Synthesis of D-Glucosamine Building Block as Required for Generating Diverse Sulfation Patterns

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

By
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CERTIFICATE

This is to certify that this dissertation entitled "**Synthesis of D-Glucosamine Building Blocks as Required for Generating Diverse Sulfation Patterns"** 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 Akhil N B at Indian institute of Science Education & Research, Pune under the supervision of **Dr. Raghavendra Kikkeri**, Assistant Professor, Department of Chemistry during the academic year 2016-2017.

Date:

Place: Pune

Dr.Raghavendra Kikkeri

Assistant Professor

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DECLARATION

I hereby declare that the matter embodied in the report entitled "**Synthesis of D-Glucosamine Building Blocks as Required for Generating Diverse Sulfation Patterns**" 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:

Place: Pune

Akhil N B

20121027

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

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CONTENTS

ABBREVIATIONS

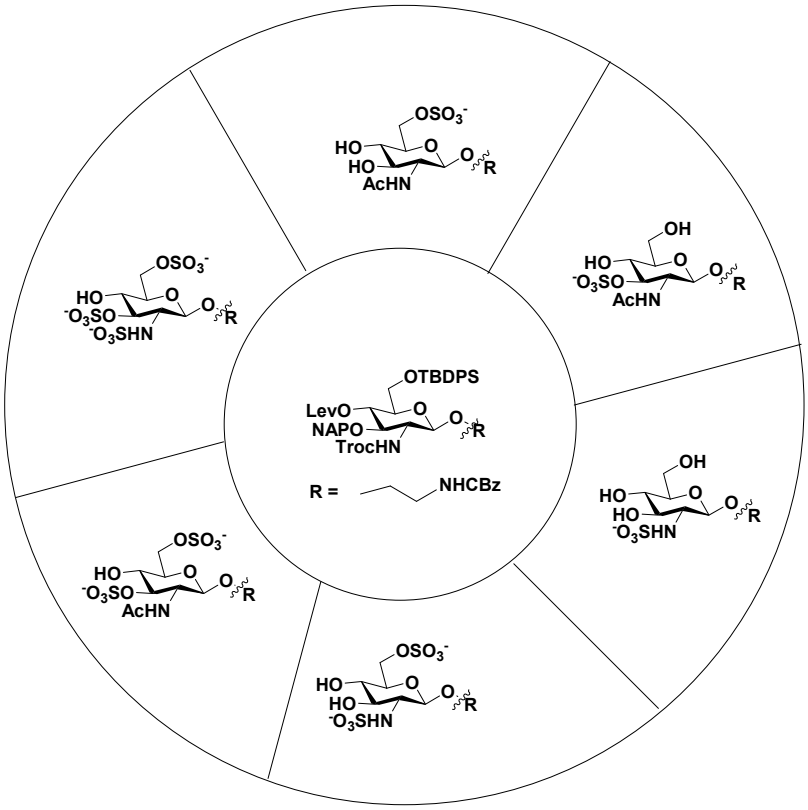
Ac_2O	Acetic anhydride
Py	Pyridine
r.t	Room temperature
H	Hours
K_2CO_3	Pottasium carbonate
TBDPSiCl	Teritary butyl diphenyl silyl chloride
SO_3	Sulphur trioxide
DCM	Dichloromethane
HCl	Hydrochloric acid
$CuSO_4$	Copper sulphate
NaOMe	Sodium Methoxide
Ml	Milli litre
M.S	Molecular Sieves
J	Coupling constant
$CHCl_3$	Chloroform
EtOAc	Ethyl Acetate
Aº	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
TolSH	Thiotoluene
$NaHCO_3$	Sodium bicarbonate
CAN	Acetonitrile
Mol	Mole
G	Gram
mmol	Milli mole
NAP-Br	Naphthalene Bromide
LevCl	Levlunic acid
NIS	N Iodo succinimide
µl	Micro litre
Et_3N	Triethylamine
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
DMAP	Dimethyl aminopropylamine
THF	Tetrahydrofuran
TrocCl	2, 2, 2 Trichloro ethylchloroformate
Pd/C	Palladium on Charcoal

TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TfOH	Trifluoromethanesulfonic acid
TLC	Thin Layer Chromatography
HRMS	High Resolution Mass Spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
p-TSA	Para-toluene sulphonic acid
NMR	Nuclear Magnetic Resonance
DMF	N, N Dimethyl formamide
DCC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide

ABSTRACT

Heparan sulfate is extracellular irregularly sulfonated macromolecules that influence the activities of various proteins on the cell surfaces. Hence, obtaining well-defined sequences of heparan sulfate is a huge challenging to understand structure-functional activities. Herein, we present the design and synthesis of heparan sulfate analogs that decipher the role of sulfated patterns of heparan sulfate in biological structure and functions. To achieve this, I have synthesized glucosamine building block in such a way as to generate a library of sulfated-glucosamine patterns with amine linker. These molecules will be immobilization on microarray slides to study lectin, cytokines and chemokines binding affinities and its subsequent biological responses.



INTRODUCTION

Heparan sulfate (HS) has been extensively used as an anticoagulant for more than 60 years.[1,2] HS is a linear polymer irregularly sulfated at disaccharide units consisting of α(1-4) linker of uronic acid and D-glucosamine unit (Figure 1). The molecular weight of these polymers varies from 4 to 40 kDa. The uronic acid unit of HS is typically either L-iduronic acid or its C_5 epimer D-glucuronic acid. HS express variably sulfated patterns from sequence to sequence and render acidic nature of the macromolecules. *O*-sulfation normally occurs at 3rd and 6th position of glucosamine and 2nd position of the uronic acids unit.[1] Also, amine part of the glucosamine could be sulfated or acetylated. These sulfated patterns and the variable uronic acid units result in diversity in the structure and functions of HS. HS binds to several extracellular signalling proteins including growth factors, cytokines and chemokines. These interactions are important for the growth control, inflammation, cell-signalling and cell-adhesion.[1,3]

It has been reported that fibroblast growth factors (FGFs) bind to the extracellular matrix of target tissues biological process by interacting with HS as one of the key components.[4] There are 23 different types of FGF-proteins, which are independently or collectively involved in the physiological and pathological process of cells, including cell proliferation, migration, differentiation etc.[6,7] FGF-1 (acidic FGF) and FGF-2 (basic FGF) are the most well studied FGF. HS are essential components to regulate the FGF signaling and cell-specific tyrosine kinase receptors complexations.[6] High-resolution X-crystal structure of these complexes revealed that tetra or hexa-saccharides of HS with specific sulfated patterns are sufficient to form the complexes with high affinity. However, long chain HS oligomers are essential for the dimerization and activation of FGFs.[8,9] Hung et al., has demonstrated that *2-O*-sulfated iduronate and *N*-sulfated glucosamine are critical modification necessary for FGF-1 and FGF-2 binding and signalling. Furthermore, *6-O*-sulfated glucosamine has shown critical for FGF-1 binding. [5]

Chemokines are small proteins secreted by immune cells and endothelial and must cells, which are necessary for the lymphocyte migration to the inflammation site.[10] The binding between the chemokines and HS provides insight mechanism of chemokine molecular level functions.[8] Seeberger et al., has shown that CCL21,

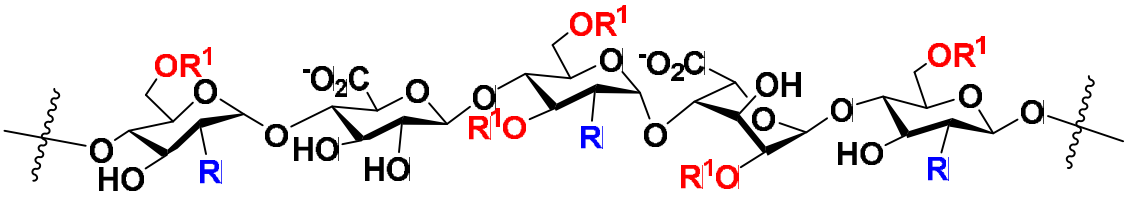
CCL13 chemokines prefer hexasaccahrides of *N*-sulfated glucosamine over iduronic acid at the non-reducing end. While the CXCL12 or CCL19 displayed a strong affinity to long HS oligosaccharides compared to hexa or tetra-saccharides.[11] IL-8 profile clearly showed that *2-O*-sulfated iduronic acid is crucial for the specific binding. CCL25 and CCL28 displayed similar binding pattern, indicating that minimum of *2-O*-sulfated idoA and *N*-sulfated glucosamine constitute a general recognition scaffold for these chemokines.[11]

Antithrombin III (AT III) is one of the most well established HS binding proteins, which involved in the interaction of thrombin and factor XA in the blood coagulation process.[12,15] Recently, it has been confirmed by NMR spectroscopy and X-crystallography that HS pentasaccharides sequence and $^{2}S_{0}$ conformation of L-iduronic acid are necessary for the binding to AT-III. [13,14] Overall, it has been showed that the sulfated patterns and reducing and non-reducing end sugar structures and the conformation of L-iduronic acid within the oligomers play a significant role in the protein binding and subsequent biological functions.[1] However, most of the HS sequences used for these studies were obtained from either enzymatic and chemical depolymerisation methods. Therefore, it is essential to further investigate these HS-protein interactions using chemically defined sequences. Herein, we proposed to synthesized heparin sugars ranging from monosaccharide to hexa-saccharides with various degrees of *O*-sulfation and *N*-sulfated patterns to validate the significance of these two groups in HS structure and functions.

The main objective of this work was to develop a common building block to synthesized different sulphated derivatives of glycosamine and functionalize them on the microarray to study the carbohydrate-protein interactions.

The particular aims are:

1. Synthesis of glucosamine building block.
2. Synthesis of different sulphated patterns.
3. Conjugation of these analogs on microarray slides.
4. Evaluate the sulphated-pattern specific carbohydrate-protein interactions.



R= $NHSO_3^-$ / NHAc R^1= SO_3^-

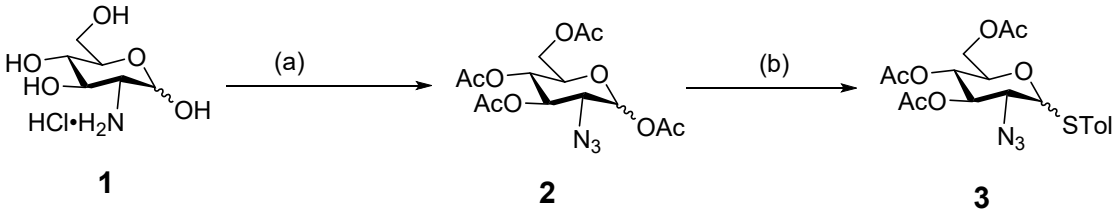
Figure 1. Structure of Heparan sulphate

Results & Discussions

Synthesis of Glucosamine Building Blocks

Our strategy for the synthesis of sulphated glucosamine is based on selective de-protection of the glucosamine building block to introduce *O*-sulfation at 3rd, 4th and 6th position and *N*-sulfation at amine group of D-glucosamine. Recently, Hung et al., developed an elegant and high yield synthetic strategy to synthesize glucosamine building block. We have employed the same method in our synthesis. Techniques such as ^{1}H-NMR, ^{13}C-NMR, MALDI-TOF and HRMS were used to the characterization the intermediates and final compounds.

Synthesis of peracetylated glucosamine donor building block:



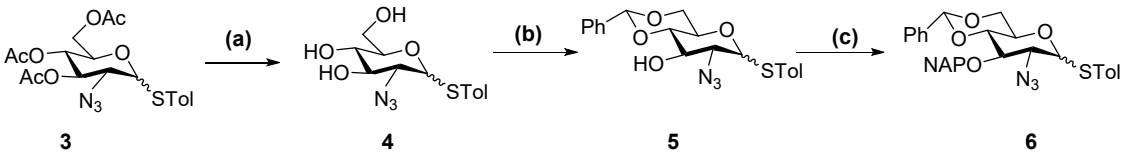
1 **2** **3**

Reagents: a) imidazole sulphonyl azide, K_2CO_3, $CuSO_4$, MeOH; Ac_2O, Py, 0ºC to rt, 12h, 81% (α:β =1:1); b) TolSH, BF_3-Et_2O DCM, 0ºC to rt, 12h, 90% (α:β =1:1).

Commercially available D-glucosamine (**1**) was used to prepare the required building block**.** In the first step, amine group of D-glucosamine was protected with azide with freshly prepared imidazole sulphonyl azide in the presence of potassium carbonate and copper (II) sulphate in methanol. After 6h, the solvent was evaporated and dried under reduced pressure. The crude product was treated with acetic anhydrate and pyridine to protect all hydroxyl groups of 2-azidodeoxyglucose with base labile acetyl group. Once the reaction was completed excess solvents was removed and subsequent purified by column chromatography yielded 81% of comp **2**. The four singlet between 2.0-1.8 ppm corresponds to acetate proton of **2**. Comp **2** 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 **3** as a white solid. The presence of multiplet between 7.52-7.13 ppm in aromatic region and a singlet at 4.33 ppm corresponds to α-proton of thio-cresol and 4.38 ppm *β*-proton of thio-cresol.

Synthesis of Benzylidine and NAP protected glycosamine building block:

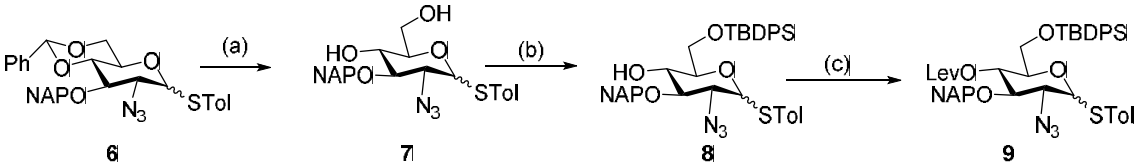


3 **4** **5** **6**

Reagents: a) NaOMe, MeOH, 94% (α:β =1:1); (b) $Ph(OMe)_2$, CSA, ACN, rt, 1h, 84% (α:β =1.5:1); (c) NAP-Br, NaH, DMF, 93% (α:β =1.5:1).

Sugar **3** was then subjected to de-acetation under basic conditions at 0ºC resulting in the compound **4** as α:β =1:1 mixture with 94% yield. Furthermore, the C-4 and C-6 hydroxyl group of **4** was protected with benzaldehyde dimethyl acetal and CSA as reagents in acetonitrile solvent to afford **5** as a white solid in 80% yield. In this case, we have separated α and β-conformation of **5**. The singlet at 5.57 ppm for the benzylidine proton and a doublet at 5.51 ppm with coupling constant *J* = 5.5 Hz indicating the product is in alpha confirmation in ^{1}H NMR and double at 4.47 ppm with coupling constant *J* = 10.2 Hz indicating the product is in beta confirmation. Finally, C-3 hydroxyl group of **5** was protected with base labile NAP-Br so that the C-4 hydroxyl group can be accessible further glycosylation. The doublet of doublet at 5.12 ppm with coupling constant *J* = 11.2 Hz indocating the CH_2 proton of NAP protection.

Synthesis of multi labile glucosamine Building block:

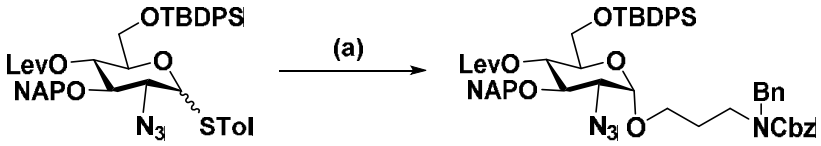


6 **7** **8** **9**

Reagents: a) p-TSA, DCM:MeOH (2:1), 84% (α:β =1:1); (b) TBDPSCl, Et_3N, DMAP, DCM, 75%; (c) LevCl, DCC, DMAP, 40% (α:β =1:1).

The benzylidene ring opening of **6** was carried out with *p*-toluene sulfonic acid in DCM:MeOH (2:1) mixture for 3h resulted in **7** as a white solid with 84% yield. C-6 of **7** was protected with silyl derivative by treating TBDPSCl in the presence of trietyl amine and DMAP, yielded 75% of comp **8**. Finally, C-4 of **8** was protected with labile Levulinic acid protection in the presence of LevCl, DCC and DMAP, yielded 40% of comp **9**.

Synthesis of glucosamine building block with linker.



9 (α:β = 1.5:1) **10**

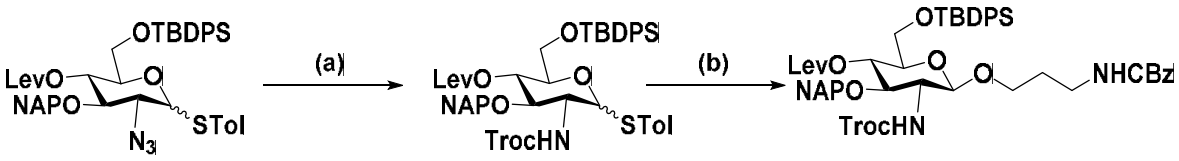
Reagents: a) Linker, NIS, TMSOTf, DCM.

Compound **9** was mixed with NH-Cbz protected propanol amine and *N*-Iodosuccinimide in DCM as solvent. The mixture was cooled to -40^{o}C and promoter TMSOTf (--) was added. After 1 h, the reaction mixture was quenched with triethyl amine and product was purified by column chromatography. ^{1}H-NMR of the product clearly showed α:β =1:1 with same R_f value. To improve the α:β ratio, we have changed the solvent to acetonitrile. Although there was a slight increase in the yield, but α:β ratio was remained same. Furthermore, pure α-form of **9** was also used to as a donor to synthesize comp **10**. But again we got a mixture, which was difficult to separate by the column chromatography. Hence, we decided to change the linker with much more bulky group. We have synthesized N(Bn)Cbz-protected propanol amine linker and performed glycosylation at different temperature. Interesting, at -20^{o}C, we observed the formation of the product and we also noticed a solvent effect. In the presence of diethyl ether and DCM as a solvent we observed 1.5:1 and 1.2:1 ratio of α:β mixture. While in the presence of acetonitrile, we got 1:1.5 ratio of α:β mixture. We also observed that the bulkiness of the linker cleared improved the prospect of separating the two compound by column chromatography.

Donor (9) (Equivalent)	Acceptor (Linker) Equivalent	Temp (^{o}C)	Solvent	α:β (Yield) of 10
1	1.2 (R = Bn)	-60	DCM	NR
		-40		NR
		-20		1.2:1** (58%)
1	1.2 (R = Bn)	-60	Diethyl ether	NR
		-40		NR
		-20		1.5:1** (60%)
1	1.2 (R = Bn)	-40	Acetonitrile	NR
		-20		1:1.5** (56%)
1	1.2 (R = H)	-40	DCM	1:1* (57%)
1	1.2(R = H)	-40	Acetonitrile	1:1* (60%)
1 (α-form)	1.2(R = H)	-40	DCM	1:1* (60%)

Table 1. Glycosylation of 9 in the present of linker at different conditions. * = Not separable; ** = Easy to separate.

Synthesis of *β*-glycosylated glucosamine linker.



9 11 12

Reagent: (a) Zn dust, AcOH; TrocCl, TEM, THF. (b) Linker, NIS, TMSOTf, DCM.

To improve the *β*-linkage system, comp **9** was treated with --- to convert azide to amine and the crude product was treated with TrocCl resulted as a white solid of **11** with 84% yield. Comp **11** was glycosylated with *N*-Cbz linker in the presence of NIS and TMSOTf, yielded 67% of comp **12**.

Conclusions.

In summary, we have synthesized glycosamine building block protected with TBDPS, Lev, NAP, Troc and azide protection. These protecting groups can be selectively cleaved at different labile conditions to introduce sulfation pattern at different positions of glucosamine. In addition, we have also optimized the glycosylation condition to obtain selective *α* and *β*-glycosylated linker. Currently, we are in the process of optimizing the sulfation, deprotection of the final compounds and synthesizing homopolymer of glucosamine. Finally, these compounds will be immobilized on the microarray slides to study the carbohydrate-protein interactions.

Materials

- All the chemicals for synthesis were purchased from Sigma Aldrich, Spectrochem and Alfa Acer
- All the NMR data were recorded on JEOL 400MHz and Bruker 400MHz using TMS as internal slandered. Chemical shifts were expressed in ppm units downfield from TMS
- ESI HRMS data was done by using Water Synapt G2 spectrometer
- Monitoring of all reactions are done by using Thin Layer Chromatography(TLC) with UV light using staining agents such as CAM, Ninhydrin and KMNO4
- All reactions are carried out at inert atmosphere using nitrogen and dry solvents
- Heidolph Rotary evaporator was used for solvent evaporation
- Silica used for column chromatography are silica (100-200) and silica (60-120)

Experimental Procedures

Compound **2**: Commercially available D-(+)-Glucosamine hydrochloride (48.7g, 226.511 mmol) and Imidazole-1-sulphonyl azide (46.731 g, 271.691 mmol) were dissolved in methanol and catalytic amount of $CuS0_4$ (.563g, 1.31 mmol) and K_2CO_3 (46.707 g, 338.451 mmol) were added to the reaction flask respectively and stirred for 12h. Completion of the reaction was monitored by TLC and after the completion of reaction, Methanol was evaporated using rota evaporator. Kept for high vacuum for long time. The vacuum dried compound **1** was dissolved in pyridine (300 ml, 3.5 mol) and acetic anhydride (170 ml, 1.78 mol) was added slowly using additional funnel at 0 ºC and stirred to r.t for 12h. Completion of the reaction was monitored by TLC and after completion the pyridine was evaporated using high vacuum in rota evaporator and co-evaporated with toluene (200 ml x 3). Then the reaction mixture was extracted with 1M HCl and Brine. For further purification did a flash Silica gel column chromatography (EtOAc: Hexane) and pure product was obtained and dried it in high vacuum. Yield: (80% 81.106 g).

Compound **3**: The vacuum dried compound **2** was divided into two batches (40.553 g in one reaction flask). Then vacuum dried compound **2** was dissolved in dry DCM and stirred at 0 ºC. Then Thiophenol (32.746 g, 264.079 mmol) was added at 0 ºC and $BF_3.Et_2O$ (57 ml, 421.334 mmol) were added to both the reaction flask slowly by using dropping funnel at 0 º and stirred to r.t. Reaction mixture was stirred around 12h at N_2 atmosphere. Reaction was monitored using TLC. After the completion of reaction, $BF_3.Et_2O$ was neutralized using Et_3N and the reaction mixture was extracted with $NaHCO_3$ and brine. The collected organic layer was dried under anhydrous Na_2SO_4 and evaporated the solvent using rota evaporator. For further purification did a silica gel column chromatography (EtOAc: Hexane) and pure product was obtained and dried it in high vacuum. Yield: (82% 78.758 g).

Compound **4**: The vacuum dried compound **3** (78.758 g, 180.224 mmol) was dissolved in Methanol (500 ml) and stirred at room temperature. Then NaOMe (50 g, 925,925 mmol) was added to the reaction mixture and stirred for 4h in N_2

atmosphere at RT. Reaction was monitored using TLC. After completion of reaction NaOMe was neutralized using IR 120 H+ resin. Then Filter the resin using filter paper and evaporated using rota evaporator. For further purification did a flash column gel chromatography (DCM: MeOH) and pure product was obtained and dried it in high vacuum. Yield (85% 48 g).

Compound **5**: The vacuum dried compound **4** (48 g, 154.340 mmol) was dissolved in dry ACN and stirred at r.t. Then catalytic amount of PTSA (17.834 g, 93.75 mmol) and $Ph(OMe)_2$ (74.579 ml, 496.899 mmol) were added in to the reaction mixture and stirred it in nitrogen atmosphere for 8h. Reaction was monitored using TLC. After completion of reaction PTSA was neutralized using Et3N. Evaporated the ACN using rota evaporation and the reaction mixture was dissolved in EtOAc and extracted with water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum. Yield (65% 41 g).

Compound **6**: Vacuum dried compound **5** (41 g, 102.5 mmol) was dissolved in DMF and stirred it in 0 ^{0}C. Then 2-(Bromomethyl)naphthalene (26.52 g, 120 mmol) and NaH (60% 6 g, 666.666 mmol) was added. Then the reaction mixture was stirred for 12h in N_2 atmosphere. The reaction was monitored using TLC. After the completion of reaction NaH is neutralized by adding some methanol. Then DMF was evaporated and the reaction mixture was dissolved in DCM and extracted by water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum. Yield (89% 51g).

Compound **7**: The vacuum dried compound **6** (30 g, 54.151 mmol) was dissolved in 2:1 DCM (100 ml) Methanol (50 ml) and stirred at room r.t. Then PTSA (12.2 g, 64.136 mmol) was added in to the reaction mixture and stirred in N_2 atmosphere for 6h. The reaction was monitored using TLC. After the completion of reaction PTSA was neutralized using Et_3N and evaporated the solvent using rota evaporator. The

reaction mixture was dissolved in EtOAc and extracted with water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum. Yield (83% 20 g).

Compound **8**: The vacuum dried compound **7** (20g 44.345 mmol) was dissolved in DCM and stirred at 0 ^{0}C. Then Et_3N (18.5 ml, 183.5 mmol) and catalytic amount of DMAP (2.70g, 22.17 mmol) was added in to reaction flask at 0 c. Then TBDPSiCl (14.62 ml, 53.21 mmol) was added to the reaction mixture slowly using syringe. The reaction was stirred around 8h in N_2 atmosphere. The reaction was monitored using TLC. The reaction mixture was extracted with water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum. Yield (76% 23.552 g).

Compound **9**: The vacuum dried compound **8** (23.552g, 34.1 mmol) was dissolved in DCM and stirred at r.t. Then DCC (12.32g, 59.82 mmol) and catalytic amount of DMAP (2.08 g, 17.04 mmol) was added in to the reaction flask. Then levlunic Acid (3.55 ml, 40.94 mmol) was added slowly using syringe. Up on addition of levulinic acid a white precipitate was formed. That was the side product urea. The reaction was monitored using TLC. After the completion of reaction the side product urea was filtered using filter paper. Then evaporated the solvent using rota evaporator. For further purification did a silica gel column chromatography (EtOAc: Hexane) and pure product was obtained and dried it in high vacuum. Yield (80% 21.38g).

Compound **10**. The vacuum dried compound **11** (2 g, 2.1 mm0l) and Linker N(Bn)Cbz-protected propanol amine (.489 g, 2.56 mmol) were dissolved in different solvents and 4A^0 MS was added in to the reaction flask and stirred at r.t for 1h in nitrogen atmosphere. Then maintained the temperature to -78 to -20 ^{0}C using acetone and dry ice and stirred for 20 to 30 minute. Then NIS (.72 g, 3.2 mmol) and TfOH (38 µl, .256 mmol) were rapidly added to the reaction flask. The colour

changed to dark pinkish colour. The reaction was monitored using TLC. After the completion of reaction MS was filtered using celite filtration funnel. The reaction mixture was extracted with sodium thiosulphte and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum

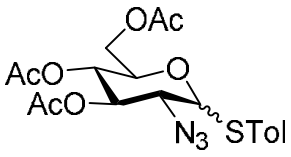
Compound **11**: The vacuum dried compound **9** (7 g, 8.86 mmol) was dissolved in AcOH 80 ml and stirred at r.t. Then Zn (5 g, 76.9 mmol) was added into the reaction flask and stirred for 4h in N_2 atmosphere. The reaction was monitored using TLC. After the completion of reaction the Zn was filtered using ciliate filtration funnel. The reaction mixture was dissolved in EtOAc and extracted with water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator and dried it in high vacuum. Yield (84% 5.5g). The vacuum dried compound A9 (5.5 g, 7.21 mmol) was dissolved in 2:1 THF (100 ml) H_2O (50 ml) and stirred at r.t. Then $NaHCO_3$ was added in to the reaction flask. After 10 minutes TrocCl was slowly added to the reaction flask using syringe and stirred for 10h in N_2 atmosphere. The reaction was monitored using TLC. After the completion of reaction THF was evaporated using rota evaporator. The reaction mixture was dissolved in EtOAc and extracted with water and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum. Yield (80% 5.8 g).

Compound **12**: The vacuum dried compound **11** (2 g, 2.1 mm0l) and Linker NCbz-protected propanol amine (.489 g, 2.56 mmol) were dissolved in DCM and $4A^0$ MS was added in to the reaction flask and stirred at r.t for 1h in nitrogen atmosphere. Then maintained the temperature to -20 ^{0}C using acetone and dry ice and stirred for 20 to 30 minute. Then NIS (.72 g, 3.2 mmol) and TfOH (38 µl, .256 mmol) were rapidly added to the reaction flask. The colour changed to dark pinkish colour. The reaction was monitored using TLC. After the completion of reaction MS was filtered using celite filtration funnel. The reaction mixture was extracted with sodium

thiosulphte and brine. The organic layer was collected and dried using anhydrous Na_2SO_4 and evaporated using rota evaporator. The reaction mixture was further purified by silica gel column chromatography (EtOAc: Hexane). Pure product was obtained and dried it in high vacuum

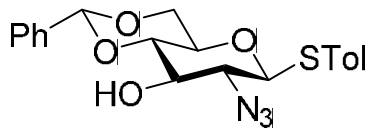
Characterization Data

Compound 3



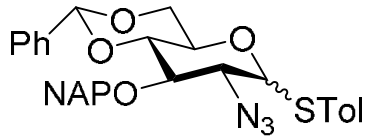
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 37.4, 7.9 Hz, 3H), 7.15 (t, *J* = 9.4 Hz, 3H), 5.57 (d, *J* = 5.5 Hz, 1H), 5.34 (t, *J* = 9.9 Hz, 1H), 5.11-4.87 (m, 2H), 4.62 (dd, *J* = 10.2, 3.6 Hz, 1H), 4.46-4.26 (d, 2H), 4.20 (dd, *J* = 11.2, 3.2 Hz, 1H), 4.09 – 4.00 (m, 2H), 3.72 – 3.63 (m, 0.42H), 3.36 (t, *J* = 9.9 Hz, 0.41H), 2.38 (s, 1H), 2.33 (s, 3H), 2.10 (s, 3H), 2.09 (s, 1H), 2.06 (s, 4H), 2.04 (s, 3H), 2.01 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48, 170.46, 169.82, 169.77, 169.65, 139.30, 138.43, 134.65, 132.82, 130.00, 129.86, 128.56, 126.13, 86.84, 85.69, 77.43, 77.11, 76.79, 75.67, 74.44, 72.00, 68.77, 68.39, 68.06, 62.43, 62.01, 61.97, 61.60, 21.21, 21.12, 20.70, 20.64, 20.64, 20.61, 20.60, 20.55. HRMS (ESI) $C_{19}H_{23}O_7SN_3Na$ Caluculated-460.114**;** Expected**:** 460.114

Compound 5



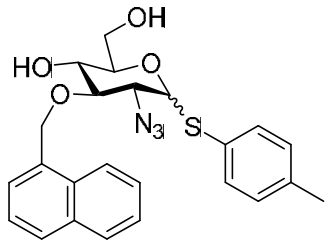
^{1}H NMR of β anomer (400 MHz, $CDCl_3$) δ 7.53 – 7.34 (m, 7H), 7.20 (d, *J* = 7.9 Hz, 2H), 5.53 (s, 1H), 4.45 (d, *J* = 10.1 Hz, 1H), 4.38 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.74 (dt, *J* = 17.7, 9.3 Hz, 2H), 3.42 (dt, *J* = 9.5, 6.6 Hz, 2H), 3.31 (t, *J* = 9.6 Hz, 1H), 3.01 (s, 1H), 2.40 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.13, 136.75, 134.30, 129.94, 129.47, 128.44, 126.79, 126.27, 101.95, 86.82, 80.25, 74.08, 70.24, 68.45, 65.01, 21.24. HRMS (ESI) $C_{20}H_{21}O_4SNa$ Calculated: 422.1150**;** Expected**:** 422.1152

Compound 6



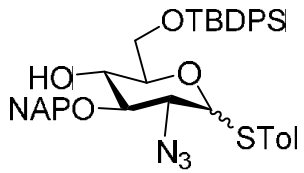
^{1}H NMR of α **anomer** (400 MHz, Chloroform-d) δ 7.84 – 7.74 (m, 4H), 7.55 – 7.44 (m, 5H), 7.44 – 7.36 (m, 5H), 7.14 (d, *J* = 8.1 Hz, 2H), 5.61 (s, 1H), 5.51 (d, *J* = 5.0 Hz, 1H), 5.17 – 4.96 (m, 2H), 4.47 (td, *J* = 10.0, 4.9 Hz, 1H), 4.24 (dd, *J* = 10.3, 4.9 Hz, 1H), 4.07 – 3.96 (m, 2H), 3.78 (td, *J* = 10.3, 9.7, 2.9 Hz, 2H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.37, 137.17, 135.22, 133.31, 133.13, 130.01, 129.15, 129.09, 128.36, 128.20, 128.03, 127.70, 127.03, 126.12, 126.09, 126.04, 125.95, 101.60, 88.18, 82.77, 77.94, 75.21, 68.65, 63.75, 63.74, 21.17. ^{1}H NMR of **β anomer** (400 MHz, $CDCl_3$) δ 7.82 – 7.74 (m, 4H), 7.47 (dd, *J* = 11.6, 8.9 Hz, 6H), 7.41 – 7.37 (m, 4H), 7.14 (d, *J* = 7.9 Hz, 2H), 5.58 (s, 1H), 5.05 (d, *J* = 11.2 Hz, 1H), 4.94 (d, *J* = 11.2 Hz, 1H), 4.42 (d, *J* = 10.3 Hz, 1H), 4.38 (dd, *J* = 10.6, 5.0 Hz, 1H), 3.78 (t, *J* = 10.3 Hz, 1H), 3.72 – 3.60 (m, 2H), 3.44 (td, *J* = 9.8, 5.0 Hz, 1H), 3.35 (dd, *J* = 10.1, 8.7 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.14, 137.12, 135.05, 134.50, 133.24, 133.13, 129.92, 129.13, 128.34, 128.22, 127.98, 127.68, 127.23, 126.59, 126.17, 126.15, 126.07, 126.03, 126.00, 101.35, 86.69, 81.34, 80.91, 75.19, 70.46, 68.54, 64.67, 21.21. HRMS (ESI) $C_{31}H_{29}O_4SNa$ Calculated: 562.1776**;** Expected**:** 562.1774

Compound 7



^{1}H NMR of α **anomer** (400 MHz, Chloroform-*d*) δ 8.04 – 7.77 (m, 4H), 7.62 – 7.41 (m, 5H), 7.16 (d, *J* = 7.9 Hz, 2H), 5.12 (d, *J* = 11.4 Hz, 1H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.44 (d, *J* = 9.8 Hz, 1H), 3.83 (ddd, *J* = 45.3, 12.0, 4.2 Hz, 2H), 3.58 (t, *J* = 9.2 Hz, 1H), 3.45 – 3.23 (m, 3H), 2.66 (s, 1H), 2.37 (s, 3H). 1.81 – 1.66 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 138.98 , 135.12 , 134.03 , 133.15 , 129.93 , 128.65 , 127.99 , 127.77 , 127.13 , 127.06 , 126.35 , 126.23 , 125.82 , 86.44 , 84.67 , 79.30 , 75.56 , 70.32 , 64.84 , 62.46 , 21.19 . ^{1}H NMR of β **anomer** (400 MHz, Chloroform-*d*) δ 7.97 – 7.80 (m, 4H), 7.60 – 7.46 (m, 3H), 7.44 – 7.36 (m, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 5.56 – 5.41 (m, 1H), 5.15 (d, *J* = 11.4 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.32 – 4.10 (m, 1H), 3.95 – 3.63 (m, 5H), 2.77 (d, *J* = 14.0 Hz, 1H), 2.36 (s, 3H), 1.81 – 1.66 (m, 1H).^{13}C NMR (101 MHz, Chloroform-*d*) δ ^{13}C NMR (101 MHz, Chloroform-*d*) δ 178.96 , 138.14 , 136.34 , 127.90 , 124.59 , 123.41 , 123.12 , 123.04 , 122.76 , 118.62 , 112.59 , 73.86 , 73.10 , 72.40 , 72.13 , 71.52 , 70.14 , 67.00 , 64.90 , 61.77 , 21.04 HRMS (ESI) $C_{24}H_{25}N_3O_4SNa$ Calculated: 474.176 Expected: 474.146

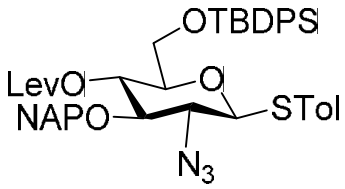
Compound 8



^{1}H NMR of α **anomer** (400 MHz, Chloroform-*d*) δ 7.96 – 7.82 (m, 4H), 7.75 (ddd, *J* = 8.1, 4.3, 1.6 Hz, 4H), 7.59 – 7.38 (m, 11H), 7.09 (d, *J* = 7.9 Hz, 2H), 5.15 – 4.94 (m, 2H), 4.41 (d, *J* = 10.0 Hz, 1H), 4.01 – 3.93 (m, 2H), 3.76 (td, *J* = 9.2, 2.7 Hz, 1H), 3.54 – 3.29 (m, 3H), 2.63 (d, *J* = 2.7 Hz, 1H), 2.35 (s, 3H),1.12 (s, 9H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 180.65 , 178.70 , 173.06 , 135.94 , 135.71 , 128.42 , 124.28 , 123.17 , 122.86 , 122.54 , 120.95 , 115.20 , 113.90 , 86.10 , 77.46 , 73.84 , 73.79 ,

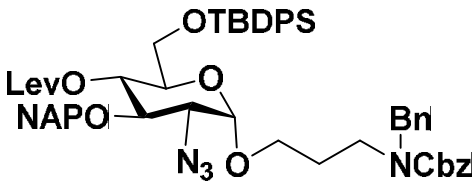
73.69 , 72.99 , 72.67 , 72.17 , 72.05 , 72.03 , 71.89 , 71.41 , 70.09 , 70.00 , 69.96 , 65.71 , 64.29 , 64.25 , 61.22 , 31.71 , 26.94 , 21.30 , 19.35 . ^{1}H NMR of β **anomer** (400 MHz, Chloroform-*d*) δ 7.94 – 7.82 (m, 4H), 7.68 (ddd, *J* = 9.2, 8.0, 1.4 Hz, 4H), 7.55 – 7.30 (m, 11H), 7.03 (d, *J* = 8.0 Hz, 2H), 5.47 (d, *J* = 5.3 Hz, 1H), 5.16 – 5.00 (m, 2H), 4.28 (dt, *J* = 9.1, 4.4 Hz, 1H), 3.96 – 3.71 (m, 5H), 2.72 (s, 1H), 2.31 (s, 3H), 1.07 (s, 9H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 135.78 , 132.81 , 129.96 , 128.54 , 127.87 , 127.17 , 126.16 , 87.74 , 77.47 , 76.83 , 75.67 , 73.07 , 71.87 , 64.40 , 26.96 , 19.36 , 14.27 .HRMS (ESI) $C_{40}H_{43}N_3NaO_4SSi$ Calculated: 712.264 Expected: 712.264

Compound 9



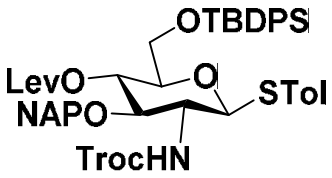
^{1}H NMR (400 MHz, Chloroform-d) δ 7.97 – 7.64 (m, 8H), 7.59 – 7.35 (m, 11H), 7.06 (d, *J* = 7.9 Hz, 2H), 5.12 (td, *J* = 9.7, 1.7 Hz, 1H), 4.99 (d, *J* = 11.5 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.42 (dd, *J* = 10.0, 1.3 Hz, 1H), 3.85 – 3.67 (m, 2H), 3.63 – 3.37 (m, 3H), 2.52 – 2.40 (m, 2H), 2.34 (s, 3H), 2.26 (ddd, *J* = 8.2, 6.6, 1.4 Hz, 2H), 2.06 (s, 3H),1.09 (d, *J* = 1.6 Hz, 9H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 206.08 , 171.26 , 135.82 , 135.12 , 134.29 , 129.96 , 128.29 , 127.78 , 126.13 , 83.00 , 77.45 , 76.82 , 75.45 , 69.72 , 64.67 , 62.56 , 37.67 , 29.80 , 26.81 , 19.33 .HRMS (ESI) $C_{45}H_{49}N_3NaO_6SSi$ Calculated; 810.301 Expected: 810.301

Compound 10



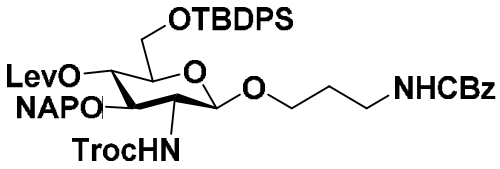
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.71 (m, 6H), 7.68 – 7.55 (m, 6H), 7.51 – 7.26 (m, 22H), 5.45 – 5.36 (m, 1H), 5.14 – 5.01 (m, 5H), 4.96 – 4.85 (m, 3H), 4.72 (d, *J* = 10.8 Hz, 1H), 4.15 – 3.21 (m, 15H), 2.47 – 2.14 (m, 7H), 2.09 – 2.00 (m, 2H), 2.00 (s, 3H), 1.01 (s, 9H). $C_{56}H_{62}N_4NaO_9Si$ Calculated; 985.418 Expected: 985.418

Compound 11



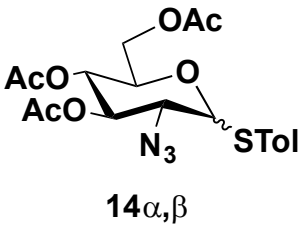
^{1}H NMR of (400 MHz, Chloroform-*d*) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.61 (m, 8H), 7.51 – 7.27 (m, 11H), 6.99 (d, *J* = 8.0 Hz, 2H), 5.19 – 5.02 (m, 2H), 4.82 – 4.69 (m, 2H), 4.63 – 4.47 (m, 1H), 4.28 – 4.08 (m, 1H), 3.80 – 3.67 (m, 2H), 3.56 (s, 1H), 2.46 (t, *J* = 6.8 Hz, 2H), 2.36 – 2.15 (m, 5H), 2.08 – 1.96 (m, 3H), 1.57 (s, 1H), 1.03 (d, *J* = 7.3 Hz, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 206.11, 171.38 , 138.00 , 135.75 , 133.28 , 132.22 , 130.00 , 128.51 , 128.10 , 127.69 , 126.84 , 125.89 , 95.46 , 94.30 , 77.46 , 76.82 , 73.65 , 62.76 , 54.79 , 37.79 , 29.82 , 27.94 , 26.83 , 21.22 , 19.37 .HRMS (ESI) $C_{48}H_{52}Cl_3NNaO_8SSi$ Calculated: 958.215 Expected: 958.215

Compound 12



^{1}H NMR of (400 MHz, Chloroform-*d*) δ 7.84 (td, *J* = 7.2, 6.6, 3.2 Hz, 3H), 7.78 – 7.66 (m, 5H), 7.53 – 7.46 (m, 2H), 7.46 – 7.30 (m, 12H), 5.60 (d, *J* = 8.0 Hz, 1H), 5.20 – 5.04 (m, 4H), 4.82 (d, *J* = 2.5 Hz, 2H), 4.60 (s, 2H), 3.94 (dt, *J* = 10.3, 5.6 Hz, 2H), 3.76 (t, *J* = 4.3 Hz, 2H), 3.57 – 3.37 (m, 4H), 3.23 (dq, *J* = 13.8, 5.8 Hz, 1H), 2.54 (td, *J* = 6.6, 2.4 Hz, 2H), 2.36 (t, *J* = 6.6 Hz, 2H), 2.08 (s, 3H), 1.70 (s, 3H), 1.06 (s, 9H).HRMS (ESI) $C_{52}H_{59}Cl_3N_2NaO_{11}Si$ Calculated: 1043.285 Expected: 1043.285

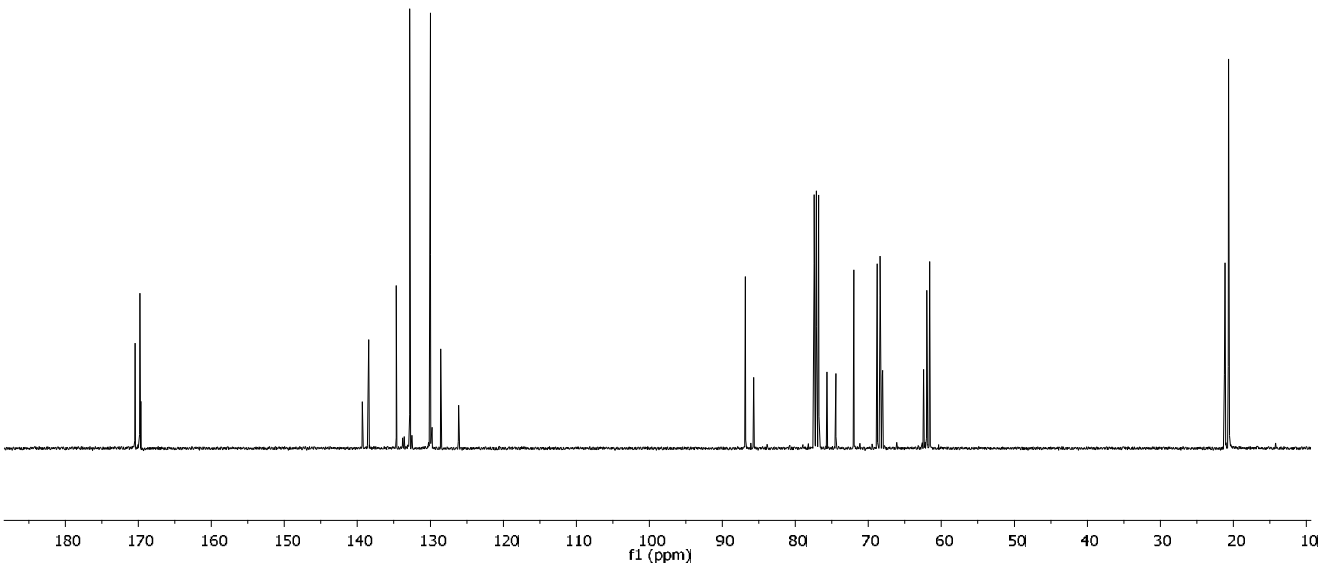
NMR SPECTRA



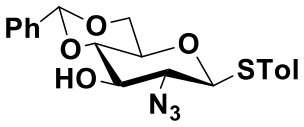
14α,β

^{1}HNMR

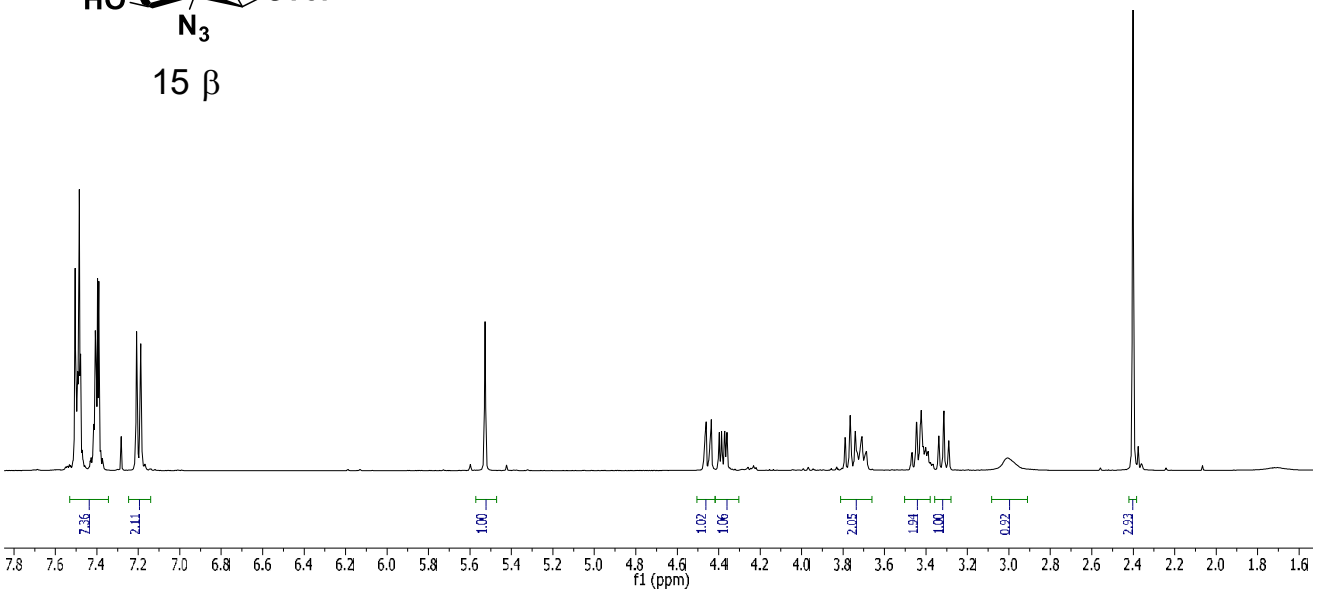
^{13}C NMR



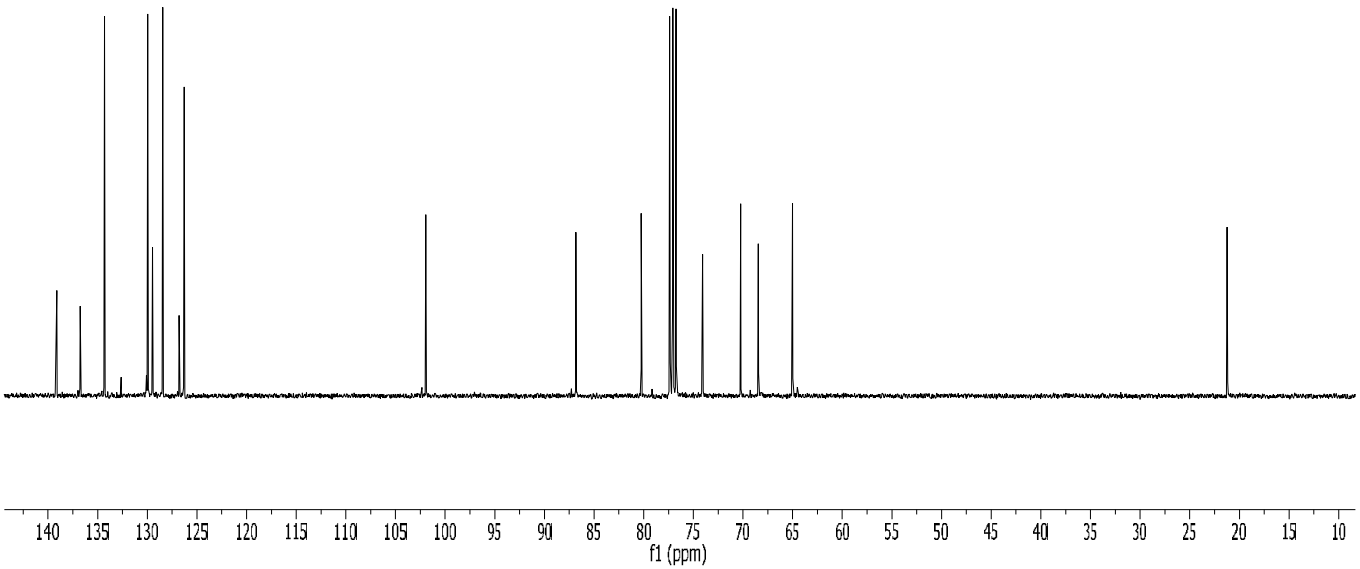
^{1}H NMR

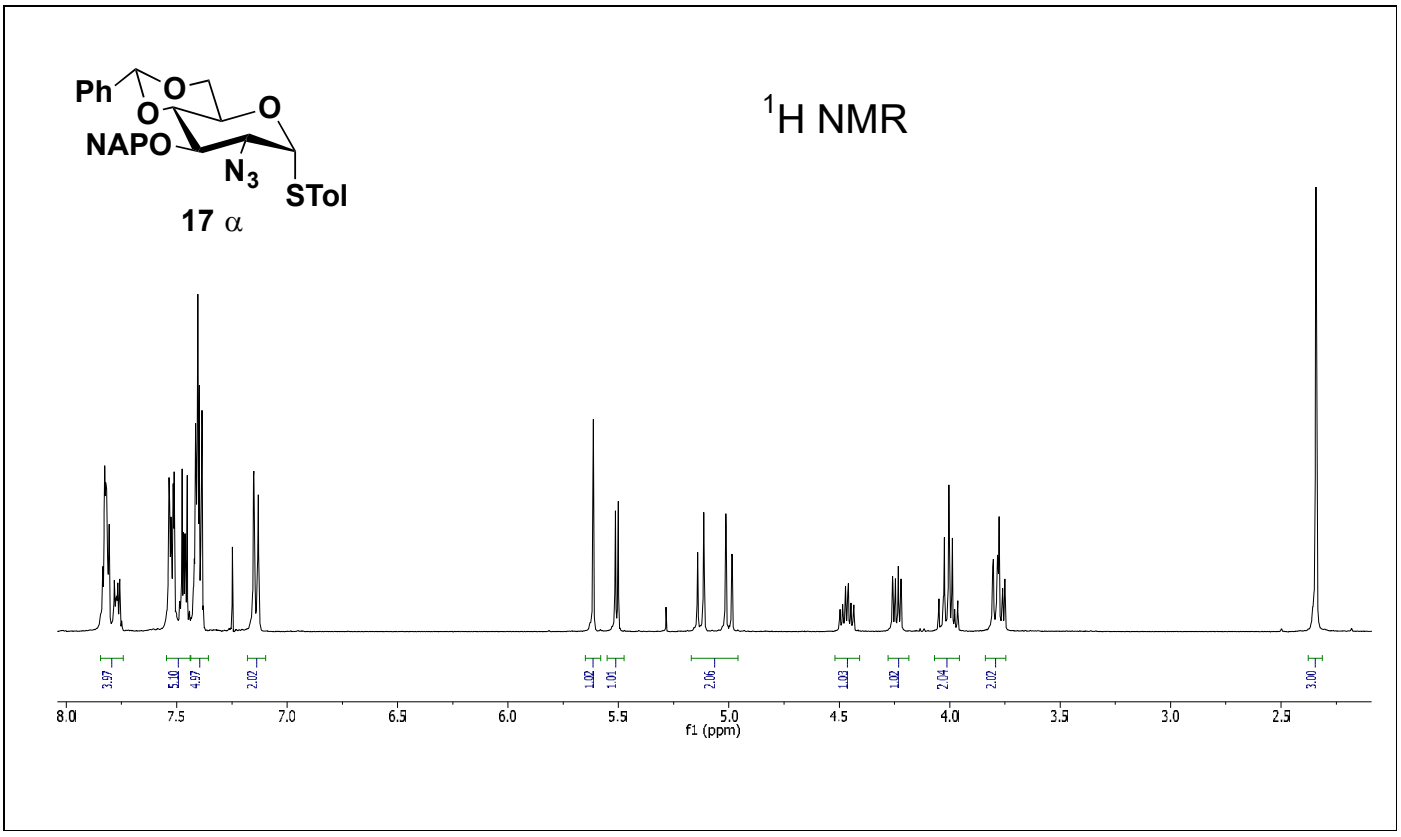


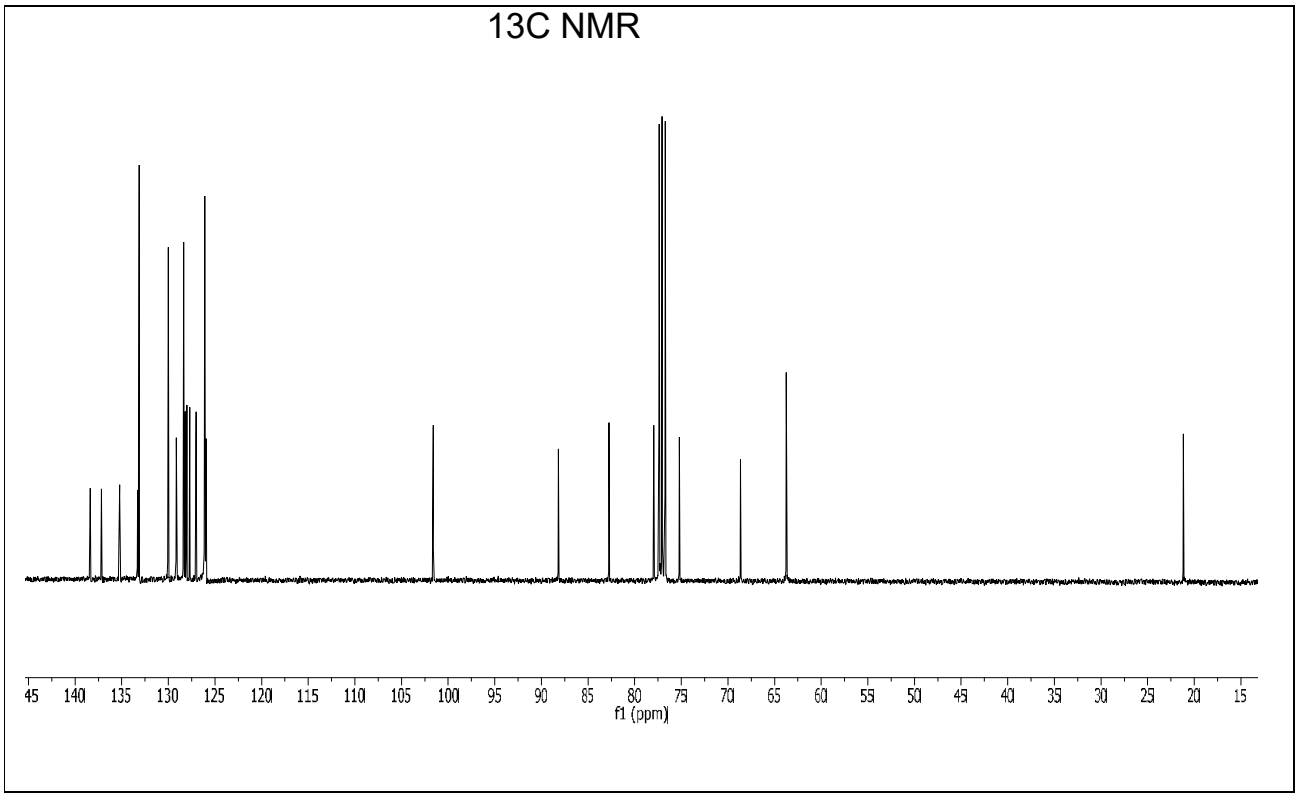
15 β



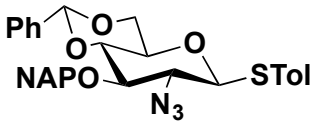
^{13}C NMR



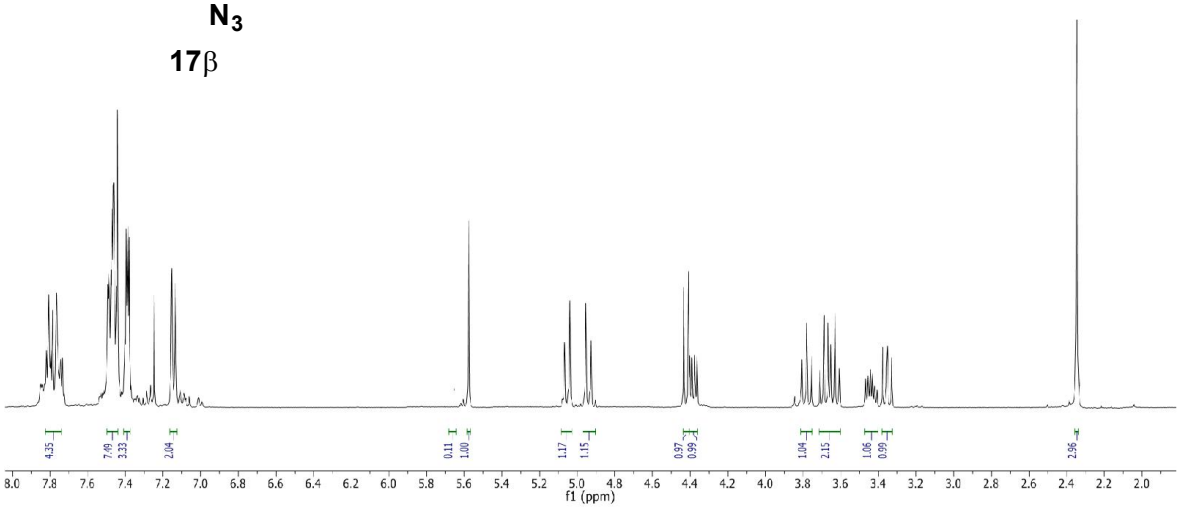

Ph
O
O
O
NAPO
N3
STol
17 α
1H NMR
f1 (ppm)


13C NMR
f1 (ppm)

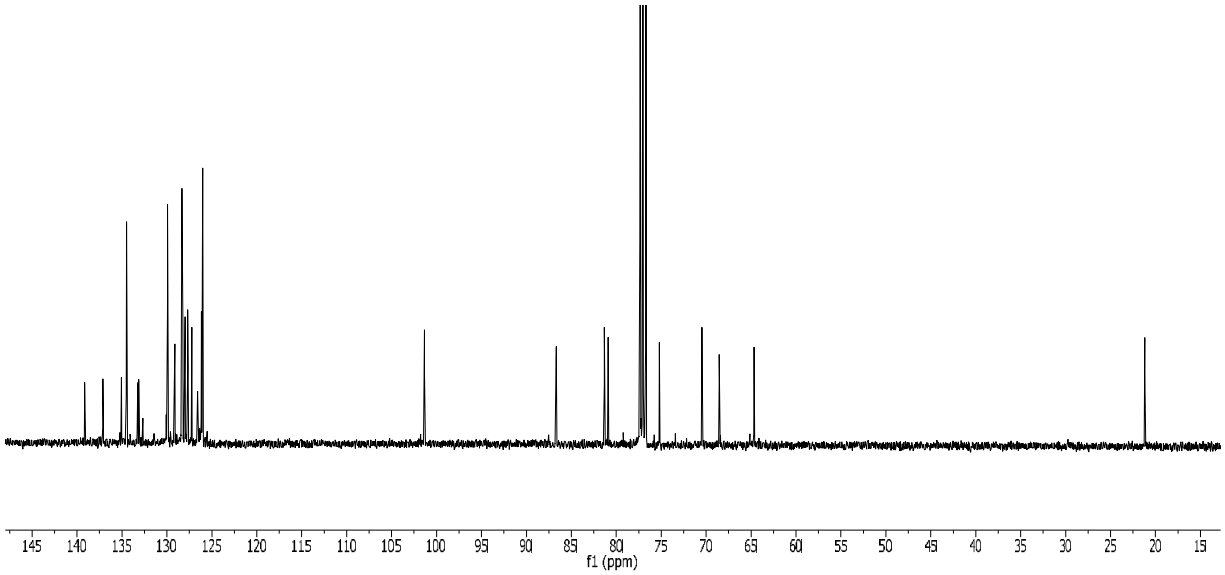
^{1}H NMR



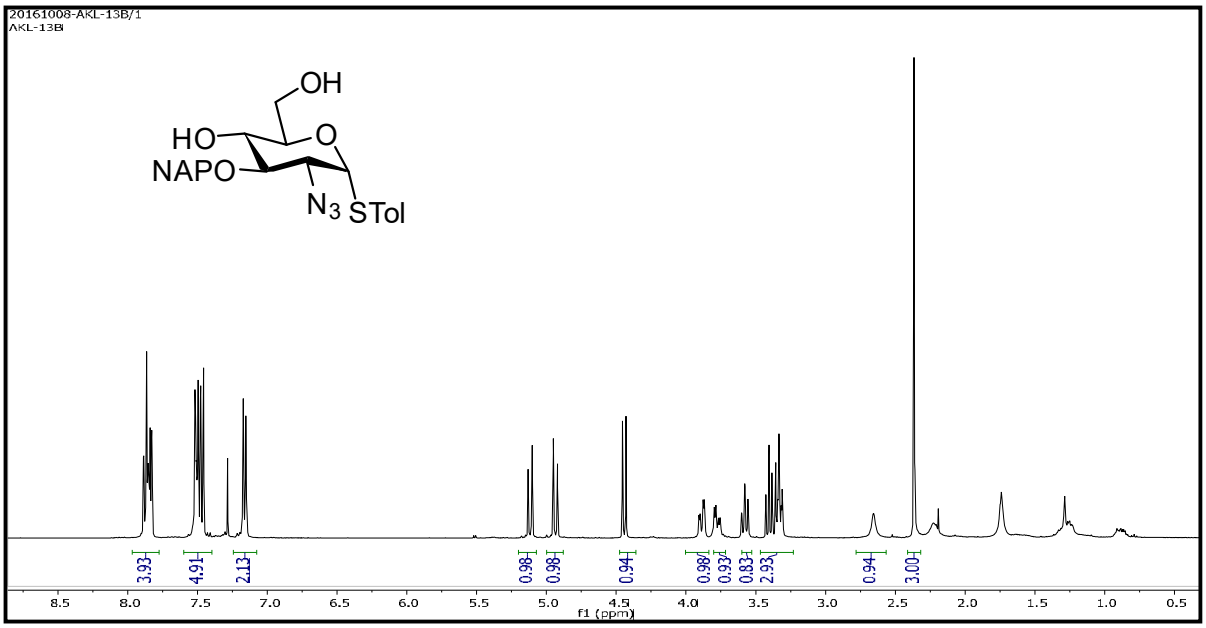
17β



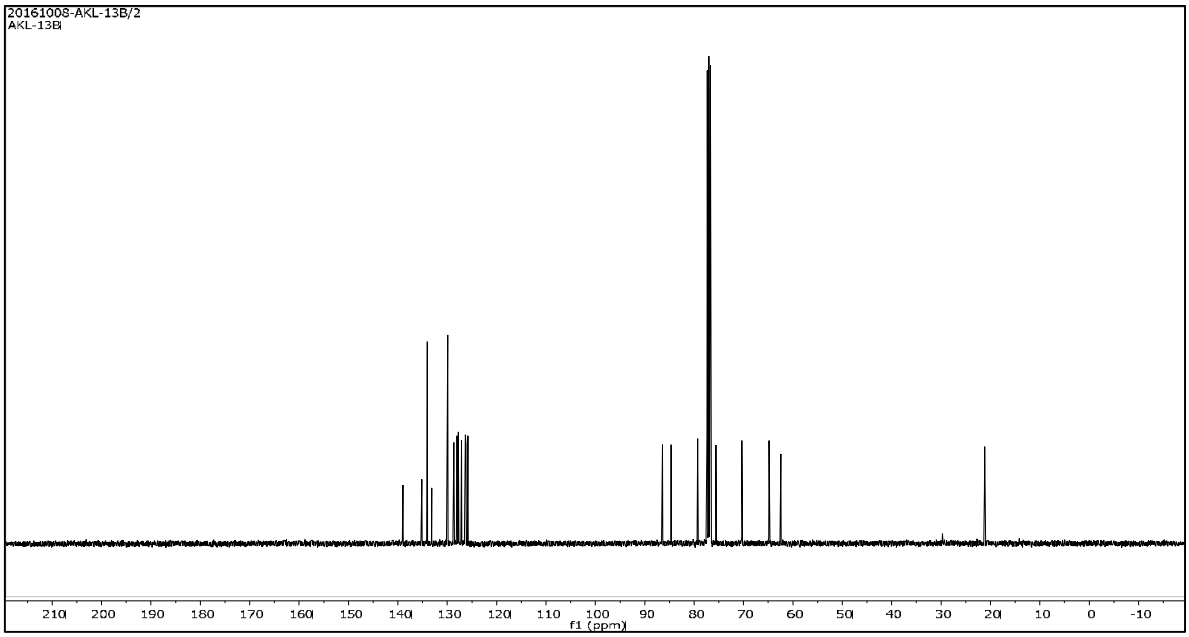
^{13}C NMR



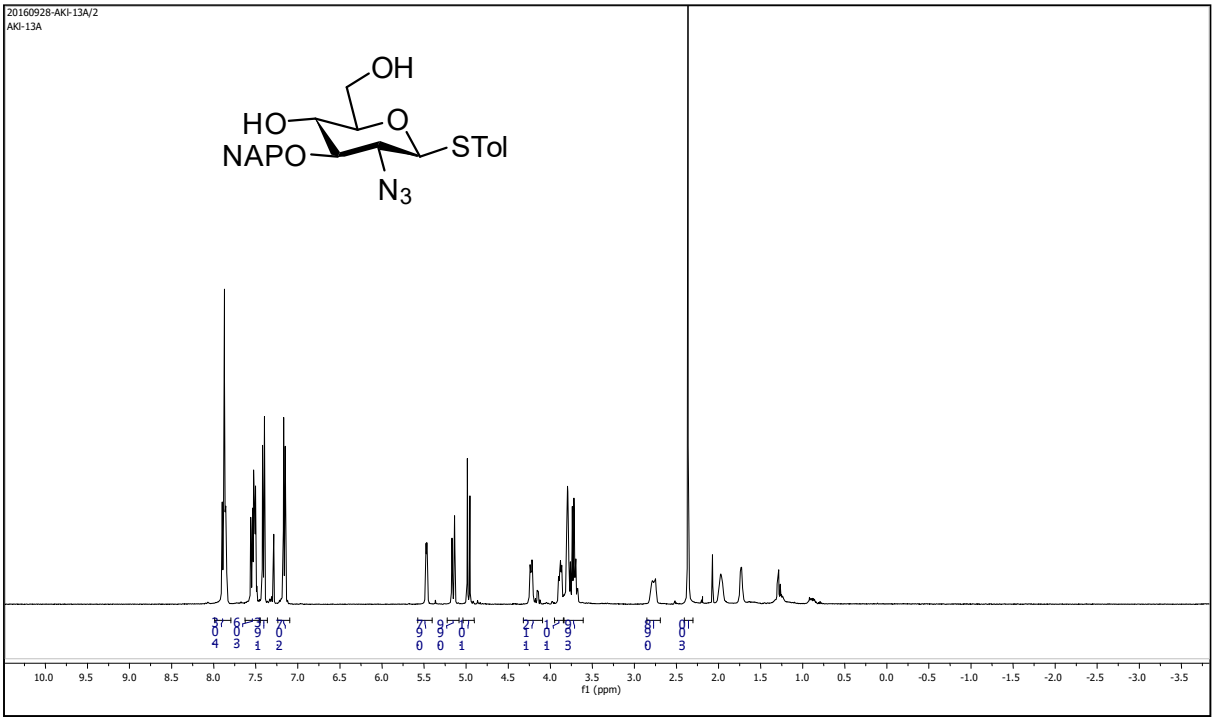
^{1}H NMR



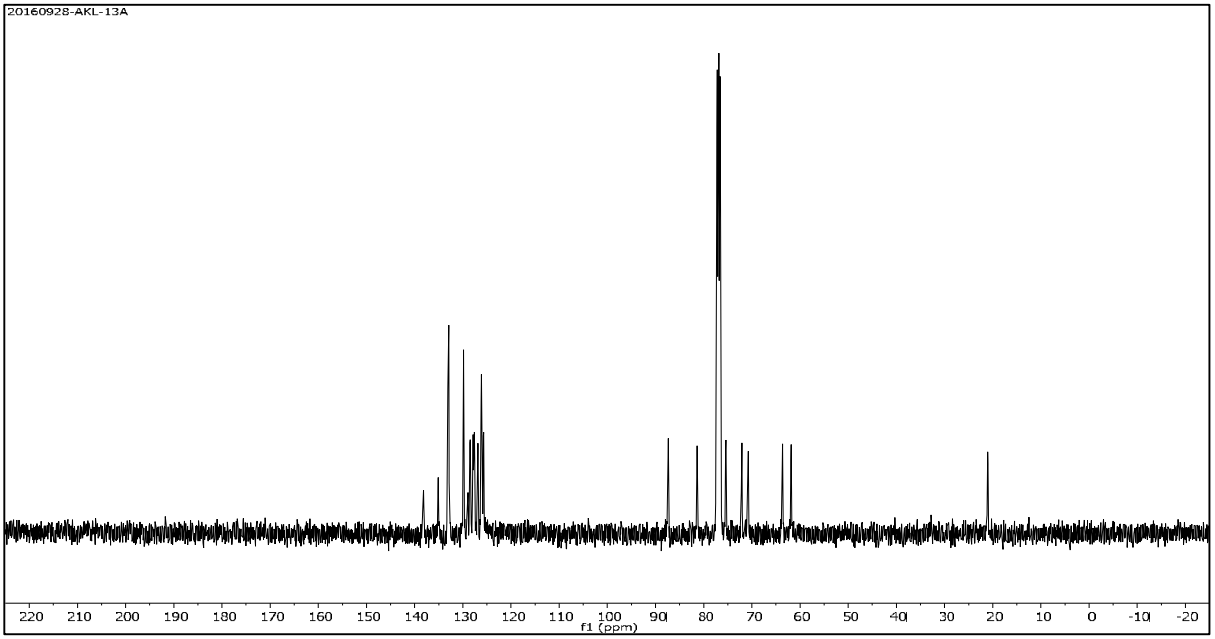
^{13}C NMR



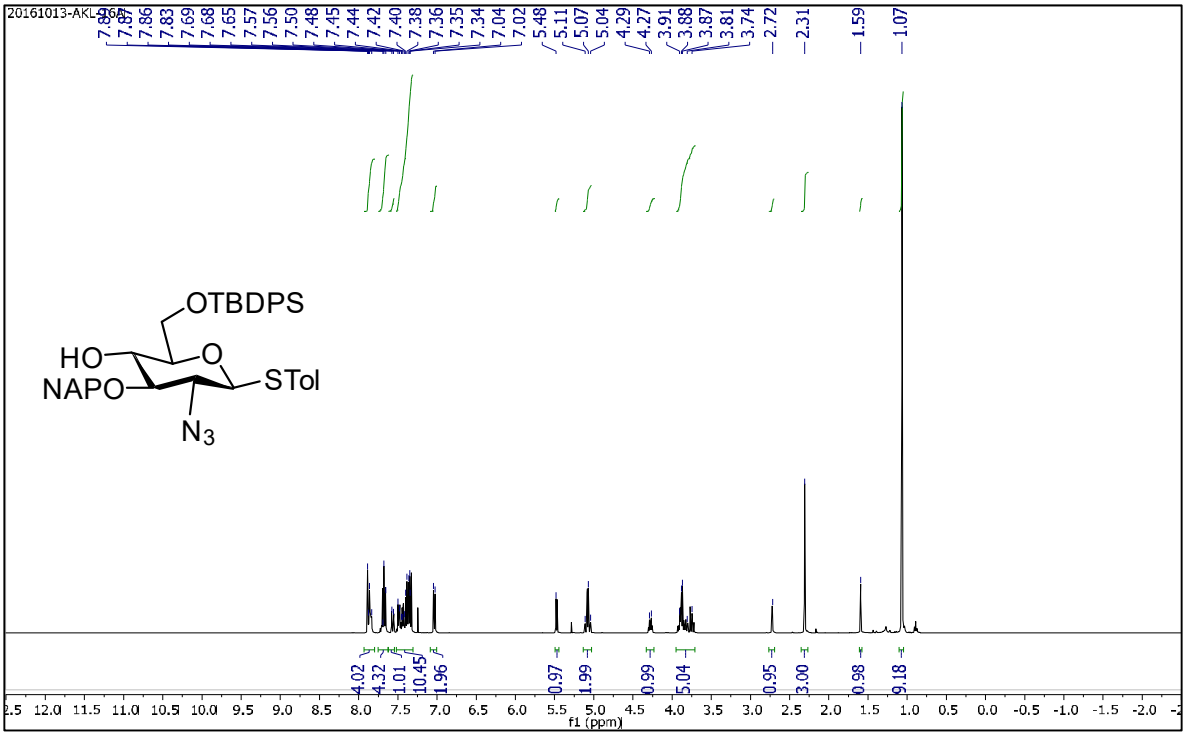
^{1}H NMR



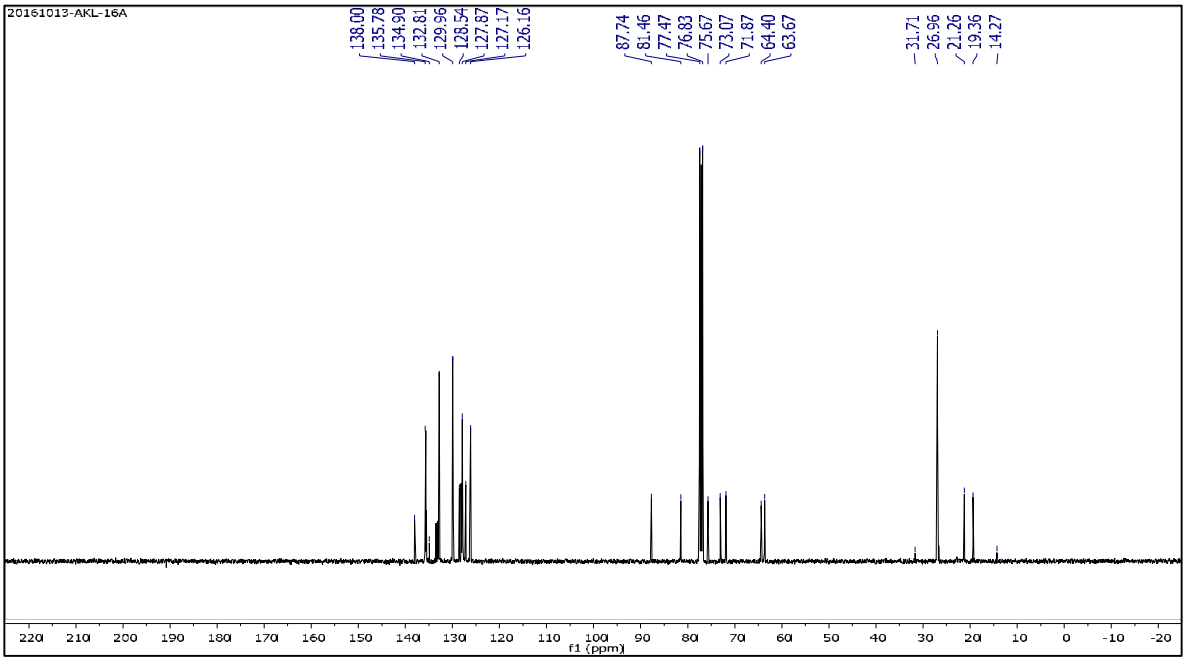
^{13}C NMR



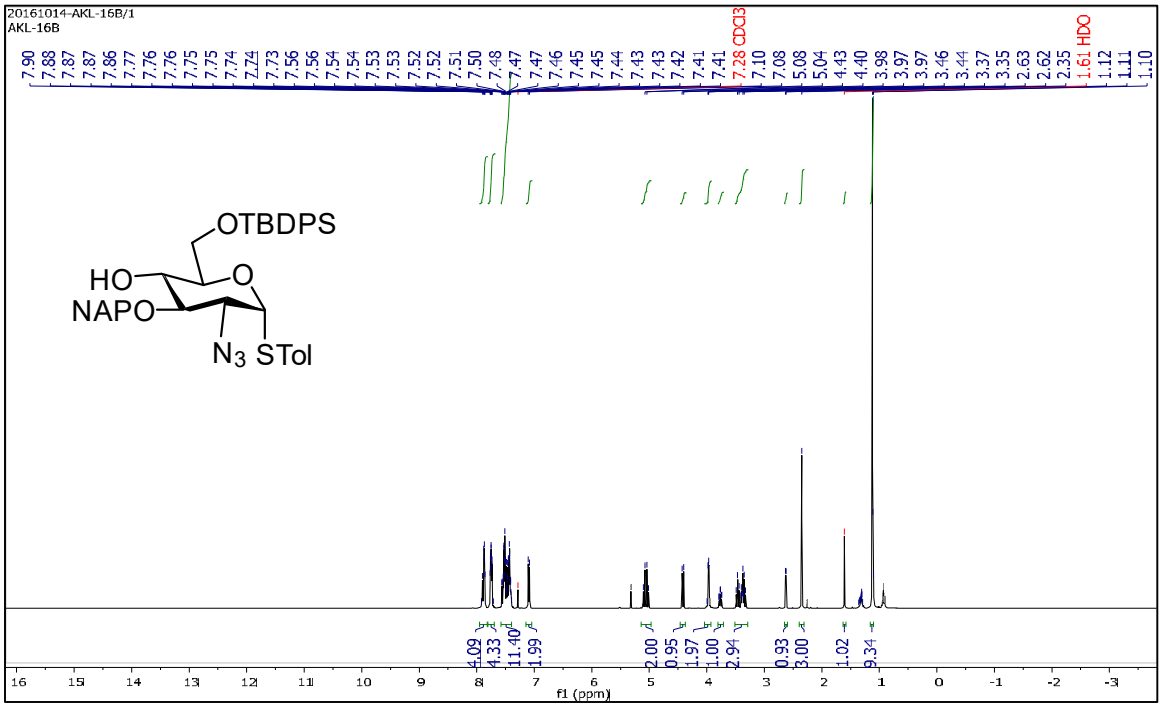
^{1}H NMR



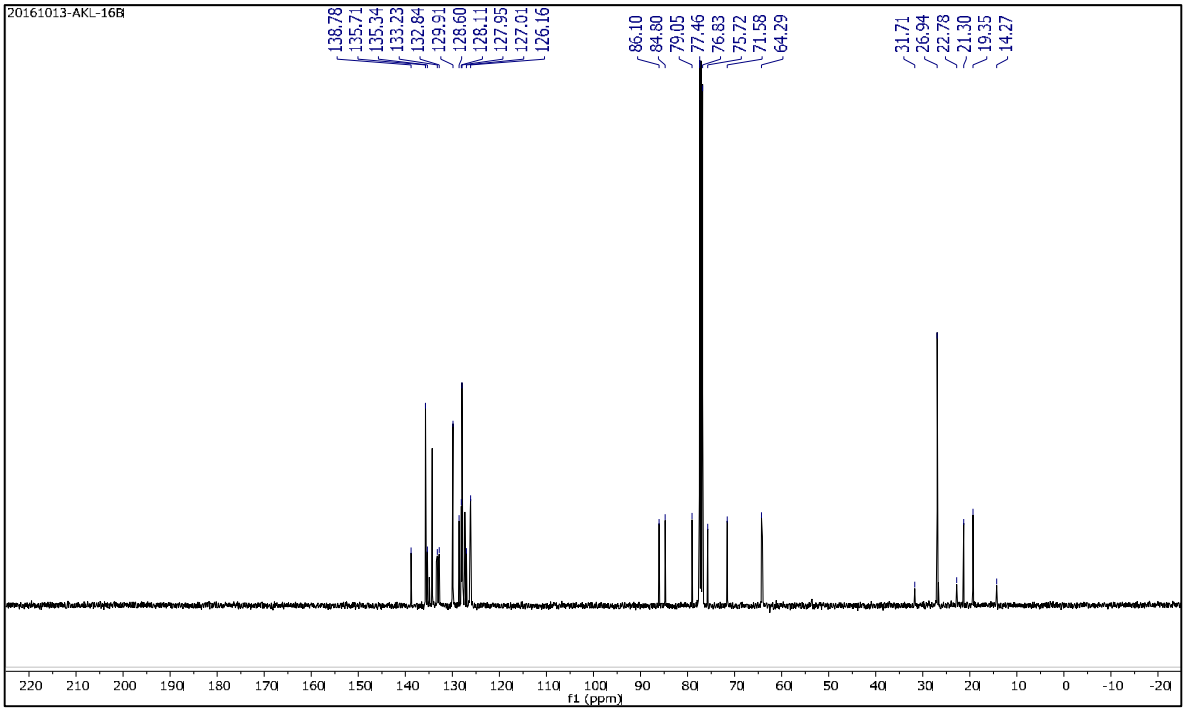
^{13}C NMR



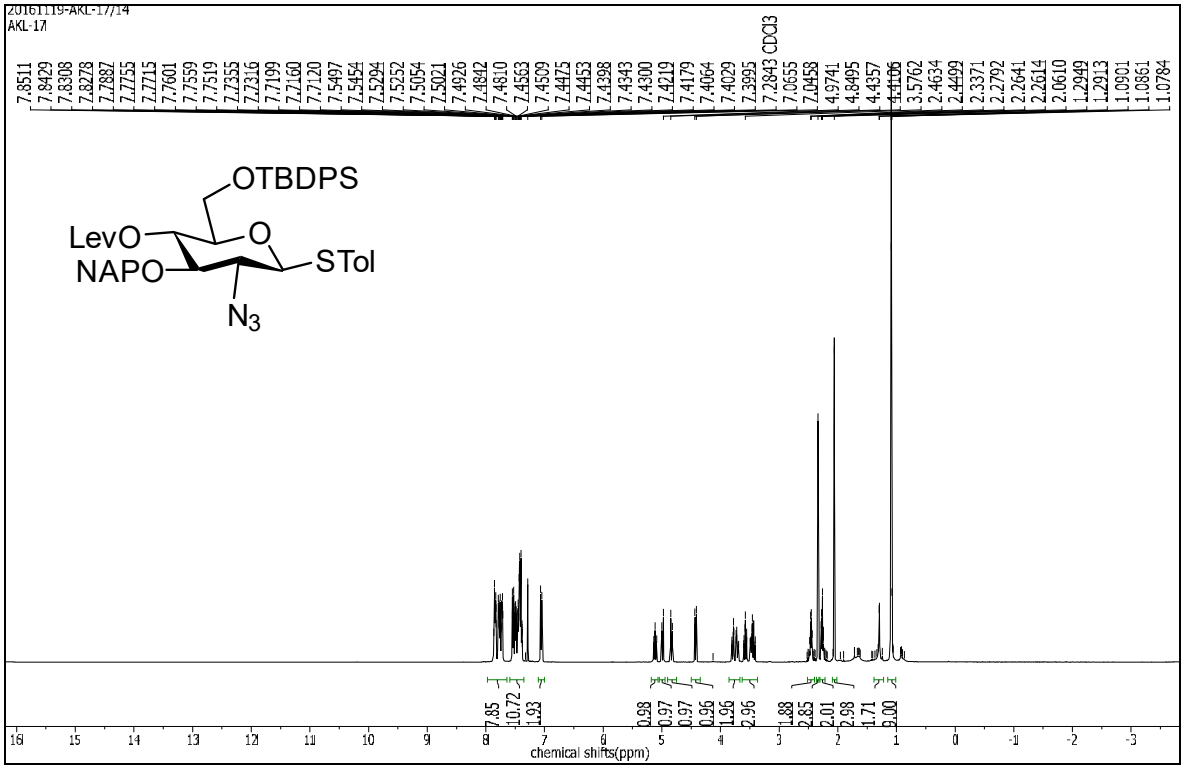
^{1}H NMR



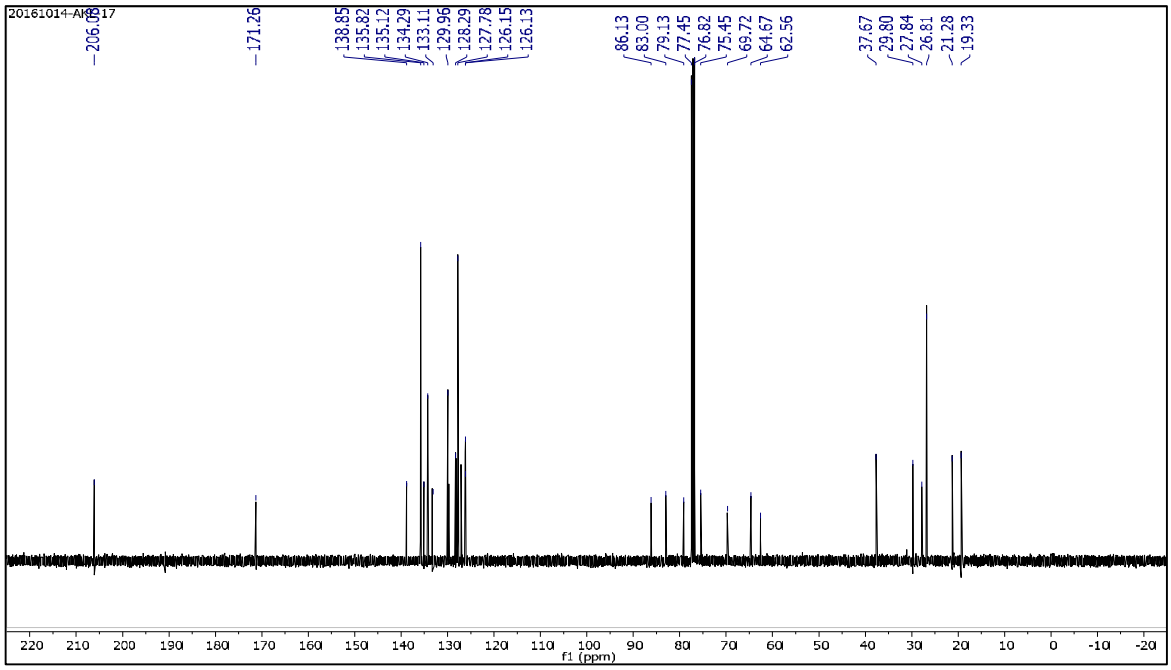
^{13}C NMR



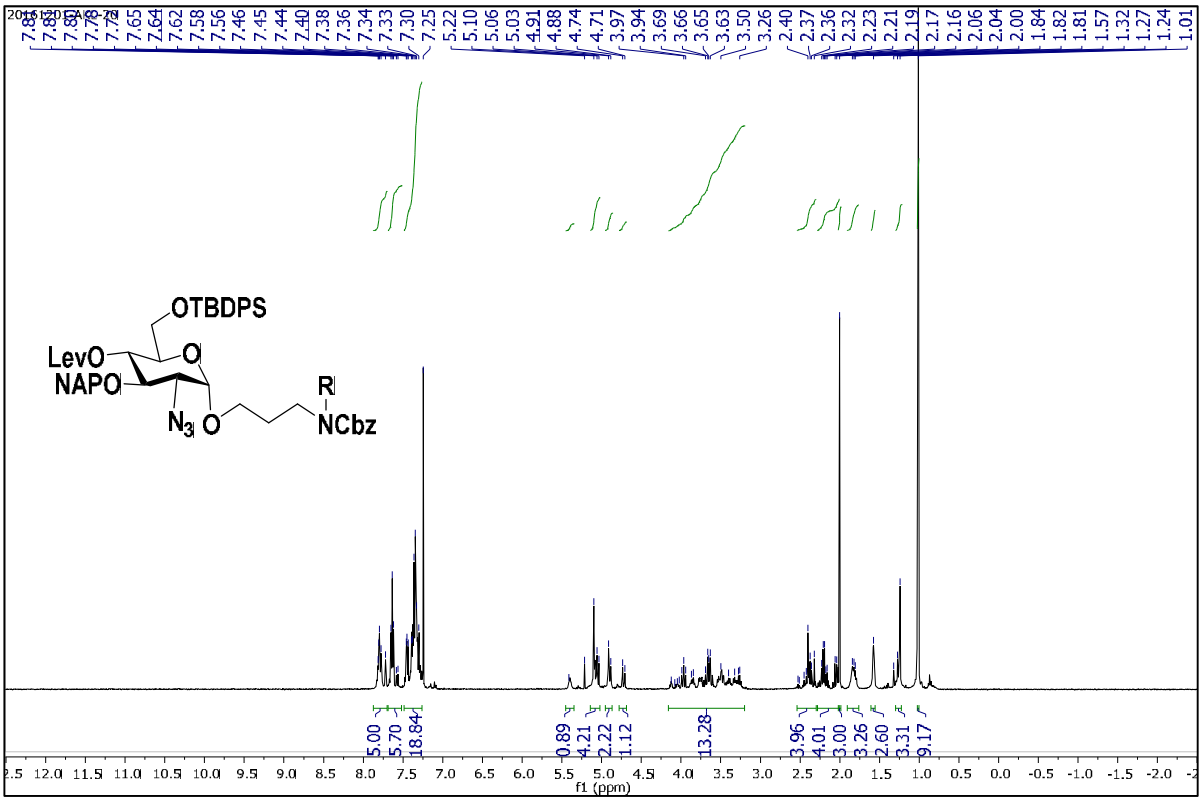
^{1}H NMR



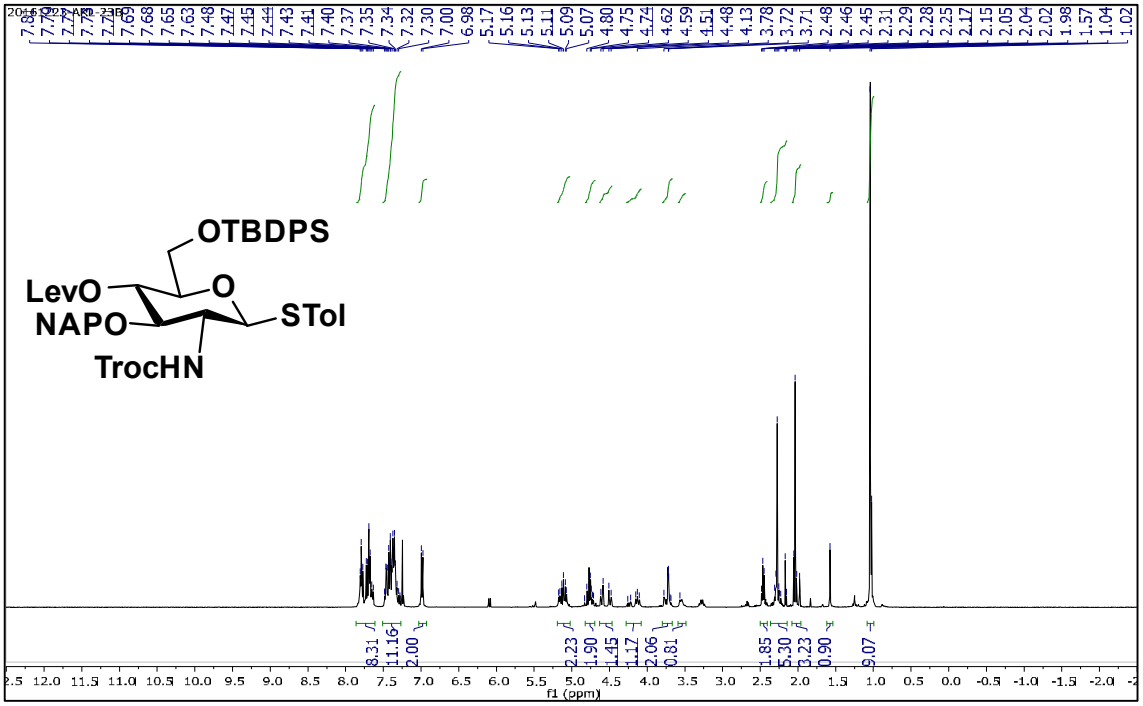
^{13}C NMR



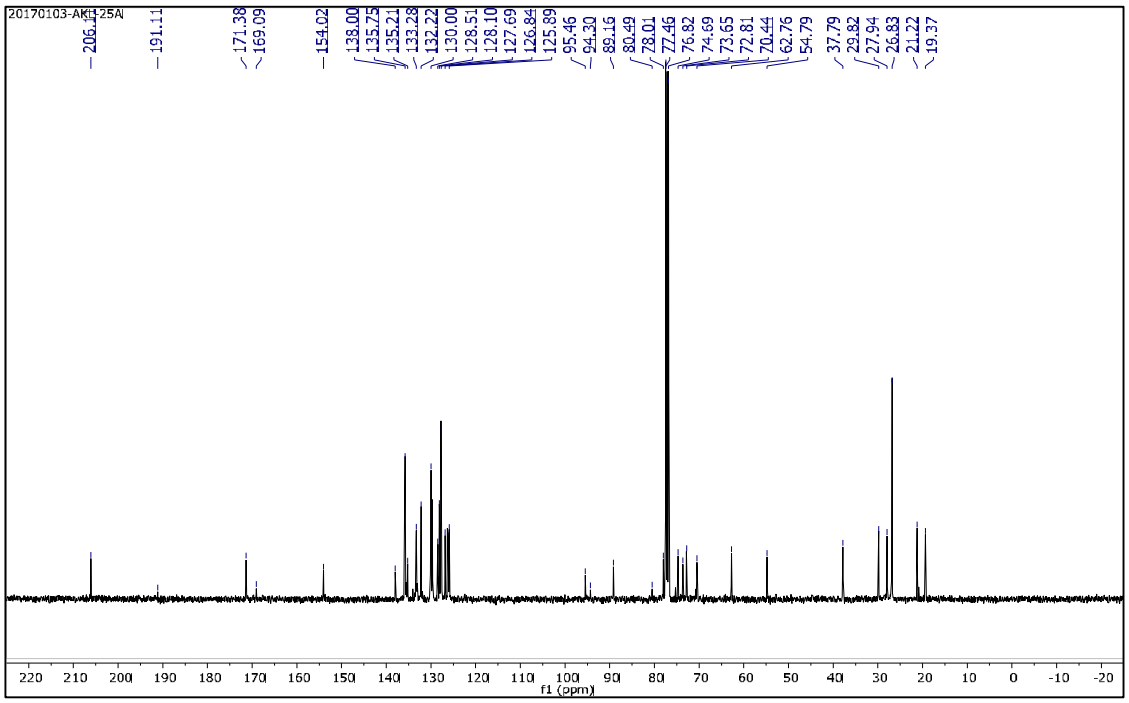
^{1}H NMR



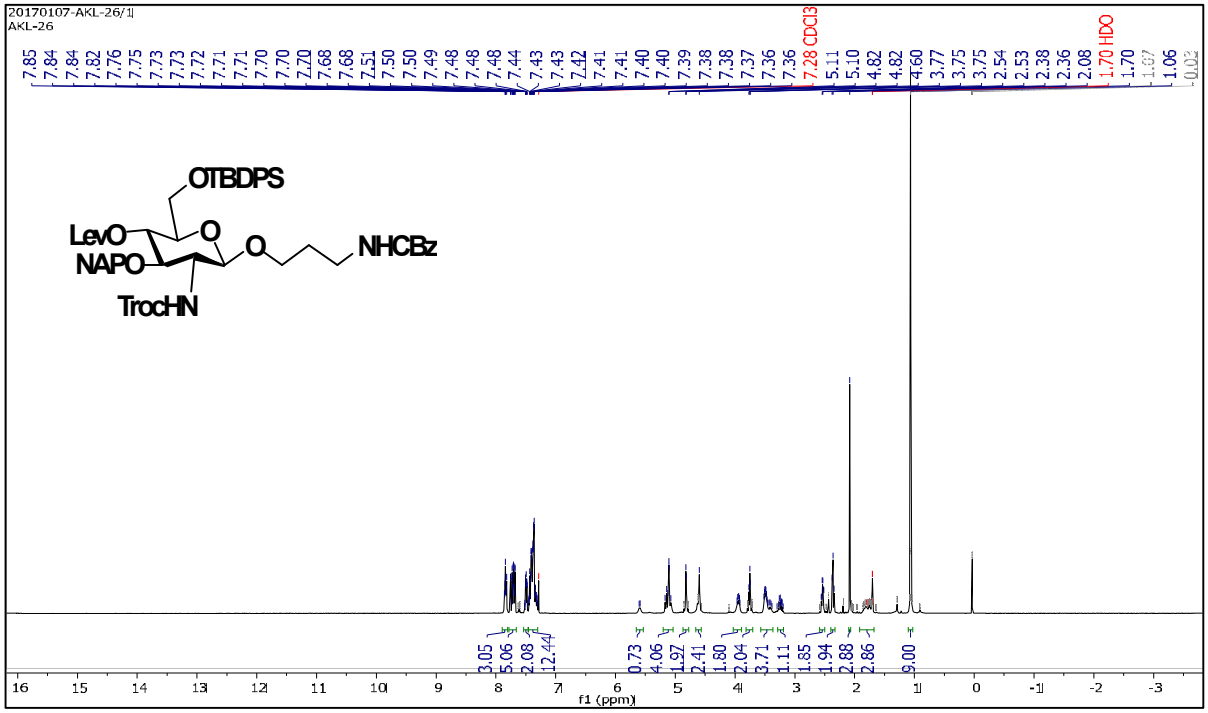
^{1}H NMR



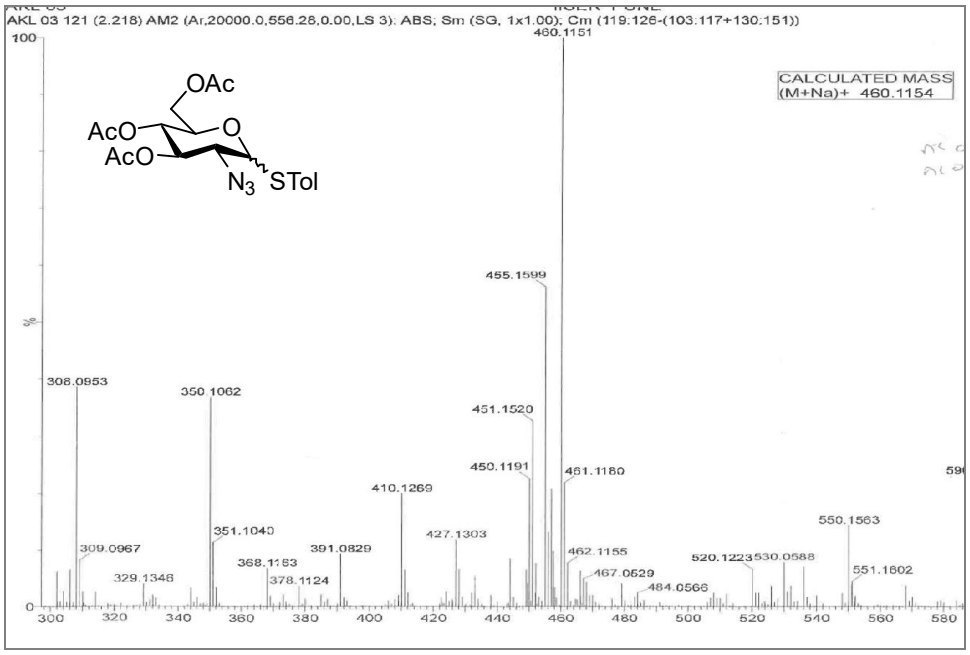
^{13}C NMR

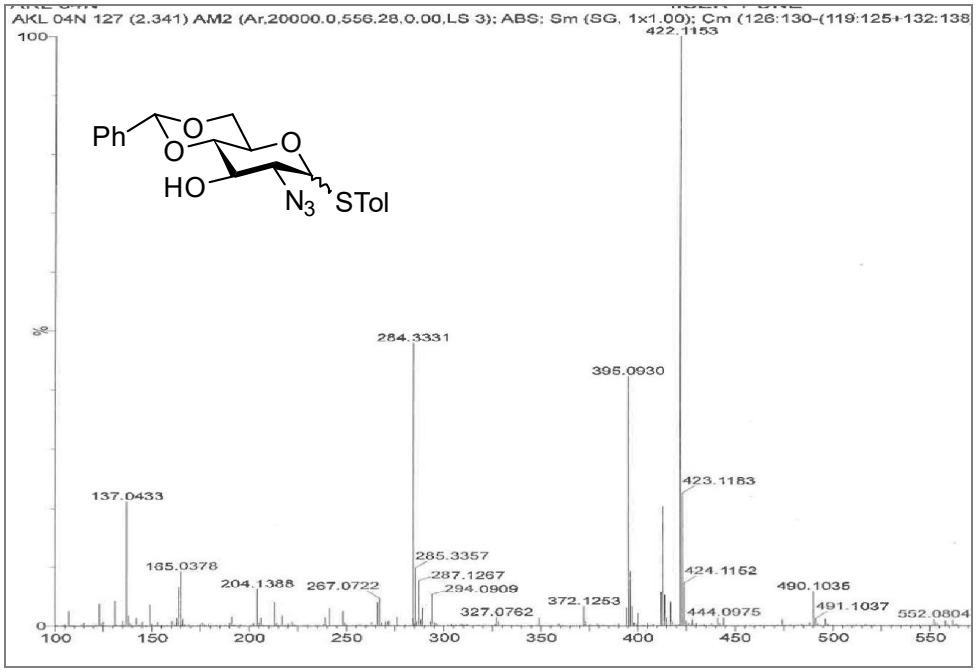


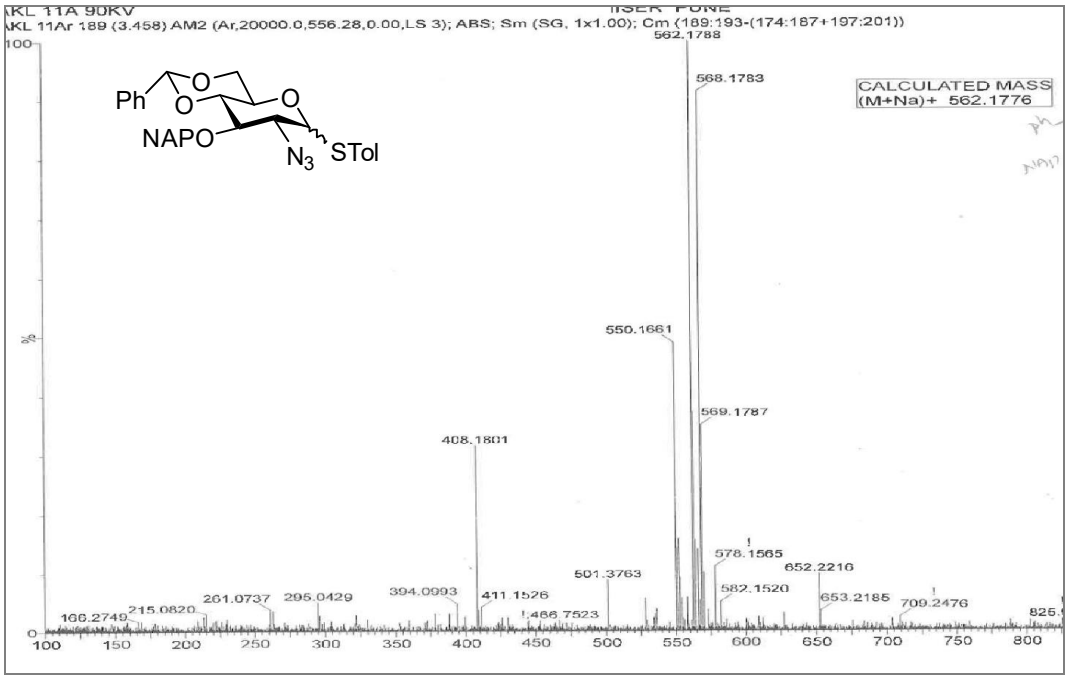
^{1}H NMR

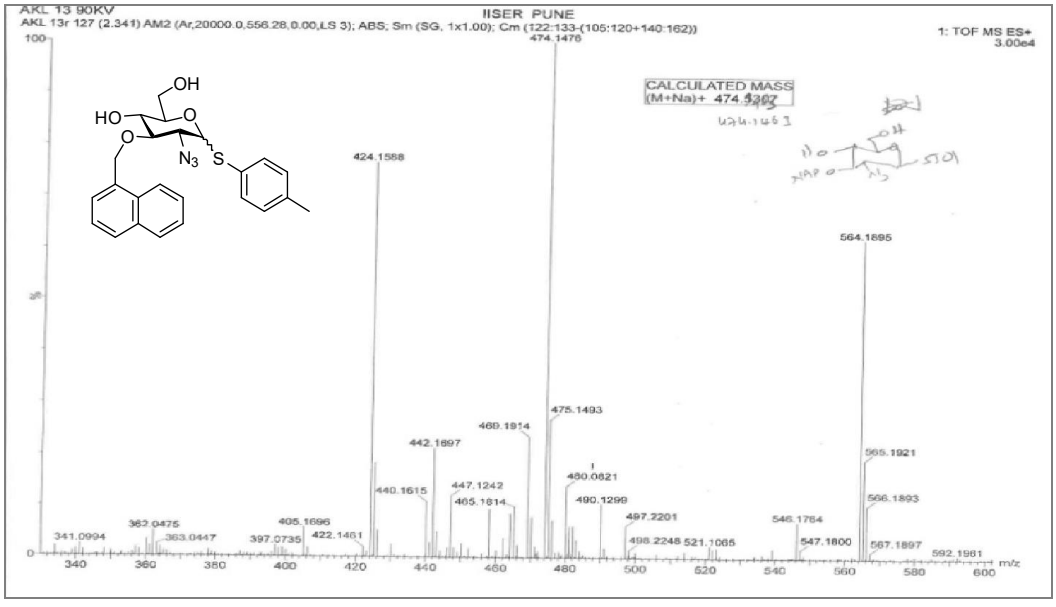

20170107-AKL-26/1
AKL-26
OTBDPS
LevO
NAPO
TrocHN
O
O
NHCBz
f1 (ppm)

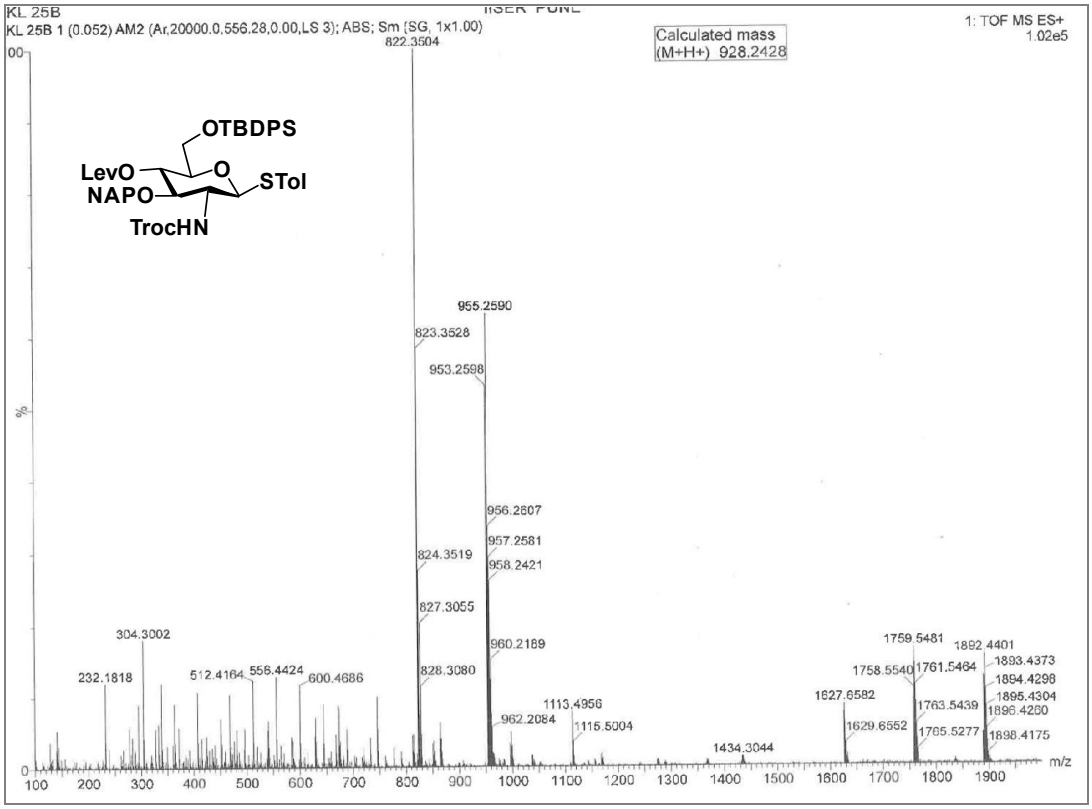
HRMS Data


AKL 03 121 (2.218) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (119:126-(103:117+130:151))
CALCULATED MASS
(M+Na)+ 460.1154
OAc
AcO
AcO
O
N3
STol
460.1151
455.1599
308.0953
350.1062
451.1520
450.1191
461.1180
410.1269
351.1040
309.0967
427.1303
391.0829
550.1563
462.1155
520.1223
530.0588
368.1153
329.1348
378.1124
467.0629
551.1602
484.0566


AKL 04N 127 (2.341) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (126:130-(119:125+132:138
Ph
O
O
O
HO
N3
STol
422.1153
284.3331
395.0930
423.1183
137.0433
285.3357
165.0378
287.1267
424.1152
204.1388
267.0722
294.0909
490.1035
372.1253
327.0762
444.0975
491.1037
552.0804


IISER PUNE
AKL 11Ar 189 (3.458) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (189:193-(174:187+197:201))
Ph
O
O
O
NAPO
N3
STol
CALCULATED MASS
(M+Na)+ 562.1776
562.1788
568.1783
550.1661
569.1787
408.1801
578.1565
652.2216
501.3763
582.1520
653.2185
709.2476
394.0993
411.1526
486.7523
261.0737
295.0429
215.0820
166.2749
%
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800


AKL 13 90KV
IISER PUNE
AKL 13r 127 (2.341) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (122:133-(105:120+140:162))
1: TOF MS ES+
3.00e4
OH
HO
O
O
N3
S
CALCULATED MASS
(M+Na)+ 474.1476
474.1476
424.1588
564.1895
475.1493
469.1914
442.1897
565.1921
480.0821
440.1615
447.1242
465.1814
490.1299
566.1893
497.2201
546.1764
547.1800
567.1897
592.1981
498.2248
521.1065
405.1696
422.1461
397.0735
362.0475
363.0447
341.0994
%
340
360
380
400
420
440
460
480
500
520
540
560
580
600
m/z


KL 25B
KL 25B 1 (0.052) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00)
Calculated mass
(M+H+) 928.2428
1: TOF MS ES+
1.02e5
OTBDPS
LevO
O
STol
NAPO
TrocHN
822.3504
955.2590
823.3528
953.2598
956.2607
957.2581
824.3519
958.2421
827.3055
304.3002
960.2189
232.1818
512.4164
556.4424
600.4686
828.3080
1759.5481
1892.4401
1893.4373
1758.5540
1761.5464
1894.4298
1627.6582
1763.5439
1895.4304
1896.4280
962.2084
1113.4956
1115.5004
1629.6552
1765.5277
1434.3044
1898.4175
%
m/z
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900

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