diol (8): To a hermiacetals **7** (6.03 g, 35.43 mmol) in methanol (150 mL) at 0 °C, sodium tetrahydroborate (1.34 g, 35.43 mmol) was added in portions over 20 min. After stirring for 1 h, the reagent was quenched by adding ice-cold water, and the reaction solvent was evaporated on the rotary evaporator. The reaction mixture was extracted multiple times with ethyl acetate. The extract was washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The compound was purified using a silica gel chromatography (hexane:ethyl acetate) system to afford desired compound **8** (5.5 g, 90% over two steps) as a colourless liquid. R_f = 0.3 (ethyl acetate:hexane 60:40). $[\alpha]_D^{25}$ = -23.939 (*c* = 0.33, $CHCl_3$) IR ($CHCl_3$); 3375, 2915, 1722, 1647, 1405, 1254, 1063 cm^{-1}; ^1H NMR (400.31 MHz, $CDCl_3$) δ 6.02 – 5.94 (m, 1H), 5.93 – 5.81 (m, 1H), 5.52 – 5.44 (m, 1H), 5.28 – 5.21 (m, 1H), 5.20 – 5.13 (m, 1H), 4.19 – 4.12 (m, 2H), 4.09 – 4.02 (m, 1H), 4.02 – 3.96 (m, 1H), 3.88 – 3.74 (m, 2H), 2.68 (brs, 2H), 1.17 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 134.8, 134.3, 130.0, 117.3, 78.3, 69.6, 69.2, 58.5, 18.7. HRMS (ESI-MS): m/z calcd. for $[C_9H_{17}O_3Na]^+$: 195.0997; found: 195.1029.



Synthesis of (2*S*,3*R*,*Z*)-3-(allyloxy)-6-(bis(4-methoxyphenyl)(phenyl)methoxy)hex-4-en-2-ol (9): To a diol compound **8** (1.52 g, 8.83 mmol) in dry pyridine, dimethoxytrityl chloride (3.14 g, 9.27 mmol) was added and stirred the reaction mixture for 2 h at room temperature. After completion of the reaction, pyridine was evaporated on a rotary evaporator, the resultant residue was purified by silica gel column chromatography using (hexane:ethyl acetate) to afford the corresponding desired compound **9** (3.3 g, 78% over two steps) as a pale yellow colourless liquid. R_f = 0.45 (ethyl acetate:hexane 30:60). $[\alpha]_D^{25}$ = 8.182 (*c* = 0.33, $CHCl_3$) IR ($CHCl_3$); 3477, 2916, 1605, 1506, 1453, 1299, 1246, 1174, 1040 cm^{-1}; ^1H



NMR (400.31 MHz, $CDCl_3$) δ 7.48 – 7.44 (m, 2H), 7.38 – 7.33 (m, 4H), 7.32 – 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 6.87 – 6.81 (m, 4H), 6.08 – 5.99 (m, 1H), 5.87 – 5.76 (m, 1H), 5.49 (ddt, *J* = 11.0, 9.3, 1.6 Hz, 1H), 5.19 – 5.11 (m, 1H), 5.11 – 5.06 (m, 1H), 4.01 (ddt, *J* = 12.8, 5.1, 1.5 Hz, 1H), 3.80 (s, 6H), 3.78 – 3.69 (m, 4H), 3.62 (ddd, *J* = 11.9, 5.6, 1.8 Hz, 1H), 2.23 (d, *J* = 4.0 Hz, 1H), 1.06 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 158.6(2C), 145.0, 136.2, 136.2, 134.8, 132.9, 130.1(2C), 130.1(2C), 128.5, 128.2(2C), 127.9(2C), 126.9, 117.0, 113.2(4C), 86.4, 78.0, 69.4, 69.3, 60.1, 55.3(2C), 17.9. HRMS (ESI-MS): m/z calcd. for $[C_{30}H_{34}O_5Na]^+$: 497.2304; found: 497.2303.

Coupling of linker to Merrifield Resin

The linker **9** (1.377 g, 0.88 mmol, 3.3 equiv.) and DMF (10 mL) were added to a round bottom flask. Then, upon cooling to 0 ^oC, NaH (60% dispersion in mineral oil, 0.069 g, 1.1 mmol, 3.3 equiv.) was added slowly, and the solution was kept under stirring for 1 h. Merrifield resin (1% crosslinked: 0.8 g, 1.1 mmol,1 equiv.) and tetrabutylammonium iodide 0.037g, 0.88 mmol, 0.1 equiv.) was added at the same temperature. After shaking for 1 h at 0 ^oC the reaction mixture was warmed to room temperature and again shaken for 12 h. Capping of the unreacted sites was performed using methanol (0.1 mL) and NaH (60% dispersion in mineral oil, 0.1 g) for 4 h. Finally, methanol (5 mL) was added, and resin was washed with 10 mL each of 1:1 MeOH:DMF, 3X (DMF, THF, and CH_2Cl_2). After drying under vacuum, afforded 0.95 g resin. DMT functionalized resin deprotection was performed using 3x20 mL of 2% trifluoroacetic acid\$CH_2Cl_2$ solution. Further, the resin was neutralized by washing with 3x20 mL of CH_2Cl_2, 1% TEA\$CH_2Cl_2$, CH_2Cl_2, and then dried under vacuum to afford 0.89 g of metathesis cleavable linker attached resin **10**.

Loading value Determination

An aliquot of DMTr protected linker attached support (1 mg) is accurately weighed into a 50 mL volumetric flask. The flask was filled with 2% trifluoroacetic acid in dichloromethane to release the dimethoxytrityl (DMT) cation. Then, UV absorption was measured on a spectrophotometer at 495 nm for the resulting orange colour solution. The loading value of resin in mmol/g was then calculated as follows:

For λ_{495}, [DMTr] = Absorbance at 495 nm x dilution factor/Molar extinction coefficient

Where, A_{495} is the absorbance at 495 nm, and the molar extinction coefficient is used as 71700 $M^{-1}cm^{-1}$. After taking the average of the two readings and calculating the corresponding loading value was found as 0.395 mmol/g.

2A.9.2 General procedures and set-up for manual solid-phase oligosaccharide synthesis

Preparation of Solutions and Reagents for SPOS

Preparation of building block solution: 4 equivalents of building block (e.g., glycosyl alkynyl carbonate donor) into an Eppendorf tube and dissolved in 2 mL of anhydrous CH_2Cl_2.

Activator reagents: Solid gold phosphite (10 mol%) and AgOTf (15 mol%) were weighed into individual eppendorf tubes, and the tubes were sealed with parafilm until their utilization.

Protocols for synthesis

Protocol 1 – Swelling of resin:

The functionalized resin was loaded into the reaction vessel; dry CH_2Cl_2 was added and kept for 5 min for swelling of the resin. The solvent was drained before starting the reaction.

Protocol 2 - Glycosylation with carbonate donor:

The optimisation of the gold-catalyzed glycosylation reaction condition has been discussed in detail in chapter 3. The building block donor solution (4 equiv. 0.125 mmol) in 2 mL dry CH_2Cl_2 was delivered under a nitrogen atmosphere to the reaction vessel. The resin was then mixed with donor solution for 10 minutes by bubbling N_2 gas. After that, with the slight interruption of N_2 bubbling, solid gold phosphite (10 mol %); Silver triflate (15 mol%) were added to the reaction vessel. The reaction mixture was then left for 30 min under Nitrogen bubbling. The solution was drained, and the resin was washed with CH_2Cl_2, DMF, and CH_2Cl_2 (3x with 2 mL for 15 s sequentially). Using CH_2Cl_2 solvent, the resin was then transferred to another reaction vessel and washed with 2 mL dry CH_2Cl_2 three times. The resin was then dried using the vacuum pump.

Post-synthesis Protocols

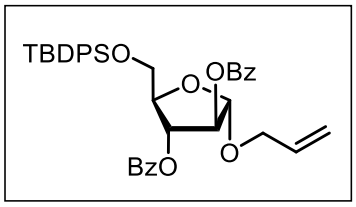
Protocol III – Metathesis-cleavage: Substrate attached resin was swelled in a reaction vessel with 10 mL of dry CH_2Cl_2. The resulting suspension in a closed vessel fitted with septum and syringe needle was degassed for 30 min using argon gas. The Grubbs I catalyst (9 mol%) was added, and then the reaction mixture was stirred using an argon stream for 5h at room temperature. The reaction solvent was collected in another vessel. Further, the resin was washed three times with 2 mL CH_2Cl_2, and combined filtrates were evaporated at low temp (0 °C) using a rotary evaporator. The resin was dried for 2h under a high vacuum, and then similar

steps were repeated one more time to perform the complete cleavage reaction. The filtrate obtained each time was analyzed by TLC and MALDI analysis.

Protocol IV - Purification of protected oligosaccharide: after cleavage of the protected monomer from the solid support, it was purified by silica gel column chromatography using a hexane:ethyl acetate system for complete characterization.

2A.9.3 NMR of the RCM cleaved product 16 from the solid support

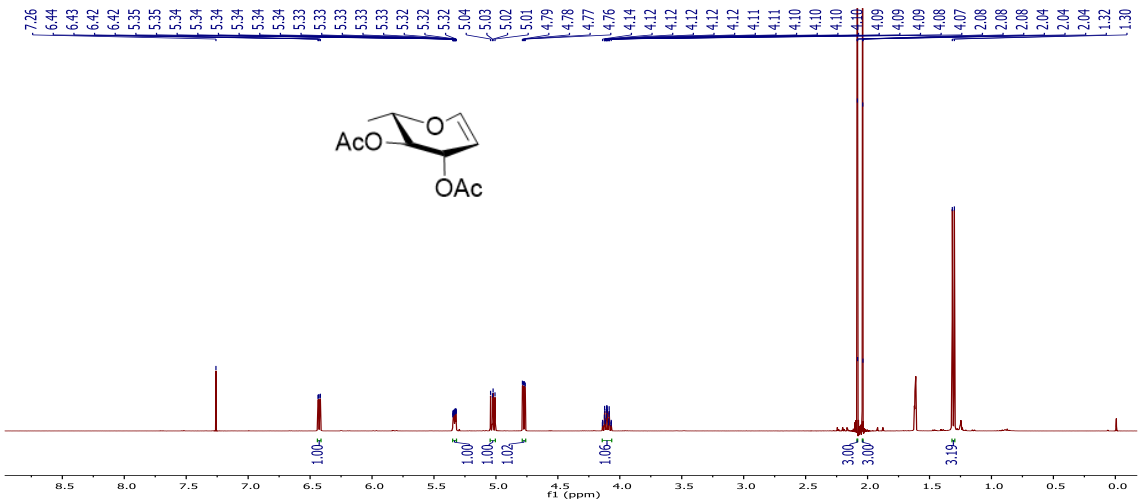
Allyl 2,3-di-*O*-benzoyl-5-*O*-^{t}butyldiphenylsilyl-α-D-arabinofuranoside (16):



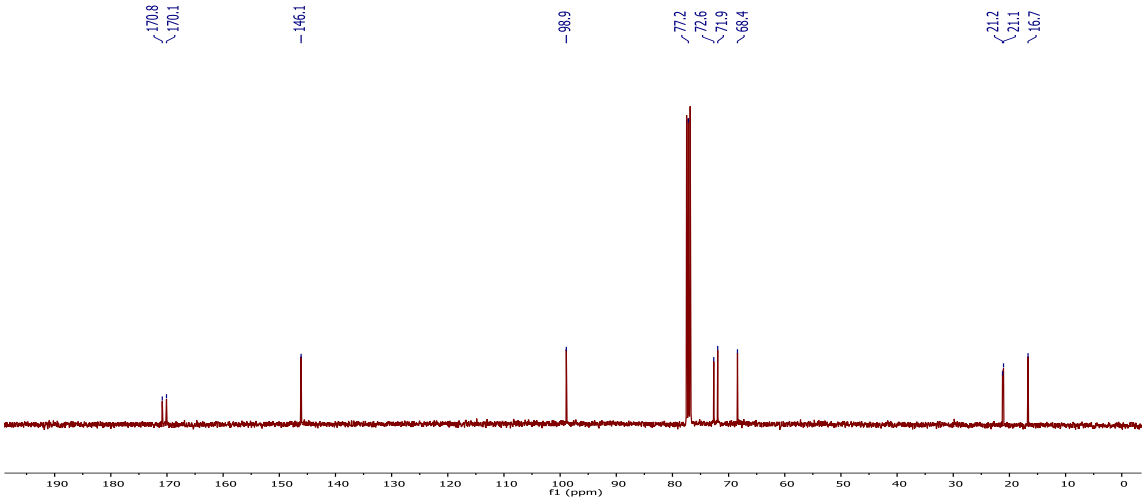
$[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): -17.5; IR (cm^{-1}): 3439, 3029, 2930, 1599, 1454, 1214, 701; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 8.14 – 8.07 (m, 2H), 8.06 – 8.01 (m, 2H), 7.79 – 7.74 (m, 4H), 7.65 – 7.57 (m, 2H), 7.52 – 7.46 (m, 2H), 7.46 – 7.35 (m, 8H), 6.01 (m, 1H), 5.67 (dd, *J* = 5.2, 1.6 Hz, 1H), 5.54 (d, *J* = 1.6 Hz, 1H), 5.45 – 5.38 (m, 1H), 5.31 (s, 1H), 5.27 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.45 (q, *J* = 4.7 Hz, 1H), 4.34 (ddt, *J* = 13.1, 5.0, 1.6 Hz, 1H), 4.16 (ddt, *J* = 13.1, 6.0, 1.5 Hz, 1H), 4.06 (d, *J* = 4.6 Hz, 2H), 1.10 (s, 9H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 165.64, 165.50, 135.7(4C), 134.0, 133.4, 133.3, 133.3, 133.3, 130.0(4C), 129.7(2C), 129.5, 129.3, 128.4(4C), 127.7(4C), 117.3, 105.0, 83.0, 82.5, 77.5, 67.9, 63.6, 26.8(3C), 19.3. HRMS (ESI-MS): m/z calcd for $[C_{38}H_{40}O_7NaSi]^+$: 659.2441; found: 659.2445.

2A.10 Spectral charts

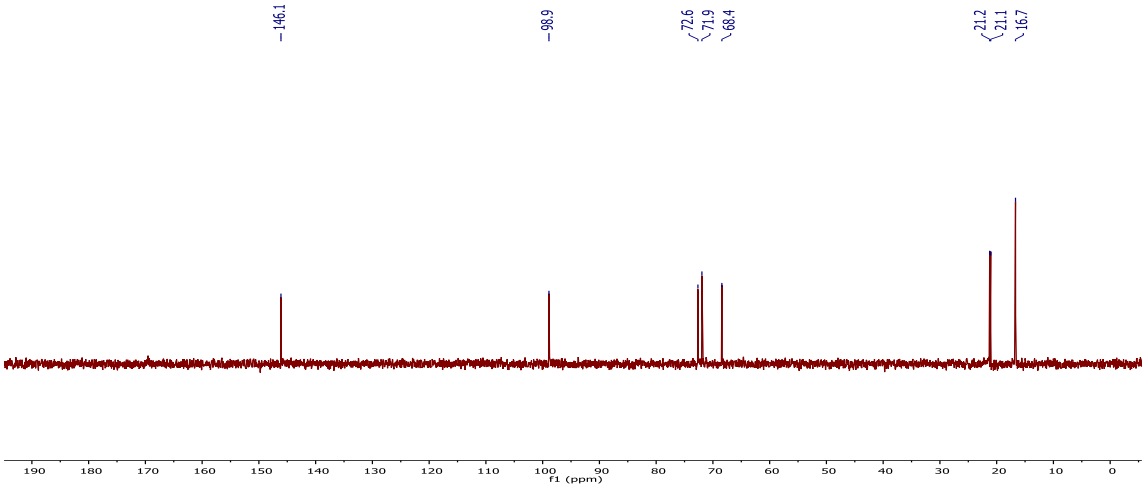
^{1}H NMR Spectrum (399.78 MHz, $CDCl_3$) of Compound **3**



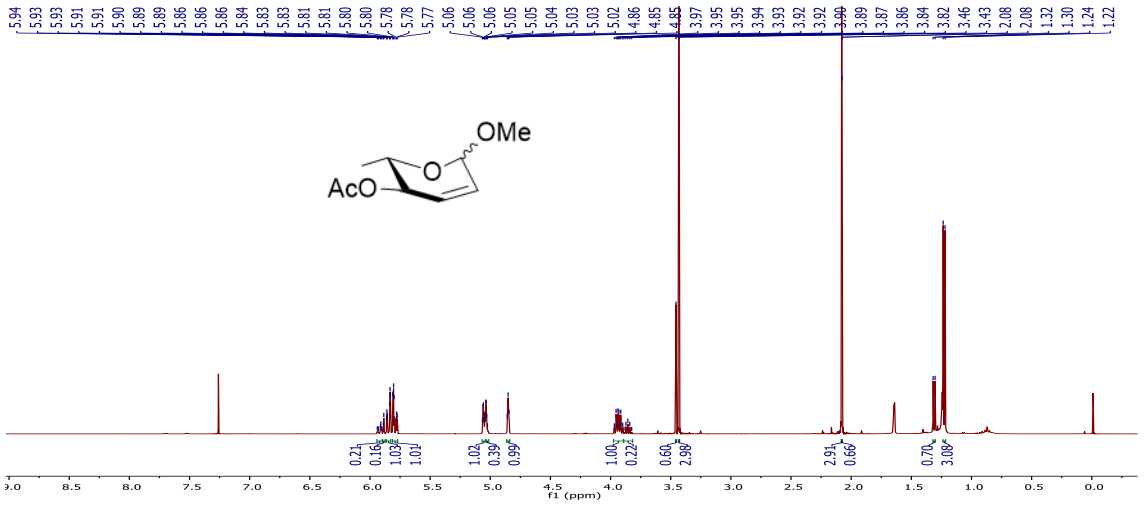
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **3**



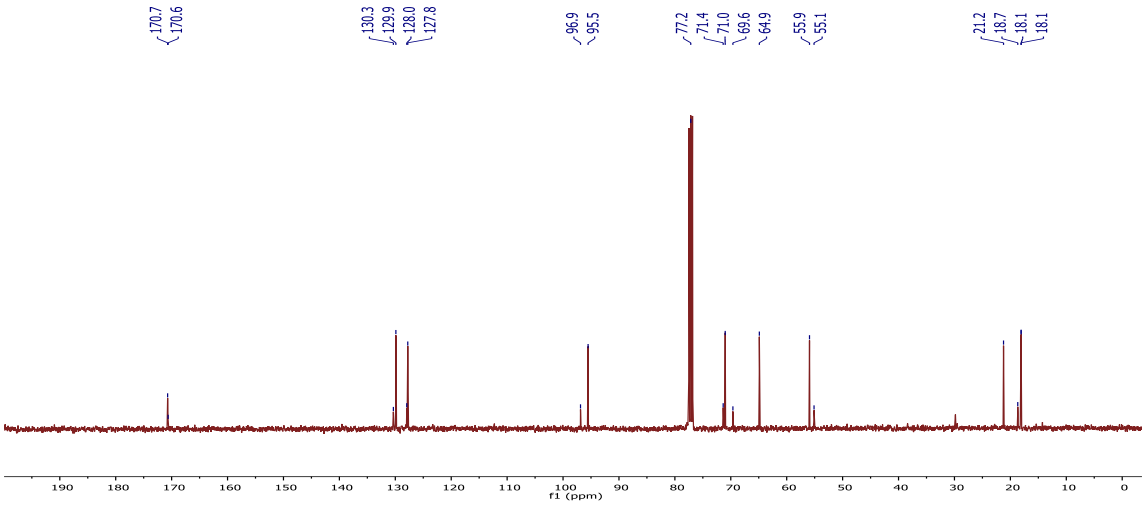
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **3**



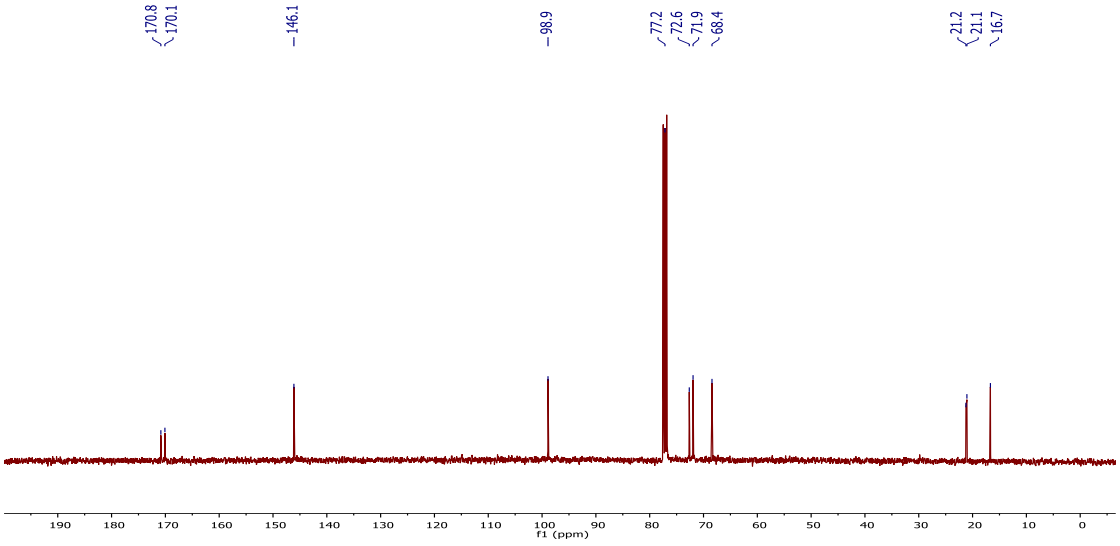
^{1}H NMR Spectrum (399.78 MHz, $CDCl_3$) of Compound **4**



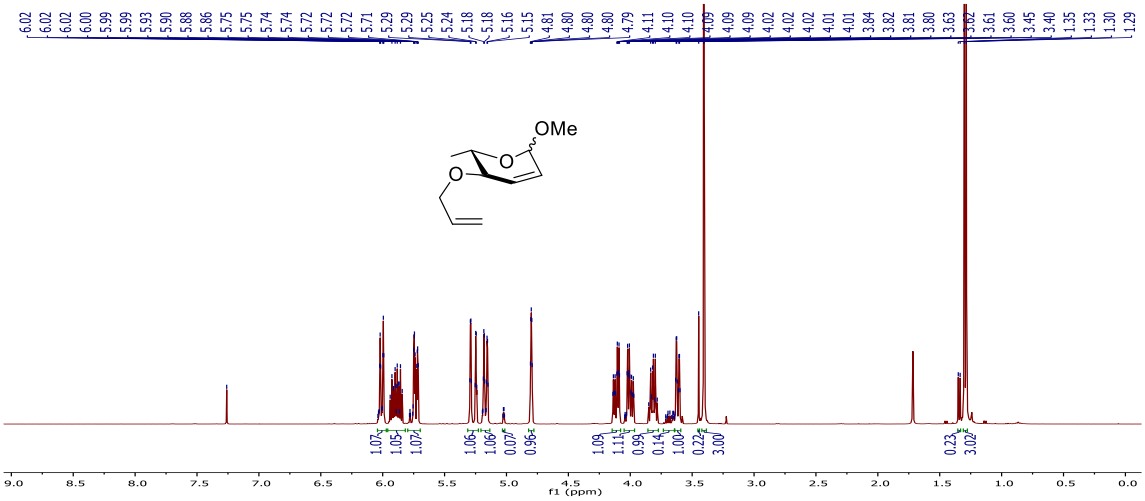
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **4**



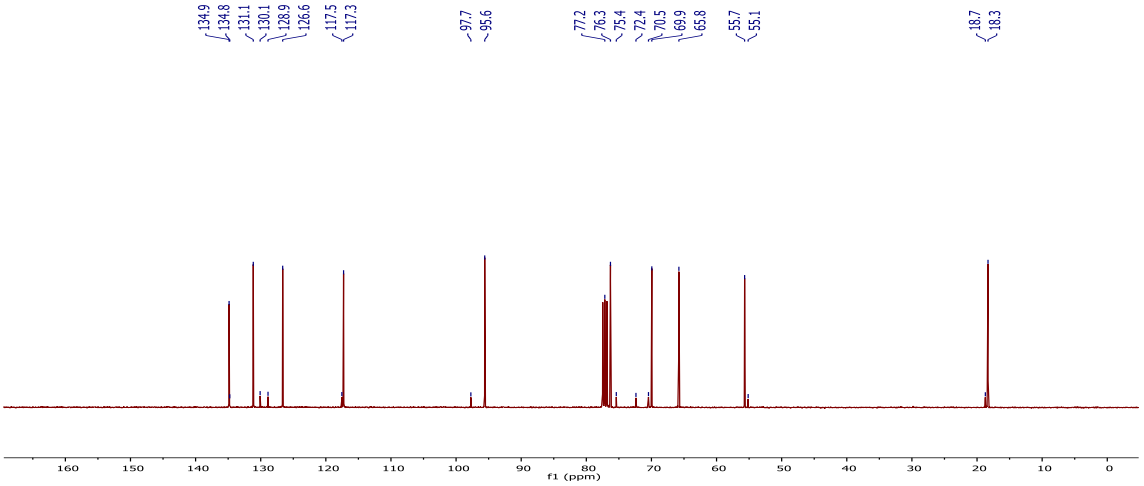
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **4**



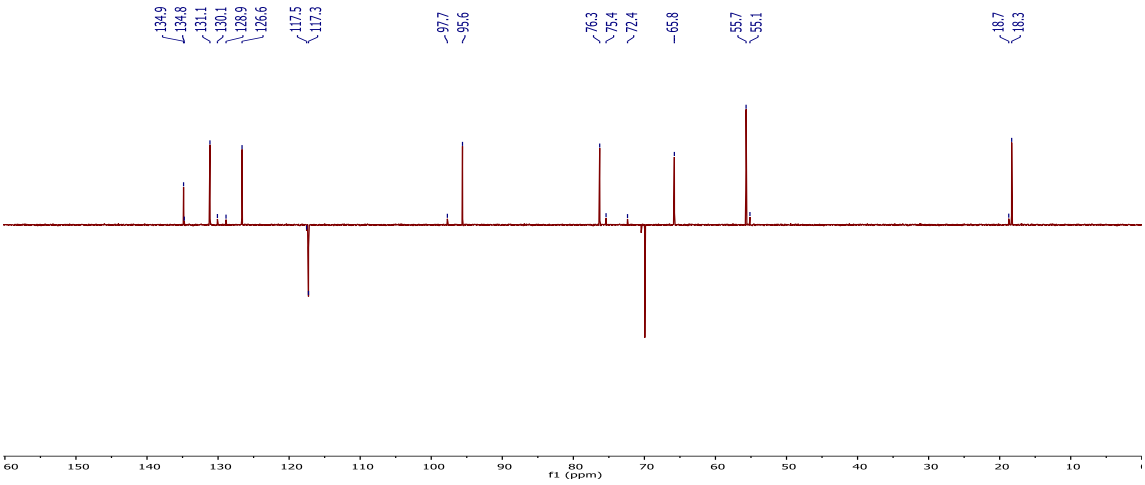
^{1}H NMR Spectrum (400.31 MHz, $CDCl_3$) of Compound **6**



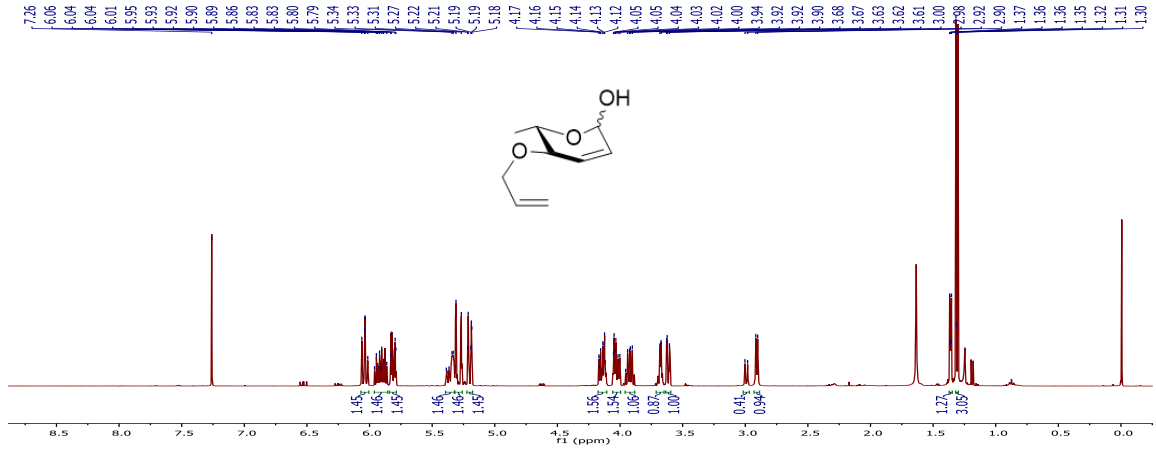
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **6**



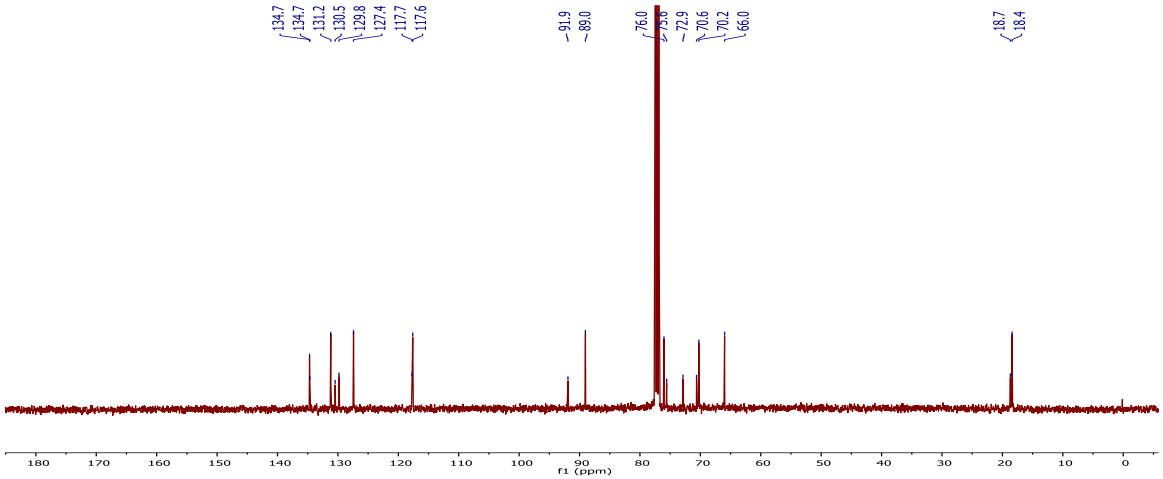
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **6**



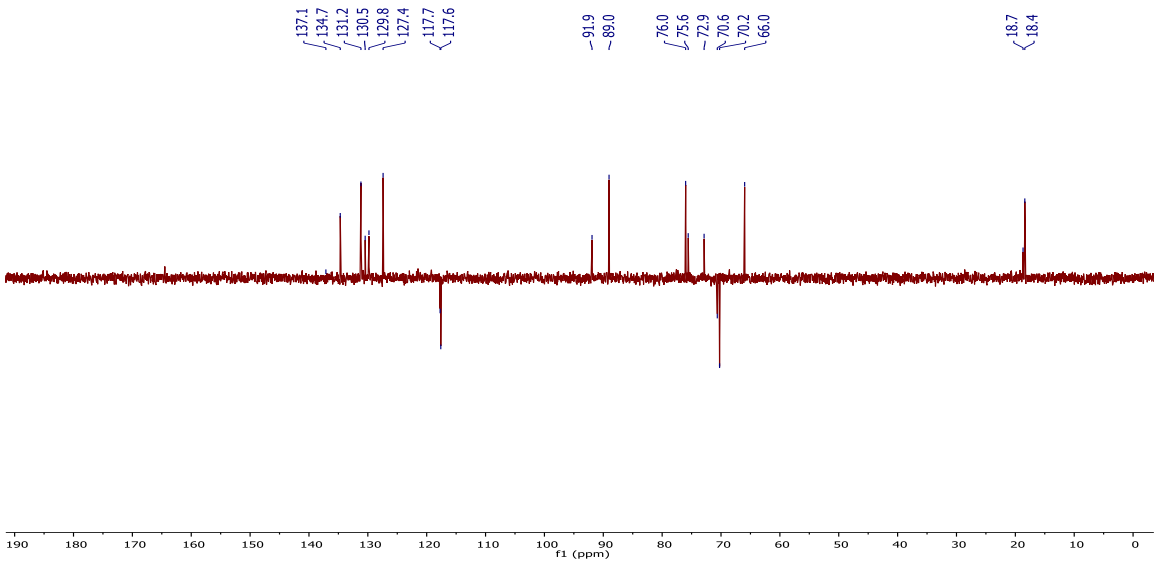
^{1}H NMR Spectrum (399.78 MHz, $CDCl_3$) of Compound **7**



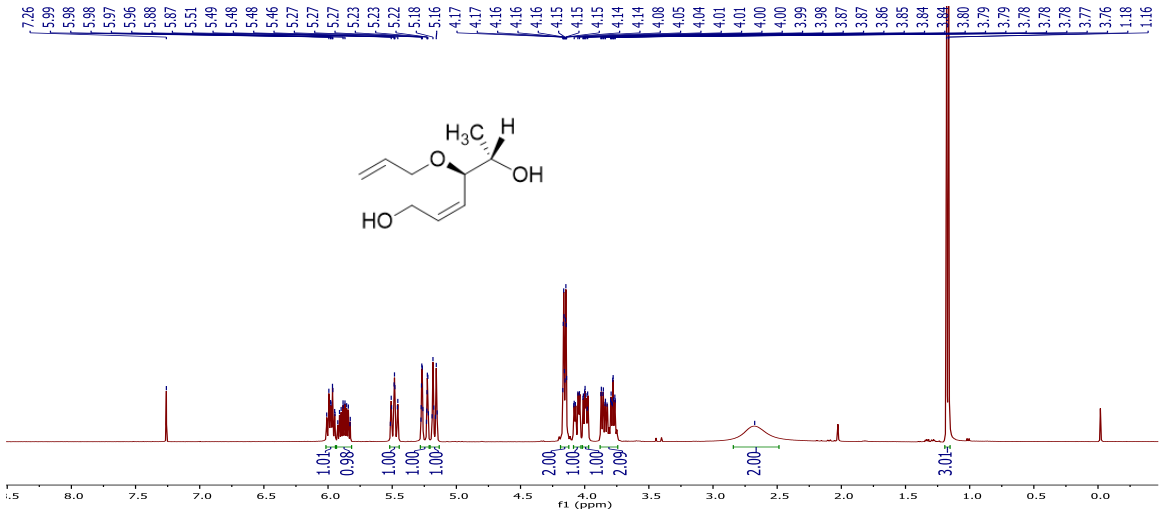
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **7**



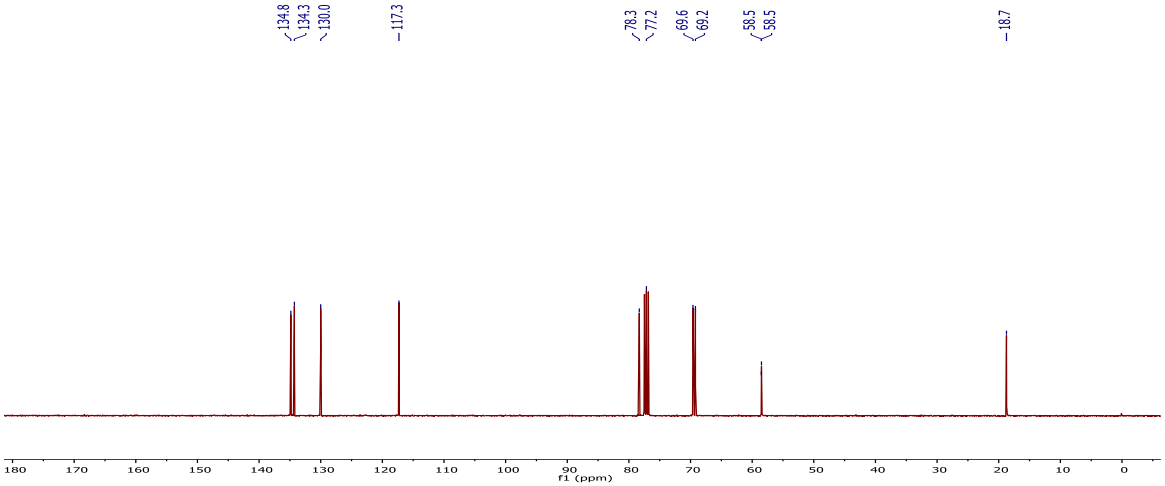
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **7**



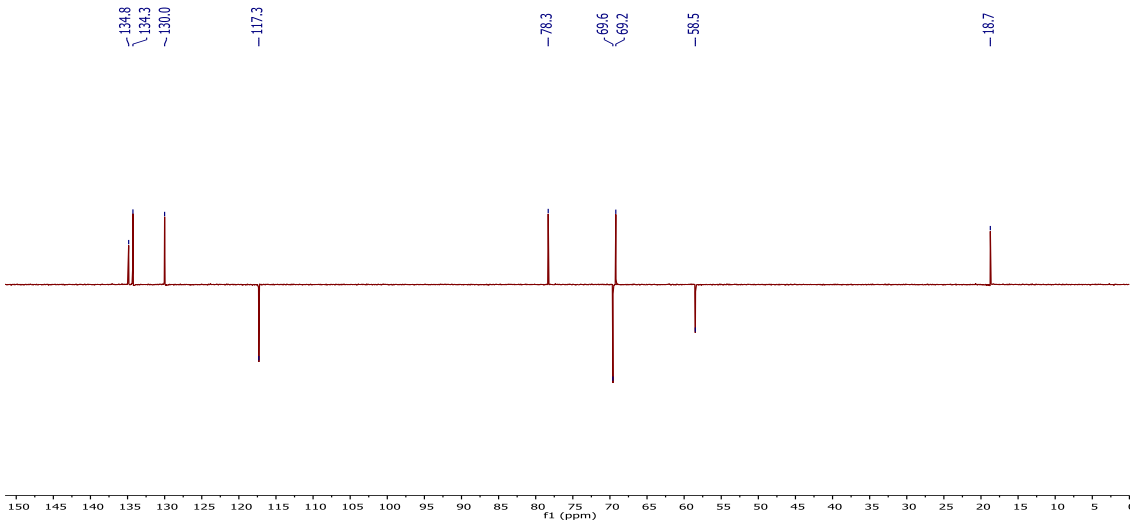
^{1}H NMR Spectrum (400.31 MHz, $CDCl_3$) of Compound **8**



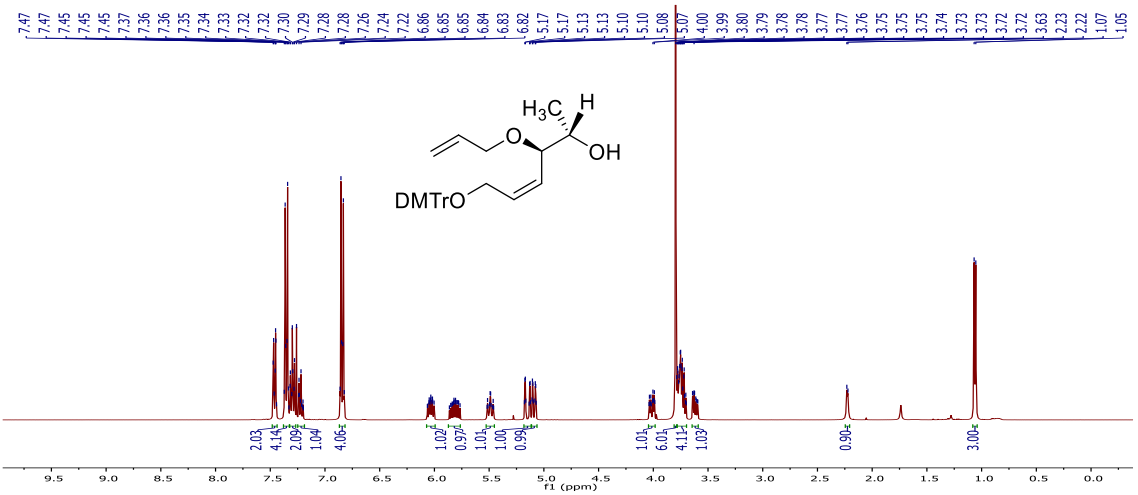
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **8**



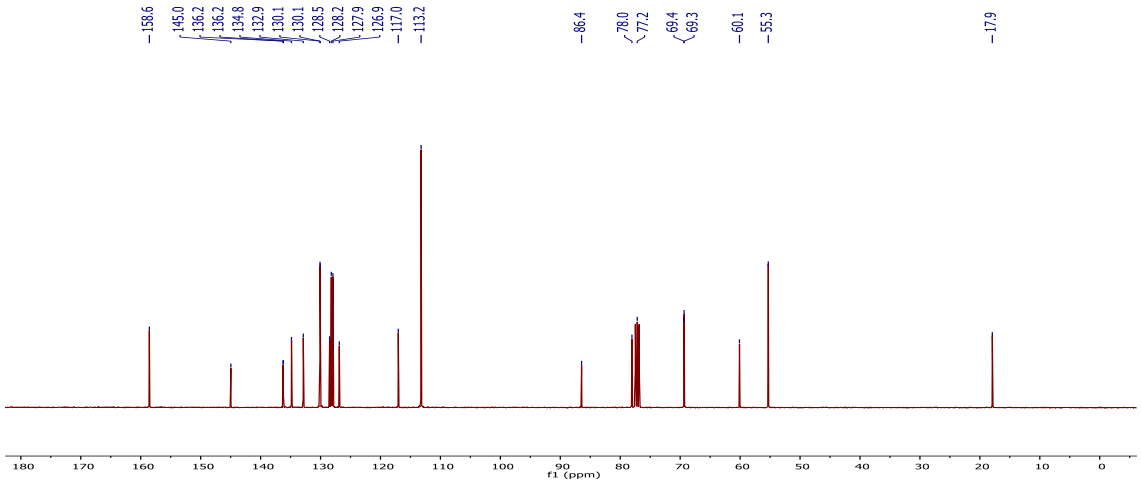
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **8**



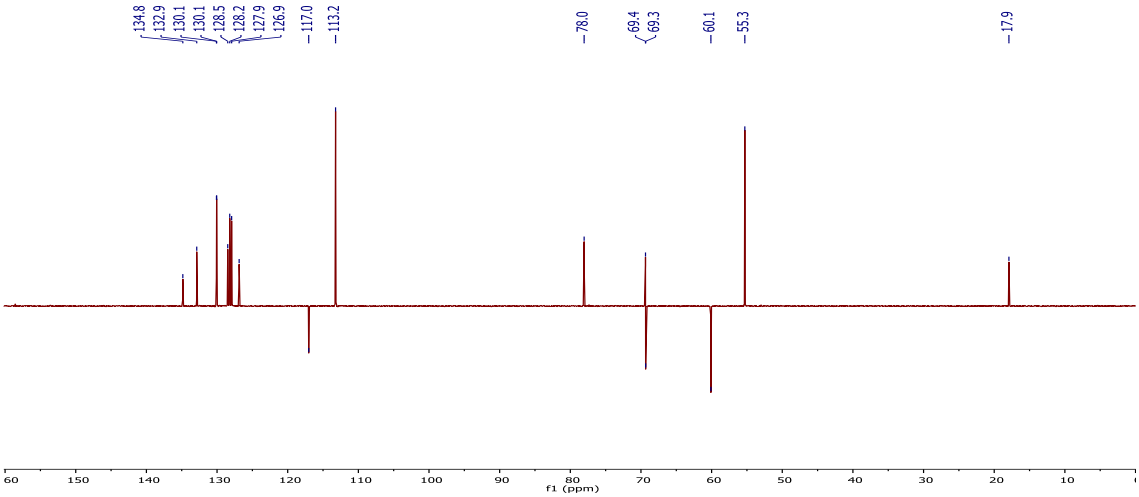
^{1}H NMR Spectrum (400.31 MHz, $CDCl_3$) of Compound **9**



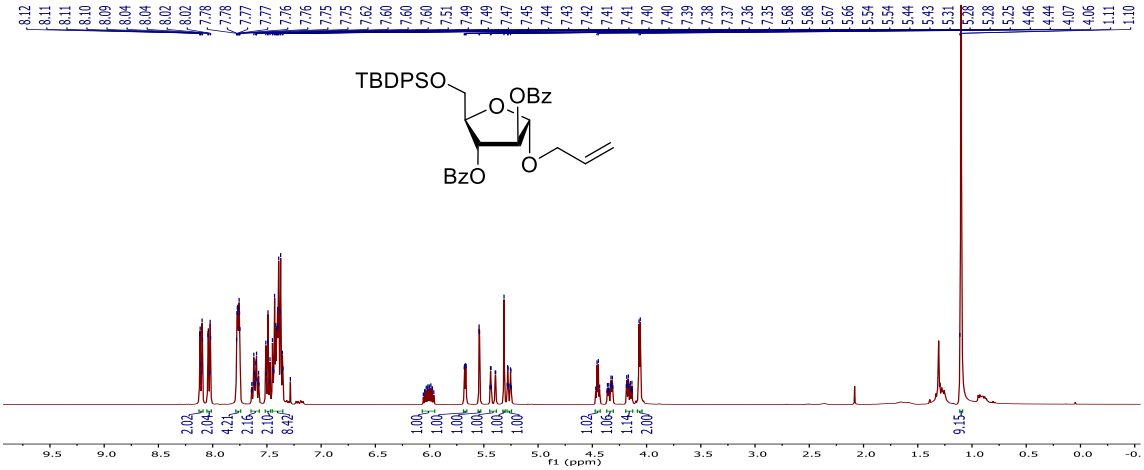
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **9**



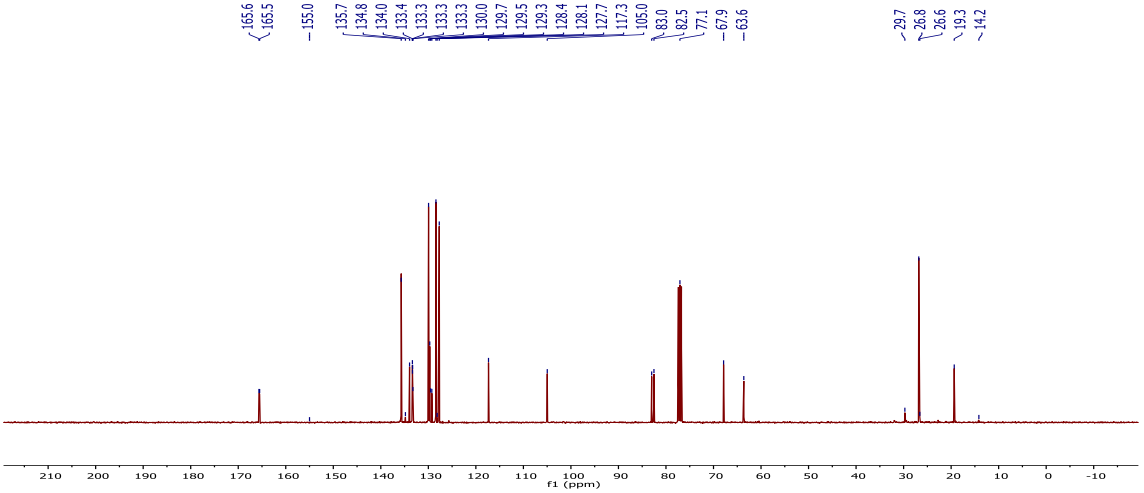
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **9**



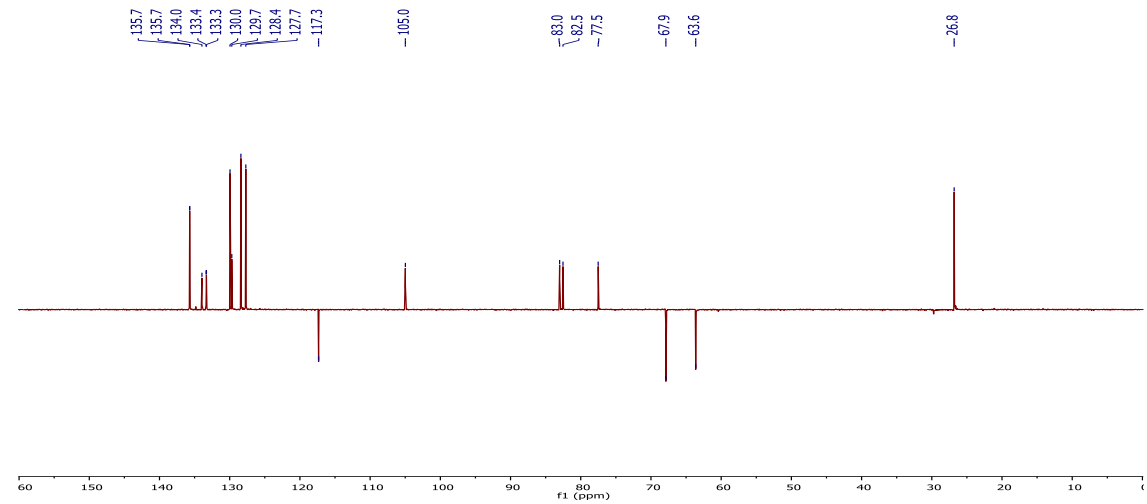
^{1}H NMR Spectrum (400.31 MHz, $CDCl_3$) of Compound **16**



^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **16**



DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **16**



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2B. Synthesis and Utility of Modified Photocleavable Linkers for Gold-catalysed Solid Phase Glycosylation Reaction

Chapter 2B

2B.1 Introduction:

Fulfilling all the characteristics to act like a universal linker has remained unbeatable in solid-phase chemistry. Among all linkers, only a few have shown primarily good impression as synthetically well-accepted linkers. Still, usage of any linker for various purposes has always thrown minor flaws in their utility. The photocleavable linker is quite distinguishable and very impressive because of its unique mode of cleavage condition. The usefulness of a photocleavable linker is immense because: i) it cleaves the synthesized molecule simply by radiation, ii) the reaction happens under mild conditions without the use of any reagent and offers an environmentally benign process, iii) it provides a cleaved product without less contamination and becomes beneficial for biological reactions as well.

In recent decades, many *o*-nitrobenzyl based photocleavable linkers have been discovered. The first report of *o*-nitrobenzyloxy-based resin was by Rich and Gurwara in 1973.[1] This linker was used initially for peptide synthesis. Later, Merrifield et al. designed the multidetachable resin support.[2] In contrast, Barany and Albericio demonstrated the usefulness of the three dimensional orthogonal protection strategy for Solid-Phase Peptide Synthesis.[3] Greenberg and co-workers showed the ability of photocleavable linker in oligonucleotide synthesis.[4,5] In the field of carbohydrate chemistry, the use of photocleavable linker was explored by Zehavi et al. in 1983.[6] Further, the use of *o*-nitrobenzyloxy linker was explored by Nicolaou et al. and Kantchev et al. for oligosaccharide synthesis in higher yield. To reduce the cleavage reaction time, Damha et al. introduced the branching and the lengthening of the hydroxyl handle side chain, which further facilitated the solid phase synthesis in practice.[7] This change in the reduced reaction time was found because of the difference in the release mechanism compared to the *o*-nitrobenzyloxy linker. Rich and Gurwara[8] developed a *o*-nitrobenzyl-based linker which upon cleavage releases amides and further similar type of linker was developed by Seeberger and coworkers for the synthesis of glucosaminoglycans using automation.[9] The *o*-nitrobenzyl-based linker has some disadvantages such as generation of nitrosobenzaldehyde which sometimes affect the efficiency of the cleavage reaction. To tackle this problem, Ajayaghosh and Pillai developed α-substituted nitrobenzyl-derived photolabile linker and further they established the protocol for the linker synthesis by using which they synthesized longer peptides.[10,11] Later on in 1997, Geysen and co-workers illustrated the use of the hydroxyl functionalized variant of this type for the synthesis of the oligosaccharide.[12] Yoo and Greenberg introduced *o*-nitroveratryl linker[13] to solve the issue of unsubstituted *o*-nitrobenzyl linker, such as low cleavage yield and long reaction time.[14]

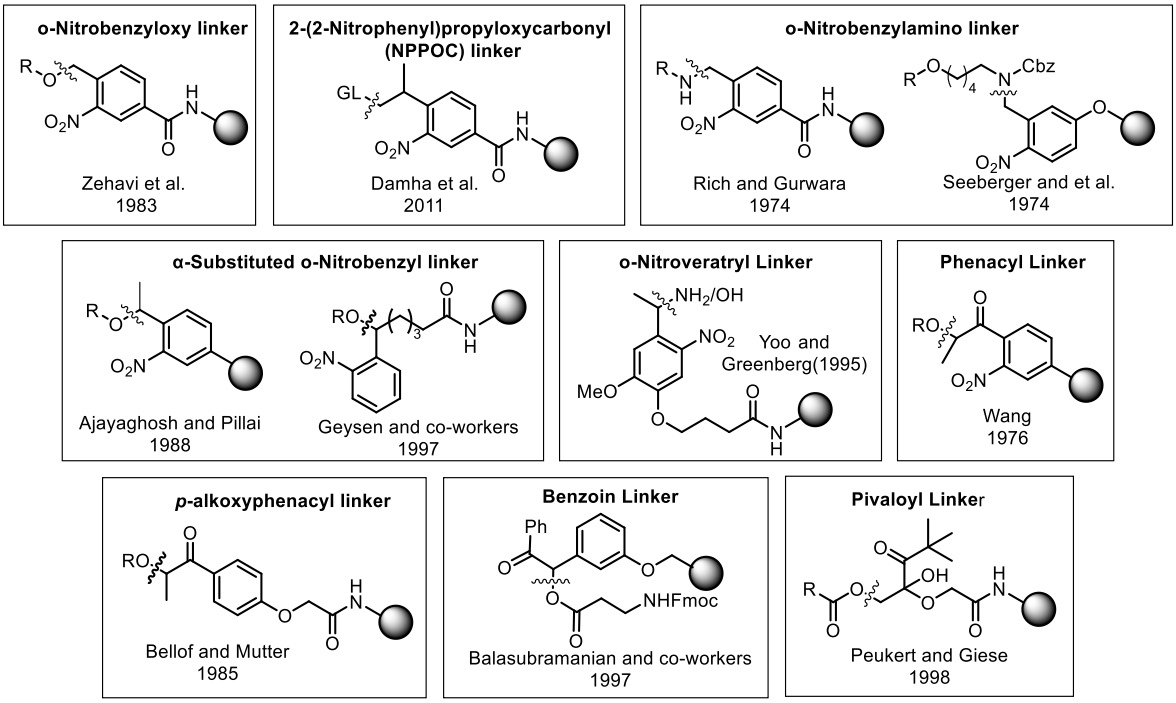


Figure 2B.1: Photocleavable linkers

This type of linkers with α-hydroxy substituent were used initially for the oligonucleotide synthesis whereas the α-amino substituent was used for the oligopeptide synthesis. Holmes and coworkers[15] have done systematic study of the presence of –OMe substituent on the aromatic ring where they found that increase in the cleavage rate. Additionally, they have also studied the positive effect of alkyl substituent at the α-position of amine group. Recently, the solvent mediated bidetachable linker system has been introduced by the Qvortrup et al. for the release of hydroxamic acids.[16]

The *o*-nitroveratryl linker[17], phenacyl linker[18], *p*-alkoxy phenacyl linker[19], benzoin linker[20] and pivaloyl linker[21] have been well explored for the synthesis of long peptide molecules. Few of them are very useful for the oligonucleotide molecule. Among all the other derivatives of *o*-nitrobenzylhydroxy and *o*-nitrobenylamino type of photocleavable linkers, the Seeberger's linker has shown the profound outcome of cleavage reaction.[22,23] Moreover, the development of this type of linker has given rise to many other useful linkers e.g. traceless photocleavable linker[24] that has helped the exploration of the solid phase oligosaccharide synthesis.[25] The structural and the functional group benefits of the *o*-nitrobenzylamino linkers have been well studied by Damha et al. Therefore, we thought that synthesizing such type of photocleavable linker and their derivatives could help in producing applicable outcome in the field of solid phase oligosaccharide chemistry.

2B.2 The rationale behind choosing thiol and hydroxyl-containing linker:

The photolytically cleavable linkers have shown a significant impact in support-based glycan synthesis. So, we envisaged that by taking advantage of it, we can explore them for various glycosylation conditions for support-based chemistry. Therefore, we choose the typical –OH-containing photocleavable linker, which has a butanol side chain with a photocleavable nitrobenzylamine moiety attached to the solid support. The structure and the usefulness of this linker is shown below.

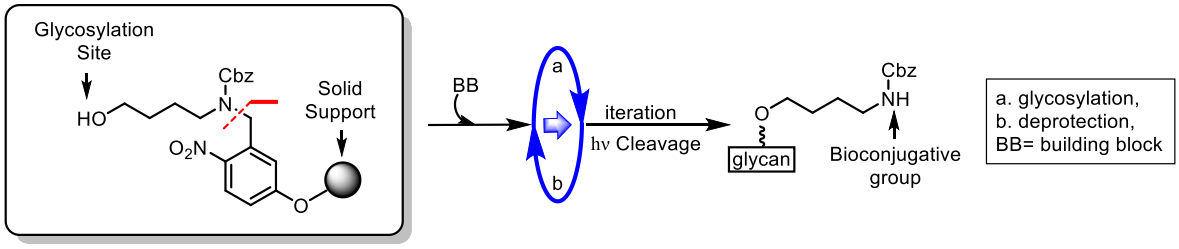


Figure 2B.2: Hydroxyl-containing photocleavable linker and its cleavage outcome

Additionally, synthesizing various types of linkers has breadth in i) synthesizing new linkers with simple, scalable strategies and ii) their utilization to tackle the biologically important glycans. The life-saving drug, "Fondaparinux" (Arixtra®), has been synthesized successfully by many other groups, including the Hotha group in the solution phase. There are the reports for the heparan sulphate disaccharide synthesis using solid support[26,27] but, the reports for the generating the –OMe terminus containing Fondaparinux are not known. In the context of developing the fast and facile synthetic protocol for synthesizing biologically important molecule with the ease in purification using the solid support, we envisioned that the thioglycoside-based linker would be helpful for obtaining the methyl glycoside at the reducing end of the glycan (shown in figure **3**). We imagined that the thiol functional group on solid support would result in a thioglycosidic product first, and subsequent iterative deprotection and glycosylation would lead us to protected Fondaparinux. Employing the reported thio linker cleavage method by Schmidt[28] could offer the desired Fondaparinux bearing –OMe at the reducing end. Additionally, the photocleavage at the benzylic position of the *o*-nitrobenzyl linker could offer the anomeric sulphur containing aliphatic N-terminous functionality that can help in performing microarrays.

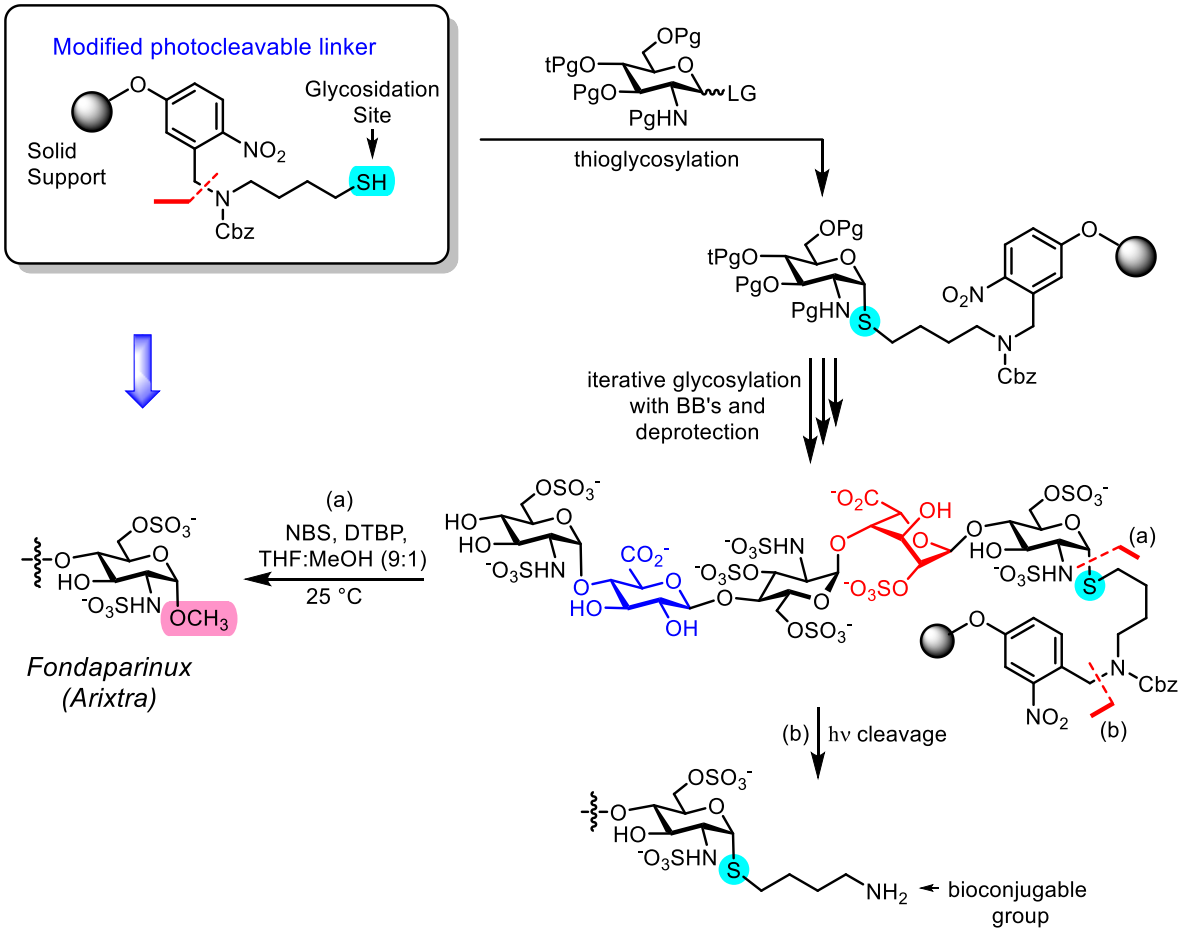


Figure 2B.3: Thiol-containing photocleavable linker and its cleavage outcome

2B.3 Present work:

After understanding the advantage of the photocleavable linker and its design for glycan synthesis, we first synthesized the –OH containing photocleavable linker attached solid support. We followed the reported protocol published by the Seeberger group to synthesize this linker.[29]

❖ Synthesis of photocleavable linker

The essentials of solid-phase oligosaccharide chemistry include the choice of solid support and linker. The Merrifield (divinylbenzene cross-linked polystyrene) resin (loading value 1.1 mmol/ g) is popular in carbohydrate chemistry. So, here we choose the same support for our goal too. The well-known, most helpful, stable, and easily cleavable photo-cleavable linker **1** and its attachment to the Merrifield resin are given below and synthesized by known protocol.[29]

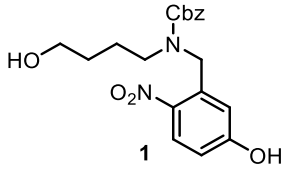
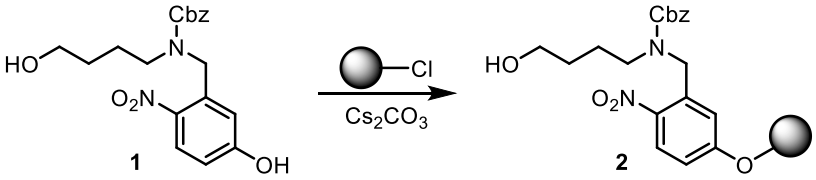


Figure 2B.4: Structure of photocleavable linker

Synthesis of linker attached solid support and calculation of loading value:



Scheme 2B.1: Attachment of linker to the solid support

Merrifield resin and photocleavable linker **1** were mixed in anhydrous DMF solvent, and then on adding an excess of solid Cs_2CO_3 and TBAI, the resultant solution was stirred at 60 °C overnight. After the removal of excess reagents by the washing and the subsequent capping was performed using CsOAc in DMF to block remaining unreacted sites. Finally, again on washing with appropriate solvents and drying, the photocleavable linker attached to solid support **2** was accomplished. Notably, after the attachment of linker **1** to the Merrifield resin, the increment in the final weight of the starting resin was observed. Then, the loading value for the linker attached resin was determined using the known protocol of FmocCl protection and its subsequent deprotection using the DBU solution strategy.[9] The loading value was determined using a UV spectrophotometer at two wavelengths, 294 and 304 nm, and in the multitude of experiments, it was found as 0.94 mmol/g.

Glycosylation reaction using support 2: The glycosylation reaction using the gold phosphite and the silver triflate catalyst is optimized well for this linker attached solid support **2** and is discussed in detail in chapter 3.

❖ Synthesis of modified photocleavable linker

After synthesis of photocleavable linker attached solid support **2,** we switch our attention towards the synthesis of second linker i.e. thiol containing photocleavable linker.

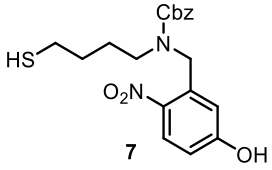
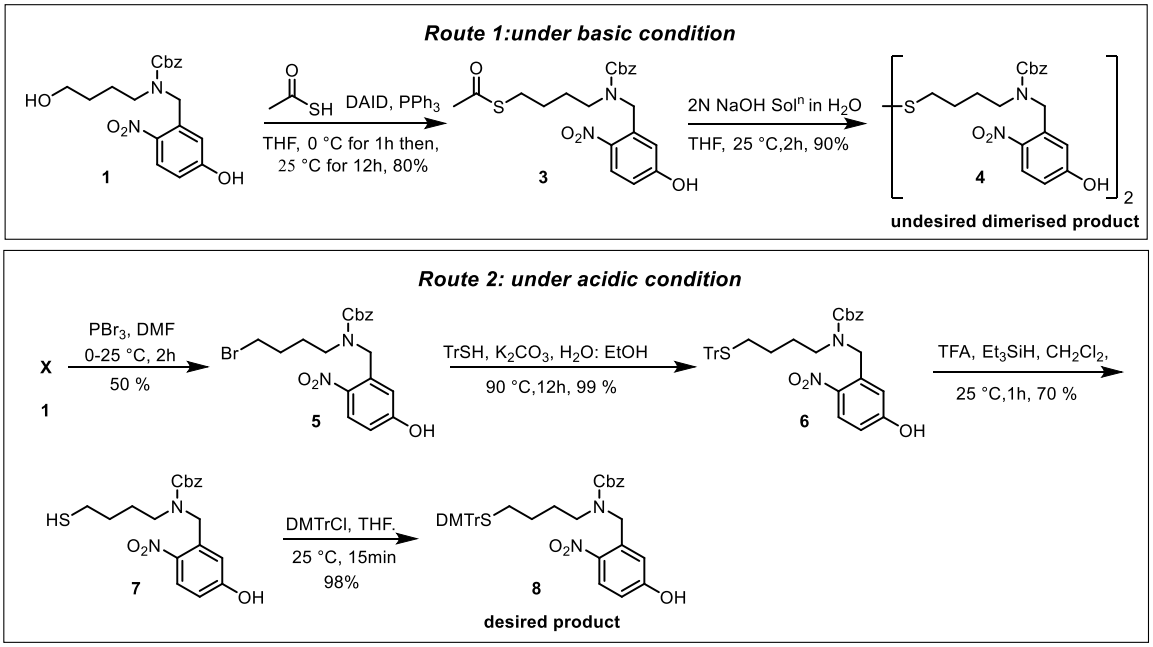


Figure 2B.5: Structure of modified photocleavable linker attached solid support

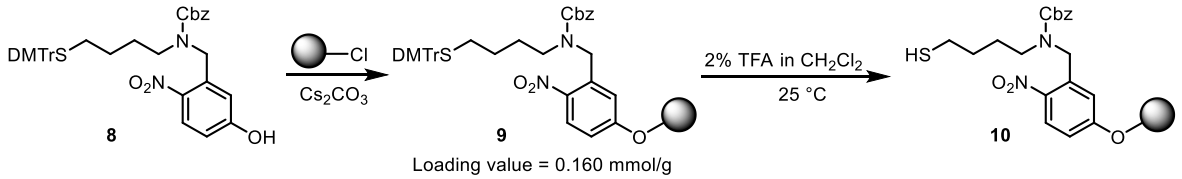


Scheme 2B.2: Synthetic routes for modified photocleavable linker

To synthesize the thiol-containing photocleavable linker **7**, we choose molecule **1** as a starting precursor. In the beginning, we imagined that the conversion of the hydroxyl functionality to thioacetate by the Mitsunobu reaction would produce molecule **3**, and then deacetylation under basic conditions would result in the desired thiol-containing linker **7**. With this thought, we performed the Mitsunobu reaction between thioacetic acid and molecule **1** in the presence of DIAD and PPh_3 to yield molecule **3** in 80%.[30] ^1H and ^{13}C NMR spectra confirmed the formation of product **3**. Further, the resultant molecule **3** in THF was treated with the 2*N* NaOH solution in water at room temperature to afford the deacetylated product **4**.[31] Then, we tried to confirm this compound **4** using ^1H and ^{13}C NMR spectroscopy. The aromatic ring -CH- protons in ^1H NMR spectra appeared at δ 8.07 (d, *J* = 8.9 Hz, 1H), 7.44 – 7.08 (m, 5H), 6.83 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.75 – 6.63 (m, 1H), and those of two benzylic $–CH_2-$ at δ 5.10 (d, 2H), 4.78 (d, *J* = 14.1 Hz, 2H). The aliphatic four methylene groups were found in the expected upfield region, but the thiol (-SH-) proton, which should be a triplet around δ 0.7 to 1.5 ppm, which did not find at all in DMSO-D_6 solvent. In ^{13}C NMR spectra, the signals for the Cbz and the benzylamine ring carbons were found at δ 163.1(2C), 155.9, 155.6, 139.3, 139.1, 137.9, 137.5, 136.8(2C), 128.5(3C), 128.4(3C), 127.9, 127.8, 127.5(2C), 127.1(2C), 114.4(2C), 113.5(2C) ppm. The remaining $–CH_2-$ group showed signals at δ 66.7, 66.3, 48.6, 48.3, 47.2, 46.8, 37.4(2C), 26.8, 26.3, 25.8(2C) ppm in DMSO-D_6 solvent. Further, we checked the HRMS value for this compound, *m*/*z* calcd. for $[C_{38}H_{43}N_4O_{10}S_2]^+$: 779.2421; found: 779.2433, indicated the mass of dimerized product **4**. So, based on this analysis, we concluded that under

the basic condition, the compound undergoes dimerization to yield compound **4** in 90%. Therefore, we redesigned the synthetic plan by preferring either acidic or neutral condition. We performed the Appel reaction by treating compound **1** with PBr_3 to get the bromosubstituted derivative **5**.[32] Further, we transformed it to the trityl derivative **6** by treating it with trityl thiol and K_2CO_3 base at room temperature to yield the desired compound **6** in 99%.[33] Subsequently, we performed the detritylation reaction at room temperature using trifluoroacetic acid and triethylsilane in dichloromethane to afford the compound **7** in 70% yield.[34] The formation of this compound was confirmed by ^1H and ^{13}C NMR spectroscopy. In ^1H NMR spectrum, the peaks for the Cbz and nitrobenzyl group were seen at δ 8.23 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.45 – 7.22 (m, 6H), 7.11 (dd, *J* = 6.7, 3.0 Hz, 4H), 6.91 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.84 (d, *J* = 2.7 Hz, 1H), 6.67 (d, *J* = 8.6 Hz, 2H) ppm and the remaining signals for the methylene protons were found at their respective regions. Importantly, the peak for the free thiol group was traced at δ 1.32 ppm. The ^{13}C NMR spectra showed seven inverted peaks in DEPT-135 spectra, and all other benzene ring carbons, along with the Cbz carbonyl, were seen in the expected downfield region. The typical unpleasant smell of thiol compound was experienced after isolation. Moreover, the HRMS analysis, *m*/*z* calcd. for $[C_{19}H_{23}N_2O_5S]^+$: 391.1328; found: 391.1327 confirms the formation of desired compound **7**. Further, we performed the DMTr protection in THF solvent using DMTrCl reagent and trimethylamine base at room temperature to afford compound **8** in 98% yield.[35]

Attachment of linker to solid support and calculation of loading value: After confirmation of the synthesized compound **8**, we performed the scale-up and connected the synthesized compound **8** to the Merrifield solid support using the excess of Cs_2CO_3, a stoichiometric amount of TBAI and DMF solvent at 60 °C. After washing with polar and nonpolar solvents, the resin was treated with the CsOAc to cap the unreacted site.



Scheme 2B.3: synthesis of thiol acceptor containing linker attached solid support

Finally, the DMTr group was removed using 2% trifluoroacetic acid to get the desired free thiol-containing linker attached support **10**. Furthermore, we calculated the loading value for the linker attached support **9.** On treating support **9** with the 2% TFA solution in CH_2Cl_2, the

spectrophotometer measured the resultant trityl ion concentration at 495 nm wavelength.[36] The loading value calculated for resin **9** was 0.160 mmol/g.

Synthesis of required donors: The synthesis of alkynyl glycosyl carbonate donor **11** is well established by Maidul and co-authors.[37]

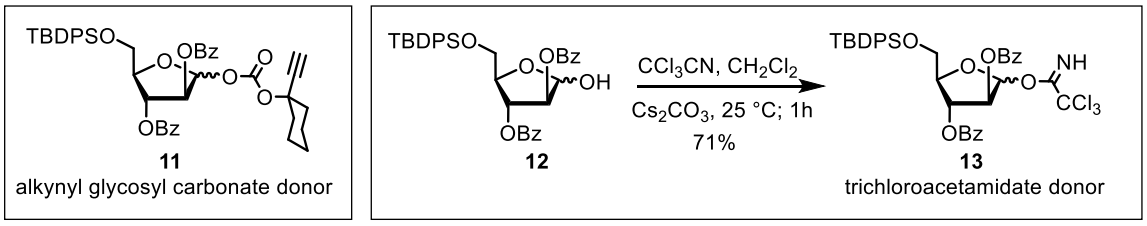


Figure 2B.6: Structure of the required donors

Another trichloroacetimidate donor **13** was synthesized from compound **12**, which is also the same precursor used for synthesizing donor **11** and whose synthesis is also well reported by Hotha group.[38] Compound **12** in CH_2Cl_2 was treated with the trichloroacetonitrile and the cesium carbonate at room temperature to afford compound **13** in 71% yield.

2B.4 Reagent preparation and general protocol for synthesis

Reagent Preparation

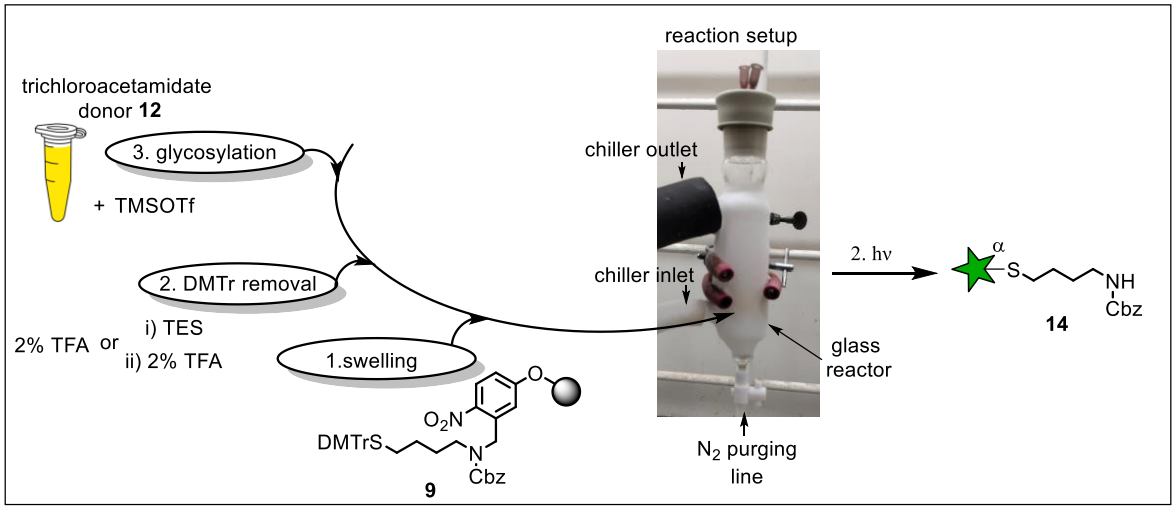
The donor solution, the building blocks and activator reagents were weighed accurately, and then dry CH_2Cl_2 was added to enclose Eppendorf tubes just before their use. The activators, gold phosphite and silver triflate, were used as such in their dry solid form. The 2% TFA solution, 0.25M triethylsilane solution, and 0.25M TMSOTf solutions were prepared all the time freshly using dry CH_2Cl_2.

General Protocol for Synthesis

After loading the functionalized resin into the reaction vessel, dry CH_2Cl_2 was added and kept for 5 min for swelling under the nitrogen flow. After that, draining of the solvent was performed using nitrogen pressure. The solution of the building blocks (4 or 3 eq. in CH_2Cl_2) was added to the reaction vessel, and then the resulting biphasic mixture was stirred using N_2 flow. After attaining the desired temperature after 5 min, the activators were added to the reaction vessel under the inert atmosphere. Then the reaction mixture was mixed for a specific time using nitrogen bubbling. The excess liquid reagents were drained and washed successively with washing solvents (according to the protocol given in the experimental section **2B.6.4**) to get a reagent-free substrate attached to the solid support. The reaction progress at each step was

monitored by TLC analysis. The deprotection of temporary protecting groups can be performed using appropriate reagents. This way, the desired synthesis can be achieved by performing an iterative glycosylation and deprotection cycles. Finally, the synthesized glycan was cleaved from the solid support using a light of wavelength 365 nm with 4 h time or continued until complete cleavage. The resultant impure components can be isolated using silica gel column chromatography for further characterization.

General set up for the glycosylation reaction



Glycosylation reaction over the solid support

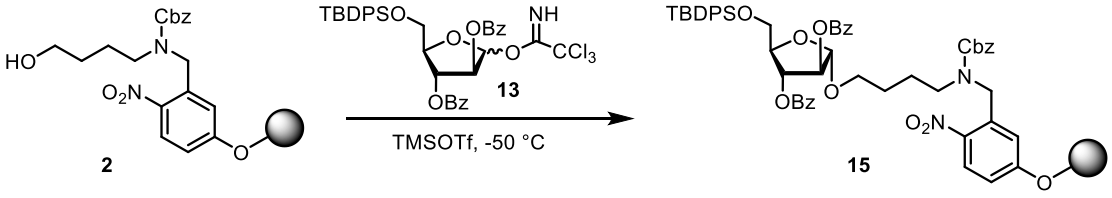
After the synthesis of required donors **11**, **13** and linker attached solid support **9**, we tried to perform the solid phase glycosylation reaction for support **10** using known protocol of thioglycosylation utilizing TMSOTf at -50 °C. The reaction vessel fitted with the temperature-adjusting outer jacket was charged with the functionalized resin **9** (80 mg; loading 0.16 mmol/g; 0.013 mmol), and CH_2Cl_2 (2 mL) was added for swelling. To start the synthesis, the resin was washed with dry CH_2Cl_2 (2x), and coupling was performed, as depicted in Table 1.

Table 1: First glycosylation over the solid support

Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	4	1 mL of 2% TFA solution dry CH_2Cl_2	10 min	2
	3	3 eq. of donor **13** in 2 mL dry CH_2Cl_2 + 90 µL of 0.25M TMSOTf solution	1h	2
Resin was transferred to another flask				

After performing the glycosylation reaction, we followed protocol I to cleave the molecule from the solid support and further checked the TLC and the mass analysis, indicating the absence of the desired product. On repetition of a similar experiment and also with the usage of higher equivalents of the TMSOTf and the donor, the reaction did not afford the thioglycosidic product over the solid support. Therefore, to address the reaction failure, we thoroughly investigated the reaction conditions employed in both steps, i.e., glycosylation and the DMTr removal.

i) Cross-checking of glycosylation reaction condition using –OH containing solid support **2**



Scheme 2B.4: Glycosylation reaction TCA donor and the –OH containing solid support

We carried out the glycosylation reaction using the solid support **2** and the glycosyl donor **13** under the same conditions employed for the support **10**. The designed reaction vessel was charged with the functionalized resin **2** (50 mg; loading 0.94 mmol/g; 0.047 mmol), and CH_2Cl_2 (2 mL) was added for swelling. The synthesis was performed, as depicted in Table 2.

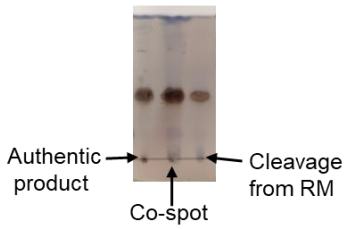
Table 2: First glycosylation over solid support

Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	3	3 eq. of donor **13** in 2 mL dry CH_2Cl_2 + 57 µL of 0.25M TMSOTf solution	1h	2
Resin was transferred to another flask				

After performing glycosylation reaction followed by the cleavage using the protocol I, we checked the TLC and the mass for the resultant cleaved molecule. The TLC analysis showed the exact matching of the Rf value with the authentic and the mass analysis also showed the exact mass; HRMS (ESI-MS): m/z calcd. for $[C_{47}H_{51}O_9NSiNa]^+$: 824.3231; found: 824.3230.

TLC of the first *O*-glycosylation

TLC image for the reaction



ii) Investigation of the free –SH functionality on solid support

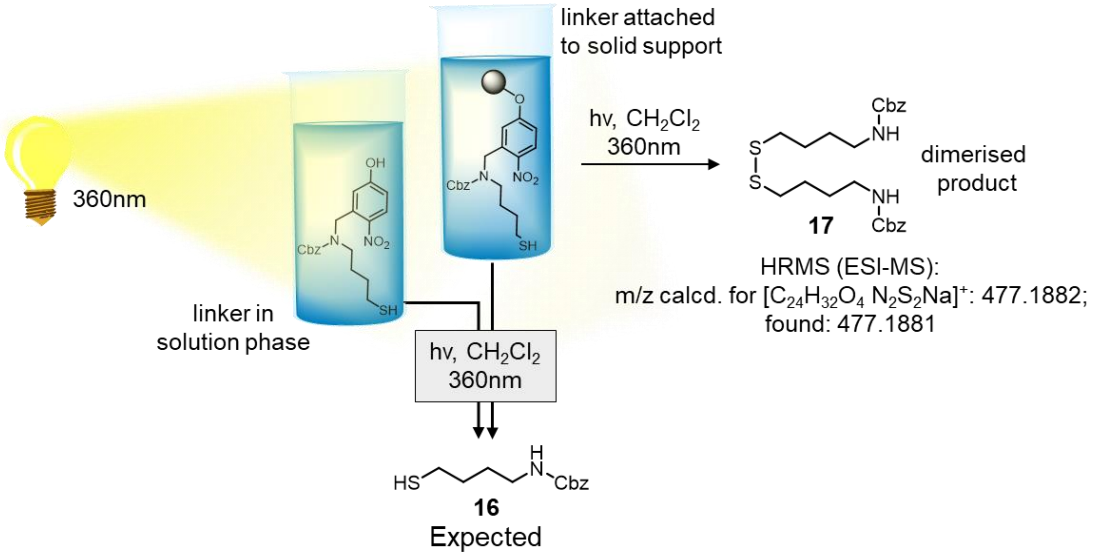


Figure 2B.7: Photoclevage reaction of thiol containing linker in solution and the solid phase

After removing the DMTr group from support **9** using the 2% trifluoroacetic acid in CH_2Cl_2 solution, we investigated the availability of free thiol functionality over the solid support. For that, we performed the photocleavage reaction for this linker attached support **10** and the linker **7** alone. The cleavage product obtained from support **10** was the unexpected S-S dimerized product **17**, which was different from the expected product **16**. By performing TLC analysis, we confirmed the formation of the dimerized molecule.

Further, to avoid the dimerization of the linker over the solid support, we performed the DMTr deprotection reaction using triethylsilane and 2% trifluoroacetic acid solution method, which we had already used for the DMTr removal in the solution phase. The utilization of this method in the solution phase is well-known for small organic molecules. Therefore, we

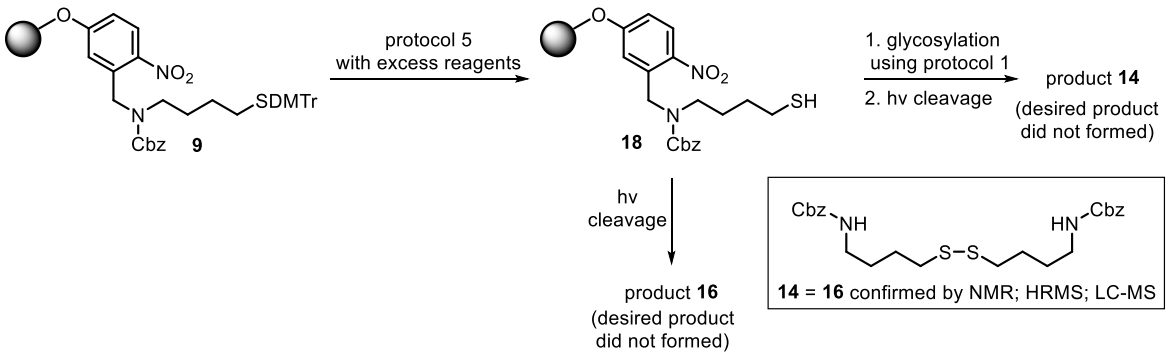
imagined that applying a similar reaction condition for the solid support may help to solve the dimerization issue. In that perspective, we performed the DMTr removal reaction in the presence of the 3 equivalents of donor and the excess amount of the reagents (TES and TFA).

The reaction vessel was charged with the functionalized resin **9** (100 mg; loading 0.16 mmol/g; 0.016 mmol), and CH_2Cl_2 (2 mL) was added for swelling. To start the synthesis, the resin was washed with dry CH_2Cl_2 (2x), and coupling was performed at -50 °C under an N_2 atmosphere, as depicted in Table 3.

Table 3: First glycosylation over solid support

Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	5	1 mL 0.025M TES solution dry CH_2Cl_2 +1 mL of 2% TFA solution dry CH_2Cl_2	10 min	2
	3	3 eq. of donor **13** in 2 mL dry CH_2Cl_2 + 1 mL of 0.25M TMSOTf solution	1h	2
Resin was transferred to another flask				

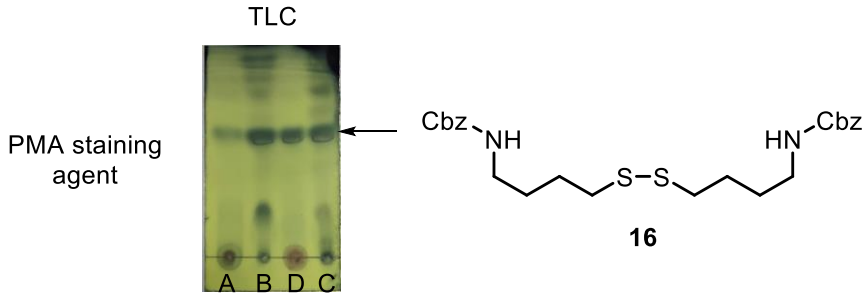
The DMTr removal and the glycosylation reaction time were kept very less, and immediately after the two steps, the cleavage reaction was performed using protocol I.



Scheme 2B.5: DMTr deprotection and the glycosylation reaction over the solid support

On performing the cleavage protocol I for resin **18**, after and before the glycosylation, the resultant products were run over the TLC; showed the matching of the Rf value and the HRMS value. Further, we performed the LC-MS run for the resulting cleaved product.

TLC for the cleavage products in CH_2Cl_2 solution



A = cleavage of thiol linker without DMTr

B = cleavage of thiol linker after DMTr removal using 2% TFA in CH_2Cl_2

C = cleavage of linker from the support after DMTr removal using 2% TFA + TES in CH_2Cl_2

D = cleavage of product from solid support after 1st glycosylation

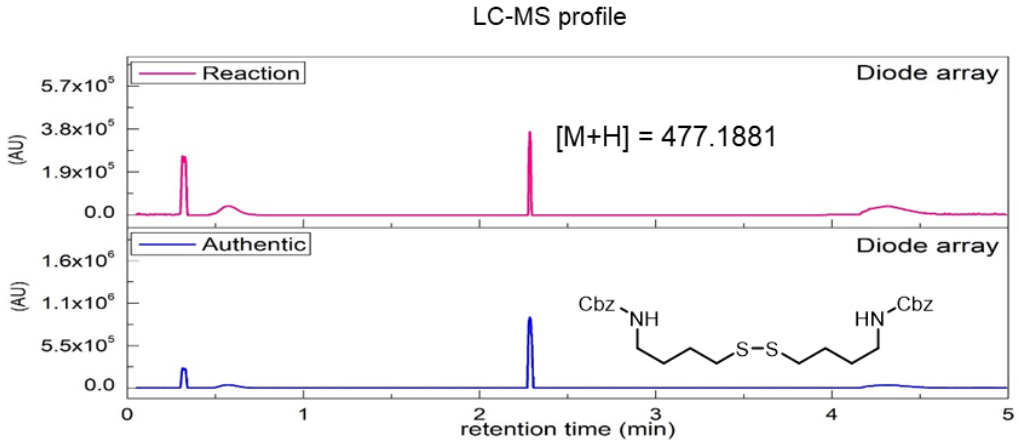


Figure 2B.8: LC-MS profile of the dimerised cleaved product

Cleavage, purification and analysis of product **14 or 16**:

Finally, after confirmation of the product by TLC analysis, the whole resin was cleaved using protocol I and purified using protocol II. The confirmation of products **14** and **16** was carried out by ^{1}H and ^{13}C NMR spectroscopy. The signal for the Cbz group appears at δ 7.39 – 7.28 (m, 5H), 5.09 (s, 2H) in as well as the remaining methylene protons were found at δ 3.21 (q, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 1.70 (q, *J* = 7.2 Hz, 2H), 1.60 (p, *J* = 6.9 Hz, 2H). The same functional groups were identified in the expected region in ^{13}C NMR spectroscopy. Further, it was supported by HRMS (ESI-MS): m/z calcd. for $[C_{24}H_{33}O_4N_2S_2]^+$: 477.1882; found: 477.1881.

Remark for the S-S bond cleavage over the solid support: Performing the S-S bond cleavage in the solution phase many times requires the appropriate pH, solvent and the reagents for the effectiveness of reaction. Additionally, the formed thiol product is air sensitive is another drawback. Particularly for the solid support chemistry, synthesizing free thiol compound over the support and holding it in the –SH form is a cumbersome task where intramolecular dimerization is very much feasible. But, if we modify the reaction conditions under appropriate inert atmosphere then we could easily prepared designed thiol containing linker.

2B.5 Conclusions:

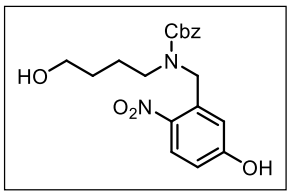
The hydroxyl containing photocleavable linker was successfully synthesized by using known protocols and then connected to the Merrifield resin. This linker attached solid support can be used as a photocleable support for carrying out the silver assisted gold catalysed glycosylation. The glycosylation and the cleavage reaction happened efficiently.

The thiol containing photocleavable linker i.e. modified photocleavable linker was designed for obtaining –OMe at the reducing terminus required in the fondaparinux molecule. Unfortunately, the DMTr removal from the linker attached solid support underwent spontaneous dimerization under 2% trifluroacetic acid. The presence of the triethylsilane also failed to produced thiol over the solid support. Therefore, modified linker fails to offer free thiol over the solid support, instead spontaneously converts to unwanted dimerised product.

2B.6 Experimental section:

2B.6.1 Synthesis of the photocleavable linker

Synthesis of Benzyl (5-hydroxy-2-nitrobenzyl)(4-hydroxybutyl)carbamate (1): A solution of 5-Hydroxy-2-nitrobenzaldehyde (2.44 g, 14.58 mmol) and 4-aminobutanol (1.3 g, 14.58 mmol) in anhydrous methanol (45 mL) at 25 °C was stirred for 2.5 h under argon atmosphere. The reaction mixture was cooled to 0 °C, and $NaBH_4$ (0.55 g, 14.58 mmol) was added portion-wise and brought to 25 ºC over 30 min. After 1 h, excess $NaBH_4$ was quenched by adding acetone (50 mL) and stirred for 5 min. The solvents were evaporated to furnish the secondary amine, which was then re-dissolved in anhydrous MeOH (300 mL), triethylamine (4.09 mL, 43.70 mmol), and Cbz-Cl (6.21 mL, 36.42 mmol) and stirred for 1 h at 25 ºC. K_2CO_3 (9.68 g) was added to the reaction mixture and stirred for an hour. The reaction mixture was then filtered through a bed of Celite®, and the filtrate was evaporated to dryness. The crude residue was redissolved in CH_2Cl_2 and washed with 0.1 M HCl and water. Combined organic layers were dried over anhydrous Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo* to obtain a residue that was purified by silica gel column chromatography (ethyl acetate:hexane) to obtain photocleavable linker **1** in 76% yield (4.12 g) as tanish green colored liquid. R_f = 0.28 (ethyl acetate:hexane 60:40); IR (cm^{-1}): 3611, 3212, 2934, 1676, 1584, 1516, 1460, 1310, 1249, 1132, 1067, 982, 838, 745, 695; ^{1}H NMR (400.31 MHz, $CDCl_3$, mixture of rotamers[2]): δ 8.16-7.98 (m,1H), 7.37 – 7.04 (m, 5H), 6.85 – 6.64 (m, 2H), 5.15-5.05 (m, 2H), 4.90-4.87 (m, 2H), 3.60 (m, 2H), 3.36 – 3.29 (m, 2H), 2.54 – 2.00 (brs, 1H), 1.72 – 1.59 (m, 2H), 1.55 – 1.46 (m, 2H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 162.9(2C), 157.3(2C), 140.4, 139.8, 137.5, 137.0, 136.2, 135.9, 129.1, 128.8, 128.7(4C), 128.3(2C), 128.1, 127.6(3C), 115.1, 114.8, 114.3, 113.3, 68.0, 67.9, 62.4, 62.3, 49.7, 49.6, 48.7, 48.1, 29.8, 29.3, 25.0, 24.8. HRMS (ESI-MS): m/z calcd. for $[C_{19}H_{22}O_6N_2Na]^+$: 397.1376; found: 397.1375.



Coupling Linker 1 to Merrifield Resin: To a suspension of Merrifield resin (2.0 g, 2.2 mmol, loading 1.1 mmol/g) in CH_2Cl_2 (20 mL), the photocleavable linker **1** (4.12 g, 2.0 mmol) in CH_2Cl_2 (5 mL) was added and subsequently anhydrous DMF (20 mL) was injected into the flask. Solid Cs_2CO_3 (1.7 g, 8.8 mmol) and TBAI (3.25 g, 8.8 mmol) were added, and the resulting solution was stirred overnight on the rotavap at 60 °C and washed successively with DMF/water (1:1), DMF, THF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2 (2 times each). The resin was again transferred into a flask containing CsOAc (0.844 g, 1.57 mmol) in DMF (20 mL) and stirred overnight on rotavap at 60 °C for capping the unreacted resin. The resin was washed

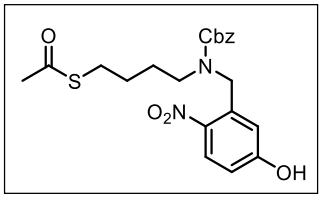
successively with DMF/water (1:1), DMF, THF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2 (2 times each) and dried under a high vacuum to obtain resin **2**. The loading value (0.94 mmol/g) was determined as described in the below procedure.

Loading value determination: Dry resin **7** (50 mg, theoretical loading: 1.1 mmol/g, 0.055 mmol) was placed in a syringe equipped with a frit. CH_2Cl_2 (3 mL) was added for swelling the resin, CH_2Cl_2 was drained, and FmocCl (149.60 mg, 0.60 mmol) in pyridine (0.14 mL, 1.80 mmol) and CH_2Cl_2 (2 mL) was added. The reaction mixture was mixed by bubbling N_2 gas for 6 h, solvents were drained, and the resin was washed with CH_2Cl_2, MeOH, and CH_2Cl_2 (2 times each). Subsequently, a freshly prepared DBU solution (2% in DMF, v/v; 2 mL) was added to the resin and stirred for 1 h. The solution was drained into a vial and washed with 1 mL of DBU to ensure complete transfer of dibenzofulvene. An aliquot of this solution (45 µL) was diluted with acetonitrile to a total volume of 10 mL, and the UV absorption of this solution was measured at 294 and 304 nm. The loading of the resin was calculated as follows:

For λ_{304}, [Fmoc] = Absorbance at 304 nm x dilution factor/ Molar extinction coefficient
Similarly, for wavelength 294 nm was calculated. The average of these two loading values was found as 0.94 mmol/g.

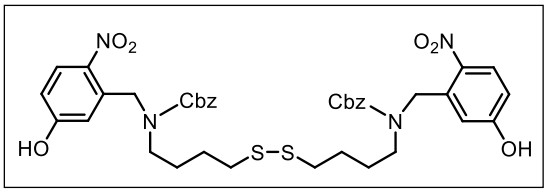
2B.6.2 Synthesis of the modified photocleavable linker

Synthesis of (4-(((benzyloxy)carbonyl)(5-hydroxy-2-nitrobenzyl)amino)butyl) ethanethioate (3): To a solution of triphenylphosphine (33.39 mmol, 8.76 g) in THF (100 mL), diisopropyl azodicarboxylate (DIAD) (33.39 mmol, 6.55 mL) was added at 0 ºC and stirred for 1 h. A THF (2 mL) solution of alcohol compound **1** (13.35 mmol, 5.0 g) and thioacetic acid (33.39 mmol, 2.35 mL) was added at 0 °C and then stirred the reaction mixture at room temperature for 12 h. On completion of the reaction, the reaction mixture was quenched with the $NaHCO_3$ solution. The compound was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4; the solvent was then evaporated on a rotary evaporator. The mixture was purified using silica gel column chromatography using ethyl acetate:hexane system to obtain the desired product **3** (4.65g, 80 % yield) as an orange solid. R_f = 0.2 (ethyl acetate:hexane 30:70); IR (cm^{-1}): 3220, 2925, 1673, 1590, 1515, 1441, 1307, 1245, 1118, 1069, 958; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.48 (s, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 8.9 Hz, 1H), 7.45 – 7.32 (m, 3H), 7.28 – 7.23 (m, 4H), 7.15 – 7.07 (m, 3H), 6.93 – 6.81 (m, 3H), 6.74 – 6.64 (m, 1H), 5.19 (s, 1H), 5.09 (s, 3H), 4.91 (d, *J* = 12.1 Hz, 4H), 3.41 –

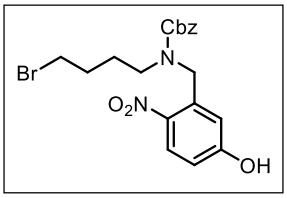


3.26 (m, 4H), 2.82 (t, *J* = 7.1 Hz, 4H), 2.33 (s, 6H), 1.71 – 1.61 (m, 4H), 1.60 – 1.49 (m, 4H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 196.9, 196.8, 162.9, 162.1, 157.4, 157.2, 140.4, 139.9, 137.3, 136.9, 135.9, 135.7, 129.1, 128.8(2C), 128.6(4C), 128.5, 128.3, 128.0, 127.5(2C), 115.1, 114.8, 113.9, 113.0, 68.2, 67.9, 49.6, 49.5, 48.2, 47.9, 30.7(2C), 28.7(2C), 27.5, 27.1(2C), 27.0. HRMS (ESI-MS): m/z calcd. for $[C_{21}H_{25}N_2O_6S]^+$: 433.1433; found: 433.1431.

Formation of dibenzyl (disulfanediylbis(butane-4,1-diyl))bis((5-hydroxy-2-nitrobenzyl)carbamate) (4): To a solution of thioacetate compound (8.79 mmol, 3.8 g) in THF (30 mL), a 2*N* aq. NaOH solution (150 mL) was added portion-wise, and simultaneously, the reaction was monitored using TLC. On completion of the reaction after 2h, the reaction mixture was slowly quenched using 1N HCl solution under constant stirring. The reaction mixture was then extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. Finally, the whole organic volume was evaporated using a rotary evaporator. The compound was purified using silica gel column chromatography using ethyl acetate:hexane solvent system to obtain pure compound **4** (3.1g, 90% yield) as a white solid. R_f = 0.4 (ethyl acetate:hexane 40:60); IR (cm^{-1}): 3301, 2921, 2856, 1683, 1594, 1515, 1456, 1375, 1316, 1249, 1177, 1107; ^{1}H NMR (399.78 MHz, DMSO-D_6) δ 10.97 (s, 1H), 8.07 (d, *J* = 8.9 Hz, 1H), 7.44 – 7.08 (m, 5H), 6.83 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.75 – 6.63 (m, 1H), 5.10 (d, 2H), 4.78 (d, *J* = 14.1 Hz, 2H), 3.29 (d, *J* = 5.9 Hz, 2H), 2.65 (d, 2H), 1.60 (s, 4H). ^{13}C NMR (100.53 MHz, DMSO-D_6) δ 163.1(2C), 155.9, 155.6, 139.3, 139.1, 137.9, 137.5, 136.8(2C), 128.5(3C), 128.4(3C), 127.9, 127.8, 127.5(2C), 127.1(2C), 114.4(2C), 113.5(2C), 66.7, 66.3, 48.6, 48.3, 47.2, 46.8, 37.4(2C), 26.8, 26.3, 25.8(2C). HRMS (ESI-MS): m/z calcd. for $[C_{38}H_{43}N_4O_{10}S_2]^+$: 779.2421; found: 779.2433.

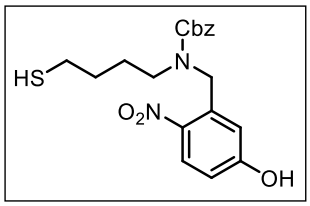


Synthesis of Benzyl (4-bromobutyl)(5-hydroxy-2-nitrobenzyl)carbamate (5): To a stirred solution of alcohol compound **1** (5.37 mmol, 2.01 g) in DMF (20 mL), PBr_3 (6.44 mmol, 0.61 mL) was added dropwise at 0 °C. The reaction mixture was gradually shifted to room temperature and kept under stirring for 6 h. On completion of the reaction, the reaction mixture was quenched using ice-cold water, and then the reaction mass was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The organic volume was evaporated using a rotary evaporator. The compound was purified using silica gel column chromatography using ethyl acetate:hexane system to obtain pure compound **5** (1.18g, 50% yield) as an off-white solid. R_f = 0.7 (ethyl acetate:hexane 30:70); IR (cm^{-1}): 3211, 2923,



2858, 1674, 1587, 1519, 1445, 1304, 1248, 1157, 1114, 1072, 978; ^{1}H NMR (399.78 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 8.11 (s, 1H), 7.98 (d, *J* = 8.9 Hz, 1H), 7.41 – 7.28 (m, 5H), 7.26 – 7.20 (m, 4H), 7.12 – 7.06 (m, 2H), 6.88 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.70 – 6.59 (m, 1H), 5.18 (s, 1H), 5.10 (d, *J* = 15.0 Hz, 3H), 4.93 (s, 2H), 4.90 – 4.85 (m, 2H), 3.38 – 3.23 (m, 8H), 1.83 – 1.69 (m, 8H). ^{13}C NMR (100.53 MHz, $CDCl_3$) δ 162.9, 162.0, 157.5, 157.2, 140.5, 139.9, 137.0, 136.5, 136.1, 135.6, 129.3, 128.8, 128.7(2C), 128.6, 128.4, 128.1, 127.8, 127.7, 127.5, 120.5, 120.5, 115.1, 114.9, 114.2, 112.9, 68.3, 68.0, 49.1, 49.0, 47.6, 47.2, 33.1, 33.0, 29.9, 29.8, 27.0, 26.7. HRMS (ESI-MS): m/z calcd. for $[C_{19}H_{22}N_2O_5Br]^+$: 437.0712; found: 437.0712.

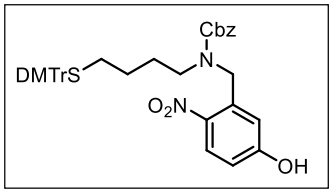
Synthesis of Benzyl (5-hydroxy-2-nitrobenzyl)(4-mercaptobutyl)carbamate (7): Bromo compound **5** (1.18 g, 2.70 mmol), tritylmercaptan (0.89 g, 3.24 mmol), and potassium carbonate (1.31 g, 9.44 mmol) were dissolved in a mixture of water and ethanol 1:1 (30 mL) solvents. The reaction mixture was refluxed at 90 °C for 12 h. After the completion of the reaction, the reaction mass was neutralized with 1N HCl and then extracted with dichloromethane. The organic volume was washed with water, then brine, and dried over anhydrous Na_2SO_4. The organic solvent was then evaporated using a rotary evaporator to afford a crude compound **6** (1.7 g, 99% yield) as an off-white solid and used as such for the next step.



To a stirred solution of trityl thiol crude compound **6** (3.0 g, 4.74 mmol) in (30 mL) dry CH_2Cl_2, trifluoro acetic acid (2.45 mL, 18.96 mmol) and triethylsilane (1.97 mL, 12.33 mmol) were added dropwise at room temperature under an argon atmosphere. The reaction mixture was kept under stirring in an inert atmosphere and monitored by TLC. After 1h, on completion of the reaction, water was added and extracted the reaction mass into CH_2Cl_2, washed with brine, dried over sodium sulphate, and then evaporated the organic volume using a rotary evaporator. The compound was then purified using silica gel column chromatography under the isocratic flow of a mixture of solvents (0.2% methanol in CH_2Cl_2), dichloromethane, and methanol. Pure compound **7** (1.3g, 70% yield) was isolated as a pale yellow solid. R_f = 0.45 (methanol:dichloromethane 05:95); IR (cm^{-1}): 3227, 2921, 2856, 1673, 1590, 1514, 1455, 1308, 1249, 1167, 1119, 1071, 980; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 8.92 (s, 2H), 8.23 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.45 – 7.22 (m, 6H), 7.11 (dd, *J* = 6.7, 3.0 Hz, 4H), 6.91 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.84 (d, *J* = 2.7 Hz, 1H), 6.67 (d, *J* = 8.6 Hz, 2H), 5.20 (s, 2H), 5.10 (s, 2H), 4.98 – 4.88 (m, 4H), 3.38 – 3.22 (m, 4H), 2.55 – 2.45 (m, 4H), 1.77 – 1.66 (m, 4H), 1.62 – 1.49 (m, 4H), 1.36 – 1.30 (m, 2H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 163.0, 161.9, 157.6, 157.2, 140.6, 139.9, 137.1, 136.6, 135.8, 135.6, 129.3, 128.9, 128.7(3C), 128.6,

128.5(2C), 128.1, 127.5(3C), 115.2, 114.9, 114.2, 112.9, 68.3, 68.0, 49.2, 49.0, 48.0, 47.6, 31.1, 31.0, 27.0, 26.7, 24.3(2C). HRMS (ESI-MS): m/z calcd. for $[C_{19}H_{23}N_2O_5S]^+$: 391.1328; found: 391.1327.

Synthesis of Benzyl (4-((bis(4-methoxyphenyl)(phenyl)methyl)thio)butyl)(5-hydroxy-2-nitrobenzyl)carbamate (8): To a thiol compound **7** (1.30g, 3.33 mmol) in dry THF, dimethoxytrityl chloride (1.24g, 3.66 mmol), followed by triethyl amine (0.51 mL, 3.66 mmol) was added at room temperature under the argon atmosphere. After 15 min, dry methanol was added to quench reagents, solvents were evaporated on the rotary evaporator. Then the purification was performed using triethyl amine treated silica and an isocratic flow of a mixture of (0.3% methanol in CH_2Cl_2) solvents dichloromethane and methanol. The pure compound **8** (2.27 g, 98% yield) was isolated as a pale yellow solid. R_f = 0.55 (methanol:dichloromethane 05:95); IR (cm^{-1}): 3245, 2925, 2854, 1674, 1597, 1506, 1453, 1308, 1249, 1175, 1116, 1034; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 8.72 (s, 2H), 8.10 (d, *J* = 9.0 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.30 – 7.24 (m, 4H), 7.23 – 7.16 (m, 15H), 7.15 – 7.05 (m, 7H), 6.97 (tt, *J* = 5.0, 2.7 Hz, 2H), 6.76 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.72 – 6.67 (m, 8H), 6.64 (d, *J* = 2.6 Hz, 1H), 6.55 – 6.46 (m, 2H), 5.04 (s, 2H), 4.93 (s, 2H), 4.76 (s, 2H), 4.72 (s, 2H), 3.68 (d, *J* = 3.0 Hz, 12H), 3.01 (dt, *J* = 14.8, 7.4 Hz, 4H), 2.05 (t, *J* = 7.2 Hz, 4H), 1.44 (p, *J* = 7.8 Hz, 4H), 1.25 – 1.20 (m, 4H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 162.9, 162.0, 158.0(4C), 157.4, 157.0, 145.6(2C), 140.4, 139.8, 137.4(4C), 137.2, 136.7, 135.9, 135.7, 130.8(8C), 129.5(4C), 129.2, 128.8, 128.7(3C), 128.5, 128.3(2C), 128.0(2C), 127.9(4C), 127.5(2C), 126.6(2C), 115.1, 114.8, 113.9, 113.2(8C), 113.0, 68.1, 67.9, 65.8, 65.7, 55.3(4C), 49.2(2C), 48.2, 47.8, 31.7, 31.6, 27.6, 27.3, 26.1, 26.0. HRMS (ESI-MS): m/z calcd. for $[C_9H_{17}O_3]^+$: 173.1178; found: 173.1186.



Coupling Linker 8 to Merrifield resin: To a suspension of Merrifield resin (1.1 g, 1.21 mmol, loading 1.1 mmol/g) in CH_2Cl_2 (10 mL), the DMTr protected photocleavable linker **9** (3.35 g, 4.84 mmol) in CH_2Cl_2 (2.5 mL) was added. Subsequently, anhydrous DMF (10 mL) was injected into the flask. Solid Cs_2CO_3 (0.93 g, 4.84 mmol) and TBAI (1.56 g, 4.84 mmol) were added, and the resulting solution was stirred overnight on the rotavap at 60 °C and washed successively with DMF/water (1:1), DMF, THF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2 (2 times each). The resin was again transferred into a flask containing CsOAc (0.7 g, 3.63 mmol) in DMF (10 mL) and stirred overnight on rotavap at 60 °C for capping of the unreacted resin. The resin was then washed successively with DMF/water (1:1), DMF, THF, MeOH, CH_2Cl_2,

MeOH, and CH_2Cl_2 (2 times each) and dried under a high vacuum to obtain resin **9**. The loading value (0.16 mmol/g) was determined as described below.

Loading value determination: An aliquot of DMTr protected linker attached support **9** (1 mg) is accurately weighed into a 50 mL volumetric flask. The flask was then filled using 2% trifluoroacetic acid in dichloromethane to release the dimethoxytrityl (DMT) cation. Then, the UV absorption was measured on a spectrophotometer at 495 nm for the resulting orange color solution. The loading value of resin in 0.16 mmol/g, was then calculated as follows:

For $\lambda_{495,}$ [DMTr] = Absorbance at 495 nm x dilution factor/ Molar extinction coefficient

A495 is the absorbance at 495 nm, and the molar extinction coefficient is 71700 $M^{-1}cm^{-1}$. After taking the average of the two readings, the corresponding loading value was found as 0.16 mmol/g.

2B.6.3 Synthesis of required donor

Synthesis of 2,3-di-O-benzoyl-5-O-^{t}butyldiphenylsilyl-α-D-arabinofuranoside trichloroacetimidate (13): To a solution of compound **12** (340 mg, 0.57 mmol) in CH_2Cl_2 (2 mL), powdered 4Å molecular sieves and trichloroacetonitrile (0.114 mL, 2.0 mmol) were added at room temperature. The reaction was stirred for 30 minutes under an N_2 atmosphere, and Cs_2CO_3 (93 mg, 0.285 mmol) was added. The reaction mixture was stirred for 1h, and on completion, it was passed through the Celite® pad. The filtrate was concentrated at 25 °C using a rotary evaporator. The crude product was purified using neutralized silica gel chromatography using ethyl acetate:hexane system to obtain pure compound **13** (300 mg, 71% yield) as a sticky liquid. R_f = 0.7 (ethyl acetate:hexane 20:80); IR (cm^{-1}): 3333, 3062, 2930, 2861, 1725, 1672, 1597, 1454, 1257, 1102, 967; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.15 – 8.10 (m, 2H), 7.98 – 7.94 (m, 2H), 7.72 – 7.67 (m, 4H), 7.64 – 7.55 (m, 2H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.42 – 7.30 (m, 8H), 6.58 (s, 1H), 5.78 (dt, *J* = 3.9, 0.9 Hz, 1H), 5.73 (d, *J* = 1.0 Hz, 1H), 4.61 (td, *J* = 5.1, 3.8 Hz, 1H), 4.08 (dd, *J* = 11.0, 4.8 Hz, 1H), 3.99 (dd, *J* = 11.0, 5.4 Hz, 1H), 1.03 (s, 9H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 165.5, 165.2, 160.7, 135.8(2C), 135.8(2C), 133.7, 133.6, 133.2, 133.1, 130.2(2C), 130.1(2C), 129.9, 129.9, 129.4, 128.9, 128.6(2C), 128.5(2C), 127.9(4C), 103.4, 91.2, 86.3, 81.1, 76.9, 63.4, 26.9(3C), 19.4.

2B.6.4 General procedures for manual solid-phase oligosaccharide synthesis

Preparation of solutions and reagents for SPOS

Preparation of building block solution: 4 equivalents of building block (e.g., glycosyl alkynyl carbonate donor) into an eppendorf tube and dissolved in 2 mL of anhydrous CH_2Cl_2.

Carbonate donor activators: Solid gold phosphite (10 mol%) and AgOTf (15 mol%) were weighed into individual eppendorf tubes, and the tubes were sealed with parafilm until their utilization.

TMSOTf solution for TCA donor activation: 0.25M trimethylsilyl triflate solution was prepared freshly every time using dry CH_2Cl_2 solvent.

TFA solution for DMTr deprotection: 2% trifluoro acetic acid solution was prepared freshly using dry CH_2Cl_2 solvent.

Triethylsilane solution: Triethylsilane 0.25M solution was prepared freshly every time by dissolving it in a dry CH_2Cl_2 Solvent.

Protocol 1 – Swelling of resin:

The functionalized resin was loaded into the reaction vessel; dry CH_2Cl_2 was added and kept for 5 min for swelling of the resin. The solvent was drained before starting the reaction.

Protocol 2 - Glycosylation with carbonate donor:

The building block donor solution (4 equiv, 0.125 mmol) in 2 mL dry CH_2Cl_2 was delivered under a nitrogen atmosphere to the reaction vessel containing the resin. Then, the resin was mixed with the donor solution for 10 minutes by bubbling N_2 gas. After that, with the slight interruption of N_2 bubbling, solid gold phosphite (10 mol %); Silver triflate (15 mol%) were added to the reaction vessel. The reaction mixture is then left for 30 min under Nitrogen bubbling. The solution is drained, and the resin is washed with CH_2Cl_2, DMF, and CH_2Cl_2 (3x with 2 mL for 15 s sequentially). With the use of CH_2Cl_2 solvent, the resin was then transferred to another reaction vessel and washed with 2 mL dry CH_2Cl_2 three times. The resin is then dried using a vacuum pump.

Protocol 3 - Glycosylation with trichloroacetimidate donor:

Under the N_2 atmosphere, the building block donor solution (3 equiv, 0.125 mmol) in 2 mL dry CH_2Cl_2 was delivered to the resin-containing reaction vessel whose temperature was pre-

set at -50 °C. The resin and the donor solution were mixed up for 10 min by bubbling N_2 gas. After that, using a syringe, 0.25M TMSOTf (0.3 equiv) solution was added to the reaction vessel under the same atmosphere. The reaction mixture is then left for 1h under Nitrogen bubbling. The solution was drained, and the resin was washed with CH_2Cl_2 (3x with 2 mL for 15 s sequentially). With the use of CH_2Cl_2 solvent, the resin was transferred to another reaction vessel, and after washing with 2 mL dry CH_2Cl_2 three times, the resin was dried using a vacuum pump.

Protocol 4 – DMTr deprotection using 2% TFA solution:

The resin was washed with dry CH_2Cl_2 two times, and then dry CH_2Cl_2 (1.5 mL) was added. The resin was agitated using N_2 bubbling for 5 min, then 2% TFA solution was added dropwise under an inert atmosphere at room temperature for 10min. The reaction vessel was emptied into the waste and washed with CH_2Cl_2 (3x2mL). The resin was then dried under a high vacuum.

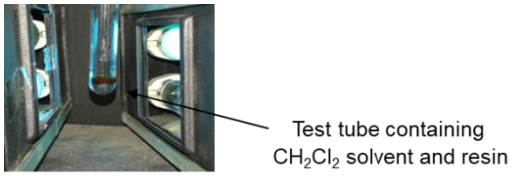
Protocol 5 - DMTr deprotection using 2% TFA and 0.25M triethylsilane solution:

The resin was washed with dry CH_2Cl_2 two times, and then dry CH_2Cl_2 (1.0 mL) was added. The resin was agitated using N_2 bubbling for 5 min, then 0.25M triethyl silane solution (0.5 mL) was added dropwise under an inert atmosphere at room temperature for 5 min. After that, 2% TFA solution (1.0 mL) was added slowly in a dropwise manner. Then, after 5 min, the reaction vessel was emptied into the waste and washed quickly with dry CH_2Cl_2 (3x2mL). The resultant resin was used immediately for the glycosylation.

Post-synthesis protocols

Protocol I - Photocleavage: The resin ready for deprotection was transferred to the 15 mL glass test tube fitted with a rubber septum, dry CH_2Cl_2 (1 mL) was added under nitrogen atmosphere and then kept under the UV-visible cabinet equipped with a light of wavelength range 365 nm for 4 h. The resin was then carefully filtered through a column with a frit. The resin was washed with CH_2Cl_2 (5x2 mL), and combined filtrates were evaporated under vacuum to obtain a residue that was redissolved in CH_2Cl_2, and UPLC-MS was performed.

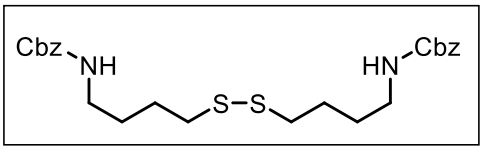
Photocleavage reaction setup



Protocol II - Purification of protected oligosaccharide: After cleavage from the solid support, protected oligosaccharides were purified by silica gel column chromatography using a hexane:ethyl acetate system for complete characterization.

2B.6.5 Characterization of the cleaved product from the support after DMTr removal and the glycosylation reaction

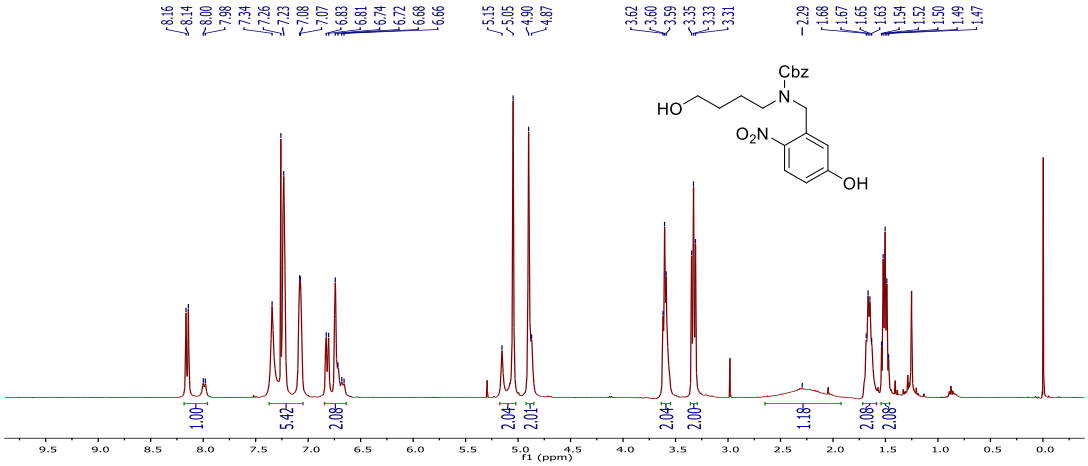
Isolation of dibenzyl (disulfanediylbis(butane-4,1-diyl))dicarbamate from the resin 18:



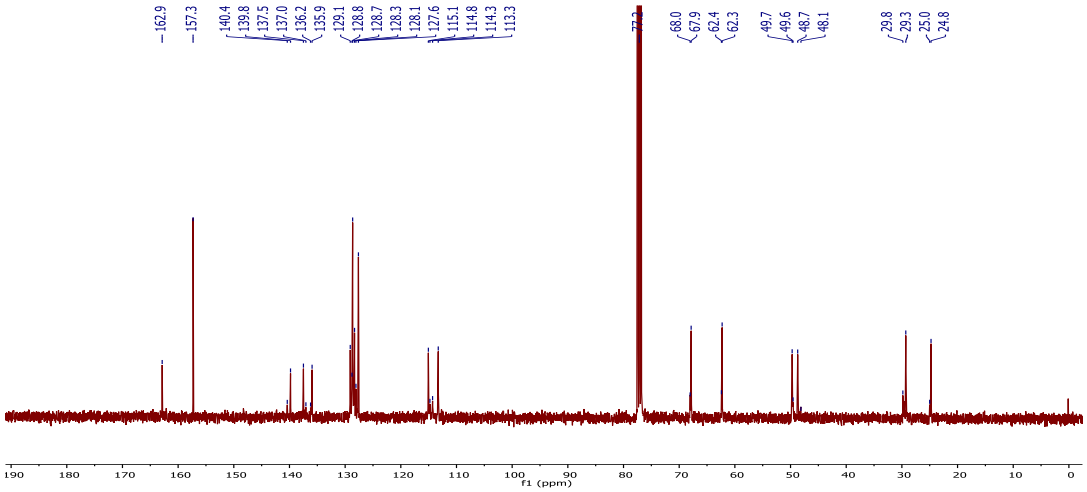
R_f = 0.5 (ethyl acetate:hexane 40:60); IR (cm^{-1}): 3330, 2923, 2859, 1698, 1526, 1454, 1368, 1244, 1132, 1016; ^{1}H NMR (400.31 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H), 5.09 (s, 2H), 4.93 – 4.84 (m, 1H), 3.21 (q, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 1.70 (q, *J* = 7.2 Hz, 2H), 1.60 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 156.6, 136.7, 128.6(3C), 128.2(2C), 66.8, 40.7, 38.5, 28.9, 26.3. HRMS (ESI-MS): m/z calcd. for $[C_{24}H_{33}O_4N_2S_2]^+$: 477.1882; found: 477.1881.

2B.7 Spectral charts

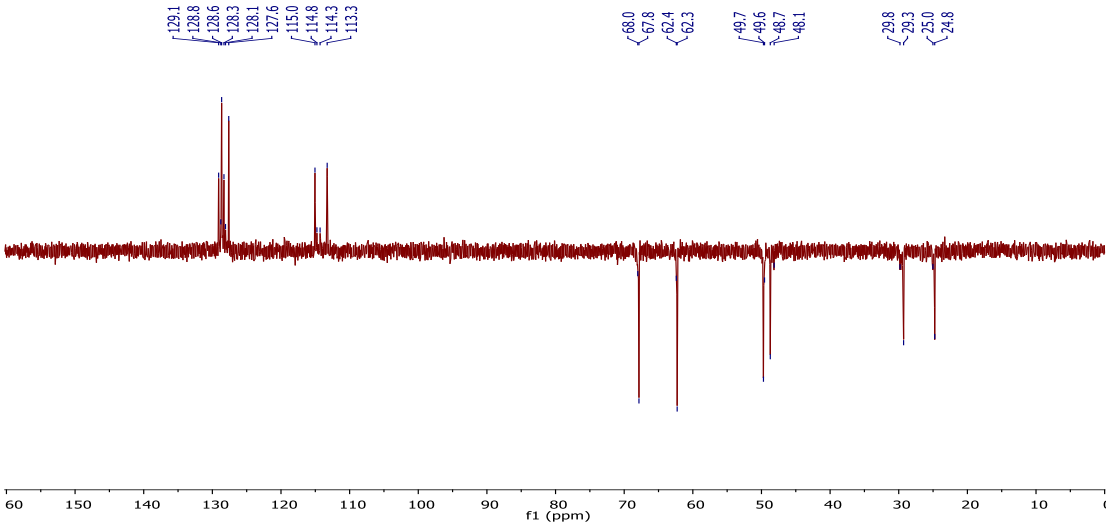
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **1**



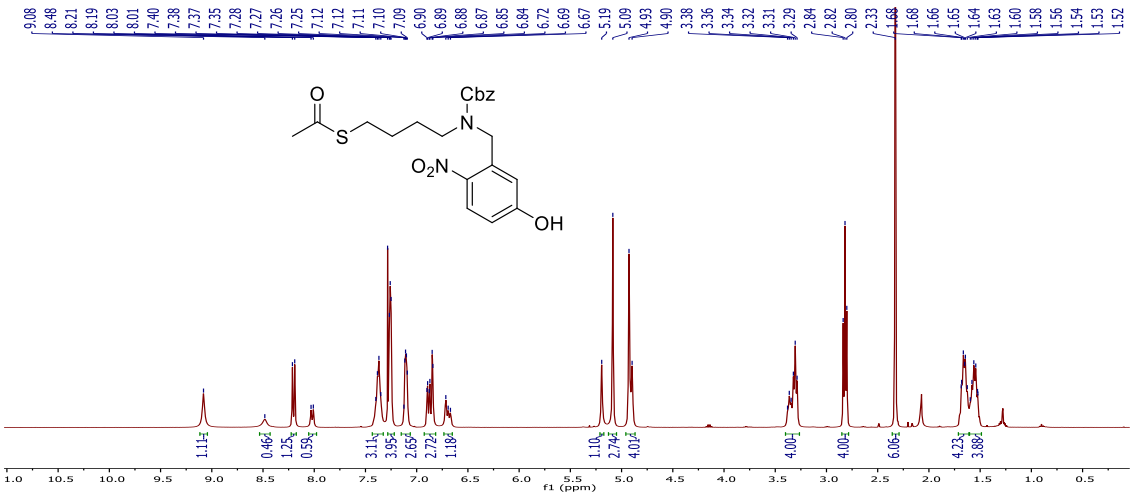
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **1**



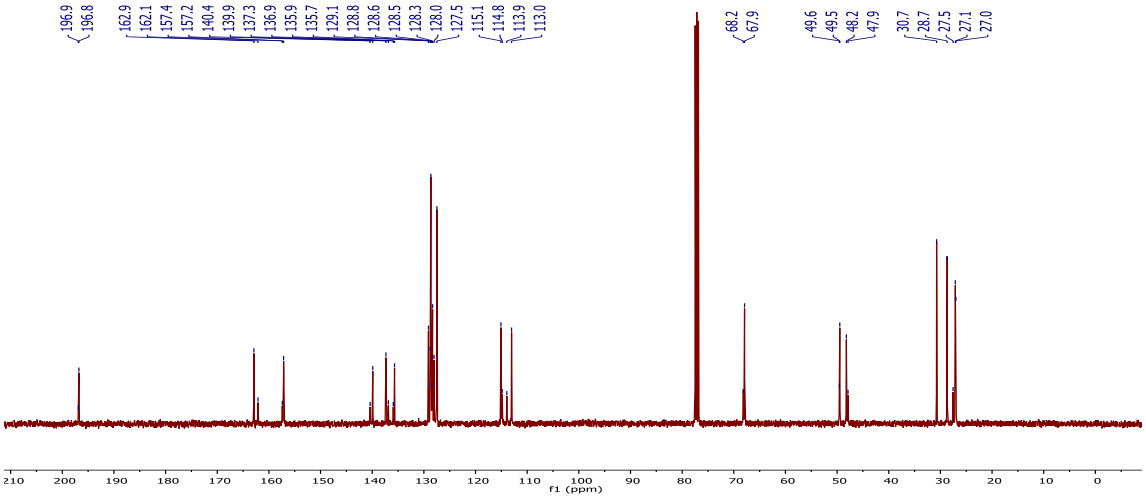
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **1**



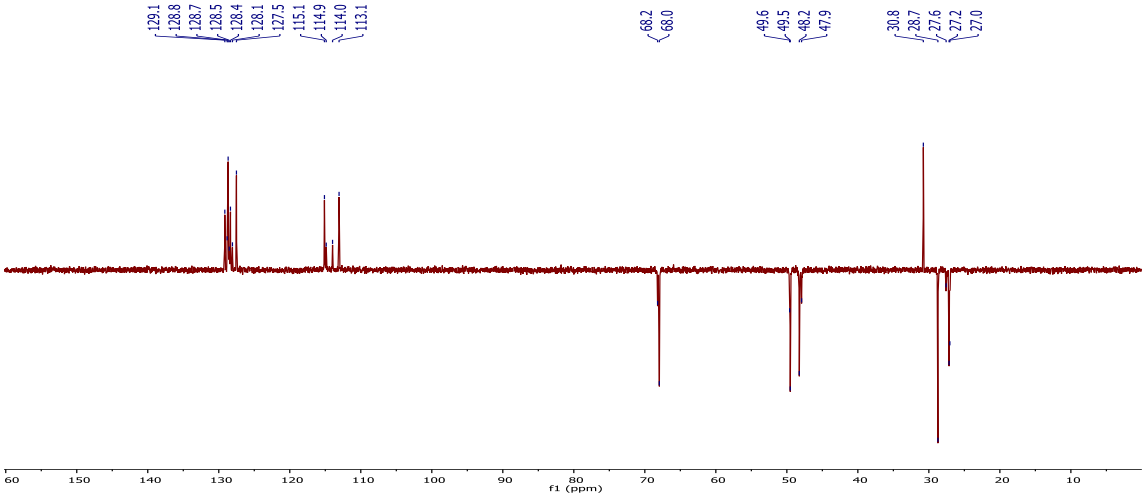
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **3**



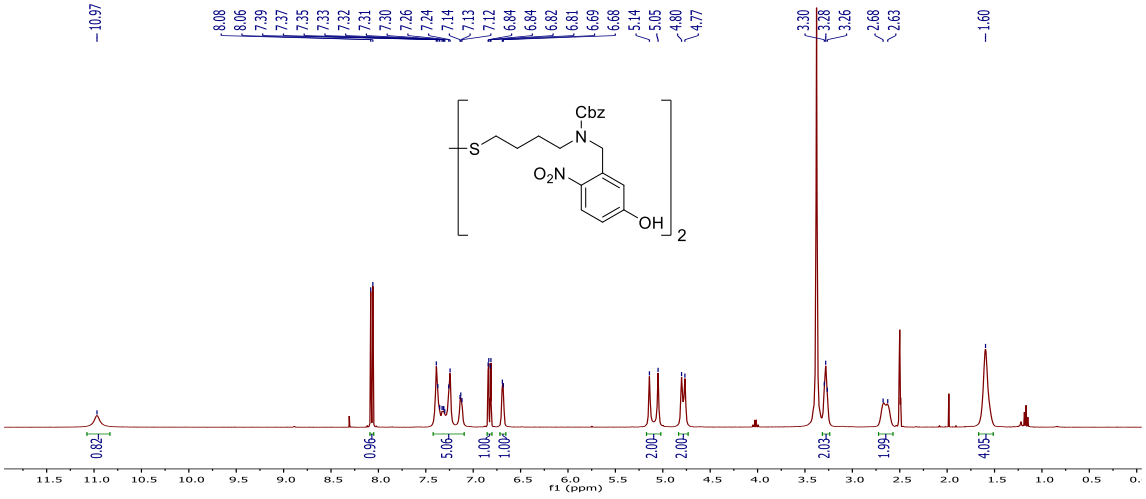
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **3**



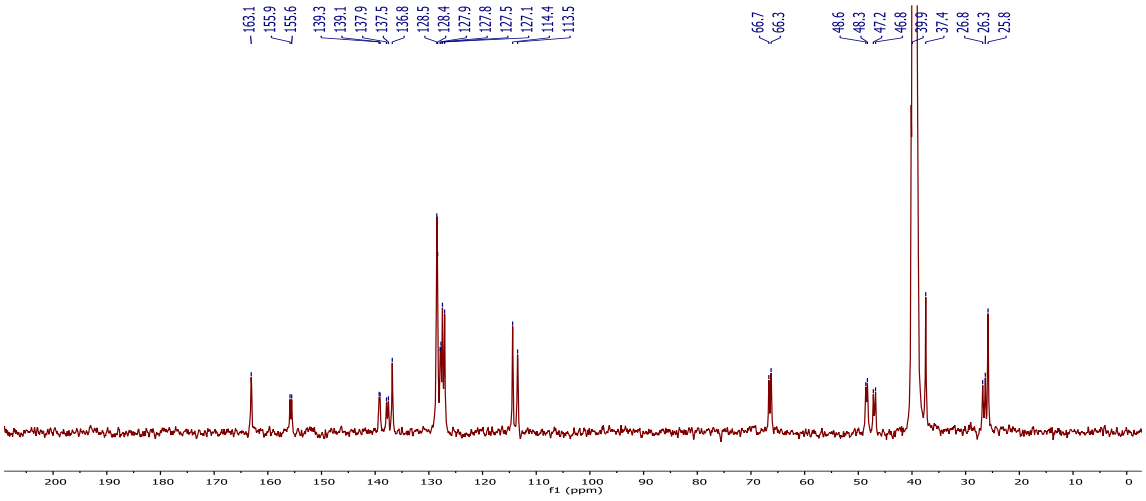
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **3**



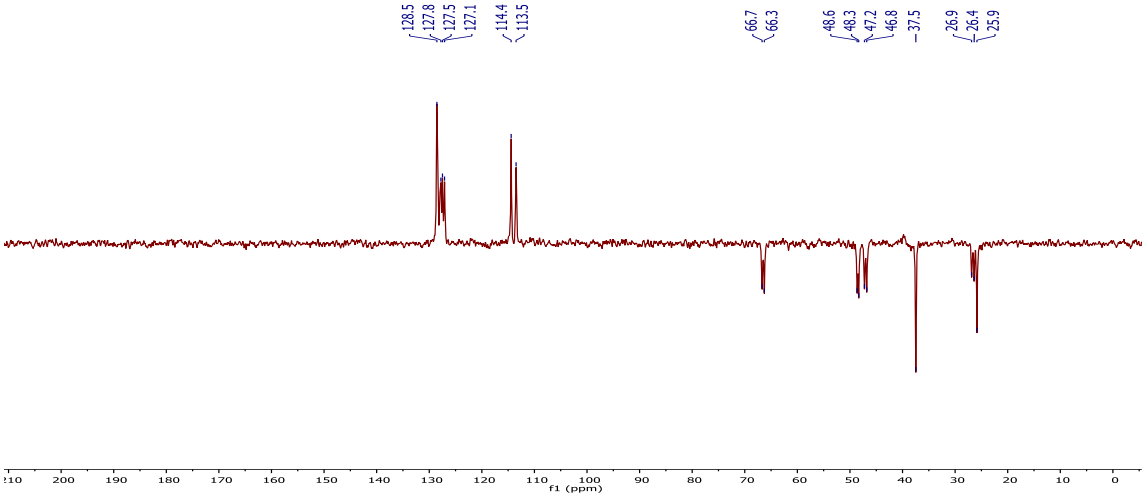
^{1}H NMR Spectrum (399.78 MHz, $CDCl_3$) of Compound **4**



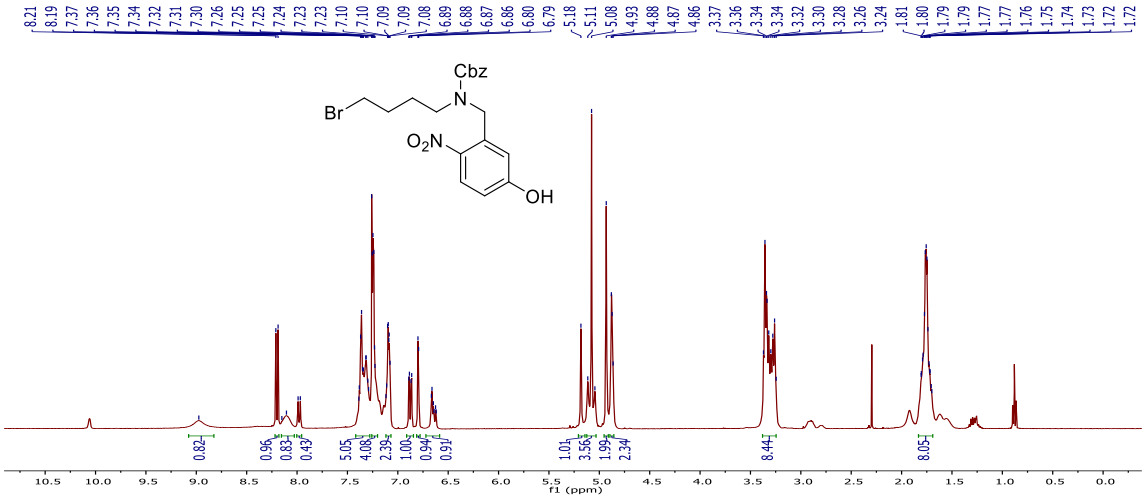
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **4**



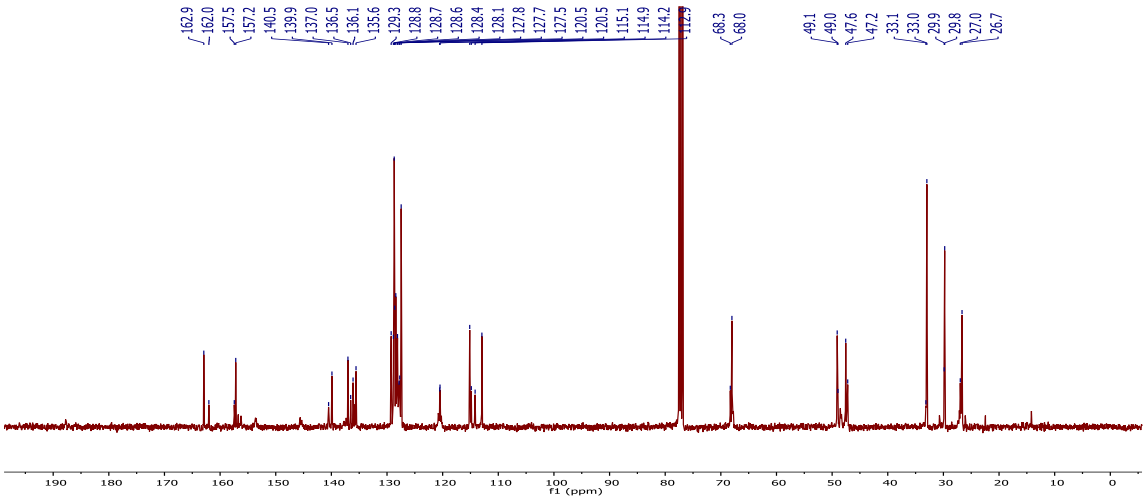
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **4**



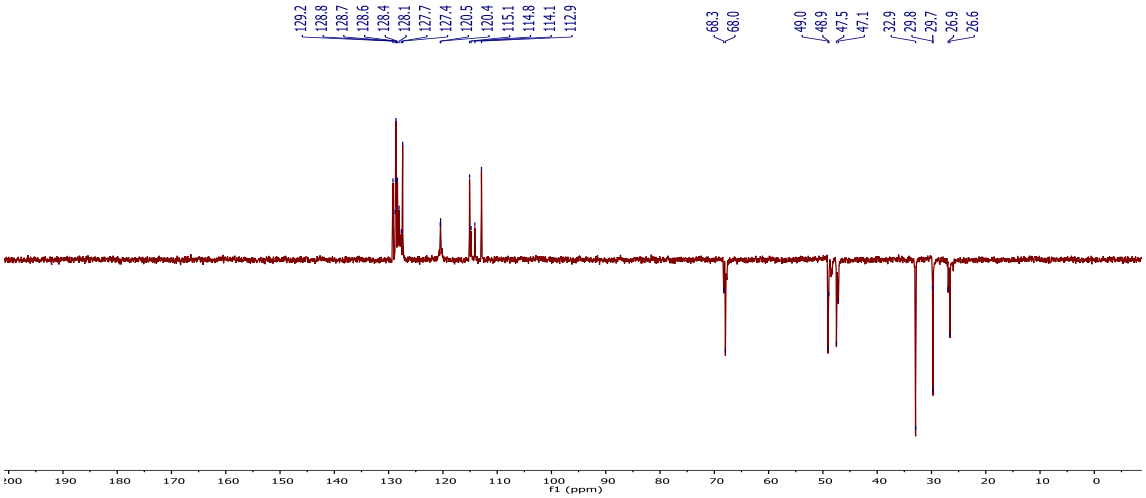
^{1}H NMR Spectrum (399.78MHz, $CDCl_3$) of Compound **5**



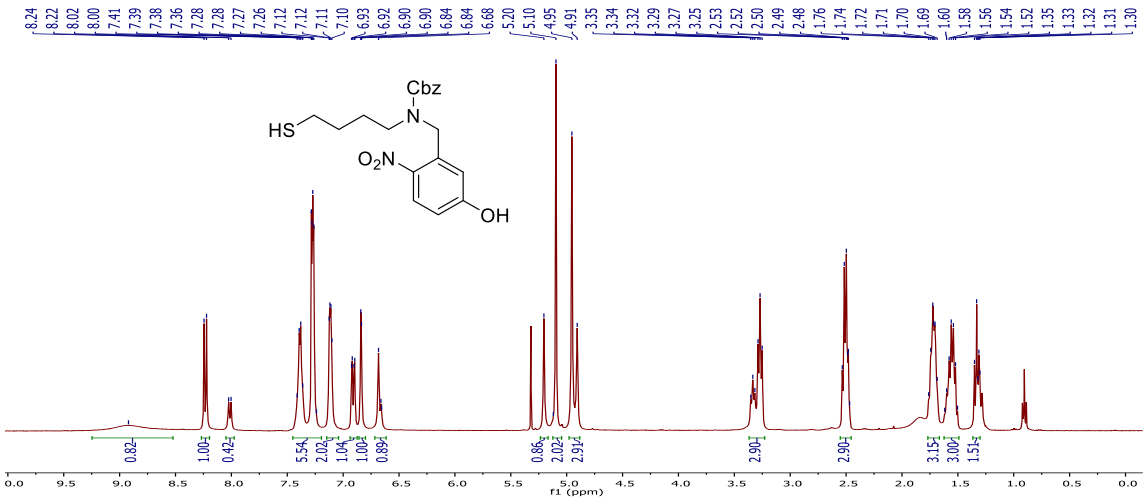
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **5**



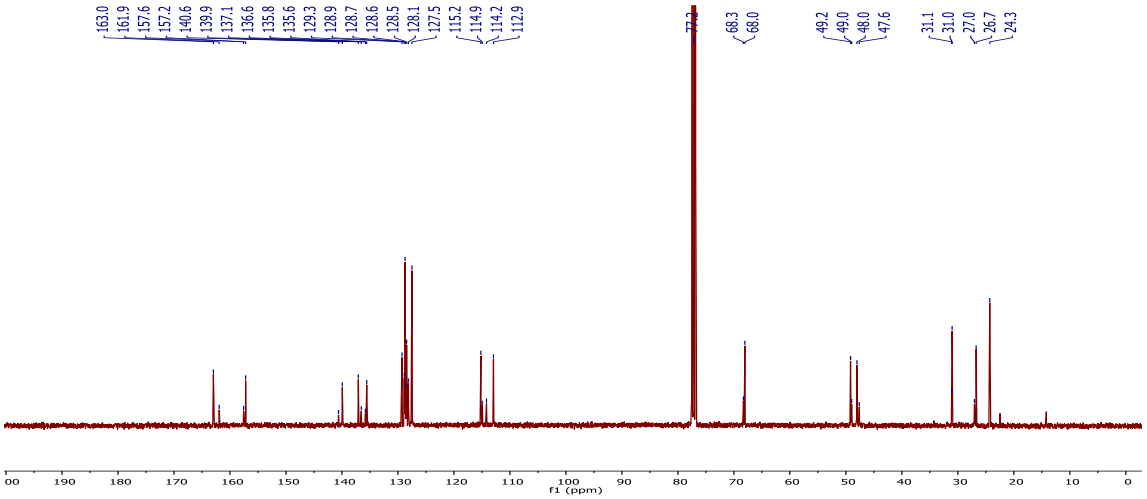
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **5**



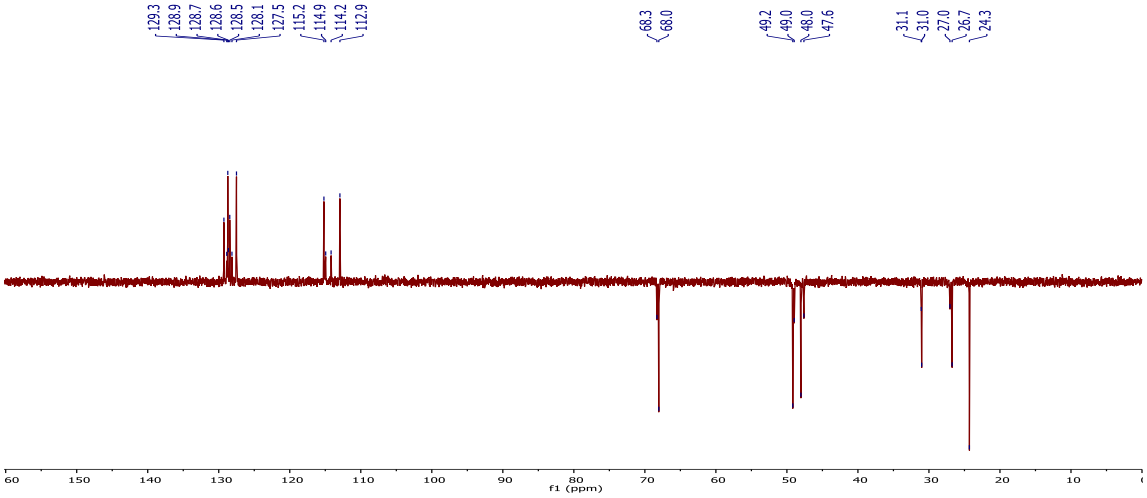
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **7**



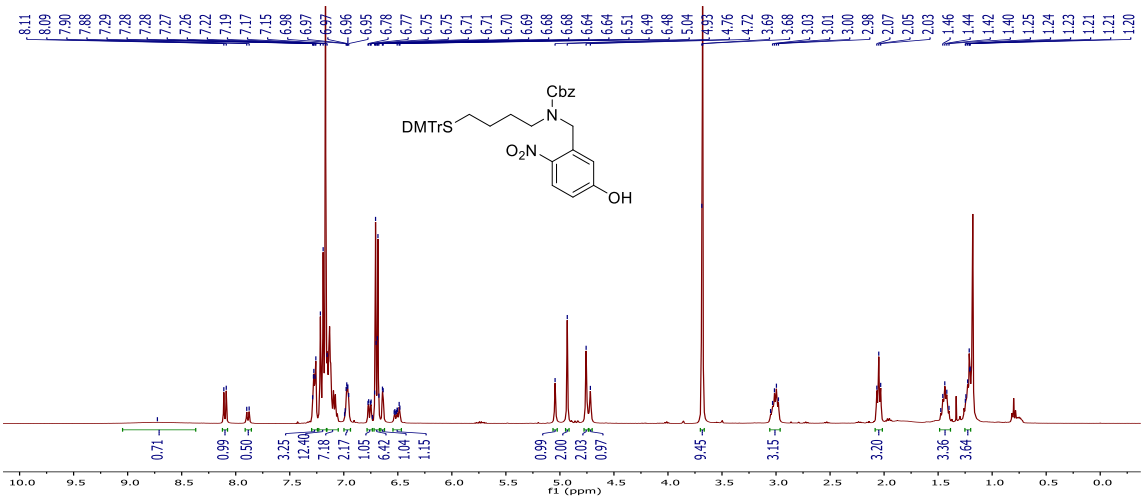
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **7**



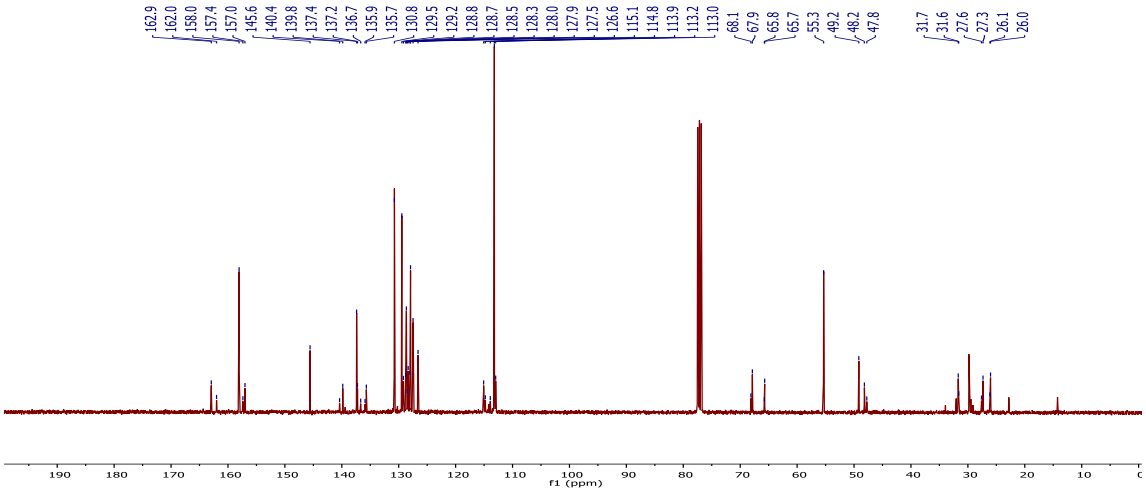
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **7**



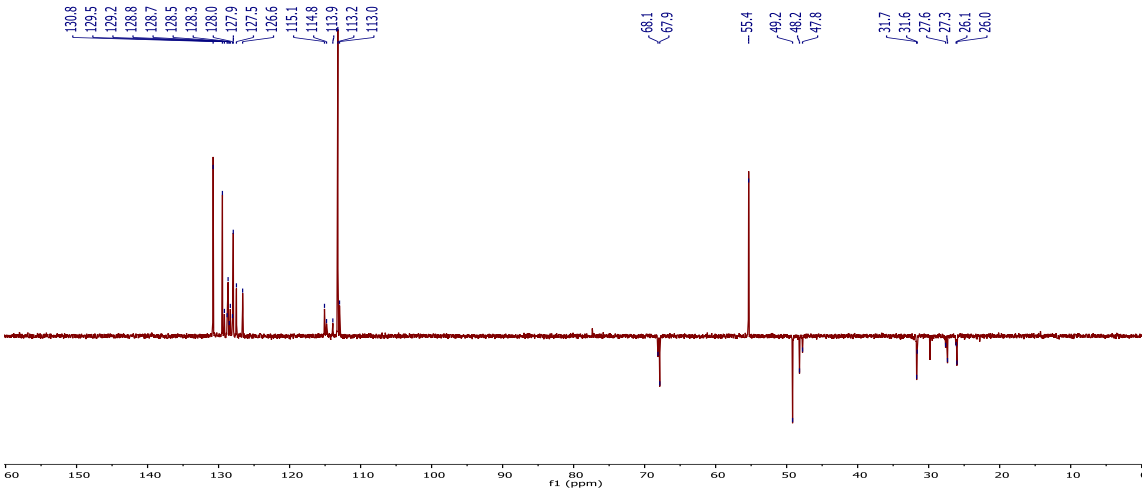
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **8**



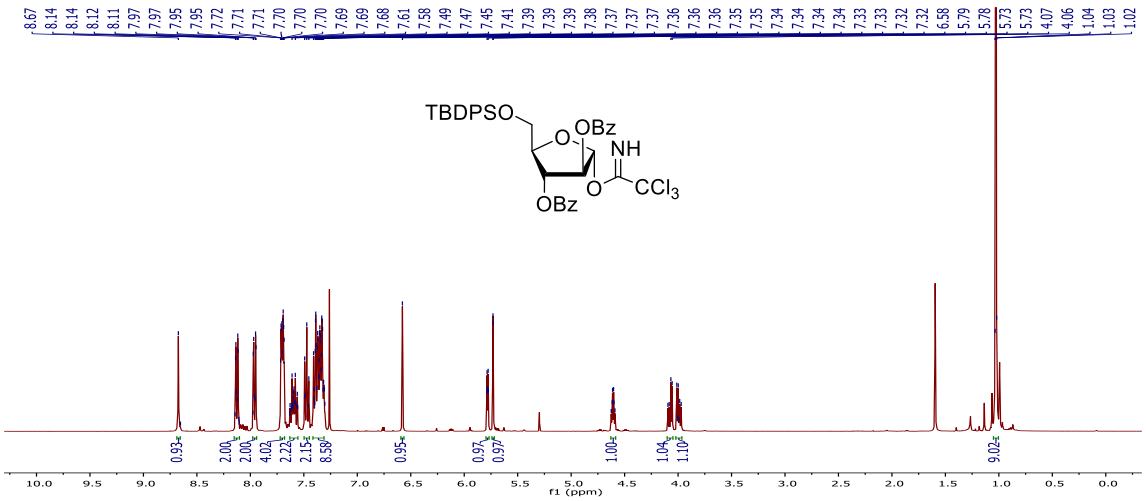
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **8**



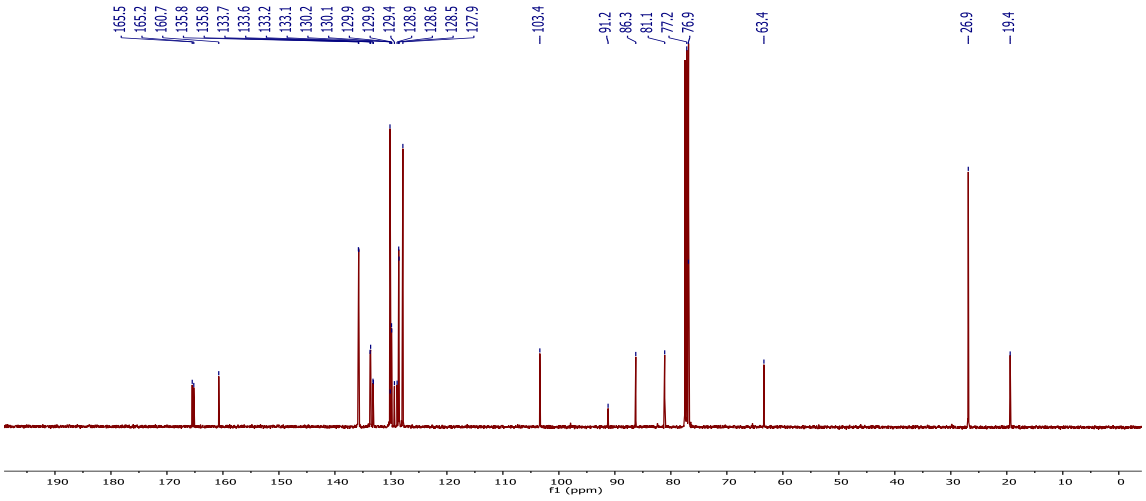
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **8**



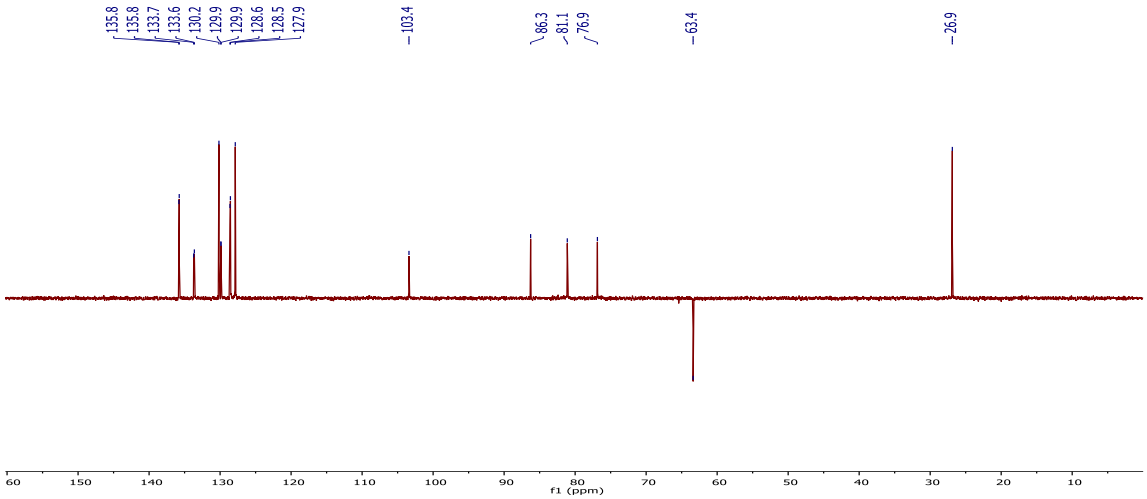
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **13**



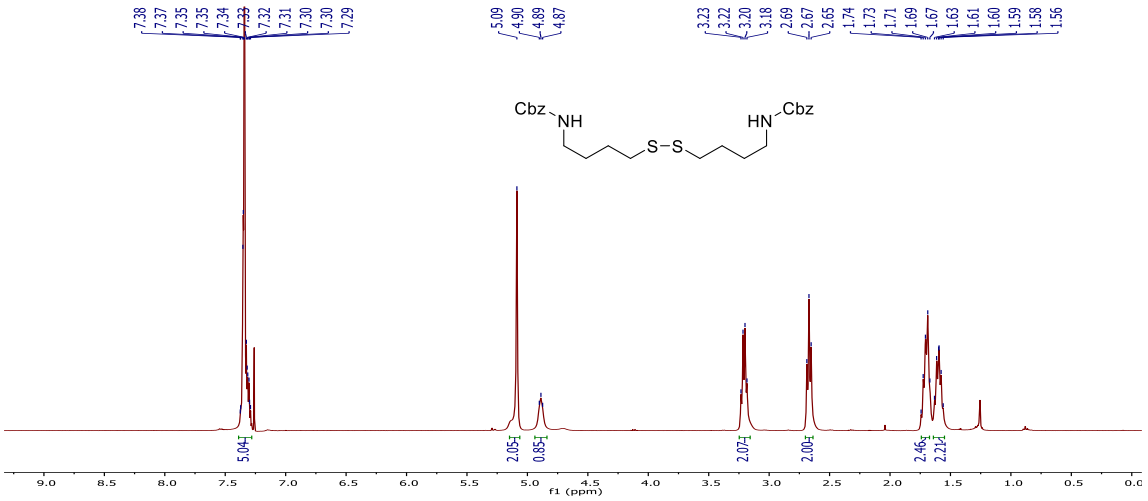
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **13**



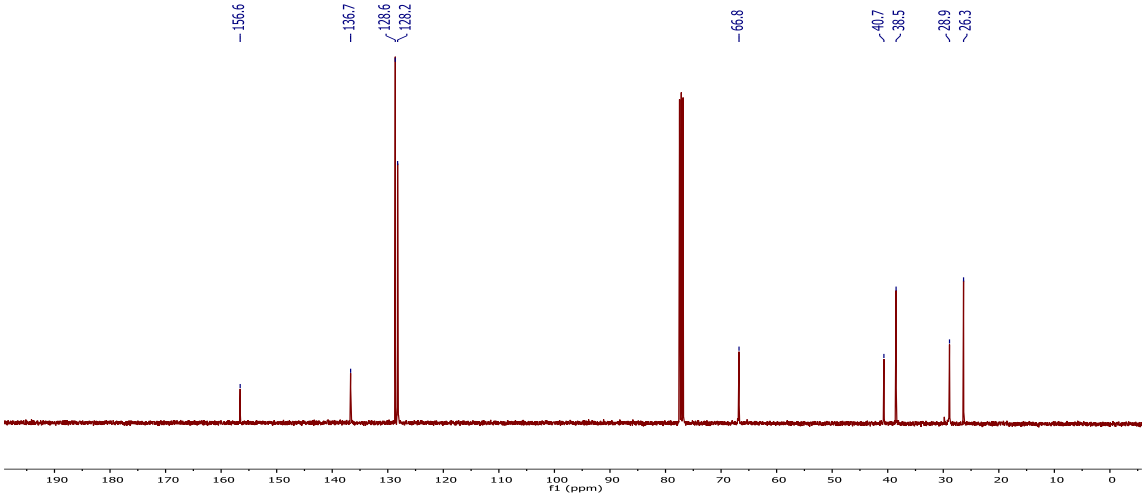
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **13**



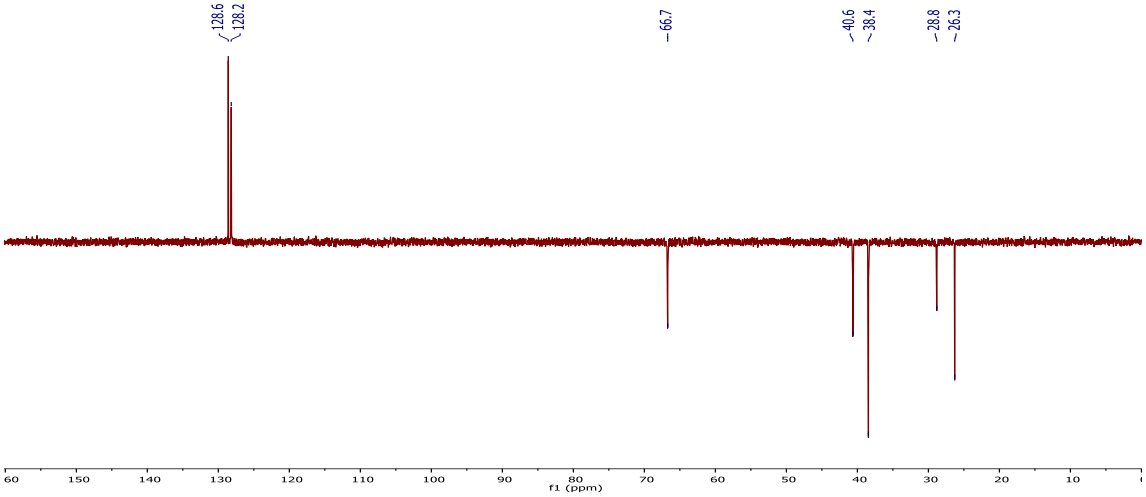
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **14 or 16**



^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **14 or 16**



DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **14 or 16**



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Chapter: 3

Silver-assisted Gold-catalysed Solid-Phase Synthesis of Linear and Branched Arabinans

Chapter 3

3.1 Introduction

Tuberculosis (TB) is still one of the prime killers of communicable diseases and one of the leading causes of death worldwide in human history. Ancient human skeleton studies have shown that humans have been affected by it for thousands of years.[1] The cause of tuberculosis was unknown until Dr. Robert Koch discovered it in 1882 and named it the bacillus *Mycobacterium tuberculosis*.[2] It is estimated that about 2 billion people worldwide are infected with M. tuberculosis so far. WHO reported in 2019 that TB ranked 13th on the list of the leading cause of deaths worldwide and was the most common infectious agent toppling HIV/AIDS. In 2021, TB mortality was projected to be much higher than in 2020 in all of the 16 modelled countries; by 2022, TB incidence is projected to be above the level of 2020, which is consistent with forecasted models published in 2020.[3-5]

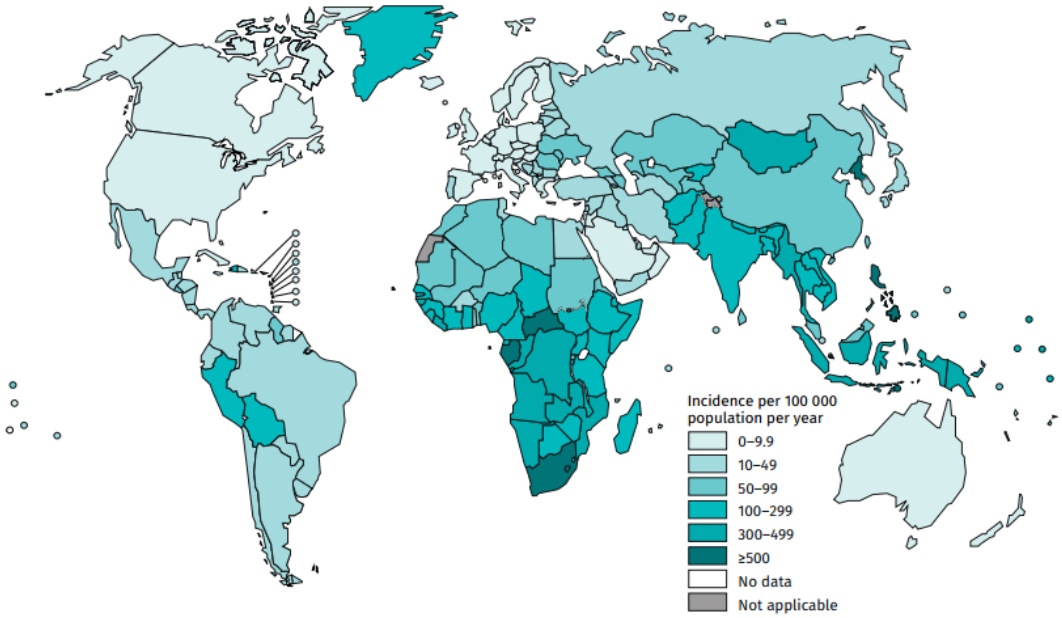


Figure 3.1 Estimated TB incidence rates, 2020 (Source: GLOBAL TUBERCULOSIS REPORT 2021)

Disease tuberculosis and treatment: TB is caused by an etiological agent called *Mycobacterium tuberculosis*,[6] which spreads when TB-infected people expel bacteria into the air (e.g., by coughing). The disease can also affect other organs, along with the typical effect on the lungs (pulmonary TB). TB can affect anyone, regardless of age or gender. There are more cases among men (about 90%) than women and disease primarily affects adults more. However, HIV positive patients, patients with diabetes, smokers, and those who consume alcohol are more susceptible to develop TB. The most frontline drug recommended is isoniazid and rifampicin. The Bacille Calmette-Guérin (BCG) vaccine is licensed for the prevention of TB disease. This

was developed almost 100 years ago and has been widely used to prevent severe forms of TB in children. Currently, no vaccine is effective in preventing TB disease in adults before or after TB infection.

3.2 Mycobacterium tuberculosis (Mtb)

Historical existence and identification of Mtb:

The origin of Tb was found 4000 years ago in soil, and it was thought that some of its species evolved to live in mammals. Majorly isolated strains from human and animal sources representing wild diversity are M. tuberculosis, M. africanum strains, M. bovis strains, M. bovis strains, M. microti strains, and M. canettii strains. Except for the proteic poly-γ-glutamate (PGA) capsule of Bacillus antiracist, most bacterial cell walls are associated with exopolysaccharides (EPS) that form slime, which is the crucial reason for the survival of bacteria.

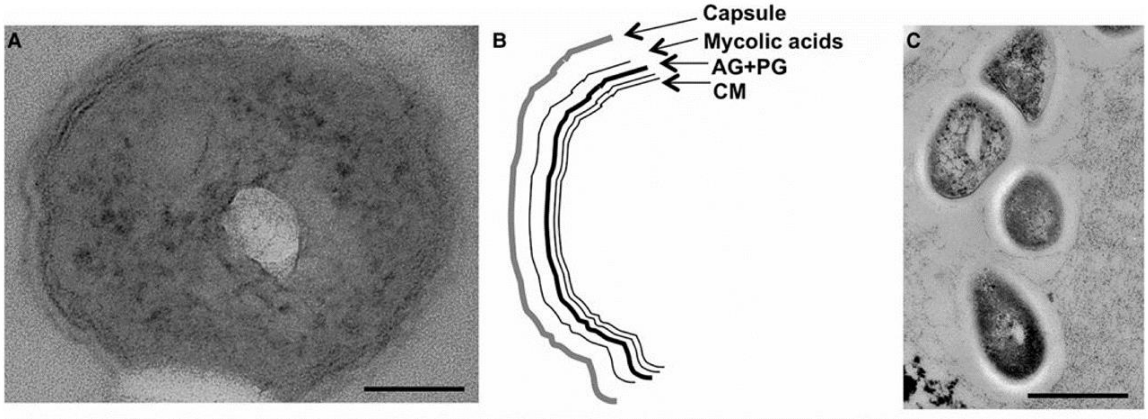


Figure 3.2: A) Transmission electron microscopy (TEM) image of ultra-thin sections of Mtb grown in the absence of detergent for 1 day. B) Model of the Mtb cell envelope as observed from (A). C) TEM image of ultra-thin sections of Mtb grown in the absence of detergent for 5 days. (**Source:** R. Kalscheuer, A. Palacios, I. Anso, J. Cifuente, J. Anguita, W. R. Jacobs Jr, M. E. Guerin and R. J. B. J. Prados-Rosales, *Biochem J.*, 2019, *476*, 1995-2016.**)**

The mycobacterial capsule could be defined as a peripheral capsule that can remain weakly attached to the cell surface and can be shed to the culture medium while retaining similar physicochemical properties.[7] The bacterial cell's outermost layer is the outcome of the secretion machinery that organizes outer polymeric substances with the coding sequence in operons, including both the genes encoding the polysaccharide synthesizing enzymes, which makes it a serotype capsule.[8] This Mtb capsular pathogen has the ability to infect and persist in

humans for decades despite the presence of a completely functioning immune system. The visualization of conventional or pathogenic samples of the bacterial capsule was initially carried out by using transmission electron microscopy (TEM) but later on, high-pressure freeze-substitution[9] or cryo-electron microscopy[10] (Cryo-EM) have been used. An early study showed that bacterial capsule/ bacillus is associated with extracellular material.[11, 12] Based on electron micrographs, this extracellular material was referred to as an "electron transparent zone."[13] The biological significance and nature of this 'extracellular material' have remained unspecified till today.[14],[11],[12]

Pathogenesis of Mtb:

When the infected person's tubercle bacilli enter the non-infected person through the air, it first reaches the alveoli. Subsequently, it gets phagocytized by alveolar macrophages to cause the killing of bacteria. Suppose the macrophages fail to kill the bacteria infection in the initial stage.

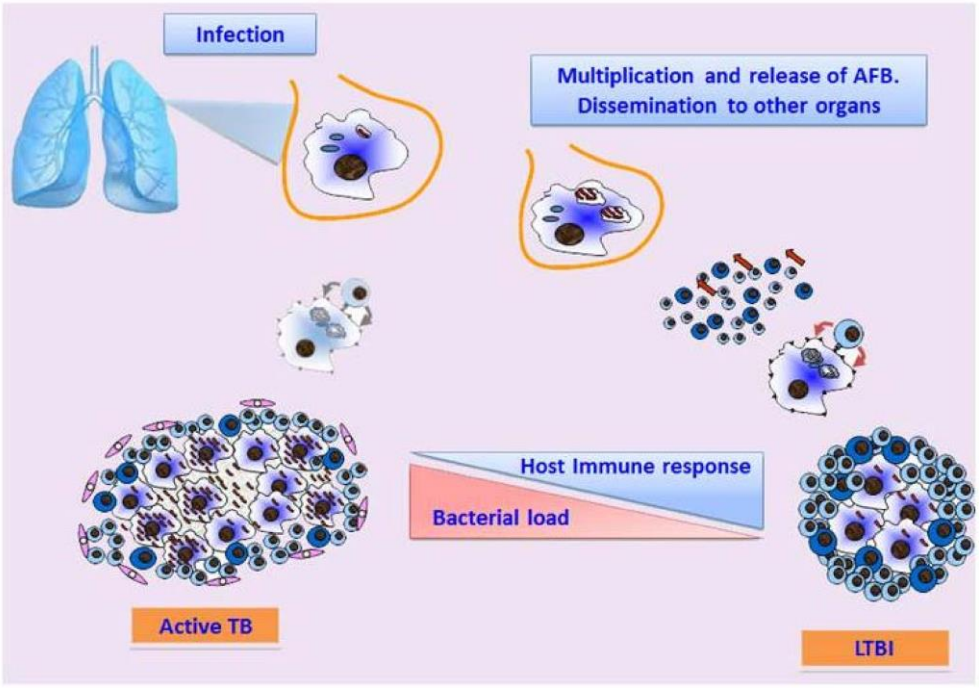


Figure 3.3: TB pathogenesis (**Source**: G. Delogu, M. Sali and G. Fadda, *Mediterr J Hematol Infect Dis* **2013**, *5*, e2013070)

In that case, replication starts rapidly from the initial lesion and diffuses to other body parts through lymphatic and circulatory systems.[15] After that, the immune cell migrates to the site of primary infection to build an adaptive immune response. It forms a cellular infiltrate that later gives rise to a typical granuloma structure containing encapsulated bacilli.[16] Later, the bacilli

remain encapsulated in the calcified granuloma and become protected by the immune response. Finally, after one or two months, the tuberculin skin test (TST) and interferon-gamma release assay (IGRA) become positive once the host develops immunity. Primary TB progression is a function of age and immunogenic response; thus, after infection in infants or immunocompromised hosts, infection progression in lungs sometimes result in miliary tuberculosis or central nervous system disease within 2 to 6 months. Adolescents may develop adult-type (infectious) pulmonary TB or extrapulmonary TB within the first 8-24 months of infection. If the host resistance and disease tolerance mechanism work well against the infection, 90 to 95% of cases will become disease-free but extremely limited. Much evidence suggests that Mtb resides in different organs, tissue types, and cell types during LTBI without showing typical granulomatous lesions. Additionally, these diverse lesions were seen in a patient with, and those lesions responded to chemotherapy differently, indicating that lesion reflects distinct Mtb subsets.[17]

Structure of Mtb cell wall:

The bacterial cell wall is commonly termed as the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex. In general, it represents three major components i) peptidoglycan and ii) lipopolysaccharides (arabinogalactan, lipoarabinomannan, and lipomannan) iii) outer membrane (mycolic acid, various glycolipids and phthiocerol dimycocerosates) which are explained briefly below.

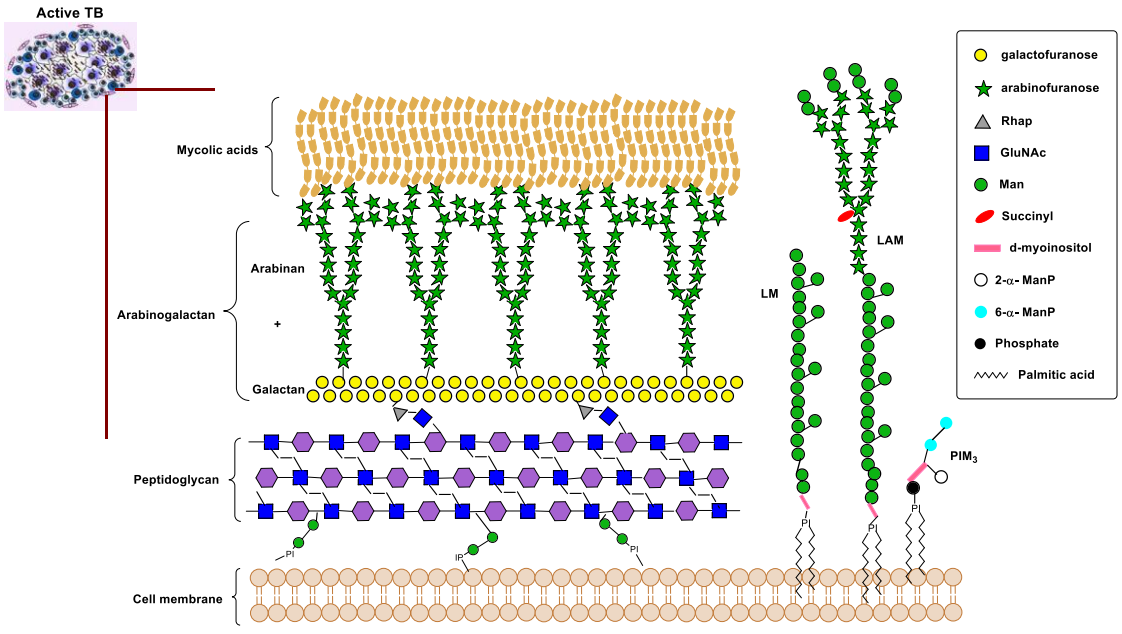


Figure 3.4: Structure of Mtb cell wall

Peptidoglycan: The peptidoglycan is located outside of the mycobacterial inner membrane or the plasma membrane. It is composed of alternating N-acetylglucosamine and muramic acid (either N-acetylated or N-glycolylated) residues linked by (1→4) bonds.[18] The L-alaninyl-D-isoglutaminylmeso-diaminopimelyl-D-alanine chain of a tetrapeptide chain is linked to the either side of the corresponding peptide chain of a glycan chain. These cross linkage are of two types i) meso-diaminopimelic acid and D-alanine to form a 3→4 linkages; ii) two meso-diaminopimelate residues to form a 3→3 linkages.[19, 20]

LM, LAM and PIMs: The inner membrane of mycobacterial cell wall includes the mixture of three lipoglycans such as lipomannan (LM), lipoarabinomannan (LAM) and phosphatidyl-myo-inositol mannosides (PIMs). The PIMs are identified by number of Man*p* residues on the molecule and which is substituted with up to six Man*p* residues. Thus, phosphatidyl-myo-inositol with one Man*p* residue is PIM_1. The phosphatidylinositol is the simplest glycolipid form with two Man*p* residues at C-2 and C-6 of myoinositol (PIM_2). PIM_3 contains Man*p* residue linked to the C-6 position of the 6-linked Man*p* residue, while PIM_5 and PIM_6 contain Man*p* residue linked to α-(1 → 2)-position. Palmitate and tuberculostearic acids are esterified to the glycerol to form the diacyl form for PIM_1 and PIM_2 as well as the triacyl and tetraacyl forms for PIM_2 with additional palmitate moiety.[21]

PIMs are mostly associated with the two mannose units, LM and LAM. LM is the long chain of the mannan or branching with the α-D-(1→2) monopyranose unit. LAM is the longest glycopolymer chain, which is composed of D-mannan and D-arabinan anchor to a phosphatidyl-myo-inositol (PI) moiety in the mycobacterial cell wall. The highly branched D-mannan structure consists of α-(1→6) linked mannopyranose (Man*p*) backbone and is substituted at C-2 by single Man*p* units. Similar to that of the arabinogalactan, the arabinan part contains a linear α-(1→6) Ara*f* backbone with the branching produced by 3,5-*O*-linked α-D-Ara*f* residues. The lateral side chains can either be present as a linear tetra-arabinofuranosides or a bi-antennary hexa-arbinofuranosides.[22, 23]

AG: Arabinogalactan is heteropolysaccharide which is covalently bound to the muramic acid residue of peptidoglycan *via* phosphodiester bond. It is positioned between the cytoplasmic membrane and the outer mycolic acid layer of the Mtb cell. It is composed of arabinose and galacose sugar in their furanoside form. The component of AG is composed of approximately 30 linear alternating β-(1→5) and β-(1→6) Gal*f* residues as D-galactose part and further attached to the rhamnosyl residue of the linker unit. The galactan region consists of linear

alternating 5- and 6-linked β-D-Gal*f* residues. Three tricosarabinoside (23mer) moieties are attached to the C5 hydroxyl of β(1→6) linked Gal*f* unit. This arabinan part is highly branched and contains a linear α(1→5) linked polysaccharide backbone unit with branching introduced by 3,5-α-D-Araf residue. Further the non-reducing termini consist of a structural motif [β-D-Ara*f*-(1→2)-α-D-Ara*f*]$_2$-3,5-α-D-Ara*f*-(1-5)-α-D-Ara*f*, which is a distinctive hexaarabinose residue. Approximately one-third of the arabinan tricosamer, AG of M. tuberculosis further contains succinyl residue and galacosamine (GalN). The mycolic acids are located on the terminal hexaarbinofuranoside, but only 2/3 of these are mycolated.

As a part of the outer membrane, trehalose-containing glycolipids and phthiocerol dimycocerosates are interspersed with mycolic acid. These constituents also play a crucial role in the immunity response when they interact with the host.

3.3 Rationale behind choosing arabinofuranosides as a synthetic target

The assumption has been made by many groups that the MTb fragment of the *Mycobacterium tuberculosis* bacilli could help us in finding a new vaccine as a potential solution for this malignant disease Mtb. In this perspective, i) the synthesis of such types of glycan fragments, in particular, furanoside fragments, may help in finding drug candidates, ii) Expeditious synthesis of these furanosides or pyranosides complex glycan requires unified, simple, fast, and less moisture sensitive or moisture free method for automation in the current scenario. ***As a drug candidate:*** Many carbohydrate-based drug molecules have been approved as diagnostic agents and are naturally occurring isolated carbohydrates from microorganisms and plants or their derivatives. For example, streptomycin was isolated from the soil organism *Streptomyces griseus*, and was used as an antibiotic agent. The drug candidate could be a sole carbohydrate polysaccharide or the major structural unit of the approved carbohydrate-based drug. The mycobacterial cell wall is composed of the constituents such as galacto-furanose (Gal*f*) and arabinofuranose (Ara*f*).[24] In addition, hyper-mannosylated derivatives (lipomannans and lipoarabinomannans) and phosphatidylinositol mannosides (PIMs) are also present on the outer surface of the capsular cell wall. The *in vitro* study of PIMs has shown that its derivatives may help with the adaptive immune response required for protection.[25] Similarly, many groups have recently synthesized and studied other complex fragments of AG and LAM. Wang's group synthesized tetra-, hepta-, and undecasaccharides of LAM oligosaccharides with a free amino group and their protein-linked conjugated BSA and KLH derivatives. Out of these, an immunological study in mice reveals that KLH conjugates were partially immunologically active because of their carbohydrate antigen structures.[26] So, we envisioned synthesizing such

types of a library of compounds of the arabinofuronosides may pave the way for finding vaccine candidates for tuberculosis. ***Finding a unified method:*** Making a library of such compounds of furanosides and pyranosides remains a daunting task for the carbohydrate chemists. There are various approaches for synthesizing glycans, including the solution phase method, solid phase method, electrochemical method, microwave method, and some non-conventional methods. Out of which, the solid phase technique (discussed in chapter 1) has its unique existence and superiority in terms of purifications and synthetic protocol. So, synthesizing various linkages in oligosaccharides, the potential of a unified glycosylation method is required under which all the furanosides and pyranosides could be accomplished. Along the same line, the glycosylation reaction conditions should be easily implementable to the solid phase chemistry for the synthetic ease and amenable for automation.

A glimpse of glycosyl donors used in solid phase oligosaccharide synthesis:

Among the plethora of leaving groups developed for the oligosaccharide synthesis in the solution phase, only a few donors are explored for the solid supported synthesis.

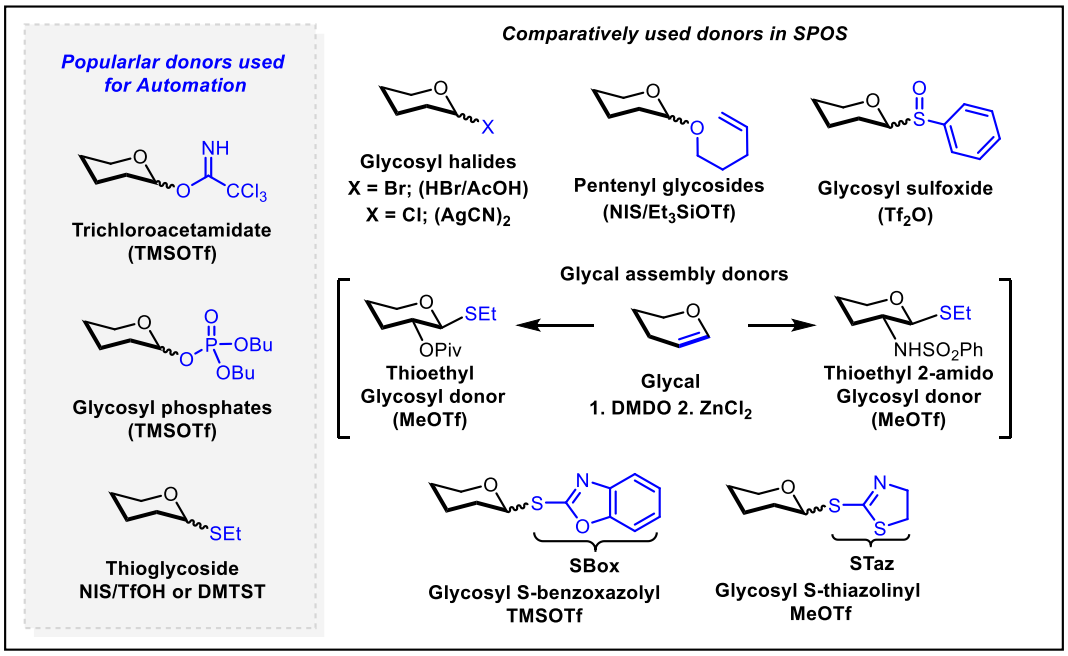


Figure 3.5: Donors used in solid-phase oligosaccharide synthesis

The first report of the synthesis of di- and trisaccharides on solid supports was illustrated by Fréchet and Schuerch in 1971 under very harsh conditions using a glycosyl bromide donor.[27] Later on, to improve the yield and scope of the donor usage for oligosaccharide syntheses, many research groups have put enormous efforts into improving glycosylation conditions as

well as in the direction of the choice of support and linker used for the reaction. But it did not support much because of the strong acidic conditions employed to generate glycosyl bromide donor. Glycosyl halide donors remain at a standstill for oligosaccharide synthesis except for one report by Van boom et al. for the synthesis of an immunogenic heptasaccharide.[28] Another class of donors, i.e., 1,2- anhydrosugars or glycals, have been used as influential glycosyl donors for obtaining β-glycosidic linkages on solid supported reactions. Converting this glycal into an epoxide derivative using DMDO and treating it with a glycosyl acceptor using mild activator $ZnCl_2$ offers good conversion.[29] This epoxide ring opening always generates C2 hydroxy on a newly formed product that may help to synthesize branched glycans (e.g., Blood group H-type 2 determinant synthesis)[30] or that can act as a competitor acceptor in the next glycosylation. So, this later occasion mandates protection of that exposed -OH group. Glycals can also be converted to their thioethyl glycosyl donor by treating its epoxy derivative with ethanethiol (EtSH) and cat. TFA and subsequently with a pivaloyl protecting group. This is a substantially improved donor compared to the simple glycals, which can be easily activated using methyl triflate and an equivalent amount of nonnucleophilic base di-*tert*-butylpyridine (DTBP). But again, this donor usage in its strategy necessitates additional treatment of reagent (DTBP) to prevent the decomposition of the glycal bond resident in the acceptor.[31] The construction of N-acetylaminoglucosidic linkage using these two glycal donors was a severe shortcoming. To overcome this obstacle, a new strategic donor called 'thioethyl 2-amidoglycosyl donor' came into the picture. The glycal on treatment with iodonium *sym*-collidine perchlorate forms intermediate iodosulfonamide and subsequently converts to thioethyl 2- amidoglycosyl donor through *trans*-diaxial displacement using ethanethiol agent.[32] However, while synthesizing higher oligomers, this donor sometimes results in unexpected side products, though the reaction temperature is set to -40 °C in the presence of an excess of activator MeOTf and base DTBP as well as all other required reagents. Thioglycosides are shelf-stable and can be easily converted into sulphoxide donors, because of which they remain attractive donors. Additionally, they are slow reactive donors, so their activation requires more than a stoichiometric promoter NIS/TfOH[33] or NIS/TMSOTf. Along the same line, another thiophilic agent, dimethyl(methylthio)-sulfonium triflate[34] (DMTST), has been used as an activator. The high toxicity of activators is the frontline drawback for this donor. Kahne and coworkers[35] have reported that thioglycosides can be converted to their sulfoxide derivatives. Then they proved that these glycosyl sulfoxide donors could be used as effective glycosylating agent with the excess of 2,6-di-*tert*-butyl-4-methylpyridine (DTBP) base and of triflic anhydride (~0.5 eq.) as an activator on the solid support.[36] This reaction generally completes

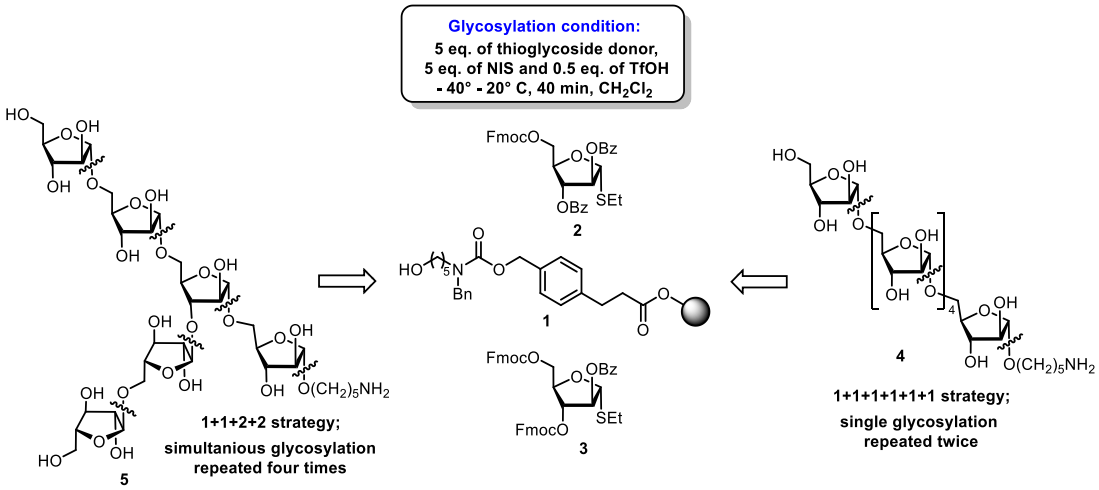
within 30 min. at -60 °C on the Merrifield solid support, but the synthesis of larger oligosaccharides using sulphoxide donors is not much explored. Activation of *n*-Pentenyl glycoside requires a stoichiometric amount (1.2 eq.) of NIS and (0.3 eq.) of TESOTf for activation.[37] The glycosyl trichloroacetimidate donor has been used as the most efficient donor for almost all sorts of solid supports, including CPG support and the linker system used for this chemistry. Using this donor, the reaction generally completes in one hour with the use of (3eq. x 2) of donor and a catalytic amount of activator TMSOTf (0.2 eq.) at a low-temperature range (-78 °C to -20 °C).[38, 39] This donor meets the fulfilment of *O*-glycan synthesis over peptide attached solid support. However, it mandates the use of low temperatures for their utilization.[40] Glycosyl phosphate is one more potential donor for SPOS because it involves non-toxic activator and generally does not require molecular sieves. The reaction completes in high yield within one hour at -50 °C using (2 x 3eq.) of the donor.[41] Overall, the phosphate and imidate donors can be activated by non-catalytic and catalytic amounts, respectively. Still, they are relatively less stable and require very low glycosylation temperatures (-70 °C to -20 °C), making them less convenient for their use and subsequently vulnerable to their use in automation. Despite all these facts, within the cluster of available donors, only thioglycosides, glycosyl phosphates, glycosyl trichloroacetimidates, and glycosyl N-phenyl trifluoroacetimidates are very frequently used donors for the automated platform.

Automation as an expeditious tool for glycan synthesis: As per the discussion above about the solid phase technique (in chapter 1), which involves integral steps such as washing, deprotection, and glycosylation that constitute the whole process and become a major reason for time consumption. The carrying out expeditious synthesis of glycans is the main reason for rising automation over the last two decades. Unlike traditional solution-phase chemistry, automation in solid-phase chemistry has been associated with enormous challenges regarding the experimental arrangement required for performing each required step. Despite that, many groups constructed various glycan synthesizers over the last two decades to accelerate and make the whole process smooth. Seeberger and co-workers introduced the first automated glycan synthesizer in 2001, called 'AB 433 peptide synthesizer'. Subsequently, they developed its modified version, the "first fully automated solid-phase oligosaccharide synthesizer," and they converted it to the most sophisticated machine, "Glyconeer 2.1". Very recently, with a few prerequisite changes, such as a variable-bed flow reactor (VBFR) system and a dual temperature regulation reaction (DTRR) system, they have updated their synthesis platform into the latest version. Along the same line, Demchenko's (STICS) technology-based Omnifit

column-fitted unmodified HPLC instrument has proven its strength and is noteworthy for expeditious glycan synthesis.

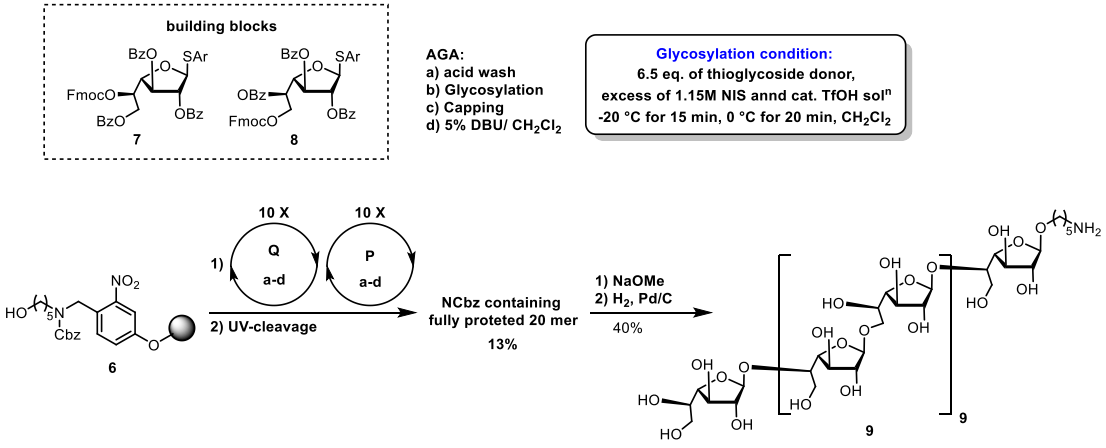
3.4 approaches for the arbinofuranoside fragments synthesis

1st approach: Using AGA synthesizer, Seeberger and co-workers[42] have amalgamated linear and branched arabinofuranoside fragments of arabinogalactan of mycobacterial cell envelope by employing linker **1** and arabinofuranosyl thioglycoside donor **2** and **3**. For assembling fragments **4** and **5** on support, they performed 6 and 4 glycosylations and yielded 69% and 63% on subsequent hydrogenolysis, respectively.



Scheme 3.1: Automated synthesis of oligosaccharide D, E

2nd approach: Seeberger and co-workers[43] have recently synthesized a segment, 20 mer of arabinogalactan **9** of Mycobacterium tuberculosis in a linear sequential fashion using AGA and prerequisite component benzoyl protected galalactofuranosyl thioglycosides donor **7, 8** and the photocleavable linker **6** in 13% yield. Subsequently, complete deprotection of residing groups yielded 40% over two steps.



Scheme 3.2: Syntheis of *β*-(1 →5)- and *β*-(1 →6)- linked linear 20 mer of arabinogalactan motif using AGA

3.5 The gold catalysis in the solution phase synthesis

Despite the range of oxidation states (-1 to +5) of gold, its usage in organic reaction transformations was not so common. It was used as a heterogeneous catalyst for reactions like the hydrogenation of olefins, nucleophilic addition of water and alcohol onto alkyne, cycloisomerization of furan-ynes as well as in the total synthesis of citreoviral.

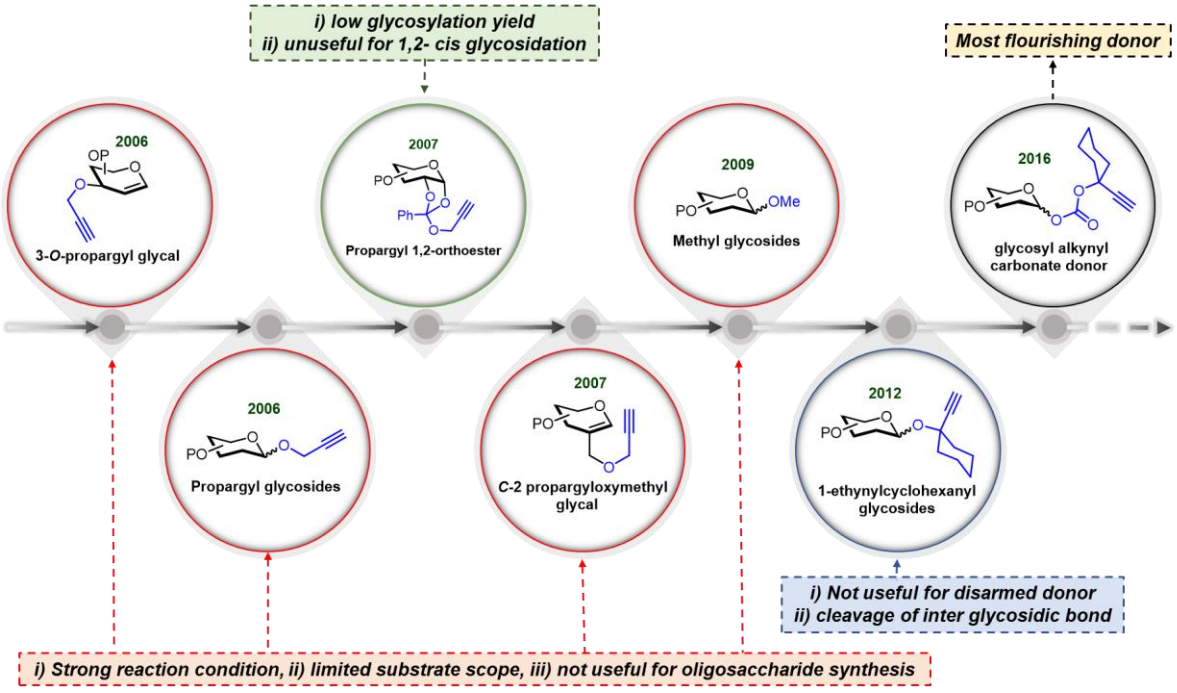


Figure 3.6: Donor development history map of Hotha group and their disadvantages

The Hotha group discovered the gold catalyzed glycosylation reaction in 2006. Their first report showed the Ferrier-like reaction using Au(III) catalyst for 3-*O*-propargyl glucal substrate.[44] In the same year, a very attentive result they obtained, wherein they identified that propargyl glycosides are very stable glycosyl donors and offer good reaction yield under elevated temperature (60 °C) and a catalytic amount of $AuCl_3$. Using this quite harsh condition, they have demonstrated its usefulness for disaccharide synthesis with limited substrates.[45] In 2007, Sureshkumar and Hotha introduced propargyl 1,2-orthoesters as versatile glycosyl donors, which was helpful for the synthesis of 1,2-*trans* glycosidic product under the milder condition in the presence of catalytic (10 mol%) amount of $AuBr_3$.[46] The great advantage of this donor is that it can be selectively activated in the presence of propargyl and *n*-pentenyl glycosides.[47, 48] Later on, they showed that 1,2 orthoester donor is not only beneficial for the synthesis of oligosaccharides but also in the preparation of glycosyl carbamates[49], furanosides[50], pyrimidine nucleosides[51], aminooxy glycosides[52], thioglycosides[53] and glycosyl acrylate/ acrylamides.[54-56] At the same time it has a significant drawback like i) 1,2-*cis*

glycosylation and glycosylation of C-2 *deoxy* sugar were beyond the scope, ii) sterically hindered aglycons always result in low glycosylation yield, iii) reaction is also associated with unwanted side product because the propargyl alcohol generated in the reaction become a competitor for the incoming nucleophile iv) synthesis of the donor in case of higher oligosaccharide is also inconvenience. Around the same year, Hotha et al. demonstrated the synthesis of C-2 methylene glycosides from C-2 propargyloxymethyl glycals by exploiting 5 mol % $AuCl_3$ catalyst.[57] Later in 2009, Rao and Hotha identified methyl glycoside donors for synthesizing oligosaccharides under quite harsh conditions. In this report, they have proved the alkynophilic character of $AuBr_3$.[58] After doing a systematic investigation for leaving ability of various alkyne bearing appendages at the anomeric position and screening of variable catalysts, co-catalysts and solvents, they found that 1-ethynylcyclohexanyl (ECH) glycosyl donor as excellent donors for disaccharide synthesis at the ambient temperature.[59] The primary limitation was that the disarmed sugar donor remained inactive under reaction conditions. Albite, the reaction condition was applicable for armed sugar donors; a longer oligosaccharide couldn't be achieved because of the cleavage of the interglycosidic bond owing to the oxophilic nature of Au(III). In 2016, Hotha et al. introduced a most flourishing donor called an alkynyl glycosyl carbonate donor. The superiority of this donor includes room temperature glycosylation within 15 min in the presence of the catalytic amount of gold and silver catalyst.[60] This donor fulfils all the flaws associated with the previously synthesized donors from the Hotha group.

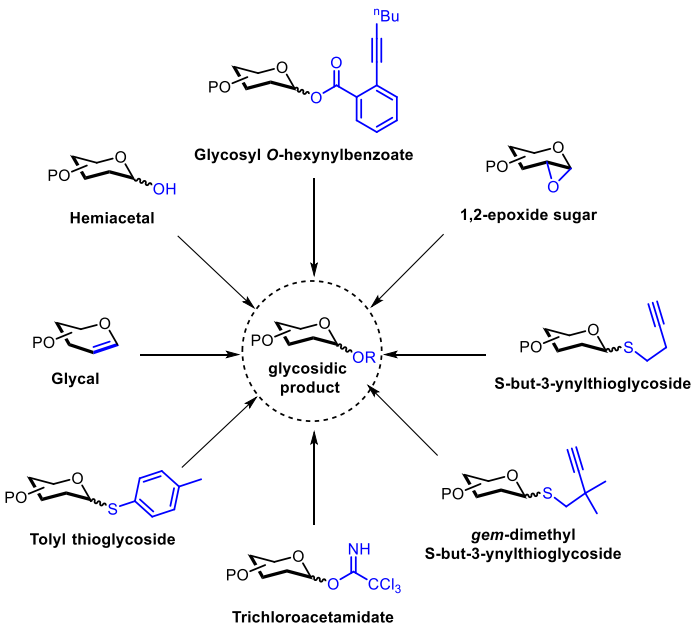


Figure 3.7: Few other donors for solution phase gold catalysis

A new glycosylation technique was reported by Biao Yu in 2008, using glycosyl ortho-alkynylbenzoates as donors and $Ph_3PAuOTf$ as a catalyst for the synthesis of various glycans and complex glycoconjugates.[61] Later, they showed that this $Ph_3PAuOTf$ could also be used as an effective catalyst for the glycosidation of 1,2-anhydrosugars instead of conventional $ZnCl_2$.[62] Zhu et al. reported activation of *gem*-dimethyl *S*-but-3-ynyl thioglycoside and *S*-but-3-ynyl thioglycoside donors using Au(I) or Au(III)/AgOTf catalysts.[63] Whereas, in 2015, Vankar et al. demonstrated the ambient temperature activation of trichloroacetimidate donor using $AuCl_3$–phenylacetylene.[64] Later, Peng Peng and R. Schmidt[65] introduced a new concept of 'catalyst-acceptor adduct' with the substitution for phenylacetylene usage to improve the yield. They proposed that the reaction proceeds through a hydrogen bond mediated S_N2- type transition state at a very low temperature (-60 °C). Further, in 2016, Sureshan et al. introduced an $AuCl_3$-catalyzed glycosylation strategy for activating thioglycoside donors.[66] Galan et al. exemplified direct and stereoselective glycosylation of glycal enol ethers using (p-$CF_3Ph)_3P$)AuCl and AgOTf catalyst.[67] Recently, in 2022, they have epitomized a highly stereoselective method for direct activation of hemiacetal to synthesize 2-*deoxy* trehalose analogs using a catalytic amount of $AuCl_3$.[68]

Power of alkynyl glycosyl carbonate donor in solution phase:

Among all existing glycosyl donors, Hotha's alkynyl glycosyl carbonate donor has been successfully utilized for the synthesis of various fragments of Mtb cell wall such as tridecasaccharide[60] (13mer), nonadecaarabinofuranoside[69] (19mer), heneicosaarabino-mannan[70] (21mer), Pentacosafuranoside[71] (25mer) in solution phase using standard glycosylation condition. While synthesizing these epitopes of Mtb cell wall, they have achieved sterically hindered α-(1→5)-Ara*f* and α-(1→2)-Ara*f* linkages as well as β-(1→2)-Ara*f* linkage.

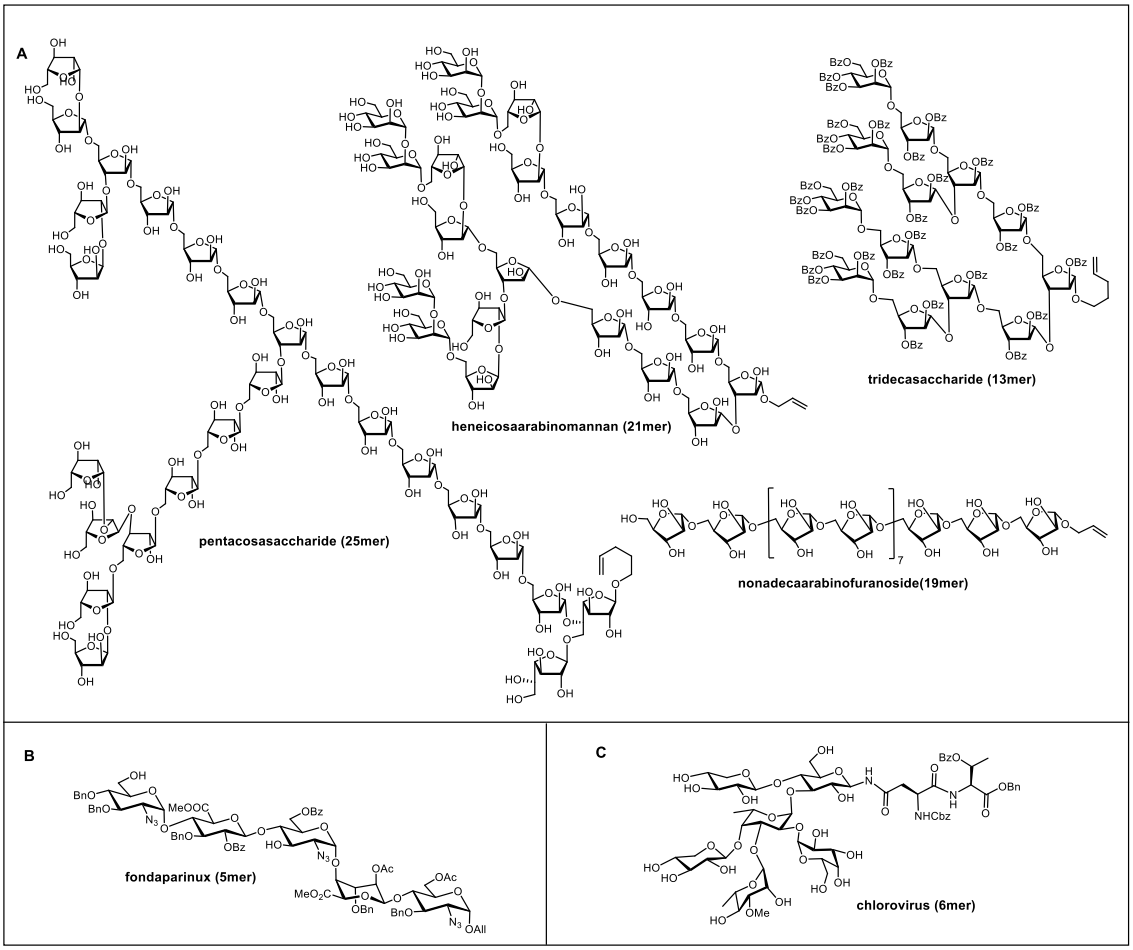


Figure 3.8: Power of alkynyl glycosyl donor in presence of gold and silver catalyst in solution phase a) Mtb cell wall fragments b) Fondaparinux molecule c) hyper-branched chlorovirus motif

Further, this donor has also been used to synthesize the very challenging life-saving molecule fondaparinux,[72] which contains β-1,4-linked D-glucuronic and α-1,4-linked L-iduronic acid with D-glucosamine in its structure. In 2021, the most sterically hindered hyperbranched chlorovirus molecule[73] was synthesized. They successfully installed D-galactose sugar on the central Fuc*p* at the C2 position with 1,2-*cis* selectivity using [Au]/[Ag]-catalyzed alkynyl carbonate donor at ambient temperature. Further, they have developed excellent reaction conditions by carrying out the reaction at the branched di- and trisaccharide acceptor levels. So the synthesis of such a complex linear and branched oligosaccharides exploiting alkynyl glycosyl carbonate donor chemistry in the presence of gold and silver catalyst spurred us to think about its usage for solid phase chemistry. Moreover, this glycosylation method offers an excellent yield for oligosaccharide synthesis in almost all cases. Unique characteristic properties like shelf stability, ease to synthesize, provides all types of glycosidic linkages, room

temperature reaction, a catalytic amount of gold phosphite and silver triflate as an activator for its mechanical action, and no extreme-dry condition, triumph it among all other existing carbonate donors.

…….. necessitate a finding of a new glycosylation method?

Although significant advancement has occurred in developing a powerful glycosylation method for solid-phase glycan synthesis, there is no versatile and satisfactory method available to date which could fulfil all the requirements and allow the process of synthesizing oligosaccharides. Synthesizing oligosaccharides with their stereospecificity is one of the formidable tasks, independent of which technique is used. Furthermore, the array of most useful donors, such as thioethyl glycosides, trichloroactimidate, and phosphate donors, have already proven their eminent strength in solid phase chemistry accompanying some flaws. Moreover, their accessible easiness in the glycosylation method for solid phase chemistry has shown substantial pitfalls in designing and developing glycan synthesizers. Therefore, seeking a new glycosylation condition for SPOS is still knocking the area for its higher yield and ongoing development in automation for greater glycan accessibility.

3.6 Present work

The synthesis of oligosaccharides is required for understanding carbohydrates because they are found responsible for mediating diverse physiological and pathological processes such as cell signalling, tumor progression and metastasis, and so on. Additionally, one of the deadly diseases, *Mycobacterium tuberculosis*, heightens the urgent requirement for finding a curative drug or vaccine as a potential solution. So, the glycoconjugate segment of AG and LAM present on the bacterial cell wall may help to find a new therapeutic agent; therefore, their synthesis is crucial. The core structure fragments contain arabinofuranoside residue majorly, and its synthesis has been tackled by the Seebergers group using thioglycoside or thioaryl glycoside donors utilizing AGA in recent years. Few other potential donors have shown their strength in AGA. Still, the versatility in their usage concerning the generation of stereogenic centers and direct application of the glycosylation method for expeditious synthesis through automation majorly limits the complete fulfilment of the perfect search. The primary flaws associated with these glycosylation methods are i) longer reaction time, ii) use of toxic activating agents, iii) use of a stoichiometric amount of activators, iv) low reaction temperature, and v) complexity in implementation to automation.

So, to diminish the pitfalls exerted by already existing donors and to shallow the process of oligosaccharide synthesis, we first envisioned that a recently developed alkynyl glycosyl carbonate donor in our group might help in that regard. Additionally, this donor has already proven its strength in the solution phase eminently because of its stunning properties like high yielding under the use of the catalytic amount of Au/Ag activator, a very rapid reaction that completes within 15 min., room temperature reactions, and less moisture sensitive. Therefore, we imagine that an alkynyl glycosyl carbonate donor could help to explore solid-support chemistry.

3.6.1 Prerequisites for SPOS

Choice of solid support and linker: Before starting actual solid-phase chemistry for the oligosaccharides, the essentials, including the choice of solid support and linker, were carried out first. The Merrifield (divinylbenzene cross-linked polystyrene) resin (loading value 1.1 mmol/ g) is popular in solid phase carbohydrate chemistry. So, we choose here the same support for our goal too. Linker search accomplishment happened with the most useful, stable, and easily cleavable photo-cleavable linker, whose synthesis and characterization and its attachment

to the Merrifield resin is already discussed in chapter 2. The loading value calculated for this linker attached substrate is 0.94 mmol/g and is also mentioned in chapter 2.

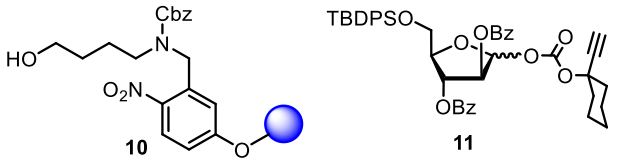


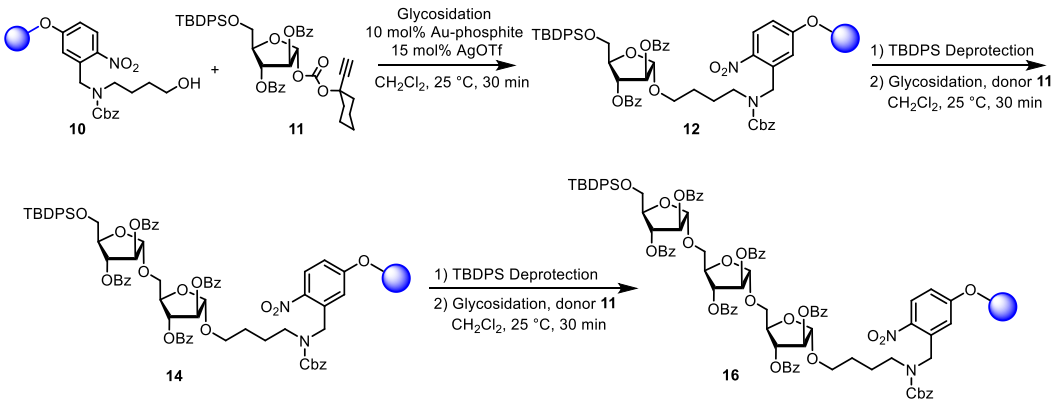
Figure 3.9: Structure of the linker attached solid support

Synthesis of building blocks: Building block **11** was prepared using a well-established protocol by Maidul Islam in our lab.[70]

3.6.2 Optimization of glycosidation and deprotection:

Glycosidation reaction on solid support

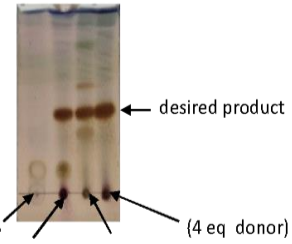
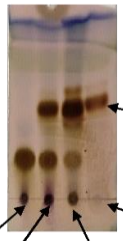
The first glycosidation reaction on the solid support was performed in dry CH_2Cl_2 using 5 equivalents of the donor (5eq. in 2mL) and 10 mol% of solid gold-phosphite, 15 mol% of silver triflate at room temperature for 30 min. While proceeding the reaction, aliquots of resin beads were removed at regular intervals, subjected to the photocleavage reaction, and subsequent TLC analysis was performed. The product obtained from the solid support was confirmed by MALDI and TLC analysis implied that the reaction condition was settled moderately. After that, we performed the reaction with the lesser equivalents of donor (i.e., with 2, 3, 4 eq. of donors in 2ml CH_2Cl_2), where 3eq. donor usage showed complete conversion for the first glycosylation but failed to produce the same result in subsequent di and tri-saccharide synthesis. The multiple-time donor treatment was also performed for second and third glycosylation, but the single-time 4eq. of donor treatment afforded significant results.



Scheme 3.3: Optimisation of gold catalysed glycosylation and TBDPS deprotection reaction for the solid support

Additionally, the effect of concentration of the reaction mixture was also studied by adding variable concentrations of donor **11** (approximately 300 mg) in 0.5, 1.0, 1.5, 2.0, and 2.5 mL of CH_2Cl_2, where we observed a profound effect on the conversion of reaction. In this study, we observed that 2.0 mL solvent was the best. Further, to locate the minimal glycosylation reaction time, we performed the experiments with a time interval of 15 min. Here we noticed that 30 min was the least and sufficient for the completion of the reaction.

Finally, after doing these many experiments, we concluded that (i) reduction of the number of the equivalents of donors was found to be detrimental to the effective reaction. (ii) a concentration of 0.3 g per 2.0 mL of the solvent yielded the best results, and (iii) 30 min for the glycosylation reaction was the best. In this way, we successfully optimized the glycosylation reaction condition for oligosaccharide synthesis for solid support.

<table>
<tr><td>TLC of the first glycosylation</td><td>Optimization of the second glycosylation with number of equivalents of donor</td></tr>
<tr><td> 
Monosaccharide with TBDPS</td><td> 
desired product
Monosaccharide without TBDPS (authentic)
(3 eq donor)
(3+2 eq donor)
(4 eq donor)</td></tr>
<tr><td colspan="2">Optimization of the third glycosylation with number of equivalents of donor</td></tr>
<tr><td colspan="2"> 
desired product
Disaccharide without TBDPS (authentic)
(3 eq donor)
(3+2 eq donor)
(4 eq donor)</td></tr>
</table>

TBDPS and Bz deprotection standardization: For the cleavage of the support attached TBDPS moiety, the variable concentrations of HF•pyridine in anhydrous pyridine were tested. We found that treatment of 0.4 mL of HF•pyridine in 1 mL anhydrous pyridine for 15 h was suitable for the complete removal of TBDPS functionality, and reaction monitoring was carried out by TLC analysis. Similarly, for saponification of benzoates, the resin was treated with commercially available 0.5 M NaOMe in methanol solution (1 mL) for different intervals. Here, in this case, we monitored the reaction progress using TLC analysis and observed that debenzoylation requires approximately 1 h for the complete saponification of benzoates.

3.6.3 Reagent preparation and general protocol for synthesis

Reagent Preparation

After successful optimization of i) gold-catalyzed glycosylation reaction and required ii) silyl ether and benzoyl group deprotection on solid support, the very first task of preparation of all essential reagents was performed. For that, every time, the building block and activator reagents were weighed accurately and enclosed into the Eppendorf tubes just before their use. The donor solution was prepared by adding dry CH_2Cl_2 into an accurately weighed sticky donor, and the activators, gold phosphite and silver triflate, were used as such in their dry solid form. HF•py solution was always prepared freshly using dry pyridine diluent according to the ratio above. The hydrazine acetate solution for the levulinoate deprotection was prepared according to the standard protocol by dissolving solid hydrazine acetate in the mixture of pyridine:AcOH (4:1) solvent. A commercially available solution of 0.5 M NaOMe in MeOH was used directly for the saponification reaction.

General Protocol for Synthesis

After loading the functionalized resin into the reaction vessel, dry CH_2Cl_2 was added and kept for 5 min for swelling under the nitrogen flow. After that, draining of the solvent was performed using nitrogen pressure. The solution of the building block (4 eq. in CH_2Cl_2) was added to the reaction vessel, and then the resulting biphasic mixture was stirred using N_2 flow. After 5 min, both the activators, gold phosphite (10 mol %); silver triflate (15 mol%), were added to the reaction vessel in their solid form under the inert atmosphere. Then the reaction mixture was mixed by nitrogen bubbling for 30 min. The excess liquid reagents were drained and washed successively with washing solvents (CH_2Cl_2, DMF, and CH_2Cl_2) to get a reagent-free substrate attached to the solid support. Then, after drying the resin using a high vacuum, the subsequent swelling followed by deprotection of the temporary protecting group was performed by adding appropriate reagents to get the free –OH over the solid support. The reaction progress at each step was monitored by TLC and UPLC-MS analysis. This way, the desired synthesis was achieved by performing iterative glycosylation and deprotection reaction. Finally, the protected or completely deprotected glycan was cleaved from the solid support using a light of wavelength 365 nm with 4 h time or continued until complete cleavage ensures. The resultant impure components were isolated using silica gel column chromatography or HPLC technique and further characterized using NMR, MALDI/HRMS techniques.

Synthetic targets and their retrosynthetic disconnection approach

First, we chose the linear pentaarabinofuranoside as a synthetic target, which is the fragment of arabinogalactan part of the Mtb cell wall. In its composition, the AG part has variable connectivity within a chain of similar units of arabinofuranoside sugar residue. It contains predominantly observed α-Ara*f*(1→5)Ara*f* linkage and other two α-Ara*f*(1→3)-α-Ara*f* and β-Ara*f*(1→2)-α-Ara*f* linkages. Out of these, the majorly found connection in its composition is α-Ara*f*(1→5)Ara*f*. Therefore, we set it as the first synthetic target.

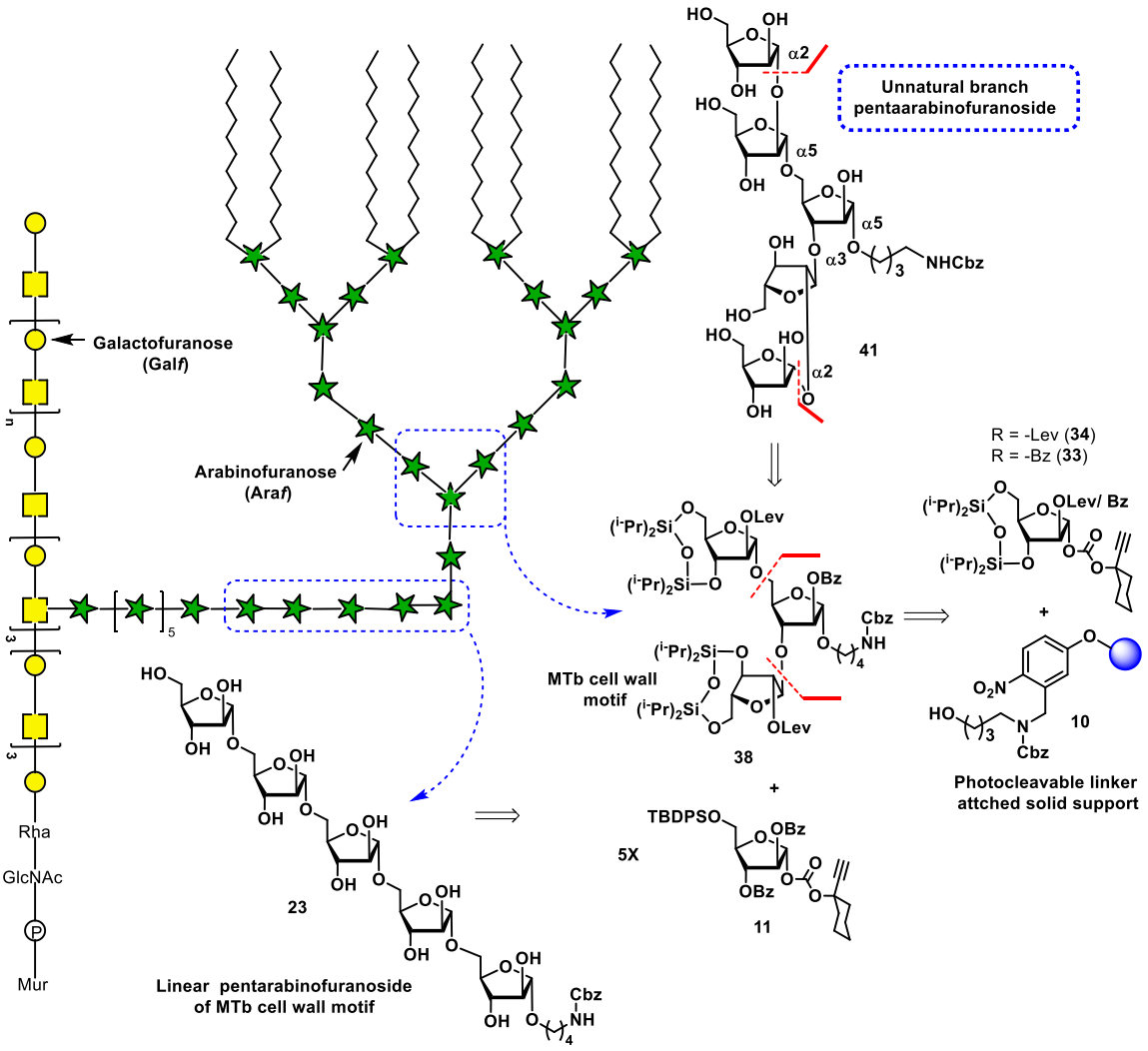


Figure 3.10: Representation of arabinogalactan part of Mtb cell wall as a synthetic target and their retrosynthesis

For synthesizing first target **41**, i.e., linear pentaarabinofuranoside, which contains all trans 1→5 linkages, we performed the disconnection approach systematically, reflected the requirement of only one type of building block **11** and linker attached solid support **10**. The synthetic strategy was the linear connection of the same building block through five glycosylations. The building block design was based on the alkynyl glycosyl carbonate donor as we envisioned that gold-catalyzed chemistry could help to explore the solid support

chemistry. Similarly, for synthesizing the second target **41**, i.e., unnatural branched pentaarbinofuranoside, we performed disconnection in such a way that the building block will have a similar type of protecting group with appropriate orthogonality to get branching at the required site and also will cause minimal deprotection steps. The synthetic planning was based on the simultaneous glycosylation reaction. Performing the first glycosylation and subsequent removal of the disilane group would cause the generation of dihydroxy functionality on the support. Then the resultant substrate could further produce trisaccharide by performing two simultaneous glycosylations. Further subsequent removal of temporary protecting groups and simultaneous glycosylation could produce pentasaccharide. The synthesis of this unnatural branched pentasaccharide **41** contains a small trisaccharide branched fragment, the tiny constituent of the Mtb cell wall. The retrosynthetic scheme implies that the forward synthesis includes two types of rigid building blocks possessing the C2 variant Lev/Bz group. The strategy consists of effortless removal of the Lev group after trisaccharide synthesis and extended to pentasaccharide. The target synthesis was planned to have a photocleavable linker attached to a solid support **10** which offers orthogonal cleavage condition.

General procedures and set-up for manual solid-phase oligosaccharide synthesis

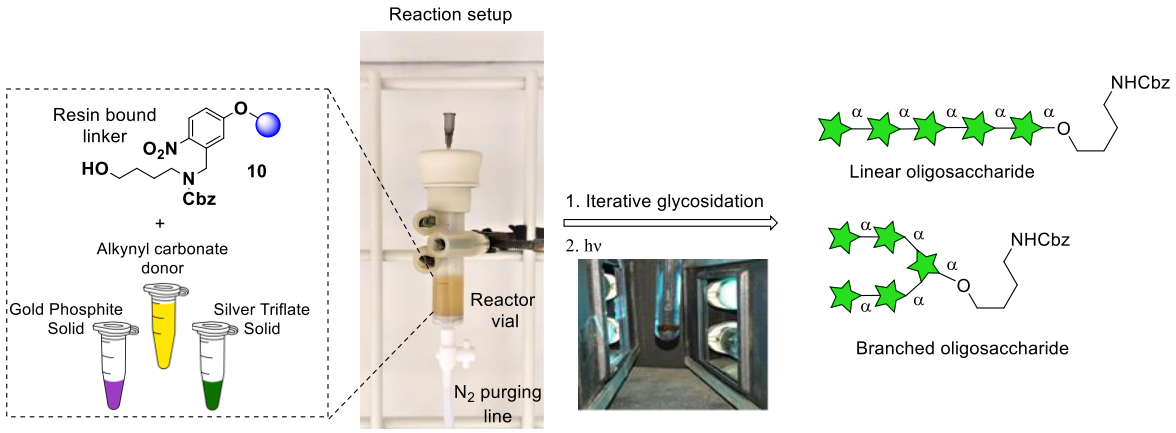
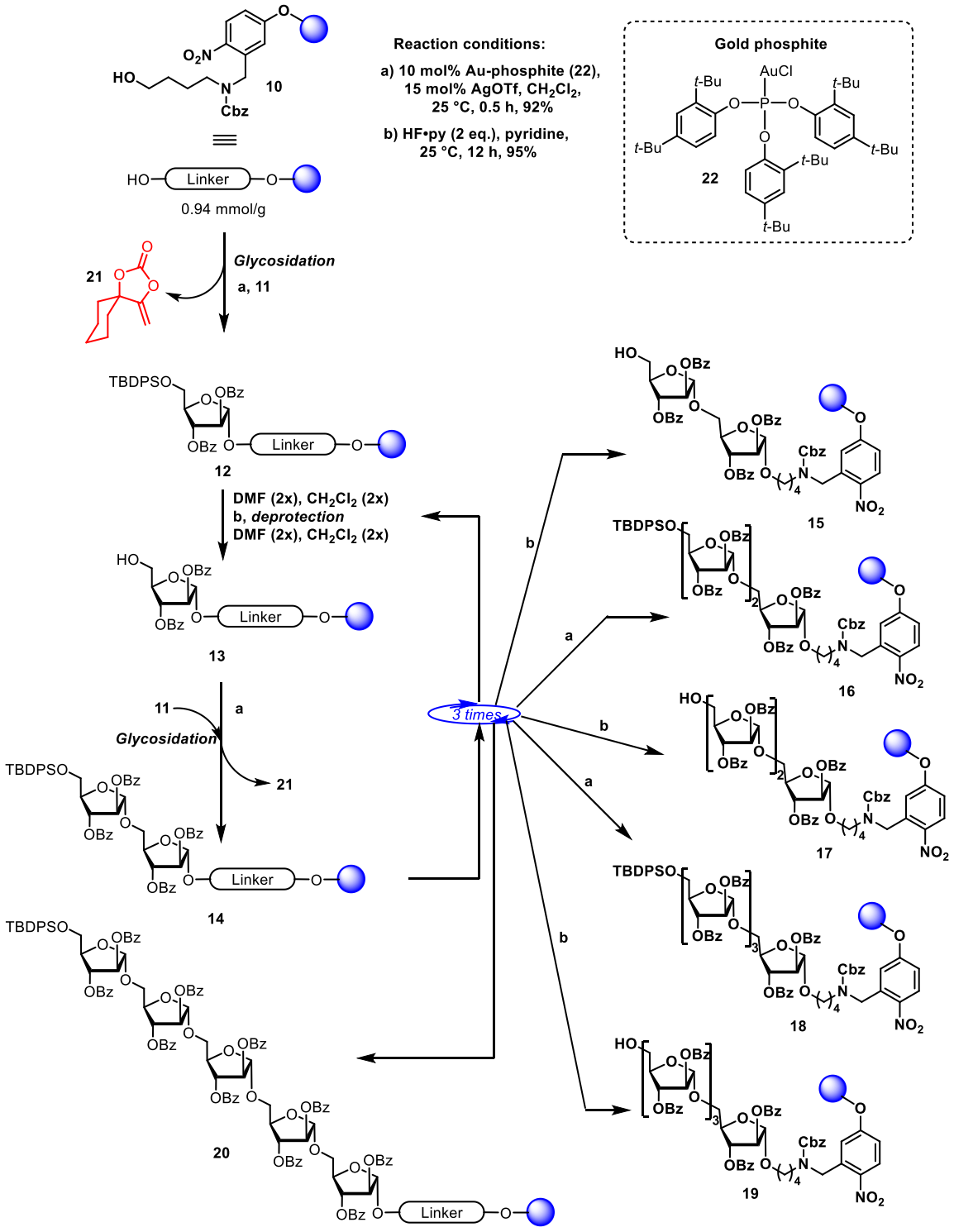


Figure 3.11: Set-up for manual solid-phase oligosaccharide synthesis

Synthesis of linear pentaarabinofuranoside



Scheme 3.4: Synthesis of the linear pentaarbinofuranoside on solid support

To start the actual synthesis of linear pentasaccharide, at first, the reaction vessel (10 mL PTFE vial) was charged with the functionalized resin **10** (150 mg; loading 0.94 mmol/g; 0.141 mmol)

and CH_2Cl_2 (2 mL) was added for swelling. To start the synthesis, the resin was washed with dry CH_2Cl_2 (2x) and then coupling/deprotection cycle were performed as depicted in Table 1. This cycle was repeated 5 times to produce pentasaccharide **20**.

Supplementary Table: All Coupling/ deprotection cycle were carried out at room temperature.

Table 1: general protocol for linear pentasaccharide **20** synthesis.

Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	2	4 eq of donor **11** in 2 mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				
2 disaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	3	1 mL dry Py + 70% HF/Py 0.4 mL	15 h	1
	2	4 eq of donor **11** in 2 mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				
3 trisaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	3	1 mL dry Py + 70% HF/Py 0.4 mL	15 h	1
	2	4 eq of donor **11** in 2 mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				
4 tetrasaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	3	1 mL dry Py + 70% HF/Py 0.4 mL	15 h	1
	2	4 eq of donor **11** in 2mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				
5 pentasaccharide synthesis	1	2 ml dry CH_2Cl_2	5 min	2
	3	1 mL dry Py + 70% HF/Py 0.4 mL	15 h	1
	2	4 eq of donor **11** in 2 mL dry CH_2Cl_2	30 min	1
Resin was transferred to another flask				

At every stage of the glycosylation and deprotection cycle, 3 mg of the resin was removed and subjected to the photocleavage (Protocol **I**) and the crude filtrate was subjected to UPLC to understand efficiency of the reaction.

Sample preparation: Photocleaved product obtained from the 3 mg of the resin was filtered, concentrated, redissolved in 300 µL of acetonitrile and transferred to septum sealed, screw capped 1 mL Wheaton vial.

Experimental:

UPLC conditions

UPLC system: *Acquity* UPLC H-Class with PDA detector

Sample manager: Flow-through needle

Column: ACQUITY UPLC BEH C18 1.7 µm (2.1x 50 mm column)

Mobile Phase A: Water+ Formic acid (0.1% solution)

Mobile Phase B: Acetonitrile + Formic acid (0.017% solution)

Column temp.: 25 °C

Sample temp.: 25 °C

Flow rate: 0.5 mL/min

Run time: 30 min

Injection volume: 5 µL

UV detection: 190 nm – 500 nm (20 points/sec)

Gradient:

Time (min)	%A	%B
0.0	100	0.0
5.0	50.0	50.0
8.0	20.0	80.0
11.0	10.0	90.0
23.0	0.0	100.0
24.0	40.0	60.0
25.0	70.0	30.0
26.0	100	0.0
30.0	100	0.0

Before running the reaction mixture samples, we developed a unique gradient for all the oligoarabinofuranosides with N-Cbz protected amino linker at the reducing end. The observation f UPLC and TLC analysis showed that all the glycosylation and deprotection steps were completed with high efficiency (< 90%). After completing the synthesis of linear pentasaccharide, the total weight of substrate attached resin was found to be 320 mg which was higher than the starting amount. This total amount was divided into two portions (70 mg and 250 mg). Resin (70 mg) was exposed to UV light to identify fully protected linear pentasaccharide **20.** Another portion of 250 mg resin was subjected to deprotection of silyl- and Bz- moieties as described above in table 1. Subsequently, resin-bound fully deprotected glycan with NHCbz linker was exposed to the UV light to obtain desired glycan **41**.

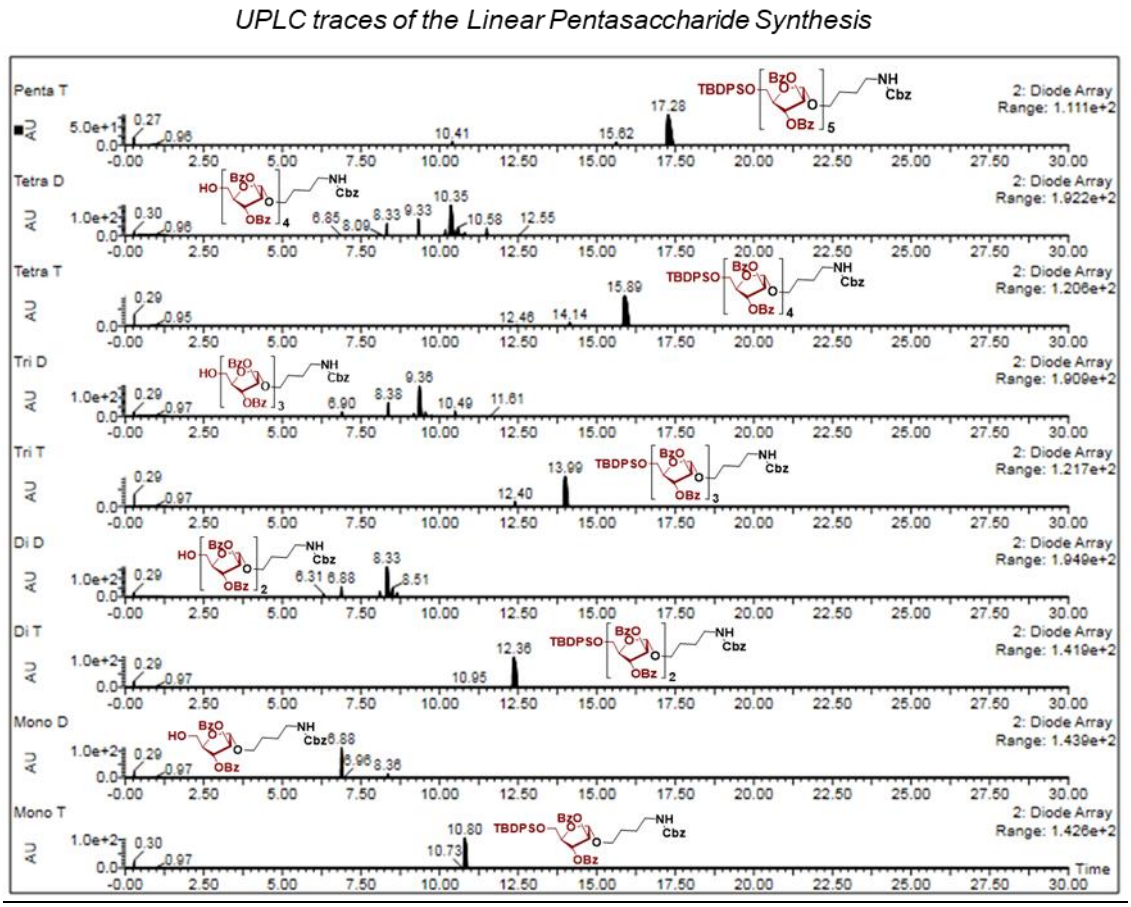
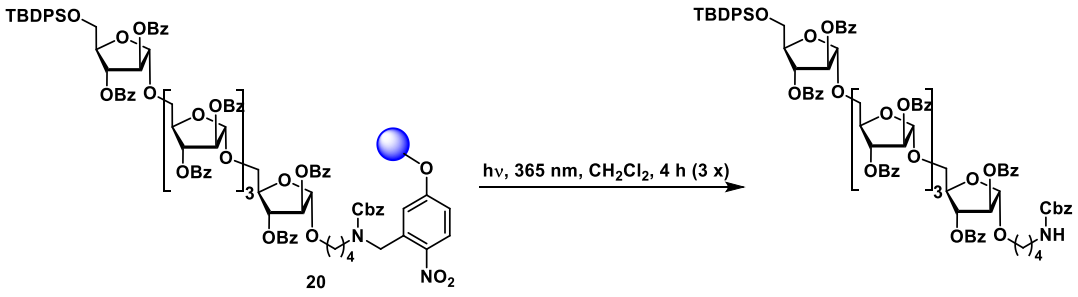


Figure 3.12: UPLC runs for SPOS cleavage products obtained while synthesizing compound 20

*Cleavage, purification and analysis of protected linear pentasaccharide **20**:*

Pentasaccharide **20** was cleaved off from the solid support as delineated in protocol **I**, concentrated, and the residue was purified by normal phase silica gel column chromatography (hexane:ethyl acetate) to give the linker attached fully protected arabinofuranosyl pentasaccharide **20** (13.4 mg, 20% yield, 0.031 mmol) as a white solid.

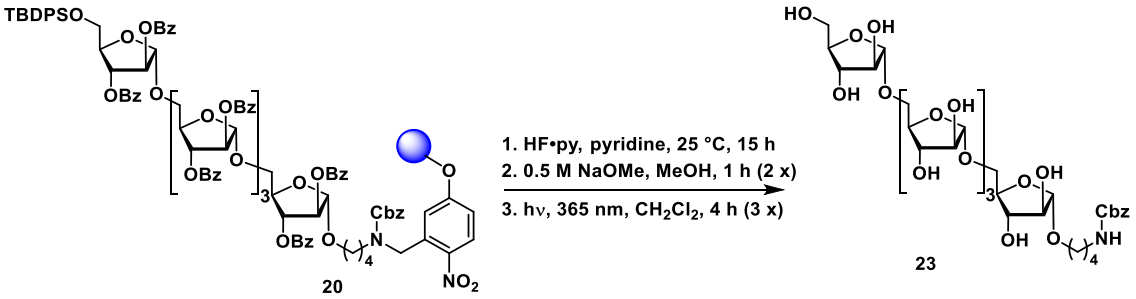


Scheme 3.5: Cleavage of the protected pentaarabinofuranoside from the solid support

In the ^{1}H NMR spectrum, the five anomeric protons were observed at δ 5.56 (5H), and the corresponding ^{13}C value for the anomeric carbon was seen at δ 105.9(2C), 105.9, 105.8, 105.6. Four $–CH_2$ signals of linker appeared at δ 3.50 (2H), 3.22 (2H), 1.60 (4H) in proton NMR and reflected in carbon NMR as well at δ 63.4, 40.8, 26.8, 26.7 ppm The remaining $–CH_2$ signals were found at δ 67.0, 66.6, 66.0, 65.8, 65.8, 65.7 ppm, clearly indicates that molecule pentaarabinofuranoside is flank with the linker. The rest of the expected ^{1}H and ^{13}C NMR signals for the TBDPS, BZ, and Cbz functional groups were traced in their expected region. This confirms the synthesized molecule **20** is desired, and further, it was confirmed by MALDI-TOF analysis, (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{123}H_{115}NO_{33}SiNa]^+$: 2184.7018; found: 2184.7018.

Synthesis of partially deprotected linear pentasaccharide 23:

To get fully deprotected glycan over the solid support, a portion of the functionalized resin **20** was kept aside and treated with HF•py in pyridine, followed by 0.5 M NaOMe solution to remove all temporary protecting groups. Finally, pentasaccharide **23** was cleaved from the solid support using protocol **I,** and further, the desired pentasaccharide **23** was purified by semi-preparative HPLC (protocol **III**) to afford compound **23** (8.9 mg, 9% yield, 0.110 mmol).



Scheme 3.6: On resin removal of Bz and TBDPS group and cleavage of the pentasaccharide from resin

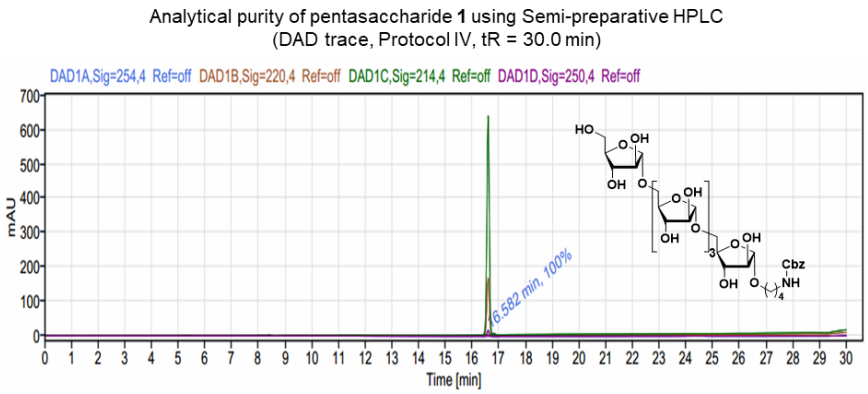
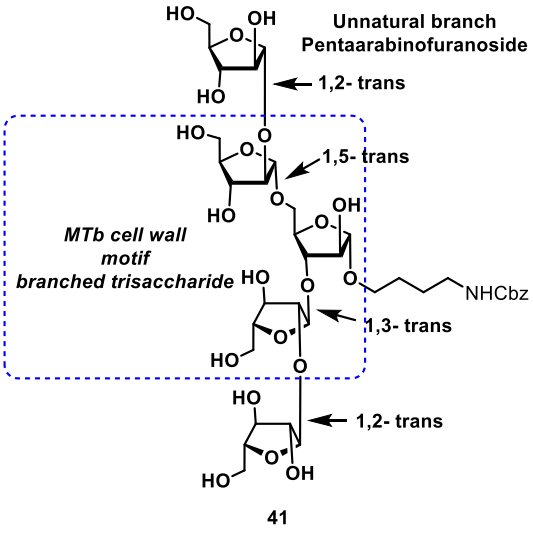


Figure 3.13: Analytical purity by HPLC for pentasaccharide 23

Then we performed NMR analysis of compound **23**, we found the signals for five anomeric protons at δ 4.96 in ^{1}H-NMR spectra, and their corresponding ^{13}C values were found at δ 109.7(3C), 109.6, 109.5 ppm. All methylene carbon showed δ value at 68.5, 68.2, 68.2, 68.1(2C), 67.3, 63.1, 41.5, 27.9, 27.7 which were inverted in DEPT-135 spectra. The Cbz functionality remained intact after the cleavage reaction and was identified by benzyl group protons and their respective carbon values in the downfield region. It was further confirmed by MALDI analysis; (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{37}H_{57}NO_{23}Na]^+$: 906.3225; found: 906.3230.

Synthesis of branched pentasaccharide

After successful synthesis of linear pentaarabinfuranoside over the solid support, we choose another target, i.e., branched pentasaccharide **41**, which contains α-Ara*f*(1→5)Ara*f* linkage and other two α-Ara*f*(1→3)-α-Ara*f* and α-Ara*f*(1→2)-α-Ara*f* linkages. The overall structural composition reflects that the central trisaccharide branched fragment is the motif that belongs to the Mtb cell wall, and that is attached to another arabinonofuranoside sugar residue through 1,2-*trans* linkage that makes it collectively as the unnatural branched pentasaccharide **41**.



Scheme 3.7: Structure of unnatural branch Pentaarabinofuranoside

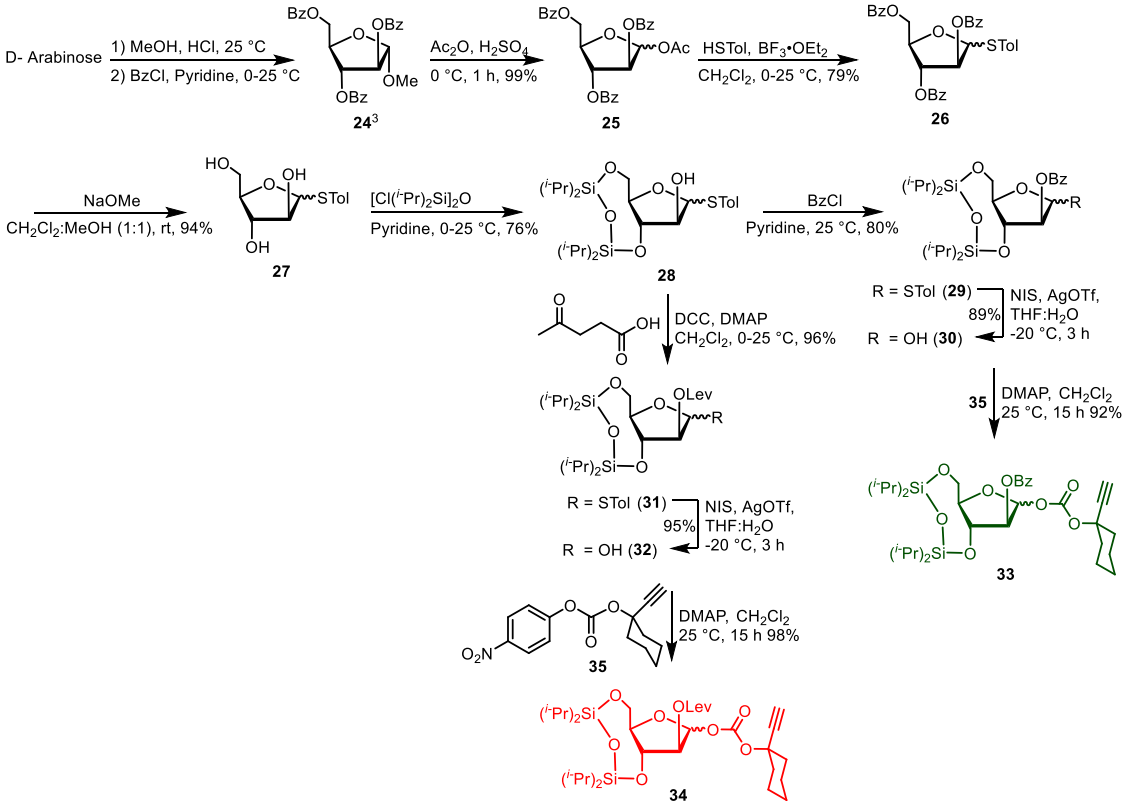
Retrosynthetic analysis of branched pentaarabinofuranoside

Retrosynthetic analysis of branched pentaarbinofuranoside is shown in the figure **3.11** mentioned above. The synthetic plan was based on the 1+2+2 strategy and included easiness for synthesizing building blocks. The protecting group installation over the building blocks was managed in such a way that their simultaneous removal will generate two hydroxyl functionalities at a time that would help in producing simultaneous glycosidations to result in

desired product **41** under gold-catalyzed conditions. The retrosynthetic plan showed that **41** synthesis requires building blocks **33, 34** and the linker attached solid support **10**.

Synthesis of required building blocks

In order to start our synthetic efforts, we synthesized the crucial arabinofuranoside derivative **28**. At first, we performed the synthesis of *O*-methyl glycoside from D-arabinofuranoside using MeOH**.**HCl, which was generated from AcCl and MeOH and then perbenzoylation of remaining –OH group was carried out using BzCl/Py to get desired derivative **24** in quantitative yield. Further we converted it to **25** using Ac_2O/ H_2SO_4 at 0 °C in 99% yield.[74] Subsequently, we treated it with *p*-cresol in presence of $BF_3.OEt_2$ at low temperature to obtain compound **26** in 79% yield. Later on, by performing Zémplen reaction followed by disilane protection using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine, we successfully synthesized required intermediate compound **28**.[75] The formation of this compound was confirmed by ^1H and ^{13}C NMR spectroscopy and that was further supported by HRMS analysis. m/z calcd for $[C_{24}H_{42}O_5SSi_2H]^+$: 499.2370; found: 499.2365.

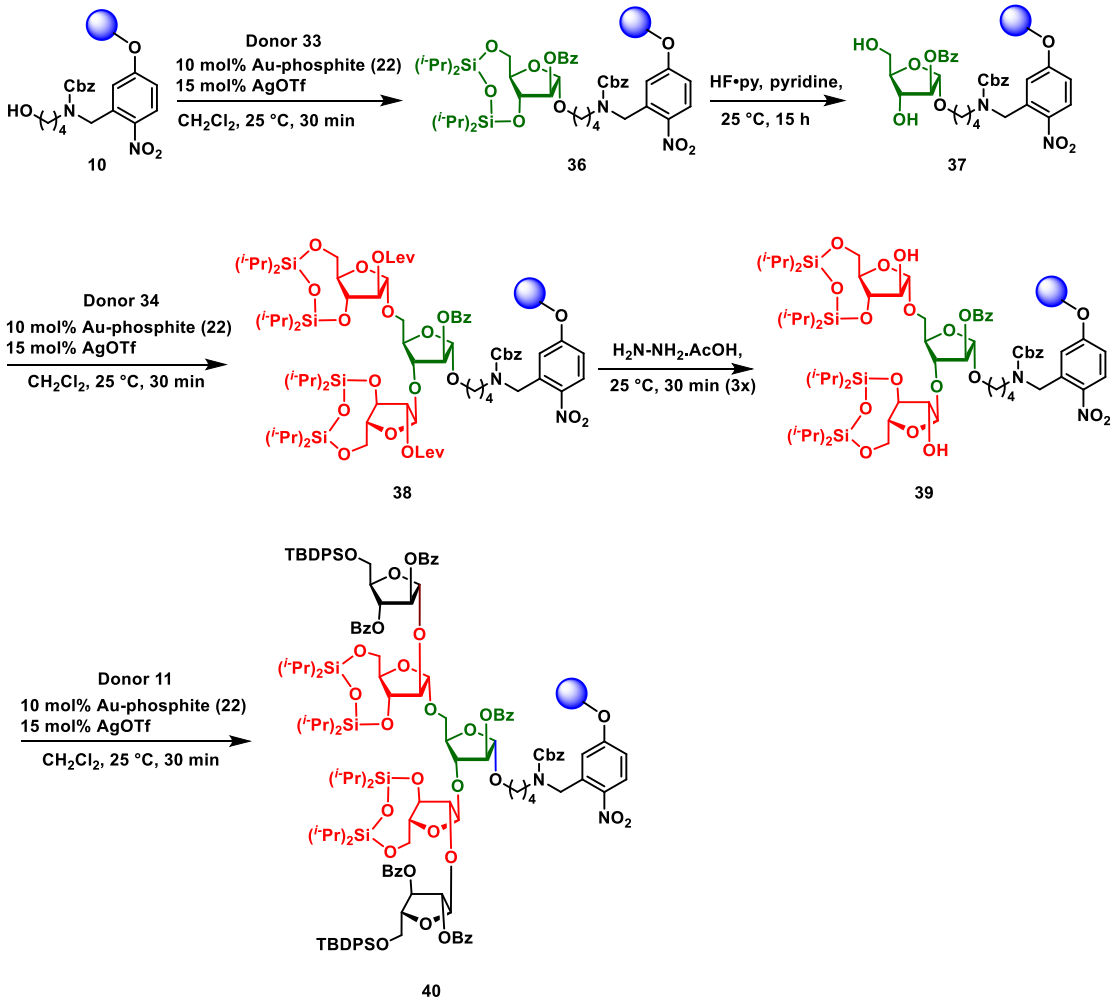


Scheme 3.8: Synthetic strategy for the synthesis of required building blocks for Unnatural branch Pentaarabinofuranoside

To synthesise building block **33** and **34**, we split whole amount of compound **28** into two portions. For one portion, we performed benzoyl protection[76] at C2 position using standard protocol of Bz/Py at room temperature to yield **29** in 80% and subsequently converted to hemiacetal derivative using NIS/AgOTf at -20 °C for 3h to yield compound **30** in 89%.[77] Finally, we treated it with carbonate reagent **35** and DMAP at 25 °C for 15h to afford the desired compound 4 as α:β (1.74:1) mixture with 92% yield.[60] The formation of compound **33** was confirmed by ^{1}H NMR spectroscopy, wherein the peaks in the region of δ value from 1.26 to 2.23 corresponds to cyclohexyl ring and alkyne CH was found at δ 2.63 (s, 1H) and 2.38 (s, 1H) for the two isomers. The eight isopropyl group protons belonging to disilane functionality form the mixture of (α:β) isomer were appeared at δ 1.18 –0.95 (m, 56H). In ^{13}C spectra the peak at δ 151.3, 151.0 ppm indicated the carbonyl of Bz and δ 165.8, 165.5 ppm indicated the presence of carbonyl of carbonate reagent. The two signals of the anomeric carbons were noticed at δ 101.9, 96.0 and remaining carbons of all other functional groups have appeared in their respective regions confirming the formation of compound **33**. Further its formation was supported by HRMS analysis as (ESI-MS): m/z calcd for $[C_{33}H_{50}O_9Si_2K]^+$: 685.2630; found: 685.2639.

The second portion of **28** was treated with the levulinic acid and N,N'-dicyclohexylcarbodiimide to protect C2-hydroxyl moiety as a Levulinoate[71] and then subsequently converted to its hemiacetal compound **32** using NIS/AgOTf at -20 °C for 3h to yield 89%. Finally, the resultant hemiacetal derivative was converted to its carbonate form compound **34** using the similar conditions as stated above. The formation of this donor **5** was also confirmed by ^{1}H and ^{13}C spectroscopy. The signals for the Lev carbonyls appeared at δ 206.2, 206.1, 172.1, 171.8 ppm and that for anomeric sugar ring carbon appeared at δ 101.7, 95.8. The remaining ^{13}C NMR signals for the disilane and the carbonate functionality were found in their respective regions similar to that of compound **33**. Additionally, it was confirmed by HRMS analysis as (ESI-MS): m/z calcd for $[C_{31}H_{52}O_{10}Si_2K]^+$: 679.2736; found: 679.2738.

After synthesizing all the required donors, we started the synthesis of branched pentasaccharide by using the already prepared linker attached solid-support. For that the functionalized resin **10** (100 mg; loading 0.94 mmol/g; 0.094 mmol) was loaded into the reaction vessel (10 mL PTFE vial) and allowed to swell by adding 2 mL of CH_2Cl_2. Coupling/deprotection cycles were performed as depicted in Table 2.



Scheme 3.9: Synthesis of branched pentasaccharide 40 on solid support

Importantly, double glycosidation using donor **34** was also completed in a stereoselective manner within 0.5 h to give compound **38**. This cycle was repeated to furnish branched pentasaccharide **40**.

Supplementary Table: All Coupling/deprotection cycle were carried out at room temperature.

Table 2: General protocol for branched pentasaccharide **40** synthesis.

Glycosidation sequence	Protocol	Details	Time	Cycle
1 monosaccharide synthesis	1	2 mL dry CH_2Cl_2	5 min	2
	2	4 eq of donor **33** in 2 mL dry CH_2Cl_2	30 min	1
Transfer resin to another flask using CH_2Cl_2				
2 trisaccharide synthesis	3	2 mL dry Py + 70% HF/Py 0.8 mL	15 h	1
	1	2 mL dry CH_2Cl_2	5 min	2
	2	8 eq of donor **34** in 2 mL dry CH_2Cl_2	30 min	1
Transfer resin to another flask using CH_2Cl_2				

3 pentasaccharide synthesis	5	1 mL Hydrazine Acetate 0.15 M solution	1 h	3
	1	2 mL dry CH_2Cl_2	5 min	2
	2	8 eq of donor **11** in 2 mL dry CH_2Cl_2	30 min	1

At every stage of the glycosylation and deprotection cycle, 3 mg of the resin was removed and subjected to the photocleavage (Protocol **I**) and the crude filtrate was subjected to UPLC to understand efficiency of the reaction.

Sample preparation: Photocleaved product obtained from the 3 mg of the resin was filtered, concentrated, redissolved in 300 µL of acetonitrile and transferred to septum sealed, screw capped 1 mL Wheaton vial.

Experimental:

UPLC conditions

UPLC system: *Acquity* UPLC **H**-Class with PDA detector

Sample manager: Flow-through needle

Column: ACQUITY UPLC BEH C18 1.7µm (2.1x 50 mm column)

Mobile Phase A: Water+ Formic acid (0.1% solution)

Mobile Phase B: Acetonitrile + Formic acid (0.017% solution)

Column temp.: 25 °C

Sample temp.: 25 °C

Flow rate: 0.5 mL/min

Run time: 30 min

Injection volume: 5 µL

UV detection: 190 nm – 500 nm (20 points/sec)

Gradient:

Time (min)	%A	%B
0.0	100	0.0
5.0	50.0	50.0
8.0	20.0	80.0
11.0	10.0	90.0
23.0	0.0	100.0
24.0	40.0	60.0
25.0	70.0	30.0
26.0	100	0.0
30.0	100	0.0

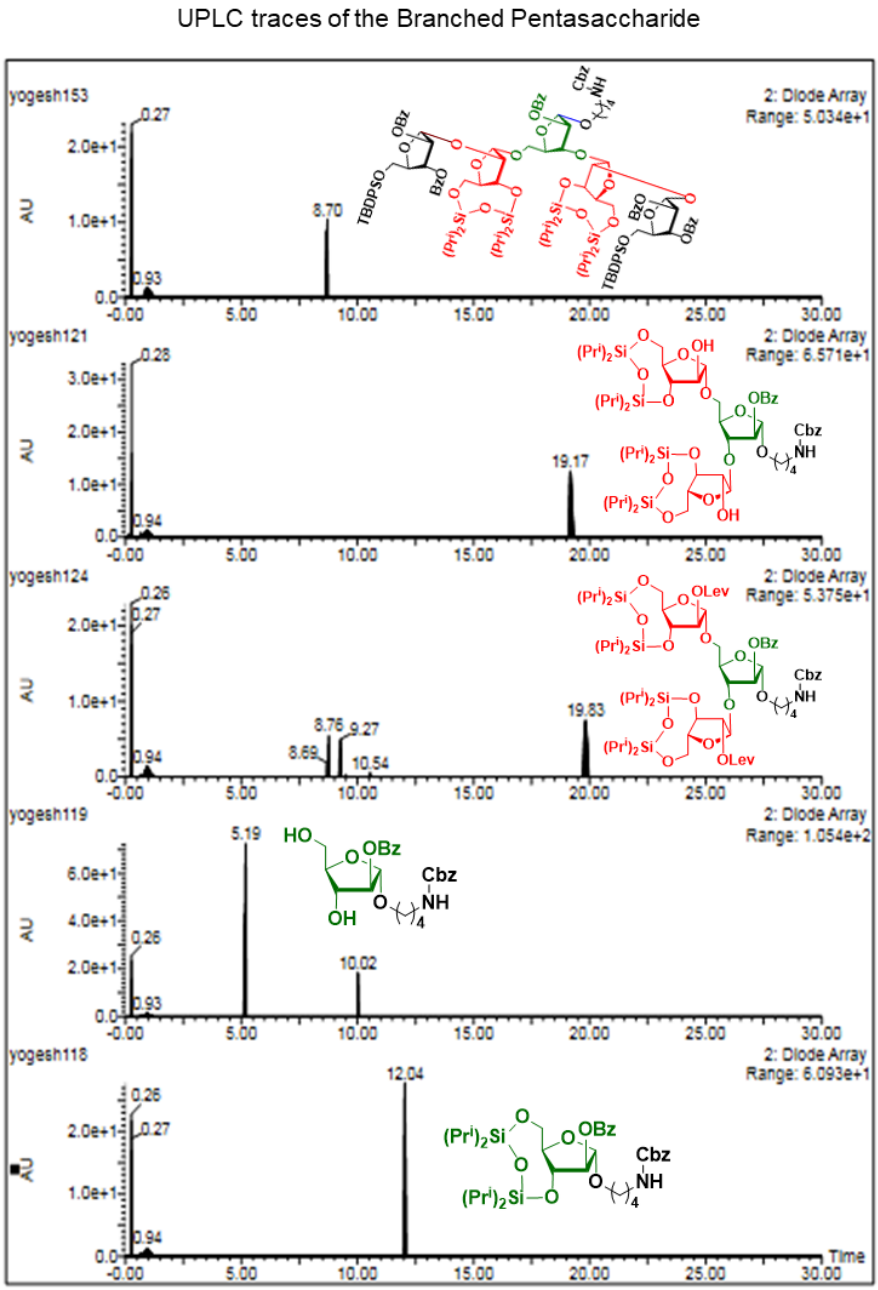


Figure 3.14: UPLC runs for SPOS cleavage products obtained while synthesizing compound 40

After completion of the synthesis of the branched trisaccharide, the total weight of substrate attached resin was found to be 150 mg from which 60 mg of the resin was exposed to UV light to identify fully protected trisaccharide **38** in protected form.

*Cleavage, purification and analysis of protected branched oligosaccharide **38**:*

Compound **38** was then cleaved from solid support as described in protocol **I**. The crude product was purified by normal phase chromatography (Silica, hexane: ethyl acetate) to give linker

attached fully protected trisaccharide arabinofuranoside **38** (13.8 mg, 26% from 0.038 mmol) as a colourless liquid which was confirmed by NMR. The formation of compound **38** was confirmed by ^{1}H and ^{13}C spectroscopic technique. In ^{1}H NMR spectra the signal for anomeric protons were observed at δ 5.14 (d, *J* = 1.4 Hz, 1H), 5.11 (m, 1H), 4.94 (d, *J* = 1.1 Hz, 1H) and that for the Lev at δ 2.73 – 2.68 (m, 4H), 2.61 – 2.54 (m, 4H), 2.14 (s, 3H), 2.11 (s, 3H). The TIPDS group was identified by signal at δ 1.10 – 0.98 (m, 56H). In ^{13}C NMR spectrum, the anomeric carbons appeared at δ 106.0, 105.3, 104.4 ppm and carbonyls from lev functionality were traced at δ 206.4, 206.3, 172.0, 171.7 ppm. The Cbz and Bz groups were confirmed by signals at δ 156.7 and 165.8 ppm, whereas δ 17.6- 12.6 ppm showed total of 24 carbon signal count indicating the presence of TIPDS group. Further, the formation of compound **38** was confirmed by MALDI-TOF analysis, $[M+Na]^+$ m/z calcd for $[C_{68}H_{109}NO_{22}Si_4Na]^+$: 1426.6416; found: 1426.6420.

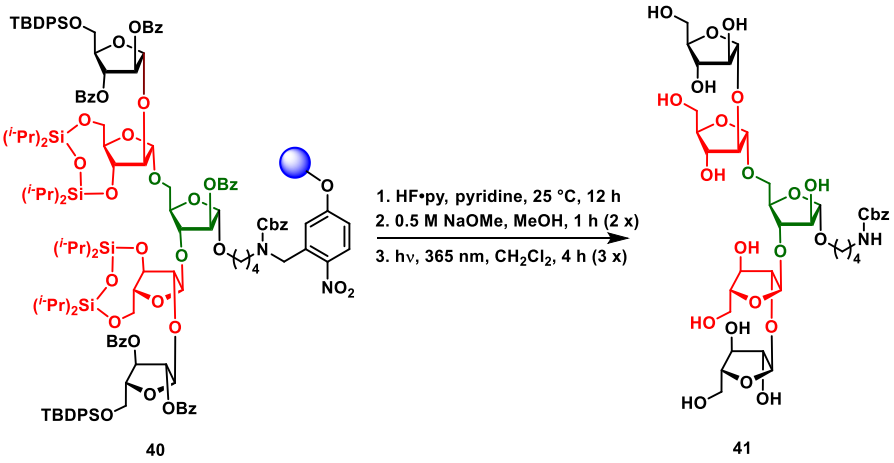
Remaining resin-bound fully protected trisaccharide was subjected to the glycosidation to afford branched pentasaccharide **40**; resulting resin of 130 mg was divided into two portions (50 mg and 80 mg), 50 mg resin was exposed to UV light to identify fully protected branched pentasaccharide **40** and another portion of 80 mg resin was treated with reagents to carry out on resin deprotection of all silyl- and Bz- groups. Subsequently, we irradiated the resin with UV light to obtain N-Cbz protected aminobutyl pentaarabinofuranoside **41**.

Cleavage, purification and analysis of protected branched oligosaccharide **40**:

Compound **40** was then cleaved off from the solid support as described in protocol **I**. The crude product was purified by normal phase chromatography (Silica, hexane: ethyl acetate) to give linker attached fully protected trisaccharide arabinofuranoside **40** (14.0 mg, 27% from 0.022 mmol) as a colourless liquid which was confirmed by NMR spectral studies. In the ^{1}H NMR spectra of compound **40**, the signals for the anomeric protons were observed at δ 5.39 (d, *J* = 3.7 Hz, 2H), 5.36 (d, *J* = 2.2 Hz, 1H), 5.06 (d, *J* = 2.0 Hz, 1H), 4.91 (s, 1H). The TBDPS and TIPDS groups $-CH_3$ and –CH protons were found in the upfield region at δ 1.07 – 0.97 (m, 60H), 0.95 – 0.92 (m, 14H). The presence of Bz functionality was identified by carbonyl ester signals at δ 165.7, 165.6, 165.2, 165.2(2C) ppm and that of Cbz by δ 156.3 ppm. All other very distinguishable carbon signals for the $-CH_3$, -CH, $-CH_2$ belongs to TBDPS, TIPDS and linker motif were observed in their appropriate regions, stipulated the formation of desired compound 18. This was then comfirmed by MALDI-TOF analysis as well; $[M+K]^+$ m/z calcd for $[C_{128}H_{165}NO_{30}Si_6Na]^+$: 2386.9930; found: 2386.9927.

Synthesis of partially deprotected branched pentasaccharide 41

To get fully deprotected glycan over the solid support, portion of functionalized resin **40** was treated with HF•Py, pyridine followed by 0.5 M NaOMe solution to remove all temporary protecting group. After that pentasaccharide **41** was released from the solid support by employing above delineated protocol **I**. The desired product **41** was purified by using semi-preparative HPLC (protocol **III**) to afford compound **2** (3.4 mg, 11% from 0.035 mmol).



Scheme 3.10: On resin removal of Bz, TIPDS, TBDPS group and the cleavage of pentasaccharide from resin

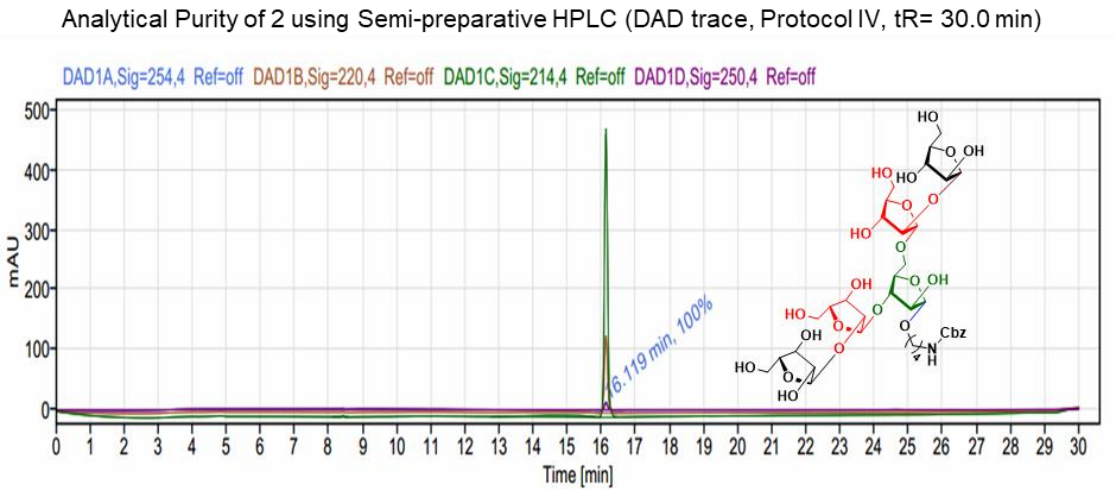


Figure 3.15: UPLC runs for SPOS cleavage products obtained while synthesizing compound 41

*Cleavage, purification and analysis of partially deprotected branched oligosaccharide **41**:*

Then we performed NMR analysis of compound **41**, we found a signal for five protons at δ 5.23 (d, *J* = 1.6 Hz, 1H), 5.11 (d, *J* = 1.2 Hz, 1H), 5.08 (d, *J* = 1.8 Hz, 1H), 5.07 (s, 1H), 5.05 (d, *J* = 1.9 Hz, 1H) in ^{1}H-NMR spectra, and their corresponding ^{13}C NMR values were found at δ 107.7, 108.0, 109.3, 109.6, 109.9 ppm. All methylene carbon belonging to sugar and linker

showed δ values at 68.3, 67.9, 67.3, 63.1, 62.9, 62.8, 62.5, 41.6, 27.9, 27.7 ppm which were inverted in the DEPT-135 spectra indicating that the all the residues remained intact after reagent treatment. The Cbz functionality remained with with amino group after the cleavage reaction and was identified by benzyl group protons and their respective carbon values in their expected downfield region. Further it was confirmed by MALDI analysis; (MALDI-TOF) m/z calcd for $[C_{37}H_{57}NO_{23}Na]^+$: 906.3219; found: 906.3230.

3.7 Conclusions

In conclusion, an efficient solid-phase glycosylation method was developed for synthesizing linear and branched pentaarabinofuranosides and was accomplished using i) Merrifield resin, ii) photo-cleavable linker, and iii) orthogonally protected building blocks. On bead deprotection of silyl and benzoate protecting group was performed to get fully deprotected arabinofuranosides over the solid support. All the glycosylations were performed using well optimized silver-assisted gold-catalyzed glycosidation method, which is the key feature of this endeavour. All the glycosidations were performed using four equivalents of donor and a catalytic amount of gold phosphite 10 mol% and silver triflate 15 mol% in dry CH_2Cl_2 solvent at room temperature. It has been noticed that, one time coupling with four equivalents for single glycosidation and eight equivalents of the donor for simultaneous two glycosylation was sufficient to afford excellent coupling efficiency. Thus, after the cleavage of the synthesized glycans from the solid support are equipped with Cbz protected amino functionality that can be exploited for ligating biomolecules such as proteins after its removal.

This method was simple and efficient for 1,2 trans glycosylations using arabinofuranoside donors. Therefore, this method can be easily exploited for synthesizing various pyranoside glycan and challenging glycosidic linkages.

3.8 Experimental section

Acetyl 2,3,5-tri-*O*-benzoyl-α/β-D-arabinofuranose [α:β(4.4:1)](25): To a solution of compound **24** (30.0 g, 62.96 mmol) in Ac_2O (190 mL), conc. H_2SO_4 (1.7 mL, 31.48 mmol) was added dropwise at 0 °C, stirred for 1 h. After completion of the reaction as adjudged by TLC analysis, solid $NaHCO_3$ was dumped and a few pieces of ice were added carefully while vigorously stirring. The compound **25** was extracted into ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give crude residue which was subjected to silica gel column chromatography to yield compound **25** (31.44 g, 99.0%) as a sticky colourless liquid. R_f = 0.46 (ethyl acetate:hexane 20:80); IR (cm^{-1}): 3440, 3066, 2952, 1724, 1451, 1366, 1264, 1177, 1105, 1022, 959, 710; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 8.11 – 8.00 (m, 12H), 7.60 (p, *J* = 7.6 Hz, 4H), 7.54 – 7.36 (m, 10H), 7.30 (t, *J* = 7.8 Hz, 4H), 6.66 (d, *J* = 4.7 Hz, 1H), 6.49 (s, 1H), 6.02 – 5.97 (m, 1H), 5.81 (dd, *J* = 6.8, 4.8 Hz, 1H), 5.66 (s, 1H), 5.64 (d, *J* = 3.9 Hz, 1H), 4.82 – 4.70 (m, 3H), 4.70 – 4.64 (m, 2H), 4.58 (dd, *J* = 10.1, 5.7 Hz, 1H), 2.19 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 169.2(2C), 166.2, 166.1, 165.9, 165.6, 165.4, 165.2, 133.8(2C), 133.8, 133.7, 133.2, 133.2, 130.0(3C), 129.9, 129.9(4C), 129.8, 129.8(3C), 129.7, 129.6, 129.0, 128.8, 128.7, 128.7, 128.6(3C), 128.6(5C), 128.4, 128.4(3C), 99.5, 93.7, 83.2, 81.3, 80.0, 77.5, 76.1, 75.5, 64.8, 63.6, 21.1, 20.9; HRMS (ESI-MS): m/z calcd. for $[C_{28}H_{24}O_9Na]^+$: 527.1318; found: 527.1317.

***p*-Tolyl 2,3,5-tri-*O*-benzoyl 1-thio-α/β-D-arabinofuranoside [α:β (5.75:1.00)] (26):** $BF_3.OEt_2$ (17.39 mL, 140.93 mmol) was added slowly to a solution of compound **25** (35.55 g, 70.47 mmol) in anhydrous CH_2Cl_2 (350 mL) at 0 °C. The reaction mixture was warmed to 25 °C and stirred. After 1 h, the reaction mixture was cooled to 0 °C and $BF_3.OEt_2$ was neutralized by adding Et_3N (18 mL), diluted with water, extracted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to obtain a crude residue which was subjected to silica gel column chromatography (hexane:ethyl acetate) to yield pure desired product **26** (59.12 g, 79%) as a colourless liquid. R_f = 0.47 (ethyl acetate:hexane 20:80); IR (cm^{-1}): 3440, 3069, 2977, 1723, 1601, 1451, 1104, 1069, 1026, 995, 879, 766, 684; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 8.16-8.12 (m, 4H), 8.1-8.0 (m, 8H), 7.65 – 7.56 (m, 4H), 7.55 – 7.38 (m, 14H), 7.35 – 7.29 (m, 4H), 7.15-7.10 (m, 4H), 5.95 (dd, *J* = 4.6, 3.5 Hz, 1H), 5.83 (d, *J* = 4.9 Hz, 1H), 5.80 (d, *J* = 3.6 Hz, 1H), 5.78 (s, 1H), 5.73 (d, *J* = 1.2 Hz, 1H), 5.67 (dd, *J* = 4.8, 0.7 Hz, 1H), 4.88 (dd, *J* = 8.8, 4.7 Hz, 1H), 4.84 (d, *J* = 3.7 Hz, 2H), 4.83 – 4.80 (m, 1H), 4.75 (dd, *J* = 11.9, 5.1 Hz, 1H), 4.51 (dd, *J* = 9.7, 5.6 Hz, 1H), 2.33 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 166.3, 166.2, 165.6, 165.4, 165.3, 165.3, 138.2, 138.1, 133.7, 133.6,

133.1, 133.0(2C), 132.7, 130.2, 130.1(4C), 129.9(4C), 129.9(4C), 129.8, 129.8(4C), 129.7(2C), 129.5(2C), 129.0(2C), 128.9(2C), 128.7, 128.6(4C), 128.6(4C), 128.5, 128.3(3C), 128.3, 91.7, 90.2, 82.5, 81.2, 81.1, 78.1 ,77.1, 78.0, 64.4, 63.6, 21.2, 21.2; HRMS (ESI-MS): m/z calcd. for $[C_{33}H_{28}O_7SNa]^+$: 591.1453; found: 591.1450.

***p*-Tolyl 1-thio-α/β-D-arabinofuranoside [α:β (12.61:1)] (27):** Solid sodium methoxide (2.53 g, 46.83 mmol) was added to a solution of the tri-*O*-benzoate **26** (26.63 g, 46.83 mmol) in 300 mL of 1:1 MeOH:CH_2Cl_2 and stirred for 12 h. After ensuring the completion of reaction, it was neutralized with IR-120 (H^+) resin, filtered and the filtrate was evaporated to obtain a crude residue that was purified by silica gel column chromatography using hexane, ethyl acetate as mobile phase to afford compound **27** (11.25 g, 94%) as a colourless liquid. The major isomer that was isolated and characterized as α-isomer.[4] R_f = 0.33 (ethyl acetate:hexane 80:20); IR (cm^{-1}): 3338, 2923, 1639, 1027, 860, 804, 697; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 7.35 (d, *J* = 8.1 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 2H), 5.30 (d, *J* = 4.0 Hz, 1H), 5.09 (brs, 1H), 4.78 (brs, 1H), 4.15 – 3.96 (m, 3H), 3.77 (dd, *J* = 12.3, 2.7 Hz, 1H), 3.69 (dd, *J* = 12.4, 2.7 Hz, 1H), 3.37 (brs, *1*H), 2.26 (s, 3H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 137.9, 132.6(2C), 130.0(2C), 129.9, 92.0, 82.8, 81.9, 76.5, 60.9, 21.2. HRMS (ESI-MS): m/z calcd for $[C_{12}H_{16}O_4SNa]^+$: 279.0667; found: 279.0669.

***p*-Tolyl 3,5-*O*-(tetra-isopropylsiloxane-1,3-diyl)-1-thio-α/β-D-arabinofuranoside [α:β (16.0:1)] (28):** To a solution of triol **27** (16.73 g, 65.27 mmol) in pyridine (180 mL) at 0 °C, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (22.65 g, 71 80 mmol) was added dropwise over 30 min. The reaction mixture was warmed to 25 °C and stirred for 2 h. After completion of the reaction as adjudged by the TLC, disiloxane was quenched by addition of excess amount of methanol and water, volatiles were evaporated *in vacuo*, extracted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography using hexane, ethyl acetate as mobile phase to give the 3,5-*O*-tetraisopropyldisiloxane **28** (24.14 g, 76%) as a colourless syrup. R_f = 0.61 (ethyl acetate:hexane 10:90); IR (cm^{-1}): 3445, 2939, 2867, 2356, 1646, 1150, 1093, 1032, 864, 695; Data of major isomer α: ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.1 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.25 (d, *J* = 5.5 Hz, 1H), 4.26 – 4.16 (m, 2H), 3.98 (d, *J* = 3.4 Hz, 2H), 3.94 (dt, *J* = 6.6, 2.3 Hz, 1H), 2.40 (d, *J* = 4.2 Hz, 1H), 2.32 (s, 3H), 1.11 – 1.02 (m, 28H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 137.6, 132.0(2C), 130.9, 129.8(2C), 91.2, 81.9, 80.6, 76.4, 61.4, 21.2, 17.6, 17.4(2C), 17.4, 17.2, 17.2(2C), 17.1, 13.6,

13.3, 12.9, 12.7. HRMS (ESI-MS): m/z calcd for $[C_{24}H_{42}O_5SSi_2H]^+$: 499.2370; found: 499.2365.

***p*-Tolyl 2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-α/β-D-arabinofuranoside [α:β (4.9:1.0)] (29):** Benzoyl chloride (3 .43 g, 24.38 mmol) was added to a vigorously stirred solution of compound **28** (7.47 g, 16.26 mmol) in pyridine (50 mL) at 0 °C. The reaction mixture was stirred for 3 h at 25 °C, diluted with water, extracted with CH_2Cl_2, washed with 1 M aqueous HCl followed by saturated aqueous $NaHCO_3$ solution and treated with brine solution. Combined organic phases were pooled and dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness under diminished pressure to obtain a residue that was purified by silica gel column chromatography using hexane and ethyl acetate to furnish the titled compound **29** (7.35g, 80 %, α/β:4.9:1.0) as a sticky liquid. R_f = 0.48 (ethyl acetate:hexane 5:95); IR (cm^{-1}): 3611, 2942, 2868, 1730, 1461, 1389, 1102, 1035, 806, 701; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 8.13 (d, *J* = 0.7 Hz, 1H), 8.11 (d, *J* = 1.5 Hz, 1H), 8.05 (d, *J* = 0.8 Hz, 1H), 8.03 (d, *J* = 1.5 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.52 – 7.48 (m, 2H), 7.48 – 7.41 (m, 4H), 7.36 – 7.33 (m, 2H), 7.12 - 7.06 (m, 4H), 5.77 (d, *J* = 6.0 Hz, 1H), 5.59 (dd, *J* = 5.2, 3.8 Hz, 1H), 5.55 (d, *J* = 6.6 Hz, 1H), 5.46 (d, *J* = 3.7 Hz, 1H), 4.71 (t, *J* = 6.6 Hz, 1H), 4.56 (dd, *J* = 7.9, 5.3 Hz, 1H), 4.22 (dt, *J* = 7.7, 3.7 Hz, 1H), 4.15 – 4.10 (m, 2H), 4.10 – 4.00 (m, 2H), 3.95 (ddd, *J* = 8.0, 6.5, 4.2 Hz, 1H), 2.31 (s, 6H), 1.18 – 0.91 (m, 56H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 165.9, 165.6, 137.9, 137.6, 133.5, 133.5, 133.1(2C), 132.3(2C), 130.8(2C), 130.0(2C), 129.9(2C), 129.8, 129.8, 129.7(2C), 129.5, 129.5, 128.6(2C), 128.6(2C), 89.8, 88.3, 83.3, 82.5, 81.0, 80.3, 77.0, 75.6, 65.1, 61.5, 21.2(2C), 17.7, 17.6, 17.6, 17.6, 17.5, 17.5(2C), 17.2, 17.1(2C), 17.0(2C), 17.0(2C), 17.0(2C), 13.6, 13.5, 13.5, 13.3, 13.0, 13.0, 12.6, 12.6. HRMS (ESI-MS): m/z calcd for $[C_{31}H_{46}O_6SSi_2NaK]^+$: 664.2088; found: 664.2096 .

***p*-Tolyl 2-*O*-Levulinoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-1-thio-α/β-D-arabinofuranoside [α:β (14.1:1)] (31):** Compound **28** (6.76 g, 13.55 mmol) was dissolved in anhydrous CH_2Cl_2 (60 mL), DMAP (3.31 mg, 2.71 mmol), Levulinic acid (2.07 mL, 20.33 mmol) and *N*,*N'*-Dicyclohexylcarbodiimide (4.16 mL, 16.26 mmol) were added at 0 °C under N_2 atmosphere. The reaction mixture was stirred at 25 °C for 2 h. After completion of reaction, the compound was extracted into CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ solution, brine and dried over anhydrous Na_2SO_4. Organic solvent was evaporated on rotary evaporator and then desired compound was isolated by silica gel column chromatography using hexane:ethyl acetate system to afford corresponding levulinoate ester **31** (7.80 g, 96%, α/β:14.1:1) as colourless liquid. R_f = 0.43 (ethyl acetate:hexane 20:80). Data of the major α-

isomer: IR (cm^{-1}): 3615, 2941, 2869, 2353, 1736, 1465, 1369, 1145, 1034, 783, 695; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.1 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 5.32 – 5.27 (m, 2H), 4.36 (dd, *J* = 7.9, 5.0 Hz, 1H), 4.14 – 4.09 (m, 1H), 4.03 (dd, *J* = 12.7, 3.1 Hz, 1H), 3.96 (dd, *J* = 12.6, 4.6 Hz, 1H), 2.79 – 2.74 (m, 2H), 2.64 – 2.58 (m, 2H), 2.31 (s, 3H), 2.19 (s, 3H), 1.12 – 0.99 (m, 28H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 206.2, 171.9, 137.6, 132(2C), 130.7, 129.7(2C), 89.5, 83.1, 80.9, 75.6, 61.5, 37.9, 30.0, 27.9, 21.2, 17.6, 17.4(3C), 17.1, 17.0(3C), 13.6, 13.3, 12.9, 12.6; HRMS (ESI-MS): m/z calcd for $[C_{29}H_{48}O_7SSi_2Na]^+$: 619.2557; found: 619.2565.

General procedure for deprotection of –STol: To a solution of thioglycoside (12.93 mmol) in THF−H_2O (40:1, 93 mL), NIS (22.49 mmol) and AgOTf (0.11 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 3 h (the reaction mixture turned to brown) and neutralized by the addition of excess amount of Et_3N. All volatiles were evaporated, the residue was diluted with 50 mL of dichloromethane and 50 mL of water, extracted with CH_2Cl_2 and washed with a saturated aq. solution of $Na_2S_2O_3$ and then washed with aqueous brine solution. Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain a crude residue that was purified by silica gel column chromatography using hexane and ethyl acetate to accomplish the corresponding hemi acetals **30**, **32**.

2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α/β-D-arabinofuranose [α:β(0.85:1)] (30): Colourless sticky liquid; Yield = 89% ; R_f = 0.31 (ethyl acetate:hexane 20:80); IR (cm^{-1}): 2939, 1729, 1451, 1362, 1264, 1100, 756, 708; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 8.08 (dt, *J* = 8.5, 1.5 Hz, 2H), 8.03 (dt, *J* = 8.5, 1.5 Hz, 2H), 7.62 – 7.55 (m, 2H), 7.49 – 7.42 (m, 4H), 5.63 (t, *J* = 4.5 Hz, 1H), 5.37 (dd, *J* = 4.5, 1.5 Hz, 1H), 5.31 (dd, *J* = 4.8, 1.7 Hz, 1H), 5.14 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.78 (dd, *J* = 7.8, 6.0 Hz, 1H), 4.55 (dd, *J* = 7.0, 4.8 Hz, 1H), 4.24 (ddd, *J* = 7.1, 5.5, 3.4 Hz, 1H), 4.12 – 4.01 (m, 2H), 4.00 – 3.89 (m, 3H), 3.42 (d, *J* = 4.6 Hz, 1H), 3.11 (d, *J* = 4.6 Hz, 1H), 1.16 – 0.99 (m, 56H); ^{13}C NMR (100.67 MHz, $CDCl_3$): δ 166.4, 166.3, 133.6, 133.5, 130.0(2C), 129.9(2C), 129.5, 129.4, 128.6(2C), 128.6(2C), 100.4, 93.7, 85.3, 82.2, 80.9, 80.1, 75.7, 75.3, 65.5, 62.2, 17.7, 17.6, 17.6(2C), 17.5, 17.5(2C), 17.5, 17.2, 17.1, 17.1, 17.1, 17.1 (3C), 17.0, 13.6, 13.6, 13.4, 13.4, 13.1, 12.9, 12.6(2C); (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{24}H_{40}O_7Si_2Na]^+$: 519.2210 ; found: 519.2216.

2-*O*-levulinoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α/β-D-arabinofuranose [α:β (1:1)] (32): Colourless sticky liquid; Yield = 95 % ; R_f = 0.14 (ethyl acetate:hexane 30:70); IR (cm^{-1}): 3613, 3439, 2942, 2869, 1728, 1463, 1369, 1242, 1148, 1029; ^{1}H NMR (400.31 MHz,

$CDCl_3$): δ 5.45 (d, *J* = 4.3 Hz, 1H), 5.21 (s, 1H), 5.05 (d, *J* = 4.8 Hz, 1H), 4.88 (dd, *J* = 7.7, 4.4 Hz, 1H), 4.57 (t, *J* = 7.0 Hz, 1H), 4.34 (dd, *J* = 6.9, 5.1 Hz, 1H), 4.17 – 4.11 (m, 1H), 4.00 (ddd, *J* = 14.8, 11.9, 3.3 Hz, 2H), 3.93 – 3.80 (m, 3H), 2.81 – 2.73 (m, 4H), 2.68 – 2.56 (m, 4H), 2.18 (s, 6H), 1.12 – 0.98 (m, 56H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 207.1, 206.5, 172.6, 172.5, 100.1, 93.5, 85.0, 82.0, 80.7, 79.9, 75.7, 75.5, 65.7, 62.2, 38.1, 37.9, 29.9(2C), 27.9, 27.9, 17.6, 17.6, 17.5(2C), 17.5, 17.5, 17.5, 17.4, 17.1(5C), 17.0(3C), 13.5, 13.5, 13.4, 13.4, 13.0, 12.9, 12.6(2C); HRMS (ESI-MS): m/z calcd for $[C_{22}H_{42}O_8Si_2H]^+$: 491.2496; found: 491.2496 .

General procedure for Synthesis of carbonate donors 33 and 34: To a solution of hemiacetal **30** or **32** (9.80 mmol) in anhydrous CH_2Cl_2 (50 mL), DMAP (12.74 mmol) and 1-Ethynylcyclohexyl 4-nitrophenyl carbonate **35** (14.71 mmol) were added portion wise and stirred for 6 h at room temperature. After consumption of the starting material, the reaction mixture was concentrated *in vacuo* to obtain an oily residue which was partially purified by silica gel column chromatography. The eluents of the column fractions contained trace quantity of *p*-nitrophenol and hence, the crude residue was redissolved in minimum volume of CH_2Cl_2 (30 mL) and washed several times with aqueous saturated $NaHCO_3$ solution until aqueous layer becomes completely colourless. Finally, organic layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to obtain pure alkynyl arabinofuranosyl donors **33** and **34**.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-2-*O*-benzoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α/β-D-arabinofuranose [α:β (1.74:1)] (33): Colourless solid; Yield = 92% ; R_f = 0.42 (ethyl acetate:hexane 5:95); IR (cm^{-1}): 3616, 3292, 2941, 2868, 1748, 1460, 1377, 1238, 1104, 1029, 892, 778, 700; ^{1}H NMR (399.78 MHz, $CDCl_3$): δ 8.08-8.00 (m, 4H), 7.62-7.54 (m, 2H), 7.48 – 7.41 (m, 4H), 6.30 (d, *J* = 4.3 Hz, 1H), 6.08 (d, *J* = 1.4 Hz, 1H), 5.61 (dd, *J* = 5.0, 1.7 Hz, 1H), 5.42 (dd, *J* = 8.3, 4.2 Hz, 1H), 4.81 (dd, *J* = 8.2, 6.1 Hz, 1H), 4.57 (dd, *J* = 7.5, 4.9 Hz, 1H), 4.25 – 4.19 (m, 1H), 4.12 – 4.01 (m, 3H), 4.01 – 3.89 (m, 2H), 2.63 (s, 1H), 2.38 (s, 1H), 2.23 - 2.13 (m, 1H), 2.09 – 1.99 (m, 1H), 1.98 – 1.79 (m, 4H), 1.78 – 1.59 (m, 5H), 1.58 – 1.40 (m, 7H), 1.38-1.26 (m, 2H), 1.18 – 0.95 (m, 56H); ^{13}C NMR (100.53 MHz, $CDCl_3$): δ 165.8, 165.5, 151.3, 151.0, 133.6, 133.4, 130.1(2C), 129.9(2C), 129.3(2C), 128.6(2C), 128.4(2C), 101.9, 96.0, 83.5, 83.2, 82.8, 82.7, 82.3, 78.3, 78.2, 77.9, 76.4, 75.3, 75.0, 74.9, 65.4, 61.9, 36.9, 36.8, 36.7, 36.6, 25.1, 25.0, 22.6, 22.6, 22.4, 22.4, 17.6, 17.6, 17.5, 17.5, 17.5(2C), 17.4(2C), 17.2, 17.1, 17.0(5C), 17.0, 13.5, 13.5, 13.4, 13.3, 13.0, 12.9, 12.6, 12.5. HRMS (ESI-MS): m/z calcd for $[C_{33}H_{50}O_9Si_2K]^+$: 685.2630; found: 685.2639.

1-*O*-(((1-ethynylcyclohexyl)oxy)carbonyl)-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-2-*O*-(4-oxopentanoyl)-α/β-D-arabinofuranose [α:β (1.5:1)] (34): Colourless solid; Yield = 98% ; R_f = 0.51 (ethyl acetate:hexane 30:70); IR (cm^{-1}): 3294, 2940, 2867, 1756, 1720, 1361, 1269, 1240, 1153, 1116, 1070, 1036, 1004; ^{1}H NMR (400.31 MHz, $CDCl_3$): δ 6.11 (d, *J* = 4.3 Hz, 1H), 5.92 (s, 1H), 5.32 (dd, *J* = 4.7, 1.3 Hz, 1H), 5.16 (dd, *J* = 8.3, 4.3 Hz, 1H), 4.61 (dd, *J* = 8.2, 6.3 Hz, 1H), 4.37 (dd, *J* = 7.3, 4.8 Hz, 1H), 4.16 – 4.09 (m, 1H), 4.06 – 3.82 (m, 5H), 2.83 – 2.67 (m, 4H), 2.66 – 2.57 (m, 6H), 2.17 (s, 6H), 2.15 – 2.05 (m, 3H), 1.97 – 1.82 (m, 4H), 1.70 – 1.48 (m, 11H), 1.38 – 1.28 (m, 2H), 1.14 – 0.98 (m, 56H); ^{13}C NMR (100.66 MHz, $CDCl_3$): δ 206.2, 206.1, 172.1, 171.8, 151.3, 151.1, 101.7, 95.8, 83.3, 83.3, 83.0, 82.8, 82.2, 78.2, 78.0, 77.9, 76.4, 75.2, 75.1, 74.7, 65.3, 62.0, 38.0, 37.9, 37.1, 36.9, 36.8, 36.5, 30.0, 29.9, 27.8, 27.7, 25.1, 25.1, 22.6(2C), 22.5(2C), 17.6, 17.6, 17.5, 17.5, 17.5, 17.5, 17.4(2C), 17.1, 17.0(6C), 17.0, 13.5, 13.5, 13.4, 13.3, 13.0, 12.9, 12.6, 12.5; HRMS (ESI-MS): m/z calcd for $[C_{31}H_{52}O_{10}Si_2K]^+$: 679.2736; found: 679.2738

3.8.1 General procedures for manual solid-phase oligosaccharide synthesis

Preparation of Solutions and Reagents for SPOS

Preparation of the solution of building blocks: 4 equivalents of building block (e.g. glycosyl alkynyl carbonate donor) into an Eppendorf tube and dissolved in 2 mL of anhydrous CH_2Cl_2.

Activator reagents: Solid gold phosphite (10 mol%) and AgOTf (15 mol%) were weighed into individual Eppendorf tubes and the tubes were sealed with parafilm until their utilization.

HF.py solution for TBDPS and Disiloxane deprotection: HF•Pyridine/Pyridine in (0.4:1 mL proportion) was prepared.

Saponification of Benzoates: Commercially available solution of 0.5 M NaOMe in MeOH was used.

Levulinoate deprotection solution: Hydrazine acetate (550 mg) was dissolved in a mixture of Pyridine:AcOH (40 mL; 4:1) and used as a stock solution (0.15 M solution).

Protocol 1 – Swelling of resin:

The functionalized resin was loaded into the reaction vessel, dry CH_2Cl_2 was added and kept for 5 min for swelling of the resin. The solvent was drained before starting the reaction.

Protocol 2 - Glycosylation with carbonate donor:

The building block donor solution (4 equiv, 0.125 mmol) in 2 mL dry CH_2Cl_2 was delivered under nitrogen atmosphere to the reaction vessel containing the resin. The resin was then allowed to mix with donor solution for 10 min by bubbling N_2 gas. After that, with the small interruption of N_2 bubbling, solid gold phosphite (10 mol %); Silver triflate (15 mol%) was added to the reaction vessel. The reaction mixture is then left for 30 min under Nitrogen bubbling. The solution is drained and the resin is washed with CH_2Cl_2, DMF and CH_2Cl_2 (3x with 2 mL for 15 s sequentially). With the use of CH_2Cl_2 solvent resin was then transferred to another reaction vessel and washed it with 2 mL dry CH_2Cl_2 three times. Resin is then dried using vacuum pump.

Protocol 3 – TBDPS deprotection using HF/Pyridine:

The resin was washed with dry CH_2Cl_2 two times and then dry Pyridine (1.5 mL) was added. The resin was agitated using N_2 bubbling for 5 min then 0.6 mL of HF.Pyridine solution (70% HF in Pyridine) was added drop wise under inert atmosphere at room temperature for 15 h. The reaction vessel was emptied into the waste, washed with CH_2Cl_2, DMF, and CH_2Cl_2 (3x2mL). Resin was then dried under high vacuum.

Protocol 4 - Bz deprotection using NaOMe solution:

To the swollen resin in CH_2Cl_2 (2 mL), NaOMe solution (0.5 N NaOMe in MeOH) in 1 mL was added. Resin was agitated using N_2 bubbling for 1 h, the solution was drained. Washed resin with MeOH and CH_2Cl_2, (3x2 mL each). Resin was then dried under high vacuum.

Protocol 5 - Levulinoate deprotection using Hydrazine Acetate solution:

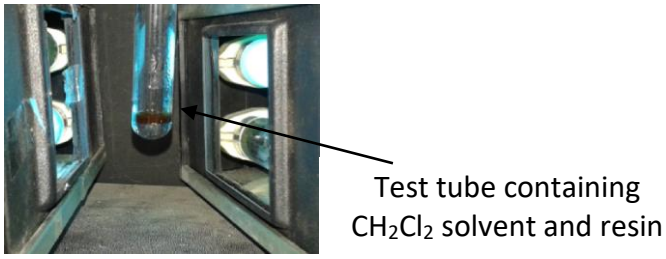
The resin is washed with CH_2Cl_2 (3x2 mL), swollen in 1.5 mL CH_2Cl_2, at the room temperature. For Lev deprotection, 0.8 mL of 0.15 M solution of hydrazine acetate in pyridine/acetic acid (stock solution) was added. After 30 min, the reaction solution was drained and the resin was washed with 0.2 M acetic acid in CH_2Cl_2 and CH_2Cl_2 (6x2 mL). The entire procedure is repeated twice.

Post-synthesis Protocols

Protocol I - Photocleavage: The resin ready for deprotection was transferred to the 15 mL glass test tube fitted with a rubber septum, dry CH_2Cl_2 (1 mL) was added under nitrogen atmosphere and then kept under the UV-visible cabinet fitted with a light of wavelength range

365 nm for 4 h. The resin was then carefully filtered through a column with a frit. The resin was washed with CH_2Cl_2 (5x2 mL), combined filtrates were evaporated under vacuum to obtain a residue which was redissolved in CH_2Cl_2 and UPLC-MS was performed.

Photocleavage reaction setup



Protocol II - Purification of protected oligosaccharide: Protected oligosaccharides after cleavage from the solid support were purified by silica gel column chromatography using hexane:ethyl acetate system for complete characterization.

Protocol III - HPLC purification of partially protected oligosaccharide: The crude oligosaccharide mixtures were purified by semi-preparative HPLC (Agilent 1260 infinity II series) recording on DAD at a wavelength of 214 nm. Column: RP-C_{18} (10µ 250 × 10 mm, 110 Å). Eluent A: 0.1% TFA in water/CH_3CN (95:5) and B: 0.1% TFA in CH_3CN/water (95:5) were used in a linear gradient of 0 to 40% (10 min) to 60% (25 min) to 100% (30 min) at a flow rate of 2 mL/min.

Protocol IV HPLC purity analysis: Purity of compounds **23** and **41** was ascertained by re-injecting the purified sample into semi preparative HPLC (Agilent 1260 infinity II series) and was recorded by DAD using a flow of 2 mL/min on a RP-C_{18} column (5 µm, 250 mm, 4.6 mm, 110 Å). Eluents A (0.1% TFA in water) and B (0.1% TFA in CH_3CN) were used in a linear gradient of 0 to 40% (10 min) to 60% (25 min) to 100% (30 min) at a flow rate of 2 mL/min.

***N*-benzyloxycarbonyl 4-aminobutyl 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*-^{t}butyldiphenylsilyl-α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranoside (20):** $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): +1.0; IR (cm^{-1}): 3845, 3740, 3671, 3615, 2924, 2860, 2355, 1720, 1523, 1461, 1262, 1107, 969, 706; ^{1}H NMR (600.40 MHz, $CDCl_3$): δ 8.00 (dd, *J* = 10.8, 7.9 Hz, 7H), 7.96 (dd, *J* = 7.0, 5.2 Hz, 4H), 7.88 (dt, *J* = 16.6, 8.4 Hz, 7H), 7.68 (t, *J* = 6.2 Hz, 4H), 7.56 – 7.35 (m, 23H), 7.34 – 7.27 (m, 14H), 7.25 – 7.20 (m, 6H), 5.62 (dd, *J* = 11.3, 4.6 Hz, 7H), 5.54 (s, 1H), 5.47 (s, 1H), 5.37 (dd, *J* = 9.6, 8.0

Hz, 4H), 5.19 (s, 1H), 5.05 (s, 1H), 4.86 (s, 1H), 4.58 (dd, *J* = 8.1, 3.9 Hz, 3H), 4.47 (q, *J* = 4.6 Hz, 1H), 4.42 (d, *J* = 2.7 Hz, 1H), 4.20 – 4.12 (m, 4H), 3.98 – 3.87 (m, 6H), 3.78 – 3.72 (m, 1H), 3.50 (d, *J* = 12.4 Hz, 3H), 3.22 (d, *J* = 5.8 Hz, 2H), 1.60 (s, 4H), 0.99 (s, 9H); ^{13}C NMR (150.99 MHz, $CDCl_3$): δ 165.6, 165.6, 165.6, 165.6, 165.5, 165.5, 165.2, 165.2, 165.1, 165.1, 136.6, 135.7(2C), 135.6(2C), 133.4(2C), 133.4, 133.4, 133.3, 133.3, 133.2, 133.2, 133.2, 133.1(2C), 133.0, 129.9(2C), 129.8(11C), 129.8(9C), 129.6(2C), 129.3, 129.2(2C), 129.1(3C), 129.1, 129.0(2C), 129.0, 128.5(10C), 128.4(2C), 128.3(2C), 128.3(2C), 128.2(4C), 128.2(2C), 128.1, 128.1, 127.6(4C), 105.9(2C), 105.9, 105.8, 105.6, 83.1, 82.1(4C), 81.9, 81.9, 81.5, 81.5, 81.5, 77.2(5C), 67.0, 66.6, 66.0, 65.8, 65.8, 65.7, 63.4, 40.8, 26.8, 26.7(3C), 26.7, 19.3; (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{123}H_{115}NO_{33}SiNa]^+$: 2184.7018; found: 2184.7018.

***N*-benzyloxycarbonyl 4-aminobutyl 5-*O*-[5-*O*-[5-*O*-[5-*O*-[α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranoside (23):** $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): +92.0; ^{1}H NMR (400.31 MHz, CD_3OD): δ 7.38 – 7.28 (m, 5H), 5.08 (s, 2H), 4.96 (s, 5H), 4.11-4.07 (m, 3H), 4.03 – 4.00 (m, 5H), 3.97 – 3.95 (m, 1H), 3.93 - 3.88 (m, 5H), 3.88 – 3.81 (m, 5H), 3.76 (dd, *J* = 11.9, 3.3 Hz, 1H), 3.70 – 3.62 (m, 6H), 3.48 - 3.42 (m, 1H), 3.16 (t, *J* = 6.5 Hz, 2H), 1.65 – 1.56 (m, 4H); ^{13}C NMR (150.99 MHz, CD_3OD): δ 158.9, 138.5, 129.4(2C), 128.9, 128.8(2C), 109.7(3C), 109.6, 109.5, 85.9, 84.1(3C), 83.6, 83.5, 83.2(3C), 83.1, 79.1(3C), 79.1, 78.7, 68.5, 68.2, 68.2, 68.1(2C), 67.3, 63.1, 41.5, 27.9, 27.7. (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{37}H_{57}NO_{23}Na]^+$: 906.3225; found: 906.3230.

***N*-benzyloxycarbonyl 4-aminobutyl 2-*O*-benzoyl-3,5-di-*O*-[2-*O*-levulinoyl-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)-α-D-arabinofuranosyl]-α-D-arabinofuranoside (38):** $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): +0.037**;** ^{1}H NMR (600.40 MHz, $CDCl_3$): δ 8.03 (d, *J* = 7.4 Hz, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.27 (m, 5H), 5.29 – 5.19 (m, 4H), 5.14 – 5.08 (m, 4H), 4.94 (d, *J* = 1.1 Hz, 1H), 4.30 – 4.25 (m, 2H), 4.23 (s, 2H), 4.00 – 3.90 (m, 6H), 3.87 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.75 (s, 1H), 3.72 – 3.69 (m, 1H), 3.52 – 3.46 (m, 1H), 3.26 (d, *J* = 5.4 Hz, 2H), 2.73 – 2.68 (m, 4H), 2.61 – 2.54 (m, 4H), 2.14 (s, 3H), 2.11 (s, 3H), 1.63 – 1.59 (m, 4H), 1.10 – 0.98 (m, 56H); ^{13}C NMR (150.97 MHz, $CDCl_3$): δ 206.4, 206.3, 172.0, 171.7, 165.8, 156.7, 137.0, 133.4, 130.0, 129.6, 128.6(2C), 128.6(4C), 128.2, 128.1, 106.0, 105.3, 104.4, 84.0, 84.0, 82.6, 81.3, 81.2, 81.1, 80.9, 76.1, 75.9, 67.1, 66.5, 66.5, 61.6, 61.6, 41.0, 38.0, 37.9, 29.9, 29.9, 28.0, 27.9, 26.7, 26.7, 17.6(2C), 17.5(3C), 17.5(3C), 17.1, 17.1, 17.1(2C), 17.1(2C), 17.0(2C), 13.6, 13.5, 13.3(2C), 12.9, 12.9, 12.6, 12.6; (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{68}H_{109}NO_{22}Si_4Na]^+$: 1426.6416; found: 1426.6420.

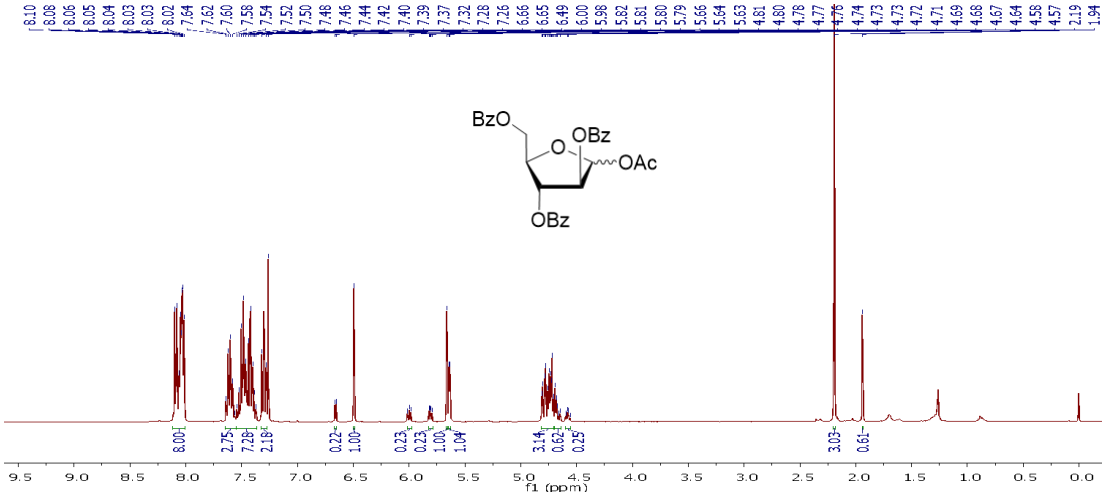
***N*-benzyloxycarbonyl 4-aminobutyl 2-*O*-benzoyl-3,5-di-*O*-[2-*O*-[2,3-di-*O*-benzoyl-5-*O*-^{t}-butyldiphenylsilyl-α-D-arabinofuranosyl]-3,5-*O*-(tetraisopropylsiloxane-1,3-diyl)- α-D-arabinofuranosyl]-α-D-arabinofuranoside (40):** (14.0 mg, 27% from 0.022 mmol); $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): +0.036; IR (cm^{-1} $CHCl_3$); 3845, 3740, 3671, 3616, 2925, 2861, 2354, 1917, 1726, 1523, 1462, 1261, 1106, 1042, 881, 792, 702. ^{1}H NMR (600.40 MHz, $CDCl_3$) δ 8.08 – 8.06 (m, 2H), 8.05 – 8.02 (m, 2H), 7.96 – 7.92 (m, 4H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.69 – 7.64 (m, 9H), 7.59 – 7.51 (m, 5H), 7.45 – 7.38 (m, 7H), 7.37 – 7.32 (m, 7H), 7.32 – 7.28 (m, 8H), 7.25 – 7.21 (m, 4H), 5.74 (d, *J* = 4.5 Hz, 1H), 5.63 (d, *J* = 4.7 Hz, 1H), 5.49 (s, 1H), 5.47 (d, *J* = 1.1 Hz, 1H), 5.39 (d, *J* = 3.7 Hz, 2H), 5.36 (d, *J* = 2.2 Hz, 1H), 5.21 (s, 1H), 5.06 (d, *J* = 2.0 Hz, 1H), 5.03 (s, 1H), 4.91 (s, 1H), 4.84 (t, *J* = 5.5 Hz, 1H), 4.60 (dd, *J* = 8.9, 4.5 Hz, 1H), 4.41 (q, *J* = 4.5 Hz, 1H), 4.26 – 4.22 (m, 3H), 4.21 – 4.17 (ddd, *J* = 8.0, 6.2, 2.2 Hz, 2H), 4.15 (dd, *J* = 7.3, 4.7 Hz, 1H), 4.06 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.00 – 3.94 (m, 4H), 3.94 - 3.88 (m, 4H), 3.83 – 3.73 (m, 4H), 3.70 (dd, *J* = 11.0, 2.4 Hz, 1H), 3.50-3.46 (m, 1H), 3.22 – 3.17 (m, 1H), 2.99 (d, *J* = 5.8 Hz, 1H), 1.64 – 1.61 (m, 4H), 1.07 – 0.97 (m, 60H), 0.95 – 0.92 (m, 14H). ^{13}C NMR (150.97 MHz, $CDCl_3$) δ 165.7, 165.6, 165.2, 165.2(2C), 156.3, 136.8, 135.6(8C), 133.3(3C), 133.3, 133.2, 133.1(2C), 133.1, 133.1, 129.9(11C), 129.8(3C), 129.8(3C), 129.7, 129.6, 129.6, 129.6(3C), 129.5, 129.4, 129.2, 129.2, 128.4, 128.4, 128.4(2C), 128.3(2C), 128.0, 127.9, 127.7(4C), 127.6(3C), 106.5, 106.2, 105.8, 105.6, 104.3, 88.6, 88.4, 83.4, 83.3, 82.3, 82.3, 82.1, 81.9, 80.5, 79.8, 79.6, 77.6, 77.3, 75.9, 75.6, 66.6, 66.5, 66.4, 63.3, 63.2, 61.0, 60.8, 40.6, 26.7(6C), 26.4, 26.4, 19.3, 19.3, 17.5, 17.5, 17.4, 17.3(3C), 17.3(2C), 17.1(2C), 17.0, 17.0, 16.9, 16.9, 16.9(2C), 13.5, 13.4, 13.1, 13.1, 12.8, 12.7, 12.4, 12.4. (MALDI-TOF) $[M+K]^+$ m/z calcd for $[C_{128}H_{165}NO_{30}Si_6Na]^+$: 2386.9930; found: 2386.9927.

***N*-benzyloxycarbonyl 4-aminobutyl 3,5-di-*O*-[2-*O*-[α-D-arabinofuranosyl]-α-D-arabinofuranosyl]-α-D-arabinofuranoside (41):** $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1.0): +92.00; ^{1}H NMR (600.40 MHz, CD_3OD): δ 7.38 – 7.26 (m, 5H), 5.23 (d, *J* = 1.6 Hz, 1H), 5.11 (d, *J* = 1.2 Hz, 1H), 5.08 (d, *J* = 1.8 Hz, 1H), 5.07 (s, 1H), 5.05 (d, *J* = 1.9 Hz, 1H), 4.17 (dd, *J* = 3.1, 1.5 Hz, 1H), 4.14 (td, *J* = 5.7, 3.0 Hz, 1H), 4.09 (dd, *J* = 3.4, 1.3 Hz, 1H), 4.04 (dd, *J* = 6.4, 3.1 Hz, 1H), 4.02 (dd, *J* = 4.3, 1.6 Hz, 1H), 4.00 (dd, *J* = 4.0, 1.8 Hz, 1H), 3.99 – 3.98 (m, 2H), 3.97 – 3.93 (m, 5H), 3.92 – 3.87 (m, 3H), 3.86 – 3.81 (m, 3H), 3.80 – 3.74 (m, 5H), 3.73 – 3.69 (m, 2H), 3.66 – 3.61 (m, 5H), 3.44 (dd, *J* = 10.6, 5.7 Hz, 1H), 3.15 (t, *J* = 6.6 Hz, 2H), 1.62 – 1.58 (m, 4H); ^{13}C NMR (150.99 MHz, CD_3OD): δ 158.9, 138.5, 129.5(2C), 128.9(2C), 128.8, 109.9, 109.6, 109.3, 108.0, 107.7, 90.9, 89.6, 85.5(2C), 84.7, 84.5, 83.9, 83.8(2C), 82.5, 82.2, 78.6,

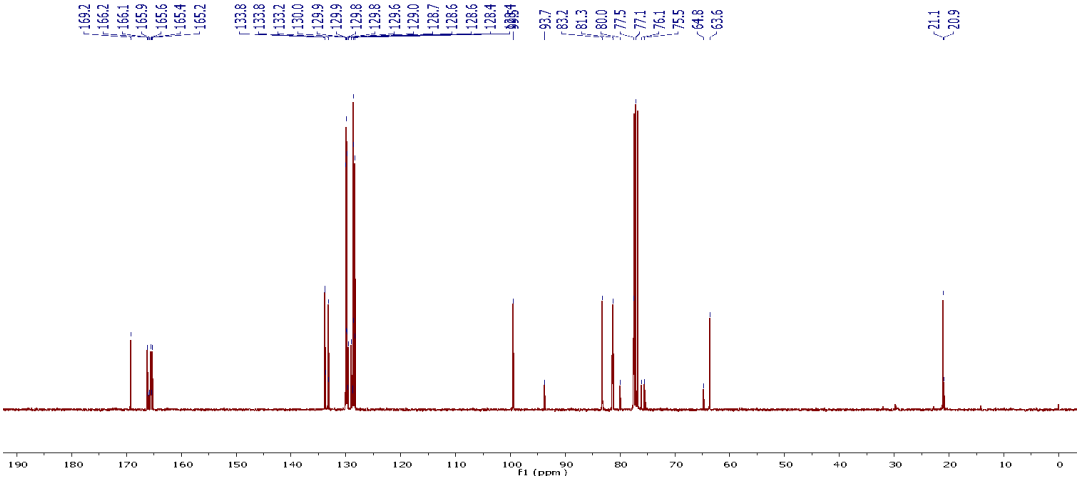
78.6, 77.5, 77.2, 68.3, 67.9, 67.3, 63.1, 62.9, 62.8, 62.5, 41.6, 27.9, 27.7; (MALDI-TOF) $[M+Na]^+$ m/z calcd for $[C_{37}H_{57}NO_{23}Na]^+$: 906.3219; found: 906.3230.

3.9 Spectral charts

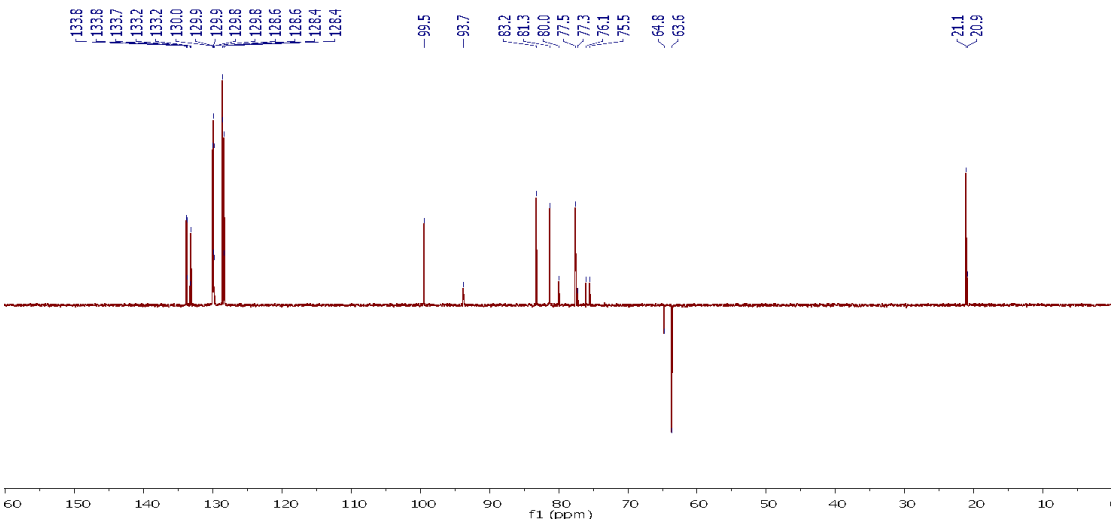
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **25**



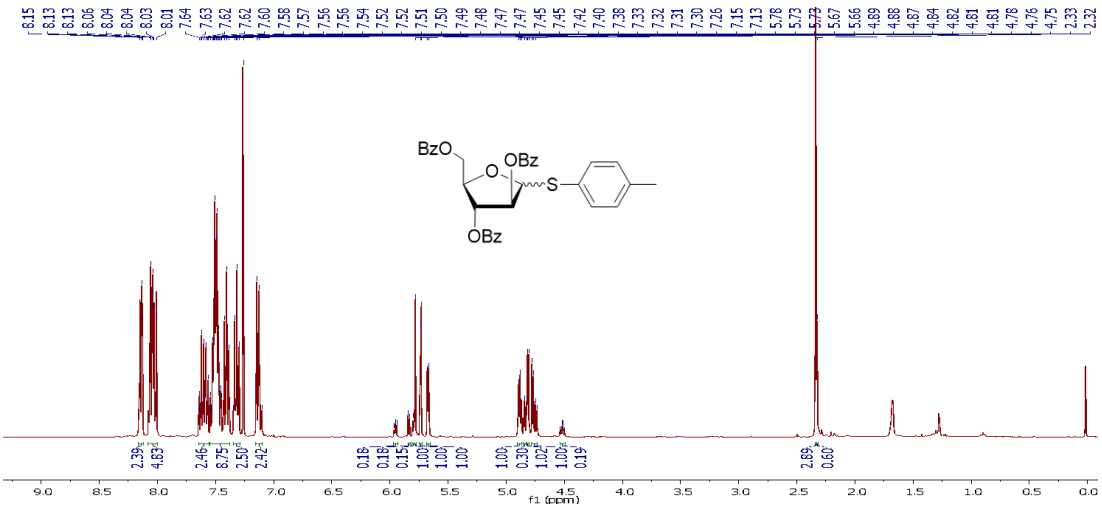
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **25**



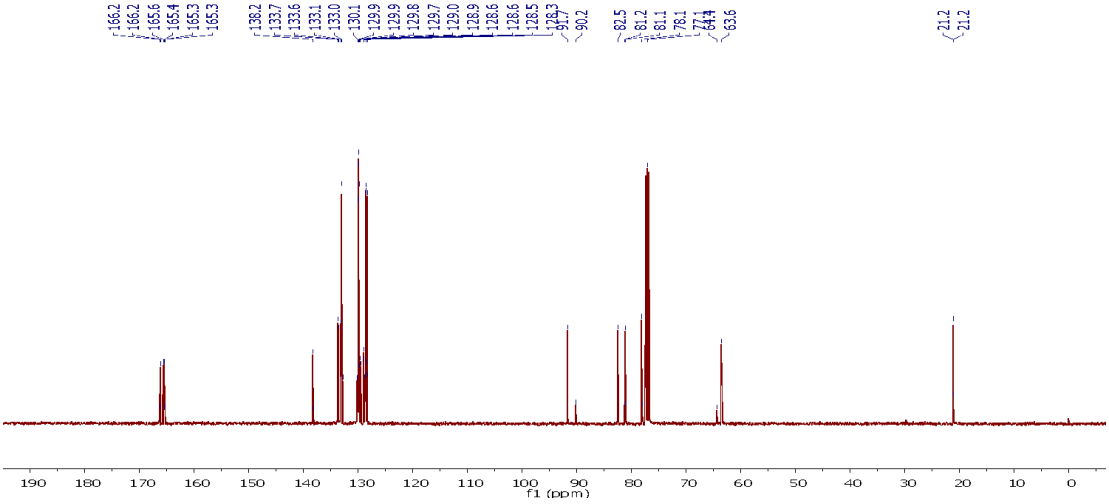
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **25**



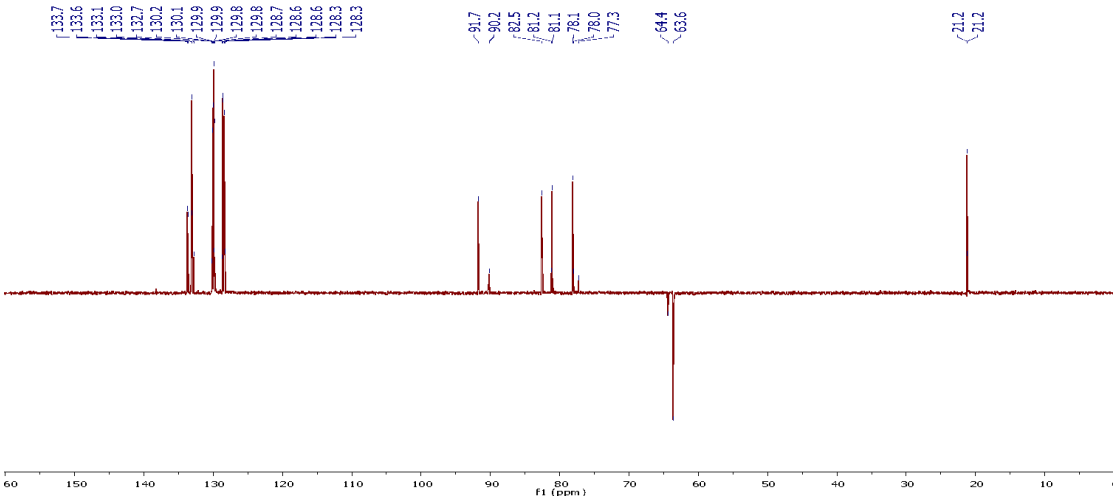
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **26**



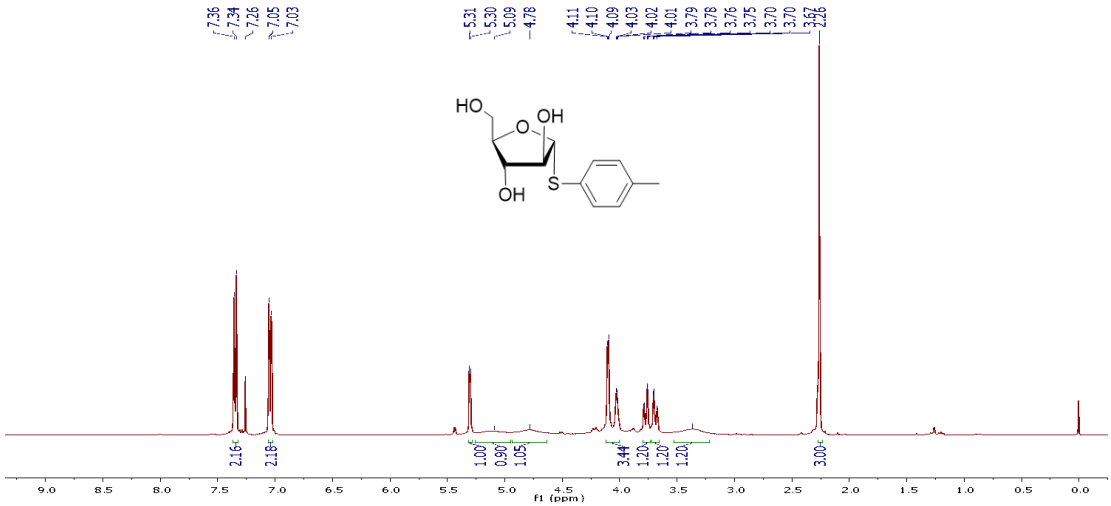
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **26**



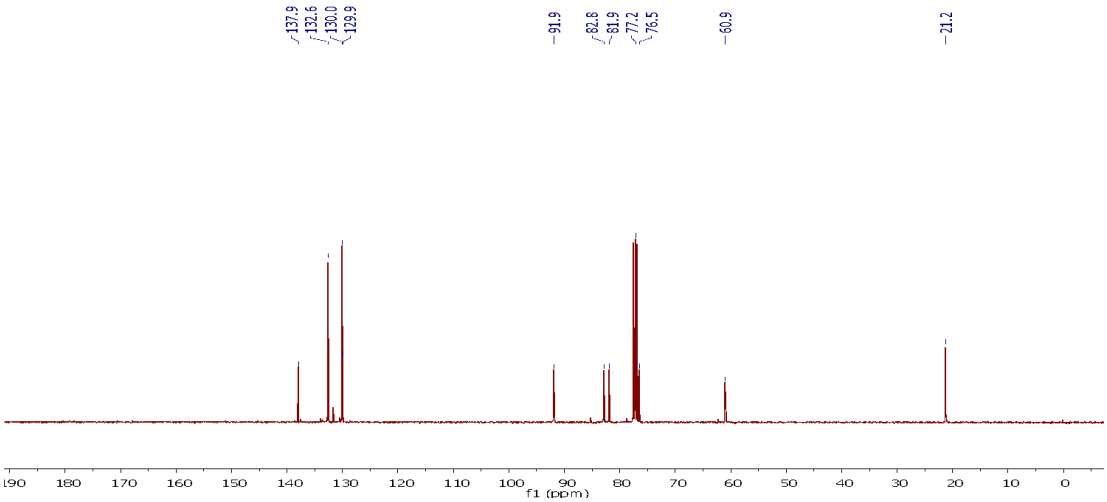
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **26**



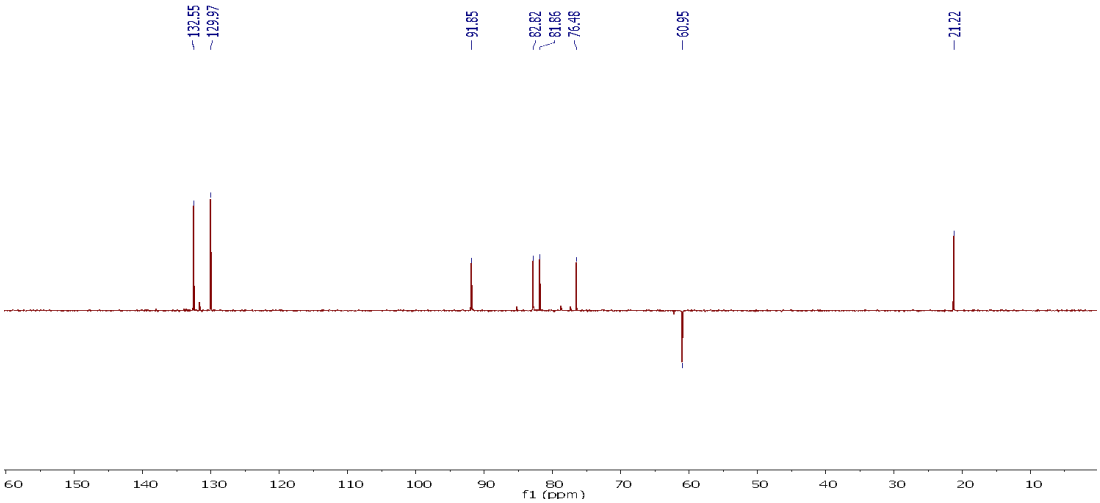
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **27**



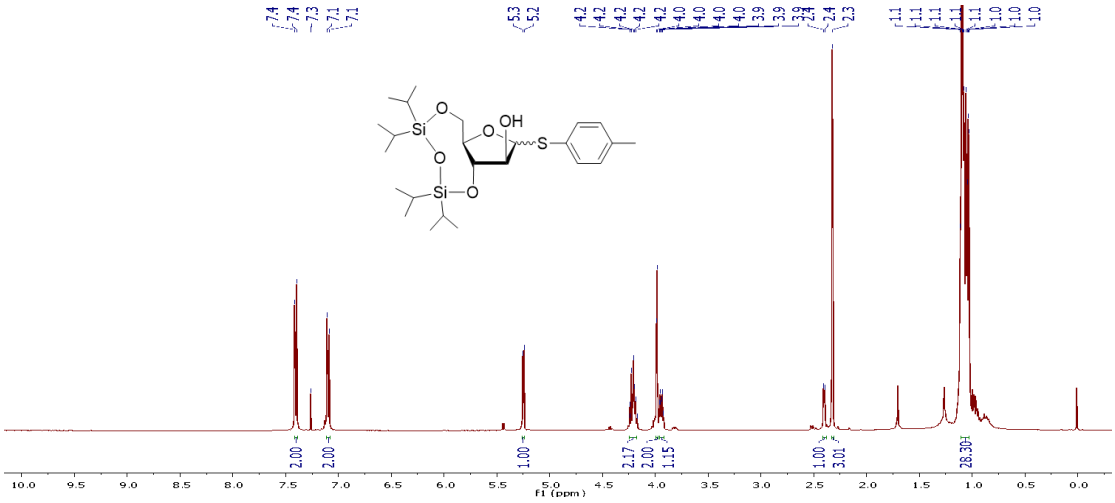
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **27**



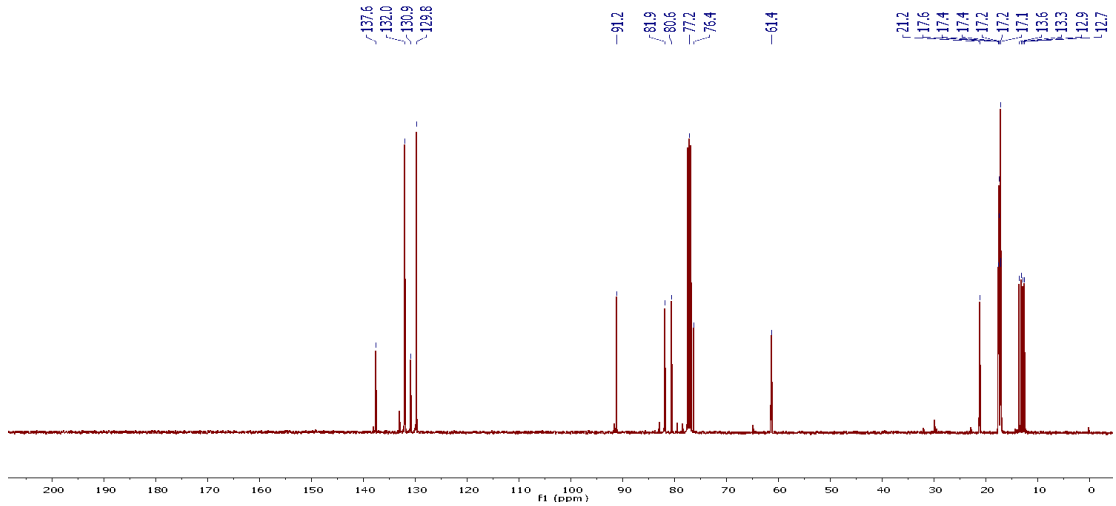
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **27**



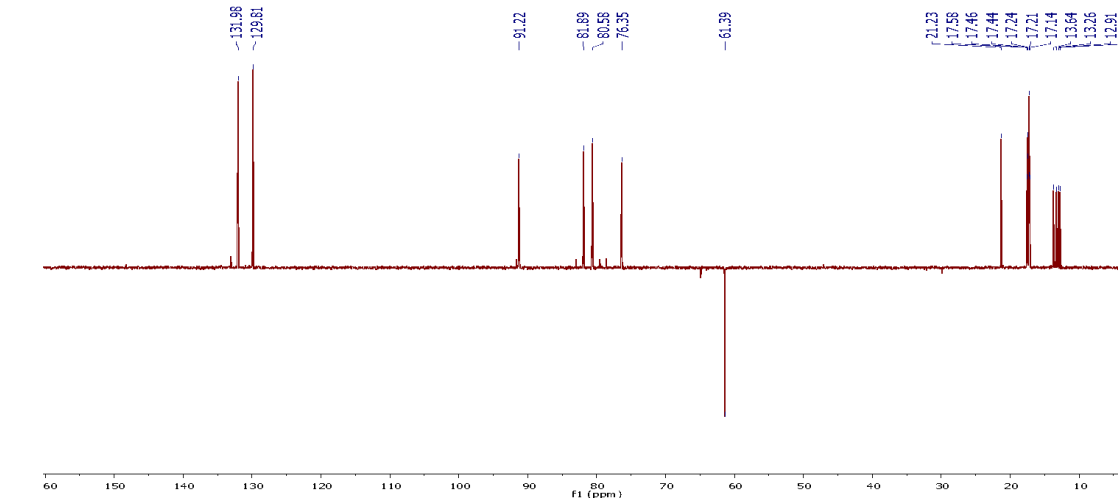
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **28**



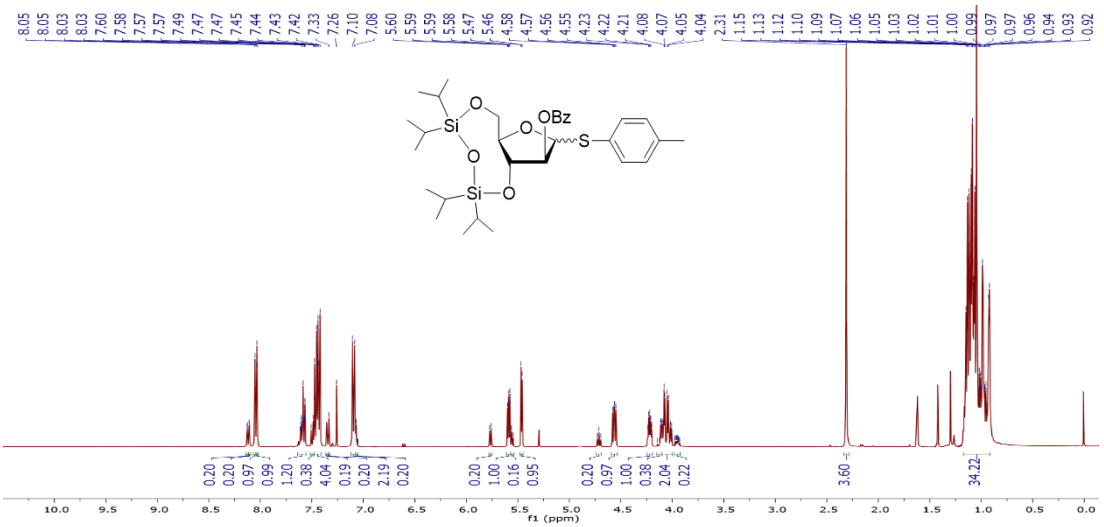
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **28**



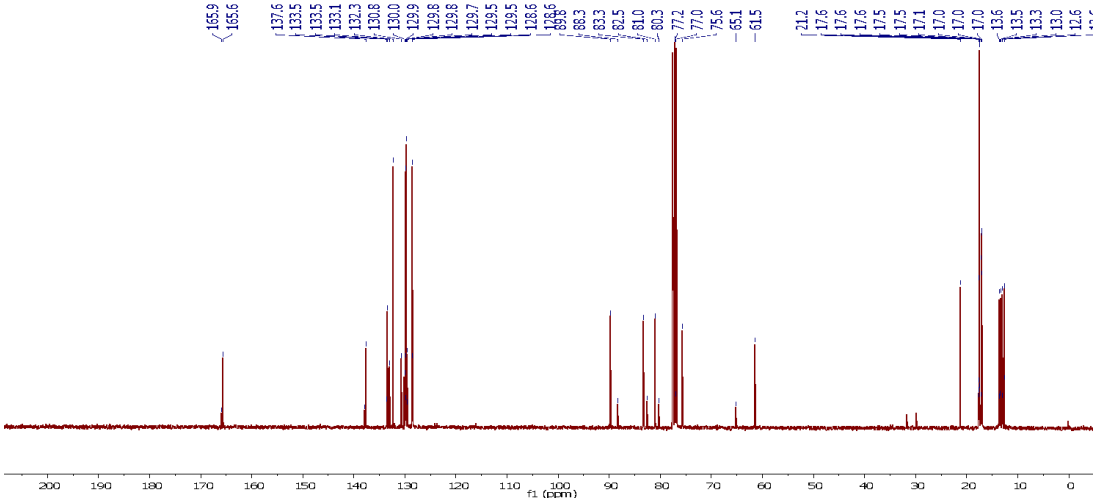
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **28**



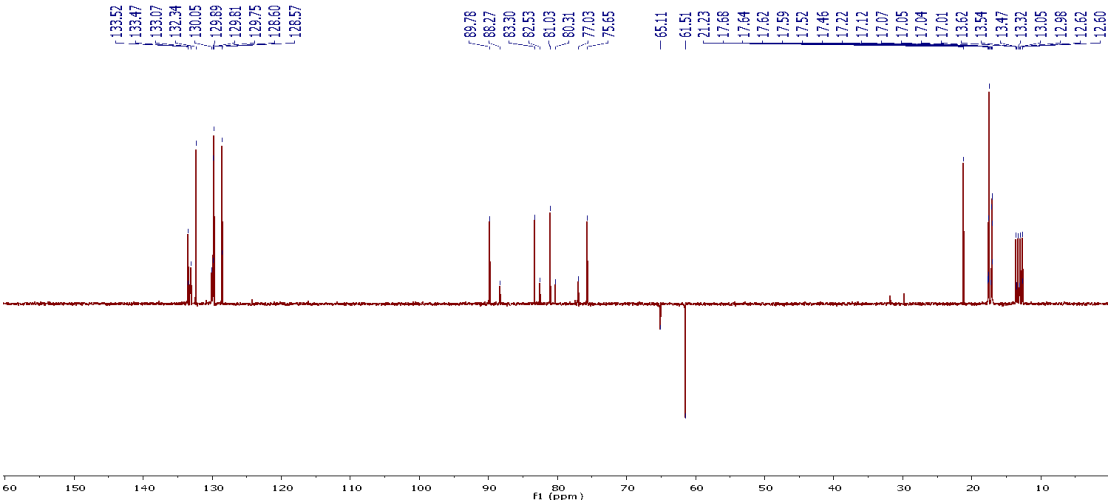
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **29**



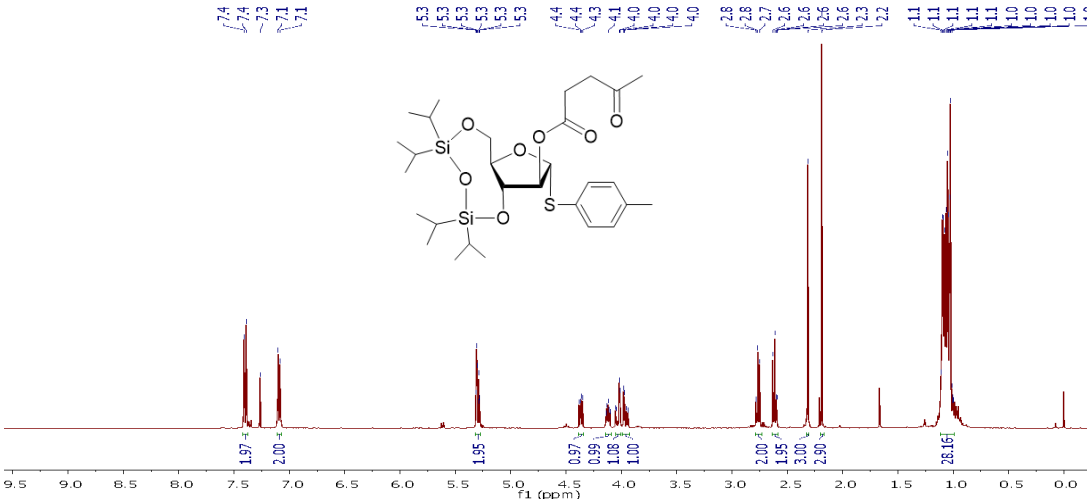
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **29**



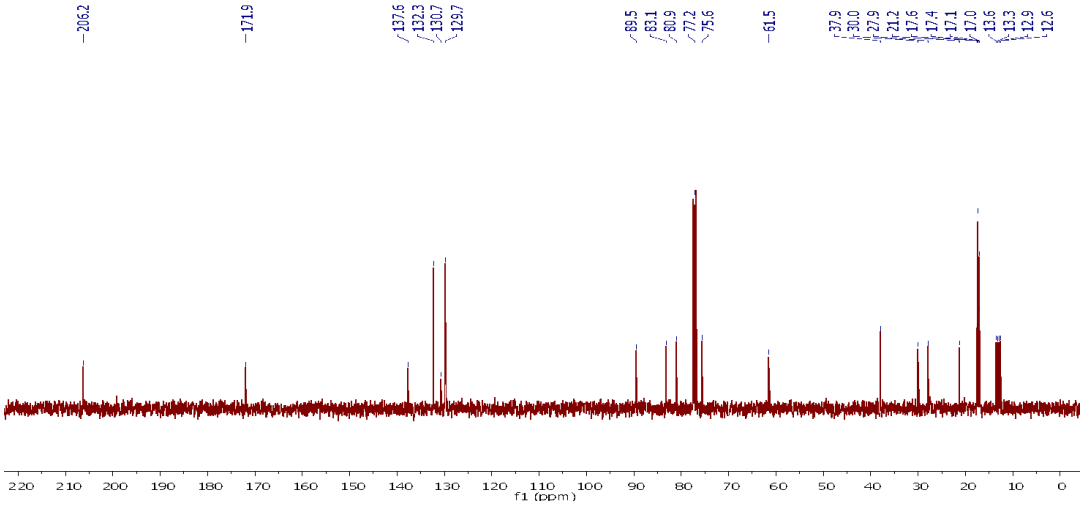
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **29**



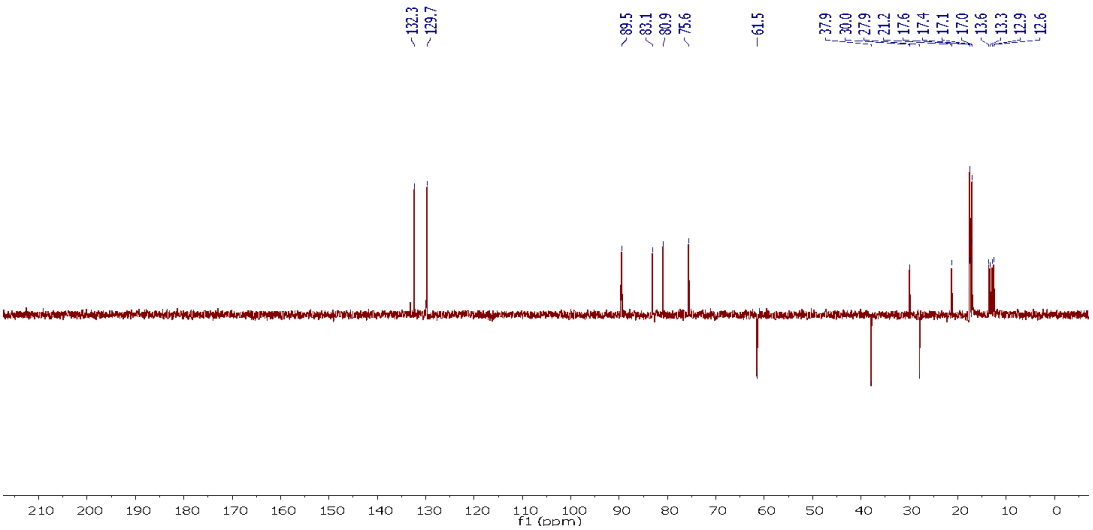
^{1}H NMR Spectrum (399.78 MHz, $CDCl_3$) of Compound **31**



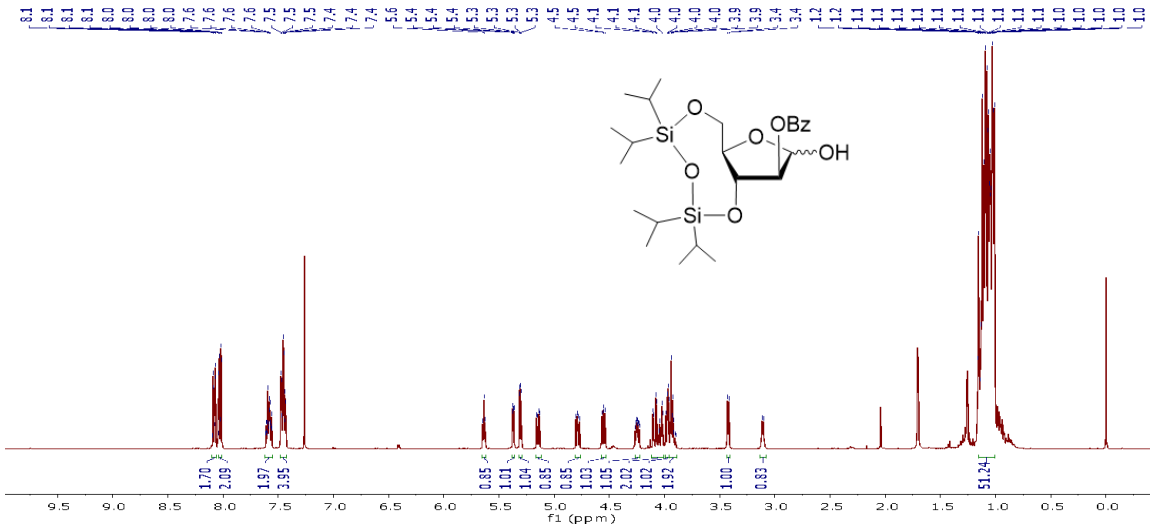
^{13}C NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **31**



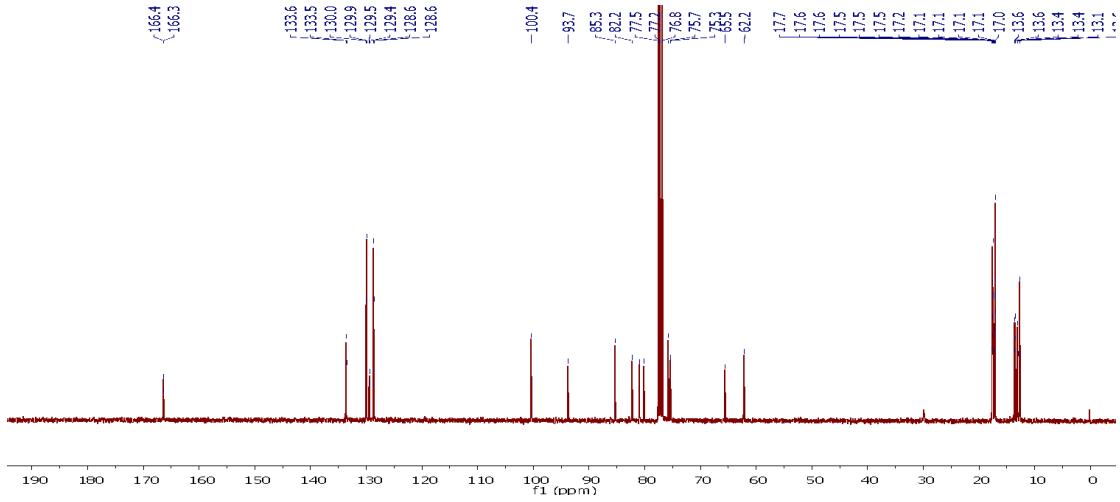
DEPT-135 NMR Spectrum (100.53 MHz, $CDCl_3$) of Compound **31**



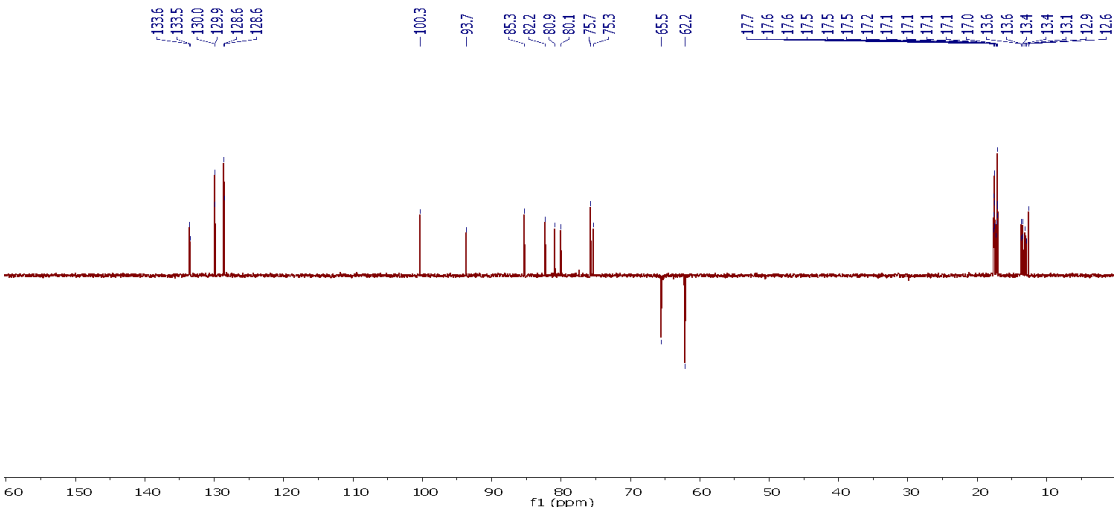
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **30**



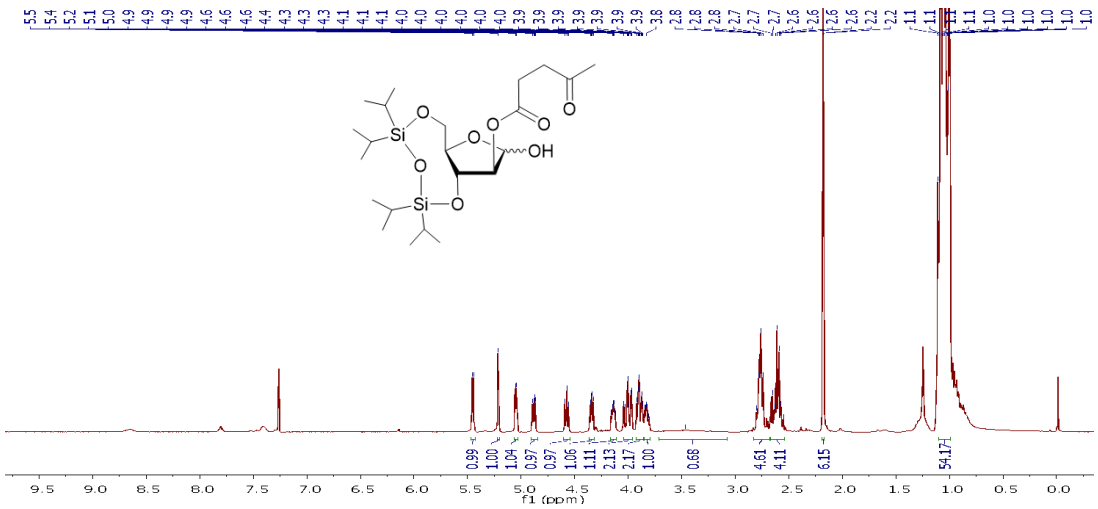
^{13}C NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **30**



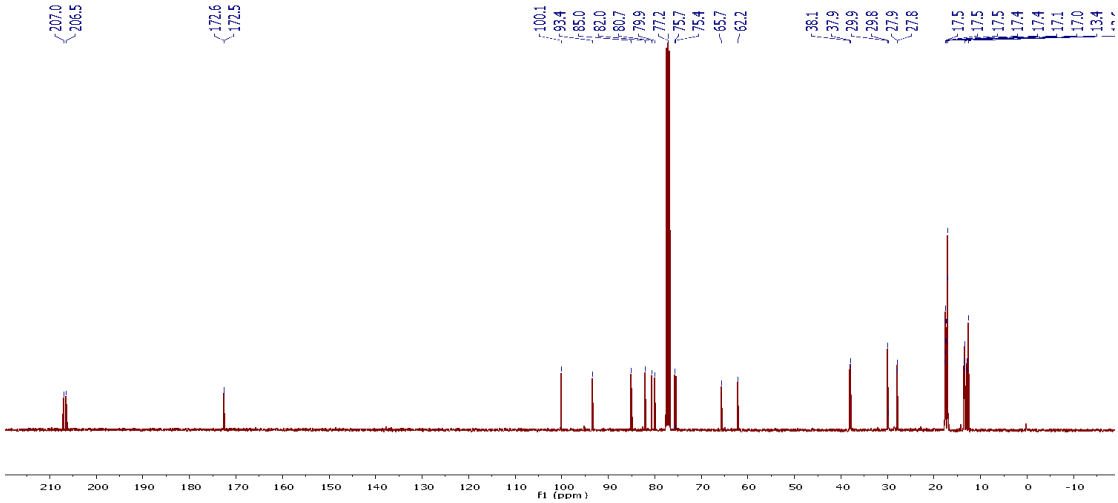
DEPT-135 NMR Spectrum (100.67 MHz, $CDCl_3$) of Compound **30**



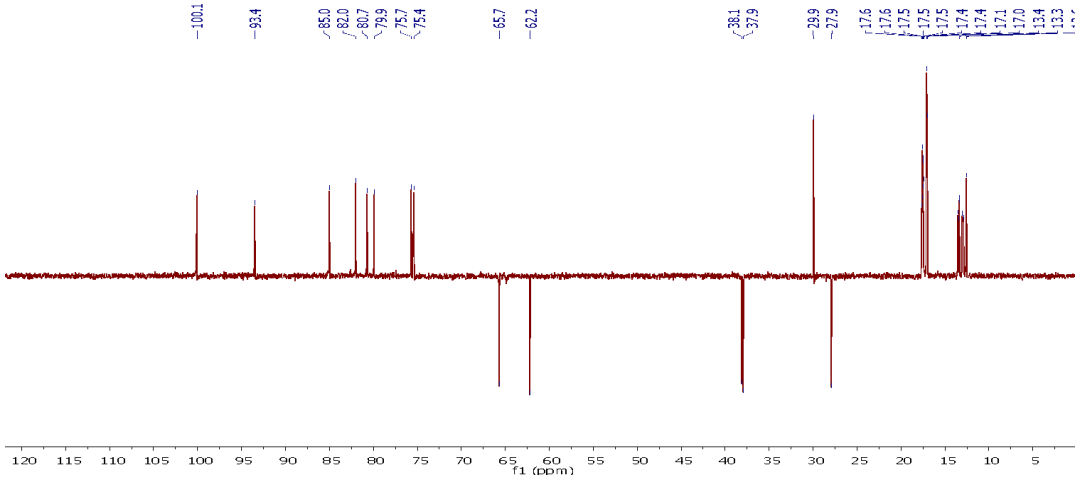
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **32**



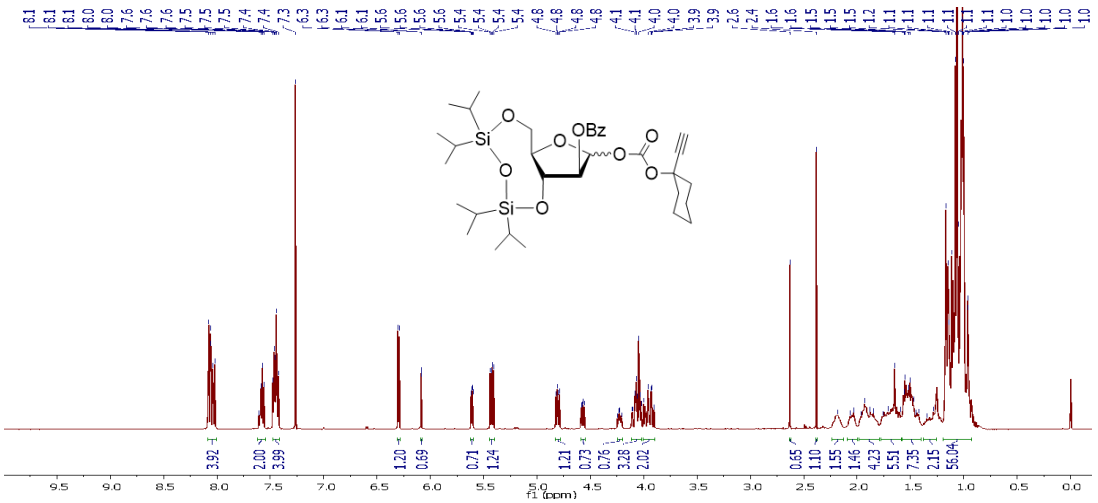
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **32**



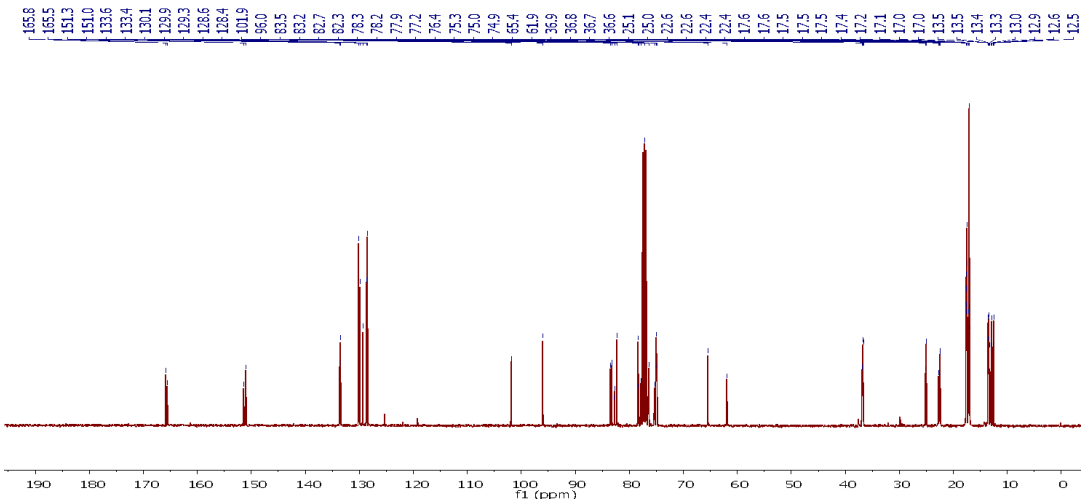
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **32**



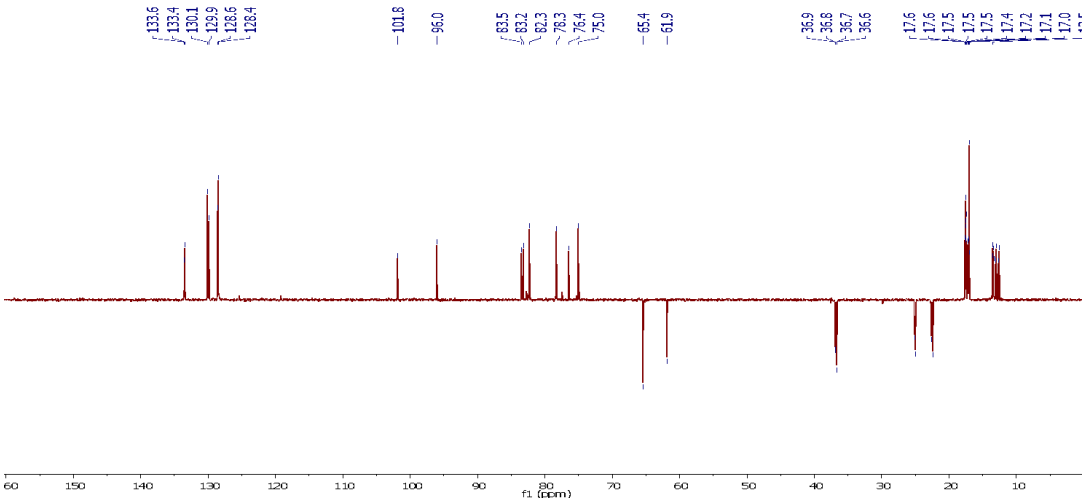
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **33**



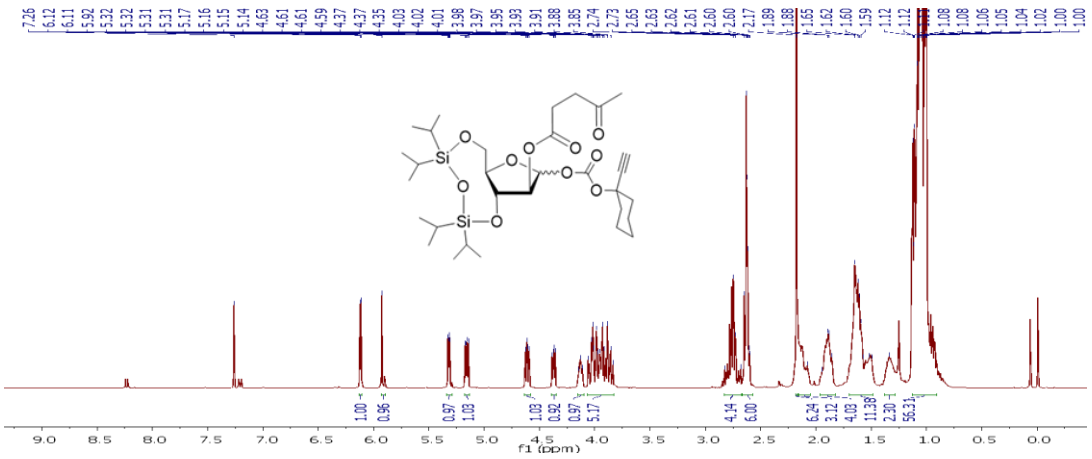
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **33**



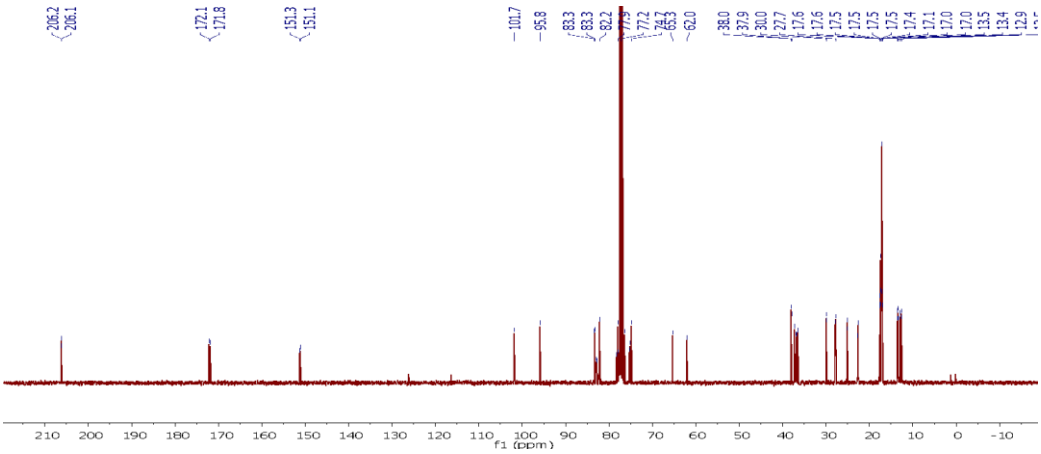
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **33**



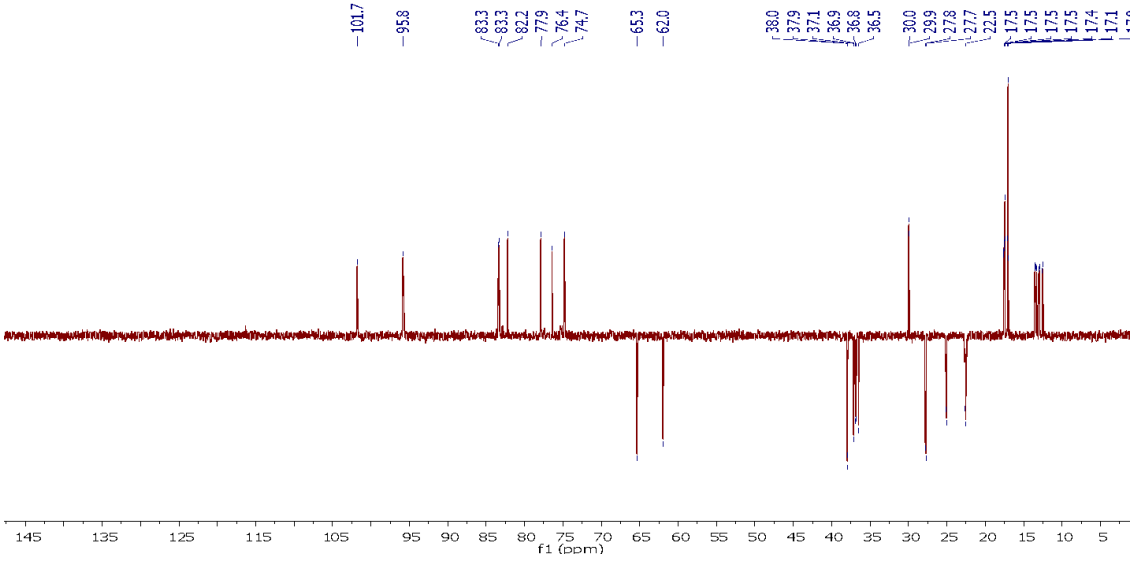
^{1}H NMR Spectrum (400.31MHz, $CDCl_3$) of Compound **34**



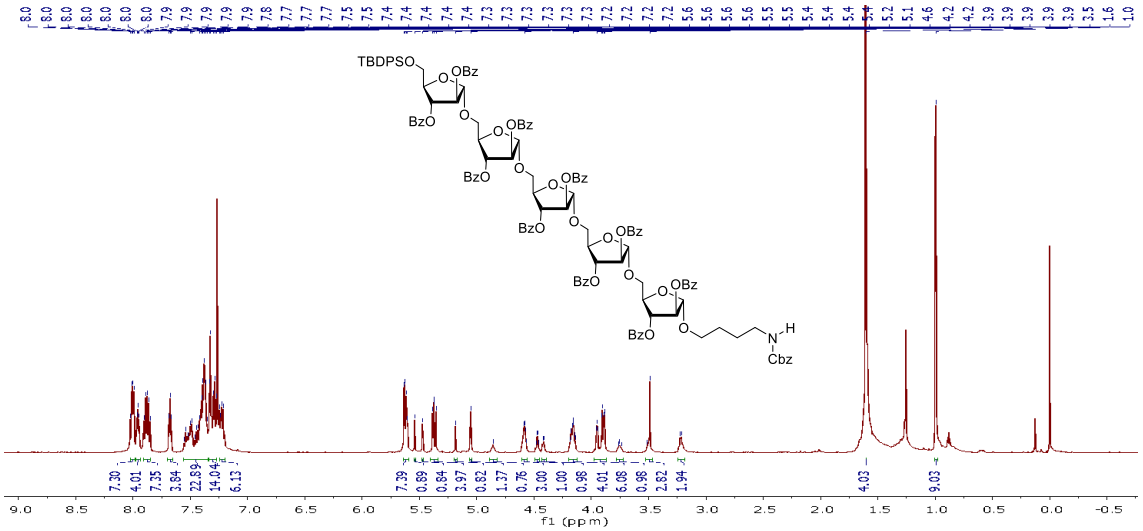
^{13}C NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **34**



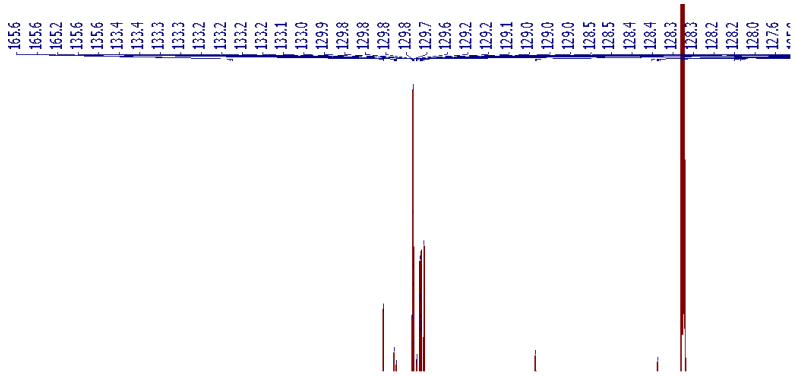
DEPT-135 NMR Spectrum (100.66 MHz, $CDCl_3$) of Compound **34**



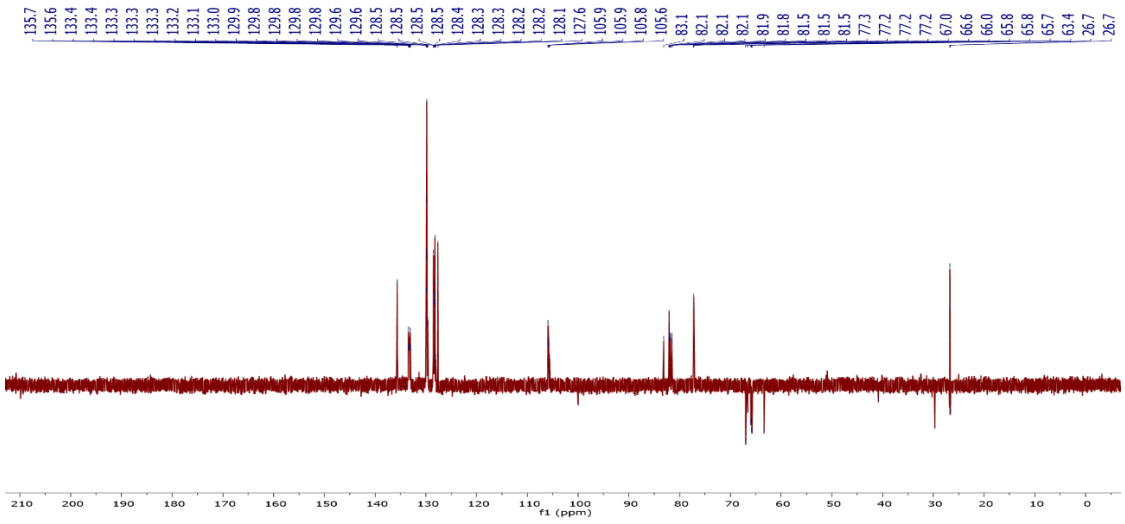
^{1}H NMR Spectrum (600.40 MHz, $CDCl_3$) of Compound **20**



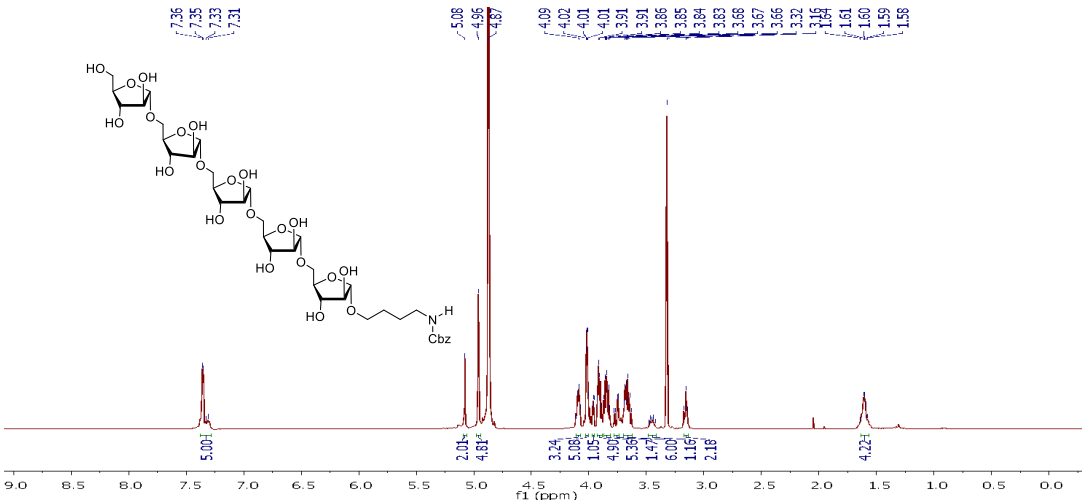
^{13}C NMR Spectrum (150.99 MHz, $CDCl_3$) of Compound **20**



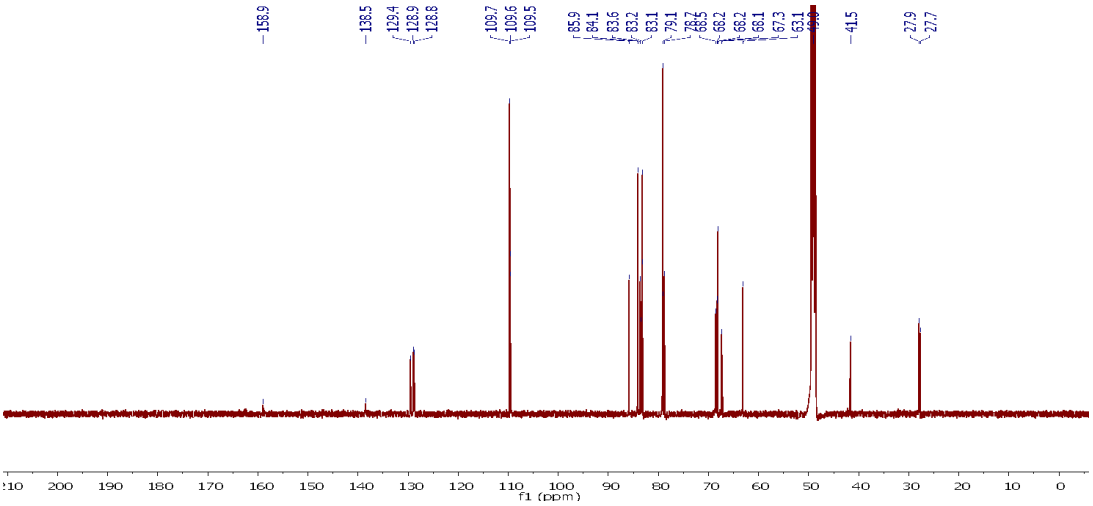
DEPT-135 NMR Spectrum (150.99 MHz, $CDCl_3$) of Compound **20**



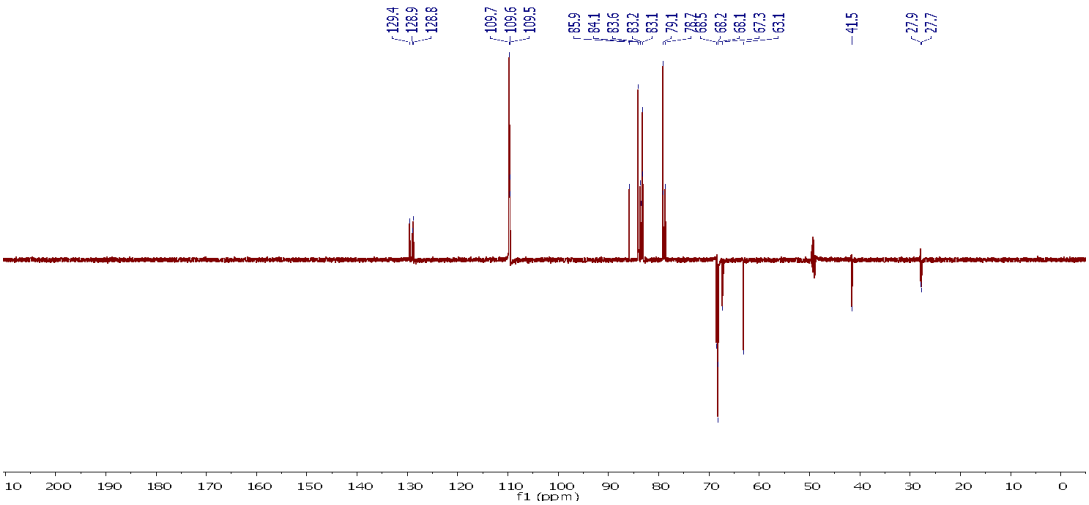
^{1}H NMR Spectrum (400.31MHz, CD_3OD) of Compound **23**



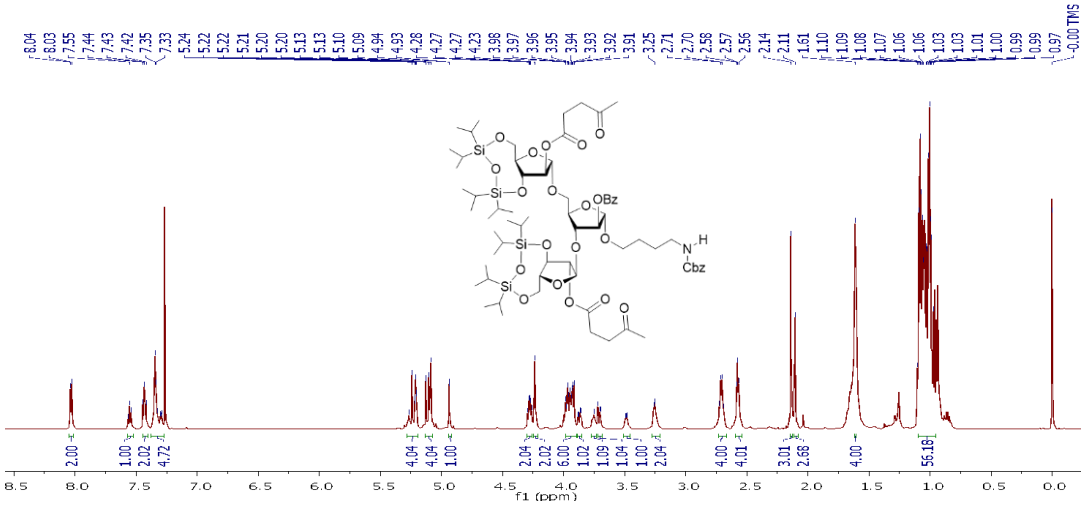
^{13}C NMR Spectrum (150.99 MHz, CD_3OD) of Compound **23**



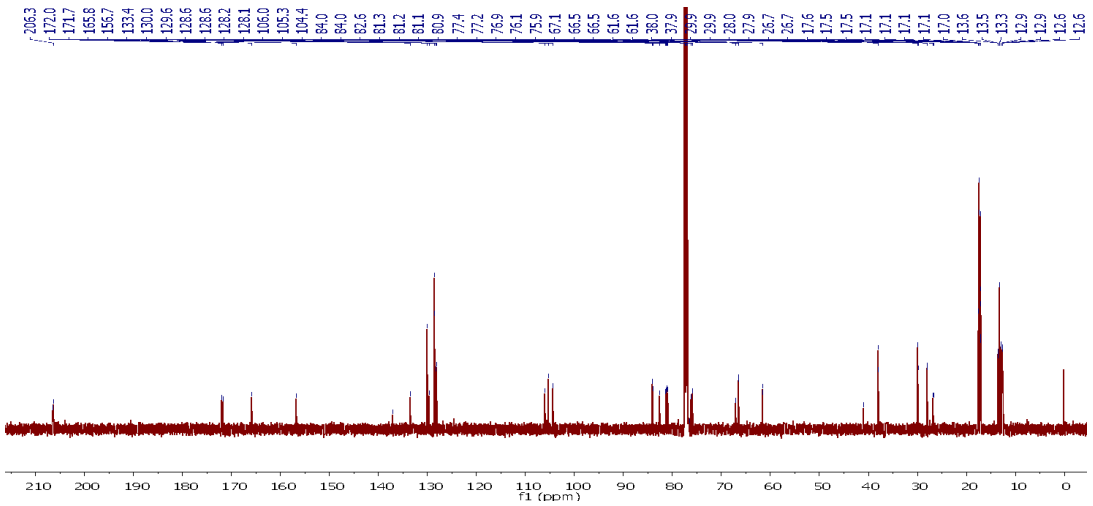
DEPT-135 NMR Spectrum (150.99 MHz, CD_3OD) of Compound **23**



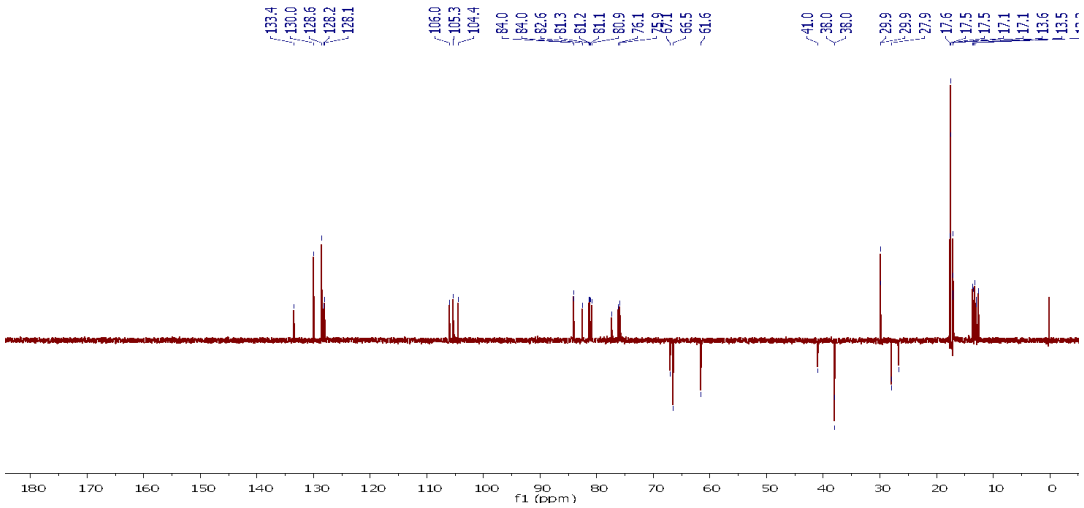
^{1}H NMR Spectrum (600.40 MHz, $CDCl_3$) of Compound **38**



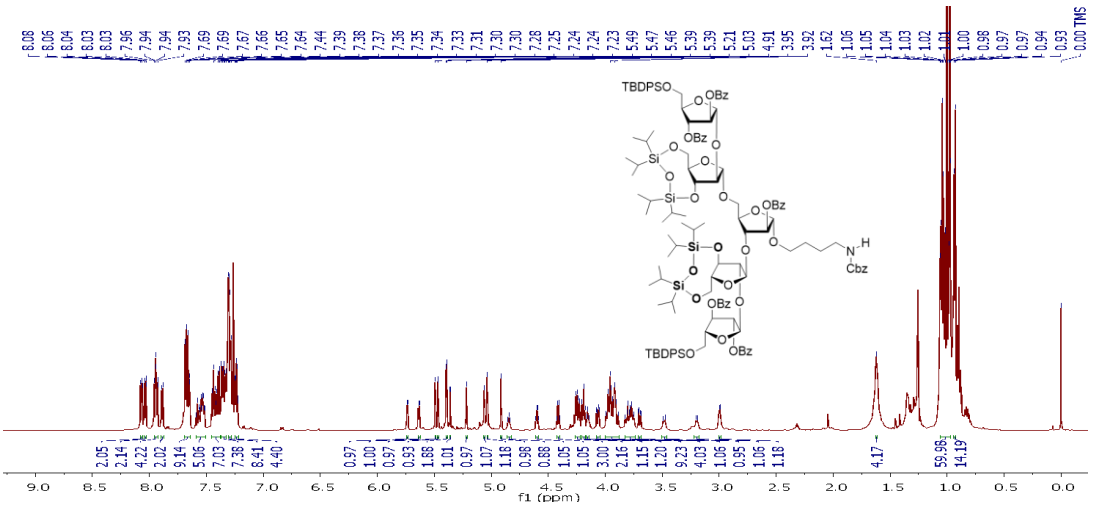
^{13}C NMR Spectrum (150.97 MHz, $CDCl_3$) of Compound **38**



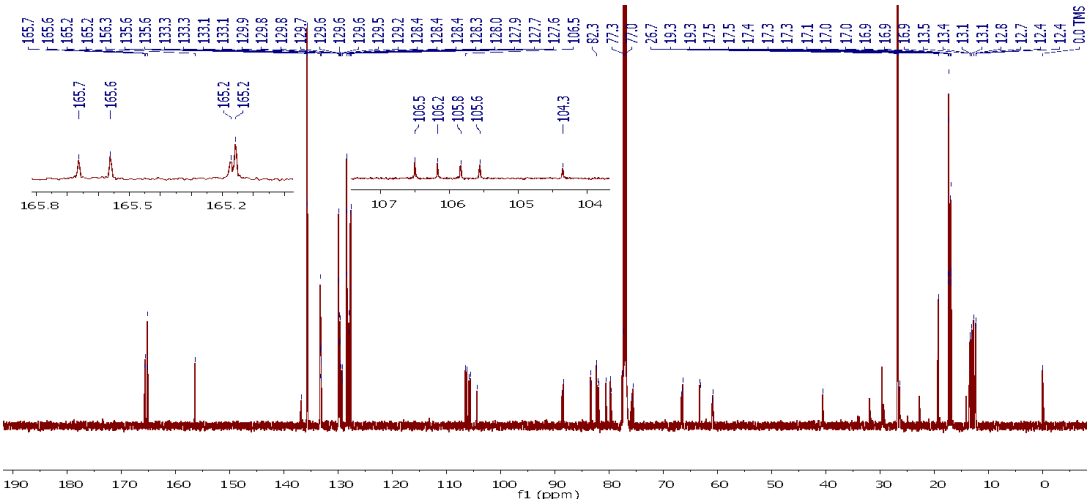
DEPT-135 NMR Spectrum (150.97 MHz, $CDCl_3$) of Compound **38**



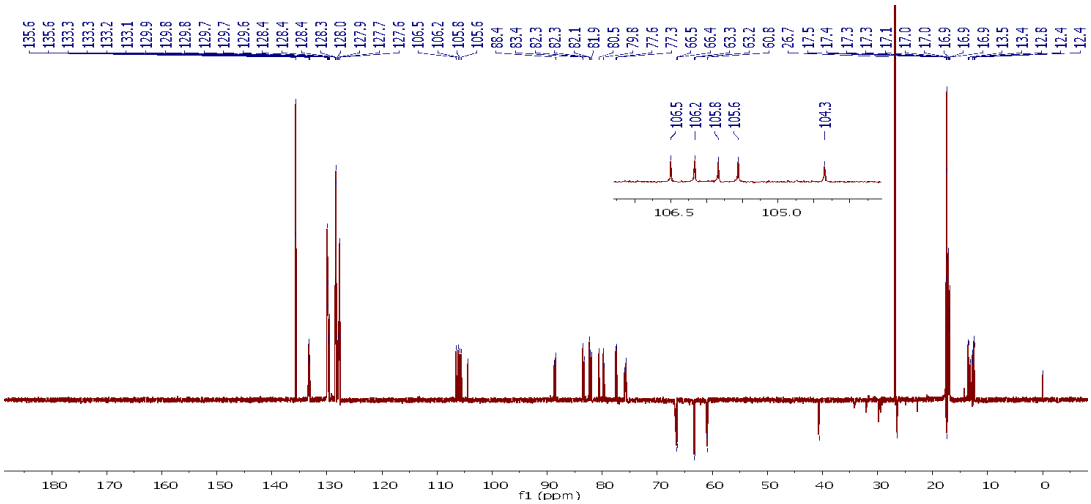
^{1}H NMR Spectrum (600.40 MHz, $CDCl_3$) of Compound **40**



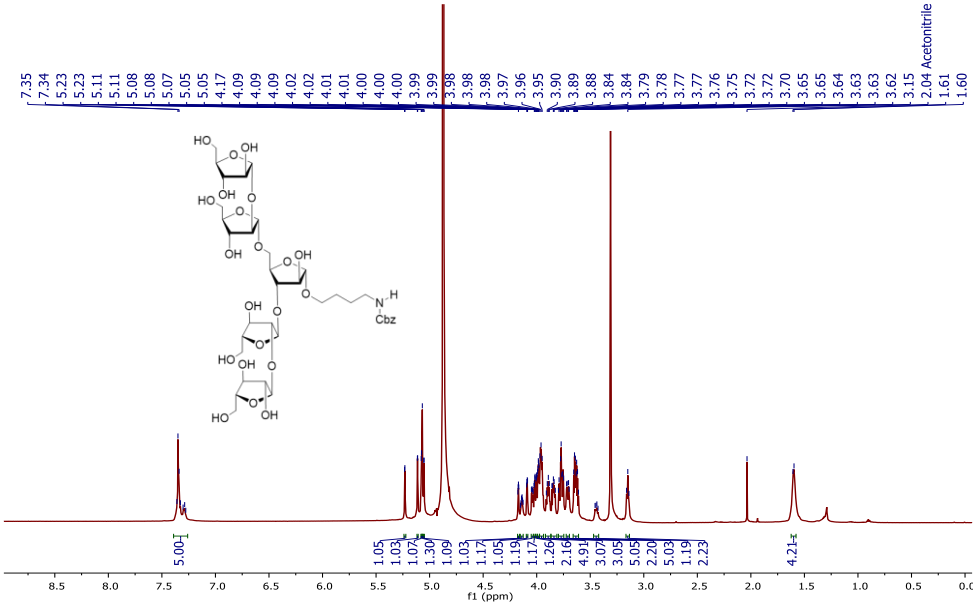
^{13}C NMR Spectrum (150.97 MHz, $CDCl_3$) of Compound **40**



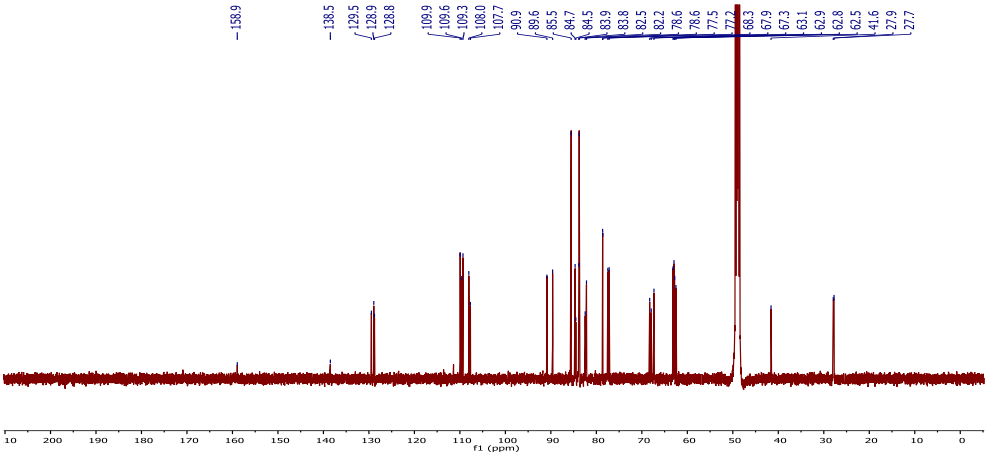
DEPT-135 NMR Spectrum (150.97 MHz, $CDCl_3$) of Compound **40**



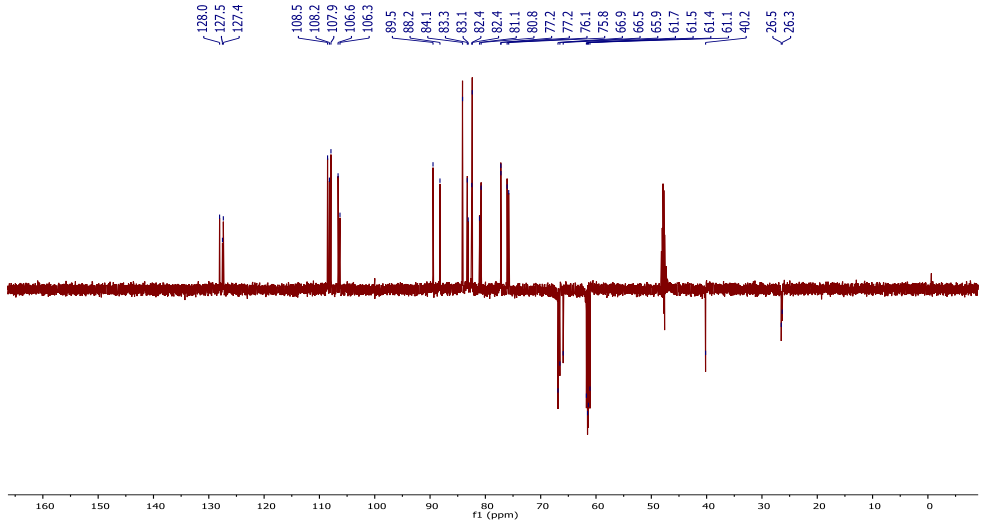
^{1}H NMR Spectrum (600.40 MHz, CD_3OD) of Compound **41**



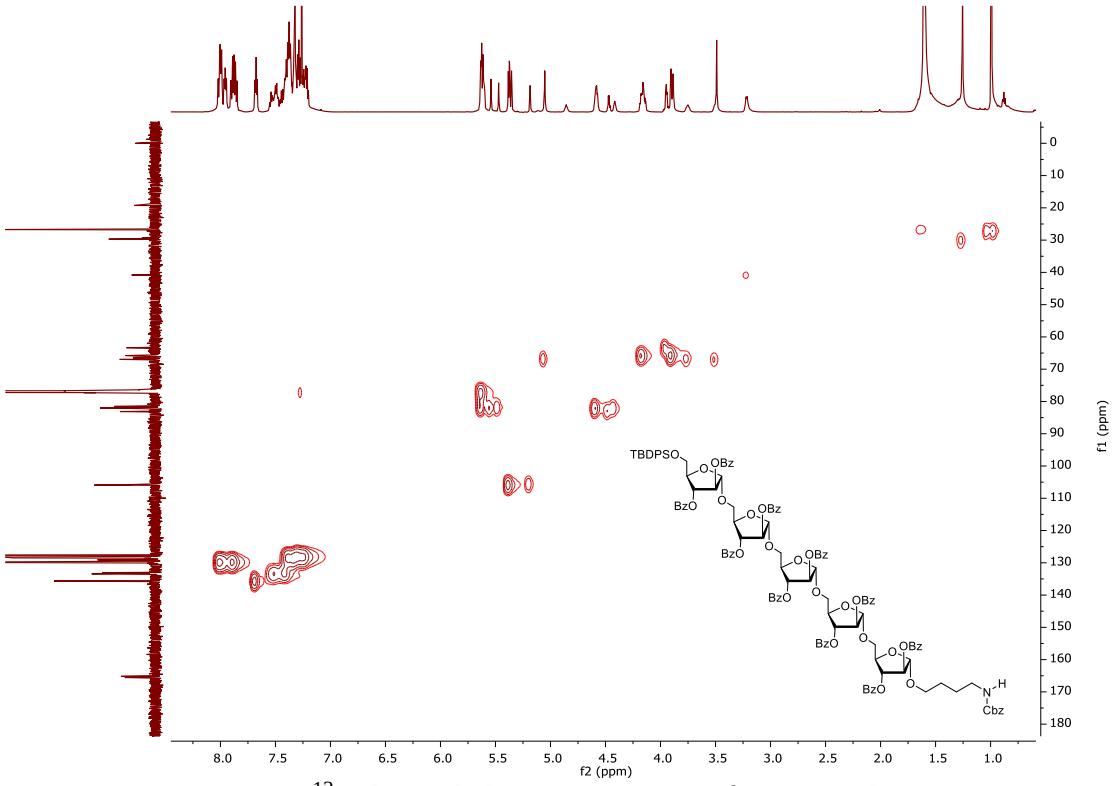
^{13}C NMR Spectrum (150.99 MHz, CD_3OD) of Compound **41**



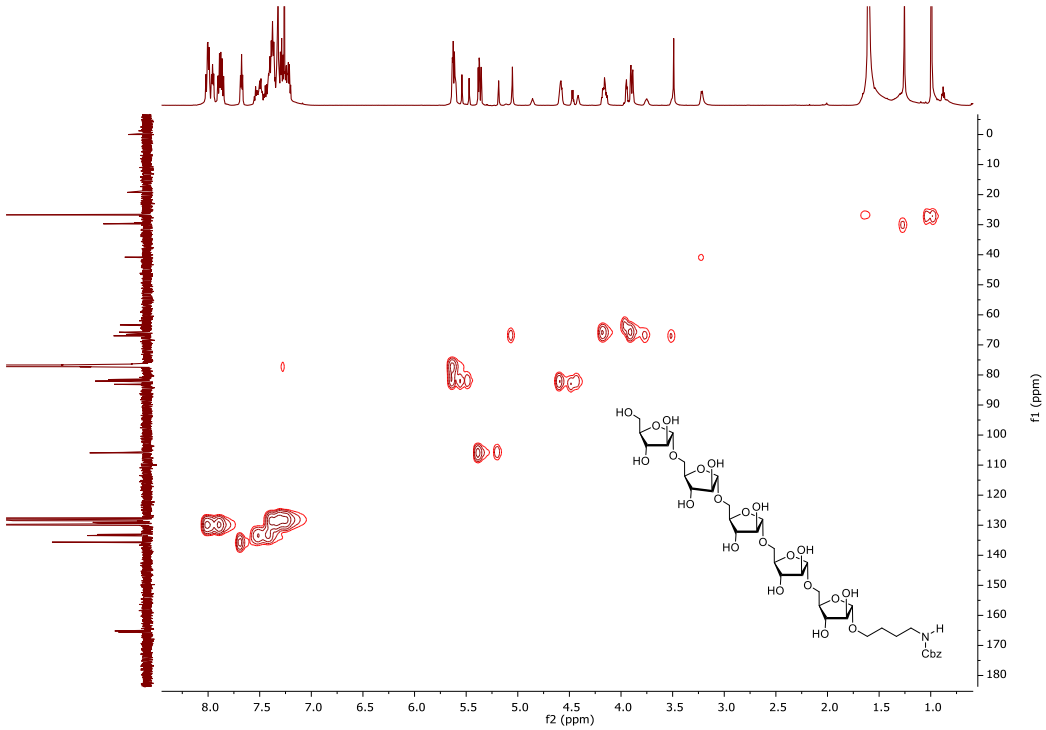
DEPT-135 NMR Spectrum (150.99 MHz, CD_3OD) of Compound **41**



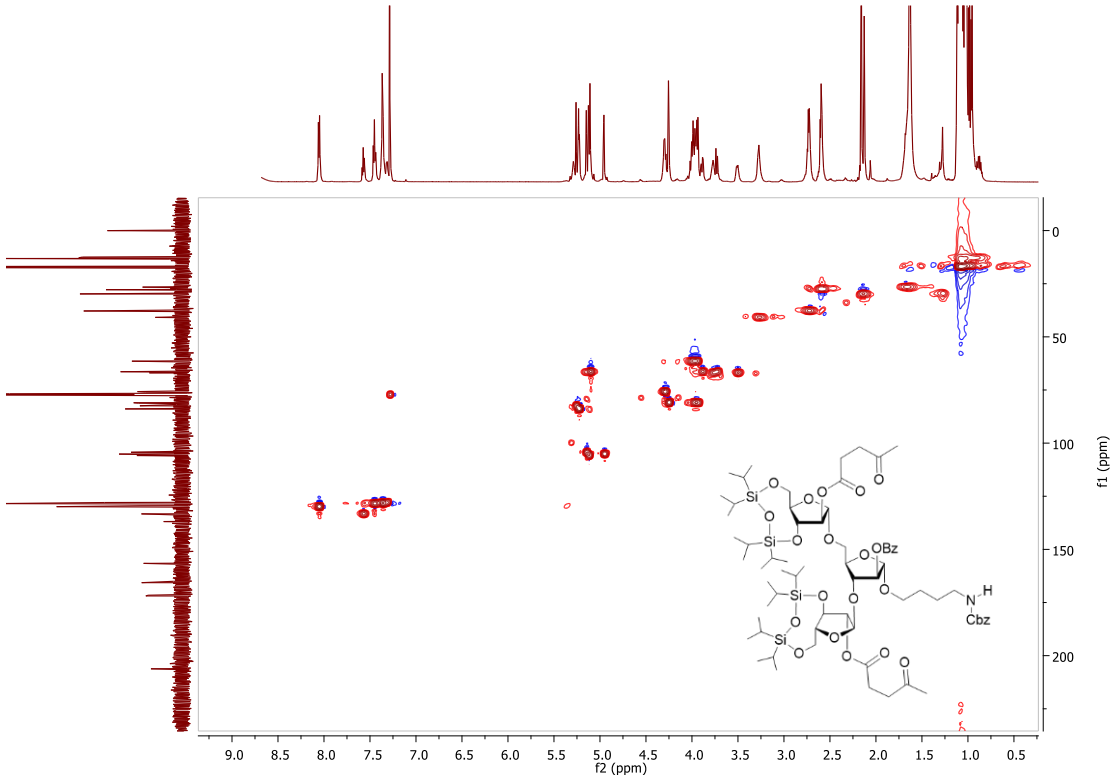
HSQC ^{13}C decoupled NMR spectrum of compound **20**



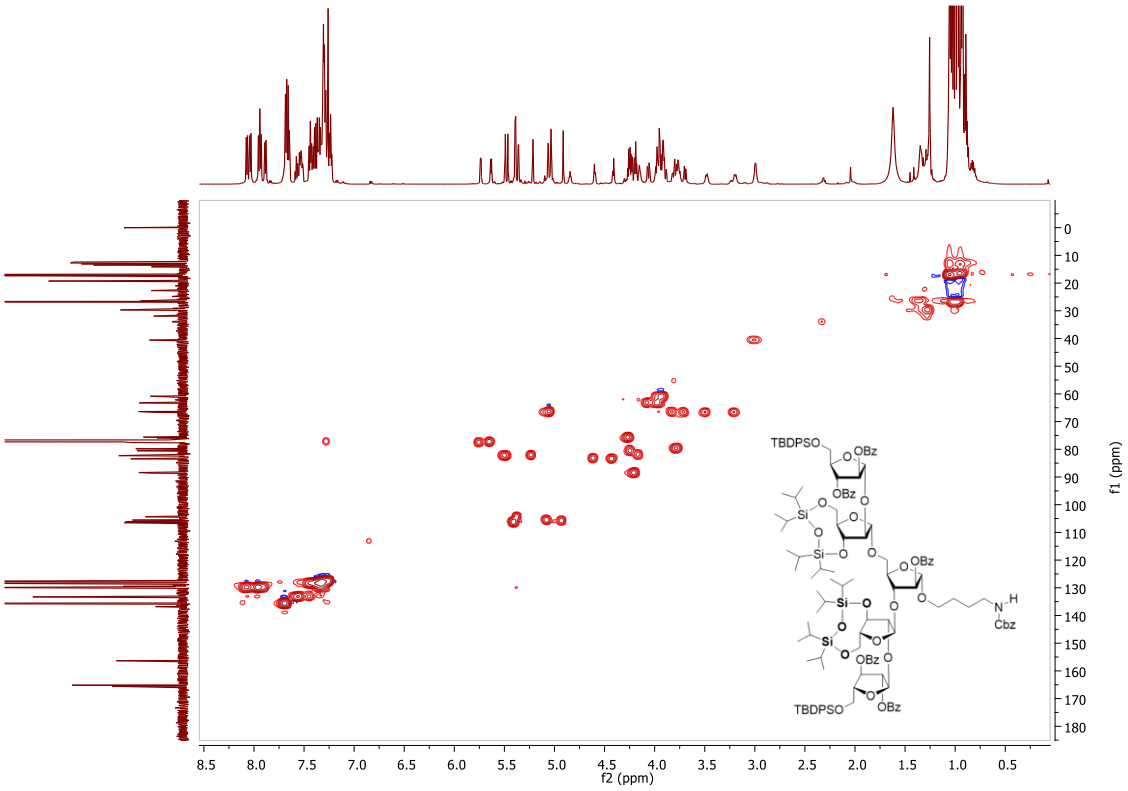
HSQC ^{13}C decoupled NMR spectrum of compound **23**



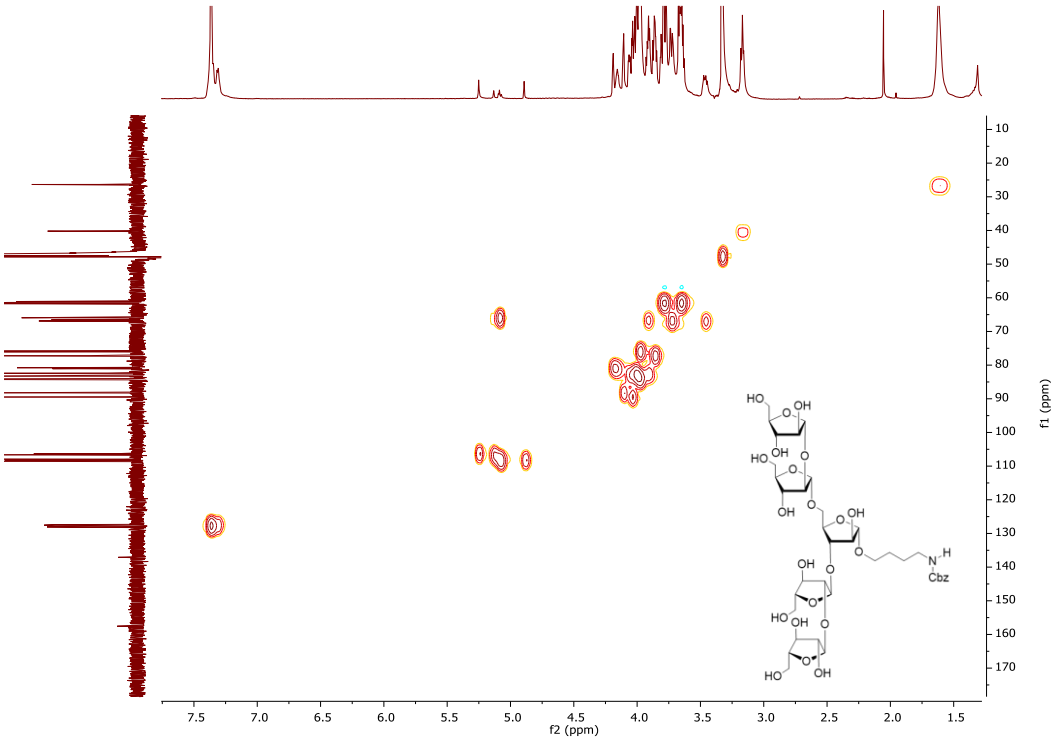
HSQC ^{13}C decoupled NMR spectrum of compound **38**



HSQC ^{13}C decoupled NMR spectrum of compound **40**

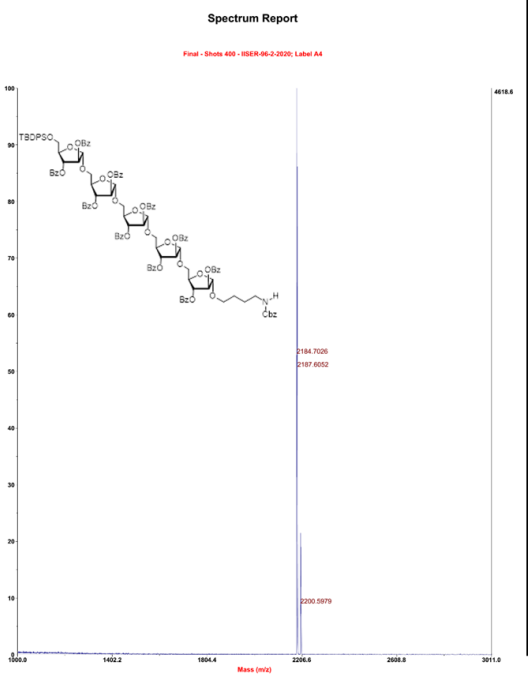


HSQC ^{13}C decoupled NMR spectrum of compound **41**

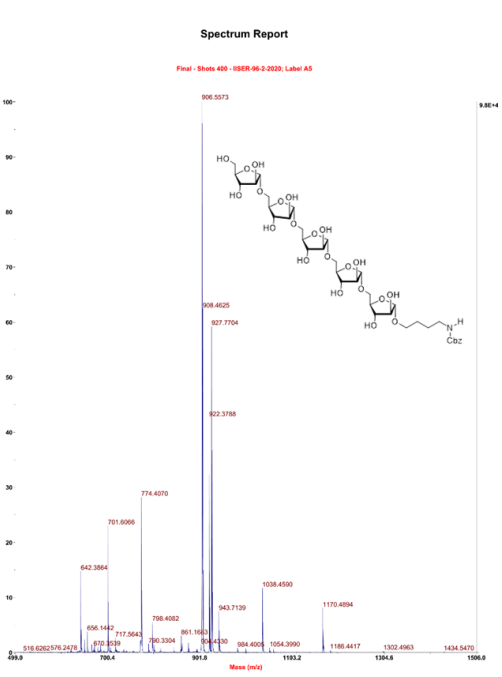


MALDI-TOF Mass spectral charts of compounds

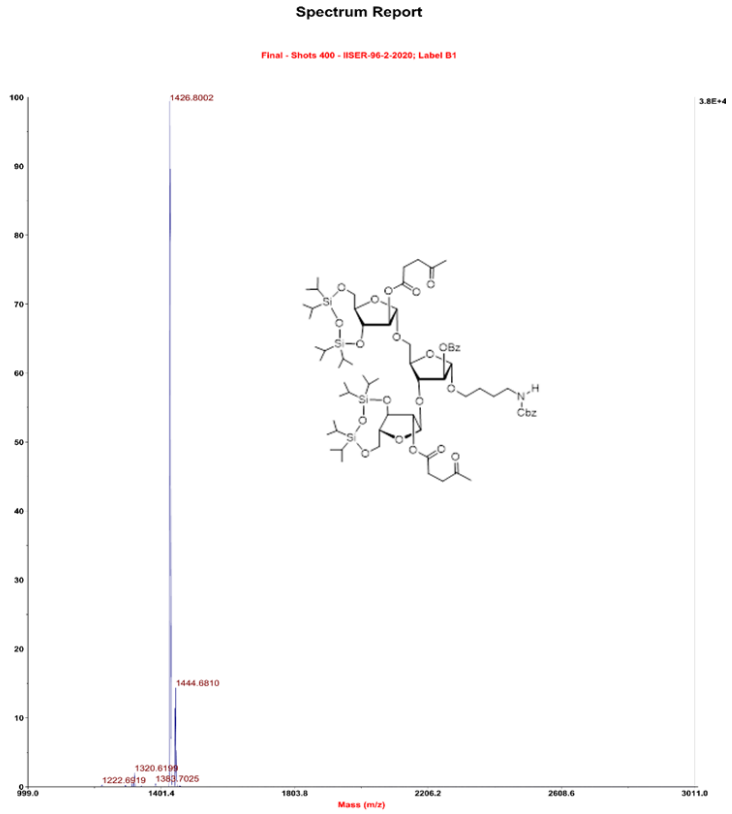
MALDI-TOF Mass Spectrum of Compound **14**



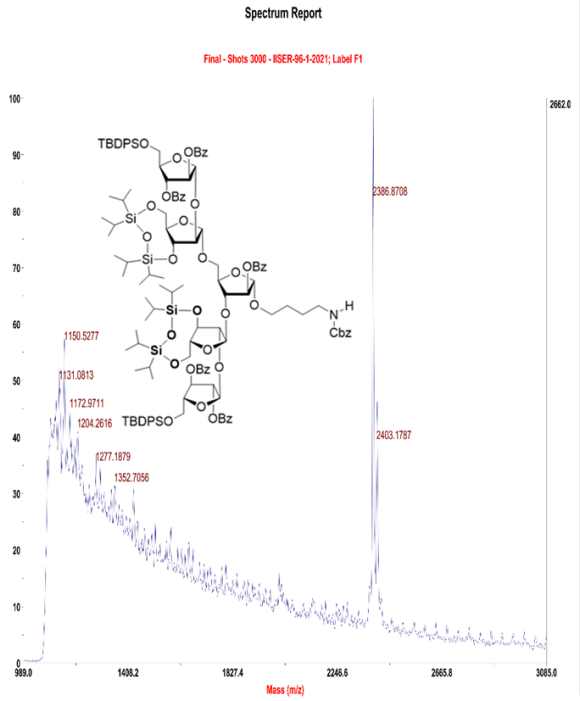
MALDI-TOF Mass Spectrum of Compound **1**



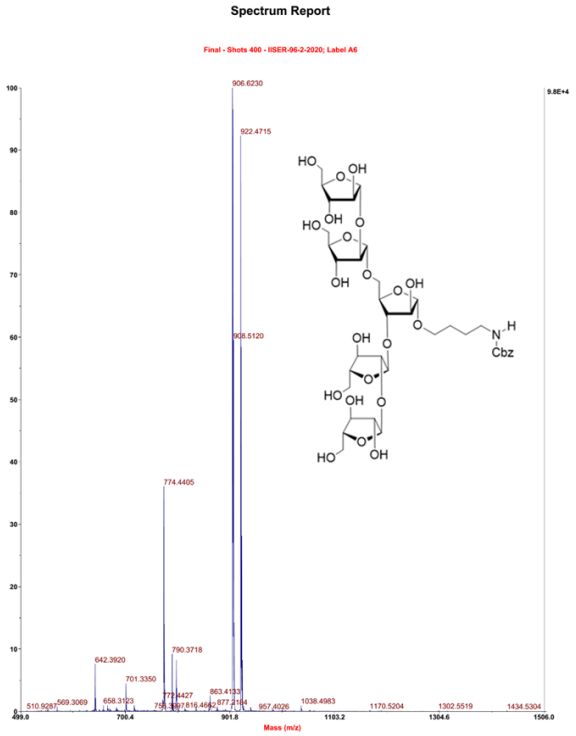
MALDI-TOF Mass Spectrum of Compound **38**



MALDI-TOF Mass Spectrum of Compound **40**



MALDI-TOF Mass Spectrum of Compound **41**



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List of Publications

1. Silver-assisted Gold-catalyzed Solid Phase Synthesis of Linear and Branched Pentaarabinofuranosides.***Yogesh Sutar***, Madhuri Vangla* and Srinivas Hotha* *Chem.Commun.*, **2022**, *58*, 641–644.
2. Silver-Assisted Gold-Catalysed Formal Synthesis of Anticoagulant Fondaparinux Pentasachharide. Gulab Walke, Niteshlal Kasdekar, ***Yogesh Sutar*** and Srinivas Hotha* *Communications Chemistry* **2021**, *4*, 15.
3. Silver Assisted Gold Catalysis for the Preparation of Fondaparinux Pentasachharides and Intermediates. Srinivas Hotha, Gulab Walke, Niteshlal Kasdekar and ***Yogesh Sutar***. Patent No. 202021050409.
4. Glycosylation in the green chemistry era, Sujit Manmode, Saptashwa Chakraborty, ***Yogesh Sutar***, Madhuri Vangala and Srinivas Hotha*, In *Carbohydrate Chemistry,* pp. 131-156, **2021**. (Book Chapter)
5. Transition-Metal-Free C−H Hydroxylation of Carbonyl Compounds. Moreshwar B. Chaudhari, ***Yogesh Sutar***, Shreyas Malpathak, Anirban Hazra, and Boopathy Gnanaprakasam*, *Org. Lett.* **2017**, *19*, 13, 3628–3631.
6. Establishing shortest chemical route for the synthesis of Molnupiravir from Ribose and Cytosine. Saptashwa Chakraborty, ***Yogesh Sutar***, Jayashree Rajput and Srinivas Hotha* (Manuscript under preparation)

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Silver-assisted gold-catalyzed solid phase synthesis of linear and branched oligosaccharides†

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Unlike solid phase synthesis of peptides, synthesis of oligosaccharides by solid phase methods is lagging behind owing to inherent challenges faced while executing glycosidations. In this communication, silver-assisted gold-catalyzed glycosidations are found to be excellent for solid phase oligosaccharide synthesis. Glycosidations under catalytic conditions, one time coupling with four equivalents of donor, reactions in less than 30 min at 25 °C and on-resin deprotection of silyl and benzoate protecting groups are the salient features. Photocleaved glycans possess a protected amino functionality that can be utilized for bioconjugation. The versatility of this approach is established by synthesizing linear and branched pentaarabinofuranosides.

Recent and rapid studies in glycobiology have heightened the need for pure and homogenous glycoconjugates; thereby the demand for versatile platforms to synthesize glycans has been felt now more than ever.[1–3] Synthesis of oligosaccharides in the solution phase is still a challenging task owing to the formation of unwanted side products that increase the number of tedious purifications in the iterative glycosidation–deprotection–glycosidation cycles.[4] Solution phase synthesis offers good quality control in all steps though it suffers from inherent difficulties with chromatographic separations. In this context, Merrifield pioneered solid phase synthesis through carboxylic acid activation that transformed the syntheses of peptides;[5,6] however, adopting glycosidation for solid phase synthesis is a very challenging task as the universal glycosidation chemistry is yet to be established.[7] Solid phase synthesis demands highly efficient and fast reactions with no side products from the solid phase bound substrate.[8] Unlike peptides, carbohydrates have multiple hydroxyls, whose pK_a values are very similar and hence, multiple orthogonally cleavable protecting groups such as esters and ethers are invoked.[9] This adds to the overall complexity of performing these reactions on solid-supports. Nonetheless, automation of the glycan synthesis by parallel or sequential synthesizers either by chemical or enzymatic approaches in solution or the solid phase has been explored by several investigators. Among them, commercially available glycan synthesizer Glyconeer 2.1 by the Seeberger group[10] and a HPLC-A set-up by Demchenko[11] are unique, whereas Pohl's[12a] adaptation of the ChemSpeed parallel synthesis platform and Nokami's[12b] automated solution-phase synthesis of oligosaccharides exploiting the electrochemical assembly of thioglycosides are noteworthy. Some of these automated platforms require trained human resources and are often expensive; and therefore, a robust manual solid-phase synthesis platform is highly desirable.

A number of conditions are to be fulfilled in order for a reaction to be suitable for adapting to solid phase synthesis due to the reduced mass transfer and difficulties in purification of compounds on solid-supports.[13] We envisioned that the recently reported silver-assisted gold-catalyzed glycosidation repertoire is suitable for the solid phase synthesis of oligosaccharides since most of the reactions are completed in under 15 min, produce the desired glycosides in more than 90% yields, are homogenous reactions, and are not super moisture-sensitive, and the catalysts are highly soluble in organic solvents.[14] Therefore, [Au]/[Ag]-catalyzed glycosidations qualify for manual solid phase oligosaccharide synthesis. In addition, gold-catalyzed glycosidations have been rarely explored for solid phase synthesis to the best of our knowledge.

To demonstrate the power of silver-assisted gold-catalyzed glycosidations by solid phase methods, oligoarabinofuranosides (**1** and **2**) are considered due to our continued interest in the chemical synthesis of cell wall epitopes of *Mycobacterium tuberculosis* (MTb).[15–18] In addition, the polysaccharide component of the MTb cell wall is well established though the biophysical significance is not fully studied perhaps due to the lack of a combinatorial library of glycans. We have targeted to synthesize a linear pentaarabinofuranoside (**1**) that is present in the cell wall of MTb and an unnatural branched pentasaccharide (**2**) from monosaccharide donors **3–5** (Fig. 1).

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Silver-assisted gold-catalyzed formal synthesis of the anticoagulant Fondaparinux pentasaccharide

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Clinically approved anti-coagulant Fondaparinux is safe since it has zero contamination problems often associated with animal based heparins. Fondaparinux is a synthetic pentasaccharide based on the antithrombin-binding domain of Heparin sulfate and contains glucosamine, glucuronic acid and iduronic acid in its sequence. Here, we show the formal synthesis of Fondaparinux pentasaccharide by performing all glycosidations in a catalytic fashion for the first time to the best of our knowledge. Designer monosaccharides were synthesized avoiding harsh reaction conditions or reagents. Further, those were subjected to reciprocal donor-acceptor selectivity studies to guide [Au]/[Ag]-catalytic glycosidations for assembling the pentasaccharide in a highly convergent [3 + 2] or [3 + 1 + 1] manner. Catalytic and mild activation during glycosidations that produce desired glycosides exclusively, scalable route to the synthesis of unnatural and expensive iduronic acid, minimal number of steps and facile purifications, shared use of functionalized building blocks and excellent process efficiency are the salient features.

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Glycosylation in the green chemistry era

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According to the IUPAC definition, Green Chemistry or sustainable chemistry is the ''design of chemical product and processes that reduce or eliminate the use or generation of substances hazardous to humans, animals, plants and the environment.'' John. C. Warner and Paul Anastas formulated 'twelve principles' in 1998 to guide the practice of green chemistry. Traditional carbohydrate synthesis generally employs usage of multiple protection and deprotection steps, harmful chemicals, solvents and many harsh conditions which unpleasantly impact health and environment. So new greener methods for the syntheses of glycoconjugates are in dire need and therefore gaining prominence recently. Though the majority of carbohydrate-based compounds are synthesized *via* chemical methods, a greener alternative which is an enzymatic method for synthesis has also been investigated over past two decades. Polar green solvents, such as water, supercritical fluid, ionic liquid (IL), are generally used in greener methods for carbohydrate synthesis. This chapter aims to bring challenges in developing greener methods for the glycosylation.

1 What is green chemistry?

Green chemistry which is gaining more popularity as the sustainable chemistry in very simple terms is just an alternate way of thinking about design, development and implementation of chemical products and processes by reducing waste, conserving the energy and replacement of hazardous substances.[1] Some of the overarching goals of the green chemistry are (i) use and recycle available resources while developing a chemical process; (ii) reduce the waste generation in all processes that need chemicals; (iii) implementation of catalytic processes wherever possible or develop newer catalytic protocols wherever those are not available yet; (iv) replace all toxic reagents, intermediates and products with safer alternatives; (v) reduce the energy requirements of a process by developing energy efficient methods. In this context, Warner and Anastas have proposed twelve guiding principles[2] in order to achieve these very ambitious goals and they have become mantras or gold standards in green chemistry (Table 1).

One of the most significant issues of synthesizing glycosides is the generation of chemical waste since most of the methods to synthesize carbohydrates in large quantities depend on chemical methods which demand use of multiple protection and deprotection steps.[3] Therefore, the process generates a lot of steps, increases the waste, and decreases the atom economy and hence, process efficiency drops substantially. At this point, a brief discussion on what are glycosides/oligosaccharides and what is glycosylation is very important.

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Transition-Metal-Free C−H Hydroxylation of Carbonyl Compounds

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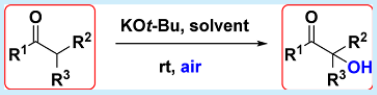
Supporting Information

ABSTRACT: Transition metal and reductant free α-C(sp3)−H hydroxylation of carbonyl compounds are reported. This method is promoted by commercially available inexpensive KO-*t*-Bu and atmospheric air as an oxidant at room temperature. This unified strategy is also very facile for hydroxylation of various carbonyl compound derivatives to obtain quaternary hydroxyl compounds in excellent yield. A preliminary mechanistic investigation, supported by isotope labeling and computational studies, suggests the formation of a peroxide bond and its cleavage by in situ generated enolate.



The oxidation reaction is a fundamentally important transformation in organic synthesis by which hydrocarbons are converted into valuable oxygenated products as feedstocks for chemical and pharmaceutical industries.[1] Of these reactions, α C−H hydroxylation of α-substituted carbonyl compounds to obtain quaternary α-hydroxyl carbonyl compounds obtains more attention in synthetic community since such a quaternary α-hydroxyl functionality is a central motif in all facets of chemistry ranging from several natural products (Figure 1) to synthetic drugs such as tephrosin,[2a] doxycycline,[2b] bicalutamide,[2c] aryloxindole,[2d] and donaxaridine.[2e] Furthermore, this type of quaternary α-hydroxyl carbonyl compounds serves as an efficient photoinitiator in the coating industry.[3a,b] Similarly, the hydroxyl derivatives of barbituric acid are responsible for improving the durability of a polarizing plate.[3c] While significant progress has been documented for the oxidation of the C−H bond by catalytic/noncatalytic approaches in the presence of various oxidant sources, there is an increasing demand for more environmentally benign approaches avoiding the use of expensive metal catalysts, hazardous stoichiometric oxidants, and reductants. Recently, the direct hydroxylation of C(sp^3)−H bond with a stoichiometric amount of oxidants such as oxaziridine, cumene hydroperoxide (CHP), PIDA, PIFA, TBHP, Oxone, and H_2O_2, etc. was reported, which suffer from the ease of handling and other hazards (Scheme 1).[4] The consumption of molecular oxygen as an oxidant accessible from air and utilization for oxygen incorporation in organic synthesis has attracted substantial attention.[5,6]

Remarkable progress has been made using transition-metal catalysis with molecular O_2 for selective C−H hydroxylation of carbonyl compounds.[7] The use of O_2 for the synthesis of quaternary α-hydroxyl carbonyl compounds was first described by Ritter and co-workers using dinuclear palladium complex with a special base hppH.[8] Interestingly, to minimize the use of metal, Jiao and co-workers developed an elegant method for C−H hydroxylation catalyzed by Cs_2CO_3, required a phosphine reductant.[9] Zhao's group reported an enantioselective C−H hydroxylation by using dimeric cinchona alkaloid-derivative in the presence of aqueous alkali and phosphine reductant.[10] Very

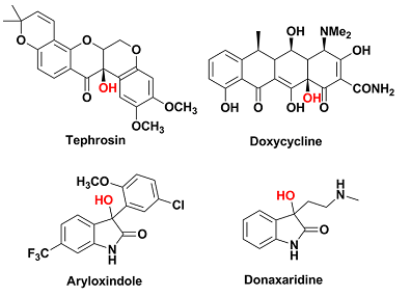
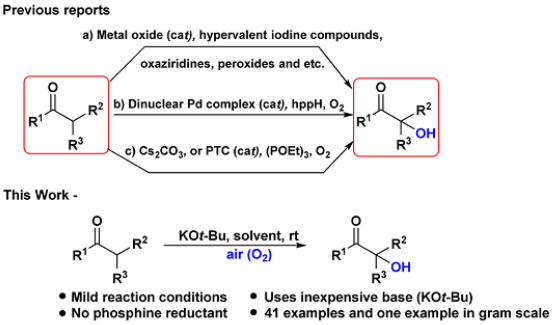


Figure 1. Quaternary hydroxyl biologically active molecules.

Scheme 1. State of the Art on C−H Hydroxylation



recently, Schoenebeck et al. developed and elucidated the use of metal oxide (Cu_2O) along with special base (hppH) for C−H hydroxylation and C−C cleavage using experimental and computational studies.[11] In general, the key methodologies to achieve the synthesis of such compounds involve either hazardous oxidants or transition metals or metal oxide and additive (phosphine as a reductant) which were the drawback for above

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