Immunomodulation of Globo-H Antigen Using Gold Nanoparticles for Breast Cancer Therapy.

A thesis submitted towards partial fulfillment of the requirement of BS-MS Dual Degree Program

By

Keerthana T V

20131052

Indian Institute of Science Education and Research Pune

Under the guidance of

Dr.Raghavendra Kikkeri

Associate Professor

Department of Chemistry

Indian Institute of Science Education and Research Pune

CERTIFICATE

This is to certify that this dissertation entitled "**Immunomodulation of Globo-H Antigen Using Gold Nanoparticles for Breast Cancer Therapy**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents the work carried out by **Keerthana T. V** at Indian Institute of Science Education and Research under the supervision of **Dr. Raghavendra Kikkeri**, Associate professor, Department of Chemistry during the academic year **2017-2018**

Date: 20-03-2018

Place: Pune

Dr.Raghavendra Kikkeri

Assistant Professor

IISER Pune.

DECLARATION

I hereby declare that the matter embodied in the report entitled "**Immunomodulation of Globo-H Antigen Using Gold Nanoparticles for Breast Cancer Therapy**" are the results of the work carried out by me at the Department of Chemistry,Indian Institute of Science Education and Research, Pune, under the supervision of **Dr.Raghavendra Kikkeri** and the same has not been submitted elsewhere for any other degree.

Date: 20-3-2018

Place: Pune

Keerthana T V

20131052

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

Dedicated to Family & Friends

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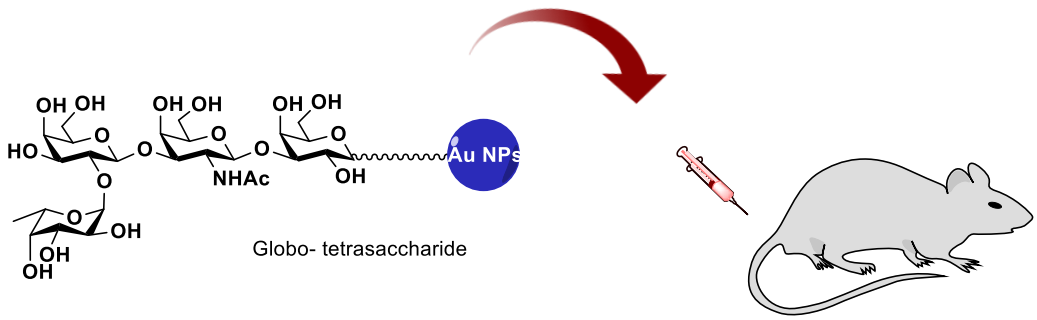
ABBREVATIONS

Ac_2O	Acetic anhydride
Py	Pyridine
RT	Room temperature
H	hour
$BF_3.OEt_2$	Boron trifluoride diethyl etherate
DCM	Dichloromethane
NaOMe	Sodium methoxide
MeOH	Methanol
$PhCH(OMe)_2$	Benzaldehyde dimethyl acetal
CAN	Acetonitrile
p-TSA	Para toluene sulfonic acid
NAPBr	2-(Bromomethyl) naphthalene
Bu_2SnO	Dibutyl tin oxide
DMF	Dimethyl formamide
BzCl	Benzoyl chloride
LevOH	Levulinic acid
THF	Tetrahydrofuran
DCC	$N,N^{l\text{-}}$Dicyclohexylcarbodiimide
DMAP	Dimethylaminopyridine
HBr	Hydrobromic acid
M.S	Molecular sieves
Zn	Zinc
NaH_2PO_4	Sodium dihydrogen phosphate
CAN	Ceric ammonium nitrate
NaN_3	Sodium azide
NaOAc	Sodium acetate
AcOH	Acetic acid
TrocCl	Trichloroethylchloroformate
CA	Chloroacetate
BnBr	Benzyl bromide

NaH	Sodium hydride
NIS	N-iodosuccinamide
TfOH	Trifluorosulfonic acid
DDQ	Dichlorodicyanobenzoquinine
CsF	Cesiumfluoride
NH_2NH_2	Hydrazine hydrate
NP	Nanoparticle
Au	Gold

ABSTRACT

Cancer is a devastating disorder and till now no medicines or therapies beaten down its spreading. Tumor associated carbohydrate antigens (TACAs) are unique antigen signatures on cancer cell surface to develop markers to target cancer cells. However, owing to their poor immunogenicity, most of the TACA as such failed to induce T-cell mediated immunity. Previously, Danishefsky et al. used KLH (keyhole limpet hemocyanin) as a carrier protein to improve the immunogenicity of TACAs. However, the TACA-KLH conjugate induced T-cell independent immune responses and produced high level of antibodies against KLH, compared to TACAs. To avoid antibody against the platform and to induce cytotoxic T-cell mediated immune responses, Herein, we proposed to conjugate TACAs on gold nanoparticles to increase the high titre IgG antibody against specific TACAs. We have used gold nanoparticles of different shapes and size as adjuvants to alter the immune responses. As a prototype, we synthesized Globo-H active tetrasaccharides, a TACA found mainly on the breast cancer cell surfaces. We believe that multivalent glyco-nanoparticles are expected to produce high level of IgG antibody against TACA for targeting breast cancer cell for immunotherapy.



INTRODUCTION

Cancer is one of the devastating health problem faced by humanity for the past few decades. According to the report by American Cancer Society, around 14.1 million new cancer cases and 8.2 million deaths are being reported worldwide in 2012. Even though there is significant progress in cancer therapy, like surgery, chemotherapy, cancers are still not cured or prevented entirely. So the need for better and improved treatment has grown impetus. For cancer prevention and treatment, the immunotherapy and vaccines came in to picture

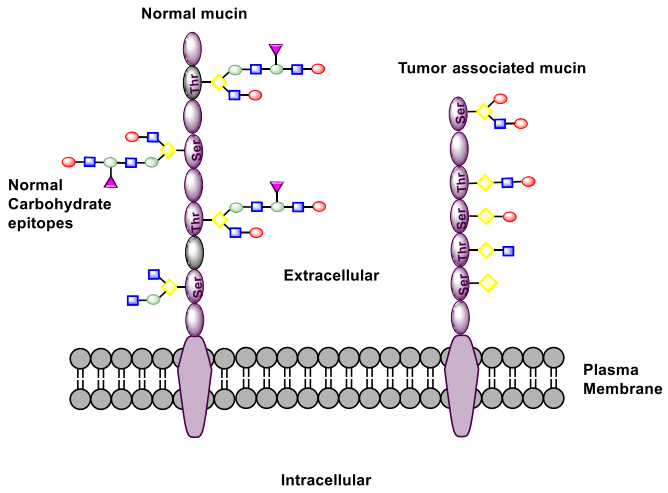


Figure 1. Carbohydrate structures of cell surface mucins in healthy and cancer cells.

Immunotherapy is a cancer treatment that exploits body's immune response to fight against cancer. Recently significant efforts have been taken in the direction of targeting immune system for therapeutic and preventive purpose. Components of immune system such as natural killer cells, B-cells, T-cells and cytokines etc. are playing pivotal role in all types of malignancies.[1] Use of these immune molecules for treatment of cancer is emerging as an immunotherapy. Recently, passive immunotherapy using antitumor monoclonal antibodies (mAbs) are into play. Even though it was successful, there were some side effects as the immune system destroyed healthy cells to a minimal extent.[3] This scenario intensifies the need for targeted and long lasting treatment for cancer. Various cancer associated antigens has been explored over the period for development of immunization strategies effective at inducing protective immune responses.[4] Tumor associated carbohydrate antigens (TACAs) presents good vaccine candidates for cancer treatment as they are abundantly present on the cancer cell surfaces.

Tumor-associated carbohydrate antigen (TACA)- The onset and progression of cancer was initated by the dramatic changes undergone by the cells in carbohydrate expression. Over the last 30 years, dozens of tumor-associated carbohydrate antigens (TACA) (carbohydrate epitopes that are highly over-expressed or uniquely expressed on tumors (**Fig.1**)) was identified [16] and these TACAs can be broadly classified into two groups. (1) Glycoprotein antigens which are carbohydrates linked to serine/threonine residues of protein. It includes antigens such as Tn, Thomsen-Friedreich (TF), and sialyl-Tn (sTn). (2) Glycolipid antigen which is carbohydrates linked to the lipid bilayer on the cell surface. It includes several families like gangliosides such as GD2, GD3, GM2, GM3, etc., globo class like Globo H, Gb3, Gb4, etc., and blood group determinants like Lewis^x, lewis and their derivatives.[5] Some mucin type TACAs are rarely present on normal cell whereas TACAs like Lewis antigens are present in both tumor cells as well as normal cells. However, it is overexpressed in the tumor cell surface. Hence these kinds of altered carbohydrate expression in tumor cells can be used as a marker to generate TACA based vaccines for antitumor therapy. Vaccines containing the TACAs would stimulate the immune response which leads to the formation of antibodies. These antibodies can then selectively destroy the cells exhibiting the specific kind of TACA and thereby eliminating the tumorcells. [5, 6]

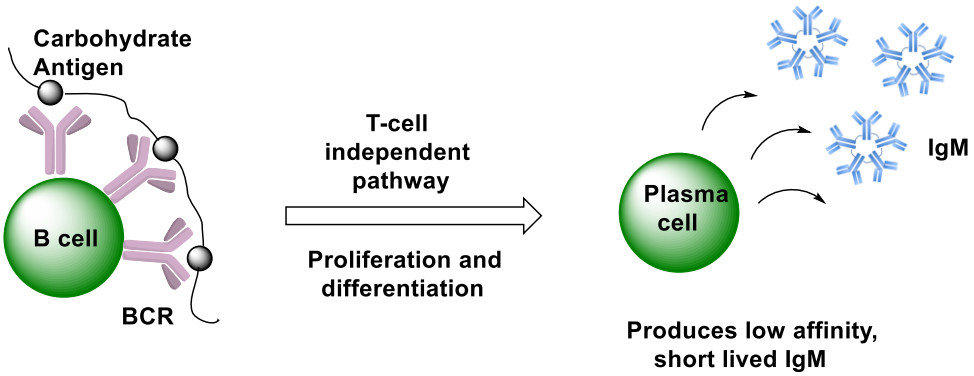


Figure 2.T cell-independent pathway of antibody production.

Our body exhibits two kinds of immune responses; T-cell dependent and T-cell independent. Carbohydrates being the T cell independent immunogen induce a less titer of IgM antibody response rather than the high-affinity IgG response as it proceeds through the T cell-independent pathway of antibody production **(Fig. 2)** which is less efficient and nonspecific. Hence to induce the T-cell mediated response

(Fig. 3), the TACAs are conjugated with some other molecules like nanoparticles, proteins, etc.

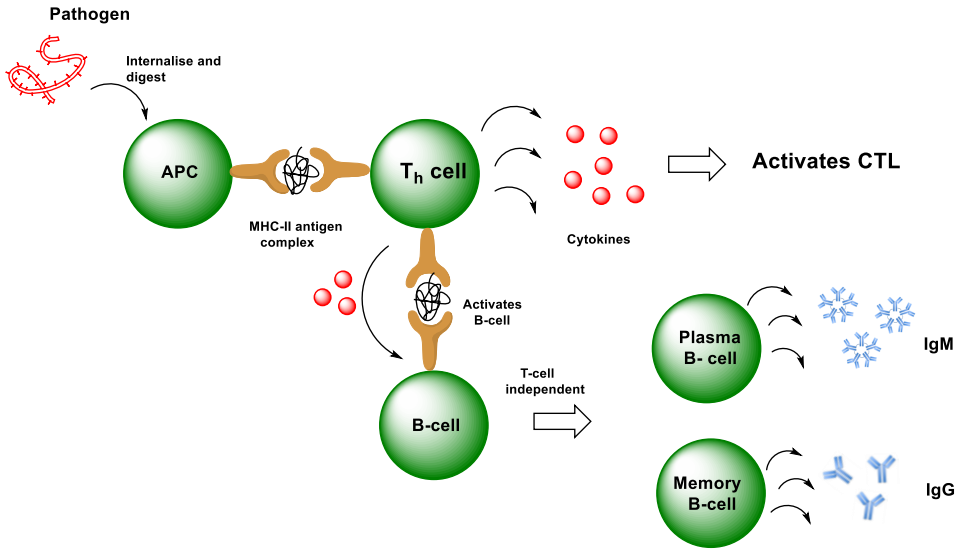


Figure 3.T cell-independent pathway of antibody production.

Due to self-antigenic nature and non-immunogenicity block the production of strong immune response against these glycans. Attempts were made to improve immune response of these TACAs by conjugation with KLH protein found to result in production of antibodies against both KLH and antigen. Danishefsky and co-workers have attached the antigens with KLH protein, which is an immunostimulant, but this effect doesn't last for long. First injection of vaccine showed a good IgG response but as the time progresses level of antibody started decreasing. [7] Recent studies have shown that polymer, or nanoparticle can also enhance vaccine efficacy *via* diverse mechanisms.[6] Examples include conjugation of antigens to polymers to provide new properties such as multi-valancy and/or controlled release; antigens conjugated to nanoparticles can also lead to changes in the pathways by which antigens are processed by APCs. Thus, bioconjugates can be tailored and functionalized according to vaccine-specific needs. Among the different nanomaterials, gold nanoparticles have already been used as antigen carriers for vaccine development without the production of anti-gold nanoparticle antibodies. Moreover, gold nanoparticles are biocompatible, easy to fabricate in terms of size and shape to alter the immune responses.[17-20] Herein, we propose to synthesize Globo-H conjugated multiple functional nanopaticles, which can induce cytotoxic T-cell (CTL) mediated immune responses and increase the high titer IgG antibody against Globo-H. Gold nanoparticles of different shapes and size as adjuvants to alter the immune

responses and increase the cross-presentation of exogenous antigens by dendritic cells. In order to simply the synthetic protocol, we synthesized the active component of Globo-H. Previously, Wang et al.[8] used microarray technique to show that terminal tetrasaccharide is actively recognizing both IgG and IgM of anti-Globo H antibodies and the fucose moiety is essential for recognition. Motivated by this result, we first targeted to synthesize terminal tetrasaccharide of Globo-H.

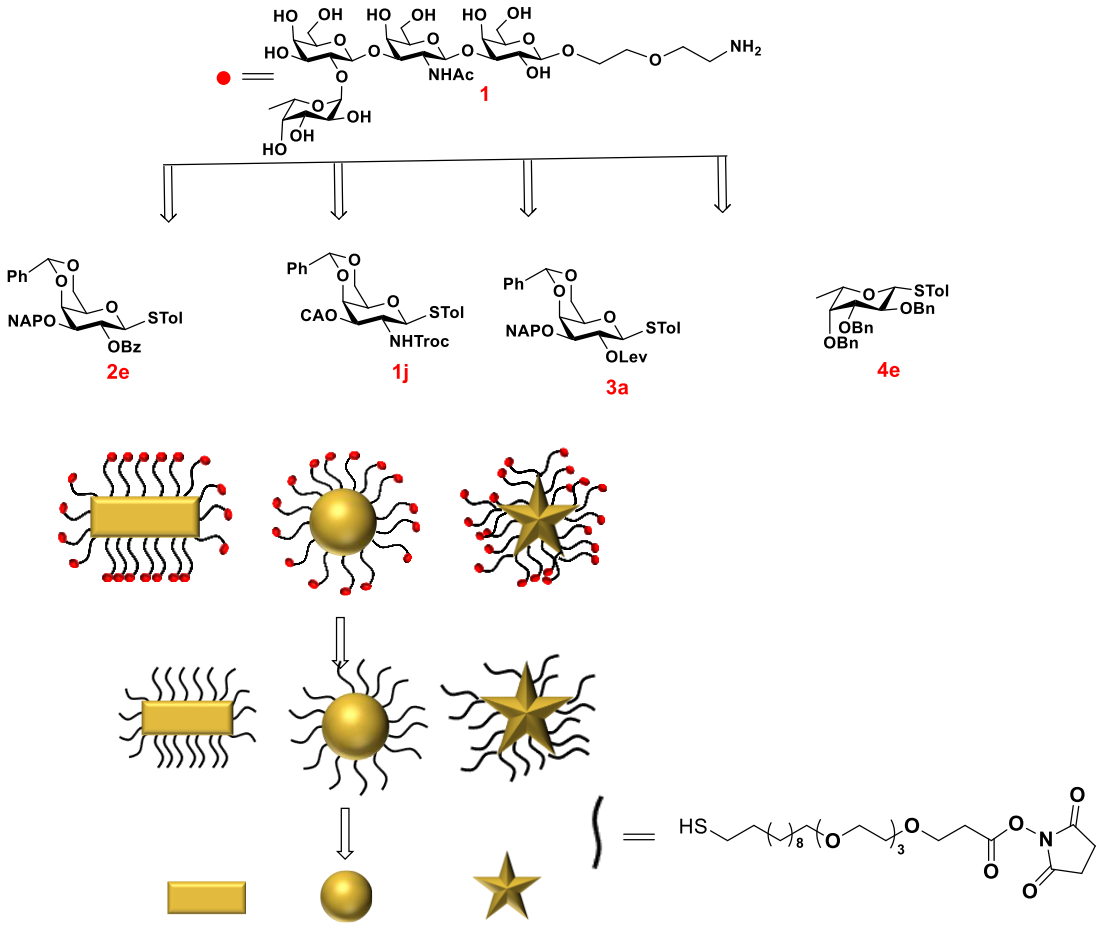
Objectives:

This work aims to generate TACA-nanoparticle based immune platform to generate next generation vaccine against breast cancer.

The objectives include:

- Synthesis of Globo-H active components.
- Synthesis of different size and shapes of gold nanoparticles
- Bioconjugation of Globo-H antigen active tratrasaccharides and check the cytokine secretion and immune responses.

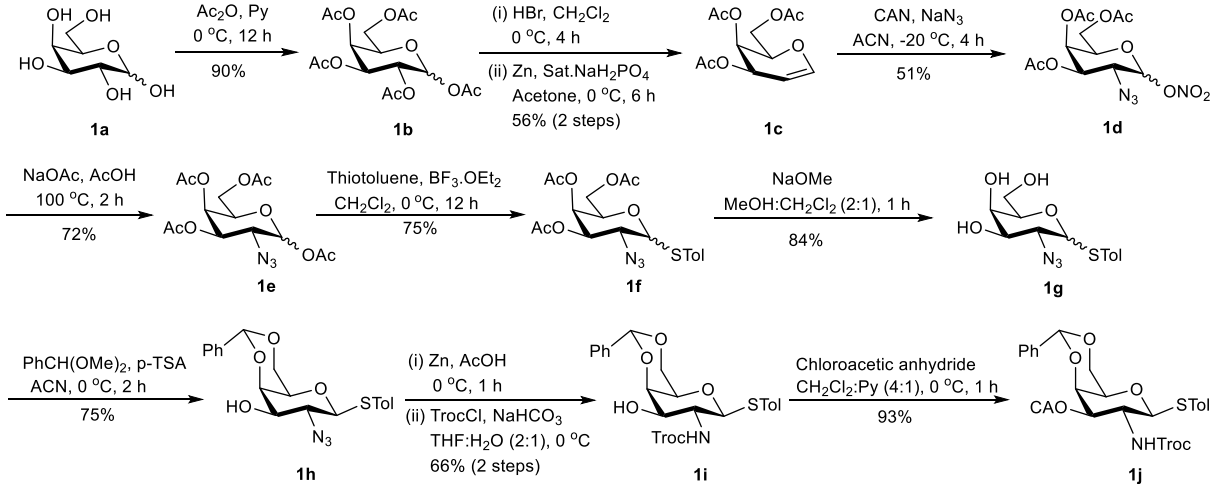
Synthesis of Globo-H tetrasaccharide conjugated gold nanoparticles:



Scheme 1. Retrosynthesis of tetrasaccharide of globo-H and Globo-H glycoconjugates.

The Globo-H active glycan (**1**) was synthesized from fully protected tetrasaccharides. **(Scheme 1).** The assembly of Globo-H antigen involved the thiofucoside, thiogalactoside and thiogalactosamine building blocks. Several high yielding procedures have already been reported for the synthesis of monomeric building blocks.[10-15] Hence we used the same strategies in the synthesis. The characterizations of the compounds were done using NMRs, MALDI-TOF and HRMS. To assess adjuvent properties of gold nanoparticles during immune responses, three different shapes of gold nanoparticles (rod, sphere and star-shapes) were synthesized and conjugated with thio-linker appended with *N*-hydroxysucciminide active ester group for glycoconjugation. Size and shapes of the nanoparticles are characterized by SEM imaging technique.

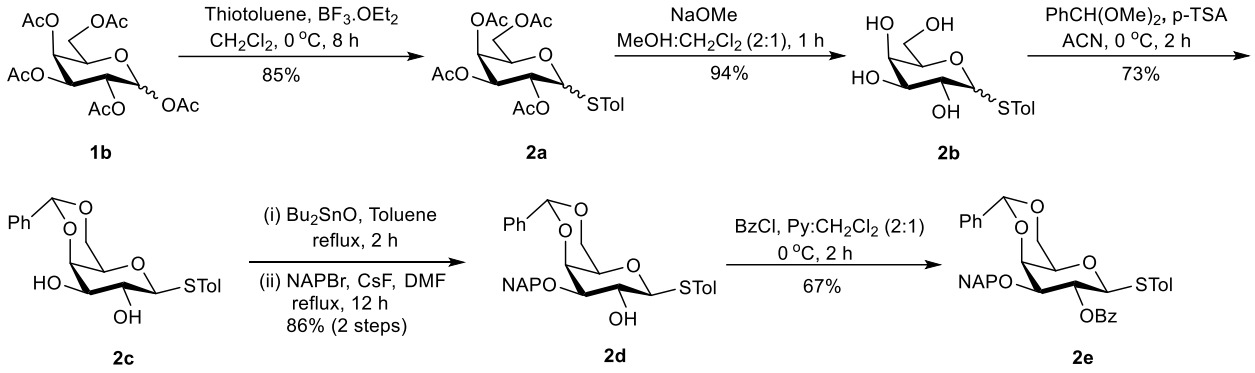
Synthesis of galactosamine building block:



Scheme 2. Synthesis of galactosamine donor

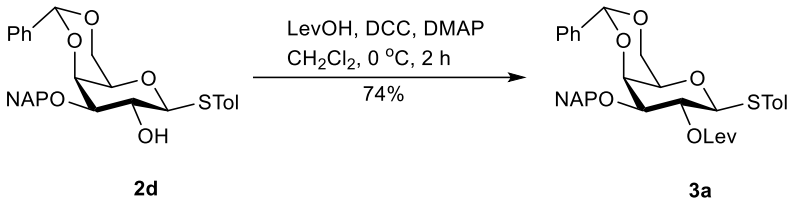
The galactosamine building block **1j** was yielded from the commercially available D-galactose **1a** in 11 steps. In the first step, all hydroxyl groups of galactose were protected with acetyl group using pyridine and acetic anhydride to get **1b** which is a white powder. The **1b** was then dissolved in DCM and bromination is done at the anomeric position using HBr stirring at 0 ^{o}C to yield the brominated product. Since this was not stable and gets degraded above 35 ^{o}C, excess solvents were removed slowly, and workup was done using cold water and then was further dissolved in equi-volume mixture of water and acetone and was converted to **1c** using Zn and NaH_2PO_4 at 0 ^{o}C. The obtained crude was further preceded for workup and column chromatography to yield pure product **1c**. Further **1c** was dissolved in ACN and reacted with CAN and NaN_3 at -20 ^{o}C to yield **1d** with 51% yield.**1d** in the presence of AcOH and NaOAc, converted to**1e**. In the fifth step, **1e** was dissolved in DCM and thiotoluene was added and stirred. Subsequently, $BF_3.OEt_2$ is added dropwise at 0 ^{o}C to yield **1f**. Subsequently, deprotectionof allacetyl groups was done followed by benzylideneacetal protection of C4 and C6 hydroxyl group to yield **1g** and **1h.** Then C2 azide of **1h** was reduced to using Zn in AcOH to obtain galactosamine, which without purification was taken in further step for Troc protection of amine using TrocCl, $NaHCO_3$to get **1i**, which is further purified to obtain pure isomers. Finally C3 hydroxyl group of **1i** was protected with chloroacetate to get final building block **1j** with 93 % yield **(Scheme.2).**

Synthesis of galactose building block:



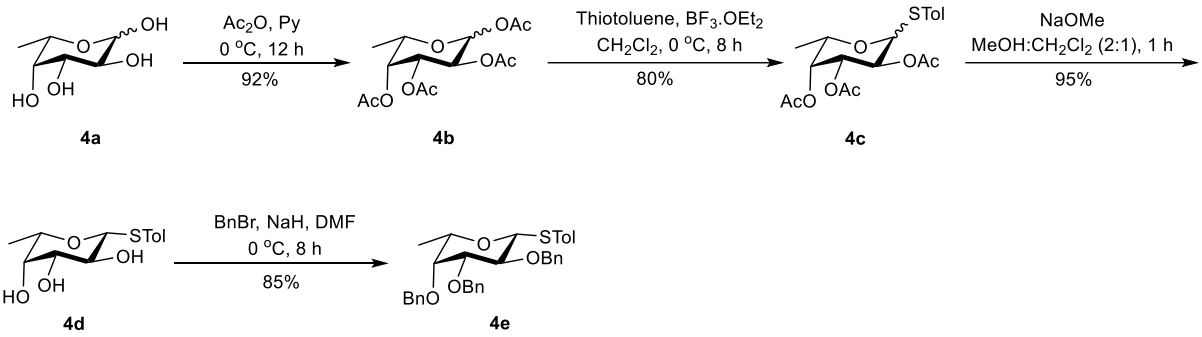
Scheme 3. Synthesis of galactose donor 1

Commercially available D-Galactose was used to prepare the required building block **2e** in six steps. The per acetylated **1b** was dissolved in DCM and the anomeric carbon was selectively glycosylated with thiocresol to yield 85% of **2a** as a solid. It was then purified by clumn chromatography by 30% EtOAc:Hexane. Next rest all acetates were deprotected using sodium methoxide and subsequently C4 and C6 hydroxyls were protected in the form of benzylideneacetal to get **2c** with 73% yield. Then **2c** C3 hydroxyl group was protected with naphthalene using 2-bromo methyl naphthalene in presence of Bu_2SnO and CsF to yield **2d**. Further **2d** was used to get two building blocks **2e** and **3a** by protecting C2 hydroxyl with benzoyl and Lev respectively (Scheme. 2), (Scheme 3). Final building blocks **G6** and **G7** were completely characterised using NMR and HRMS.



Scheme 4. Synthesis of galactose donor 2

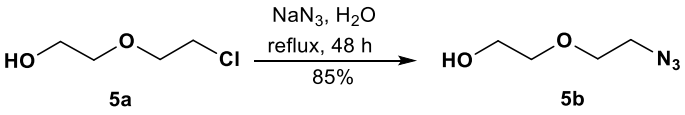
Synthesis of Fucose building block:



Scheme 5. Synthesis of Fucose donor

Commercially available L-Fucose **4a** was used to prepare the required **4e** in four steps. In the first step, all the hydroxyl groups were protected with base labile acetyl group using pyridine and acetic anhydride. Once the reaction was completed excess solvents were removed, and workup with dil.HCl yielded **4b**. **4b** was then dissolved in dry DCM and *p*-thiocresol was added and stirred at 0ºC, subsequently mild Lewis acid $BF_3.Et_2O$ was added dropwise to the reaction mixture and stirred at 0ºC 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 yielded **4c** as a white solid. The compound **4c** was then dissolved in 2:1 mixture of methanol and dichloromethane and acetyl groups were deprotected by using NaOMe to obtain **4d**. Finally, **4d** was benzylated and purified by silica column chromatography yielding the required fucose donor **4e** as a white solid (75% yield) **(Scheme 5).**

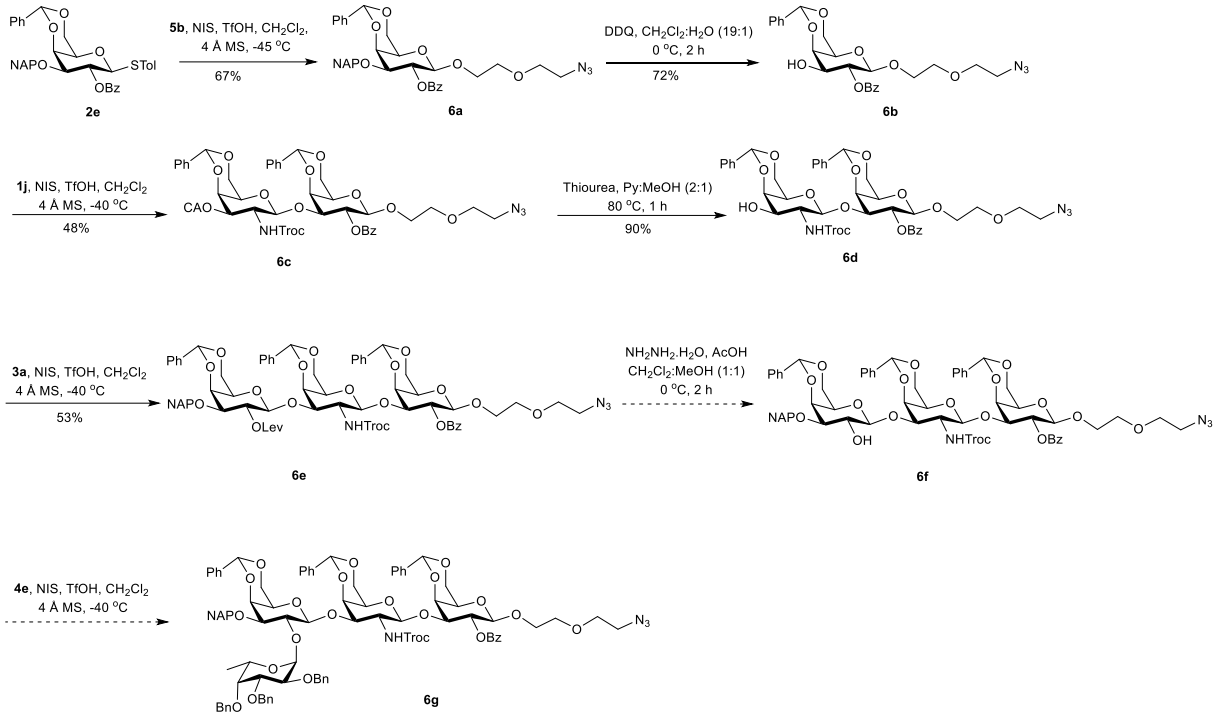
Synthesis of linker 1:



Scheme 6. Synthesis of Linker 1

Synthesis of the linker was achieved by using the starting material 2-(2-chloroethoxy) ethanol by dissolving in water and heating up to 100 ºC and adding sodium azide to it and then stirring it for 2 days **(Scheme 6)**

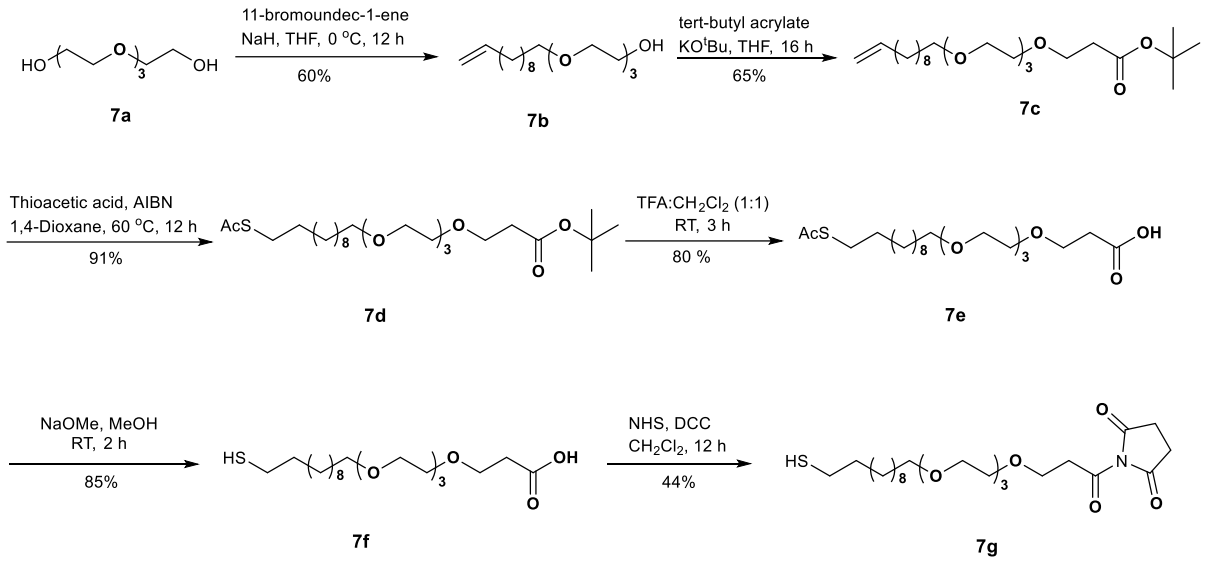
Synthesis of tetramer of globo-H:



Scheme 7. Synthesis of tetrasaccharide

Synthesis of Globo-H active tetrasaccharide will be done by step-by-step glycosylation of the donors **1j, 2e, 3a, 4e** and **5b** as acceptor. To obtain the **6a**, the **2e** was glycosylated with linker by using NIS as an activator and TfOH as a promoter. Then further the NAP protecting group at the third position of **6a** was deprotected using DDQ:H_2O in 2:1 ratio to obtain **6b**, which is an acceptor and then it was further glycosylated to the donor **1j**. Now to the disaccharide with the linker, the 3rd position chloroacetate group was deprotected using thiourea by heating in pyridine and methanol at 80 ^{o}C.Then the disaccharide **6e** was obtained by glycosylating the donor **3a** with acceptor **6d** in the presence of NIS and TfOH at -40 ^{o}C. Then the trisaccharide lev group will be deprotected using hydrazine hydrate **(6f)** and glycosylated to **4e** by using NIS and TfOH to obtain the tetrasaccharide of globo-H **6g**. The global deprotection and hydrogenation of 6g is expected to result final tetrasaccharide for glyco-goldnanoparticle conjugation.**(Scheme 7)**.

Synthesis of Linker 2:



Scheme 8. Synthesis of linker for nanoparticles conjugation

Synthesis of linker for glycoconjugation was done in 6 steps starting from the coupling reaction between triethylene glycol **(7a)** and 11-Bromoundec-1-ene by using NaH and DMF as a solvent**.** Once the coupling product **(7b)** was obtained, the alcoholic group of **7b** was protected using tert-butyloxy carbonyl group using tert-butyl acrylate and KO^{t}Bu by dissolving it in THF yielding 85% of **7c**. Then the terminal alkene of **7c** was reacted with thioacetic acid in the presence of AIBN in 1,4-Dioxane afforded **7d** with 91% yield. In subsequent steps, the tert-butyloxy carbonyl group was deprotected using 50% TFA in DCM (**7e**) followed by deprotection of acetate of thiol (**7f)** with the help NaOMe in MeOH respectively. Finally, the carboxylic acid terminal of the linker was activated with NHS using DCC in DCM affording **7g** with 44% yield

MATERIALS

- All chemicals were purchased from sigma Aldrich, Spectrochemicals, Avra and alfa aeser.
- 1H, 13C & 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 KMnO4.
- 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 40^0C unless otherwise specified.
- Silica gel (100 -200) & (60-120) mesh was used for column chromatography.

EXPERIMENTAL PROCEDURES

Compound 1 b

Commercially available D-(+)-Galactose (50g, 277.5mmol) was dissolved in anhydrous pyridine (500 mL) and allowed it to cool and stir for 10 minutes. Then acetic anhydride (262 mL, 2.78 mol) was added drop-wise using a dropping funnel and continued stirring for 12 h. The reaction mixture was then extracted with DCM, washed with 10% HCl, dried over sodium sulphate and then the solvents were evaporated yielding **1a** of 90% yield. **1a** was then carried out for the next reaction without silica gel column chromatography.

Compound 1 c

1b (40g, 102.5 mmol) was vaccum dried, dissolved in DCM and cooled to 0 ^{o}C. Then HBr (40mL) was added dropwise using dropping funnel and then kept stirring for 2 h to obtain brominated product. Subsequently, the reaction mixture was washed with cold water and dried over sodium sulphate followed by evaporation of the excess solvents under 30 ^{o}C to obtain the crude product. It is then proceeded for the next step without purification by dissolving it in Acetone and sat.NaH_2PO_4 in 2:1 (400 mL:200 mL) ratio followed by cooling to 0 ^{o}C . Then Zinc (80.5 g,1.2 moles) was added and stirred at 0 ^{0}C for 4 h. On completion of the reaction, the reaction mixture was filtered using celite and extracted with EtOAc. The EtOAc layer was further washed with brine , dried over Na_2SO_4 and then evaporated to obtain **1c**. It is then purified using silica gel column chromatography using 30% EtOAc in Hexane and vaccum dried to obtain **1c** (18 g, 56%). ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 6.44 (dd, *J* = 6.3, 1.6 Hz, 1H), 5.53 (dd, *J* = 2.6, 1.9 Hz, 1H), 5.44 – 5.37 (m, 1H), 4.74 – 4.68 (m, 1H), 4.36 – 4.14 (m, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 170.65, 170.37, 170.22, 145.49, 98.92, 81.74, 72.86, 63.97, 63.82, 62.00, 20.88, 20.83, 20.73. HRMS (ESI) m/z: calc'd for $C_{12}H_{16}O_7$: 272.0896; Found $C_{12}H_{16}O_7Na$: 295.0785

Compound 1d

The vaccum dried **1c** (18 g,57.8mmol) was dissolved in anhydrous ACN along with molecular sieves under N_2 atmosphere and was allowed to cool to -20 ^oC. Then CAN (73 g,0.138 mol) and NaN_3(5.25 g, 0.1 mol) was added and stirred for 6 h until the completion of the reaction. Once the reaction was completed, the reaction mixture is filtered using whatmann filter paper. The filtered product was the extracted with EtOAc, washed with water and brine, dried over sodium sulphate and evaporated to obtain crude **1d**. It was then further purified using silica gel column chromatography using 25% EtOAc in Hexane yielding 51% of **1d** and then vaccum dried to proceed for the next reaction (11.5 g, 30.5 mmol). The obtained product was a mixture of α, β and talo in the ratio1:0.7:0.2. The individual isomers were not isolated from crude but NMR data for each is reported separately. ***α*- isomer** ^1H NMR (400 MHz, $CDCL_3$) δ 6.36 (d, J = 4.1 Hz, 1H), 5.52 (dd, J = 3.3, 1.3 Hz, 1H), 5.27 (dd, J = 11.4, 3.2 Hz, 1H), 4.40 – 4.36 (m, 1H), 4.16 – 4.13 (m, 3H), 2.19 (s, 3H), 2.09 (s, 3H), 2.05 (d, J = 0.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29 ¬169.18 (C=O, acyl), 96.87, 69.49, 68.58, 66.62, 60.91, 55.93, 20.58 – 20.36 (3x CH3). ***β*- isomer** ^1H NMR (400 MHz, $CDCl_3$) δ 5.60 (d, J = 8.8 Hz, 1H), 5.41 (dd, J = 3.3, 1.1 Hz, 1H), 4.98 (dd, J = 10.6, 3.3 Hz, 1H), 4.13 – 4.09 (m, 2H), 4.10 – 4.05 (m, 1H), 3.84 (dd, J = 10.6, 8.8 Hz, 1H), 2.19 (s, 3zH), 2.10 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29 – 169.18 (C=O, acyl), 98.08, 71.87, 71.71, 65.82, 60.83, 57.49, 20.58 – 20.36 (3x CH3). **Talo-isomer** ^1H NMR (400 MHz, $CDCl_3$) δ 6.23 (d, J = 1.5 Hz, 1H), 5.47– 5.45 (m, 1H), 5.33 – 5.30 (m, 1H), 4.23 – 4.20 (m, 3H), 4.01 – 3.98 (m, 1H), 2.23 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.29 – 169.18 (C=O, acyl), ^{13}C NMR (100 MHz, CDCl3) δ 97.74, 69.51, 67.20, 64.81, 61.14, 55.27, 20.58 – 20.36. HRMS (ESI) m/z: calc'd for $C_{12}H_{16}N_4O_{10}$: 376.0866: Found $C_{12}H_{16}N_4O_{10}Na$:399.0756

Compound 1 e

1d (11.5 g, 0.03 moles) was dissolved in AcOH (120ml) under inert atmosphere and heated to 100 ^oC. Then NaOAc (4.6g, 0.06 moles) was added and stirred for 1 h to obtain 1e. Once the reaction was completed, the reaction mixture was cooled and then the excess solvent was rota evaporated under high vaccum and the product was extracted with DCM and washed with Sat.$NaHCO_3$ and brine, dried over Na_2SO_4 to obtain **1e**. **1e** was then further proceeded for column chromatography and the product was eluted by 30% EtOAc in Hexane (α:β (1:0.5)) to obtain **1e** (8.5g) of

yield 72%.. ***α*- isomer** ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (d, J = 3.6 Hz, 1H), 5.49 (dd, J = 3.2, 1.2 Hz, 1H), 5.33 (dd, J = 11.2, 3.2 Hz, 1H), 4.33 – 4.28 (m, 1H), 4.12 – 4.06 (m, 2H), 3.95 (dd, J = 11.0, 3.6 Hz, 1H), 2.19 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.32 – 168.52 (C=O, acyl), 90.41, 68.74, 68.66, 66.85, 61.08, 56.83, 20.91 – 20.55 (3x CH3). ***β*- isomer** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56 (d, J = 8.6 Hz, 1H), 5.39 (dd, J = 3.2, 0.8 Hz, 1H), 4.91 (dd, J = 10.8, 3.4 Hz, 1H), 4.16 – 4.12 (m, 2H), 4.04 – 4.00 (m, 1H), 3.86 (dd, J = 10.8, 8.4 Hz, 1H), 2.22 (s, 3H), 2.18 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.32 – 168.52, 92.86, 71.72, 71.29, 66.16, 60.93, 59.65, 20.91 – 20.55. HRMS (ESI) m/z: calc'd for $C_{14}H_{19}N_3O_9$: 373.1121; Found $C_{14}H_{19}N_3O_9Na$: 396.1027

Compound 1f

The vacuum dried **1e** (8.5g, 22.77mmol) was dissolved in DCM (100 mL) along with thiotoluene under N_2 atmosphere and was cooled to 0 ^{o}C. Then $BF_3.OEt_2$ (8.43 mL, 68.31 mmol) was added dropwise using a syringe and then continued stirring for 12 h till the completion of the reaction. It was then quenched with Et_3N till the reaction mixture indicates neutral when checked with litmus paper. The organic layer was further washed with Sat.$NaHCO_3$, brine, dried over sodium sulphate. It was then evaporated and proceeded for silica gel column chromatography to obtain **1f** as α-β mixture (5.4g) with 75% yield. ^{1}H NMR (400 MHz,CHLOROFORM-D) δ 7.38 (d, *J* = 8.1 Hz, 2H), 7.12 (m, 2H), 5.59 (d, *J* = 5.5 Hz, 1H), 5.45 (dd, *J* = 3.2, 1.0 Hz, 1H), 5.15 (dd, *J* = 11.1, 3.3 Hz, 1H), 4.27 (dd, *J* = 11.1, 5.5 Hz, 1H), 4.06 (d, *J* = 6.5 Hz, 2H), 2.31 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H).^{13}C NMR (101 MHz, CHLOROFORM-*D*) δ 170.44, 170.06, 169.67, 138.98, 138.36, 134.03, 133.21, 130.76, 130.02, 129.82, 128.58, 127.25, 87.34, 70.19, 67.57, 67.45, 61.82, 58.18, 21.28, 21.23, 20.75, 20.70, 20.70, 20.64. HRMS (ESI) m/z: calc'd for $C_{19}H_{23}N_3O_7S$: 437.1257; Found $C_{19}H_{23}N_3O_7SNa$: 460.0879

Compound 1g

1f (5.4g, 17.18 mmol) was dissolved in MeOH:DCM in 2:1 ratio (50: 25 mL) and NaOMe (3.71g, 68.7 mmol)was added. The reaction mixture was then stirred for 1 h. The product precipitates out in the solvent on completion of the reaction. The reaction mixture was then quenched with acidic raisin and the excess solvent is then rota evaporated, dried over sodium sulphate and preceded for silica gel column

chromatography by flashing out using 5% DCM in MeOH yielding **1g** of 2.8g (84%). HRMS (ESI) m/z: calc'd for $C_{13}H_{17}N_3O_4S$: 311.0940; Found $C_{13}H_{17}N_3O_4SNa$: 334.0823

Compound 1 h

The vaccum dried **1g** (2.8g, 9 mmol) was dissolved in ACN and benzilidene dimethyl acetal (2.7 mL,18mmol) was added to it. It was then cooled to 0 ^oC and p-TSA (0.342 g, 1.8 mmol) was added. Once the reaction was completed, it was quenched with triethylamine till the litmus paper showed neutral and then the excess reagents were removed by extracting with DCM and then washing the DCM layer with sat.$NaHCO_3$ and brine. It is then rota evaporated and preceded for column chromatography using 60% EtOAc in hexane to obtain white coloured **1h** (4.6 g. 75%).^{1}H NMR (400 MHz, CHLOROFORM-D) δ 7.62 (d, *J* = 8.1 Hz, 2H), 7.44 – 7.33 (m, 5H), 7.11 (d, *J* = 7.9 Hz, 2H), 5.50 (s, 1H), 4.36 (dd, *J* = 11.0, 8.4 Hz, 2H), 4.14 (d, *J* = 2.4 Hz, 1H), 4.01 (d, *J* = 12.5 Hz, 1H), 3.61 (td, *J* = 9.6, 3.3 Hz, 1H), 3.50 – 3.44 (m, 2H), 2.54 (t, *J* = 8.8 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 138.84, 137.47, 134.89, 129.88, 129.59, 128.34, 126.67, 126.36, 101.49, 85.09, 74.51, 73.26, 69.88, 69.34, 62.14, 21.38. HRMS (ESI) m/z: calc'd for $C_{20}H_{21}N_3O_4S$: 399.1253; Found $C_{20}H_{21}N_3O_4SNa$:422.1102

Compound 1i

The vacuum dried **1h** (4.6 g,11.5mmol) was dissolved in AcOH (50 mL) and then cooled to 0 ^oC. Once it was cooled, Zn (12.8g, .120 mol) was added to it and stirred for 2 h. On completion of the reaction, the reaction mixture was filtered using cealite and then extracted with DCM ,washed with $NaHCO_3$ solution, brine to remove excess AcOH. It was then dried over Na_2SO_4, concentrated and proceeded for the next step without purification by column chromatography. The product obtained after the Zn reaction is dissolved in THF:H_2O in 2:1 ratio (50: 25 mL) and is cooled to 0 ^oC. Then $NaHCO_3$ and TrocCl (2.8 mL) dissolved in small amount of THF was added dropwise using a syringe. It was then stirred for 2 h to RT and once the reaction is completed, the reaction mixture was extracted with DCM. The Organic layer was then washed with water and brine to remove excess water soluble reagents and was rota evaporated, preceded for silica gel column chromatography. the product obtained contained both alpha and beta product which was eluted separately by 20%

EtOAc in pet ether and 40% EtOAc in hexane respectively to yield the product of 4.2 g (both alpha and beta combined with 66 % yield. **β isomer**: ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 7.53 (d, *J* = 8.1 Hz, 2H), 7.43 – 7.35 (m, 5H), 7.11 – 7.03 (m, 2H), 5.51 (s, 1H), 5.22 (d, *J* = 7.6 Hz, 1H), 4.86 – 4.78 (m, 1H), 4.73 (q, *J* = 12.2 Hz, 2H), 4.36 (dd, *J* = 12.4, 1.4 Hz, 1H), 4.17 (d, *J* = 3.1 Hz, 1H), 4.00 (dd, *J* = 12.5, 1.6 Hz, 1H), 3.94 (t, *J* = 7.6 Hz, 1H), 3.51 (s, 2H), 2.72 (d, *J* = 9.6 Hz, 1H), 2.33 (s, 3H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 138.57, 137.61, 134.26, 132.74, 129.80, 129.49, 128.33,127.38, 126.68, 101.45, 85.02, 75.12, 74.66, 71.42, 70.01, 69.37, 53.55, 29.53, 21.36. HRMS (ESI) m/z: calc'd for $C_{23}H_{24}Cl_3NO_6S$: 547.0390;Found $C_{23}H_{24}Cl_3NO_6SNa$: 570.0276

Compound 1j

The vacuum dried **1i** (both alpha and beta)(4.2g,7.65 mmol) was separately dissolved in DCM:Py(4:1) and then cooled to 0^{o}C. Once it was cooled, Chloroacetic anhydride (2.61 g, 15.3 mmol) was added to it and stirred for 1 h. Once the reaction was completed, the Py is removed by washing the DCM layer with 10% dil. HCl and then rota evaporated to get the crude product. It was then proceeded for column to obtain **1j** (4.4 g) of yield 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (t, *J* = 10.0 Hz, 2H), 7.46 – 7.37 (m, 6H), 7.12 (dd, *J* = 24.0, 7.7 Hz, 3H), 5.55 – 5.40 (m, 2H), 5.11 (dd, *J* = 24.6, 9.0 Hz, 2H), 4.81 – 4.72 (m, 2H), 4.43 – 4.36 (m, 2H), 4.05 (t, *J* = 4.9 Hz, 3H), 3.92 – 3.79 (m, 1H), 3.65 (s, 1H), 2.37 (d, *J* = 5.0 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 167.10, 153.61, 138.60, 137.51, 134.28, 129.78, 129.24, 128.18, 126.49, 100.94, 95.43, 84.57, 74.45, 73.02, 69.56, 69.16, 50.33, 40.71, 21.22. HRMS (ESI) m/z: calc'd for $C_{25}H_{25}NO_7S$: 622.0106. Found $C_{25}H_{25}NO_7SNa$: 646.1003

Compound 2a

The vaccum dried 1b (20 g, 51.2 mmol) was dissolved in DCM (200 mL) under N_2 atmosphere and was then cooled to 0 ^{o}C. Once it was cooled, thiotoluene (9.55 g,76.9 mmol) was added and then $BF_3.OEt_2$(118.98 mL, 0.153 mol) was added dropwise using a syringe. The reaction mixture was stirred for 12 h from 0 ^{o}C-RT till the completion of the reaction and was then quenched using Et_3N till neutral. Then the organic layer was washed with Sat.$NaHCO_3$ and brine to remove excess reagents and driend over sodium sulphate followed by rota evaporation and

preceded for column to obtain pure 1f (16 g) with 75% yield. HRMS (ESI) m/z: calc'd for $C_{21}H_{26}O_9S$:454.1298 ;Found $C_{13}H_{18}O_5S$: 477.1186

Compound 2b

The vacuum dried **2a** (16 g, 36.3 mmol) was dissolved in MeOH:DCM in 2:1 ratio and NaOMe (7.8 g, 0.145 mol) was added. The reaction mixture was then stirred for 1 h . The product precipitates out in the solvent on completion of the reaction. The reaction mixture was then quenched with acidic raisin and the excess solvent was then rota evaporated and preceded for silica gel column chromatography and flashed out using 8% DCM in MeOH to yield **1g** (9.5 g)of 94%. HRMS (ESI) m/z: calc'd for $C_{13}H_{18}O_5S$:286.0875; Found $C_{13}H_{18}O_5SNa$: 309.0693

Compound 2c

The vaccum dried **2b** (9.5 g, 34.8 mmol) was dissolved in ACN (100 mL) and benzilidene dimethyl acetal (10.5 mL, 69.7 mmol) was added to it. It was then cooled to 0 ^oC and p-TSA (2.65 g, 13.9 mmol) was added.Once the reaction was completed it was quenched with triethylamine till basic and then the excess reagents were removed by extracting with DCM and then washing the DCM layer with sat.$NaHCO_3$ and brine. It is then evaporated and preceded for silica gel column chromatography to obtain 2c (9.3 g) using 5% DCM in methanol and then it was vaccum dried. ^1H NMR (400 MHz, CHLOROFORM-D) δ 7.56 (d, *J* = 8.1 Hz, 2H), 7.41 – 7.30 (m, 5H), 7.09 (d, *J* = 8.0 Hz, 2H), 5.47 (s, 1H), 4.43 (d, *J* = 8.9 Hz, 1H), 4.35 (dd, *J* = 12.4, 1.4 Hz, 1H), 4.16 (d, *J* = 2.9 Hz, 1H), 3.99 (dd, *J* = 12.5, 1.7 Hz, 1H), 3.69 – 3.58 (m, 2H), 3.50 (s, 1H), 2.68 (s, 1H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 138.65, 137.77, 134.35, 129.81, 129.43, 128.29, 126.78, 126.68, 101.41, 87.10, 75.45, 73.78, 70.08, 69.37, 68.74, 21.35. HRMS (ESI) m/z: calc'd for $C_{20}H_{22}O_5S$:374.1188; Found $C_{20}H_{22}O_5SNa$: 397.1067

Compound 2d

2c (9.2g, 24.5 mmol) was dissolved in toluene under N_2 atmosphere and refluxed to 104 ^oC by attaching a dean stark apparatus on top of the RB. Once the compound was dissolved completely, Bu_2SnO is added and stirred for 4 h. After 4 h the solvent was evaporated and then vacuum dried. It was then dissolved in DMF (80 mL) and then heated and refluxed to 140 ^oC by attaching a condenser on top. Once the

compound was dissolved, NAPBr (5.5 g, 24.5 mmol) and CsF (4.10 g, 27 mmol) was added to the reaction mixture and stirred for 8 h. Once the reaction was completed, it was quenched with MeOH and then DMF is evaporated and then product was extracted with DCM, later washing the DCM layer with brine and dried over sodium sulphate. The combined organic layer was then concentrated and was preceded for column chromatography by using 40% EtOAc in hexane yielding 9.1 g of 2d (83%) which is a yellowish white powder. ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 7.85 – 7.70 (m, 4H), 7.62 – 7.53 (m, 2H), 7.53 – 7.29 (m, 8H), 7.06 (d, *J* = 7.9 Hz, 2H), 5.39 (s, 1H), 4.87 (s, 2H), 4.45 (d, *J* = 9.4 Hz, 1H), 4.31 (dd, *J* = 12.3, 1.6 Hz, 1H), 4.11 (dd, *J* = 3.2, 0.6 Hz, 1H), 3.96 – 3.85 (m, 2H), 3.53 (dd, *J* = 9.3, 3.3 Hz, 1H), 3.37 (d, *J* = 1.1 Hz, 1H), 2.33 (s, 3H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 138.50, 137.95, 135.61, 134.47, 133.25, 133.14, 129.80, 129.16, 128.39, 128.19, 127.98, 127.81, 126.82, 126.74, 126.63, 126.29, 126.13, 125.90, 101.26, 87.22, 80.24, 73.50, 71.96, 70.08, 69.46, 67.29, 21.34.HRMS (ESI) m/z: calc'd for $C_{31}H_{30}O_5S$:514.1814; Found $C_{31}H_{30}O_5SNa$: 537.1732

Compound 2e

2 d (4 g, 7 mmol) was dissolved in Py:DCM in 2:1 ratio under inert atmosphere. Then it was cooled to 0 ^{o}C and BzCl (2.71 mL, 23.4 mmol) was added dropwise using a syringe. Once the reaction was completed, the reaction mixture was quenched with MeOH. Then the excess solvents were evaporated followed by extraction using DCM. The DCM layer wass then washed by dil.HCl and brine and dried over Na_2SO_4. It was then rota evaporated under low vacuum and then preceded to column chromatography using 30% EtOAc in Hexane to obtain 3.2 g of pure **2e** (67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.13 (m, 2H), 7.86 – 7.76 (m, 4H), 7.66 – 7.59 (m, 3H), 7.51 (t, *J* = 5.5 Hz, 6H), 7.44 – 7.37 (m, 5H), 7.08 (d, *J* = 7.8 Hz, 2H), 5.42 (s, 1H), 4.49 (d, *J* = 9.4 Hz, 1H), 4.35 (dd, *J* = 12.3, 1.6 Hz, 1H), 4.16 (dd, *J* = 3.3, 0.8 Hz, 1H), 3.95 (dt, *J* = 18.8, 5.5 Hz, 2H), 3.58 (dd, *J* = 9.3, 3.3 Hz, 1H), 3.43 (d, *J* = 1.1 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-D) δ 165.05, 138.31, 137.71, 135.26, 134.53, 133.10, 130.33, 129.96, 129.57, 129.16, 128.45, 128.25, 128.19, 127.86, 127.74, 127.54, 126.79, 126.58, 126.16, 126.01, 125.79, 101.42, 85.54, 78.21, 77.43, 77.12, 76.80, 73.21, 71.17, 70.08, 69.40, 69.16, 29.79, 27.98, 21.34. HRMS (ESI) m/z: calc'd for $C_{38}H_{34}O_6S$:618.2076; Found $C_{38}H_{34}O_6SNa$: 641. 1986

Compound 3a

The vacuum dried 2d (4 g, 8.16 mmol) was dissolved in 40 mL DCM and was cooled to 0 ^{o}C. Once it was cooled, levulinic acid (1.41 mL,16.3 mmol) was added dropwise followed by the addition of DCC (4.2 g, 20 mmol) and DMAP (498 mg, 4 mmol) . It was then stirred for 2 h and once the reaction was completed, the reaction mixture was washed with $NaHCO_3$ and brine solution followed by drying over sodium sulphate. The combined DCM layer was then evaporated at low vacuum and then vacuum dried to yield **3a** of 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.75 (m, 4H), 7.50 (ddd, *J* = 8.5, 4.6, 1.7 Hz, 5H), 7.47 – 7.42 (m, 2H), 7.41 – 7.35 (m, 3H), 7.08 (d, *J* = 7.9 Hz, 2H), 5.44 (s, 1H), 5.32 (dd, *J* = 11.5, 7.9 Hz, 1H), 4.87 – 4.78 (m, 2H), 4.59 (d, *J* = 9.8 Hz, 1H), 4.35 (dd, *J* = 12.3, 1.5 Hz, 1H), 4.18 (t, *J* = 9.5 Hz, 1H), 3.98 (dd, *J* = 12.3, 1.6 Hz, 1H), 3.68 (dd, *J* = 9.6, 3.4 Hz, 1H), 3.42 (d, *J* = 0.8 Hz, 1H), 2.81 – 2.74 (m, 2H), 2.68 – 2.63 (m, 2H), 2.36 (d, *J* = 6.6 Hz, 3H), 2.19 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 206.47, 171.17, 138.21, 137.62, 135.56, 134.21, 133.14, 133.04, 129.49, 128.98, 127.77, 127.73, 127.57, 126.67, 126.47, 126.32, 126.08, 125.87, 101.28, 85.38, 78.50, 73.31, 71.53, 69.93, 69.23, 68.70, 37.98, 29.96, 28.21, 21.26. HRMS (ESI) m/z: calc'd for $C_{36}H_{36}O_7S$: 612.2182; Found $C_{36}H_{36}O_7SNa$:635.2066

Compound 4b

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 ^{0}C and stirred to r.t for 12 h. On completion of the reaction, excess solvents were evaporated under low vacuum and the product was extracted using DCM followed by washing the DCM layer with 10% HCl and the combined DCM layer was evaporated and dried under to afford **4b** as white powdered solid which was used for the next reaction without further purification. Yield: (80%, 3.2 g). HRMS (ESI) m/z: calc'd for $C_{12}H_{12}O_9$: 332.1107; Found $C_{12}H_{12}O_9Na$: 355.1012

Compound 4c

The vacuum dried crude compound **4b** (3.2 g, 9.6 mmol) was dissolved in DCM and stirred at 0 ^{0}C. Thiotoluene was then added to the reaction flask at 0 ^{0}C and

stirred for 20 min.BF3.Et2O was added to the reaction mixture by dropping funnel and stirred to r.t for 12 h under nitrogen atmosphere. The reaction mixture was then neutralized by adding Et3N and extracted by using saturated The combined organic layers were dried under Na2SO4, concentrated under reduced pressure and purified by silica gel column chromatography (40% EtOAc: 60% Hexane) to yield **4c** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (t, *J* = 10.0 Hz, 2H), 7.46 – 7.37 (m, 6H), 7.12 (dd, *J* = 24.0, 7.7 Hz, 3H), 5.55 – 5.40 (m, 2H), 5.11 (dd, *J* = 24.6, 9.0 Hz, 2H), 4.81 – 4.72 (m, 2H), 4.43 – 4.36 (m, 2H), 4.05 (t, *J* = 4.9 Hz, 3H), 3.92 – 3.79 (m, 1H), 3.65 (s, 1H), 2.37 (d, *J* = 5.0 Hz, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 167.10, 153.61, 138.60, 137.51, 134.28, 129.78, 129.24, 128.18, 126.49, 100.94, 95.43, 84.57, 74.45, 73.02, 69.56, 69.16, 50.33, 40.71, 21.22. HRMS (ESI) m/z: calc'd for $C_{19}H_{24}O_7S$: 396.1243; Found $C_{19}H_{24}O_7SNa$: 419.1132

Compound 4d

The vacuum dried compound **4c** (3.24 g, 8.2 mmol) was dissolved in an mixture of MeOH (20 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 acidic resin. Excess solvents were evaporated and resulting crude compound was purified by silica gel column chromatography (2% MeOH:DCM) to afford required compound **4d** as white solid of yield 90% (1.96 g). 1H 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). 13C NMR (101 MHz, CDCl3) δ 138.22, 132.96, 129.78, 128.71, 88.82, 75.11, 74.77, 71.69, 69.82, 21.17, 16.68. HRMS (ESI) m/z: calc'd for $C_{13}H_{18}O_4S$: 270.0926; Found $C_{13}H_{18}O_4SNa$: 293.0813

Compound 4e

The vacuum dried compound **4d**(1.96 g, 7.27 mmol) was dissolved in 12 mL DMF and then cooled to 0 ^{0}C. Once it was cooled, NaH (60 %, 3.6 eq) was added portionwise to the reaction flask at 0 ^{0}C and stirred under nitrogen atmosphere. BnBr was then added dropwise at 0 ^{0}C 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 high pressure. The resulting residue was extracted with brine and the organic layer was dried under anhydrous Na2SO4 and concentrated under reduced pressure. The obtained crude compound was purified by silica gel column chromatography (5% EtOAc: Hexane) to afford required compound **4e** as a white solid. Yield: (75%, 2.94 g). ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.52 (d, *J* = 8.1 Hz, 2H), 7.44 – 7.30 (m, 15H), 7.04 (d, *J* = 7.9 Hz, 2H), 5.03 (d, *J* = 11.6 Hz, 1H), 4.84 – 4.68 (m, 5H), 4.57 (d, *J* = 9.6 Hz, 1H), 3.91 (t, *J* = 9.4 Hz, 1H), 3.65 (d, *J* = 2.2 Hz, 1H), 3.61 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.53 (q, *J* = 6.3 Hz, 1H), 2.32 (s, 3H), 1.29 (d, *J* = 6.4 Hz, 3H);^{13}C NMR (101 MHz, $CDCl_3$) δ 138.79, 138.50, 138.41, 137.11, 132.20, 130.47, 129.52, 128.45, 128.36, 128.34, 128.16, 127.97, 127.70, 127.59, 127.46, 87.90, 84.60, 77.18, 76.64, 75.56, 74.58, 72.87, 21.14, 17.34. HRMS (ESI) m/z: calc'd for $C_{34}H_{36}O_4S$: 540.2334; Found $C_{34}H_{36}O_4SNa$: 563.2301

Compound 5b

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 ^{0}C) for 48 h. After completion of the reaction the reaction mixture was extracted with excess of EtOAc and brine .The combined organic layers were dried under Na2SO4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (40% EtOAC: Hexane) to afford the required linker compound **5b** as golden yellow liquid of yield 18g (85%). 1H 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).13C NMR (101 MHz, CHLOROFORM-D) δ 72.53, 70.19, 61.89, 50.83. HRMS (ESI) m/z: calc'd for $C_4H_9N_3O_2$: 131.0695; Found $C_4H_9N_3O_2Na$: 149.0243

Compound 6a

The vaccum dried compounds **1j** (3 g, 4.9 mmol) and **5b** (0.5 g,3.8 mmol) were dissolved in anhydrous DCM along with molecular sieves. It was then stirred for around 1 h and then cooled to -45 ^{o}C . Then NIS (1.4 g, 6 mmol) and TfOH (88 µL,0.9 mmol) was added to the reaction mixture and stirred for 10 mints. On addition of TfOH, the reaction mixture colour changed to brownish red. Then TLC is checked to confirm the completion of the reaction. Once the reaction was completed, it was then quenched with triethyl amine and then filtered using cealite. The filtered reaction

mixture was then washed with $Na_2S_2O_4$, dried over sodium sulphate and concentrated at low vacuum and preceded for silica gel column chromatography using 30% EtOAc in hexane to yield (1.9 g) of **6a.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.81 – 7.77 (m, 1H), 7.71 (s, 1H), 7.69 – 7.57 (m, 5H), 7.51 – 7.35 (m, 8H), 5.70 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.56 (s, 1H), 4.90 – 4.77 (m, 2H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.36 (dd, *J* = 12.3, 1.4 Hz, 1H), 4.29 (d, *J* = 2.9 Hz, 1H), 4.08 (dd, *J* = 12.3, 1.7 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.82 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.74 (ddd, *J* = 11.1, 7.4, 3.5 Hz, 1H), 3.65 – 3.54 (m, 2H), 3.46 (t, *J* = 5.0 Hz, 2H), 3.44 (s, 1H), 3.13 – 2.95 (m, 2H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 165.05, 138.31, 137.71, 135.26, 134.53, 133.10, 133.04, 130.33, 129.96, 129.57, 129.16, 128.45, 128.25, 128.19, 127.86, 127.74, 127.54, 126.79, 126.58, 126.16, 126.01, 125.79, 101.42, 85.54, 78.21, 73.21, 71.17, 70.08, 69.40, 69.16, 29.79, 27.98, 21.34. HRMS (ESI) m/z: calc'd for $C_{35}H_{35}N_3O_8$: 625.2424; Found $C_{35}H_{35}N_3O_8Na$: 648.2313

Compound 6b

The vacuum dried 6a (1.9 g, 3 mmol) was dissolved in DCM and H_2O in 19:1 ratio and was allowed to stir for 1h. It was then cooled to 0 ^oC and DDQ(2.8 g, 1.3 mmol) was added to it. Once the reaction was completed, it was quenched with Sat.$NaHCO_3$. The DCM layer was then washed with $NaHCO_3$ solution and brine. It was then dried over sodium sulphate and excess DCM was rota evaporated and preceded for column chromatography to yield (1g, 72%). ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 8.07 (dt, *J* = 8.6, 1.6 Hz, 2H), 7.62 – 7.49 (m, 3H), 7.48 – 7.41 (m, 2H), 7.41 – 7.34 (m, 3H), 5.57 (s, 1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.34 (dt, *J* = 10.8, 5.4 Hz, 1H), 4.28 – 4.22 (m, 1H), 4.13 – 4.06 (m, 1H), 4.00 (dt, *J* = 11.2, 3.7 Hz, 1H), 3.95 – 3.86 (m, 1H), 3.76 (ddd, *J* = 11.1, 7.3, 3.6 Hz, 1H), 3.66 – 3.52 (m, 3H), 3.52 – 3.42 (m, 2H), 3.06 (qdd, *J* = 13.1, 5.9, 4.1 Hz, 2H), 2.65 (d, *J* = 11.0 Hz, 1H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 166.28, 137.50, 133.27, 130.03, 129.92, 129.42, 128.38, 126.52, 101.59, 101.10, 75.72, 72.91, 71.73, 70.63, 70.15, 69.05, 66.69, 50.68. HRMS (ESI) m/z: calc'd for $C_{24}H_{27}N_3O_8$: 485.1798; Found $C_{24}H_{27}N_3O_8$: 508.1686

Compound 6c

The vacuum dried compounds **1j** (1.5, 2.4 mmol) and **6b** (0.69 g, 1.4 mmol) were dissolved in anhydrous DCM along with molecular sieves. It was then stirred for around 1 h and then cooled to -40 ^{o}C. Then NIS (2.18 g, 9 mmol) and TfOH (84 µL, 0.98 mmol) was added to the reaction mixture and stirred for 10 mints. On addition of TfOH, the reaction mixture colour changed to brownish red. Then TLC is checked to confirm the completion of the reaction. Once the reaction was completed, it was then quenched with triethylamine and then filtered using celite. The filtered reaction mixture was then washed with $Na_2S_2O_4$, dried over sodium sulphate and concentrated at low vacuum and preceded for silica gel column chromatography to yield (1.2 g, 48%) of **6c.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.1 Hz, 2H), 7.61 (d, *J* = 7.1 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 11.3 Hz, 3H), 7.42 – 7.36 (m, 4H), 7.32 (t, *J* = 8.6 Hz, 3H), 5.71 – 5.62 (m, 2H), 5.47 (d, *J* = 4.5 Hz, 1H), 5.31 (dd, *J* = 12.3, 2.2 Hz, 1H), 5.16 (dd, *J* = 24.3, 7.6 Hz, 2H), 4.81 (d, *J* = 2.3 Hz, 1H), 4.75 – 4.66 (m, 1H), 4.56 – 4.50 (m, 1H), 4.46 – 4.40 (m, 1H), 4.40 – 4.31 (m, 2H), 4.20 (ddd, *J* = 15.2, 11.8, 3.3 Hz, 2H), 4.11 (dd, *J* = 12.3, 1.4 Hz, 1H), 4.06 – 3.94 (m, 4H), 3.59 – 3.49 (m, 4H), 3.41 (dd, *J* = 9.6, 5.4 Hz, 2H), 3.13 – 2.91 (m, 2H), 2.70 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.84, 165.28, 133.33, 129.88, 129.47, 129.32, 128.56, 128.44, 128.33, 128.19, 126.53, 126.31, 126.28, 115.33, 101.64, 101.28, 100.76, 73.64, 72.51, 71.17, 70.54, 69.94, 68.96, 68.69, 68.49, 67.14, 66.68, 66.16, 52.46, 50.54, 40.92, 40.59, 29.70, 29.53. HRMS (ESI) m/z: calc'd for $C_{42}H_{44}Cl_4N_4O_{15}$: 984.1557. Found $C_{42}H_{44}Cl_4N_4O_{15}K$: 1023.1923

Compound 6d

The vacuum dried 6c (1.2 g, 1.3 mmol) was dissolved in pyridine:methanol in equi-volume ratio and was then cooled and stirred to 0 ^{o}C. Then thiourea (0.214 g, 2.9 mmol) was added to the reaction mixture and stirred for around 1 h. Once the reaction was completed, the excess pyridine was removed by extracting it with DCM and washing the DCM layer with 10% HCl. DCM layer was then combined, dried over sodium sulphate and evaporated to afford 6d (90 %, 0.9 g) using silica gel column chromatography (80% EtOAc:Hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.08 (m, 2H), 7.68 – 7.57 (m, 3H), 7.49 (dd, *J* = 10.6, 4.7 Hz, 2H), 7.47 – 7.40 (m, 5H), 7.38 – 7.30 (m, 3H), 5.77 – 5.70 (m, 2H), 5.48 (s, 1H), 5.26 (d, *J* = 4.8 Hz, 1H), 4.86 (d, *J* = 7.6 Hz, 1H), 4.75 (d, *J* = 7.7 Hz, 1H), 4.67 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J*

= 3.2 Hz, 1H), 4.38 (dd, *J* = 12.4, 1.4 Hz, 1H), 4.26 (dd, *J* = 10.6, 3.4 Hz, 1H), 4.22 – 4.12 (m, 2H), 4.09 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.07 – 3.92 (m, 3H), 3.78 (ddd, *J* = 11.2, 7.5, 3.4 Hz, 1H), 3.65 – 3.53 (m, 4H), 3.48 – 3.38 (m, 3H), 3.04 (dddd, *J* = 19.4, 13.1, 5.9, 4.2 Hz, 2H), 2.78 (d, *J* = 9.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.50, 154.81, 137.40, 137.37, 133.39, 129.91, 129.86, 129.48, 129.26, 128.60, 128.48, 128.23, 126.56, 126.40, 101.77, 101.33, 98.95, 95.54, 77.36, 77.04, 76.73, 74.79, 74.32, 70.91, 70.62, 69.97, 69.33, 69.00, 68.93, 68.85, 66.70, 66.58, 55.66, 50.57, 29.71, 0.02. HRMS (ESI) m/z: calc'd for $C_{42}H_{43}Cl_3N_4O_{21}$: 908.1841. Found $C_{42}H_{43}Cl_3N_4O_{21}Na$: 933.1777

Compound 6e

The vacuum dried compounds **3a** (0.162g, 0.3 mmol)) and **6d** (160 mg, 0.2 mmol) were dissolved in anhydrous DCM along with molecular sieves. It was then stirred for around 1 h and then cooled to -40 ^{o}C . Then NIS (128 mg) and TfOH (8 µL) was added to the reaction mixture and stirred for 10 mints. On addition of TfOH, the reaction mixture colour changed to brownish red. Then TLC is checked to confirm the completion of the reaction. Once the reaction was completed, it was then quenched with triethyl amine and then filtered using celite. The filtered reaction mixture was then washed with $Na_2S_2O_4$, dried over sodium sulphate and concentrated at low vacuum and preceded for silica gel column chromatography (6% Acetone:DCM) to yield (48%) of 120 mg. HRMS (ESI) m/z: calc'd for $C_{69}H_{71}Cl_3N_4O_{21}$: 1396.3676. Found $C_{69}H_{71}Cl_3N_4O_{21}Na$: 1417.3990

Compound 7b

Triethylene glycol (10g, 67 mmol) was dissolved in THF (60 mL) and was cooled to 0 ^{o}C. After 10 mints of cooling, NaH (1.6 g, 67 mmol) was added to it portionwise. In Parallel, 11- Bromo undec-1-ene (7.81 g, 33.5 mmol) was dissolved in 20 mL of THFand was added to the reaction mixture at 0 ^{o}C and was stirred to overnight. After the completion of the reaction, the reaction mixture was quenched with water and the THF was evaporated and the product was extracted using dichloromethane, washed with water, brine, dried over sodium sulphate, evaporated and silica gel column chromatography was done using 80% EtOAc in Hexane to obtain product 7a of yield (4.3g, 60%)^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.82 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.02 – 4.92 (m, 2H), 3.74 – 3.58 (m, 13H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.09-2.02

(m, 2H), 1.59 (p, *J* = 6.8, 6.4 Hz, 2H), 1.39 – 1.28 (m, 13H)^{13}C NMR (101 MHz, $CDCl_3$) δ 139.21, 114.09, 72.55, 71.58, 70.61, 70.35, 70.01, 61.74, 33.80, 29.57, 29.52, 29.45, 29.42, 29.11, 28.92, 26.05. HRMS (ESI) m/z: calc'd for $C_{16}H_{32}O_4$: 288.2301. Found $C_{16}H_{32}O_4Na$: 311.2312

Compound 7c

The vacuum dried **7b** (4g, 13.22 mmol) was dissolved in THF(30 mL) and then ^{t}BuOK (100 µL) was added to it. Then butyl acrylate (1.94 mL,13.22 mmol) dissolved in small amount of THF was added to the reaction mixture dropwise and stirred overnight. Once the reaction was completed, the THF was evaporated and loaded to column and to obtain pure 7b of yield 65% in 80% EtOAc in Hexane (2.5 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.82 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.03 – 4.92 (m, 2H), 3.72 (t, *J* = 6.6 Hz, 2H), 3.67 – 3.57 (m, 12H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.51 (t, *J*= 6.6 Hz, 2H), 2.08 – 2.02 (m, 2H), 1.58 (p, *J* = 6.9, 6.2 Hz, 2H), 1.46 (s, 9H), 1.40 – 1.29 (m, 13H).^{13}C NMR (101 MHz, CHLOROFORM-*D*) δ 171.00, 139.31, 114.18, 80.58, 71.62, 70.69, 70.58, 70.45, 70.13, 66.97, 36.33, 33.89, 29.70, 29.62, 29.55, 29.51, 29.20, 29.00, 28.17, 26.16. HRMS (ESI) m/z: calc'd for $C_{23}H_{44}O_6$: 416.3138; Found $C_{23}H_{44}O_6Na$: 439.3130

Compound 7d

7d (2.5 g, 5.8 mmol) was dissolved in 1,4 dioxane (60 mL) and then AIBN (1.9 g, 11.6 mmol) was added to it. After that thioacetic acid (9.8 mL, 139.33 mmol) was added to it and stirred overnight at 70 ^{o}C. Once the reaction was completed, the reaction mixture was quenched with $KMnO_4$ solution, purified by silica gel column chromatography to obtain 7d of yield 91% (3 g)(15% EtOAc in Hexane) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.75 (t, *J* = 5.2 Hz, 2H), 3.63-3.58 (m, 12H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.85– 2.81 (m, 2H), 2.60 (t, *J* = 5.5 Hz, 2H), 2.30 (s, 3H), 1.57-1.49 (m, 4H), 1.33-1.23 (m, 13H).^{13}C NMR (101 MHz, CHLOROFORM-*D*) δ 196.32, 71.66, 70.67, 70.57, 70.51, 70.47, 70.28, 70.04, 66.61, 35.12, 30.74, 29.62, 29.57, 29.52, 29.24, 29.19, 28.89, 26.08. HRMS (ESI) m/z: calc'd for $C_{25}H_{48}O_7S$: 492.3121; Found $C_{25}H_{48}O_7SNa$: 515.3110

Compound 7e

The vacuum dried **7d** (3g, 5.9 mmol) was dissolved in 50% TFA in DCM (30 mL) and stirred for 3 h. Once the reaction was completed, the excess solvents were rota evaporated and thenpreceded for column chromatography. The product was eluted using10% MeOH in DCM yielding **7e** of 80 % (2 g) ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 3.75 (t, *J* = 5.2 Hz, 2H), 3.62 (d, *J* = 4.7 Hz, 10H), 3.58 (s, 3H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.87 – 2.79 (m, 2H), 2.60 (t, *J* = 5.5 Hz, 2H), 2.30 (s, 3H), 1.53 (dt, *J* = 15.5, 7.3 Hz, 4H), 1.26 (d, *J* = 26.4 Hz, 13H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 196.32, 71.66, 70.67, 70.57, 70.51, 70.47, 70.28, 70.04, 66.61, 35.12, 30.74, 29.62, 29.57, 29.52, 29.24, 29.19, 28.89, 26.08. HRMS (ESI) m/z: calc'd for $C_{21}H_{40}O_7S$: 436.2495; Found $C_{21}H_{40}O_7SNa$: 459.2498

Compound 7f

7e (2g, 7.57 mmol) was dissolved in MeOH(20 mL) and then NaOMe (800 mg, 1.13 mmol) was added to it and stirred for 2 h. On completion of the reaction, it was quenched using acidic raisins and then preceded to column chromatography using 10% MeOH in DCM yielding 85% of **7f** (1.6 g) ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (q, *J* = 6.1 Hz, 2H), 3.71 – 3.57 (m, 12H), 3.47 (dd, *J* = 14.5, 7.1 Hz, 2H), 2.73 – 2.58 (m, 3H), 1.79 – 1.50 (m, 4H), 1.45 – 1.21 (m, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.57, 70.58, 70.47, 70.40, 70.22, 70.02, 69.96, 66.57, 39.32, 39.18, 35.04, 34.85, 29.60, 29.55, 29.50, 29.22, 29.18, 28.51, 26.08, 26.01. HRMS (ESI) m/z: calc'd for $C_{19}H_{38}O_6S$: 394.2389; Found $C_{19}H_{38}O_6SNa$: 417.2367

Compound 7g

Firstly NHS (47.8 mg, 0.416 mmol) was dissolved in 25 mL of DCM and stirred. In another RB, **7f** (170 mg, 0.41 mmol) was dissolved in 5 mL of DCM and DCC (94 mg, 0.453 mmol) was added to it. Once the solution becomes clear, it was added dropwise to the NHS solution and stirred overnight. Once the reaction was completed, the excess solvents were rota evaporated and cealite filteration was done to remove urea which is a biproduct. Then it was preceded for silica gel column chromatography and the product was eluted using 50% DCM and EtOAc yielding of **7g** ^{1}H NMR (400 MHz, CHLOROFORM-D) δ 3.83 (t, *J* = 6.5 Hz, 2H), 3.66 – 3.60 (m, 10H), 3.58 – 3.54 (m, 2H), 3.43 (t, *J* = 6.8 Hz,2H), 2.89 (t, *J* = 6.5 Hz, 2H), 2.81 (d, *J* = 6.3 Hz, 4H), 2.70 – 2.63 (m, 2H), 1.65 (dt, *J* = 14.7, 7.4 Hz, 4H), 1.54 (dd, *J* = 14.0,

6.9 Hz, 2H), 1.37 – 1.24 (m, 13H).^{13}C NMR (101 MHz, CHLOROFORM-D) δ 169.03, 169.02, 166.81, 76.78, 71.63, 70.79, 70.72, 70.68, 70.66, 70.58, 70.56, 70.12, 65.80, 39.24, 32.22, 29.78, 29.71, 29.66, 29.63, 29.59, 29.58, 29.33, 29.30, 28.62, 26.17, 25.68, 25.67, 25.66, 25.65, 25.64, 25.53, 20.46, 0.08. HRMS (ESI) m/z: calc'd for 505.3002. HRMS (ESI) m/z: calc'd for $C_{23}H_{41}O_6S$: 475.2604; Found $C_{23}H_{41}O_6S$: 498.2598

Synthesis of different shapes of gold nanoparticles:

- **Sphere**

  To synthesize spherical AuNPs, clean RBs with magnetic bead and 30 mL of milli-Q water was taken. Then 300 µL of 25 mM $HAuCl_4$ solution was added. The mixture was then heated to 100 ^oC and then 600 µL of 25 mM Sodium citrate solution was added to the boiling solution at a shot. When the colour changed from golden yellow to wine red, it was cooled followed by centrifugation to obtain the spherical AuNPs

- **Rods**

Growth solution	**Seed solution**
CTAB-0.2 mM (5 mL)	CTAB-0.2 mM (5 mL)
$AgNO_3$-0.004 M (0.2 mL)	$HAuCl_4$-0.0005 M (5 mL)
$HAuCl_4$-0.001 M (5 mL)	ice cold $NaBH_4$-0.01 M (0.6 mL)
L-Ascorbic acid- 0.0788 M (0.07 mL)	

  The solutions required for both the growth and seed solutions were prepared as mentioned in the above concentrations and then 20 µL of seed solution was pipetted to the growth solution to obtain rod AuNPs

- **Star**

  HEPES buffer of pH adjusted with 1 M NaOH solution is taken and then 40 mM $HAuCl_4$ of 5 µL was added to the HEPES solution prepared.

SEM images

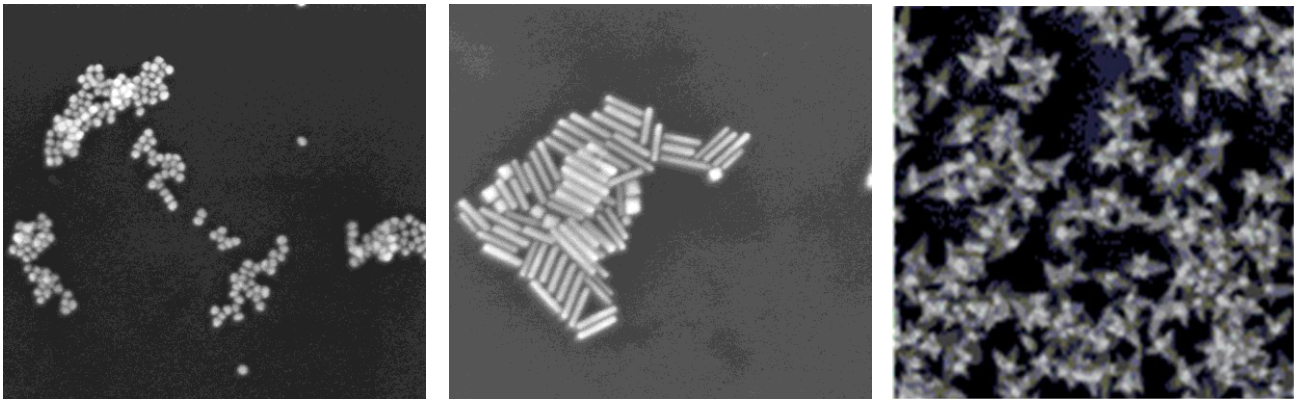


Figure 5. SEM image of Sphere, rod, star AuNPs

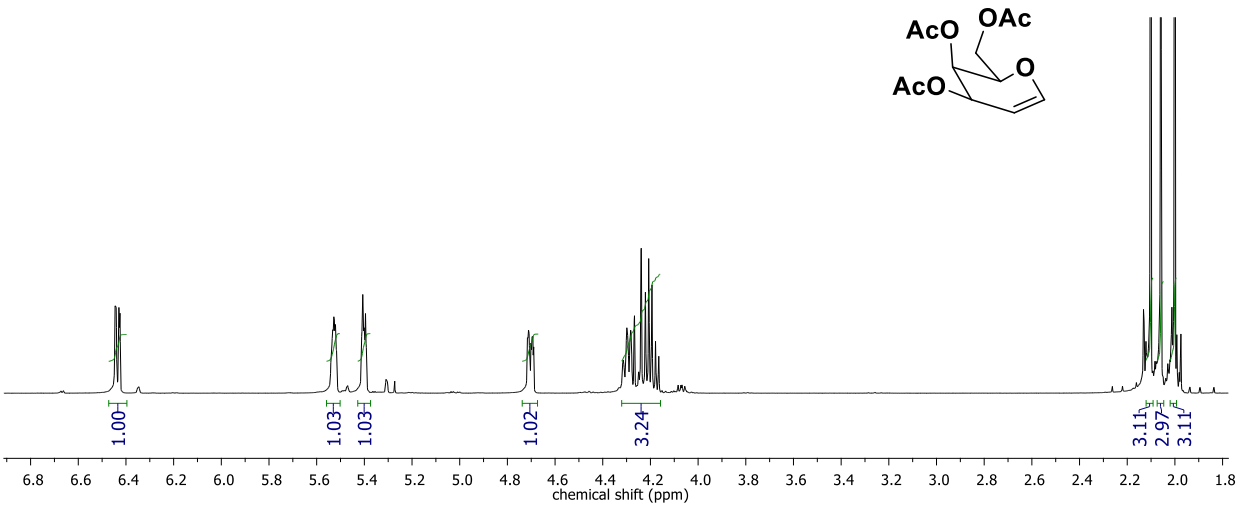
CONCLUSION AND FUTURE PLANS

We have successfully synthesized protected form of Globo-H trisaccharide. All the intermediates and final compounds were well characterized by NMR, high resolution mass-spectra. Simultaneously, we have synthesized different shapes of gold nanoparticles by different methods. SEM images of these nanoparticles have clearly showed the formation of different shapes. Currently, the final deprotection, glyco-nano conjugation and immune responses in mice is under progress. We believe that this research will result in next generation platform for vaccine again cancer.

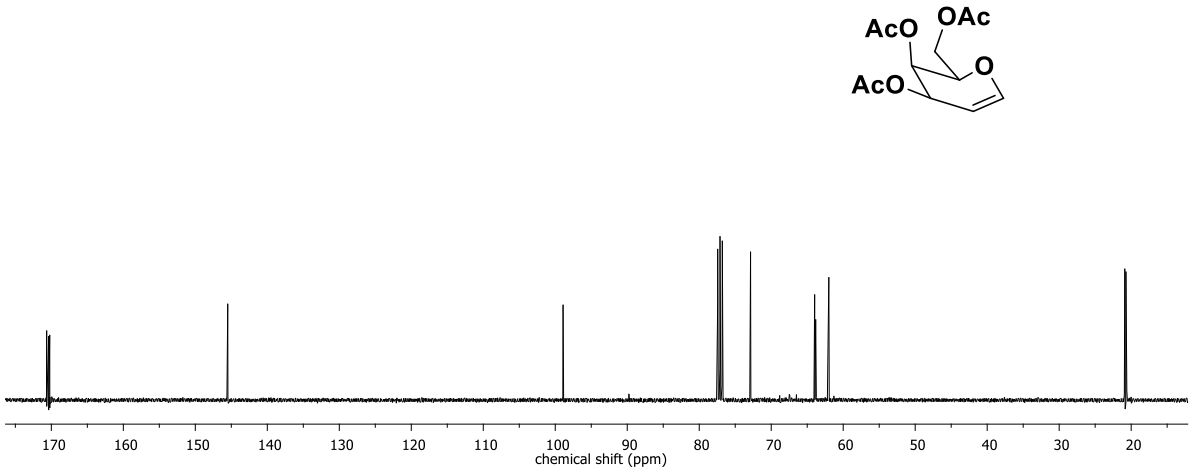
SPECTRAS

Compound 1c

^{1}H NMR

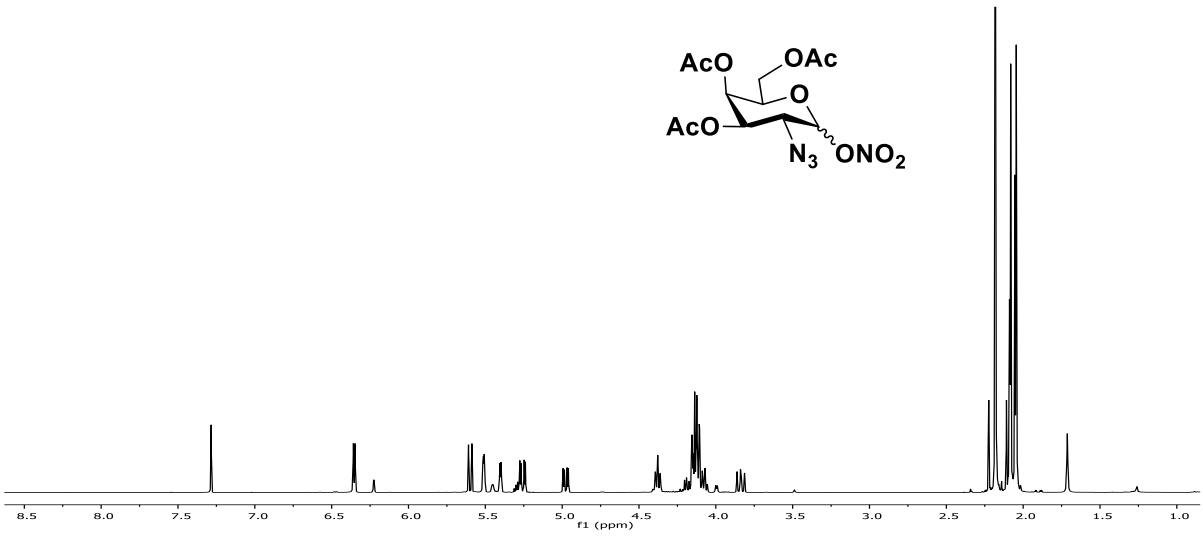


^{13}C NMR

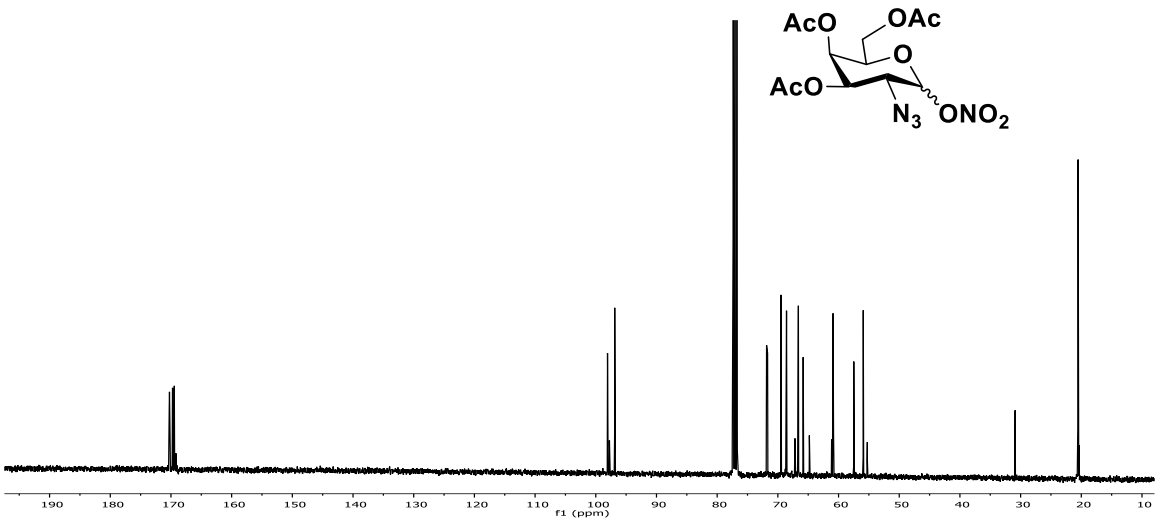


Compound 1d

^{1}H NMR

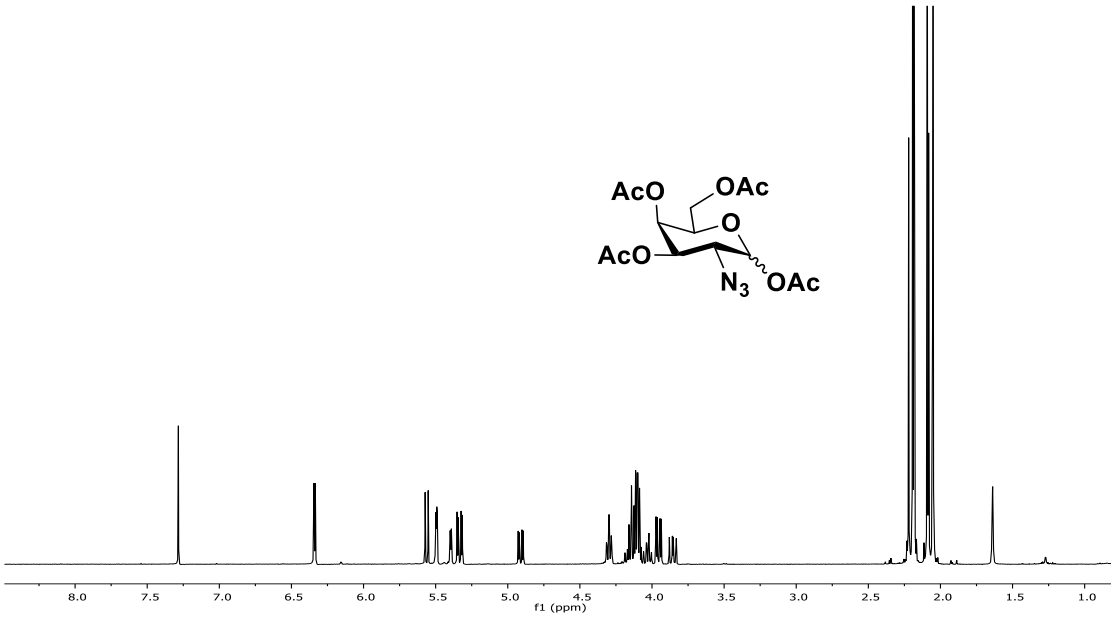


^{13}C NMR

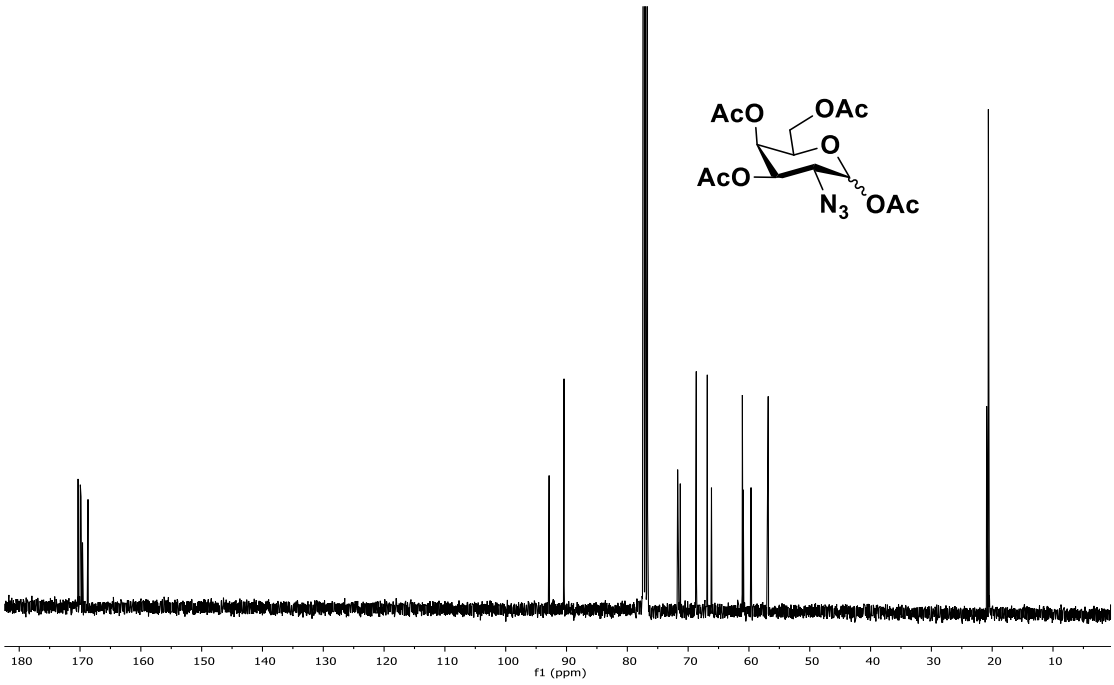


Compound 1e

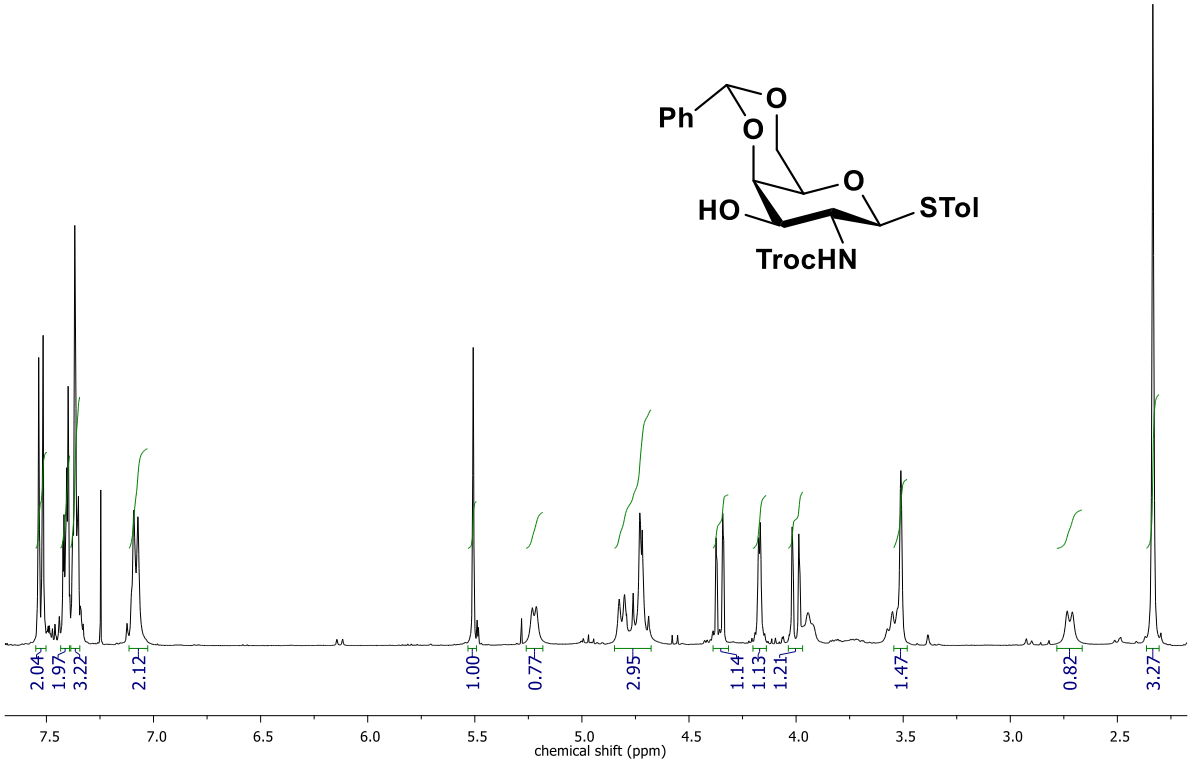
^{1}H NMR

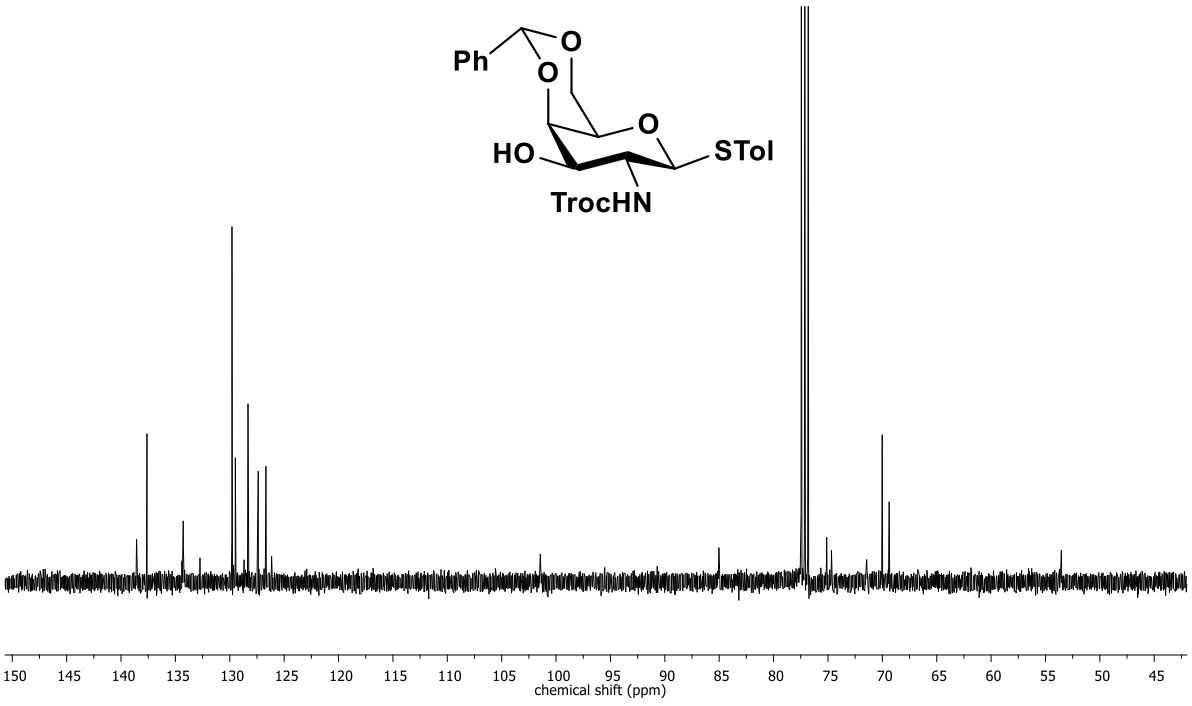


^{13}C NMR

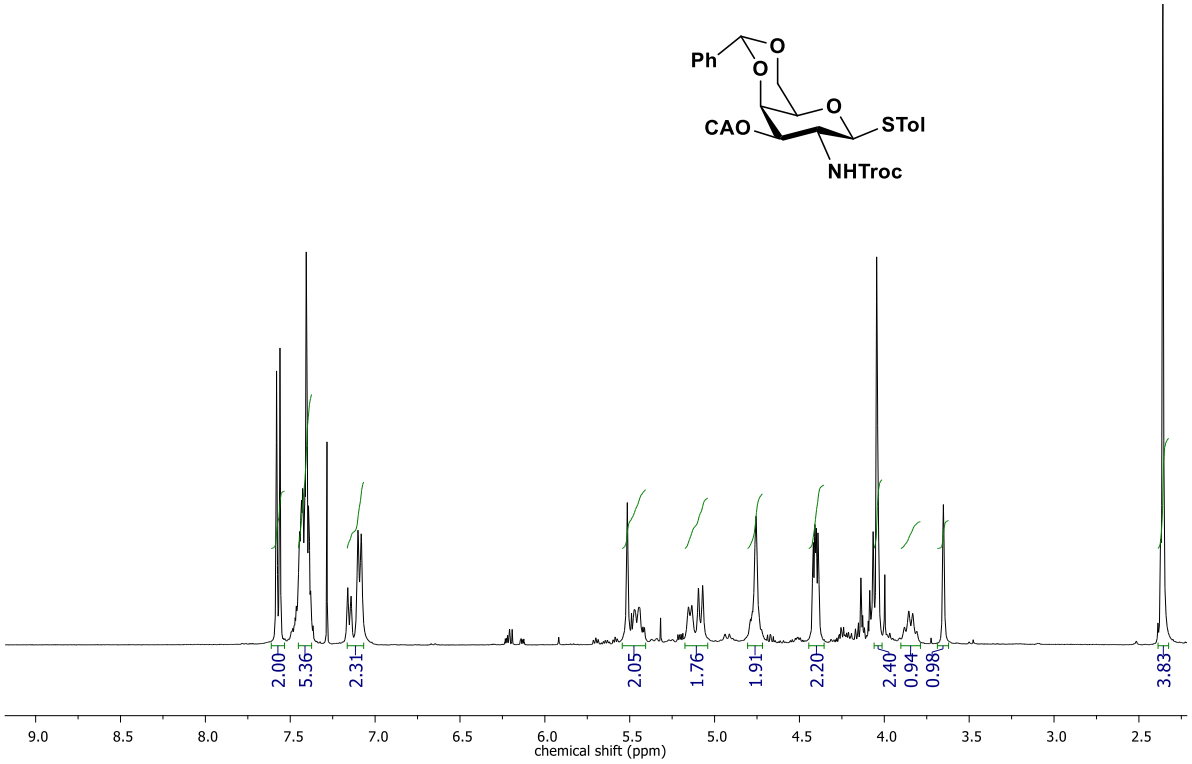


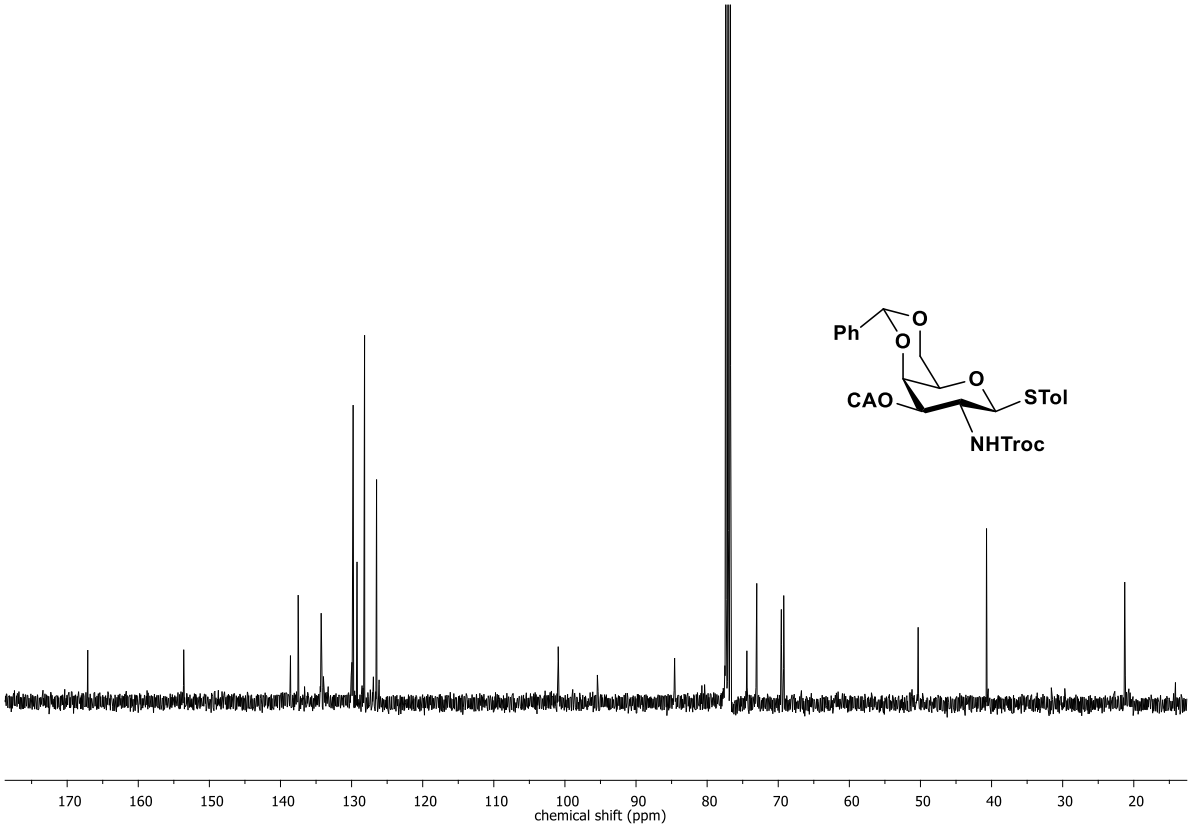
Compound 1i


Ph
O
O
O
HO
STol
TrocHN
2.04
1.97
3.22
2.12
1.00
0.77
2.95
1.14
1.13
1.21
1.47
0.82
3.27
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
chemical shift (ppm)


Ph
O
O
O
HO
STol
TrocHN
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
chemical shift (ppm)

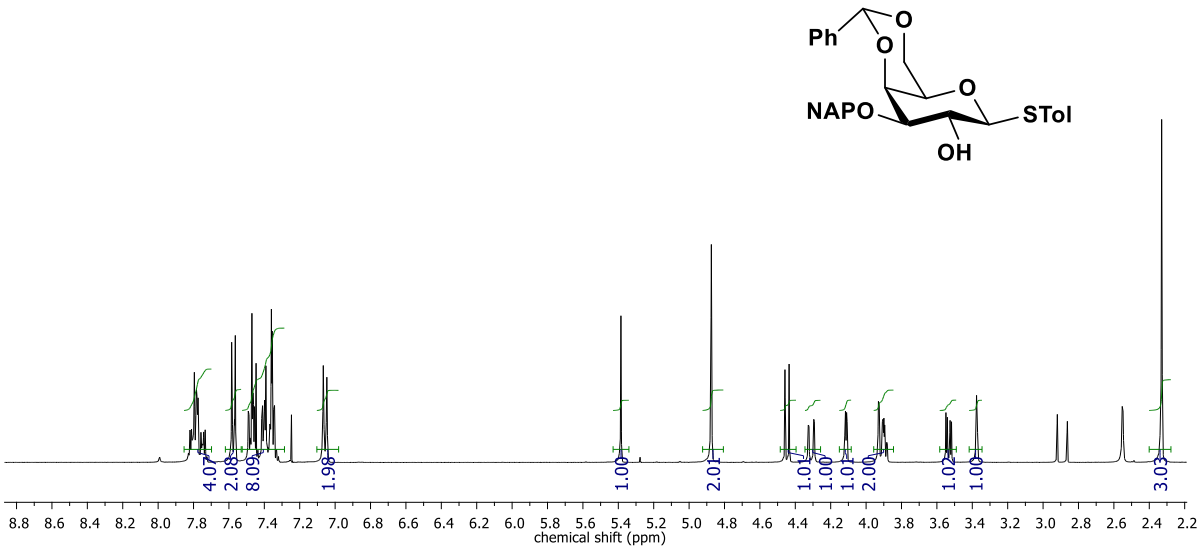
Compound 1j


Ph
O
O
O
CAO
STol
NHTroc
2.00
5.36
2.31
2.05
1.76
1.91
2.20
2.40
0.94
0.98
3.83
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
chemical shift (ppm)

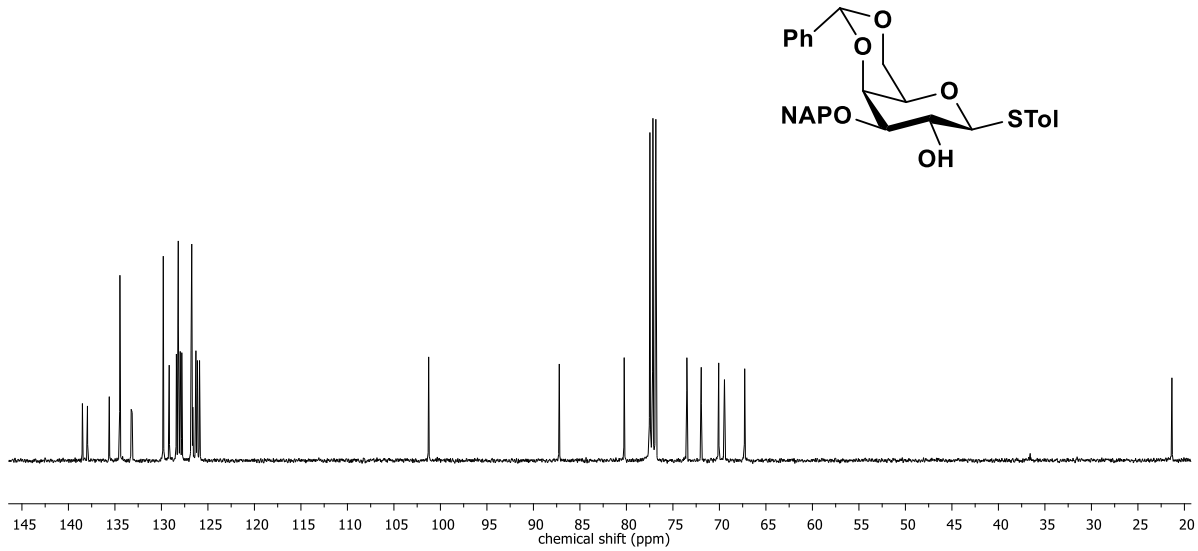

Ph
O
O
O
CAO
STol
NHTroc
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
chemical shift (ppm)

Compound 2d

^{1}H NMR

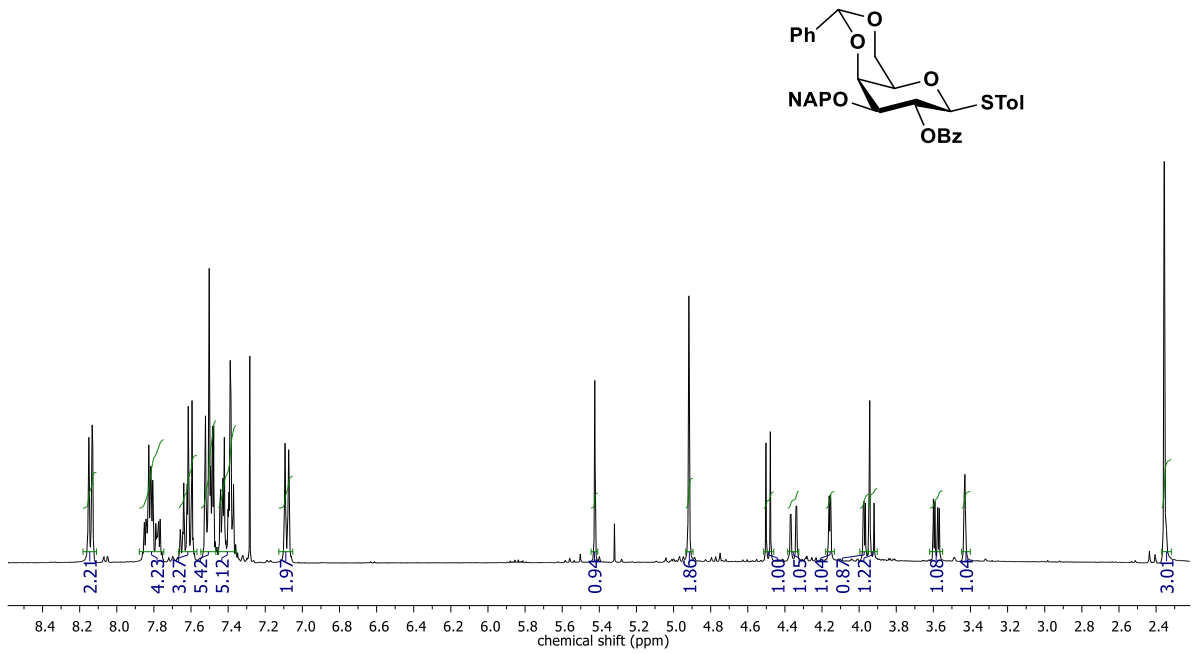


^{13}C NMR

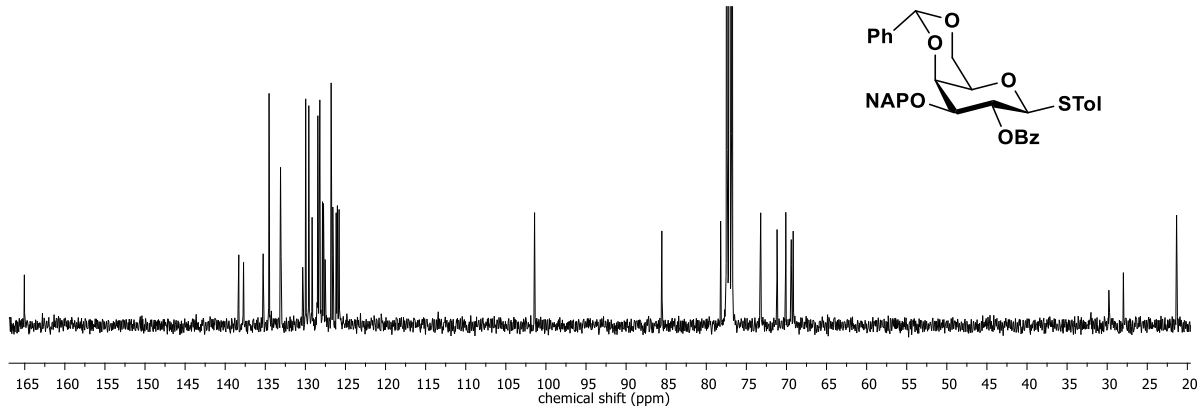


Compound 2e

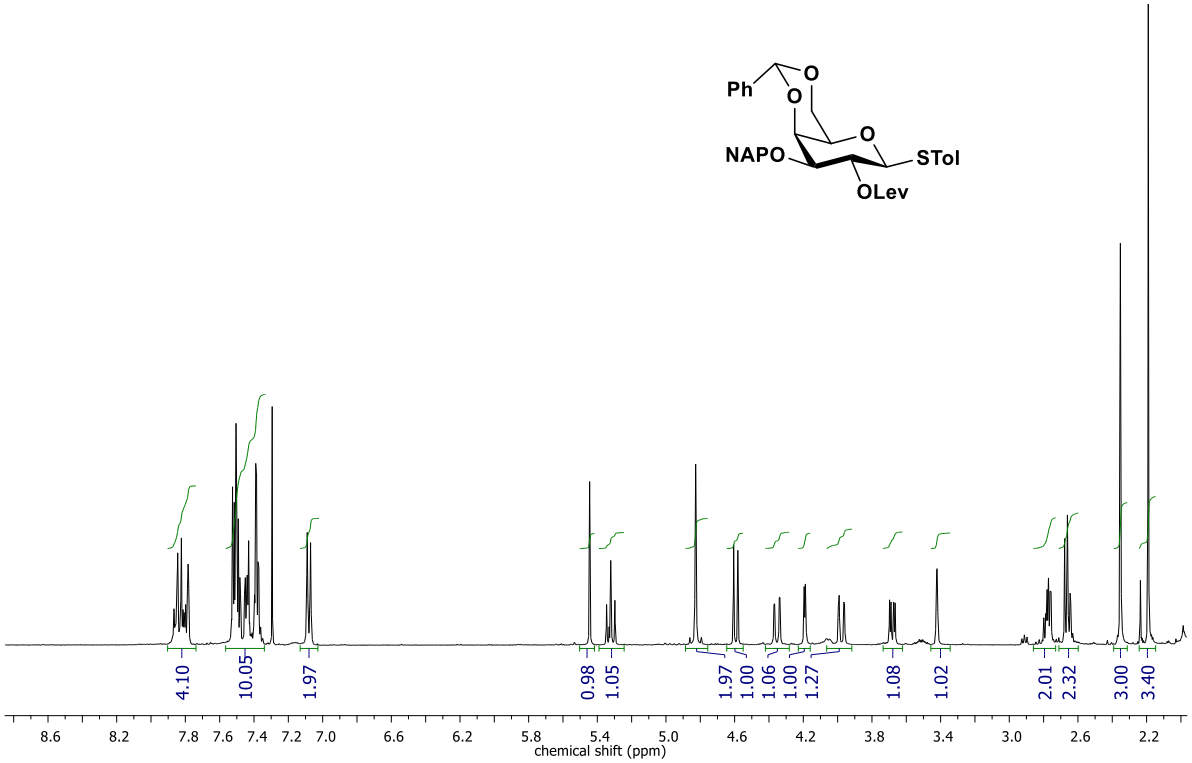
^{1}H NMR

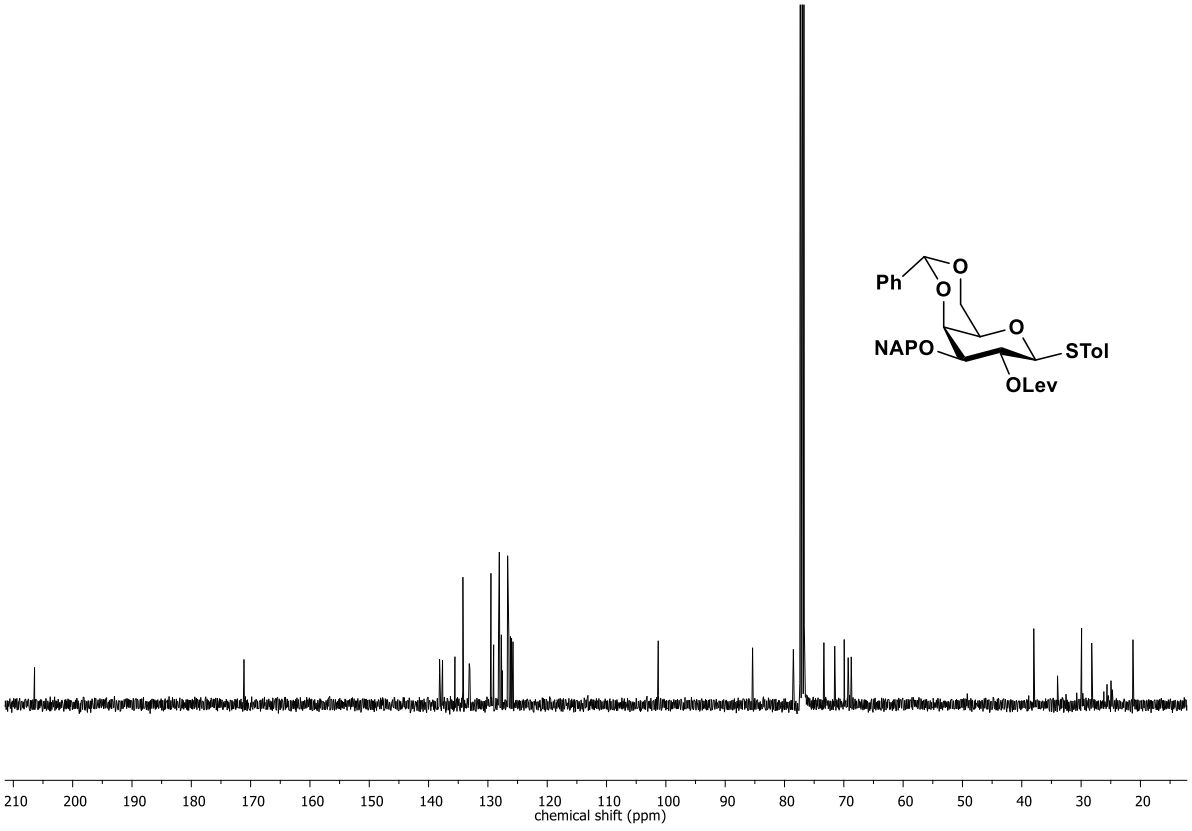


^{13}C NMR

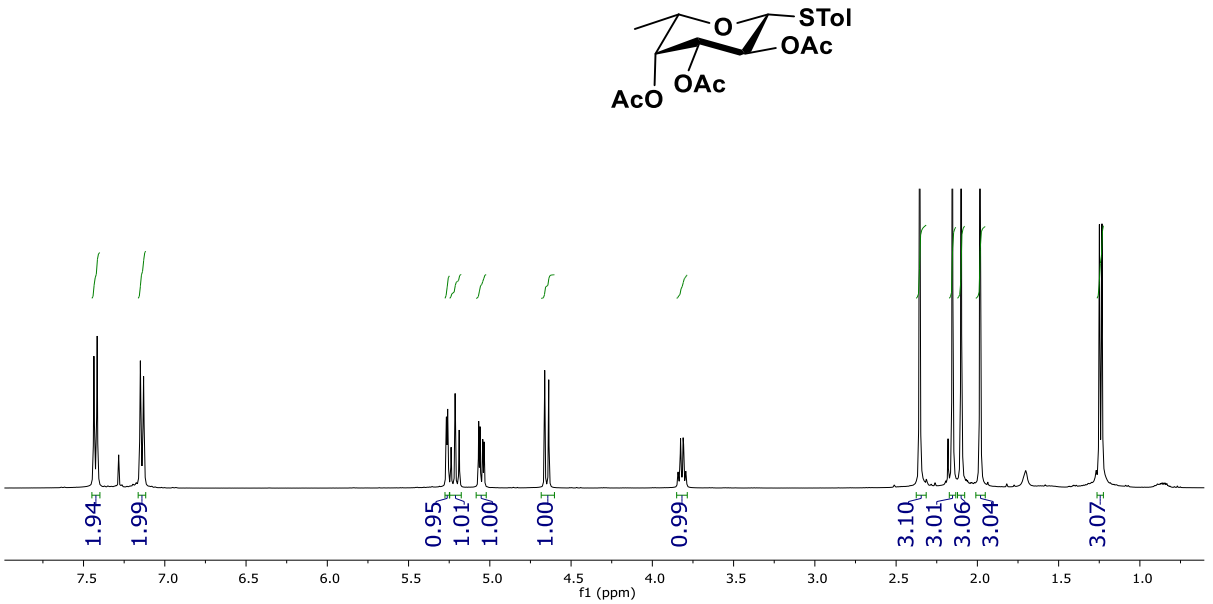


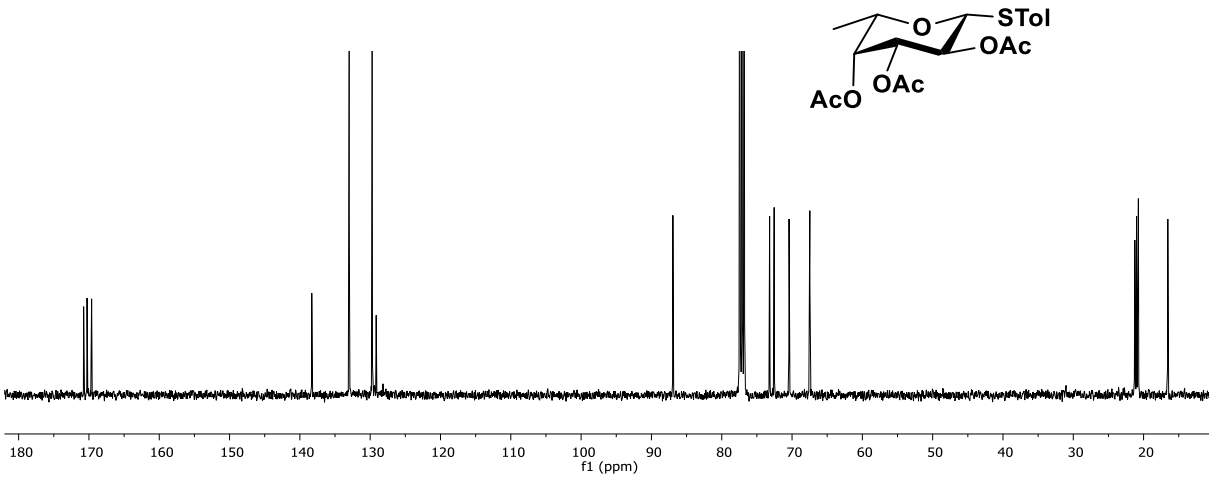
Compound 3a


Ph
O
O
O
NAPO
STol
OLev
4.10
10.05
1.97
0.98
1.05
1.97
1.00
1.06
1.00
1.27
1.08
1.02
2.01
2.32
3.00
3.40
8.6
8.2
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
chemical shift (ppm)

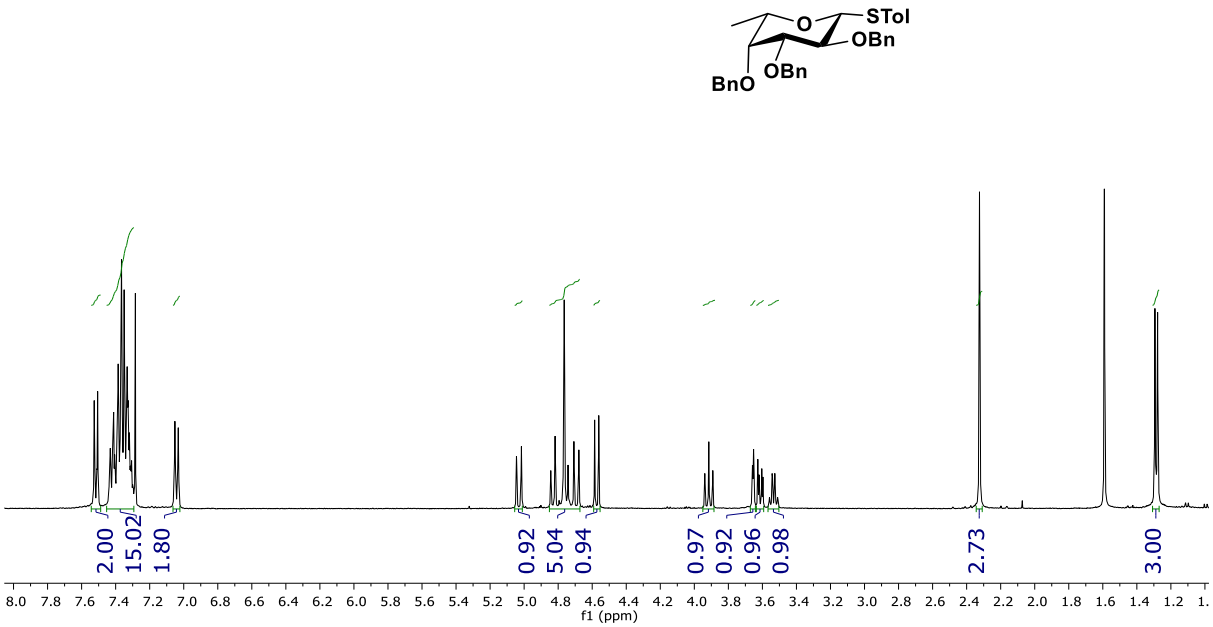

Ph
O
O
O
NAPO
STol
OLev
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
chemical shift (ppm)

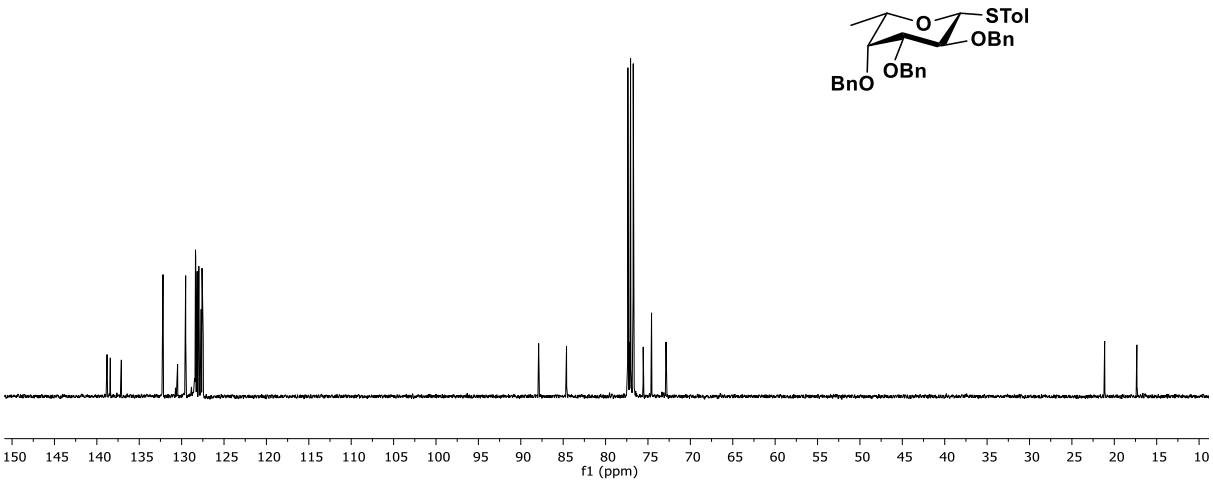
Compound 4c


O
STol
OAc
AcO
OAc
1.94
1.99
0.95
1.01
1.00
1.00
0.99
3.10
3.01
3.06
3.04
3.07
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

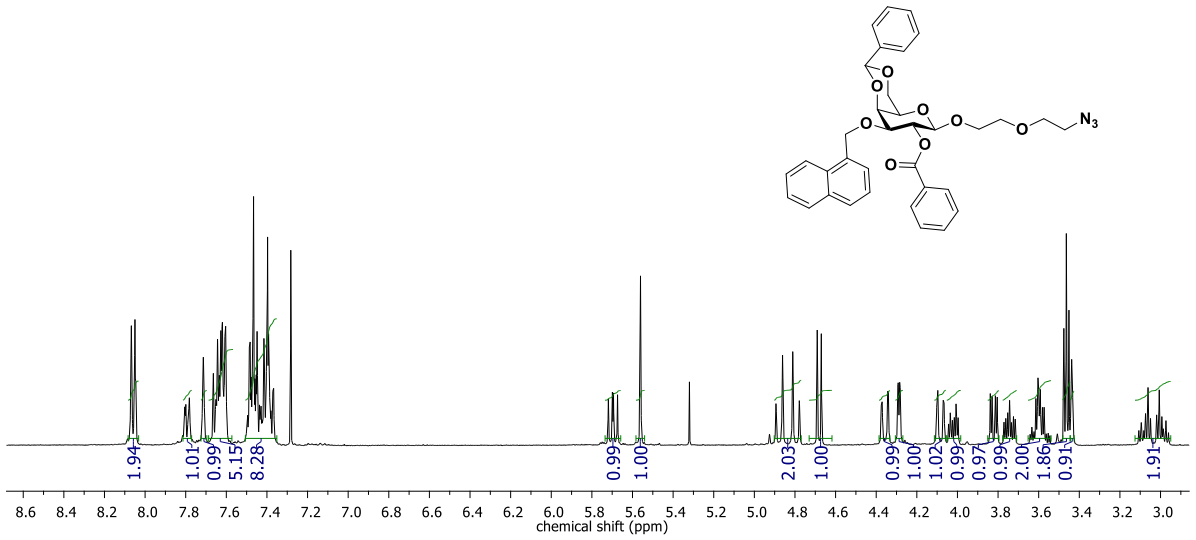

O
STol
OAc
AcO
OAc
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

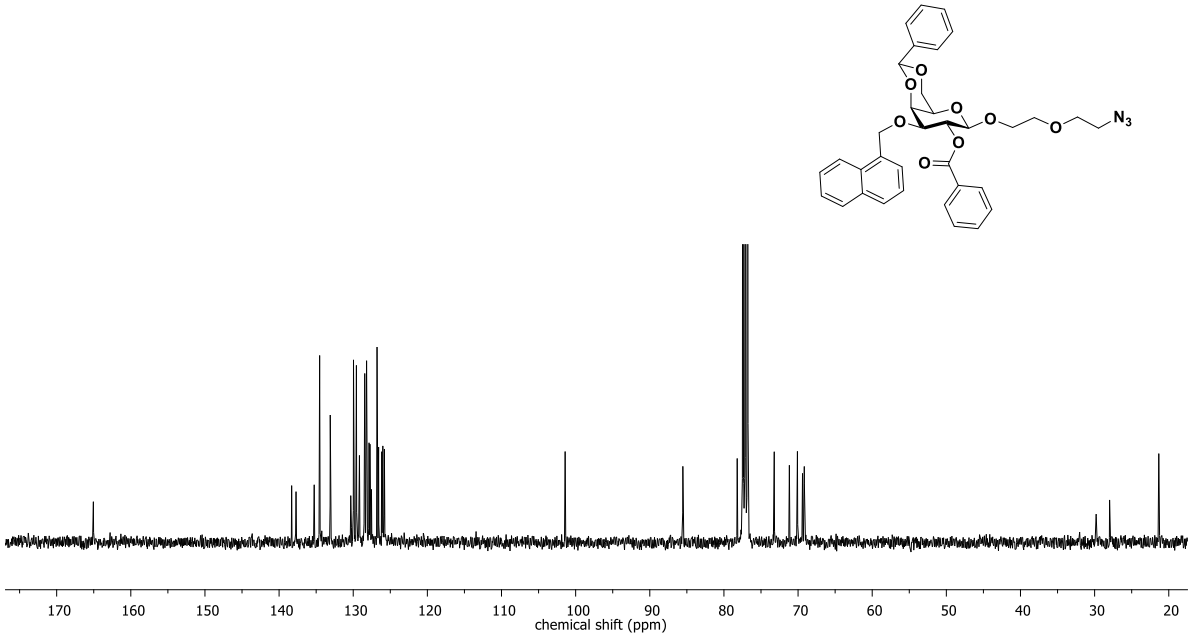
Compound 4e


O
STol
OBn
BnO
OBn
2.00
15.02
1.80
0.92
5.04
0.94
0.97
0.92
0.96
0.98
2.73
3.00
f1 (ppm)

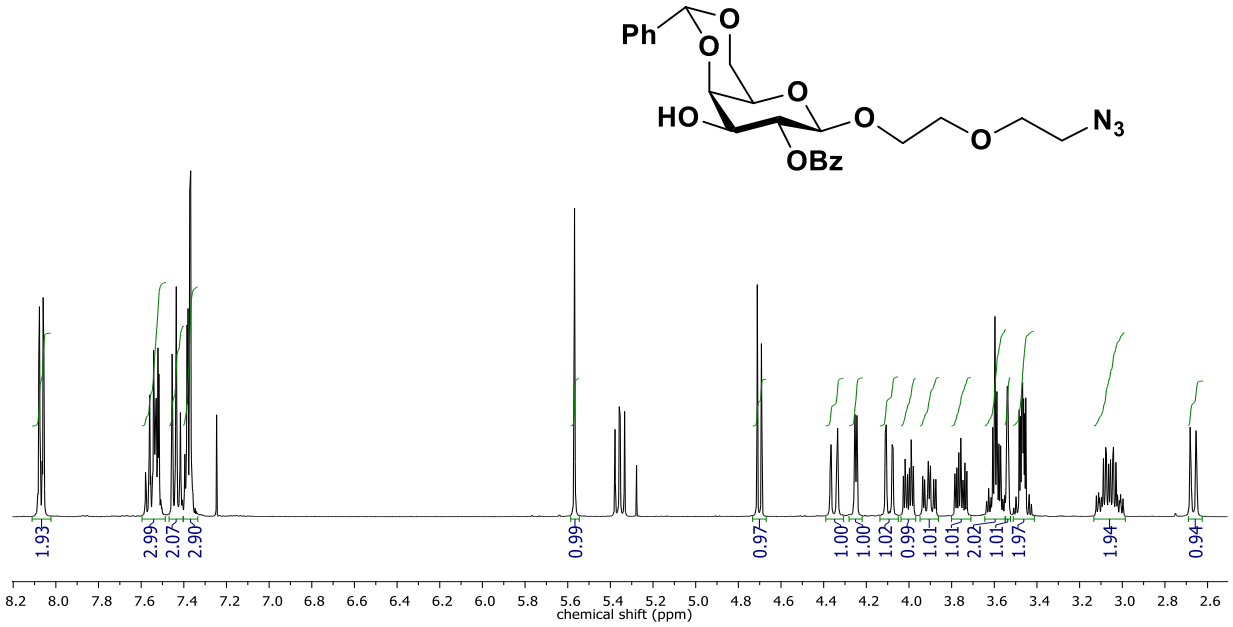

O
STol
OBn
BnO
OBn
f1 (ppm)

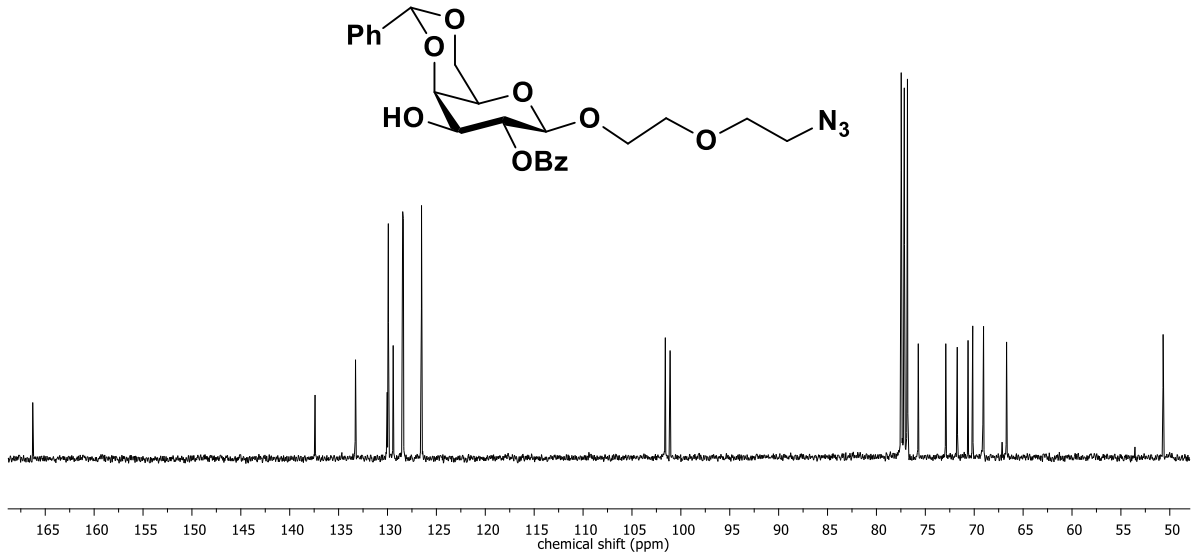
Compound 6a


1.94
1.01
0.99
5.15
8.28
0.99
1.00
2.03
1.00
0.99
1.00
1.02
0.99
0.97
0.99
2.00
1.86
0.91
1.91
chemical shift (ppm)

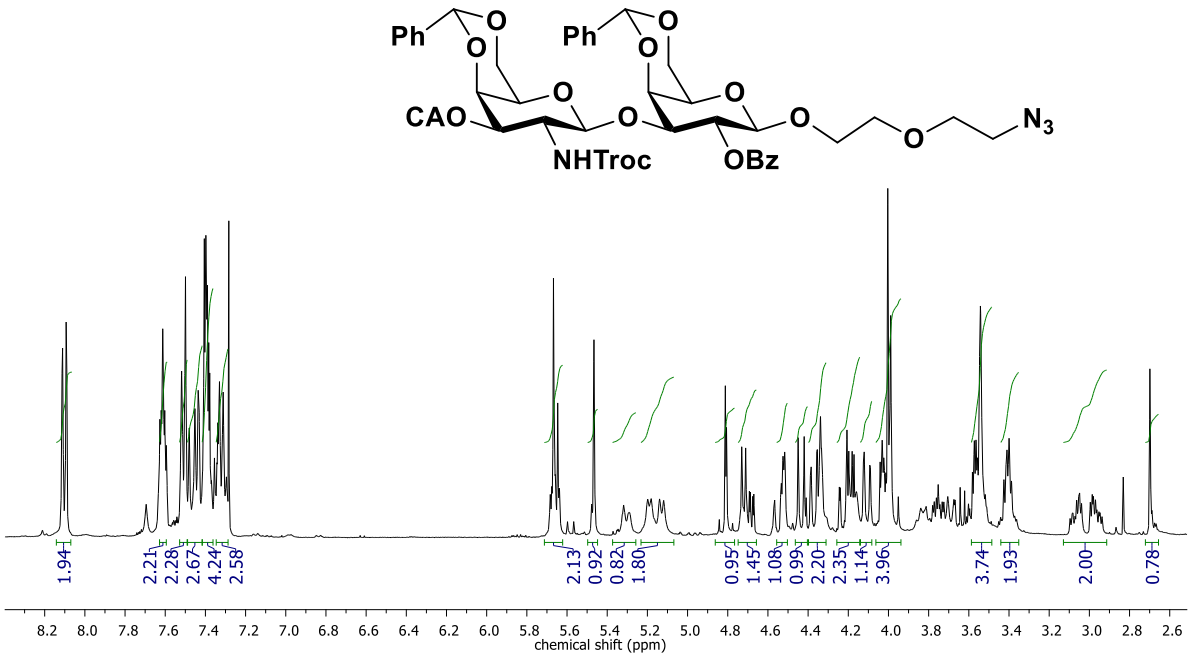

chemical shift (ppm)

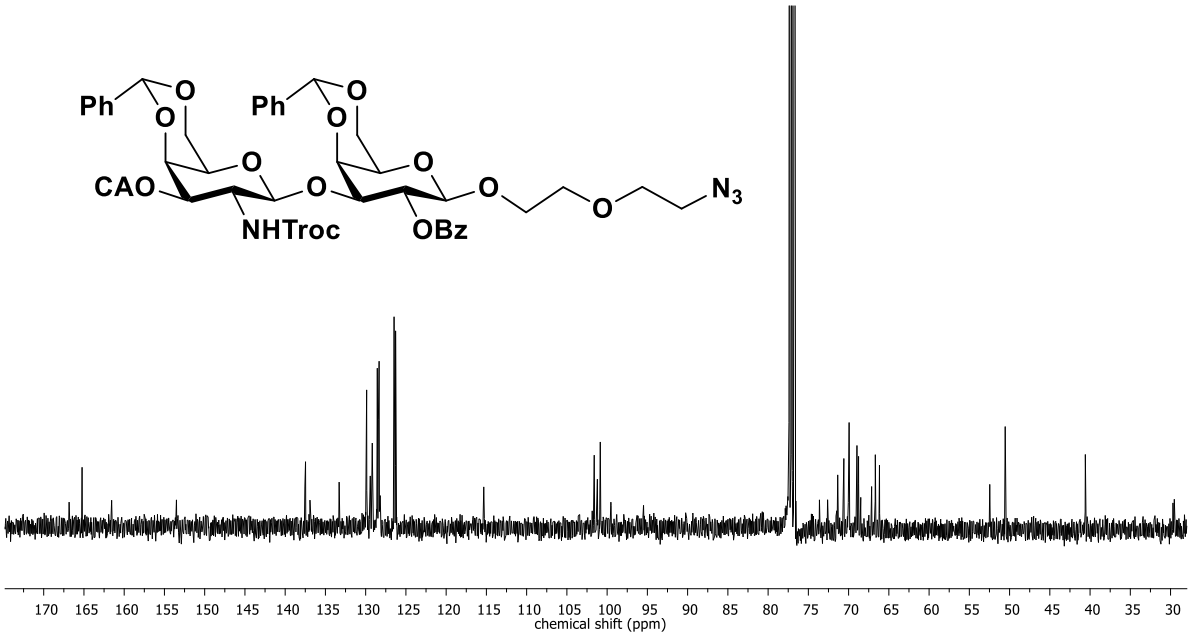
Compound 6b


Ph
O
O
O
HO
O
O
N3
OBz
1.93
2.99
2.07
2.90
0.99
0.97
1.00
1.00
1.02
0.99
1.01
1.01
2.02
1.01
1.97
1.94
0.94
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6
chemical shift (ppm)

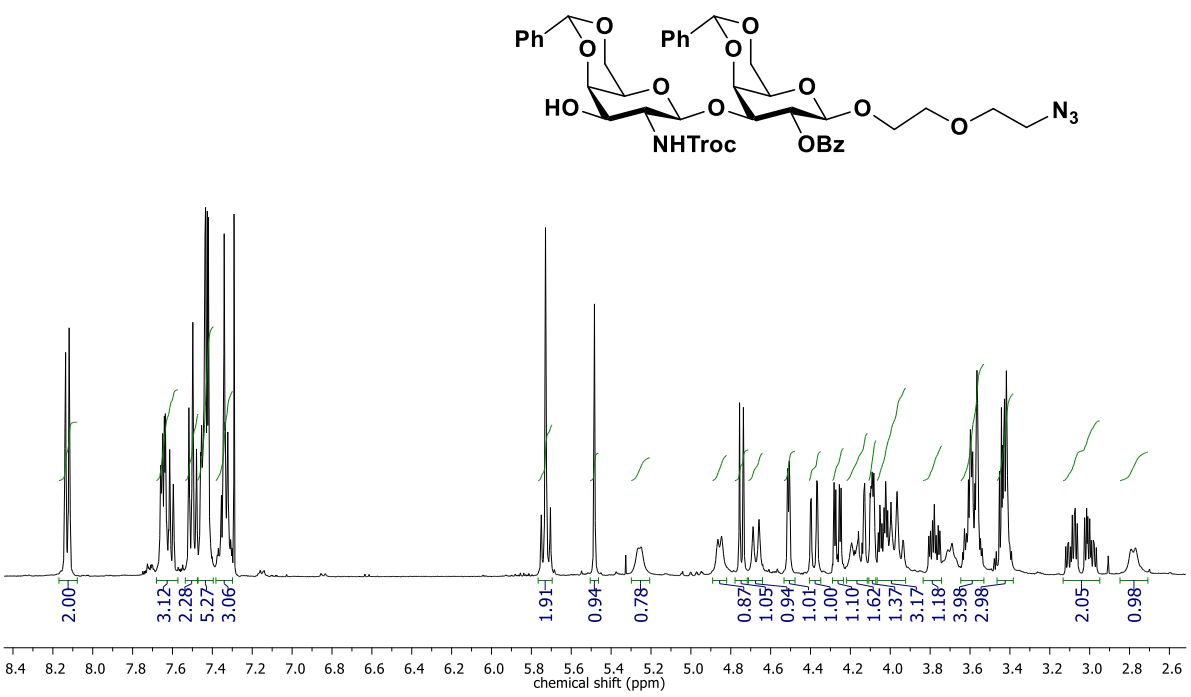

Ph
O
O
O
HO
O
O
N3
OBz
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
chemical shift (ppm)

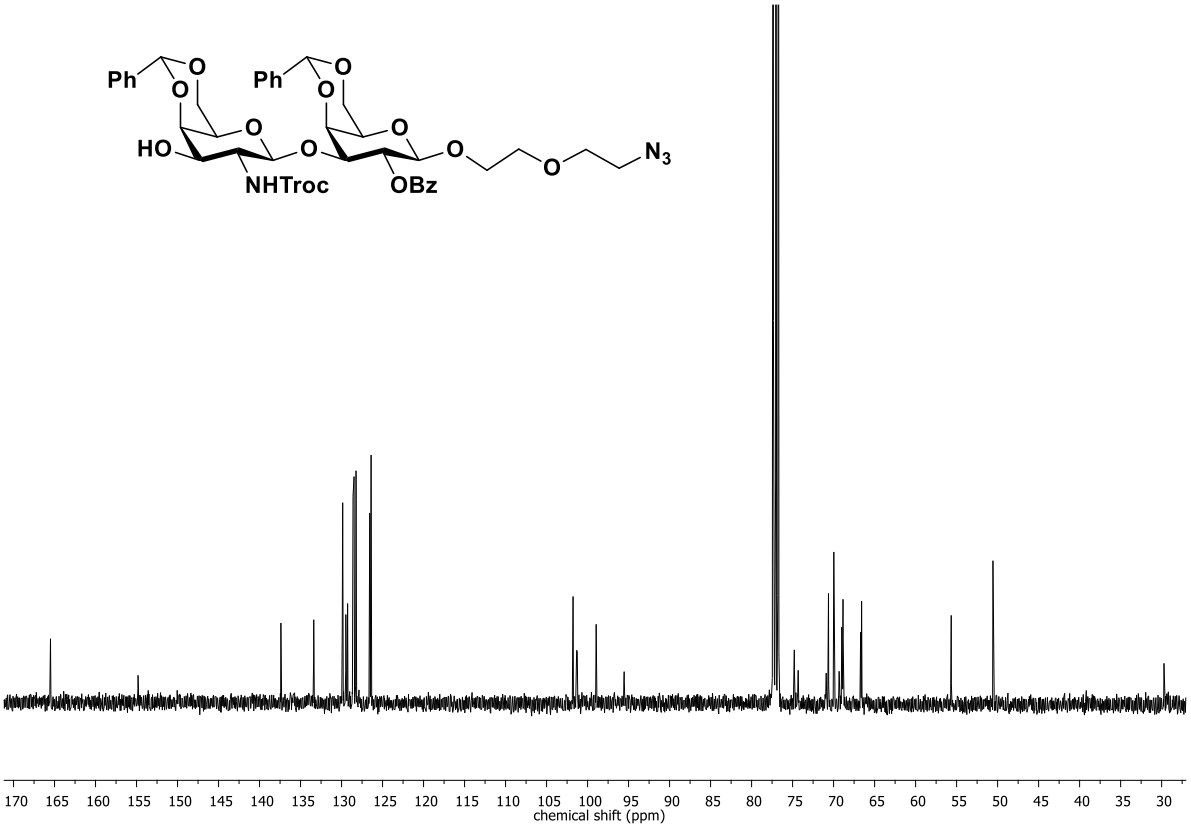
Compound 6c


Ph
O
O
Ph
O
O
CAO
O
O
O
O
N3
NHTroc
OBz
1.94
2.21
2.28
2.67
4.24
2.58
2.13
0.92
0.82
1.80
0.95
1.45
1.08
0.99
2.20
2.35
1.14
3.96
3.74
1.93
2.00
0.78
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6
chemical shift (ppm)

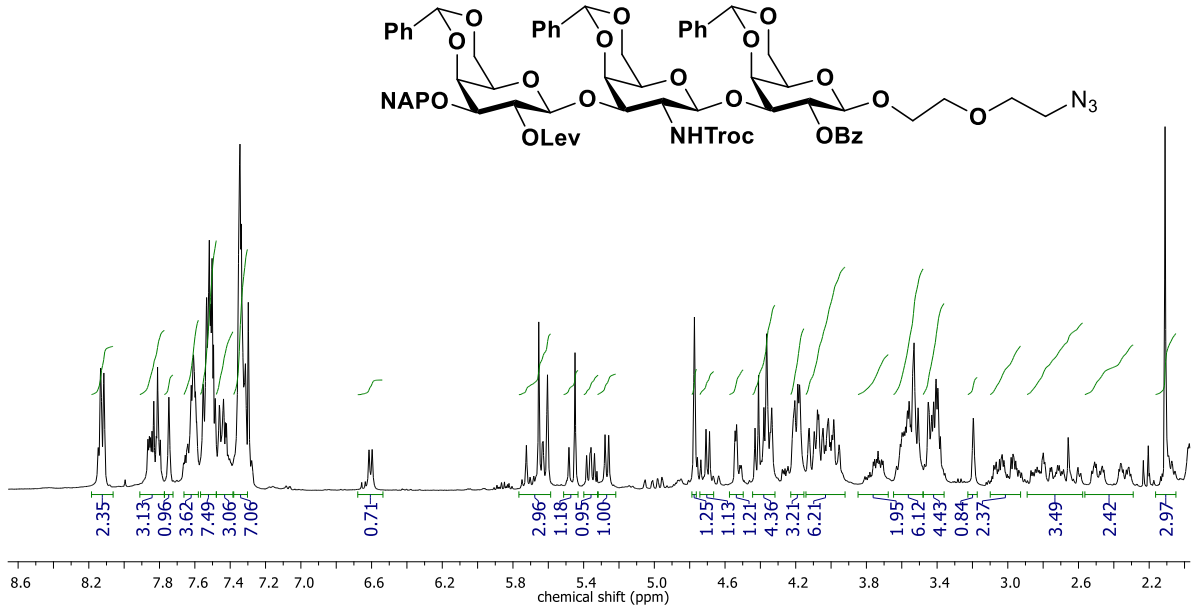

Ph
O
O
Ph
O
O
CAO
O
O
O
O
N3
NHTroc
OBz
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
chemical shift (ppm)

Compound 6d

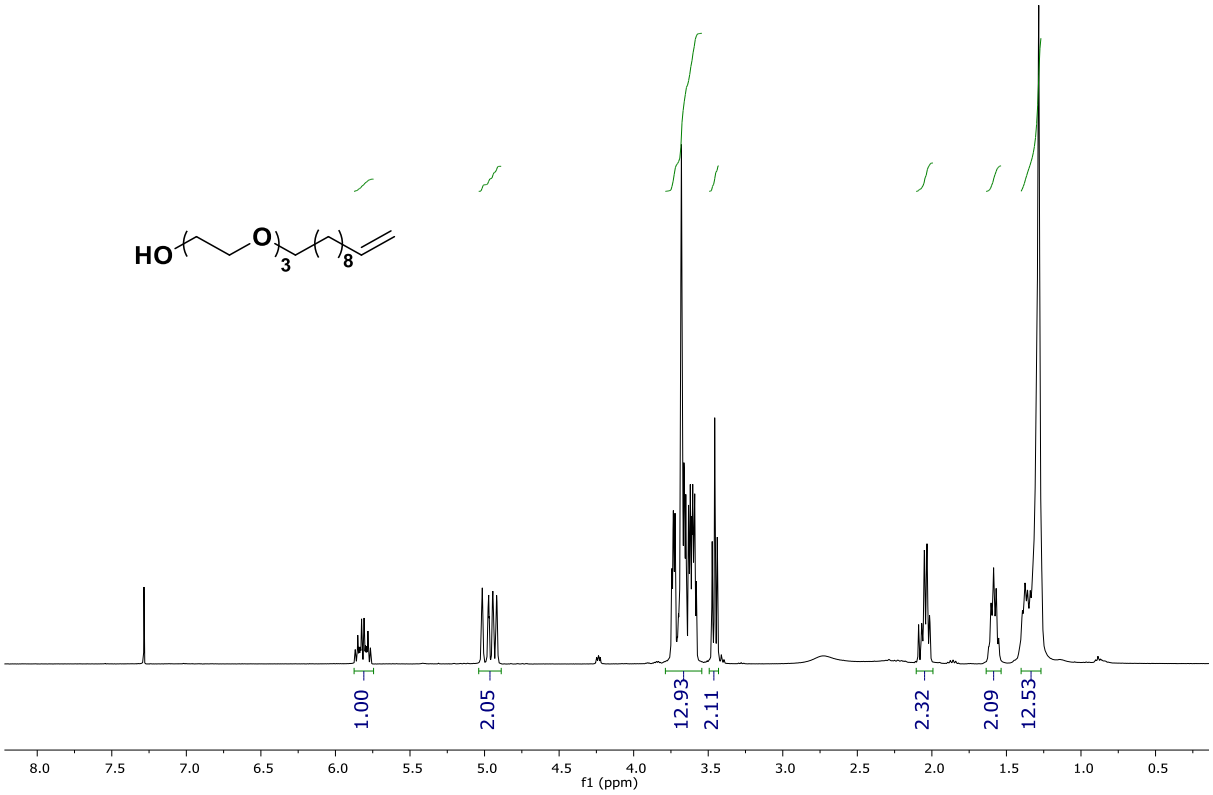

Ph
O
O
Ph
O
O
HO
O
O
O
O
O
O
N3
NHTroc
OBz
2.00
3.12
2.28
5.27
3.06
1.91
0.94
0.78
0.87
1.05
0.94
1.01
1.00
1.10
1.62
1.37
3.17
1.18
3.98
2.98
2.05
0.98
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6
chemical shift (ppm)

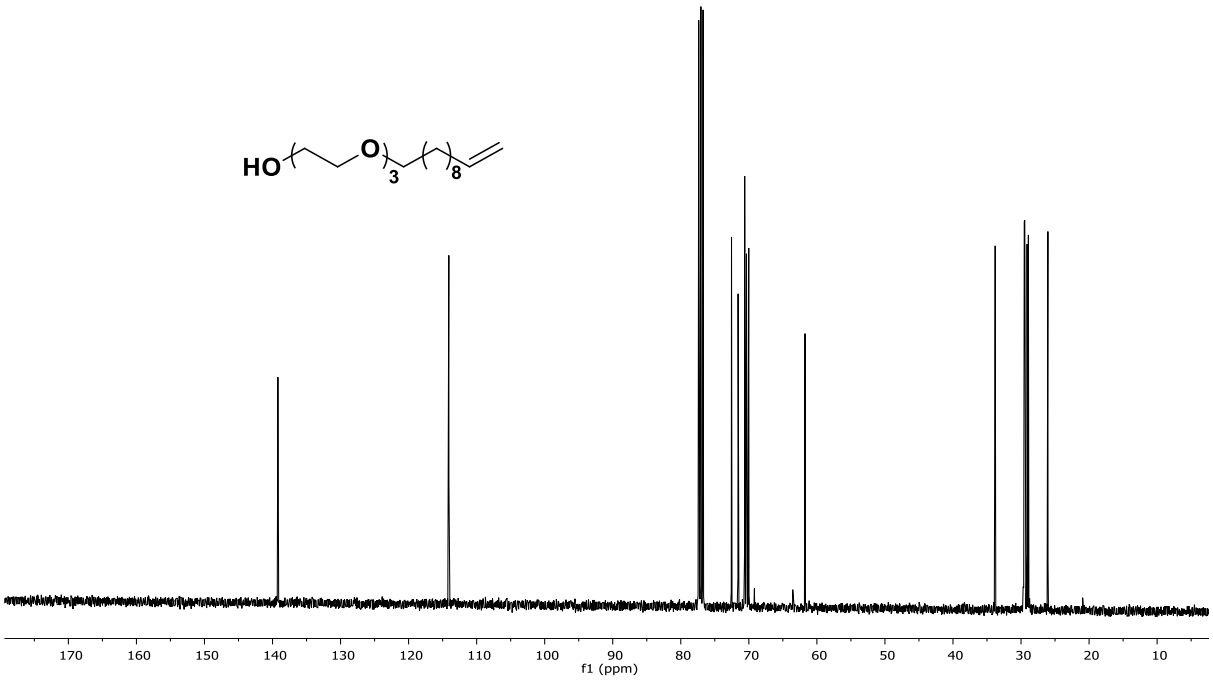

Ph
O
O
Ph
O
O
HO
O
O
O
O
O
O
N3
NHTroc
OBz
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
chemical shift (ppm)

Compound 6e

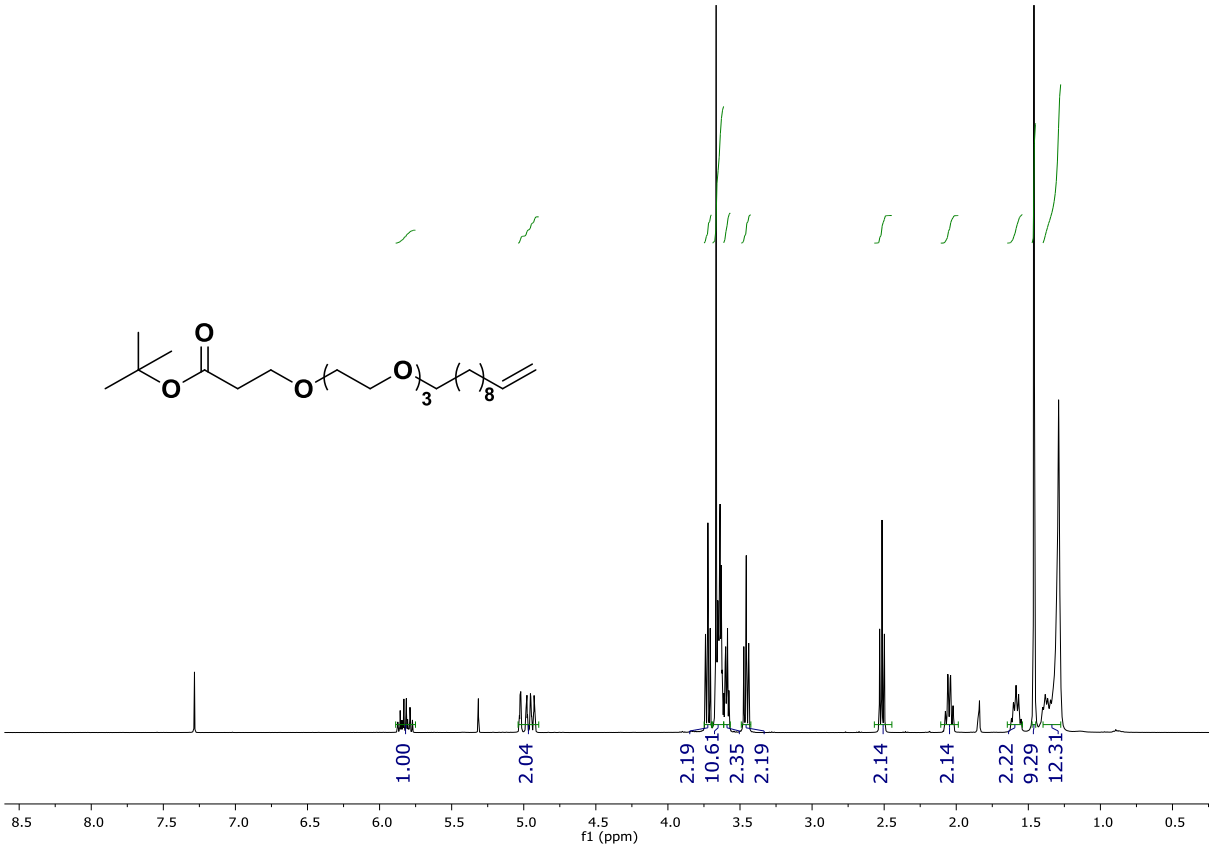

Ph
Ph
Ph
NAPO
OLev
NHTroc
OBz
N3
2.35
3.13
0.96
3.62
7.49
3.06
7.06
0.71
2.96
1.18
0.95
1.00
1.25
1.13
1.21
4.36
3.21
6.21
1.95
6.12
4.43
0.84
2.37
3.49
2.42
2.97
8.6
8.2
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
chemical shift (ppm)

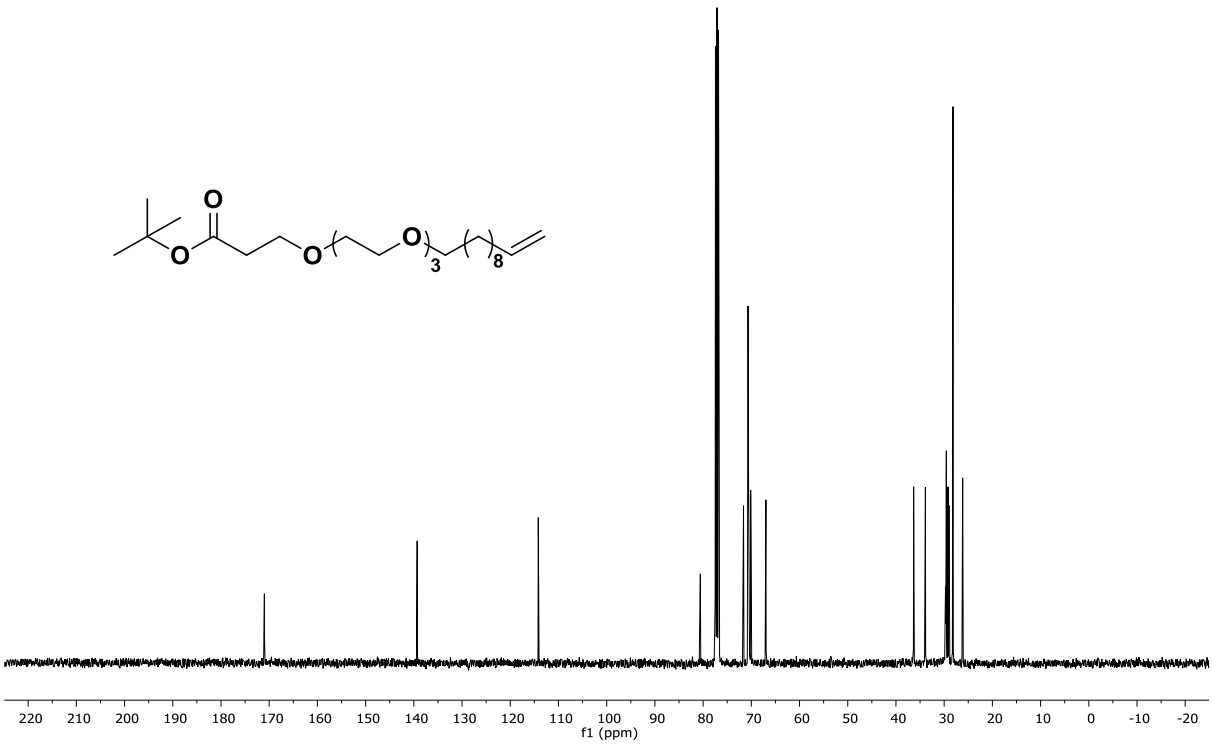
Compound 7b


HO
O
3
8
1.00
2.05
12.93
2.11
2.32
2.09
12.53
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

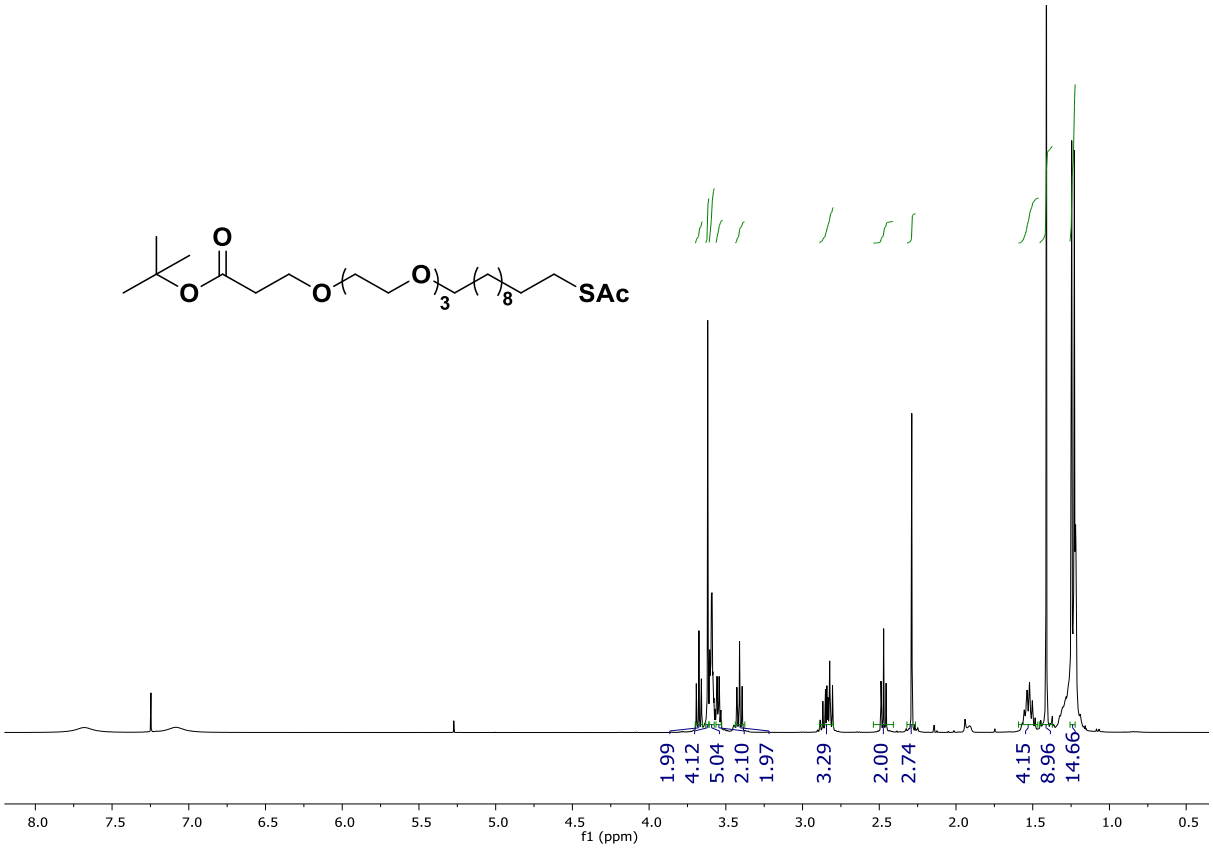

HO
O
3
8
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

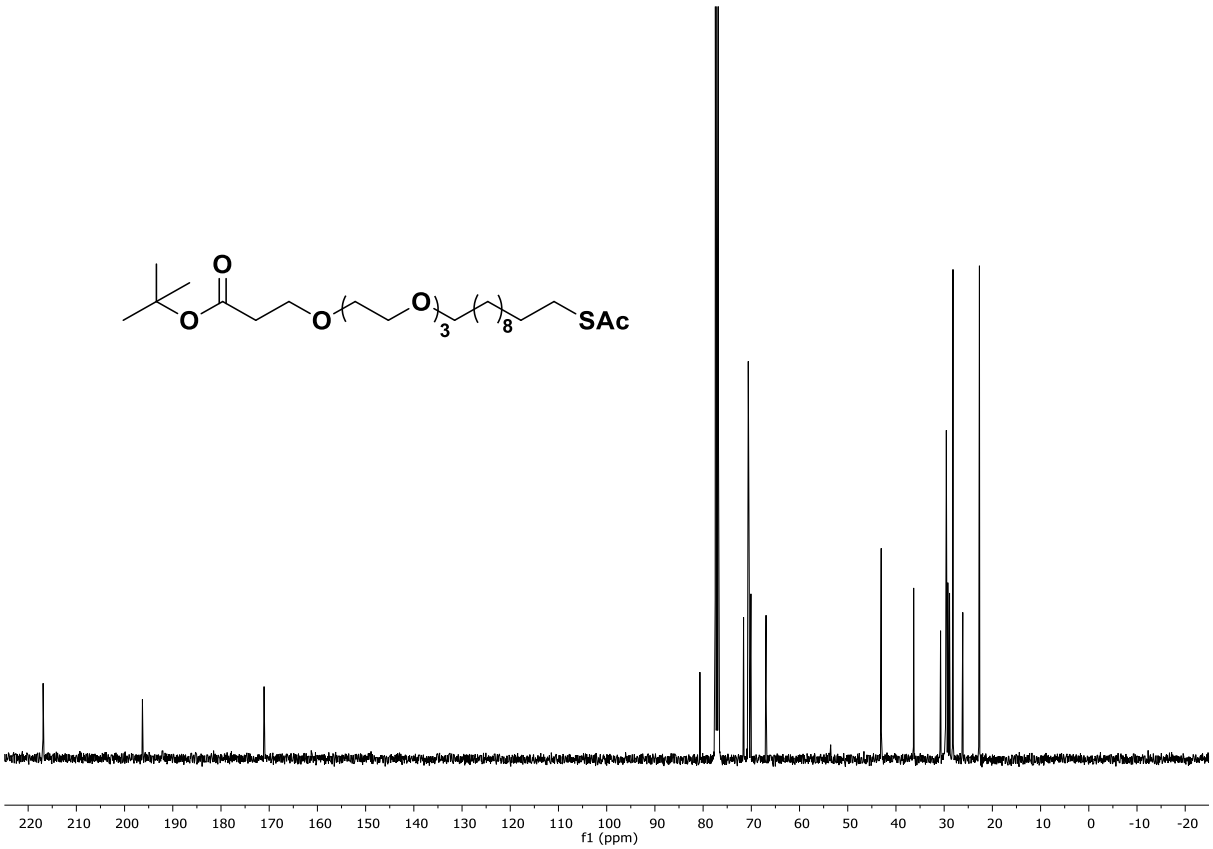
Compound 7c



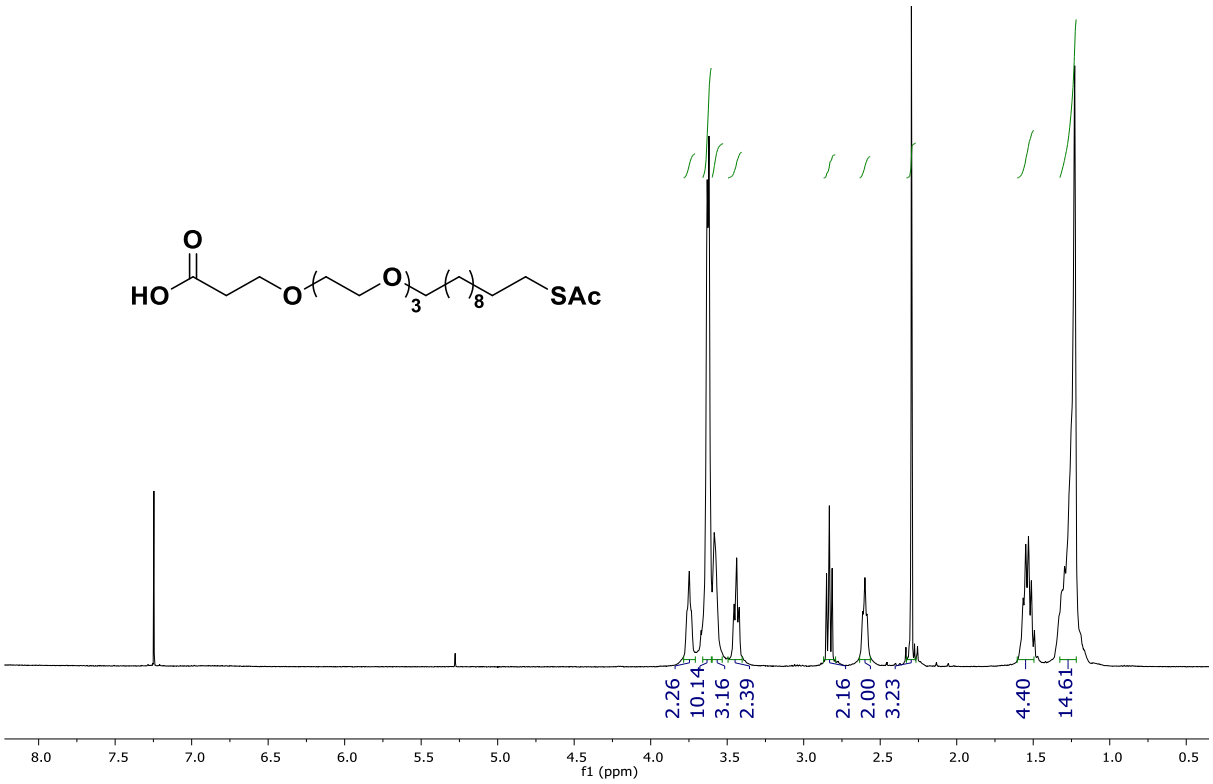


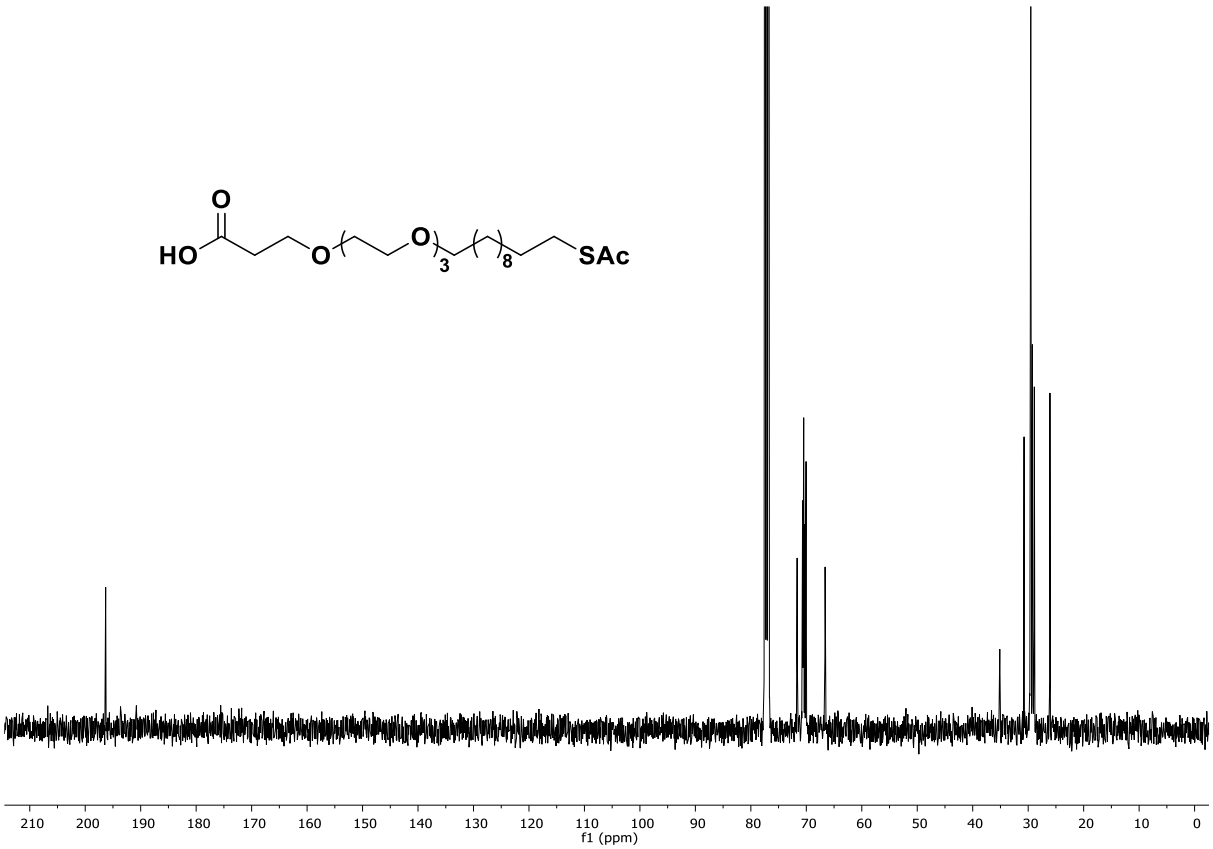
Compound 7d


O
O
O
O
3
8
SAc
1.99
4.12
5.04
2.10
1.97
3.29
2.00
2.74
4.15
8.96
14.66
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

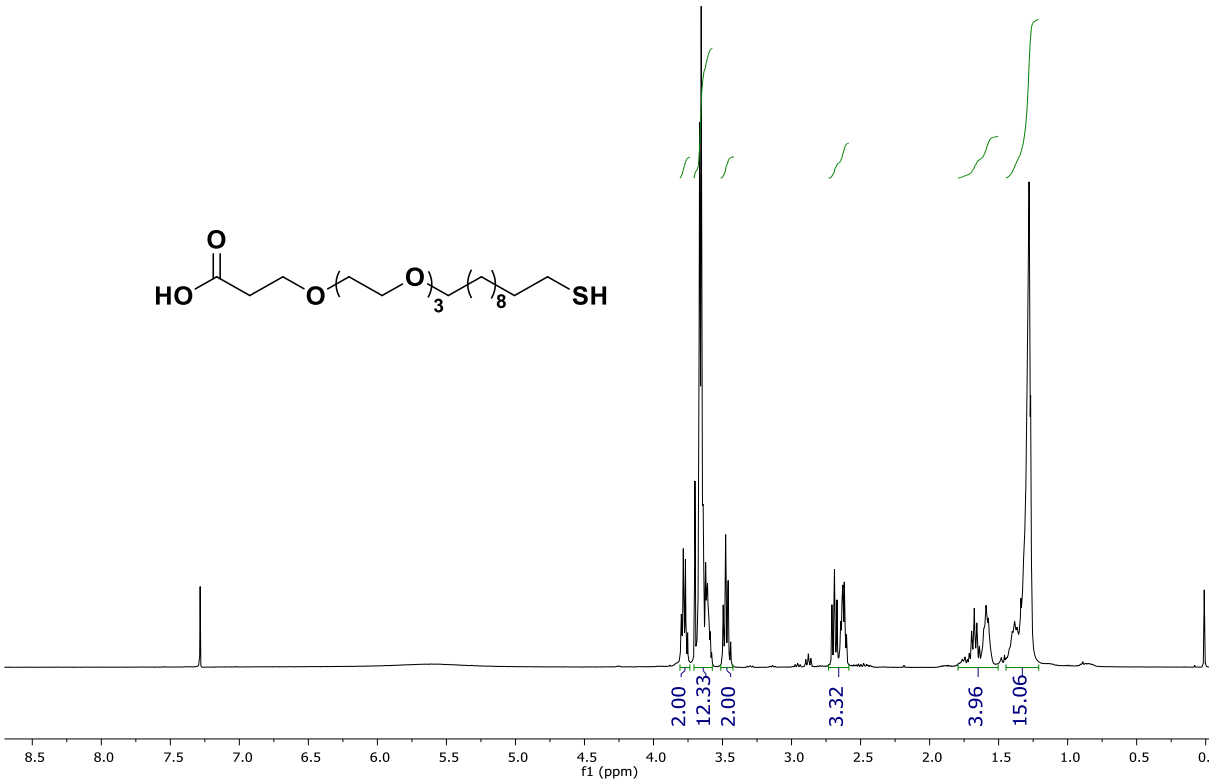

O
O
O
O
3
8
SAc
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

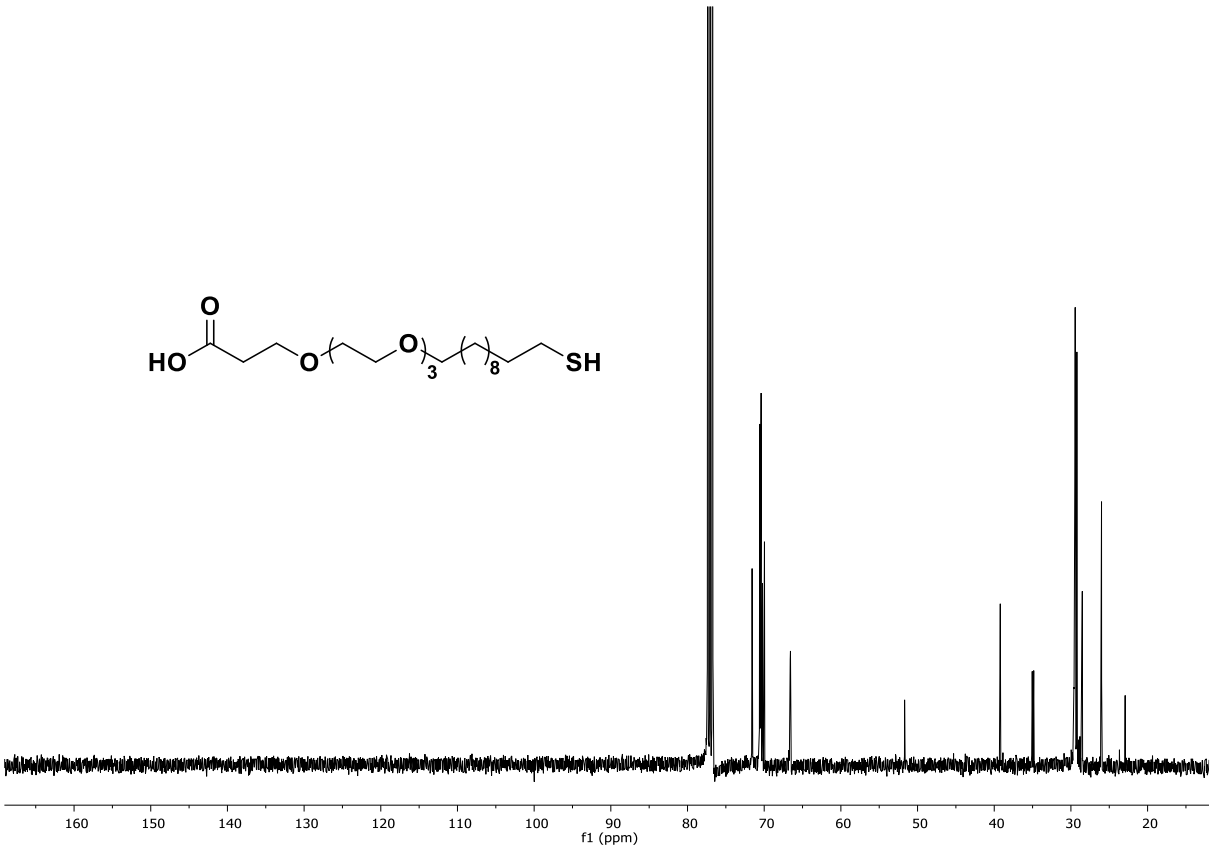
Compound 7e


HO
O
O
3
8
SAc
2.26
10.14
3.16
2.39
2.16
2.00
3.23
4.40
14.61
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

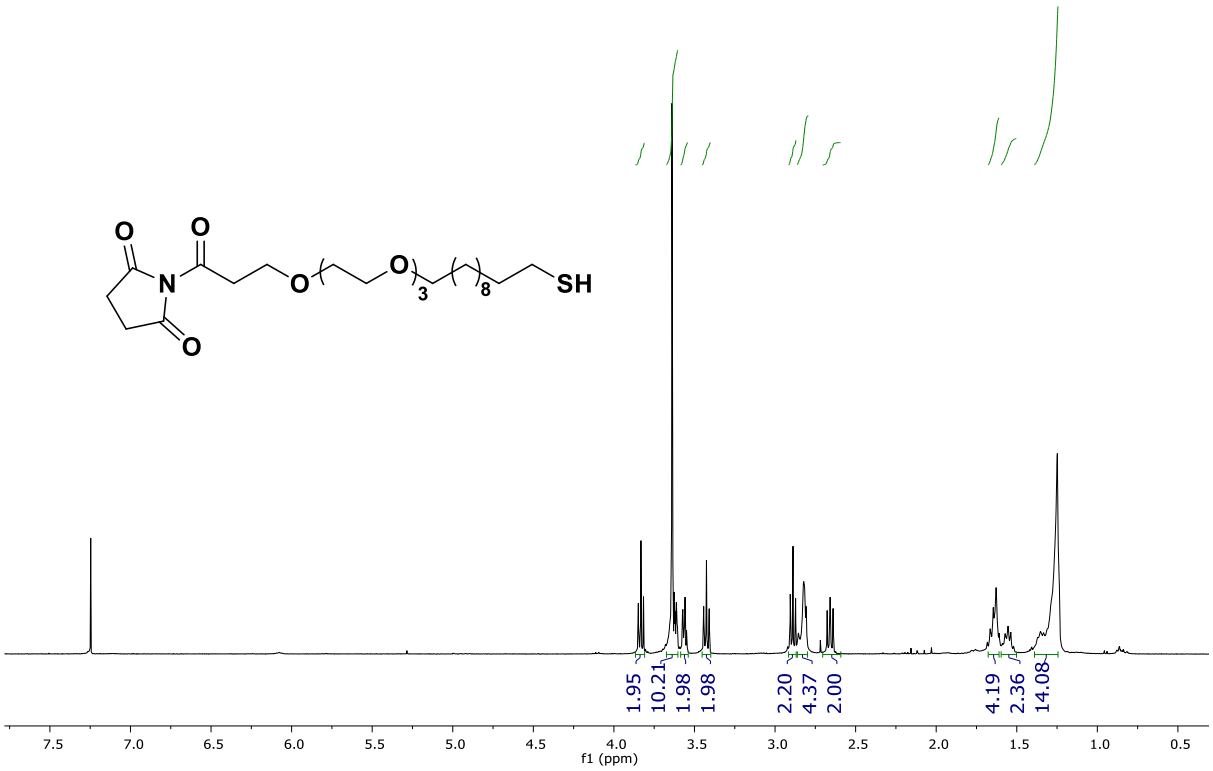

HO
O
O
3
8
SAc
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

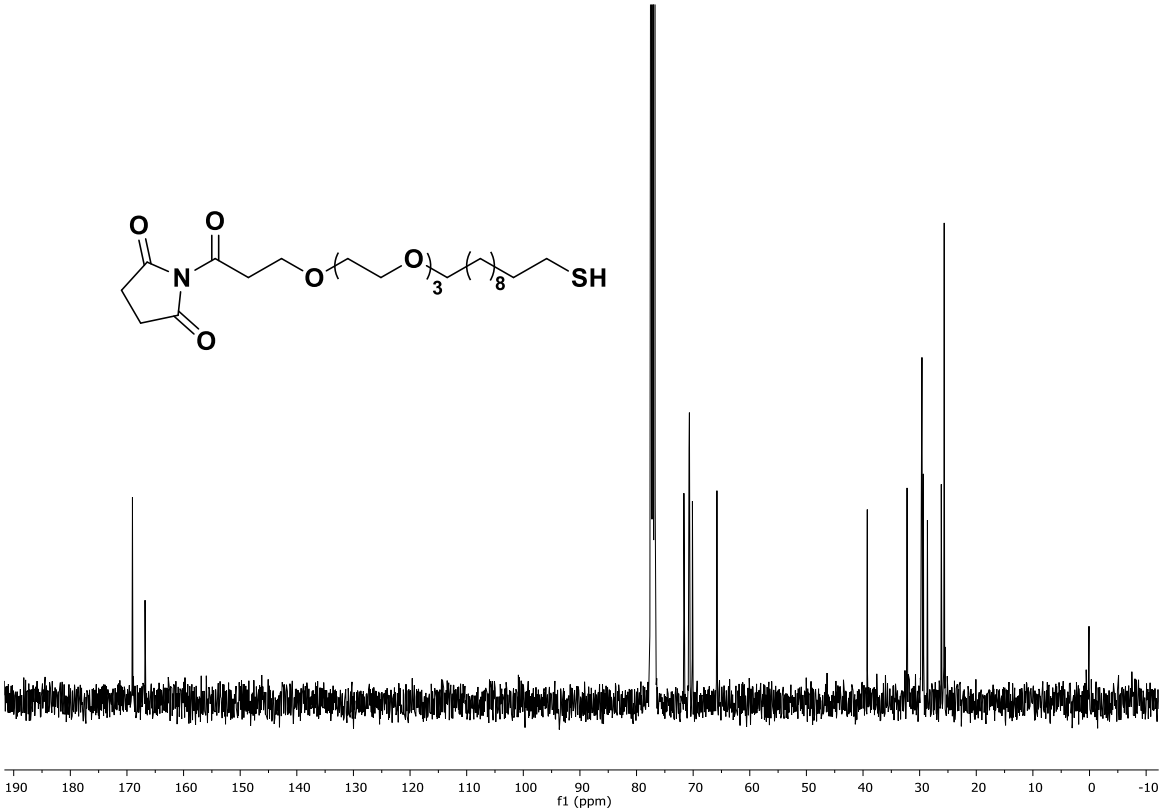
Compound 7f





Compound 7g


O
O
N
O
O
O
3
8
SH
1.95
10.21
1.98
1.98
2.20
4.37
2.00
4.19
2.36
14.08
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


O
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120
110
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80
70
60
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20
10
0
-10
f1 (ppm)

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Response to the remarks

In this MS thesis, Keerthana has attempted to synthesize a tumor associated carbohydrate antigen (TACA) that has been identified and tested by Danishefsky group. Keerthana successfully synthesized the trigalactoside and then went on to synthesize a linker and some different shapes of nanoparticles. The following are the major observations:

Comment: What is the connection between the three portions viz. synthesis of the trisaccharide, linker and gold nanoparticles?

Response: The primary aim of my thesis is to synthesize gold nanoparticles of different shapes carrying Globo-H antigen for immune modulation. Trisaccharide, linker and gold nanoparticles are essential components of final Globo-H gold nanoparticles. We now modified page-15 accordingly.

Comment: It could have been better if she has completed at least one out of the three objectives rather than uncompleted three portions.

Response: We agree with the reviewer that there is more than one way to complete the project. In my master thesis, I wanted to learn both carbohydrate and nanoparticles synthesis and if time permits immune studies. It will be an advantage for my future research in interdisciplinary areas.

Comment: The last paragraph on page 19 gives the impression that Keerthana has synthesized the tetrasaccharide. The reality is that she has not synthesized that. She has synthesized only a trisaccharide. This has to be corrected

Response: We have already included dotted link between tri to tetrasacchride. Dotted line reveals tetrasaccharide yet to synthesize.

Comment: The significance of synthesizing the linker is not apparent from the report. More clarity is required in this regard.

Response: As we mentioned above, we have included all these details in page-15

Comment: Overall, I did not understand any visible progress in the project after the December presentation. In summary, I recommend the thesis

Response: In the first six months of my master thesis (Dec presentation), I managed to synthesize building blocks required for final Globo-H synthesis. From Dec-March 2018, I tried my best to synthesize Globo-H tetrasaccharides. There are several hurdles to synthesize oligosaccharides, including low yield, separation of by column chromatography, solvent impurity, etc. The reviewer also requested to consider the shortage of solvents, dry ice, necessary institute facilities including NMR, mass spectra, etc., during this period. Taking all these into account, I tried my best to complete my project in given time.