Targeting Endothelial Inflammation by Super-paramagnetic Iron Glyco-Nano Particles.

A thesis submitted towards partial fulfilment of the requirements of BS-MS Dual Degree Program

By

KESAVA PHANEENDRA CHERUKURI

20111076

Indian Institute of Science Education and Research, Pune

Under the guidance of

Dr.Raghavendra Kikkeri

Assistant Professor

Department of Chemistry

Indian Institute of Science Education and Research Pune

CERTIFICATE

This is to certify that this dissertation entitled "**Targeting Endothelial Inflammation by Super-paramagnetic Iron Glyco-Nano Particles**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents the research carried out by **Kesava Phaneendra Cherukuri** at Indian institute of Science Education & Research, Pune under the supervision of **Dr.Raghavendra Kikkeri**, Assistant Professor, Department of Chemistry during the academic year 2015-2016.

Date: 28/3/2016

Place: Pune

Dr.Raghavendra Kikkeri

Assistant Professor

IISER Pune.

राघवेंद्र किक्केरी / Raghavendra Kikkeri
सहाय्यक प्राध्यापक, रसायनसास्त्र/ Assistant Professor, Chemistry
भारतीय विज्ञान शिक्षा एवं अनुसंधान संस्थान
Indian Institute of Science Education & Research
पुणे, भारत / Pune - 411 008 India

DECLARATION

I hereby declare that the matter embodied in the report entitled "**Targeting Endothelial Inflammation by Super-paramagnetic Iron Glyco-Nano Particles**" are the results of the investigations carried out by me at the Department of Chemistry, Indian Institute of Science Education & Research, Pune under the supervision of **Dr.Raghavendra Kikkeri** and the same has not been submitted elsewhere for any other degree.

Date:28/3/2016

Place: Pune

Kesava Phaneendra Cherukuri

5th Year B.S-M.S Dual Degree

Dedicated to my parents

ACKNOWLEDGEMENTS

First and foremost I would like to express my deep sense of gratitude to my research supervisor Dr.Raghavendra Kikkeri for his excellent guidance, continuous support & encouragement throughout the project and for very crucial discussions on diverse research topics over the time and freedom to explore various research directions like peptide chemistry, carbohydrate chemistry & nanoparticles for various material and bio applications in his lab.

I would also like to thank Prof.Krishna N Ganesh for his valuable suggestions during the midyear presentation and IISER Pune for the facilities.

I extend my heartful thanks to Sivakoti Sangabathuni for the continuous support during the peptide project and training me in my initial days of working in the lab.

My sincere thanks Rohan Yadav, Harikrishna Bavireddi and Sivakoti Sangabathuni for many crucial discussion sessions and clarifying many of my questions in the lab for the past 3.5 years.

I wholeheartedly thank all of my other lab members Chetan D S, Madhuri Gade, Catherine Alex, Prasanth Jain, Suraj, Balamurugan S, Akhil, Chinmay Tamane, Dr.Preeti Chaudhary, Dr.Raghavendra Vasudeva Murthy for their continuous support and making a pleasant atmosphere in the lab.

Lastly I would like to thank all the NMR, MALDI-TOF and HRMS operators for all the instrumentation help.

CONTENTS

ABBREVIATIONS

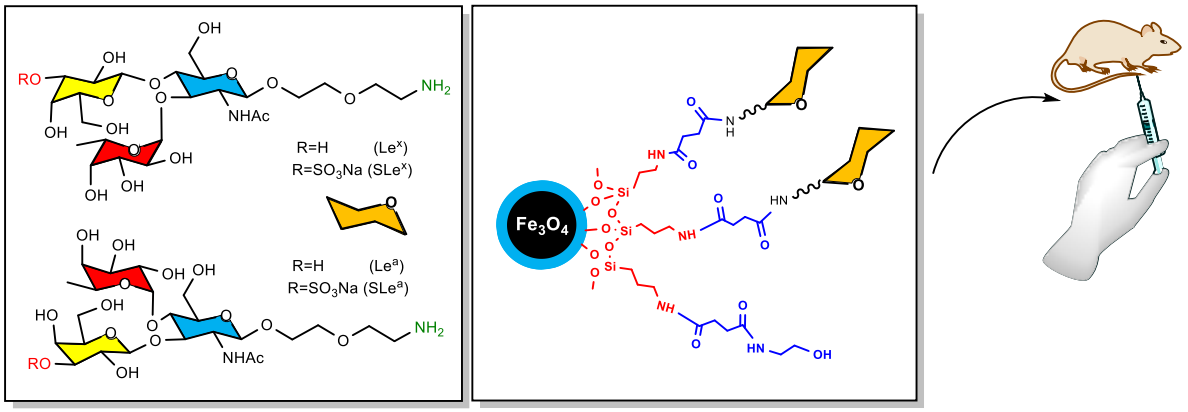
Ac_2O	Acetic anhydride
Py	Pyridine
r.t	Room temperature
h	Hours
LiOH	Lithium Hydroxide
Et_3SiH	Triethyl silane
SO_3	Sulphur trioxide
DCM	Dichloromethane
HCl	Hydrochloric acid
TFA	Trifluoro acetic acid
NaOMe	Sodium Methoxide
ml	Milli litre
M.S	Molecular Sieves
J	Coupling constant
$CHCl_3$	Chloroform
EtOAc	Ethyl Acetate
A^o	Angstrom
eq	Equivalents
Hz	Hertz
δ	Chemical Shift
MHz	Mega hertz

MeOH	Methanol
BnBr	Benzyl Bromide
NaH	Sodium Hydride
NaOH	Sodium Hydroxide
NaN_3	Sodium Azide
H_2O	Water
H_2	Hydrogen
$NaHCO_3$	Sodium bicarbonate
ACN	Acetonitrile
mol	Mole
g	Gram
mmol	Milli mole
NHS	N Hydroxysuccinimide
EtOH	Ethanol
NIS	N Iodo succinimide
µl	Micro litre
Et_3N	Triethylamine
CCl_3CN	Trichloroacetonitrile
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
DMAPA	Dimethyl aminopropylamine
THF	Tetrahydrofuran
Bu_2SnO	DiButyl Tin oxide
DBU	1, 8-Diazabicyclo [5.4.0] undec-7-ene

TrocCl	2, 2, 2 Trichloro ethylchloroformate
Pd/C	Palladium on Charcoal
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TfOH	Trifluoromethanesulfonic acid
FmocCl	Fluorenylmethyloxycarbonyl chloride
TEOS	Tetraethyl orthosilicate
IONP	Iron Oxide nanoparticles
TLC	Thin Layer Chromatography
HRMS	High Resolution Mass Spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionization-Time of flight
DEPT	Distortionless enhancement by polarization transfer
COSY	Correlation spectroscopy
CSA	(1S)-(+)-10-Camphorsulfonic acid
NMR	Nuclear Magnetic Resonance
DMF	N, N Dimethyl formamide
APS	(3-Aminopropyl) triethoxysilane
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide

ABSTRACT

The adhesion of leukocytes to vascular endothelium is a hallmark of the inflammatory process, which play a crucial role in multiple sclerosis (MS), ischemic stroke, and HIV-related dementia. Consequently, diagnoses of this activation are highly desirable. The conventional imaging techniques fail to diagnose the symptom, due to the weak permeability of the molecules through blood brain barrier (BBB). Hence, the delivery of imaging molecules across the BBB is still a major challenging aspect for presymptomatic diagnosis. Herein, we present the design and synthesis of sulfate-lewis^{x} functionalized nanoparticles that allow direct detection of endothelial marker E and P-selectin in acute inflammation. We designed silica-coated iron oxide core, which exhibits superparamagnetic and also provided multiple copies of carbohydrate to increase the avidity during specific carbohydrate-protein interactions. Magnetic resonance imaging studies of these nanoparticles are expected to target E and P selectin in the ischemic brain and potential for translation into the clinic.



INTRODUCTION

A stroke is caused by sudden interruption of the blood supply to the tissue. According to the World Health Organization, 15 million people suffer strokes worldwide each year. Of these, five million die and another five million are permanently disabled[1]. The most common type of stroke, the ischemic stroke, accounts for almost 85% of all strokes while the other 15% are hemorrhagic strokes[2]. Ischemic stroke occurs when an artery to the brain is blocked. When an artery is obstructed, the blood supply to an area of brain is stopped, leading to ischemia (lack of oxygen) and eventually necrosis (death of the tissue). If the artery remains blocked for more than a few minutes, the brain cells die via a series of events, the so-called ischemic cascade[3-5].Therefore, imaging these processes would provide valuable information on the symptoms and plan treatments more efficiently. Different targeting agents and fluorescent probes have been proposed for imaging the ischemic stroke. Among them biocompatible selectin specific carbohydrate ligands conjugated glyco-probes are promising, since they contribute to signalling, adhesion, and recognitions processes of the extra-cellular domain[6] **.**Recently, Davis group has shown *in vivo* imaging of artificial brain disease using carbohydrate-protein interactions[7]. Host response to injury or disease induces up-regulation of the carbohydrate-binding trans-membrane proteins CD62E (E-selectin) and CD62P (P-selectin) at the sites of inflammation in the brain, offering the opportunity to act as an ideal diagnostic marker. Iron nanoparticles were coated with Sialyl Lewis^x (SiaLe^x) – a targeting vector for CD62 followed by intravenous injection in the tail vein to visualize inflammation in the brain. In this work, selectin expression on activated endothelium in the brain was induced by microinjection of interleukin-1 (IL-1) into the left striatum of the rat. Since the artificial inflammation was used, it is unclear if one can extrapolate to the particular disease (i.e. ischemic stroke). To address this issue,

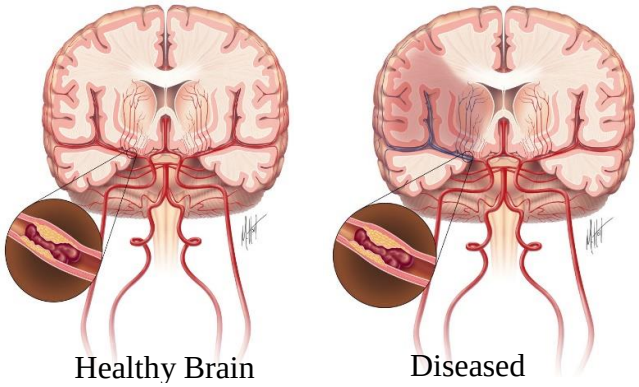


Figure: 1 Stroke caused by the blockade in brain

Seeberger et al.[8] used intraluminal thread (ILT) techniques to develop ischemic stroke mouse model. This involved the insertion of an occluding device into the internal carotid of the mice. The thread was removed from a mouse model of middle cerebral artery occlusion (MCAO) after 60 mins. Depending on the length of occlusion, the severity of the reduction in blood flow, and the collateral supply resulting impacts was located either in cortical and subcortical regions. Though these results provided valuable insights into the ischemic strokes, the weak blood brain barrier (BBB) permeability of these nanoparticles and non-specific binding affinity between SiaLe^x-selectin resulted in undesired background signal and limiting its application in translation research. Herein, we proposed to synthesize a duel functional iron nanoparticles carrying sulfate-lewis^x ligand and B6 peptides as a promising candidate with a potential to improve the BBB permeability [9, 10] and target the specific site more efficiently.

Objective:

The main objective of this work was to develop a series of hybrid iron nanoparticles carrying BBB targeting peptides and E/P-selectin targeting sugar unit. Magnetic resonance imaging (MRI) studies of these nanoparticles could yield valuable tool for early treatment of ischemic stroke.

The particular aims are:

1. Design and synthesis of sulfated-lewis^x and lewis^a derivatives
2. Synthesis of iron nanoparticles with superparamagnetic nature
3. Bio-conjugation of sugar and B6 peptides in different stoichiometric ratio.
4. Evaluate the sequestration and bio-distribution of these nanoparticles in normal and ischemic stroke mouse model.

Synthesis of Sulfated Le$^{x/a}$

Our strategy for detection of E and P-selectin exploited their affinity for sulfate-lewis$^{x/a}$ carbohydrate ligands[11-14]. Hence, we synthesized four tri-saccharides with linker amine functional to conjugate on IONP's (Figure 1). These glycan's were chemically synthesized from three important building blocks derived from D-Galactose, L-Fucose and D-Glucosamine respectively.

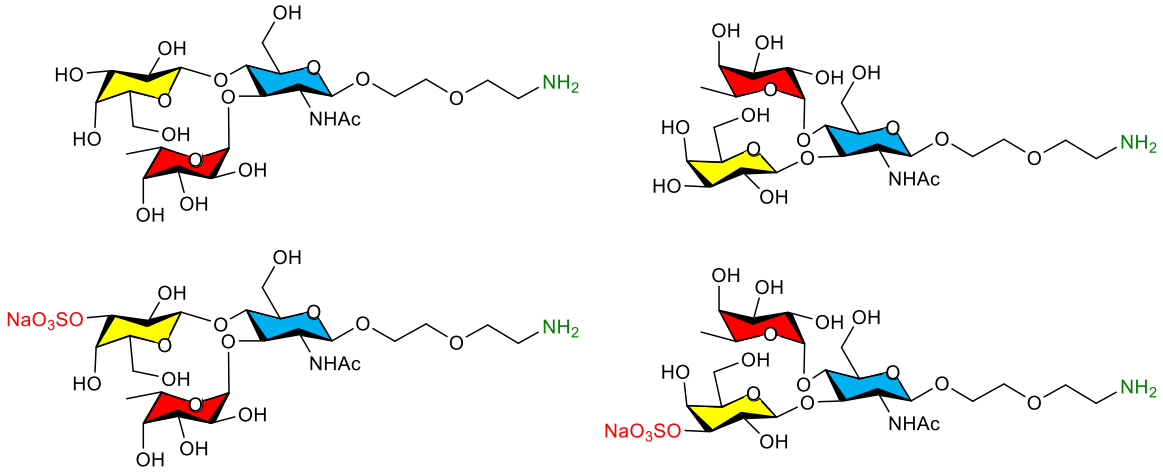
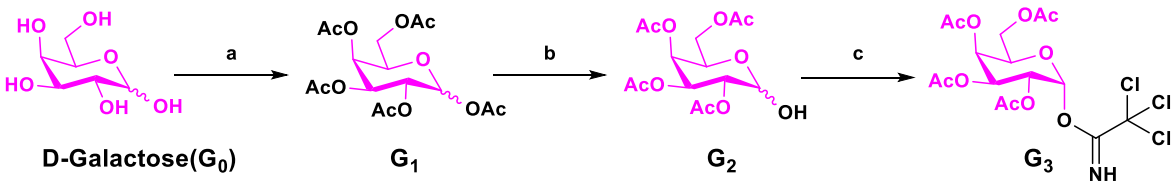


Figure: 2 Target molecules- lewis$^{x/a}$ carbohydrate ligands

Recently several elegant and high yielding synthetic strategies have been reported for the synthesis of the monomeric building blocks[15-20]. We have employed the same methods in our synthesis. Techniques like ^{1}H, ^{13}C, DEPT, HSQC, COSY NMR, MALDI-TOF and HRMS were used to the characterization the intermediates and final compounds.

Synthesis of Galactose Building Block:

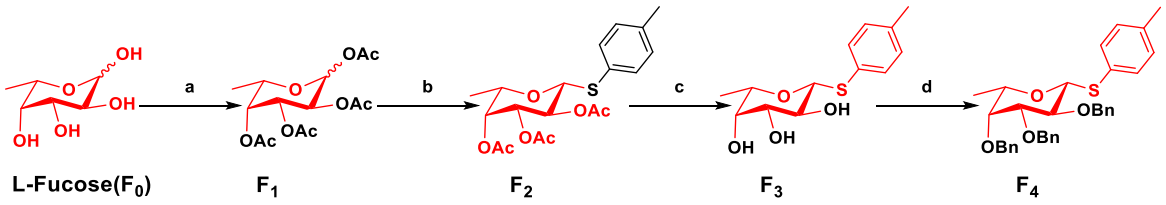


Scheme: 1 Synthesis of Galactose Donor

Reagents: a) Ac_2O, Py, 0 ºC to rt, 12h, 90% ; b) DMAPA, THF, rt, 2 h, 65% ; c) CCl_3CN, DBU, DCM, rt, 1 h, 75%.

Commercially available D-Galactose (**G_0**) was used to prepare the required building block **G_3** in three steps. In the first step all the hydroxyl groups of D-galactose were protected with base labile acetyl group using pyridine as a base and acetic anhydride as a reagent. Once the reaction was completed excess solvents were removed and subsequent precipitation in diethyl ether yielded 90% of **G_1** as a white solid. The obtained compound **G_1** was dissolved in THF and the anomeric acetyl group was selectively deprotected by using *N,N'*-dimethyl amino propyl amine[21] (DMAPA) .The excess reagent was removed by extracting the compound with 1N HCl to yield 65% of **G_2** as a semi solid. Finally, compound **G_2** was dissolved in dry DCM and mixed with DBU & CCl_3CN at rt. The clear solution turned into blackish liquid indicating the completion of reaction. Excess solvents were evaporated and purified by silica column chromatography. Further co-precipitation in hexane yielded 75% of desired galactose building block **G_3** as a white solid. The singlet at 8.66 ppm corresponds to NH proton of **G_3** and the doublet at 6.6 ppm and J = 3.4 Hz in ^{1}H NMR corresponds α-conformation of the sugar, which was further supported by ^{13}C NMR peak at 94ppm.

Synthesis of Fucose Building Block:



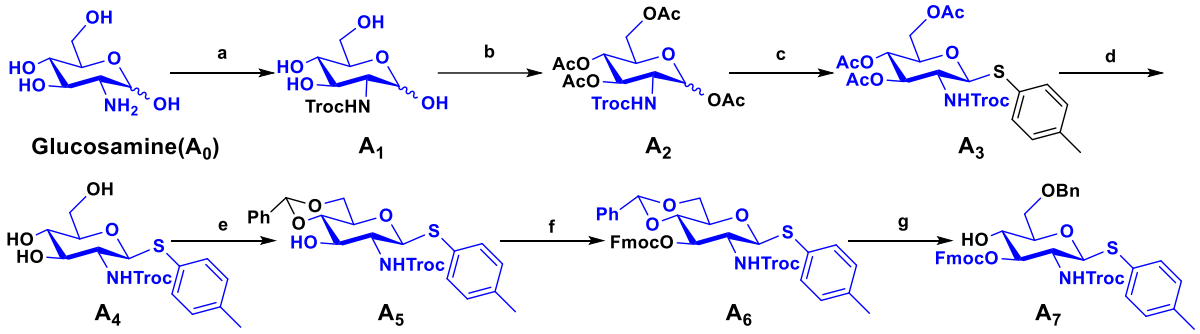
Scheme: 2 Synthesis of Fucose Donor

Reagents: a) Ac_2O, Py, 0 ºC to rt, 12 h, 90% ; b) *p*-Thiocresol, $BF_3.Et_2O$, Dry DCM, 0º C to rt, 12 h, 85% ; c) NaOMe, MeOH:DCM (1:1), rt, 2 h, 90% ; d) BnBr, NaH, DMF, 0 ºC to rt, 6 h, 75%.

Commercially available L-Fucose (**F_0**) was used to prepare the required building block **F_4** in four steps. In the first step, all the hydroxyl groups of L-fucose were protected with base labile acetyl group using pyridine and acetic anhydride as a solvent and reagent respectively. Once the reaction was completed excess solvents was removed and subsequent precipitation in diethyl ether yielded 90% of **F_1** as a white solid. The resulting compound was then dissolved in Dry DCM and *p*-

thiocresol was added and stirred at 0 ºC, subsequently mild Lewis acid $BF_3.Et_2O$ was added dropwise to the reaction mixture and stirred at 0 ºC for 12 h and once the reaction was completed, the reaction mixture was quenched and extracted with $NaHCO_3$ and purified by column chromatography to yield 85% of **F_2** as a white solid. The presence of two doublets at 7.41 ppm & 7.12 ppm in aromatic region and a singlet at 2.34 ppm indicates the incorporated thio-cresol moiety into the molecule and doublet at 4.63 ppm with coupling constant *J* = 9.9Hz in ^{1}H NMR corresponds to β-conformation of the sugar which is also further confirmed by the peak at 86.9 ppm in ^{13}C NMR. The compound **F_2** was then dissolved in equi-volume mixture of methanol and dichloromethane and acetyl groups were deprotected by using NaOMe. The disappearance of three singlets at 1.97 ppm, 2.09 ppm, 2.14 ppm in ^{1}H NMR and C=O peaks at 170.65 ppm, 170.15 ppm, 169.51 ppm and acetyl CH_3 peaks at 20.9 ppm, 20.65 ppm, 20.68 ppm in ^{13}C NMR confirmed the formation of the desired product **F_3**. Finally, **F_3** was benzylated and purification by silica gel column chromatography yielded 75% of desired fucose building block **F_4** as a white solid. The doublet at 4.55 ppm with coupling constant *J* = 9.6 Hz in ^{1}H NMR corresponds to β-conformation of the sugar which is also further confirmed by the peak at 87.88 ppm in ^{13}C NMR indicating that the conversion from **F_2** to **F_4** have not hindered the configuration of the anomeric position.

Synthesis of Glucosamine Building Block:

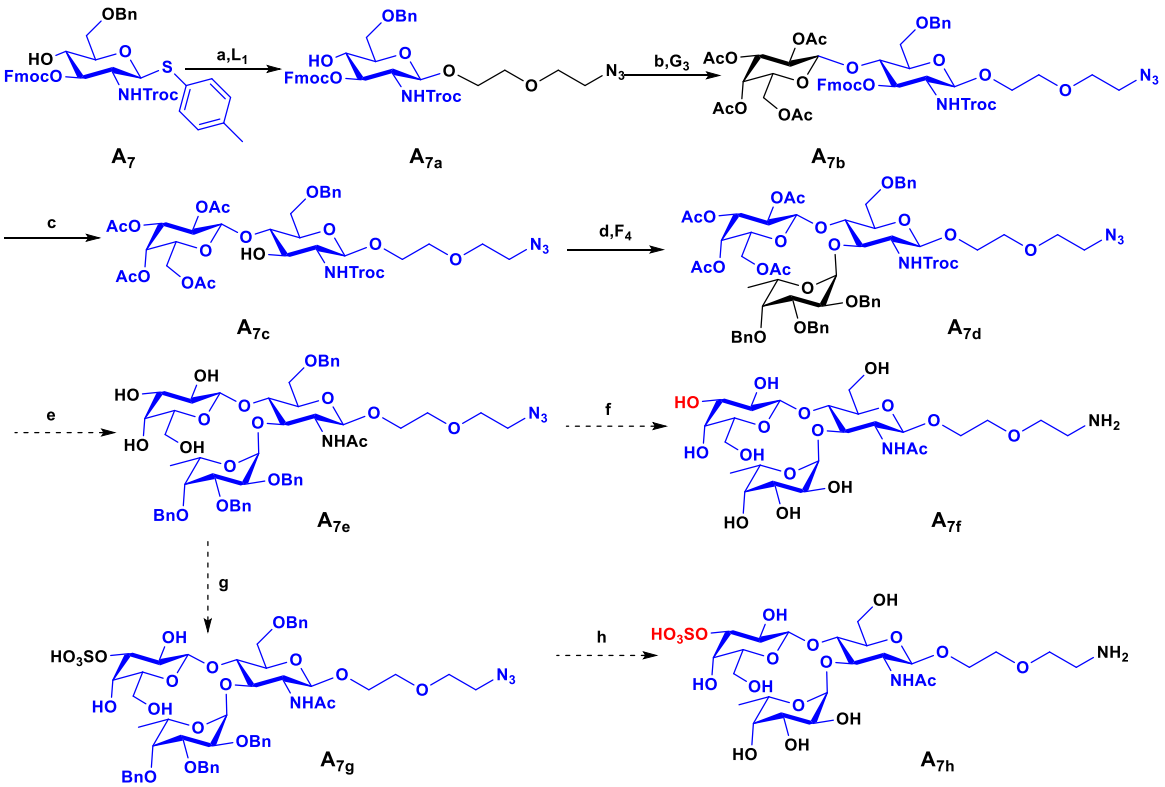


Scheme: 3 Synthesis of Glucosamine building blocks for Le^x & Le^a

Reagents: a) TrocCl, $NaHCO_3$, THF:H_2O (1:20), 0 ºC to rt, 6 h, 95%; b) Ac_2O, Py, 0 ºC to rt, 12 h, 92% ; c) *p*-Thiocresol, $BF_3.Et_2O$, Dry DCM, 0 ºC to rt, 12 h, 85% ; d) NaOMe, MeOH:DCM (1:1), 0 ºC, 2 h, 86% ; e) $Ph(OMe)_2$, CSA, ACN, rt, 1 h, 80%; f) FmocCl, Py:DCM (3:2), 0 ºC to rt, 3 h, 80% ; g) Et_3SiH, TFA, Dry DCM, 0 ºC to rt, 1 h, 83%.

Glucosamine building block **A_7** was readily synthesized from commercially available D-Glucosamine by protection of C-2 amine with 2, 2, 2-trichloro ethyl chloroformate (Troc) and hydroxyl groups with acetyl group, yielding 92% of two non-separable diastereomers as a white solid. The anomeric acetyl group was substituted with *p*-thiocresol in presence of mild lewis acid $BF_3.Et_2O$ resulting in 85% of **A_3** as white solid in β-confirmation at the anomeric position which was confirmed by ^1H & ^{13}C NMR. The obtained protected sugar **A_3** was then subjected to de-acetation under basic conditions at 0 ºC resulting in 90% of **A_4** as white solid. Furthermore, the C-4 and C-6 hydroxyl group of **A_4** was protected with benzaldehyde dimethyl acetal and CSA as reagents in acetonitrile solvent to afford 80% of **A_5** as white solid which was confirmed by the singlet at 5.53 ppm for the benzylidine proton and a doublet at 4.86 ppm of anomeric proton with coupling constant *J* = 10.3 Hz indicating the product is in beta confirmation in ^1H NMR. Finally, C-3 hydroxyl group of **A_5** was protected with base labile carbamate protecting group FmocCl so that the C-4 hydroxyl group can be accessible further. The selective ring opening of benzylidine in **A_6** with Et_3SiH & TFA yielded 83% of desired building block **A_7** as white solid which was used for further reactions.

Synthesis of Le^x and sulfated Le^x:

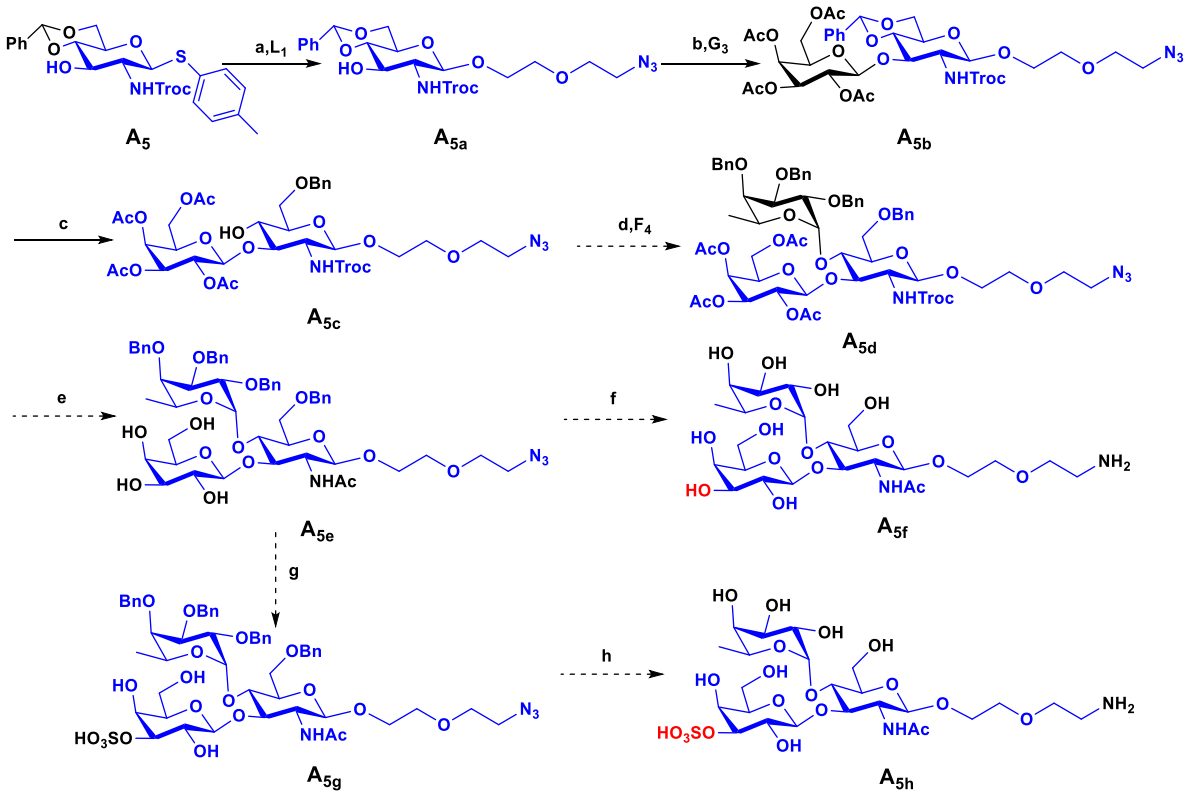


Scheme 4: Synthesis of Le^x & SLe^x Tri-Saccharides

Reagents: a) NIS, TfOH, Dry DCM, -20 ºC, 1 h, 85% ; b) TMSOTf, Dry DCM, -78 ºC to rt, 3 h, 45% ; c) Et_3N, DCM, rt, 2 h, 95% ; d) NIS, TfOH, Dry DCM, -40 ºC, 0.5 h, 70% ; e) i) $LiOH.H_2O$, $EtOH:H_2O$ (3:2) , 80 ºC, 6 h ; ii) Ac_2O, $NaHCO_3$, $MeOH:H_2O$ (1:1) , rt, 3 h ; f) H_2, Pd/C, MeOH, rt, 10 h ; g) i) Bu_2SnO, MeOH, Relux, 2 h; ii) SO_3.Py, THF, rt, 40 h ; h) H_2, Pd/C, MeOH, 15 ºC, 1 h.

The synthesis of the Le^x was achieved by step-by-step glycosylation of **G_3** and **F_4** donors with **A_{7a}** acceptor (scheme 4) The anomeric thio cresol of **A_7** was replaced by linker **L_1** using NIS & TfOH as an activator and promoter respectively at -20 ºC resulting in 85% of **A_{7a}** as white solid, which was further subjected to glycosylation reaction with the galactose donor **G_3** yielding 45% of product as a mixture of required compound **A_{7b}** (90%) and Galactose-OH (10%) which couldn't be separated by silica gel column chromatography. (Determined by NMR). This mixture was then dissolved in DCM and the carbamate protecting group (Fmoc) was deprotected by using Et_3N.Purification of this crude compound has yielded 95% of required product **A_{7c}** which was characterized by ^1H, ^{13}C, COSY to determine that the newly formed glyosidic bond was in beta conformation. Further fucose donor **F_4** was reacted with the obtained disaccharide acceptor **A_{7c}** resulting in 70% of Le^x trisaccharide **A_{7d}** in protected form as white solid.

Synthesis of Le^a and sulfated Le^a:



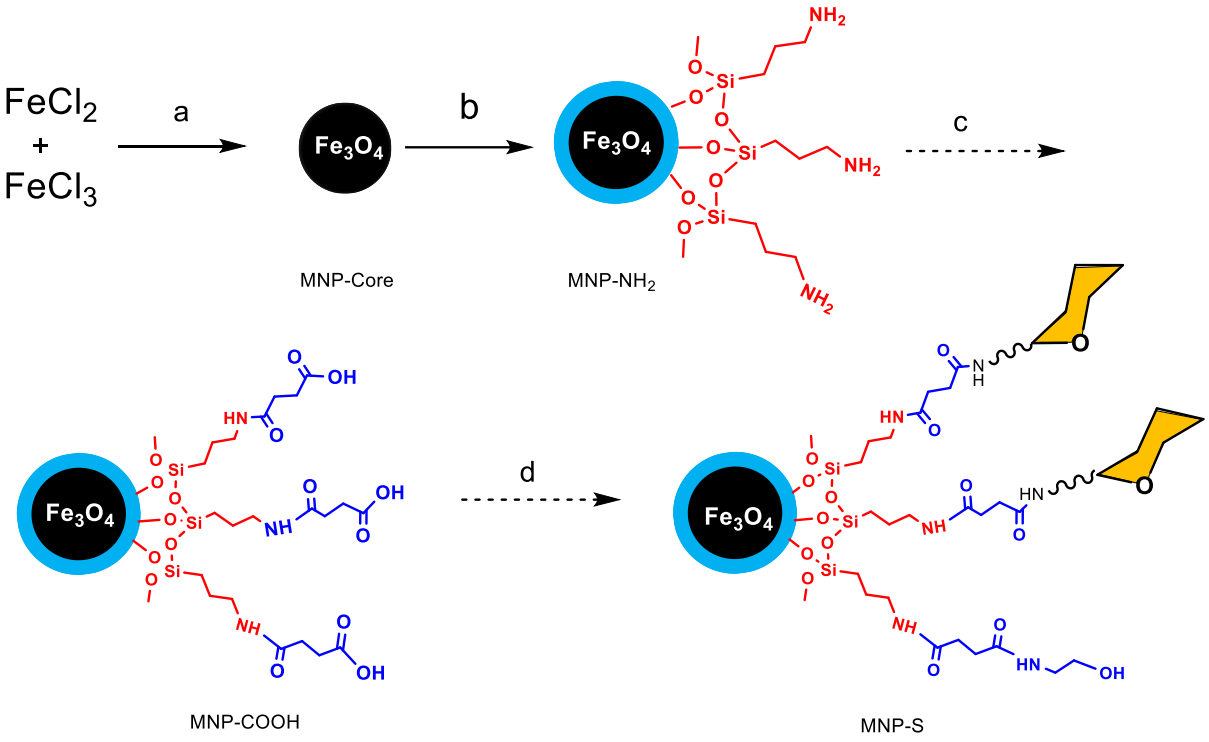
Scheme 5: Synthesis of Le^a & SLe^a Tri-Saccharides

Reagents: a) NIS, TfOH, Dry DCM, -20 ºC, 1 h, 70% ; b) TMSOTf, Dry DCM, -78 ºC to rt, 3 h, 52% ; c) Et_3SiH, TFA, Dry DCM, 0 ºC to rt, 1 h, 65% ; d) NIS, TfOH, Dry DCM, -40 ºC, 0.5 h ; e) i) $LiOH.H_2O$, EtOH:H_2O (3:2) , 80 ºC, 6 h ; ii) Ac_2O, $NaHCO_3$, MeOH:H_2O (1:1) , rt, 3 h ; f) H_2, Pd/C, MeOH, rt, 10 h ; g) i) Bu_2SnO, MeOH, Relux, 2h ; ii) SO_3.Py, THF, rt, 40 h ; h) H_2, Pd/C, MeOH, 15 ºC, 1 h.

The synthesis of the Le^a was achieved by step-by-step glycosylation of **G$_3$** and **F$_4$** donors with **A$_{5a}$** acceptor (scheme 5) The anomeric thio cresol of **A$_5$** was replaced by linker **L$_1$** using NIS & TfOH as an activator and promoter respectively at -20 ºC resulting in 70% of **A$_{5a}$** as white solid, which was further subjected to glycosylation reaction with the galactose donor **G$_3$** resulting in 52% of the product as a mixture of the required compound **A$_{5b}$** (80%) and Galactose-OH (20%) which couldn't be separated by silica gel column chromatography (determined by NMR). This mixture was then dissolved in dry DCM and the 4-6 benzylidine ring was subjected to reductive ring opening in the presence of Et_3SiH and TFA to yield 65% of required disaccharide **A$_{5c}$** as white solid which was characterized by ^1H, ^{13}C, COSY to

determine that the newly formed glyosidic bond was in β-conformation. Further fucose donor **F_4** will be reacted with the obtained disaccharide **A_{5c}** acceptor to achieve the Le^a trisaccharide **A_{5d}** in protected state similar to **A_{7d}.**

Synthesis of Iron Glyco Nano Particles:



Scheme: 6 Synthesis of IONP's

Reagents: a) 1.5M NaOH ; b) i) TEOS, EtOH:H_2O (4:1), 50 ºC, 12 h ; ii) APS, DMF:Toluene (3:2), rt, 24 h ; c) Succinic anhydride, DCM:Py (1:1), rt, 18 h ; d) i) NHS, EDC, DMF, rt, 18 h ; ii) Amine, Et_3N, DMF, rt, 48 h ; iii) Ethanolamine, DMF, rt, 4 h.

Synthesis of the Glyco-IONPs was achieved by co-precipitation method in basic conditions slightly modified from the literature protocol[22-24]. Commercially available $FeCl_2$ (0.5 g, 3.94 mmol) and $FeCl_3$ (1.28 g, 7.89 mmol) were mixed in deionized & deoxygenated water in the molar ratio of 1:2.This orange colour solution was added dropwise into 1.5 M NaOH solution with vigorous stirring (P^H=11-12) for 20 min. Once the black precipitate was obtained the magnetic nature of the particles was checked by using an external magnet. The solution was washed with deoxygenated & deionized water 3-4 times and once with ethanol by using magnetic separation and centrifugation. The obtained sample was dried under vacuum to obtain naked

Fe_3O_4 particles. Small amount was separated for analysis. Rest of the sample was dispersed in a solution of Ethanol: Water (4:1) and P^H was adjusted to 9 by addition of NaOH solution. TEOS (3 ml) was then added to the flask and stirred at r.t for 10 h and at 50 ºC for additional 12 h. The sample was washed with water and ethanol several times and dried under vacuum resulting in silica coated iron oxide nanoparticles. A small amount was separated for analysis and the rest sample was washed with DMF once and then dispersed in a mixture of DMF:Toluene (3:2) and APS (5 ml) was then added dropwise and stirred at r.t for 24 h. Obtained sample was subjected to washings with excess toluene, centrifuged, dried to obtain the amine coated Iron Oxide nanoparticles. These nanoparticles would be converted into acid functional by addition of succinic anhydride in DCM:pyridine (1:1) mixture. Further activation of these COOH-MNP's with N-hydroxy succinamide, EDC and reacting with respective carbohydrate compound will yield the required IONP's. Unreacted sites on the nanoparticle surface will be capped with ethanol amine.

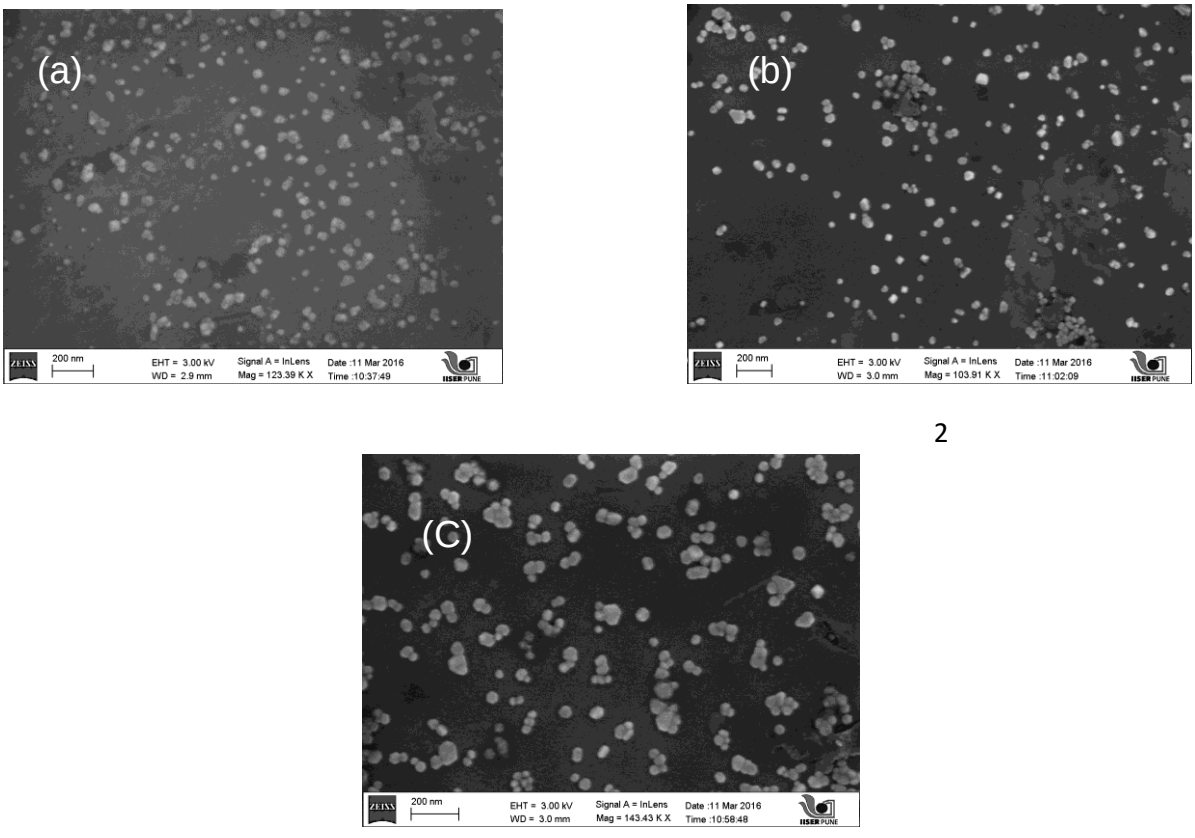


Figure: 3 (a) MNP-core; (b) MNP-SiO_2; (c) MNP-NH_2.

MATERIALS

- All chemicals were purchased from sigma Aldrich, Spectrochemicals and alfa aeser.
- ^{1}H, ^{13}C & DEPT were recorded on JEOL 400 MHz and Bruker 400 MHz with TMS as internal standard. Chemical shifts were expressed in ppm units downfield from TMS.
- ESI HRMS data was recorded on Waters Synapt G2 spectrometer.
- All reactions were monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV-light and staining with CAM, ninhydrin and $KMnO_4$.
- All reactions were carried out under nitrogen atmosphere with dry solvents purchased from Merck and Finar unless specified.
- All solvent evaporations were carried out under reduced pressure on Heidolph rotatory evaporator below 40ºC unless otherwise specified.
- Silica gel (100 -200) & (60-120) mesh was used for column chromatography.

Compound G_1: Commercially available D-(+)-Galactose (50 g, 277.5 mmol) was dissolved in pyridine (269 ml, 3.33 mol) and acetic anhydride (157ml, 1.66mol) was added to the reaction flask by dropping funnel at 0 ºC and stirred to r.t for 12 h. After the completion of the reaction excess solvents were evaporated under reduced pressure and the reaction mixture was co-evaporated by using toluene (250 ml x 3) and dried under vacuum which was precipitated in diethyl ether to afford required compound **G_1** as white powdered solid which was used without further purification. Yield: (90%, 97.4 g).

Compound G_2: The vacuum dried compound **G_1** (10 g, 25.6 mmol) was dissolved in THF (50 ml) and DMAPA (4.8 ml, 38.46 mmol) was added to it and stirred for 2 h at r.t (TLC). After completion of the reaction the excess solvents were evaporated under reduced pressure and the resulting reaction mixture was extracted with 1M HCl and brine. The combined organic layers were dried under anhydrous Na_2SO_4 and concentrated under reduced pressure to afford required compound **G_2** as white semi solid which was used without further purification. Yield: (65%, 5.8 g).

Compound G_3: The vacuum dried compound **G_2** (5.8 g, 16.66 mmol) was dissolved in anhydrous DCM and DBU (0.75ml, 5mmol) was added to the reaction flask followed by CCl_3CN (6.7 ml, 66.66 mmol) and stirred under nitrogen atmosphere for 1 h. After completion of the reaction excess solvents were evaporated and purified by silica gel column chromatography (EtOAc: Hexane) with 0.01% Et_3N in mobile phase to afford required compound **G_3** as a white solid after precipitation in hexane. Yield: (75%, 6.15 g).

Compound F_1: Commercially available L-(-)-Fucose (2 g, 12.18 mmol) was dissolved in pyridine (9.8 ml, 121.18 mmol) and acetic anhydride (5.75 ml, 61 mmol) was added to the reaction flask by dropping funnel at 0 ºC and stirred to r.t for 12 h. After the completion of the reaction excess solvents were evaporated under reduced pressure and the reaction mixture was co-evaporated by using toluene (10ml x 3) and dried under vacuum which was precipitated in diethyl ether to afford required compound **F_1** as white powdered solid which was used without further purification. Yield: (80%, 3.2 g).

Compound F_2: The vacuum dried crude compound **F_1** (3.2 g, 9.6 mmol) was dissolved in anhydrous DCM and stirred at 0 ºC .*p*-thiocresol was then added to the reaction flask at 0 ºC and stirred for 20 min.$BF_3.Et_2O$ was added to the reaction mixture by dropping funnel and stirred to r.t for 12 h under nitrogen atmosphere resulting in a pinkish solution. The reaction mixture was then neutralized by adding Et_3N and extracted by using saturated $NaHCO_3$ solution and brine. The combined organic layers were dried under anhydrous Na_2SO_4, concentrated under reduced pressure and purified by silica get column chromatography (EtOAc: Hexane) to afford required compound **F_2** as a white solid. Yield: (85%, 3.24 g).

Compound F_3: The vacuum dried compound **F_2** (3.24 g, 8.2 mmol) was dissolved in an equi-volume mixture of MeOH (10 ml) and DCM (10 ml). NaOMe (2 g, 37 mmol) was then added to the reaction flask and stirred at r.t for 2h.After the completion of the reaction the reaction mixture was neutralized by IR 120 H^+ resin. Excess solvents were evaporated and resulting crude compound was purified by silica gel column chromatography (MeOH: $CHCl_3$) to afford required compound **F_3** as white solid. Yield: (90%, 1.96 g).

Compound F_4: The vacuum dried compound **F_3** (1.96 g, 7.27 mmol) was dissolved in anhydrous DMF and stirred at 0 ºC for 10 min. NaH (60 %, 3.6 eq) was then added to the reaction flask at 0 ºC and stirred under nitrogen atmosphere for another 10 min. BnBr was then added dropwise at 0 ºC and stirred to r.t for 12 h. After the completion of the reaction it was quenched with few drops of MeOH and the excess solvents were evaporated under reduced pressure. The resulting residue was extracted with brine and the organic layer was dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained crude compound was purified by silica gel column chromatography (EtOAc: Hexane) to afford required compound **F_4** as a white solid. Yield: (75%, 2.94 g).

Compound L_1: Commercially available 2-(2-Chloroethoxy) ethanol (20 g, 0.16 mol) was dissolved in water and NaN_3 (15.6 g, 0.24 mol) was added to it and the reaction flask was stirred at reflux conditions (100 ºC) for 48 h. After completion of the reaction the reaction mixture was extracted with excess of EtOAc (500 ml x 5) and brine (50 ml x 2). The combined organic layers were dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by

silica gel column chromatography (EtOAC: Hexane) to afford the required linker compound **L_1** as transparent liquid. Yield: (85%, 18 g).

Compound A_1: Commercially available D-(+)-Glucosamine hydrochloride (25 g, 116 mmol) was added to a stirred mixture of $NaHCO_3$ (24.3 g, 290 mmol) and water (400 ml) at 0 ºC. TrocCl (16 ml, 117 mmol) which was pre-dissolved in THF (20 ml) was added into the reaction flask by using dropping funnel and stirred to r.t for 6 h resulting in a while foam like precipitate which was filtered by buchner flask and washed with cold diethyl ether to remove the excess reagent. The obtained white solid was then co-evaporated with toluene (150 ml x 3) and dried under vacuum to afford required compound **A_1** as white powder which was used without further purification. Yield: (95%, 39 g).

Compound A_2: The vacuum dried crude compound **A_1** (39 g, 110 mmol) was dissolved in pyridine (89 ml, 1.1 mol) and acetic anhydride (52 ml, 0.55 mol) was then added to the reaction flask by dropping funnel at 0 ºC and stirred to r.t for 12 h. Excess solvents were evaporated under reduced pressure and co-evaporated by using toluene (200 ml x 3) and dried under vacuum to afford required compound **A_2** as white solid which was used without further purification. Yield: (92%, 52 g).

Compound A_3: The vacuum dried crude compound **A_2** (52 g, 100 mmol) was dissolved in anhydrous DCM and stirred at 0ºC. *p*-thiocresol (13.6 g, 109 mmol) was then added to the reaction flask at 0 ºC and stirred for 20 min.$BF_3.Et_2O$ (28 ml, 229 mmol) was then added to the reaction mixture by dropping funnel and stirred to r.t for 12 h under nitrogen atmosphere resulting in a pinkish solution. The reaction mixture was then neutralized by adding Et_3N and extracted by using saturated $NaHCO_3$ solution and brine. The combined organic layers were dried under anhydrous Na_2SO_4, concentrated under reduced pressure and purified by silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_3** as white solid. Yield: (85%, 49 g).

Compound A_4: The vacuum dried compound **A_3** (20 g, 34 mmol) was dissolved in an equi-volume mixture of MeOH (50 ml) and DCM (50 ml) and stirred at 0 ºC. NaOMe (829 mg, 15 mmol) was then added to the reaction flask and stirred at 0 ºC for 1 h while monitoring with TLC. After the completion of the reaction the reaction mixture was neutralized by using IR 120 H^+ resin. Excess solvents were evaporated

and resulting crude compound was purified by silica gel column chromatography (MeOH: $CHCl_3$) to afford required compound **A_4** as white solid. Yield: (86%, 13 g).

Compound A_5: The vacuum dried compound **A_4** (13 g, 28 mmol) was dissolved in ACN (75 ml) and $Ph(OMe)_2$ (8.9 ml, 60 mmol) was added to the flask subsequently, after stirring for few minutes CSA (1.96 g, 8.5 mmol) was added to the reaction flask and stirred for 1 h under nitrogen atmosphere resulting in formation of turbid solution indicating the completion of the reaction. The reaction mixture was dissolved in EtOAc and extracted with $NaHCO_3$ and brine. The combined organic layers were dried under anhydrous Na_2SO_4 and the solvents were evaporated under reduced pressure. The resulting crude compound was purified by silica gel column chromatography (EtOAc: $CHCl_3$) to afford required compound **A_5** as white solid. Yield: (80%, 12.4 g).

Compound A_{5a}: The vacuum dried compounds **A_5** (4.0 g, 7.3 mmol) and **L_1** (1 g, 7.65 mmol) with activated 4 Aº MS were dissolved in anhydrous DCM (160 ml) and stirred at r.t for 1 h under nitrogen atmosphere. The reaction flask was then cooled to -20 ºC and stirred for another 30 min. NIS (2.44 g, 11 mmol) and TfOH (65 µl, 0.73 mmol) were added to the reaction flask and stirred at -20 ºC for 1 h. After completion of reaction it was quenched with Et_3N, filtered through celite, extracted with $Na_2S_2O_3$, $NaHCO_3$ and brine. The combined organic layers were dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_{5a}** as white solid. Yield: (70%, 2.83 g).

Compound A_{5b}: The vacuum dried compounds **A_{5a}** (360 mg, 0.64 mmol) and **G_3** (426.1 mg, 0.86 mmol) with activated 4 Aº MS were dissolved in anhydrous DCM (3 ml) and stirred at r.t for 1h under nitrogen atmosphere. The reaction flask was then cooled to -78 ºC and stirred for 30min. TMSOTf (20.3 µl,0.112 mmol) was then added drop wise as 10% solution in DCM to the reaction mixture at -78 ºC and warmed to room temperature over a period of 3 h. Quenched with Et_3N, filtered through celite and extracted with $NaHCO_3$ and brine. The combined organic layers were dried under anhydrous Na_2SO_4 and evaporated under reduced pressure. The obtained crude compound was purified by silica gel column chromatography (EtOAc:

Hexane) to afford required compound **A_{5b}** as white solid. Unreacted starting material **A_{5a}** was recovered. Yield: (52%, 297 mg).

Compound A_{5c}: The vacuum dried compound **A_{5b}** (100 mg, 0.113 mmol) was dissolved in anhydrous DCM and stirred at 0 ºC for 10 min. Et_3SiH (90 µl,0.565 mmol) was added to the reaction flask and subsequently TFA (87 µl,1.13 mmol) was added drop wise at 0 ºC and stirred it to r.t under nitrogen atmosphere. After completion of reaction (1 h) the reaction mixture was quenched with dilute $NaHCO_3$ and extracted with brine and water. The combined organic layers were dried under anhydrous Na_2SO_4 and solvents were evaporated under reduced pressure. The crude product was purified through silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_{5c}** as white solid. Yield: (65%, 65 mg).

Compound A_6: The vacuum dried compound **A_5** (4 g, 7.28 mmol) was dissolved in DCM: Pyridine (1:1) mixture and cooled to 0 ºC. FmocCl (2.26 g, 8.74 mmol) was then added to the reaction flask portion wise for 30 min and stirred to room temperature under nitrogen atmosphere for 2-3 h. Upon completion of the reaction the solvents were evaporated under reduced pressure and the crude compound was purified through silica gel column chromatography (EtOAc: $CHCl_3$) to afford required compound **A_6** as white solid. Yield: (80%, 4.5 g).

Compound A_7: The vacuum dried compound **A_6** (4.5 g, 5.83 mmol) was dissolved in anhydrous DCM and stirred at 0 ºC for 10 min. Et_3SiH (4.65 ml, 29.1 mmol) was added to the reaction flask and subsequently TFA (4.46 ml, 58.35 mmol) was added drop wise at 0 ºC and stirred it to r.t under nitrogen atmosphere. After completion of reaction (1 h) the reaction mixture was quenched with dilute $NaHCO_3$ and extracted with brine and water. The combined organic layers were dried under anhydrous Na_2SO_4 and solvents were evaporated under reduced pressure. The crude product was purified through silica gel column chromatography (EtOAc: Hexane) to afford compound **A_7** as white solid. Yield: (83%, 3.76 g).

Compound A_{7a}: The vacuum dried compounds **A_7** (3.76 g, 4.86 mmol) and **L_1** (669 mg, 5.1 mmol) with activated 4 Aº MS were dissolved in anhydrous DCM and stirred at r.t for 1 h under nitrogen atmosphere. The reaction flask was then cooled to -20 ºC and stirred for another 30 min. NIS (1.63 g, 7.29 mmol) and TfOH (43 µl, 0.486 mmol) were added to the reaction flask and stirred at -20 ºC for 1 h. The reaction

mixture was quenched with dilute $NaHCO_3$, filtered through celite, extracted with $Na_2S_2O_3$, $NaHCO_3$ and brine. The combined organic layers were dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_{7a}** as white solid. Yield: (85%, 3.2 g).

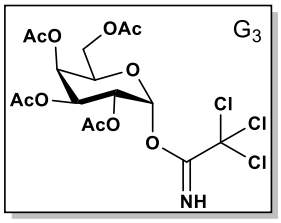
Compound A_{7b}: The vacuum dried compounds **A_{7a}** (300 mg, 0.38 mmol) and **G_3** (250 mg, 0.51 mmol) with activated 4 AºMS were dissolved in anhydrous DCM and stirred at r.t for 1 h under nitrogen atmosphere. The reaction flask was then cooled to -78 ºC and stirred for 30 min. TMSOTf (9.28 µl, 0.05 mmol) was then added drop wise as 10% solution in DCM to the reaction mixture and warmed to room temperature over a period of 3 h. Quenched with $NaHCO_3$ solution, filtered through celite and extracted with $NaHCO_3$ and brine. The combined organic layers were dried under Na_2SO_4 and evaporated under reduced pressure. The obtained crude compound was purified by silica gel column chromatography (EtOAc: Hexane) to afford the required compound **A_{7b}** as white solid. Yield: (45%, 192 mg).

Compound A_{7c}: The vacuum dried compound **A_{7b}** (530 mg, 0.477 mmol) was dissolved in anhydrous DCM and stirred under nitrogen atmosphere. Et_3N (2 ml, 14.32 mmol) was then added to the reaction flask and stirred for 2 h. After the completion of the reaction the solvents were evaporated under reduced pressure and purified through silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_{7c}** as white solid. Yield: (95%, 400 mg).

Compound A_{7d}: The vacuum dried compounds **A_{7c}** (80 mg, 0.09 mmol), **F_4** (81 mg, 0.15 mmol) and activated 4Aº MS were dissolved in anhydrous DCM and stirred at r.t for 1 h under nitrogen atmosphere. The reaction flask was then cooled to -60 ºC and stirred for 30 min. NIS (50.4 mg, 0.225 mmol) and TfOH (1.33 µl, 0.015 mmol) were added to the flask at -60 ºC and the flask was warmed to -40 ºC and stirred for 30 min. Quenched with Et_3N filtered through celite. Resulting solution was extracted with $Na_2S_2O_3$, $NaHCO_3$ and brine. The resulting organic layers were dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (EtOAc: Hexane) to afford required compound **A_{7d}** as white solid. Yield: (70%, 80 mg).

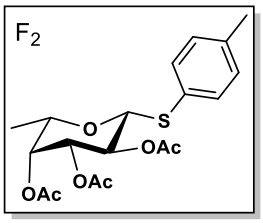
CHARACTERIZATION DATA

Compound G_3:



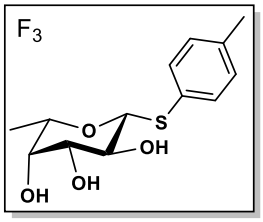
^{1}H NMR (400 MHz, Chloroform-d) δ 8.66 (s, 1H), 6.60 (d, *J* = 3.4 Hz, 1H), 5.56 (dd, *J* = 3.0, 1.1 Hz, 1H), 5.42 (dd, *J* = 10.8, 3.1 Hz, 1H), 5.36 (dd, *J* = 10.9, 3.5 Hz, 1H), 4.46 – 4.41 (m, 1H), 4.16 (dd, *J* = 11.3, 6.6 Hz, 1H), 4.08 (dd, *J* = 11.3, 6.7 Hz, 1H), 2.17 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 170.46, 170.26, 170.24, 170.13, 161.07, 93.64, 90.88, 69.10, 67.63, 67.48, 67.02, 61.39, 20.82, 20.78, 20.76, 20.70.MALDI-TOF m/z calculated for $[C_{16}H_{20}Cl_3NO_{10}+Na]^+$: 514.0050 found: 513.9841 m/z calculated for $[C_{16}H_{20}Cl_3NO_{10}+K]^+$: 529.9790 found: 529.9650.

Compound F_2:



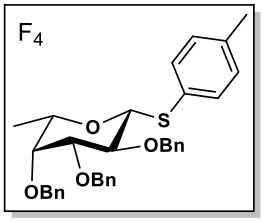
^{1}H NMR (400 MHz, Chloroform-d) δ 7.41 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 7.9 Hz, 2H), 5.25 (dd, *J* = 3.4, 1.0 Hz, 1H), 5.20 (t, *J* = 9.9 Hz, 1H), 5.03 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.63 (d, *J* = 9.9 Hz, 1H), 3.80 (qd, *J* = 6.4, 1.1 Hz, 1H), 2.34 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 1.97 (s, 3H), 1.23 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.64, 170.16, 169.51, 138.23, 132.94, 129.63, 129.08, 86.87, 73.13, 72.48, 70.37, 67.43, 21.16, 20.90, 20.68, 20.65, 16.48.HRMS m/z calculated for $[C_{19}H_{24}O_7S+Na]^+$: 419.1140 found: 419.1137 m/z calculated for $[C_{19}H_{24}O_7S+K]^+$: 435.0880 found: 435.0883.

Compound F_3:



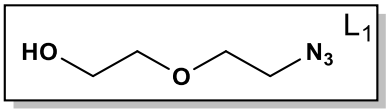
^{1}H NMR (400 MHz, Chloroform-d) δ 7.44 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 7.9 Hz, 2H), 4.45 (d, *J* = 8.6 Hz, 1H), 3.81 – 3.74 (m, 1H), 3.63 (dt, *J* = 5.8, 2.1 Hz, 4H), 3.29 (s, 1H), 2.80 (s, 1H), 2.33 (s, 3H), 1.88 (s, 1H), 1.33 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.22, 132.96, 129.78, 128.71, 88.82, 75.11, 74.77, 71.69, 69.82, 21.17, 16.68. HRMS m/z calculated for $[C_{13}H_{18}O_4S+Na]^+$:293.0823 found: 293.0830. M/z calculated for $[C_{13}H_{18}O_4S+K]^+$: 309.0563 found: 309.0532.

Compound F_4:



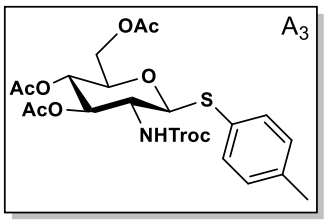
^{1}H NMR (400 MHz, Chloroform-d) δ 7.49 (d, *J* = 8.1 Hz, 2H), 7.42 – 7.27 (m, 15H), 7.02 (d, *J* = 7.9 Hz, 2H), 5.01 (d, *J* = 11.6 Hz, 1H), 4.80 (d, *J* = 10.2 Hz, 1H), 4.73 (d, *J* = 9.8 Hz, 3H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 9.6 Hz, 1H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.63 (dd, *J* = 2.9, 1.0 Hz, 1H), 3.59 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.51 (q, *J* = 6.3 Hz, 1H), 2.30 (s, 3H), 1.26 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.22, 138.92, 138.84, 137.55, 132.64, 130.89, 129.96, 128.89, 128.81, 128.78, 128.60, 128.41, 128.15, 128.13, 128.03, 127.90, 88.33, 85.03, 77.61, 77.05, 76.00, 75.01, 73.31, 21.58, 17.77. HRMS m/z calculated for $[C_{34}H_{36}O_4S+Na]^+$: 563.2232 found: 563.2231.

Compound L_1:



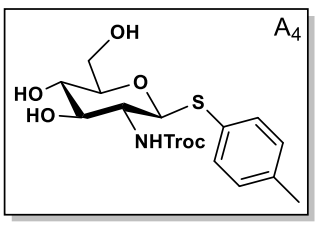
^{1}H NMR (400 MHz, Chloroform-d) δ 3.77 – 3.73 (m, 2H), 3.69 (ddd, *J* = 7.9, 2.8, 1.3 Hz, 2H), 3.65 – 3.58 (m, 2H), 3.45 – 3.38 (m, 2H), 2.23 (s, 1H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 72.53, 70.19, 61.89, 50.83. HRMS m/z calculated for $[C_4H_9N_3O_2+Na]^+$: 154.0592 found: 154.0594.

Compound A_3:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.41 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 5.27 (t, *J* = 9.8 Hz, 1H), 5.20-5.15 (m, 1H), 5.02 (t, *J* = 9.7 Hz, 1H), 4.82 – 4.71 (m, 3H), 4.15-4.25 (m, 2H), 3,67-3.72 (ddt, *J* = 10.1, 5.0, 2.6 Hz, 1H), 3.67 – 3.59 (m, 1H), 2.35 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H). MALDI-TOF m/z calculated for $[C_{22}H_{26}Cl_3NO_9S+Na]^+$: 608.0262 found: 610.0734 m/z calculated for $[C_{22}H_{26}Cl_3NO_9S+K]^+$: 624.0031 found: 626.0374.

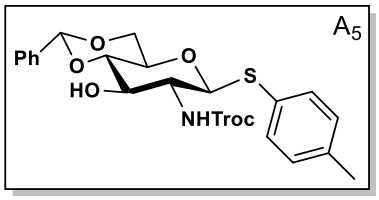
Compound A_4:



^{1}H NMR (400 MHz, Methanol-d4) δ 7.40 (d, *J* = 7.2 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 2H), 4.92 – 4.88 (m, 1H), 4.75 – 4.66 (m, 2H), 3.86 (dd, *J* = 12.2, 1.9 Hz, 1H), 3.68 (dd, *J* = 11.9, 5.5 Hz, 1H), 3.45 (dt, *J* = 8.5, 3.9 Hz, 2H), 3.31 (tdd, *J* =

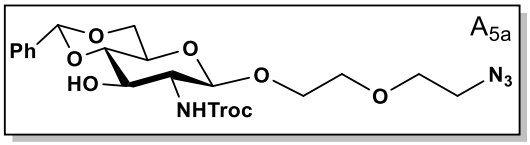
12.5, 9.9, 4.8 Hz, 3H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 155.39, 137.31, 131.82, 130.38, 129.15, 95.79, 87.62, 80.63, 75.90, 74.22, 70.51, 61.50, 56.88, 19.70. HRMS m/z calculated for $[C_{16}H_{20}Cl_3NO_6S+Na]^+$: 481.9975 found: 481.9967.

Compound A_5:



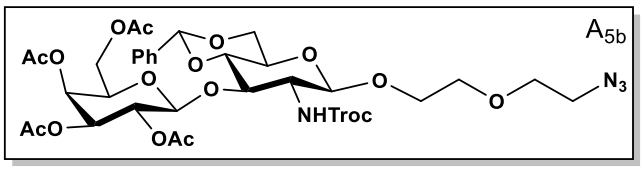
^{1}H NMR (400 MHz, Chloroform-d) δ 7.47 (d, *J* = 4.5, 2H), 7.42 – 7.33 (m, 5H), 7.14 (d, *J* = 7.8 Hz, 2H), 5.53 (s, 1H), 5.27 (bd, *J* = 8.0 Hz, 1H), 4.87 (d, *J* = 10.4 Hz, 1H), 4.82 (d, *J* = 12.1 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.40 – 4.34 (m, 1H), 4.08 – 4.00 (m, 1H), 3.77 (bt, *J*=9.8,1H), 3.49 (dd, *J* = 6.8, 4.1 Hz, 2H), 3.44-3.37 (m, 1H), 2.96 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 154.44, 138.85, 136.95, 133.64, 129.96, 129.48, 128.49, 127.91, 126.38, 101.97, 86.80, 81.25, 77.31, 74.75, 72.37, 70.34, 68.61, 57.23, 21.28.HRMS m/z calculated for $[C_{23}H_{24}Cl_3NO_6S+Na]^+$: 570.0288 found: 570.0294

Compound A_{5a}:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.52 – 7.47 (m, 2H), 7.42 – 7.33 (m, 3H), 5.88 (s, 1H), 5.56 (s, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 4.72 (t, *J* = 9.6 Hz, 2H), 4.35 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.98 (dt, *J* = 11.7, 3.6 Hz, 2H), 3.87 – 3.76 (m, 2H), 3.75 – 3.68 (m, 2H), 3.64 (ddt, *J* = 13.3, 8.5, 5.0 Hz, 2H), 3.55 (dd, *J* = 15.5, 8.5 Hz, 2H), 3.51 – 3.39 (m, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 155.35, 136.98, 129.29, 128.34, 126.34, 101.92, 95.44, 81.31, 77.36, 77.04, 76.72, 74.79, 71.93, 71.09, 70.02, 69.39, 68.58, 66.22, 59.01, 50.92. HRMS m/z calculated for $[C_{20}H_{25}Cl_3N_4O_8+Na]^+$: 577.0636 found: 577.0631.

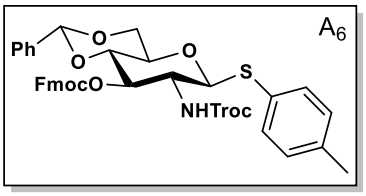
Compound A_{5b}:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.47 (dd, *J* = 6.8, 2.9 Hz, 2H), 7.36 (dd, *J* = 5.1, 2.0 Hz, 3H), 5.62 (s, 1H), 5.54 (s, 1H), 5.50 (t, *J* = 3.1 Hz, 1H), 5.46 (dd, *J* = 3.4, 1.3 Hz, 1H), 5.39 (d, *J* = 3.4 Hz, 1H), 5.28 (d, *J* = 1.1 Hz, 1H), 5.15 (dd, *J* = 10.7, 3.4 Hz, 1H), 4.92 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.86 (d, *J* = 8.4 Hz, 1H), 4.65 (d, *J* = 8.1 Hz, 1H), 4.48 – 4.43 (m,

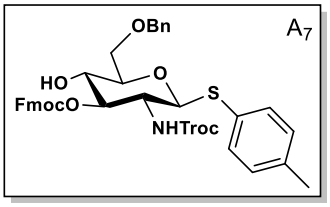
1H), 4.32 (dd, *J* = 10.5, 4.9 Hz, 1H), 4.14 (dd, *J* = 7.0, 1.4 Hz, 1H), 4.09 (dd, *J* = 6.6, 4.1 Hz, 2H), 3.82 – 3.75 (m, 2H), 3.69 – 3.60 (m, 4H), 3.49 – 3.38 (m, 5H), 2.14 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 170.40, 170.34, 170.26, 170.15, 169.55, 154.22, 137.09, 129.30, 128.39, 126.10, 101.54, 101.21, 95.60, 80.07, 74.58, 71.07, 70.58, 69.96, 69.36, 69.17, 68.71, 68.35, 68.26, 67.29, 67.19, 66.85, 66.31, 66.22, 61.89, 60.88, 57.85, 51.03, 20.94, 20.82, 20.75, 20.70, 20.66. HRMS m/z calculated for $[C_{34}H_{43}Cl_3N_4O_{17}+Na]^+$: 907.1586 found: 907.1579.

Compound A_6:



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.54 (dd, *J* = 10.8, 7.8 Hz, 2H), 7.46 – 7.36 (m, 6H), 7.53-7.29 (m, 4H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 2H), 5.54 (s, 1H), 5.38 (d, *J* = 9.1 Hz, 1H), 5.24 (t, *J* = 9.7 Hz, 1H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.43 – 4.28 (m, 3H), 4.22 (t, *J* = 7.4 Hz, 1H), 3.38-3.72 (m , 3H), 3.58-3.52(m, 1H), 2.35 (s, 3H);^{13}C NMR (101 MHz, $CDCl_3$) δ 155.01, 154.00, 143.13, 143.08, 141.27, 141.22, 138.77, 136.75, 133.49, 129.88, 129.16, 128.24, 128.04, 127.92, 127.91, 127.23, 126.19, 125.15, 125.09, 120.07, 120.06, 101.52, 95.33, 87.66, 78.42, 76.35, 74.56, 70.53, 70.50, 68.46, 55.71, 46.50, 21.20.MALDI-TOf m/z calculated for $[C_{38}H_{34}Cl_3NO_8S+Na]^+$:792.0968 found:792.1572 m/z calculated for $[C_{38}H_{34}Cl_3NO_8S+K]^+$: 808.0708 found: 808.1159.

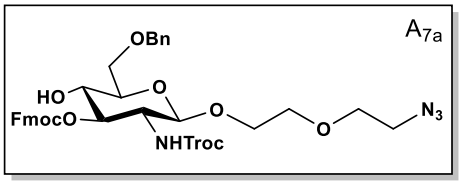
Compound A_7:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 7.44 – 7.28 (m, 11H), 7.06 (d, *J* = 7.8 Hz, 2H), 5.29 (d, *J* = 9.1 Hz, 1H), 4.97 (t, *J* = 9.8 Hz, 1H), 4.79 (d, *J* = 10.3 Hz, 1H), 4.72 – 4.51 (m, 4H), 4.38 (d, *J* = 7.4 Hz, 2H), 4.23 (t, *J* = 7.4 Hz, 1H), 3.81 (dh, *J* = 9.8, 5.2, 4.6 Hz, 3H), 3.69 (q, *J* = 10.0 Hz, 1H), 3.57 (dt, *J* = 9.5, 4.6 Hz, 1H), 2.91 (s, 1H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.61, 153.98, 143.17, 143.10, 141.30, 141.26, 138.44, 137.58, 133.28, 129.78, 128.52, 128.44, 127.96, 127.94, 127.78, 127.26, 125.19, 125.16, 120.10, 95.40, 86.86, 79.96, 77.95, 77.35, 77.23, 77.03, 76.71,

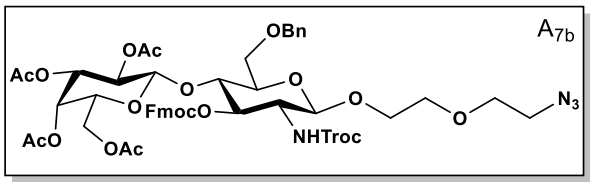
74.52, 73.82, 70.53, 70.20, 54.93, 46.59, 34.68, 31.61, 29.08, 22.67, 22.64, 21.16, 14.14, 11.45.HRMS: m/z calculated for $[C_{38}H_{36}Cl_3NO_8S+Na]^+$: 794.1125 found: 794.1120.

Compound A_{7a}:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.0 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.37 – 7.28 (m, 7H), 5.65 (d, *J* = 9.2 Hz, 1H), 4.94 (t, *J* = 10.0 Hz, 1H), 4.70 (d, *J* = 7.9 Hz, 1H), 4.68 – 4.51 (m, 3H), 4.46 – 4.32 (m, 2H), 4.26 (t, *J* = 7.5 Hz, 1H), 3.94 (dt, *J* = 12.0, 3.6 Hz, 1H), 3.88 – 3.75 (m, 4H), 3.75 – 3.69 (m, 1H), 3.55-3.68 (m,6H), 3.49 – 3.34 (m, 2H), 2.97 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.62, 154.37, 143.28, 143.16, 141.29, 141.25, 137.49, 128.55, 127.99, 127.93, 127.82, 127.25, 125.24, 120.07, 101.52, 95.54, 79.05, 74.43, 73.81, 72.40, 71.13, 70.84, 70.48, 70.19, 69.93, 69.05, 56.05, 50.98, 46.61.MALDI-TOF m\z calculated $[C_{35}H_{37}Cl_3N_4O_{10}+Na]^+$: 801.1473 found: 801.39 m/z calculated $[C_{35}H_{37}Cl_3N_4O_{10}+K]^+$: 817.1212 found: 815.35.

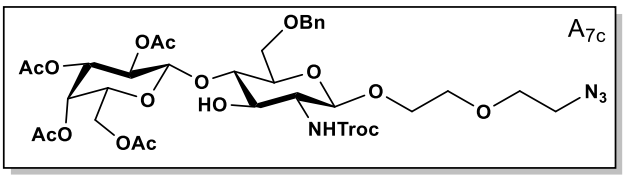
Compound A_{7b}:



^{1}H NMR (400 MHz, Chloroform-d) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.61 (dd, *J* = 14.2, 7.4 Hz, 2H), 7.45 – 7.29 (m, 9H), 5.69 (d, *J* = 9.3 Hz, 1H), 5.21 (d, *J* = 3.2 Hz, 1H), 5.09 (dd, *J* = 10.3, 2.4 Hz, 1H), 5.00 (t, *J* = 9.7 Hz, 1H), 4.86 (dd, *J* = 10.5, 3.3 Hz, 1H), 4.75 (d, *J* = 12.0 Hz, 1H), 4.65 (dd, *J* = 10.2, 7.1 Hz, 2H), 4.60 – 4.44 (m, 3H), 4.39 (d, *J* = 12.1 Hz, 1H), 4.27 (t, *J* = 7.6 Hz, 1H), 4.21 – 4.14 (m, 1H), 4.06 – 3.99 (m, 3H), 3.95 (dt, *J* = 11.5, 3.3 Hz, 1H), 3.77 (ddd, *J* = 16.4, 8.0, 5.1 Hz, 4H), 3.71 – 3.57 (m, 5H), 3.52 (d, *J* = 9.7 Hz, 1H), 3.41 (q, *J* = 5.5, 4.9 Hz, 2H), 1.95 (s, 9H), 1.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.21, 170.17, 170.04, 169.17, 154.80, 154.37, 143.48, 143.03, 141.29, 141.22, 137.91, 128.56, 127.98, 127.94, 127.28, 127.24, 125.55, 125.17, 120.07, 120.05, 101.48, 101.36, 100.82, 90.71,77.24, 75.75, 74.63, 74.49, 73.78, 71.17, 70.92, 70.40, 70.37, 70.22, 69.94,

69.10, 69.03, 68.29, 68.20, 67.55, 67.23, 66.55, 66.28, 61.80, 61.45, 60.34, 56.37, 50.99, 46.56, 31.59, 22.66, 20.84, 20.74, 20.72, 20.68, 20.66, 20.64, 20.56, 20.47, 14.13.MALDI-TOF m/z calculated $[C_{49}H_{55}Cl_3N_4O_{19}+K]^+$: 1147.2163 found: 1147.07

Compound A_{7c}:

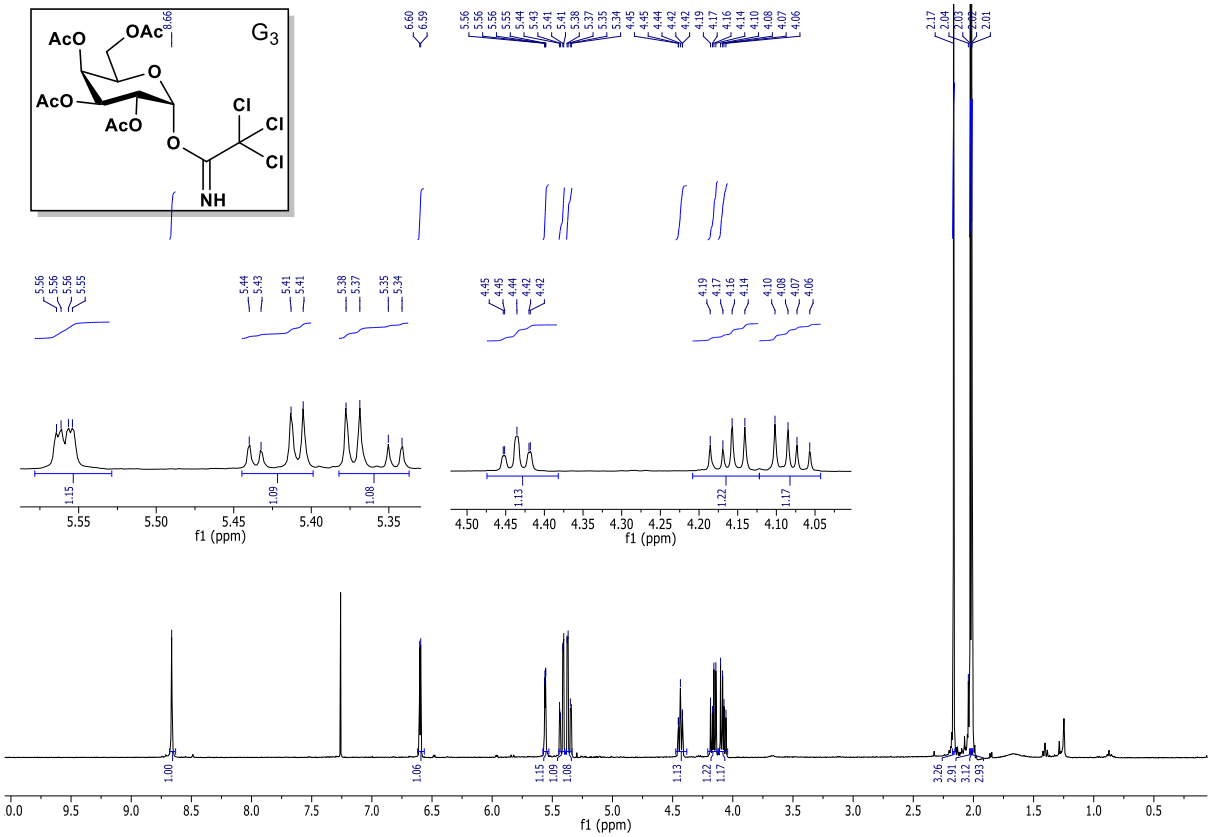


.^{1}H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.29 (m, 5H), 5.52 – 5.46 (m, 1H), 5.34 (dd, *J* = 3.5, 1.1 Hz, 1H), 5.17 (dd, *J* = 10.5, 8.0 Hz, 1H), 4.92 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.71 (d, *J* = 12.1 Hz, 1H), 4.69 – 4.59 (m, 2H), 4.50 – 4.46 (m, 2H), 4.11 (dd, *J* = 6.6, 1.4 Hz, 2H), 4.00 – 3.93 (m, 2H), 3.90 (td, *J* = 6.6, 1.2 Hz, 1H), 3.76 (ddd, *J* = 11.2, 7.5, 3.3 Hz, 2H), 3.72 – 3.60 (m, 7H), 3.52 – 3.44 (m, 2H), 3.41 (dd, *J* = 5.5, 4.4 Hz, 2H), 2.14 (s, 3H), 2.06 (s, 3H), 1.98 (s, 6H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 170.66, 170.25, 170.10, 169.32, 154.61, 138.10, 128.69, 128.09, 128.01, 101.46, 95.75, 81.24, 77.37, 74.68, 74.11, 73.82, 72.74, 71.31, 71.19, 70.85, 70.13, 69.09, 68.84, 68.00, 66.96, 61.54, 57.61, 51.09, 34.25, 22.48, 20.85, 20.75, 20.73, 20.66, 14.21. HRMS m\z calculated $[C_{34}H_{45}Cl_3N_4O_{17}+Na]^+$: 909.1743 found: 909.1755

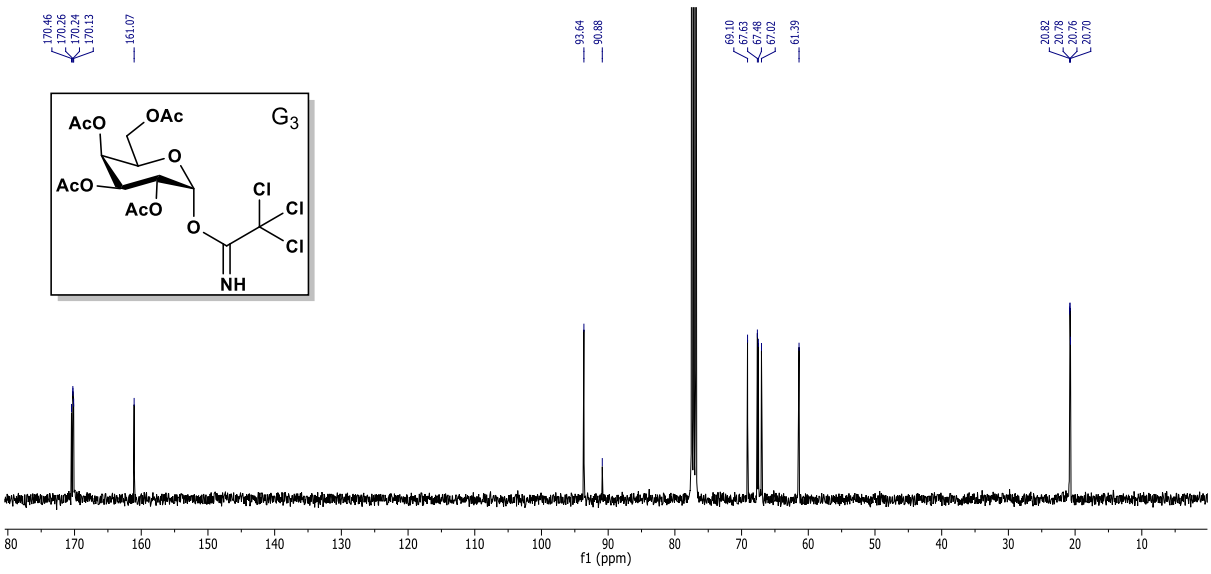
CONCLUSION & FUTURE PLAN

We have successfully synthesized protected form of lewis^{x} trisacchardie and lewis^{a} disaccharide glycan's. All the intermediates and final compounds were well characterized by NMR, high resolution mass-spectra. Simultaneously, we also synthesized superparamagnetic iron oxide nanoparticles by coprecipitation method in basic conditions. SEM images of these nanoparticles have clearly showed monodispersity with size of around 30-40 nm. Currently, the final deprotection, conjugation and further biological studies of these glyco-magnetic nanoparticles is under progress. We believe that this research will result in next generation biomarkers to detect ischemic strokes and inflammations.

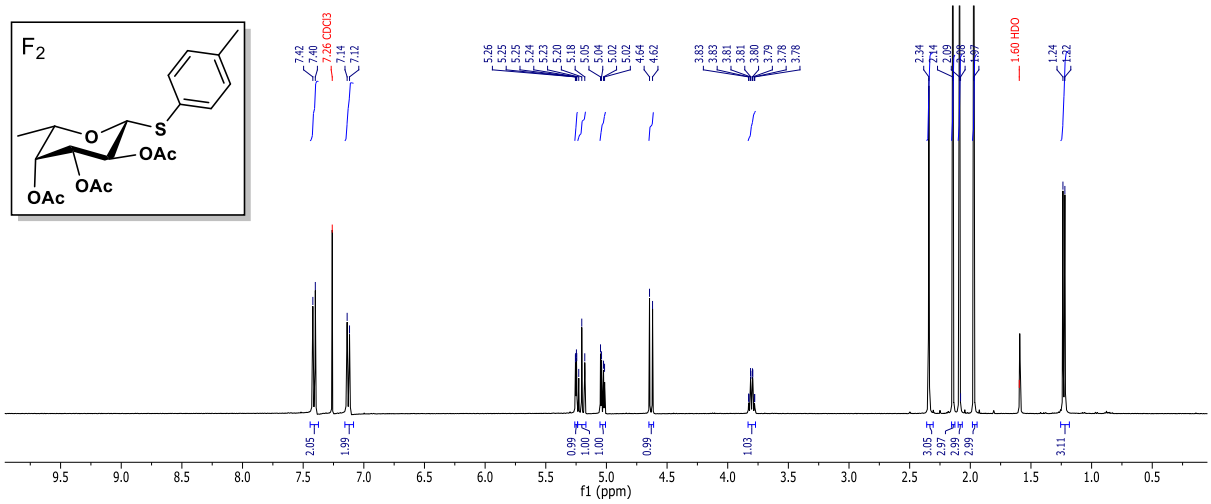
^{1}H NMR of Compound **G_3**



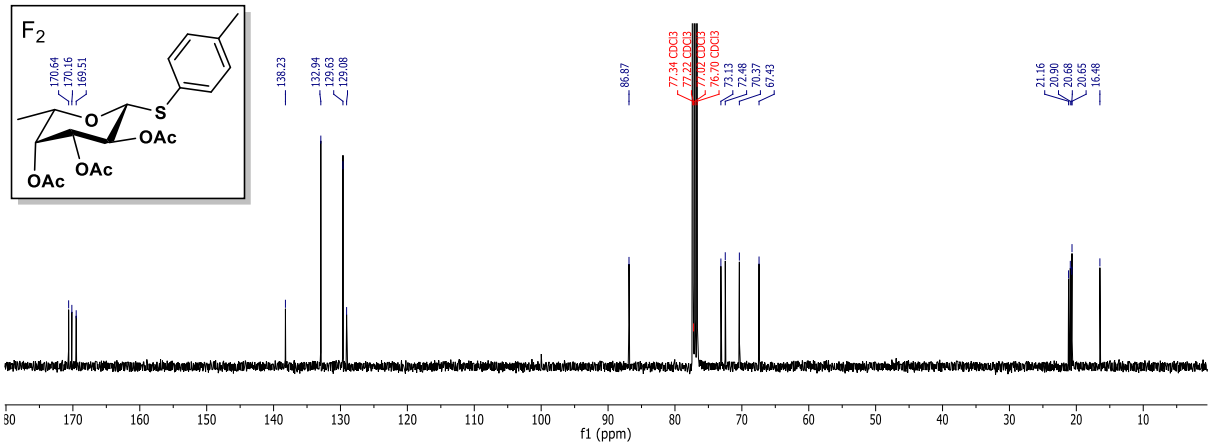
^{13}C NMR of Compound **G_3**



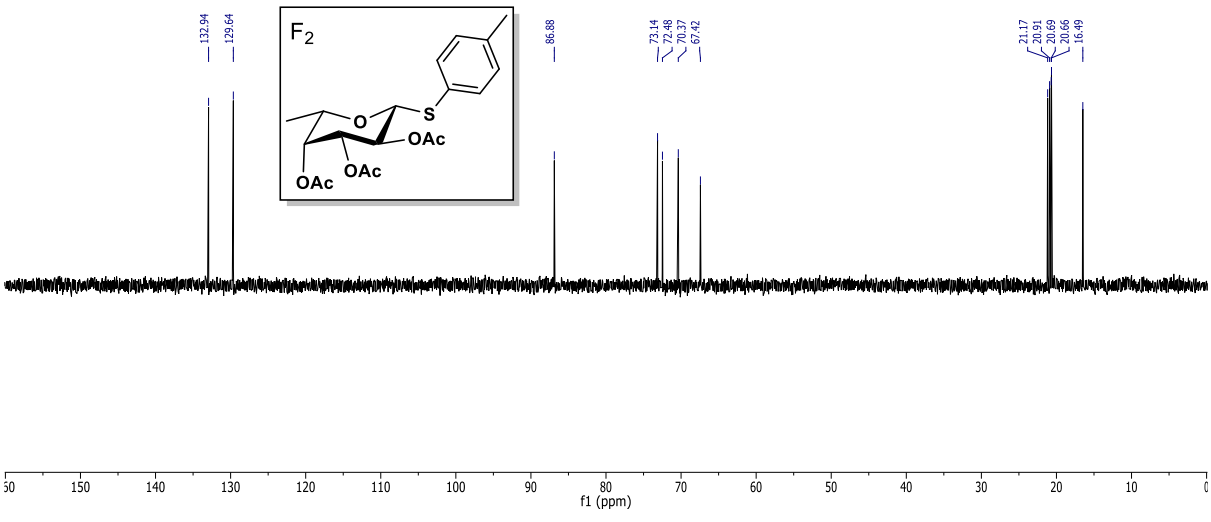
^{1}H NMR of Compound **F2**



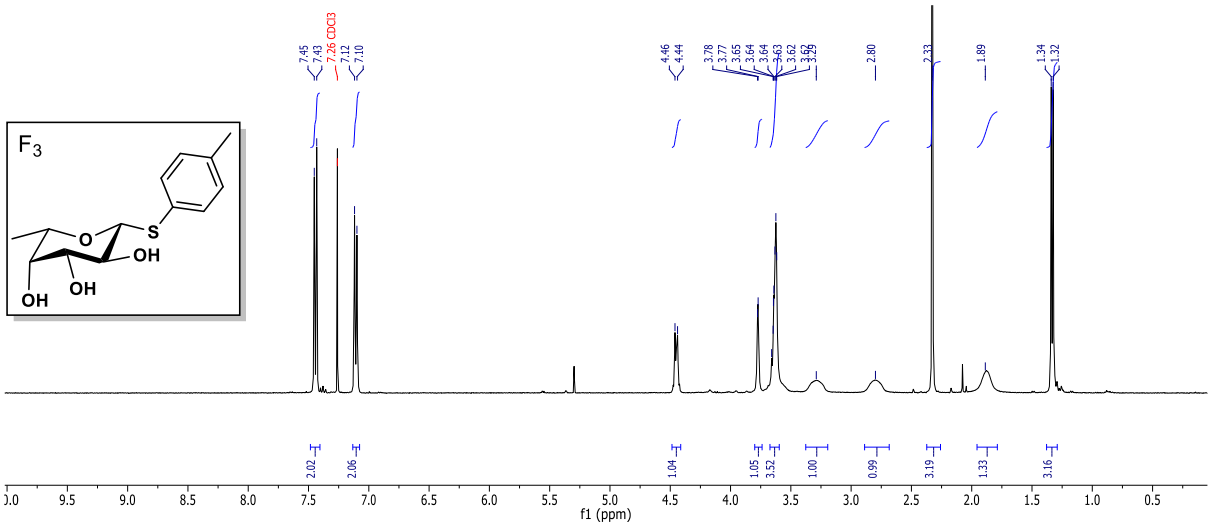
^{13}C NMR of Compound **F2**



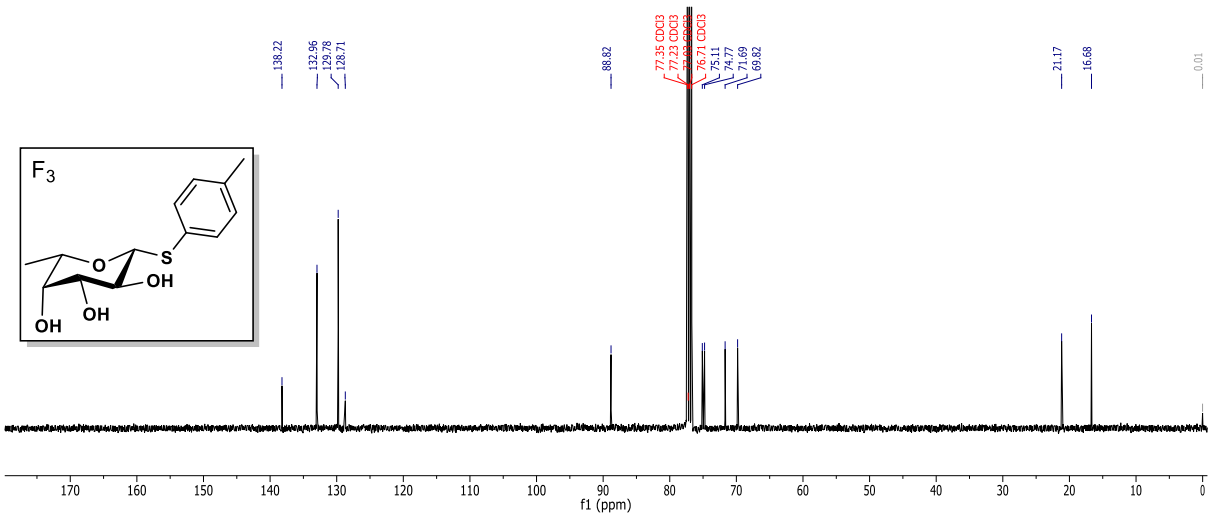
DEPT of Compound **F2**



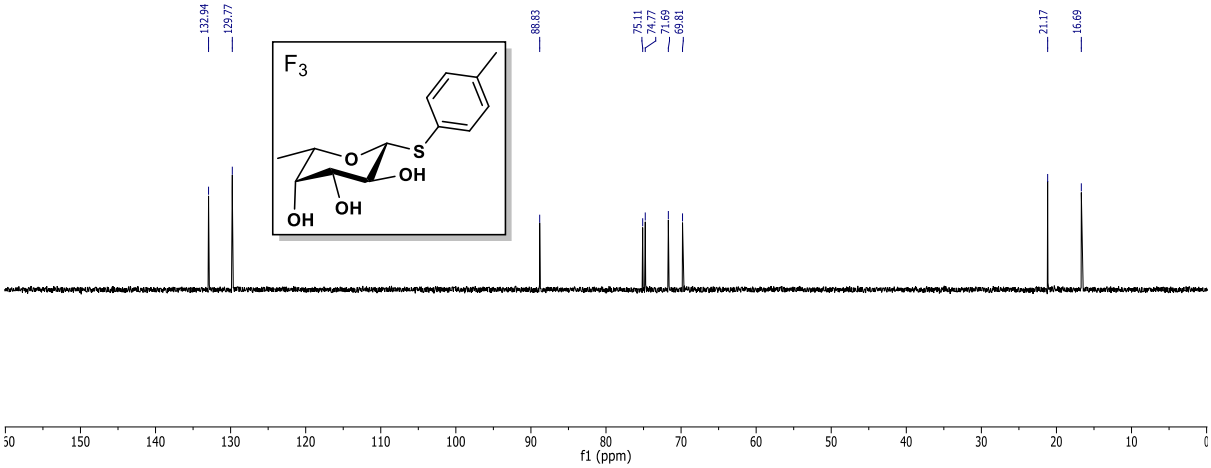
^{1}H NMR of Compound **F$_3$**



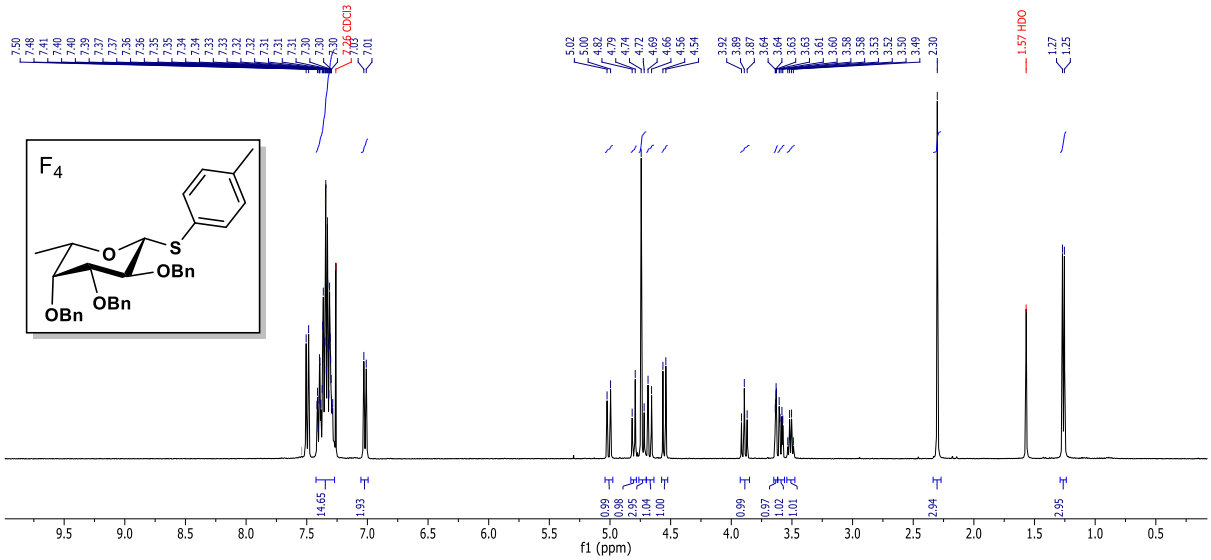
^{13}C NMR of Compound **F$_3$**



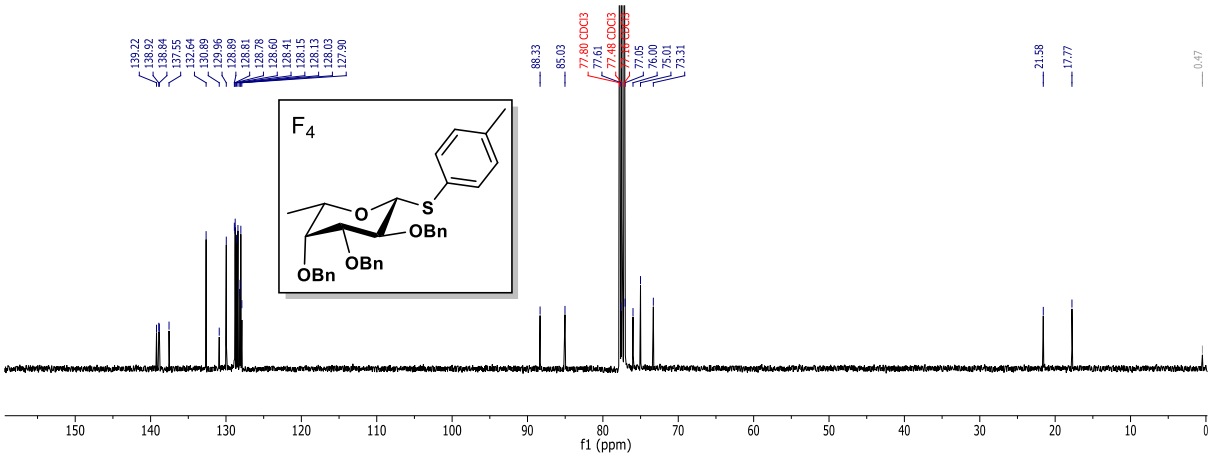
DEPT of Compound **F$_3$**



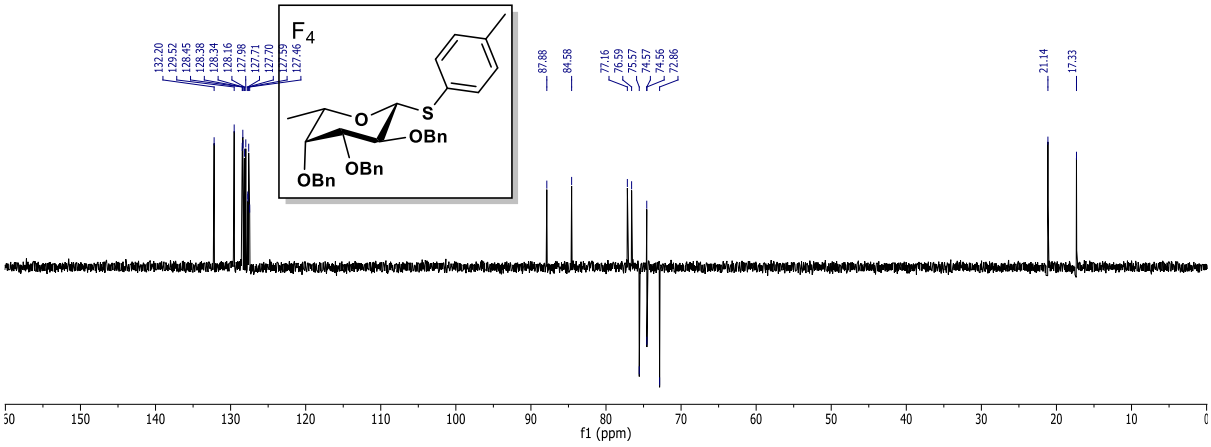
^{1}H NMR of Compound **F4**



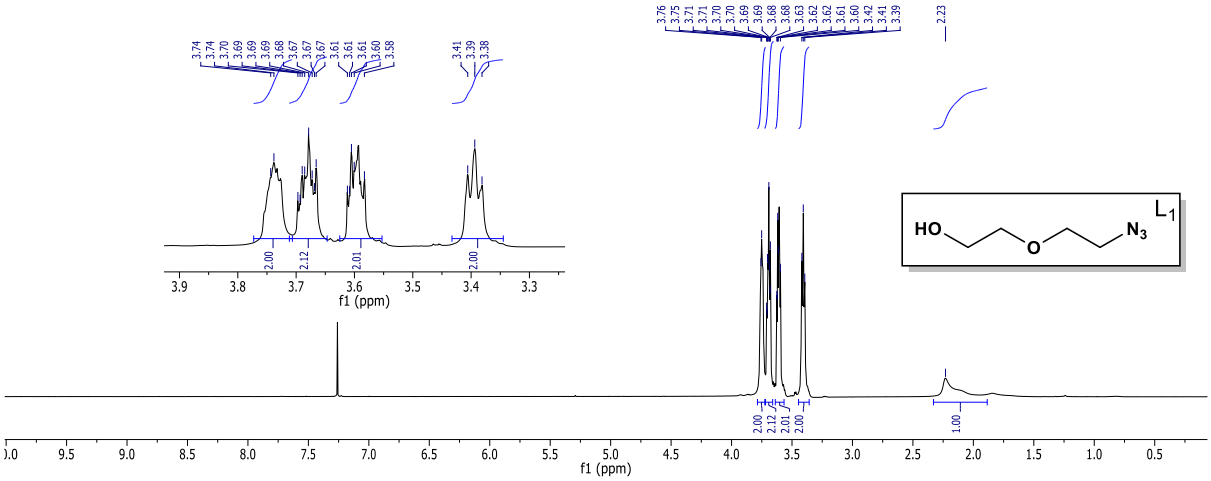
^{13}C NMR of Compound **F4**



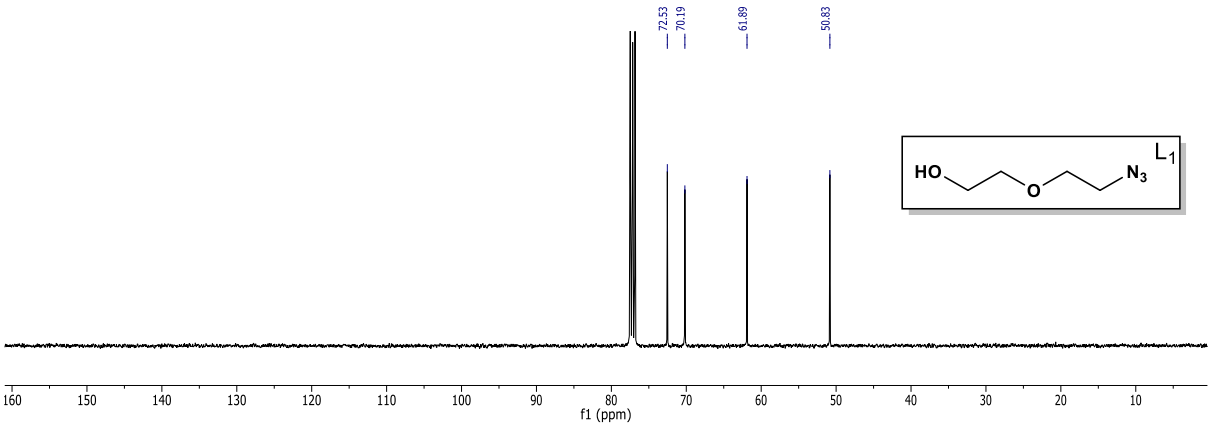
DEPT of Compound **F4**



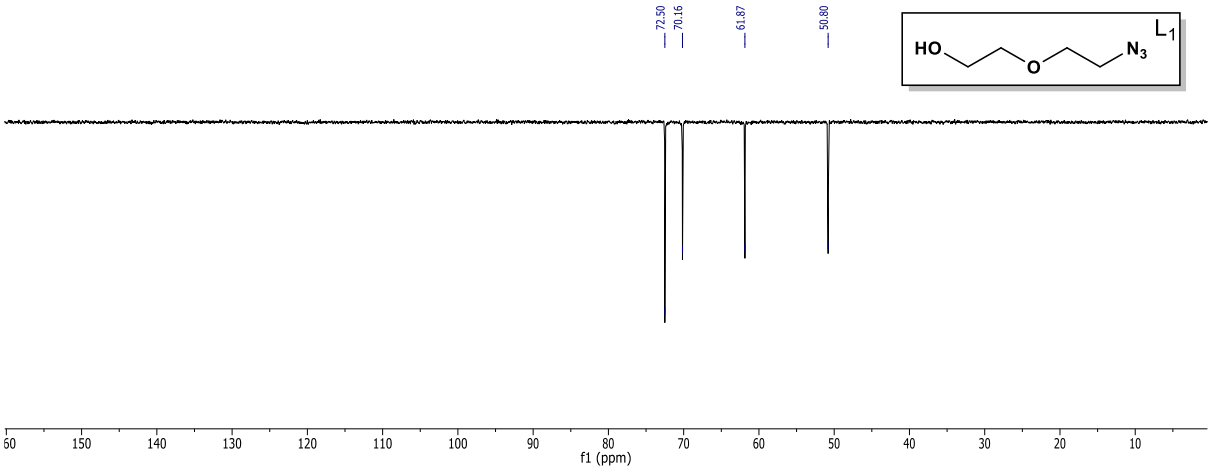
^{1}H NMR of Compound **L_1**



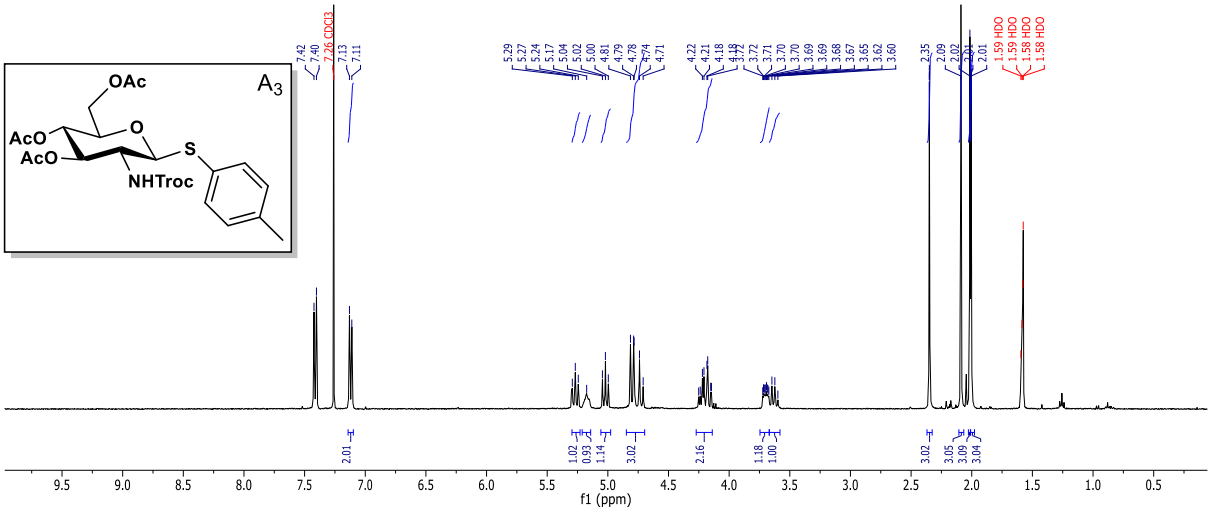
^{13}C NMR of Compound **L_1**



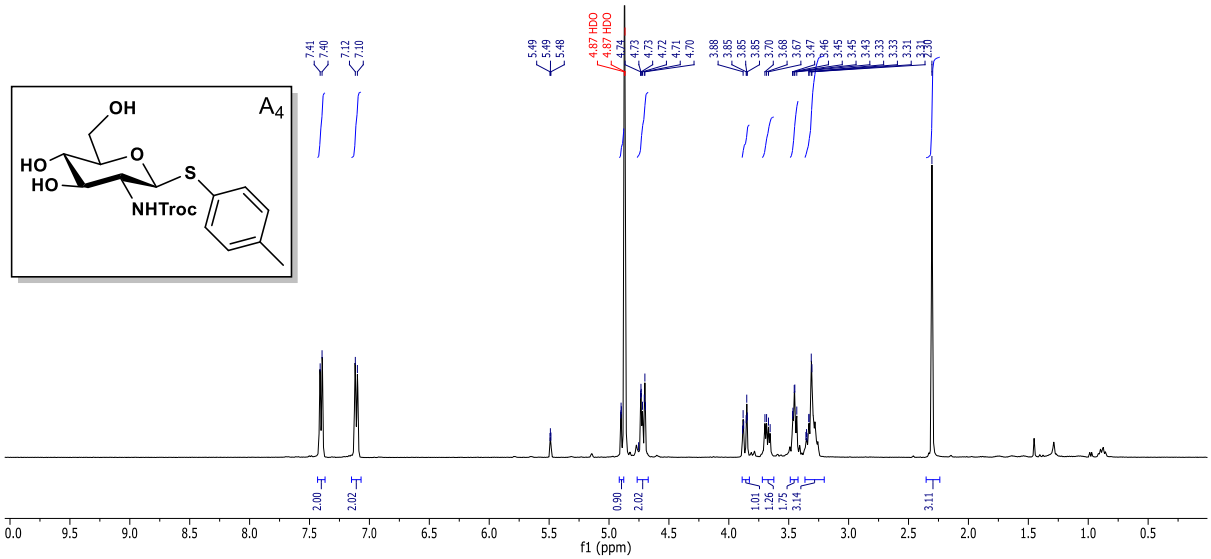
DEPT of Compound **L_1**



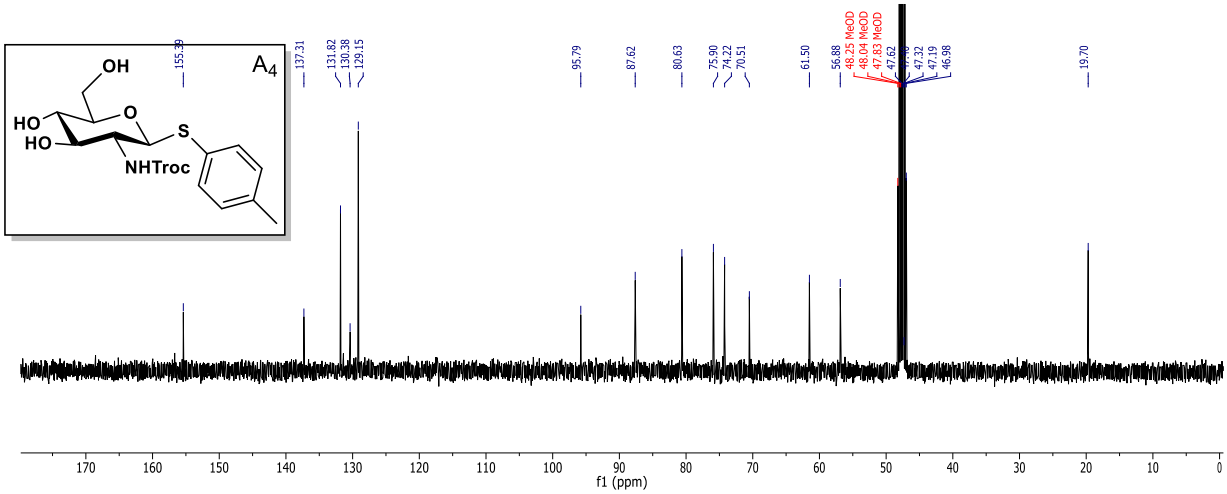
^{1}H NMR of Compound **A_3**



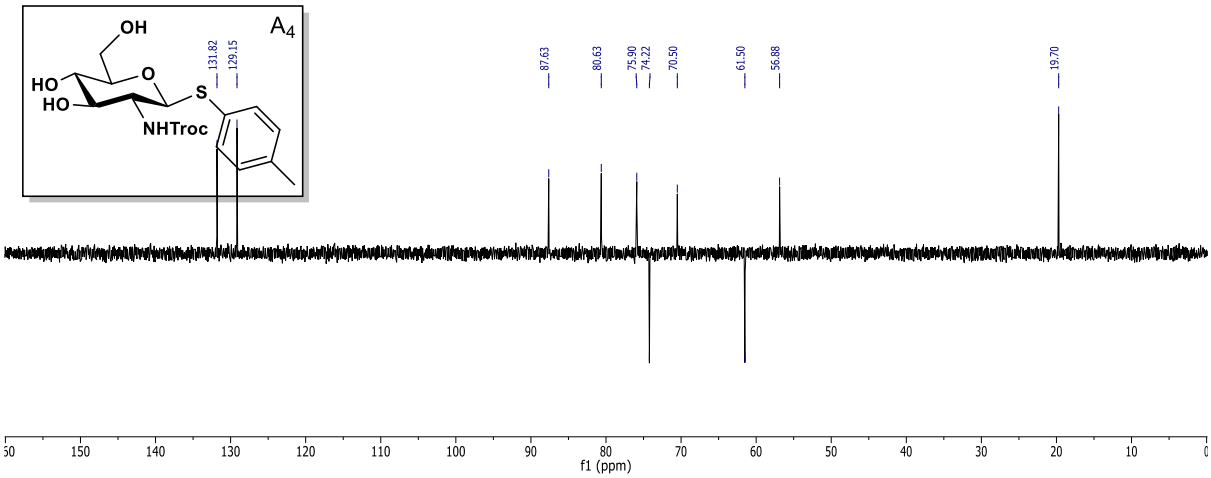
^{1}H NMR of Compound **A4**



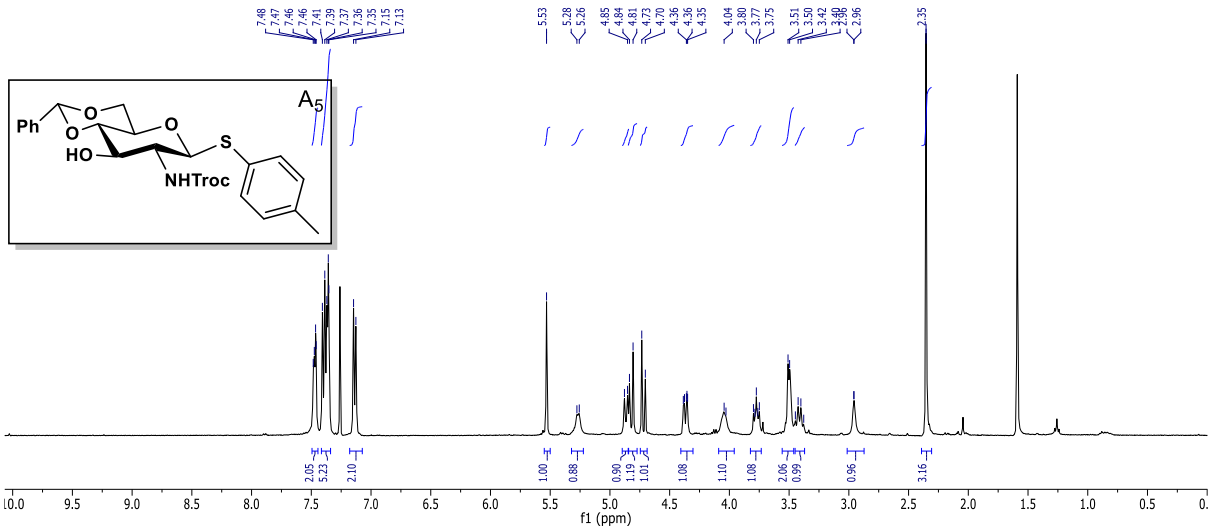
^{13}C NMR of Compound **A4**



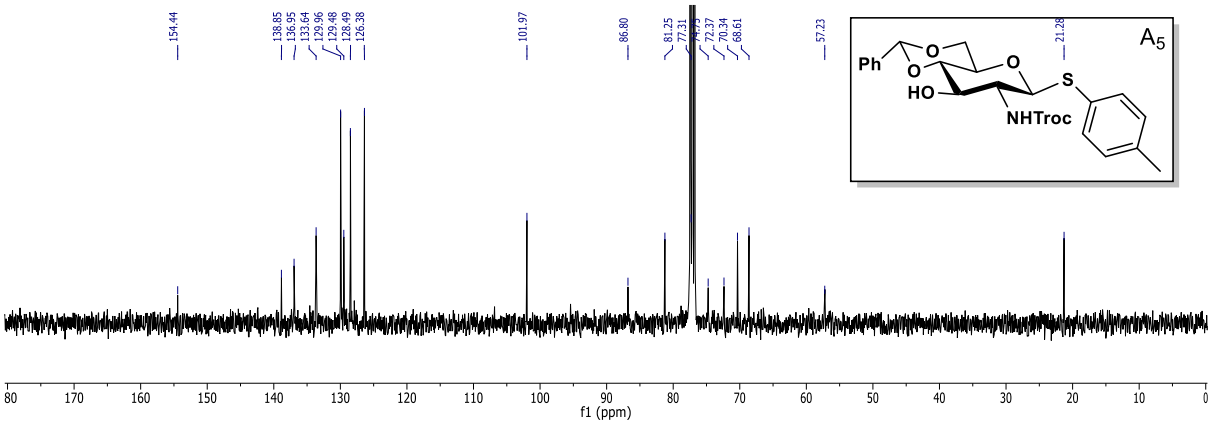
DEPT of Compound **A4**



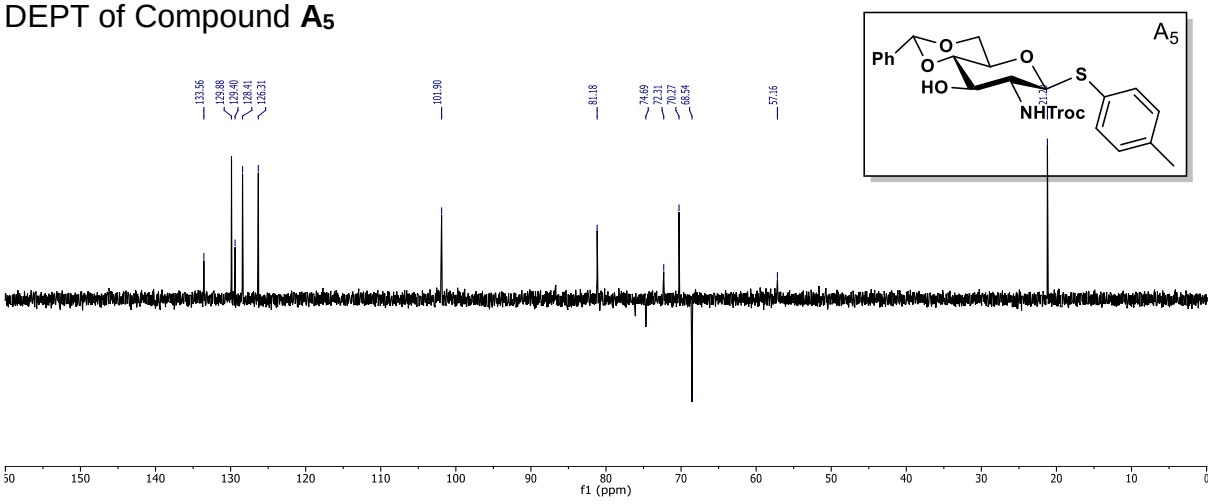
^{1}H NMR of Compound **A_5**



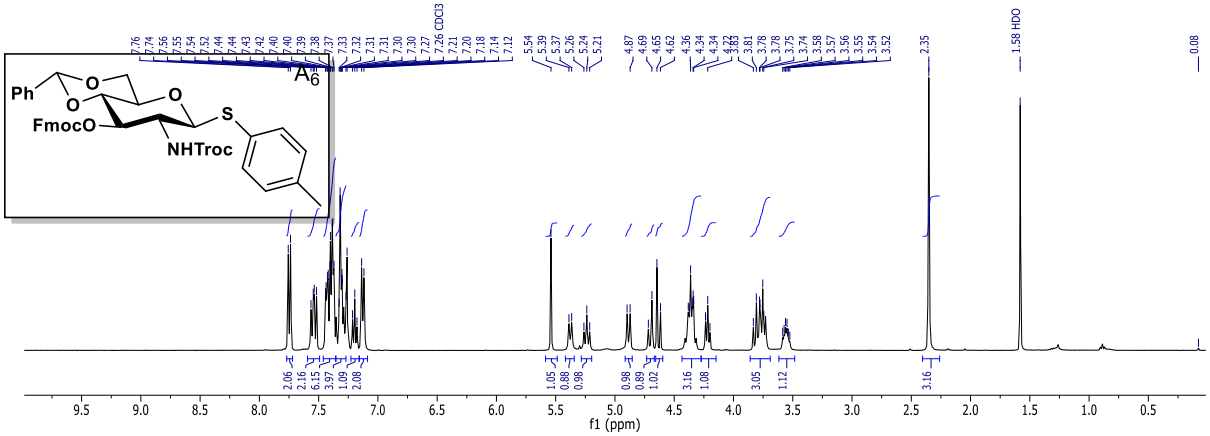
^{13}C NMR of Compound **A_5**



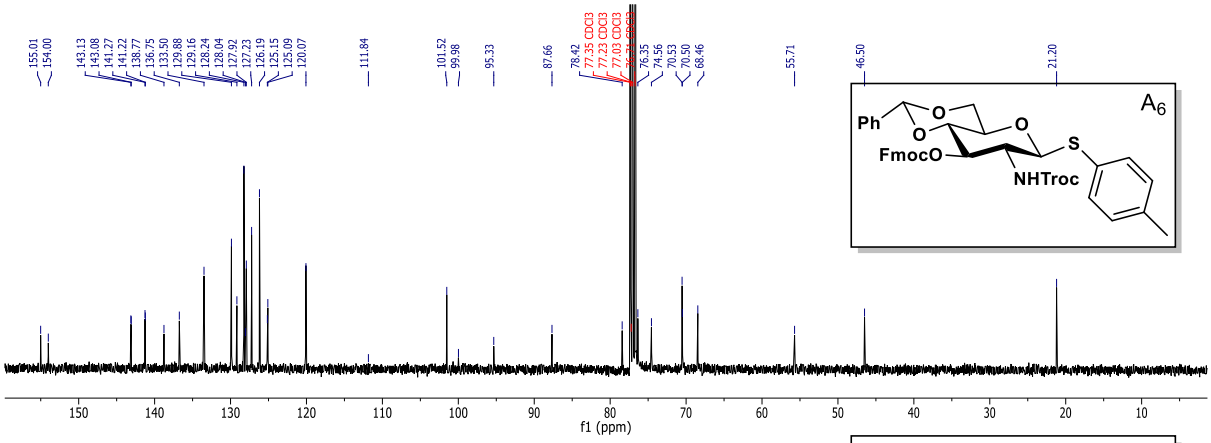
DEPT of Compound **A_5**



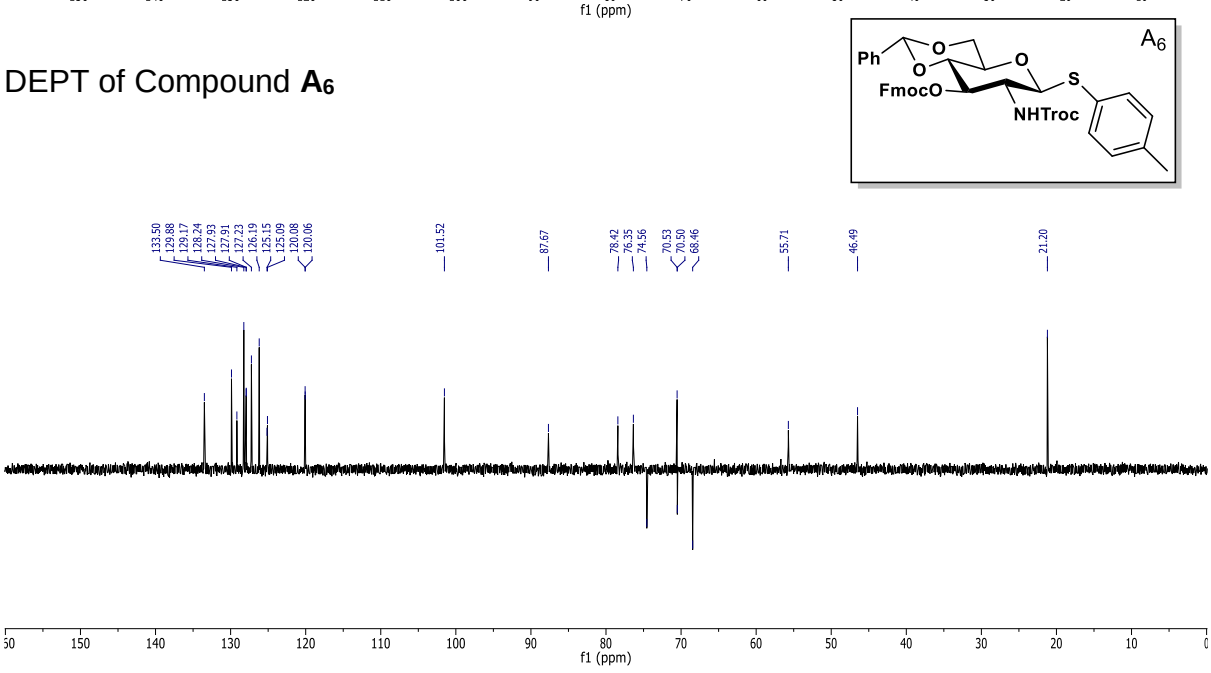
^{1}H NMR of Compound **A$_6$**



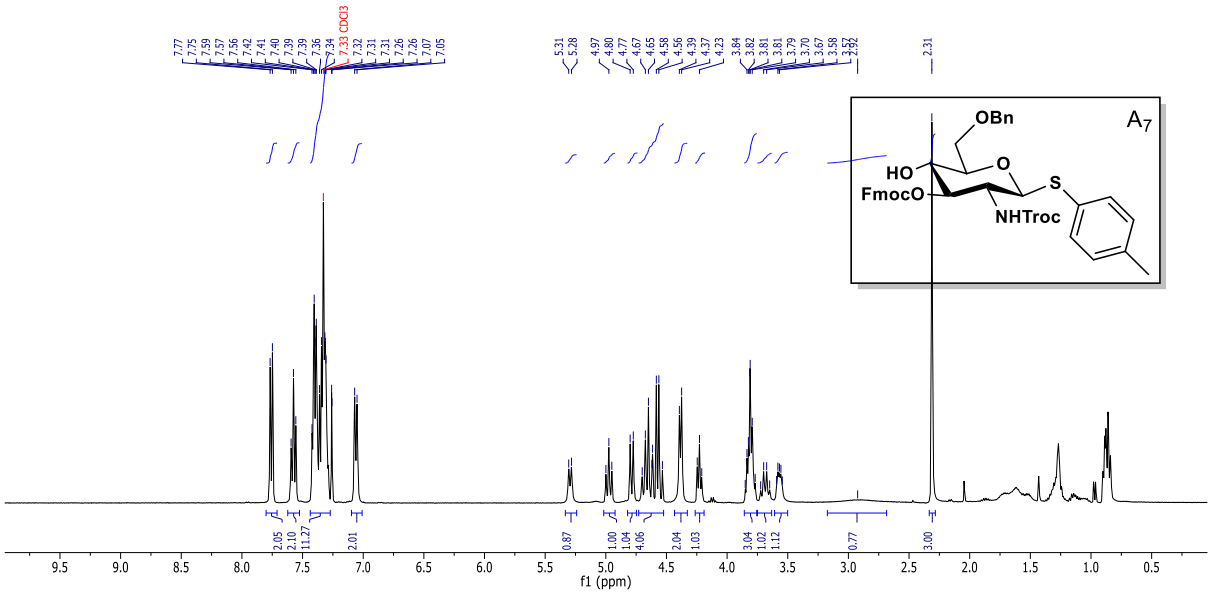
^{13}C NMR of Compound **A$_6$**



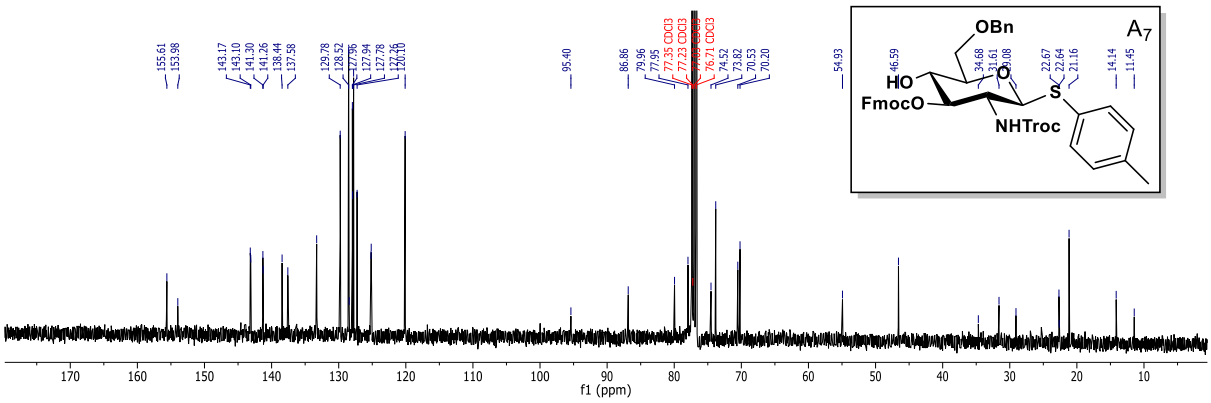
DEPT of Compound **A$_6$**



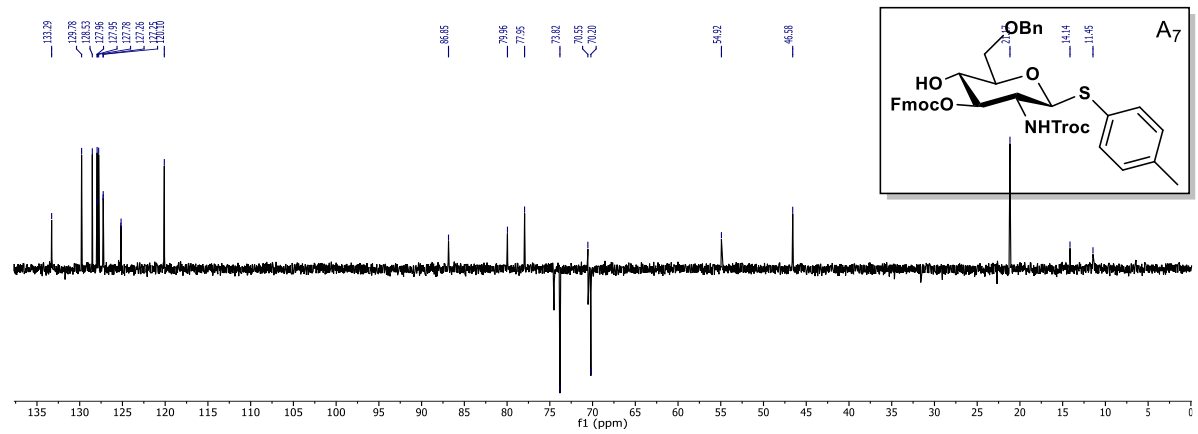
^{1}H NMR of Compound **A$_7$**



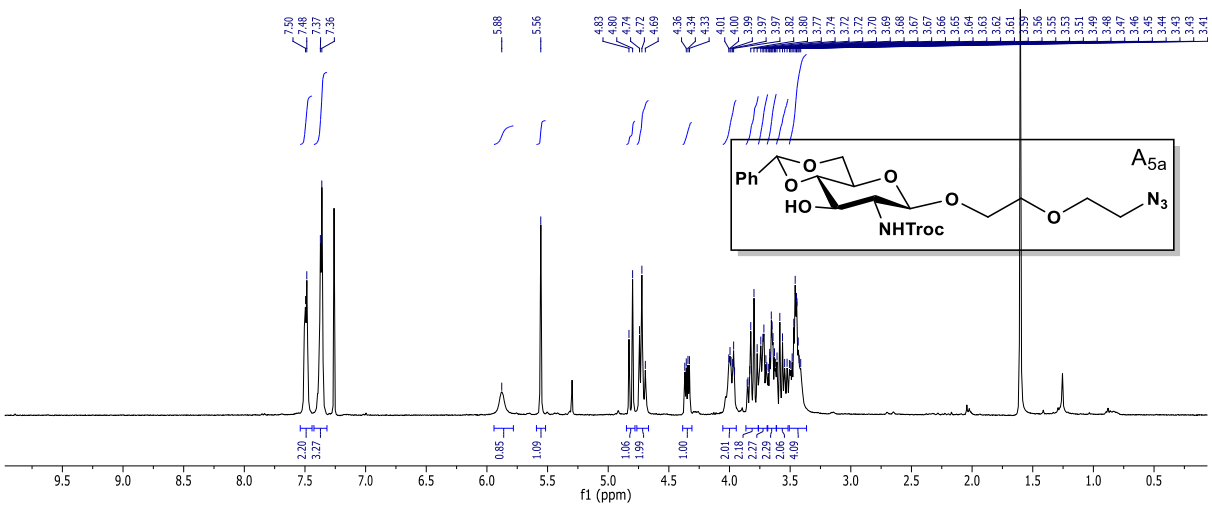
^{13}C NMR of Compound **A$_7$**



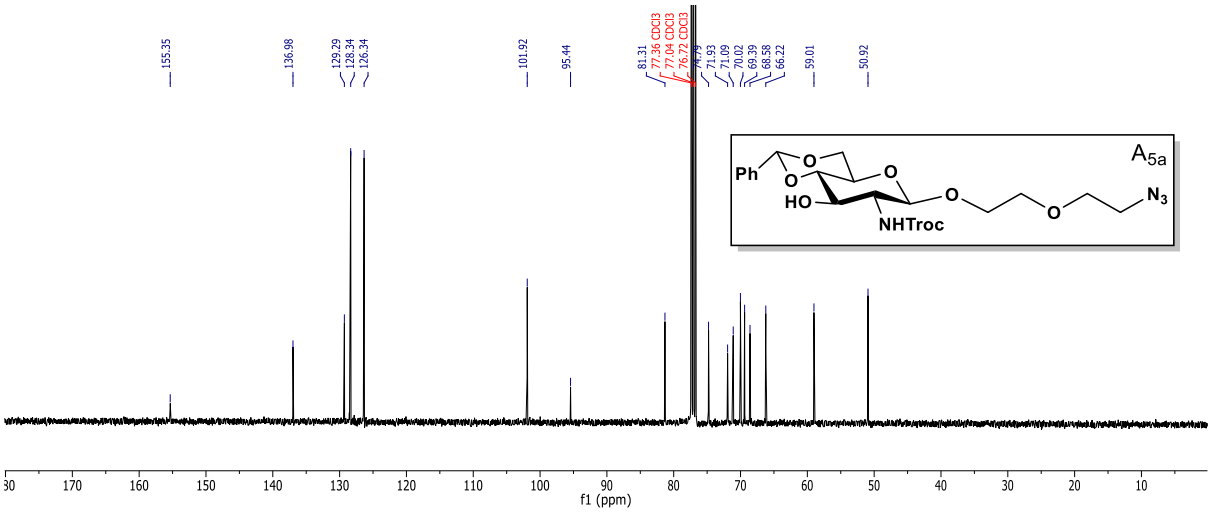
DEPT of Compound **A$_7$**



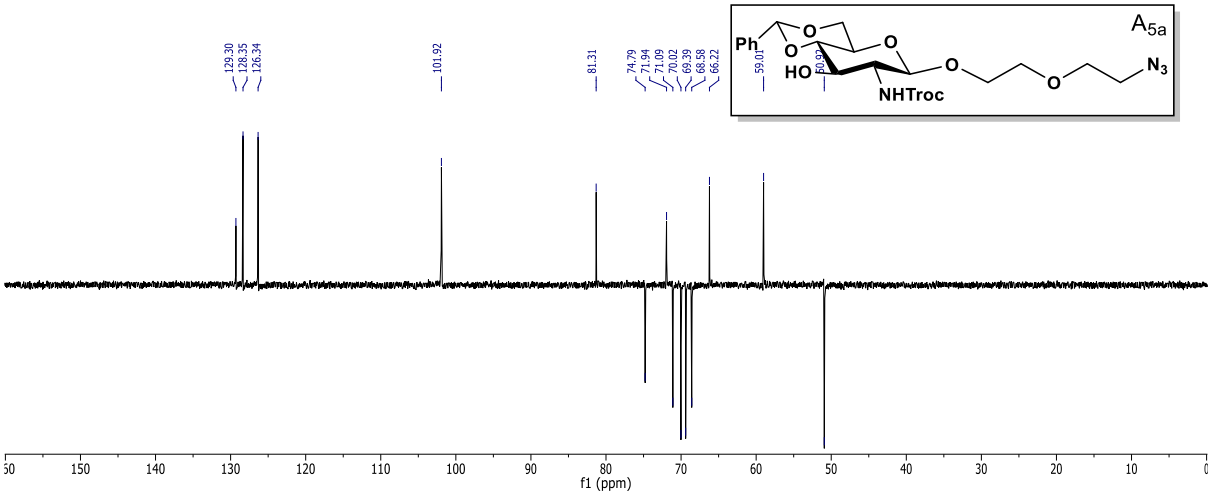
^{1}H NMR of Compound **A$_{5a}$**



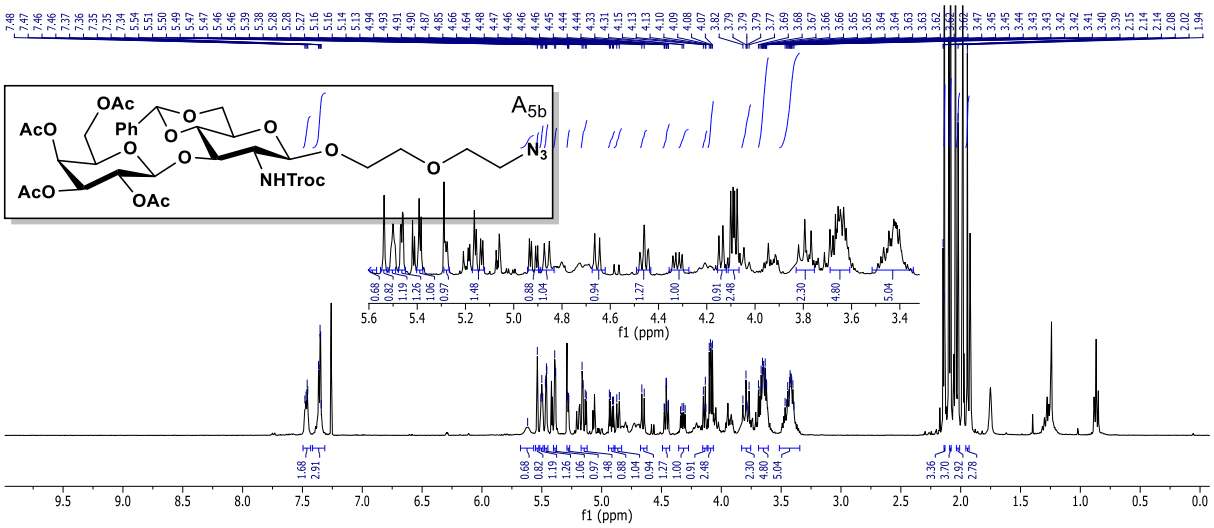
^{13}C NMR of Compound **A$_{5a}$**



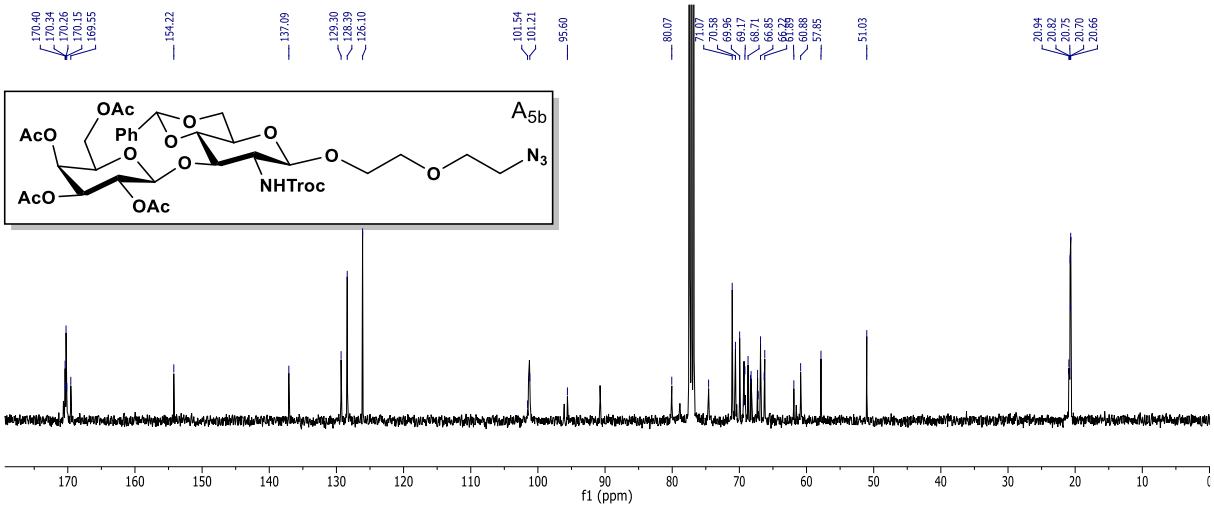
DEPT of Compound **A$_{5}$**



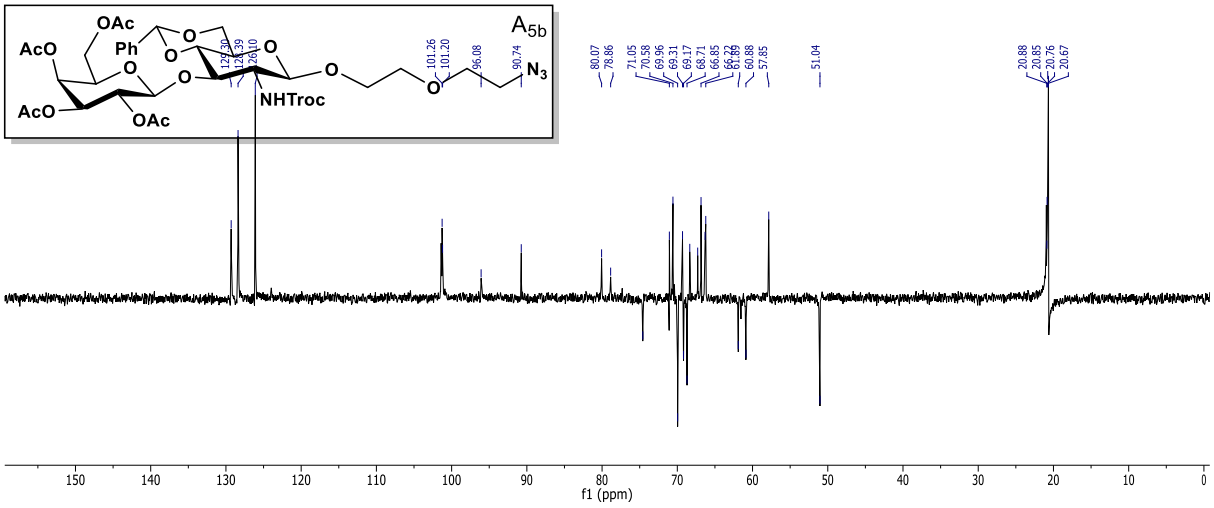
^{1}H NMR of Compound **A$_{5b}$**



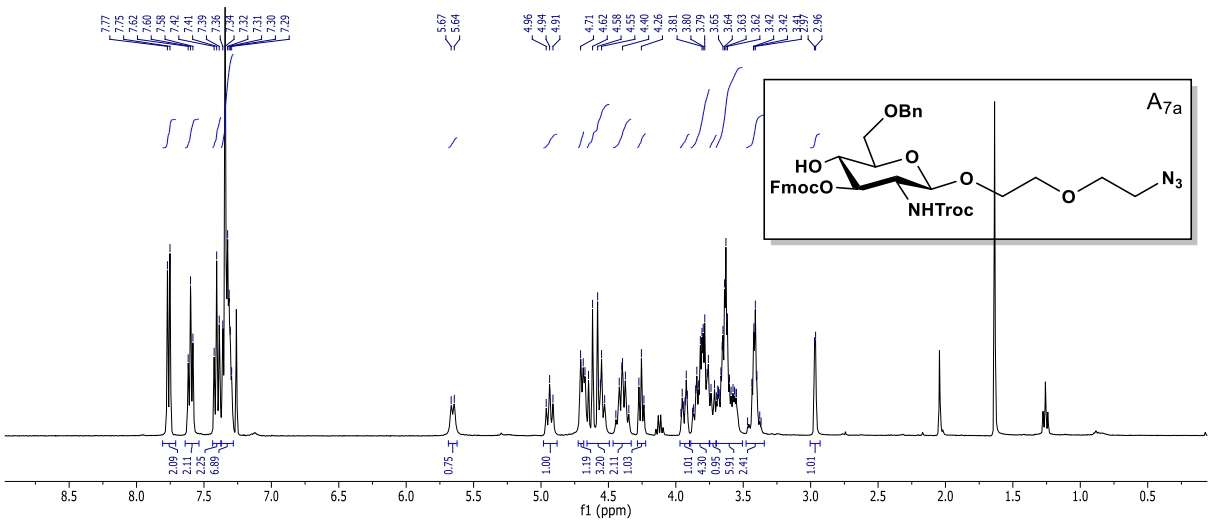
^{13}C NMR of Compound **A$_{5b}$**



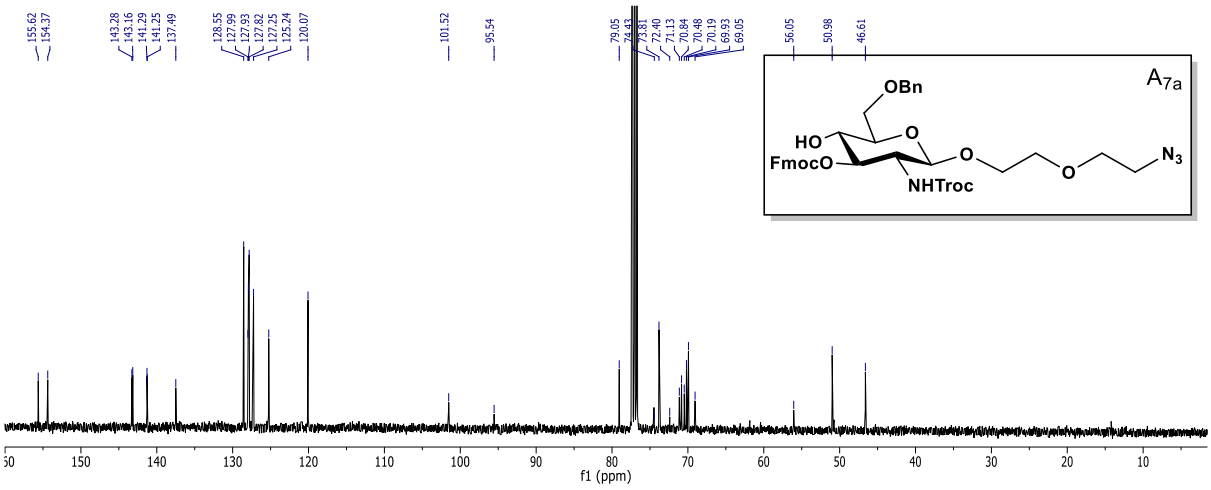
DEPT of Compound **A$_{5b}$**



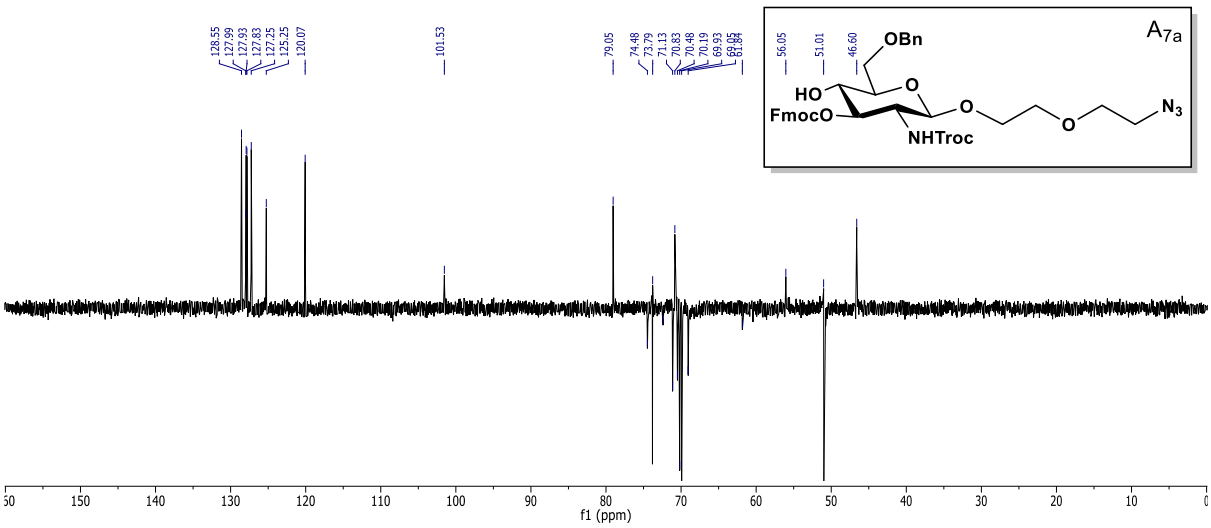
^{1}H NMR of Compound **A$_{7a}$**



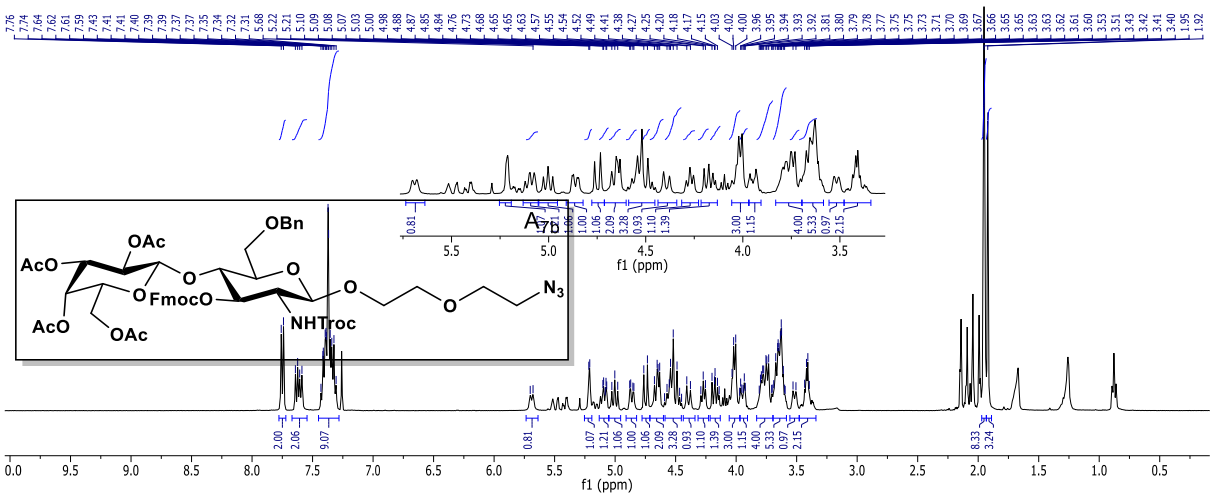
^{13}C NMR of Compound **A$_{7a}$**



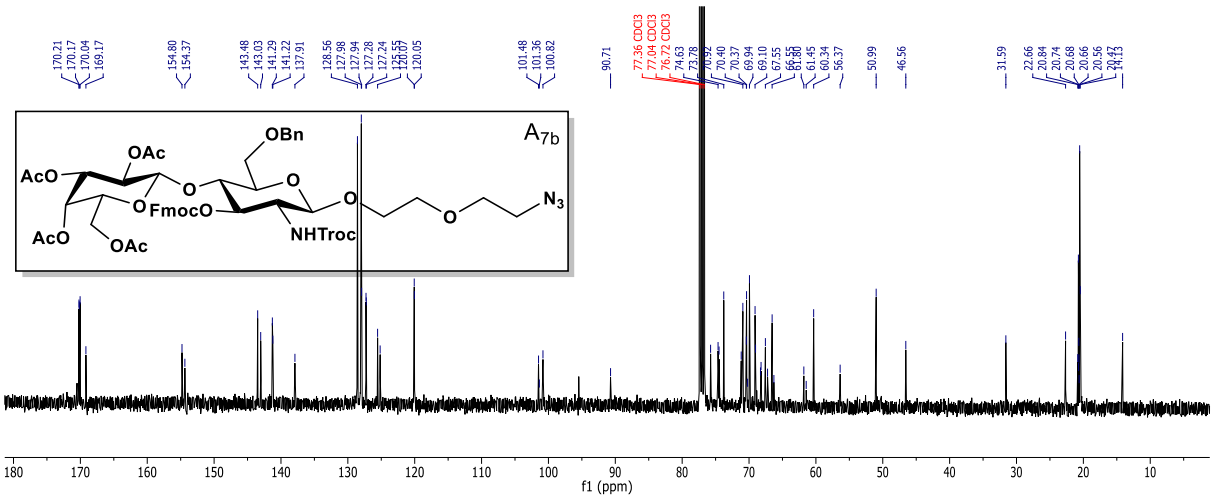
DEPT of Compound **A$_{7a}$**



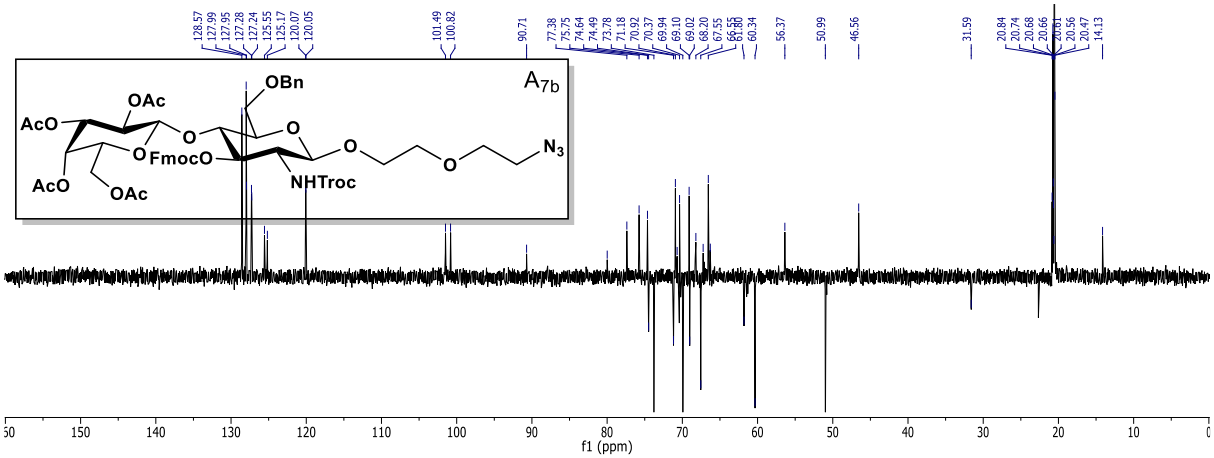
^{1}H NMR of Compound **A_{7b}**



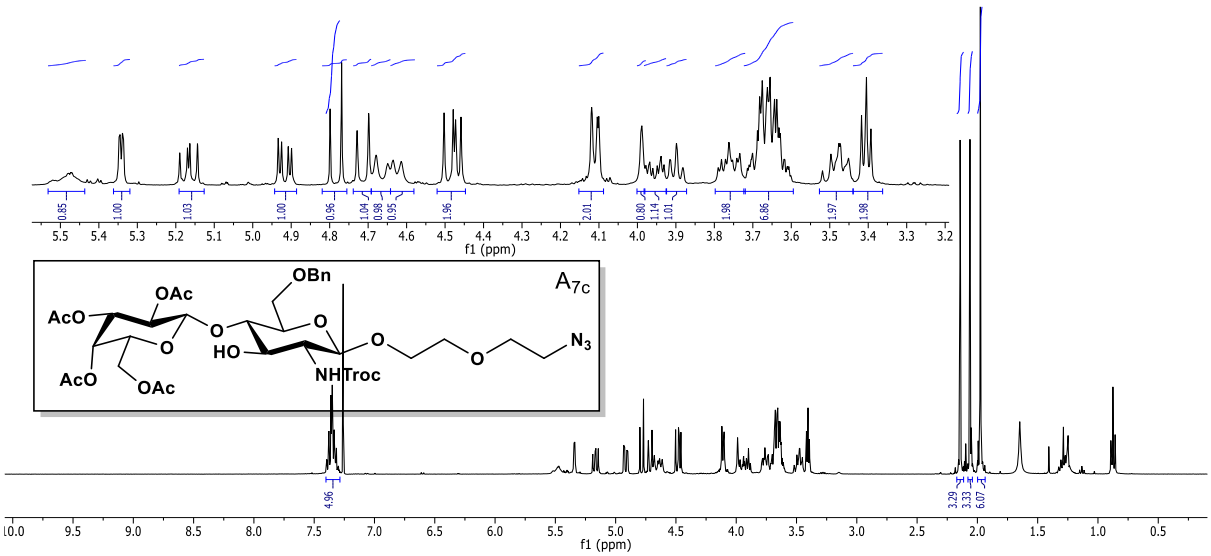
^{13}C NMR of Compound **A_{7b}**



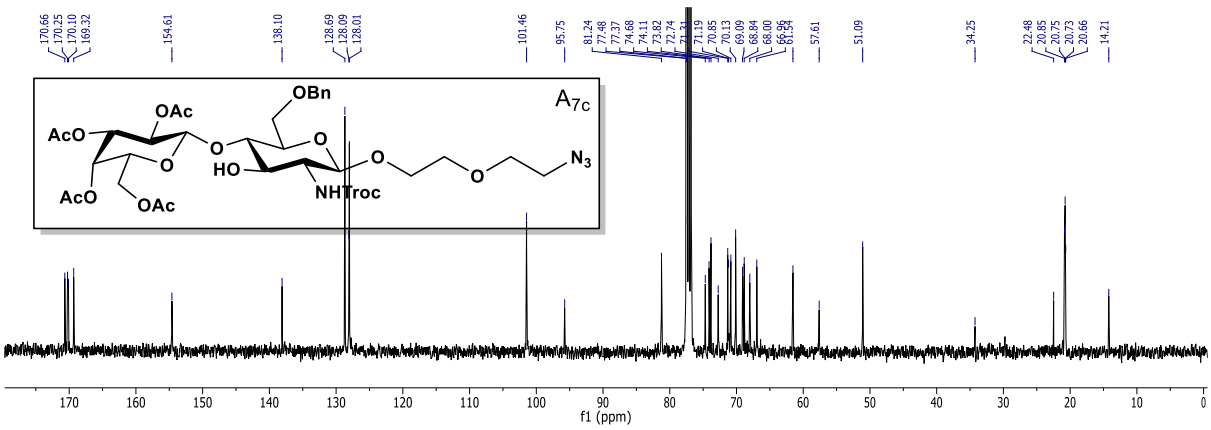
DEPT of Compound **A_{7b}**



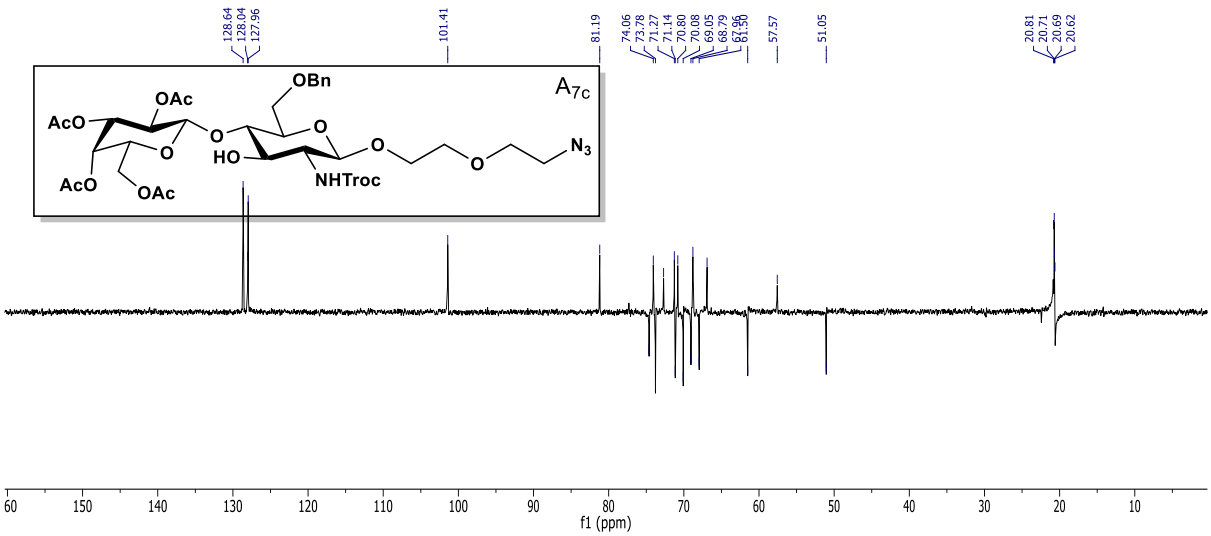
^{1}H NMR of Compound **A$_{7c}$**



13C NMR of Compound **A$_{7c}$**



DEPT of Compound **A$_{7c}$**



REFERENCES

1. a).http://www.emro.who.int/health-topics/stroke-cerebrovascular-accident/index.htmlb).http://www.strokecenter.org/patients/about-stroke/ischemic-stroke/

2. Shaheen E Lakhan, Annette Kirchgessner and Magdalena Hofer. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *Journal of Translational Medicine*, **2009**, 7:97.

3. Ulrich Dirnagl, Costantino Iadecola and Michael A. Moskowitz.Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*.**1999**, *22*, 391–397.

4. Lipton, Peter. Ischemic Cell Death in Brain Neurons. *Physiol. Rev.,* **1999***, 79* 1431–1568.

5. Rong Jin & Lin Liu & Shihao Zhang & Anil Nanda & Guohong Li. *J.of Cardiovasc. Trans. Res*., **2013**, 6, 834–851.

6. Jan Holgersson, Anki Gustafsson and Michael E Breimer, Characteristics of protein–carbohydrate interactions as a basis for developing novel carbohydrate-based antirejection therapies. *Immunology and Cell Biology*, **2005, 83**, 694–708.

7. Sander I. van Kasteren, Sandra J. Campbell, Se´bastien Serres, Daniel C. Anthony, Nicola R. Sibson and Benjamin G. Davis. Glyconanoparticles allow pre-symptomatic in vivo imaging of brain disease *PNAS*, **2009**, *106*, 1, 18-23.

8. Tracy D. Farr, Chian-Hui Lai, Dan Grünstein, Guillermo Orts-Gil, Cheng-Chung Wang, Philipp Boehm-Sturm, Peter H. Seeberger, and Christoph Harms. Imaging Early Endothelial Inflammation Following Stroke by Core

Shell Silica Superparamagnetic Glyconanoparticles That Target Selectin. *Nano Lett***, 2014**, *14*, 2130−2134.

9. Tiantian Yin, Licong Yang, Yanan Liu, Xianbo Zhou, Jing Sun, Jie Liu. Sialic acid (SA)-modified selenium nanoparticles coated with a high blood-brain barrier permeability peptide-B6 peptide for potential use in Alzheimer's disease. *Acta Biomaterialia*, **2015**, *25*, 172-183.

10. Albert Y Jin, Ursula I Tuor, David Rushforth, Jaspreet Kaur, Robert N Muller, Jodie Lee Petterson, Sébastien Boutry, Philip A Barber. Reduced blood brain barrier breakdown in P-selectin deficient mice following transient ischemic stroke: a future therapeutic target for treatment of stroke. *BMC Neuroscience*, **2010**, 11:12.

11. Christine Galustian, Robert A. Childs, Chun-Ting Yuen, Akira Hasegawa, Makoto Kiso, Andre´ Lubineau,Gray Shaw, and Ten Feizi. Valency Dependent Patterns of Binding of Human L-Selectin toward Sialyl and Sulfated Oligosaccharides of Le^a and Le^x Types: Relevance to Anti-Adhesion Therapeutics *Biochemistry*, **1997**, *36,* 5260-5266.

12. Chun-Ting Yuen, Karel BezouBk, Jacqui O'Brient, Mark toll, Remy Lemoinell, Andre Lubineaull, Makoto Kisoll, Akira Hasegawall, and Nicholas J. Bockovich Ky-riacos. Nicolaou and Ten Feizit Sulfated Blood Group $Lewis^a$ A SUPERIOR OLIGOSACCHARIDE LIGAND FOR HUMAN E-SELECTIN. *The Journal of Biological Chemistry*, **1994***, 269*, No. 3, pp. 1595-1598.

13. Chun-Ting Yuen, Alexander M. Lawson, f Wengang Chai,f Margot Larkin,: Mark S. Stol,t Ashley C. StuartJ Francis X. Sullivan, Tim J. Ahern, and Ten Feizi'J. Novel Sulfated Ligands for the Cell Adhesion Molecule E-Selectin Revealed by the Neoglycolipid Technology among O-Linked Oligosaccharides on an Ovarian Cystadenoma Glycoprotein *Biochemistry*, **1992***,31*,9126-9131

14. William J. Sanders, Tamiko R. Katsumoto, Carolyn R. Bertozzi, Steven D. Rosen, and Laura L. Kiessling. L-Selectin-Carbohydrate Interactions: Relevant Modifications of the Lewis x Trisaccharide *Biochemistry* **1996,** *35,* 14862-14867.

15. Yongmin Zhang, Pierre Sinay.Synthesis and characterization of a sulphated pentasaccharide containing the Lewis^{x} motif. *Carbohydrate Research*, **2003**, *338*, 1793-1795.

16. K. C. Nicolaou, N. J. Bockovich, and D. R. Carcanague. Total Synthesis of Sulfated Le^{x} and Le^{a}-Type Oligosaccharide Selectin Ligands. *JACS*, **1993**, *115*, 8843-8844.

17. Andre Lubineau, Joelle Le Gallic and RBmy Lemoine, First Synthesis of the 3'-Sulfated Lewisa Trisaccharide, Putative Ligand for the Leukocyte Homing Receptor *J. CHEM. SOC., CHEM. COMMUN*., **1993**, 1419-1420.

18. Graham E. Ball, Roger A. O'Neill,J Joanne E. Schultz,John B. Lowe, Brent W. Weston,Jon O. Nagy,Edward G. Brown, Christopher J. H, Mark D. Bednarski. Synthesis and Structural Analysis Using 2-D NMR of Sialyl Lewis X *(SW)* and Lewis X (Le")Oligosaccharides: Ligands Related to E-Selectin [ELAM-11 Binding *JACS,* **1992**, *114,* 5449-5451.

19. Gerd Hummel, Richard R Schimdt A versatile synthesis of the lactoneo-Series Antigens-Synthesis of Sialyl Dimer Lewis X and of Dimer Lewis Y. *Tetrahedron letters*, **1997**,38,7,1173-1176,

20. Bin-Ling Tsai a,b, Jeng-Liang Han a, Chien-Tai Ren a, Chung-Yi Wua,⇑, Chi-Huey Wonga,b Programmable one-pot synthesis of tumor-associated carbohydrate antigens Lewis X dimer and KH-1 epitopes *Tetrahedron Letters,**2011***, 52 ,2132–2135.

21. Sofie Meng Andersen, Mads Heuckendorff, and Henrik H. Jensen. 3-(Dimethylamino)-1-propylamine: A Cheap and Versatile Reagent for Removal of Byproducts in Carbohydrate Chemistry. *Org. Lett*. **2015**, *17*, 944−947.

22. Young Soo Kang, Subhash Risbud, John F. Rabolt, and Pieter Stroeve. Synthesis and Characterization of Nanometer-Size Fe_3O_4 and *γ*-Fe_2O_3 Particles.*Chem. Mater*, **1996**, *8,* 2209-2211.

23. Xianqiao Liu, Zhiya Ma, Jianmin Xing, Huizhou Liu. Preparation and characterization of amino–silane modified superparamagnetic silica nanospheres. *Journal of Magnetism and Magnetic Materials*, **2004**, *270*, 1–6.

24. Y P He, S Q Wang, C R Li, Y M Miao, Z Y Wu and B S Zou. Synthesis and characterization of functionalized silica-coated Fe_3O_4 superparamagnetic nanocrystals for biological applications. *J. Phys. D: Appl. Phys*, **2005**, *38*, 1342–1350.