Gold-catalyzed Glycosidation for Glycoaminoacids and Glycopolypeptides

THESIS

Submitted to the

UNIVERSITY OF PUNE

For the degree of

DOCTOR OF PHILOSOPHY

In CHEMISTRY

By

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(Research Guide)

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APRIL 2013

Dedicated to

Mother, Father & Sisters….

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CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "***Gold-catalyzed Glycosidation for Glycoaminoacids and Glycopolypeptides***" which is being submitted to the ***University of Pune*** for the award of ***Doctor of Philosophy*** in ***Chemistry*** by ***Mr. Ashif Yasin Shaikh*** was carried out by him under my supervision at the National Chemical Laboratory, Pune. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

April 2013
Pune

Dr. Srinivas Hotha
(Research Guide)

Candidate's Declaration

I hereby declare that the research work incorporated in the thesis entitled "***Gold-catalyzed Glycosidation for Glycoaminoacids and Glycopolypeptides***" submitted for the degree of ***Doctor of Philosophy*** in ***Chemistry*** to the ***University of Pune***, has been carried out by me at CSIR- National Chemical Laboratory, Pune, India, from August 2007 to April 2013 under the supervision of Dr. Srinivas Hotha. This work has not been submitted in part or full by me for a degree or diploma to this or any other University or Institution.

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Acknowledgments

We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for humanit. This thesis would not have been completed without the encouragement and co-operation of my teachers, parents, friends, well-wishers and relatives. I take this opportunity to express my deep gratitude to one and all.

Let me take this opportunity to express my genuine gratitude from bottom of heart to my research supervisor Dr. Srinivas Hotha who introduced me into this fascinating realm of sweet (carbohydrate) chemistry and freedom throughout my research. I am very much grateful to him for his valuable guidance, suggestion how to improve presentation skills and everlasting encouragement throughout my course. I am certain that the ethics and moral values which I learnt from him will go a long way in making me a better human being.

I wholeheartedly extend my sincere thanks to active collaborator in CSIR-NCL Dr. Sayam Sen Gupta introducing me to fascinating field of Glycopolypeptide Chemistry, suggestion, discussion and to improve independent thinking of application of research work before starting project. I would like to thanks all group members of Dr. Sayam Sen Gupta for their support & help and especially to Debasis Pati for guiding me to ho to do NCA-polymerization reactions.

I thank our Head, Division of Organic Chemistry, Head, Chemical Engineering & Process Development and Director CSIR-NCL for providing infrastructure facilities. I sincerely acknowledge CSIR, New Delhi for the award of research fellowship. I wish to deep sense of gratitude to my M.Sc. project guide Dr. S.P. Chavan & Dr. U. R. Kalkote for their valuable guidance, encouragement, stimulating discussion and gain initial knowledge of peptide coupling, orgnanic synthesis & purification techniques during the course of my project. I thank Mahesh Sonar, Dhanajay, Preeti, Sharad, Sachin & Sayali for their kind cooperation and guidance during the course of the M.Sc project and treating me as friend. I also want to give my sincere thank to Dr. A.R. A. S. Deshmukh and Dr.V.K Gumaste for their support & encouragement during my project assistance stay in CSIR-NCL and for introduction to β-lactam chemistry.

I also thank all DOC students, staff members for their timely help throughout. The work would not have been complete without the help provided by the analytical groups like NMR, IR, microanalysis, mass spectroscopy and HRMS. I sincerely thank Dr. P. R. Rajmohanan for smooth running of NMR facility which is primary source of getting information about what we have synthesized without one cannot

proceed further. Help of Digital Information Resource Center (DIRC) and library staff members has been also acknowledged. I also thank Prof. Kusurkar for their helpful discussions and suggestions.

I am thankful to Director Indian Institute of Science Education and Research (IISER-Pune) for allowing to work a nd use infrastructure facilities during my stay. Also I thanks to all my friends in IISER-Pune for their help and cheerful atmosphere in lab.

I am thankful to my mentors from School and College for their inspirational teaching, ethics and discipline. I sincerely thank Dr. Md. Qurdratullah (late), Dr. G. M. Nazeruiddin, Dr.(Mrs.) S. T. Mulla, Prof. M. Rafique Sarkhawas, Prof. Alamgir Shaikh, Prof. Iqbal, Prof.Harun, Prof. Iliyas, Prof. H Sonawane & other staff from chemistry department, Poona College (Affiliated to University of Pune) Camp, Pune for their encouragement. I would like give my special thanks to Prof. M. S. Wadia for teaching us organic chemistry and I have never seen very enthusiastic and passionate teacher in my life. I am also thankful to my lecturers and professors from Tuljaram Chaturchand College, Baramati (M.S. Affiliated to University of Pune) . I would like also thanks especially to Prof. S. Salunke Prof. S. Kale, Prof. K. Dhamal, Prof. B. Torne & Prof. H. M. Wadde (HOD) for inspiration and teaching organic chemistry. I would like thanks Prof. M. Godse for teaching 'Unity and Discipline' & friends during my stay in National Cadet Corps (NCC)

It is hard to find a lab better well-knit as a unit than the one I was blessed with an opportunity to work in. I had wonderful seniors in Sudhir, Ashish, G. Suresh Kumar and Srinivas Rao (srini) who, apart from tuning my skills, made me feel at home right from day one in the lab. The co-operation and support extended by Abhijeet, Shivaji (Maharaj), Venkateshwara Rao, Maidul Islam, Abhik and Bijoynand have been exemplary, to say the least, and I cherished their company throughout.

I was lucky to have some really nice seniors in all of NCL during my stay here, Aarif, Merajuddin, Noor, Shabab, Shadab, Ansari, Asad, Imran, Mujahid (Mujju Kha), Ruby, Rehaman & Rita, Kishor, Pinnak, Prakash, Vaijyanti, Prasanna, Nishant, Rama, Malli, Pushpesh, Abhijit, Ravi, Manmath, Deepak, Ganesh, Gauri, Amol, Arup, Ramesh, Mahesh, Abasaheb, Kishor Harale, Lalit, Rahul, Sujit, Swaroop, Kannan, Somesh, Rishi to name just a few. I am also thankful to my friends Ankush, Sangram, Tukaram, Roshana, Kheria,Vijay, Nilesh, Sumanta, Prakash, Pradeep, Sutar, Anand, Krishanu,Pankaj & Jyoti, Laha, Krunal, Indra, Anuj, Ajay, Rupesh, Dipesh, Roshan, Saikat, Nilofer, Pankaj, Swati, Kiran, Bhaushaheb & all my other colleagues for their cheerful support, co-operation and making my stay at NCL very comfortable and memorable one.

My stay here was made livelier by GJ hostel, a hostel unique in ways more than one. It provided the perfect atmosphere to spend leisure time and I thoroughly enjoyed the gardening in hostel & planted tree to make green environment, which will help to compensate pollution created during my Ph.D. work. It was a

pleasure to share room with Pitamber and Kundan.I owe a big "thanks" to all my friends who made GJH such a wonderful place to stay in.

Friends make things go easy and life beautiful. Indeed I am bleseed to have friends like Abhijeet, Ankush, Mandeep (3idiot), Varun (3idiot), Brij, Saikat and Krunal. Manndeep is very special kind of person who will never listen anyone and whereever he goes makes atmosphere cheerful & enjoyable like 'sardar' always do. Also I would supporting Varun to me while debating with Manndeep . When it comes to Briju, Saikat and Ankush I am lost for words to describe them as individuals and it should suffice to say that I am simply blessed to have them ever by my side; I've spent the best of times with them around, especially Abhijeet, I always loved his deep general knowledge and explanation.

I am thankful to my graduation and post-graduation friends Javed, Vijay, Kiran, Rifahat, Lalamuhammd, Abrar, Aftab, Sikandar, Nusurat, Rumana, Gazala, Quwsar, Asiya, Nitin, Vikrant, Navanath, Yogendra, Virkram, Rishi and others for their support and co-operation.

I would like to express my deep and invaluable thanks to Nikhat for her inspiration, constructive criticism and support from post-graduation until now, hope will continue in my entire life. I would like especially thanks to my school friend Kumar, Trishala and graduation Sujit, Javed, Mushtaq, Imtiyaz and Bilal for their constant encouragement and moral support.

I am grateful to the employees of Golden Jublee Hostel (GJS) for providing good meals especially to head cook Chakru who can make any dish in the world.

No word would suffice to express my gratitude and love to my mother, father and sister for their continuous showering of boundless affection on me and supporting me in whatever I choose or did. It is my late grandfather's, late grandmother's, constant struggle and relentless hard work to overcome the odds of life, which has inspired me to pursue life with a greater optimism. The warmth and moral value of my parents have stood me in good stead throughout my life and I would always look up to them for strength no matter what I have to go through. This Ph. D. thesis is a result of the extraordinary will, efforts and sacrifices of my family. I would like to dedicate this moment of joy to my family. My success is dedicated to them now and always.

*I wish to express my gratitude towards "**Allaha-almighty**", who gave me the strength and courage to fulfil my dreams and has showered upon me his choicest blessings.*

.....Ashif

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- ^{1}H NMR spectra were recorded on AV-200 MHz, AV-400 MHz, JEOL ECX 400 MHz and DRX-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C NMR spectra were recorded on AV-50 MHz, AV-100 MHz, JEOL ECX 100 MHz and DRX-125 MHz spectrometer.
- Mass spectra was recorded on Applied Biosystems API QSTAR Pulsar Mass Spectrometer (Electro spray ionization, direct infusion method, solvents used acetonitrile/methanol). EI Mass spectra were recorded on Finngan MAT-1020 spectrometer at 70 *eV* using a direct inlet system. Mass spectra were recorded on Waters LCMS-UPLC system.
- Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and are measured in cm^{-1}.
- Optical rotations were measured with a JASCO DIP 370 digital polarimeter.
- All reactions are monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV light, I_2, and anisaldehyde in ethanol as developing agents.
- All ring opening polymerization (ROP) reaction of *N*-Carboxyanhydrides carried in glove box (Dry Box).
- Gel Permeation Chromatography of the glycopolypeptides was performed using an instrument equipped with a Waters 590 pump with a Spectra System RI-150 RI detector and Light Scattering. Separations were effected by 105 and 103 A ° Phenomenex 5 m columns using 0.1 M LiBr in DMF eluent at 60 °C at a sample concentration of 5 mg ml_1. A constant flow rate of 1 ml min_1 was maintained, and the instrument was calibrated using polystyrene standards. UV-VIS spectra were obtained.
- CD (190−250 nm) spectra of the glycopolypeptides (0.50 mg/mL in deionized water) were recorded (JASCO CD SPECTROPOLARIMETER, Model Name J-815) in a cuvette with a 1 mm path length.
- All reactions were carried out under nitrogen or argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise specified.
- All evaporations were carried out under reduced pressure on Büchi rotary evaporator below 45 °C unless otherwise specified.
- Silica gel (60–120), (100-200), and (230-400) mesh were used for column chromatography.

Ac	Acetyl
Ac_2O	Acetic anhydride
AcOH	Acetic acid
aq	Aqueous
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Bz	Benzoyl
BnCl	Benzyl chloride
BzCl	Benzoyl chloride
BnBr	Benzyl bromide
Cat	Catalytic
Conc	Concentrated
CuAAC	Copper catalyzed Alkyne Azide Cycloaddition
Cbz	Carbobenzoloxy
DBU	1,8-Diazabicycloundec-7-ene
DGPLL	D-Glucose-Poly-L-Lysine
DEAM-PS	Dietheylaminomethyl-Polystyrene
DIPEA	N,N-Diisopropylethylamine
DMAP	N,N-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl (methylthio) sulfonium triflate
DEPT	Distortionless Enhancement by Polarization Transfer
eq	Equivalents
Fmoc	9-Flourenylmethoxy Carbonyl
GPC	Gel Permeation Chromatography
g	Gram
H	Hour
Hz	Hertz
J	Coupling constant
NIS	*N*-Iodosuccinimide

mL	Millilitre
mol	Mole
mmol	Millimole
Me	Methyl
MeOH	Methanol
4ÅMS	4Å Molecualar sieves
MeOTf	Methyl triflate
mg	Milligram
min	Minutes
NMR	Nuclear Magnetic Resonance
PD	Polydispersity
Proton Sponge	1,8-bis(dimethylamino)naphthalene
PS	Polystyrene
PTSA, TsOH	*para*-Tolune sulphonic acid
rt	Room temperature
TBAI	Tetra-*n*-butylammonium iodide
TESOTf	Triethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
TfOH	Trifluoromethane sulphonic acid
TLC	Thin Layer Chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Ts	Tosyl

*****

Abstract

The present thesis is divided into two chapters. The first chapter highlights the utility of gold salts for synthesis of *O*-linked and carbamate linked glycoaminoacids. The second chapter is divided into two sections. In Section I, Part A describe the application of glycoaminoacids for the synthesis of glycopolypeptides using Ring Opening Polymerization (ROP) and Part B describes the gluconoric acid containing self assemblies using click chemistry approach. In section II, a new methodology for the synthesis of azido functionalized glycopolymer and its application is delineated.

Chapter 1 Gold Catalyst: Synthesis of *O*-Linked and Carbamate linked Amino Acid Glycoconjugates

Saccharides glycosylated to an aglycone are termed as glycoconjugates and they play an important role in various biological events and are classified in accordance with the type of attached aglycone. For example, if the aglycone is a long chain fatty acid the glycoconjugate is called a glycolipid whereas a protein attached to a saccharide is known as a glycoprotein. Glycoprotein's have immense significance in a variety of biological processes including cell signaling, inflammatory responses, neuronal development and immune surveillance. In mammalians, blood group determinants, tumor associated antigens and a host of other significant biological events involve glycoproteins. Thus, synthetic glycopeptides are required for improving our current understanding of those biological events which involve them.

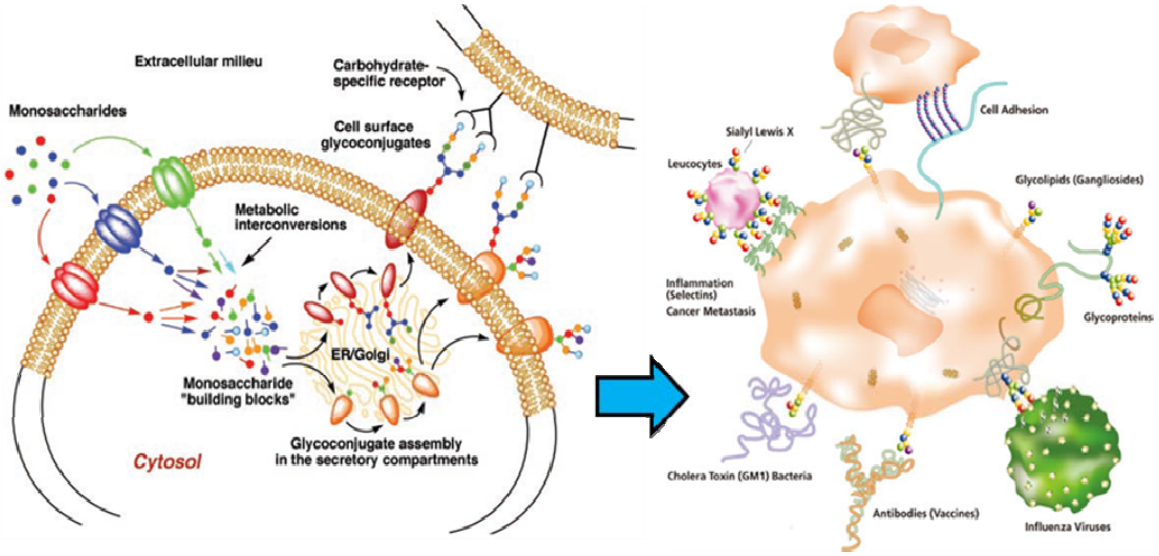
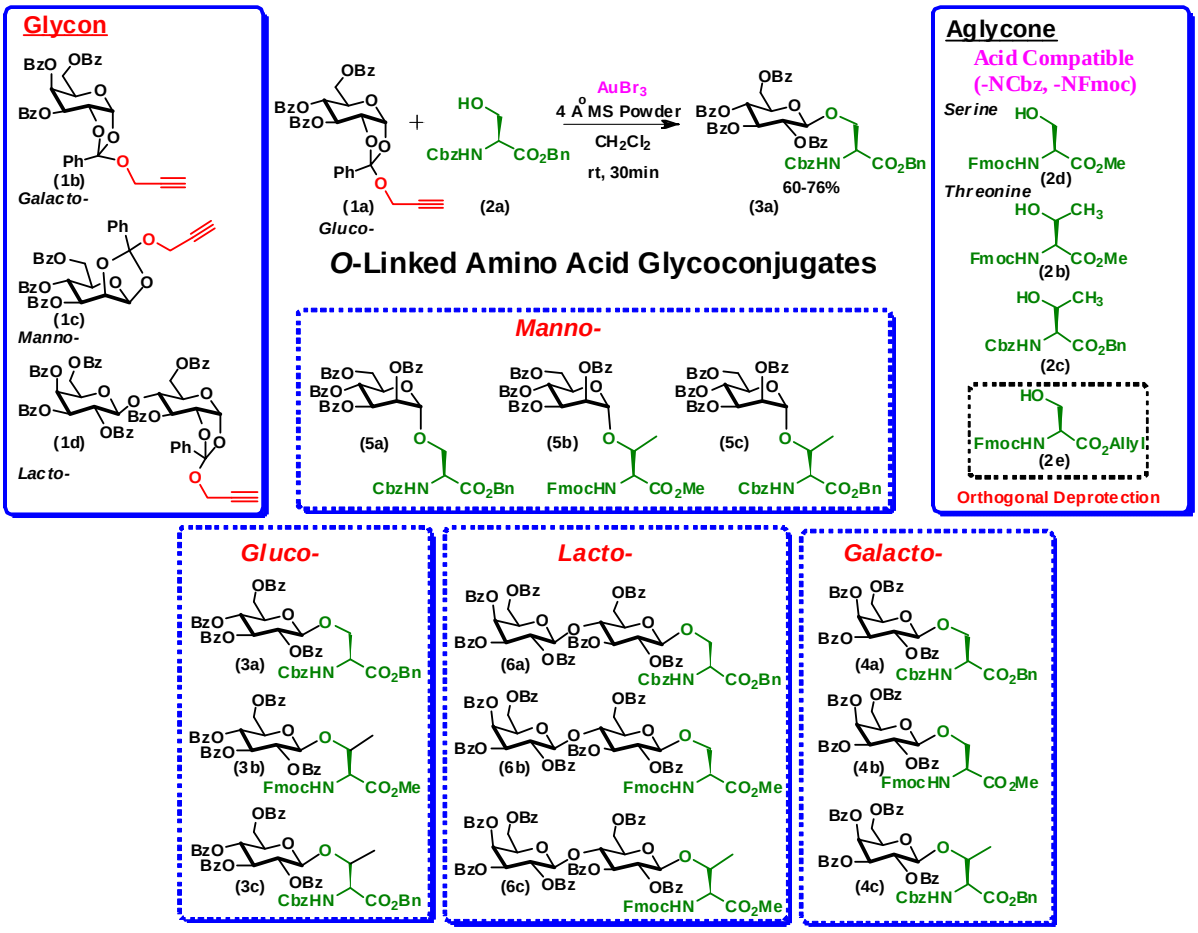


Figure 1:- Glycoconjugate Biosynthesis and Cell Surface Recognition

The glycocalyx is very complex; it is very difficult to isolate in pure and sufficient form from biological system due to its microheterogeneous nature for the biological study. So, there is need to have method which will give access to these synthetic glycoconjugates to understand the biological role of carbohydrates. Carbohydrates has huge diversity compared to amino acids and nucleotide because of which Nature has chosen carbohydrate as protecting wall or differentiation between pathogen, bacteria and host cell (**Figure 1**).

At the heart of any glycopeptide synthesis, the attachment of the sugar residue(s) to the amino acid as a glycoside from a suitable saccharide and a protected amino acid precursor is the key event. However, only a limited number of approaches are available for the attachment of a serine/threonine to saccharides. Recent observations from our laboratory led to the identification of propargyl glycosides as novel glycosyl donor. In continuation of the programme on gold catalyzed glycosylations, we got interested in the exploitation of propargyl 1,2-*O*-orthoesters for the synthesis of amino acid glycoconjugates. To begin our investigation, initially glucose propargyl 1,2-*O*-orthoester (**1a**) was allowed to react with

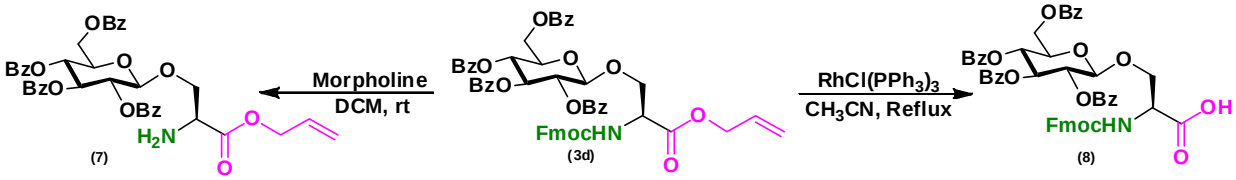


Scheme 1:- O-Linked Glycoaminoacids

CbzSer(OH)Bn (**2a**) in the presence of 7 mol% of $AuBr_3/CH_2Cl_2$/4 Å MS powder/rt/30 min to afford serine glucoside (**3a**).

Versatility of the glycosylation methodology with gold catalyst was further extended to other glycosyl donors. For example, galactosyl **(1b)**, mannosyl **(1c)** and lactosyl **(1d)** 1,2-orthoesters were allowed to react with serine and threonine derived aglycons (**2a-2d**) to obtain corresponding galactosides **(4a-4c)**, mannoside **(5a-5c)** and lactosides **(6a-6c)** respectively in good yields (**Scheme 1**). In all cases, we identified glycoside formation in 1,2–*trans* fashion.

Also we have used orthogonally deprotectable groups such as *N*-Fmoc and *O*-allyl ester of serine for glycosylation reaction, which can be orthogonally deprotected using suitable condition to get corresponding functional groups. These kinds of amino acid linked carbohydrates are very useful for incorporation of carbohydrate moiety into peptides to get synthetic glycopolypeptides using solid phase synthesis (**Scheme 2**).

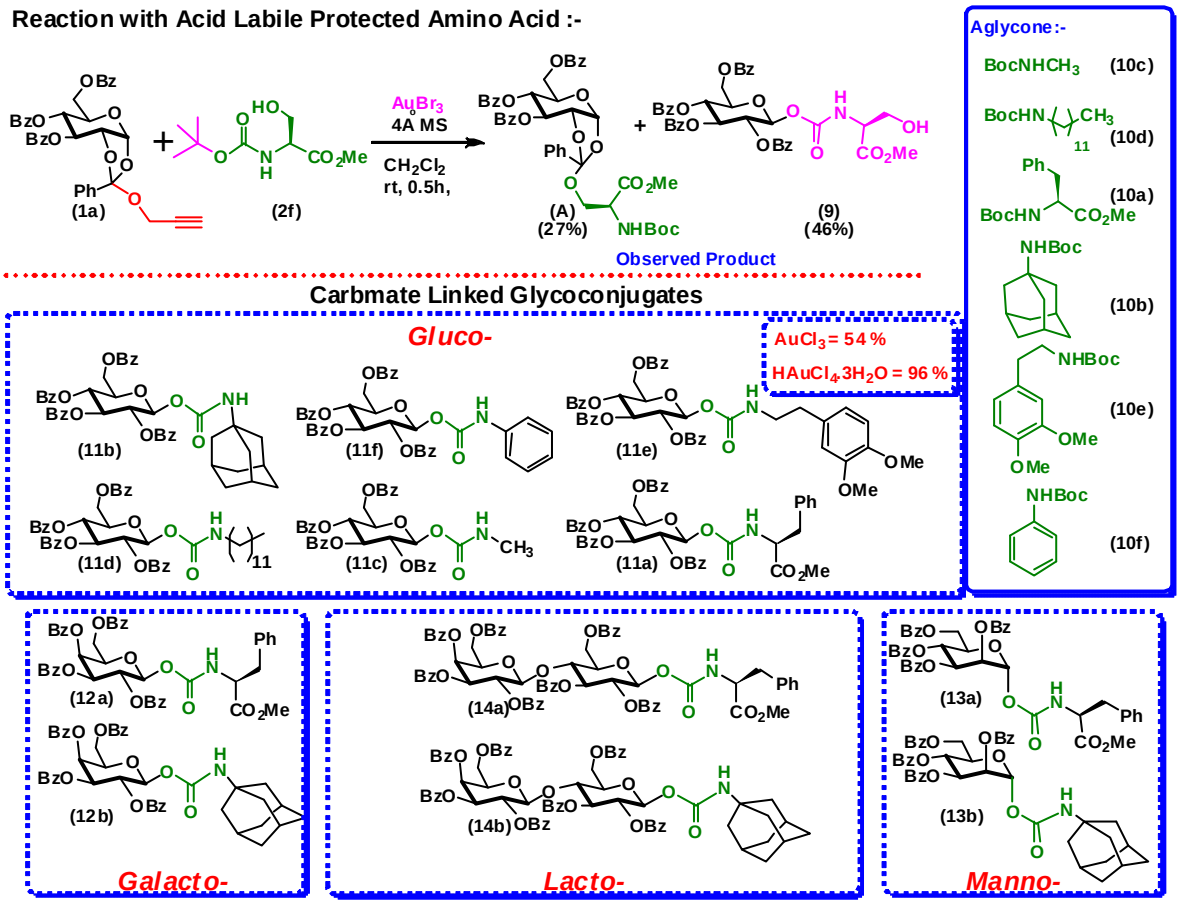


Scheme 2:- Orthogonal Deprotection for Solid Phase Glycopeptie Synthesis

tert-Butoxy carbamates (*t*-Boc) of serine/threonine are also frequently used in the glycopeptide synthesis. Thus a model gold catalyzed glycosylation was performed between propargyl 1,2-*O*-orthoester (**1a**) and BocSer(OH)OMe (**2f**). The much anticipated amino acid glucoside was not observed and instead, surprisingly an orthoester **A** was obtained in (**27%)** yield; the major compound (**46%**) being an *O*- linked glucosyl carbamate (**9**). To avoid side product formation, we have chosen simple *t*-Boc protected phenylalanine (**10a**) which doesn't have hydroxyl group. Optimized reaction conditions showed $HAuCl_4.3H_2O$ gave glycosyl carbamates in excellent yields (**96%**).

The generality of the methodology has been verified by using a panel of *t*-Boc protected amines. *t*-Boc-protected amino compounds of alicyclic (**10b**), aliphatic (**10c**, **10d)** and aromatic (**10e, 10f**) reacted with glucose 1,2-orthoester **1a** to afford their respective carbamates **11a–11f**. Furthermore, galactosyl- (**1b**), mannosyl- (**1c**) and lactosyl- (**1d**) derived propargyl 1,2-orthoesters (**1d**) also participated successfully in the glycosylation

reaction to afford the corresponding 1,2-*trans* glycosides **12a–b, 13a–b** and **14a–b** in near quantitative yields (**Scheme 3**).



Scheme 3:- Carbamate Linked Glycoaminoacid

<u>Chapter 2</u>. Application of Gold Catalyzed Glycosidation: Synthesis of Glycopolypeptide & new method for synthesis of controlled azide functionalization Glycopolypeptide

Chapter 2 is divided into two sections. Section IA presents the application of glycoaminoacids for synthesis of glycopolypeptide and IB describes the use of click chemistry for synthesis of carbohydrate modified polypeptide and its application in self-assembly. Section II describes the synthesis of partially azido functionalized and fully azido functionalized glycopolypeptide using Ring Opening polymerization.

Glycopolymers featuring synthetic macromolecules with pendant carbohydrate moieties, have found widespread application in various fields such as macromolecular drug delivery systems, hydrogels, matrices for controlled cell culture, and as models of biological systems. Majority of these glycopolymers currently explored are acrylate based and controlled radical polymerization is used to synthesize polymers with defined molecular weight, glycosylation

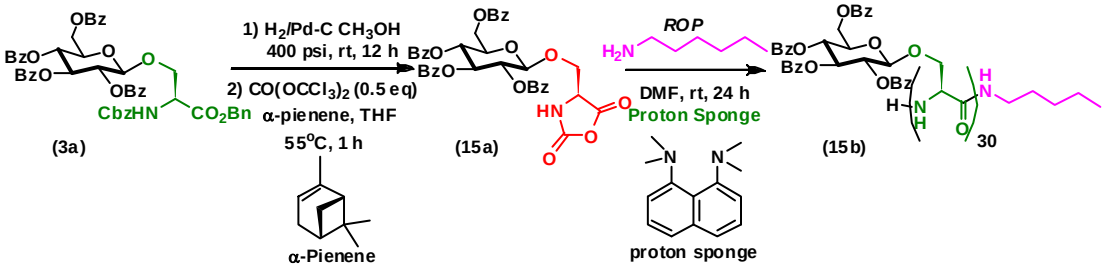
density and position attributes which are necessary for biological recognition processes. However, these polymers do not have well-defined higher order structures and often adopt a random-coil conformation, which inevitably renders some of the side-chain bioactive moieties inaccessible toward biological active sites.

On the other hand, glycopolypeptides (glycopolymers with pendant carbohydrates on a polypeptide backbone) not only have the ability to fold into well-defined secondary structures (e.g. helix or sheet) but also mimic the molecular composition of proteoglycans. Therefore it is desirable to develop methodologies that give access to easy and well defined synthetic glycopolypeptides.

Section IA

Syntheis of Glycopolypeptide and Glycosylated Polyproline using Ring Opening Polymerization

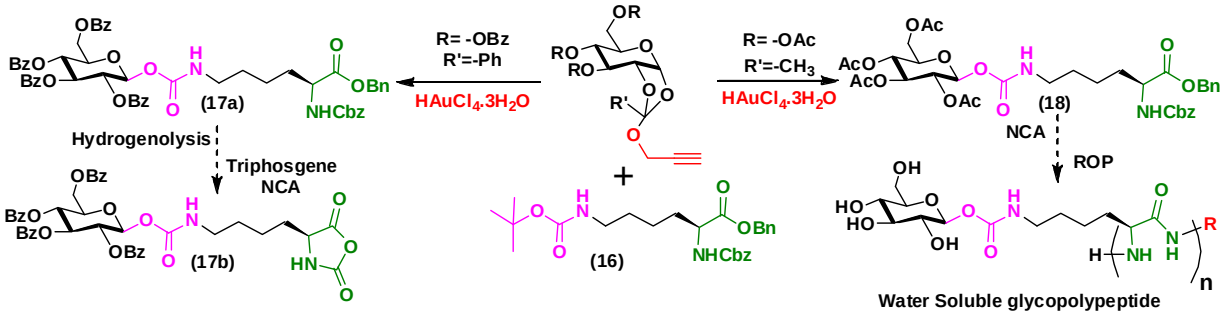
In this section, we showed a novel and simple methodology for the synthesis of *O*-glycosylated serine NCA and their application to subsequent Ring Opening Polymerization(ROP) to afford glycopolypeptides. We attempted the synthesis of the natural D-glucose-L-serine polypeptide (**15b**) from the serine *O*-glucoside **3a**. Polymerization of **15a** was attempted using n-hexylamine as the initiator (M/I = 20) in dry DMF (**Scheme 4**). The structure of the resulting polymer **15b** was identified by ^{1}H and ^{13}C NMR spectral analysis. The overall strategy can be applied to obtain all glycosyl NCAs and eventually to prepare glycopolypeptides.



Scheme 4:- Ring Opening Polymerization for Glycopolypeptide

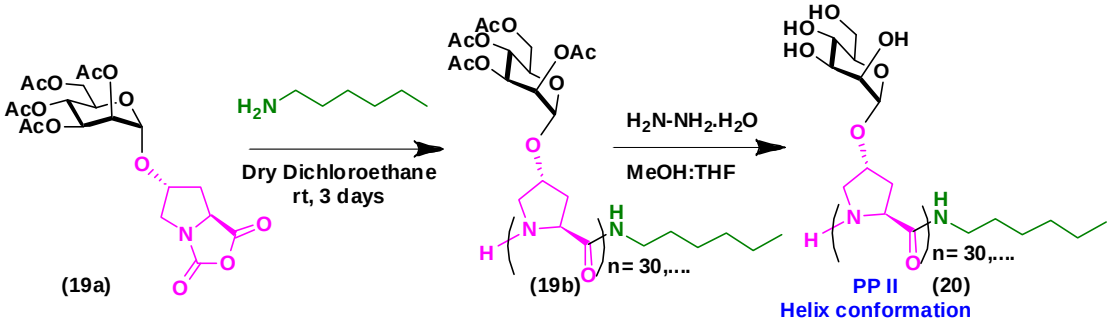
We have tried different methods to deprotect per-*O*-benzoylated glycopolypeptide but failed to get completely deprotected glycopolypeptide. Then we changed to acetate protected 1,2-propargyl orthoester and did glycosidation using $HAuCl_4.3H_2O$ to get corresponding acetate protected carbamate linked glycoaminoacid. Having obtained N-

Carboxyanhydride with acetate on the sugar moiety we explored its application for water soluble glycopolypeptide (**Scheme 5**)



Scheme 5:- Application to Water Soluble Glycopolypeptide

Proline is one of the most important amino acid present in biological system such as collagen. 4-Hydroxy N-Cbz-proline-OBn was glycosylated with mannose using gold catalyst to get mannose proline in good yield. Hydrogenolysis of *N*-Cbz-mannose-OBn proline was crried out by using H_2/Pd-C at 400 psi pressure 12 h. Subsequently, it was converted to mannose Proline NCA (**19a**) using triphosgene and *N,N'* diethyl aminopolystyrene resin. Again we have confirmed the NCA formation by IR and NMR (**Scheme 6**).



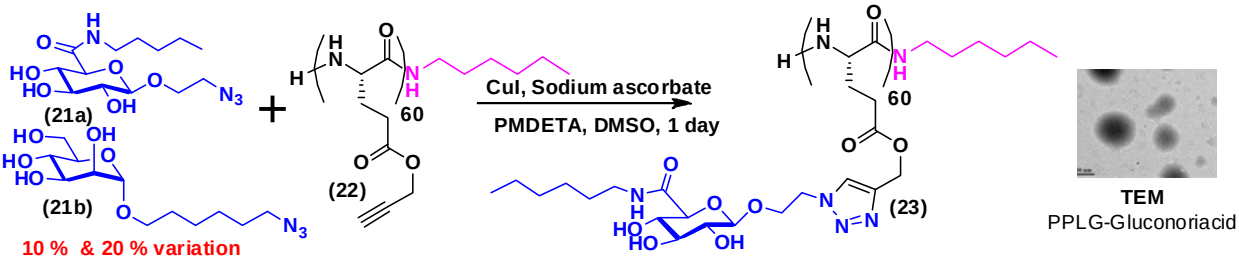
Scheme 6:- Glycopolyproline by ROP

We tried polymerization of mannose proline NCA using our previous method, which we have developed in our lab. Progress of reaction was monitored by IR and GPC. We did temperature dependent study on these glycopolyproline (**20**) and showed that carbohydrate residue on backbone is giving extra stability to helix. The conformation shown by glycopolyproline was PPII helix conformation confirmed by circular dichroism measurement. We are standardizing the thing and work is under progress.

Section IB

Gluconoric acid application

After successful synthesis of glycopolypeptide, we studied this efficacy for self-assembly. For this we have chosen gluconoric acid as starting material, so that we can modify at 6-position in a modular fashion. We "clicked" azido containing gluconoric acid derivatives with propargyl modified polyglutamate using well-known 'click' reaction under CuAAC condtion (**Scheme 7**).



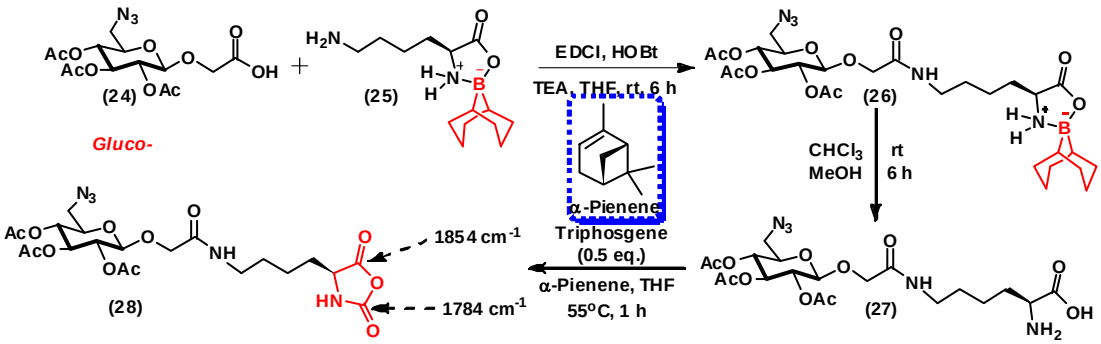
Scheme 7:- Click Chemistry for Modification of Propargylated Polypeptide

Section II

Synthesis of 6-Azido and 6-Amino functionalized Glycopolypeptide for Hydro gel and Sortase activity

Understanding the importance of the glycoaminoacid, we thought to introduce diversity in the system to get complex glycopolymer. Our previous work showed details about the normal glycosylated amino acid polymerization, introduction of azide or amine functionalities on carbohydrate to get 6-azido or 6-amino functionalized glycoaminoacids would facilitate synthesis of diverse glycopolymers.

Accordingly, we have synthesized carboxylic acid containing 6-Azido derivative of glucose. Lysine monohydrochloride was reacted with 9-BBN to get protected 9-BBN-Lysine complex (**25**). One specialty of these complexes is that these are highly soluble in organic solvents

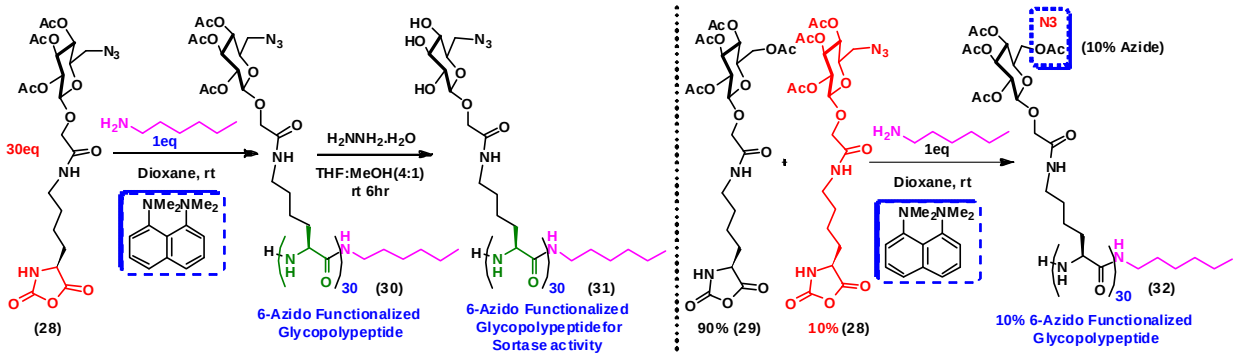


Scheme 8:- Synthesis of 6-Azido functionalized Glucose N-carboxyanhydride

used for amide coupling. Treatment of L-lysine-9-BBN complex (**25**) with 6-azido glucose carboxylic acid (**24**) using standard amide coupling EDCI and HOBt in THF gives 6-Azido-

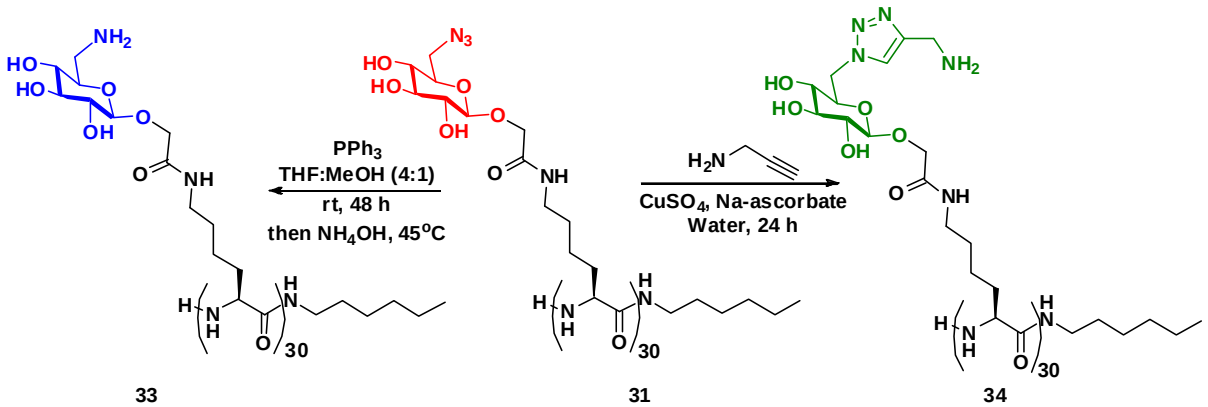
glucose-Lysine 9-BBN complex (**26**), which was purified by using simple gravity column chromatography.

9-BBN serves as temporary protecting group for α-amino acid, which avoid cross coupling reaction during attachment of amino acid to the carbohydrate. It was easily removed by stirring the reaction mixture in chloroform and methanol for 12 h. Excess 9-BBN was removed by treatment with hot hexane and diethyl ether. 6-Azido glucose Lysine NCA (**28**) was prepared by our previous method and also polymerization was carried out (**Scheme 9**). A fluorescent tag was clicked to glycopolypeptides (**32**) and also varied amount of azido functionalization in glycopolypeptides.



Scheme 9:- 6-Azido and Partially Azido Functionalized Glycopolypeptide

6-Azido functionalized glycopolypeptide **(31)** is very good substrate for sortase activity, cross-linked polymers and for synthesis of brush glycopolypeptide. Staudinger reduction of **(31)** was done using PPh_3 in THF:H_2O mixture and then treatment with aq. NH_4OH at 45°C



Scheme 10: Cationic Charged Helical Glycopolypeptide

gives 6-Amino functionalized glycopolypeptide for chemical ligation and sortase activity **(Scheme 10)**. These glycopolypeptide have cationic charge at side chain and surprisingly gives α-helical conformation. It is known in literature that cationic charge destabilizes α-

helical conformation and prefer random coil conformation. Again cationic charge was introduced by clicking propargyl amine with 6-azido glycopolypeptide using $CuSO_4.5H_2O$ and sodium ascorbate in water which also shows peaks in CD at 222 nm and 208 nm corresponds to α-helical conformation at pH 7.4 **(Figure 2)**. So side chain amine could be used for functionalization with other alkyne containing polymer to get brush polymer or amine could be used for cross-linked with epoxy PEG (PolyEthyleneGlycol) to get hydrogels.

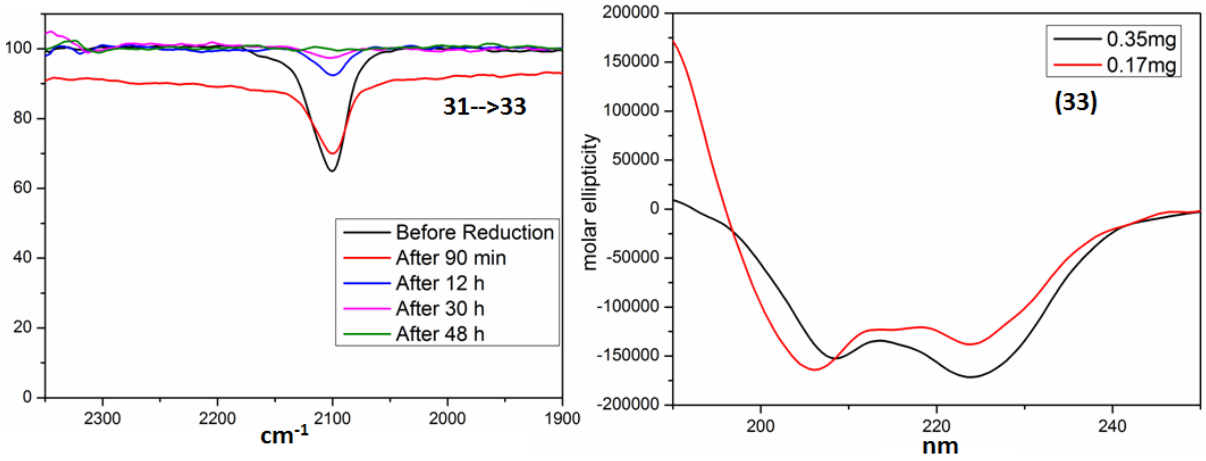


Figure 2: IR and CD spectra of Cationic Charged Glycopolypeptide at pH 7.4

In conclusion, we have successfully synthesized *O*-linked and carbamate linked Glycoaminoacid in excellent yields using $AuBr_3$ and $HAuCl_4.3H_2O$ catalysts respectively. The generality of the methodology was evaluated using various glycosyl donors and aglycons. Also the glycosylated amino acid was used for synthesis of *N*-carboxyanhydride and controlled ring opening polymerization (ROP) of NCA gave glycopolypeptide. Explored application of gold catalyzed glycosidation for the synthesis of water-soluble glycopolypeptide. Synthesized polymers were characterized thoroughly by various spectroscopic techniques. We have achieved the complete control over amount of functionalization on glycopolypeptide by varying amount of normal and azido functionalized glyco-*N*-Carboxyanhydride monomer. Cationic charged helical glycopolypeptides were synthesized for sortase activity and for brush glycopolypeptide synthesis. Also we have introduced azide functionality as a very good bio-orthogonally reacting partner for click reaction and Staudinger ligation.

Note: Compound numbers in abstract are different from those in the thesis

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CHAPTER 1

Gold Catalysis: Synthesis of *O*- and Carbamate- linked Amino Acid Glycoconjugates

Carbohydrates are like water, ubiquitously found on our planet. Carbohydrates are synthesised in plants and cyanobacteria by "photosynthesis" which is essential for all living beings on the earth. They are primary bio-molecules, which can get metabolized to monosaccharides, oligo- and polysaccharides. The photosynthetically produced sugar serves as energy source for photosynthesizing as well as for non-photosynthesizing organisms by direct consumption or indirect consumption, where the photosynthesis process is essentially reverses in very complex oxidative carbohydrate metabolism (Fig.1). Apart from source of energy to living organisms, carbohydrates are also present as major constituents of the shells of insects, crabs, and lobsters, and the supporting tissue of plants. Carbohydrates are present as part of all cell walls, spanning from the world of microbes to mammals.

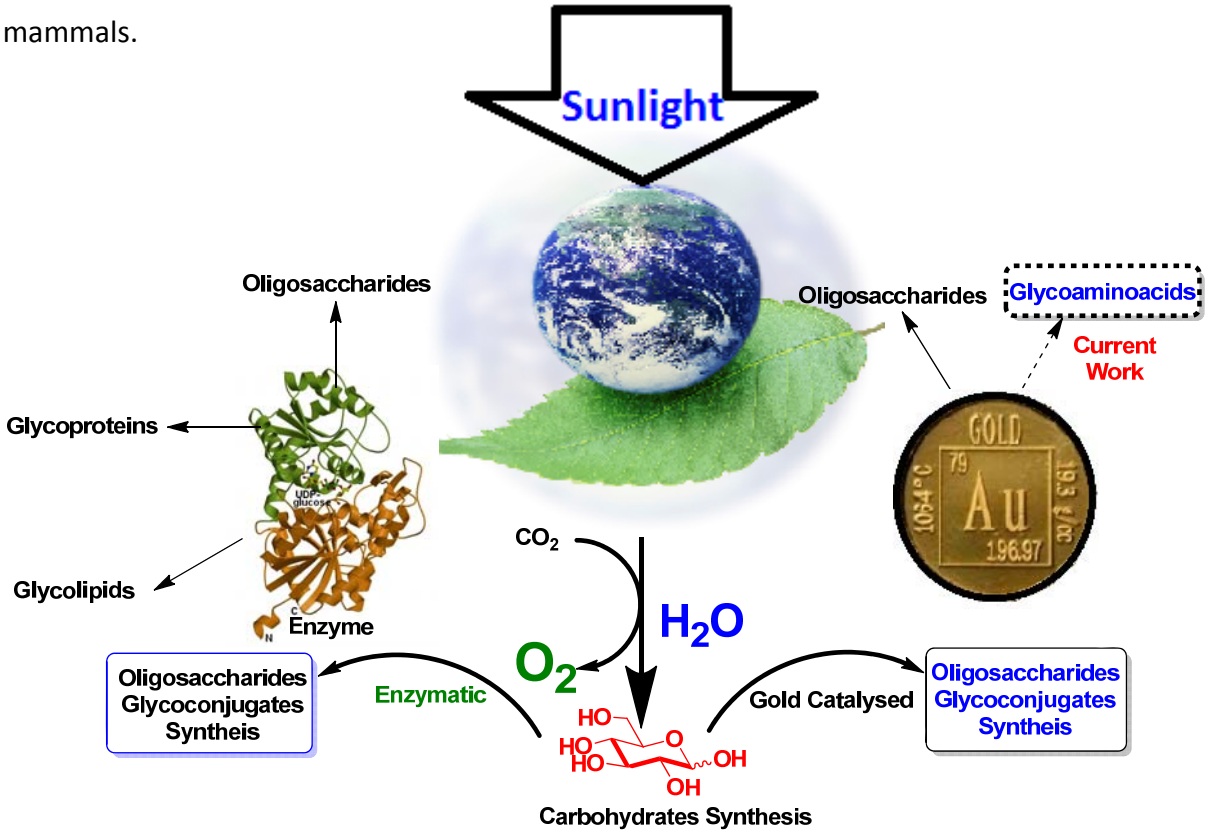


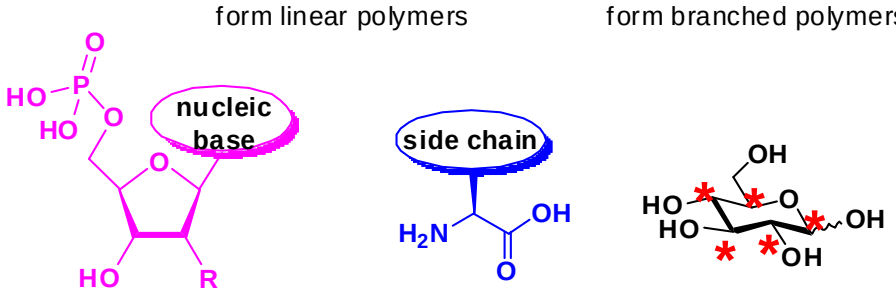
Figure 1:- Carbohydrate Synthesis and Utilization

Synthetic carbohydrate chemistry is a challenging field for organic chemists because of the structural complexity associated with carbohydrates such as several hydroxyls per monosaccharide, branched and linear oligomers and carry different kinds of functional groups. Emil Fischer has made distinctive contribution in the area of carbohydrates,

nucleotide and peptide chemistry, who solved many problems of structure of carbohydrates and basic question about the stereochemistry. Many aspects of the roles played by the carbohydrates in the storage and supply of energy in biochemical system and the mechanism of biosynthesis and biodegradation of carbohydrates were understood in 1960. Further, the isolation of biologically active compounds from microorganisms, such as antibiotics, which contain unusual saccharides motivated organic chemists to learn the art of chemical transformation of monosaccharides & oligosaccharides.

Diversity in Carbohydrates:-

A unique kind of diversity embedded in the carbohydrates, which allows them to form very complex branched and linear oligomers compared to other two major classes of biologically important biopolymers, proteins and nucleic acids.[1-2] Let us consider the oligomers of amino acids and nucleic acids, which have only two sides available to form polymers whereas in case of carbohydrates which have more than two functional groups participating in an oligomerization. In addition, the anomeric carbon can lead to two different stereoisomers, the α-glycoside and β-glycoside. The carbohydrate oligomers diversity can be enhanced by derivatizations of the sugars rings such as *O*-methylation, *O*-acetylation, *O*-sulfatation, *O*-phosphorylation or oxidation.



Pentamer	Nucleotide	Amino Acid	Carbohydrates
Homooligomeric	**1**	**1**	**17872**
Heteroooligomeric	**120**	**120**	**2144640**

Figure 2:- Constitutional stereoisomers of Carbohydarte, Amino acid & Nucleotide

One more thing about the diversity of carbohydrates is that many more constitutional stereoisomers can be constructed from monosaccharides than from amino acids or nucleotide from which only linear oligomers can be designed (Fig.2).[3] Modern glycosciences until now is not able understand, How is the extremely large structural diversity of

oligosaccharides utilized in nature is still a daunting question. Four monomeric building blocks are sufficient for DNA and RNA to form the molecular basis of the genomic information of an organism. DNA and RNA perform transformation of genetic code and the synthesis of many different proteins structures, which are required for the organism to function. Like nucleic acid, carbohydrates do not code the biological information in single monomer unit but in many oligosaccharides, three-dimensional structures and their molecular dynamics in water concludes its function.[4] May be the biodiversity in carbohydrates is very vast because of which Nature has chosen them as protective wall and to differentiate between cells of different organism or within same.

Glycoconjugates:-

Carbohydrates present on cell surfaces are frequently attached to other non-carbohydrates natural product of different kind, which are called "glycoconjugates". The size of glycoconjugates varies from relatively small molecules to large biopolymers. Many small glycoconjugates possess antibiotic activity are glycosylated with oligosaccharides of varying complexity. These saccharides are important for biological storage and transport, for pharmacokinetics and pharmacodynamics and influence the properties of the molecule such as solubility, efficacy, and selectivity among the other.[1]

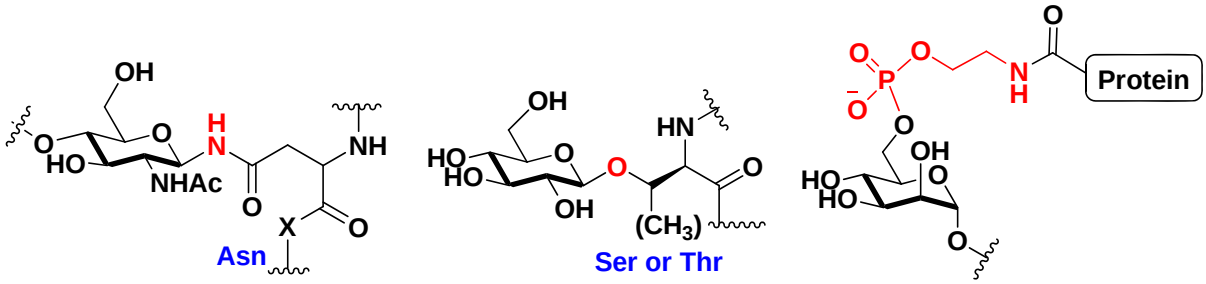
On the other hand, even more complexity arises when carbohydrates are linked to proteins and lipids producing a large number of different glycoconjugates called glycoproteins, proteoglycans, glycolipds and GPI-anchors, respectively. Complex glycoconjugates are found in dissolved form or membrane bound and as part of a nano-dimensioned, macromolecular super-system, which is part of living cell surfaces and is called the 'glycocalyx'. The biology of a complex carbohydrate 'super-system' is not fully understood until today and this forms the basis for a modern research field, named '*glycobiology*' which requires an interdisciplinary endeavour to unravel the secrets of carbohydrates-based cellular communication and to eventually utilize this new knowledge and understanding in a novel therapeutic context.

Carbohydrate content in glycosylated proteins such as enzymes, antibodies, hormone, cytokines and receptor protein varies from 1% (in collagen) to 99% (in glycogen).Why carbohydrate content varies in nature? Consider glycogen stores glucose in animal livers and

muscles. Starch and glycogen serve as energy for plants and animals, respectively. Both these polymers are readily hydrolyzed into glucose monomer, which in turn can be further degraded to liberate their stored energy. But if it is glucose that is actually needed for fuel, why must it be stored as a polymer? The reason is that 1,000 glucose molecules would exert 1,000 times the osmotic pressure of a single glycogen molecule, causing water to enter the cell that eventually causes cell burst. The carbohydrate portion of the proteins can alter their biological and physicochemical properties such as their stability against protease or the activity of enzymes, and they can direct the folding of proteins towards certain three-dimensional structure.[5-11]

Glycoproteins are present in blood (on the basis blood group decided), in cytosol or in subcellular organelles and they are basic constituents of all cell membranes.[12] In eukaryotic cell, they are integrated into the lipid bilayer, so that the biologically important carbohydrate moieties are exposed to extracellular matrix for biological process or event.[13] Membrane bound glycoconjugates form the carbohydrate coat on cell surface, which called 'glycocalyx' is about more than 100nm size connected to extracellular matrix. The carbohydrate coat of particular cell is characteristic for its type and for its developmental, physiological and even pathological status. In embryonic cells or in cancer cells, the glyco-coat varies significantly, compared with adult or healthy cells, respectively. Thus, certain oligosaccharides can usually be associated with degenerative cell growth and used in cancer diagnostics as so-called tumor-associated antigens.[14-19]

Glycoproteins may contain one or several oligosaccharide side chains and most commonly three chemical linkages found in glycoproteins are 1) *N*-glycosidic (An aspargine of protein attached to N-acetyl glucosamine), 2) *O*-glycosidic (mostly serine and threonine via –OH side chain) and 3) via ethanolamine phosphate (anchors protein in cell membranes).



***N*-glycosidic** ***O*-glycosidic** **via ethanolamine phosphate**

Figure 3:- Glycosidic linkages Generally Observed in Glycoproteins

Mucin or O-Glycan

Mucin-type glycans are the oligosaccharide components of glycoproteins initially found in mucs. Generally they are in the form of membrane-associated or secreted in gel form. Mucins form a major part of the protective biofilm on the surface of epithelial cells, where they provide a barrier to particulate matter bind microorganisms. In mucin type of glycoproteins mostly the serine and threonine residue of protein are linked through *O*-linkage of side chain hydroxyl and with different chain lengths leading to significant heterogeneity of these molecules. A characteristic feature of all mucins is the linkage of sugars portion to the protein backbone, which is always a α-*O*-glycosidic bond to *N*-acetyl-galactosamine (GalNAc). There is no common core of oligosaccharide present in *O*-glycoprotein as in case of N-glycans where a pentasaccharidic unit is common to all.

Glycoconjugates consist of very large diverse family of *O*-glycan. Apart from the above, there are several other structural patterns within *O*-glycan exist. Glycosylation may occur with different carbohydrates such as galactose, glucose, xylose and arabinose and even with mannose or L-fucose residue which are shown below (Fig.4).

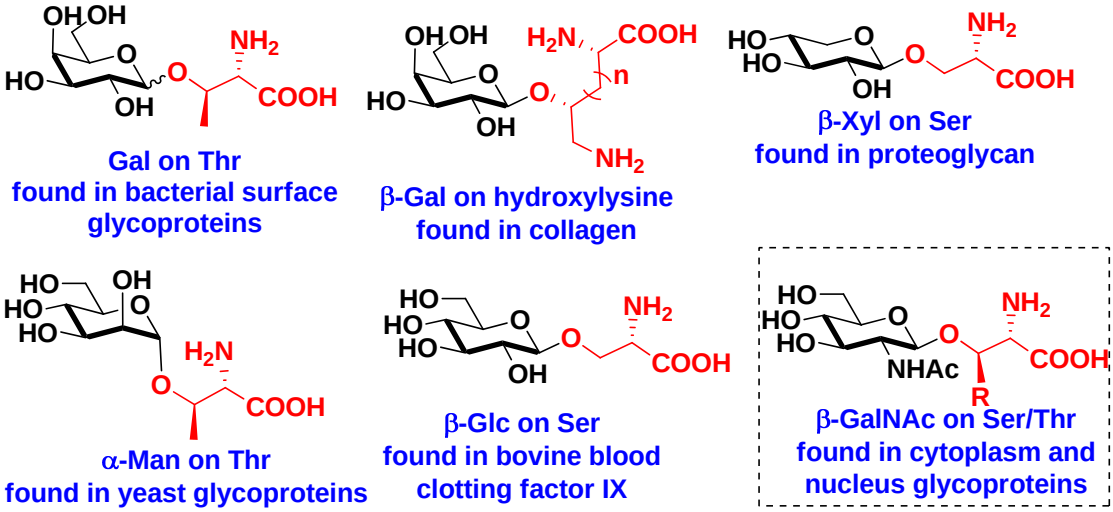


Figure 4:- Glycoaminoacid Units Found in *O*-linked Glycoproteins

Also *O*-glycosidic linkage is present in glycolipids where the hydrophobic part containing hydroxyl groups such as ceramide (Cer) are attached to hydrophilic carbohydrate moiety. Several hundred different glycosphingolipids structures are known. More complex glycosphingolipids are called gangliosides. Most gangliosides have common β-lactosyl moiety in the ceramide, the first two glycosylation steps in the biosynthesis of gangliosides are same for all members of this group.

Enzymatic Glycosylation

Before going to detailed study about the chemical glycosylation, it is better to briefly understand biosynthetic pathways of glycosylation work? The enzyme, which catalyzes the biosynthesis of oligosaccharides, is called 'Leloir enzymes' after the scientist who discovered their mechanism.[20] They use nucleoside diphosphate or monophosphates for the stereo- and regiospecific transfer of the respective monosaccharide onto an acceptor saccharide (Fig.5).

Glycosyl transfer is facilitated by the superior leaving group properties of sugar nucleotides. The ester bond between the phosphate residue and the carbon atom in the sugar is a high-energy bond and thus transfer of the sugar residue to an acceptor hydroxyl group of another sugar or on a serine or threonine residue is energetically favored. Each glycosyltransferase is specific for both the donor sugar nucleotide and the acceptor molecule.

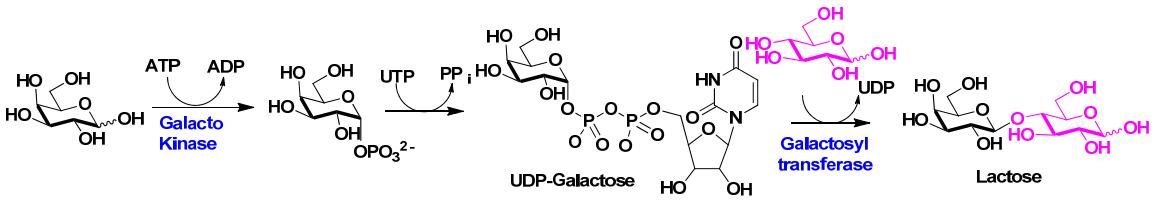


Figure 5:- Biosyntheis of UDP-Galactose and Enzyme Catalyzed Glycosylation in Nature

Like the biosynthesis of protein guided by the genetic code, the structure of oligosaccharide is determined by the action of enzymes, and therefore oligosaccharides can be called as '*secondary gene products*'. If the glycoconjugates are synthesized in nature then why can't it be isolated and used in medicinal chemistry and medicine. The problem is the '*microheterogeneity*' associated with it, which arises because of the reaction conditions of specific enzymatic glycosylation and small differences in the biosynthesis of oligosaccharides. So, it is very difficult to isolate biologically important Leloir enzymes in single & pure form. Also we don't have known universal enzyme, which will do the enzymatic glycosylation as they are very specific to every carbohydrate monomer. Here the carbohydrate chemist can play a very crucial role about the synthesis of glycoconjugates in laboratory and connect with the biology by checking its biological function in living organisms. This interconnection between the biology and carbohydrate chemistry opened new branch of chemistry called chemical glycobiology.

O-Glycosidation

21st century carbohydrate chemists have confronted with major challenge of synthesizing glycosides[21-23] which deals with two main problems: 1) the regio- and stereoselective formation of glycosidic linkages for the synthesis of oligosaccharides and 2) the selective protection and deprotection of saccharide building blocks prior to and after the linkage step. Nature does all these things very selectively, without any protection-deprotection, efficiently and in an ecofriendly manner. Still the carbohydrate chemists are in search of a method, which will compete with nature. Very efficient method for oligosaccharide synthesis using robotics is not available. Many carbohydrate chemists have concentrated their research areas on the improvement in glycosylation methods and new reactions to get stereo- and regio- specific glycosidic linkage formation. The advances made in this sphere have been due to improvements made in recent years in the chemical, physical & enzymatic methods of structure analysis.

Our laboratory is involved in solving the problems of glycosylation reaction using Gold Catalysts, which will be discussed in detail later. The term glycoside here is referring normally to an *O*-glycoside. However, there are other glycosides such as *N*-, *S*- and *C*-glycosides present in nature but they are beyond scope of our work.

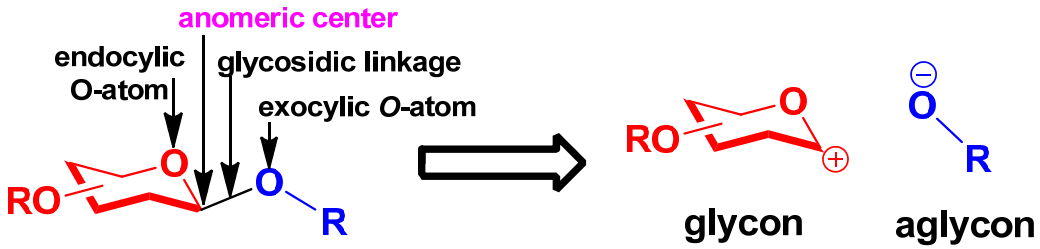


Figure 6:-General Terms in Glycosylation

Glycosides in the carbohydrate chemistry can no longer be termed as a reducing sugar as the cyclic hemiacetal could be further functionalized at its anomeric center. The standard retro-synthetic analysis cleaves a glycoside at the glycosidic linkage into an electrophilic 'glycosyl donor' (glycon) synthon and a nucleophilic 'glycosyl acceptor' equivalent (aglycon) (**Figure 6**).

The aglycon part may a simple nucleophile such as methanol or ethanol, in case of methyl or ethyl glycoside respectively or it can be more complex hydroxylated natural product. When

a hydroxyl group of another monosaccharide is used to form glycoside with glycosyl donor, the product is called disaccharide and consequently glycoside, which consist of three, four or more monosaccharides unit are called tri-, tetra- and oligosaccharides respectively. Fischer glycosylation can be used for the synthesis of simple glycosides when the alcohol is not too much costly, complex and excess alcohol can be removed by evaporations after the reaction has been completed. To do the glycosylation efficiently one has to protect anomeric hydroxyl group by a better leaving group, which can be activated by a suitable catalyst and promoter, whereas the other hydroxyl groups of the sugar ring need to be protected. The beauty of nature is that it can do glycosylation in stereo- and regioselective manner without any protection of other hydroxyl group of monosaccharide by bringing both glycosyl donor and acceptor in close proximity by the use of enzymes.

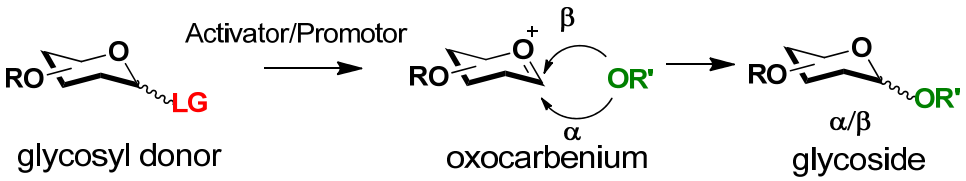


Figure 7:- General Way of Glycoside Bond Formation

The following are essential requirement for many glycosidation methodology 1) the derivatization of sugar into a sufficiently protected glycosyl donor equipped with a leaving group 'LG' at anomeric center; and 2) activation of this glycosyl donor by a suitable promoter, which activates the leaving group to allow coupling to a partially protected glycosyl acceptor leading to an glycosidic linkage (Figure 7). Now days many glycosylation methods are available, however it is difficult to predict which method is most suitable to synthesize a specific glycosidic linkage[24-25] (Figure 8). Here I want to quote words of Hans Paulsen[1] for this situation: "Each oligosaccharides synthesis remains an independent problem whose resolution requires considerable systematic research and a good deal of know-how. There are no universal reaction conditions for oligosaccharide synthesis."

Despite the availability of a variety of glycosyl donors, glycosylation methods used to date can be classified into three sub-catergories:

1**) 'Koenigs-Knorr'** type reaction using glycosyl halide
2) Trichloroacetimidate method employing '**Glycosyl Trichloroacetimidates'**, and
3) Use of stable glycoside such as **Thioglycosides** and **n-Pentenyl Glycosides** as glycosyl donors.

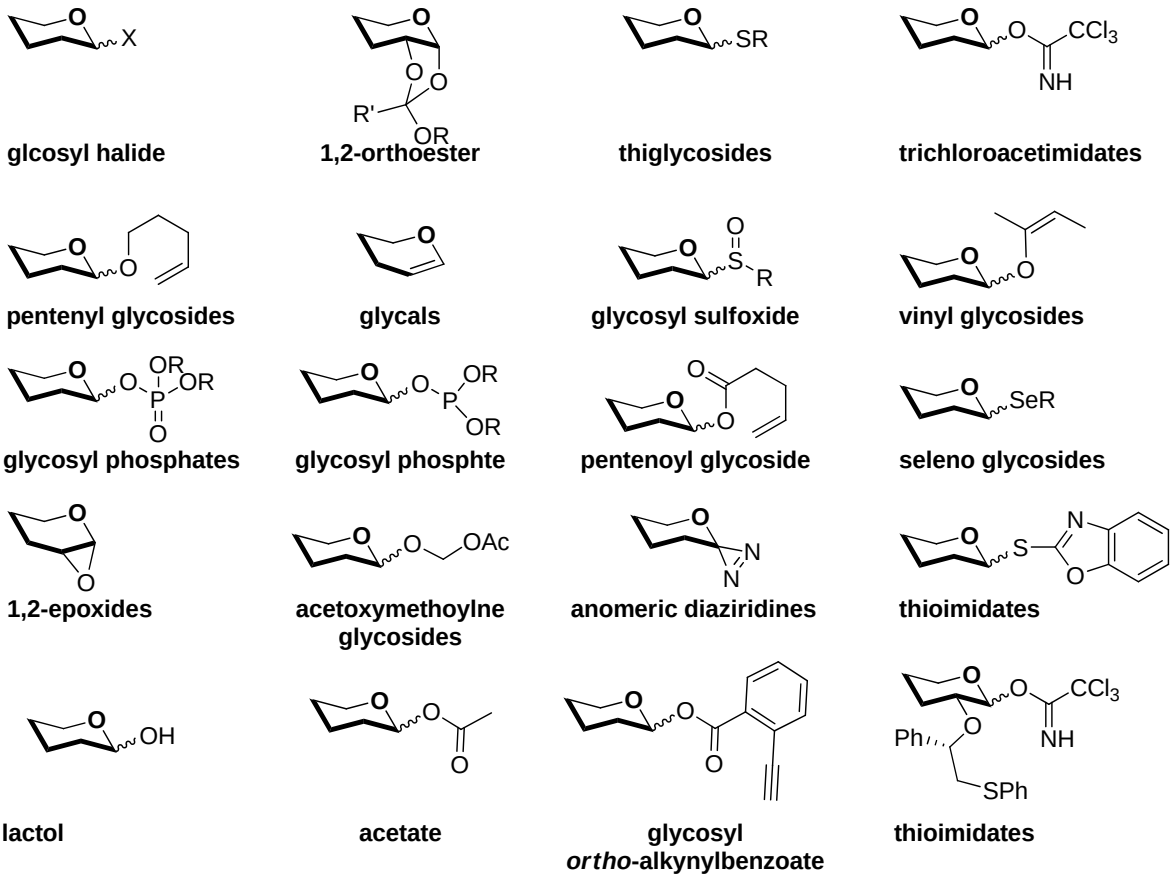


Figure 8:- Glycosyl Donors Used in Glycosylation Reaction

Two major principal problems are frequently associated with the stereochemical outcome of glycoside synthesis. These are 1) the regiochemistry of the glycosidic linkage, which is formed and 2) the conformation of the new glycosidic bond. Regiochemistry problem can be halted with by placing the appropriate protecting group on the glycosyl acceptor, leaving only one hydroxyl group unprotected. The stereochemical course of a glycosidation procedure determines whether a β-or α-glycoside will be the product of this reaction which was found to be highly dependent on the neighboring group at C-2. Participating neighboring group at C-2 leads normally to a 1,2-*trans* glycoside in a stereospecific reaction, whereas glycosylation with non-participating C-2 substituent will result in the formation of both 1,2-*trans* as well as 1,2-*cis* glycosides with more or less *stereoselectivity*. The recent observations on the neighboring group from Demchenko[26] group showed that sometimes C-3, C-4 and C-5 group can also participate and gives raise to stereoselective glycoside products. The detailed mechanism and factors affecting of formation of 1,2-*trans* and 1,2-*cis* glycoside will be discussed in the following section.

Anomeric Effect[27]

Two stereogenic isomers are generally possible at anomeric center for any monosaccharide. They are termed as α- and β-isomers or 1,2-cis and 1,2-trans isomers. In general the equatorial substituent of cyclohexyl chair rings are most energetically favoured as compared to their axial counterparts because of steric reasons and 1,3-diaxial interactions. However, in D-pyranosides especially carbohydrate derivatives with electronegative group at anomeric center, axial isomers are often more stable than equatorial ones. The unusual preference of sterically unfavoured axial position over equatorial position at C-1 carbon has been termed as "anomeric effect". The anomeric effect is explained by R U Leimeux on the basis of intramolecular electrostatic interaction of two dipoles next to the anomeric center (Figure 9).

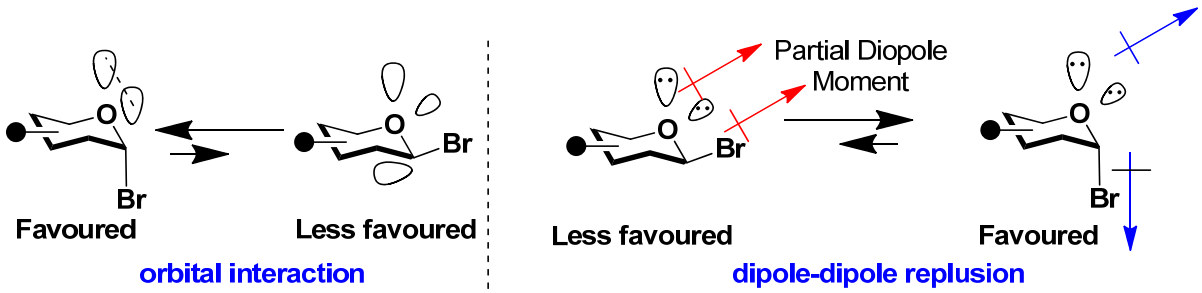


Figure 9:- Favoured Conformation Due to Anomeric Effect

The anomeric effect was discovered in the case of carbohydrates but has been found to be of general importance for molecules where two heteroatoms are bound to a tetrahedral center.[28] So, the essential group for the appearance of an anomeric effect is -C-Y-C-X- where Y= N, O, S and X= Br, Cl, F, N, O, S. Anomeric configuration, where the two nearly perpendicular dipoles partially neutralize each other (an energetically more stable arrangement as in axial substituent) are favoured over the diastereomers where the anomeric configuration leads to intramolecular addition of the two parallel dipoles (an energetically unfavourable arrangement as in equatorial substituent). The anomeric effect is different for each case and strongly influenced by the substituent at C-2 position. When the substituent is an equatorial position as in the case of glucose and galactose, the anomeric effect is weakened, whereas the anomeric effect is enhanced in C-2 axial substituent of mannose.

The nature of group at C-1 has crucial influence for the anomeric effect, as it is directly depends upon the electronegativity. Solvents also influence the anomeric effect, increased polarity of the solvent decreases the influence of the anomeric effect on the equilibration of the two alternative conformers in solution. If the substituent at the anomeric center is clearly electropositive then, it leads to the stabilization of the anomer with the equatorially positioned anomeric group (Figure 10). This effect is not different from the anomeric effect, and hence been termed as the "reverse anomeric effect".[29-30]

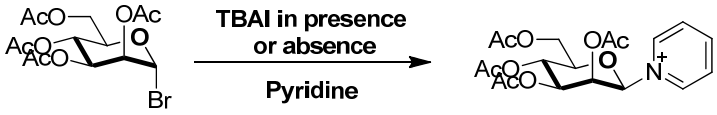


Figure 10:- Reverse Anomeric Effect

Neighbouring Group Participation of the C-2 Ester Functionality

The substituent present at C-2 of glycosyl donor plays an important role in the stereoselective synthesis of 1, 2-*trans* glycosides. When esters such as benzoate, acetate etc are attached on C-2 of glycosyl donor, the promoter activates leaving group resulting in the formation of oxocarbenium ion which is in equilibrium with the stable dioxolenium ion formed by neighbouring group participation of carbonyl group of ester functionality that allows unidirectional attack of alcohols from *trans* side die to the steric influence exerted by this five membered ring offering 1,2-*trans* glycoside. Therefore, β-linked products are formed in these types of glucosyl donors, whereas mannosyl type donors provide α-mannosides.

Effect of Solvent

Solvent has also an effect on the anomeric outcome of glycosidations.[31] The influence of ether and acetonitrile on the anomeric outcome of glycosylation reactions under S_N1-type

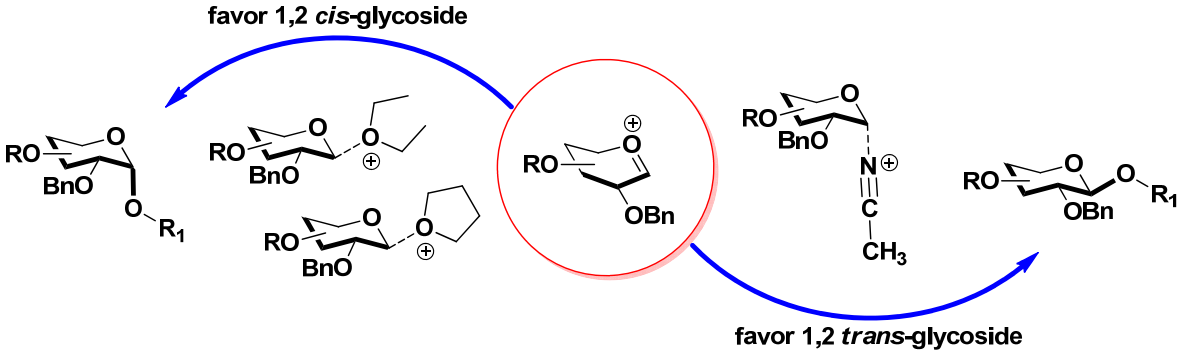


Figure 11:- Effect of Solvent on Glycoside Formation

conditions that is without a participating neighboring group, has been extensively invistigated.[32] Ethers such as diethyl ether or THF favor the formation of 1, 2-*cis* glycosides, where as 1, 2-*trans* glycosides are the major products of glycosylation in acetonitrile in the absence of a neighboring group at C-2 (Figure 11).

1,2-*trans* Glycosidation

1,2-trans glycoside can be obtained by using suitable glycosyl donor which is activated in presence of a which initiates departure of the anomeric leaving group leaving behind a cation, which is stabilized as oxocarbenium ion. The ester group bound at C-2 will exert an anchimeric effect leading to an acyloxonium intermediate which is formed initially from the oxocarbenium ion. Nucleophilic attack on dioxolane ring at C-1 leads to *trans*-cleavage yielding 1, 2-*trans* oriented *O*-glycosidic linkage (Figure 12).

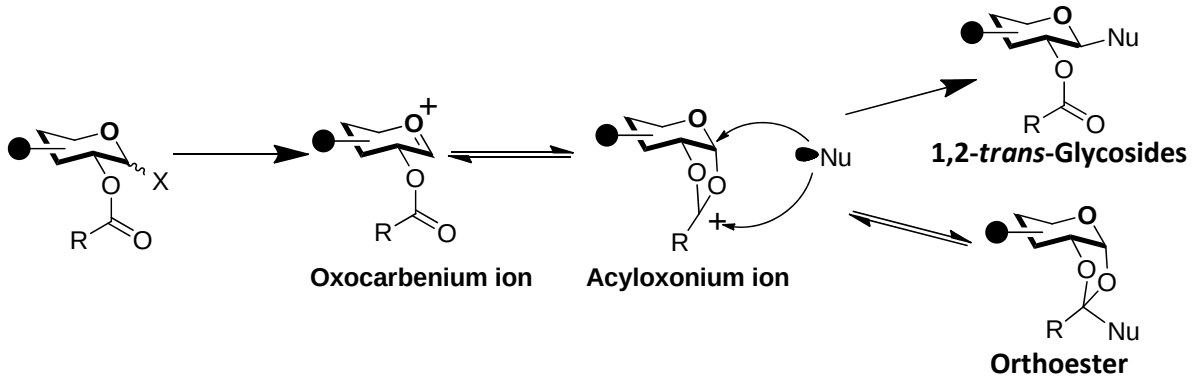


Figure 12:- 1,2 *trans*-Glycosylation with Neighbouring Group Participation

Sometimes we can observe formation of orthoesters when the nucleophilic attack took place at the dioxiolane ring carbon instead of C-1. Orthoester formation can be main reaction when neutral or basic reaction conditions are applied. Using benzoates or pivaloates as protecting groups in C-2 strongly reduces the tendency for orthoester formation compared to that when acetyl groups are used.

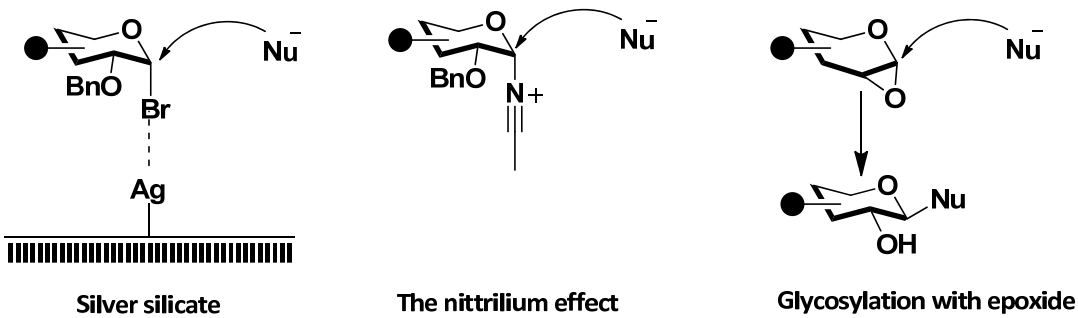


Figure 13:- 1,2 trans-Glycosylation Without Neighbouring Group Participation

1,2-*trans* glycosidation product are observed in some cases which are shown below (Figure 13). When glycosyl halides are treated with insoluble silver salts and no neighboring group participation group present, we could get the 1,2-*trans* glycoside as major product. Solvent also play role important role when no NGP group present and polar solvent such as acetonitrile gives *trans* glycosidation product. Nucleophilic 2,3-glycosyl epoxide opening also gives trans glycoside. These are some cases where no NGP present and other reaction condition favours *trans* glycosidation.

1,2-*cis* Glycosidation

Synthesis of 1,2-*cis* glycoside is much more difficult than 1,2-trans glycosides. For this one has to choose suitable group at C-2 as a non-neighboring group such as an *O*-alkyl ether. This can direct an S_N2 reaction at the anomeric center of β-glycosyl bromide that eventually would furnish α-glycoside. However this is not practical since β-pyranosyl halide, especially bromides are greatly destabilized by the anomeric effect. R. Lemeuix and co-workers[33], showed that α-pyranosyl bromides reacted in the presence of tetraalkylammonium bromide with anion to produce the β-pyranosyl bromide *in situ.* The highly reactive β-pyranosyl

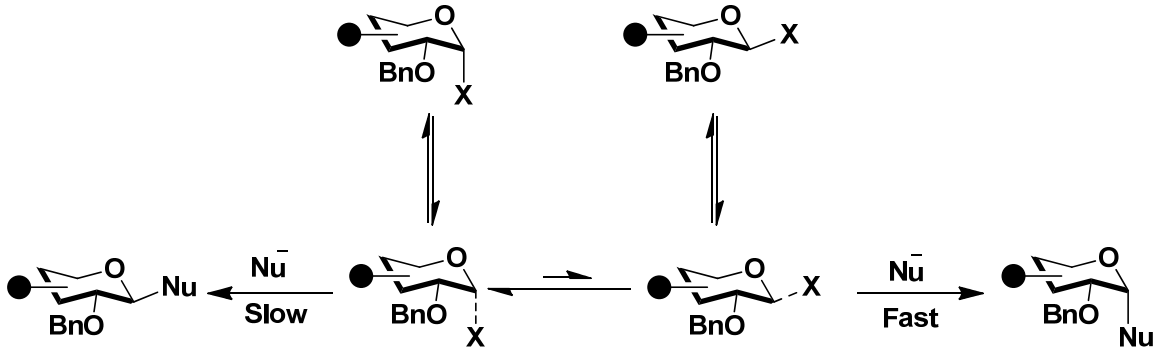


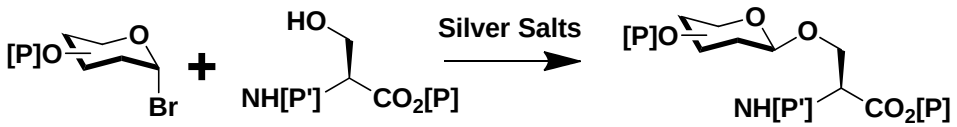
Figure 14:- 1,2-*cis*-Glycosyaltion as Major product by *in situ* Anomerization

bromide reacts much faster than its α-analog to give the α-glycoside in large proportions in a kinetically-controlled reaction.[1,34] This method is called '*in situ* anomerization' (Figure 14). Which works well with galactose or fucose as donor, it is less effective for glucose and β-mannoside synthesis. Acceptor hydroxyl also plays important role in the proportion of α-glycoside as it decreases with lower donor activity or diminished reactivity of hydroxyl group. Also one can take advantage of "*reverse anomeric effect"* for the synthesis of 1,2-*cis* *O*-glycosides.[35] Very few methods were forced to be suitable for the synthesis of glycosyl amino acid and reported methods for the synthesis O-linked glycoaminoacid are:

1) *Glycosyl Halide Koenigs-Knorr method*
2) *Glycosyl trichloroacetimidate (by R. Schmidt)*
3) *Thioglycosides*
4) *N- Pentenyl Glycosides*

The Koenigs-Knorr method[36-38]

In 1901 *Wilhelm Koenigs* and the *Eduard Knorr* from the University of Munich introduced a very classical method for the synthesis of 1,2-*trans* glycosides using glycosyl halide as glycosyl donor. This very old method of glycosylation, generally glycosyl bromide and glycosyl chlorides are used in the presence of a halide ion acceptor (promoter) usually a silver[23] or mercury salts as both insoluble Ag_2O, Ag_2CO_3 as well as soluble silver triflate (AgOTf)[39] and silver perchlorate ($AgClO_4$)[40] are employed. Later on Helferich introduced modification to the promoter by using mixture of mercury salts such as $HgBr_2$ and $Hg(CN)_2$.[41]



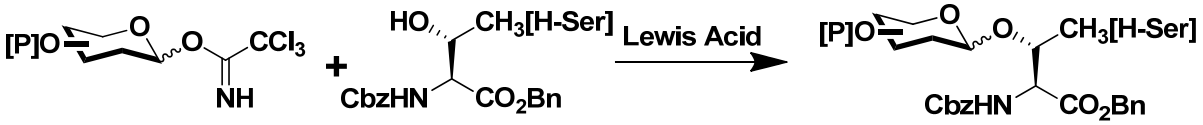
Koenigs-Knorr reaction normally leads to the formation of 1,2-*trans* glycosides with participating group at the C-2 position, especially when insoluble silver salts are used as . The reason for this is that silver salts provide surface for glycosidation thereby assisting the formation of the *trans* glycoside by shielding the α-face of the sugar. During the course of reaction, silver bromide formed as byproduct which is precipitated out of the reaction mixture.

Sometimes base used in combination with plays a major role in glycosylation reaction such as combination of silver trifluoromethanesulfonate (AgOTf) and 2,4,6-collidine which leads to the formation of glycosyl orthoester. When it is replaced by *N,N,N',N'*-tetramethylurea the course of the reaction is different and goes through 1,2-orthoester intermediate leading to 1,2-*trans* glycoside as a major product.

However the reaction suffers from two disadvantages: first is the lability of glycosyl halides, with the β-glycosyl bromide being unstable due to anomeric effect, and the second the use of heavy metals salts in the range of equimolar amounts. Sometimes mixture of 1,2-*cis* and *trans* glycoside products observed using glycosyl halide as donor.[42-43]

The trichloroacetimidate method[22,41,44-46]

R.R. Schmidt and co-workers have developed the most widely used glycosylation method in oligosaccharides synthesis by employing trichloroacetimidate as glycosyl donors, that is superior to the Koenigs-Knorr method.[41] The general significance lies in their ability to act as strong glycosyl donors (armed donor) under relatively mild acid catalysis. This reaction is very simple to carry out and amount of Lewis acid varies from case to case. This may happen because of the formation of orthoester as intermediate, thus more catalyst is required in this case to isomerize orthoester to corresponding direct glycoside. For the synthesis of glycosyl donor, one has to selectively deprotect the anomeric-protecting group and base treatment (K_2CO_3, NaH or DBU) with trichloroacetonitrile to form glycosyl trichloroacetimidate.



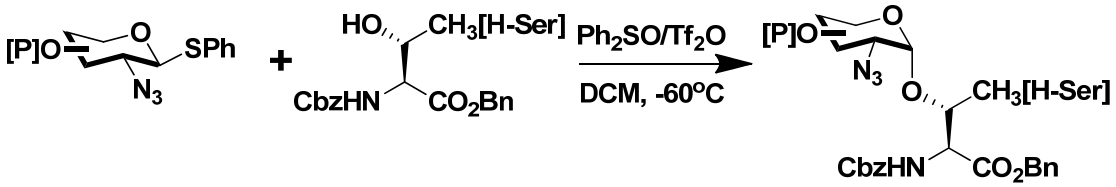
Glycosyl trichloroacetimidates give very good yields in small as well as large scales. Frequently glycosyl trichloroacetimidate are more stable than respective glycosyl bromides and can be stored at lower temperatures for many months. Ether protected trichloroacetimidate are more reactive than their ester protected counterparts, as ether groups stabilize the oxonium ion, which occurs as intermediate of the glycosylation reaction. Reactive donors are handled at lower temperature because the high reactivity leads to side reaction or even decomposition of the glycosyl donor before the glycosylation happens. When the acceptor alcohol is very unreactive, the donor may rearrange to the corresponding glycosyl trichloroactamide, which is reported to have no donor activity leading to lower glycoside yields. In such cases Inverse glycosylation procedure will be used to improve the yields and stereocontrol, where the glycosyl acceptor and the catalyst are first dissolved together and the glycosyl donor is then added.

Neighboring group participation effect of C-2 protecting group in trichloroacetimidate method is dominating the anomeric stereocontrol during glycosylation, giving rise to 1,2-*trans* glycosides. When non-participating protecting groups are selected, S_N2-type reaction can be carried out by use of non-polar solvents, low reaction temperature, and weak Lewis acid catalyst ($BF_3.Et_2O$). Strong acid catalyst (TMSOTf, TfOH), higher temperature and more

polar solvents support the formation of the thermodynamically more stable glycosylation products, which are the α-manno and β-gluco type. One can perform one-pot tri- or oligosaccharide synthesis by employing different glycosyl donors with tuned donor activities to allow multiple glycosylation in a single pot.

Thioglycosides[39-40,47-48]

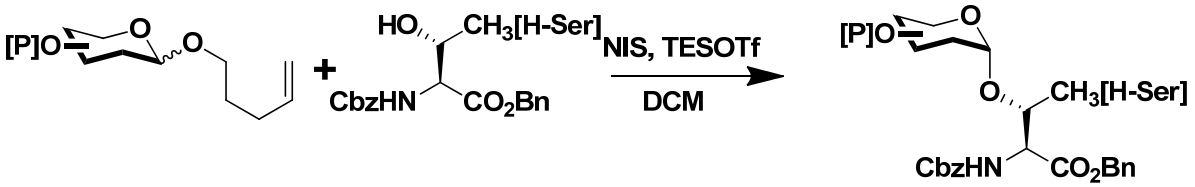
Thioglycosides as glycosyl donors was first introduced by Lonn and coworkers in 1980's wherein, the promoter activates the sulphur of thioglycosides producing an intermediate sulfonium ion which in turn forms oxocarbenium ion and subsequently, trapped with the glycosyl acceptor to offer glycosides. Promoters used for such reaction are MeOTf, DMTST,



Iodinium dicollidine perchlorate, and NIS-TfOH. The advantages of using thioglycosides are their stability under a wide range of reaction conditions. Additionally, thioglycosides act as temporary protecting groups at the anomeric position that helps to synthesize both glycosyl donor as well as glycosyl acceptor for the synthesis of oligosaccharides. But the unpleasant odour of thiols and its effect on health limits use of them as glycosyl donors.

n-Pentenyl glycosides[49-54]

Fraser-Reid and coworkers introduced pentenyl glycoside as stable glycosyl donor in which the pentenyl group is activated by an electrophilic addition of the iodinium ion to double bond of the glycosyl donor followed by an intramolecular displacement through an oxygen atom present at the anomeric center and the simultaneous removal of cyclized product to form an oxocarbenium ion which is then trapped by glycosyl acceptor. The s used for these

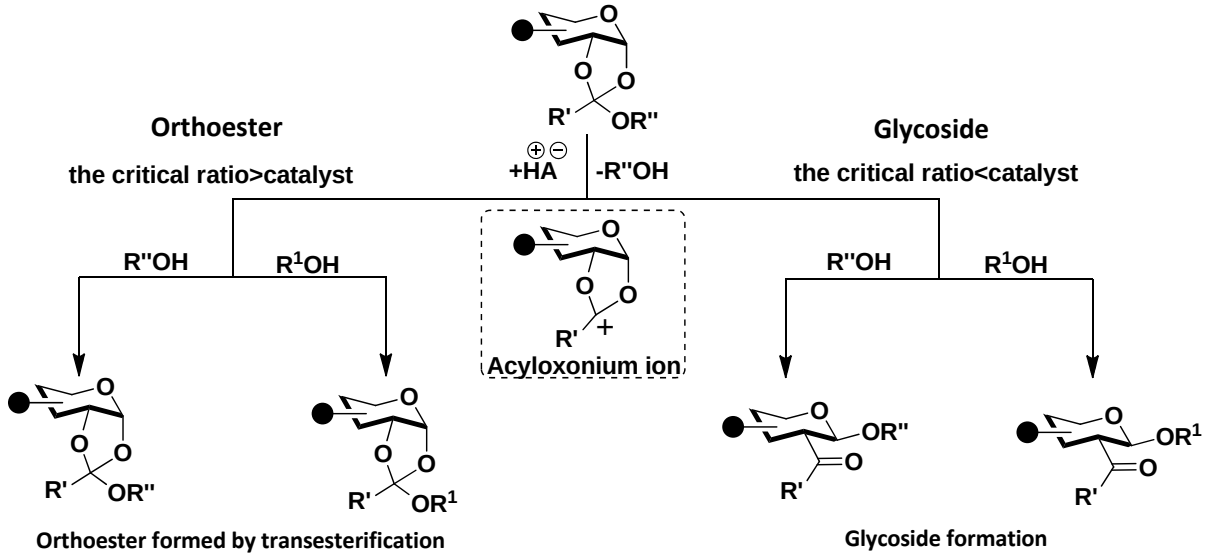


reactions are NIS alone or NIS/Et_3SiOTf, NIS/TfOH and NIS/$Yb(OTf)_3$. Later pentenyl 1,2-orthoesters was also employed for the synthesis of oligosaccharides by Fraser-Reid.[49] But the major limitation is the excess use of N-iodosuccinimide (NIS).

The potential of the *n*-petenyl glycoside methodology is further increased by the observation that acyl and ether protected n-pentenyl glycosides displays different reactivities. This finding leads to new concept in glycosylation known as the armed-disarmed glycosylation.[51]

1,2-Orthoesters[55-63]

Kochetkov and coworkers systematically studied 1,2-orthoester and applied for the stereoselective synthesis of 1,2-*trans* glycosides. In 1964 they published first paper on glycoside synthesis using 1,2-methyl orthoester as glycosyl donor and cholesterol as acceptor[55] and they have also studied leaving ability of different orthoesters such as ethyl, isopropyl, tert-butyl and cyano 1,2-orthoester. Taking advantage from pentenyl glycoside as glycosyl donor Fraser-Reid and coworkers introduced the pentenyl 1,2-orthoesters as glycosyl donor in the presence of NIS/$Yb(OTf)_3$ to get 1,2-*trans* glycosides.[64-66]



When the orthoester is reacted in the presence of suitable catalyst, there are two possibilities. One is the formation of *trans* orthoester or the direct glycoside which depends upon the ratio of catalyst to orthoester, i.e the critical ratio.[56] Glycosylation predominates above the critical ratio, and trans- esterification below. At high catalyst concentration (H+A-) its anion mask the positive charge of the acyloxonium, thus hindering nucleophilic attack of glycosyl acceptor which then takes place at the glycoside C-1. For a reaction without addition of co-catalyst, dichloromethane as solvent and addition catalyst of $HgBr_2$ minor amount (0.001moles) results in formation of transorthoester product. On the other hand use of polar solvent nitromethane and high catalyst ratio of $HgBr_2$ (0.008-0.1 moles) gives

trans glycoside as major product. In presence of co-catalyst such as p-toluenesulfonic acid (TsOH, 0.00025 mole), $HgBr_2$ (0.001-0.008 mole) per mole of orthoester & nitromethane as solvent gives *trans* glycoside whereas less than above ratio gives either mixture of trans orthoester & trans glycoside or trans orthoester as major product.[55-56] At low concentration direct attack and fresh orthoester formation becomes more likely. However, at higher catalyst concentration the formation of other by products such as the acetate of glycosyl acceptor and dialkyl ether becomes very pronounced *cis* and *trans* glycosides can also result. Even solvent-catalyst combination has characteristic critical value. In polar solvents, like nitromethane, $HgCl_2$ and $HgBr_2$ are examples of suitable catalysts, whereas in nonpolar solvents like chlorobenzene and dichloromethane, the perchlorates of pyridine and lutidine represent better choice. Actually the last combination gives best yields. The orthoester method has been successfully applied to numerous glycoside syntheses.

With this brief introduction to O-glycoside synthesis we will discuss the present work on the synthesis of amino acid glycoconjugates using Gold Catalyst and Propargyl 1,2-orthoesters as glycosyl donor and hydroxyl group containing amino acid such as serine, threonine etc. Also the interesting result we got when the *t*-Boc protected amino acid reacted with Propargyl 1,2-orthoester in presence of Gold catalyst.

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Carbohydrates are easily available from natural resources, which are one of the starting building blocks in current research work. Availability of carbohydrates in bulk quantity as well as low price makes carbohydrates as very good starting material to carry out research work in carbohydrate field. As carbohydrates are available from nature, they are embedded with chirality, because nature only synthesizing one isomer of any biological important biomolecule such as in case of amino acid, carbohydrates, alkaloids and other natural products.[67-69] Apart from the configuration at C-1 carbon, stereochemistry of the all chiral centers in carbohydrates are fixed, which is very good source of chirality. Selective protection and deprotection strategies are heart of synthetic carbohydrate chemistry. Many research groups are involved in control stereochemistry and regioselectivity of the glycosidic bond of carbohydrates. Controlling only single carbon stereochemistry is very difficult; one could imagine what will be the situation if one has to controlled stereochemistry at rest of carbon, which will much more difficult. But nature can do this entire thing very easily with taking care of stereochemistry at each center and ecofriendly by taking the help of enzymes. Isolation of most of the biological important complex carbohydrates is difficult as they are present in small quantities as well as in micro-heterogeneous form. So it is desirable to have synthetic route, which will give access to synthesize these materials in large quantities and study its biological properties.

Biological importance of the glycosylated amino acids in glycoprotein, antifreeze compounds[70-74] & in glycopolypeptide chemistry[75-76] makes synthesis of glycosylated amino acids one of the challenging research areas for synthetic chemists. We thought to use gold catalyzed glycosylation methodology developed in our laboratory using gold salts as Lewis acid catalyst to attach hydroxyl group containing amino acid to carbohydrates. For this we have chosen the side chain hydroxyl group containing amino acids as other starting biomolecule of our research work, both these are available from Nature very easily and commercially very cheap. There are different methods for attachment of amino acid to carbohydrates, which we discussed in introduction part of this chapter, but each method has its own advantages and disadvantages. So carbohydrate community always associated with solving the major problem of stereo- and regio- selectivity in glycosylation using

different leaving group varying Lewis acid catalyst combination & by different protecting group on carbohydrates.

To begin our current work, I would like to give brief introduction about how we arrive at point where one could do glycosylation by using gold salts. Gold is known for very long time as precious metal and has unique properties (malleable, corrosion resistance, excellent electrical conductance & biologically not hazardous), which are well documented. Such as very classical example of nanotechnology in ancient days is the Lycurgus Cup 1400 year old was coloured with colloidal gold and silver. It was also used by Ethruscan peoples in sventh century BC to secure substitute teeth. Michael Faraday's 1857 report conjectured that gold could exist in solution as finely 'divided metallic state' rather than as an oxide, and exactly 100 years ago the Nobel prize winner Ernest Rutherford published date on 'gold foil' experiment proof of the existence of the nucleus and hence structure of the atom. Gold has become extremely important in science and technology because of the pioneering work done by Faraday and Rutherford. In 1960s firstly Bell Laboratories developed the first gold-bonded microchips followed by NASAs use of gold to protect sensitive instrument from radiation in space. These applications highlight bulk properties of gold. In 1970s Geoff Bond and colleagues published breakthrough describing gold having some catalytic activity n certain reactions. Up to 1980s researchers showed both theoretically and experimentally that gold can acts as superior catalyst in a range of reaction including acetylene hydrochlorination and CO oxidation. Gold is active in the form of its halide salts or complex. Gold is used in various applications such as monetary exchange, investment, jewelary, medicine, food and drinks, industry, electronics etc. Most often it occurs as a native metal, typically metal solid solution with silver i.e. gold silver alloy.

Recently nanotechnology has been benefitted because of gold's unique properties.[77-79] Also numerous applications of gold catalysis has been shown in field of key reaction in total synthesis and synthesis of diverse library of molecule for high-throughput screening.[80-82] Organic chemists believe that as gold is precious metal and its use in organic synthesis may be more expensive. But if one considers the organometallic complexes of Ru, Pt, Ir and Rh are actually more expensive than gold complexes. So gold compounds can be used in organic synthesis by taking advantage of catalytic activity of gold complex.

O-glycoconjugates plays important role in various biological processes. As evident from introduction part, which we have discussed problems associated with the synthesis and isolation of single & pure form of glycoconjugates. This led to us think about new method for the synthesis of *O*-linked glycoconjugates.

Last five year's gold catalyzed glycosidations have gained attention was foremost goal is the glycosidation. While synthesizing the diverse library of molecules for biological activity from our group, propargyl group was found to behave as leaving group in the presence of $AuCl_3$. Generally Diels-Alder reaction is used for diverse library of compounds, when the 1,2:5,6-bis-O-(1-methylethylidene)-3-C-(5-methyl-2-furanyl)-3-O-prop-2-ynyl-allofuranose **1** is reacted to get Diels-Alder product between the propargyl and the furan substituent at C-3 in presence of 3 mole% of $AuCl_3$ in acetonitrile as solvent. However, the required Diels-Alder product **2** along with the 5 % of propargyl ether hydrolyzed product **3** at C-3 carbon were observed (Figure 15).[83] To check leaving group ability of propargyl group, 3-*O*-prop-2-ynyl-4,6-di-O-benzyl-1,2-glucal **4** was treated with methanol in the presence of 3 mole % of $AuCl_3$ in acetonitrile and found an unexpected methyl 2,3-dideoxy glucoside **5** (Scheme 15).[84-85] The initial coordination of gold with the triple bond of alkyne assist the cleavage of propargyloxy moiety and simultaneous attack of alcohols from α-face lead to stereoselective formation of α-glycoside. Further $AuCl_3$ mediated activation of propargyloxy moiety was utilized for the synthesis of C-2 methylene glycoside **7** using per-*O*-benzylated C-2 propargyloxy methyl glycals **6** as glycosyl donors.[85]

Deprotection of propargyl ether in carbohydrates was already reported by different groups using samarium iodide and water,[86] dicobaltoctacarbonyl followed by treatment with trifluoroactic acid,[87] titanium and metal catalyzed isomerization.[88] Unfortunately they have never explored these methods for glycosidation and oligosaccharide synthesis. Beyond that, propargyl glycosides can used in Husigen's 1, 3-dipolar azide-alkyne cycloaddition (Click Chemistry) to synthesize bioconjugates or surface modification of polymer or nanoparticle.[89-90]

For the first time, propargyl glycosides were activated in the presence of $AuCl_3$ for the synthesis of glycosides and disaccharides. In this approach, initial treatment of armed prop-2-ynyl 2,3,4,6-tetra-O-benzyl-α:β-D-glucopyranosides **8** with water in presence of 3 mole %

$AuCl_3$ in acetonitrile for 12 h at room temperature gave a mixture of per-*O*-benzylated lactols **9** (Figure 15).[91] Encouraging results prompted to use other nucleophilic alcohols in

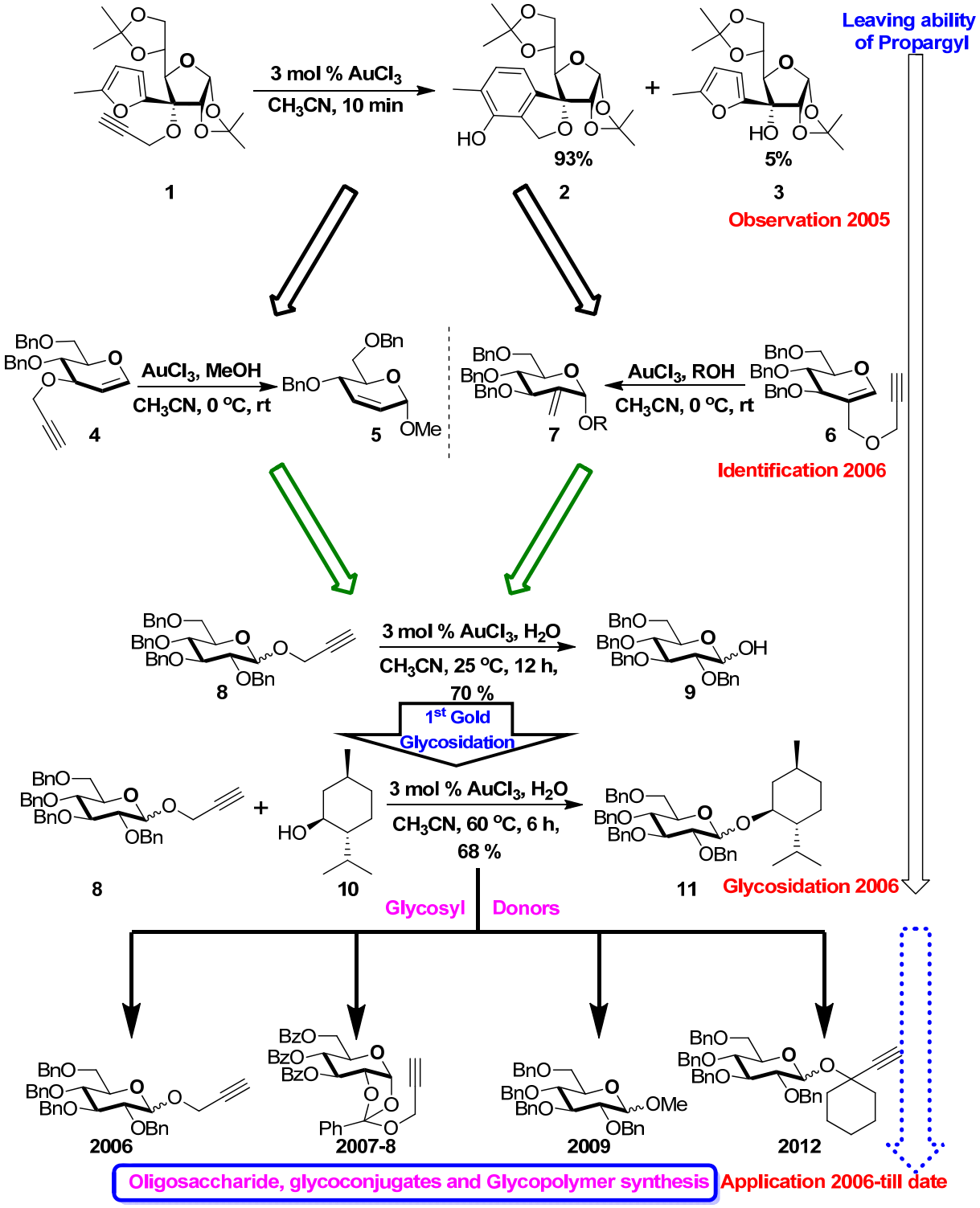


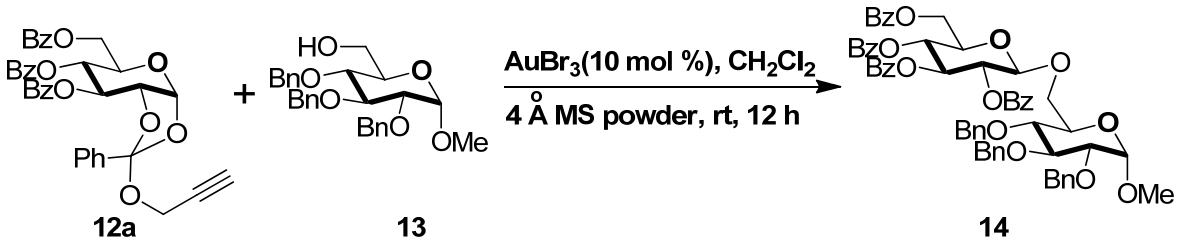
Figure 15:- Development in Gold Catalyzed Glycosidation by Activating Propargyl Group

place of water to facilitate transglycosylation for the synthesis of glycosides and disaccharides. The glycosylation performed between the propargyl glycoside **8** and menthol **10** in the presence of 3 mole % $AuCl_3/CH_3CN$/60°C/6 h resulted into a mixture of α,β-

menthyl glucosides **11** in 68 % yield.[91] The glycosylation was failed when other alkyne activators namely $PtCl_2$, $Cu(OAc)_2$, $Co_2(CO)_8$ and $RuCl_3$ catalyst were employed. Further, glycosylation failed to give 1,2-*trans* glycosides, when the glycosylation was attempted with disarmed C-2 ester functionalized propargyl per-O-benzoylated or per-*O*-acetylated glucosides and an aglycon in the presence of 3 mole % $AuCl_3/CH_3CN$/60°C. This experimental study confirms that $AuCl_3$ will work well with armed glycosides and thus was applied for other monosaccharides such as mannose, galactose and glucose with various aglycons to get corresponding glycosides or disaccharides respectively.[51] In case of glucoside and galactoside we get mixture of α,β-glycosides were observed. Whereas mannosyl donor provided the α-isomer due to strong anomeric effect as well as bulky substituent at C-2 position.

The common advantage of propargyl glycosides are 1) stable towards the environmental conditions and protecting group manipulations, 2) glycosyl donor which required 3 mole % of $AuCl_3$ for the activation of propargyloxy group, 3) easily accessible and 4) widely useful in bioconjugation or functionalization via copper catalyzed cycloaddition reaction with azide derived from peptides, lipids, proteins and polymers.

Unfortunately, a mixture of products formed during glycosylation, when a propargyl glucoside and galactoside were used in presence of $AuCl_3$ in acetonitrile at higher temperature. In order to get stereoselective 1,2-*trans* glycosylation, propargyl 1,2-orthoester can be used as glycosyl donor in which the C-2 ester group will be participated to stabilize the oxonium ion and nucleophilic attack will give rise to a 1,2-*trans* glycoside. Already orthoesters are explored to give stereoselective 1,2-*trans* glycoside product as we discussed in introductory part by *Kochetkov* & *Bert-Fraser-Reid* groups, who showed its



Scheme 1:- Application of Gold Complexes for Stereoselective 1,2-*trans* Glycoside Formation

application to 1,2-*trans* glycoside synthesis using different orthoesters. Gold Catalysis repature was found to be suitable for the synthesis of 1,2-trans glycosides from orthoester.

Accordingly propargyl 1,2-orthoester **12a** are used for synthesis of 1,2-*trans* glycosides and disaccharides **14** (Scheme 1).[92-93] Controlled study on glycosylation has been done using different Lewis catalyst in combination with Brønsted acid & base, which confirms that gold salts are required to get glycosidation reaction happen; addition of bases such as triethylamine or pyridine doesn't give any glycosidic product due to quenching of acidic reaction medium.

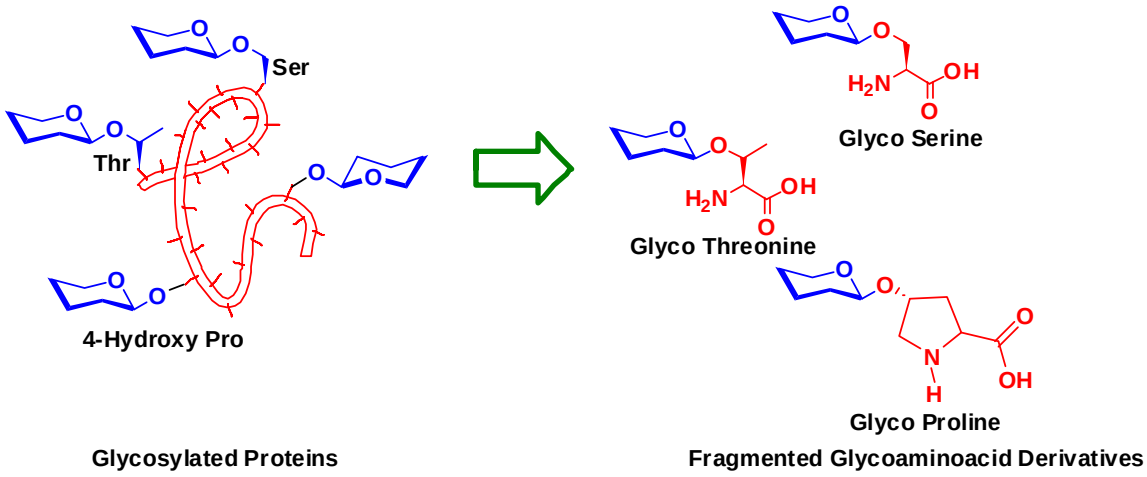
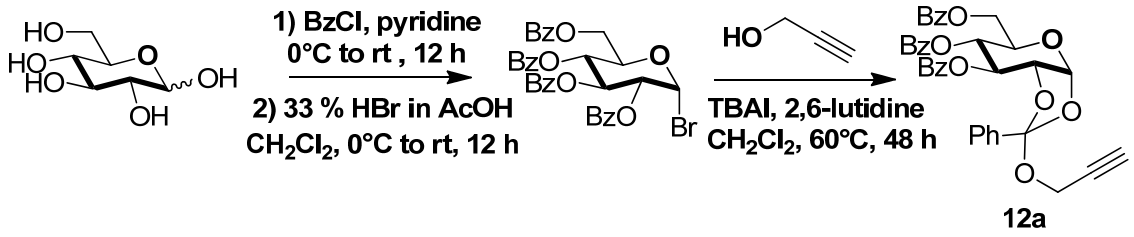


Figure : Glycoproteins and Importance of Glycoaminoacid Derivatives in Glycopeptide Synthesis

Thus, we thought of applying glycosylation methodology for the synthesis of glycosylated amino acid glycoconjugates, which are important class of building blocks for glycopolypeptide synthesis and solid phase glycopeptide synthesis. For this we have chosen side chain hydroxyl group containing amino acids such as serine or threonine in place of simple alcohol or carbohydrate to get glycosylated amino acid glycoconjugates. Synthesis starts with propargyl 1,2-Orthoester, which has been synthesized from per-*O*-benzyolated monosaccharide such as glucose, galactose and mannose in two steps.

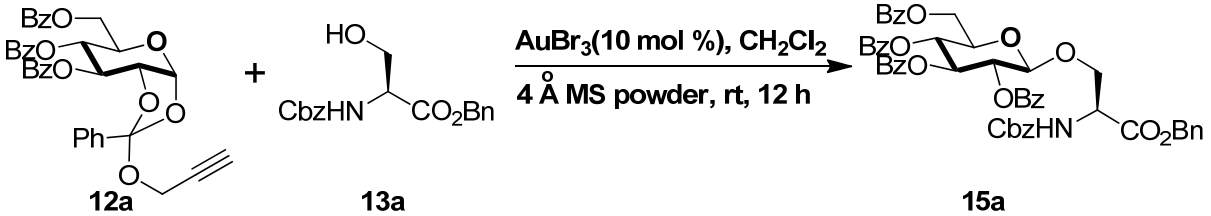


We have chosen benzoyl as protecting group for carbohydrates because it is easily available in comparison to acetic anhydride which comes under narcotic drug act. Benzolyation of all the monosaccharides used in present work was carried out by using benzoyl chloride and pyridine as the reaction solvent. The treatment of per-*O*-benzoylated glucose with 33% HBr

in AcOH formed 2,3,4,6-tetra-*O*-benzyol-α-D-glucopyranosyl bromide that was subsequently reacted with propargyl alcohol, 2,6-lutidine and a catalytic amount of TBAI in dichloromethane at 65°C for 2 days to give propargyl 1,2-orthoester **12a**. In the ^{1}H NMR spectrum of compound **12a**, resonances at δ_H 2.40 ppm as triplet and δ_H 3.99 ppm as a doublet clearly indicate the presence of propargyl group and a doublet at δ_H 6.11 ppm (J=5.31 Hz) which is a characteristic for β–configured anomeric proton of orthoester were noticed. Rest of signals in spectrum were in complete agreement with the assigned structure **12a**. In addition, ^{13}C NMR spectrum revealed the characteristic signals corresponding to α-anomeric carbon and the quaternary carbon at δ 97.7 (C1) and 121.1 ppm respectively while rest of the other signals were in accordance to the assigned structure 3,4,6-tri-O-benzoyl-α-D-glucopyranose-1,2(prop-2-ynyl orthobenzoate) **12a**. The structure was further supported by mass spectroscopic analysis (Mol.Wt.Calcd. 634.62, Found: 656.21(M^++Na)).

Only a limited number of approaches are available for the attachment of a serine/threonine.[94-104] For example, one of the earliest and widely used methods couples the CbzSer(OH)Bn and an acetobromo sugar by means of environmentally detrimental mercury salts. A notable and significant improvement was reported by Cameron's group[94] taking advantage of reaction conditions developed by Field et al.[96] In this premise, methods that enable preparation of amino acid glycosides from stable glycosyl donors using catalytic and eco-friendly reagents are essential.[105]

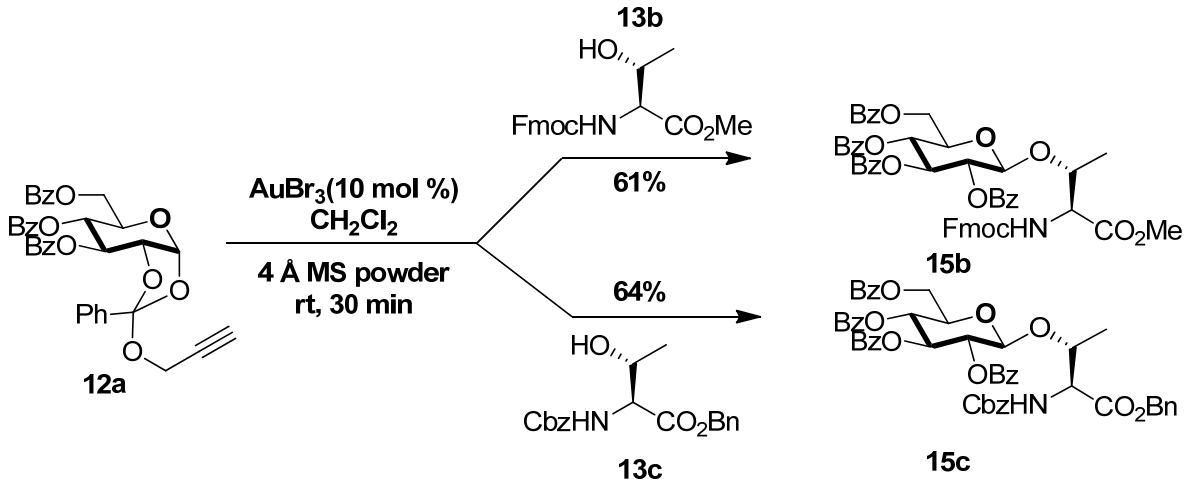
In continuation of the programme on gold catalyzed glycosylations, we got interested in the exploitation of propargyl 1,2-orthoesters[57,92-93] for the synthesis of amino acid glycoconjugates. To begin our investigation, initially propargyl 1,2-orthoesters, Fmoc- and Cbz-protected serine/threonine were considered. Selective protection of amino acid was done by following literature procedure[106-107] to get desired hydroxyl containing amino acid



Scheme 2 :- Gold catalyzed Glycosidation for Glycoaminoacid derivatives

derivative for glycosidation reaction. Accordingly, per-*O*-benzoylated glucose 1,2-*O*-orthoester **12a** was allowed to react with serine derived aglycon. Among various naturally occurring amino acids, hydroxyl containing serine and threonine and their Fmoc-, Cbz- and Boc-protected derivatives are the most widely used in peptide synthesis. Thus, CbzSer(OH)Bn **13a** reacted with 3,4,6-tri-O-benzoyl-α-D-glucopyranose-1,2(prop-2-ynyl orthobenzoate) **12a** in the presence of 7 mol% of $AuBr_3$ as catalyst and 4 Å MS powder to make sure anhydrous medium in dry dichloromethane at room temperature for 30 min to afford Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-b-D-glucopyranosyl)-L-serinate **15a** (Scheme 2).[108]

The literature indicated that several attempts have been made to synthesize amino acid glycosides that included use of toxic Hg salts, very extensive and laborious purification protocols, which led to a poor overall yield of the resulting glycoconjugate.[94,97,109] However, $AuBr_3$ catalyzed glycosylation resulted in the isolation of Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-b-Dglucopyranosyl)-L-serinate **15a** in 76% yield. Simple gravity flow and conventional silica gel column chromatography would be sufficient to separate propargyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (sole byproduct)[92-93] from the required serine glucoside **15a**. 1,2-*trans* Glycosidic linkage was confirmed by NMR spectral studies wherein the anomeric proton was noticed at δ_H 4.78 ppm (d, 1H, J = 7.6 Hz) and the anomeric carbon was observed at the δ_c 101.3 ppm.[108]

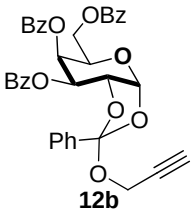
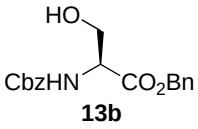
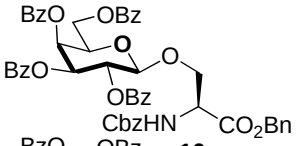
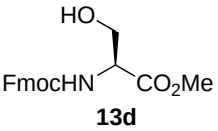
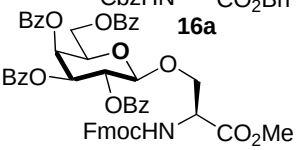
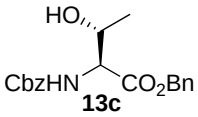
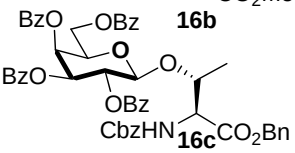
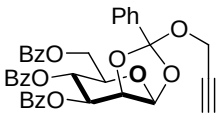
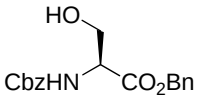
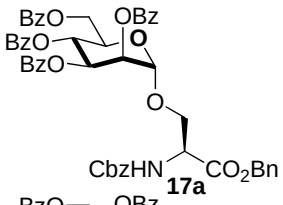
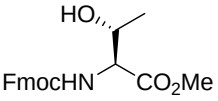
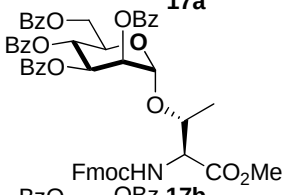
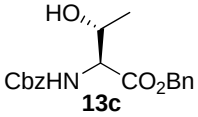
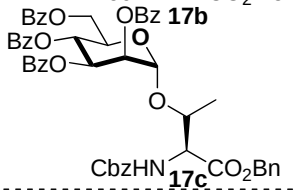
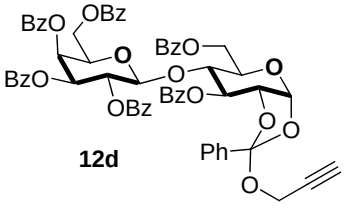
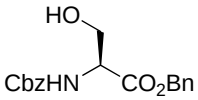
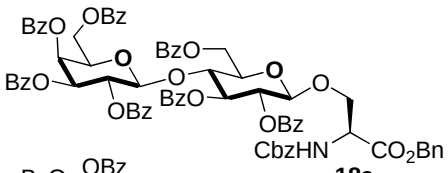
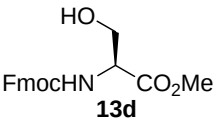
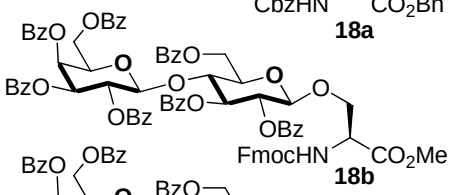
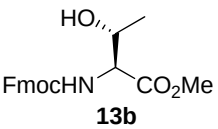
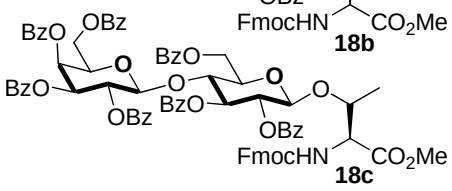


Scheme 3 :- Reaction with different aminoacid derivatives

We then continued our synthesis endeavour with 1,2-orthoester **12a** that was allowed to react with FmocThr(OH)OMe **13b** to give the threoninyl glucoside **15b** in good yield.

Furthermore, CbzThr(OH)Bn **13c** reacted with the glucosyl orthoester donor **12a** resulting in the formation of threoninyl glucoside **15c** (Scheme 3). It is interesting to note that the *N*-

Table 1

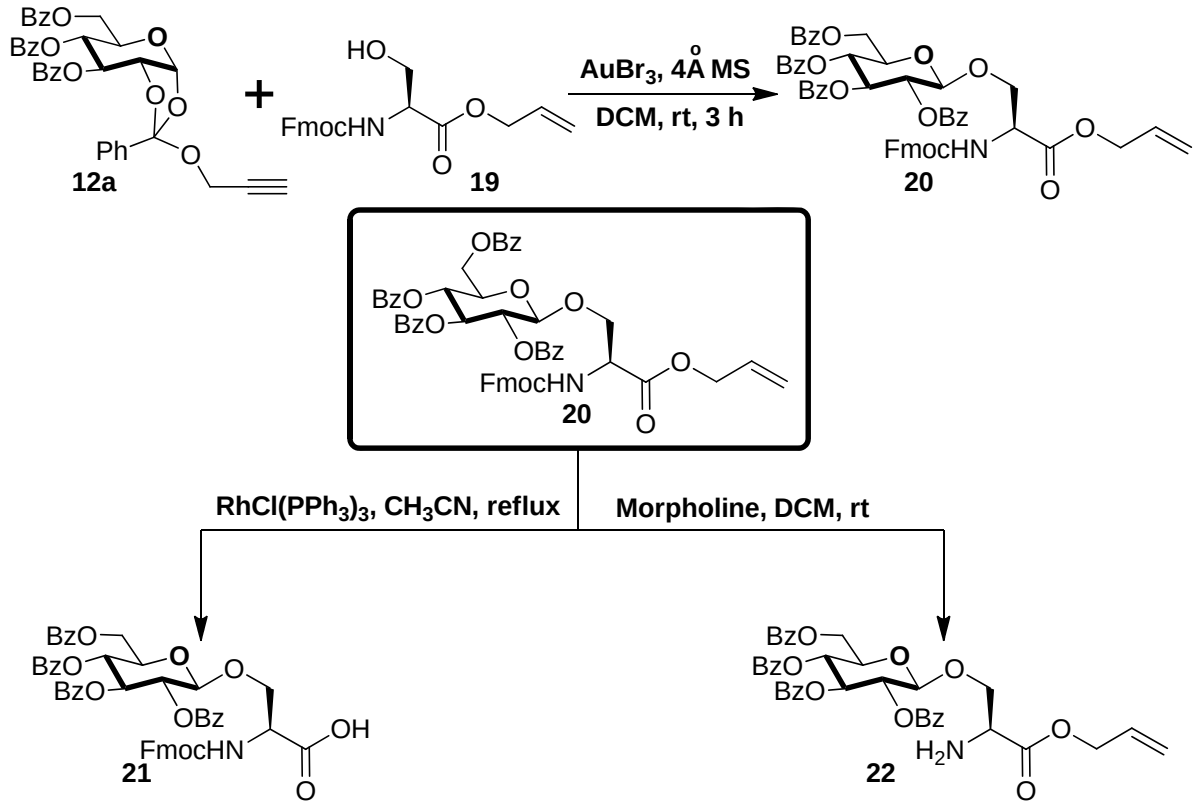
Propargyl 1,2-othoesters	Glycosyl acceptors	1,2-trans glycoaminoacid	Time & Yield
 **12b**	 **13b**	 **16a**	**63 %/ 1h**
	 **13d**	 **16b**	**67 %/ 2h**
	 **13c**	 **16c**	**69 %/ 1h**
 **12c**	 **13a**	 **17a**	**62 %/ 2h**
	 **13b**	 **17b**	**66 %/ 2h**
	 **13c**	 **17c**	**62 %/ 2h**
 **12d**	 **13a**	 **18a**	**61 %/ 2h**
	 **13d**	 **18b**	**63 %/ 2h**
	 **13b**	 **18c**	**64 %/ 2h**

Reagents and Conditions: $AuBr_3$ (10 mol %)/CH_2Cl_2/4 Å MS powder/rt, 30 min

Cbz- or *N*-Fmoc-groups and benzyl/methyl esters were intact during the gold catalyzed glycosylation.[108]

Versatility of the glycosylation methodology with gold reagents was further extended to the other glycosyl donors to get glycoamino acids. For example, galactosyl (**12b**), mannosyl (**12c**) and lactosyl (**12d**) 1,2-orthoesters were allowed to react with serine and threonine derived aglycons (**13a–13d**) to obtain corresponding galactosides (**16a–16c**), mannosides (**17a–17c**) and lactosides (**18a–18c**) respectively in good yields (Table 1). In all the cases, we identified formation of 1,2-*trans* selective glycosides only.[108]

Glycosylated amino acids are very useful in the synthesis of carbohydrate modified peptides. These glycopeptides shows antifreeze properties[73-74] and carbohydrate moiety gives extra stability to helical conformation of peptide backbone.[110] Only few methods are reported for synthesis of glycoaminoacid derivatives, which can be used in solid peptide phase synthesis. Here, we are reporting gold catalyzed method for synthesis of glycoaminoacid. For this, we synthesized amino acid derivatives, which can be selectively deprotected to get amino or acid group using suitable condition. This kind of selectively

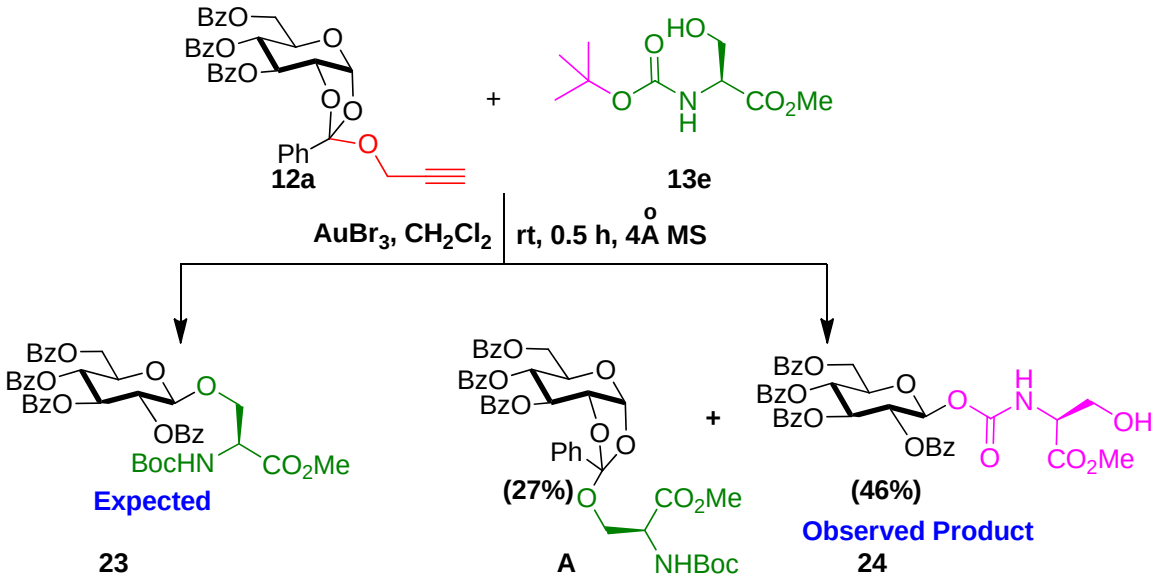


Scheme 4 :- Orthogonal Deprotection of Glycoaminoacid for Solid Phase Peptide Synthesis

deprotected compounds are useful for amide coupling or to attach resin in case of solid phase synthesis. Amino and the acid groups are protected as Fmoc and allyl respectively, so that they can be orthogonally deprotected.

Synthesis of glycopeptides demands the use of differentially and orthogonally protected glyco-serine/threonine conjugates.[111-114] In order to synthesize a free carboxylic acid or amine, aglycon FmocSer(OH)COOAllyl ester **19** was designed and reacted with the glucosyl propargyl 1,2-orthoester **12a** to obtain the desired amino acid glycoside **20** in 70%. Orthogonal deprotection of amino and acid group was carried out by Rh-mediated isomerization of the double bond using Wilkinson's catalyst[115] followed hydrolysis led to the amino acid glycoside as a free carboxylic acid **21** in 85% yield whereas the deprotection of 9-fluorenylmethoxycarbonyl (Fmoc-) group by the use of diethylamine afforded the free amine **22** (Scheme 4).[116]

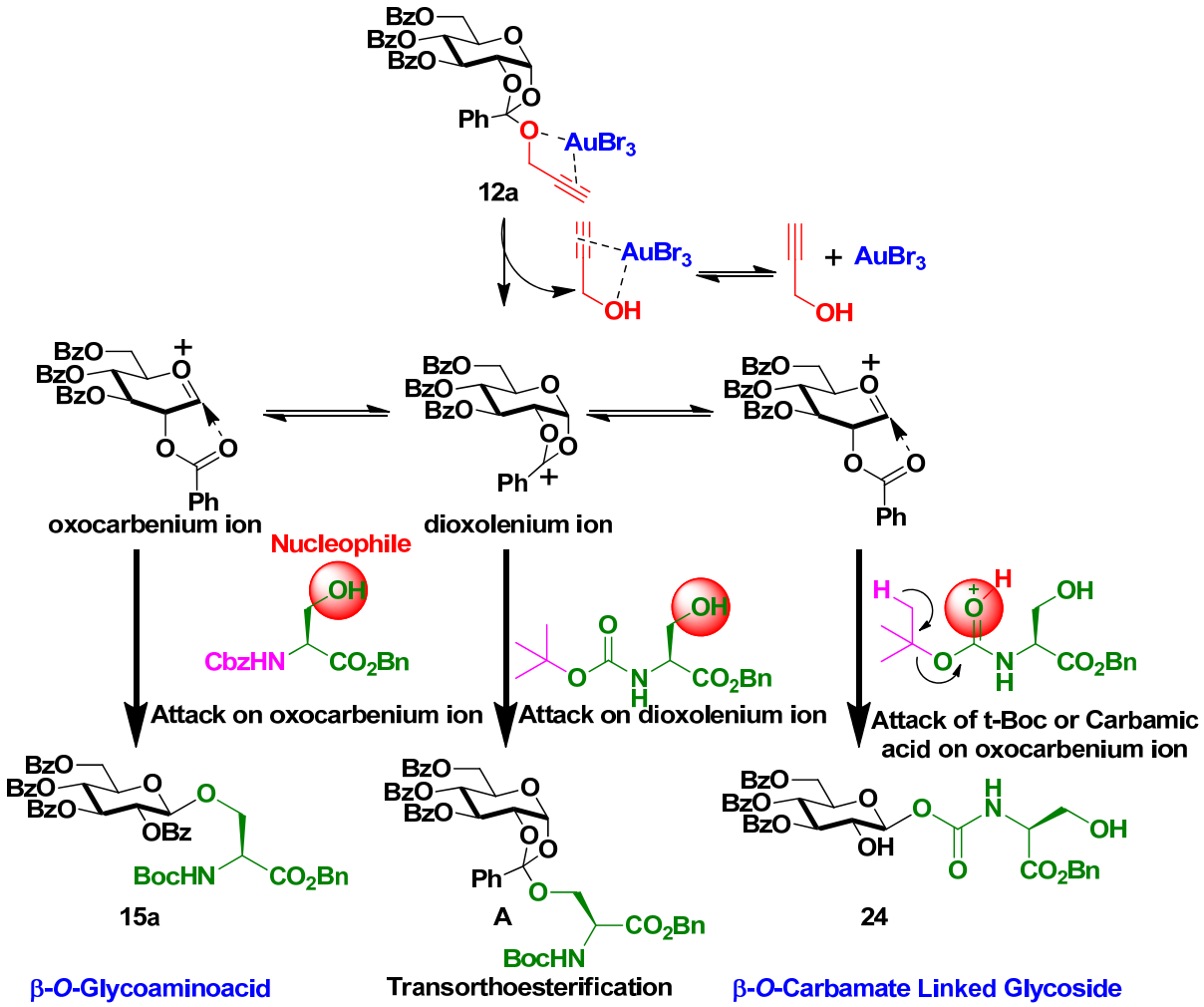
tert-Butoxy carbamates (*t*-Boc) of serine/threonine are also frequently used in the glycopeptide synthesis.[111-113] Thus a model gold catalyzed glycosylation was performed between 1,2-orthoester **12a** and BocSer(OH)OMe **13e** using standard condition. The required amino acid glucoside was not observed and instead, surprisingly an orthoester **A** was obtained in 27% yield; the major compound being a *O*-linked glucosyl carbamate **24**.



Scheme 5 :- Unusal Glycosyl Carbamate from t-Boc protected Primary Amine

The structures of orthoester **A** and carbamate **24** were assigned after thorough characterization using ^{1}H & ^{13}C NMR and LC-MS spectral analysis (Scheme 5). For example,

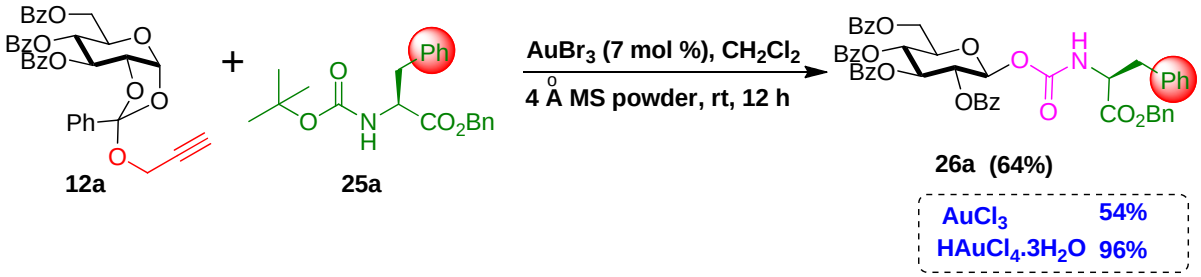
anomeric proton of compound **A** was noticed at δ_H 6.01 ppm as a doublet (J = 5.2 Hz) along with anomeric and quaternary carbons at δ_C 97.6 and 121.1 ppm respectively. Structural integrity of compound **24** was confirmed as the 1,2-*trans* *O*-carbamate, since the anomeric proton was identified in a more deshielded region at δ_C 6.06 ppm as a doublet (J = 8.4 Hz) and concurrently the anomeric carbon was noticed at δ_C 93.5 ppm.[117] This unexpected set back was attributed to acidic reaction conditions and hence it has been envisioned that strong Lewis and Brønsted acids would facilitate the glycoside formation.[93] But the presence of the acid labile *t*-Boc group posed a serious hurdle in this direction.



Activation of propargyl orthoesters led to the formation of oxocarbenium ion which is in equilibrium with dioxolenium ion intermediate. These intermediates were trapped with protected amino acid derivatives in different ways. Such as if the aglycon amino acid was protected with acid compatible group the gives β-*O*-glycoaminoacid, direct attack of *t*-Boc protected amino acid on dioxolenium ion gives transorthoester product **A** in case of low Lewis acid catalyst addition, whereas if catalyst contains both Lewis and Brønsted acid then

preferentially 1,2-trans carbamate linked glycoaminoacid derivatives formation was observed. Possible mechanism for formation of high yielding carbamate linked glycoside are direct trapping of oxocarbenium ion by carbonyl group of *t*-Boc and then cleavage of *t*-Boc into isobutylene group led to formation of carbamate linked glycoside **24**. Other possibility of formation of glucosyl carbamate was the reaction conditions that facilitated the cleavage of the tertiary butyl group thereby producing a reactive carbamic acid.[117] Usually carbamic acids liberate carbon dioxide to afford corresponding amines; however, in this situation, carbamic acid trapped the *in situ* generated oxocarbenium ion from to give **12a**.

This peculiar observation was further corroborated, as the *t*-Boc protected phenylalanine has also reacted with the glycosyl donor **12a** to give glucosyl carbamate **26a** in 65% yield. In previous case (Scheme 5), there was competition between the aglycon because of which mixture of product formation was observed to overcome this aglycon **25a** was designed and reacted with **12a**. Less Brønsted acidic and alkynophilic $AuCl_3$ did not improve the performance of the reaction as the release of HCl will not be facile in an aprotic medium; nevertheless, near quantitative yield of compound **26a** was obtained in 2 h with a catalytic amount of $HAuCl_4.3H_2O$ was used (Scheme 6). Similar cleavage of the *t*-butyl group and

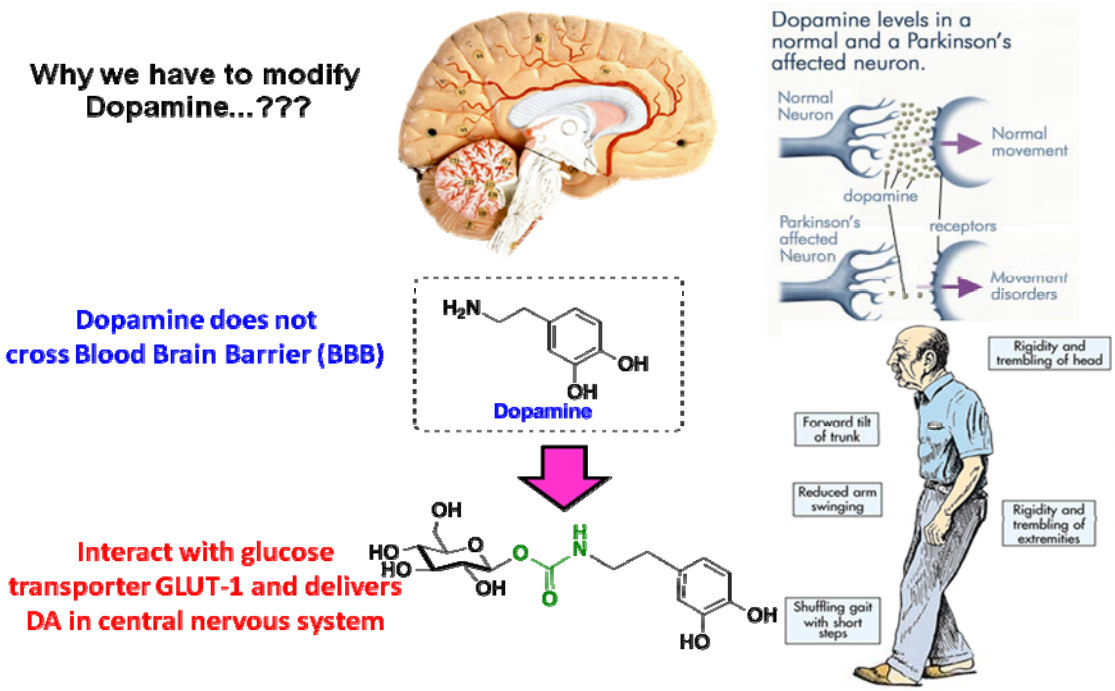


Scheme 6:- Optimization of Glycosyl Carbamate formation using different Gold Complexes

intramolecular cyclization was observed, when *N*-Boc protected alkynylamines were treated with gold reagents to give alkylidene 2-oxazolidinones.[118-119]

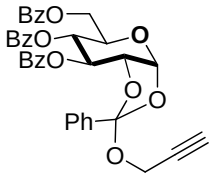
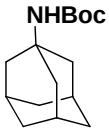
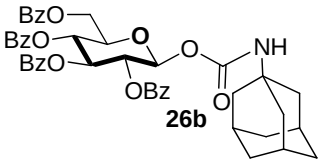
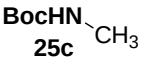
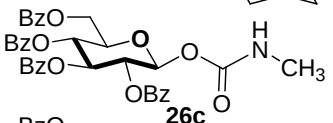
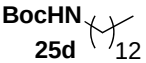
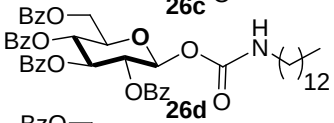
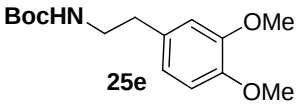
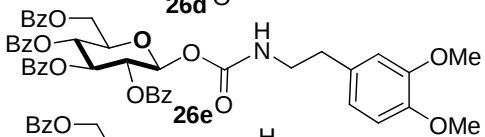
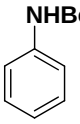
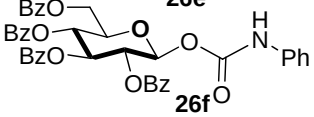
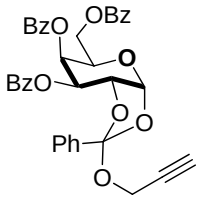
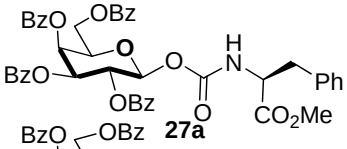
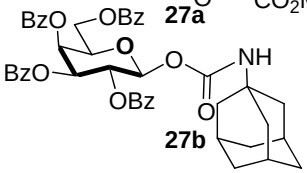
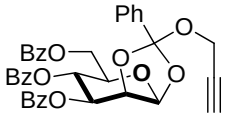
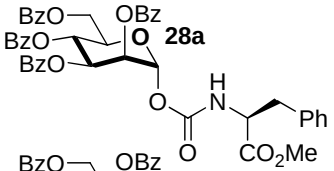
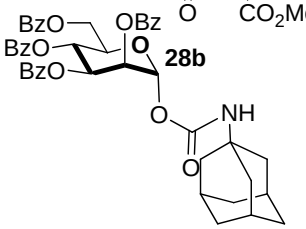
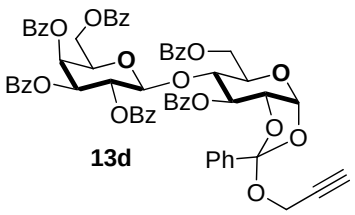
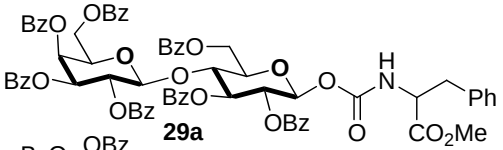
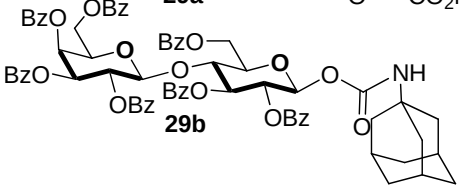
The unusual formation of the carbamate linkage needs a special mention. Glycosyl carbamates are important because of their stability to alkaline conditions[117] and some of them are reported to act as glycosyl donors.[120-121] In addition, glycosyl carbamates are studied as dopamine prodrugs (Parkinson's)[122-123](Figure 16), nitric oxide (NO) donors for biomedical research[124] and surfactants.[125] Recently, carbamate linkages were explored for studying carbohydrate-protein interactions[126] and also for ligation.[127] Glycosyl carbamates

can be prepared by a reaction of corresponding lactol and an isocyanate[128] in the presence of a base[120] or from glycosyl carbonates.[129] TMSOTf catalyzed reaction of glycosyl trichloroacetimidates perhaps gives easy access but in moderate to good yields depending on the type of substrate.[117] Recently Markus Oberthür and coworker showed the synthesis of glycosyl carbamate under non-acid conditions.[130]



Thus, methods that enable easy synthesis of glycosyl carbamates without the use of toxic isocyanates from stable glycosyl donors are invaluable. The foregoing discussion clearly highlights the merit of $HAuCl_4.3H_2O$ catalyzed synthesis of glucosyl carbamate from stable propargyl 1,2-orthoester as a glycosyl donor. This reaction works very well when the *t*-Boc protected primary amine were used whereas *t*-Boc protected secondary or tertiary amines could not gives desired glycosyl carbamates. The generality of the methodology has been verified by using a panel of *t*-Boc protected amines and propargyl 1,2-orthoesters. *t*-Boc protected amino compounds of alicyclic (**25b**), aliphatic (**25c**, **25d**) and aromatic (**25e,25f**) reacted with glucose 1,2-orthoester **12a** to afford their respective carbamates **26b–26f** (Table 2). Furthermore, galactosyl- (**12b**), mannosyl- (**12c**) and lactosyl- (**12d**) derived propargyl 1,2-orthoesters also participated successfully in the glycosylation reaction to afford the corresponding 1,2-*trans* glycosides **27a–b**, **28a–b** and **29a–b** in near quantitative yields (Table 2).

Table 2

Propargyl 1,2-othoesters	Glycosyl acceptors	1,2-*trans* Glycosyl carbamate	Yield
 **12a**	 **25b**	 **26b**	**98 %**
	 **25c**	 **26c**	**96 %**
	 **25d**	 **26d**	**99 %**
	 **25e**	 **26e**	**94 %**
	 **25f**	 **26f**	**95 %**
 **12b**	**25a**	 **27a**	**97 %**
	25b	 **27b**	**98 %**
 **12c**	**25a**	 **28a**	**98 %**
	25b	 **28b**	**95 %**
 **13d**	**25a**	 **29a**	**95 %**
	25b	 **29b**	**96 %**

Reagents and Conditions: $HAuCl_4$ (7 mol %)/CH_2Cl_2/4 Å MS powder/rt, 30 min

In conclusion, a new method for the synthesis of aminoacid glycoconjugates was described which is more applicable to Cbz- and Fmoc-protected amino acid Me/benzyl esters. *t*-Boc protected serine derivative was found to give serine 1,2-orthoester in poor yield and the major compound was found to be the glycosyl carbamate. Optimized reaction conditions showed $HAuCl_4.3H_2O$ gives glycosyl carbamates in excellent yields. The generality of this methodology was evaluated using various glycosyl donors, aglycons and building blocks. Gold salts are found to be of immense benefit to synthesize diverse glycosidic linkages and glycoaminoacid derivatives. Transformation of these glycoaminoacid derivatives to polymerizable monomers and their application to glycopolypeptide synthesis can be envisioned.

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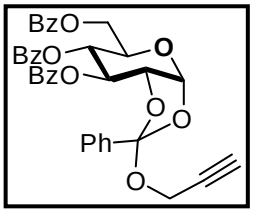
General Procedure for Propargyl 1,2-Orthoester:



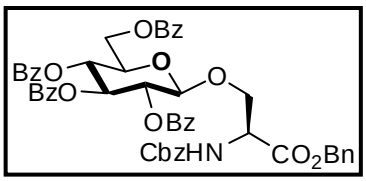
In 500mL round bottom flask α-D-Glucose (10 g, 0.056mol) and DMAP (0.05g, 0.4mmol) were dissolved in pyridine (125mL) and to this solution benzoyl chloride (100mL) was added slowly added using dropping funnel. The resultant reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. The solid residue was dissolved in CH_2Cl_2 and water was carefully added with cooling and vigorous stirring to decompose the excess of benzoyl chloride. Then solvent was removed on rota vap to give crude pentabenzoate glucose (35.0 g, 90 % yield). The crude per-O-benzyolated glucose was dissolved in CH_2Cl_2 and acetic anhydride was added to maintain anhydrous conditions. The reaction mixture was cooled to 0°C for 1 h. Then temperature of reaction mixture was raised to room temperature and stirred for 12 h. The reaction mixture was then diluted with cold CH_2Cl_2 and cold water. After this organic layer was washed 3-4 times with cold water and then with saturated $NaHCO_3$ to remove acetic acid. Organic layer was kept on anhydrous Na_2SO_4 and then solvent was evaporated on rotavap to get the crude glycosyl bromide 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl bromide (32.3 g, 98 % yield). To a solution of per-benzoylated glucosyl bromide (20 g, 30.3mmol) in anhydrous CH_2Cl_2 (100mL) was added 2,6-lutidine (15mL), propargyl alcohol (9mL, 15.2mmol) followed by a catalytic amount of tetra-n-butylammonium iodide (50mg) at room temperature under argon atmosphere. Then, the reaction mixture was refluxed at 65°C for 48 h, diluted with aqueous oxalic acid solution and extracted with CH_2Cl_2 (2X100). A combined organic layer was washed with water, brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting brownish black residue was purified by silica gel column chromatography using petroleum ether-ethyl acetate as the mobile phase to afford corresponding 3,4,6-tri-O-benzoyl-a-D-glucopyranose-1,2-(pro-2-ynyl orthobenzoate) **12a** (16.3g, 85%) as white solid. Exactly similar procedure was used for synthesis of propargyl 1,2-orthoester of D-galactose, D-mannose and Lactose

General procedure for the Gold Catalyzed Glycosylations:

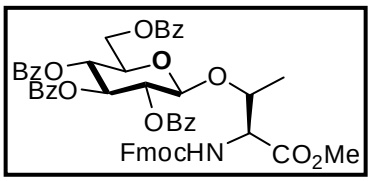
To a solution of 1,2-orthoester (0.1 mmol), glycosyl acceptor (0.11 mmol) and activated 4Å molecular sieves powder (50 mg) in anhydrous CH_2Cl_2 (5 mL) was added $AuBr_3$ (7 mol%) under argon atmosphere at room temperature. The reaction mixture was stirred at room temperature for the specified time and the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography using ethyl acetate-petroleum ether as the mobile phase.

General procedure for the synthesis of glycosyl carbamates from propargyl 1,2-orthoesters:

To a solution of glycosyl donor (0.1 mmole) and *t*-Boc protected amine (0.1 mmole) in anhydrous CH_2Cl_2 (5 mL) was added 4 Å MS powder (50mg 0.1 mmol-1) and 5 mol% of $HAuCl_4.3H_2O$ under argon atmosphere at room temperature. The resulting mixture was stirred till the completion of the reaction as judged by TLC analysis (2 h). The reaction mixture was concentrated *in vacuo* to obtain a crude residue, which was purified by conventional silica gel column chromatography using ethyl acetate−petroleum ether as mobile phase.

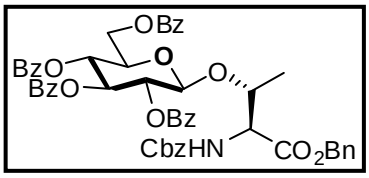


Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-serinate (15a): $[\alpha]_{25}^{D}$ +14.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.91(dd, *J* = 3.4, 10.3 Hz, 1H), 4.01(ddd, *J* = 3.3, 4.9, 8.3 Hz, 1H), 4.32-4.56(m, 3H), 4.61(dd, *J* = 3.2, 12.2 Hz, 1H), 4.78(d, *J* = 7.6Hz, 1H), 4.99(dd, *J* = 12.3, 19.4 Hz, 2H), 5.13(dd, *J* = 12.3, 14.3 Hz, 2H), 5.45(dd, *J* = 7.8, 9.5 Hz, 1H), 5.61(m, 2H), 5.86(t, *J* = 9.6 Hz, 1H), 7.20-7.58(m, 22H), 7.78-8.05(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 54.2, 62.9, 66.9, 67.4, 69.3, 69.4, 71.7, 72.2, 72.5, 101.3, 128.0-129.8, 133.1, 133.2, 133.3, 133.4, 135.2, 136.2, 155.8, 165.1, 165.1, 165.7, 166.1, 169.2; Anal. calcd for $C_{52}H_{45}NO_{14}$: C, 68.79; H, 5.00; N, 1.54); found: C, 67.87; H, 5.21; N, 1.66; MS (ESI) calcd for $C_{52}H_{45}NO_{14}$ $[M+Na]^+$, 930.902; found, 930.671.

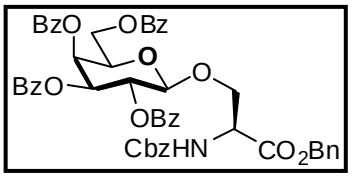


Methyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-threoninate (15b): $[\alpha]_{25}^{D}$ +12.0°(*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.14(d,

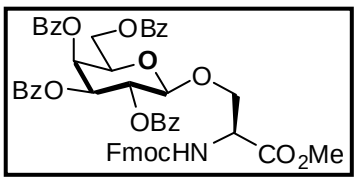
3H, *J* = 6.3Hz), 3.71(s, 3H), 4.05-4.58(m, 7H), 4.65(dd, *J* = 3.3, 12.2 Hz, 1H), 4.87(d, *J* = 7.7 Hz, 1H), 5.48(dd, *J* = 8.0, 9.7 Hz. 1H), 5.69(m, 2H), 5.91(t, *J* = 9.7 Hz, 1H), 7.20-7.65(m, 20H), 7.72-8.06(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 16.9, 47.0, 52.5, 58.4, 62.8, 67.3, 69.4, 71.8, 72.1, 72.6, 75.1, 99.3, 119.9, 125.2, 127.0-128.8, 133.2, 133.3, 133.3, 133.4, 141.1, 141.2, 143.6, 143.9, 156.7, 165.1, 165.1, 165.7, 166.1, 170.5; Anal. calcd for $C_{54}H_{47}NO_{14}$: C, 69.44; H, 5.07; N, 1.50; found: C, 69.18; H, 5.22; N, 1.80; MS (ESI) calcd for $C_{54}H_{47}NO_{14}$ $[M+Na]^+$, 956.939; found, 956.694.



Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-threoninate (15c): $[\alpha]_{25}^{D}$ +4.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.11(d, *J* = 6.3 Hz, 3H), 3.86(td, *J* = 3.5, 4.1, 9.4 Hz, 1H), 4.30-4.59(m, 4H), 4.74(d, *J* = 8.0 Hz, 1H), 5.04(s, 2H), 5.18(s, 2H), 5.38(dd, *J* = 8.0 Hz, 1H), 5.62(m, 2H), 5.80(t, *J* = 9.5Hz, 1H), 7.20-7.58(m, 22H), 7.79-8.05(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 17.0, 58.4, 62.6, 66.9, 67.2, 69.2, 71.9, 72.0, 72.5, 75.0, 99.0, 127.8-129.7, 133.1, 133.2, 133.3, 133.4, 135.4, 136.2, 156.7, 165.0, 165.0, 165.7, 166.0, 169.8; Anal. calcd for $C_{53}H_{47}NO_{14}$: C, 69.05; H, 5.14; N, 1.52; found: C, 68.64; H, 5.76; N, 1.77; MS (ESI) calcd for $C_{53}H_{47}NO_{14}$ $[M+Na]^+$, 944.928; found, 944.722.

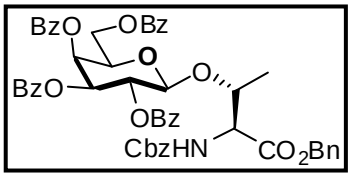


Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-L-serinate (16a): $[\alpha]_{25}^{D}$ +58.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.98(dd, 1H, *J* = 3.2, 10.4Hz), 4.19(m, 2H), 4.32-4.80(m, 4H), 5.00(q, 2H, *J* = 12.3, 17.7Hz), 5.17(q, 2H, *J* = 12.4, 17.3Hz), 5.56(m, 1H), 5.73(m, 2H), 5.96(m, 1H), 7.20-7.68(m, 22H), 7.74-8.11(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 54.3, 61.8, 66.9, 67.0, 67.4, 67.9, 69.4, 69.5, 71.4, 101.8, 127.9-130.0, 133.2, 133.3, 133.3, 133.6, 135.2, 136.1, 155.8, 165.2, 165.4, 165.5, 166.0, 169.3; Anal. calcd for $C_{52}H_{45}NO_{14}$: C, 68.79; H, 5.00; N, 1.54; found: C, 67.86; H, 5.18; N, 1.62; MS (ESI) calcd for $C_{52}H_{45}NO_{14}$ $[M+Na]^+$, 930.902; found, 930.671.

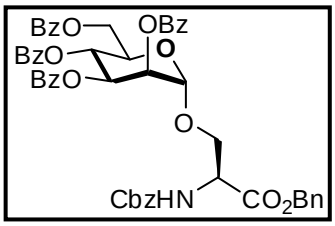


Methyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-L-serinate (16b): $[\alpha]_{25}^{D}$ +66.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.68(s, 3H), 3.94(dd, *J* = 3.4, 10.4 Hz, 1H), 4.14(t, *J* = 6.9 Hz, 1H), 4.25-4.52(m, 6H), 4.63(dd, *J* = 6.5, 11.1 Hz, 1H), 4.73(d, *J* = 7.6 Hz, 1H), 5.52(d, *J* = 8.1 Hz, 1H), 5.60(dd, *J* = 3.3, 10.2 Hz, 1H), 5.75(dd,

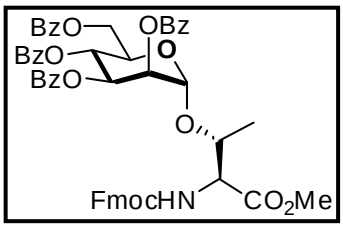
J = 7.8, 10.2 Hz, 1H), 6.00(d, *J* = 3.3 Hz, 1H), 7.20-7.66(m, 20H), 7.75-8.11(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 47.1, 52.7, 54.3, 61.9, 66.7, 67.9, 69.4, 69.4, 71.4, 71.4, 101.8, 119.9-130.0, 133.2, 133.3, 133.3, 133.6, 141.3, 141.3, 143.7, 143.8, 155.7, 165.2, 165.5, 165.5, 166.0, 169.8; Anal. calcd for $C_{53}H_{45}NO_{14}$: C, 69.20; H, 4.93; N, 1.52; found: C, 67.94; H, 4.02; N, 1.66; MS (ESI) calcd for $C_{53}H_{45}NO_{14}$ $[M+Na]^+$, 942.913; found, 942.643.



Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-L-threoninate (16c): $[\alpha]_{25}^{D}$ +36.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.14(d, *J* = 6.4 Hz, 3H), 4.06(t, *J* = 6.4 Hz, 1H), 4.31(dd, *J* = 7.1, 11.3 Hz, 1H), 4.43(dd, *J* = 2.2, 9.3 Hz, 1H), 4.48-4.61(m, 2H), 4.71(d, *J* = 7.8 Hz, 1H), 5.07(s, 2H), 5.24(s, 2H), 5.51(dd, *J* = 3.3, 10.4 Hz, 1H), 5.61-5.75(m, 2H), 5.92(d, *J* = 3.8 Hz, 1H), 7.19-7.66(m, 22H), 7.75-8.08(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 17.4, 58.5, 61.6, 67.0, 67.3, 67.8, 69.8, 71.1, 71.3, 75.6, 99.9, 127.8-130.0, 133.2, 133.3, 133.3, 133.5, 135.4, 136.2, 156.7, 165.2, 165.5, 165.5, 165.9, 169.9; Anal. calcd for $C_{53}H_{47}NO_{14}$: C, 69.05; H, 5.14; N, 1.52; found: C, 66.49; H, 4.95; N, 1.35; MS (ESI) calcd for $C_{53}H_{47}NO_{14}$ $[M+Na]^+$, 944.289; found, 944.785.

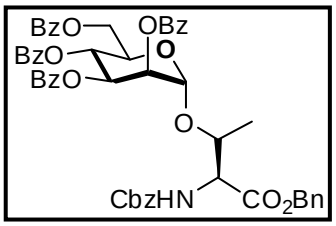


Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-L-serinate (17a): $[\alpha]_{25}^{D}$ -42.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 4.14(d, *J* = 2.7 Hz, 2H), 4.32-4.68(m, 4H), 4.99(d, *J* = 1.6 Hz, 1H), 5.14(d, *J* = 1.5 Hz, 2H), 5.32(dd, *J* = 12.1, 20.5 Hz, 2H), 5.59(dd, *J* = 1.8, 3.2 Hz, 1H), 5.79(dd, *J* = 3.2, 10.1 Hz, 1H), 5.96(d, *J* = 7.9 Hz, 1H), 6.09(t, *J* = 10.1 Hz, 1H), 7.20-7.65(m, 22H), 7.81-8.14(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 54.5, 62.6, 66.5, 67.2, 67.8, 67.9, 69.4, 69.7, 70.1, 98.4, 128.1-129.9, 133.0, 133.1, 133.4, 133.5, 135.0, 136.0, 155.9, 165.2, 165.3, 165.4, 166.1, 169.5; Anal. calcd for $C_{52}H_{45}NO_{14}$: C, 68.79; H, 5.00; N, 1.54; found: C, 68.21; H, 5.58; N, 1.49; MS (ESI) calcd for $C_{52}H_{45}NO_{14}$ $[M+Na]^+$, 930.902; found, 930.671.



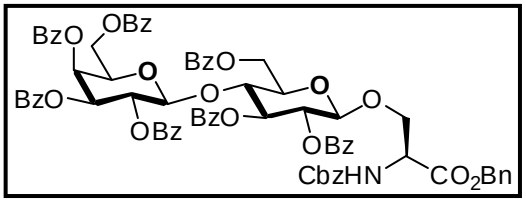
Methyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-L-threoninate (17b): $[\alpha]_{25}^{D}$-30.0°(*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.43(d, *J* = 6.2 Hz, 3H), 3.90(s, 3H), 4.33(dd, *J* = 7.1, 14.4 Hz, 2H), 4.28-4.75(m, 6H), 5.17(d, *J* = 1.5 Hz, 1H), 5.50(dd, *J* = 1.8, 3.1 Hz, 1H), 5.75-5.95(m, 2H), 6.10(t, *J* =

9.6 Hz, 1H), 7.20-8.10(m, 28H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 18.2, 47.1, 53.0, 58.6, 62.9, 66.9, 67.5, 69.4, 69.6, 70.5, 77.8, 99.0, 119.9, 125.2, 127.1-129.8, 133.1, 133.2, 133.5, 133.5, 141.3, 141.3, 143.7, 143.8, 156.7, 165.3, 165.5, 165.5, 166.1, 170.5; Anal. calcd for $C_{54}H_{47}NO_{14}$: C, 69.44; H, 5.07; N, 1.50; found: C, 66.42; H, 4.20; N, 1.34; MS (ESI) calcd for $C_{54}H_{47}NO_{14}$ $[M+Na]^+$, 956.289; found, 956.820.



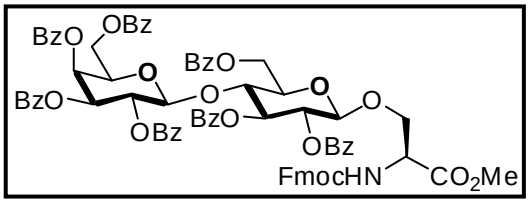
Benzyl *N*-(benzyloxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-L-threoninate (17c): $[\alpha]_{25}^{D}$ -40.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.42(d, *J* = 6.2 Hz, 3H), 4.42-4.55(m, 3H), 4.57-4.71(m, 2H), 5.07(d, *J* = 1.8 Hz, 1H), 5.19(s, 2H), 5.31(s, 2H), 5.45(dd, *J* 1.8, 3.1 Hz, 1H), 5.72-5.85(m, 2H), 6.06(t, *J* = 9.9 Hz, 1H), 7.21-7.68(m, 22H), 7.82-8.10(m, 8H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 18.3, 58.8, 62.9, 66.8, 67.3, 67.9, 69.6, 69.7, 70.5, 77.9, 99.0, 128.1-129.9, 133.1, 133.1, 133.5, 133.5, 135.0, 136.1, 156.7, 165.2, 165.2, 165.4, 166.1, 169.9; Anal. calcd for $C_{53}H_{47}NO_{14}$: C, 69.05; H, 5.14; N, 1.52; found: C, 69.51; H, 4.67; N, 1.02; MS (ESI) calcd for $C_{53}H_{47}NO_{14}$ $[M+Na]^+$, 944.928; found, 944.785.

Benzyl *N*-(benzyloxycarbonyl)-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-serinate (18a): $[\alpha]_{25}^{D}$ +46.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR

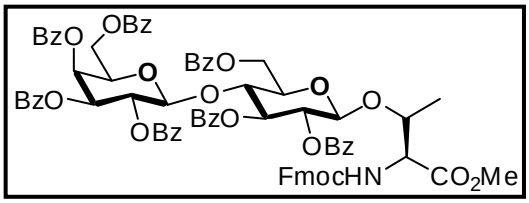


(200.13 MHz, $CDCl_3$): δ 3.59(d, *J* = 9.7 Hz, 1H), 3.70(d, *J* = 6.5 Hz, 2H), 3.78-3.92(m, 2H), 4.18(t, *J* = 9.7 Hz, 1H), 4.26(dd, *J* = 2.6, 10.4 Hz, 1H), 4.36-4.59(m, 4H), 4.81-4.98(m, 3H), 5.06(s, 2H), 5.32-5.45(m, 2H), 5.52(d, *J* = 8.0 Hz, 1H), 5.69(d, *J* = 9.2 Hz, 2H), 5.75(d, *J* = 6.2 Hz, 1H), 7.11-7.64(m, 31H), 7.69-8.02(m, 14H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 54.2, 61.0, 62.1, 66.9, 67.3, 67.5, 69.4, 69.8, 71.3, 71.5, 71.7, 72.5, 73.0, 75.6, 100.9, 101.3, 128.0-130.0, 133.1, 133.2, 133.3, 133.4, 133.4, 133.5, 133.5, 135.1, 136.2, 155.8, 164.8, 165.1, 165.2, 165.3, 165.4, 165.6, 165.8, 169.2; Anal. calcd for $C_{79}H_{67}NO_{22}$: C, 68.64; H, 4.89; N, 1.01; found: C, 68.36; H, 4.38; N, 1.12; MS (ESI) calcd for $C_{79}H_{67}NO_{22}$ $[M+Na]^+$, 1405.361; found, 1404.813 (M^++1).

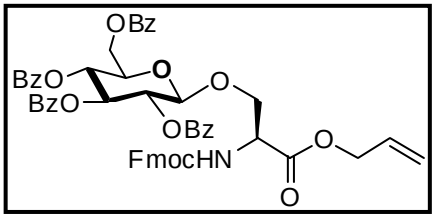
Methyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-serinate (18b): $[\alpha]_{25}^{D}$+48.0° (*c* 1.0,



$CHCl_3$); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 3.58(s, 3H), 3.71(d, *J* = 6.4 Hz, 2H), 3.80(m, 2H), 3.91(t, *J* = 6.7 Hz, 1H), 4.11(t, *J* = 6.7 Hz, 1H), 4.20-4.35(m, 3H), 4.41(m, 1H), 4.48(dd, *J* = 4.3, 12.3 Hz, 2H), 4.59(m, 2H), 4.88(d, *J* = 7.9 Hz, 1H), 5.38-5.45(m, 2H), 5.48(d, *J* = 7.9 Hz, 1H), 5.73-5.85(m, 3H), 7.11-8.02(m, 43H); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 47.1, 52.6, 54.2, 61.0, 62.1, 66.8, 67.4, 69.4, 69.8, 71.3, 71.4, 71.6, 72.5, 73.0, 75.7, 101.0, 101.3, 120.0, 125.0, 125.1, 126.9-130.0, 133.2, 133.3., 133.4, 133.4, 133.5, 141.2, 141.3, 143.6, 143.7, 155.7, 164.8, 165.1, 165.2, 165.2, 165.4, 165.5, 165.8, 169.7; Anal. calcd for $C_{80}H_{67}NO_{22}$: C, 68.79; H, 5.00; N, 1.54; found: C, 68.21; H, 5.58; N, 1.49; MS (ESI) calcd for $C_{80}H_{67}NO_{22}$ $[M+Na]^+$, 1416.405; found, 1416.882.

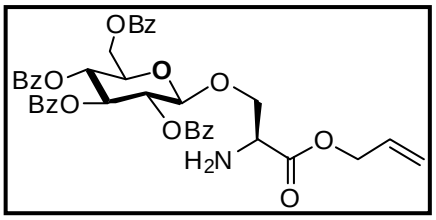


Methyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-threoninate (18c): $[\alpha]_{25}^{D}$ +42.0^o (*c* 1.0, $CHCl_3$); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.11(d, *J* = 6.4 Hz, 3H), 3.57(s, 3H), 3.70-3.86(m, 3H), 3.94(t, *J* = 6.6 Hz, 1H), 4.13-4.45(m, 6H), 4.52(dt, *J* = 3.9, 12.9 Hz, 1H), 4.61(ABq, *J* = 14.9Hz, 1H), 4.85(ABq, *J* = 8 Hz, 2H), 5.44(m, 2H), 5.69(d, *J* = 9.3 Hz, 1H), 5.73-5.87(m, 3H), 7.12-8.13(m, 43H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 16.8, 47.0, 52.3, 58.4, 61.0, 62.1, 67.3, 67.5, 69.9, 71.4, 71.7, 72.6, 73.0, 74.9, 75.7, 77.2, 99.0, 101.0, 119.9, 125.2, 127.0-129.9, 133.2, 133.3, 133.4, 133.5, 133.5, 141.1, 141.2, 143.6, 143.9, 156.7, 164.8, 165.2, 165.2, 165.3, 165.4, 165.5, 165.8, 170.4; Anal. calcd for $C_{81}H_{69}NO_{22}$: C, 69.08; H, 4.94; N, 0.99; found: C, 67.51; H, 5.02; N, 0.69; MS (ESI) calcd for $C_{81}H_{69}NO_{22}$ $[M+Na]^+$, 1431.400; found, 1431.034.

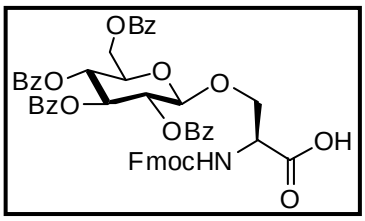


Allyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-Serinate (20): $[\alpha]_{25}^{D}$ +15.0^o (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.91(dd, *J* = 3.2, 10.1 Hz, 1H), 4.13(m, 2H), 4.08-4.70(m, 8H), 4.79(d, *J* = 7.7 Hz, 1H), 5.13-5.33(m, 2H), 5.43-5.98(m, 5H), 7.20-8.08(m, 28H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 47.1, 54.3, 62.9, 66.3, 66.9, 69.5, 71.7, 72.3, 72.6, 101.4, 118.7, 120.0, 120.0, 125.1, 125.2, 127.0-129.8, 131.3, 133.2, 133.3, 133.4, 133.5, 141.2, 141.3, 143.7,

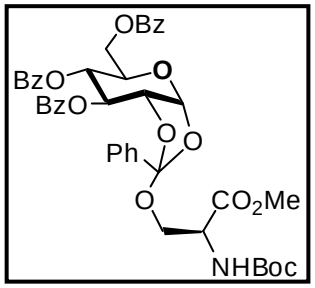
143.8, 155.8, 165.0, 165.1, 165.7, 166.1, 169.0; Anal. calcd for $C_{55}H_{47}NO_{14}$: C, 69.83; H, 5.01; N, 1.48; found: C, 69.50; H, 4.97; N, 1.51; MS (ESI) calcd for $C_{55}H_{47}NO_{14}$ $[M+Na]^+$, 968.289; found, 968.196.



Allyl *O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-L-Serinate (22): $[\alpha]_{25}^{D}$ +13.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.47(bs, 1H), 3.71(dd, *J* = 3.5, 9.3 Hz, 1H), 4.02(m, 2H), 4.14-4.34(m, 1H), 4.36(m, 2H), 4.49(dd, *J* = 2.8, 11.8 Hz, 1H), 4.77(d, *J* = 7.8 Hz, 1H), 4.93-5.14(m, 2H), 5.37(dd, *J* = 7.9, 9.5 Hz, 1H), 5.53(t, *J* = 9.6 Hz, 1H), 5.66(m, 1H), 5.75(t, *J* = 9.6 Hz, 1H), 7.08-7.45(m, 12H), 7.68(d, *J* = 7.6 Hz, 2H), 7.75(d, *J* = 7.5 Hz, 2H), 7.81(d, *J* = 7.6 Hz, 2H), 7.88(d, *J* = 7.2 Hz, 2H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 54.6, 63.0, 65.7, 69.5, 71.7, 71.7, 72.3, 72.8, 101.3, 118.5, 128.2-129.9, 131.7, 133.1, 133.2, 133.3, 133.4, 165.0, 165.1, 165.8, 166.1, 172.6; MS (ESI) calcd for $C_{40}H_{37}NO_{12}$ $[M+Na]^+$, 746.221; found, 746.306.



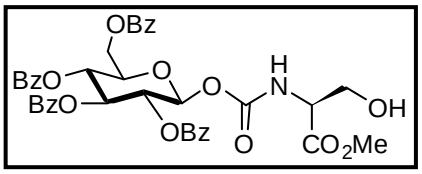
***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-Serine (21):** $[\alpha]_{25}^{D}$ +8.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 4.08(m, 3H), 4.25(m, 2H), 4.39(dd, *J* = 4.9, 8.7 Hz, 2H), 4.53(dd, *J* = 4.3, 12.1 Hz, 1H), 4.66(dd, *J* = 2.6, 12.1 Hz, 1H), 5.10(d, *J* = 8.0 Hz, 1H), 5.50(t, *J* = 8.1 Hz, 1H), 5.72(t, *J* = 9.8 Hz, 1H), 6.02(t, *J* = 9.5 Hz, 1H), 7.23-8.04(m, 28H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 56.0, 64.2, 67.8, 70.4, 71.1, 73.1, 73.3, 74.6, 79.5, 102.0, 120.9, 121.0, 126.3, 126.4, 128.1-130.9, 134.4, 134.4, 134.5, 134.7, 142.5, 142.6, 145.1, 145.3, 158.1, 166.7, 166.7, 166.7, 167.0, 167.5; MS (ESI) calcd for $C_{52}H_{43}NO_{14}$ $[M+Na]^+$, 928.886; found, 928.480.



3,4,6-tri-*O*-Benzoyl-1,2-*O*-[(2*S*-(2-*tert*-butoxycarbonylamino)-3-methoxy-3-oxopropoxy) phenylmethylene] -α-D-glucopyranoside (A): $[\alpha]_D^{25}$ = + 3.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 1.41(s, 9 H), 3.60(dd, *J* = 3.2, 9.9 Hz, 1 H), 3.69((m, 1 H), 3.71(s, 3 H), 4.09(ddd, *J* = 3.1, 4.9, 7.9 Hz, 1 H), 4.37(dd, *J* = 5.0, 11.7 Hz, 2 H), 4.52(dd, *J* = 2.8, 11.7 Hz, 1 H), 4.74(t, *J* = 4.2 Hz, 1 H), 5.31(d, *J* = 8.8 Hz, 1 H), 5.47(d, *J* = 8.8 Hz, 1 H), 5.73(bs, 1 H), 6.01(d, *J* = 5.2 Hz, 1 H), 7.21-7.66(m, 12 H), 7.70(d, *J* = 7.3 Hz, 2 H), 7.91(d, *J* = 7.8 Hz, 2 H), 7.94(d, *J* = 8.2 Hz, 2 H), 8.08(d, *J* = 7.3 Hz,

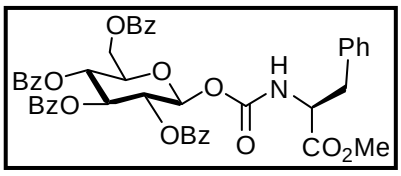
2 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 28.2(3C). 52.5, 53.4, 63.9, 64.2, 67.6, 68.3, 69.0, 72.2, 80.1, 97.6, 121.1, 126.2, 128.2-130.1, 133.0, 133.5, 133.7, 134.5, 155.3, 164.5, 165.1, 166.0, 170.7; HRMS (MALDI-TOF): m/z Calcd for $C_{43}H_{43}NO_{14}$+Na, 820.2581; Found, 820.2577.

Methyl *N*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyloxycarbonyl)-L-serinate (24): $[\alpha]_D^{25}$ =

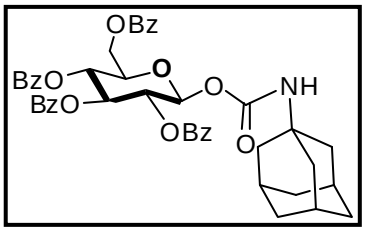


+32.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 2.44(bs, 1 H), 3.61(s, 3 H), 3.94(ddd, *J* = 3.2, 11.4, 14.8 Hz, 2 H), 4.29-4.38(m, 2 H), 4.51(dd, *J* = 4.8, 12.3 Hz, 1 H), 4.65(dd, *J* = 2.9, 12.3 Hz, 1 H), 5.68(dd, *J* = 8.3, 9.5 Hz, 1 H), 5.77(t, *J* = 9.5 Hz, 1 H), 5.97(m, 2 H), 6.06(d, *J* = 8.4 Hz, 1 H), 7.25-7.61(m, 12 H), 7.85(d, *J* = 7.4Hz, 2 H), 7.91(d, *J* = 7.4 Hz, 2 H), 7.96(d, *J* = 7.4 Hz, 2 H), 8.05(d, *J* = 7.4 Hz, 2 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 52.6, 56.0, 62.6, 62.6, 68.9, 70.7, 72.8, 73.0, 93.5, 128.3-130.0, 133.2, 133.3, 133.5, 133.5, 153.5, 165.1, 165.2, 165.6, 166.2, 170.2; HRMS (MALDI-TOF): m/z Calcd for $C_{39}H_{35}NO_{14}$+Na, 764.1955; Found, 764.1960.

Methyl *N*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyloxycarbonyl)-L-phenylalaninate



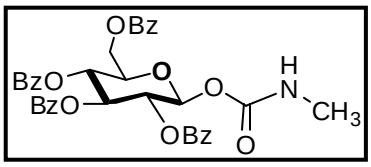
(26a): $[\alpha]_D^{25}$ = + 46.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 3.07(ddd, *J* = 5.6, 13.8, 19.4 Hz, 2 H), 3.54(s, 3 H), 4.29(ddd, *J* = 3.2, 4.5, 7.6 Hz, 1 H), 4.44-4.71(m, 3 H), 5.44(d, *J* = 8.3 Hz, 1 H), 5.63(dd, *J* = 8.3, 9.5 Hz, 1 H), 5.74(t, *J* = 9.5 Hz, 1 H), 5.92(t, *J* = 9.6 Hz, 1 H), 6.02(d, *J* = 8.2 Hz, 1 H), 6.98-7.61(m, 17 H), 7.76-8.10(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 37.8, 52.2, 55.0, 62.7, 69.1, 70.6, 72.9, 72.9, 93.3, 127.2, 128.3-130.0, 133.1, 133.3, 133.4, 133.5, 135.2, 153.1, 165.1, 165.1, 165.6, 166.1, 171.0; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{39}NO_{13}$+Na, 824.2319; Found, 824.2320.



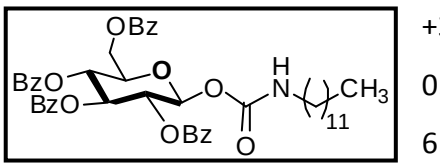
***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-glucopyranosyloxycarbonyl)-adamantylamine (26b):** $[\alpha]_D^{25}$ = + 34.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 1.61(m, 6 H), 1.82(m, 6 H), 2.01(m, 3 H), 4.26(qd, *J* = 3.1, 4.7, 8.0 Hz, 1 H), 4.48(dd, *J* = 4.9, 12.3 Hz, 1 H), 4.62(dd, *J* = 3.0, 12.3 Hz, 1 H), 4.75(s, 1 H), 5.61(dd, *J* = 8.6, 9.4 Hz, 1 H), 5.72(t, *J* = 9.5 Hz, 1 H), 5.88-6.03(m, 2 H), 7.23-7.61(m, 12 H), 7.78-8.08(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 29.3(3C), 36.1(3C), 41.3(3C), 51.2, 62.7, 69.2, 70.9, 72.8, 73.0, 92.6, 128.2-

130.0, 133.0, 133.2, 133.4, 133.5, 151.2, 165.1, 165.3, 165.6, 166.1; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{43}NO_{11}$+Na, 796.2734; Found, 796.2730.

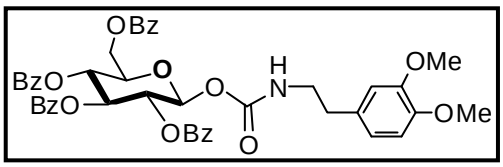
***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-glucopyranosyloxycarbonyl)-methylamine (10c):** $[\alpha]_D^{25}$ = + 47.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 2.74(d, *J* = 4.9Hz, 3 H), 4.31(qd, *J* = 3.0, 4.3, 7.3 Hz, 1 H), 4.48(dd, *J* = 4.4, 12.3 Hz, 1 H), 4.64(dd, *J* = 2.8, 12.3 Hz, 1 H), 4.92(q, *J* = 4.7, 9.6 Hz, 1 H), 5.66(dd, *J* = 8.3, 9.6 Hz, 1 H), 5.76(t, *J* = 9.6 Hz, 1 H), 5.97(t, *J* = 9.6 Hz, 1 H), 6.05(d, *J* = 8.1 Hz, 1 H), 7.24-7.61(m, 12 H), 7.79-8.01(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 27.4, 62.6, 69.0, 70.8, 72.7, 72.9, 93.1, 128.2-130.0, 133.1, 133.2, 133.4, 133.5, 154.3, 165.1, 165.2, 165.6, 166.1; HRMS (MALDI-TOF): m/z = $C_{36}H_{31}NO_{11}$Na, 676.1795; Found, 676.1790.



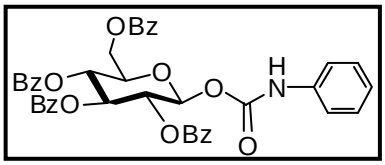
***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-glucopyranosyloxycarbonyl)-dodecylamine (26d):** $[\alpha]_D^{25}$ = +34.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 0.88(t, *J* = 6.1 Hz, 3 H), 1.15-1.45(m, 20 H), 3.10(ddd, *J* = 1.4, 6.8, 8.7 Hz, 2 H), 4.30(qd, *J* = 3.0, 4.1, 7.2 Hz, 1 H), 4.48(dd, *J* = 4.5, 12.3 Hz, 1 H), 4.65(dd, *J* = 2.9, 12.4 Hz, 1 H), 4.92(t, *J* = 5.9 Hz, 1 H), 5.64(dd, *J* = 8.3, 9.6 Hz, 1 H), 5.75(t, *J* = 9.6 Hz, 1 H), 5.96(t, *J* = 9.6 Hz, 1 H), 6.05(d, *J* = 8.3 Hz, 1 H), 7.23-7.68(m, 12 H), 7.79-8.08(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 14.1, 22.6, 26.6, 29.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.9, 41.1, 62.7, 69.1, 70.9, 72.8, 72.9, 93.1, 128.2-130.0, 133.1, 133.2, 133.4, 133.4, 153.6, 165.1, 165.2, 165.6, 166.1; HRMS (MALDI-TOF): m/z Calcd for $C_{47}H_{53}NO_{11}$+Na, 830.3516; Found, 830.3520.



***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-glucopyranosyloxycarbonyl)-2-(3,4-dimethoxyphenyl) ethylamine (26e):** $[\alpha]_D^{25}$ = +30.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 2.66(t, *J* = 7.1 Hz, 2 H), 3.35(ddd, *J* = 1.5, 6.1, 8.6 Hz, 2 H), 3.79,3.82(2s, 6 H), 4.31(qd, *J* = 2.9, 4.2, 7.9 Hz, 1 H), 4.48(dd, *J* = 4.6, 12.4 Hz, 1 H), 4.65(dd, *J* = 2.9, 12.4 Hz, 1 H), 4.97(t, *J* = 6.0 Hz, 1 H), 5.62(dd, *J* = 8.4, 9.6 Hz, 1 H), 5.75(t, *J* = 9.6 Hz, 1 H), 5.96(t, *J* = 9.6 Hz, 1 H), 6.05(d, *J* = 8.4 Hz, 1 H), 6.55-6.74(m, 3 H), 7.23-7.61(m, 12 H), 7.75-8.08(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 35.1, 42.3, 55.6, 55.7, 62.6, 69.0, 70.8, 72.7, 72.9, 93.1, 111.2, 111.6, 120.5, 128.2-129.8, 130.7, 133.0, 133.2, 133.4, 133.4, 147.5, 148.8, 153.6,

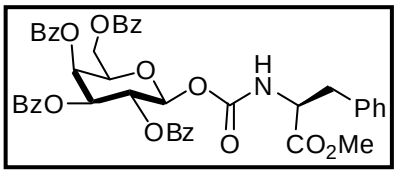


165.0, 165.1, 165.5, 166.0; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{41}NO_{13}$+Na, 826.2476; Found, 826.2480.



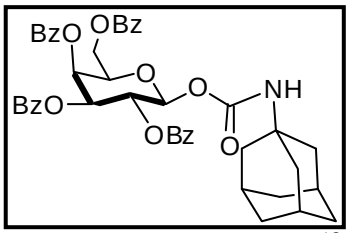
***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-glucopyranosyloxycarbonyl)-aniline (26f):** $[\alpha]_D^{25}$ = +29.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 4.35(qd, *J* = 3.1, 4.7, 7.7 Hz, 1 H), 4.50(dd, *J* = 4.7, 12.3 Hz, 1 H), 4.65(dd, *J* = 3.1, 12.3 Hz, 1 H), 5.72(dd, *J* = 8.2, 9.6 Hz, 1 H), 5.77(t, *J* = 9.7 Hz, 1 H), 6.00(t, *J* = 9.7 Hz, 1 H), 6.12(d, *J* = 8.2 Hz, 1 H), 6.92-7.20(m, 2 H), 7.23-7.61(m, 16 H), 7.80-8.08(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 62.6, 69.0, 70.8, 72.9, 73.0, 93.2, 118.9, 124.1, 128.2-130.0, 133.1, 133.3, 133.5, 133.6, 136.8, 150.7, 165.1, 165.3, 165.6, 166.1; HRMS (MALDI-TOF): m/z Calcd for $C_{41}H_{33}NO_{11}$+Na, 738.1951; Found, 738.1950.

Methyl *N*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyloxycarbonyl)-L-phenylalaninate

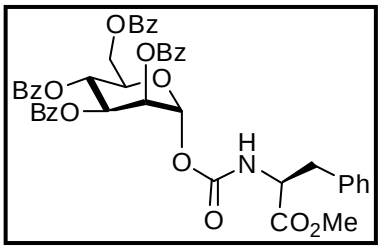


(27a): $[\alpha]_D^{25}$ = +96.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 3.10(ddd, *J* = 5.7, 13.9, 19.6 Hz, 2 H), 3.53(s, 3 H), 4.35-4.78(m, 4 H), 5.46(d, *J* = 8.2 Hz, 1 H), 5.65(dd, *J* = 3.3, 10.2 Hz, 1 H), 5.90(dd, *J* = 8.3, 10.2 Hz, 1 H), 6.01-6.08(m, 2 H), 7.09(m, 2 H), 7.18-7.70(m, 15 H), 7.73-8.15(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 37.7, 52.1, 54.9, 61.7, 67.8, 68.6, 71.7, 72.0, 93.6, 127.2, 128.2-130.0, 133.2, 133.3, 133.3, 133.6, 135.2, 153.1, 165.2, 165.4, 165.4, 165.9, 171.0; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{39}NO_{13}$+Na, 824.2319; Found, 824.2320.

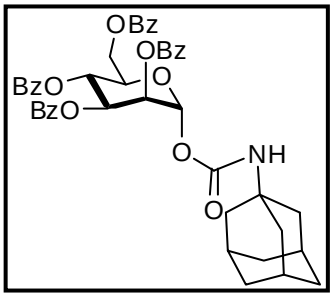
***N*-(2,3,4,6-tetra-*O*-Benzoyl-β-D-galactopyranosyloxycarbonyl)-adamantylamine (27b):**



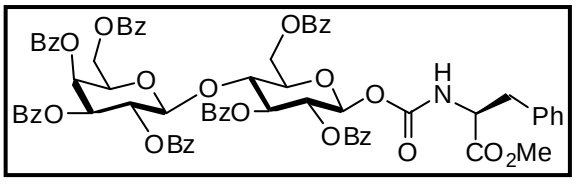
$[\alpha]_D^{25}$ = +97.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 1.61(m, 6 H), 1.83(m, 6 H), 2.01(m, 3 H), 4.34-4.52(m, 2 H), 4.67(dd, *J* = 4.2, 8.6 Hz, 1 H), 4.79(s, 1 H), 5.66(dd, *J* = 3.4, 10.1 Hz, 1 H), 5.87(t, *J* = 8.3 Hz, 1 H), 5.95-6.07(m, 2 H), 7.20-7.68(m, 12 H), 7.75-8.16(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 29.2(3C), 36.0(3C), 41.3(3C), 51.2, 61.7, 67.9, 68.9, 71.7, 71.9, 92.9, 128.2-130.0, 133.1, 133.3, 133.4, 133.5, 151.2, 165.3, 165.4, 165.5, 165.9; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{43}NO_{11}$+Na, 796.2734; Found, 796.2730.

Methyl *N*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxycarbonyl)- L-phenylalaninate (28a): 

$[\alpha]_D^{25}$ = -17.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 3.19 (d, *J* = 5.9 Hz,2 H), 3.75(s, 3 H), 4.45(dd, *J* = 3.6, 9.7 Hz, 1 H), 4.50(m, 1 H), 4.61-4.82(m, 2 H), 5.60(d, *J* = 8.3 Hz,1 H), 5.77(dd, *J* = 2.0, 3.3 Hz, 1 H), 5.89(dd, *J* = 3.3, 10.2 Hz, 1 H), 6.10 (t, *J* = 10.2 Hz, 1 H), 6.29(d, *J* = 2.0 Hz, 1 H), 7.15-7.63(m, 17 H), 7.80-8.14(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 38.1, 52.4, 54.9, 62.3, 66.1, 69.2, 69.8, 70.5, 91.7, 127.3, 128.2-130.0, 133.0, 133.3, 133.4, 133.6, 135.3, 152.5, 165.1, 165.2, 165.6, 166.0, 171.6; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{39}NO_{13}$+Na, 824.2319; Found, 824.2320.

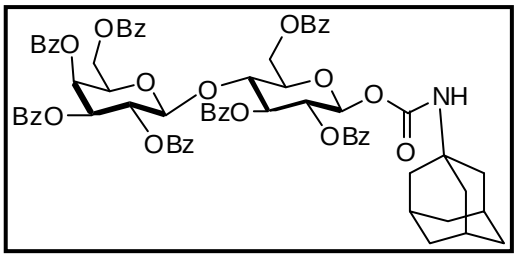
***N*-(2,3,4,6-tetra-*O*-Benzoyl-α-D-mannopyranosyloxycarbonyl)-adamantylamine (28b):** 

$[\alpha]_D^{25}$ = -41.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ = 1.61(m, 6 H), 1.83(m, 6 H), 2.01(m, 3 H), 4.34-4.52(m, 2 H), 4.67(dd, *J* = 4.2, 8.6 Hz,1 H), 4.79(s, 1 H), 5.66(dd, *J* = 3.4, 10.1 Hz, 1 H), 5.87(t, *J* = 8.3 Hz, 1 H), 5.95-6.07(m, 2 H), 7.20-7.68(m, 12 H), 7.75-8.16(m, 8 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 29.4(3C), 36.2(3C), 41.5(3C), 51.4, 62.7, 66.4, 69.6, 70.0, 70.3, 90.7, 128.3-130.0, 133.0, 133.3, 133.5, 133.5, 150.5, 165.2, 165.3, 165.7, 166.1; HRMS (MALDI-TOF): m/z Calcd for $C_{45}H_{43}NO_{11}$+Na, 796.2734; Found, 796.2730.

Methyl *N*-(2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl- β-D-galactopyranosyl)-β-D-glucopyranosyloxycarbonyl)-L-phenylalaninate (29a): $[\alpha]_D^{25}$ = +54.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR 

(200.13 MHz, $CDCl_3$): δ = 3.04(ddd, *J* = 5.8, 13.9, 19.4 Hz, 2 H), 3.52(s, 3 H), 3.58-4.05(m, 4 H), 4.29(t, *J* = 9.6 Hz, 1 H), 4.43-4.65(m, 3 H), 4.85(d, *J* = 7.9 Hz, 1 H), 5.35(dd, *J* = 3.4, 6.5 Hz, 1 H), 5.39(s, 1 H), 5.56(dd, *J* = 8.4, 9.7 Hz, 1 H), 5.72(dd, *J* = 7.9, 10.3 Hz, 2 H), 5.83(t, *J* = 9.4 Hz, 1 H), 5.87(d, *J* = 8.4 Hz, 1 H), 6.95-7.78(m, 26 H), 7.86-8.07(m, 14 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 37.8, 52.2, 54.9, 60.9, 62.0, 67.4, 69.7, 70.4, 71.3, 71.7, 72.9, 73.5, 75.6, 93.2, 101.0, 127.1, 128.2-130.0, 133.1, 133.2, 133.3, 133.3, 133.4, 133.4, 133.5, 135.2, 153.0, 164.7, 165.2, 165.2, 165.3, 165.4, 165.5, 165.8, 171.0; HRMS (MALDI-TOF): m/z Calcd for $C_{72}H_{61}NO_{21}$+Na, 1298.3634; Found, 1298.3630.

N-(2,3,6-tri-O-Benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyloxycarbonyl)-L-adamantylamine (29b): $[\alpha]_D^{25}$ = +48.0 (*c* = 1.0, $CHCl_3$); ^1H NMR (200.13 MHz, $CDCl_3$): δ = 1.58(m, 6 H), 1.78(m, 6 H), 1.99(m, 3 H), 3.60-3.98 (m, 4 H), 4.29(t, *J* = 9.6 Hz, 1 H), 4.48(dd, *J* = 3.7, 12.4 Hz, 1 H), 4.59(dd, *J* = 1.7, 12.4 Hz, 1 H), 4.71(s, 1 H), 4.84(d, *J* = 7.9 Hz, 1 H), 5.35(dd, *J* = 3.3, 10.2 Hz, 1 H), 5.54(t, *J* = 9.0 Hz, 1 H), 5.72(dd, *J* = 7.8, 10.2 Hz, 2 H), 5.79-5.95(m, 2 H), 7.06-7.80(m, 21 H), 7.75-8.06(m, 14 H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 29.2(3C), 36.0(3C), 41.3(3C), 51.1, 60.9, 62.2, 67.4, 69.7, 70.8, 71.3, 71.7, 72.9, 73.5, 75.7, 92.5, 101.0, 128.2-130.0, 133.1, 133.2, 133.3, 133.4, 133.5, 133.6, 133.6, 151.1, 164.8, 165.2, 165.2, 165.4, 165.4, 165.5, 165.8; HRMS (MALDI-TOF): m/z Calcd for $C_{72}H_{65}NO_{19}$+Na, 1270.4048; Found, 1270.4050.



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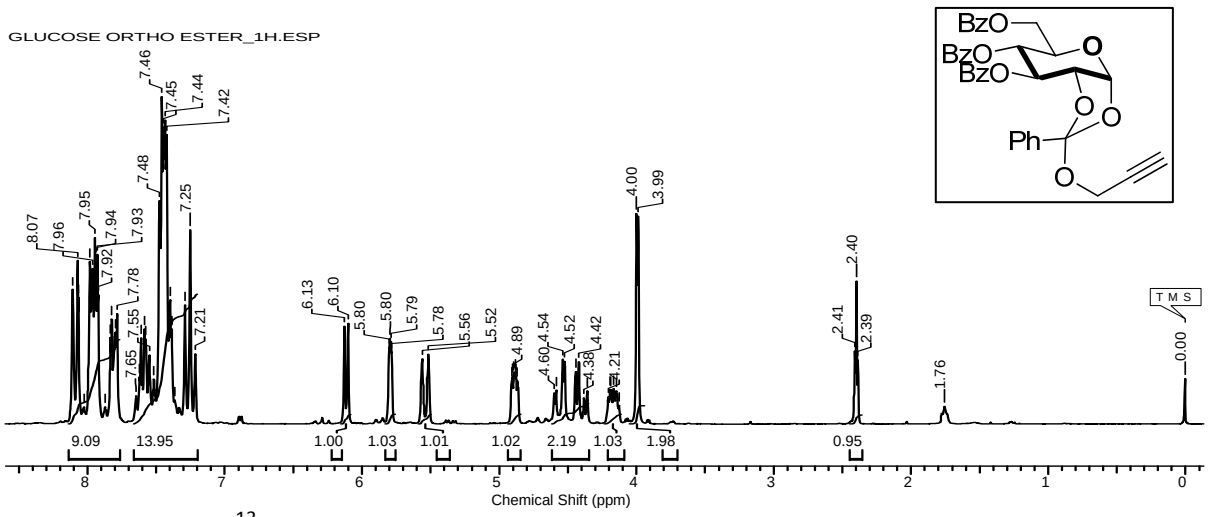
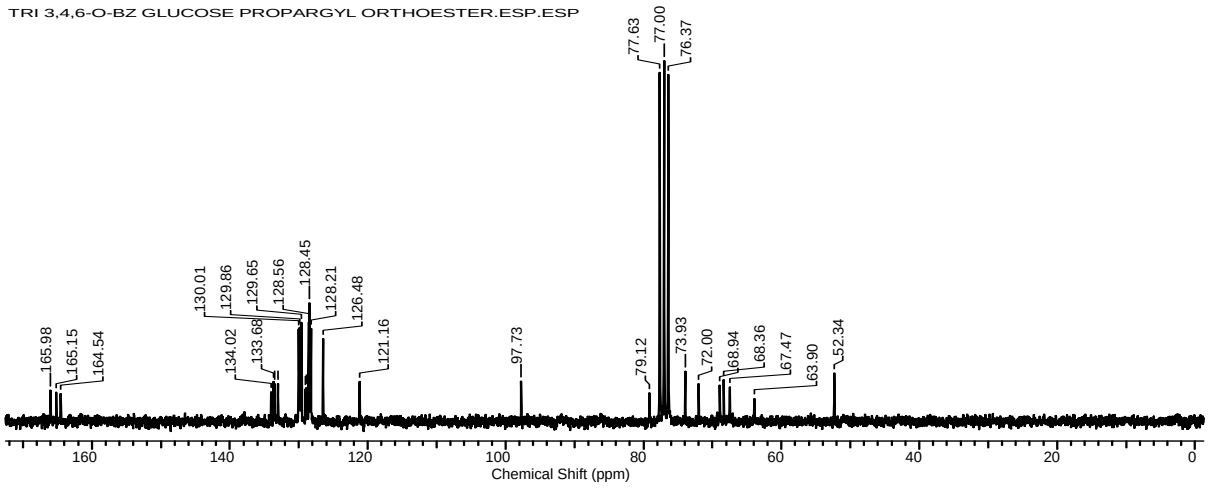
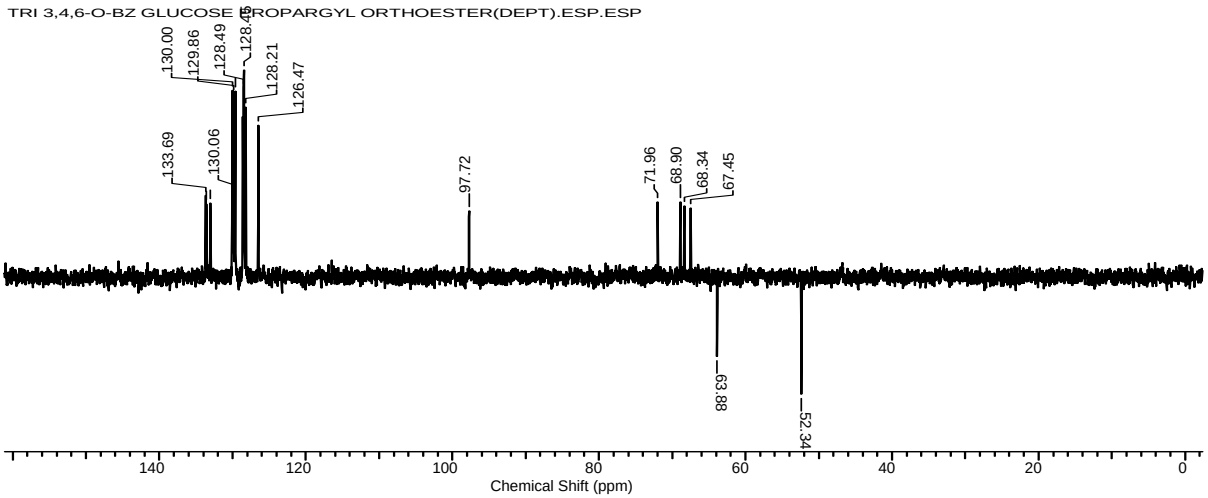
^{1}H NMR ($CDCl_3$**,** 200.13MHz) Spectrum of Compound **12a**



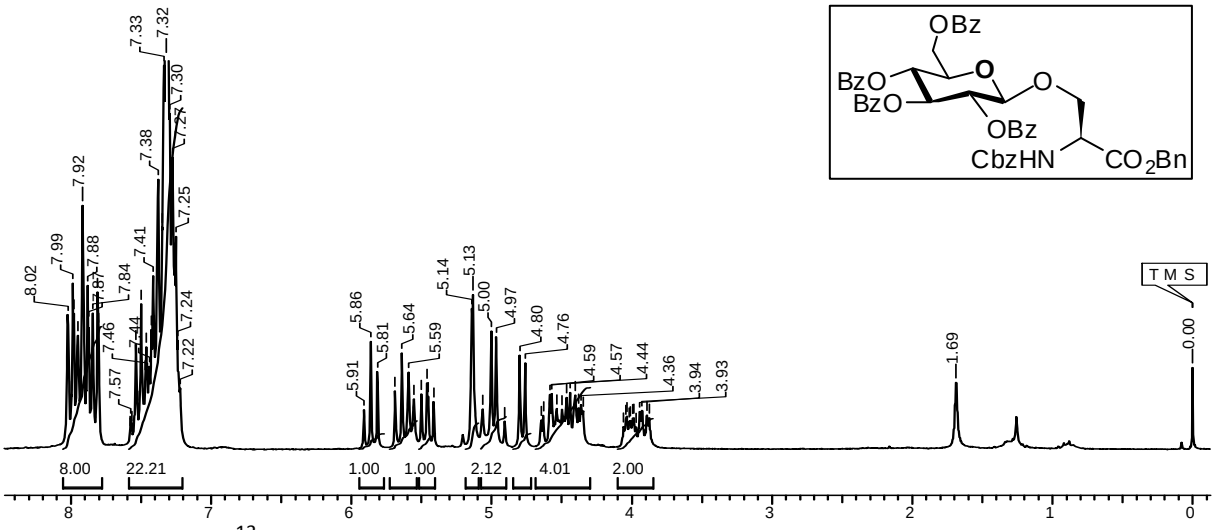
^{13}C NMR ($CDCl_3$**,** 50.32 MHz) Spectrum of Compound **12a**



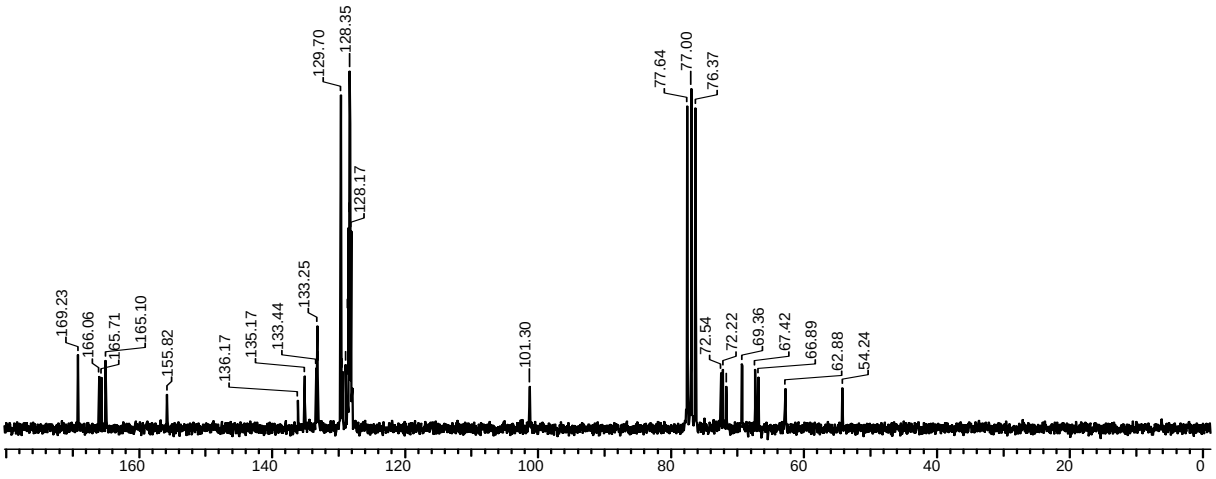
DEPT NMR ($CDCl_3$**,** 50.32 MHz) Spectrum of Compound **12a**



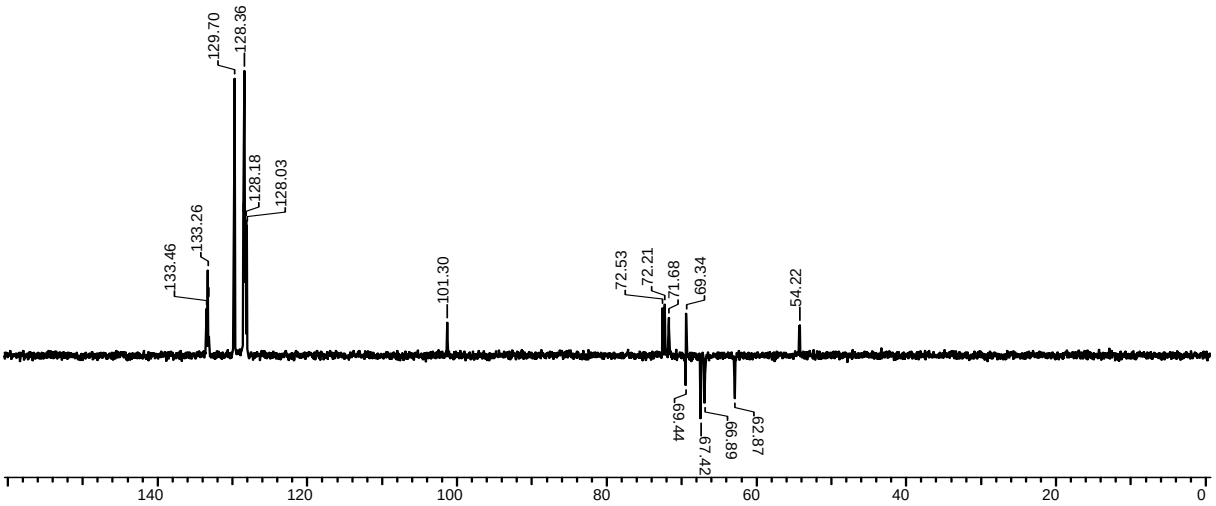
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **15a**



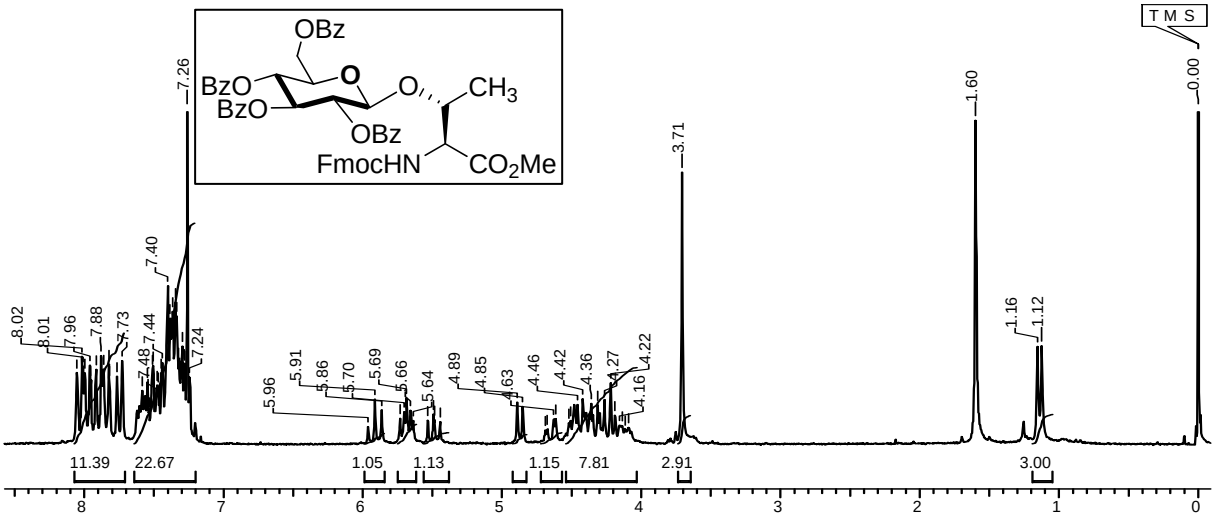
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15a**



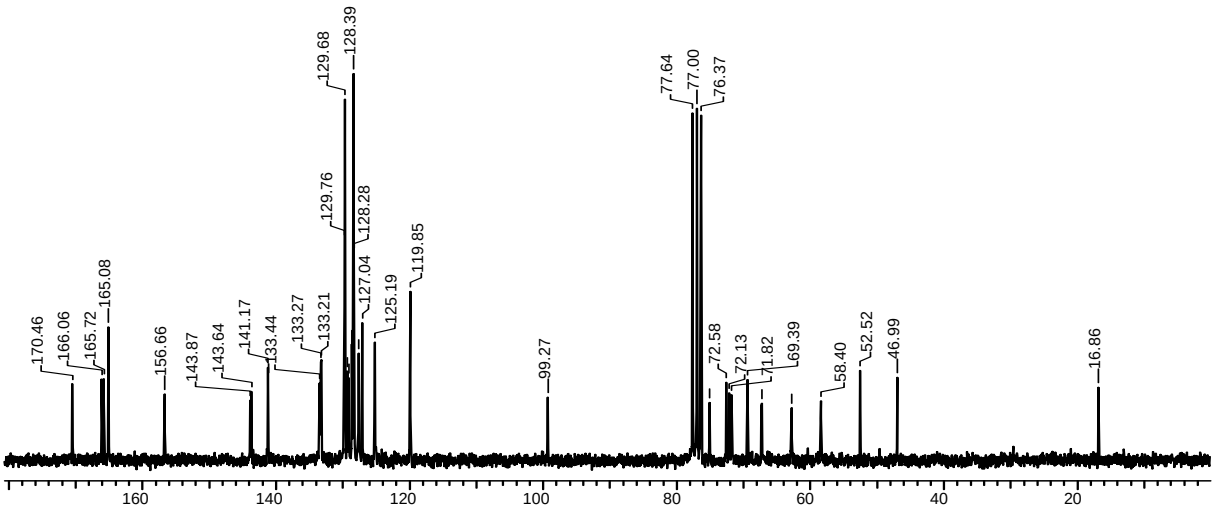
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15a**



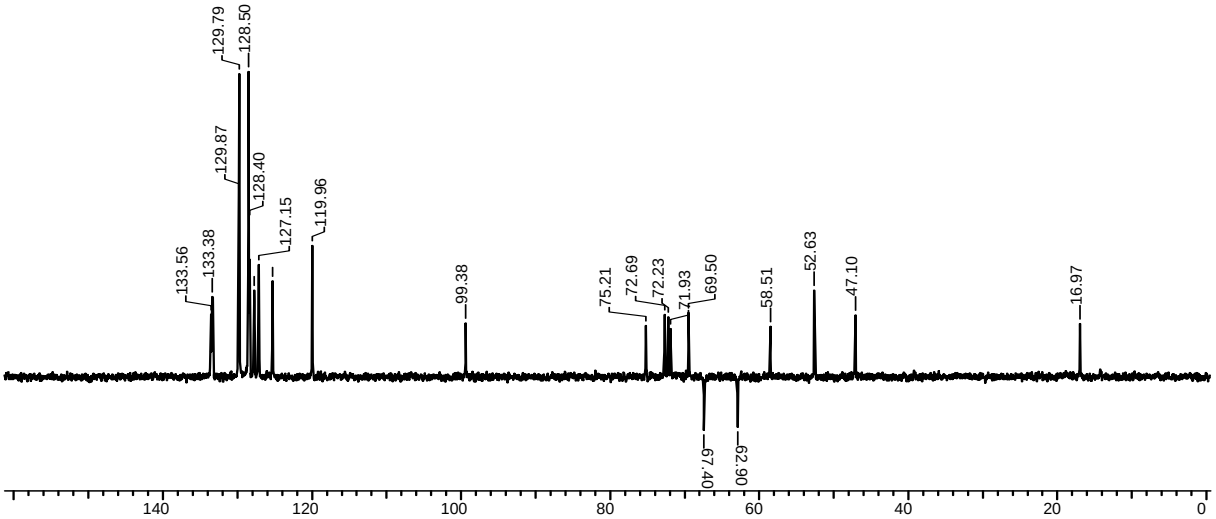
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **15b**



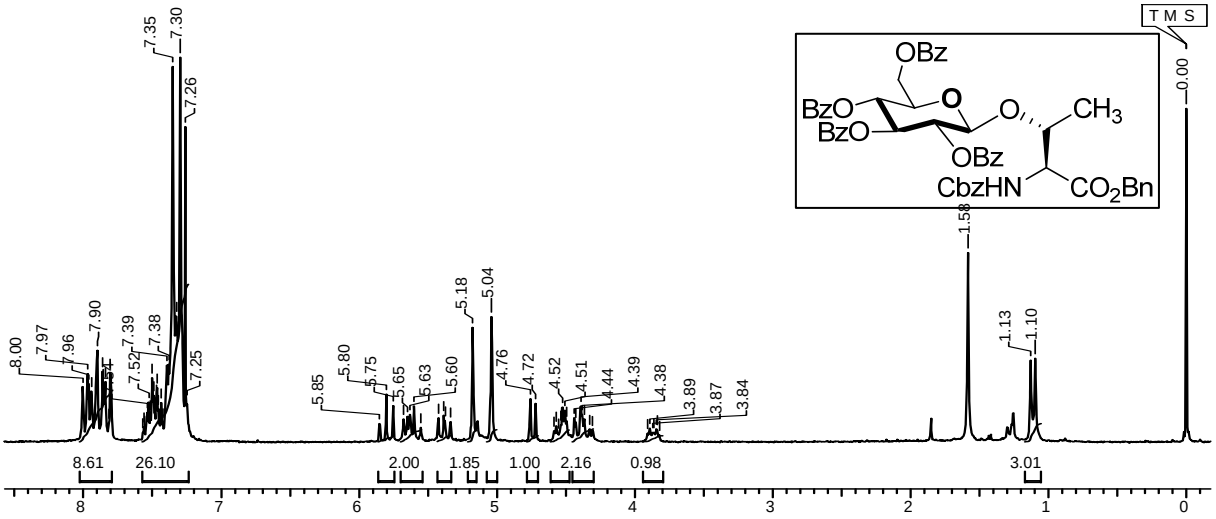
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15b**



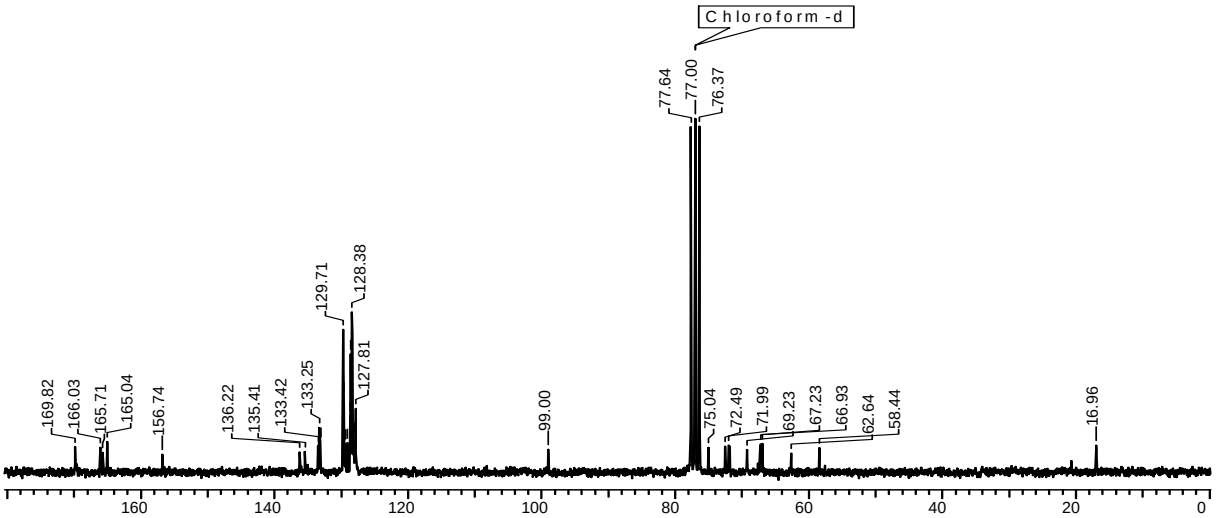
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15b**



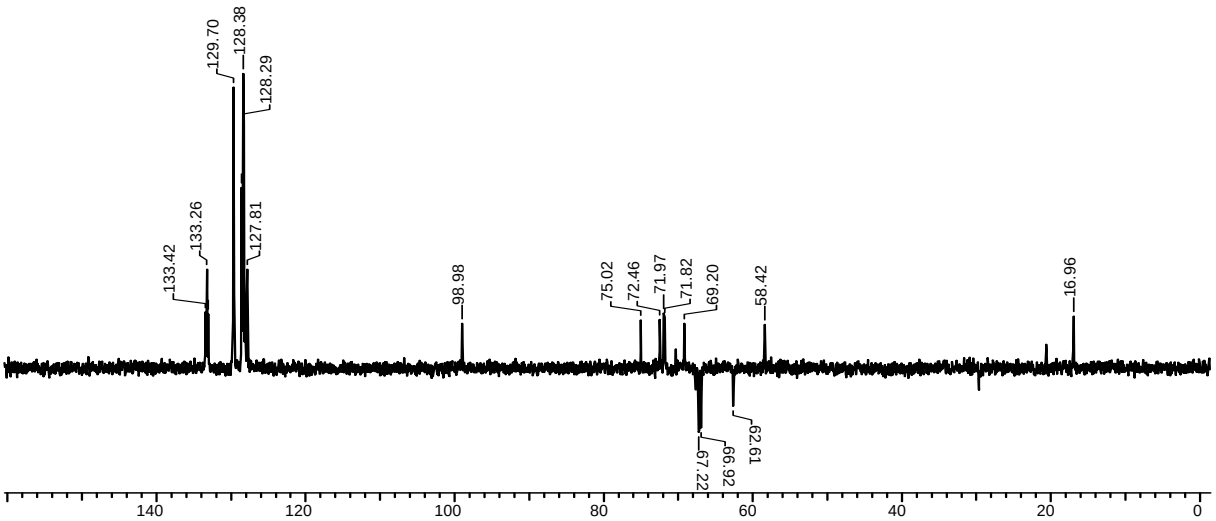
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **15c**



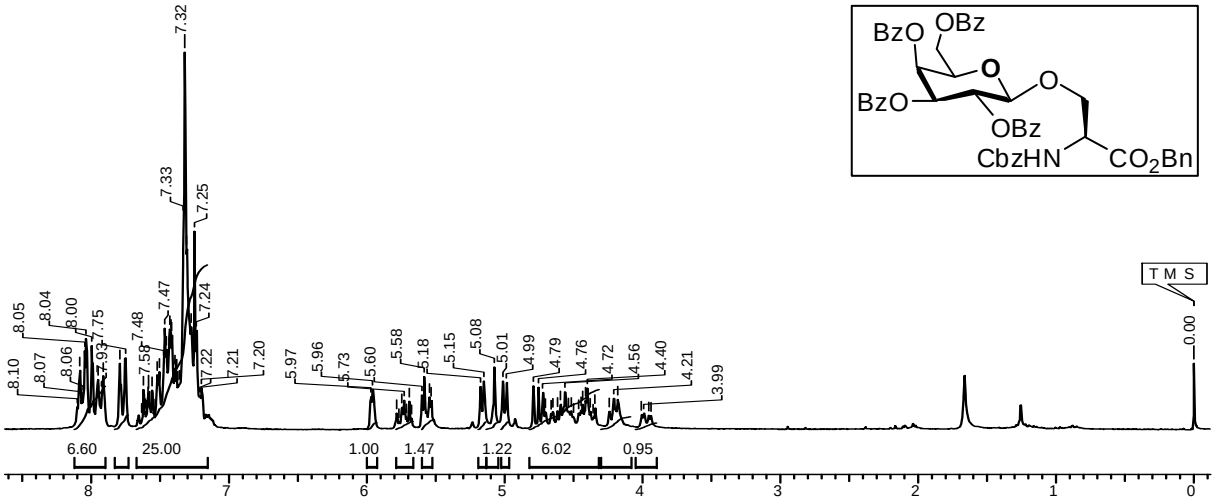
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15c**



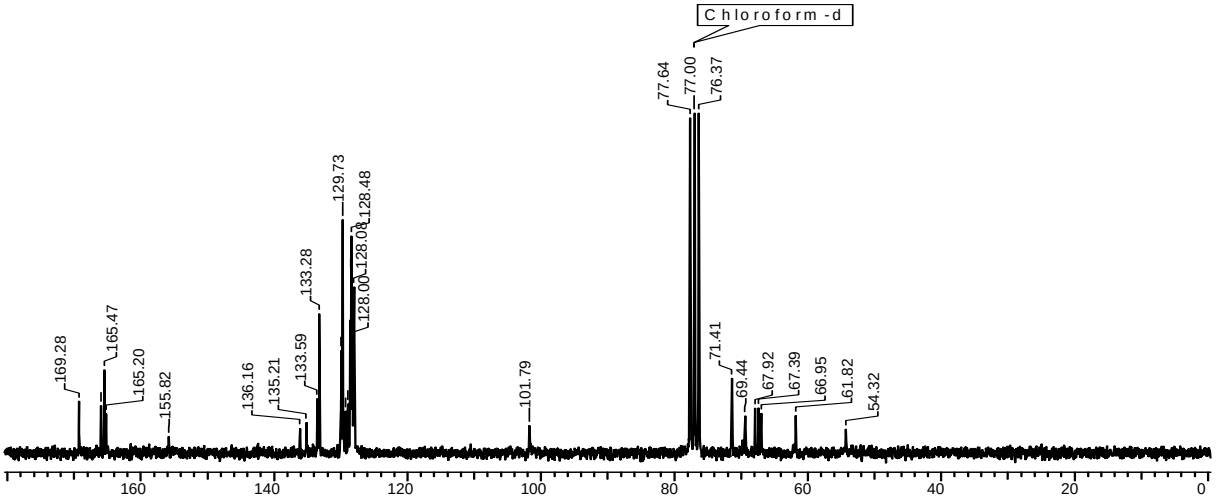
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **15c**



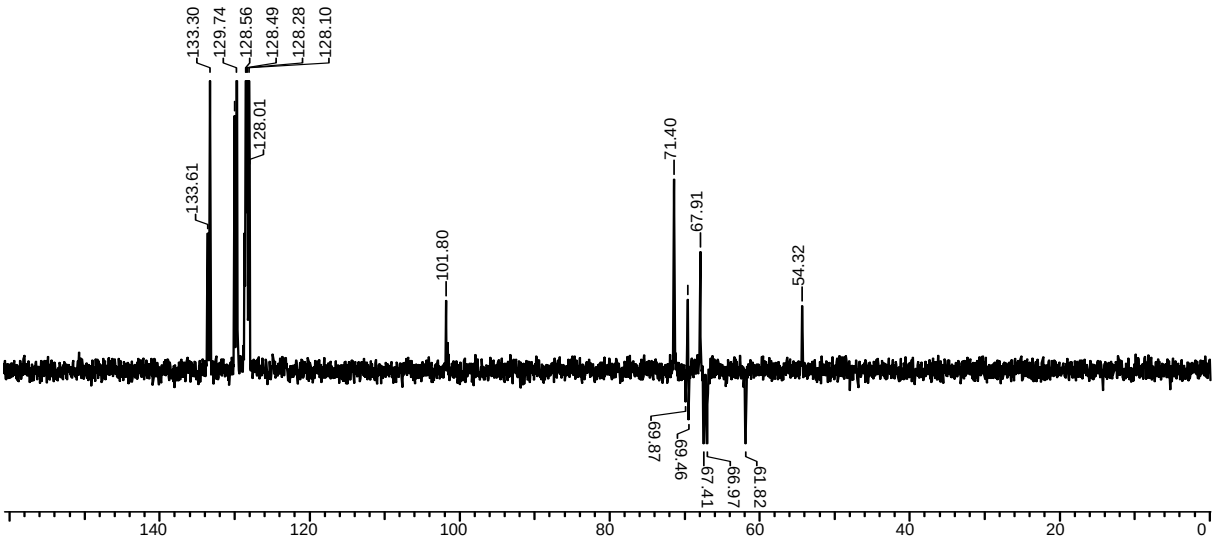
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **16a**



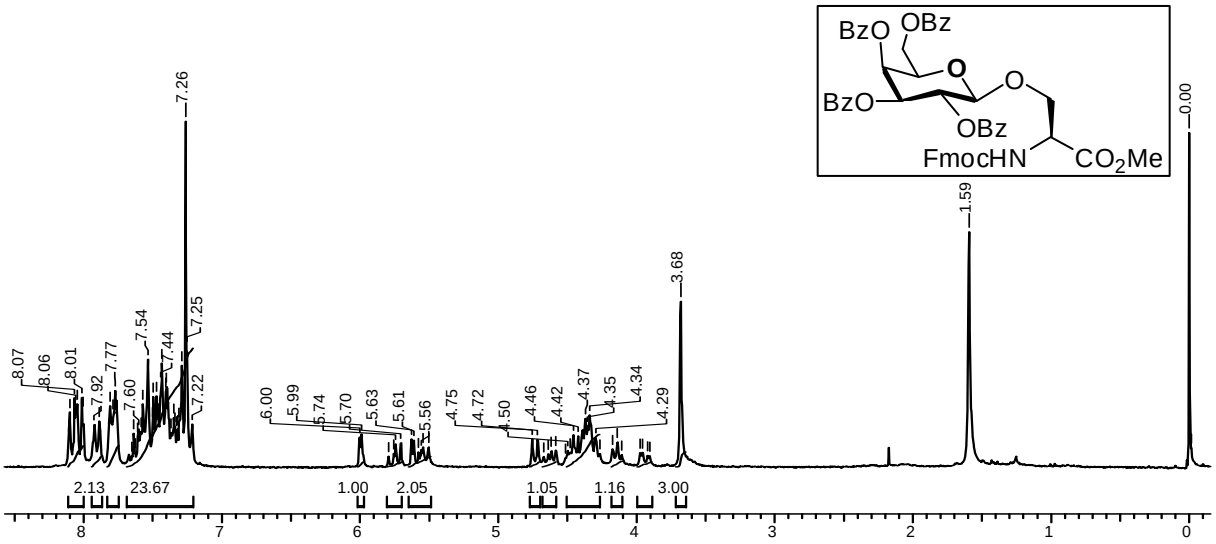
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **1a**



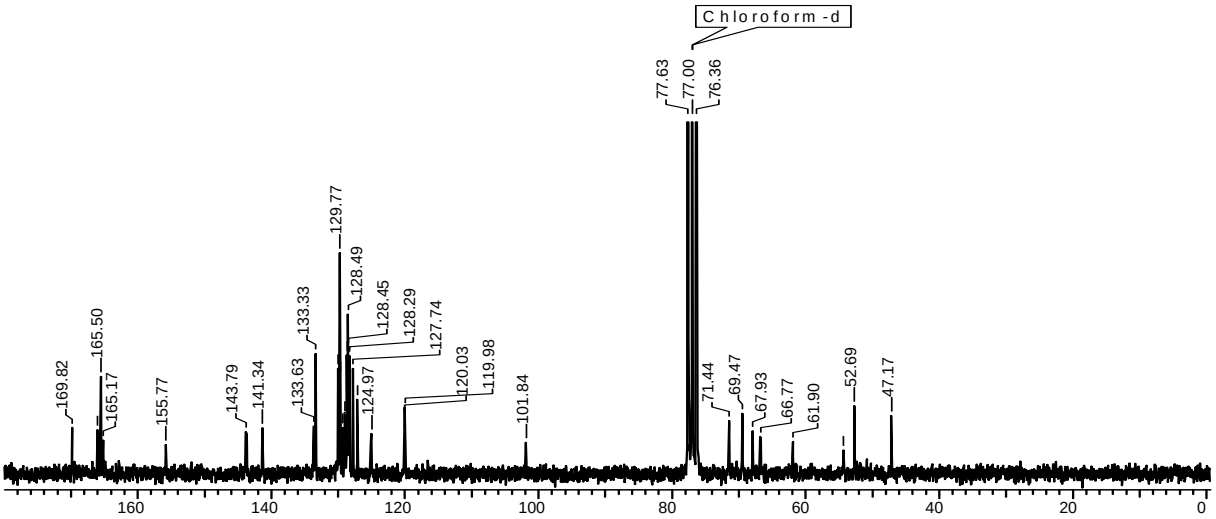
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **16a**



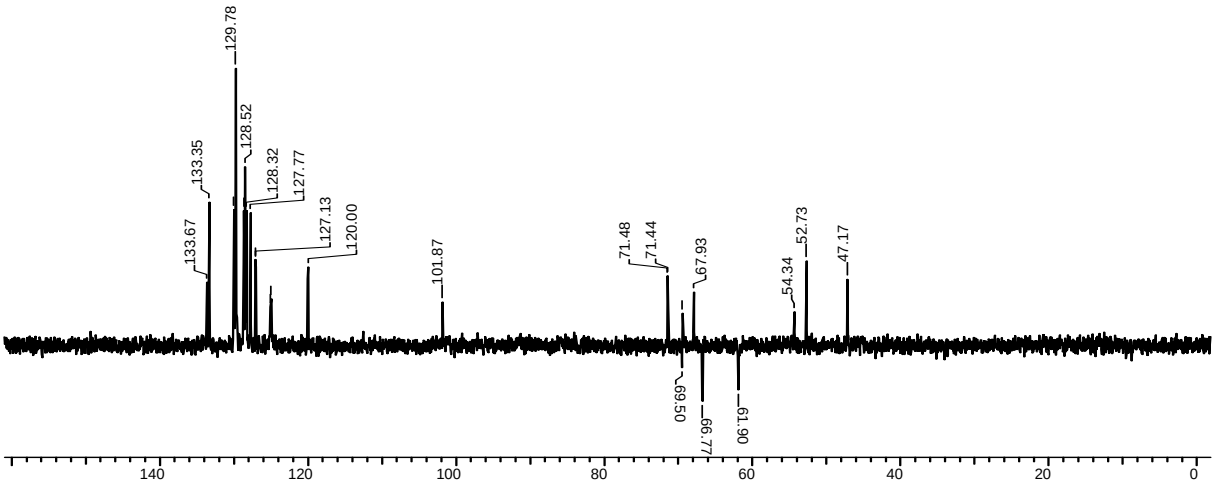
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **16b**



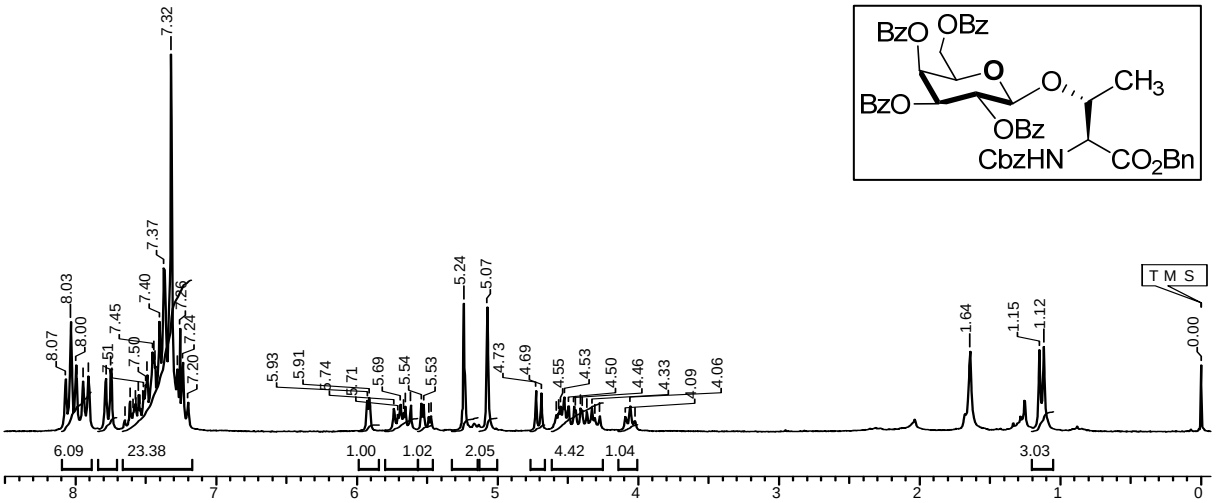
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **16b**



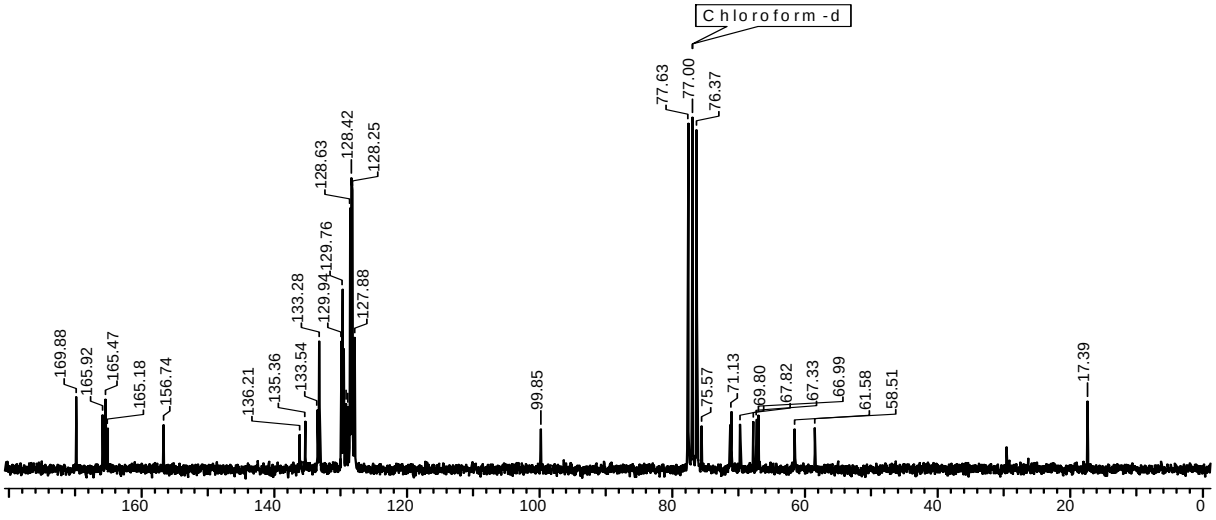
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **16b**



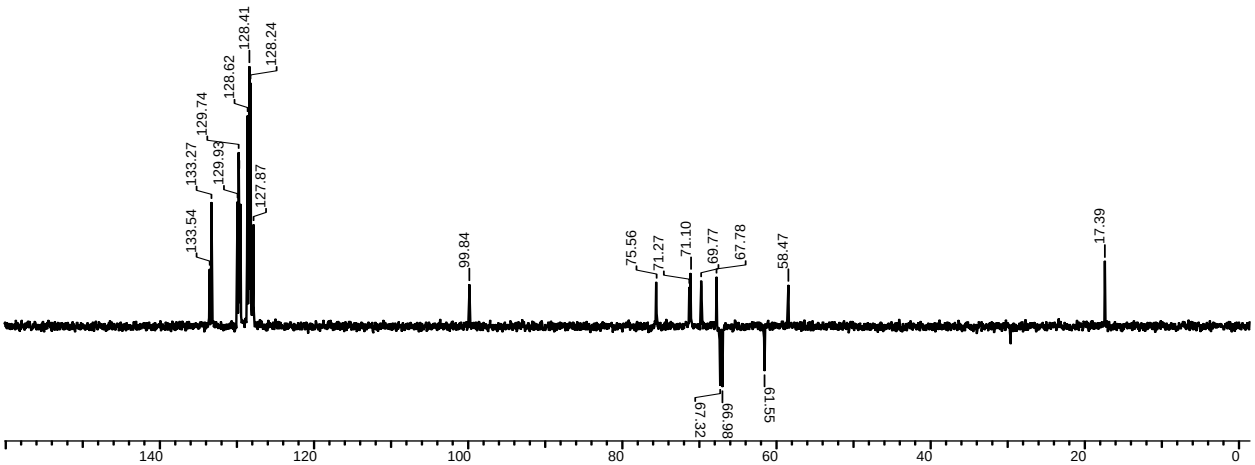
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **16c**



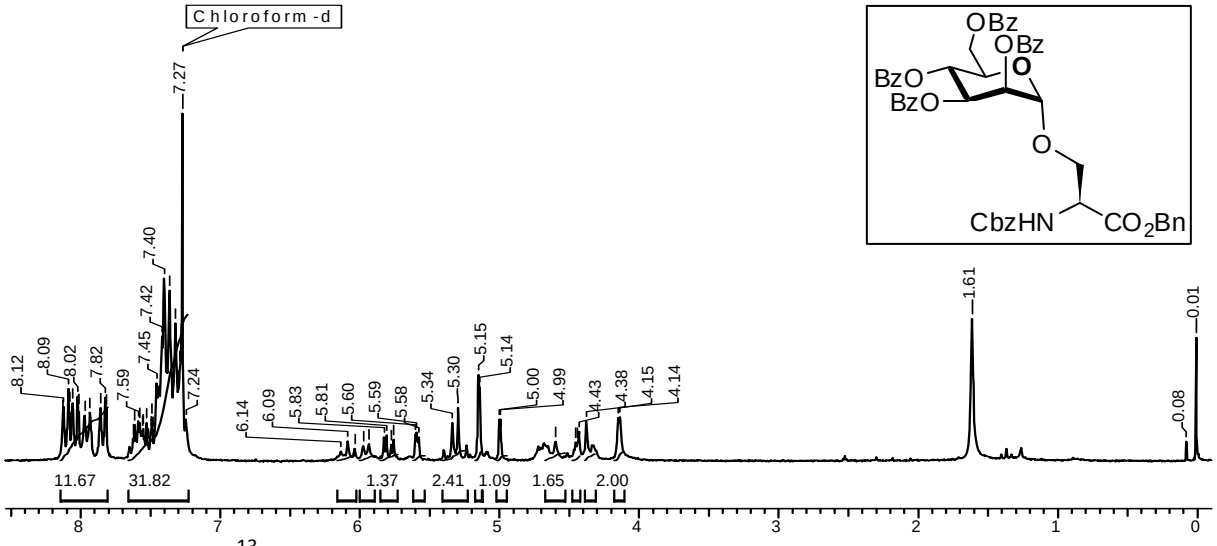
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **16c**



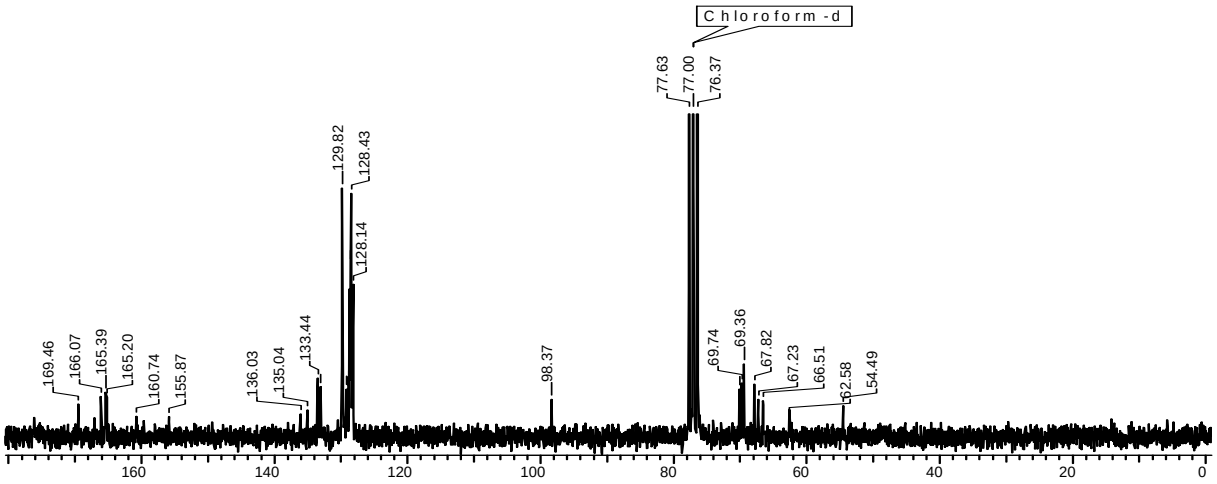
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **16c**



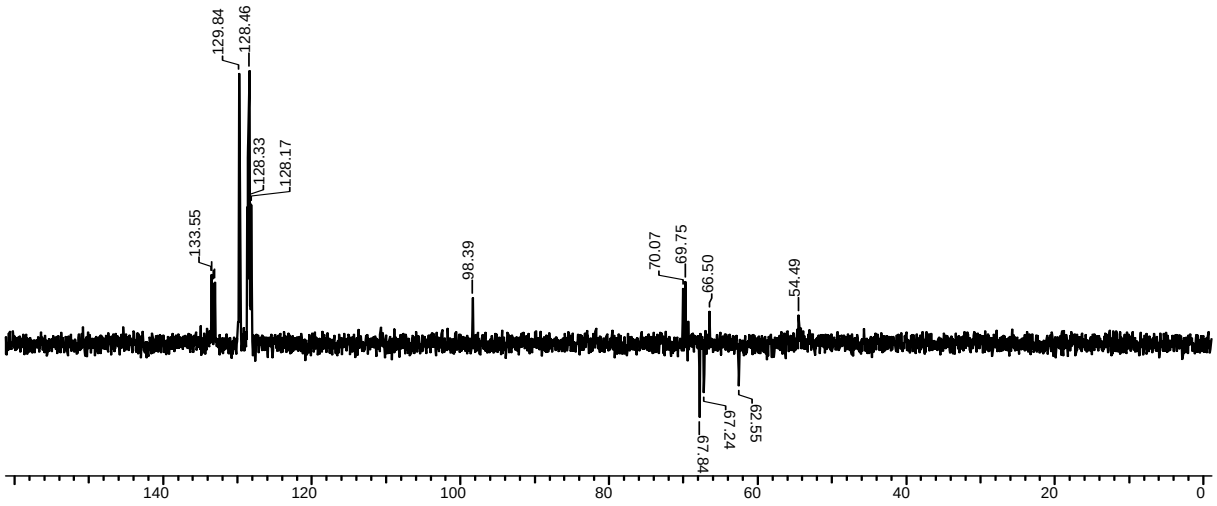
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **17a**



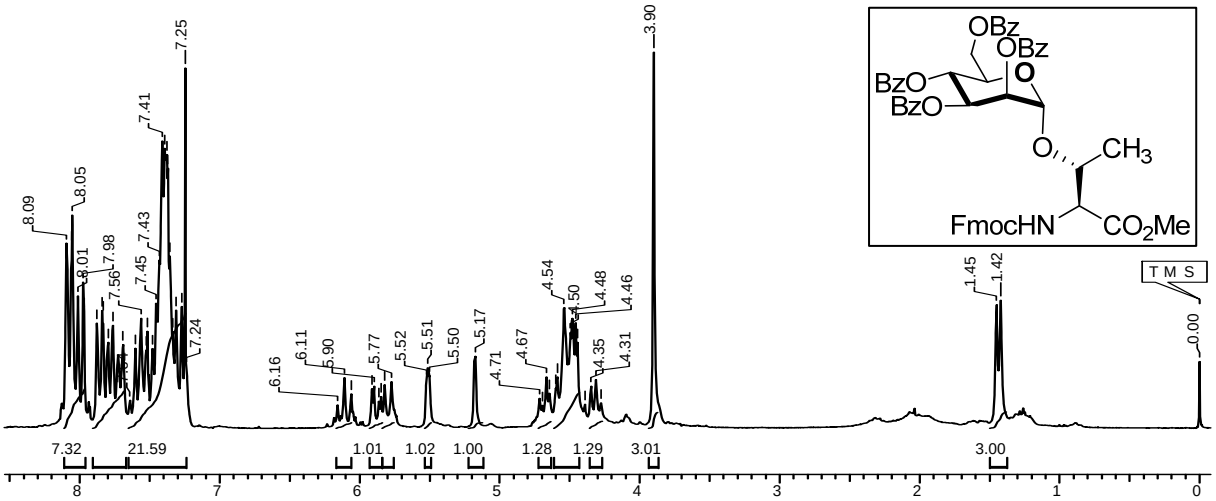
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17a**



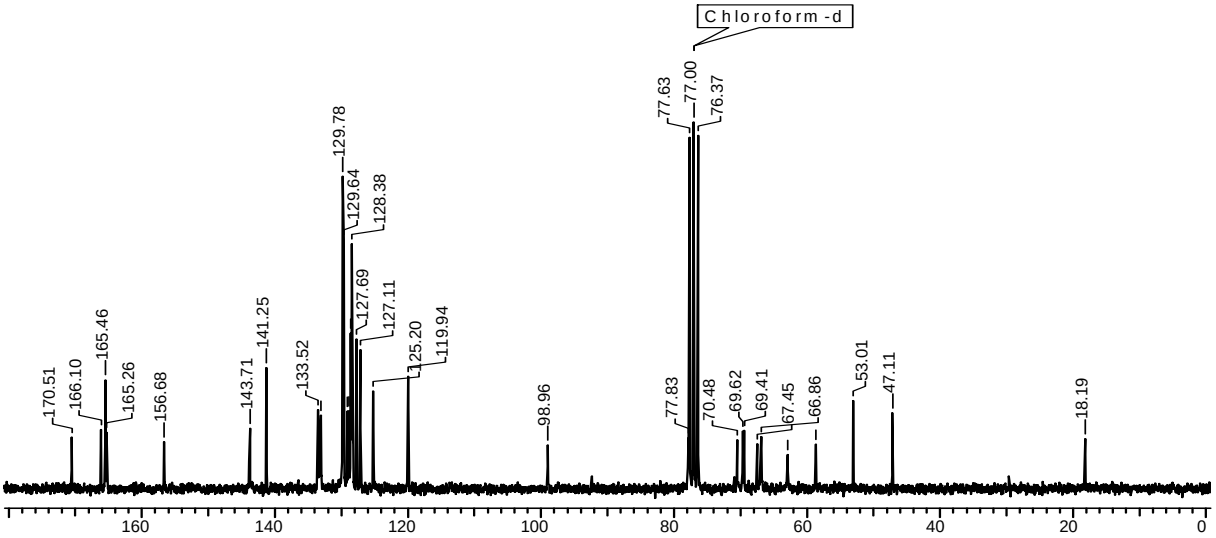
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17a**



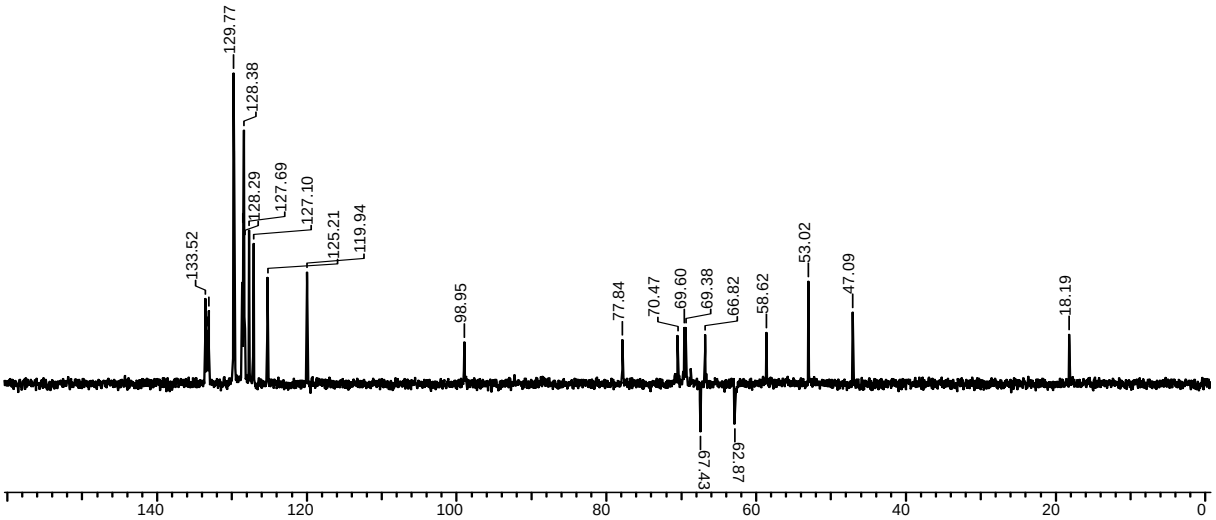
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **17b**



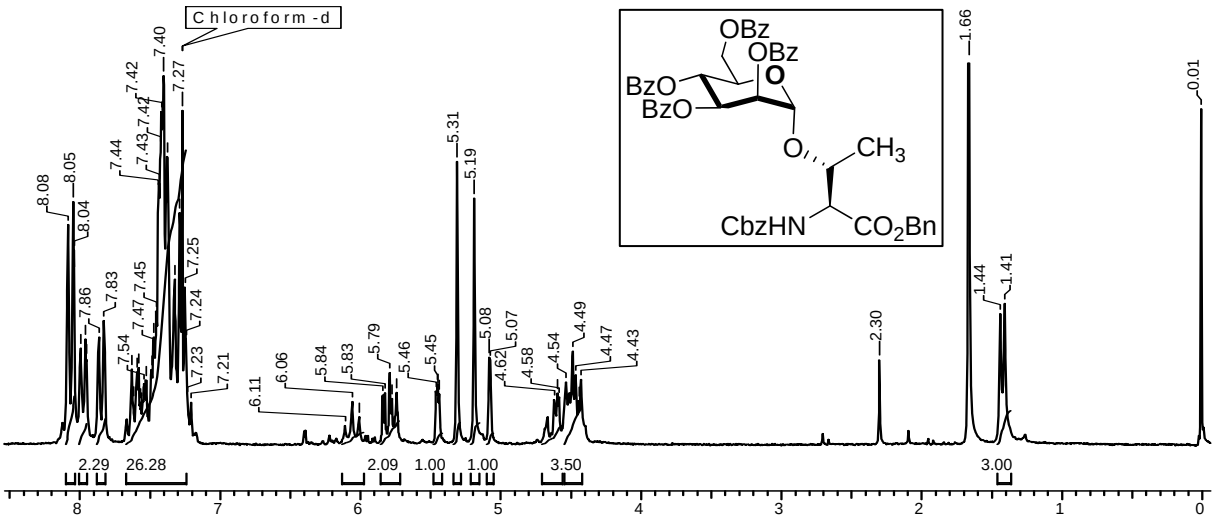
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17b**



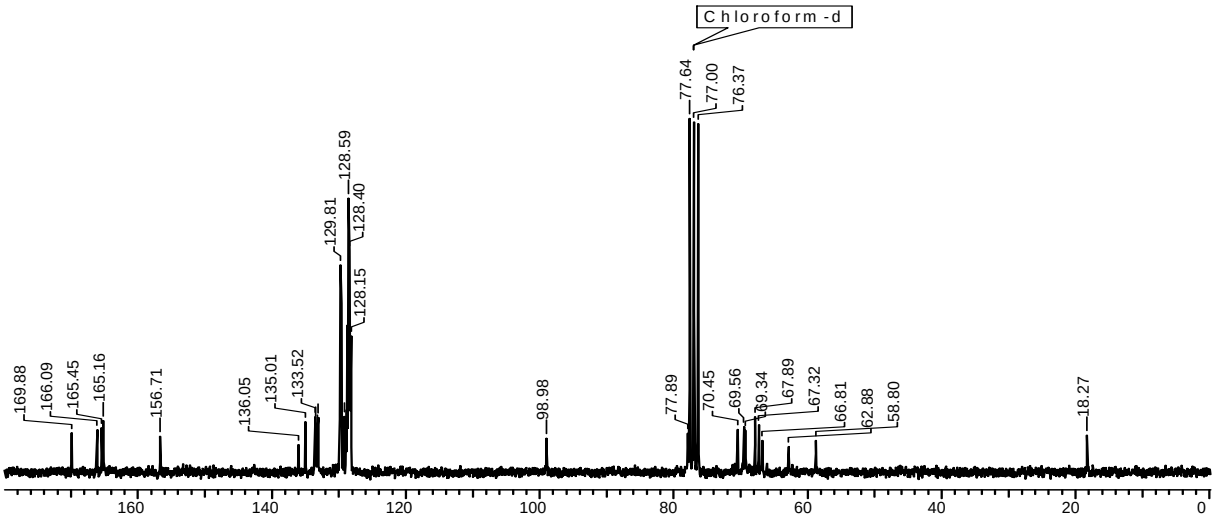
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17b**



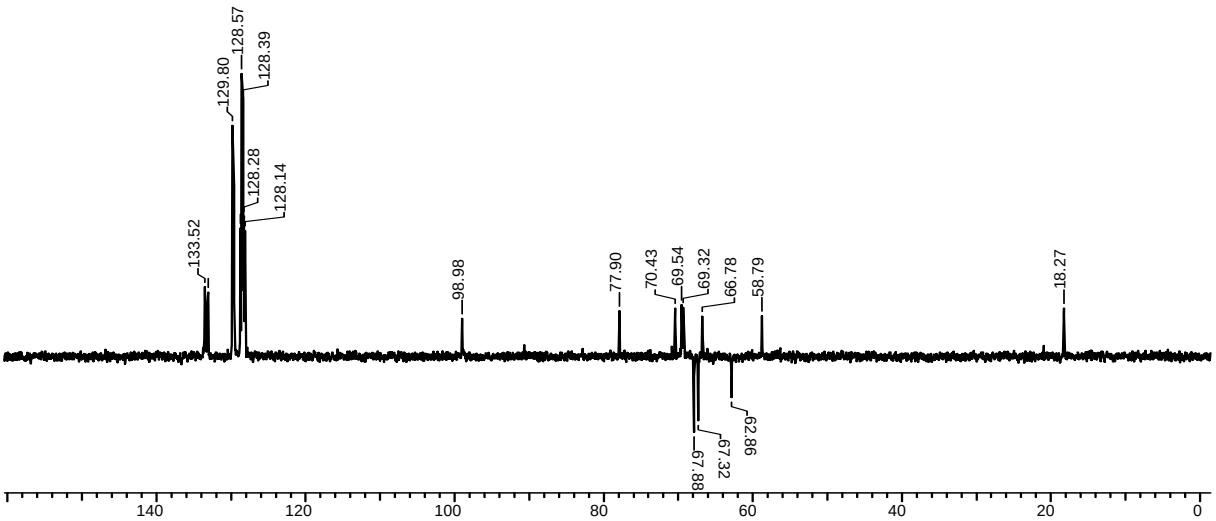
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **17c**



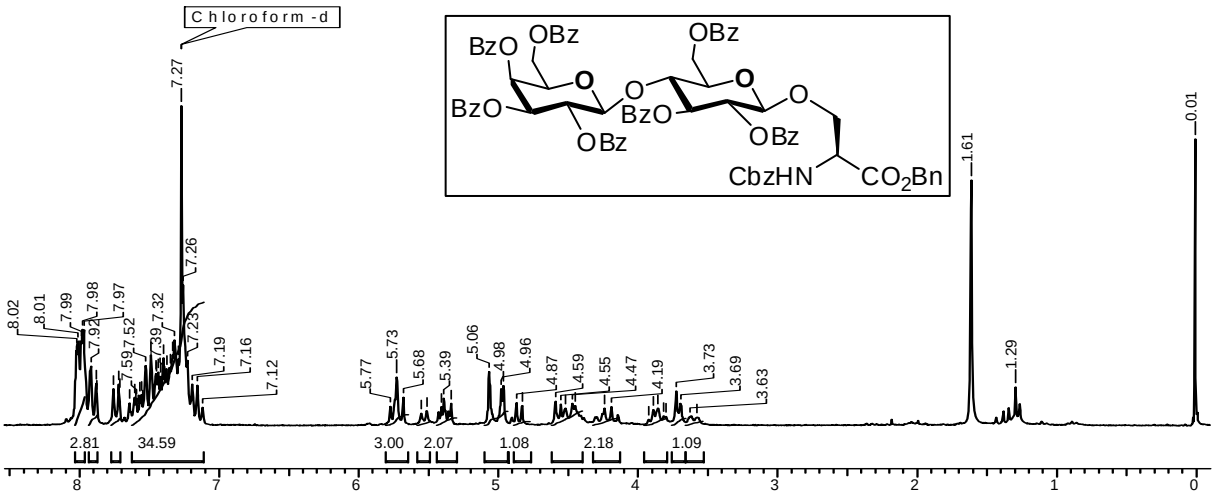
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17c**



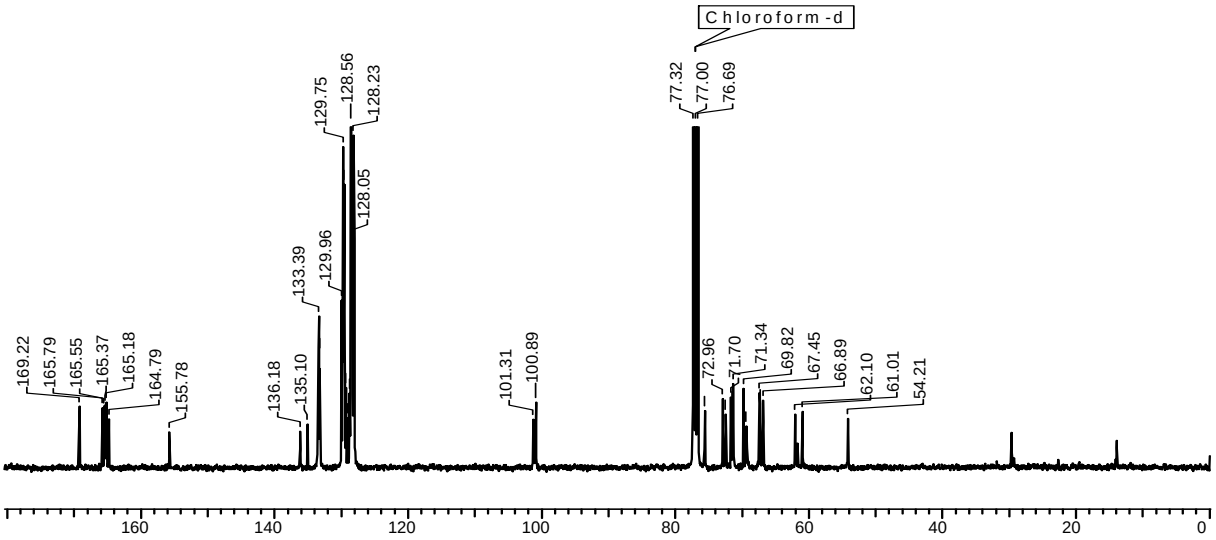
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **17c**



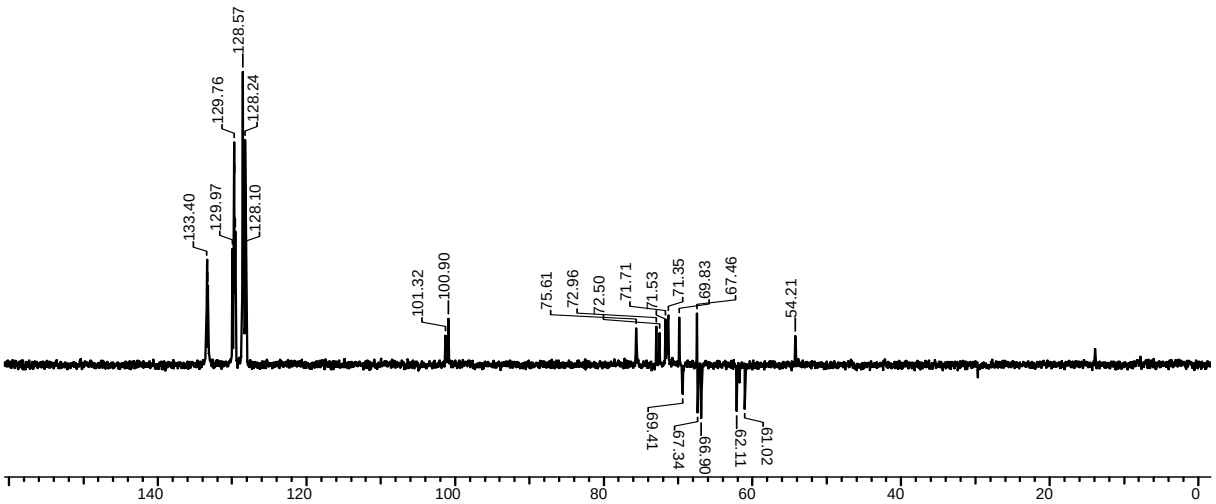
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **18a**



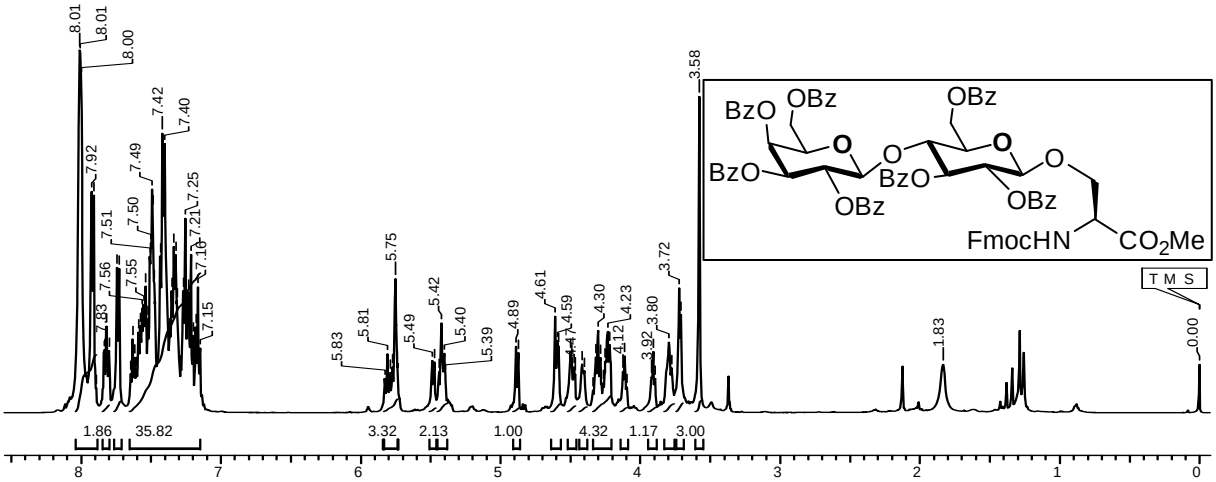
^{13}C NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **18a**



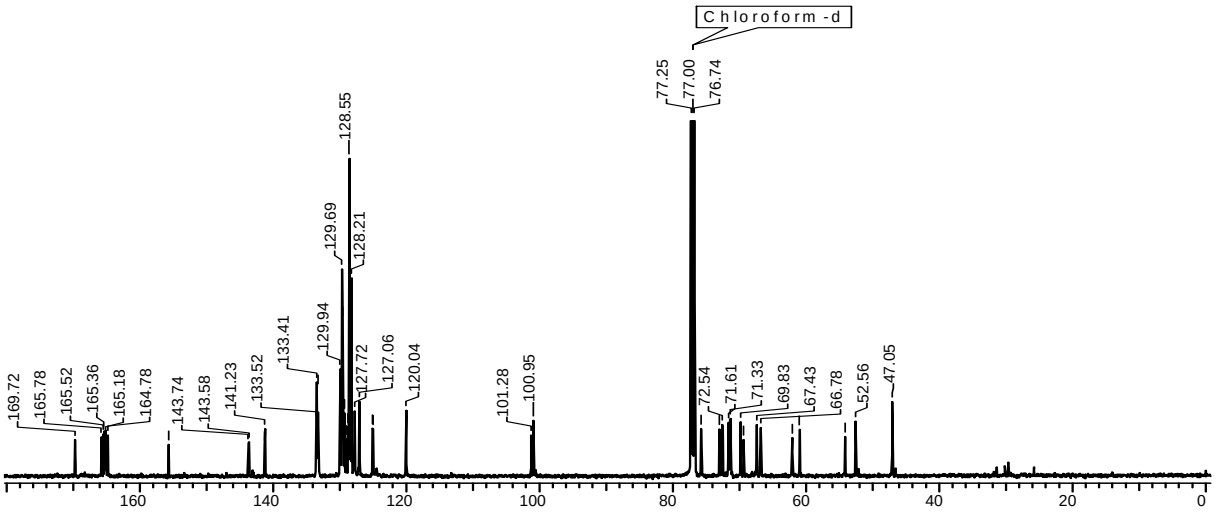
DEPT NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **18a**



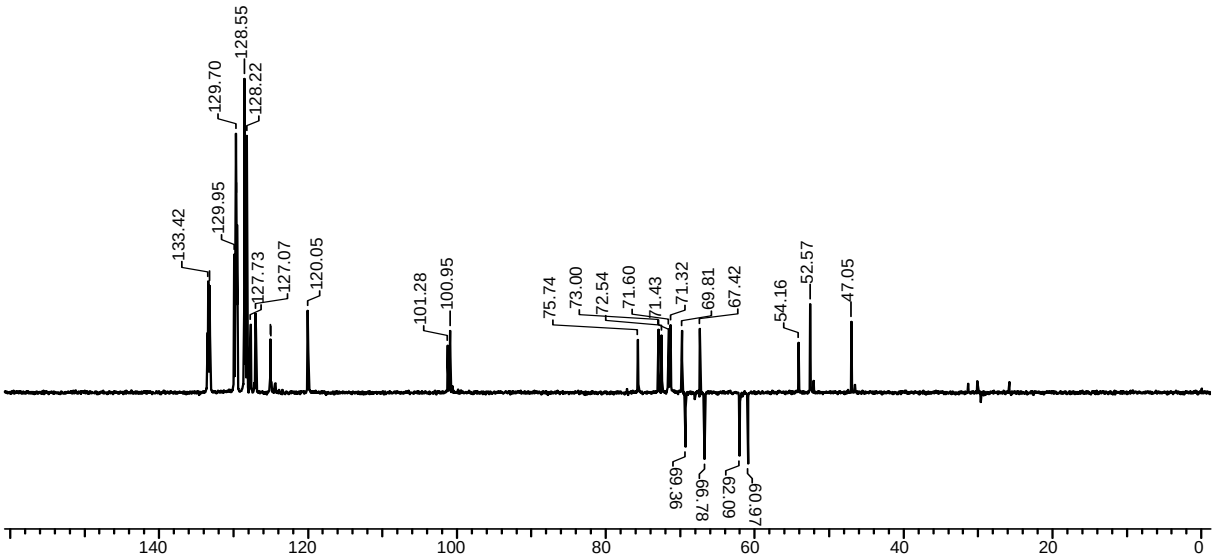
^{1}H NMR ($CDCl_3$, 500.13MHz) Spectrum of Compound **18b**



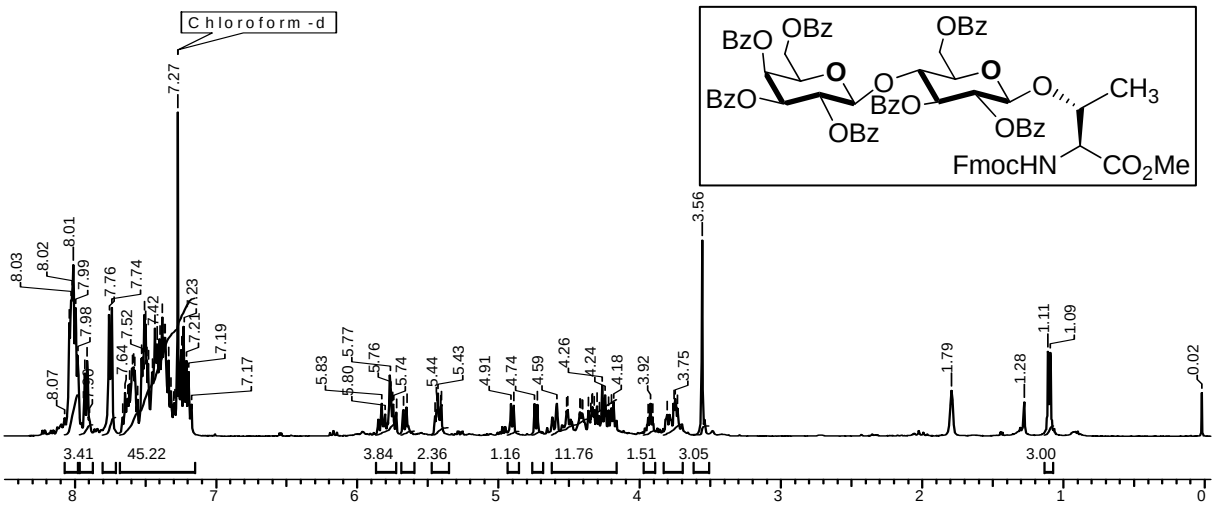
^{13}C NMR ($CDCl_3$, 125.76 MHz) Spectrum of Compound **18b**



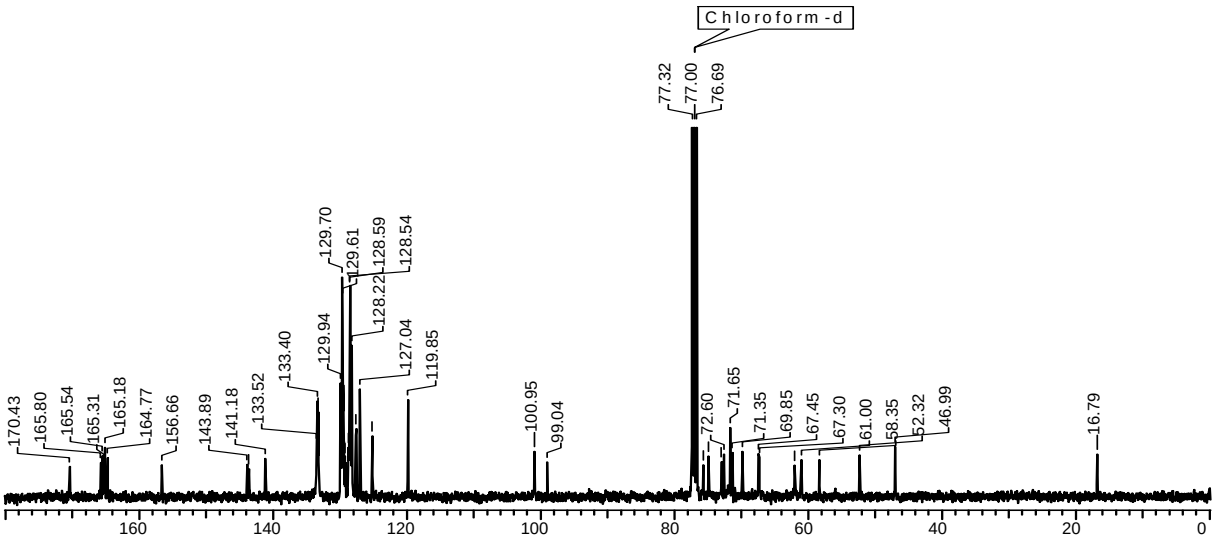
DEPT NMR ($CDCl_3$, 125.76 MHz) Spectrum of Compound **18b**



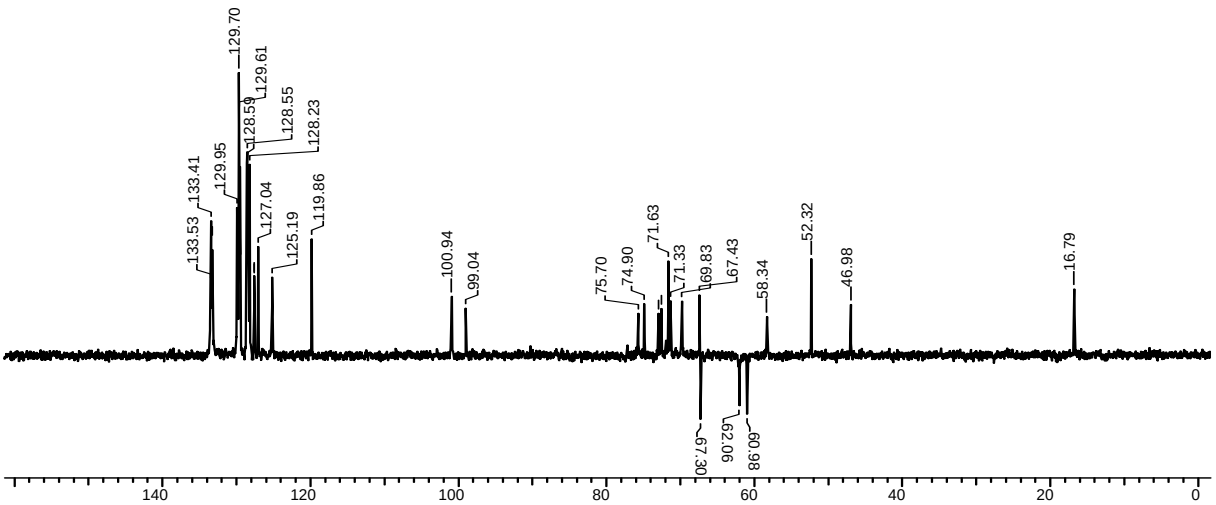
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **18c**



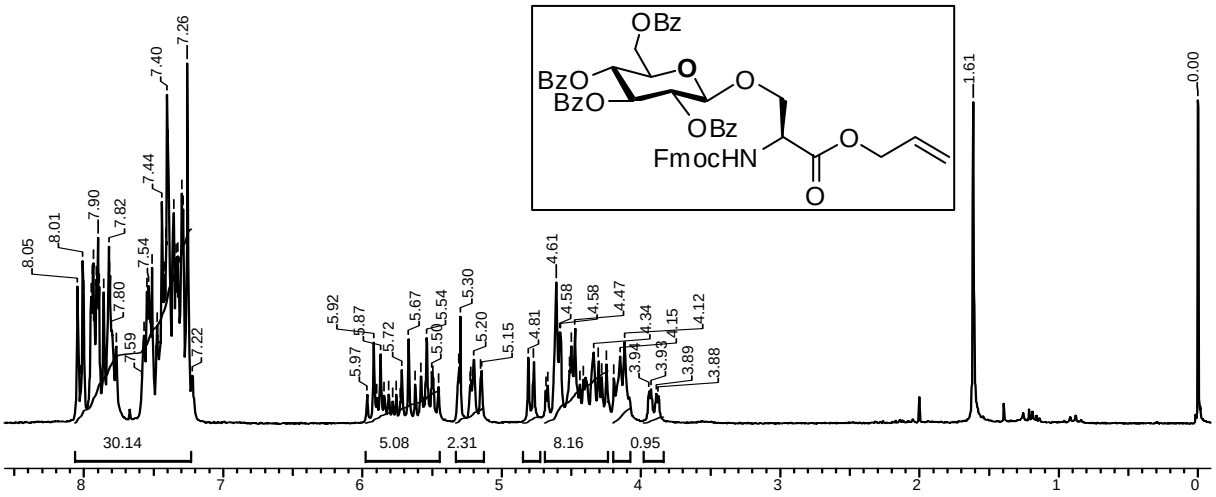
^{13}C NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **18c**



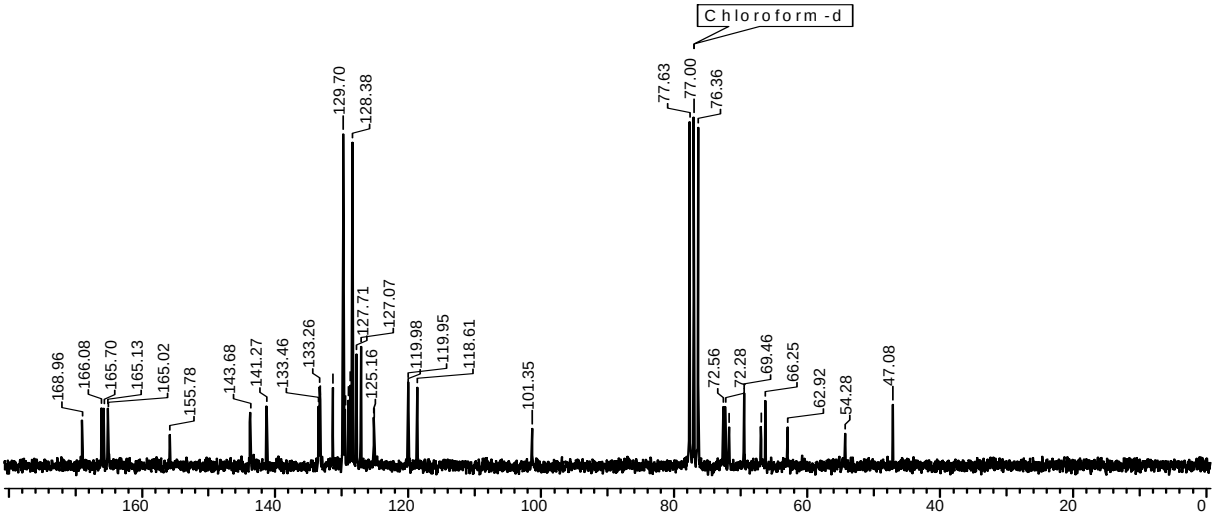
DEPT NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **18c**



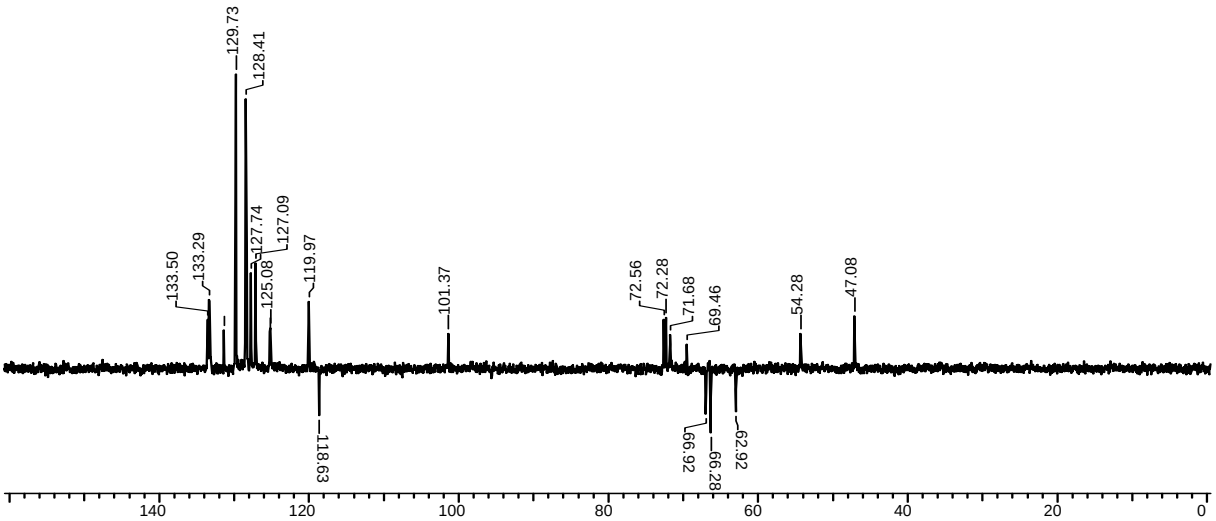
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **20**



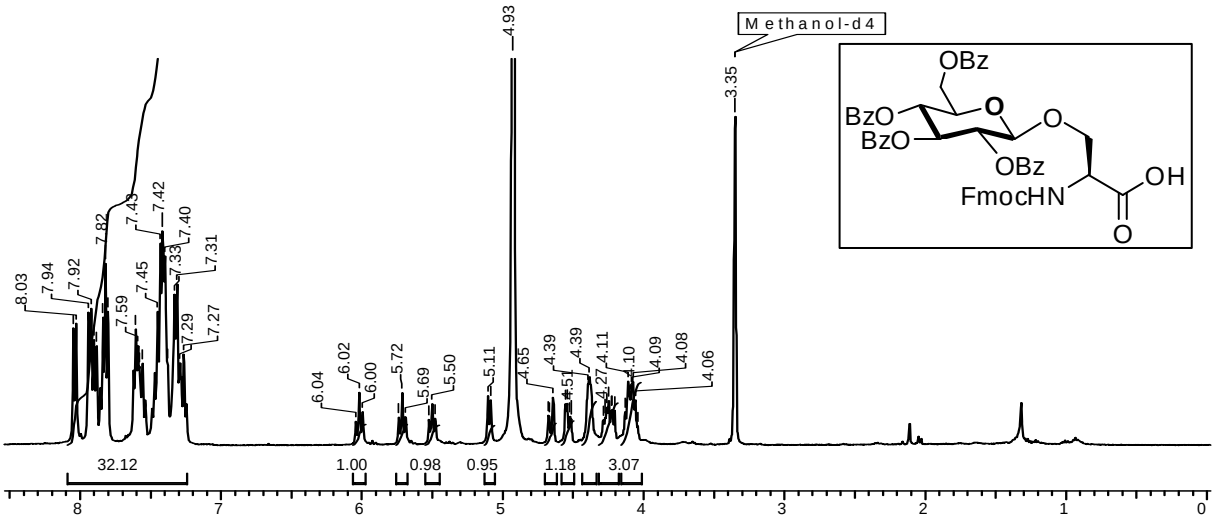
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **20**



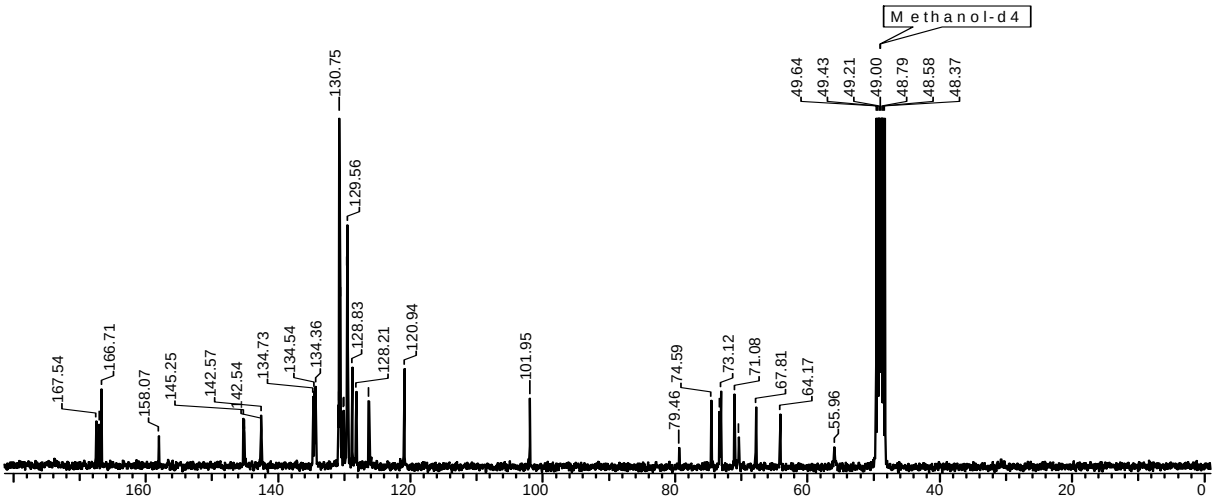
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **20**



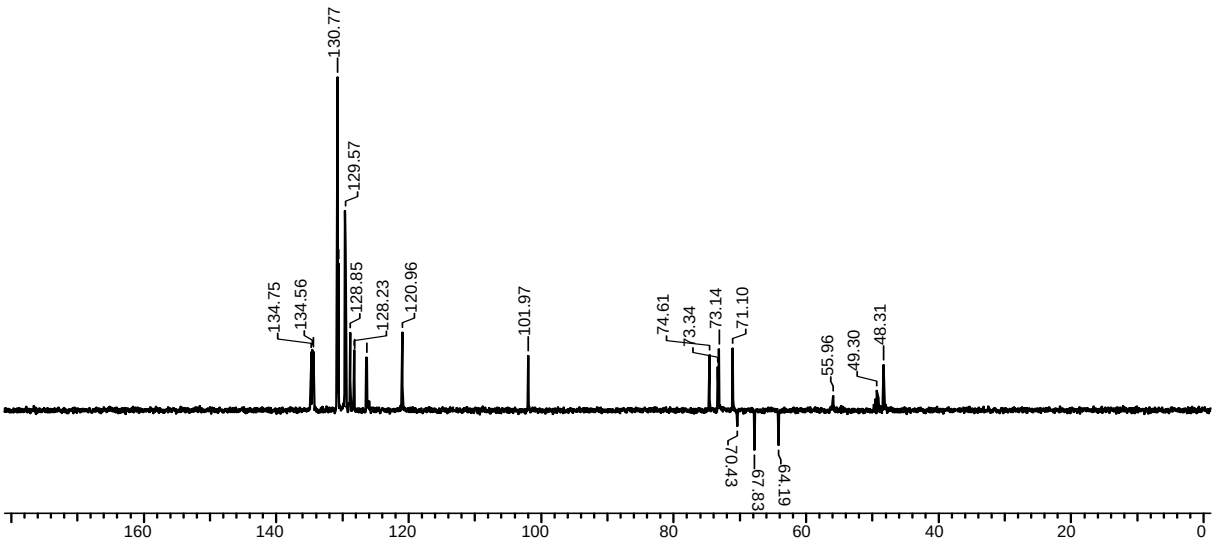
^{1}H NMR (CD_3OD, 200.13MHz) Spectrum of Compound **21**



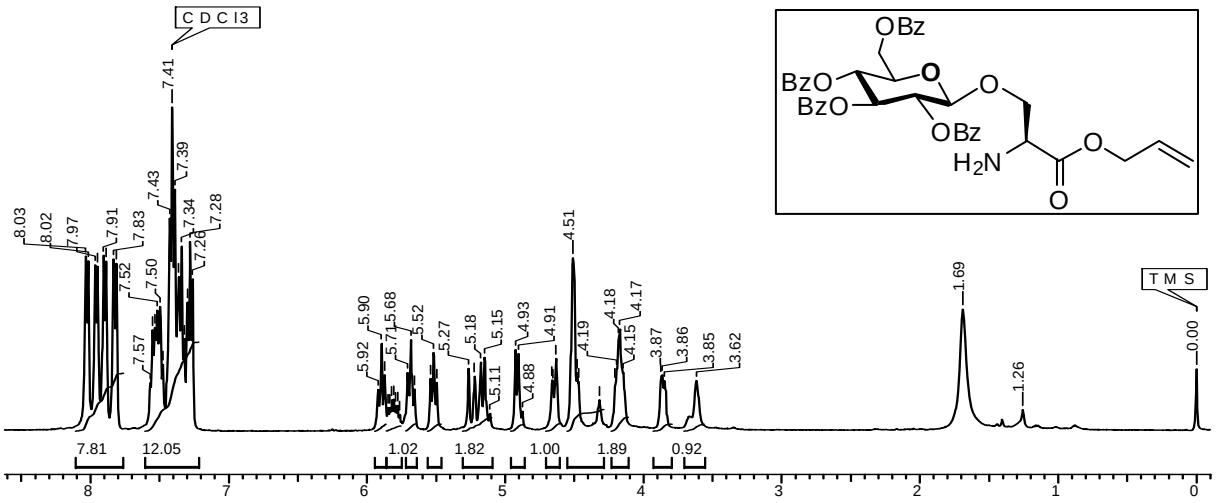
^{13}C NMR (CD_3OD, 100.61 MHz) Spectrum of Compound **21**



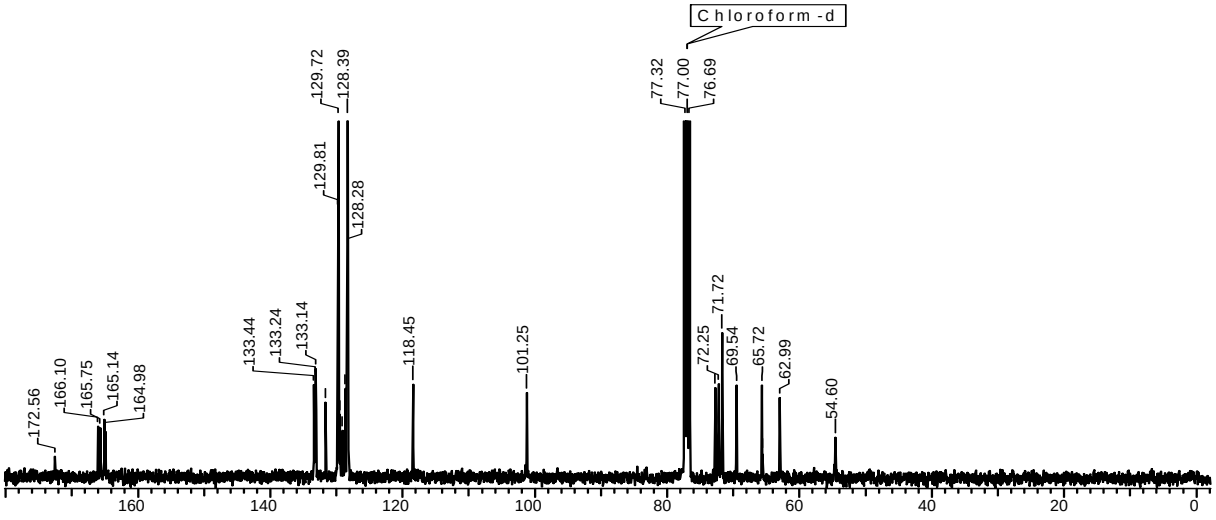
DEPT NMR (CD_3OD, 100.61 MHz) Spectrum of Compound **21**



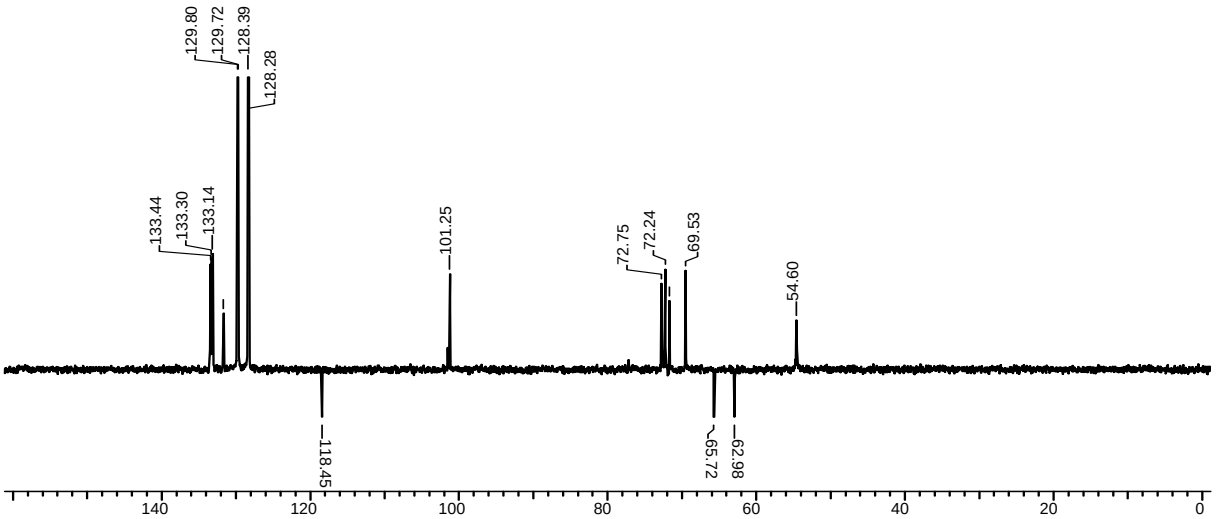
^{1}H NMR ($CDCl_3$, 200.13MHz) Spectrum of Compound **22**



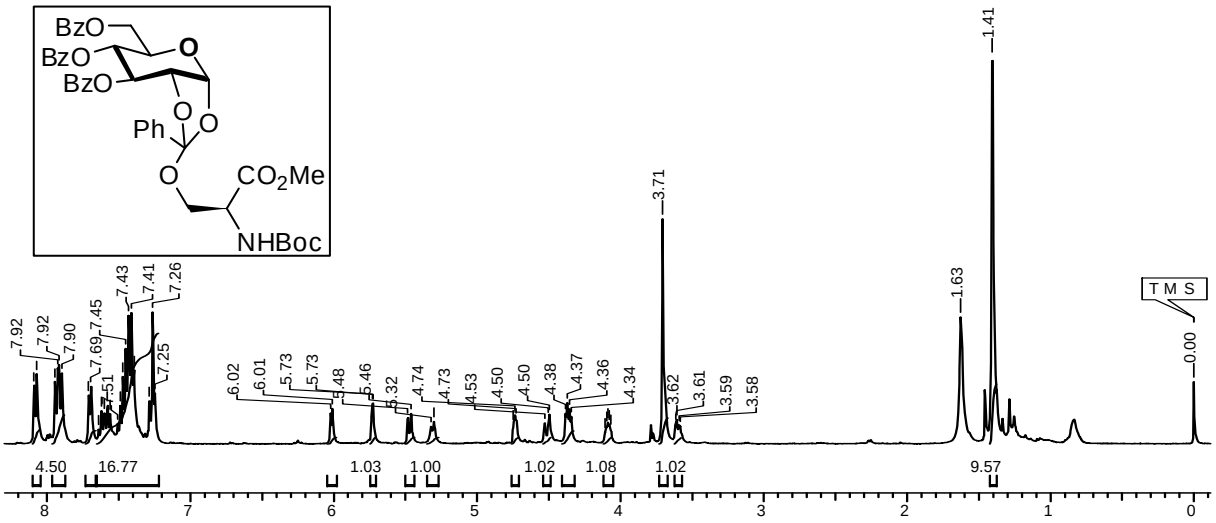
^{13}C NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **22**



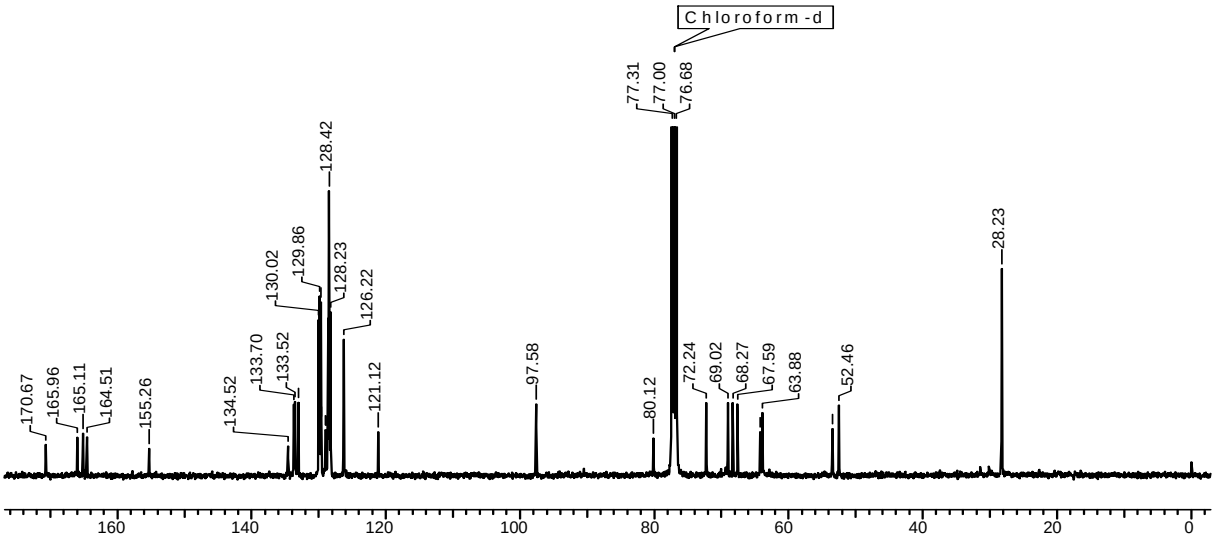
DEPT NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **22**



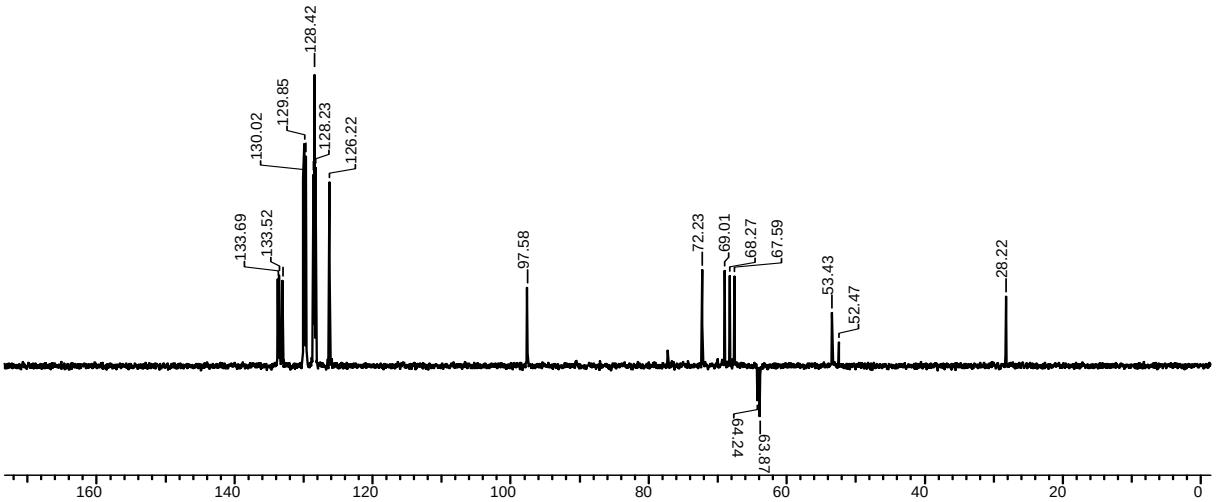
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **A**



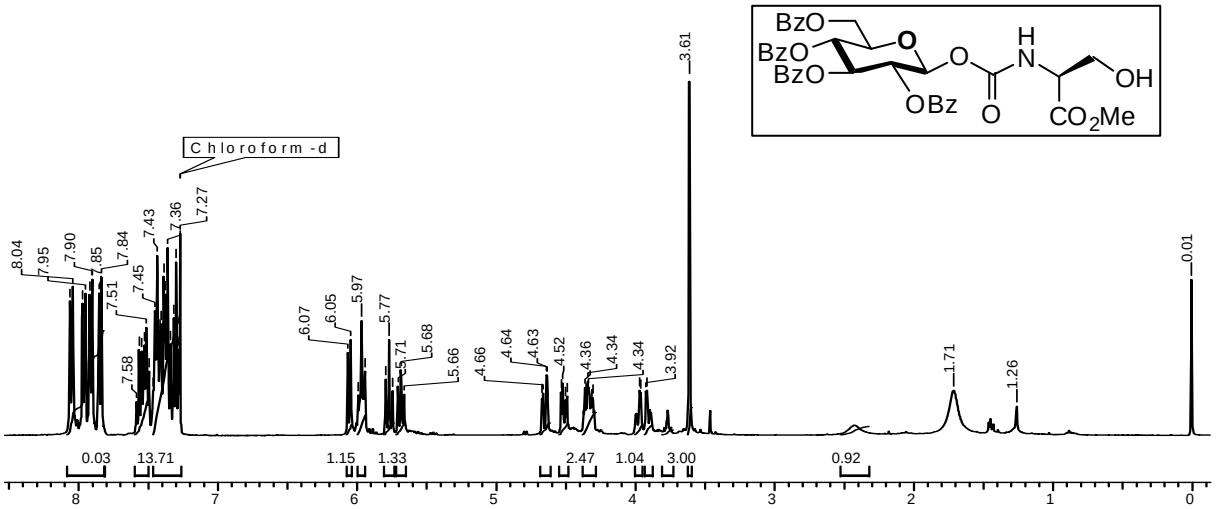
^{13}C NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **A**



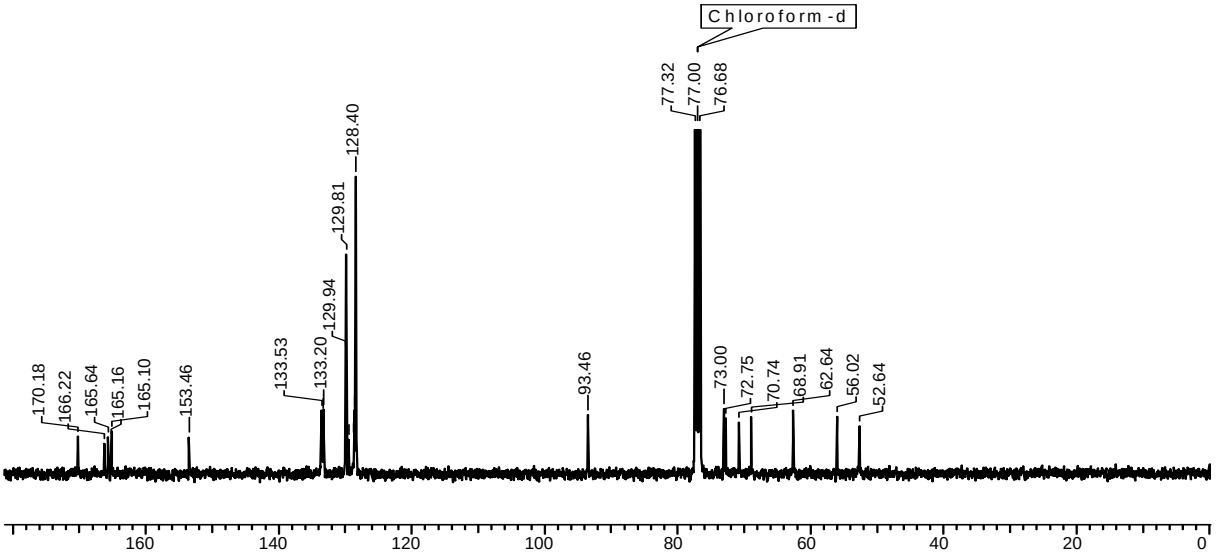
DEPT NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **A**



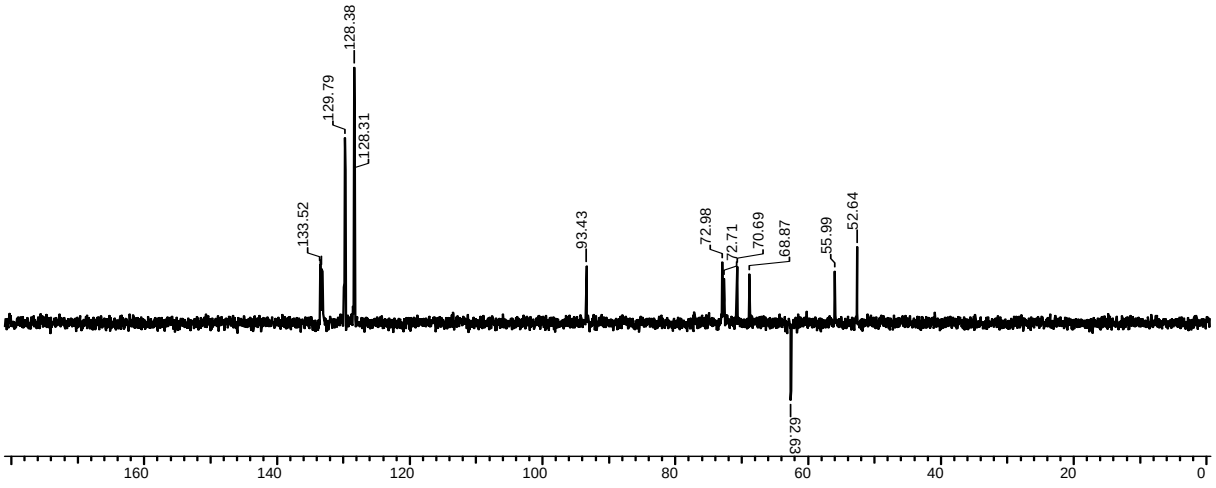
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **24**



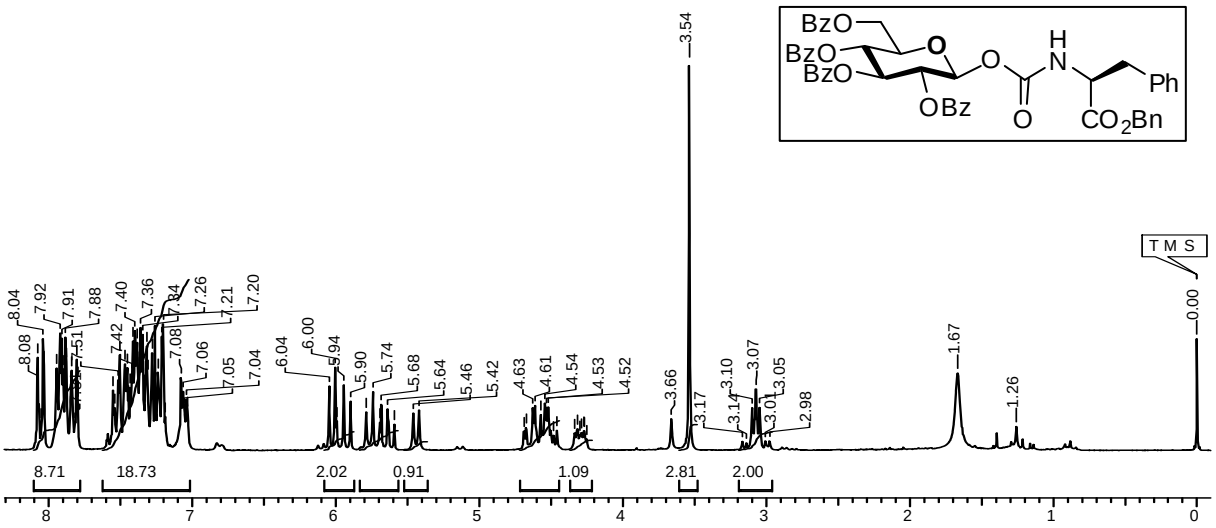
^{13}C NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **24**



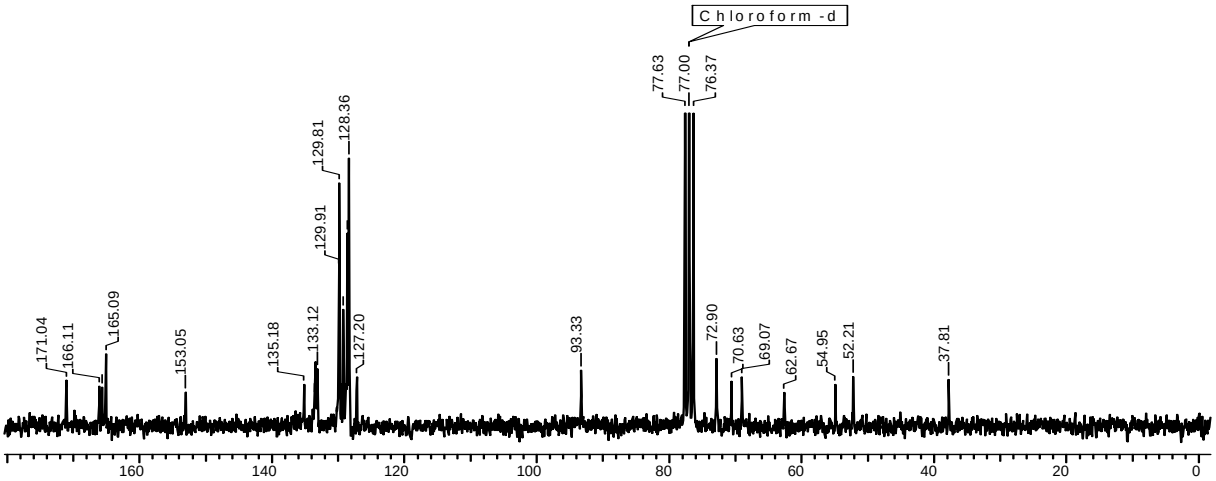
DEPT NMR ($CDCl_3$, 100.61 MHz) Spectrum of Compound **24**



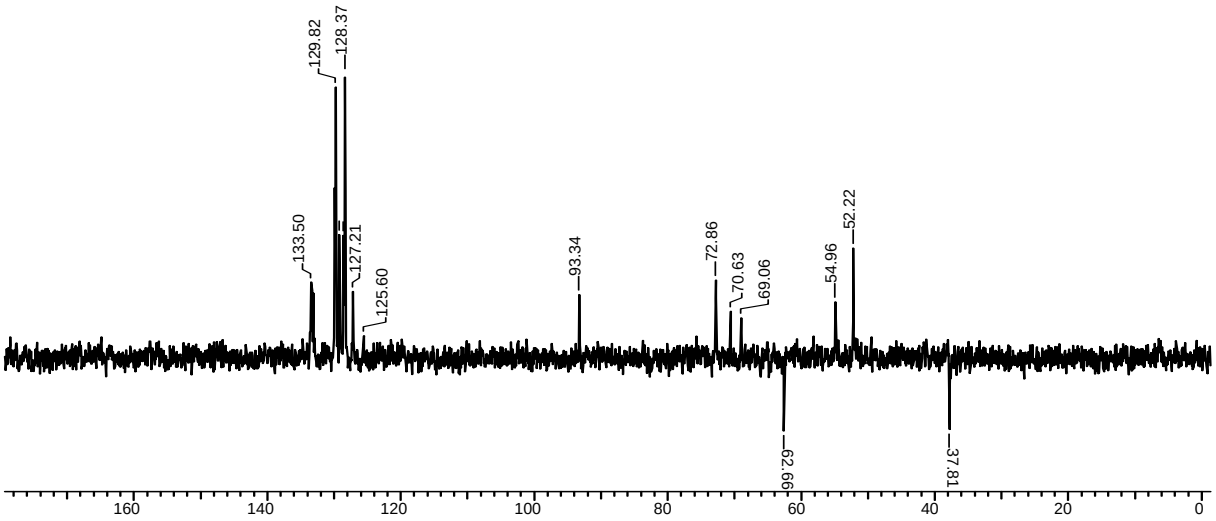
^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **26a**



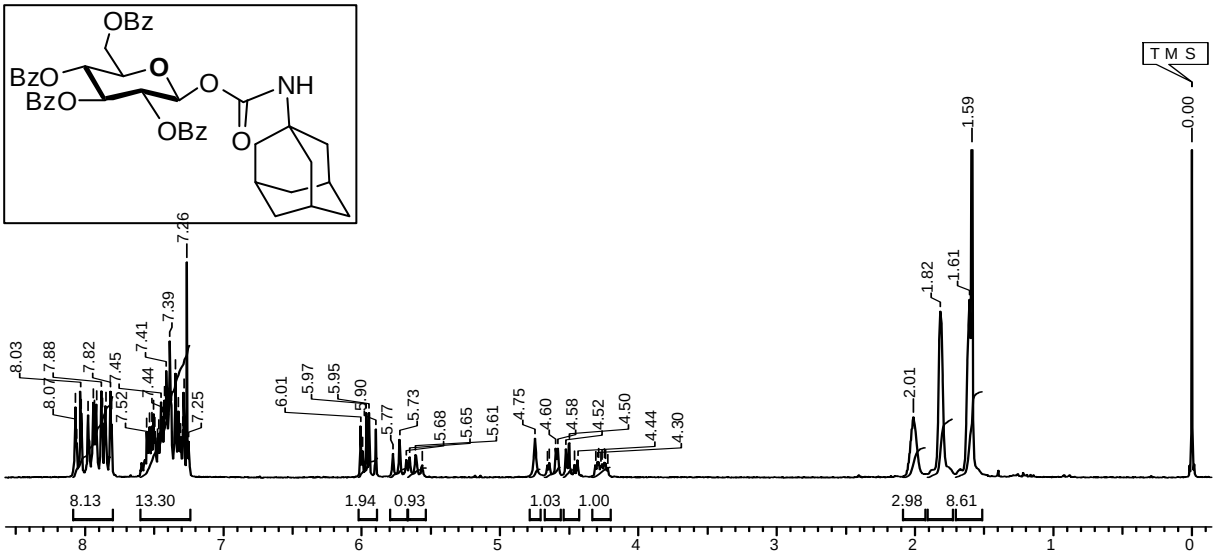
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26a**



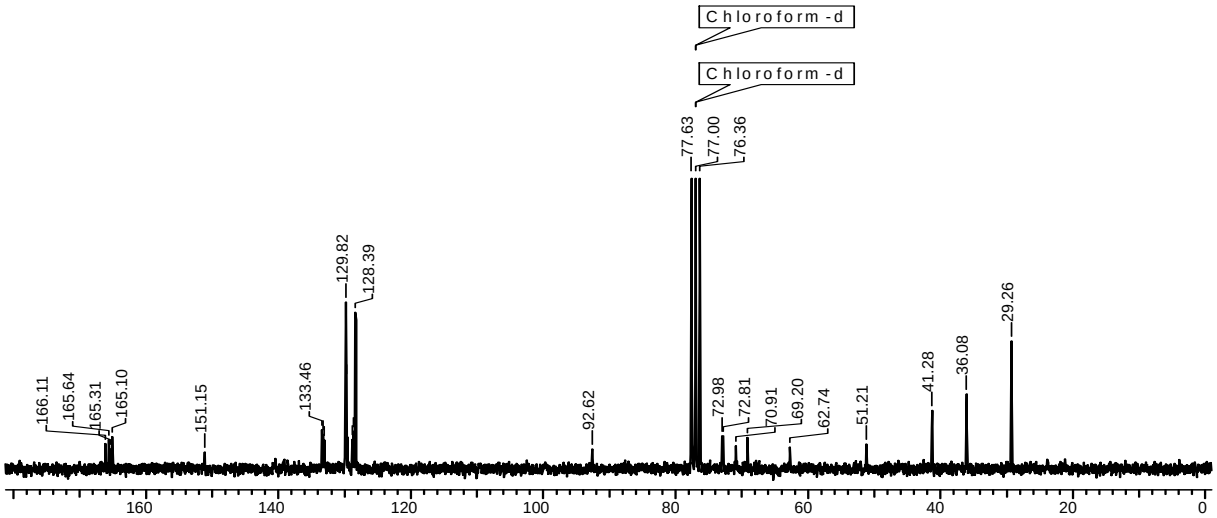
DEPT NMR ($CDCl_3$, 50.32MHz) Spectrum of Compound **26a**



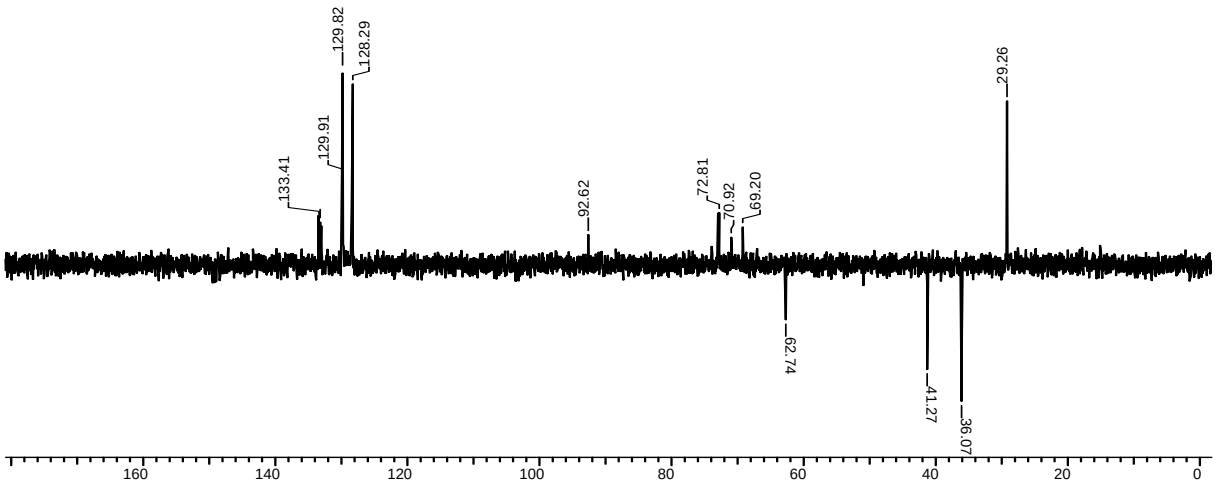
^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **26b**



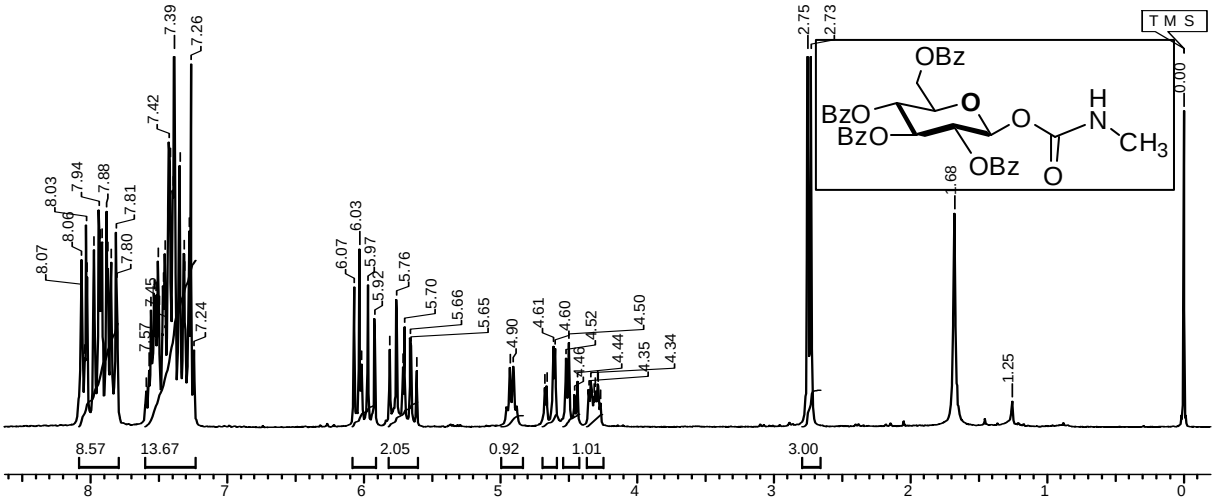
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26b**



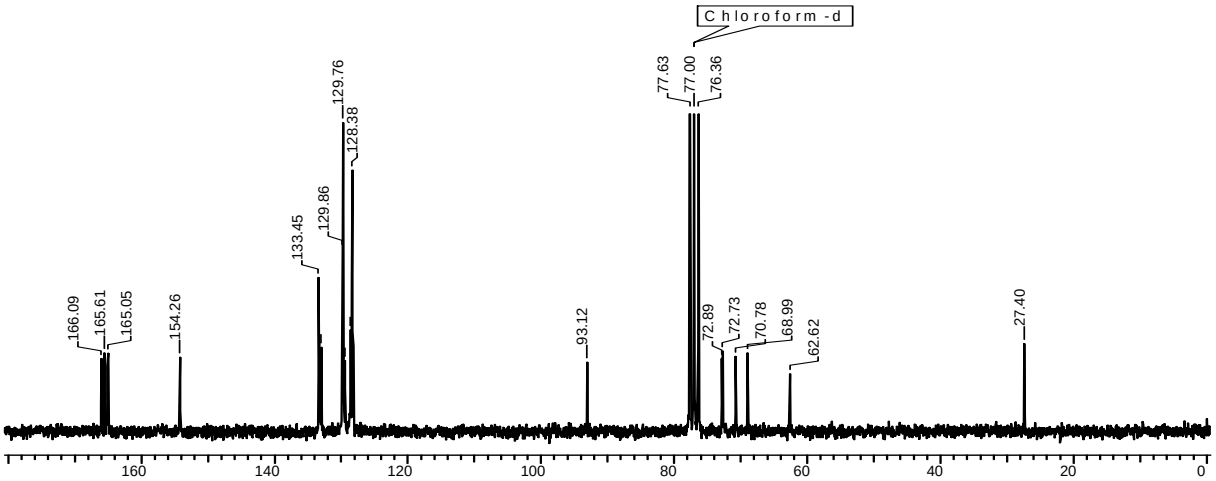
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26b**



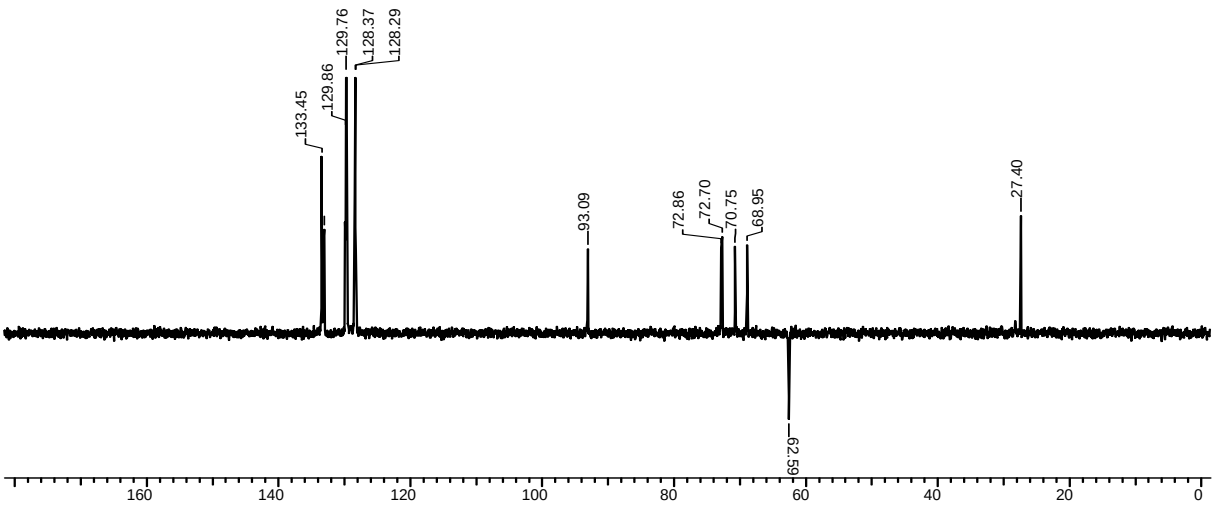
^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **26c**



^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26c**



DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26c**



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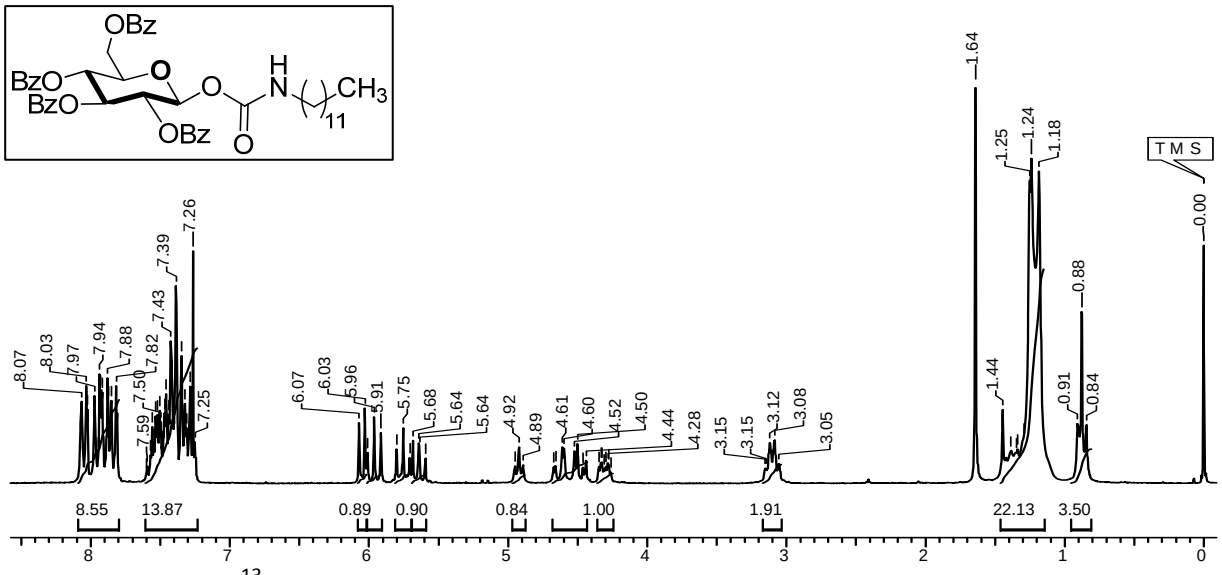

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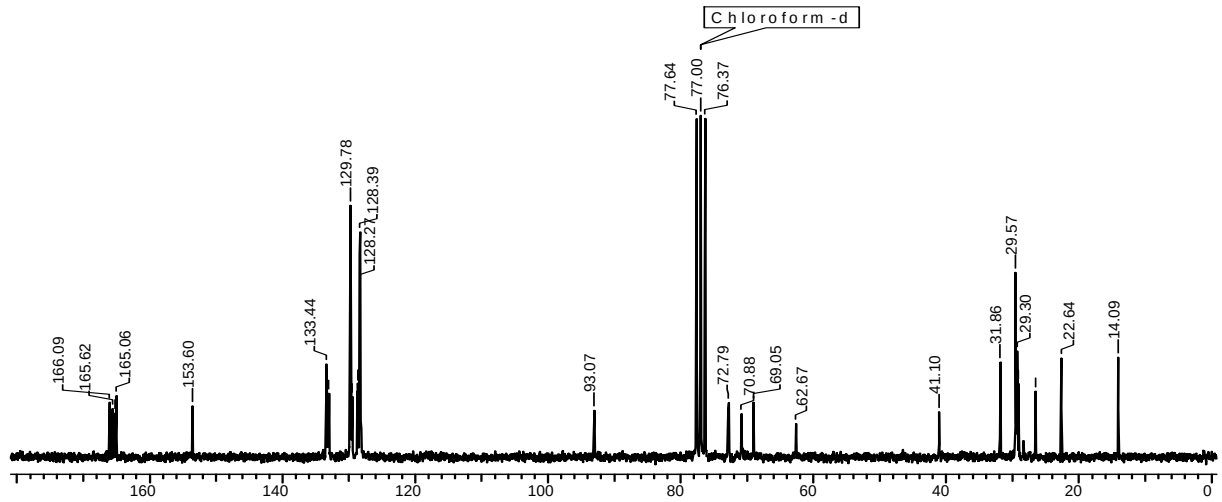
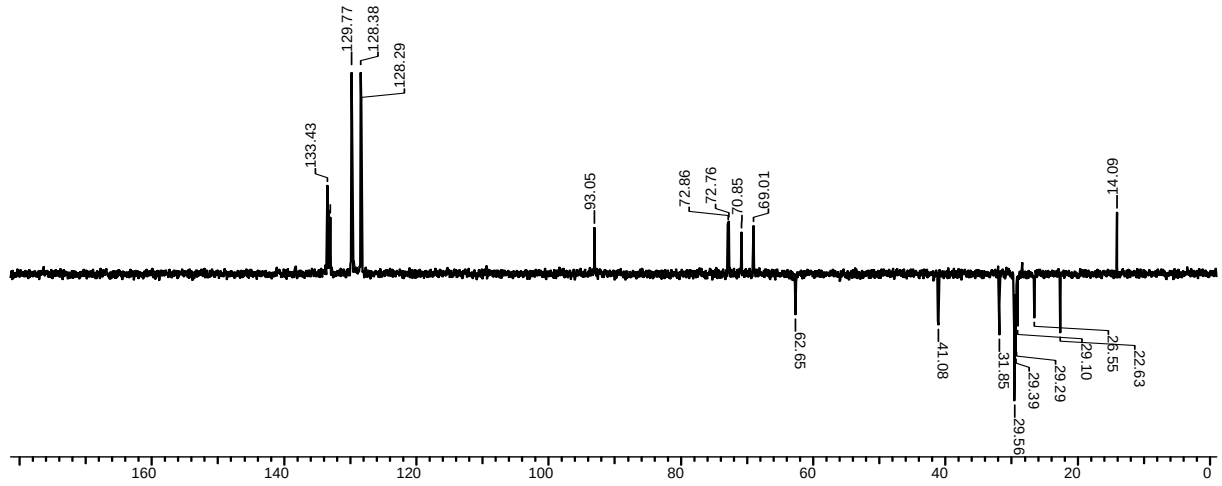


DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26d**



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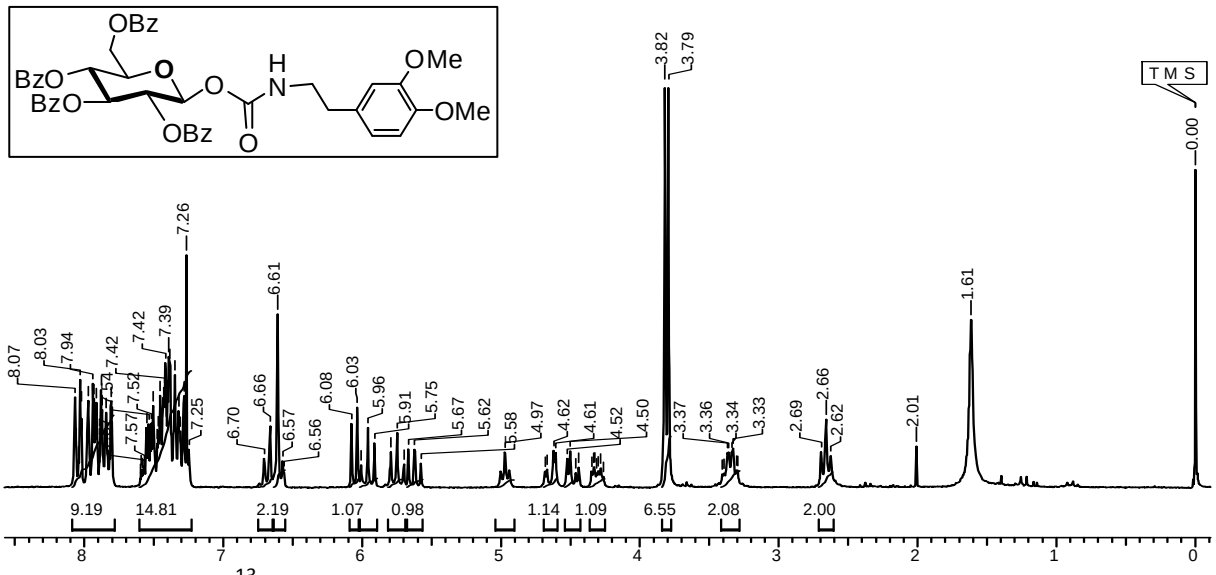
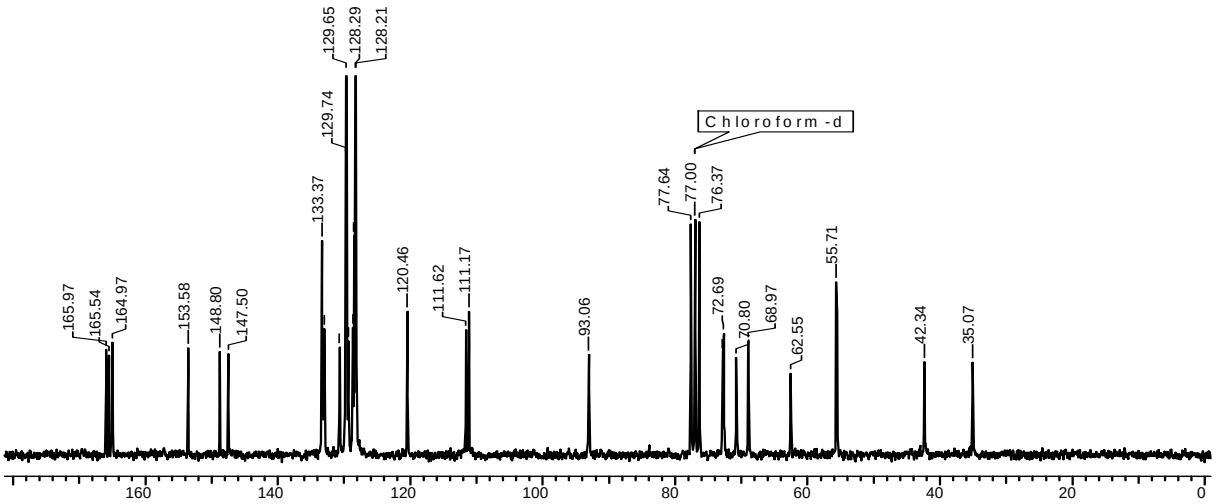
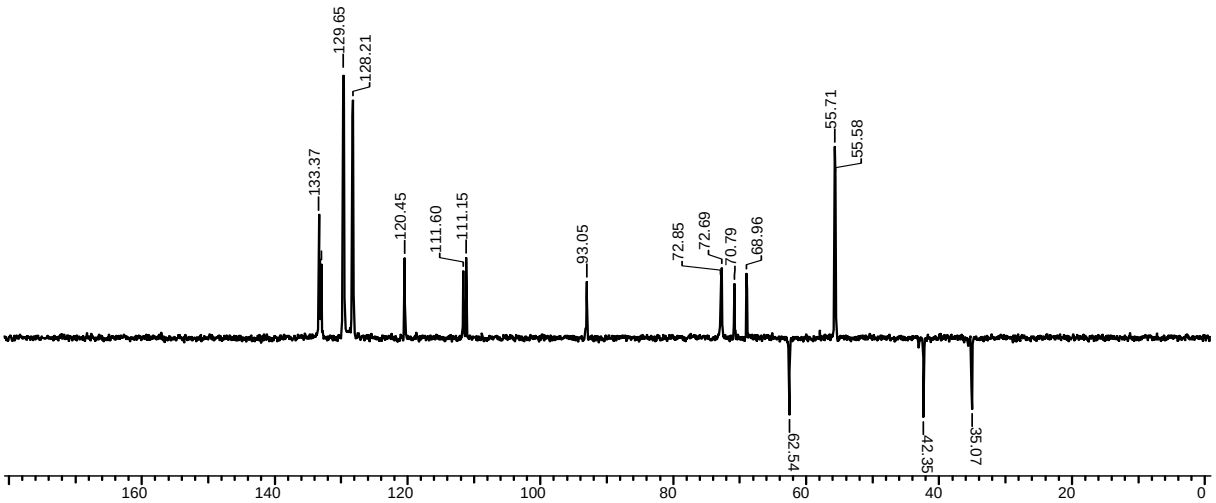


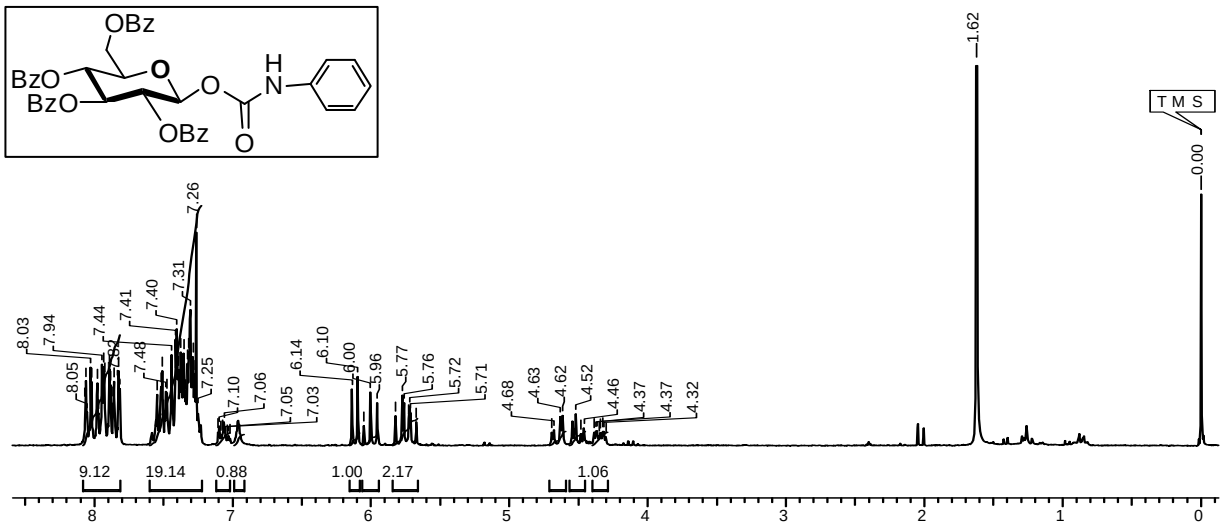
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26e**



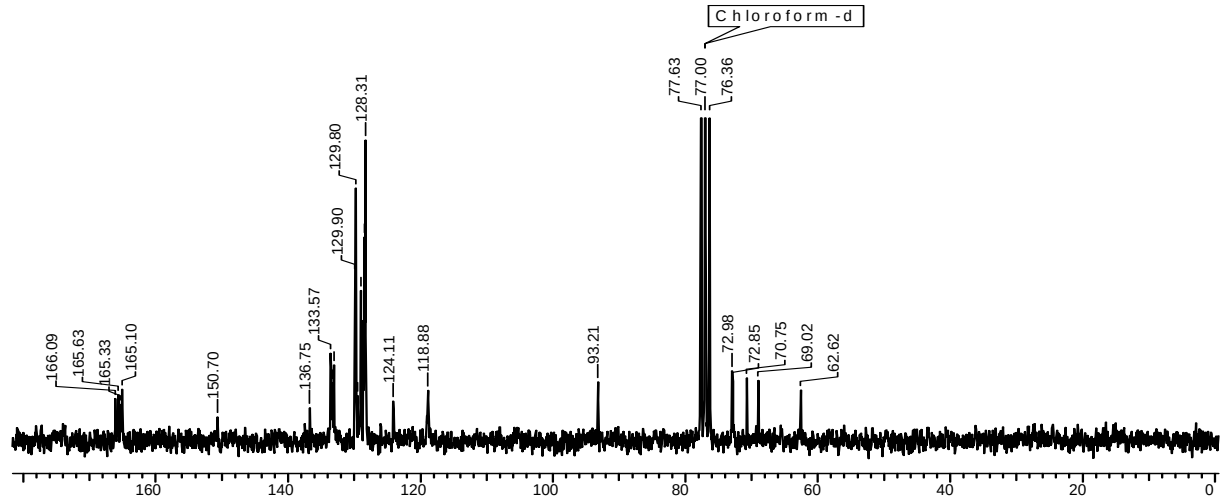
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26e**



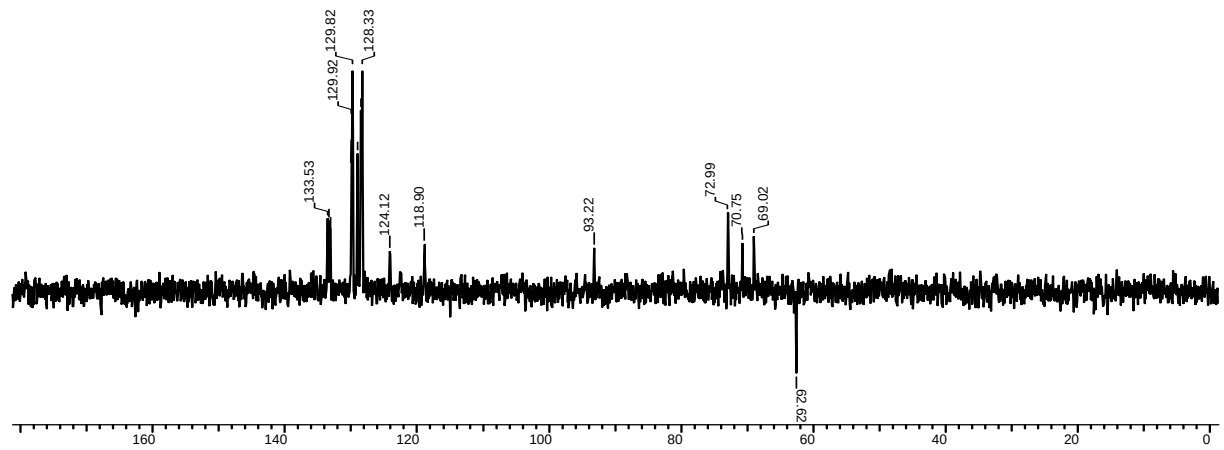
^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **26f**



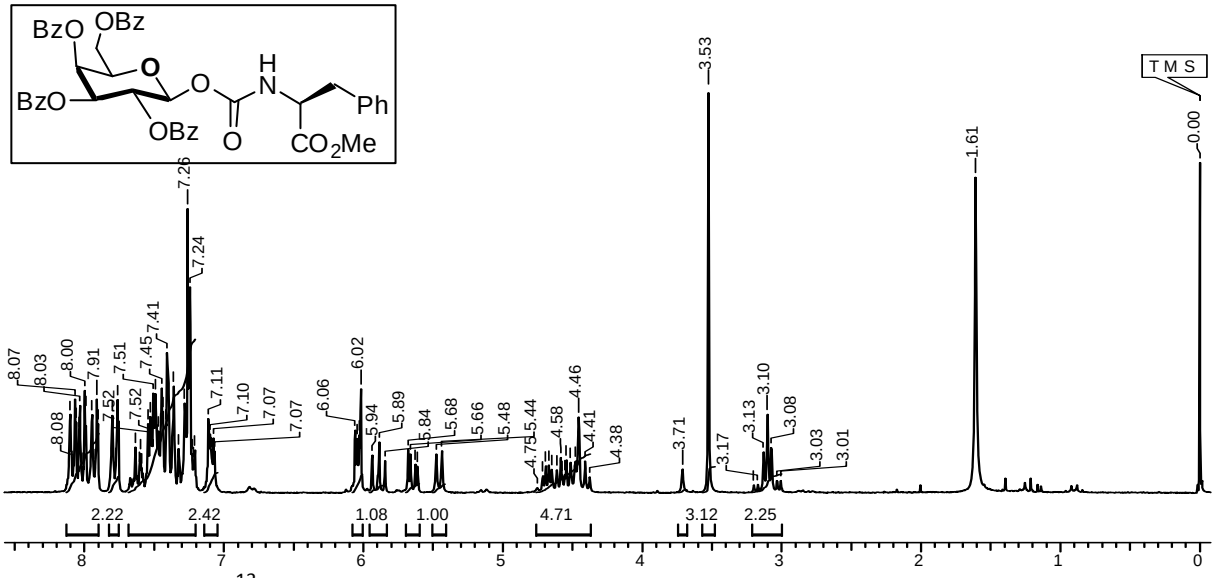
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26f**



DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **26f**



^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **27a**



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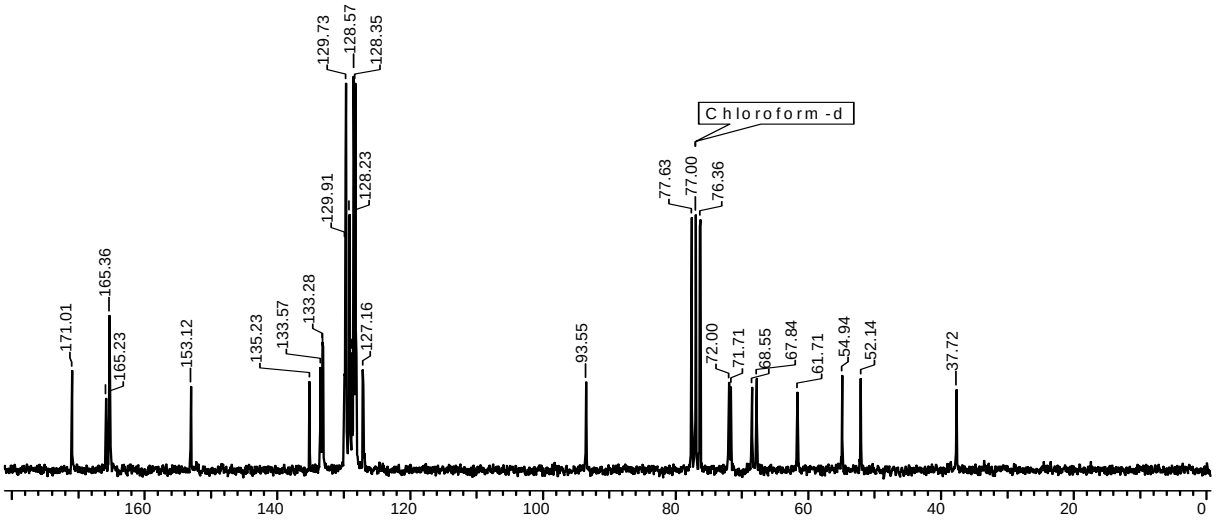
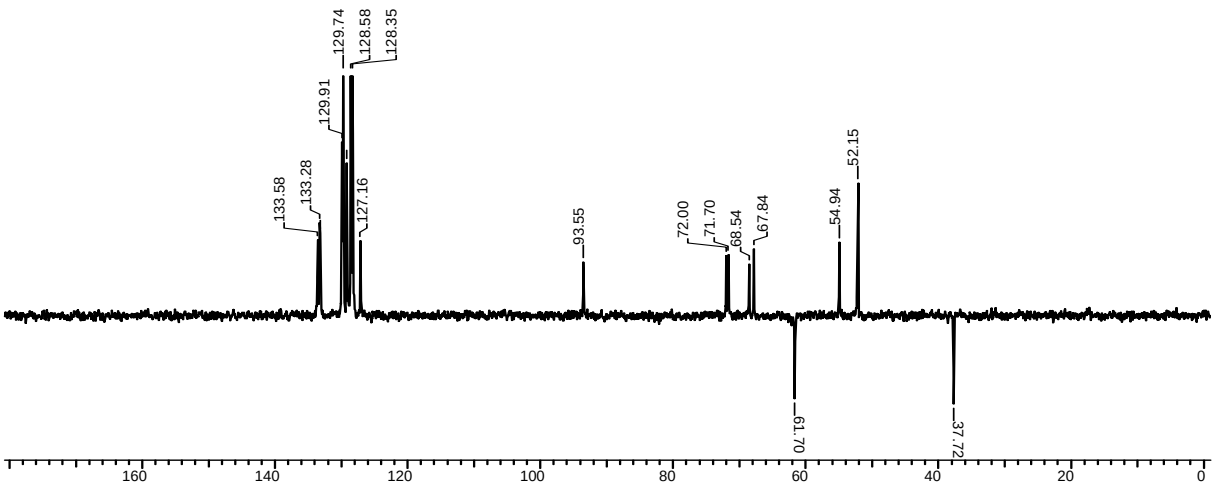
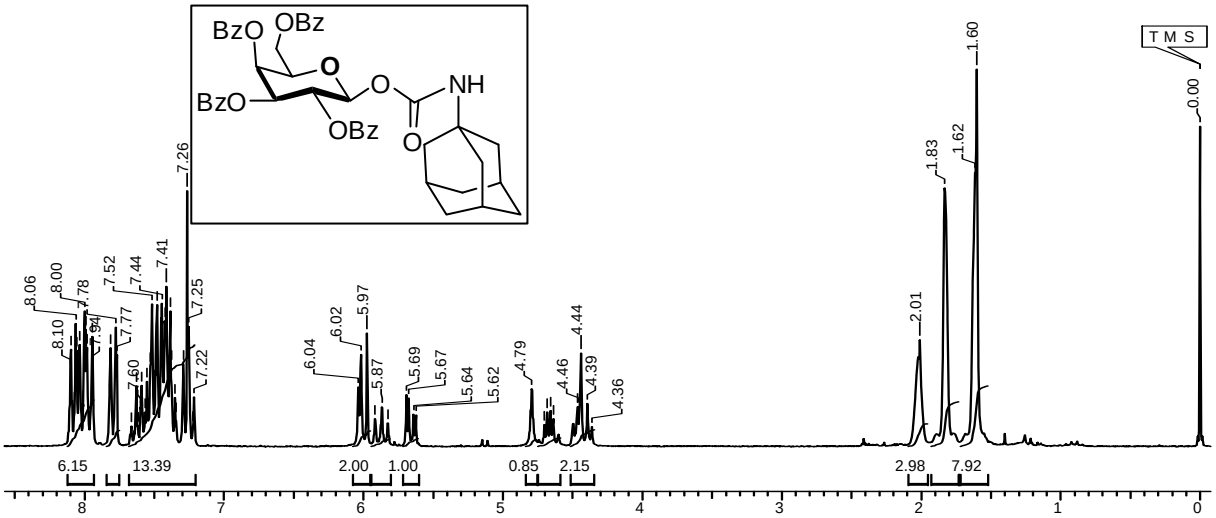


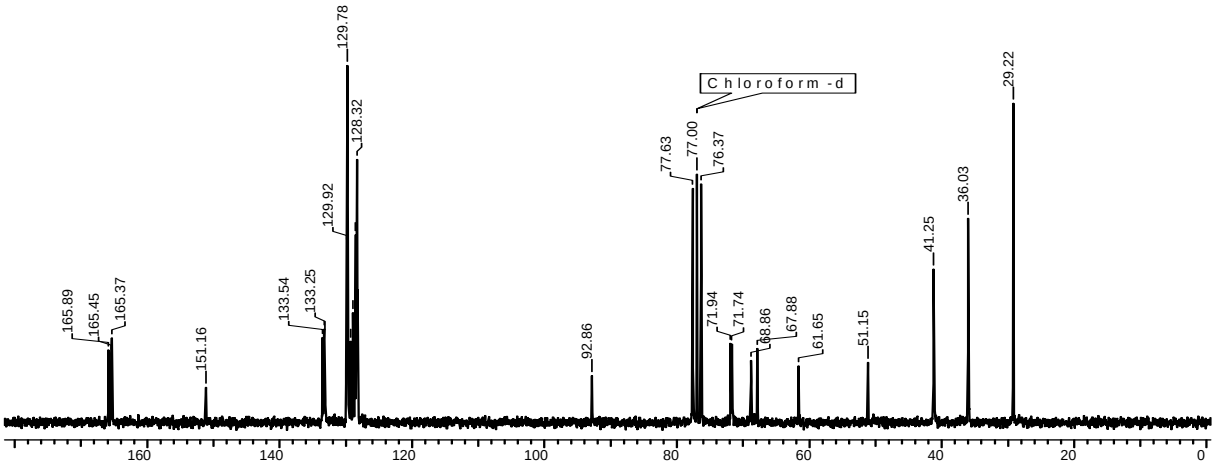
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **27a**



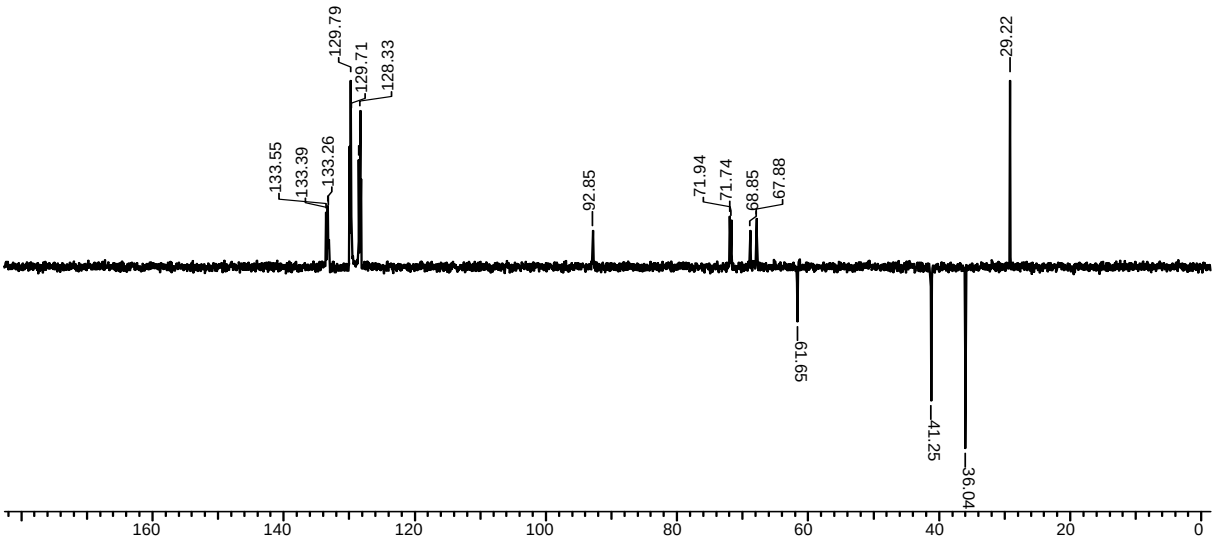
^{1}H NMR ($CDCl_3$, 200.12MHz) Spectrum of Compound **27b**



^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **27b**



DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **27b**



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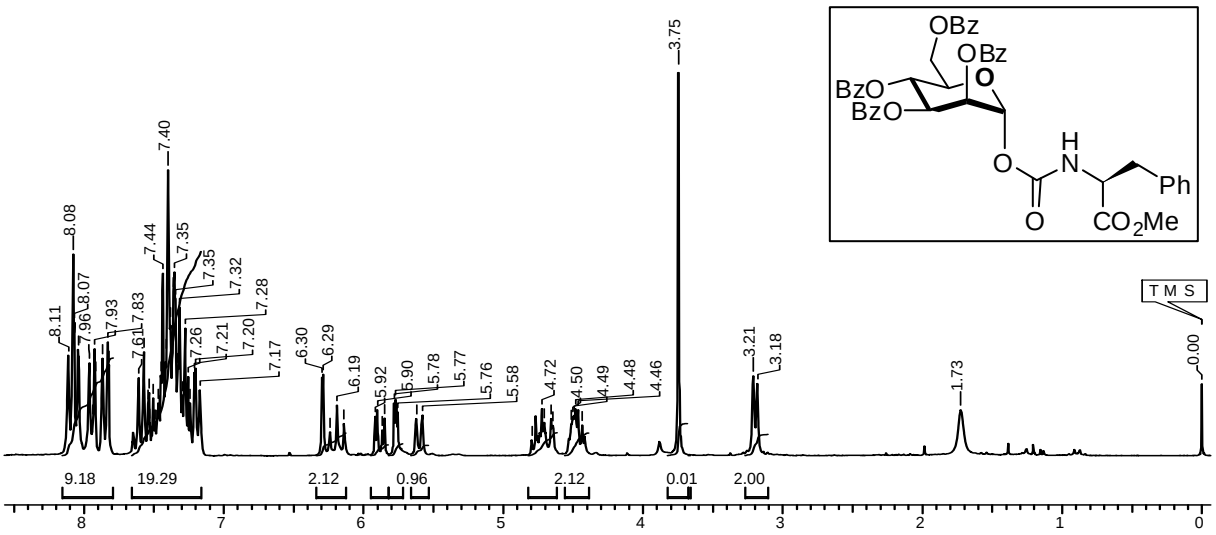

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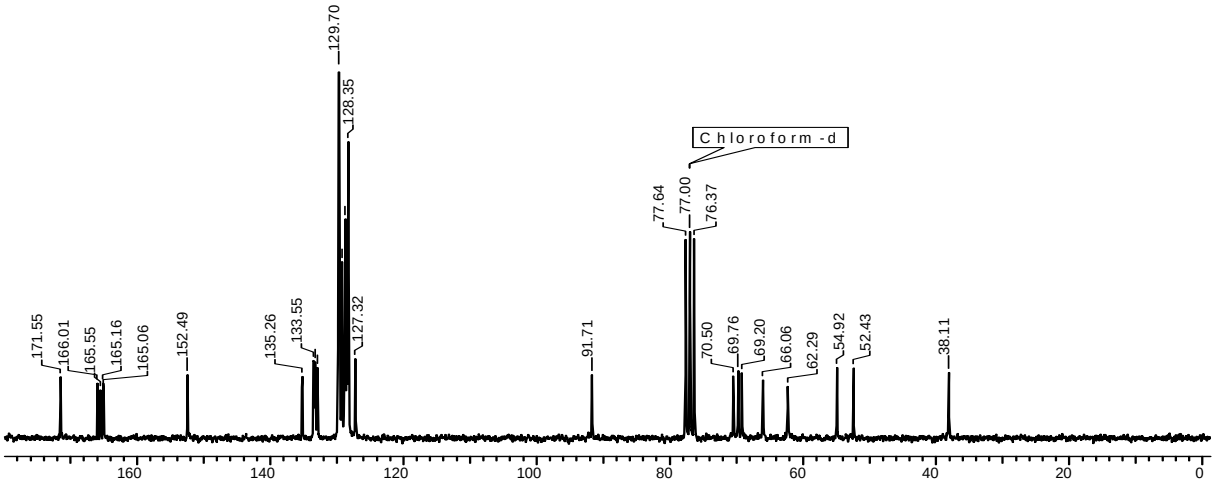
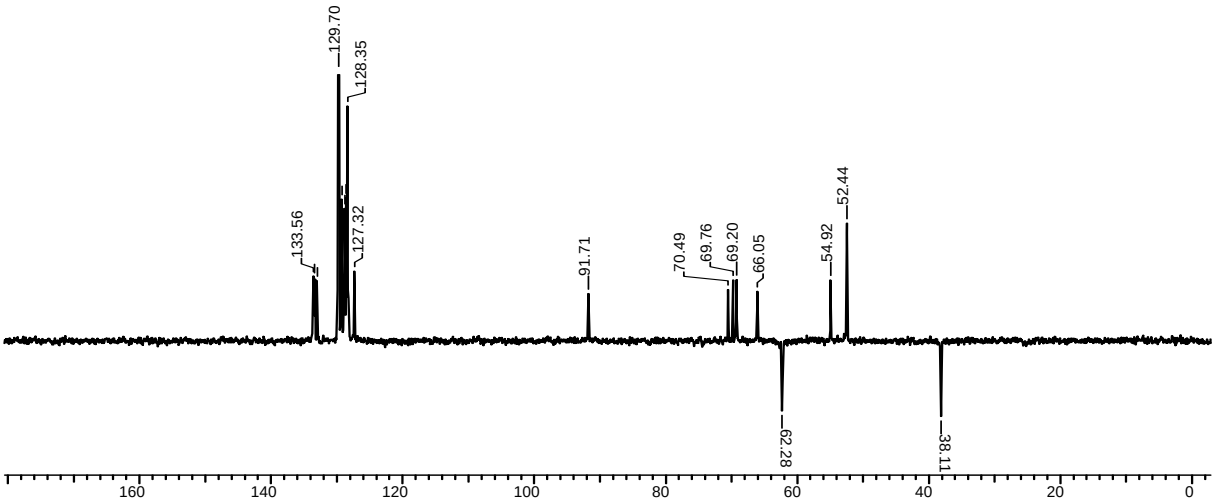


DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **28a**



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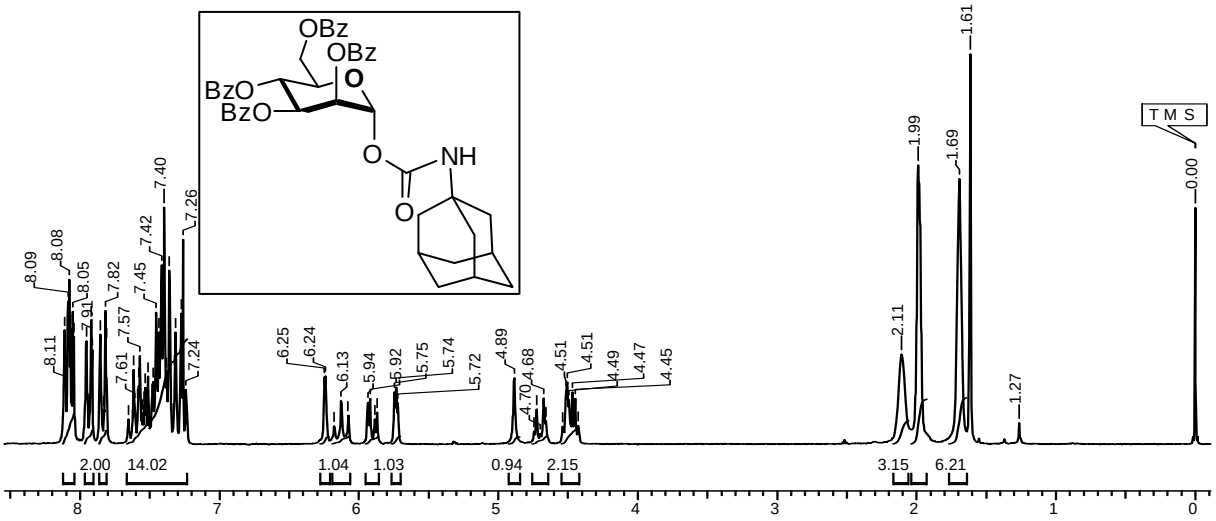

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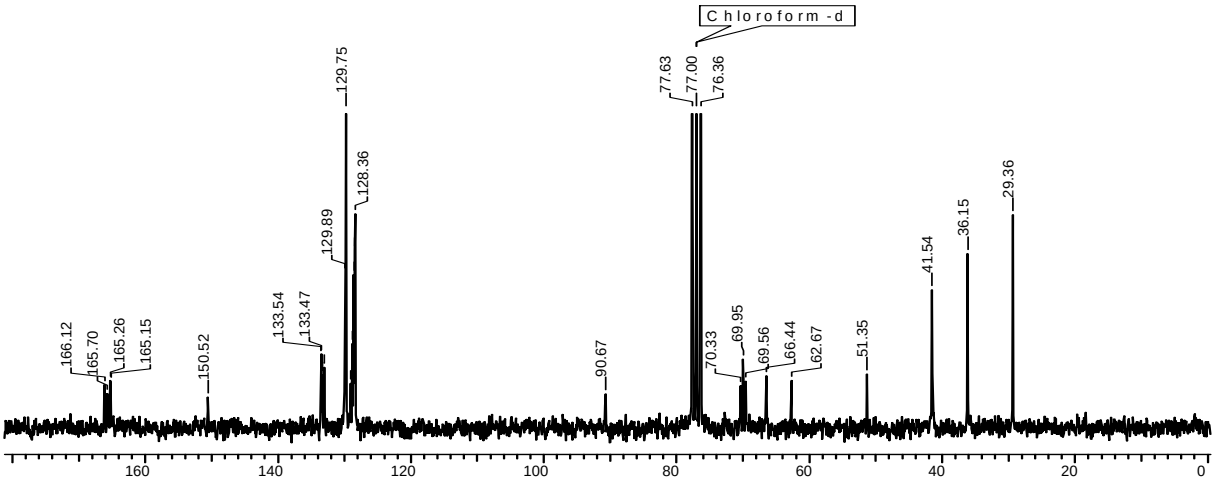
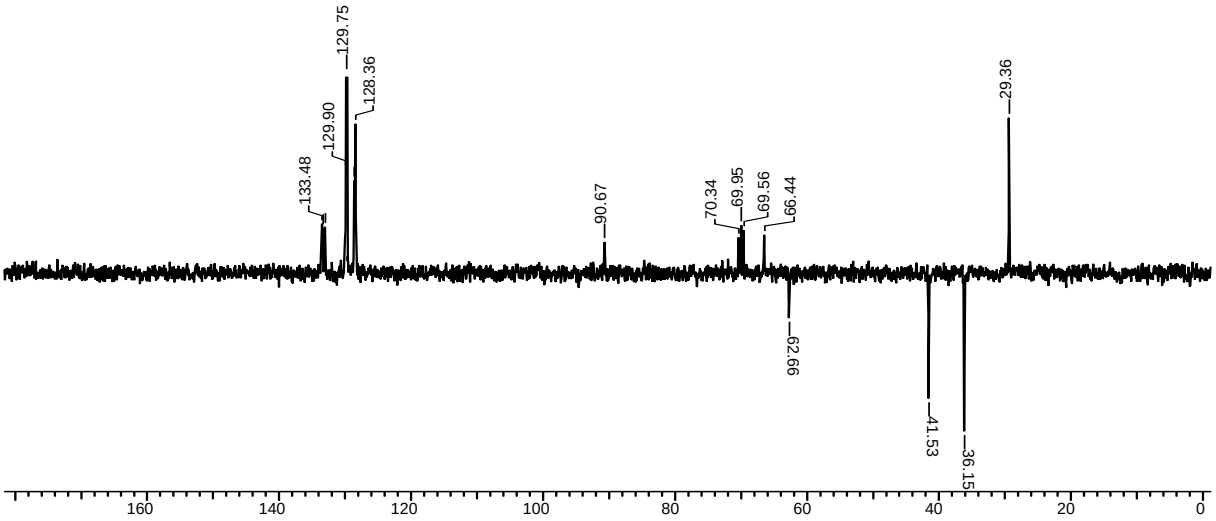


DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **28b**



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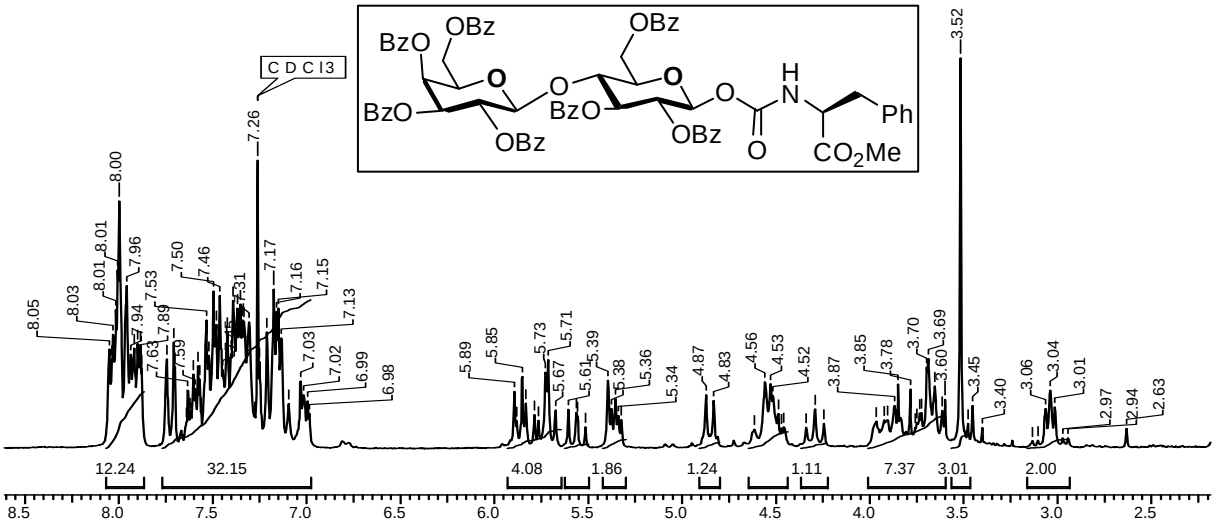

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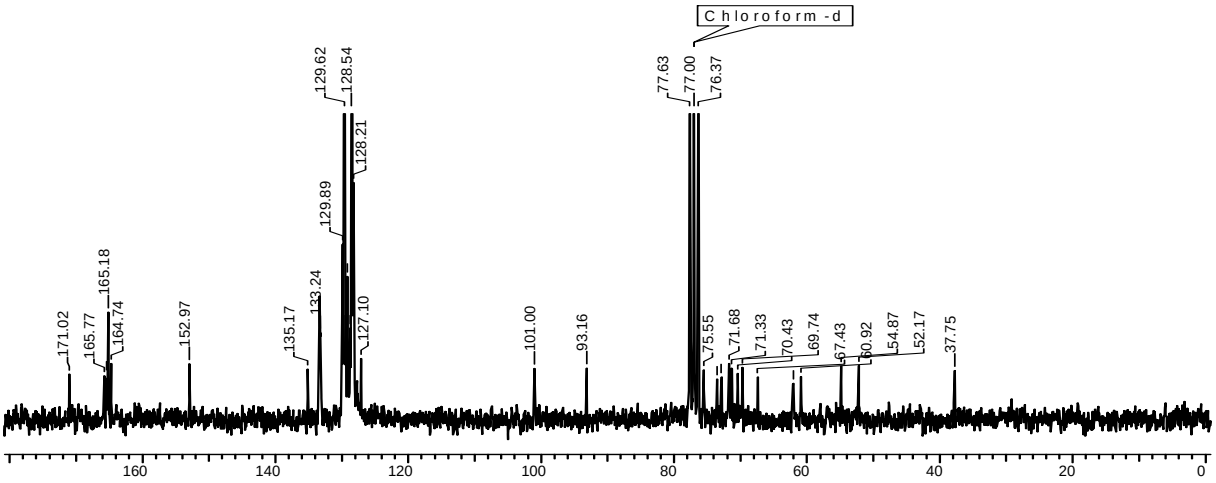
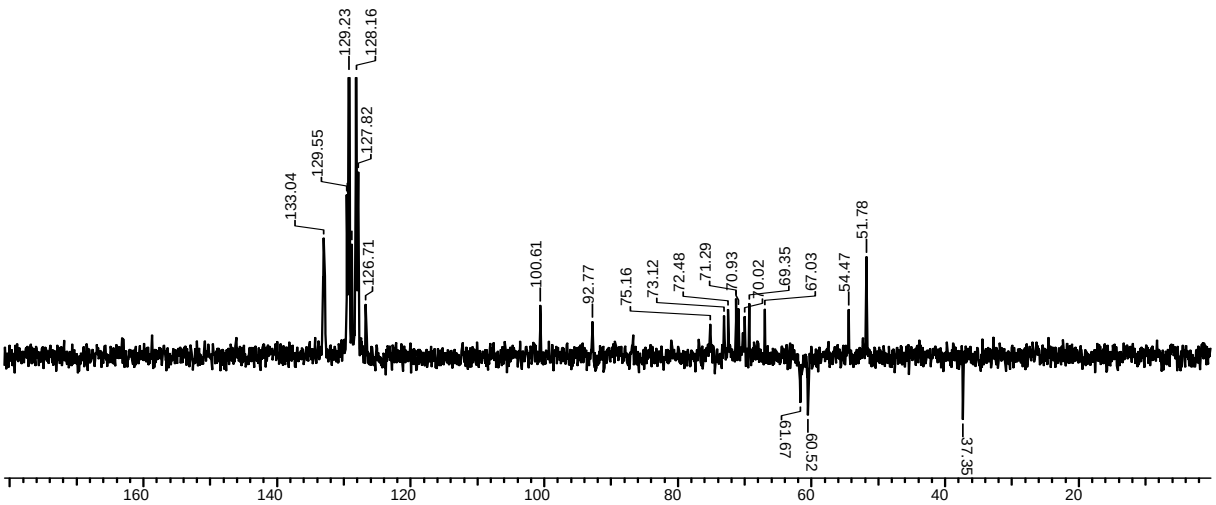


DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **29a**



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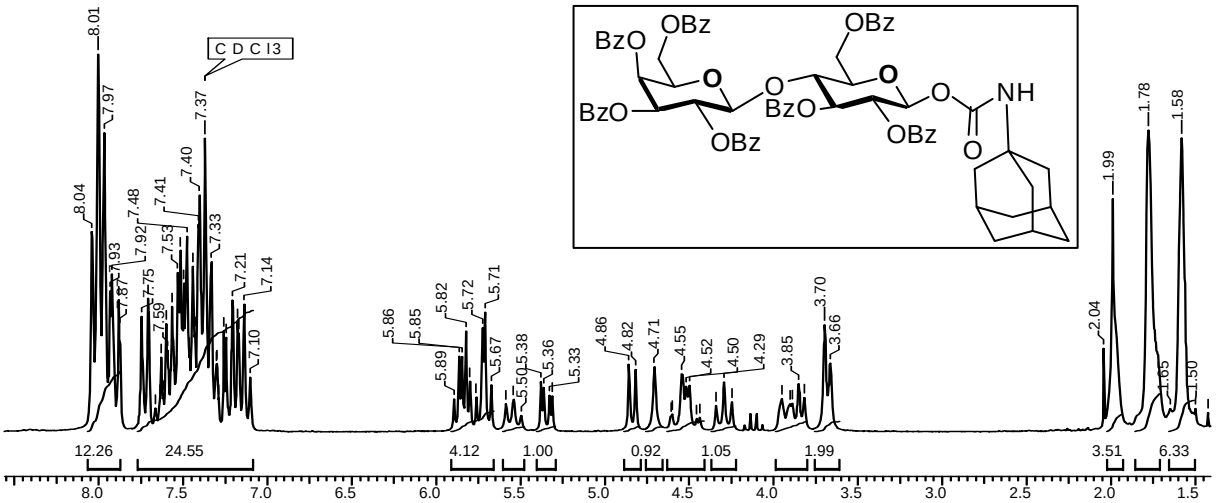

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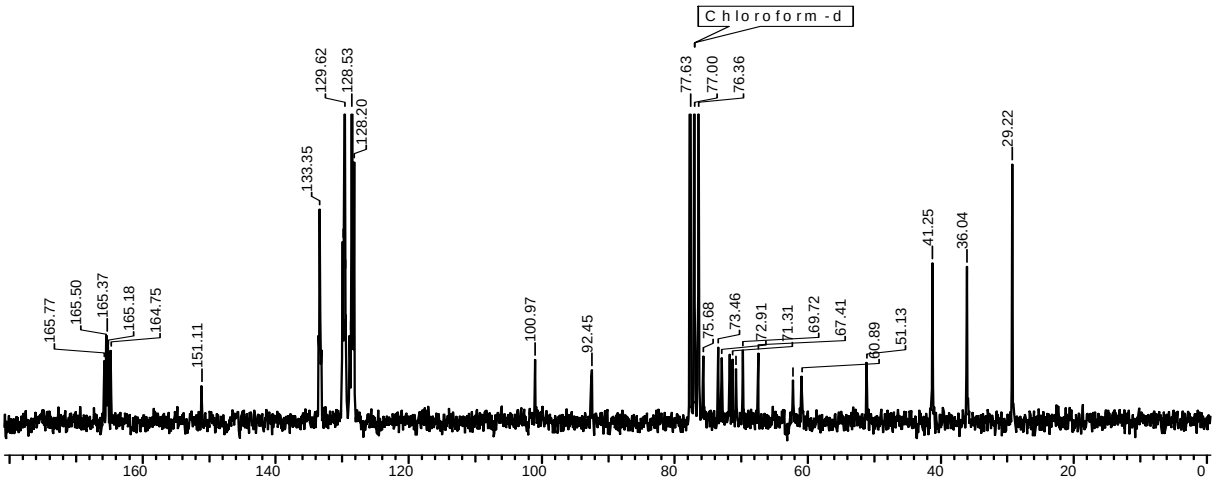
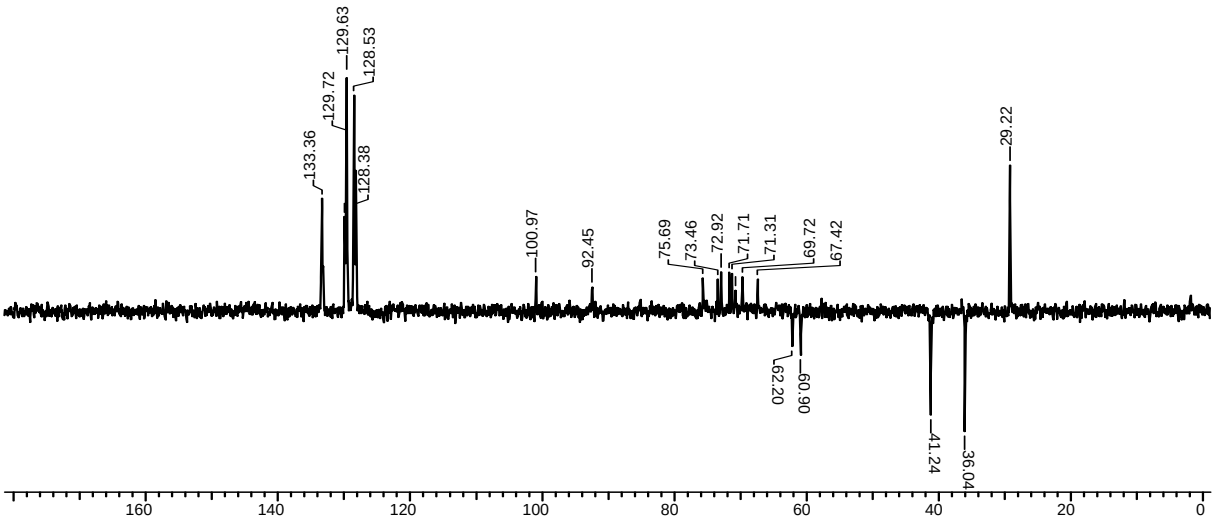


DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **29b**



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CHAPTER 2

Application of Gold Catalyzed Glycosidation: Synthesis of glycopolypeptides & new method for synthesis of controlled azide functionalization of glycopolypeptide

In Nature most of the biological systems produce proteins that possess the ability to self-assemble into complex and higher ordered secondary, tertiary & quaternary structures. Amino acid composition and sequences control these secondary conformations which is characteristic feature of polypeptides. Lot of efforts are going on development of synthetic routes for preparation of these natural polymers as well as de novo designed polypeptides sequences to make products for application in biotechnology (e.g., artificial tissue and implants), biomineralization (e.g. resilient, lightweight and ordered inorganic composite), drug delivery and analysis (e.g. biosensors and medical diagnostics).

To achieve all the above practical applications, it is important that material could self-assemble into precisely defined structures. Peptide-based polymers have many advantages over the conventional synthetic polymers, since they are able to hierarchically self assemble into stable and ordered conformation. Depending on the substituent's on amino acid side chain, polypeptides are able to adopt a multitude of conformationally stable, regular secondary structures (e.g., helices, β-sheet, and turns), tertiary structures (e.g. the β-strands-helix-β-strand unit found in β-barrels), and quaternary assemblies (e.g., collagen microfibrils). The synthesis of polypeptides that can assemble into non-natural structures is an attractive challenge for polymer chemists.

Polymer chemistry has more than 100 years of history starting from synthetic polymers to its numerous applications in different fields. In late 1800 polymer chemist replace the bilayards ball made up of a single elephant's tusks and wood by polymeric material Bakelite. At that time USA announced the prize for who will replace the wood and ivory around 10000 USD but after successful synthesis of synthetic material, people are not given the award prize. Polymer chemistry has changed life of humans and enriched their living style.

Synthetic peptide-based polymers are known for very long time such as homopolymers but have only seen limited use as structural materials. Developments in chemical synthesis made possible the preparation of new and complex glycopolypeptide sequences of controlled molecular weight that display properties far superior to ill-defined homopolypeptides.[1] This kind of polypeptides are useful in polymer assembly and functional domains need to be at length scales ranging from nanometers to micrometers. Synthesis of

simple hydrophilic/hydrophobic hybrid diblock copolymers, when dispersed in water, forms peptide-base micelles and vesicles potentially useful for drug and gene delivery application.[2-3] The regular secondary structures obtainable with the polypeptide block provide opportunities for hierarchical self-assemblies that are unobtainable with typical block copolymer or small-molecule surfactants.

Long chain peptides can be made either by classical peptide coupling reaction or by Ring Opening Polymerization (ROP) of *N*-carboxyanhydrides.[4] The problem in classical polypeptide synthesis is that as peptide length increases, the coupling efficiency goes down and yields would be less because of folding. Advantage of peptide coupling is good control over the sequences of amino acid which is not possible in ROPs of NCAs. Solid phase peptide synthesis solved these problems little not completely because it is not useful or practical to go beyond peptide length 100 units. ROPs can give access to polypeptide of length more than 100 amino acid residues but we don't have choice for selection of individual amino acid in sequence. It only gives homopolypeptides or block copolypeptides.

N-carboxyanhydride chemistry is around 100 years old. Between 1906 and 1908, three successive papers were published by the Hermann Leuchs et al. describing the synthesis and properties of the α-amino acid *N*-carboxyanhydrides (Leuchs anhydrides).[5-7] Leuchs discovered the NCAs by coincidence when he attempted to purify *N*-ethoxycarbonyl or *N*-methoxycarbonyl amino acid chlorides by distillation (Figure 1). Hermann Leuchs was mentored by Emil Fischer very influential organic chemist in Germany at that time and completed his thesis in 1902.[8]

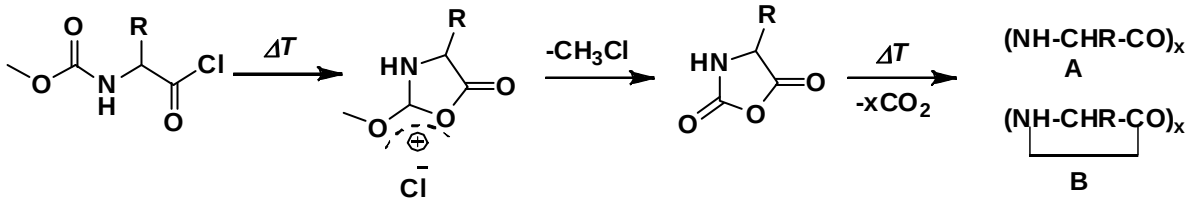


Figure 1: First report on synthesis of *N*-Carboxyanhydride and Polypeptide

At first polymerization products of NCAs are called the "anhydrides of amino acids", meaning a dehydrated form of amino acids and gave formula **A**. However in his third paper in 1907, Leuchs et al concluded, the amorphous character and from the lack of a melting point (in contrast to crystalline cyclic dipeptides), that his products were polymeric

modification of α-lactams and depicted the formula **B** (Figure 1). Leuchs abandoned the work on NCAs after 1907 for the following reasons:

1) Peptide chemistry was the long-term research area of his former supervisor Emil Fischer

2) The polypeptides resulting from the water or alcohol-initiated polymerizations of NCAs proved to be insoluble in common inert solvents and were difficult to characterize with the analytical methods available around 1910

3) Ring-Opening Polymerizations (ROPs) yield high-molar-mass polymers were outside the experimental and mental scope of almost all chemist at that time, with exception of Hermann Staudinger

Almost no chemist believed in the existence of polymers with a covalent backbone and molecular weights around or above 10000 Da. Emil Fischer also denied the existence of biopolymers and synthetic polymers with molar masses above 6000 Da. The "career" of the NCAs as monomer for the preparation of (poly)peptides by ROP continued after 1921 with the publication of Curtius et al.[9-11] and Wessely et al.,[12-19] who used water, alcohol or primary amine as initiator and assumed for the first time that the reaction products of NCAs are high-molar-mass polypeptides. In the years between 1920 and 1930 Staudinger concepts of macromolecules became fully acknowledged by the international scientific community and he was the first who coined the term "Macromolecules". At that time only the viscosity measurement was toll to check the molecular weights of polymers, so what he did was the hydrogenation of natural rubber because it contains isoprene unit. Chemists at that time believed that natural rubber is formed by the electrostatic interaction of double present in each unit. If the double present in natural rubber was removed by

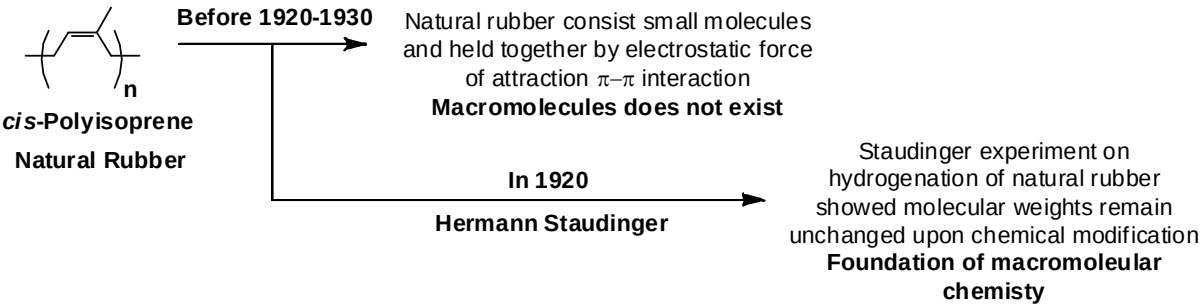
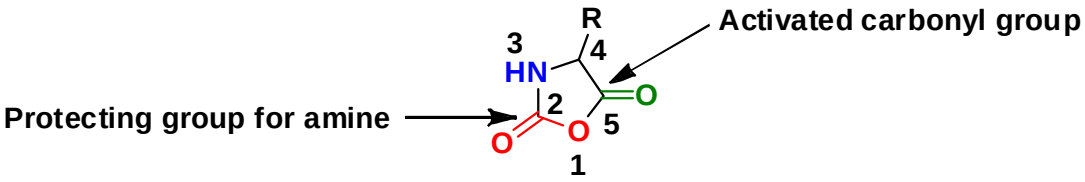


Figure 2: Experimental evidence for existance of high molecular weigh polymers

hydrogenations then there will no more interactions and molecules will be separated. Staudinger first time showed that even after the hydrogenation of the natural rubber there is no change in their physical properties such as viscosity which is almost similar to the natural rubber without hydrogenation. So if they are held by electrostatic interaction between the double bond then there will be drastic change in their viscosity after hydrogenation (Figure 2).[20]



As early as 1926, Staudinger emphasized the significance of macromolecules for biochemistry and biology. Although NCAs were most widely used as monomer for the preparation of polyamino acid through ROP, they also proved to be useful as synthons for stepwise synthesis of oligopeptides and complex polypeptides. NCAs have the advantage that their ring structure combines activation of CO group C5 with protection of the amino group. Their characteristic disadvantage is a high sensitivity to moisture and a low stability upon storage. However, a team of American Merck Company headed by Denkewalter and Hirschmann had succeeded to prepare the enzyme ribonuclease-S exclusively by stepwise

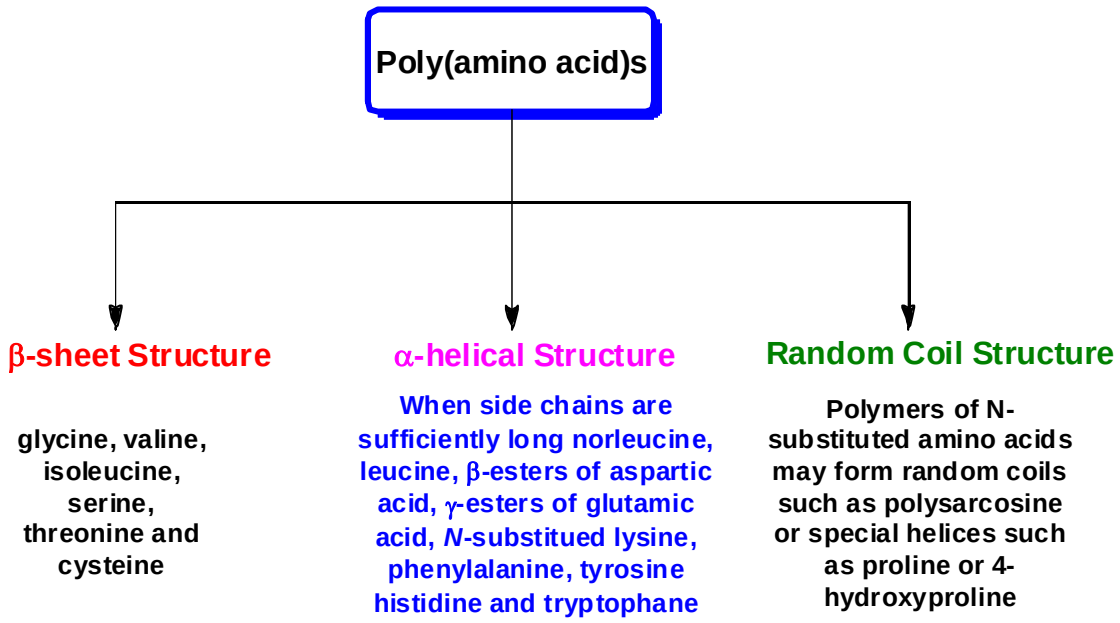


Figure 3: Tentative classification of amino acid upon forming oligomer to primary and secondary conformation

syntheses with NCAs.[21-27]

In recent years, homo and copoly(amio acid)s obtained by ROP of NCAs became important as models of natural polypeptides and proteins with regard to relationships between primary and secondary structure. Based on some of the experimental studies such as IR spectroscopy and wide-angle X-ray scattering (WAXS) or small-angle X-ray scattering (SAXS) subdivided the poly(amino acid) into three classes (Figure 3).[28-29]

Important observation and understanding of ROPs of NCAs was the finding that the oligomers of all the helicogenic amino acids adopt the β-sheet structure when the degree of polymerization (P_n) is below 10±1 (depending on solvent and side chain).[30-33] Synthetic polypeptides prepared from NCAs also played key role in the elucidation of the relationship between the primary structure of natural peptide and their immunogenic reaction.[34-37] Polymers of *N*-alkylamino acid showed negligible immunogenic properties. The presence of H-bond forming peptide groups is important. Poly(γ-benzyl-L-glutamate) (poly(γ-Bzl-L-Glu) was the first polymer that was found to form a liquid-crystalline (LC) phase.[38] Elliot and Ambrose reported that concentrating a solution of poly(γ-Bzl-L-Glu) in helicogenic solvents results in a birefringent phase with a high optical rotation.

N-carboxyanhydrides can be opened by two different ways 1) Anionic polymerization and 2) Ring Opening Polymerization (Living Polymerization) (Figure 4). Living polymer term was coined by M. Swarc in connection with anionic polymers of vinyl monomers. Living polymers combine two properties of polymers, first the reactive end group responsible for the chain growth remains unchanged (alive) when the polymerization stops owing to either 100% conversion of monomers, cooling or precipitation. Second, the molecular weight distribution (MWD) is narrow with polydispersities (PDs) below 1.2 or better below 1.1 (the PD is defined as the quotient of weight-average molecular weight, M_w and number average of the molecular weight, M_n). Polypeptide with a living end group such as primary amine could be obtained from ROPs initiated by protic nucleophiles such as water, alcohol, or by primary amines.[4] Low PD can be obtained when the initiation step is faster than the growing step, and this requirement is only met by sterically unhindered primary aliphatic amines.[39-42] This is one of the reasons why primary amine initiated ROPs are most widely used approach to the preparation of polypeptides from NCAs. The second reason is one can easily control the

degree of polymerization (P_n and thus M_n) by variation of the feed ratio NCA/amine (i.e monomer/initator ratio, M/I). *n*-hexylamine and benzyl amines are frequently used amines.

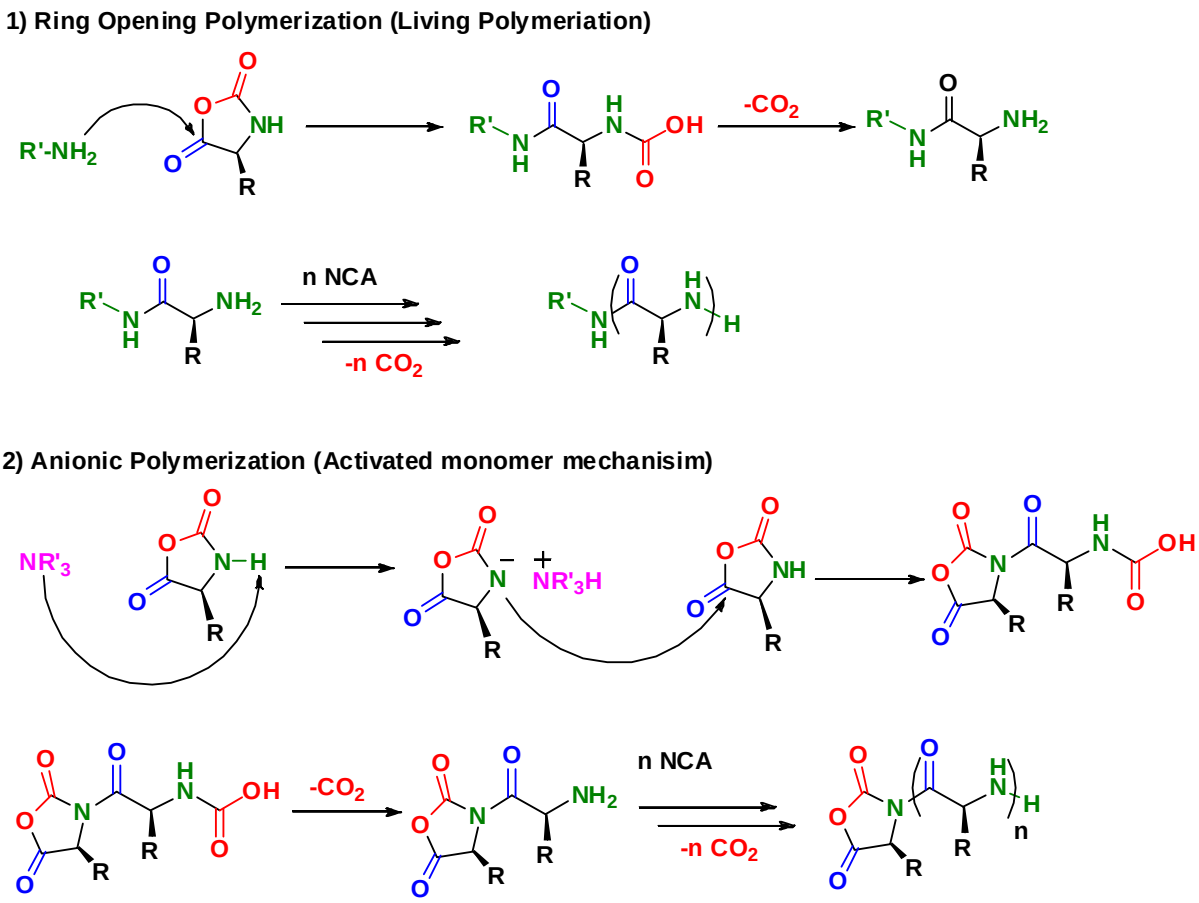


Figure 4: Mechanisim of ROP by two different reaction pathways

The third reason is the introduction of functional end group to the CO chain end of the polypeptides.

Low PD does not depend only on the reactivity of the initiator, but on the solubility and secondary structure of the growing peptide chains are also important. Generally the high PD are obtained when the rapid precipitation occurred of oligo- and polypeptides from the reaction medium. MALDI-TOF technique is used to determine high or low PD. Here the MALDI-TOF spectra are not designed for exact mass determination of the molecular weights or MWDs.[39-40] However the insolubility of most of the homopolypeptides in inert solvents prevents reliable characterisation with gel permeation chromatography. So the mass spectroscopy tool allows determination of a qualitative picture of the frequency distribution (Figure 5).

Narrow distributions are only obtained when the propagation rates of all growing chains are identical. The back-folding of growing chains on the surface of β-sheet lamellae also creates a serious problem for the preparation of high-molar-mass polypeptides. In such case the

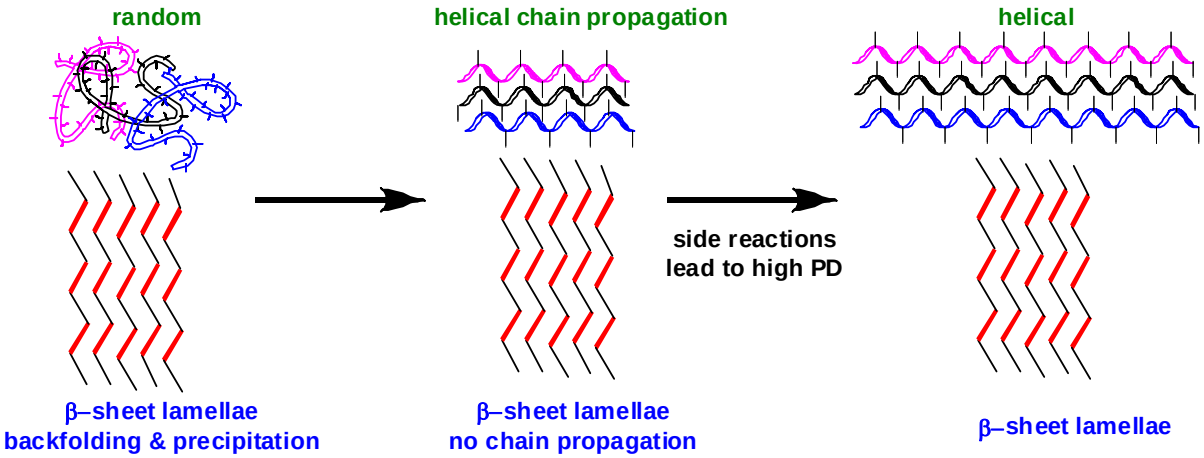
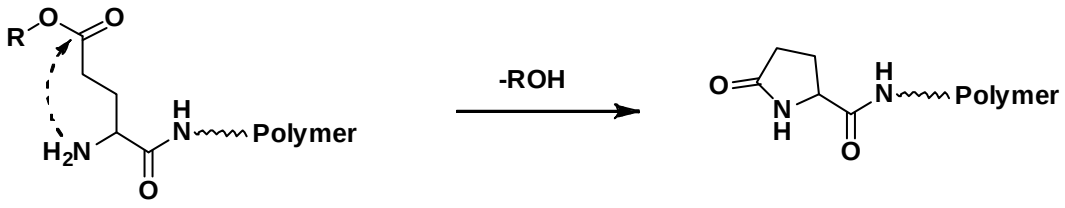


Figure 5: Effect of secondary conformation on the chain growth of polypeptide formation

chain growth stops before all monomers were consumed causes the physical death of polymerization. Because of all these factors high-molar-mass polymers (M_n>20 000 Da) of exclusively β-sheet forming amino acids have never been achieved. Long chains with low PDs can only be obtained from soluble and mostly helix-forming peptides. Therefore, more than 90% of all recent publications dealing with polypeptide derived from NCAs concentrate on Poly(γ-alkyl-Glu) and pol(Nε-Z-Lys).[4]

Recent observation by Kanazawa et al showed primary amine initiated polymerization of NCAs in solid state.[43-45] NCA crystals are suspended in *n*-hexane and polymerization was initiated by *n*-butylamine at 20-30°C. Reaction was monitored by the influence of amino acids on the crystal lattice using X-ray analysis and the secondary structures of the resulting polypeptide was examined by IR method. However this solid state polymerization does not results in low PDs.



Intramolecular termination step that is typical for poly(γ-O-alky-L-glutamate)

There is one more problem in ROP of NCAs initiated by primary amine, the side reaction of the amino group at end is intramolecular termination step, which is characteristic for polyglutamates.[42] It is possible to achieve true living polymerization by carrying out the reaction at low temperature as in the case of Nε-TFA-L-Lys-NCA. When side reactions are absent and the polypeptides are isolated in solid state, the reactive amino end group can survive for decades.[39]

Recent progress in elimination of these side reactions has been a major breakthrough for the polypeptide materials field. Ring opening polymerization by primary amine as initiator gives easy access to polypeptides and also by changing the end group of the initiator such as azide or alkyne to get end functionalized polypeptides which can be coupled by very efficient 'click' reaction. These end functionalized polypeptides have very wide range of applications in biology as well as in material science. Also one can synthesize self-assembled structures from modified block copolymer with one block helical and other with random coil, which can be used in drug delivery and surface modification giving access to targeted drug delivery.

Glycopolypeptide:-

Remarkable advances in separation and analysis techniques of complicated components reveal the molecular level understanding of functions played by naturally occurring glycoconjugates such as glycoproteins and glycolipids in animals and plants. Especially the sugar portion of these glycoconjugates have been found to play essential roles as recognition sites between cells or as factors of controlling the generation of biological functions.[46-48] Most naturally occurring glycoconjugates are of complicated structure, and biochemical studies seem to be mainly directed towards structural analyses and their relation to the biological functions of these complex glycoconjugates.

The definition of glycopolymer has not yet clearly been established. In a very broad sense, glycopolymers may include chemically modified natural polymers, such as cellulose and chitin grafted to synthetic polymers.[49-50] On the other hand, in narrower sense glycopolymers refer to synthetic polymers containing sugar moieties which act as specific biological role similar to those of naturally occurring glycoconjugates.[51-53]

In most cases, the terminal sugar units of oligosaccharides chains of natural glycoconjugates play a decisive role in molecular recognition processes in which the oligosaccharides chain participate. Therefore, from the standpoint of polymer material science, it is fundamentally important to develop suitable and simple synthetic methods to incorporate key sugar units necessary for molecular recognition to polymers, without affecting materials properties inherent to the polymer. One of the most important features of glycopolymers lies in their capability of specifically interacting with lectins and cells depending on the pendant sugar moieties.[54]

Recent developments in polymerization have made it possible to design macromolecules of controlled structure, which are needed not only to precisely correlate the functions of glycopolymers with their structures on a molecular level, but also to bestow on glycopolymers more sophisticated functions that may surpass those of natural glycoconjugates. There have been published several excellent reviews on glycoconjugates including glycopolymers, glycodendrimers, glycoclusters and so on.[55]

Methodologies for the syntheses of glycopolymers can be roughly classified into two main categories: (1) polymerizations of sugar-bearing monomers[46,56] and (2) chemical

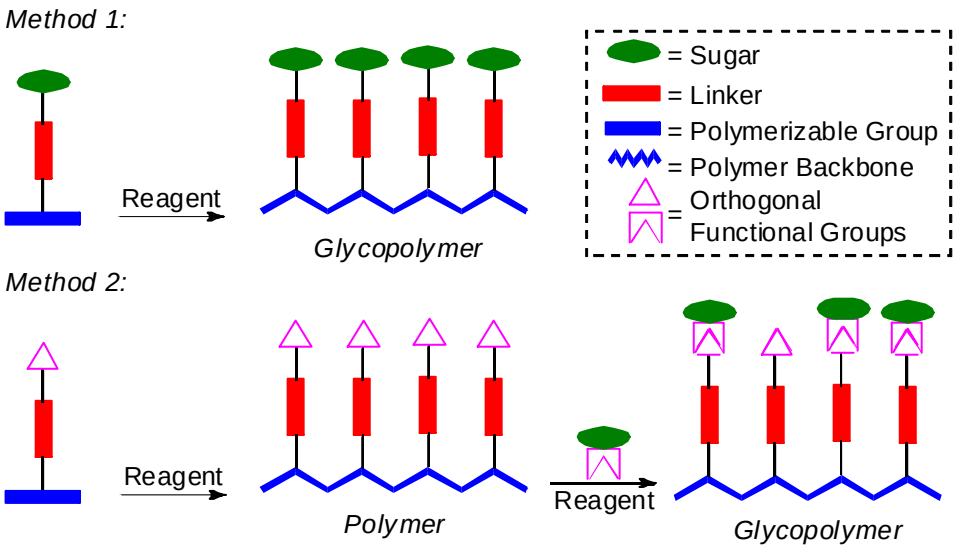


Figure 6:Different approches for syntheis of Glycopolypeptide

modifications of preformed polymers with sugar-containing reagents.[57-59] In general, the latter polymer reaction method is more simple and convenient than the former polymerization method, because the synthesis of sugar-bearing monomers often requires

tedious multi-step reactions. However, the polymer reaction method frequently results in glycopolymers having less regular structures because of incomplete reactions due to steric hindrance. Therefore, it is often better to use polymerizations of sugar-carrying monomers for synthesizing linear glycopolymers of well-defined architecture (Figure 6).

Polymerization of sugar bearing monomer was synthesized by different research groups and their properties are studied. In Nature most of the biological events are carried out by the multivalent interaction of the ligands, with surface receptors and therefore there is need to synthesize polymeric materials which will give rise to multivalent interactions. Glycopolymer synthesized by the radical or some other methods don't have polypeptide backbone; therefore, there may be possibility of some of biologically active carbohydrates being not

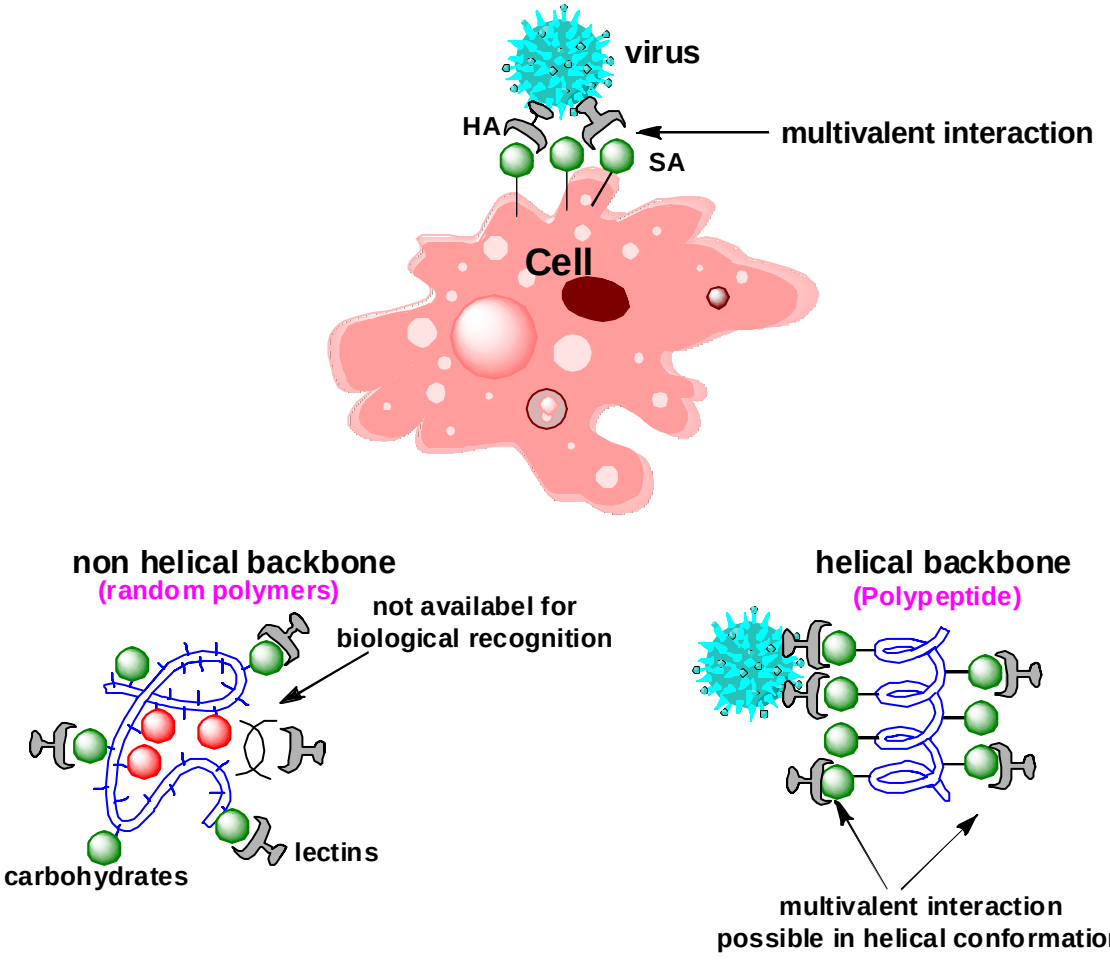


Figure 7: Multivalent interaction with lectin and conformational effect

exposed to surface for binding and encapsulated in such way that they are not available for biological recognition.[58] Similarly such kind of polymers without polypeptide backbone doesn't show any secondary conformation. So there is need to have a good method which

will give access to glycopolymers with polypeptide backbone (glycopolypeptide) which can show secondary conformation (Figure 7).

There are broadly two methods for the synthesis of glycopolypeptide 1) Solid phase synthesis[60] and 2) Ring Opening Polymerization of *N*-carboxyanhydride.[46] Solid phase method is not useful for synthesis of long chain glycopolypeptide and it has limitations which are discussed above. So the best way to synthesize glycopolypeptide is the ROPs of the *N*-carboxyanhydride. It works in two different mechanistic ways? 1) Activated Monomer (AM) and 2) Ring Opening Polymerization by primary amine (ROP). In case of activated monomer type of polymerization is initiated by the deprotonation of NCA, which then acts as nucleophile that initiates chain growth. It is important to note that a given system can switch back and forth between the amine and AM mechanisms many times during a polymerization: a propagation step for one mechanism is a side reaction for the other, and vice versa.

There are only few reports on anionic ring opening polymerization of the carbohydrate modified *N*-carboxyanhydrides. Although the monomer synthesis had been established in 1970s,[61] the polymerization of sugar carrying NCAs had not been reported until Okada et al published on the anionic ring-opening polymerization of acetylated *O*-glycosylated serine NCA (glyco-NCAs).[46] *O*-glycosylated serine moieties of naturally occurring glycoproteins play a significant role in various phenomena. For example, an *N*-acetyl-D-glucosamine (GlcNAc)-linked serine appears to be highly dynamic and responsive to cellular stimuli in fashion analogous to phosphorylation.[62]

Okada et al published papers on the synthesis of glycopolypeptides using ring opening polymerization of glycosylated *N*-carboxyanhydrides. They have synthesized the glycoaminoacid derivatives using glycosyl halide as glycosyl donor and $Hg(CN)_2$ (Helfrich method) as catalyst. Glycosylated serine derivative was transformed into glycosylated *N*-carboxyanhydride by using triphosgene as an acid activator. Anionic ring-opening polymerization was carried out in dichloromethane or acetonitrile at 25°C by using *n*-hexyl amine as an initiator. In contrast to general tendency that anionic polymerization of NCAs carrying a bulky substituent is often accompanied by side reactions such as hydantoic acid formation,[48] glyco-NCAs undergo anionic polymerization without side reaction, irrespective

of the bulky sugar moiety, gave the corresponding polypeptides of narrow molecular weight distribution. Kinetic analysis of the ring-opening polymerization of glyco-NCAs with primary amine as initiators reveals that the rate of initiation is much faster than the rate of propagation.

Spectroscopic analysis of glyco-NCAs showed the existence of the hydrogen bonding between the hydrogen of the ring nitrogen and the oxygen atom of the acetyl group at either O(2) or O(6) of the sugar residue. Therefore, it seems that this hydrogen bonding retards deprotonation from the NCA ring, thus prevents the occurrence of side reactions. The deacetylation of these polymer was carried out by hydrazine in methanol at 0°C to give the poly(L-serine) with controlled chain lengths and a β-D-glucose or GlcNAc unit in each repeating unit. They didn't observe racemisation of the asymmetric carbon nor the cleavage of the glycosidic linkages during the deacetylation process.[46]

Also they have showed that its living polymerization by synthesizing block copolymer of glyco-NCAs, and second monomer L-alanine NCAs. Graft polymers with controlled glycopeptides branch length were also synthesized by the macromonomer method.[47-48] Glycopeptide macromonomer wre prepared by ring opening polymerization of glyco-NCAs using *p*-vinylbenzyl amine as an initiator, followed by deacetylation.[47] Water soluble glycoconjugates having glycopolypeptide branch were prepared by copolymerization of the glycopeptides macromonomer with acrylamide.

The major problem with all these glyco-NCAs is the synthesis of glycosylated amino acid derivatives. Glycosylation by mercury cyanide as catalyst is not environmentally friendly, use of stiochiometric amount of catalyst and very low glycosylation yield. Overall yield of the polymerization was only 15 %. So there is a need to have good synthetic method which will give access to glycosylated amino acid derivatives in excellent yield. In chapter 1 of this thesis we have showed excellent methodology for attachment of suitably protected amino acid derivatives to carbohydrate derivatives using gold catalyst.

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This chapter is divided into two sections, section (IA) presents the application of glycoaminoacids synthesized using gold catalyst for the synthesis of glycopolypeptides using ring opening polymerizations (ROP) and section (IB) describes the use of click chemistry for the synthesis of carbohydrate modified polypeptides and its application in self-assembly. Section (II) describes the new approach for the synthesis of partially azido functionalized and fully azido functionalized glycopolypeptides using ring opening polymerization.

Glycopolymers having pendent carbohydrates moieties found to have widespread application in various field such as macromolecular drug delivery system, hydrogels and matrices for controlled cell culture and as models of biological system. Majority of these glycopolymers currently explored are acrylate based and controlled radical polymerization is used to synthesize polymers of well defined molecular weight, glycosylation density and position which are necessary for various recognition processes. However these polymers don't have well-defined higher order structures and often adopt a random-coil conformation, which inevitably renders some of the side-chain bioactive moieties inaccessible towards active sites (Figure 6).

On the other hand, glycopolypeptides (glycopolymers with pendant carbohydrates on a polypeptide backbone) not only have the ability to fold into well-defined secondary structures (e.g. helix or sheet) but also mimic the molecular composition of proteoglycans. Therefore it is desirable to develop new methodologies that give access to easy and well defined synthetic glycopolypeptides.

The present work in this chapter of thesis is basically focusing on the application of carbohydrates to glycopolymer and self-assemblies for therapeutics. Glycoaminoacids are synthesized by using gold catalyzed glycosylation and synthesized glycopolypeptides using ring opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides.

Section IA

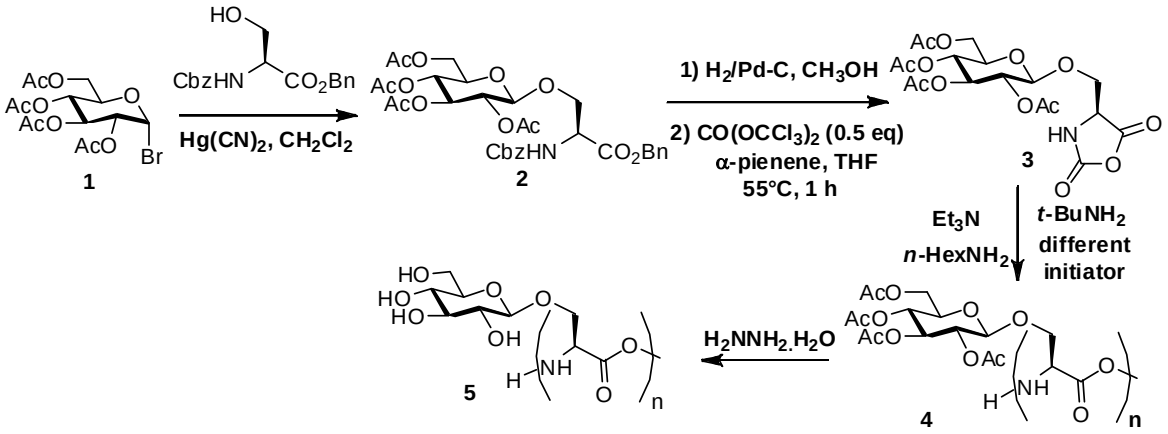
Synthesis of Glycopolypeptide and Glycosylated Polyproline using Ring Opening Polymerization (*ROP*)

Glycosylated peptides and proteins play major role in various cellular biological process such as recognition events, protection from proteases, survival of Antarctic ocean fish (Antifreeze properties), ion transport and lubricants in eye and joints. Similarly one could expect synthetic glycopolypeptides will show great potential in biomedical materials as well as valuable tools for probing carbohydrate-protein interaction. Although some research groups already showed that block and copolypeptides are cable of forming vesicles, fibrils and other structures are readily prepared by ring opening polymerization of amino acid *N*-carboxyanhydrides, the synthesis of well defined glycopolypeptides material is still a challenging research area.

Saccharides carrying NCAs have been prepared by Rude and co-worker but the detail study on the polymerization behaviour were not reported. In 1994 Okada and co-workers published first paper on the synthesis of high molecular weight glycopeptides and monodisperse living glycopeptides by the NCA method.[46] Generally polymerization of NCAs proceeds via two different mechanisms by the nature of initiator used. Primary amine initiated polymerizations give chain growth via amino end group, on the other hand tertiary amine initiated polymerization propagation proceeds via "activated monomer mechanism" (Figure 4). It's all about the nucleophilicity and basicity of the initiator, primary amines being more nucleophilic than basic, are good initiator for the chain growth polymerization vai amino end group. Whereas the tertiary amines, alkoxides and other initiator are more basic than nucleophilic give very high molecular weight polymers which could not be synthesized by primary amine as initiators (Scheme 1).

First series of polymerization was carried out by using triethylamine as initiator and using different solvents. They have observed differences in polymerization reaction as well as yield of reaction in different solvents. In acetonitrile polymerization proceeds with good yield and high molecular weight polymer which is confirmed by analyzing samples using GPC, ^{1}H NMR and ^{13}C NMR. Dichloromethane and dioxane gives low yields as well as low

molecular weight polymers. Broad molecular weight distribution by GPC analysis suggested the polymerization reaction proceeds via anionic activated monomer mechanism. They also deprotected polymer using hydrazine hydrate without any cleavage glycosidic linkage and racemisation at chiral centre and deprotected polymer showed random coil conformation in water by circular dichroism measurement.[46]



Scheme 1: Okada's approach towards Glycopolypeptide using ROP of Glyco-*N*-carboxyanhydride

The initiation with primary amine as initiator such as *n*-hexylamine gives the monodisperse glycopolypeptide. Molecular weight distribution by GPC were reasonably narrow measured by using Poisson distribution signifies the living nature of polymerization of carbohydrate bearing monomer. This was first application of the living polymerization of carbohydrate-derivatized monomer to synthesize a glycopolypeptide having sugar-peptide linkage. So the living polymerization of carbohydrate-carrying NCAs monomer can be utilized to design various types of glycoprotein models and synthetic glycopolymers for block and graft copolymer.[47] They have shown living nature of polymerization by synthesizing the AB-type of block copolymers. Conventional polycondensation and polymer reaction have some difficulties in the synthesis of these types of glycosylated peptides. The method developed by Okada and co-workers for the synthesis of glycopolypeptides has following draw backs I) use of highly toxic and non-environmentally friendly reagent $Hg(CN)_2$ for attachment of carbohydrate to amino acid in glycosylation step,[61] II) use of $Hg(CN)_2$ in stoichiometric quantity for glycosylation & III) the overall yield of polymerization was around 15 % including glycosylation step. Glycosylation step which lowers the overall yield in glycopeptides synthesis, so there is needed to develop good methodology for attachment of carbohydrate to amino acid in very good yield, eco- as well as environmentally friendly way.

In first part we have shown that gold catalyst can be used to prepare *O*-glycosylated as well as carbamate linked glycoaminoacid acid derivatives in excellent yield.

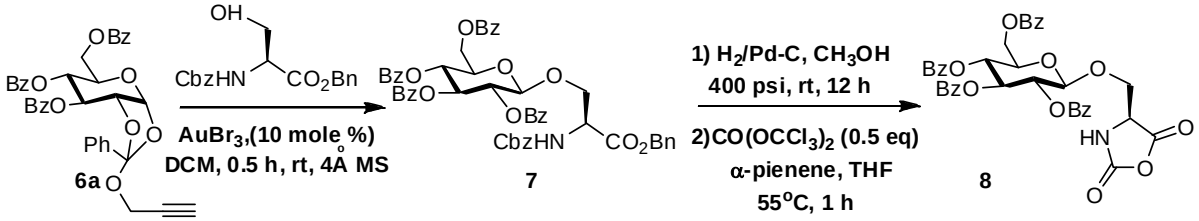
Apart from the direct polymerization of glycosylated NCAs, other strategies to prepare glycopolypeptides include the addition of carbohydrate to existing polypeptide. Such as copper catalyzed azide alkyne or thiol-ene click chemistry but these methods suffers from one drawback incomplete sugar functionalization. Also appearance of carbohydrate in non native forms (i.e. ring opened) or incorporation of triazole group could limit their use in biology. While performing and standardising the polymerization of glycosylated *N*-carboxyanhydrides of serine and L-lysine derivatives at same time similar kind or work was published by Deming and co-workers on the synthesis of glycopolypeptides.[56] In 2010 Deming's group synthesized the glycopolypeptides by living polymerization of *N*-carboxyanhydrides of *C*-glycosylated-L-lysine derivatives. These NCAs were shown to undergo living polymerization to give well-defined and high molecular homo glycopolypeptides as well as block glycopolypeptides. They tried to solve the problem of glycosylated monomer synthesis, purification and polymerization and polypeptides with 100 %. They have purified the glycosylated *N*-carboxyanhydrides by flash column chromatography in glove box (Dry box) using vacuum dried silica gel at 150°C for 48 h. Deprotection of acetate protected glycopolypeptides was carried out by using hydrazine hydrate to get water soluble glycopolypeptide. *C*-linked glycosides are very stable to these deprotection conditions and prevent deglycosylation. These water soluble glycopeptides have huge applications in tissue engineering, vesicles for drug delivery as well as for preparation of structurally defined sugars presenting polymers for glycomics research.

We have synthesized large number of serine and threonine glycoaminoacid derivatives in chapter 1 using gold catalyzed methodology for glycoaminoacid. For primary standardization of the ring opening polymerization reaction of the *N*-carboxyanhydrides, we have chosen serine derivative of glucose as a model substrate since it is not sterically hindered, and also was previously synthesized & polymerized by Okada group. There are few reports on the syntheses of glycosylated amino acid after Okada, but nobody showed its application to synthesize glycopolypeptides using ring opening polymerization of glyco-NCAs until Deming work. Cameron group reported the synthesis of Serine-*O*-glyco-NCA by using glycosyl iodide as donor, selective *N*-Boc protecting serine derivatives as acceptor and

iodine as catalyst to give serine glycoside, which was transformed into *N*-carboxyanhydride but they have not reported the polymerization of NCAs.[63]

Our method of synthesis for glycosylated amino acid derivatives is easy and exploits simple gravity column chromatography to purify required compound. Stereoselective formation of *O*-glycosidic linkage, use of catalytic amount of gold catalyst and good yield are key features of our glycosylation methodology. Glucose propargyl orthoester **6** was reacted with *N*-Cbz-Serine-OBn ester using 7 mol % $AuBr_3$ & 4Å molecular sieves in dry dichloromethane at room temperature for 30 min. Serine glycoside **7** was obtained by directly subjecting the reaction mixture to the silica gel column chromatography and eluting with ethyl acetate-petroleum ether as mobile phase in 75 % yield (Scheme 2).

To synthesize glyco-*N*-carboxyanhydrides, it is necessary to do deprotection of tetra-O-benzyol-glucose-*N*-Cbz-serine-OBn-ester to get glyco amino acid derivative. Deprotection of *N*-Cbz & OBn protected serine glycoaminoacid was carried out by the hydrogenolysis using 10 % palladium on charcoal in high pressure reactor at 400 psi for 12 h gave **A**. We have tried to attempt deprotection at lower pressure of hydrogen but we always ended up with mixture of partial deprotected glycoaminoacid derivatives. Complete deprotection is required to transform this glycoaminoacid derivative into polymerizable monomer glyco-*N*-carboxyanhydride **8**. This reaction condition did not harm the glycosidic linkage and it is intact during progress of deprotection (Scheme 2).



Scheme 2: Synthesis of *N*-carboxyanhydrides of Glycoaminoacid

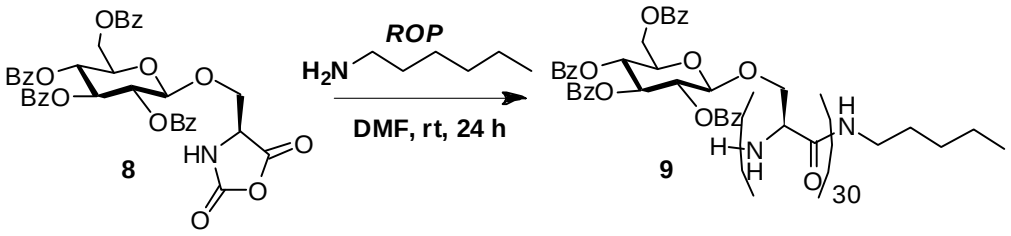
Different methods are available for transformation of amino acid derivatives into *N*-carboxyanhydrides. Such as PCl_5, PCl_3, $COCl_2$ could be also used but these might harm the glycosidic linkage, also a phosgene ($COCl_2$) is gas, which is difficult to handle and highly toxic and trichloromethyl chloroformate ($ClCO_2CCl_3$; diphosgene) is liquid and its transport and storage may cause industrial accident. This transformation could be done by using Bis(trichloromethyl) carbonate ($CO(OCCl_3)_2$; triphosgene) is a crystalline, stable solid which

is easy to transport, to store and weighed on balance. Triphosgene was first prepared in 1880 by Councler[64-68] by chlorination of dimehyl carbonate. By considering above facts, we decided to use triphosgene for transformation of glycosylated amino acid derivatives into the glyco-*N*-carboxyanhydrides as a monomer.

Glycosylated *N*-carboxyanhydrides **8** derivative was synthesized by treatment of completely deprotected per-*O*-benzoylated-D-glucose-L-serine with triphosgene and α-pinene as acid quencher in dry tetrahydrofuran at 50°C for 1 h. Purification of resultant gluco-*N*-carboxyanhydride was done by the three times precipitation in tetrahydrofuran by the addition of nonpolar dry petroleum ether and removing traces of any solvent by applying high vacuum for 1-2 h below 50°C. Formation of per-*O*-benzoylated-D-glucose-L-serine *N*-carboxyanhydride **8** was confirmed by the ^{1}H NMR analysis and in ^{13}C NMR peak at *δc* 151.5 ppm corresponds to cyclic carbamate carbonyl group, also the presence of carbohydrate was confirmed by resonance of anomeric carbon in ^{13}C NMR spectrum at *δc* 101.2 ppm. FT-IR analysis of Glyco-N-Carboxyanhydride showed carbonyl stretches at 1787 (C2 of ring) and 1852 (C5 of ring) cm^{-1}, which are characteristic of the NCA ring. For primary studies we took *n*-hexylamine as initiator which is sterically unhindered and thus the primary initiation step will be much faster than chain growth step, which are the requirement to get glycopolymers with low PD ≈ 1.1-1.2. Also extent of incorporation of n-hexylamine in glycopolypeptide could be easily determined by the methyl ^{1}H NMR signals in desired glycopolymer NMR spectrum.

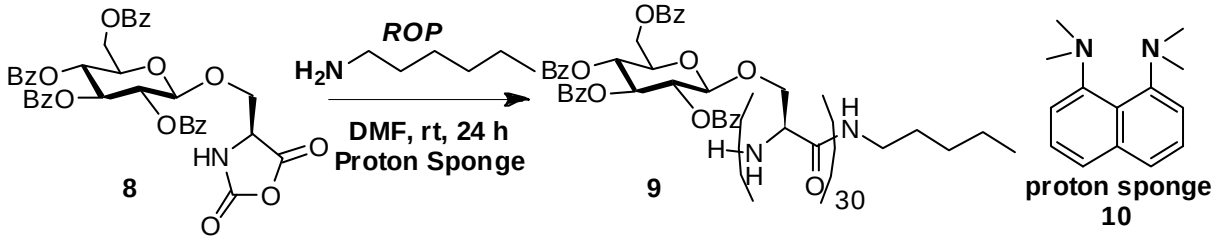
Ring Opening Polymerization of per-*O*-benzoylated-D-glucose-L-serine *N*-carboxyanhydride **8** was attempted by using reported procedure and *n*-hexylamine as micro initiator in glove box (Dry Box) in anhydrous dimethyl formamide but failed unable to get desired molecular weight glycopolypeptide (Scheme 3). The reaction was monitored by the taking IR of aliquots of reaction mixture and checking the disappearance of stretch at 1857 cm^{-1}. But we failed to observe the disappearance of 1857 cm^{-1} signal even after keeping the reaction mixture for longer time. Also different anhydrous solvents were used to carry out the reaction such as dimethyl formamide, tetrahydrofuran, dioxane and dichloromethane. After struggling for ROPs of NCAs, we tried to figure out why the polymerization was not happening? Then we concluded after a lot of trial and errors, that it may be due to the residual HCl left inside the NCAs while synthesizing them using triphosgene, which could

quenche our *n*-hexylamine initiator preventing the polymerization. We could not see the HCl impurities in ^{1}H NMR as well as ^{13}C NMR of Glyco-*N*-carboxyanhydrides **8**.



Scheme 3: Ring Opening Polymerization of *N*-Carboxyanhydrides using *n*-Hexylamine as initiator

Use of proton sponge need a special mention as it traps the residual HCl. Proton sponge is nothing but 1,8-bis(dimethylamino)naphthalene **10,** which is a non-nucleophilic base. Sometimes it may be possible that the proton sponge would initiate polymerization instead of *n*-hexylamine as discussed earlier. But this possibility is rule out by using different initiator such as propargyl amine or azido end functionalized amine. Also controlled experiments were done by variying amounts of proton sponge to the solution of glyco-*N*-carboxyanhydride **8** in anhydrous dimethyl formamide at room temperature and checked the change in FT-IR stretch. No change in FT-IR signals at 1785 and 1857 cm^{-1} and it is stable under reaction condition and also does not initiate polymerization via activated monomer mechanism. This concludes that one can use proton sponge in reaction mixture to quench the residual HCl, which could facilitate initiation of polymerization by *n*-hexylamine via ring opening polymerization (ROP).



Scheme 4: Ring Opening Polymerization of *N*-Carboxyanhydrides using n-Hexylamie as initiator in presence of proton sponge

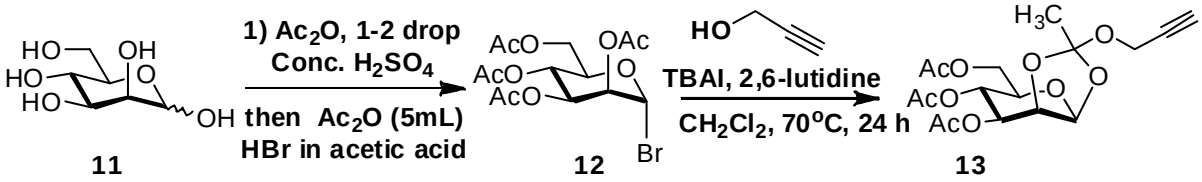
Controlled ring opening polymerization (ROP) of glyco-*N*-carboxyanhydride **8** was carried out by using *n*-hexylamine as initiator (M/I = 20) in anhydrous dimethylformamide to which one equivalent of proton sponge **10** was added a before the addition of initiator *n*-hexylamine to quench residual HCl and reaction was done in a glove box (dry box) to avoid contamination by atmospheric moisture (Scheme 4). The progress of polymerization was

monitored by measuring in intensity of anhydride stretch in FT-IR for an aliquot after different intervals of time was taken out from the glove box when complete disappearance of anhydride stretches were observed in FT-IR spectrum. Purification of resultant glycopolypeptide **9** was done by adding 2N dil. HCl solution to remove the proton sponge, successive washing with water and the remaining residue was vacuum dried in vacuum oven at 50°C for 12 h. Further analysis of glycopolypeptide was carried out by ^{1}H NMR, ^{13}C NMR and gel permeation chromatography (GPC).

The relative molecular weight of the glycopolypeptide **9** was estimated to be 13, 600 Da by GPC (expected molecular weight 13, 400, polystyrenes are used for standardization of GPC) and a molecular weight distribution was found to be i.e PDI around 1.1. The presence of *n*-hexylamine and monosaccharides was confirmed by ^{1}H NMR and ^{13}C NMR analysis. Methyl protons of *n*-hexylamine showed resonance at δ_H 0.84 ppm and carbohydrate protecting group at around δ_H 7-8 ppm whereas in ^{13}C NMR spectrum showed at δc 22 ppm and glycosidic carbon δc 101-102 ppm.

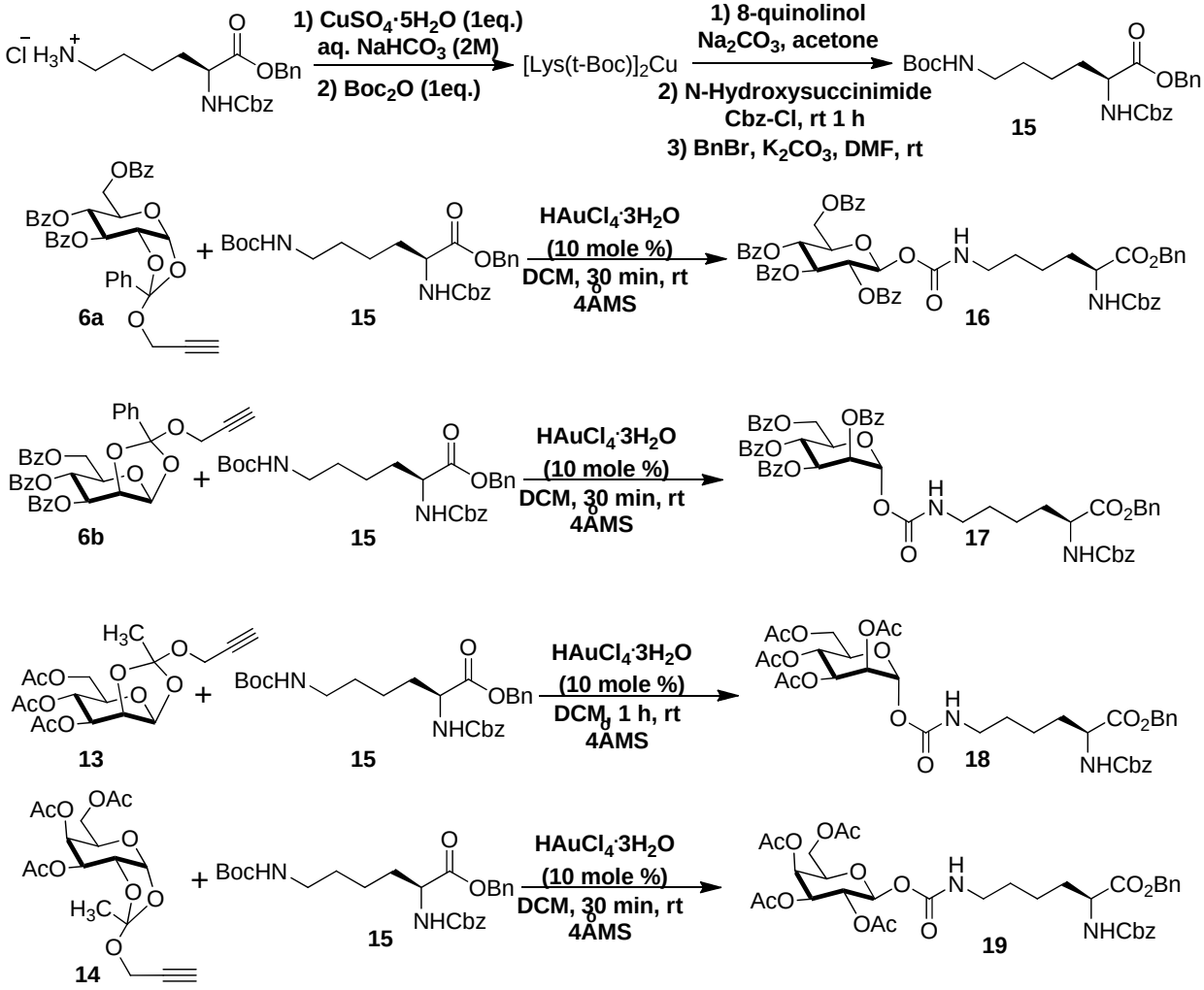
Our main goal was to synthesize water soluble glycopolypeptides because they have widespread applications in various fields such as drugs and drug delivery system, hydrogels, matrices for controlled cell culture and as models of biological systems. We have tried various methods for complete deprotection of per-*O*-benzoylated serine glycopolypeptide **9** but our attempts to do so were failed. Having failed to deprotect benzoates, much more susceptible acetates were considered. Pertinent to note that deprotection of acetates can be carried out by NaOMe, NH_3, NH_2NH_2 etc. in a easier manner. There are two reasons for not considering -Ac in the beginning. Firstly gold catalyzed glycosylation methodology was already standardized in our group for per-*O*-benzoylated propargyl orthoester and secondary is that one of the way to protect carbohydrate with acetate is use of acetic anhydride whose consumption is monitored under narcotics act.

Acetate protection of monosaccharides was done by using acetic anhydride; treatment with HBr in acetic acid in dichloromethane gave per-*O*-acetylated mannosyl bromide **12**. Mannosyl bromide **12** was transformed into per-*O*-acetylated mannosyl-propargyl-1,2-orthoester **13** by using propargyl alcohol, tetra-*n*-butylammonium iodide (catalytic amount), 2,6-lutidine in dry dichloromethane at 70°C for 24 h. After completion of the reaction,



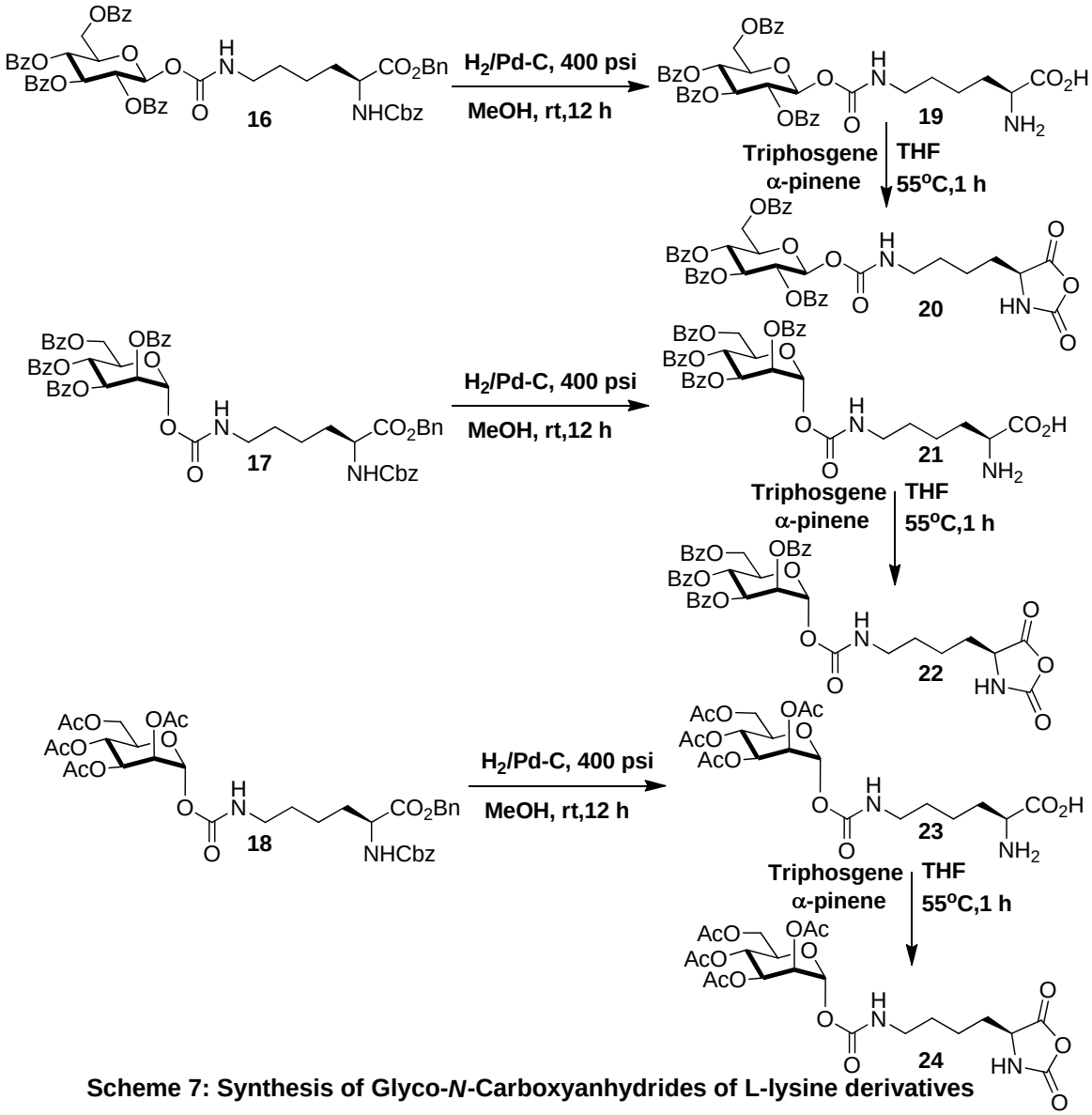
Scheme 5: Synthesis of Acetate Protected Glucose Propargyl-1,2-Orthoester

reaction mixture was diluted with cold water and washed with cold oxalic acid to remove the 2,6-lutidine; strong acid wash causes rearrangement of orthoester to direct β-*O*-propargyl glycoside. Simple gravity column chromatography was performed to separated per-*O*-acetylated-mannose-propargyl-1,2-ortheoester **13** from the crude product using ethyl acetate and petroleum ether as eluent (Scheme 5). Similarly reaction sequence per-*O*-acetylated-galctose-propargyl-1,2-ortheoester **14** also synthesized.



Scheme 6: Synthesis of Per-*O*-Benzoyl and *O*-Acetate Glycosylated Amino Acid Derivative Using Gold Catalyst

While synthesizing the acetate protected propargyl orthoester, we thought to use our second methodology for attachment of carbohydrate moiety to the amino acid. Good yields in glycosylation step are significant to improve overall yield of glycopolypeptide synthesis which in turn can be done by using good synthetic glycosylation methodology. Formation of carbamate linkage between carbohydrate and *t*-Boc protected primary amine was quantitative without any side product with wide substrate scope by using $HAuCl_4.3H_2O$ as a catalyst. For this we chose L-lysine amino acid as it has ε-side chain amino group, which can be protected by *t*-Boc group and polylysine is known to form long chain α-helical structure, that is good for chain growth of desired polypeptide polymer and also for high molecular weight polypeptide synthesis. Selective side chain protection of L-lysine as well as amino

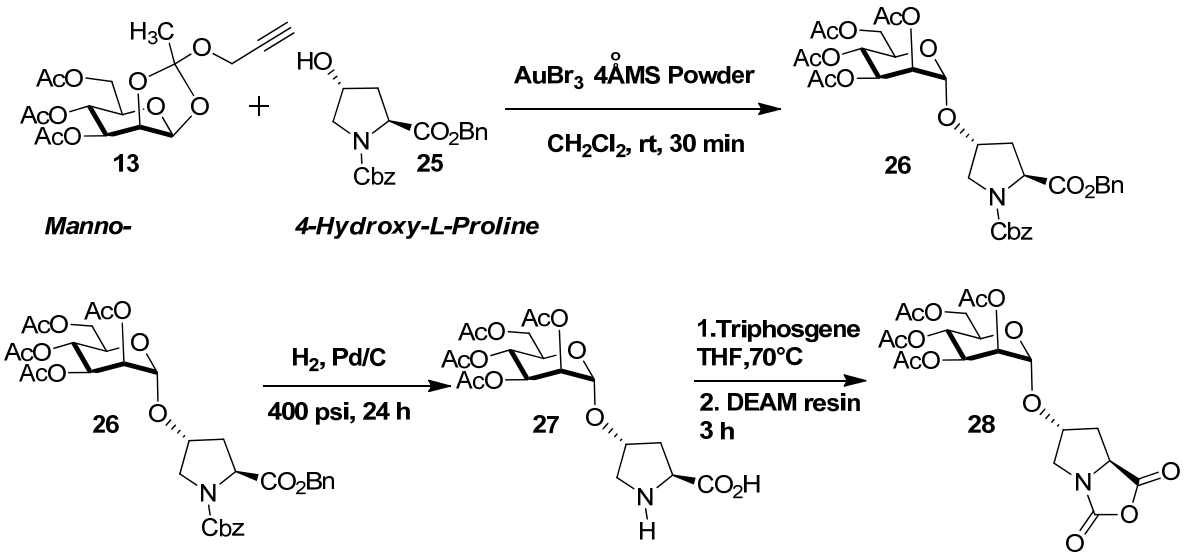


Scheme 7: Synthesis of Glyco-*N*-Carboxyanhydrides of L-lysine derivatives

acid fragment was carried out by reported literature procedure to get desired L-lysine derivative in bulk quantities (Scheme 6).[69]

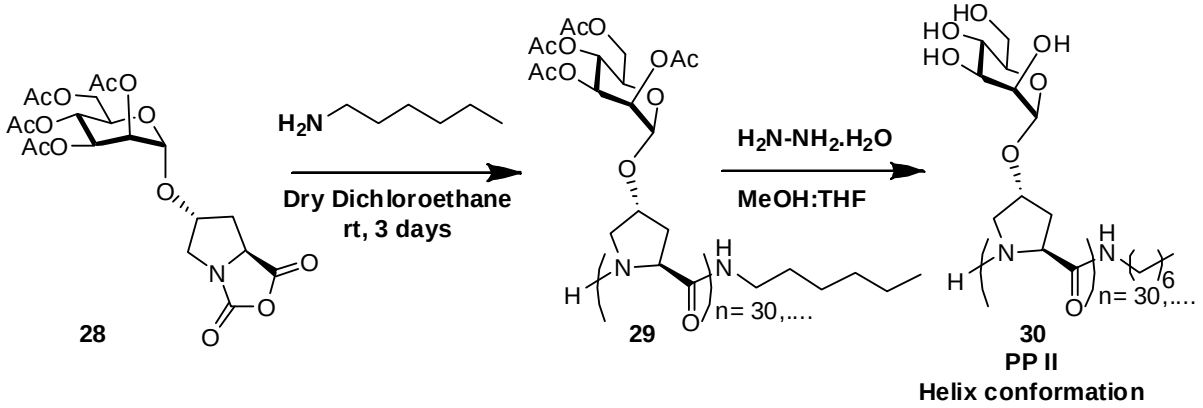
Glycosylated carbamate linked amino acid synthesis was carried out by reaction between per-*O*-benzyol-glucose-propargyl-1,2-orthoester **6a,** per-*O*-benzyol-mannose-propargyl-1,2-orthoester **6b** & per-*O*-acetylated-mannose-propargyl-1,2-orthoester **13** with N_{ε}-Boc protected N_{α}-Cbz-Lys-OBn **15** in the presence of $HAuCl_4.3H_2O$ and 4Å molecular sieves powder in anhydrous dichloromethane at room temperature to desired glucosyl carbamate **16** & **17** in excellent yield 95 %. Similar procedure was applied to attach acetylated glucose and galactose residue to L-lysine amino (92 % yield, **18, 19**) to check its biological recognition by the lectin ConA. Hydrogenolysis was carried out at 400 psi to deprotect *N*-Cbz and –OBn ester group using palladium catalyst (10 mol %) to get glycosylated-L-lysine free amino acid derivatives **19**, **21** & **23**. These glycosylated-L-lysine derivatives upon treatment with triphosgene transformed into corresponding glyco-L-lysine-*N*-carboxyanhydrides **20**, **22** & **24,** which are starting monomers for ring opening polymerization reaction. Overall yield of synthesizing *N*-carboxyanhydride reported by our approach is 3 times higher than methods reported by earlier researcher (Scheme 7). Application work of synthesized glycosylated L-lysine-NCA monomer into synthesis of water soluble glycopolypeptide and lectin binding assay was done in our collaborator's laboratory Dr. Sayam Sengupt and Debasis Pati.

Polyprlines are interesting because they exist in two conformation viz PPI and PPII,



Scheme 8: Synthesis of Mannose-L-Proline-*N*-Carboxyanhydrides For ROP

glycosylated polyprolines would be ideal for increasing their solubility as well as for lectin binding studies. Incidentally, polymerization of polyprolines was not thoroughly studied except a report by Deming et al quit recently on a simple proline. Polyprolines are found in collagen and they are very much important in biological perspective.[70] Accordingly 4-hydroxyproline **25** which upon glycosylation with per-*O*-acetyl-mannose-propargyl-1,2-orthoester **13** using gold catalyst reported in earlier work of this chapter and deprotection using hydrogenolysis give glycosylated proline derivative **26**. At same one report published on the synthesis of glycosylated-*N*-carboxyanhydrides of proline and we used same



Scheme 9: Ring Opening Polymerization of *N*-Carboxyanhydrides of Mannose Proline derivative using *n*-Hexylamine as initiator

procedure to get desired mannose-*N*-carboxyanhydride derivative of proline using N, N' diethyl aminopolystyrene resin for final cyclization.[71] Mannose-Proline-NCA **28** was characterised by using FT-IR and ^{1}H, ^{13}C NMR (Scheme 8).

We tried polymerization of mannose-proline-NCAs **28** using proton sponge. Yet, we could have not successfully standardize the reaction conditions fully for polymerization. Thus obtained glycopolyproline polymer was characterised by FT-IR, ^{1}H & ^{13}C NMR and GPC.

Progress of the polymerization of Mannose proline NCAs was monitored by FT-IR where IR

30_DMPP_Hexam **50_DMPP_Hexam** **30_PBLG_DMPP_Hexam**

GPC

peaks corresponds to NCAs at 1857 cm^{-1} and 1785 cm^{-1} were disappeared as the reaction proceeded to completion as shown below (Figure 1).

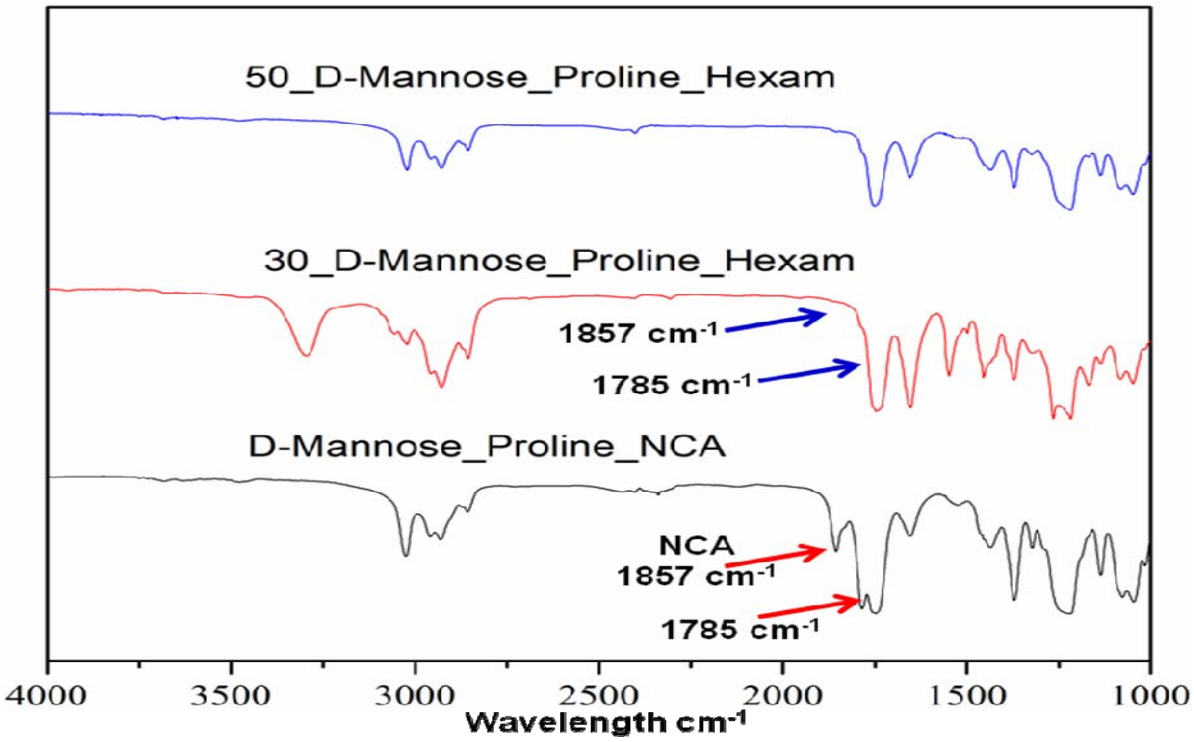
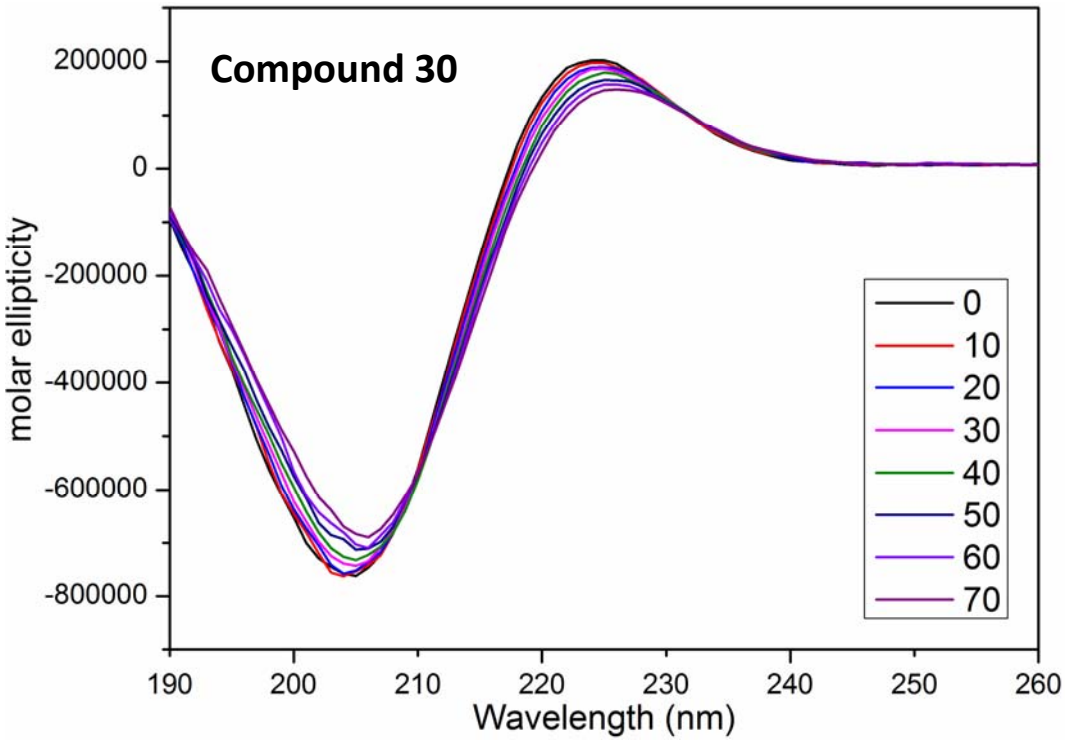


Figure 1: FI-IR of Polymerization of Mannose-proline NCAs

Run no	Monomer (M)	M/I	M_w	Mw/Mn	DP	Yield
1	α-manno-*O*-Pro	30	12 100	1.11	28	90%
2	α-manno-*O*-Pro	50	20 500	1.10	48	90%
3	γ-Bn-L-glu/α-manno-*O*-Pro	30:20	23 500	1.12	33:22	95%

Homoglycopolymer of target length 30 unit and 50 unit and block copolymer with γ-benzyl glutmate NCAs were synthesized, which was characterised by the GPC. GPC data shows expected molecular weight distribution and good polydispersity that has been targeted. Relative molecular weights of resultant glycopolypeptide were obtained by the GPC curve with reference to PS as an internal standard.



Acetate deprotection of resultant mannose polyproline **29** was done by hydrazine hydrate in THF:MeOH system to get water soluble PPII helix forming polymer **30**. Also we have checked the circular dichroism measurement of deprotected water soluble polyproline shows PPII helix conformation negative minima at 205 nm and positive maxima at 225 nm, which matches with literature report. Temperature dependent circular dichroism study showed that carbohydrate moieties give extra stability to PP II helix conformation as shown in above CD spectra where PPII conformation is quite stable upto 70°C. Further study on optimization of polymerization is under progress.

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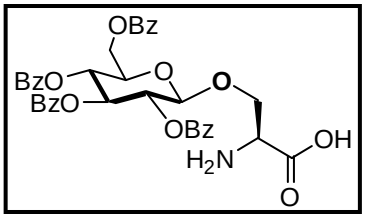
General procedure for the glyco *N*-carboxyanhydrides (8)

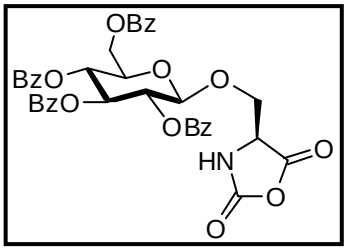
Hydrogenolysis of compounds **7** was carried out using 10% Pd/C in MeOH−EtOAc (9 : 1) at 400 psi for 12 h. After completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to afford per-*O*-benzoylated-D-glucose-L-serine in almost quantitative yield. The resulting compounds were directly used for NCA synthesis without any further purification.



***O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-Serine (A):** $[\alpha]_{25}^{D}$ +30.8° (*c* 1.0, CH_3OH); ^{1}H NMR (200.13 MHz, CD_3OD): δ 4.28-4.88(m, 7H), 5.36(d, *J* = 7.8 Hz, 1H), 5.65(t, *J* = 8.5 Hz, 1H), 5.83(t, *J* = 9.0 Hz, 1H), 6.10(t, *J* = 9.7 Hz, 1H), 7.28-7.70(m, 12H), 7.85(d, *J* = 7.2 Hz, 2H), 7.97(d, *J* = 7.3 Hz, 2H), 8.03(d, *J* = 7.5 Hz, 2H), 8.12(d, *J* = 7.2 Hz, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 54.1, 64.0, 67.6, 70.7, 73.0, 73.4, 74.6, 101.5, 129.4-130.8, 134.5, 134.6, 134.8, 134.8, 166.7, 166.9, 167.0, 167.6, 169.0; MS (ESI) calcd for $C_{37}H_{33}NO_{12}$ $[M+Na]^+$, 706.647; found, 706.363.

To a solution of per-*O*-benzoylated-D-glucose-L-serine (0.1 mmol) in freshly distilled anhydrous tetrahydrofuran (30 mL) was added a solution of triphosgene (0.05 mmol) in anhydrous tetrahydrofuran (5 mL) under argon atmosphere. α-Pinene (0.15 mmol) was added and the reaction mixture was heated to 50°C for 2 h and cooled to room temperature, poured into dry hexane. The white precipitate of the *N*-carboxyanhydride (**8**) was vacuum filtered quickly and reprecipitated (2 times) by dissolving in ethyl acetate followed by addition of light petroleum. The resulting precipitate was filtered and dried under vacuum (Yield 80%).

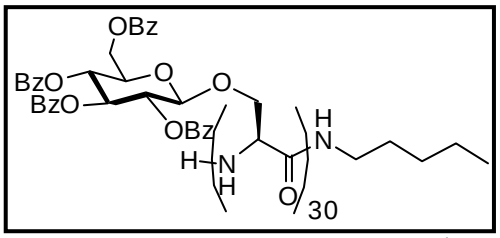
***O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-*N*-carboxy-L-serine anhydride (8)**



IR(cm^{-1}): 1854, 1789; ^{1}H NMR (500.13 MHz, $CDCl_3$): *δ* = 3.92(dd, *J* = 5.8, 11.0 Hz, 1H), 4.11(t, *J* = 9.0 Hz, 2H), 4.26−4.35(m, 2H), 4.85(d, *J* = 7.6 Hz, 1H), 4.90(d, *J* = 12.2 Hz, 1H), 5.40(t, *J* = 9.5 Hz, 1H), 5.66(t, *J* = 9.5 Hz, 1H), 5.84(t, *J* = 9.8 Hz, 1H), 6.69(s, 1H),

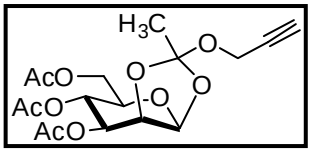
7.15−7.54(m, 12H), 7.70(d, *J* = 7.4 Hz, 2H), 7.86(d, *J* = 7.2 Hz, 2H), 7.87(d, *J* = 7.2 Hz, 2H), 8.03(d, *J* = 7.6 Hz, 2H); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 58.5, 62.1, 68.6, 68.7, 71.2, 72.4, 73.2, 101.2, 128.3−130.0, 133.3, 133.5, 133.6, 133.6, 151.5, 165.1, 165.2, 165.7, 166.7, 167.1; MS (ESI): *m*/*z*: calcd for $[C_{38}H_{31}NO_{13}+Na]^+$: 732.641; found: 732.145.

Procedure for the synthesis of glycopolymer (9)



To a 2 mL solution of glyco-*N*-carboxyanhydride **8** (200 mg, 0.28 mmol) in anhydrous DMF was added 1,8-bis(dimethylamino)naphthalene **10** (0.07 mmol; 1 M) ('proton sponge') as an additive and hexylamine (14 µL, 1.0 mmol mL^{-1}) as an initiator inside the glove box. The progress of the polymerization was monitored by FT-IR spectroscopy by comparing with the intensity of the initial NCA's anhydride **8** stretching at 1789 cm^{-1} and 1852 cm^{-1}. At the end of the reaction (24 h), an aliquot was removed for GPC analysis. The solvent was removed under reduced pressure and the residue was re-dissolved in CH_2Cl_2 and the polymer was precipitated out by the addition of methanol. The precipitated polymer was collected by centrifugation and dried to afford white glycopolypeptide **9** in 90% yield.

Synthesis of 3,4,6-tri-O-acetyl-b-D-glucopyranose-1,2-(prop-2-ynyl ortho acetate) (13)



To a suspension of D-glucose (5 g, 27.75 mmol) in glacial acetic acid (20 mL) was added acetic anhydride (18 mL, 180.39 mmol) followed by Conc. H2SO4 (4 drops) and the reaction mixture was stirred at room temperature for 30 min. Then 33% HBr in glacial acetic acid was added at 0C and the resulting solution was stirred for additional 5 h at room temperature. After completion of the reaction as judged by TLC, the reaction mixture was poured into ice and extracted with dichloromethane (2x100 mL). Combined organic layers were washed with water and concentrated *in vacuo* to give 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (10.24 g, 90%) that was redissolved in anhydrous dichloromethane (50 mL). To taht 2,6-lutidine (10 mL), propargyl alcohol (7.3 mL, 124.02 mmol) followed by a catalytic amount of tetra-n-butylammonium iodide (0.2 g) were added at room temperature under argon atmosphere. The reaction mixture was stirred for 24 h at 70C under argon atmosphere, quenched with a saturated solution of oxalic acid and extracted with

dichloromethane (2X100 mL). Combined organic layers were washed with water, brine dried over anhydrous sodium sulphate and concentrated *in vacuo* to obtain the brownish black residue which was purified by silica gel column chromatography using petroleum ether-ethyl acetate as the mobile phase to give 3,4.6-tri-O-acetyl-β-D-glucopyranose-1,2-(pro-2-ynyl orthoacetate) **13** (6.0 g, 63%).

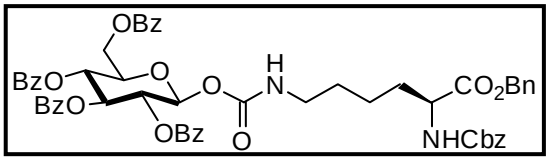
$[\alpha]^{D}_{25}$ ($CHCl_3$, c 1.0) = +3.59

^{1}H NMR ($CDCl_3$, 200.13 MHz): δ 1.79 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.39 (t, *J* = 2.46 Hz, 1H), 3.69 (ddd, *J* = 2.84, 4.84, 9.21 Hz, 1H), 4.15-4.29 (m, 1H), 4.16 (d, *J* = 2.87 Hz 1H), 4.19 (d, *J* = 2.47 Hz, 2H), 4.66 (dd, *J* = 2.66, 3.83 Hz, 1H), 5.16 (dd, *J* = 3.89, 9.90 Hz, 1H), 5.27 (d, *J* = 9.45 Hz, 1H), 5.51 (d, *J* = 2.63 Hz, 1H)

13C NMR (CDCl3, 50.32 MHz): δ 20.6, 20.7, 20.7, 24.4, 50.8, 62.3, 65.3, 70.2, 71.4, 73.6, 76.3, 79.4, 97.4, 124.1, 169.4, 170.3, 170.8

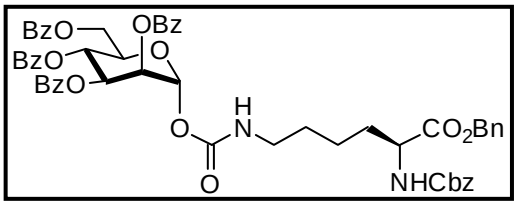
General procedure for the synthesis of amino acid glycosyl carbamates (16, 17, 18 & 19)

To a solution of propargyl 1,2-orthoester (0.1 mmol), N_α-Cbz-Lys(N_ε-Boc)OBn (0.11 mmol) and activated 4 Å molecular sieves powder (50 mg) in anhydrous CH_2Cl_2 (5 ml) was added $HAuCl_4.3H_20$ (7 mol%) under argon atmosphere at room temperature. The reaction mixture was stirred at room temperature for the specified time and the reaction mixture was filtered and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether as the mobile phase to afford the compounds **16, 17, 18** and **19.**

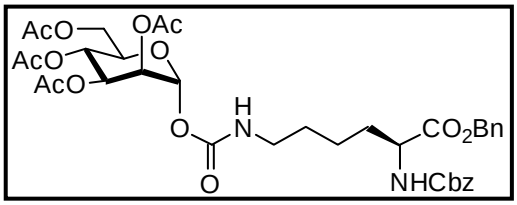


Compound 16 : $[\alpha]^{D}_{25}$ ($CHCl_3$, c 1.0) = +52.7; ^{1}H NMR ($CDCl_3$, 200.13 MHz): δ 1.03–1.78 (m, 6H), 3.02 (q, *J* = 6.2, 12.5 Hz, 2H), 4.10–4.36 (m, 2H), 4.45 (dd, *J* = 4.4, 12.5 Hz, 1H), 4.62 (dd, *J* = 2.5, 12.1 Hz, 1H), 4.93 (t, *J* = 5.7 Hz, 1H), 5.11 (m, 2H), 5.12 (s, 2H), 5.37 (d, *J* = 8.0 Hz, 1H), 5.64 (dd, *J* = 8.3, 9.5 Hz, 1H), 5.74 (t, *J*= 9.7 Hz, 1H), 5.93 (t, *J* = 9.6 Hz, 1H), 6.03 (d, *J* = 8.2 Hz, 1H), 7.21–7.59 (m, 22H), 7.80–8.06 (m, 8H); ^{13}C NMR ($CDCl_3$, 50.32MHz): δ 21.9, 28.9, 31.8, 40.4, 53.6, 62.6, 66.9, 67.1, 69.0, 70.9, 72.7, 72.8, 93.1, 128.0–129.9, 133.0, 133.2, 133.4, 133.4, 135.2, 136.2, 153.8, 155.9, 165.0, 165.2,

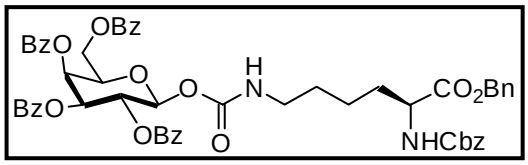
165.6, 166.0, 172.1; HRMS (MALDI-TOF): m/z: calcd for $[C_{56}H_{52}N_2O_{15}Na]^+$: 1015.3265, found: 1015.3254



Compound 17 : $[\alpha]^D_{25}$ ($CHCl_3$, c 1.0) = -29.2; ^{1}H NMR ($CDCl_3$, 200.13 MHz): δ 1.25–1.95 (m, 6H), 3.19 (q, *J* = 6.3, 12.1 Hz, 2H), 4.38–4.56 (m, 3H), 4.71 (dd, *J* = 3.6, 13.2 Hz, 1H), 5.13 (s, 2H), 5.19 (m, 3H), 5.47 (d, *J* = 8.1Hz, 1H,), 5.74 (dd, *J* = 2.4, 3.1Hz, 1H), 5.90 (dd, *J* = 3.3, 10.2 Hz, 1H,), 6.19 (t, *J* = 10.0 Hz, 1H,), 6.31 (d, *J* = 1.9 Hz, 1H), 7.21–7.66 (m, 22H), 7.78–8.15 (m, 8H); 13C NMR ($CDCl_3$, 50.32 MHz): δ 22.3, 29.0, 32.2, 40.8, 53.6, 62.4, 66.2, 67.0, 67.1, 69.4, 69.9, 70.4, 91.3, 128.0–129.9, 133.0, 133.3, 133.4, 133.5, 135.2, 136.1, 153.1, 156.0, 165.1, 165.2, 165.6, 166.0, 172.2 HRMS (MALDITOF): m/z: calcd for $[C_{56}H_{52}N_2O_{15}Na]^+$: 1015.3265, found: 1015.3254



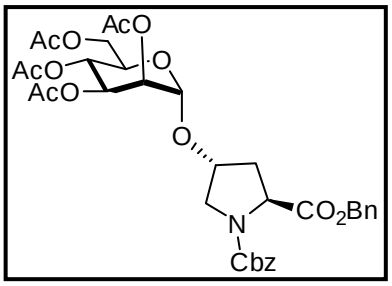
Compound 18: $[\alpha]^D_{25}$ = +22.1(*c*1.0, $CHCl_3$); ^{1}H NMR (200.13MHz, $CDCl_3$): δ 1.15-1.91(m, 6H), 1.98, 2.03, 2.04, 2.12(4s, 12H), 3.12(td, 2H, *J* = 2.1, 6.5Hz), 3.98(t, 1H, *J* = 6.5 Hz), 4.06(dd, 1H, *J* = 7.7, 11.4Hz), 4.11(d, 1H, *J* = 1.0 Hz), 4.15(d, 1H, *J* = 3.1 Hz), 4.39(m, 1H), 5.11(s, 2H), 5.05(dd, 1H, *J* = 3.5, 10.4 Hz), 5.17(d, 1H, *J* = 3.8 Hz), 5.29(dd, 1H, *J* = 8.1, 10.4 Hz), 5.39(d, 2H, *J* = 3.4 Hz), 5.62(d, 1H, *J* = 8.2 Hz), 7.32-7.38(m, 10H); ^{13}C NMR (50.32MHz, $CDCl_3$): δ 20.5(3C), 20.6, 22.2, 28.8, 32.0, 40.6, 53.5, 61.9, 65.4, 66.8, 67.0, 68.4, 68.8, 70.0, 91.1, 127.9-128.5, 135.1, 136.1, 153.0, 156.0, 169.4, 169.6, 170.0, 170.6, 172.1; MALDI-TOF(m/z): Calcd for $C_{36}H_{44}KN_2O_{15}$: 783.2379, Found: 783.2373.



Compound 19: $[\alpha]^D_{25}$ = +4.4(*c*1.0, $CHCl_3$); ^{1}H NMR (200.13MHz, $CDCl_3$): δ 1.15-1.91(m, 6H), 1.98, 2.03, 2.04, 2.12(4s, 12H), 3.12(td, 2H, *J* = 2.1, 6.5, 13.5Hz), 3.98(t, 1H, *J* = 6.5 Hz), 4.06(dd, 1H, *J* = 7.7, 11.4Hz), 4.11(d, 1H, *J* = 1.0 Hz), 4.15(d, 1H, *J* = 3.1 Hz), 4.39(m, 1H), 5.11(s, 2H), 5.05(dd, 1H, *J* = 3.5, 10.4 Hz), 5.17(d, 1H, *J* = 3.8 Hz), 5.29(dd, 1H, *J* = 8.1, 10.4 Hz), 5.39(d, 2H, *J* = 3.4 Hz), 5.62(d, 1H, *J* = 8.2 Hz), 7.32-7.38(m, 10H); ^{13}C NMR (50.32MHz, $CDCl_3$): δ 20.4(3C), 20.5, 22.0, 28.7, 31.8, 40.4, 53.5, 60.8, 66.7, 66.8, 66.9, 67.7, 70.6, 71.1, 93.0, 127.9-128.5, 135.1, 136.1, 153.7, 155.9, 169.4,

169.7, 170.0, 170.2, 172.0; MALDI-TOF(m/z): Calcd for $C_{36}H_{44}KN_2O_{15}$: 783.2379, Found: 783.2333.

***O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl-L-proline glycoside (26):**

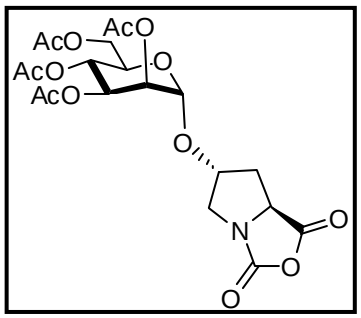


To a solution of propargyl 1,2-orthoester **13** (0.1 mmol), 4-Hydroxy-*N*-Cbz-L-Proline-OBn **25** (0.11 mmol) and activated 4 Å molecular sieves powder (50 mg) in anhydrous CH_2Cl_2 (5 ml) was added $AuBr_3$ (7 mol%) under argon atmosphere at room temperature. The reaction mixture was stirred at room temperature for the specified time and the reaction mixture was filtered and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether as the mobile phase to afford the compounds **26.**

^{1}H NMR ($CDCl_3$, 200.13 MHz): δ 1.99 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.09-2.20 (m, 1H), 2.15 (s, 3H), 2.43-2.55 (m, 1H), 3.58-3.80 (m, 1H), 3.73 (d, *J* = 3.92 Hz, 1H), 3.90–4.00 (m, 1H), 4.04-4.10 (m, 1H), 4.12 (d, *J* = 6.1, 12.0 Hz, 1H), 4.4 (m, 1H), 4.55 (m, 1H), 4.87 (d, *J*= 3.5 Hz, 1H), 4.97 (s, 1H), 5.07 (d, *J* = 2.1 Hz, 1H), 5.15-5.30 (m, 5H), 7.27–7.40 (m, 10H)

^{13}C NMR ($CDCl_3$, 50.32MHz): δ 20.5, 20.6, 20.6, 20.7, 35.9 (39.9), 51.4 (51.5), 57.7 (57.9), 62.5, 66.0, 66.8 (66.9), 67.3, 68.5 (68.6), 69.0 (69.1), 69.3 (69.4), 75.1 (76.6), 96.7 (97.2), 127.8, 127.8, 128.1, 128.3, 128.5, 135.1 (135.3), 136.1 (136.2), 154.0 (154.6), 169.6, 169.7, 169.8, 170.4, 171.8 (172.0)

***O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-*N*-carboxy-L-proline anhydride (28):**



Hydrogenolysis of compounds **26** was carried out using 10% Pd/C in MeOH−EtOAc (9 : 1) at 400 psi for 12 h. After completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to afford per-*O*-acetylated-D-mannose-L-proline **27** in almost quantitative yield. The resulting compounds were directly used for NCA synthesis without any further purification.

To a solution of per-*O*-acetylated-D-glucose-L-proline **27** (0.1 mmol) in freshly distilled anhydrous tetrahydrofuran (30 mL) was added a solution of triphosgene (0.05 mmol) in anhydrous tetrahydrofuran (5 mL) under argon atmosphere was heated to 50°C for 1 h. After 1 h the solvent was removed *in vacuo* which gave oily product of N-carbamyl chloride and high vaccum was applied to remove excess of triphosgene trapped inside, which was then redissolved into freshly distilled THF. The solution was finally added onto the polymer-supported amine resin 3 equivalent (Diethylaminomethyl-polystyrene, DEAM-PS) which had been swollen previously in dry THF and kept under argon (or nitrogen) atmosphere for 3 h at room temperature. Mannose-L-Proline NCA **28** was obtained by filtration with additional dry THF and reprecipitated (2 times) by dissolving in ethyl acetate followed by addition of light petroleum. The resulting precipitate was filtered and dried under high vacuum (Yield 70%).

^{1}H NMR ($CDCl_3$, 200.13 MHz): δ 2.00 (S, 3H), 2.06(s, 3H), 2.10 (m, 1H), 2.12 (s, 3H), 2.16 (s, 3H), 2.61 (dd, *J* = 6.0, 12.7 Hz, 1H), 3.45 (d, *J* = 12.7 Hz, 2H), 3.99 (m, 2H), 4.12 (d, *J* = 12.0 Hz, 1H), 4.25 (dd, *J* = 6.49, 12.0 Hz, 1H), 4.62 (dd, *J* = 6.2, 10.2 Hz, 1H), 4.68 (bs, 1H), 4.90 (s, 1H), 5.18 (s, 1H), 5.2 (d, *J* = 8.1 Hz, 2H)

^{13}C NMR ($CDCl_3$, 50.32MHz): δ 20.6, 20.6, 20.6, 20.8, 34.9, 52.8, 62.1, 62.7, 65.9, 68.4, 69.4, 69.6, 80.2, 97.3, 154.3, 167.8, 169.6, 169.9, 170.0, 170.1

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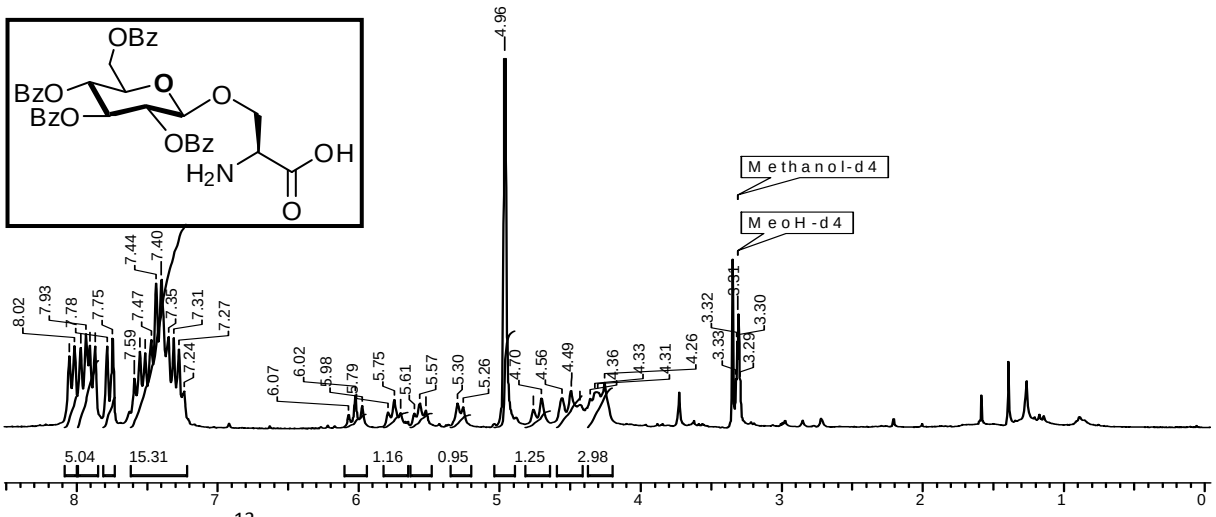
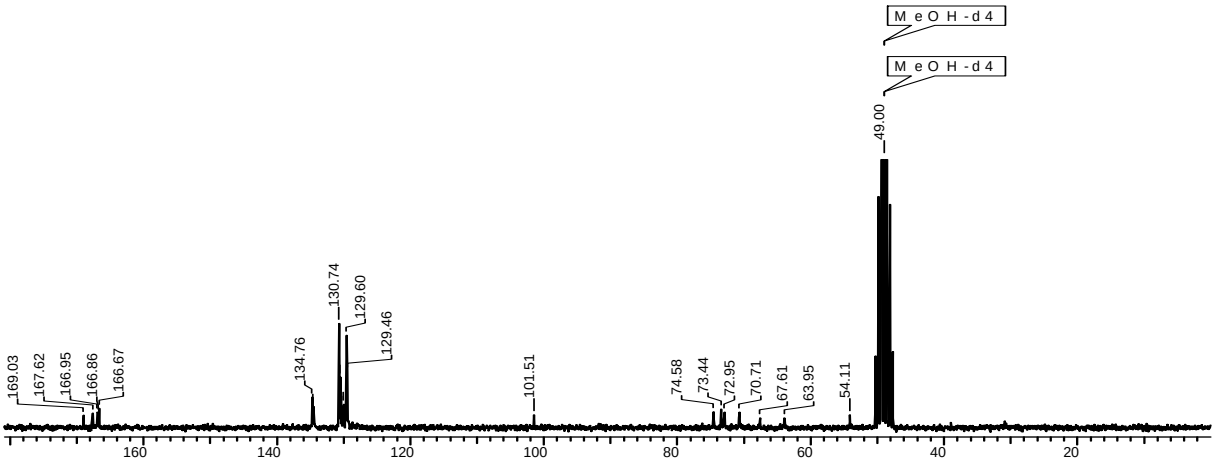
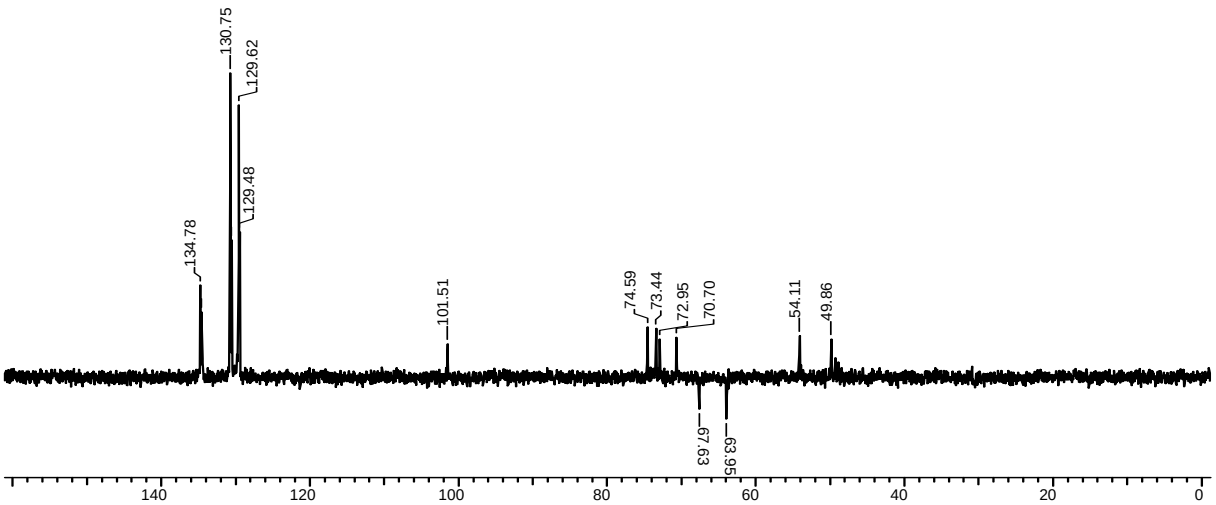
^{1}H NMR (200.13 MHz, CD_3OD) Spectrum of Compound **A**



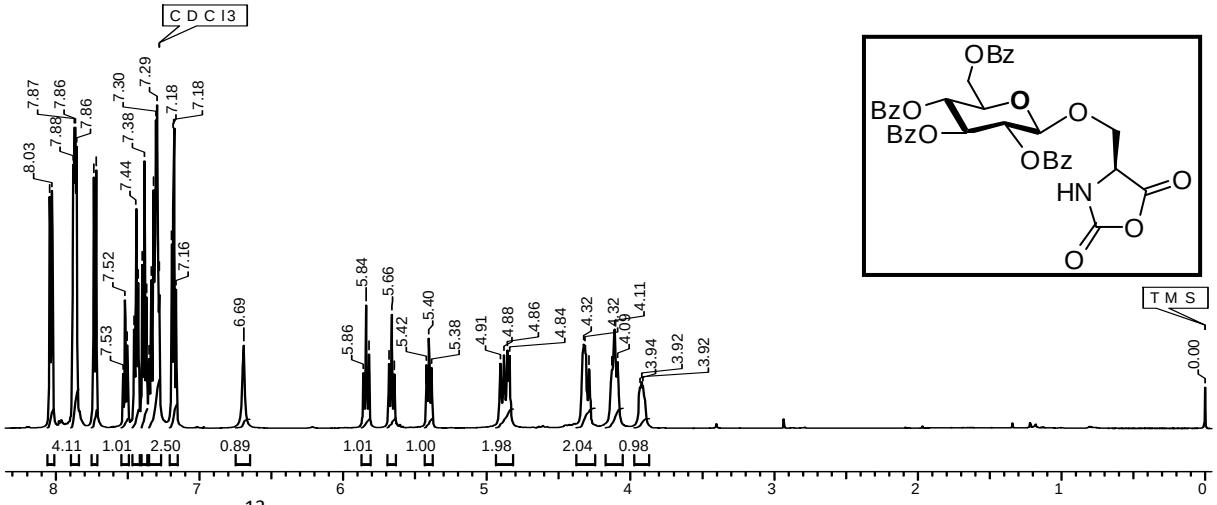
^{13}C NMR (100.61 MHz, CD_3OD) Spectrum of Compound **A**



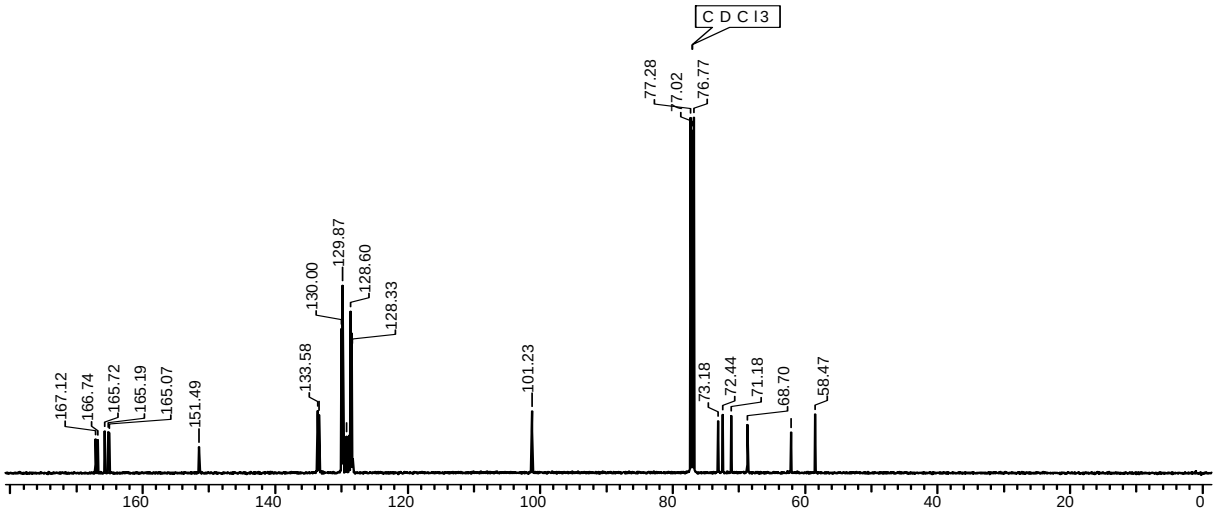
DEPT NMR (100.61 MHz, CD_3OD) Spectrum of Compound **A**



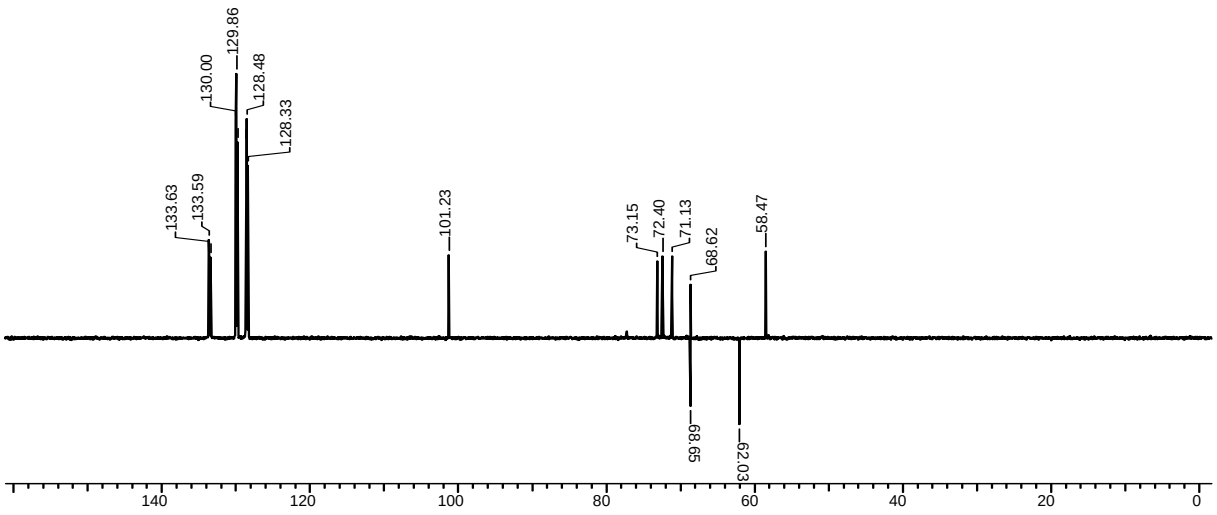
^{1}H NMR (500.13 MHz, $CDCl_3$) Spectrum of Compound **8**



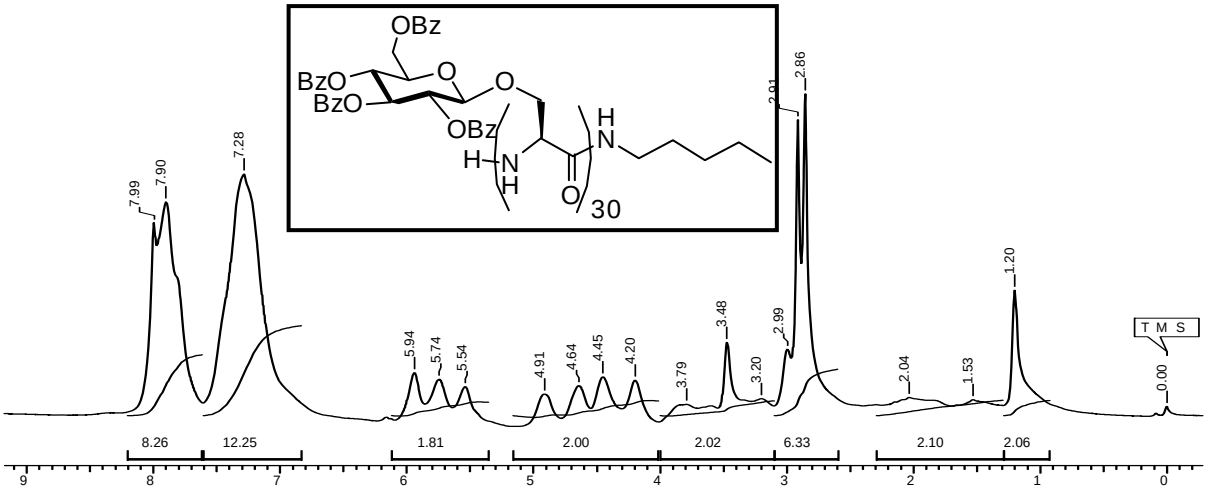
^{13}C NMR (125.76 MHz, $CDCl_3$) Spectrum of Compound **8**



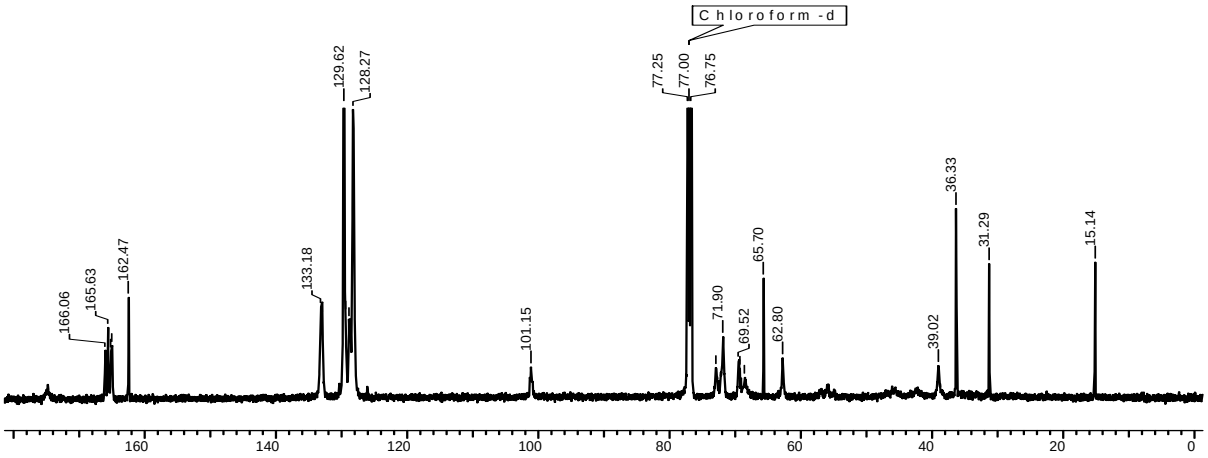
DEPT NMR (125.76 MHz, $CDCl_3$) Spectrum of Compound **8**



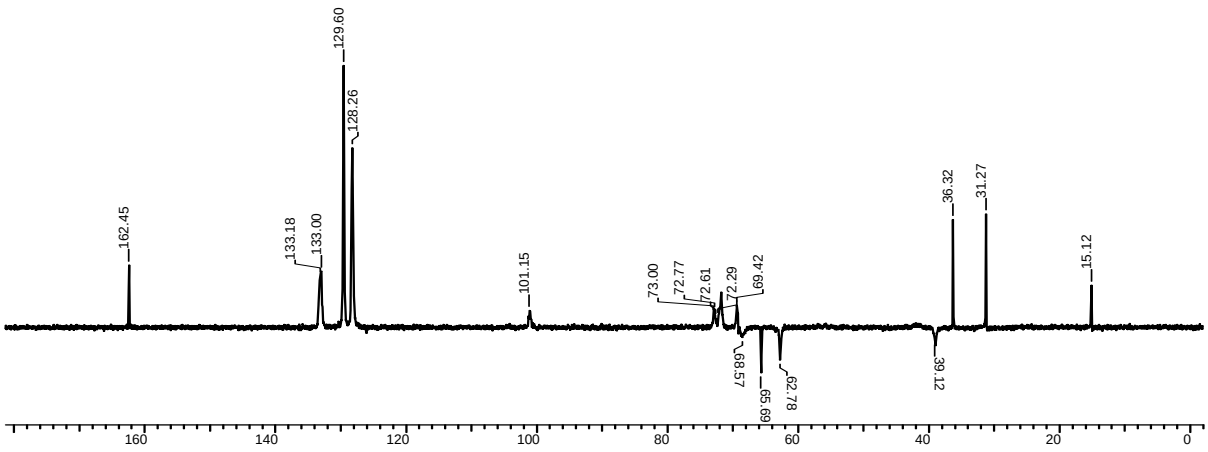
^{1}H NMR Spectrum (200.13 MHz, $CDCl_3$) of Compound **9**



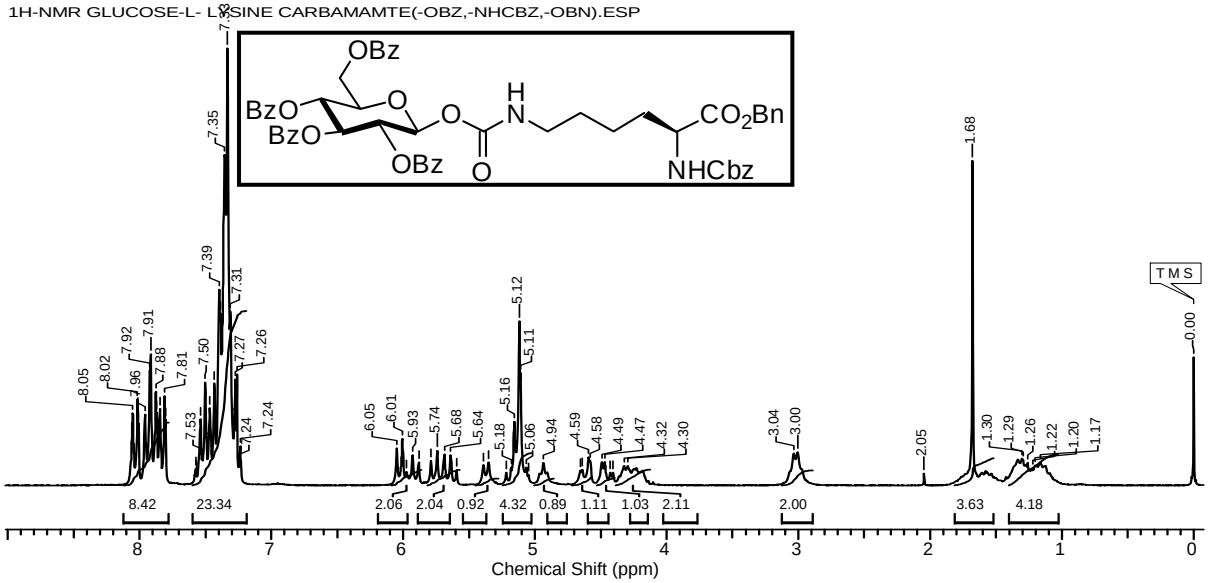
^{13}C NMR Spectrum ($CDCl_3$, 50.32 MHz, $CDCl_3$) of Compound **9**



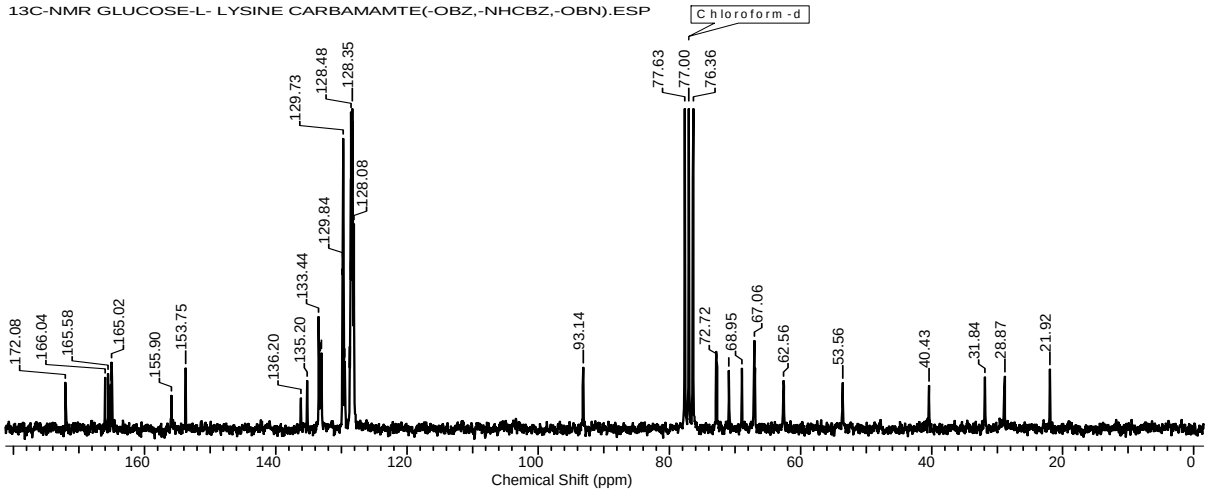
DEPT Spectrum (50.32 MHz, $CDCl_3$) of Compound **9**



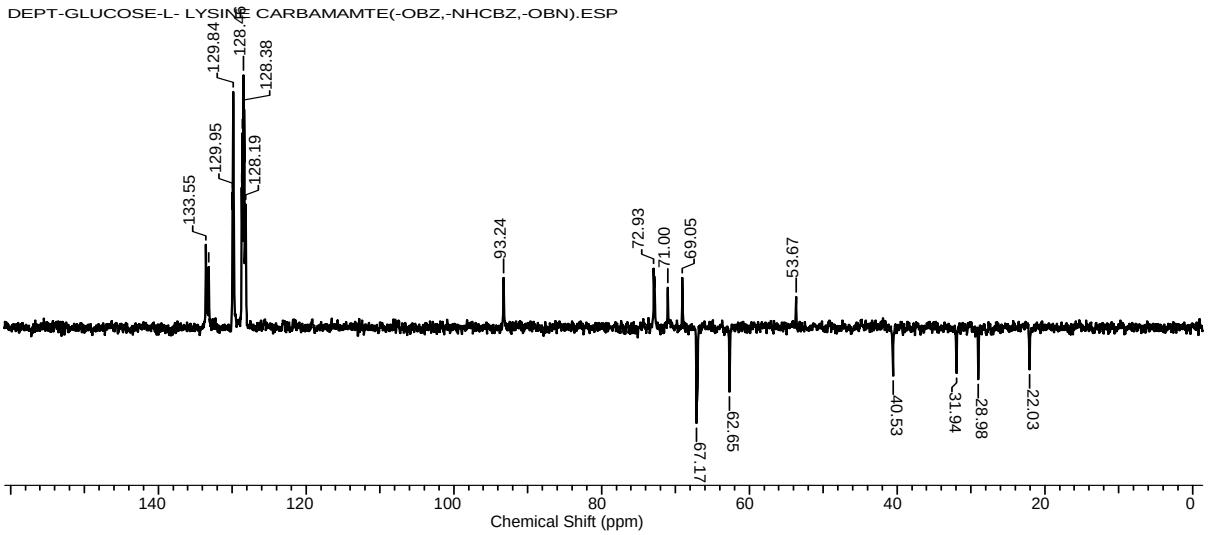
^{1}H NMR (200.13 MHz, $CDCl_3$) Spectrum of Compound **16**



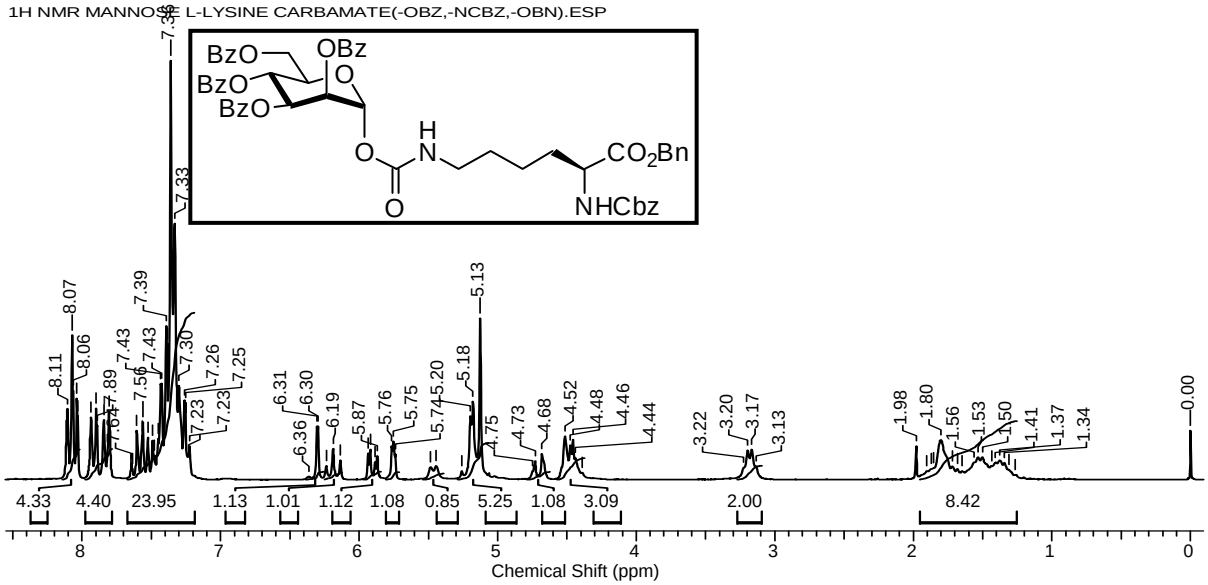
^{13}C NMR (50.32 MHz, $CDCl_3$) Spectrum of Compound **16**



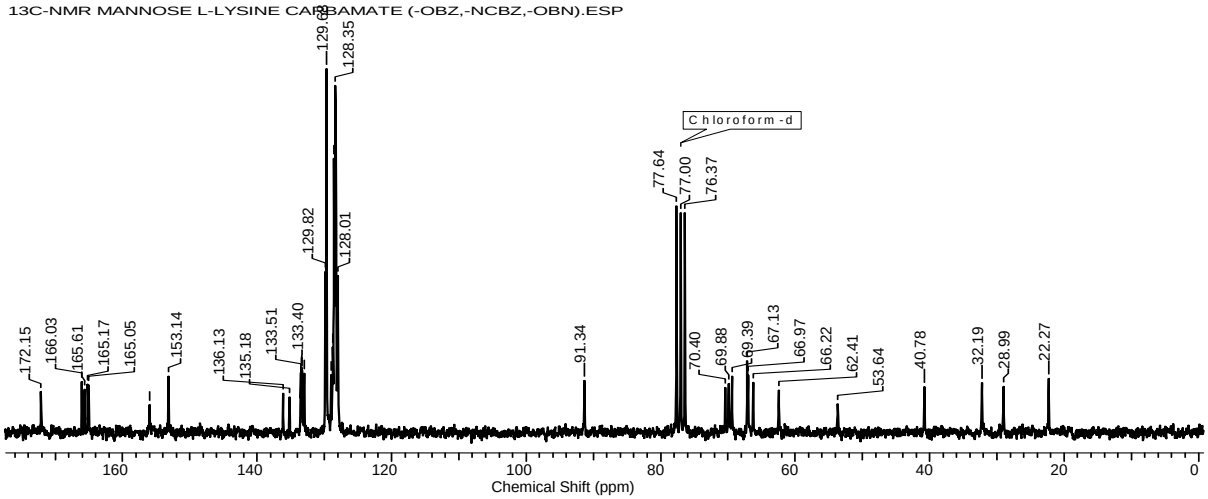
DEPT NMR (50.32 MHz, $CDCl_3$) Spectrum of Compound **16**



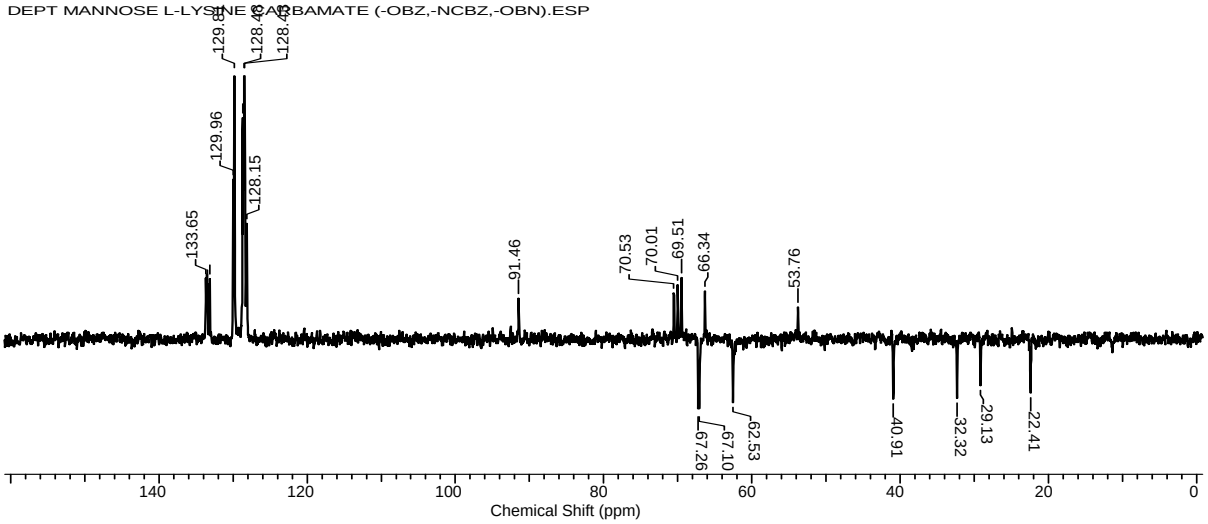
^{1}H NMR (200.13MHz, $CDCl_3$) Spectrum of Compound **17**



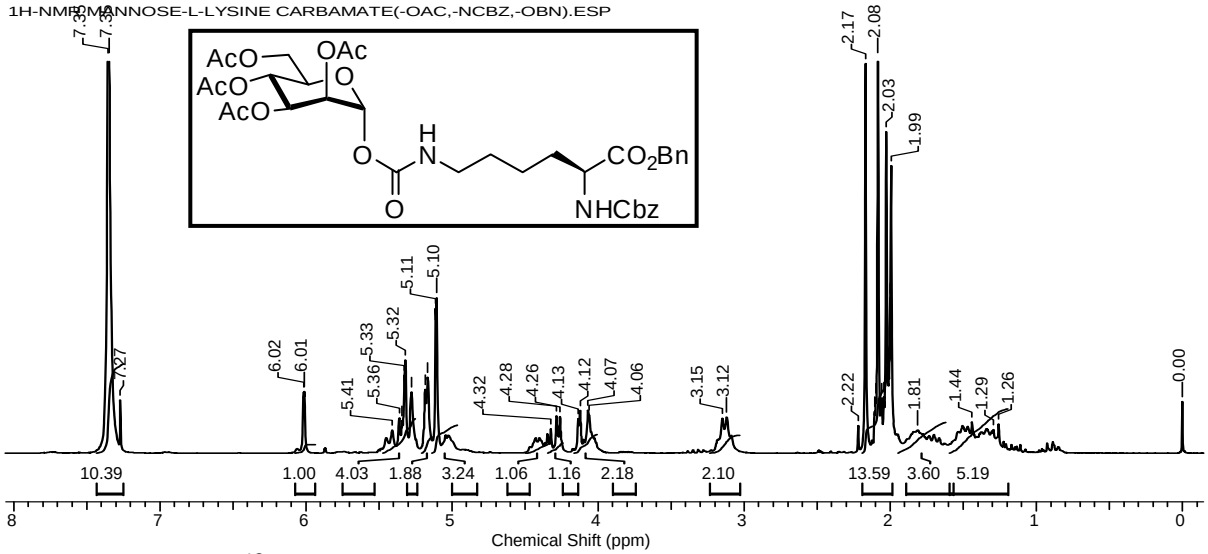
^{13}C NMR (50.32 MHz, $CDCl_3$) Spectrum of Compound **17**



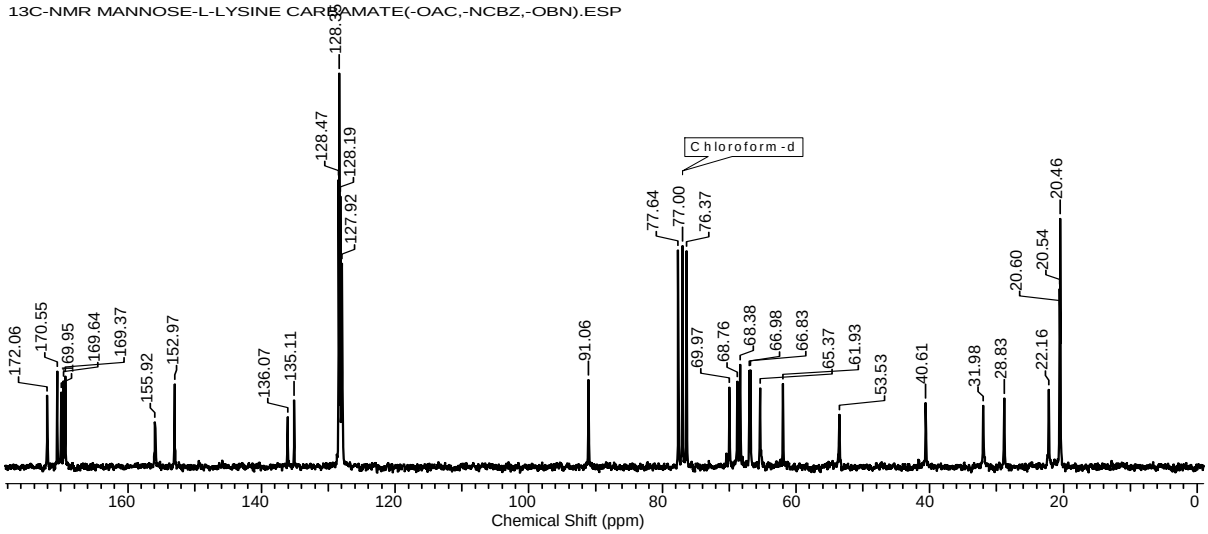
DEPT NMR (50.32 MHz, $CDCl_3$,) Spectrum of Compound **17**



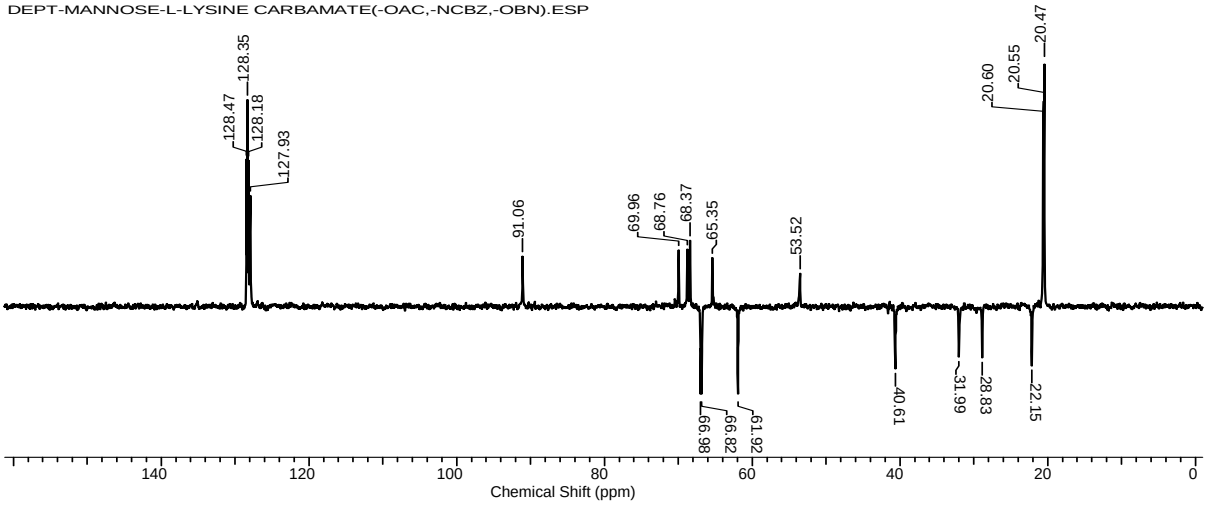
^{1}H NMR (200.13MHz, $CDCl_3$) Spectrum of Compound **18**



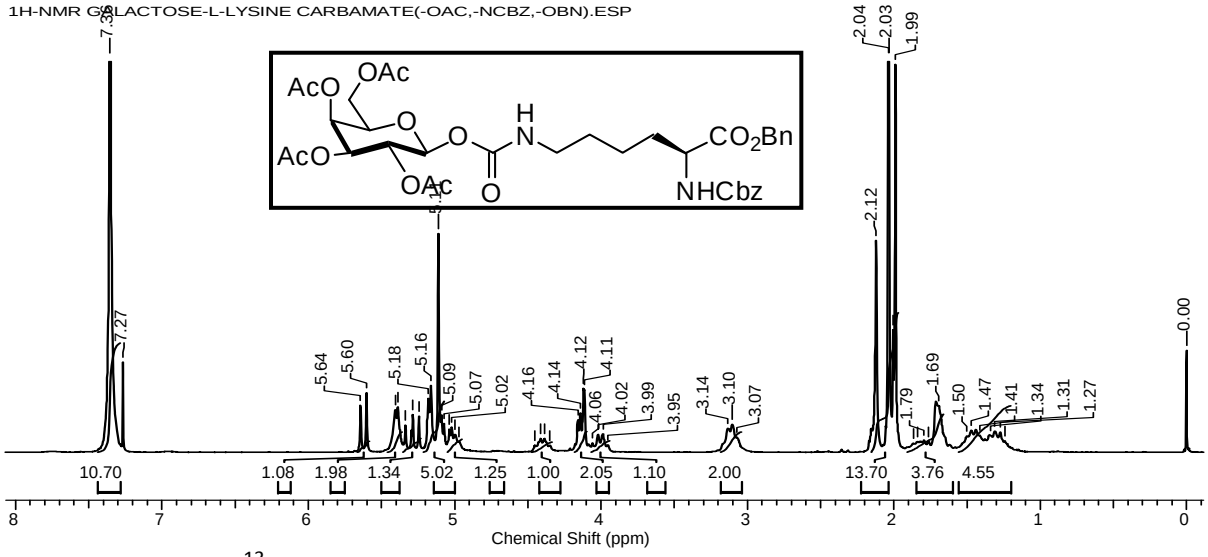
^{13}C NMR (50.32 MHz, $CDCl_3$) Spectrum of Compound **18**



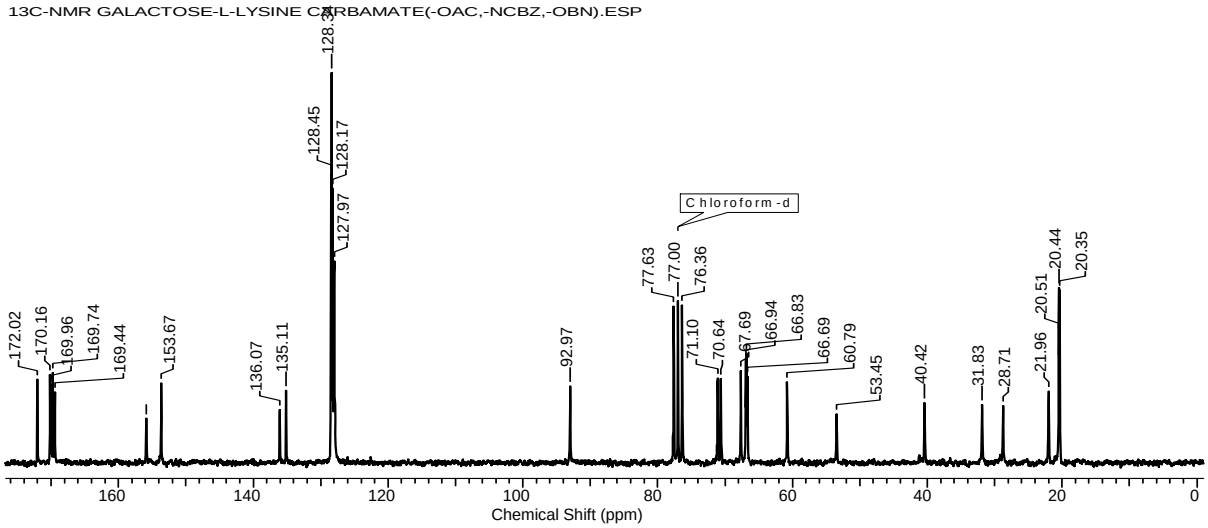
DEPT NMR (100.61 MHz, $CDCl_3$) Spectrum of Compound **18**



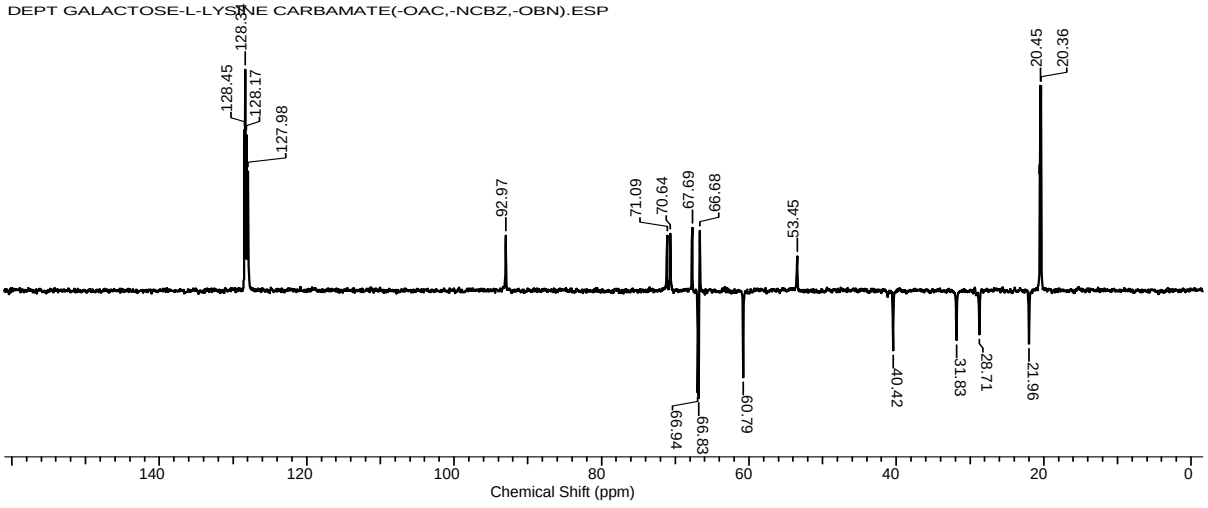
^{1}H NMR (200.13MHz, $CDCl_3$) Spectrum of Compound **19**



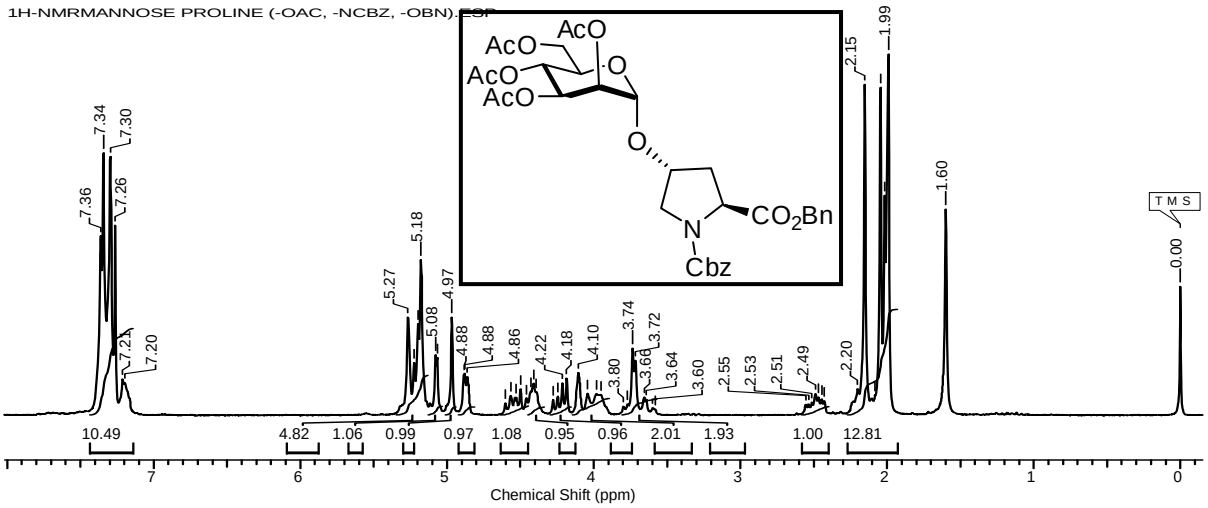
^{13}C NMR (50.32 MHz, $CDCl_3$) Spectrum of Compound **19**



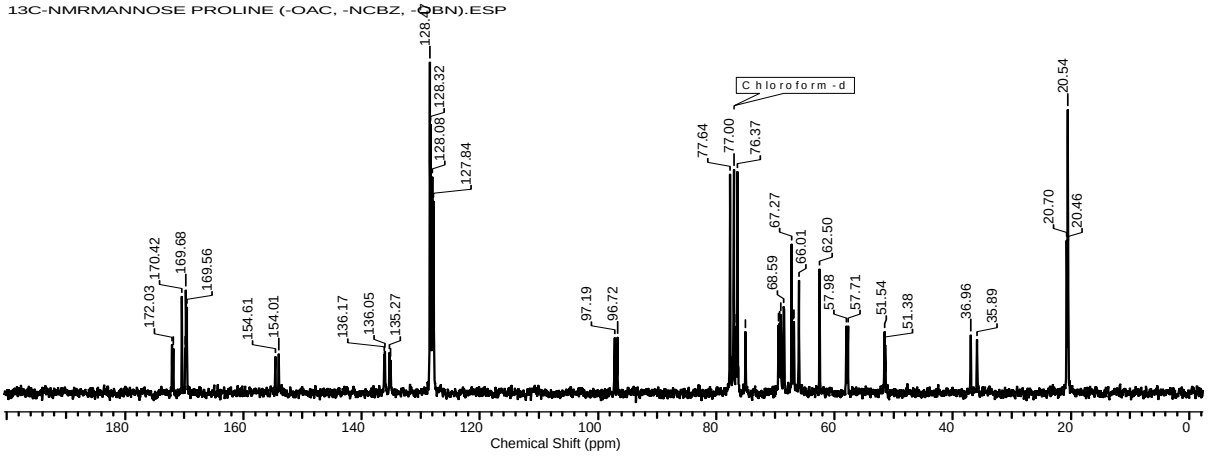
DEPT NMR (100.61 MHz, $CDCl_3$) Spectrum of Compound **19**



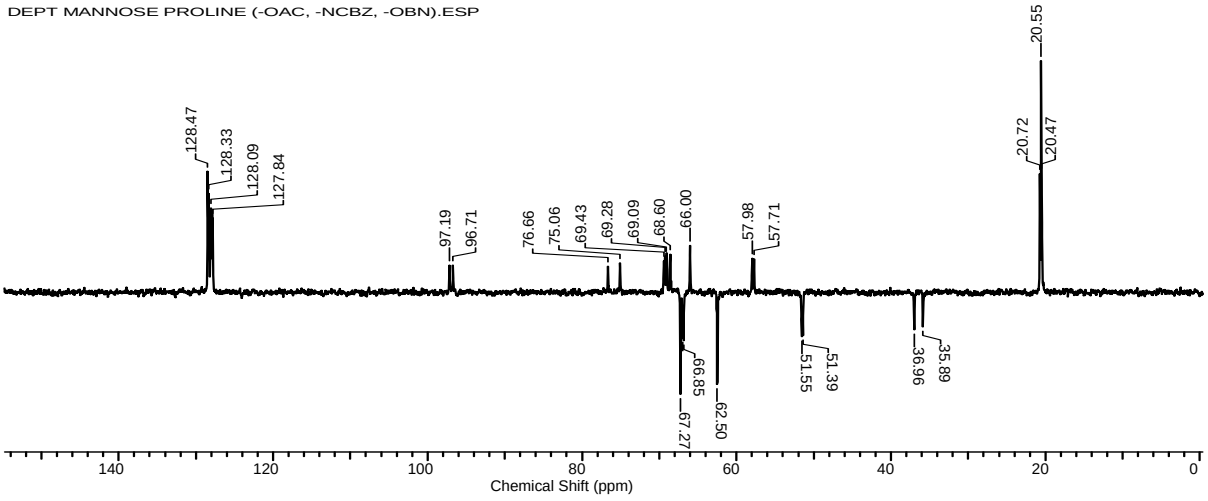
^{1}H NMR Spectrum (200.13 MHz, $CDCl_3$) of Compound **26**



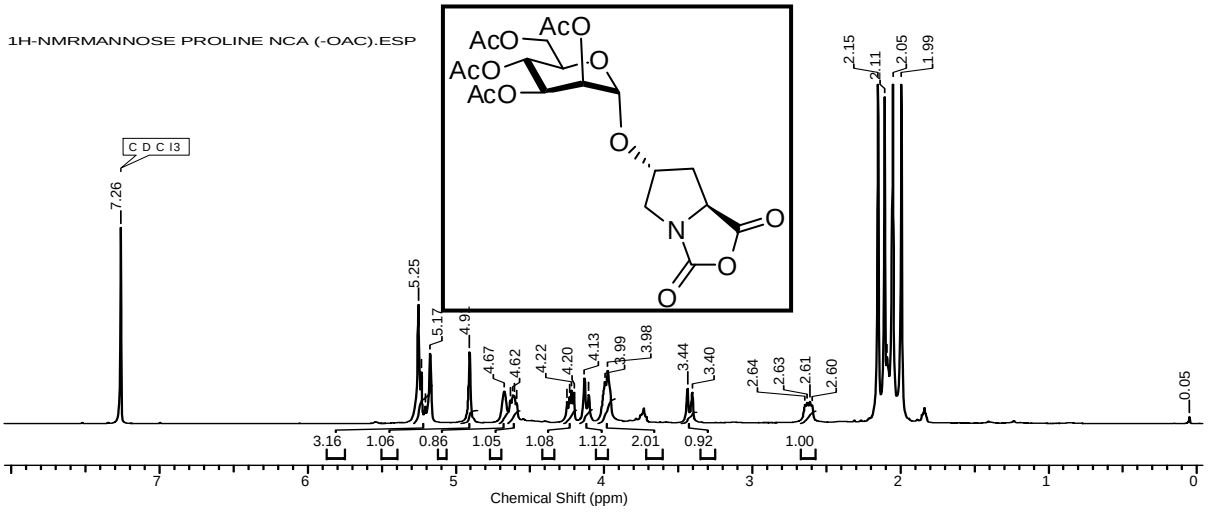
^{13}C NMR Spectrum (50.32 MHz, $CDCl_3$) of Compound **26**



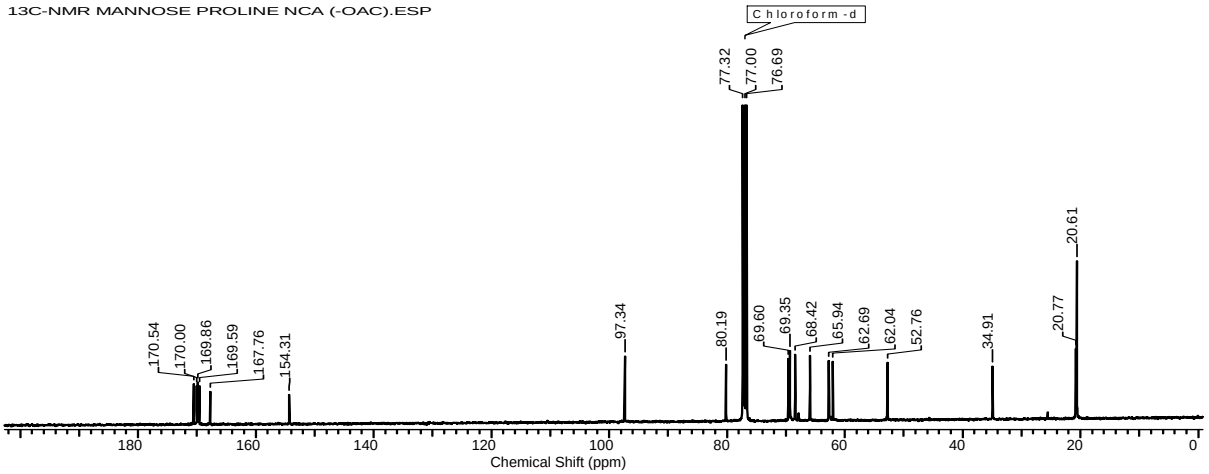
DEPT Spectrum (50.32 MHz, $CDCl_3$) of Compound **26**



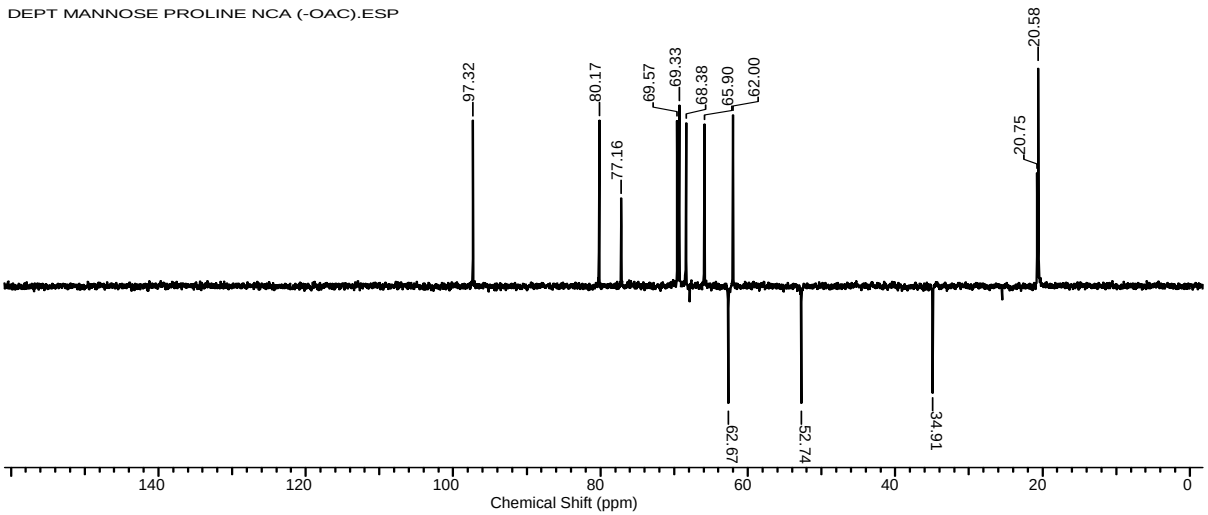
^{1}H NMR Spectrum (400.13 MHz, $CDCl_3$) of Compound **28**



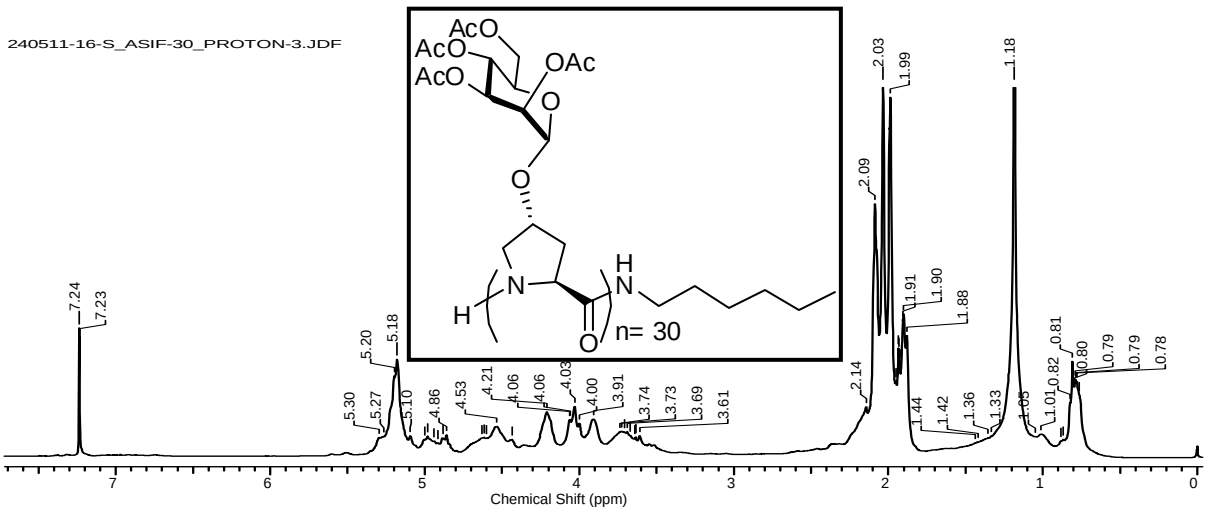
^{13}C NMR Spectrum (100.61 MHz, $CDCl_3$) of Compound **28**



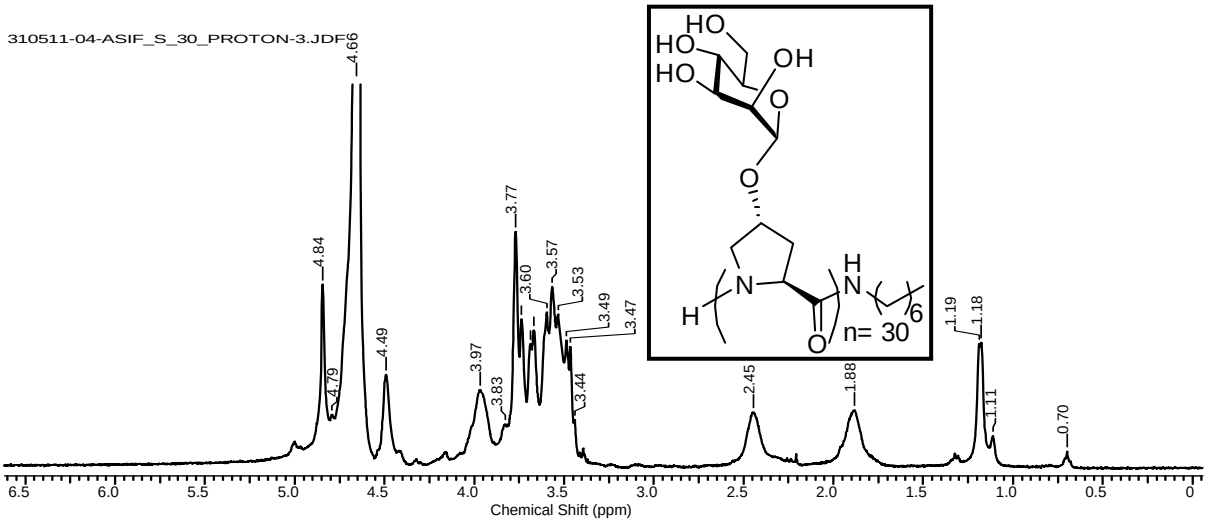
DEPT Spectrum (100.61 MHz, $CDCl_3$) of Compound **28**



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



^{1}H NMR Spectrum (399.78 MHz, D_2O) of Compound **30**



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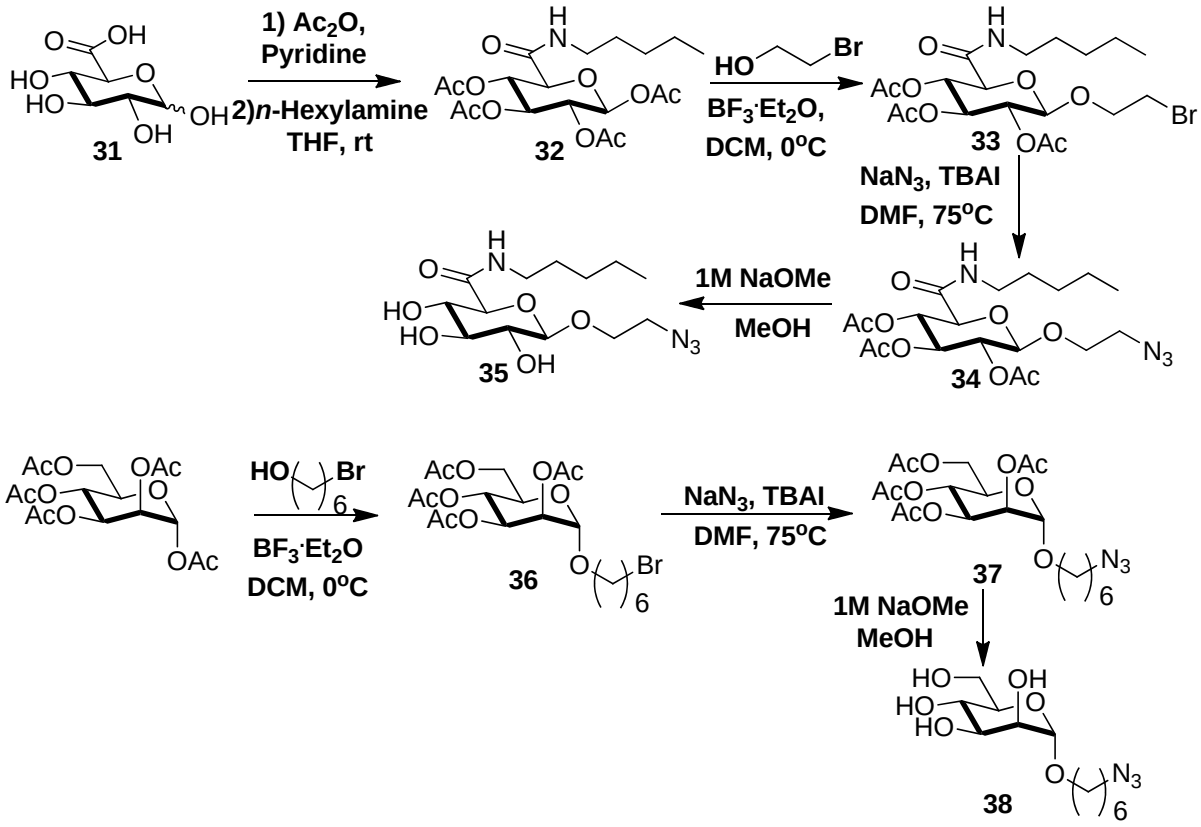
Section IB

Gluconoric acid application

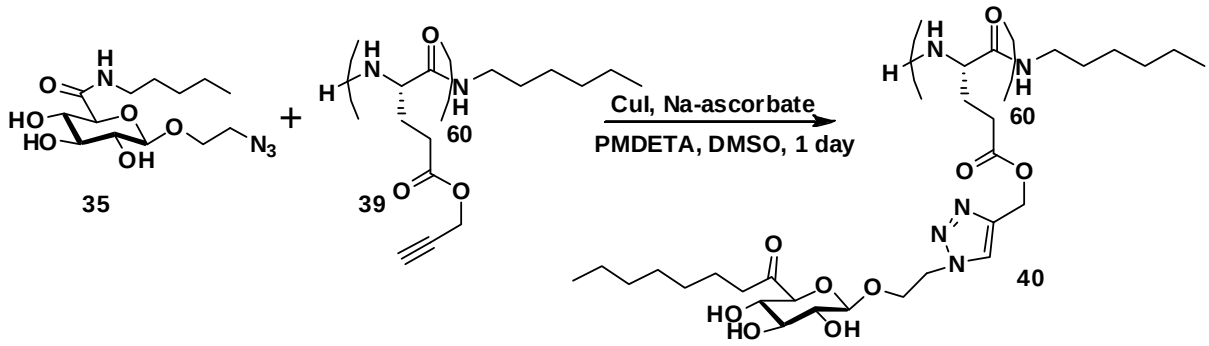
Gluconoric acid is an oxidized form of glucose at *C*-6 position, which gives handle to attach many hydrophobic as well as hydrophilic moieties to form self assemble structure. Sometimes simple modification at *C*-6 position is more difficult and required good strategy to get modified carbohydrates. In this section we used different approach to get carbohydrate functionalized glycopolypeptide for self assemblies. Conjugation of carbohydrate to the alkyne side chain functionalized glycopolypeptide can be achieved through copper catalyzed Huisgen's 1,3-dipolar cycloaddition reaction between alkyne and azide functional group. Accordingly commercially available gluconoric acid was peracetylated using acetic anhydride as reported by Paul Murphy and co-workers.[72] Treatment of per-*O*-acetylated gluconoric anhydride with aliphatic primary amine i.e. *n*-hexylamine gives corresponding hexylamide derivative of per-*O*-acetylated gluconoric acid.



Scheme 10: Synthesis of Azido derivatives of Hexyl Gluconoramide and Mannose

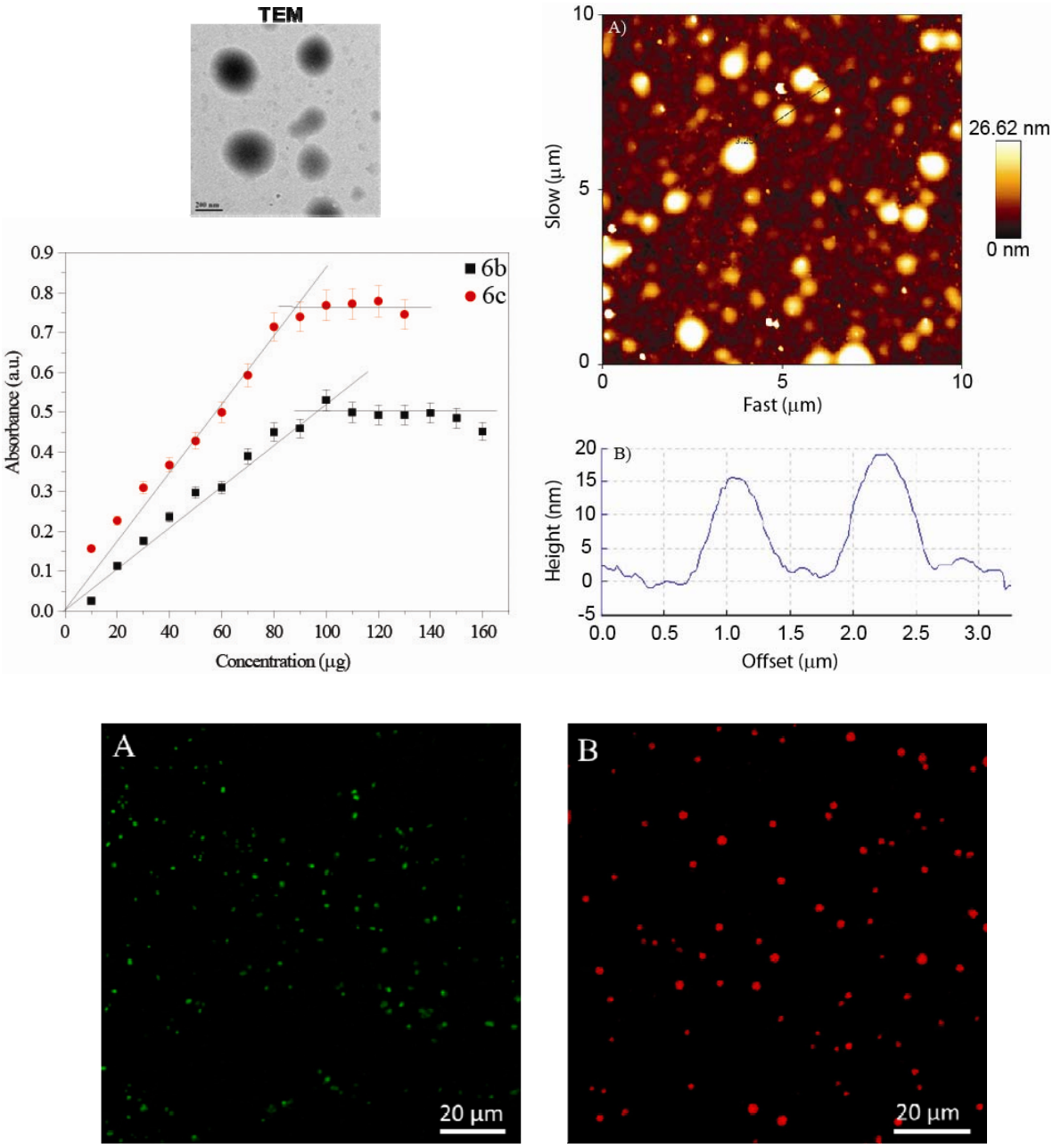
Hexyl gluconoramide derivative was converted into glycosyl bromide using HBr solution in acetic acid in dichloromethane. Then hexyl gluconoric bromide was treated with propargyl alcohol, tetra-*n*-butylammoniumiodide and 2,6-lutidine in dry dichloromethane at 70 °C for 24 h to get corresponding propargyl-1,2-orthester derivative of gluconoric acid. After this we tried glycosylation with hexyl and dodecyl derivative of propargyl orthoester using $AuBr_3$ and $HAuCl_4.3H_2O$ but we could not succeed to get direct glycoside and got *trans* orthoester in some cases. Hexyl-per-*O*-acetylated gluconoramide **32** was treated with 2-bromo-ethanol in presence of $BF_3.Et_2O$ to get glycosylated bromo derivative **33** under Fischer glycosylation condition. Displacement of bromide by azido group was carried out by treatment with NaN_3 in dimethyl formamide at 80 °C for 6 h got compound **34**. Complete acetate deprotection of azido derivative of gluconoric amide **35** was carried out by standard Zemplén condition using sodium methoxide solution in methanol to get. After complete deprotection seen on TLC, the reaction mixture was acidified to neutral pH by treatment with amberlite IR 120 H^+ and simple gravity column chromatography was performed to obtain corresponding azido functionalized hexyl derivative of gluconoric amide **35**. Similarly we have synthesized azido derivative of mannose was synthesized following same sequence of synthetic reaction explained for hexyl derivative **38** (Scheme 10).

Azido derivatives were synthesized to check self assembling properties of carbohydrate functionalized glycopolypeptide and their use in targeted drug delivery using specific



Scheme 11: Click Reaction of Azido derivatives and Poly(γ-propargyl glutamic acid)

carbohydrates. Vinitia et al. attached azido derivatives of gluconoric acid to alkyne side chain functionalized glycopolypeptide using click chemistry approach. They observed formation of micelle in case of hexyl functionalized gluconoric amide derivative using phase separation method. Formation of micelle was thoroughly characterized by the analytical methods such as TEM images, AFM images and DLS measurement as shown below.



Confocal microscope image of PPLG-Glu (**40**) nanoassembly encapsulated with (A) calcein and (B) nile red

This is also supported by the hydrophobic and hydrophilic dye experiment and analyzing sample under confocal microscope. Also we have checked the surface functionalization of micelle by mannose residue was studied using ConA binding assay following UV measurement at 280 nm. Most of the time nano assemblies only encapsulate hydrophilic drug molecule whereas current results showed that it could be possible to encapsulate both the hydrophobic as well as hydrophilic drug molecule inside micelles.

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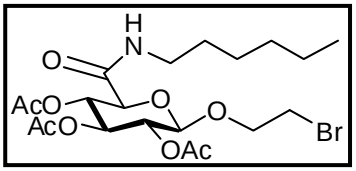
Preparation of Glucuronic acid anhydride

D-Glucuronic acid (5 g, 25.75 mmol) was suspended in acetic anhydride (75 mL) as solvent and stirred at 0 ^{o}C. Iodine (350 mg) was added slowly and the red solution was stirred for 2 h in ice, then further 3 h at room temperature. After completion of the reaction, excess acetic anhydride was mostly removed *in vacuo* and the remaining mixture was taken up in dichloromethane (70 mL). The organic layer was then washed twice with $Na_2S_2O_3$ (1 M 80 mL), dried on Na_2SO_4 and concentrated to afford peracetylated glucuronic anhydride as a white solid (10 g, 96%)

Preparation of Hexyl 1,2,3,4,-Tetra-O-acetyl glucuronic amide (32)

To a solution of anhydride (5 g, 12.37 mmol) in dry dichloromethane (50 mL) and under nitrogen atmosphere an equivalent of primary amine (1.06 mL, 14.84 mmol) was added, and the reaction mixture was stirred overnight. The product was extracted into dichloromethane and washed with HCl (dil.), $NaHCO_3$ (sat.), deionized water and dried on Na_2SO_4. The organic layer was concentrated after filtration to afford the amide **32** as a white solid, which was further, purified by column chromatography (2.75 g, 50%).

Preparation of (2-bromethyl)-hexyl-2,3,4,-Tri-O-acetyl glucuronic amide (33)



Hexyl 1,2,3,4,-Tetra-O-acetyl glucuronic amide **32** (2.5 g, 5.61 mmol) and 2-bromoethanol (0.564 mL, 6.73 mmol) in dry dichloromethane was placed in 100 mL round bottom flask in the dark and fitted with a dropping funnel. The solution was cooled to 0°C and then $BF_3.Et_2O$ (5 mL) was added dropwise over period of 25 minutes. The reaction mixture was then stirred at 0°C for 1h and then at room temperature for 12 h. Completion of reaction was monitored by TLC. The reaction mixture was diluted by dichloromethane and poured onto ice water while stirring. The organic layer was separated and washed successively with water, saturated sodium bicarbonate and brine. The organic layer was dried on Na_2SO_4, concentrated on rotary evaporator and the resulting residue was purified by column chromatography on silica gel using ethyl acetate-petroleum ether as eluent **33** (1.43 g, 50%).

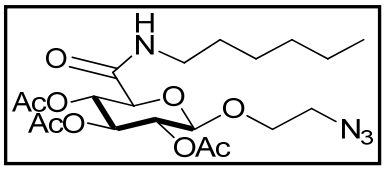
$[\alpha]^{RT}_{D}$ (c = 1.0 M, $CHCl_3$) = −12.3

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 0.89 (t, *J* = 6.4 Hz, 3H), 1.29 (m, 6H), 1.50 (m, 2H), 2.02 (s, 3H), 2.07 (s, 3H), 2.08 (s,3H), 3.22 (m, 2H), 3.48 (t, *J* = 6.0 Hz, 2H), 3.82-3.90 (m, 1H), 3.92 (d, *J* = 9.73 Hz, 1H), 4.18 (m, 1H), 4.63 (d, *J* = 7.95 Hz, 1H), 5.0 (dd, *J* = 8.1, 9.4 Hz, 1H), 5.1 (t, *J* = 9.60 Hz, 1H), 5.29 (t, *J* = 9.47 Hz, 1H), 6.42 (t, *J* = 5.60, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 13.9, 20.5, 20.7(2C), 22.5, 26.5, 29.2, 29.8, 31.4, 39.1, 69.6, 70.2, 70.9, 71.7, 72.7, 100.7, 166.2, 169.4, 169.6, 169.9

HRMS (ESI): calcd for $C_{20}H_{32}BrNNaO_9$ $[M + Na]^+$ 532.1158, found 532.1158

Preparation of (2-azidoethyl)-hexyl-2,3,4-Tri-*O*-acetyl glucuronic amide (34)



Bromo compound **33** (1.4g, 2.74 mmol)) was dissolved in dry DMF in round-bottomed flask equipped with a condenser. The solution was stirred with sodium azide (0.534 g, 8.23 mmol) and a catalytic amount of *n*-tetrabutylammoniumiodide at 70°C until the displacement reaction was complete. After completion of the reaction, the reaction mixture was diluted with diethyl ether and DMF was removed by successive washes with saturated brine solution. The organic layer was dried over Na_2SO_4 and concentrated on the rotary evaporator. Crude reaction mixture was purified by column chromatography to get azido derivative **34** (1.24 g, 96%).

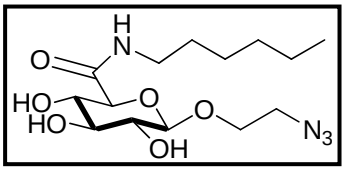
$[\alpha]^{RT}_{D}$ ($CHCl_3$, c 1.0) = −25.8

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 0.82 (t, *J* = 6.4 Hz, 3H), 1.23 (m, 6H), 1.43 (m, 2H), 1.94 (s, 3H), 1.99 (s, 3H), 2.00 (s,3H), 3.15 (m, 2H), 3.35 (m, 2H), 3.7 (m, 1H), 3.85 (d, *J* = 9.72 Hz, 1H), 3.95 (m, 1H), 4.07 (t, *J* = 7.20 Hz, 1H), 4.58 (d, *J* = 8.08 Hz, 1H), 5.0 (m, 2H), 5.2 (t, *J* = 9.40 Hz, 1H), 6.37 (t, *J* = 5.55 Hz, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 13.9, 20.5, 20.6(2C), 22.5, 26.4, 29.1, 31.4, 39.2, 50.4, 69.1, 69.6, 71.0, 71.9, 72.7, 100.6, 166.3, 169.4, 169.7, 169.9

HRMS (ESI): calcd for $C_{20}H_{32}N_4NaO_9$ $[M + Na]^+$ 495.2067, found 495.2064

Preparation of (2-azidoethyl)-hexyl glucuronic amide (35)



An ice-cold solution of the acetylated glucoside 3**4** (1.2 g, 2.54 mmol) in dry MeOH (10 mL) was treated with freshly prepared sodium methoxide (0.411g, 7.62 mmol). The reaction mixture was stirred at 0°C until the reaction was complete (after approx. 30 min), then it was neutralized with ion exchange resin (IR120, H^+)**.** The resin was filtered off and the filtrate was concentrated *in vacuo* to obtain (2-azidoethyl)-hexyl glucuronic amide **35** (0.862 g, 98%).

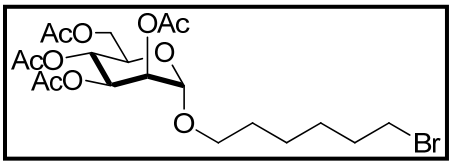
$[\alpha]^{RT}_D$ (CH_3OH, c 1.0) = −42.2

^{1}H NMR (200.13 MHz, CH_3OH-d_4): δ (ppm) 0.89 (t, *J* = 6.57 Hz, 3H), 1.29 (m, 6H), 1.51 (m, 2H), 3.24 (m, 2H), 3.42-3.74 (m, 4H), 3.79-4.02 (m, 3H), 4.50-4.90 (5H), 6.90 (t, *J* = 5.1 Hz, 1H)

^{13}C NMR (50.32 MHz, CH_3OH-d_4): δ (ppm) 13.9, 22.5, 26.5, 29.2, 29.1, 31.4, 39.2, 50.6, 68.6, 72.0, 72.7, 73.3, 75.6, 102.6, 170.1

HRMS (ESI): calcd for $C_{14}H_{26}N_4NaO_6$ $[M + Na]^+$ 369.1750, found 369.1749

Preparation of (6-bromohexyl) 2,3,4,6-tetra-*O*-acetyl-mannopyranoside (36)



$BF_3.Et_2O$ was added dropwise over a period of 25 minutes to a solution of per-*O*-acetylated mannosides (3 g, 7.69 mmol) and 6-bromohexanol (1.13 g, 9.22 mmol) in dry dichloromethane placed in the dark at 0°C. The reaction mixture was then stirred at 0°C for 1 h and at room temperature for a further 12 h. Completion of reaction was monitored by TLC. The reaction mixture was diluted by dichloromethane and poured onto ice water while stirring. The organic layer was separated and washed successively with water, saturated sodium bicarbonate and brine. The organic layer was dried on Na_2SO_4, concentrated on rotary evaporator and the resulting residue was purified by column chromatography on silica gel using ethyl acetate-petroleum ether as eluent to gave **36** (2.36 g, 60%).

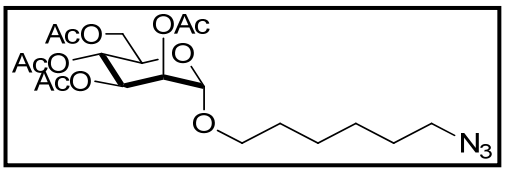
$[\alpha]^{RT}_D$ ($CHCl_3$ c 1.0) = +34.9

^{1}H NMR (200.13 MHz, CDCl3): δ (ppm) 1.39-1.70 (m, 6H), 1.88 (m, 2H), 2.00 (s, 3H), 2.05(s, 3H), 2.10 (s, 3H), 2.20 (s,3H), 3.37-3.51 (m, 3H), 3.70 (dt, *J* = 9.6, 6.5 Hz, 1H), 4.00 (m, 1H), 4.07-4.14 (m,1H), 4.30 (m, 1H), 4.80 (d, *J* = 1.52 Hz, 1H), 5.22-5.40 (m, 3H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.7(3C), 20.8, 25.3, 27.8, 29.0, 32.6, 33.7, 62.5, 66.2, 68.2, 68.4, 69.1, 69.7, 97.5, 169.7, 169.9, 170.1, 170.6

HRMS (ESI): calcd for $C_{20}H_{31}BrNaO_{10}$ $[M + Na]^+$ 533.0998, found 533.0992

Preparation of (6-azidohexyl) 2,3,4,6-tetra-*O*-acetyl-mannopyranoside (37)



Same procedure followed which is described above for **34**.

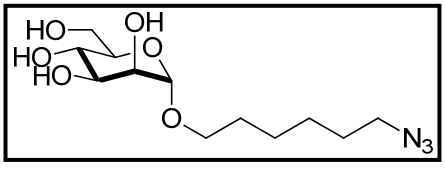
[α]$^{RT}_{D}$ ($CHCl_3$, c 1.0) = +41.5

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 1.40 (m, 4H), 1.60 (m, 4H), 2.00 (s, 3H), 2.05 (s, 3H), 2.10 (s,3H), 2.16(s, 3H), 3.2 (t, *J* = 6.70, 2H), 3.45 (m, 1H), 3.70 (m, 1H), 3.98 (m, 1H), 4.10 (dd, *J* = 12.25, 2.4 Hz, 1H), 4.30 (m, 1H), 4.80 (d, *J* = 1.39 Hz, 1H), 5.20-5.40 (m, 3H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.6(3C), 20.8, 25.6, 26.4, 28.7, 29.0, 51.3, 62.5, 66.2, 68.2, 68.4, 69.0, 69.6, 97.5, 169.7, 169.8, 170.0, 170.6

HRMS (ESI): calcd for $C_{20}H_{31}N_3NaO_{10}$ $[M + Na]^+$ 496.1907, found 496.1913

Preparation of (6-azidohexyl)-mannopyranoside (38)



Same procedure followed which is described above for **5**.

[α]$^{RT}_{D}$ (CH_3OH, c 1.0) = +49.5

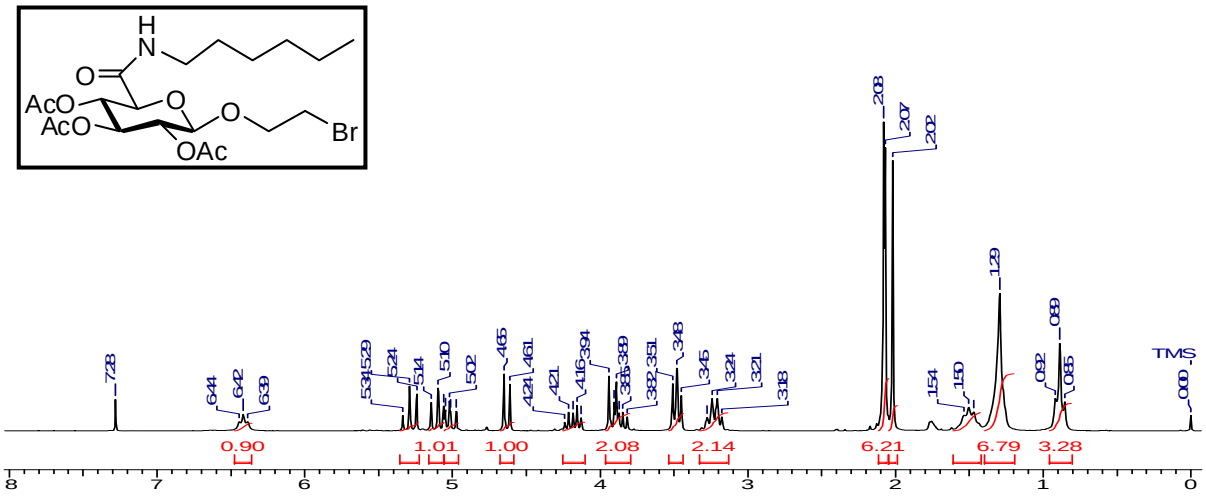
^{1}H NMR (200.13 MHz, CD_3OD): δ (ppm) 1.25-1.35 (m, 4H), 1.45-1.60 (m, 4H), 3.21 (t, *J* = 6.7 Hz, 2H), 3.30-3.44 (m, 2H), 3.53-3.90 (m,10H), 4.70 (d, *J* = 1.0 Hz, 1H)

^{13}C NMR (50.32 MHz, CDOD): δ (ppm) 25.5, 26.3, 28.5, 29.0, 51.2, 60.8, 66.2, 67.4, 70.7, 71.2, 72.1, 99.9

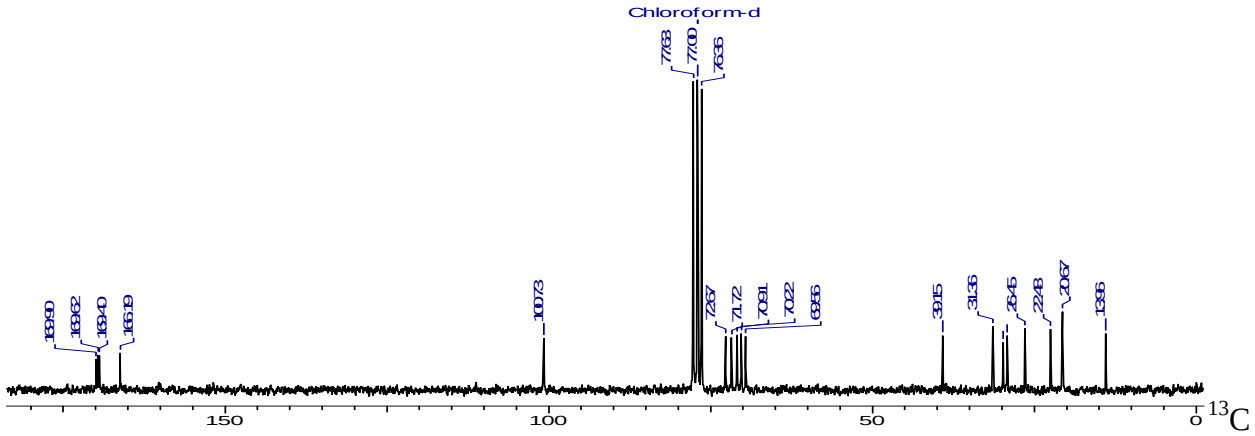
HRMS (ESI): calcd for $C_{12}H_{23}N_3NaO_6$ $[M + Na]^+$ 328.1485, found 328.1487

*****

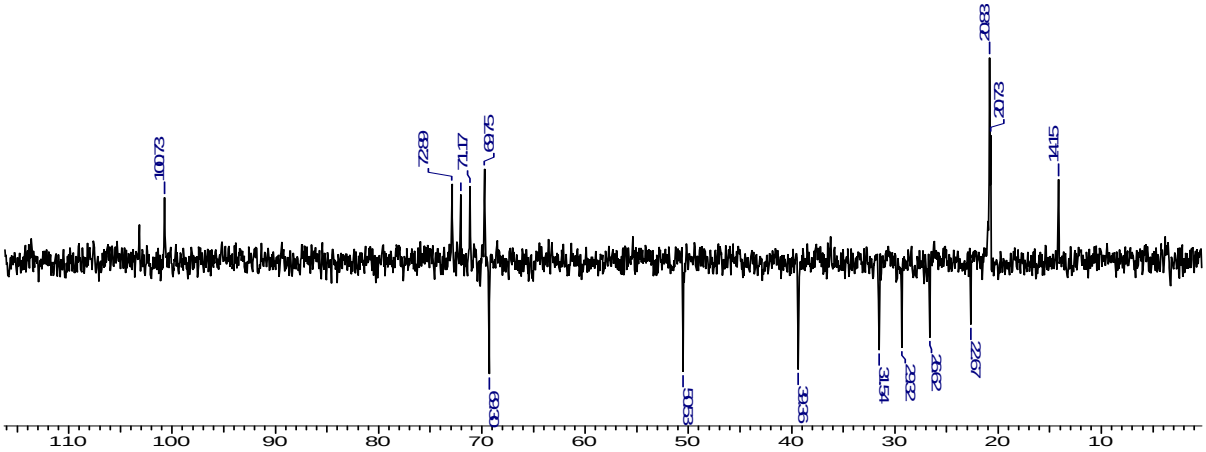
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **33**



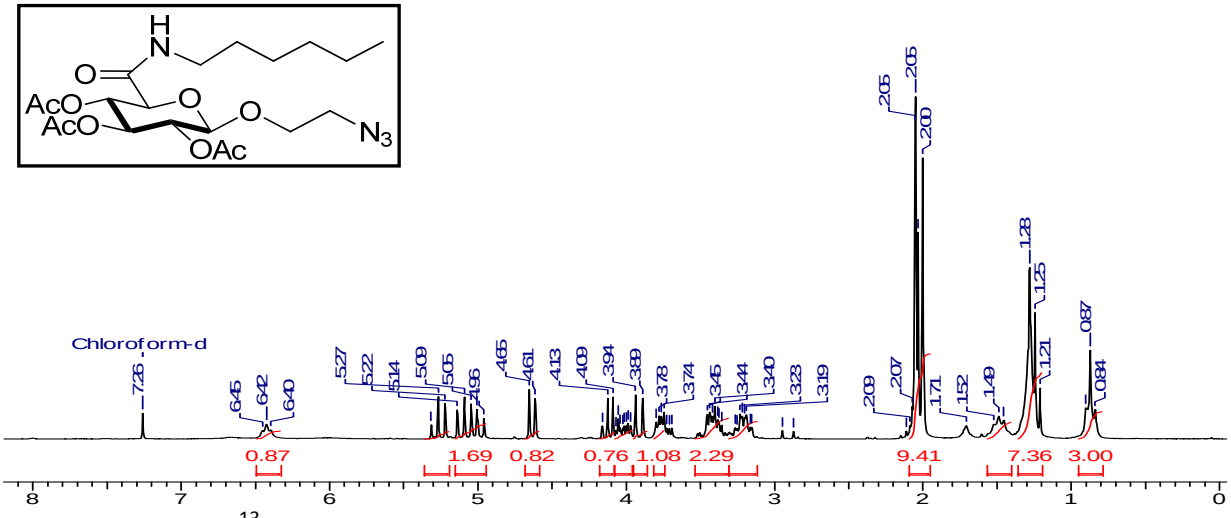
^{13}C NMR Spectrum (50.32 MHz, CDCl3) of Compound **33**



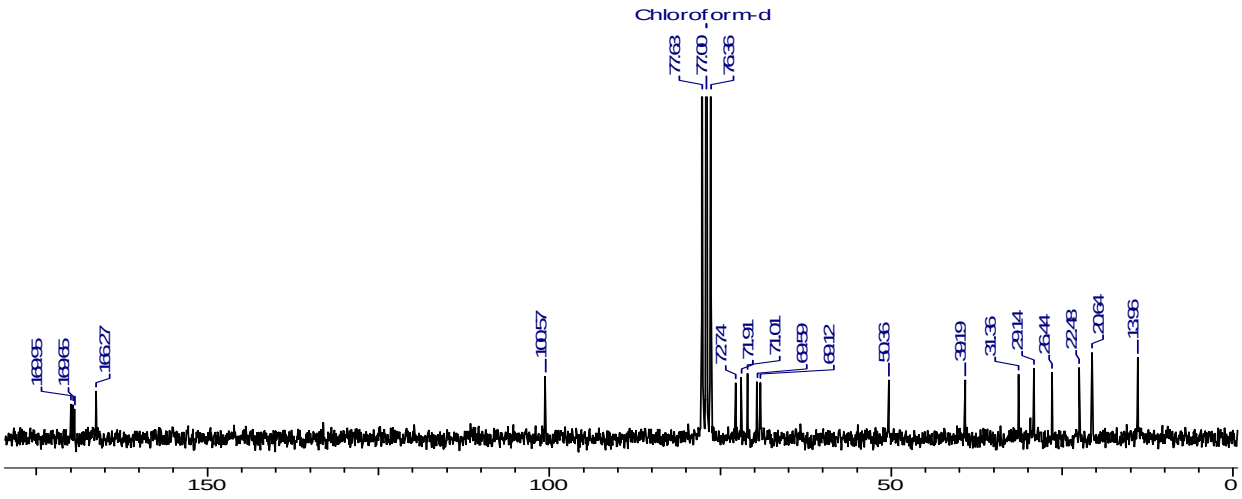
DEPT Spectrum (50.32 MHz, CDCl3) of Compound **33**



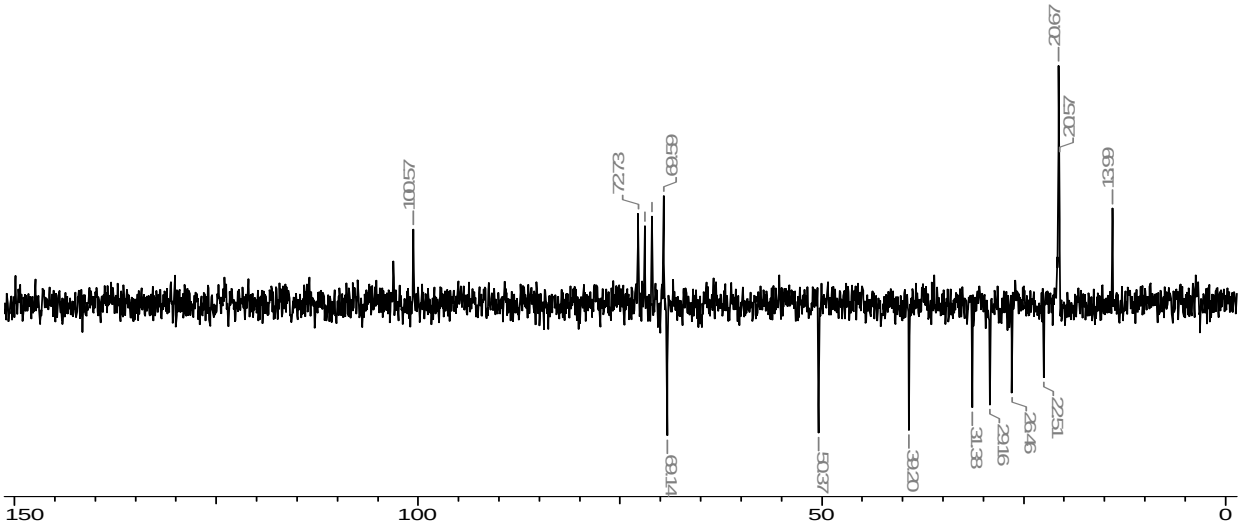
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **34**



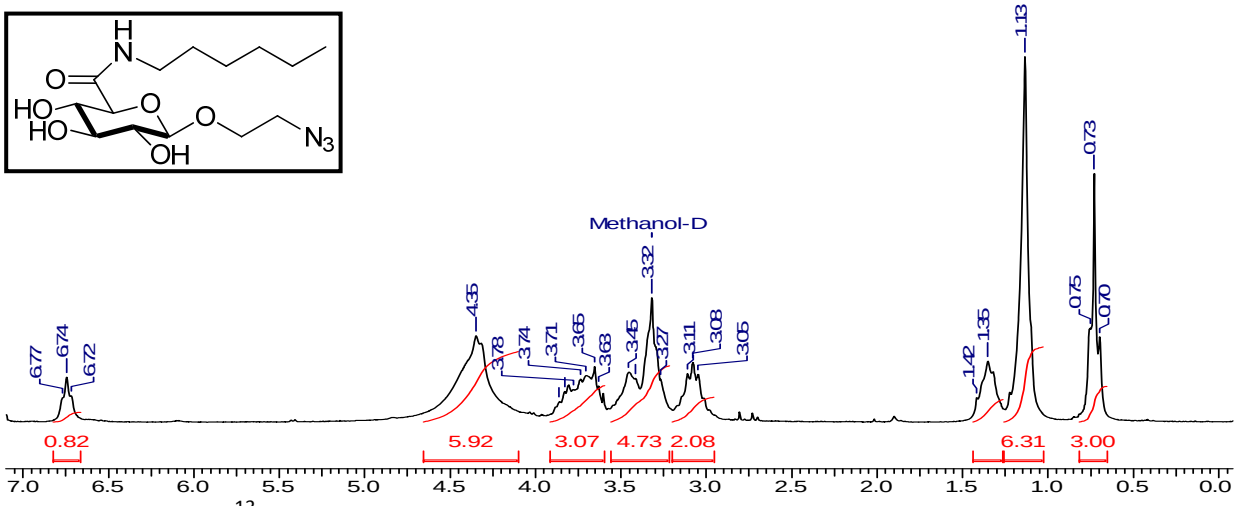
^{13}C NMR Spectrum (50.32 MHz, CDCl3) of Compound **34**



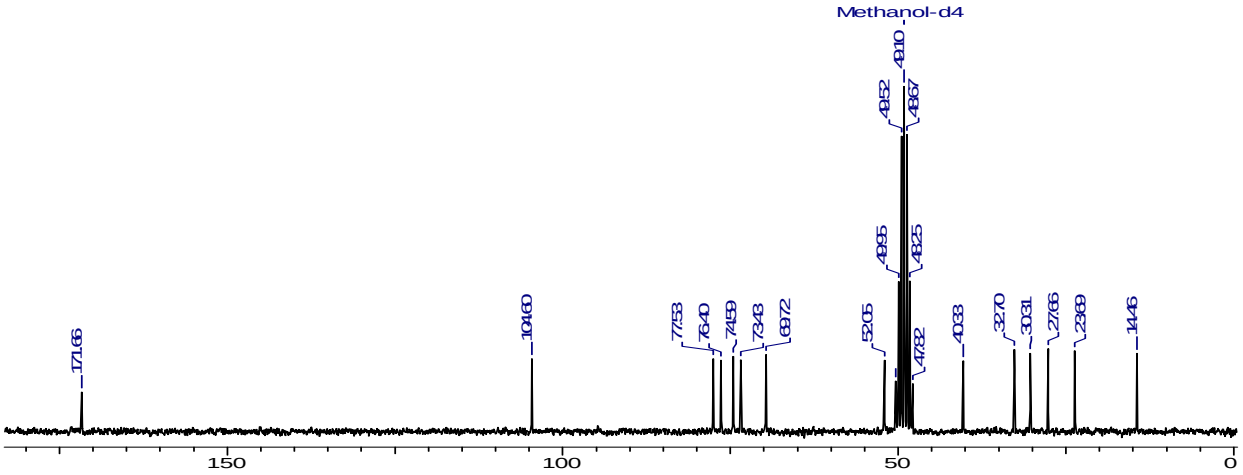
DEPT Spectrum (50.32 MHz, CDCl3) of Compound **34**



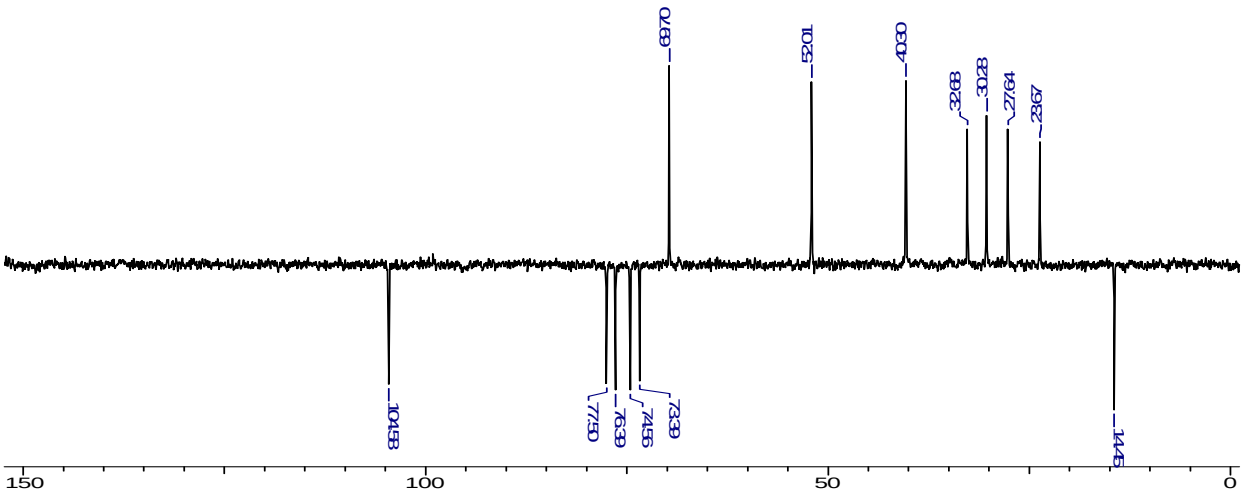
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **35**



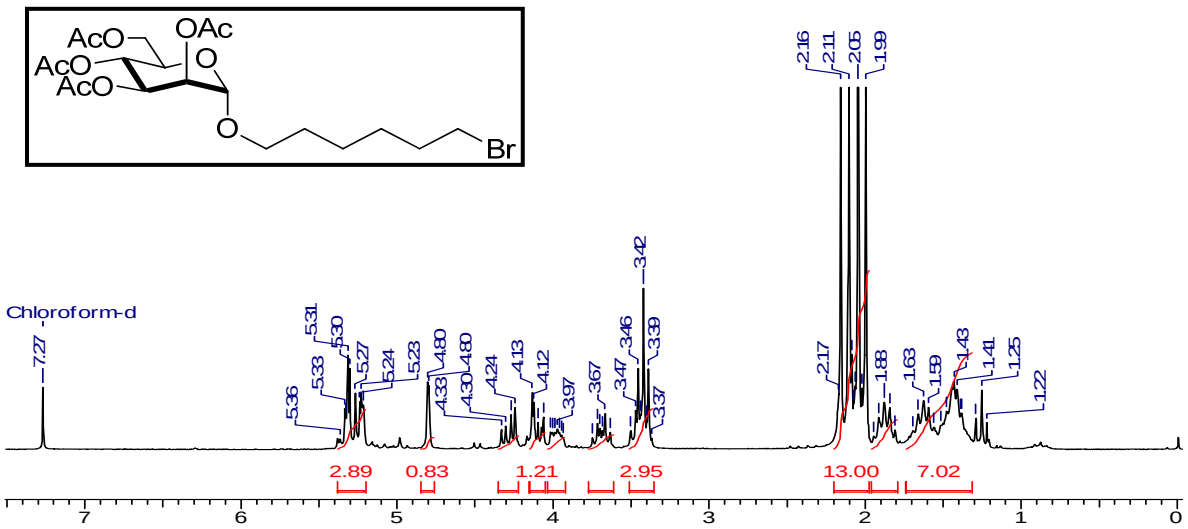
^{13}C-NMR Spectrum (50.32 MHz, CDCl3) of Compound **35**



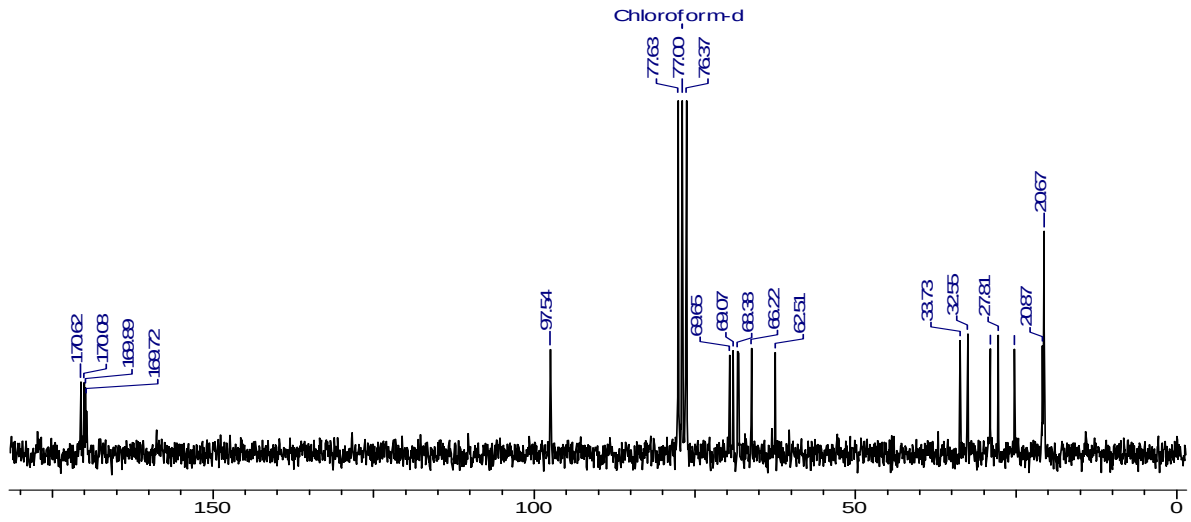
DEPT Spectrum (50.32 MHz, CDCl3) of Compound **35**



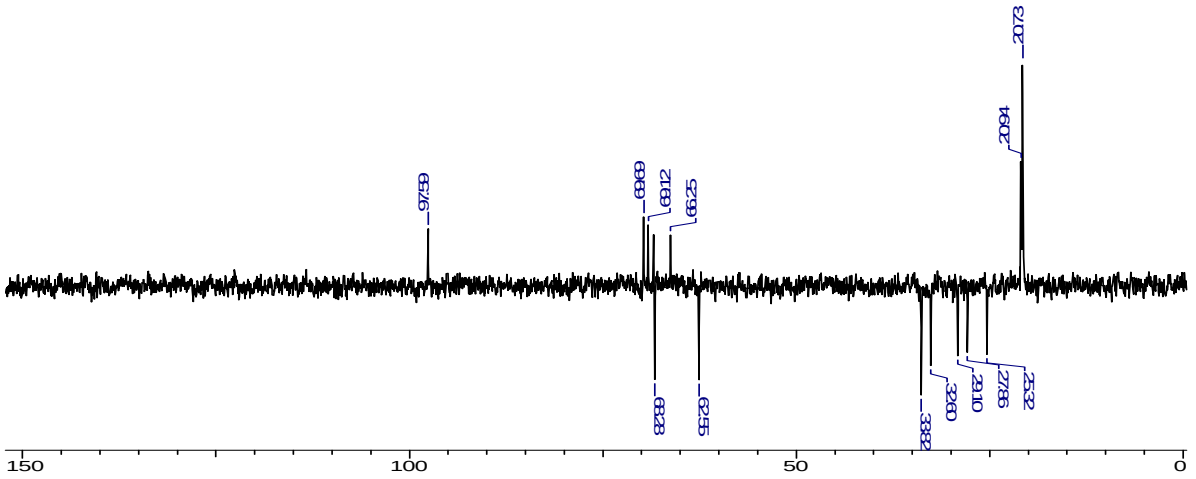
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **36**



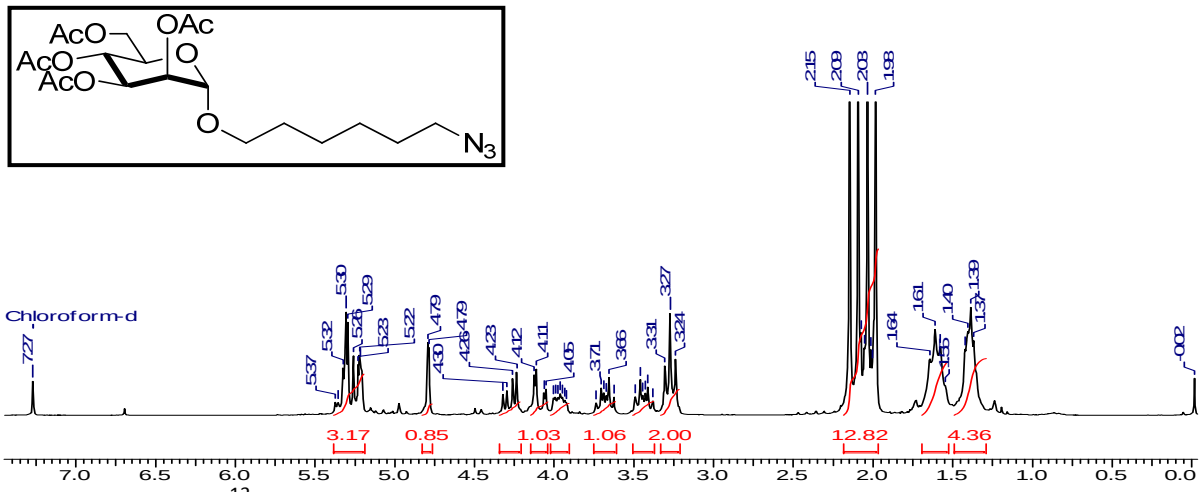
^{13}C-NMR Spectrum (50.32 MHz, CDCl3) of Compound **36**



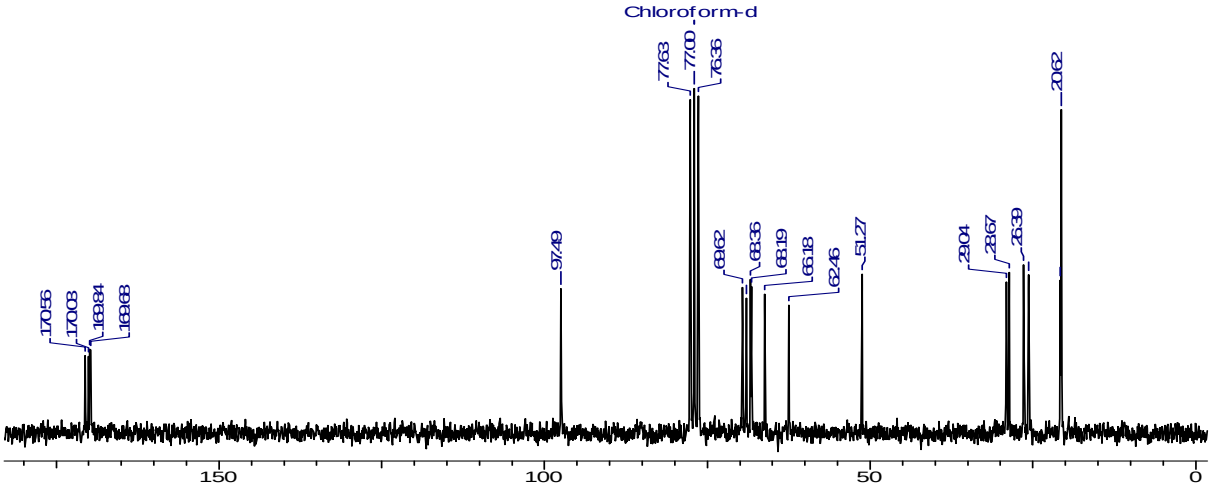
DEPT Spectrum (50.32 MHz, CDCl3) of Compound **36**



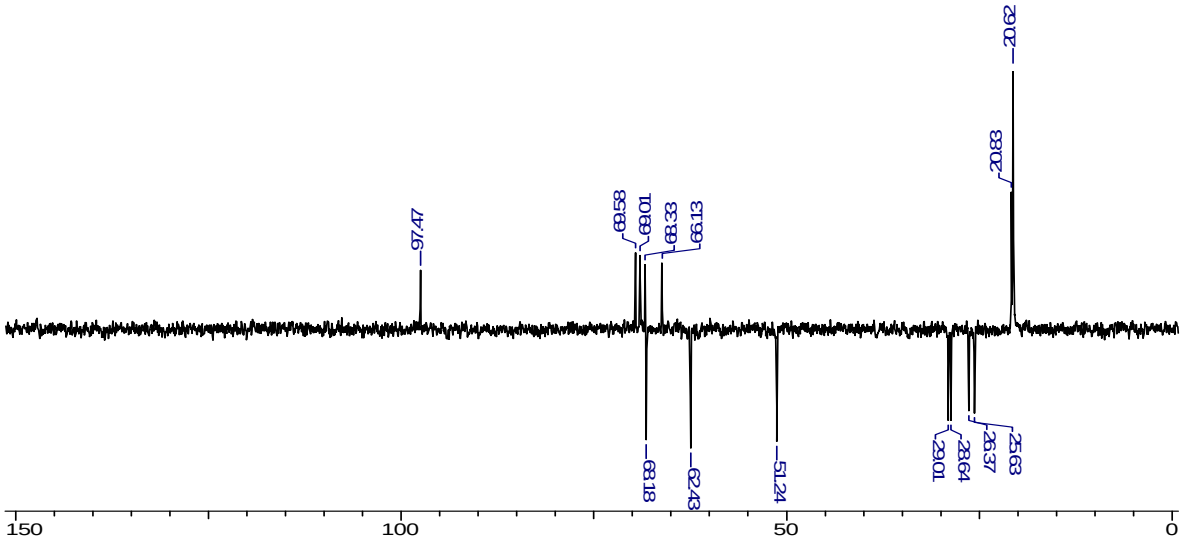
^{1}H NMR (400.13MHz, $CDCl_3$) Spectrum of Compound **37**



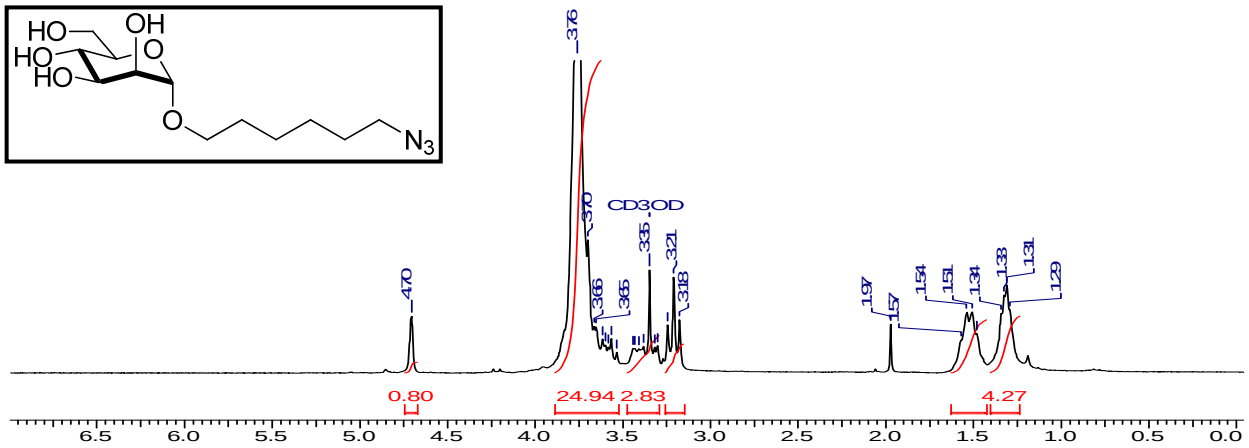
^{13}C-NMR Spectrum (50.32 MHz, CDCl3) of Compound **37**



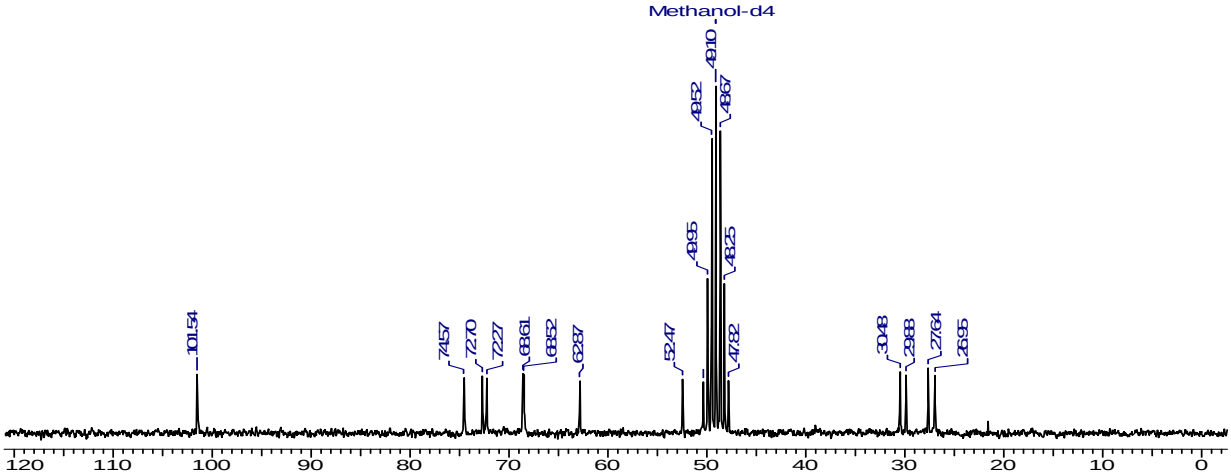
DEPT Spectrum (50.32 MHz, CDCl3) of Compound **37**



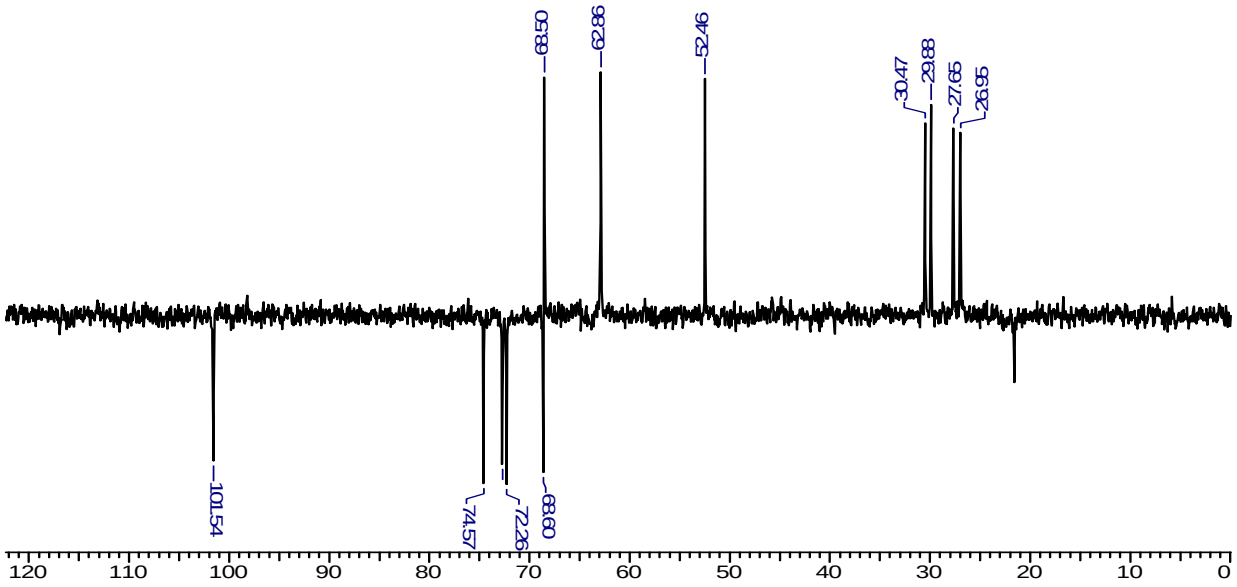
^{1}H NMR ($CDCl_3$, 400.13MHz) Spectrum of Compound **38**



^{13}C-NMR Spectrum (50.32 MHz, CDCl3) of Compound **38**



DEPT Spectrum (50.32 MHz, CDCl3) of Compound **38**



Section II

Synthesis of 6-Azido and 6-Amino Functionalized Glycopolypeptide for Hydro gel and Sortase activity

Previous sections described on synthesis of glycopolypeptides for polymer self assemblies. In this section, synthesis of azide incorporated monosaccharide into the glycopolypeptide back-bone which could be used in hydro gel formation and sortase activity. Attachment of carbohydrate to a peptide is a very difficult task for larger polypeptides and synthesis procedures available currently include tedious processes because of chain fold and incomplete functionalization. This gap can fill by using enzymes which can attach carbohydrate specifically to peptide and form covalent linkage. In addition, enzymes can perform ligation reaction very efficiently in complex system such as large proteins and GPI anchores.[73-75] Sortase enzymes are utilize as powerful tools for ligating peptides/protein to nucleic acid, lipids, and carbohydrates, and labelling proteins on living cell surfaces. Already Sortase catalyze reactions on bacterial cell wall known as "cell-wall sorting reactions", which anchors surface proteins onto bacterial cell walls. The reaction mechanism of sortase-catalyzed transpeptidation has been established Ellestad and co-worker.[76] There is one encouraging report by Roy & co-worker on peptide-sugar ligation catalyzed by transpeptidase Sortase,[77] so we thought to synthesize a glycopolymer with amino group at *C*-6 position of the carbohydrate moiety.

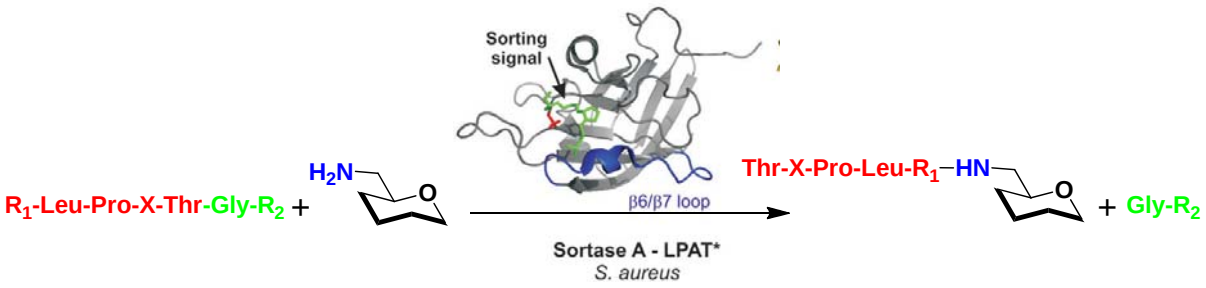


Figure 1:- Sortase A catalyzed Peptide-Sugar Ligation

How the ligations happens? Sortase A of *Staphylococcus aureus* specifically recognizes a LPXTG pentapeptide sequence motif located near the *C*-terminus of the target proteins, cleaves at Thr-Gly peptide bond, and catalyzes the formation of a new peptide bond between the threonyl carboxyl and 6-aminohexose. The transpeptidation reaction proceeds

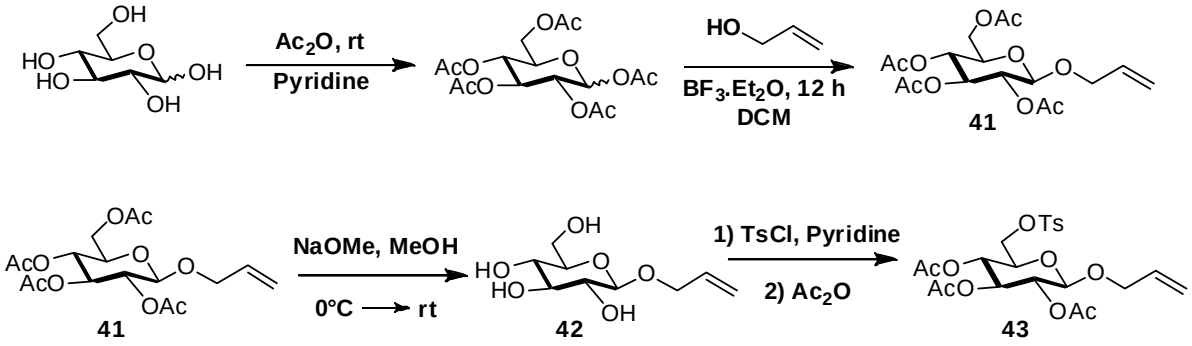
in two steps without the aid of any external molecule; the active site cysteine residue first attacks the target LPXTG substarte forming acyl-enzyme intermediate which in the second step was resolved by the nucleophilic attack of amino group of the terminal Gly residue of the peptidoglycan. In the absence of suitable amino nucleophile, the LPXTG peptide substrate was slowly hydrolyzed. The ability of sortase to ligate peptides to an aminoglycoside class is therapeutically important.[77]

Gold catalyzed methodology developed in our group gives excellent yield of glycosylation to get carbamate linked glycoaminoacid derivatives. We thought of utilizing the gold catalyzed glycosidation methodology for the synthesis of C-6 azido functionalized glyco-lysine derivative by reacting *C*-6 azido functionalized propargyl-1,2-glucose orthoester with N_{ε}-Boc-L-lysine-N_{α}-Cbz-OBn ester. For this *C*-6 azido derivative of per-*O*-acetylated glucose was synthesized but main problem was how convert anomeric –OAc into glycosyl bromide. Different approaches have been tried to get *C*-6 azido propargyl-1,2-orthoester of glucose but we could not succeed. The second problem was the deprotection of *N*-Cbz and –OBn ester of protected amino acid to get free glycosyl amino acid derivative selectively in presence of azido group.

In the meantime Deming's group published synthesis of glycopolypeptide having poly-L-lysine backbone using ring opening polymerization.[56] They have coupled side chain amine of L-lysine with the carboxylic acid derivative of carbohydrates to get carbohydrate functionalized L-lysine derivative subsequently transformed into *N*-carboxyanhydrides of glyco-L-lysine. In one experiments, we thought of using *C*-6 azido functionalized glucose residue instead of carboxylic acid.

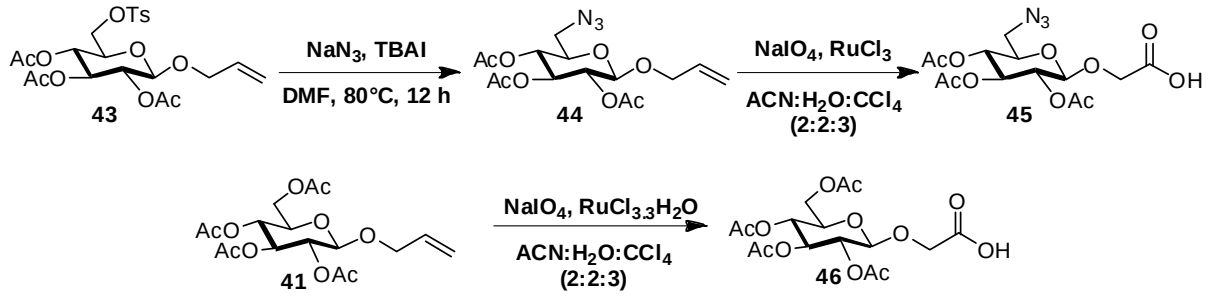
Accordingly allyl glucoside **41** of glucose derivative was obtained by carrying out reaction under Fischer conditions with allyl alcohol. The per-*O*-acetylated glucose **40** and allyl alcohol were added $BF_3.Et_2O$ slowly over period of 30 min under argon atmosphere and kept in dark for 12 h. Reaction was monitored by TLC and staining in anisaldehyde solution. After completion of reaction the reaction mixture was diluted with cold solution of dichloromethane and successive washing given to organic layer to get desired crude compound. Again the purification was carried out by column chromatography using ethyl-

acetated and petroleum ether and analysis by ^{1}H and ^{13}C NMR & mass spectroscopy (**Scheme 12**).



Scheme 12: Synthesis of 6-O-Tosyl-Allyl Glucoside derivative

Deacetylation of allyl glucoside **41** using NaOMe in methanol under Zemplén condition gave allyl 2,3,4,6-glucose **42** which was selectively protected at *C*-6 with tosyl group using p-toluenesulfonyl chloride and pyridine at 0 °C to get *C*-6 tosyl derivative **43** of ally glycoside. Further acetylation of purified *C*-6 tosyl derivative of allyl glucoside **42** using acetic anhydride and pyridine gave protected *C*-6 tosyl derivative of allyl glucoside **43**.

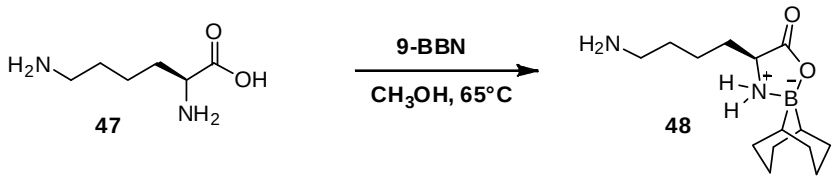


Scheme 13: Synthesis of 6-Azido-Glucose Acid derivative for Coupling with L-lysine 9-BBN Amino Acid Complex

Allyl glycoside of *C*-6 tosyl derivative of glucose **43** upon treatment with NaN_3 in dimethyl formamide at 70 °C for 6 h into corresponding *C*-6 azido derivative of glucose **44**. Further carboxylic acid derivative **45** was obtained by oxidative cleavage of the terminal double bond using $NaIO_4$ & $RuCl_3$ in acetonitrile, water and carbon tetrachloride which was developed by Sharpless & co-workers (Scheme 13).[78] The acid fragment of *C*-6 azide carbohydrate derivative **45** was directly used in coupling reaction with lysine derivative without any further purification.

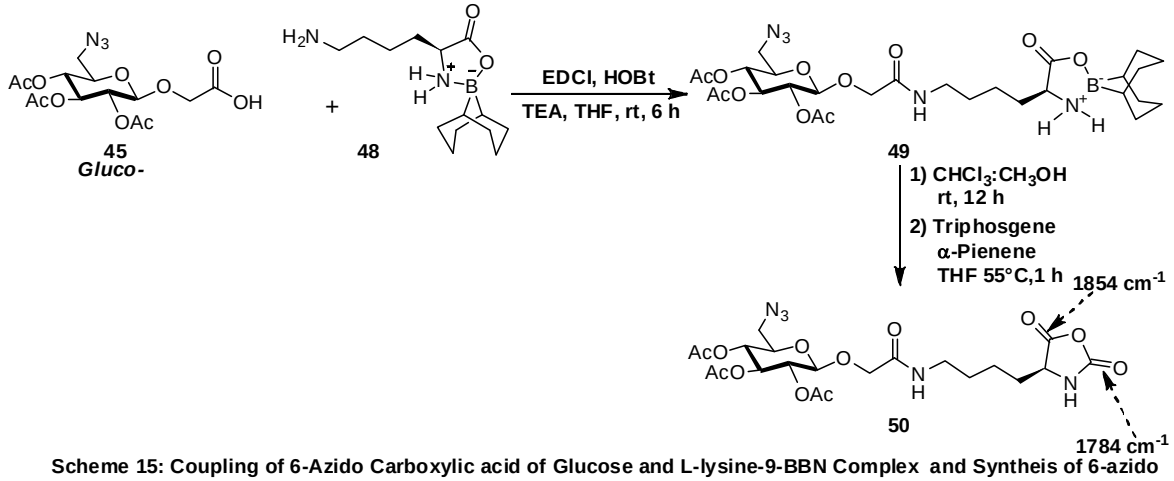
We have applied different strategy for protection of amine and acid group of lysine with single protecting group. The protecting group was chosen in such way that it could be stable

under coupling reaction conditions and selectively deprotected under mild conditions to get free amino acid carbohydrate derivative. Boron complex can serve as a very good masking agent for the amine and acid group of α-amino acid and we have chosen 9-BBN dimer for selective protection of L-lysine.[79] To a hot solution of L-lysine **47** in methanol was added 9-BBN dimer (solid) and refluxed until the complete dissolution of L-lysine. Solvents was evaporated and washed with hot tetrahydrofuran to remove excess of 9-BBN. L-Lysine complex was pure enough to use in next amide coupling reaction with acid derivative of *C*-6 azido derivative directly (Scheme 14).



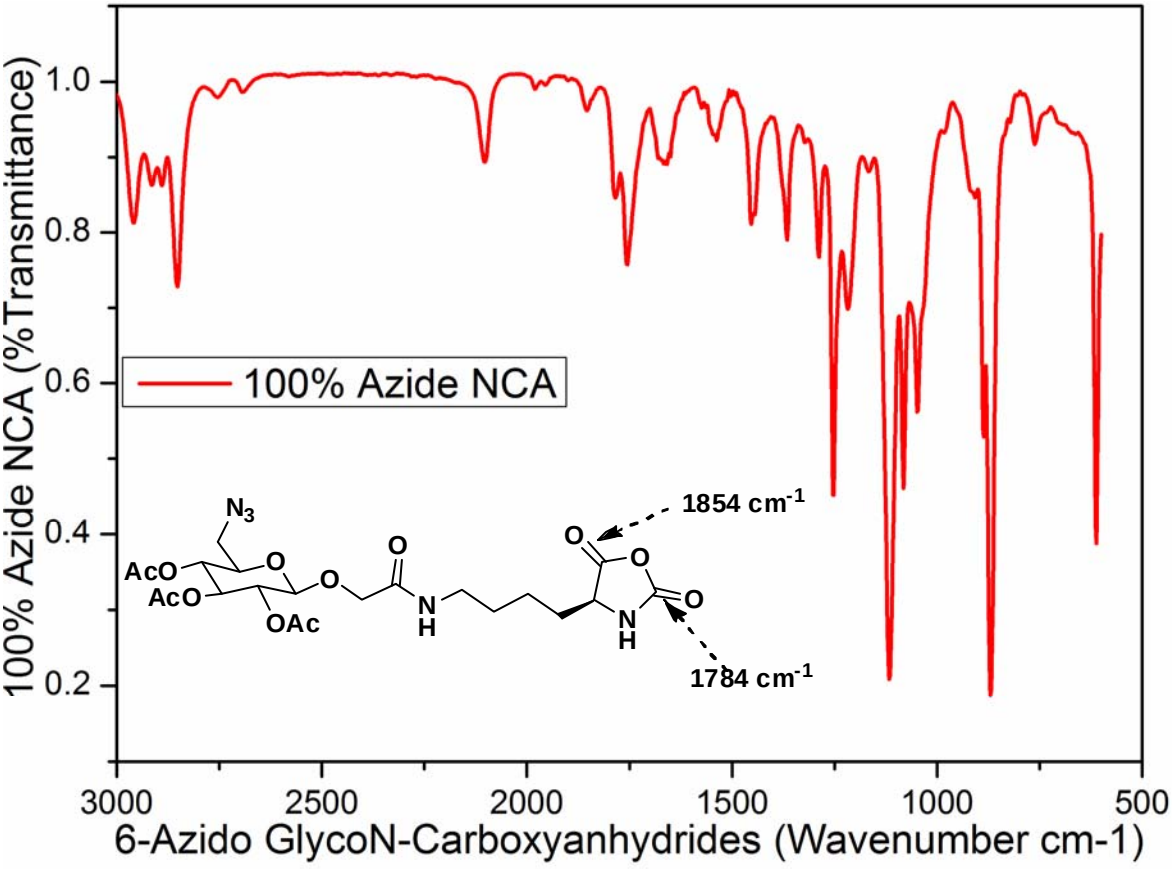
Scheme 14: Temporary Masking of α-amino acid using 9-BBN dimer

Having both the coupling partners in hand, the peptide coupling reaction between *C*-6 azido derivative of glucose **45** and the 9-BBN complex of L-lysine **48** by peptide coupling was performed using 1-ethyl-3-(3 dimethylaminopropyl)carbodiimide) (EDCI) and hydroxybenzotriazole (HOBt) to get corresponding *C*-azido carbohydrate coupled lysine derivative **49**. Purification of desired compound was done by simple gravity flow chromatography using ethyl acetate and methanol as eluent. The coupling product was confirmed by analysis of ^{1}H & ^{13}C NMR spectroscopic analysis. In ^{1}H NMR resonance at δ_H 4.54 ppm (d, J = 7.80 Hz, 1H) correspond to anomeric proton and also bridged two protons of 9-BBN resonance at 0.55 ppm due to shielding effect of boron atom and ^{13}C NMR δ_c at 100.2 ppm and methylene carbon at 22 ppm to 37 ppm respectively. Deprotection of 9-BBN

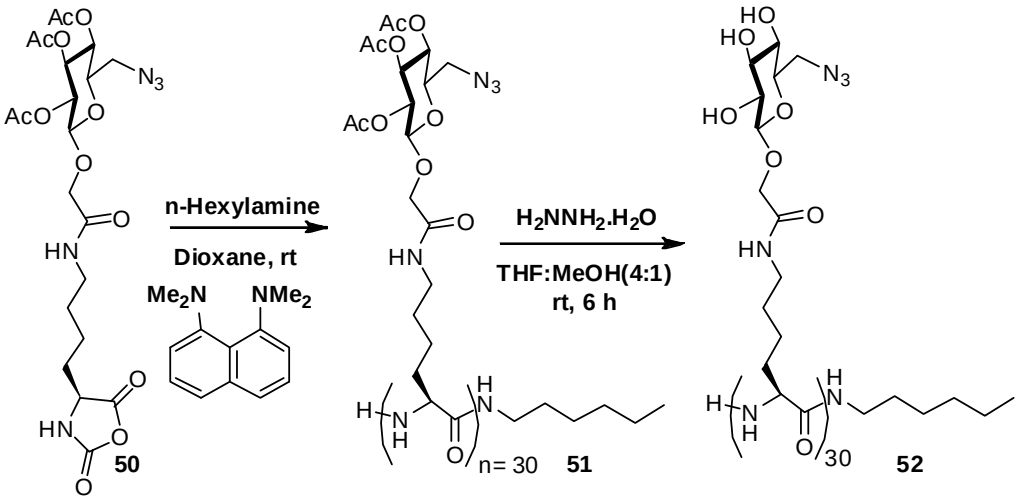


Scheme 15: Coupling of 6-Azido Carboxylic acid of Glucose and L-lysine-9-BBN Complex and Syntheis of 6-azido Glucose *N*-Carboxyanhydrides

was carried out under very mild condition using mixture of chloroform and methanol at room temperature for 12-24 h (Scheme 15). Glyco-aminoacid **49** was transformed into *N*-Carboxyanhydride **50** by treatment with triphosgene, α-pinene in tetrahydrofuran at 60 °C. Formation of *N*-Carboxyanhydride was thoroughly confirmed by FT-IR and ^{1}H & ^{13}C NMR analysis. In FT-IR stretches at 1784 cm^{-1} & 1854 cm^{-1} are characteristics peaks of carbonyl carbon of *N*-carboxyanhydride ring and ^{1}H NMR resonance at δ_H 4.54 ppm (d, J = 7.80 Hz, 1H) and ^{13}C NMR at δ_c 100.9 shows attachment of glucose moiety to NCA **50**.

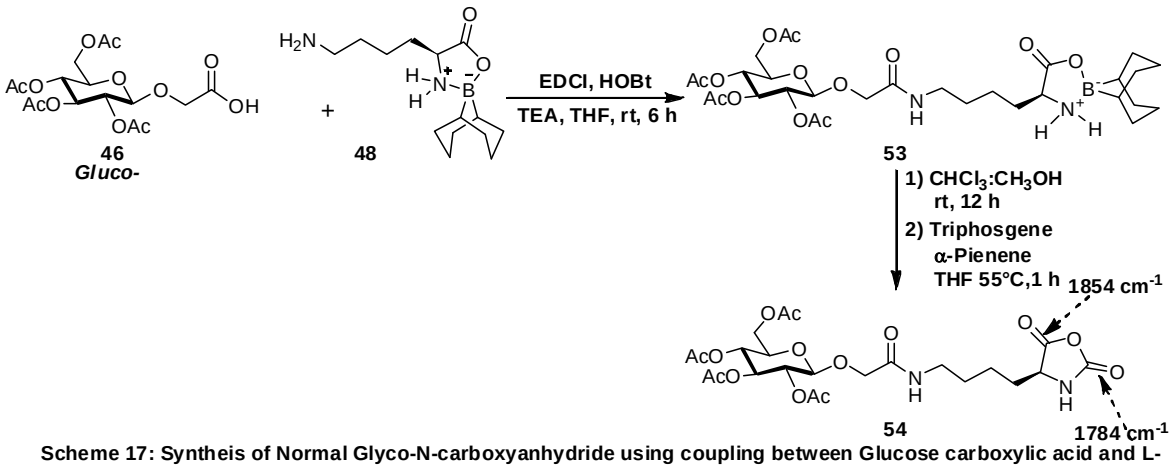


Ring opening polymerization (ROP) of *C*-6 azido carbohydrate lysine derivative *N*-carboxyanhydride **50** with *n*-hexylamine was carried out by the methodology that was delineated in the previous section. Progress of the reaction was monitored by disappearance of anhydrides stretches in FT-IR spectroscopy. After completion of the reaction, the desired azido functionalized glycopolypeptide was obtained by similar way as we did for earlier glycopolypeptide separation. Expected molecular weight of azido functionalized glycopolypeptide **51** confirmed by the GPC ^{1}H & ^{13}C NMR and azide group incorporation by FT-IR peak at 2100 cm^{-1} (Scheme 16).[80]



Scheme 16:Synthesis of 100 % 6-Azido-Gluco-Functionalized Glycopolypeptide using ROP

Further deprotection of acetate group of glycopolypeptide **51** was carried out by the use of hydrazine hydrate THF:MeOH system. Purification was done by dialyzing the reaction mixture against the deionized water using dialysis membrane 3,500 KDa molecular weight cut off for 2 days changing water after 4-6 h interval. The characterization of polymer **52** was done by ^{1}H & ^{13}C NMR analysis and IR. *C*-6 azido functionalized glycopolypeptide **52** showed α-helical conformation in water. Similarly non azido functionalized normal NCA **54** was synthesized by following same reaction sequence as for azido functionalized glyco-NCAs

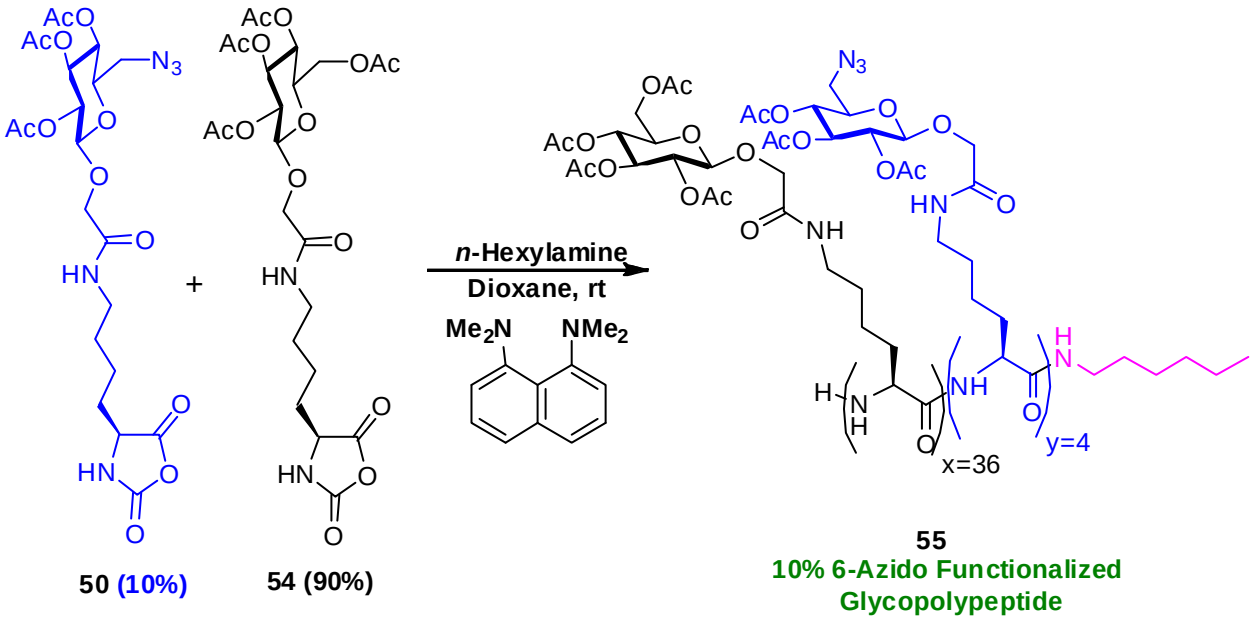


Scheme 17: Syntheis of Normal Glyco-N-carboxyanhydride using coupling between Glucose carboxylic acid and L-lysine- 9-BBN Complex

50. Amide coupling of carboxylic acid **46** with 9-BBN complex **48** using EDCI and HOBT gave compound **53**, which on 9-BBN cleavage followed by treatment with triphosgene gave normal gluco-NCAs **54**. Percentage of azide group incorporation was varied by using different ratios of Azido-NCAs and normal NCAs to get controlled azido functionalized glycopolypeptide. This gives very easy access for synthesis of azido functionalized

glycopolypeptide of which azido group can be further modified in a different ways to get different architectures and advanced materials for biomedical application (Scheme 17).

Varying the amounts of monomer ratio of the azido functionalized NCAs **50** to the normal glyco-NCAs **55**, we have synthesized 10 % azido functionalized glycopolypeptide **55** was synthesized by using ring opening polymerization (ROP) and *n*-hexylamine as initiator under standard condition. Further characterization of incorporation of azide group was done by IR and relative molecular weight by GPC (PS as reference), ^{1}H & ^{13}C NMR analysis (Scheme 18).

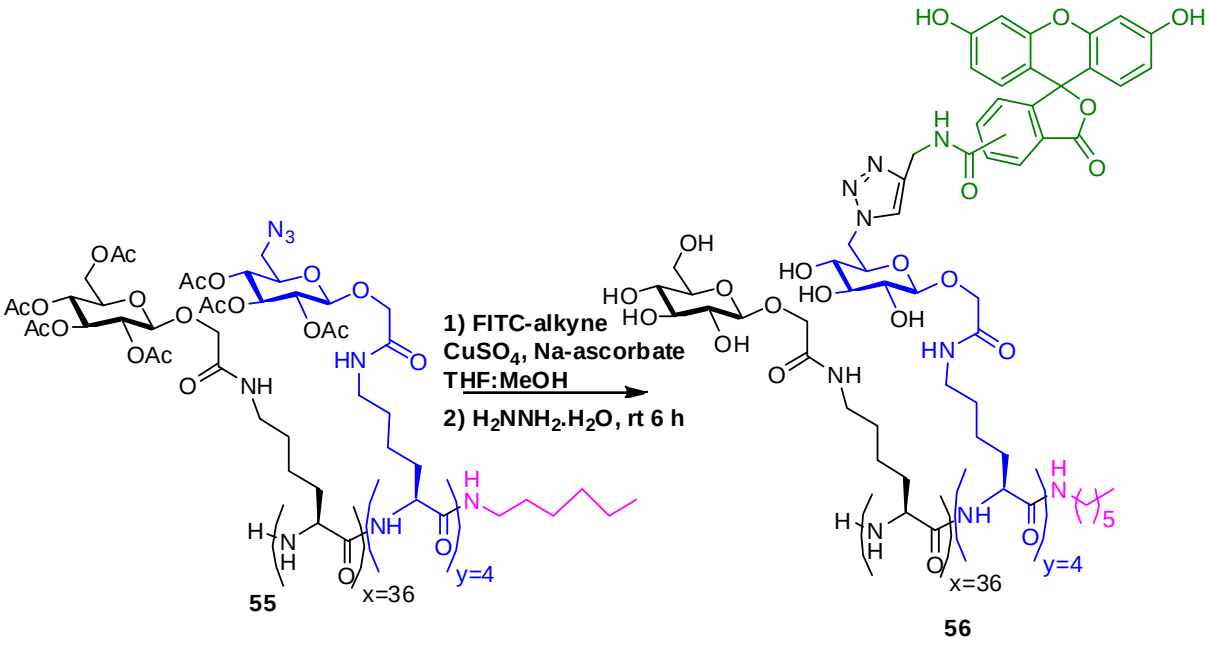


50 (10%) **54 (90%)** **55**
10% 6-Azido Functionalized Glycopolypeptide

Scheme 18: Controlled incorporation of Azido group into Glycopolypeptide

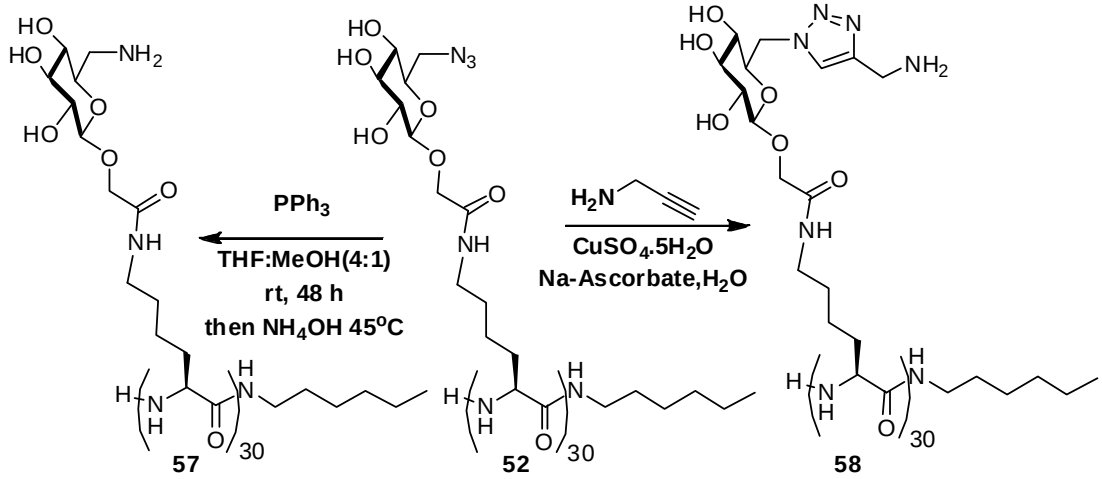
Run no	Polymer	M/I	M_w/M_n	DP(GPC)	Helicity (CD)
1	6- Azido-β-gluco-*C*-*l*-lys (**51**)	30	1.20	33	α-helix
2	6- Azido-β-gluco-*C*-*l*-lys (10 %) (**55**)	40	1.24	40	α-helix

Presence of azido group incorporation in **55** was confirmed by IR and by conjugation to a fluorescent alkyne using copper catalyzed Husigen 1,3-dipolar cycloaddition to get fluorescent glycopolypeptide **56**. These azido glycopolymers could be cross-linked to get biocompatible hydrogel and synthesis work is currently under progress (Scheme 19).



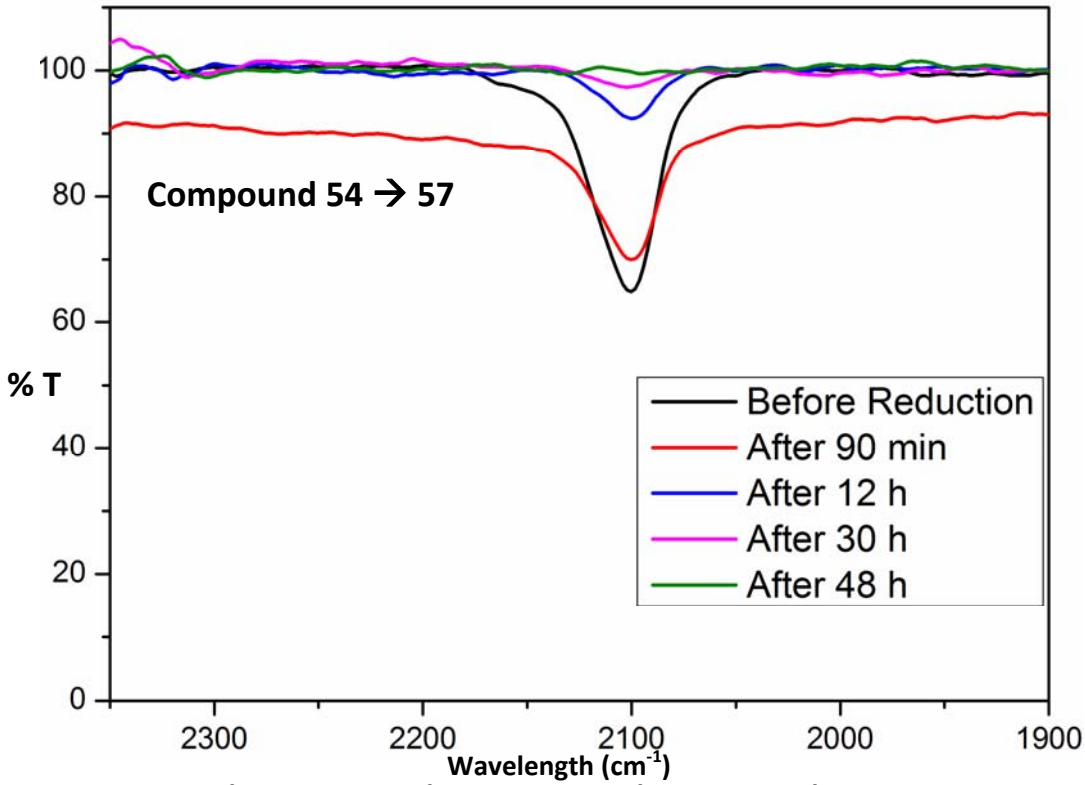
Scheme 19: Syntheis of Fluorescent Glycopolypeptide using Click Chemistry

The azide group can be reduced to amine group and kind of secondary structure can be observed for $-NH_2$ glycopolymer was helical. For this, we took acetyl deprotected 100 % azido functionalized glycopolypeptide **52** (30 Unit) and reduction of azide group to amine group was carried out by using excess triphenylphosphine in THF:H_2O system. Progress of azide reduction was monitored by the decrease in IR frequency of azide group at 2100 cm^{-1} and complete reduction to amine was observed after 48 h. Purification of the reduced glycopolypeptide **57** was carried out by extraction of excess of triphenylphosphine and other organic soluble by-product using ethyl acetate and dialyzing sample against water



Scheme 20: Reduction of 6-Azido Glycopolypeptide using triphenylphosphine to get 6-Amino Glycopolypeptide for Sortase activity

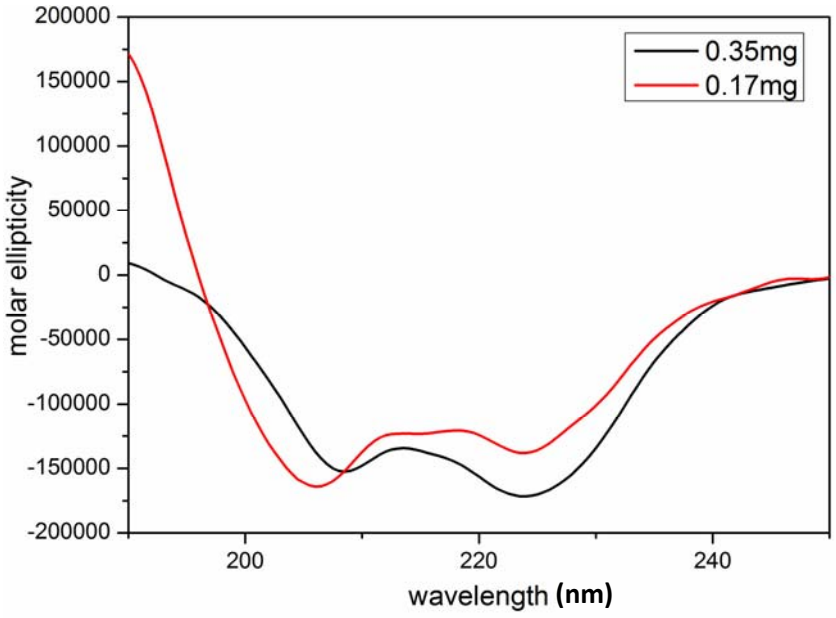
using dialysis membrane 3,500 KDa molecular weight cut off for 2 days changing water after 4-6 h interval.



Azide to Amine reduction using PPh_3 on Zn-Se plate

Characterization of desired 6-amino-glucopolypeptide **57** was done by ^{1}H & ^{13}C NMR analysis which showed peaks in aromatic region. So, the resonance correspond to the triphenylphosphine come because of the incomplete hydrolysis of iminophosphorane, which is intermediate step of azide to amine reduction mechanism. It is known that azide to amine reduction though iminophosphorane is difficult as iminotriphenylphosphorane is not easly hydrolyzed. The problem can be solved by performing the reaction in trimethylphosphine whose by-product iminotrimethylphosphorane is hydrolyzed with aq.NH_4OH at 45 °C and gives desired amino compound. Partially hydrolyzed iminophosphorane intermediate was treated with aq. NH_4OH at 45 °C and after dialysis against water using 3500 K Da molecular weight cut of membrane, ^{1}H & ^{13}C NMR was recorded to observe complete hydrolysis. In the ^{1}H NMR spectra no resonance was observed in aromatic region that suggest complete hydrolysis of iminophosphorane intermediate (Scheme 20).

Polypeptides having cationic charge at side chain are preferred to form random conformation by disturbing the helical backbone due to charge-charge repulsion, which breaks hydrogen bonding. Like polylysines have random coil conformation, there is only one report which shows that one can have helical conformation in cationic charged polypeptide if they have placed around 11- 12 bond apart from peptide backbone. They have prepared the polypeptide, which has the modified lysine derivatives. We wanted to what happens if charge is introduced into glycopolypeptide whether it give random coil or helical conformation. Until there was no report on the synthesis of *C*-6 amino functionalized glycopolypeptide and its secondary conformation.



For this reduction of azido functionalized glycopolypeptide was done using Staudinger condition to get charged glycopolypeptide. Secondary conformation analysis of these glycopolypeptide **57** was done using sample of 0.35 and 0.17 mg per mL at pH 7.4 phosphate buffer solution. Surprisingly circular dichrosim study reveals the α-helical conformation show two minima at 222 nm and 208 nm, which are characteristic peaks. Further to check the helical conformation confirmation, *C*-6 azido functionalized glycopolypeptide **52** was clicked with propargyl amine to introduce cationic charge using $CuSO_4.5H_2O$ and sodium ascorbate in water and progress of reaction was monitored by disappearance of azide peak in IR spectroscopy and clicked triazole proton resonance at δ_H 8.0 ppm in ^1H NMR. CD spectra of this propargyl amine modified glycopolypeptide **58** was recorded which again clearly shows peaks at 222 nm and 208 nm which corresponds to α-

helical conformation at pH 7.4. So both the ways reduction and propargyl amine click gives same CD signal of helical conformation, which has the cationic charge on the side chain of glycopolypeptide in first case on the *C*-6 position of carbohydrate and in second on simple amine of clicked propargyl group. Generally it's very difficult to get charged helical polypeptide and also glycopolypeptide which can be modulated to get stimuli responsive materials and block copolymer for gene delivery.

Glycopolymers having cationic charge will be very much useful in gene delivery, by encapsulating required gene into this charged block copolymer glycopolypeptide self assembled structure, which helps the them to cross the negatively charged membrane as naked gene cannot cross the membrane because of charge-charge repulsion. Azido or amino group could be used for cross-linking to get network of biocompatible polymer.

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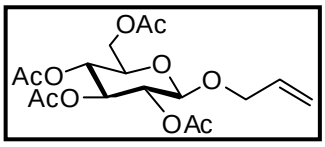
Preparation of (2-allyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (41)

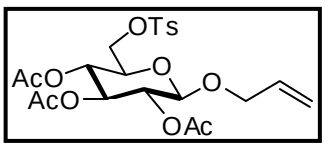


To a solution of D-glucose (5g, 22.75 mmol) in dry pyridine (20 mL) was added acetic anhydride (18mL, 180.39 mmol) and the reaction mixture was stirred at room temperature until the completion of reaction followed by TLC. After completion of reaction, most of the pyridine was removed on the rotary evaporator. The crude reaction mixture was dissolved in dichloromethane and washed with dil. HCl or $CuSO_4$ solution to remove traces of pyridine. The crude reaction mixture was purified by column chromatography to get penta-*O*-acetyl-β-D-glucopyranoside.

To a solution of penta-*O*-acetyl-β-D-glucopyranoside (10g, 25.6 mmol) and 2-bromoethanol (2.17 mL, 30.7 mmol) in dry dichloromethane was placed in 100 mL round bottom covered by aluminium foil and fitted with a dropping funnel. At 0°C $BF_3.Et_2O$ was added dropwise over period of 25 minutes. The reaction mixture was then stirred at 0°C for 1h, further at room temperature for 12 h. Completion of reaction was monitored by TLC. The reaction mixture was diluted by dichloromethane and poured onto ice water while stirring. The organic layer was separated and washed successively with water, saturated sodium bicarbonate and brine. The organic layer was dried on Na_2SO_4, concentrated on rotary evaporator and the resulting residue was purified by column chromatography on silica gel using ethyl acetate-petroleum ether as eluent to get Allyl-penta-*O*-acetyl-β-D-glucopyranoside (7.0 g, 60%) as a crystalline solid.

HRMS (ESI): calcd for $C_{17}H_{24}NaO_{10}$ $[M + Na]^+$ 411.1267, found 411.1265

Preparation of (Allyl)-6-*O*-tosyl-2,3,4,-tri-*O*-acetyl-β-D-glucopyranoside (43)



To a solution of allyl-2,4,5,6-tetra-*O*-acetyl-β-D-glucopyranoside (5g, 12.9 mmol) in anhydrous methanol was added freshly prepared solution NaOMe in MeOH (1 M, 1mL) at 0°C. The reaction mixture was stirred for 1h and progress of reaction was monitored by TLC. After complete conversion, reaction mixture was neutralized with ablerlite IR 120 H^+ resin and filtered. The crude reaction product was obtained by removal of solvent on rotary

evaporator and purified by coloumn chromatography to get desired tetraol of allyl glucoside (2.7g, 96% yield).

The above tetraol (2.7g, 12.3 mmol) was dissolved in pyridine (15mL) and to this crystallized tosyl chloride (2.8g, 14.7 mmol) was added at 0°C under argon. The reaction mixture was then allowed to attain room temperature and was further stirred for 12 h. Excess tosyl chloride was quenched by addition of methanol at 0°C and then solvents were evaporated. The crude product obtained was purified by column chromatography to get 6-*O*-tosyl-allyl glucoside (3.2g, 70%). Starting material was isolated (30%) and used again in tosyl protection reaction.

The unprotected 6-O-tosyl derivative of ally glucoside (3.2g, 8.6 mmol) was dissolved in dry pyridine and the solution was cooled to -10°C. To this ice-cold solution acetic anhydride (1.62mL, 17.1 mmol) was added slowly while stirring. The reaction mixture was kept at 4°C for 12 h and was the concentrated. The crude product was purified by column chromatography to afford Allyl-6-*O*-tosyl-2,3,4,-tri-O-acetyl-β-D-glucopyranoside (4.2g, 98%).

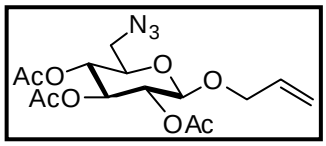
$[\alpha]^{RT}_{D}$ (c = 1.0 M, $CHCl_3$) = −1.76

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.46 (s, 3H), 3.69-3.79 (m, 1H), 3.77-4.11 (m, 3H), 4.21-4.29 (m, 1H), 4.50 (d, *J* = 7.83 Hz, 1H), 4.86-4.98 (m, 2H), 5.31-5.32 (m, 3H), 5.72-5.91 (m, 1H), 7.35 (d, *J* = 8.08, 2H), 7.78 (d, *J* = 8.08, 2H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.4(2C), 20.5, 21.6, 67.7, 68.6, 69.8, 70.1, 71.4, 72.4, 99.2, 117.6, 128.0(2C), 129.9(2C), 132.3, 133.0, 145.1, 169.2, 169.4, 170.1

HRMS (ESI): calcd for $C_{22}H_{28}NaO_{11}S$ $[M + Na]^+$ 523.1250, found 523.1253

Preparation of (Allyl)-6-azido-2,3,4,-tri-*O*-acetyl-β-D-glucopyranoside (44)



To a solution of Allyl-6-*O*-tosyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4g, 8 mmol) in dry DMF (20 mL) was added NaN_3 (1.6g, 24 mmol) and the reaction mixture was stirred at 80°C for 24 h. After completion of the reaction, the reaction was diluted by diethyl ether. The organic layer was washed with saturated brine to remove excess of DMF for 5-6 times and then by

water wash. The organic layer was dried on Na_2SO_4 and concentrated to get crude product. The crude product was purified by column chromatography to get Allyl-6-azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (2.9g, 97%).

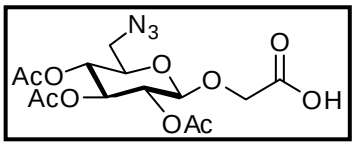
$[\alpha]^{RT}_D$ (c = 1.0 M, $CHCl_3$) = −45.1

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 2.01 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 3.15 (dd, *J* = 2.4, 13.3 Hz, 1H), 3.4 (dd, *J* = 7.58, 13.26 Hz, 1H), 3.69-3.79 (m, 1H), 4.12(qt, *J* = 1.39, 6.19, 13.26 Hz, 1H), 4.35 (qt, *J* = 1.52, 4.9, 13.26 Hz, 1H), 4.60(d, *J* = 7.96 Hz, 1H), 4.90-5.08 (m, 2H), 5.77-5.96 (m, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.5(2C), 20.6, 51.1, 69.8, 71.2, 72.5, 73.6, 99.2, 117.7, 133.1, 169.2, 169.5, 170.2

HRMS (ESI): calcd for $C_{15}H_{21}N_3NaO_8$ $[M + Na]^+$ 394.1226, found 394.1233

Preparation of 6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside ethyl carboxylic acid (45)



To a stirring solution of 6-Azido-2,3,4-allyl glucoside (2.8g, 7.5 mmol) in CCl_4 (23mL), CH_3CN (23mL) and H_2O (30mL) at 0°C in a round bottom flask was added NaIO4 (6.5g, 30.2 mmol) and $RuCl_3.H_2O$ (23mg, 0.150 mmol). After 10 min of vigorous stirring of the suspension at room temperature, the reaction mixture was concentrated on a rotary evaporator and diluted with 1M HCl (70mL) and brine (70mL). The aqueous layer was extracted with CH_2Cl_2 (4X100ml) and dried extensively over Na_2SO_4. The crude product was used in next step without any further purification.

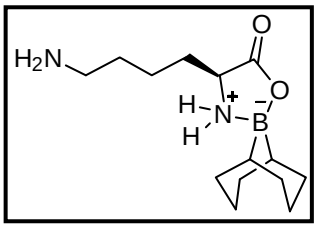
^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 2.02 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 3.21-3.48 (m, 2H), 3.66-3.77 (m, 1H), 4.29-4.30 (d, *J* = 6.76 Hz, 1H), 4.37 (s, 1H), 4.68(dd, *J* = 7.8, 11.7 Hz, 1H), 4.95-5.14 (m, 2H), 5.25 (td, *J* = 2.65, 9.35 Hz, 1H), 9.69 (bs, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.5(2C), 20.6, 50.9, 64.8, 69.4, 71.0, 72.2, 73.8, 100.1, 169.2, 169.7, 170.2, 172.7

Preparation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside ethyl carboxylic acid (46)

Same procedure followed which is described above for **45**.

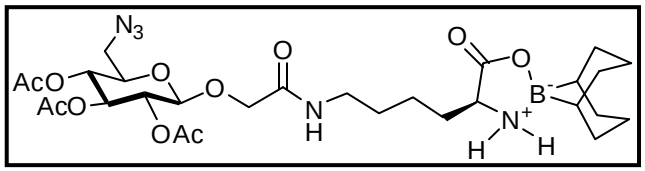
Preparation of 9-BBN complex of Lysine (48)



L-lysine mono hydrochloride salt was treated with Aq. NH_3 solution to neutralize acid salt in round bottom flask for 30 min kept at 0°C and the concentrated to remove excess of ammonia. This was directly used for 9-BBN complex formation reactions.

To 150mL methanol in a 250mL round bottom flask at room temperature under argon was added 1.1 eq. 9-BBN dimer (Aldrich). The mixture was heated at reflux until the 9-BBN was completely dissolved (30min) and to this solution 25 mmol of amino acid was added. The resultant reaction mixture was heated for additional 3 h until gas evolution ceased and the suspension became a clear homogenous solution. The methanol was removed on rotary evaporator and the residue dissolved in hot THF (100mL), filtered and the filtrate was concentrated to get a white gummy residue of 9-BBN-L-lysine complex. Excess of 9-BBN was removed by treatment with hot hexane or diethyl ether and then subjected to high vacuum for 1 h during which time it became an amorphous solid. This material was used without any further purification for coupling reaction.

Coupling of 6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside ethyl carboxylic acid with 9-BBN-L-lysine complex (49)



To a solution of 6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside ethyl carboxylic acid (1g, 2.6 mmol) and 9-BBN-L-lysine complex (0.684g, 2.57 mmol) in dry THF (10mL) was added EDCI (0.548g, 3.1 mmol) and HOBt (0.475g, 3.1 mmol)). The reaction mixture was stirred for 12 h and progress of reaction was monitored by TLC. After completion of the reaction, the solvent in reaction mixture was removed by rotary evaporator and directly loaded onto the column for further purification. The desired coupling product was obtained by using ethyl acetate/methanol as eluent (1.2g, 75%).

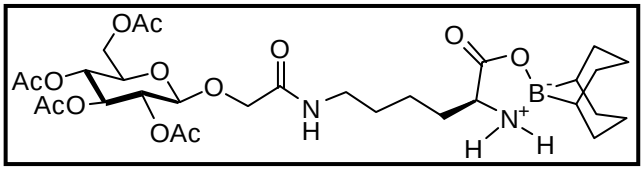
^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 0.55 (s, 2H), 1.25-1.90 (m, 18H), 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 3.25-3.40 (m, 2H), 3.71-3.80 (m, 2H), 4.03 (d, J = 14.91 Hz, 1H), 4.12 (q, J =

7.20 Hz, 1H), 4.31 (d, , *J* = 14.91 Hz, 1H), 4.55 (d, *J* = 7.96 Hz, 1H), 4.80-5.10 (m, 3H), 5.26 (t, *J* = 9.40 Hz, 1H), 6.78 (t, *J* = 6.30 Hz, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): *δ* (ppm) 20.5(2C), 20.6, 20.8, 22.2, 22.9, 23.9, 24.1, 24.3, 28.9, 29.8, 31.1, 31.2, 31.3, 31.5, 37.7, 55.2, 61.5, 68.0, 68.4, 71.4, 71.9, 72.0, 100.4, 169.2, 169.5, 169.9, 170.1, 170.2.

Coupling of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside ethyl carboxylic acid with 9-BBN-L-lysine complex (53)

Same procedure followed which is described above for **49**.



^{1}H NMR (200.13 MHz, $CDCl_3$): *δ* (ppm) 0.55 (s, 2H), 1.25-1.90 (m, 18H), 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 6H), 2.58 (bs, 1H), 3.20-3.40 (m, 2H), 3.70-3.80 (m, 2H), 4.03 (d, *J* = 14.8 Hz, 1H), 4.12 (dd, *J* = 2.0, 12.5 Hz, 1H), 4.22-4.35 (m, 2H), 4.55 (d, *J* = 7.83 Hz, 1H), 4.90-5.14 (m, 3H), 5.26 (t, *J* = 9.40 Hz, 1H), 5.50 (m, 1H), 6.78 (t, *J* = 5.94 Hz, 1H)

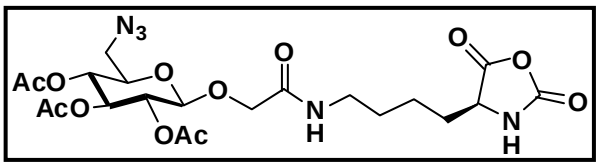
^{13}C NMR (50.32 MHz, $CDCl_3$): *δ* (ppm) 20.4(2C), 20.6, 20.8, 22.2, 22.9, 23.9, 24.1, 24.3, 28.9, 29.8, 31.1, 31.2, 31.3, 31.5, 37.7, 55.2, 61.5, 68.0, 68.4, 71.4, 71.9, 72.0, 100.4, 168.9, 169.4, 169.4, 170.1, 170.3, 170.6

Cleavage of 9-BBN complex

6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside-L-lysine **49** derivative was dissolved in MeOH:$CHCl_3$ (1:5) mixture and stirred it for 24 h at room temperature. After complete cleavage of 9-BBN complex, the reaction mixture was concentrated and treated with hot pet ether or diethyl ether to remove excess 9-BBN. If sometime complete cleavage was not occurred then 1-2 drops of conc. HCl was added for complete cleavage. The crude product was directly subjected for next reaction without any further purification.

Same procedure followed for normal glycoaminoacid derivative which is described above for **49**.

Preparation of *N*-Carboxyanhydride of 6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside-L-lysine derivative (50)



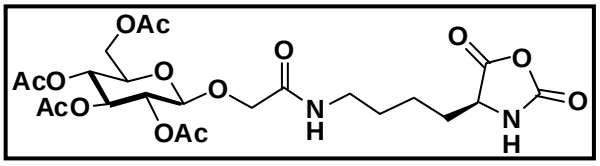
To a solution of 6-Azido-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside-L-lysine derivative (0.1 mmol) in freshly distilled anhydrous tetrahydrofuran (30 mL) was added a solution of triphosgene (0.05 mmol) in anhydrous tetrahydrofuran (5 mL) under argon atmosphere. α-Pinene (0.15 mmol) was added and the reaction mixture was heated to 50 °C for 2 h and cooled to room temperature, poured into dry hexane. The white precipitate of the *N*-carboxyanhydride (**50**) was vacuum filtered quickly and reprecipitated (2 times) by dissolving in ethyl acetate followed by addition of light petroleum. The resulting precipitate was filtered and dried under vacuum (Yield 80%).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ (ppm) 1.30-1.44 (m, 2H), 1.47-1.54 (m, 2H), 1.68-1.77 (m, 2H), 1.92 (s, 3H), 1.94 (s, 3H), 1.99 (s, 3H), 3.10-3.30 (m, 4H), 3.67-3.72 (m, 1H), 4.02 (d, *J* = 15.06 Hz, 1H), 4.2-4.3 (m, 2H), 4.54 (d, *J* = 7.80 Hz, 1H), 4.90-5.14 (m, 2H), 5.17 (t, *J* = 1.76, 9.54 Hz, 1H), 5.24 (bs, 1H), 5.27 (t, *J* = 5.27 Hz, 1H)

^{13}C NMR (50.32 MHz, $CDCl_3$): δ (ppm) 20.9(2C), 21.2, 22.2, 28.9, 31.4, 38.5, 51.5, 58.1, 69.2, 69.7, 72.1, 72.4, 74.2, 100.9, 152.5, 169.4, 170.0, 170.4, 170.7, 170.9

Preparation of *N*-Carboxyanhydride of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside-L-lysine derivative (54)

Same procedure followed for normal glycoaminoacid derivative **54** which is described above for **50**.



^{1}H NMR (399.78 MHz, $CDCl_3$): δ (ppm) 1.36-1.46 (m, 2H), 1.49-1.58 (m, 2H), 1.71-1.89 (m, 2H), 1.97 (s, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 3.16-3.36 (m, 2H), 3.66-3.76 (m, 1H), 4.05 (t, *J* = 15.0 Hz, 1H), 4.18-4.3 (m, 3H), 4.48 (d, *J* = 7.80 Hz, 1H), 4.95-5.09 (m, 2H), 5.19 (t, *J* = 9.62 Hz, 1H), 6.57 (t, *J* = 5.5 Hz, 1H),7.3 (bs, 1H)

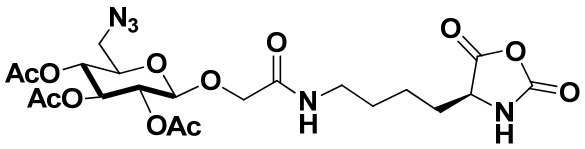
^{13}C NMR (100.53 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 20.7, 20.9, 21.3, 28.2, 30.6, 37.7, 57.4, 61.6, 68.1, 68.6, 71.6, 72.0, 72.1, 100.5, 152.1, 169.0, 169.4, 170.1(2C), 170.3, 170.7

Synthesis of Glycopolypeptide via ring opening polymerization of *N*-Carboxyanhydride of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside-L-lysine derivative

1) 100 % 6-Azido-Functionalized-Glycopolypeptide 30 unit (51)

Detail experimental and calculation of polymerization

Molecular weight of 6-Azido functionalized D-glucose-L-lysine NCA = 543.1813 (**152mg**)



50

a) n-Hexylamine (d=0.726g/mL) Mol. Wt.= 101.19

10µL diluted to 1000µL in 1,4 dioxane

mmol present in the 1000µL = 71.9 X 10^{-6} moles

b) Proton Sponge (1,8-Bis(dimethylamino)naphthalene) Mol. Wt.= 214.3

1 eq. of proton sponge is used equivalent to NCAs = 78mg

c) 30 unit with 100% azide incorporation into glycopolypeptide

0.152g of azido NCAs (Mol. Wt. 543.1813) = 279.92 X 10^{-6} moles

n-Hexylamine initiator 1/30 = 279.92/30 X10^{-6}

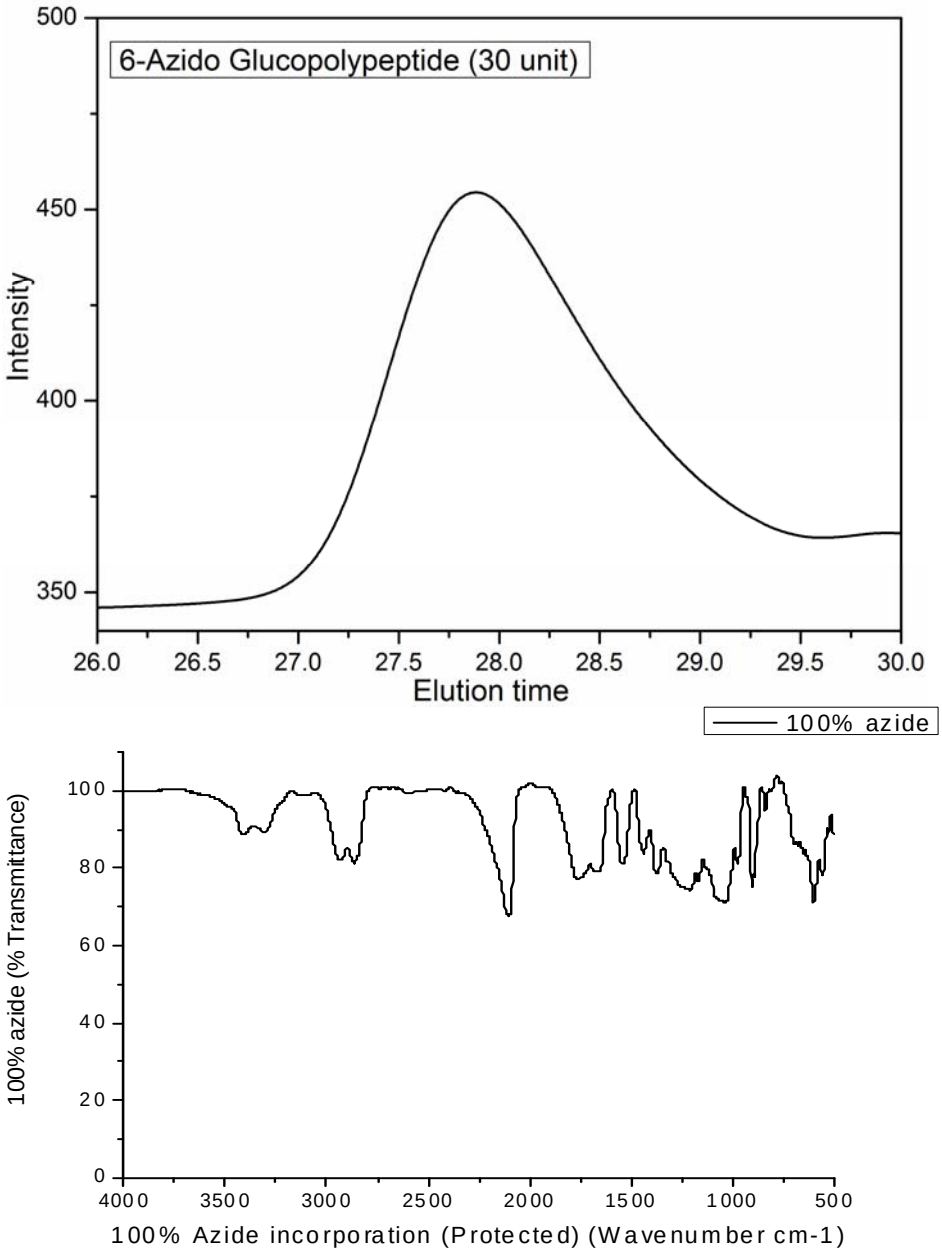
= 9.33 X 10^{-6} moles

1000µL (n-Hexylamine Stock solution) = 71.88 X 10^{-6} , So 9.33 X 10^{-6} moles is required for reaction, we took 130µL (initiator) from stock of n-hexylamine to get polymer chain length 30 unit and performed the polymerization in dry box (Glove Box). Progress of reaction was monitored by reduction in IR stretches of anhydride of NCAs at 1854 cm^{-1} and after completion the reaction mixture was taken outside the glove box. Reaction solvent 1,4

dioxane was removed and dichloromethane was added to it. To remove proton sponge used in reaction, dichloromethane layer was washed with 2N dil.HCl, by water & brine and kept on anhydrous sodium sulphate for drying. Solvent was evaporated on rotary evaporator and vacuum dried at 45 °C for 6 h.

Glycopolypeptide **51** characterization was done by ^{1}H & ^{13}C NMR analysis, IR (azide incorporation) and gel permeation chromatography analysis (GPC). 0.1M LiCl in DMF was used as mobile phase in GPC experiment coupled with RI detector to analyze sample.

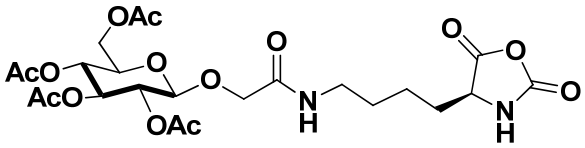
GPC of 100% azido Functionalized Glycopolypeptide



2) 10 % 6-Azido-Functionalized-Glycopolypeptide 40 unit (55)

Detail experimental and calculation of polymerization

Molecular weight of normal D-glucose-L-lysine NCA = 560.1854 (**250mg**)



54

a) *n*-Hexylamine (d=0.726g/mL) Mol. Wt.= 101.19

10µL diluted to 1000µL in 1,4 dioxane

mmol present in the 1000µL = 71.9 X 10^{-6} moles

b) Proton Sponge (1,8-Bis(dimethylamino)naphthalene) Mol. Wt.= 214.3

1 eq. of proton sponge is used equivalent to NCAs = 200mg

c) 40 unit with 10 % azide incorporation into normal glycopolypeptide

Ratio of monomer NCAs Normal: Azido = 36:4

0.250g of normal NCAs (Mol. Wt. 560.1854) = 446.3 X 10^{-6} moles

For 10 % Azide moles (Mol. Wt. 560.1854) = 446.3/10 X 10^{-6} moles

= 44.63 X 10^{-6} moles of azide required

Total moles of NCA = (446.3 + 44.63) X 10^{-6} = 491.06 X 10^{-6} moles

n-Hexylamine initiator 1/40 = 491.06/40 X10^{-6}

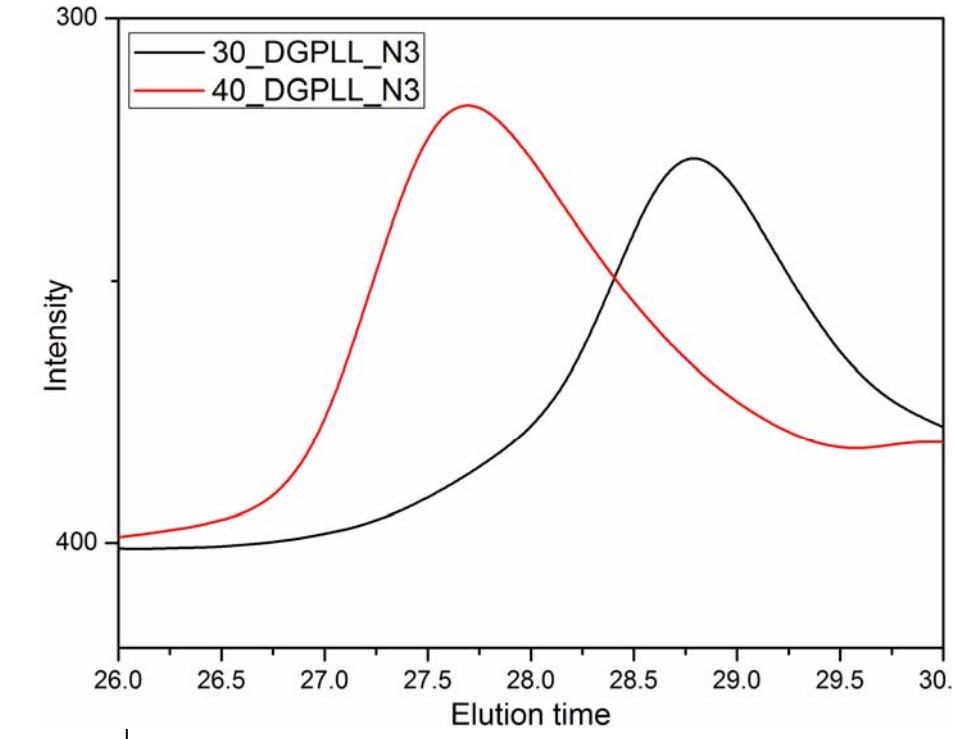
= 12.27 X 10^{-6} moles

1000µL (n-Hexylamine Stock solution) = 71.88 X 10^{-6} , So 12.27 X 10^{-6} moles is required for reaction, we took 170µL (initiator) from stock of n-hexylamine to get polymer chain length 40 unit and performed the polymerization in dry box (Glove Box). Progress of reaction was monitored by reduction in IR stretches of anhydride of NCAs at 1854 cm^{-1} and after

completion the reaction mixture was taken outside the glove box. Reaction solvent 1,4 dioxane was removed and dichloromethane was added to it. To remove proton sponge used in reaction, dichloromethane layer was washed with 2N dil.HCl, by water & brine and kept on anhydrous sodium sulphate for drying. Solvent was evaporated on rotary evaporator and vacuum dried at 45 °C for 6 h.

Glycopolypeptide **51** & **55** characterization was done by ^{1}H & ^{13}C NMR analysis, IR (azide incorporation) and gel permeation chromatography analysis (GPC). 0.1M LiCl in DMF was used as mobile phase in GPC experiment coupled with RI detector to analyze sample.

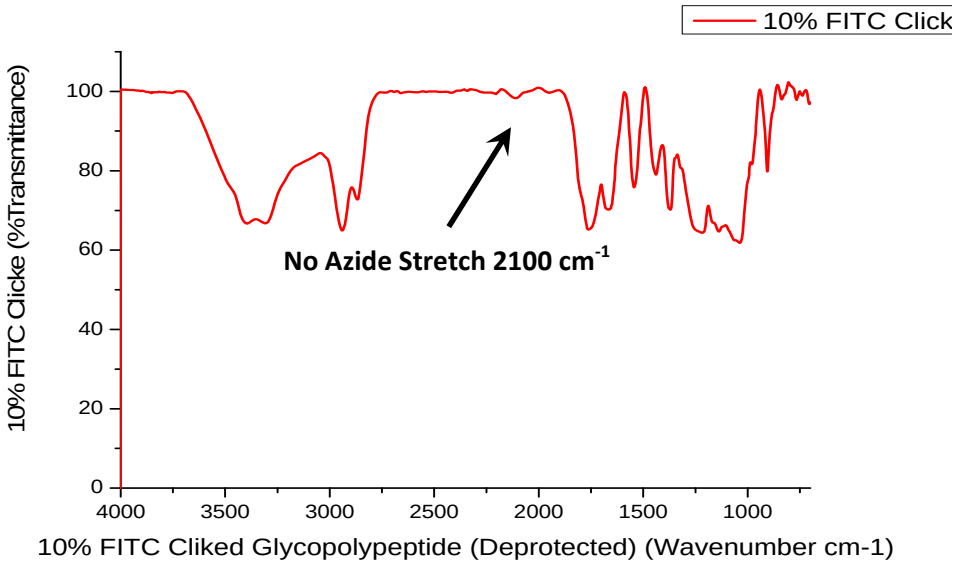
GPC 100% and 10% Azido Functionalized Glycopolypeptide 51 &55

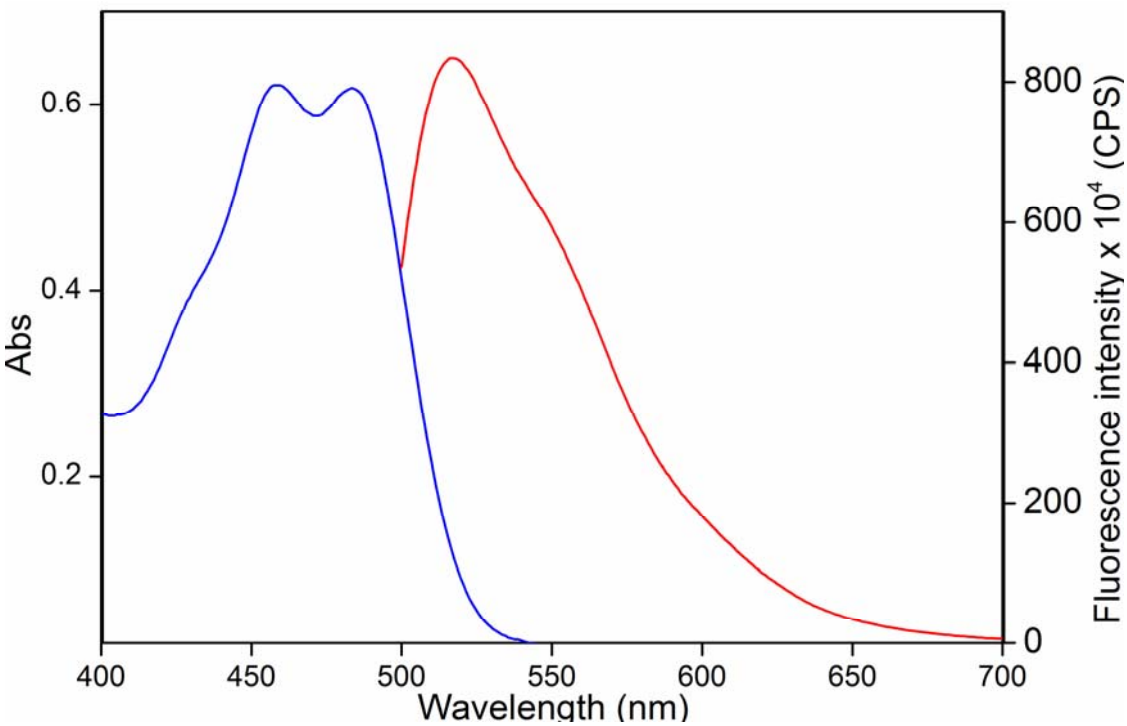


Preparation of Fluorescien-alkyne conjugated 10 % 6-Azido-Functionalized Glycopolypeptide 40 unit (56)

For FITC conjugation to the protected 10 % azido functionalized glycopolypeptide **55** was first clicked with fluorescien-alkyne synthesized using reported literature procedure by Finn et al (bioconjugation . 10 % azido glycopolypeptide (50mg, 2.2 X 10^{-6}moles) and excess fluorescien-alkyne (2mg) were dissolved in THF:MeOH (2mL+0.5mL) to this 30µL $CuSO_4.5H_20$ in MeOH (10mg in 100µL) solution was added. After this 30µL Na-ascorbate in H_2O (20mg in 200µL) was added and stirred for 3 day until the complete disappearance of azide peak at 2100 cm^{-1} in IR spectrum.

Above reaction mixture was dissolved in 5mL THF:MeOH (3:2) solution and added around 500µL of hydrazine monohydrate. Reaction mixture was stirred for 7-8 h and transformed into dialysis tubing of 3500 KDa molecular weight cut off. Dialysis was first done against 0.1M Na-EDTA salt in water to remove copper, after this with normal deionised water for 2-days and lyophilized to gave solid compound **56**. Incorporation of fluorescien group was confirmed by ^{1}H & ^{13}C NMR analysis showing triazole by measurement of fluorescence using fluoromax.

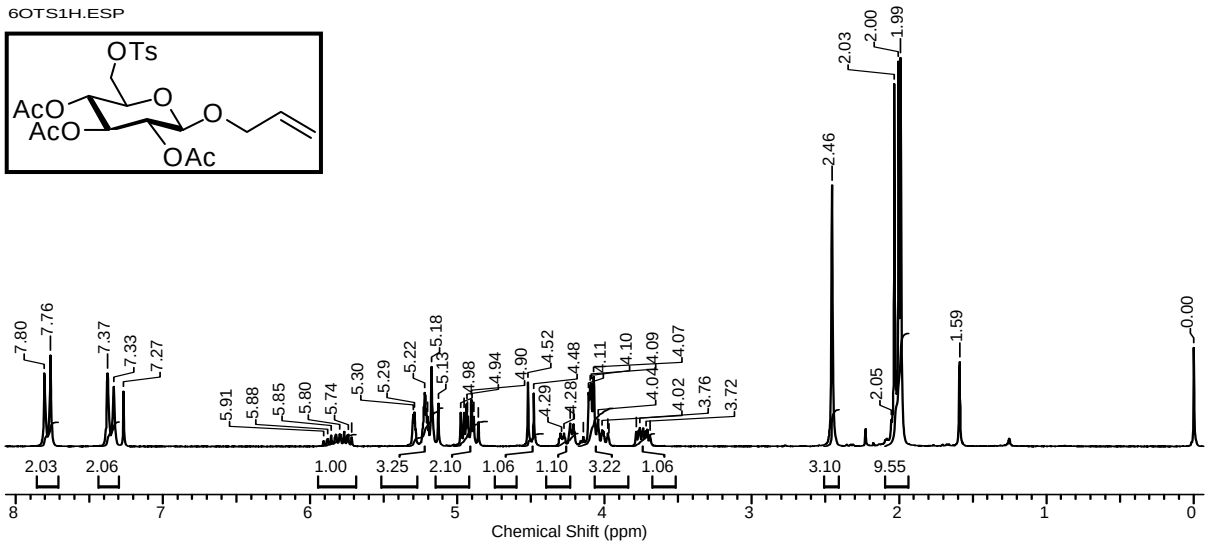




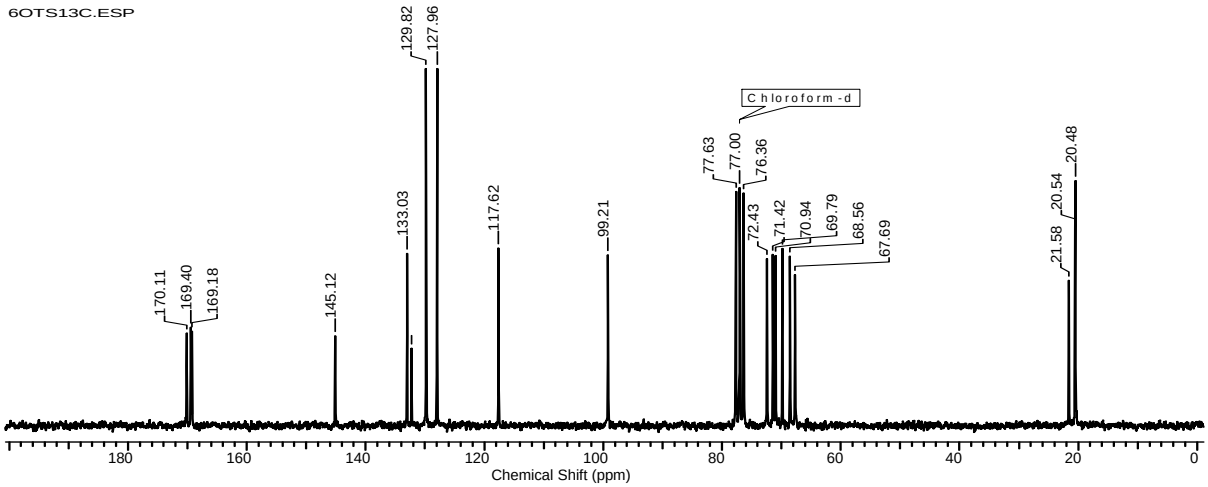
Fluorescence: 10µM solution of **58**, Excitation: 480nm, Slit width: ½, Range: 480-700nm

Absorbance: Stock solution of **58** 2mg per 1mL solution

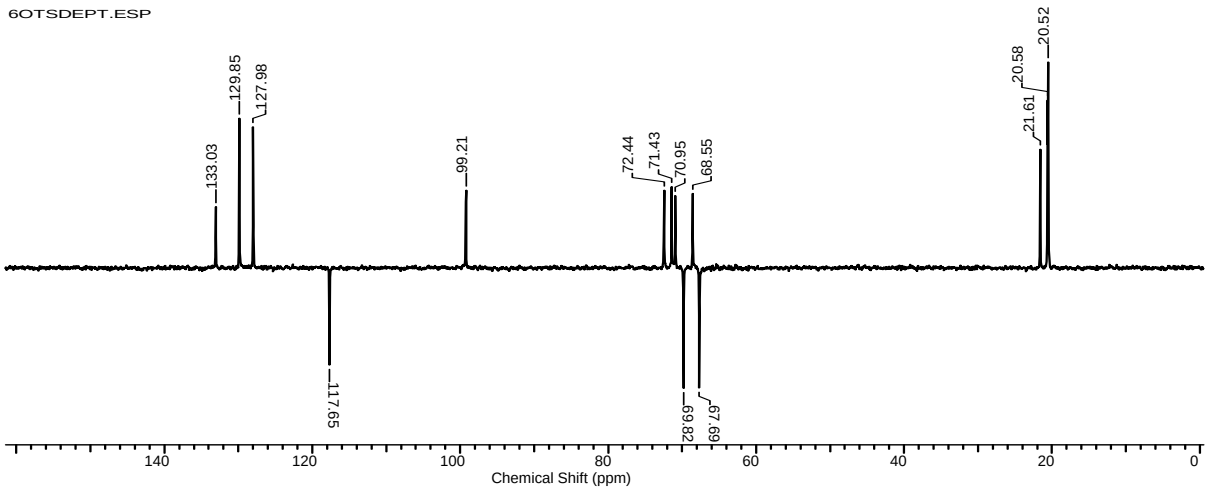
^{1}H NMR ($CDCl_3$, 200.13 MHz) Spectrum of Compound **43**



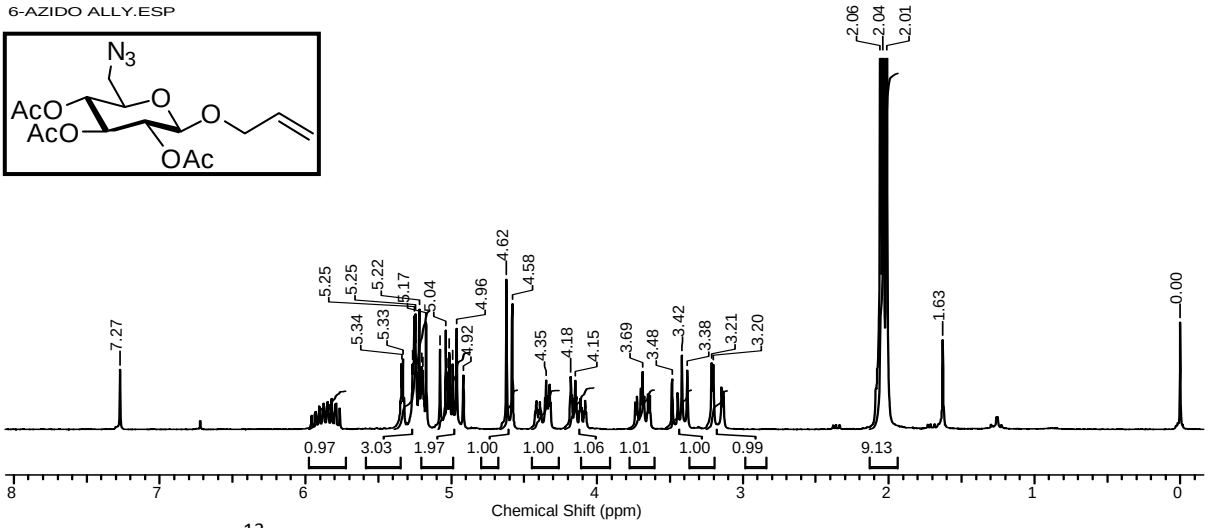
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **43**



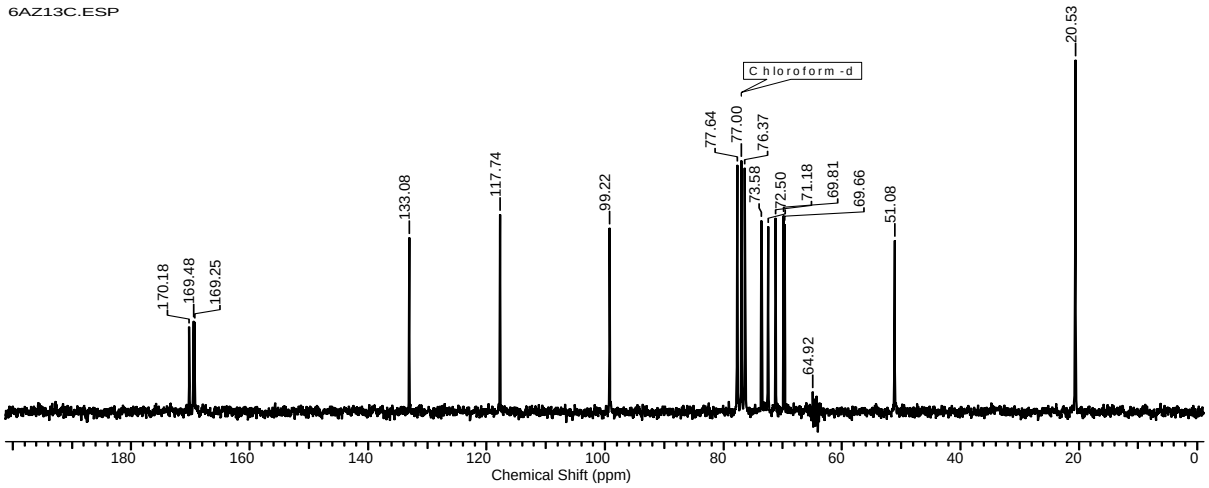
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **43**



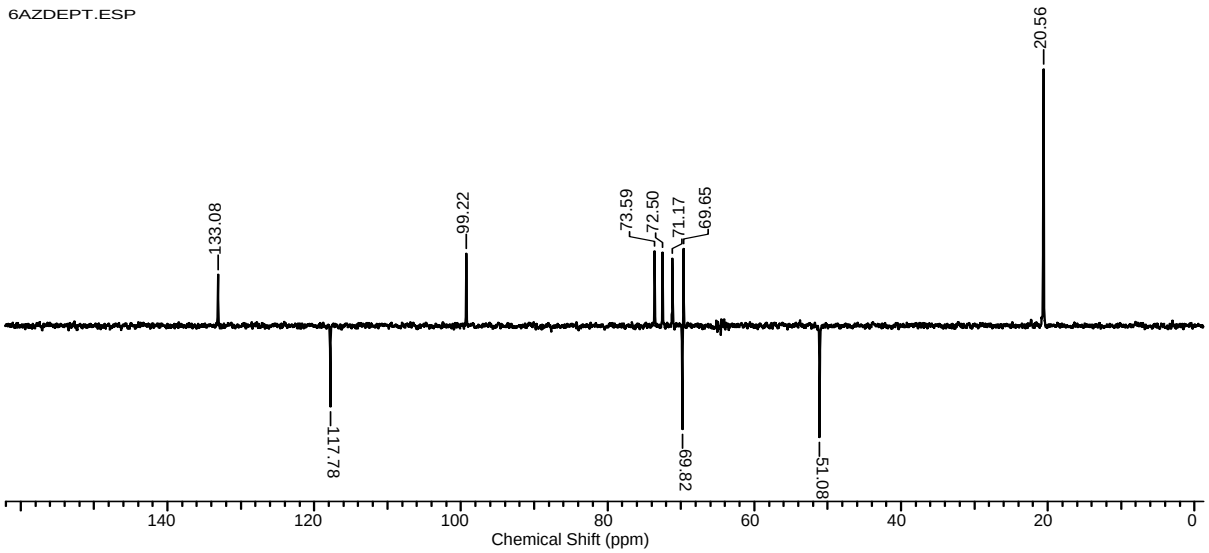
^{1}H NMR ($CDCl_3$, 200.13 MHz) Spectrum of Compound **46**



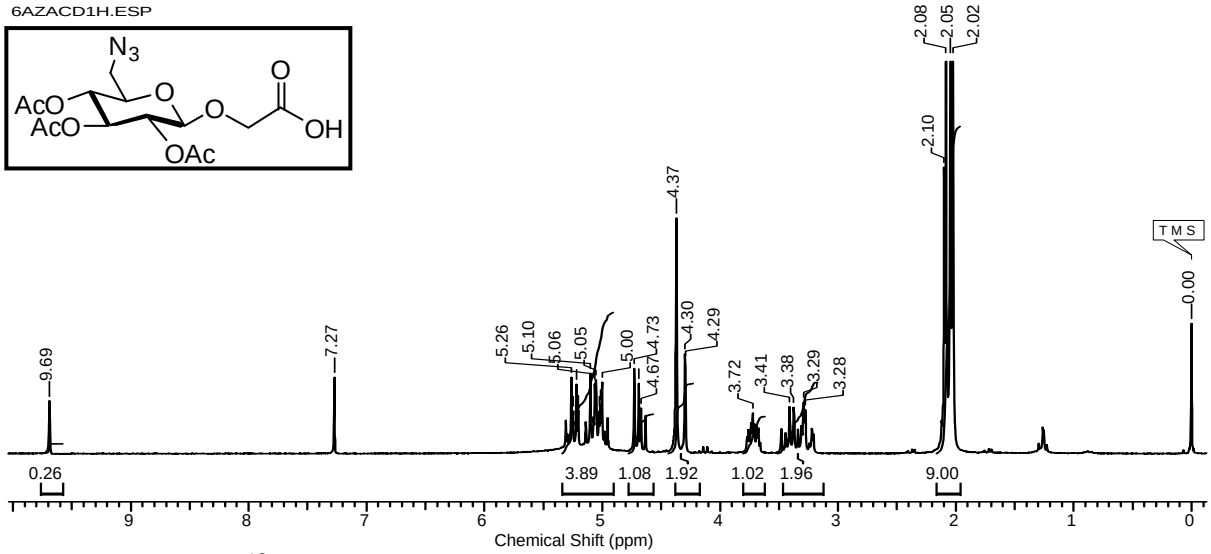
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **46**



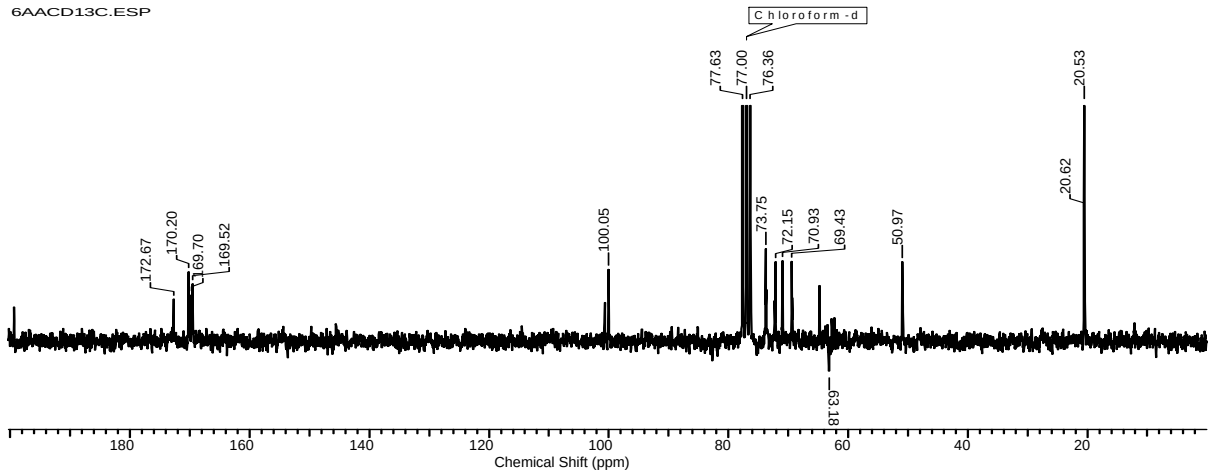
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **46**



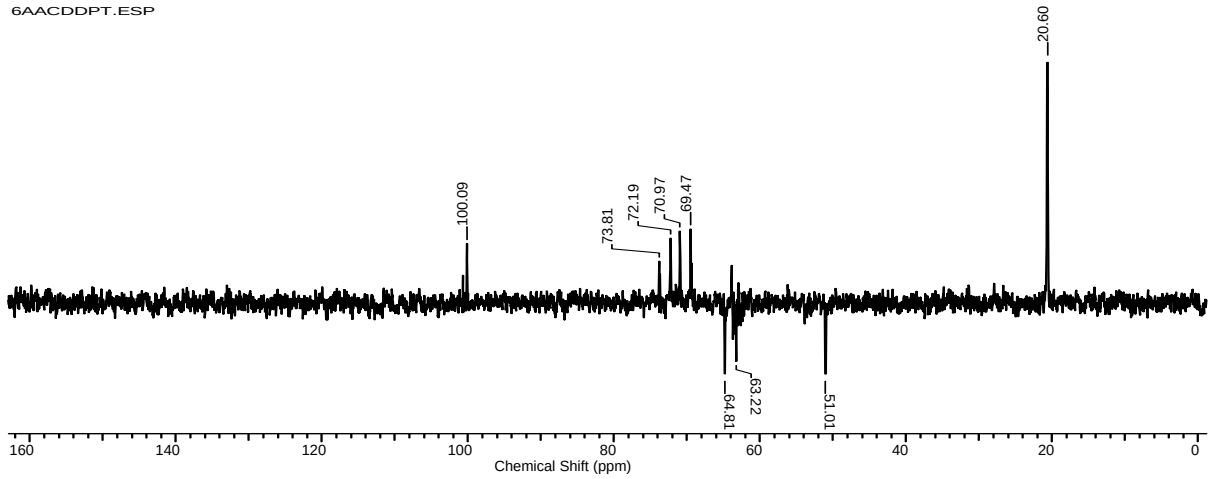
^{1}H NMR ($CDCl_3$, 200.13 MHz) Spectrum of Compound **47**



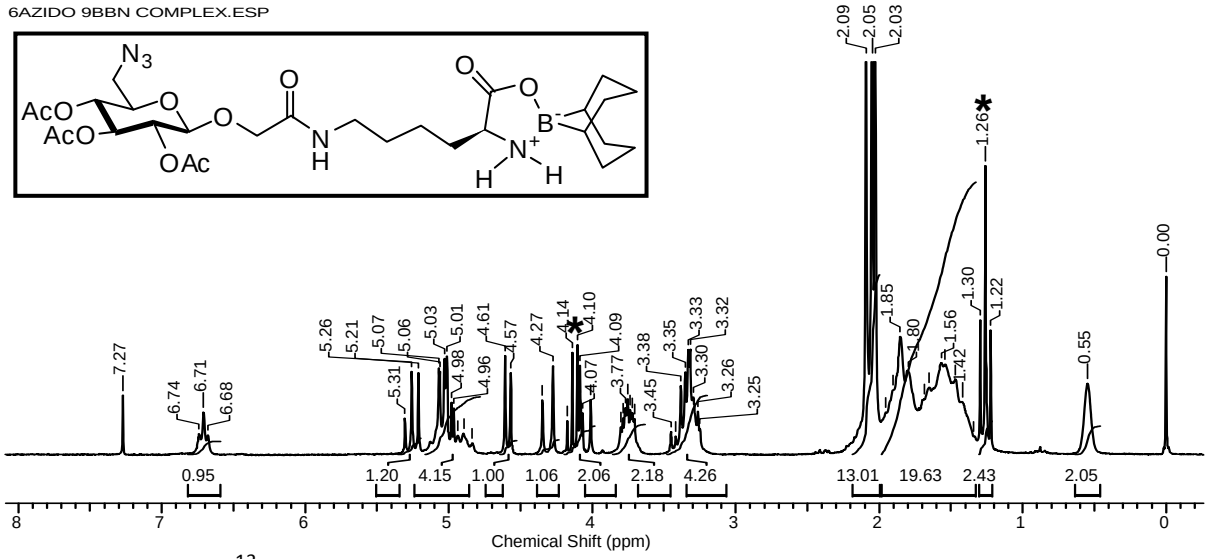
^{13}C NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **47**



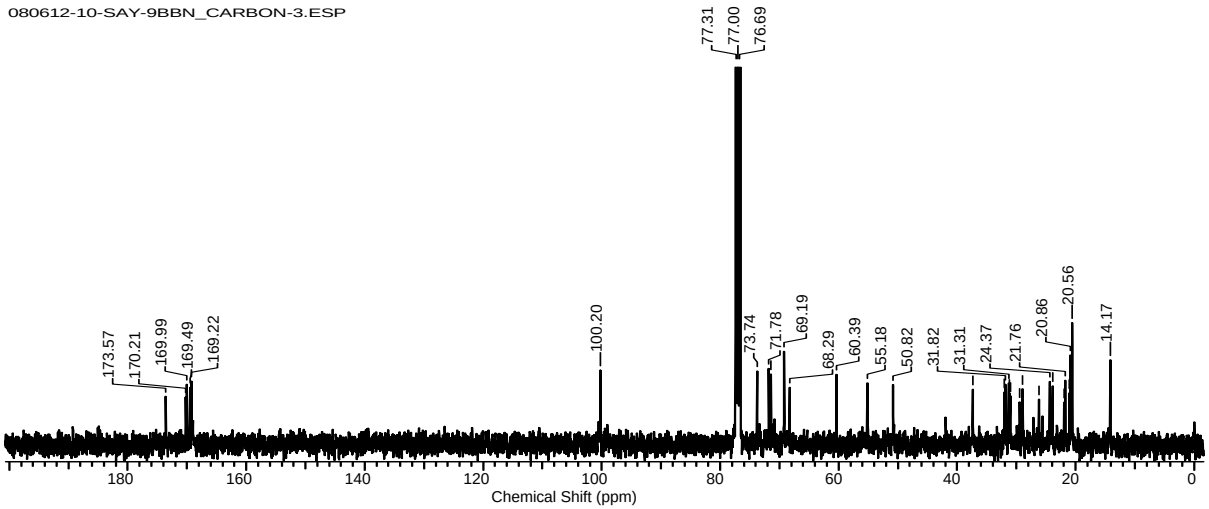
DEPT NMR ($CDCl_3$, 50.32 MHz) Spectrum of Compound **47**



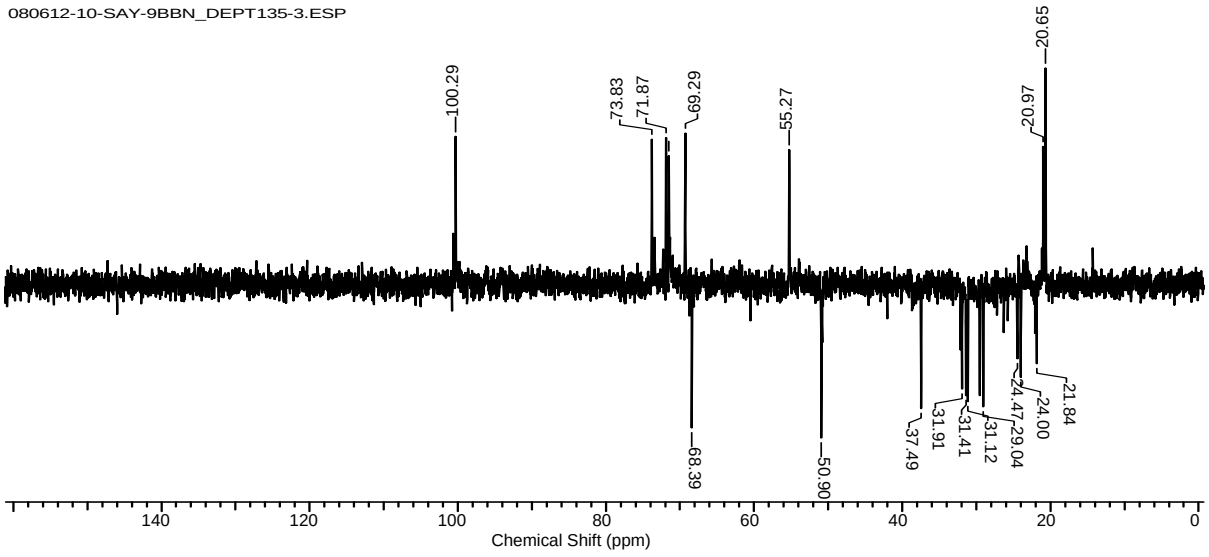
^{1}H NMR ($CDCl_3$, 399.78 MHz) Spectrum of Compound **49**



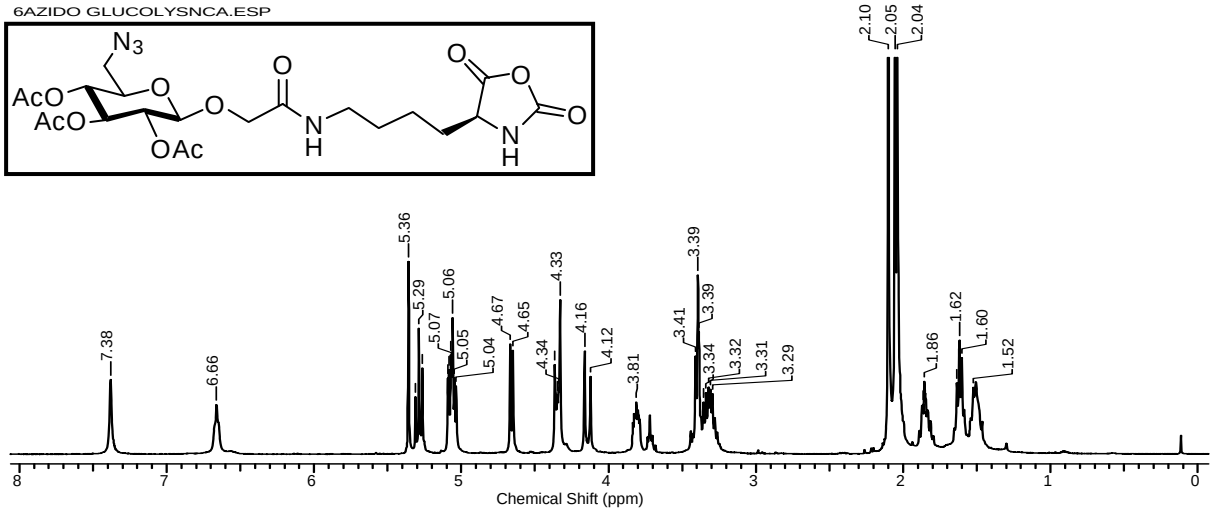
^{13}C NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **49**



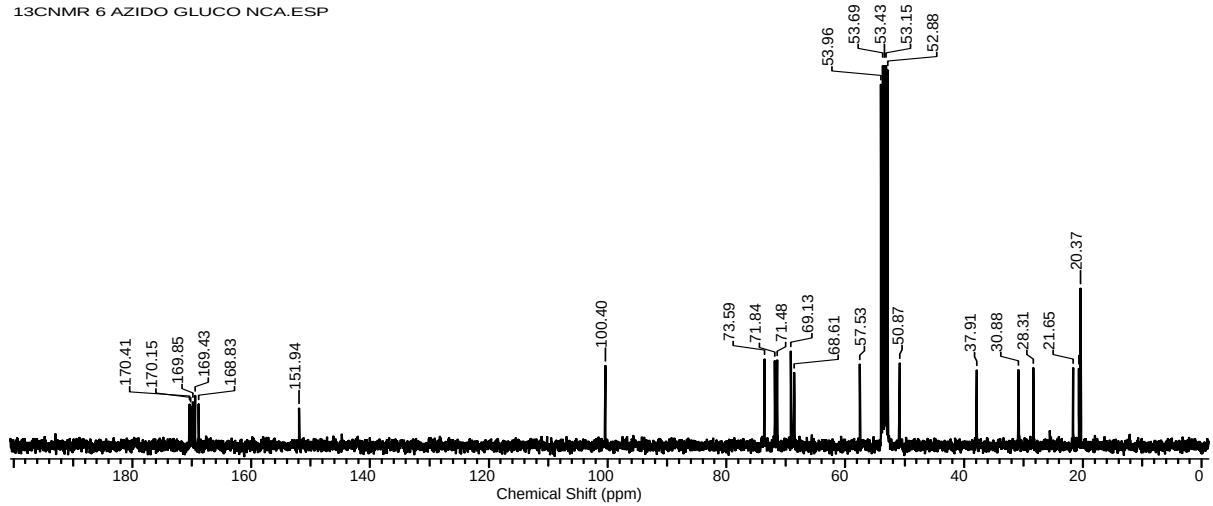
DEPT NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **49**



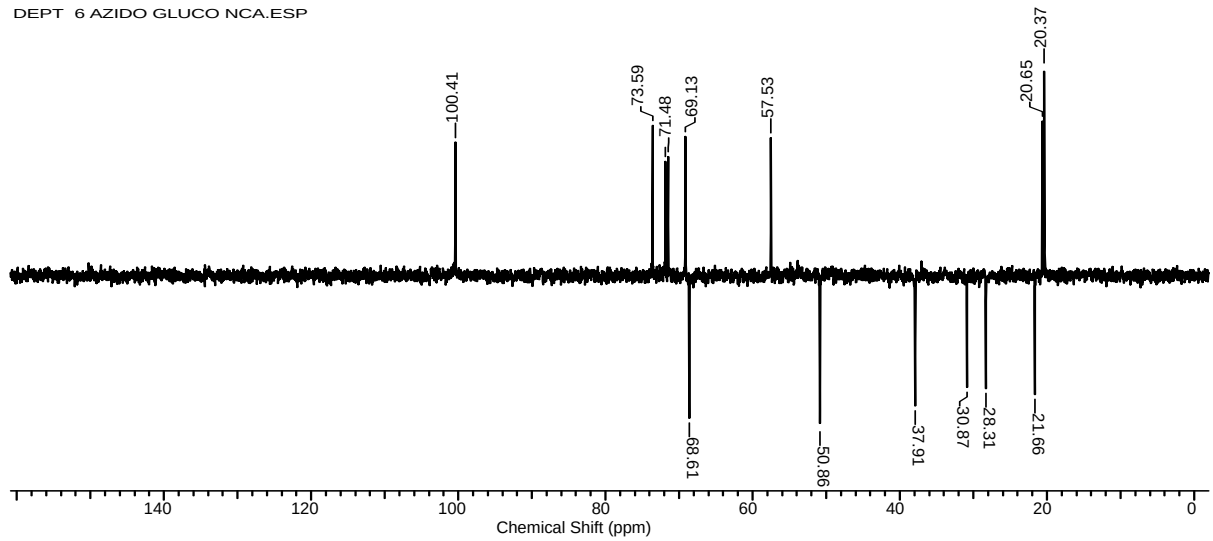
^{1}H NMR (CD_2Cl_2, 400.13 MHz) Spectrum of Compound **50**



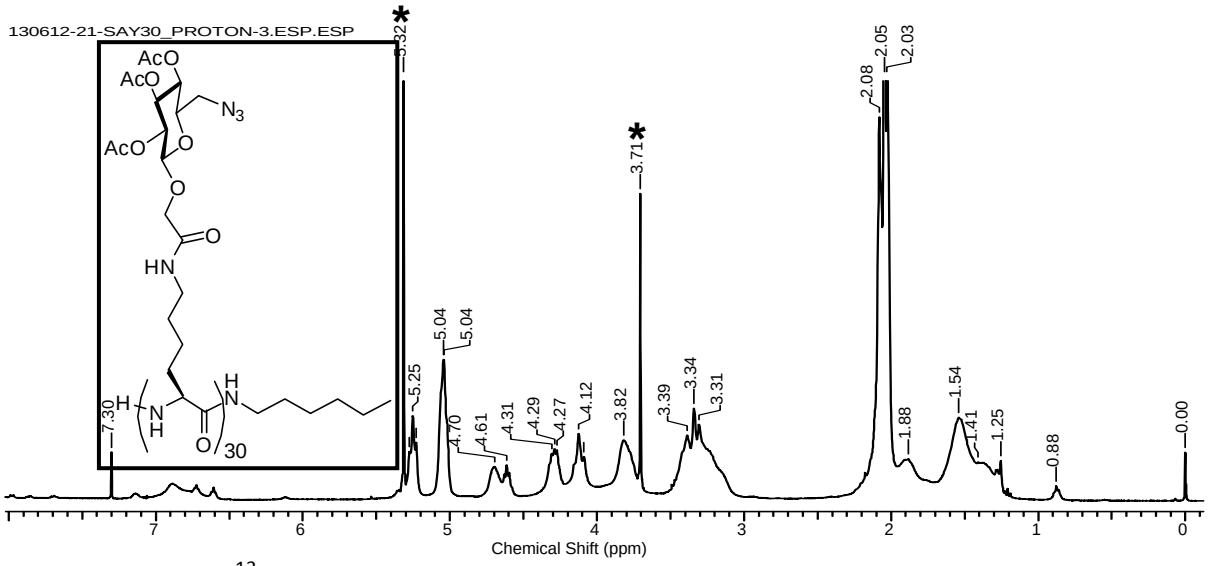
^{13}C NMR (CD_2Cl_2, 100.61 MHz) Spectrum of Compound **50**



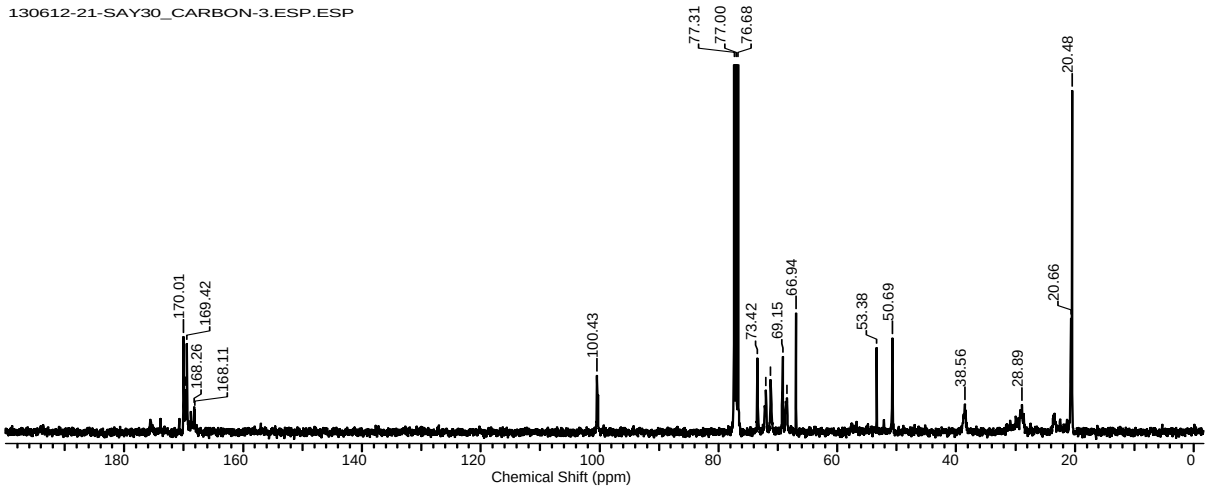
DEPT NMR (CD_2Cl_2, 100.61 MHz) Spectrum of Compound **50**



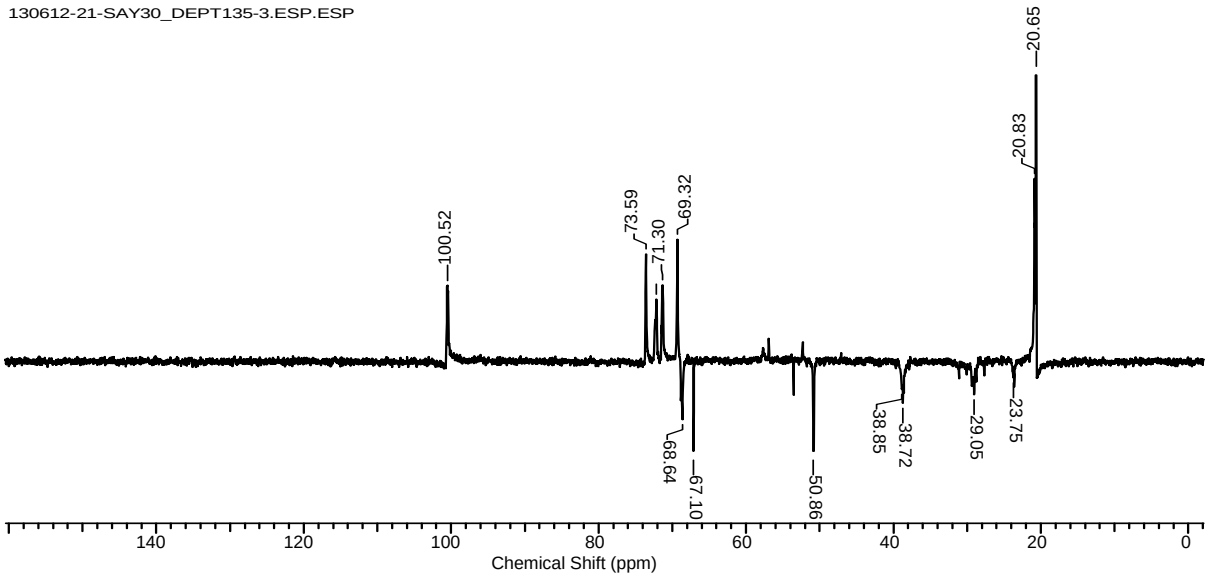
^{1}H NMR ($CDCl_3$, 399.78 MHz) Spectrum of Compound **51**



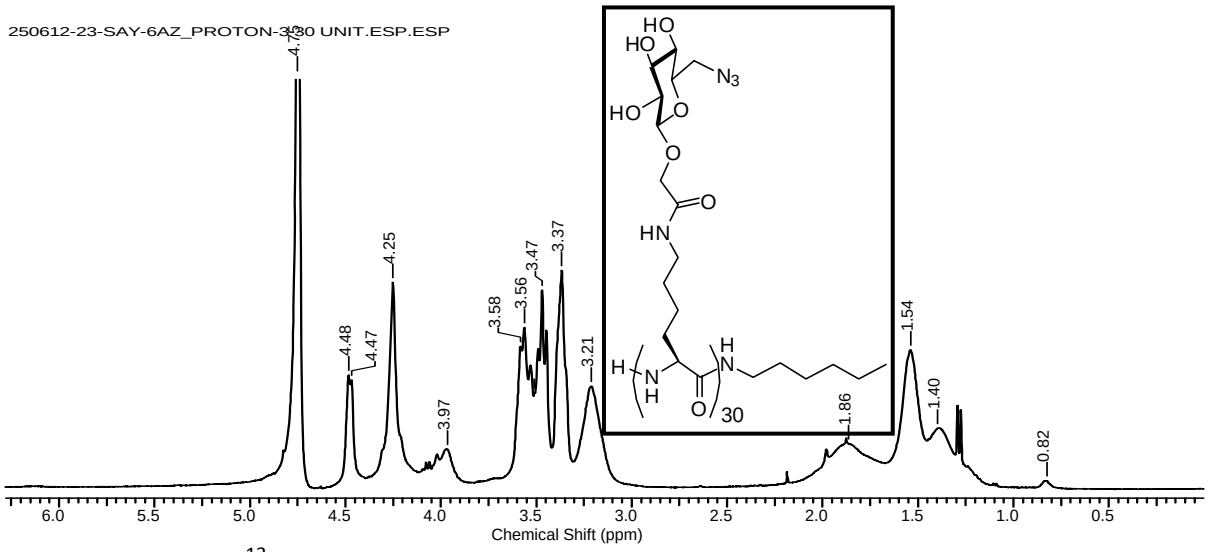
^{13}C NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **51**



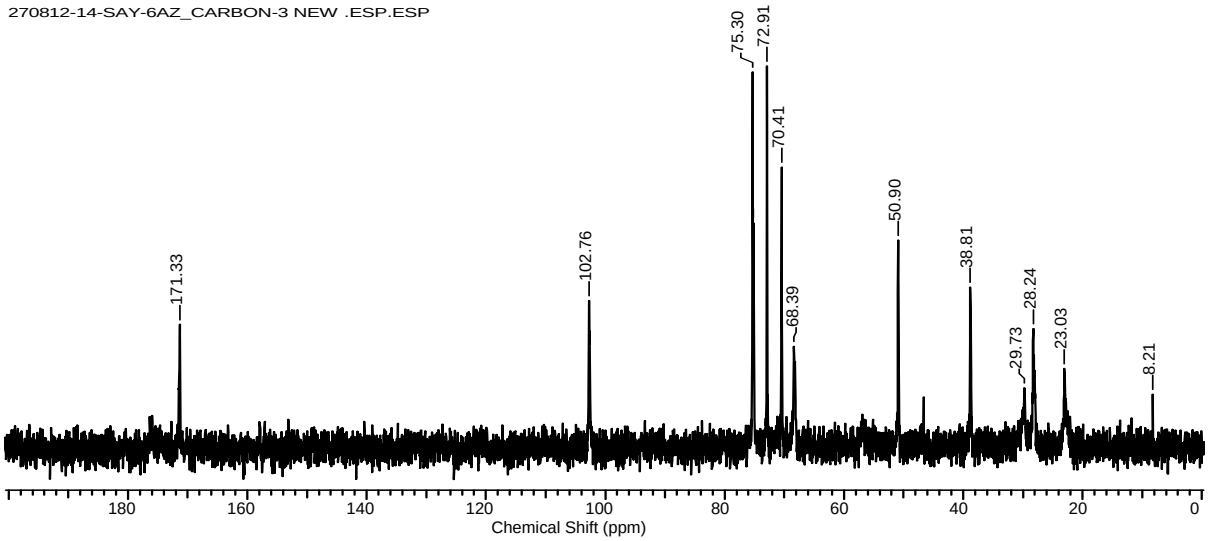
DEPT NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **51**



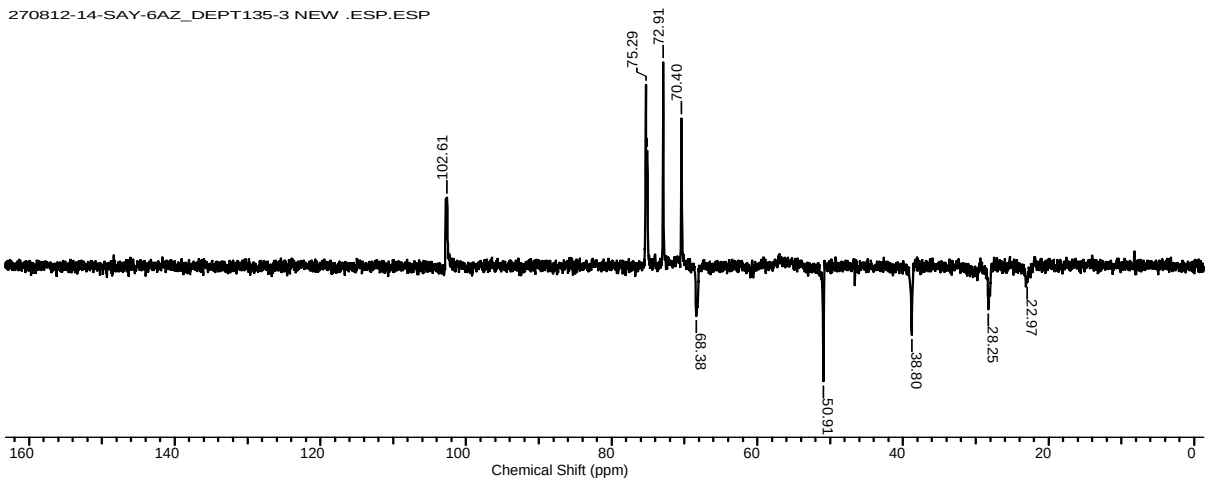
^{1}H NMR (D_2O, 399.78 MHz) Spectrum of Compound **52**



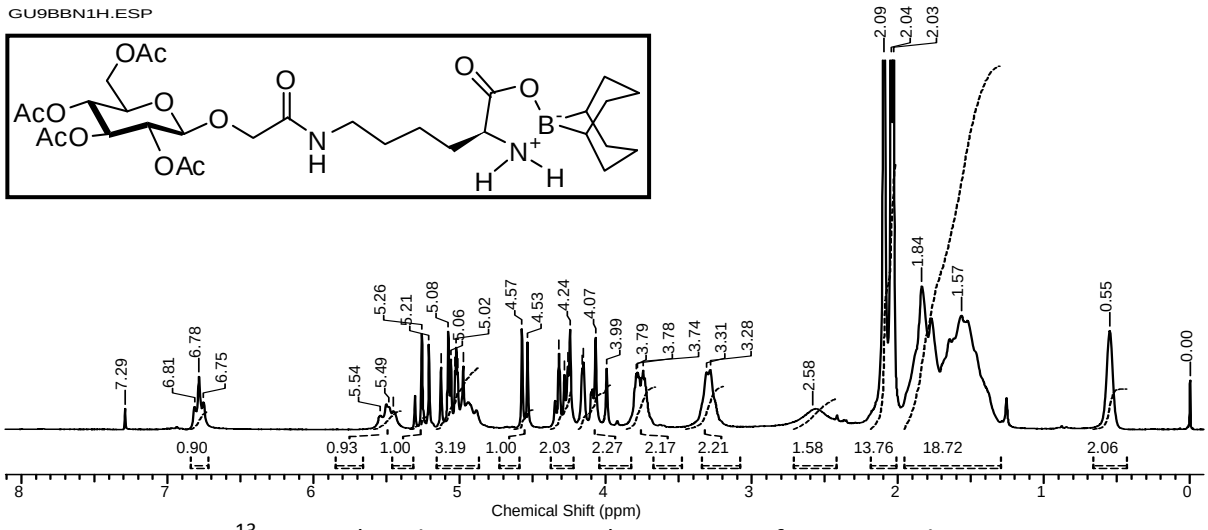
^{13}C NMR (D_2O, 100.53 MHz) Spectrum of Compound **52**



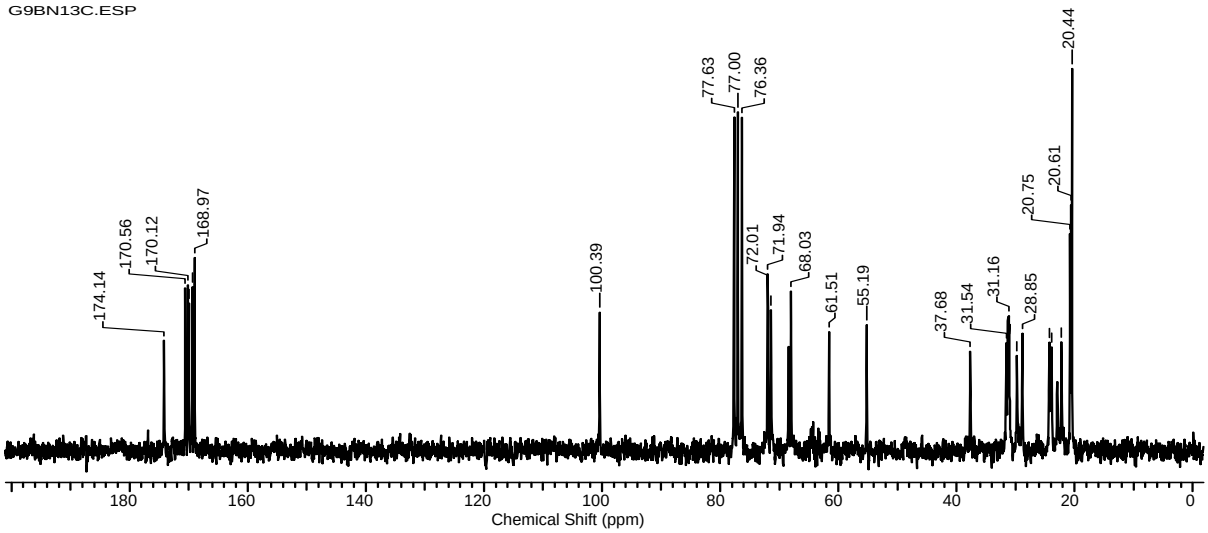
DEPT NMR (D_2O, 100.53 MHz) Spectrum of Compound **52**



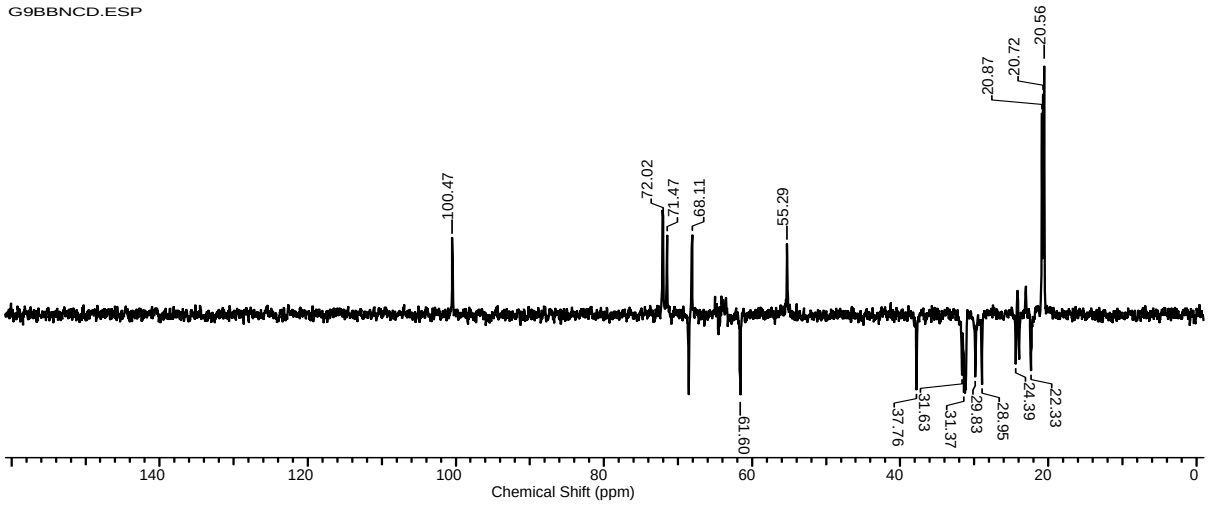
^{1}H NMR ($CDCl_3$, 399.78 MHz) Spectrum of Compound **53**



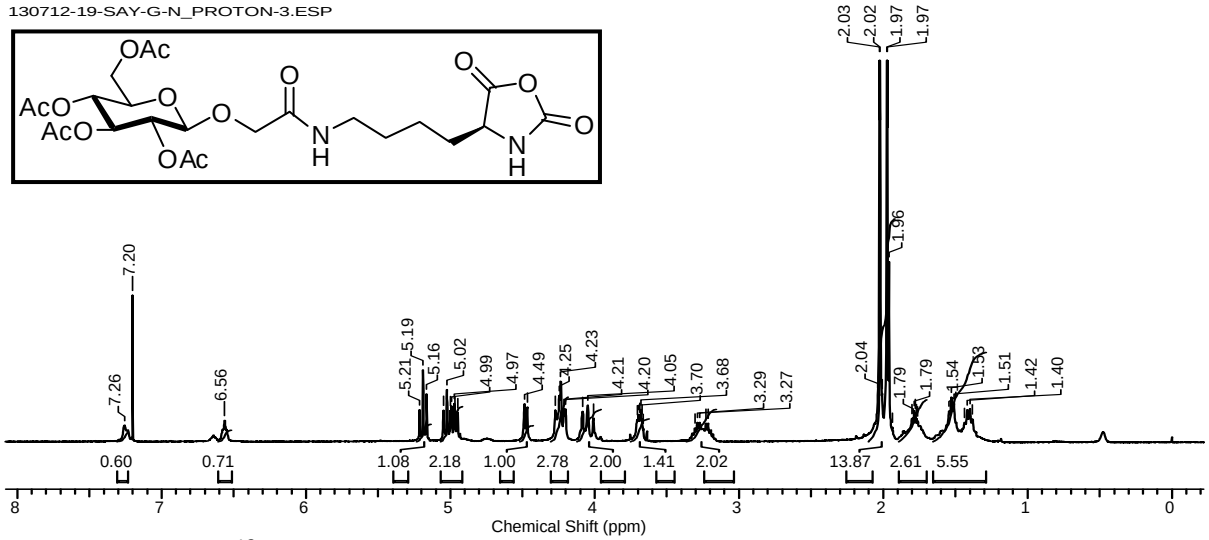
^{13}C NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **53**



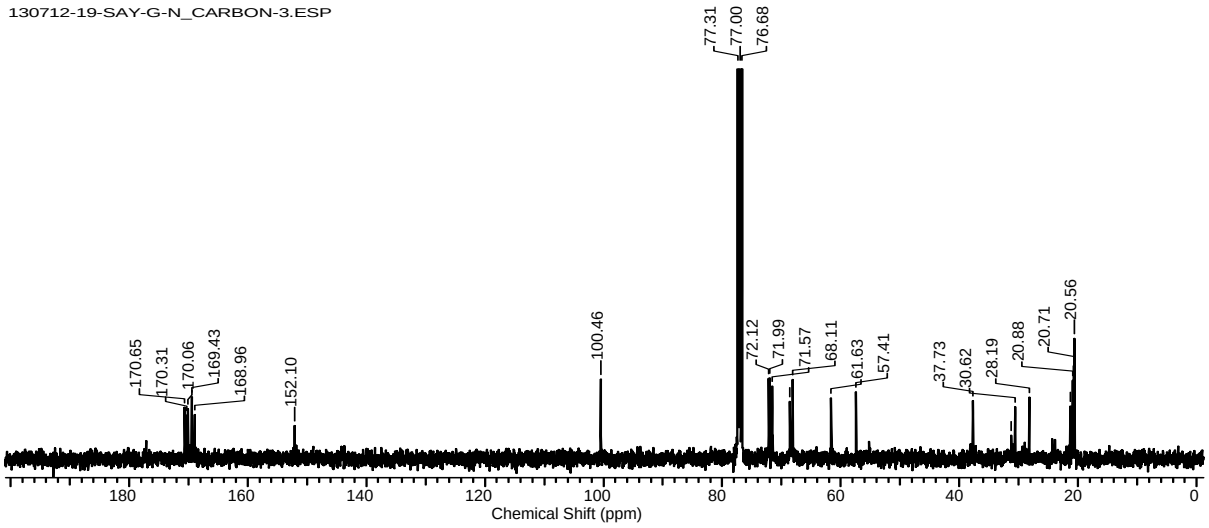
DEPT NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **53**



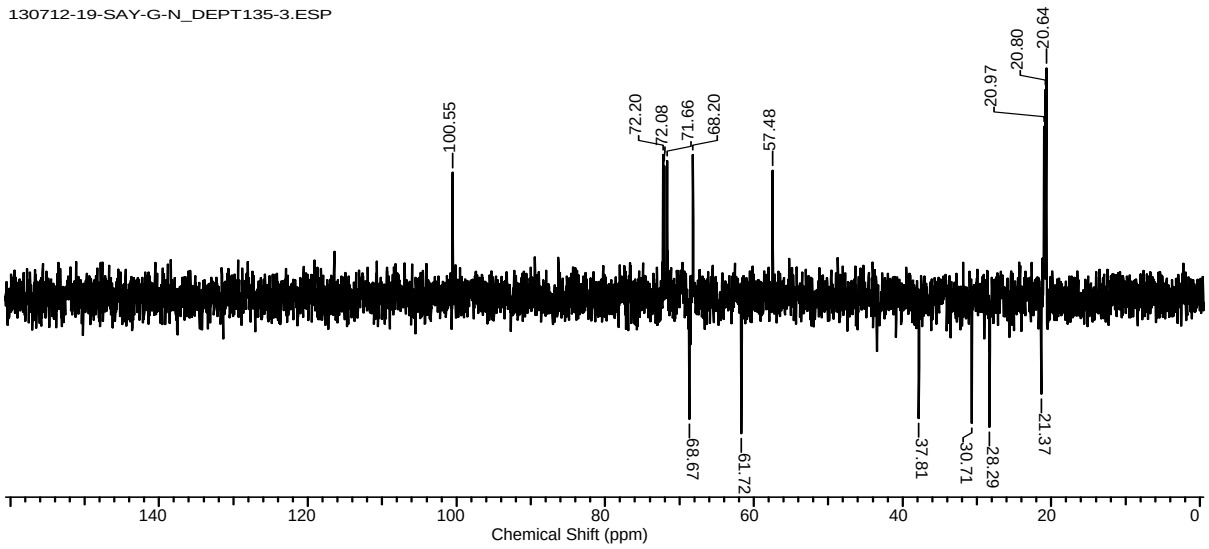
^{1}H NMR ($CDCl_3$, 399.78 MHz) Spectrum of Compound **54**



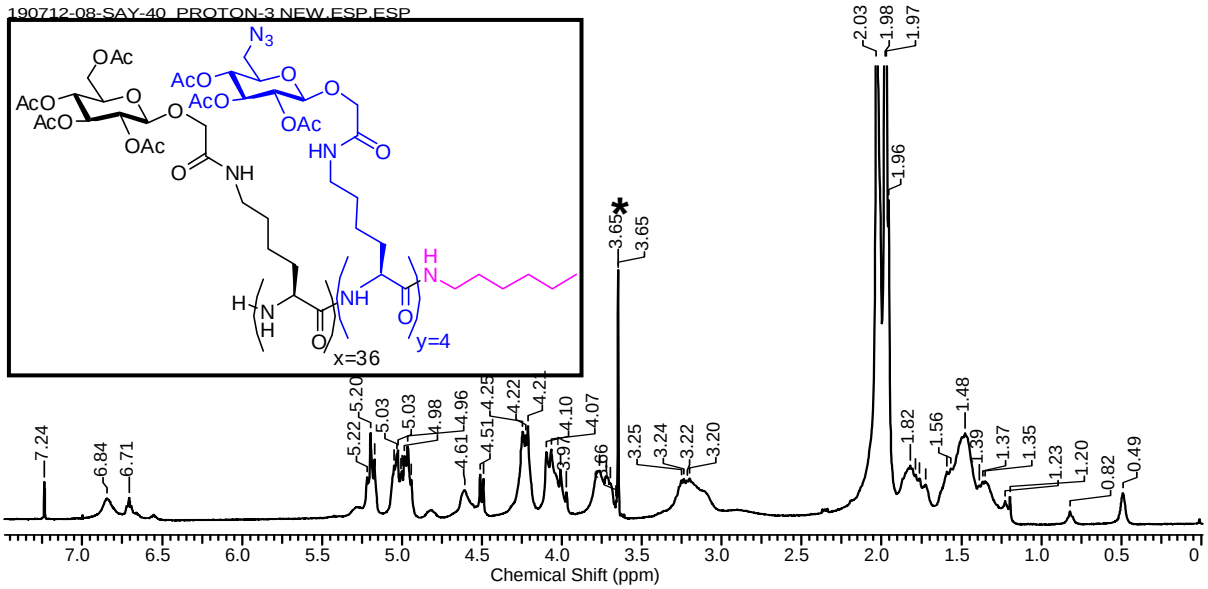
^{13}C NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **54**



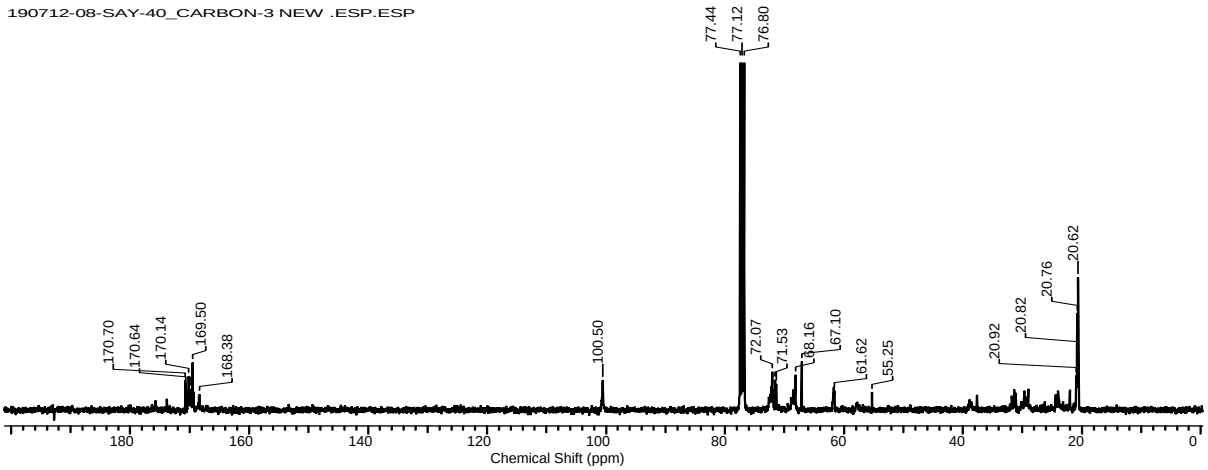
DEPT NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **54**



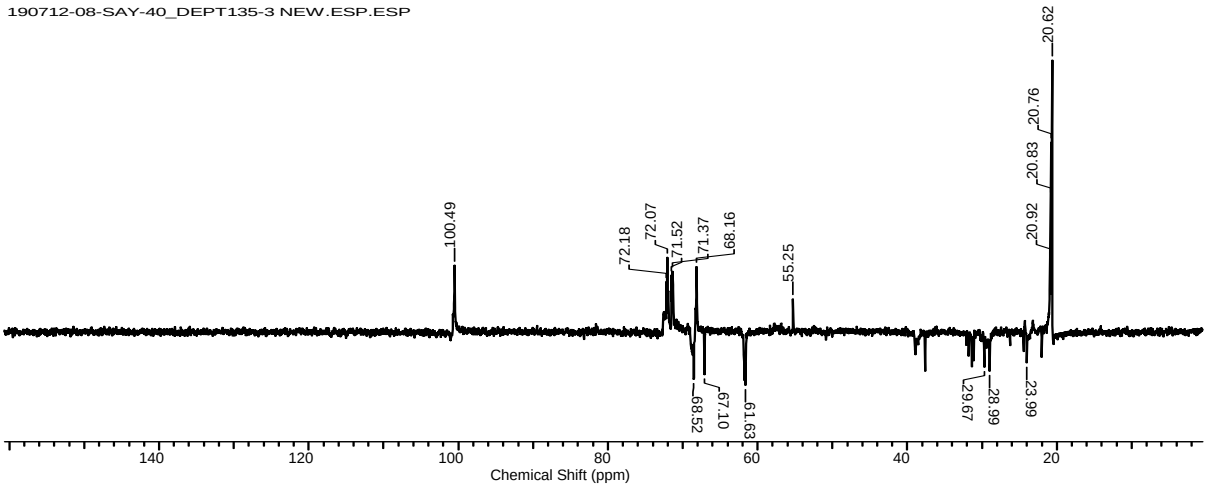
^{1}H NMR ($CDCl_3$, 399.78 MHz) Spectrum of Compound **55**



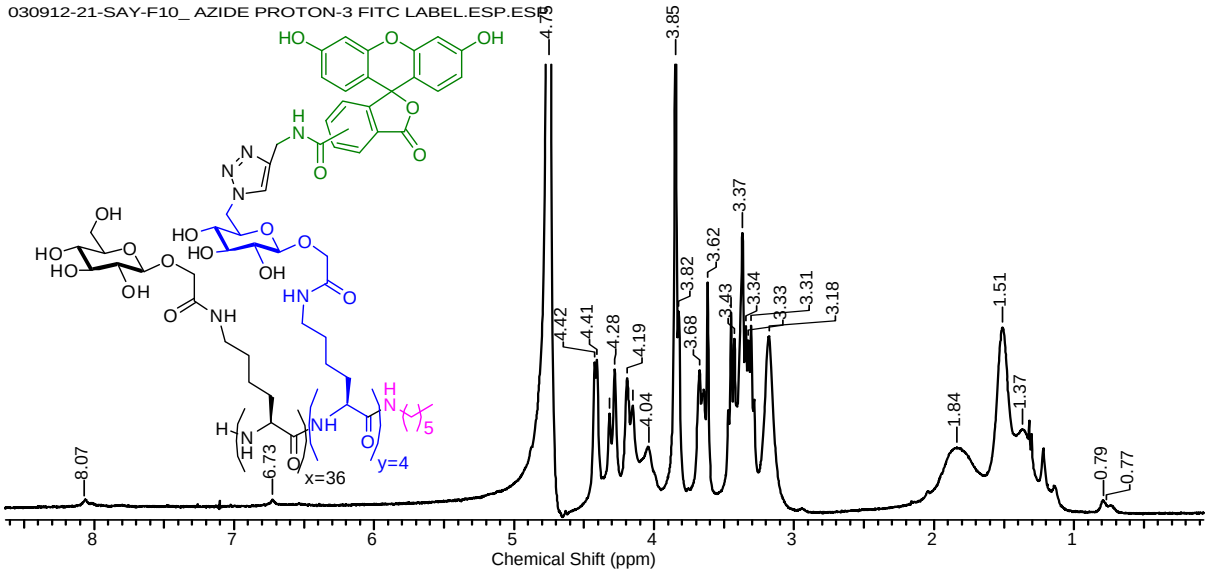
^{13}C NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **55**



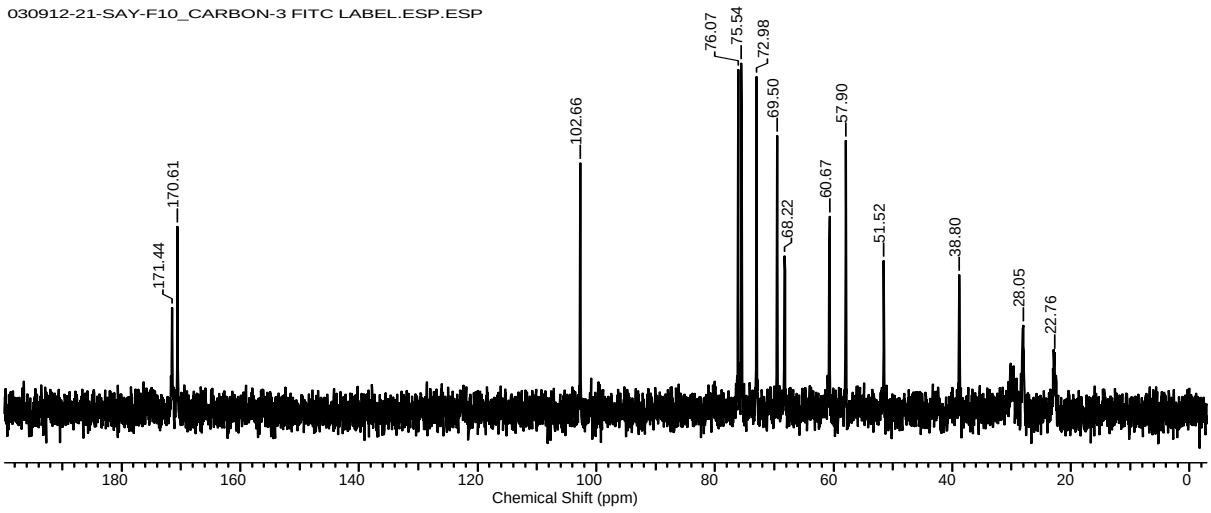
DEPT NMR ($CDCl_3$, 100.53 MHz) Spectrum of Compound **55**



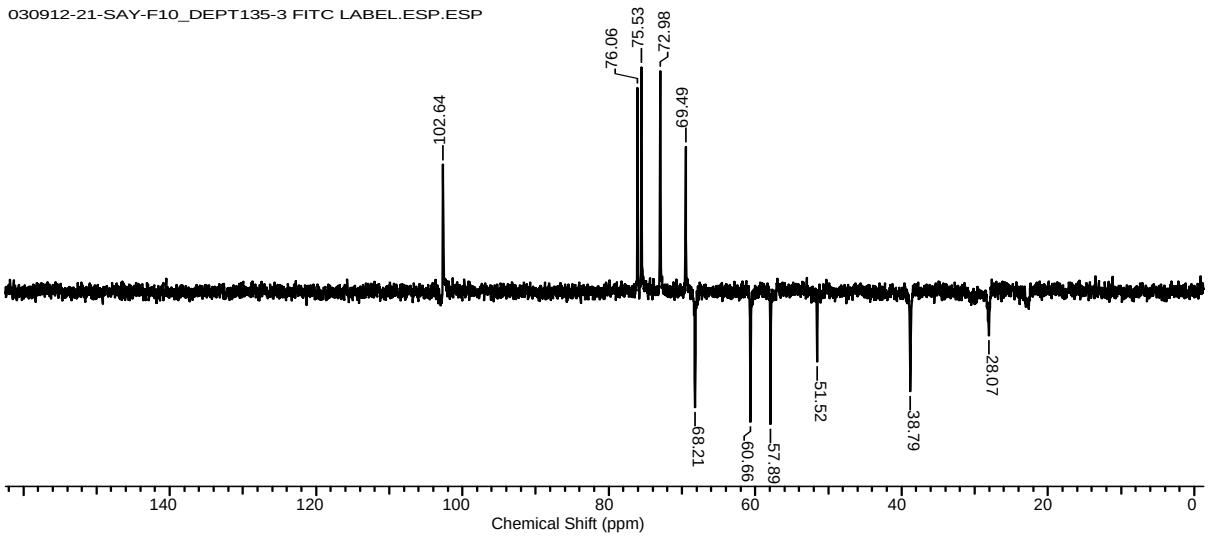
^{1}H NMR (D_2O, 399.78 MHz) Spectrum of Compound **56**



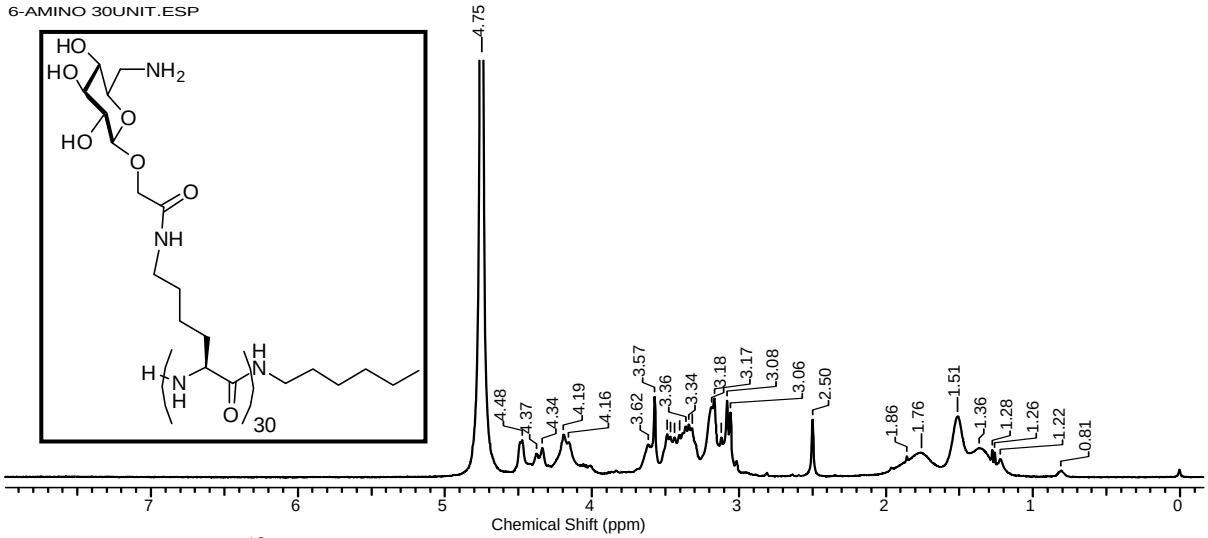
^{13}C NMR (D_2O, 100.53 MHz) Spectrum of Compound **56**



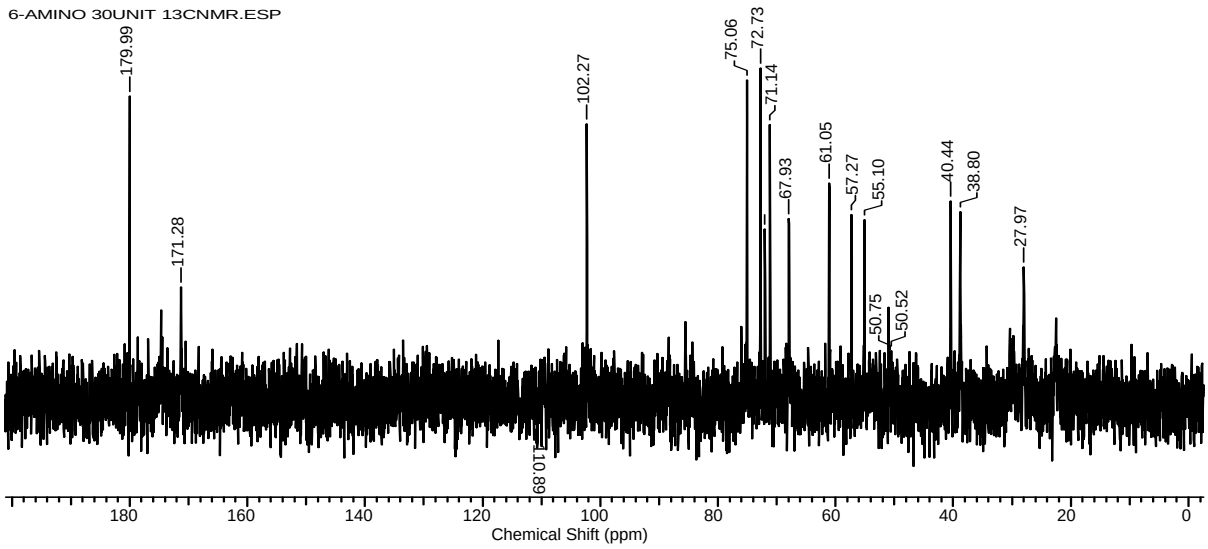
DEPT NMR (D_2O, 100.53 MHz) Spectrum of Compound **56**



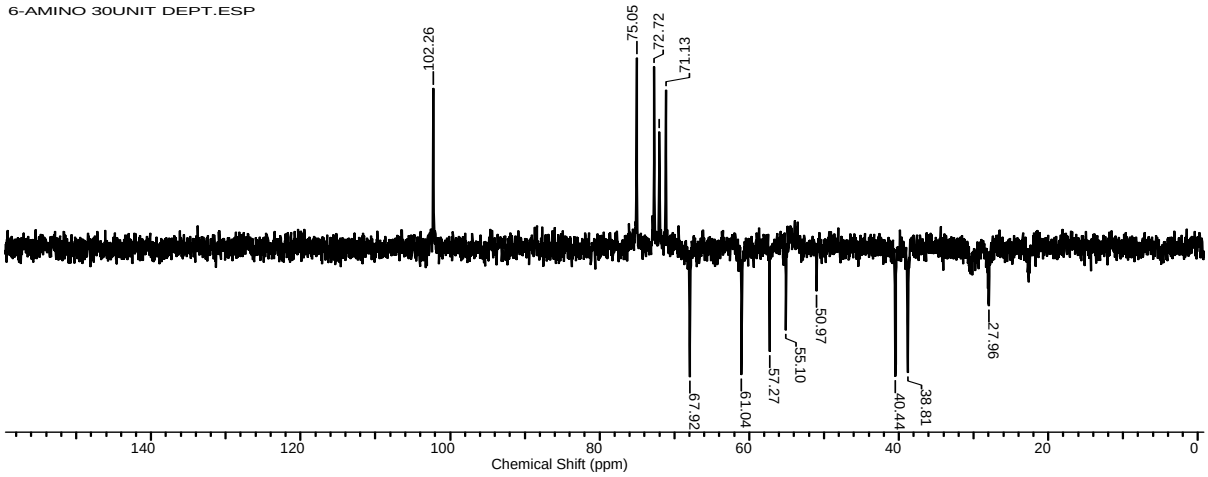
^{1}H NMR (D_2O, 399.78 MHz) Spectrum of Compound **57**



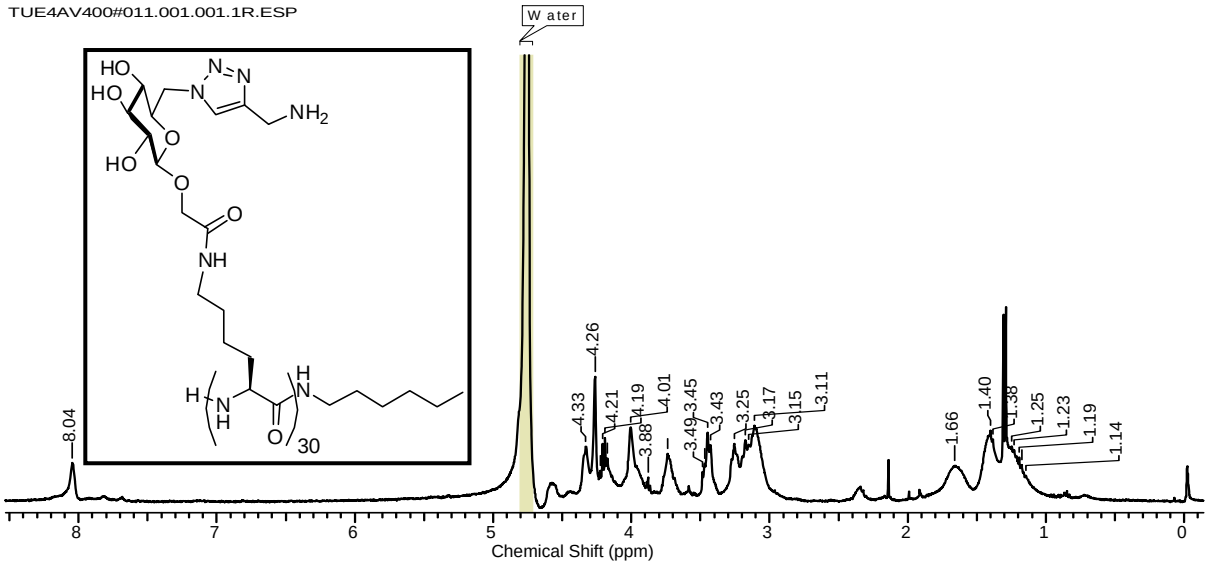
^{13}C NMR (D_2O, 100.53 MHz) Spectrum of Compound **57**



DEPT NMR (D_2O, 100.53 MHz) Spectrum of Compound **57**



^{1}H NMR (D_2O, 399.78 MHz) Spectrum of Compound **58**



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Publications

List Of Publications

1. Synthesis of Charged Helical Glycopolypeptide. **Ashif Shaikh**, Debasis Pati, Srinivas Hotha, Sayam Sen Gupta, *manuscript under preparation,* **2013**.

2. Synthesis and Self-assembly of Amphiphilic Homoglycopolypeptide. Vinita Dhaware, **Ashif Shaikh**, Mrityunjoy Kar, Srinivas Hotha, Sayam Sen Gupta, *Langmuir* , **2013,** accepted**.**

3. Controlled Synthesis of O-Glycopolypeptide Polymers and their Molecular Recognition by Lectins. Debasis Pati, **Ashif Y. Shaikh**, Soumen Das, Pavan Kumar Nareddy, Musti Joginadha Swamy, Srinivas Hotha, and Sayam Sen Gupta, *Biomacromolecules,* **2012**, *13*, 1287.

4. Synthesis of glycopolypeptides by the ring opening polymerization of O-glycosylated-α-amino acid N-carboxyanhydride (NCA). Pati, D.; **Ashif Y.Shaikh**; Hotha, S.; Sen Gupta, S*. Polym. Chem.* **2011**, 2, 805.

5. Facile synthesis of unusual glycosyl carbamates and amino acid glycosides from Propargyl 1,2,-orthoesters as Glycosyl donors. **Ashif Y. Shaikh**, Debasis Pati, Gopalsamy Sureshkumar, Sayam Sengupta and Srinivas Hotha, *Org. Biomol. Chem.***2011**, 9, 5951(**Cover Page Article**).

Erratum